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(54) **RNA-TARGETING FUSION PROTEIN COMPOSITIONS AND METHODS FOR USE**

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C12N 9/22	(2006.01)
C12N 15/90	(2006.01)

(52) **U.S. Cl.**

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(57) **ABSTRACT**

Disclosed are compositions 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. Methods of making and methods of using compositions of the disclosure are also provided. For example, compositions of the disclosure may be used in the treatment of a disease or disorder in a subject. Exemplary disease or disorders of the disclosure include genetic and epigenetic diseases or disorders.

Specification includes a Sequence Listing.

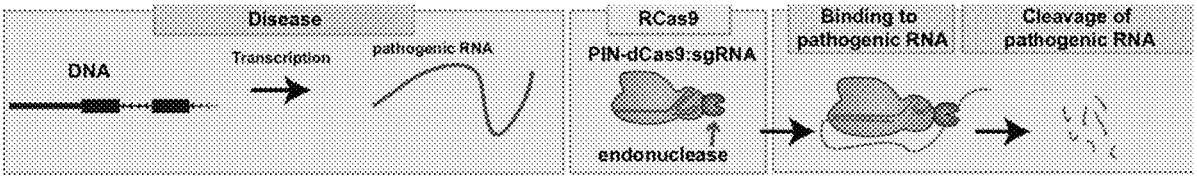


FIG. 1A

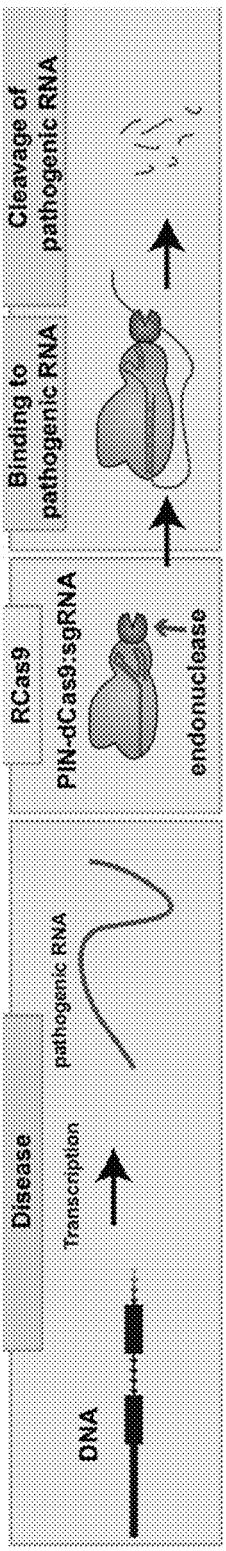

Disease
DNA
Transcription
pathogenic RNA
RCas9
PIN-dCas9:sgRNA
endonuclease
Binding to pathogenic RNA
Cleavage of pathogenic RNA

FIG. 1B

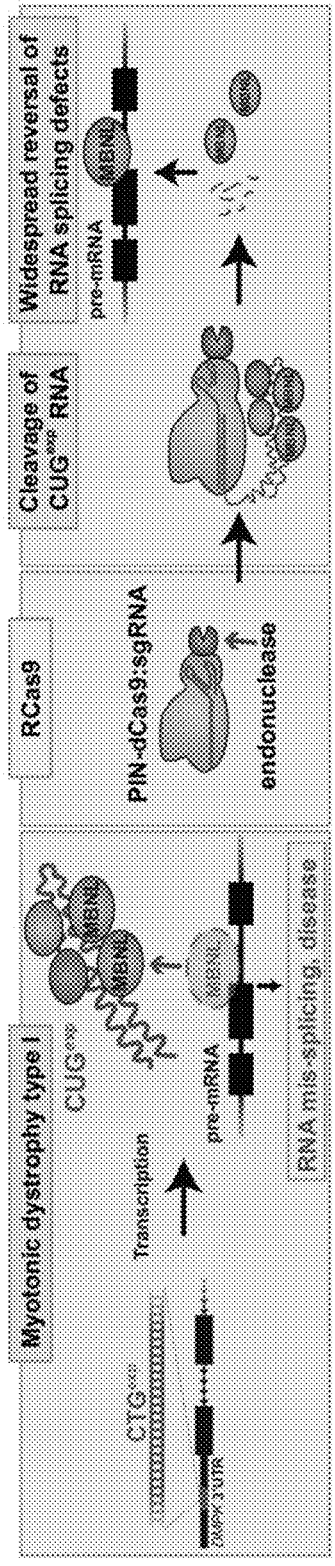

Myotonic dystrophy type I
CTG exp
Transcription
pre-mRNA
RNA mis-splicing disease
RCas9
PIN-dCas9:sgRNA
endonuclease
Cleavage of CUG exp RNA
Widespread reversal of RNA splicing defects
pre-mRNA

FIG. 2

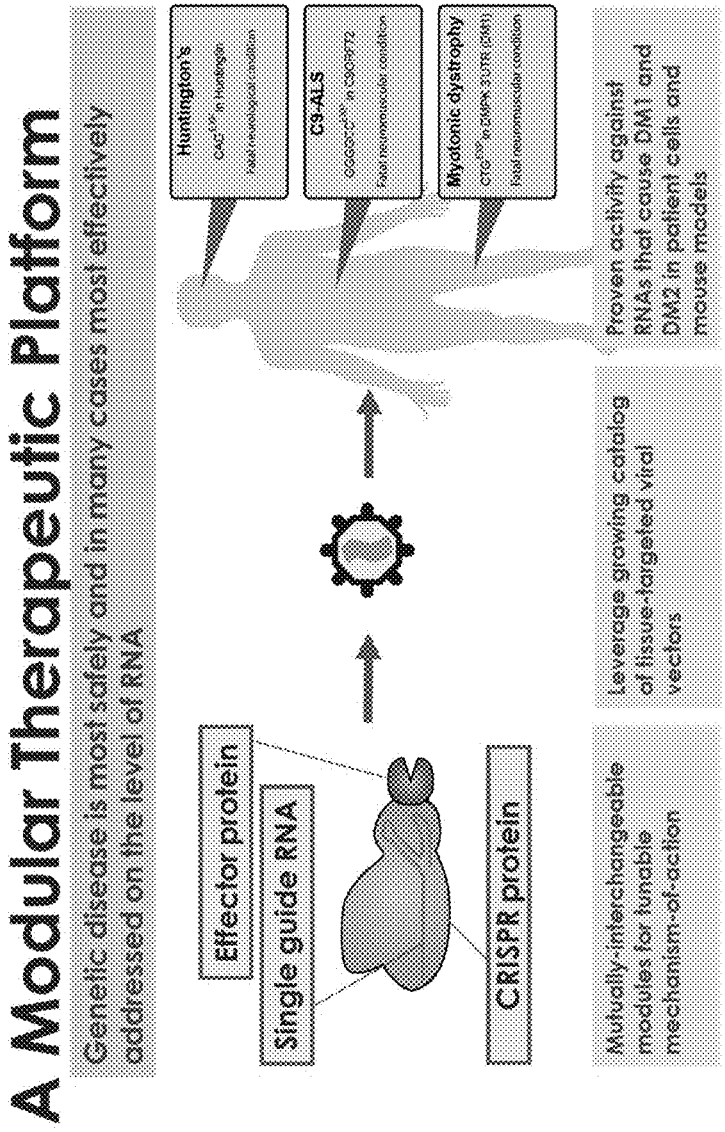
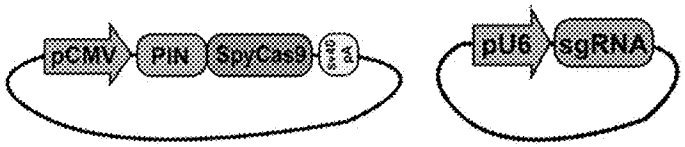

A Modular Therapeutic Platform
Genetic disease is most safely and in many cases most effectively addressed on the level of RNA
Effector protein
Single guide RNA
CRISPR protein
Mutually-interchangeable modules for tunable mechanism-of-action
Leverage growing catalog of tissue-targeted viral vectors
Proven activity against RNAs that cause DM1 and DM2 in patient cells and mouse models
Huntington's
C9-ALS
Myotonic dystrophy

FIG. 3A

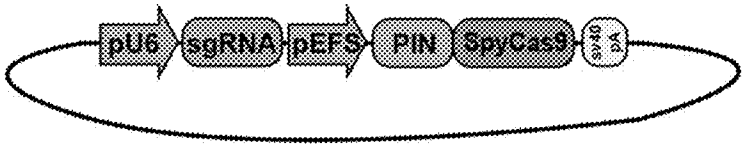
High expression positive control: "pos control"



Two plasmid system with PIN-SpyCas9 driven by a highly-active CMV promoter. This promoter is 5-20x as active as EFS.

FIG. 3B

Low expression positive control: "P13"



Single plasmid system with lower expression of the fusion. Same architecture as the fusions involving new endonucleases

FIG. 4A

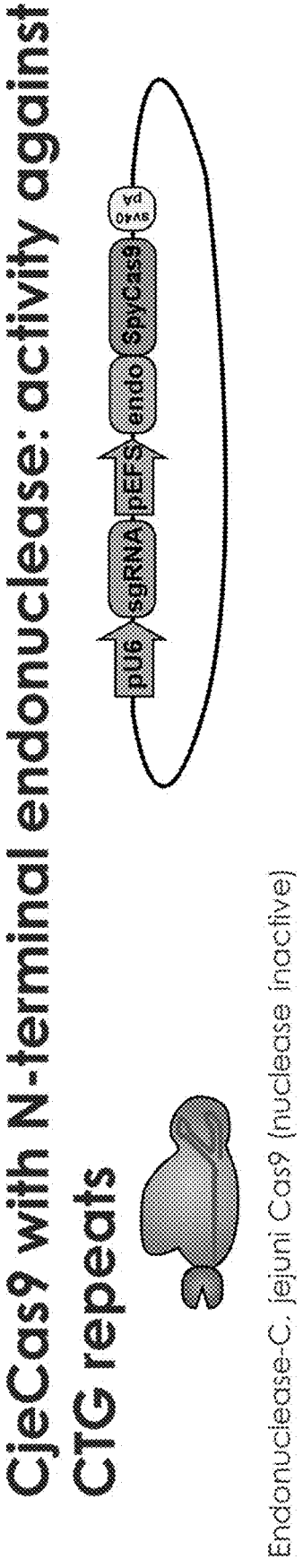

CjeCas9 with N-terminal endonuclease: activity against CTG repeats
pU6
sgRNA
pEFS
endo
SpyCas9
SV40 pA
Endonuclease-C. jejuni Cas9 (nuclease inactive)

FIG. 4B

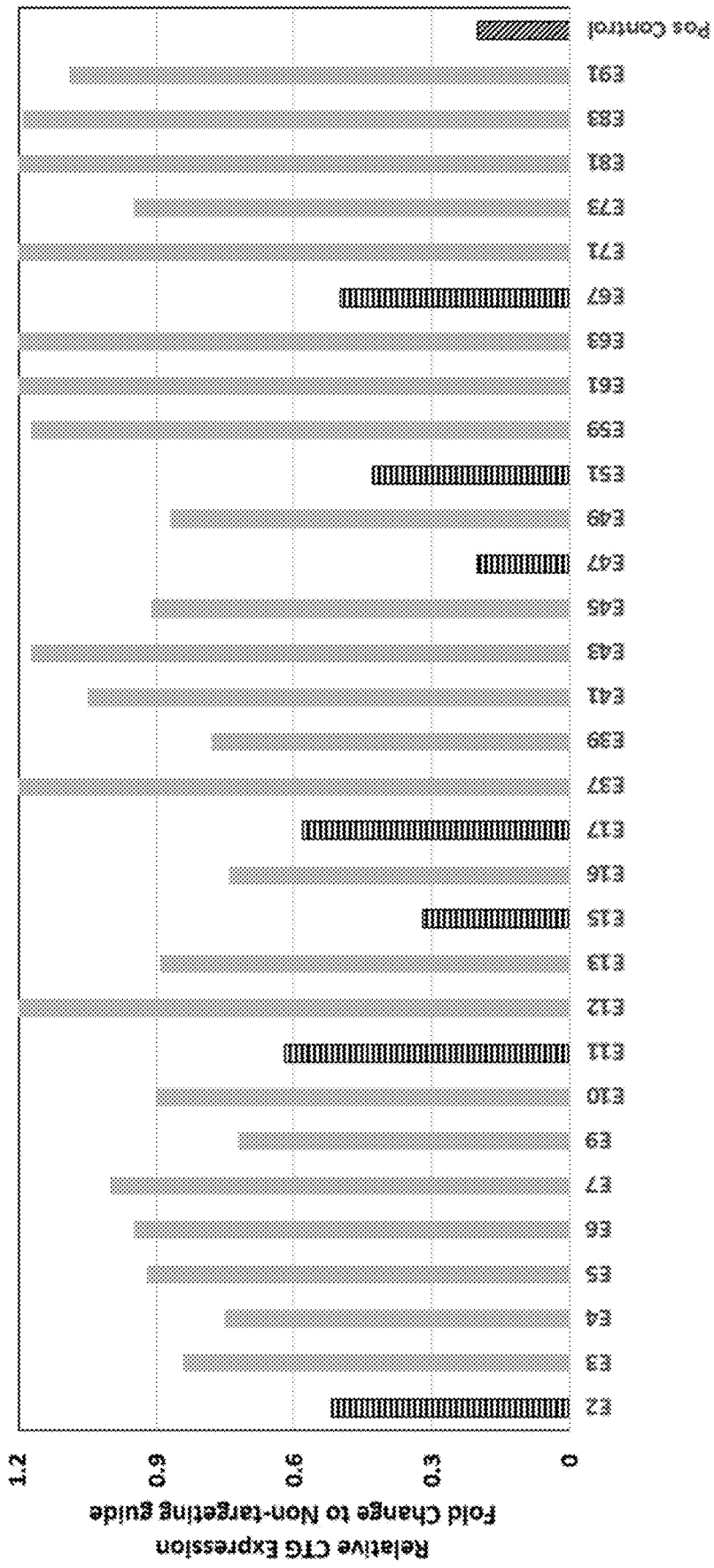

Relative CTG Expression
Fold Change to Non-targeting guide
1.2
0.9
0.6
0.3
0
E2
E3
E4
E5
E6
E7
E9
E10
E11
E12
E13
E15
E16
E17
E37
E39
E41
E43
E45
E47
E49
E51
E59
E61
E63
E67
E71
E73
E81
E83
E91
Pos Control

FIG. 5A

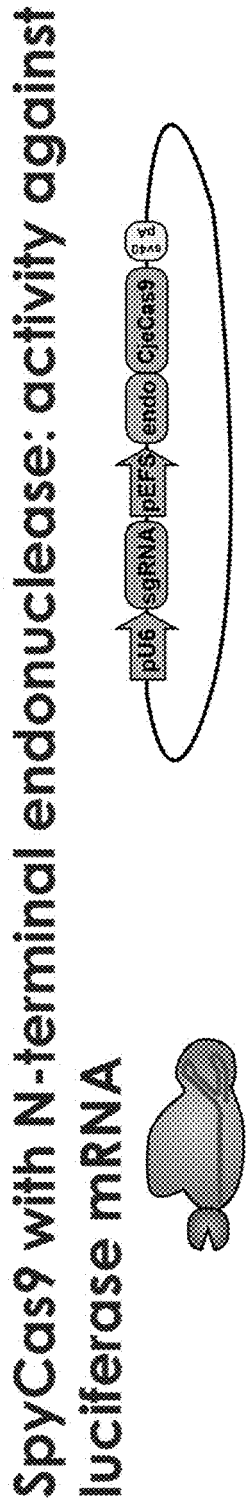

SpyCas9 with N-terminal endonuclease: activity against luciferase mRNA
hU6
sgRNA
pEFS
endo
CjeCas9
Endonuclease-C. jejuni Cas9 (nuclease inactive)

FIG. 5B

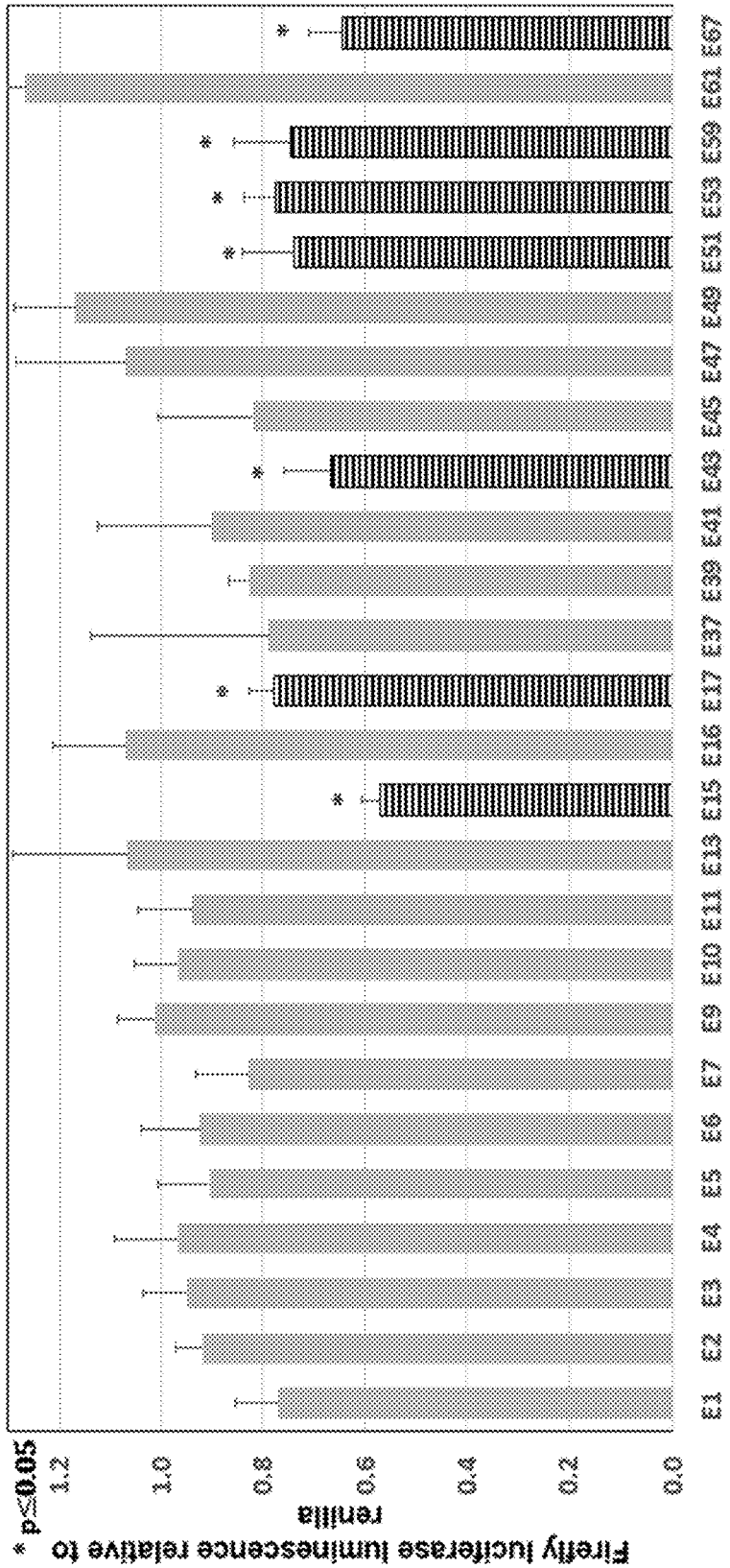

Firefly luciferase luminescence relative to renilla
* p≤0.05
1.2
1.0
0.8
0.6
0.4
0.2
0.0
E1
E2
E3
E4
E5
E6
E7
E9
E10
E11
E13
E15
E16
E17
E37
E39
E41
E43
E45
E47
E49
E51
E53
E59
E61
E67

FIG. 6

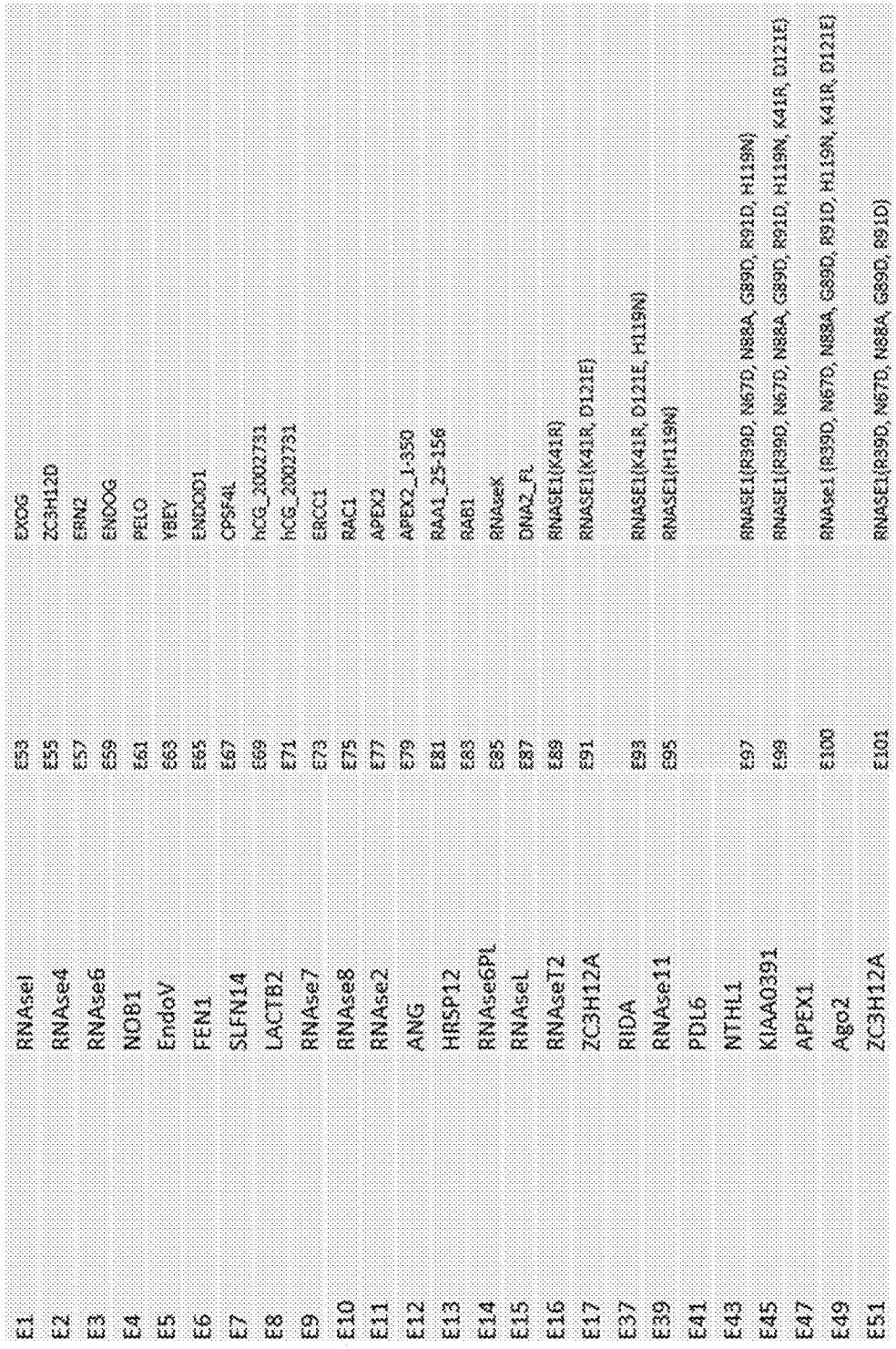

Endonuclease Name Key
E1 RNAseI
E2 RNAse4
E3 RNAse6
E4 NO81
E5 EndoV
E6 FEN1
E7 SLFN14
E8 LACTB2
E9 RNAse7
E10 RNAse8
E11 RNAse2
E12 ANG
E13 HRSP12
E14 RNAse6PL
E15 RNAseL
E16 RNAseT2
E17 ZC3H12A
E37 RIDA
E39 RNAse11
E41 PDL6
E43 NTHL1
E45 KIAA0391
E47 APEX1
E49 Ago2
E51 ZC3H12A
E53 EXOG
E55 ZC3H12D
E57 ERN2
E59 ENDOG
E61 PELO
E63 YBEY
E65 ENDOD1
E67 CPSF4L
E69 hCG_2002731
E71 hCG_2002731
E73 ERCC1
E75 RAC1
E77 APEX2
E79 APEX2_1-350
E81 RAA1_25-156
E83 RAB1
E85 RNAseK
E87 DNA2_FL
E89 RNASE1(K41R)
E91 RNASE1(K41R, D121E)
E93 RNASE1(K41R, D121E, H119N)
E95 RNASE1(H119N)
E97 RNASE1(R39D, N67D, N88A, G89D, R91D, H119N)
E98 RNASE1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)
E100 RNAse1 (R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)
E101 RNASE1(R39D, N67D, N88A, G89D, R91D)

FIG. 7A

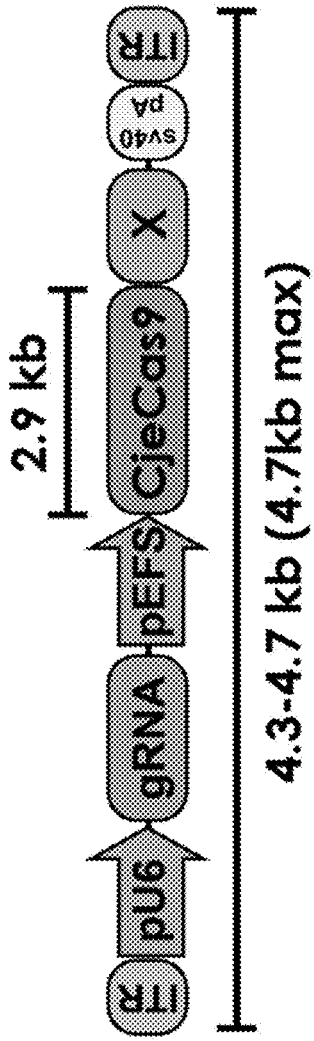

ITR
pU6
gRNA
pEFS
CjeCas9
X
SV40 pA
ITR
2.9 kb
4.3-4.7 kb (4.7kb max)
Endonuclease-
C. jejuni Cas9

FIG. 7B

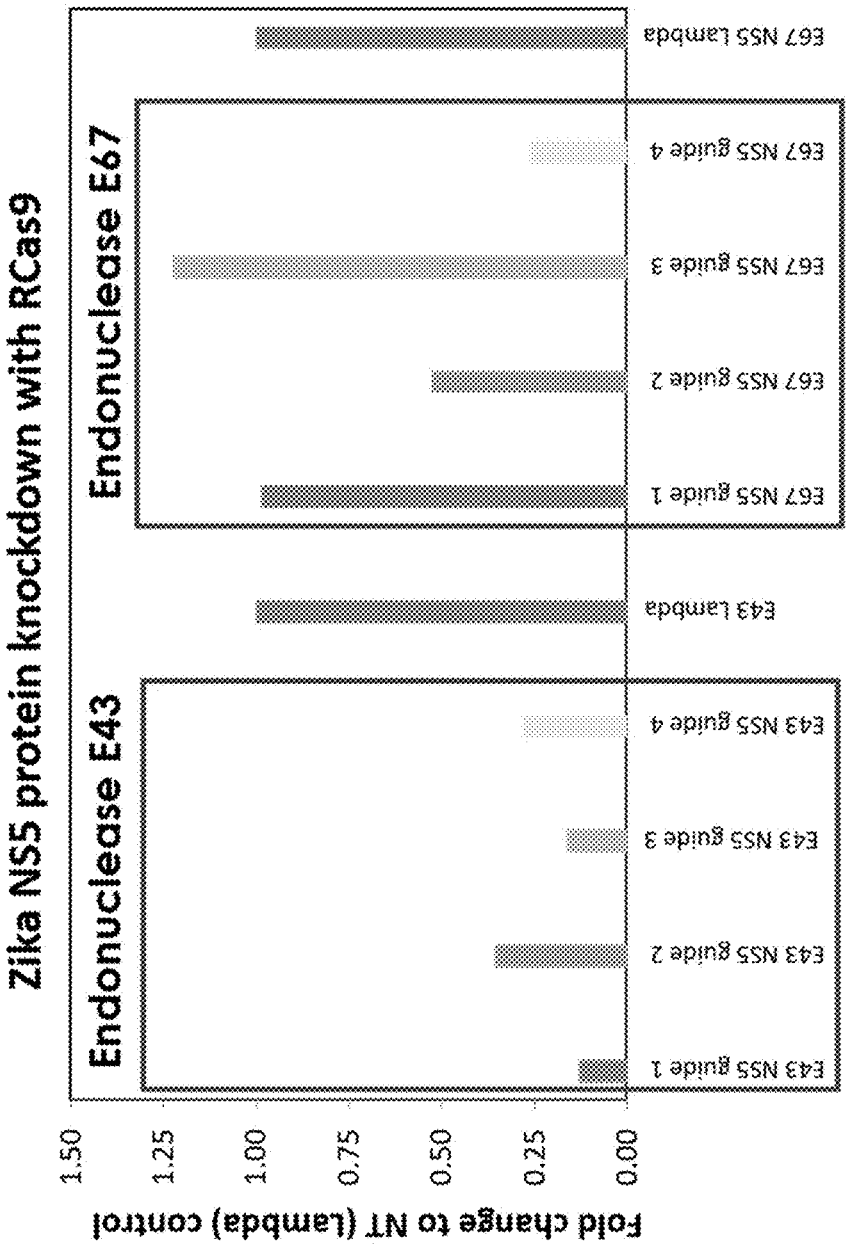
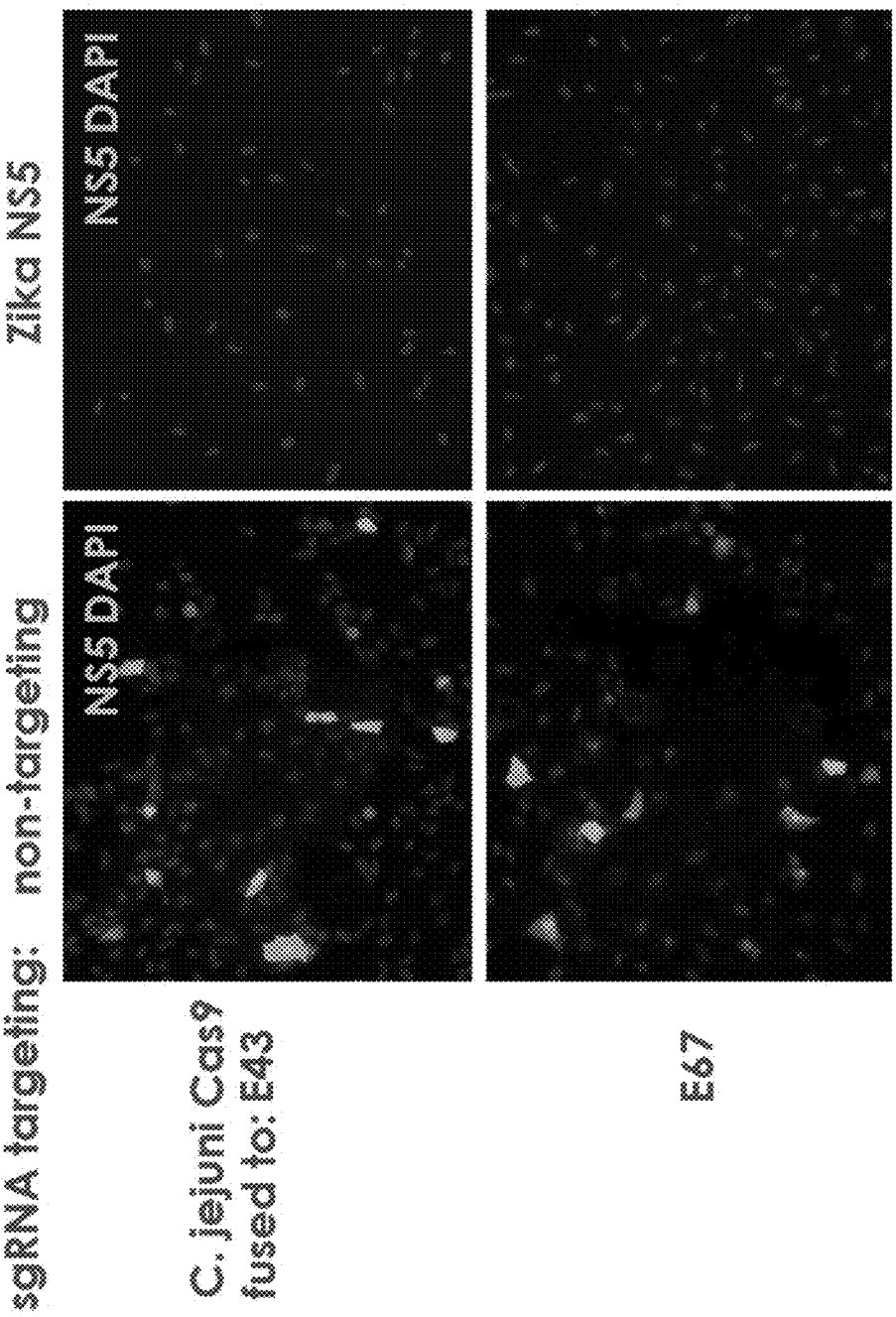

Zika NS5 protein knockdown with RCas9
Endonuclease E43
Endonuclease E67
Fold change to NT (lambda) control
1.50
1.25
1.00
0.75
0.50
0.25
0.00
E43 NS5 guide 1
E43 NS5 guide 2
E43 NS5 guide 3
E43 NS5 guide 4
E43 lambda
E67 NS5 guide 1
E67 NS5 guide 2
E67 NS5 guide 3
E67 NS5 guide 4
E67 NS5 lambda

FIG. 8A


sgRNA targeting:
non-targeting
Zika NS5
C. jejuni Cas9 fused to: E43
E67
NS5 DAPI
NS5 DAPI

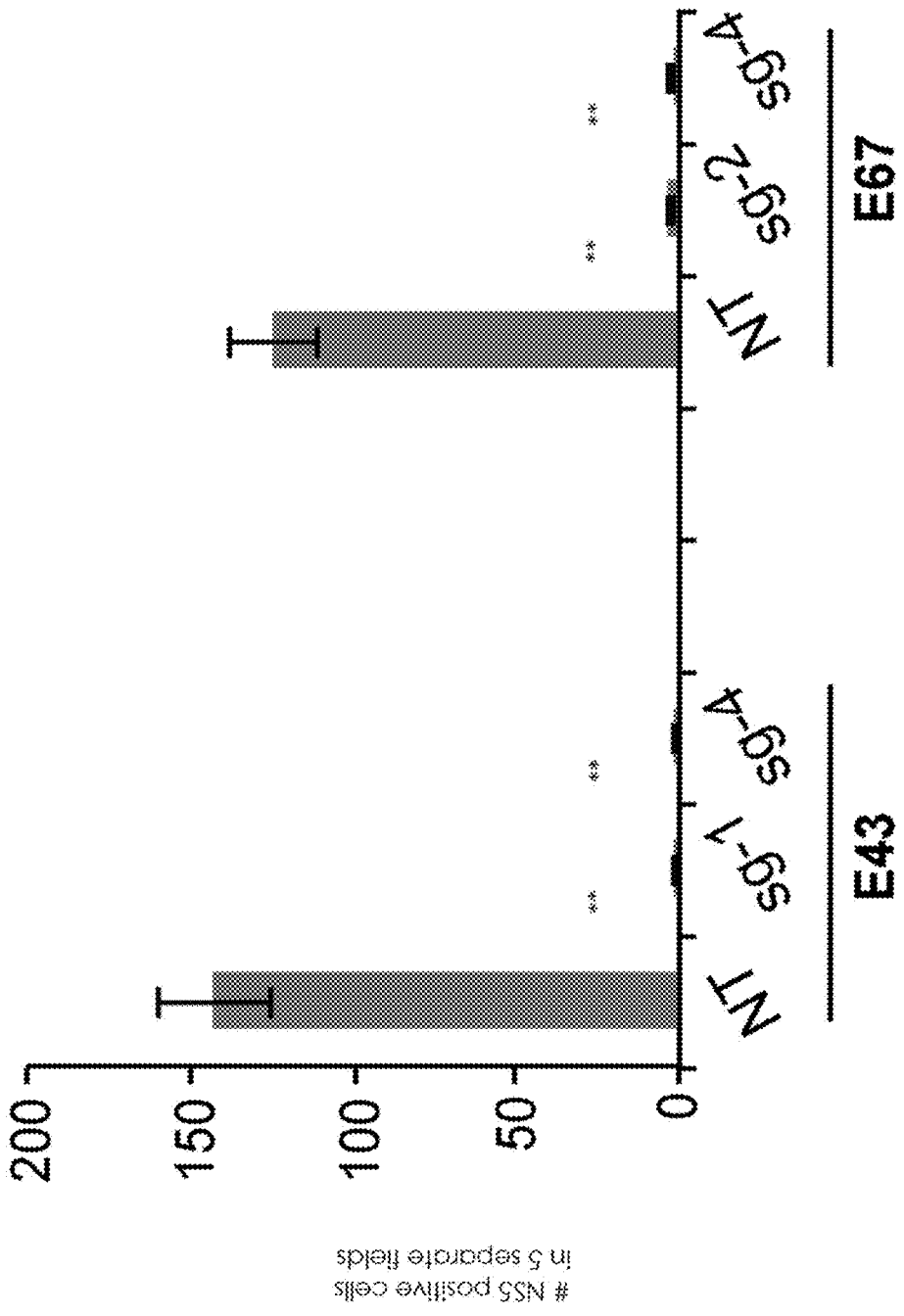
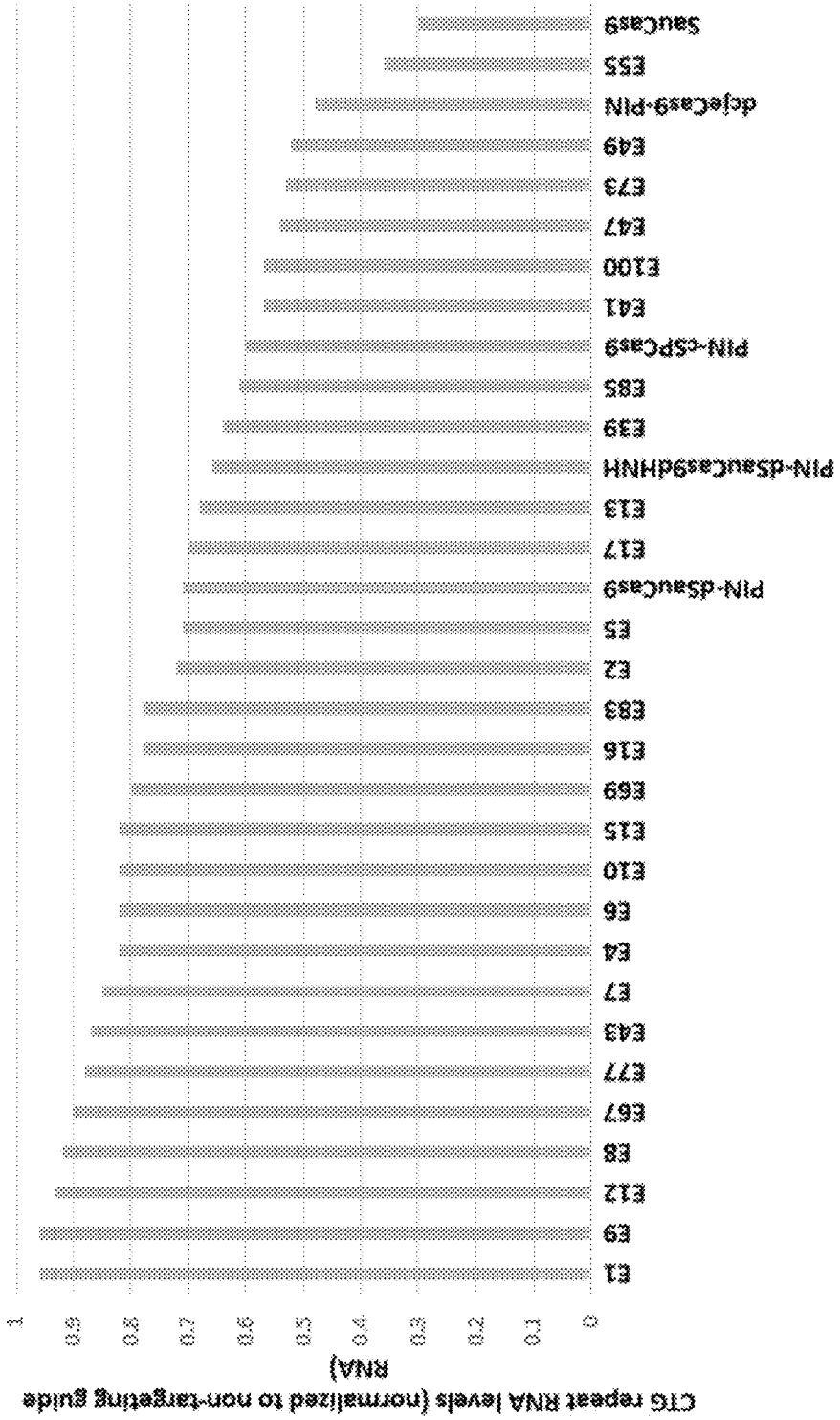

200
150
100
50
0
NS5 positive cells
in 5 separate fields
NT
sg-1
sg-4
NT
sg-2
sg-4
E43
E67
**
**
**
**

FIG. 8B

FIG. 9


CTG repeat RNA levels (normalized to non-targeting guide RNA)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
E1
E9
E12
E8
E67
E77
E43
E7
E4
E6
E10
E15
E69
E16
E83
E2
E5
PIN-dSauCas9
E17
E13
PIN-dSauCas9dHNH
E39
E85
PIN-cSPCas9
E41
E100
E47
E73
E49
dCjeCas9-PIN
E55
SauCas9

RNA-TARGETING FUSION PROTEIN COMPOSITIONS AND METHODS FOR USE

RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent Application No. 62/682,271, filed Jun. 8, 2018, the contents of which are herein incorporated by reference in their entirety. The contents of U.S. Patent Application No. 62/682,276, filed Jun. 8, 2018, are herein incorporated by reference in their entirety.

FIELD OF THE DISCLOSURE

[0002] The disclosure is directed to molecular biology, and more, specifically, to compositions and methods for modifying expression and activity of RNA molecules.

INCORPORATION OF SEQUENCE LISTING

[0003] The contents of the text file named "LOCN_002_001US_SeqList_ST25", which was created on Sep. 4, 2019 and is 773 KB in size, are hereby incorporated by reference in their entirety.

BACKGROUND

[0004] There has been a long-felt but unmet need in the art for a method of specifically binding target RNA molecules for modification of expression or activity of the RNA molecule or a protein encoded by the RNA molecule. The disclosure provides compositions and methods for specifically targeting RNA molecules in sequence-specific manner that further precludes modification of DNA sequences.

SUMMARY

[0005] 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.

[0006] The disclosure also provides a composition comprising a sequence encoding an RNA-guided 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 guided by a gRNA sequence, and wherein the second RNA-binding polypeptide comprises RNA-nuclease activity.

[0007] The disclosure additionally provides a composition comprising 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 without a gRNA sequence, and wherein the second RNA-binding polypeptide comprises RNA-nuclease activity.

[0008] In some embodiments of the compositions of the disclosure, the target sequence comprises at least one repeated sequence.

[0009] In some embodiments of the compositions of the disclosure, the sequence comprising the gRNA further comprises a sequence encoding a promoter capable of expressing the gRNA in a eukaryotic cell.

[0010] 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.

[0011] 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 is a sequence isolated or derived from a promoter capable of driving expression of a transfer RNA (tRNA). In some embodiments, the promoter 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 is isolated or derived from a valine tRNA promoter.

[0012] 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

(SEQ ID NO: 1)
UGGAGCGAGCAUCCCCCAAA,

(SEQ ID NO: 2)
GUUUGGGGGAUGCUCGCUCCA,

(SEQ ID NO: 3)
CCCUCACUGCUGGGGAGUCC,

(SEQ ID NO: 4)
GGACUCCCCAGCAGUGAGGG,

(SEQ ID NO: 5)
GCAACUGGAUCAAUUUGCUG,

-continued

(SEQ ID NO: 6)
GCAGCAAAUUGAUCCAGUUGC,

(SEQ ID NO: 7)
GCAUUCUUAUCUGGUCAGUGC,

(SEQ ID NO: 8)
GCACUGACCAGAUAAGAAUG,

(SEQ ID NO: 9)
GAGCAGCAGCAGCAGCAGCAG,

(SEQ ID NO: 10)
GCAGGCAGGCAGGCAGGCAGG,

(SEQ ID NO: 11)
GCCCCGGCCCCGGCCCCGGC,
or
(SEQ ID NO: 12)
GCTGCTGCTGCTGCTGCTGC,

(SEQ ID NO: 74)
GGGGCCGGGGCCGGGGCCGG,

(SEQ ID NO: 75)
GGGCCGGGGCCGGGGCCGGG,

(SEQ ID NO: 76)
GGCCGGGGCCGGGGCCGGGG,

(SEQ ID NO: 77)
GCCGGGGCCGGGGCCGGGGC,

(SEQ ID NO: 78)
CCGGGGCCGGGGCCGGGGCC,
or
(SEQ ID NO: 79)
CGGGGCCGGGGCCGGGGCCG.

[0013] 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.

[0014] 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

(SEQ ID NO: 14)
GUGAUAAGUGGAAUGCCAUG,

(SEQ ID NO: 15)
CUGGUGAACUUCCGAUAGUG,
or
(SEQ ID NO: 16)
GAGATATAGCCTGGTGGTTC.

[0015] 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.

[0016] 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

GUUUAAGAGCUAUGCUGGAAACAGCAUAG-
CAAGUUUAAAUAAGGCUAGUCCGUU

[0017] AUCAACUUGAAAAAGUGGCACCGAGUCG-
GUGCUUUUUUU (SEQ ID NO: 13). In some embodiments, the scaffold sequence comprises or consists of the sequence

GGACAGCAUAGCAAGUUAAAAUAAGGCUAGUC-
CGUUAUCAACUUGAAAAAGUGG

[0018] CACCGAGUCGGUGCUUUUU (SEQ ID NO: 17). In some embodiments, the scaffold sequence comprises or consists of the sequence

(SEQ ID NO: 82)
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGU

CCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU
or
(SEQ ID NO: 83)
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAA

CUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU.

[0019] 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.

[0020] In some embodiments of the compositions of the disclosure, an RNA genome or an RNA transcriptome comprises the RNA molecule.

[0021] 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.

[0022] 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.

[0023] 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 CasRx/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.

[0024] In some embodiments of the compositions of the disclosure, 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.

[0025] 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.

[0026] 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.

[0027] In some embodiments of the compositions of the disclosure, an RNA genome or an RNA transcriptome comprises the RNA molecule.

[0028] 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.

[0029] In some embodiments of the compositions of the disclosure, the sequence encoding the first RNA binding protein further comprises a sequence encoding a nuclear localization signal (NLS), a nuclear export signal (NES) or tag. 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.

[0030] 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.

[0031] 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.

[0032] 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. In some embodiments, the RNAse1 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 comprises or consists of SEQ ID NO: 21. In some embodi-

ments, the second RNA binding protein comprises or consists of an RNAse6. In some embodiments, the RNAse6 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 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 protein 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.

[0033] 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 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)) polypeptide comprises or consists of SEQ ID NO: 66. 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 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 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 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 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 SEQ ID NO: 122.

[0034] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a NOB 1 polypeptide. In some embodiments, the NOB 1 polypeptide comprises or consists of SEQ ID NO: 31.

[0035] 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 protein 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 protein 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 protein comprises or consists of SEQ ID NO: 34. In some embodiments, the second RNA binding protein comprises or consists of a Human flap endonuclease-1 (hFEN1). In some embodiments, the hFEN1 protein comprises or consists of 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 protein comprises or consists of SEQ ID NO: 64.

[0036] 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 SEQ ID NO: 123.

[0037] 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 SEQ ID NO: 36.

[0038] 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 SEQ ID NO: 37.

[0039] 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 SEQ ID NO: 38. In some embodiments, the APEX2 polypeptide comprises or consists of 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 SEQ ID NO: 125.

[0040] 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 SEQ ID NO: 40.

[0041] 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 SEQ ID NO: 41.

[0042] 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 SEQ ID NO: 42. In some embodiments, the ZC3H12A polypeptide comprises or consists of SEQ ID NO: 43.

[0043] 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 SEQ ID NO: 44.

[0044] 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 SEQ ID NO: 126.

[0045] 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 SEQ ID NO: 127.

[0046] 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 SEQ ID NO: 128.

[0047] 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 SEQ ID NO: 129.

[0048] 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 SEQ ID NO: 130.

[0049] 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 SEQ ID NO: 131.

[0050] 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 SEQ ID NO: 132.

[0051] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a YBEY metallopeptidase (YBEY) polypeptide. In some embodiments, the YBEY polypeptide comprises or consists of SEQ ID NO: 133.

[0052] 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 polypeptide comprises or consists of SEQ ID NO: 134.

[0053] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an hCG_200273 ipolypeptide. In some embodiments, the hCG_2002731 comprises or consists of SEQ ID NO: 135. In some embodiments, the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 136.

[0054] 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 SEQ ID NO: 137.

[0055] 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 SEQ ID NO: 138.

[0056] 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 SEQ ID NO: 139.

[0057] 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 SEQ ID NO: 140.

[0058] 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 SEQ ID NO: 141.

[0059] 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 SEQ ID NO: 142.

[0060] 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 SEQ ID NO: 143.

[0061] 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 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 SEQ ID NO: 145.

[0062] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Ribonuclease Kappa (RNAseK) polypeptide. In some embodiments, the RNAseK polypeptide comprises or consists of SEQ ID NO: 204.

[0063] In some embodiments, the fusion proteins of the disclosure are used in methods for treating a subject in need

thereof, the methods comprising contacting a target RNA with a fusion protein or the sequence encoding the fusion protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0064] 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.

[0065] FIGS. **1**A-B is a schematic diagram of an exemplary embodiment of a composition of the disclosure. (FIG. **1**A) An RNA-targeting Cas9 system fused to an endonuclease targets and cleaves a disease-causing RNA. (FIG. **1**B) Depicts an application of (A) in the context of myotonic dystrophy type 1, wherein an RNA-targeting Cas9 system fused to an endonuclease targets and cleaves a repetitive RNA composed of repeating CUG units. In the absence of the RNA-targeting Cas9 system, the repetitive RNA composed of repeating CUG units binds to a splicing factor MBNL and causes pathology via dysfunctional RNA splicing. Cleavage of this repetitive RNA ameliorates disease.

[0066] FIG. **2** is a schematic diagram depicting an exemplary modular therapeutic platform for treating genetic disease by targeting RNA molecules.

[0067] FIGS. **3**A-B is a pair of schematic diagrams depicting (FIG. **3**A) a "high expression" control system (also referred to as "pos control") comprising a two plasmid system comprising a cytomegalovirus promoter driving expression of the RNA endonuclease/Cas9 fusion and (FIG. **3**B) a "low expression" control system (also referred to as "P13") comprising a single plasmid system comprising a lower-expression promoter (pEFS) driving expression of the RNA endonuclease/Cas9 fusion.

[0068] FIG. **4**A is a pair of schematic diagrams depicting an exemplary RNA Endonuclease-*C. jejuni* Cas9 fusion protein (left) and a vector comprising an exemplary RNA Endonuclease-*S. pyogenes* Cas9 fusion protein (right)

[0069] FIG. **4**B is a graph depicting the ability of a variety of fusion proteins comprising either *C. jejuni* Cas9 or *S. pyogenes* Cas9, as shown in FIG. **4**A, to cleave repetitive RNA molecules.

[0070] FIG. **5**A is a pair of schematic diagrams depicting an exemplary RNA Endonuclease-*C. jejuni* Cas9 fusion protein (left) and a vector comprising an exemplary RNA Endonuclease-*S. pyogenes* Cas9 fusion protein (right)

[0071] FIG. **5**B is a graph depicting the ability of a variety of fusion proteins comprising either *C. jejuni* Cas9 or *S. pyogenes* Cas9, as shown in FIG. **5**A, to cleave mRNA molecules encoding a luciferase protein.

[0072] FIG. **6** is a table providing a key to the endonucleases shown in FIGS. **4**B, **5**B, and **9**.

[0073] FIG. **7**A is a schematic diagram depicting an exemplary RNA Endonuclease-*C. jejuni* Cas9 fusion protein.

[0074] FIG. **7**B 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 2. Zika NS5 expression is displayed as fold change relative to the endonuclease loaded with an sgRNA containing a control (Lambda) spacer sequence.

[0075] FIG. **8**A is a fluorescence microscopy image of cells transfected with CjeCas9-endonuclease fusions loaded with an sgRNA containing a Zika NS5-targeting spacer sequence.

[0076] FIG. **8**B 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 a CjeCas9-endonuclease fusions loaded with a non-Zika NS5 targeting sgRNA.

[0077] FIG. **9** is a graph depicting the cleavage efficiencies of a variety of exemplary fusion proteins (SpyCas9 fused to the annotated endonuclease).

DETAILED DESCRIPTION

[0078] The disclosure provides an RNA-guided fusion protein that selectively binds and, optionally, cleaves RNA molecules. The disclosure provides vectors, compositions and cells comprising the RNA-guided fusion protein. The disclosure provides methods of using the RNA-guided fusion protein, vectors, compositions and cells of the disclosure to treat a disease or disorder.

Guide RNA

[0079] The terms guide RNA (gRNA) and single guide RNA (sgRNA) are used interchangeably throughout the disclosure.

[0080] 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 not 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, a sequence encoding a guide RNA or single 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.

[0081] 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-hydropxymethylcytosine, isoguanine, and isocytosine.

[0082] 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).

[0083] 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).

[0084] 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.

[0085] 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).

[0086] 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.

[0087] 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.

[0088] 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 spacer 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.

[0089] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof does not comprise a nuclear localization sequence (NLS).

[0090] In some embodiments of the compositions of the disclosure, a guide RNA or a portion thereof does not comprise a sequence complementary to a protospacer adjacent motif (PAM).

[0091] 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 oligo-

nucleotide. 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'.

[0092] 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.

[0093] In some embodiments of the compositions of the disclosure, guide RNA sequence of the disclosure comprises a promoter sequence to drive expression of the guide RNA. In some embodiments, a vector comprising a guide RNA sequence of the disclosure comprises a promoter sequence to drive expression of the guide RNA. In some embodiments, the promoter to drive expression of the guide RNA is a constitutive promoter. In some embodiments, the promoter sequence is an inducible promoter. In some embodiments, the promoter is a sequence is a tissue-specific and/or cell-type specific promoter. In some embodiments, the promoter is a hybrid or a recombinant promoter. In some embodiments, the promoter is a promoter capable of expressing the guide RNA in a mammalian cell. In some embodiments, the promoter is a promoter capable of expressing the guide RNA in a human cell. In some embodiments, the promoter is a promoter capable of expressing the guide RNA and restricting the guide RNA to the nucleus of the cell. In some embodiments, the promoter is a human RNA polymerase promoter or a sequence isolated or derived from a sequence encoding a human RNA polymerase promoter. In some embodiments, the promoter is a U6 promoter or a sequence isolated or derived from a sequence encoding a U6 promoter. In some embodiments, the promoter is a human tRNA promoter or a sequence isolated or derived from a sequence encoding a human tRNA promoter. In some embodiments, the promoter is a human valine tRNA promoter or a sequence isolated or derived from a sequence encoding a human valine tRNA promoter.

[0094] In some embodiments of the compositions of the disclosure, a promoter to drive expression of the guide RNA further comprises a regulatory element. In some embodiments, a vector comprising a promoter sequence to drive expression of the guide RNA 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.

[0095] In some embodiments of the compositions of the disclosure, a vector of the disclosure comprises one or more of a sequence encoding a guide RNA, a promoter sequence to drive expression of the guide RNA and a sequence encoding a regulatory element. In some embodiments of the compositions of the disclosure, the vector further comprises a sequence encoding a fusion protein of the disclosure.

Fusion Proteins

[0096] 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.

[0097] 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.

[0098] 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.

[0099] 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.

[0100] 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.

[0101] 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, *Nitratifractor salsuginis* str. DSM 16511, *Parvibaculum lavamentivorans, Roseburia intestinalis, Neisseria cinerea*, a *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.*

[0102] Exemplary wild type *S. pyogenes* Cas9 proteins of the disclosure may comprise or consist of the amino acid sequence:

(SEQ ID NO: 147)

```
   1 MDKKYSIGLD IGTNSVGWAV ITDEYKVPSK KFKVLGNTDR HSIKKNLIGA LLFDSGETAE
  61 ATRLKRTARR RYTRRKNRIC YLQEIFSNEM AKVDDSFFHR LEESFLVEED KKHERHPIFG
 121 NIVDEVAYHE KYPTIYHLRK KLVDSTDKAD LRLIYLALAH MIKFRGHFLI EGDLNPDNSD
 181 VDKLFIQLVQ TYNQLFEENP INASGVDAKA ILSARLSKSR RLENLIAQLP GEKKNGLFGN
 241 LIALSLGLTP NFKSNFDLAE DAKLQLSKDT YDDDLDNLLA QIGDQYADLF LAAKNLSDAI
 301 LLSDILRVNT EITKAPLSAS MIKRYDEHHQ DLTLLKALVR QQLPEKYKEI FFDQSKNGYA
 361 GYIDGGASQE EFYKFIKPIL EKMDGTEELL VKLNREDLLR KQRTFDNGSI PHQIHLGELH
 421 AILRRQEDFY PFLKDNREKI EKILTFRIPY YVGPLARGNS RFAWMTRKSE ETITPWNFEE
 481 VVDKGASAQS FIERMTNFDK NLPNEKVLPK HSLLYEYFTV YNELTKVKYV TEGMRKPAFL
 541 SGEQKKAIVD LLFKTNRKVT VKQLKEDYFK KIECFDSVEI SGVEDRFNAS LGTYHDLLKI
 601 IKDKDFLDNE ENEDILEDIV LTLTLFEDRE MIEERLKTYA HLFDDKVMKQ LKRRRYTGWG
 661 RLSRKLINGI RDKQSGKTIL DFLKSDGFAN RNFMQLIHDD SLTFKEDIQK AQVSGQGDSL
 721 HEHIANLAGS PAIKKGILQT VKVVDELVKV 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 QFYKVREINN YHHAHDAYLN AVVGTALIKK YPKLESEFVY GDYKVYDVRK
1021 MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF
1081 ATVRKVLSMP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA
1141 YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDLIIKLPK
1201 YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE
1261 QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRDK PIREQAENII HLFTLTNLGA
1321 PAAFKYFDTT IDRKRYTSTK EVLDATLIHQ SITGLYETRI DLSQLGGD.
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[0103] Nuclease inactivated *S. pyogenes* Cas9 proteins may comprise a substitution of an Alanine (A) for an 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):

(SEQ ID NO: 148)

```
   1 MDKKYSIGL<u>**A**</u> IGTNSVGWAV ITDEYKVPSK KFKVLGNTDR HSIKKNLIGA LLFDSGETAE

  61 ATRLKRTARR RYTRRKNRIC YLQEIFSNEM AKVDDSFFHR LEESFLVEED KKHERHPIFG

 121 NIVDEVAYHE KYPTIYHLRK KLVDSTDKAD LRLIYLALAH MIKFRGHFLI EGDLNPDNSD

 181 VDKLFIQLVQ TYNQLFEENP INASGVDAKA ILSARLSKSR RLENLIAQLP GEKKNGLFGN

 241 LIALSLGLTP NFKSNFDLAE DAKLQLSKDT YDDDLDNLLA QIGDQYADLF LAAKNLSDAI

 301 LLSDILRVNT EITKAPLSAS MIKRYDEHHQ DLTLLKALVR QQLPEKYKEI FFDQSKNGYA

 361 GYIDGGASQE EFYKFIKPIL EKMDGTEELL VKLNREDLLR KQRTFDNGSI PHQIHLGELH

 421 AILRRQEDFY PFLKDNREKI EKILTFRIPY YVGPLARGNS RFAWMTRKSE ETITPWNFEE

 481 VVDKGASAQS FIERMTNFDK NLPNEKVLPK HSLLYEYFTV YNELTKVKYV TEGMRKPAFL

 541 SGEQKKAIVD LLFKTNRKVT VKQLKEDYFK KIECFDSVEI SGVEDRFNAS LGTYHDLLKI

 601 IKDKDFLDNE ENEDILEDIV LTLTLFEDRE MIEERLKTYA HLFDDKVMKQ LKRRRYTGWG

 661 RLSRKLINGI RDKQSGKTIL DFLKSDGFAN RNFMQLIHDD SLTFKEDIQK AQVSGQGDSL

 721 HEHIANLAGS PAIKKGILQT VKVVDELVKV MGRHKPENIV IEMARENQTT QKGQKNSRER

 781 MKRIEEGIKE LGSQILKEHP VENTQLQNEK LYLYYLQNGR DMYVDQELDI NRLSDYDVD<u>**A**</u>

 841 IVPQSFLKDD SIDNKVLTRS DKNRGKSDNV PSEEVVKKMK NYWRQLLNAK LITQRKFDNL

 901 TKAERGGLSE LDKAGFIKRQ LVETRQITKH VAQILDSRMN TKYDENDKLI REVKVITLKS

 961 KLVSDFRKDF QFYKVREINN YHHAHDAYLN AVVGTALIKK YPKLESEFVY GDYKVYDVRK

1021 MIAKSEQEIG KATAKYFFYS NIMNFFKTEI TLANGEIRKR PLIETNGETG EIVWDKGRDF

1081 ATVRKVLSMP QVNIVKKTEV QTGGFSKESI LPKRNSDKLI ARKKDWDPKK YGGFDSPTVA

1141 YSVLVVAKVE KGKSKKLKSV KELLGITIME RSSFEKNPID FLEAKGYKEV KKDLIIKLPK

1201 YSLFELENGR KRMLASAGEL QKGNELALPS KYVNFLYLAS HYEKLKGSPE DNEQKQLFVE

1261 QHKHYLDEII EQISEFSKRV ILADANLDKV LSAYNKHRDK PIREQAENII HLFTLTNLGA

1321 PAAFKYFDTT IDRKRYTSTK EVLDATLIHQ SITGLYETRI DLSQLGGD.
```

[0104] 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 ββα-metal fold or a portion thereof comprising a DNAse active site or any combination thereof.

[0105] Other exemplary Cas9 proteins or portions thereof may comprise or consist of the following amino acid sequences.

[0106] In some embodiments the Cas9 protein can be *S. pyogenes* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 149)

```
MDKKYSIGLDIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGA

LLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHR
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LEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKAD

LRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENP

INASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTP
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```
NFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAI

LLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEI

FFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLR

KQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPY

YVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDK

NLPNEKVLPKHSLLYEYFTVYNELTKVKYVIEGMRKPAFLSGEQKKAIVD

LLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFNASLGTYHDLLKI

IKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQ

LKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDD
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SLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKV

MGRHKPENIVIEMARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHP

VENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVDHIVPQSFLKDD

SIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNL

TKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI

REVKVITLKSKLVSDFRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKK

YPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEI

TLANGEIRKRPLIETNGETGEIVVVDKGRDFATVRKVLSMPQVNIVKKTE

VQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKV

EKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLP

KYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSP

EDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRD

KPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIH

QSITGLYETRIDLSQLGGD

[0107] In some embodiments the Cas9 protein can be *S. aureus* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 150)

MKRNYILGLDIGITSVGYGIIDYETRDVIDAGVRLFKEANVENNEGRRSK

RGARRLKRRRRHRIQRVKKLLFDYNLLTDHSELSGINPYEARVKGLSQKL

SEEEFSAALLHLAKRRGVHNVNEVEEDTGNELSTKEQISRNSKALEEKYV

AELQLERLKKDGEVRGSINRFKTSDYVKEAKQLLKVQKAYHQLDQSFIDT

YIDLLETRRTYYEGPGEGSPFGWKDIKEWYEMLMGHCTYFPEELRSVKYA

YNADLYNALNDLNNLVITRDENEKLEYYEKFQIIENVFKQKKKPTLKQIA

KEILVNEEDIKGYRVTSTGKPEFTNLKVYHDIKDITARKEIIENAELLDQ

IAKILTIYQSSEDIQEELTNLNSELTQEEIEQISNLKGYTGTHNLSLKAI

NLILDELWHTNDNQIAIFNRLKLVPKKVDLSQQKEIPTTLVDDFILSPVV

KRSFIQSIKVINAIIKKYGLPNDIIIELAREKNSKDAQKMINEMQKRNRQ

TNERIEEIIRTTGKENAKYLIEKIKLHDMQEGKCLYSLEAIPLEDLLNNP

FNYEVDHIIPRSVSFDNSFNNKVLVKQEENSKKGNRTPFQYLSSSDSKIS

YETFKKHILNLAKGKGRISKTKKEYLLEERDINRFSVQKDFINRNLVDTR

YATRGLMNLLRSYFRVNNLDVKVKSINGGFTSFLRRKWKFKKERNKGYKH

HAEDALIIANADFIFKEWKKLDKAKKVMENQMFEEKQAESMPEIETEQEY

KEIFITPHQIKHIKDFKDYKYSHRVDKKPNRELINDTLYSTRKDDKGNTL

IVNNLNGLYDKDNDKLKKLINKSPEKLLMYHHDPQTYQKLKLIMEQYGDE

KNPLYKYYEETGNYLTKYSKKDNGPVIKKIKYYGNKLNAHLDITDDYPNS

RNKVVKLSLKPYRFDVYLDNGVYKFVTVKNLDVIKKENYYEVNSKCYEEA

KKLKKISNQAEFIASFYNNDLIKINGELYRVIGVNNDLLNRIEVNMIDIT

YREYLENMNDKRPPRIIKTIASKTQSIKKYSTDILGNLYEVKSKKHPQII

KKG

[0108] In some embodiments the Cas9 protein can be *S. thermophiles* CRISPR1 Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 151)

MSDLVLGLDIGIGSVGVGILNKVTGEIIHKNSRIFPAAQAENNLVRRTNR

QGRRLARRKKHRRVRLNRLFEESGLITDFTKISINLNPYQLRVKGLTDEL

SNEELFIALKNMVKHRGISYLDDASDDGNSSVGDYAQIVKENSKQLETKT

PGQIQLERYQTYGQLRGDFTVEKDGKKHRLINVFPTSAYRSEALRILQTQ

QEFNPQITDEFINRYLEILTGKRKYYHGPGNEKSRTDYGRYRTSGETLDN

IFGILIGKCTFYPDEFRAAKASYTAQEFNLLNDLNNLTVPTETKKLSKEQ

KNQIINYVKNEKAMGPAKLFKYIAKLLSCDVADIKGYRIDKSGKAEIHTF

EAYRKMKTLETLDIEQMDRETLDKLAYVLTLNTEREGIQEALEHEFADGS

FSQKQVDELVQFRKANSSIFGKGWHNFSVKLMMELIPELYETSEEQMTIL

TRLGKQKTTSSSNKTKYIDEKLLTEEIYNPVVAKSVRQAIKIVNAAIKEY

GDFDNIVIEMARETNEDDEKKAIQKIQKANKDEKDAAMLKAANQYNGKAE

LPHSVFHGHKQLATKIRLWHQQGERCLYTGKTISIHDLINNSNQFEVDHI

LPLSITFDDSLANKVLVYATANQEKGQRTPYQALDSMDDAWSFRELKAFV

RESKTLSNKKKEYLLTEEDISKFDVRKKFIERNLVDTRYASRVVLNALQE

HFRAHKIDTKVSVVRGQFTSQLRRHWGIEKTRDTYHHHAVDALIIAASSQ

LNLWKKQKNTLVSYSEDQLLDIETGELISDDEYKESVFKAPYQHFVDTLK

SKEFEDSILFSYQVDSKFNRKISDATIYATRQAKVGKDKADETYVLGKIK

DIYTQDGYDAFMKIYKKDKSKFLMYRHDPQTFEKVIEPILENYPNKQIND

KGKEVPCNPFLKYKEEHGYIRKYSKKGNGPEIKSLKYYDSKLGNHIDITP

KDSNNKVVLQSVSPWRADVYFNKTTGKYEILGLKYADLQFDKGTGTYKIS

QEKYNDIKKKEGVDSDSEFKFTLYKNDLLLVKDTETKEQQLFRFLSRTMP

KQKHYVELKPYDKQKFEGGEALIKVLGNVANSGQCKKGLGKSNISIYKVR

TDVLGNQHIIKNEGDKPKLDF

[0109] In some embodiments the Cas9 protein can be *N. meningitidis* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 152)

MAAFKPNPINYILGLDIGIASVGWAMVEIDEDENPICLIDLGVRVFERAE

VPKTGDSLAMARRLARSVRRLTRRRAHRLLRARRLLKREGVLQAADFDEN

GLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGET

ADKELGALLKGVADNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYS

HTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDA

VQKMLGHCTFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDT

ERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEM

KAYHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLK

DRIQPEILEALLKHISFDKFVQISLKALRRIVPLMEQGKRYDEACAEIYG

DHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPAR

-continued

IHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKS

KDILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSF

NNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQ

RILLQKFDEDGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNG

QITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEM

NAFDGKTIDKETGEVLHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEA

DTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSA

KRLDEGVSVLRVPLTQLKLKDLEKMVNREREPKLYEALKARLEAHKDDPA

KAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVVVVRNHNGIADNATMVR

VDVFEKGDKYYLVPIYSWQVAKGILPDRAVVQGKDEEDWQLIDDSFNFKF

SLHPNDLVEVITKKARMFGYFASCHRGTGNINIRIHDLDHKIGKNGILEG

IGVKTALSFQKYQIDELGKEIRPCRLKKRPPVR

[0110] In some embodiments the Cas9 protein can be *Parvibaculum. lavamentivorans* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 153)

MERIFGFDIGTTSIGFSVIDYSSTQSAGNIQRLGVRIFPEARDPDGTPLN

QQRRQKRMMRRQLRRRRIRRKALNETLHEAGFLPAYGSADWPVVMADEPY

ELRRRGLEEGLSAYEFGRAIYHLAQHRHFKGRELEESDTPDPDVDDEKEA

ANERAATLKALKNEQTTLGAWLARRPPSDRKRGIHAHRNVVAEEFERLWE

VQSKFHPALKSEEMRARISDTIFAQRPVFWRKNTLGECRFMPGEPLCPKG

SWLSQQRRMLEKLNNLAIAGGNARPLDAEERDAILSKLQQQASMSWPGVR

SALKALYKQRGEPGAEKSLKFNLELGGESKLLGNALEAKLADMFGPDWPA

HPRKQEIRHAVHERLWAADYGETPDKKRVIILSEKDRKAHREAAANSFVA

DFGITGEQAAQLQALKLPTGWEPYSIPALNLFLAELEKGERFGALVNGPD

WEGWRRTNFPHRNQPTGEILDKLPSPASKEERERISQLRNPTVVRTQNEL

RKVVNNLIGLYGKPDRIRIEVGRDVGKSKREREEIQSGIRRNEKQRKKAT

EDLIKNGIANPSRDDVEKWILWKEGQERCPYTGDQIGFNALFREGRYEVE

HIWPRSRSFDNSPRNKTLCRKDVNIEKGNRMPFEAFGHDEDRWSAIQIRL

QGMVSAKGGTGMSPGKVKRFLAKTMPEDFAARQLNDTRYAAKQILAQLKR

LWPDMGPEAPVKVEAVTGQVTAQLRKLWTLNNILADDGEKTRADHRHHAI

DALTVACTHPGMTNKLSRYWQLRDDPRAEKPALTPPWDTIRADAEKAVSE

IVVSHRVRKKVSGPLHKETTYGDTGTDIKTKSGTYRQFVTRKKIESLSKG

ELDEIRDPRIKEIVAAHVAGRGGDPKKAFPPYPCVSPGGPEIRKVRLTSK

QQLNLMAQTGNGYADLGSNHHIAIYRLPDGKADFEIVSLFDASRRLAQRN

PIVQRTRADGASFVMSLAAGEAIMIPEGSKKGIWIVQGVVVASGQVVLER

DTDADHSTTTRPMPNPILKDDAKKVSIDPIGRVRPSND

[0111] In some embodiments the Cas9 protein can be *Corynebacter* diphtheria Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 154)

MKYHVGIDVGTFSVGLAAIEVDDAGMPIKTLSLVSHIHDSGLDPDEIKSA

VTRLASSGIARRTRRLYRRKRRRLQQLDKFIQRQGWPVIELEDYSDPLYP

WKVRAELAASYIADEKERGEKLSVALRHIARHRGWRNPYAKVSSLYLPDG

PSDAFKAIREEIKRASGQPVPETATVGQMVTLCELGTLKLRGEGGVLSAR

LQQSDYAREIQEICRMQEIGQELYRKIIDVVFAAESPKGSASSRVGKDPL

QPGKNRALKASDAFQRYRIAALIGNLRVRVDGEKRILSVEEKNLVFDHLV

NLTPKKEPEWVTIAEILGIDRGQLIGTATMTDDGERAGARPPTHDTNRSI

VNSRIAPLVDWWKTASALEQHAMVKALSNAEVDDFDSPEGAKVQAFFADL

DDDVHAKLDSLHLPVGRAAYSEDTLVRLTRRMLSDGVDLYTARLQEFGIE

PSWTPPTPRIGEPVGNPAVDRVLKTVSRWLESATKTWGAPERVIIEHVRE

GFVTEKRAREMDGDMRRRAARNAKLFQEMQEKLNVQGKPSRADLWRYQSV

QRQNCQCAYCGSPITFSNSEMDHIVPRAGQGSTNTRENLVAVCHRCNQSK

GNTPFAIWAKNTSIEGVSVKEAVERTRHWVTDTGMRSTDFKKFTKAVVER

FQRATMDEEIDARSMESVAWMANELRSRVAQHFASHGTTVRVYRGSLTAE

ARRASGISGKLKFFDGVGKSRLDRRHHAIDAAVIAFTSDYVAETLAVRSN

LKQSQAHRQEAPQWREFTGKDAEHRAAWRVWCQKMEKLSALLTEDLRDDR

VVVMSNVRLRLGNGSAHKETIGKLSKVKLSSQLSVSDIDKASSEALWCAL

TREPGFDPKEGLPANPERHIRVNGTHVYAGDNIGLFPVSAGSIALRGGYA

ELGSSFHHARVYKITSGKKPAFAMLRVYTIDLLPYRNQDLFSVELKPQTM

SMRQAEKKLRDALATGNAEYLGWLVVDDELVVDTSKIATDQVKAVEAELG

TIRRWRVDGFFSPSKLRLRPLQMSKEGIKKESAPELSKIIDRPGWLPAVN

KLFSDGNVTVVRRDSLGRVRLESTAHLPVTWKVQ

[0112] In some embodiments the Cas9 protein can be *Streptococcus pasteurianus* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 155)

MTNGKILGLDIGIASVGVGIIEAKTGKVVHANSRLFSAANAENNAERRGF

RGSRRLNRRKKHRVKRVRDLFEKYGIVTDFRNLNLNPYELRVKGLTEQLK

NEELFAALRTISKRRGISYLDDAEDDSTGSTDYAKSIDENRRLLKNKTPG

QIQLERLEKYGQLRGNFTVYDENGEAHRLINVFSTSDYEKEARKILETQA

DYNKKITAEFIDDYVEILTQKRKYYHGPGNEKSRTDYGRFRTDGTTLENI

FGILIGKCNFYPDEYRASKASYTAQEYNFLNDLNNLKVSTETGKLSTEQK

ESLVEFAKNTATLGPAKLLKEIAKILDCKVDEIKGYREDDKGKPDLHTFE

PYRKLKFNLESINIDDLSREVIDKLADILTLNIIREGIEDAIKRNLPNQF

TEEQISEIIKVRKSQSTAFNKGWHSFSAKLMNELIPELYATSDEQMTILT

RLEKFKVNKKSSKNTKTIDEKEVTDEIYNPVVAKSVRQTIKIINAAVKKY

GDFDKIVIEMPRDKNADDEKKFIDKRNKENKKEKDDALKRAAYLYNSSDK

LPDEVFHGNKQLETKIRLWYQQGERCLYSGKPISIQELVHNSNNFEIDHI

LPLSLSFDDSLANKVLVYAWTNQEKGQKTPYQVIDSMDAAWSFREMKDYV

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LKQKGLGKKKRDYLLTTENIDKIEVKKKFIERNLVDTRYASRVVLNSLQS

ALRELGKDTKVSVVRGQFTSQLRRKWKIDKSRETYHHHAVDALIIAASSQ

LKLWEKQDNPMFVDYGKNQVVDKQTGEILSVSDDEYKELVFQPPYQGFVN

TISSKGFEDEILFSYQVDSKYNRKVSDATIYSTRKAKIGKDKKEETYVLG

KIKDIYSQNGFDTFIKKYNKDKTQFLMYQKDSLTWENVIEVILRDYPTTK

KSEDGKNDVKCNPFEEYRRENGLICKYSKKGKGTPIKSLKYYDKKLGNCI

DITPEESRNKVILQSINPWRADVYFNPETLKYELMGLKYSDLSFEKGTGN

YHISQEKYDAIKEKEGIGKKSEFKFTLYRNDLILIKDIASGEQEIYRFLS

RTMPNVNHYVELKPYDKEKFDNVQELVEALGEADKVGRCIKGLNKPNISI

YKVRTDVLGNKYFVKKKGDKPKLDFKNNKK

[0113] In some embodiments the Cas9 protein can be *Neisseria cinerea* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 156)

MAAFKPNPMNYILGLDIGIASVGWAIVEIDEEENPIRLIDLGVRVFERAE

VPKTGDSLAAARRLARSVRRLTRRRAHRLLRARRLLKREGVLQAADFDEN

GLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGET

ADKELGALLKGVADNTHALQTGDFRTPAELALNKFEKESGHIRNQRGDYS

HTFNRKDLQAELNLLFEKQKEFGNPHVSDGLKEGIETLLMTQRPALSGDA

VQKMLGHCTFEPTEPKAAKNTYTAERFVWLTKLNNLRILEQGSERPLTDT

ERATLMDEPYRKSKLTYAQARKLLDLDDTAFFKGLRYGKDNAEASTLMEM

KAYHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLK

DRVQPEILEALLKHISFDKFVQISLKALRRIVPLMEQGNRYDEACTEIYG

DHYGKKNIEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPAR

IHIETAREVGKSFKDRKEIEKRQEENRKDREKSAAKFREYFPNFVGEPKS

KDILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSF

NNKVLALGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQ

RILLQKFDEDGFKERNLNDTRYINRFLCQFVADHMLLTGKGKRRVFASNG

QITNLLRGFWGLRKVRAENDRHHALDAVVVACSTIAMQQKITRFVRYKEM

NAFDGKTIDKETGEVLHQKAHFPQPWEFFAQEVMIRVFGKPDGKPEFEEA

DTPEKLRTLLAEKLSSRPEAVHKYVTPLFISRAPNRKMSGQGHMETVKSA

KRLDEGISVLRVPLTQLKLKDLEKMVNRREREPKLYEALKARLEAHKDDPA

KAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVWVHNHNGIADNATIVRV

DVFEKGGKYYLVPIYSWQVAKGILPDRAVVQGKDEEDWTVMDDSFEFKFV

LYANDLIKLTAKKNEFLGYFVSLNRATGAIDIRTHDTDSTKGKNGIFQSV

GVKTALSFQKYQIDELGKEIRPCRLKKRPPVR

[0114] In some embodiments the Cas9 protein can be *Campylobacter lari* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 157)

MRILGFDIGINSIGWAFVENDELKDCGVRIFTKAENPKNKESLALPRRNA

RSSRRRLKRRKARLIAIKRILAKELKLNYKDYVAADGELPKAYEGSLASV

YELRYKALTQNLETKDLARVILHIAKHRGYMNKNEKKSNDAKKGKILSAL

KNNALKLENYQSVGEYFYKEFFQKYKKNTKNFIKIRNTKDNYNNCVLSSD

LEKELKLILEKQKEFGYNYSEDFINEILKVAFFQRPLKDFSHLVGACTFF

EEEKRACKNSYSAWEFVALTKIINEIKSLEKISGEIVPTQTINEVLNLIL

DKGSITYKKFRSCINLHESISFKSLKYDKENAENAKLIDFRKLVEFKKAL

GVHSLSRQELDQISTHITLIKDNVKLKTVLEKYNLSNEQINNLLEIEFND

YINLSFKALGMILPLMREGKRYDEACEIANLKPKTVDEKKDFLPAFCDSI

FAHELSNPVVNRAISEYRKVLNALLKKYGKVHKIHLELARDVGLSKKARE

KIEKEQKENQAVNAWALKECENIGLKASAKNILKLKLWKEQKEICIYSGN

KISIEHLKDEKALEVDHIYPYSRSFDDSFINKVLVFTKENQEKLNKTPFE

AFGKNIEKWSKIQTLAQNLPYKKKNKILDENFKDKQQEDFISRNLNDTRY

IATLIAKYTKEYLNFLLLSENENANLKSGEKGSKIHVQTISGMLTSVLRH

TWGFDKKDRNNHLHHALDAIIVAYSTNSIIKAFSDFRKNQELLKARFYAK

ELTSDNYKHQVKFFEPFKSFREKILSKIDEIFVSKPPRKRARRALHKDTF

HSENKIIDKCSYNSKEGLQIALSCGRVRKIGTKYVENDTIVRVDIFKKQN

KFYAIPIYAMDFALGILPNKIVITGKDKNNNPKQWQTIDESYEFCFSLYK

NDLILLQKKNMQEPEFAYYNDFSISTSSICVEKHDNKFENLTSNQKLLFS

NAKEGSVKVESLGIQNLKVFEKYIITPLGDKIKADFQPRENISLKTSKKY

GLR

[0115] In some embodiments the Cas9 protein can be T7 *denticola* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 158)

MKKEIKDYFLGLDVGTGSVGWAVTDTDYKLLKANRKDLWGMRCFETAETA

EVRRLHRGARRRIERRKKRIKLLQELFSQEIAKTDEGFFQRMKESPFYAE

DKTILQENTLFNDKDFADKTYHKAYPTINHLIKAWIENKVKPDPRLLYLA

CHNIIKKRGHFLFEGDFDSENQFDTSIQALFEYLREDMEVDIDADSQKVK

EILKDSSLKNSEKQSRLNKILGLKPSDKQKKAITNLISGNKINFADLYDN

PDLKDAEKNSISFSKDDFDALSDDLASILGDSFELLLKAKAVYNCSVLSK

VIGDEQYLSFAKVKIYEKHKTDLTKLKNVIKKHFPKDYKKVFGYNKNEKN

NNNYSGYVGVCKTKSKKLIINNSVNQEDFYKFLKTILSAKSEIKEVNDIL

TEIETGTFLPKQISKSNAEIPYQLRKMELEKILSNAEKHFSFLKQKDEKG

LSHSEKIIMLLTFKIPYYIGPINDNHKKFFPDRCWVVKKEKSPSGKTTPW

NFFDHIDKEKTAEAFITSRTNFCTYLVGESVLPKSSLLYSEYTVLNEINN

LQIIIDGKNICDIKLKQKIYEDLFKKYKKITQKQISTFIKHEGICNKTDE

VIILGIDKECTSSLKSYIELKNIFGKQVDEISTKNMLEEIIRWATIYDEG

EGKTILKTKIKAEYGKYCSDEQIKKILNLKFSGWGRLSRKFLETVTSEMP

-continued

GFSEPVNIITAMRETQNNLMELLSSEFTFTENIKKINSGFEDAEKQFSYD

GLVKPLFLSPSVKKMLWQTLKLVKEISHITQAPPKKIFIEMAKGAELEPA

RTKTRLKILQDLYNNCKNDADAFSSEIKDLSGKIENEDNLRLRSDKLYLY

YTQLGKCMYCGKPIEIGHVFDTSNYDIDHIYPQSKIKDDSISNRVLVCSS

CNKNKEDKYPLKSEIQSKQRGFWNFLQRNNFISLEKLNRLTRATPISDDE

TAKFIARQLVETRQATKVAAKVLEKMFPETKIVYSKAETVSMFRNKFDIV

KCREINDFHHAHDAYLNIVVGNVYNTKFTNNPWNFIKEKRDNPKIADTYN

YYKVFDYDVKRNNITAWEKGKTIITVKDMLKRNTPIYTRQAACKKGELFN

QTIMKKGLGQHPLKKEGPFSNISKYGGYNKVSAAYYTLIEYEEKGNKIRS

LETIPLYLVKDIQKDQDVLKSYLTDLLGKKEFKILVPKIKINSLLKINGF

PCHITGKTNDSFLLRPAVQFCCSNNEVLYFKKIIRFSEIRSQREKIGKTI

SPYEDLSFRSYIKENLWKKTKNDEIGEKEFYDLLQKKNLEIYDMLLTKHK

DTIYKKRPNSATIDILVKGKEKFKSLIIENQFEVILEILKLFSATRNVSD

LQHIGGSKYSGVAKIGNKISSLDNCILIYQSITGIFEKRIDLLKV

[0116] In some embodiments the Cas9 protein can be *S. mutans* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 159)

MKKPYSIGLDIGTNSVGWAVVTDDYKVPAKKMKVLGNTDKSHIEKNLLGA

LLFDSGNTAEDRRLKRTARRYTRRRNRILYLQEIFSEEMGKVDDSFFHR

LEDSFLVIEDKRGERHPIFGNLEEEVKYHENFPTIYHLRQYLADNPEKVD

LRLVYLALAHIIKFRGHFLIEGKFDTRNNDVQRLFQEFLAVYDNTFENSS

LQEQNVQVEEILTDKISKSAKKDRVLKLFPNEKSNGRFAEFLKLIVGNQA

DFKKHFELEEKAPLQFSKDTYEEELEVLLAQIGDNYAELFLSAKKLYDSI

LLSGILTVTDVGTKAPLSASMIQRYNEHQMDLAQLKQFIRQKLSDKYNEV

FSDVSKDGYAGYIDGKTNQEAFYKYLKGLLNKIEGSGYFLDKIEREDFLR

KQRTFDNGSIPHQIHLQEMRAIIRRQAEFYPFLADNQDRIEKLLTFRIPY

YVGPLARGKSDFAWLSRKSADKITPWNFDEIVDKESSAEAFINRMTNYDL

YLPNQKVLPKHSLLYEKFTVYNELTKVKYKTEQGKTAFFDANMKQEIFDG

VFKVYRKVTKDKLMDFLEKEFDEFRIVDLTGLDKENKVFNASYGTYHDLC

KILDKDFLDNSKNEKILEDIVLTLTLFEDREMIRKRLENYSDLLTKEQVK

KLERRHYTGWGRLSAELIHGIRNKESRKTILDYLIDDGNSNRNFMQLIND

DALSFKEEIAKAQVIGETDNLNQVVSDIAGSPAIKKGILQSLKIVDELVK

IMGHQPENIVVEMARENQFTNQGRRNSQQRLKGLTDSIKEFGSQILKEHP

VENSQLQNDRLFLYYLQNGRDMYTGEELDIDYLSQYDIDHIIPQAFIKDN

SIDNRVLTSSKENRGKSDDVPSKDVVRKMKSYWSKLLSAKLITQRKFDNL

TKAERGGLTDDDKAGFIKRQLVETRQITKHVARILDERFNTETDENNKKI

RQVKIVTLKSNLVSNFRKEFELYKVREINDYHHAHDAYLNAVIGKALLGV

YPQLEPEFVYGDYPHFHGHKENKATAKKFFYSNIMNFFKKDDVRTDKNGE

IIWKKDEHISNIKKVLSYPQVNIVKKVEEQTGGFSKESILPKGNSDKLIP

-continued

RKTKKFYWDTKKYGGFDSPIVAYSILVIADIEKGKSKKLKTVKALVGVTI

MEKMTFERDPVAFLERKGYRNVQEENIIKLPKYSLFKLENGRKRLLASAR

ELQKGNEIVLPNHLGTLLYHAKNIHKVDEPKHLDYVDKHKDEFKELLDVV

SNFSKKYTLAEGNLEKIKELYAQNNGEDLKELASSFINLLTFTAIGAPAT

FKFFDKNIDRKRYTSTTEILNATLIHQSITGLYETRIDLNKLGGD

[0117] In some embodiments the Cas9 protein can be *S. thermophilus* CRISPR 3 Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 160)

MTKPYSIGLDIGTNSVGWAVTTDNYKVPSKKMKVLGNTSKKYIKKNLLGV

LLFDSGITAEGRRLKRTARRYTRRRNRILYLQEIFSTEMATLDDAFFQR

LDDSFLVPDDKRDSKYPIFGNLVEEKAYHDEFPTIYHLRKYLADSTKKAD

LRLVYLALAHMIKYRGHFLIEGEFNSKNNDIQKNFQDFLDTYNAIFESDL

SLENSKQLEEIVKDKISKLEKKDRILKLFPGEKNSGIFSEFLKLIVGNQA

DFRKCFNLDEKASLHFSKESYDEDLETLLGYIGDDYSDVFLKAKKLYDAI

LLSGFLTVTDNETEAPLSSAMIKRYNEHKEDLALLKEYIRNISLKTYNEV

FKDDTKNGYAGYIDGKTNQEDFYVYLKKLLAEFEGADYFLEKIDREDFLR

KQRTFDNGSIPYQIHLQEMRAILDKQAKFYPFLAKNKERIEKILTFRIPY

YVGPLARGNSDFAWSIRKRNEKITPWNFEDVIDKESSAEAFINRMTSFDL

YLPEEKVLPKHSLLYETFNVYNELTKVRFIAESMRDYQFLDSKQKKDIVR

LYFKDKRKVTDKDIIEYLHAIYGYDGIELKGIEKQFNSSLSTYHDLLNII

NDKEFLDDSSNEAIIEEIIHTLTIFEDREMIKQRLSKFENIFDKSVLKKL

SRRHYTGWGKLSAKLINGIRDEKSGNTILDYLIDDGISNRNFMQLIHDDA

LSFKKKIQKAQIIGDEDKGNIKEVVKSLPGSPAIKKGILQSIKIVDELVK

VMGGRKPESIVVEMARENQYTNQGKSNSQQRLKRLEKSLKELGSKILKEN

IPAKLSKIDNNALQNDRLYLYYLQNGKDMYTGDDLDIDRLSNYDIDHIIP

QAFLKDNSIDNKVLVSSASNRGKSDDVPSLEVVKKRKTFWYQLLKSKLIS

QRKFDNLTKAERGGLSPEDKAGFIQRQLVETRQITKHVARLLDEKFNNKK

DENNRAVRTVKIITLKSTLVSQFRKDFELYKVREINDFHHAHDAYLNAVV

ASALLKKYPKLEPEFVYGDYPKYNSFRERKSATEKVYFYSNIMNIFKKSI

SLADGRVIERPLIEVNEETGESVWNKESDLATVRRVLSYPQVNVVKKVEE

QNHGLDRGKPKGLFNANLSSKPKPNSNENLVGAKEYLDPKKYGGYAGISN

SFTVLVKGTIEKGAKKKITNVLEFQGISILDRINYRKDKLNFLLEKGYKD

IELIIELPKYSLFELSDGSRRMLASILSTNNKRGEIHKGNQIFLSQKFVK

LLYHAKRISNTINENHRKYVENHKKEFEELFYYILEFNENYVGAKKNGKL

LNSAFQSWQNHSIDELCSSFIGPTGSERKGLFELTSRGSAADFEFLGVKI

PRYRDYTPSSLLKDATLIHQSVTGLYETRIDLAKLGEG

[0118] In some embodiments the Cas9 protein can be *C. jejuni* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 161)

MARILAFDIGISSIGWAFSENDELKDCGVRIFTKVENPKTGESLALPRRL

ARSARKRLARRKARLNHLKHLIANEFKLNYEDYQSFDESLAKAYKGSLIS

PYELRFRALNELLSKQDFARVILHIAKRRGYDDIKNSDDKEKGAILKAIK

QNEEKLANYQSVGEYLYKEYFQKFKENSKEFTNVRNKKESYERCIAQSFL

KDELKLIFKKQREFGFSFSKKFEEEVLSVAFYKRALKDFSHLVGNCSFFT

DEKRAPKNSPLAFWVALTRIINLLNNLKNTEGILYTKDDLNALLNEVLKN

GTLTYKQTKKLLGLSDDYEFKGEKGTYFIEFKKYKEFIKALGEHNLSQDD

LNEIAKDITLIKDEIKLKKALAKYDLNQNQIDSLSKLEFKDHLNISFKAL

KLVTPLMLEGKKYDEACNELNLKVAINEDKKDFLPAFNETYYKDEVTNPV

VLRAIKEYRKVLNALLKKYGKVHKINIELAREVGKNHSQRAKIEKEQNEN

YKAKKDAELECEKLGLKINSKNILKLRLFKEQKEFCAYSGEKIKISDLQD

EKMLEIDHIYPYSRSFDDSYMNKVLVFTKQNQEKLNQTPFEAFGNDSAKW

QKIEVLAKNLPTKKQKRILDKNYKDKEQKNFKDRNLNDTRYIARLVLNYT

KDYLDFLPLSDDENTKLNDTQKGSKVHVEAKSGMLTSALRHTWGFSAKDR

NNHLHHAIDAVIIAYANNSIVKAFSDFKKEQESNSAELYAKKISELDYKN

KRKFFEPFSGFRQKVLDKIDEIFVSKPERKKPSGALHEETFRKEEEFYQS

YGGKEGVLKALELGKIRKVNGKIVKNGDMFRVDIFKHKKTNKFYAVPIYT

MDFALKVLPNKAVARSKKGEIKDWILMDENYEFCFSLYKDSLILIQTKDM

QEPEFVYYNAFTSSTVSLIVSKHDNKFETLSKNQKILFKNANEKEVIAKS

IGIQNLKVFEKYIVSALGEVTKAEFRQREDFKK

[0119] In some embodiments the Cas9 protein can be *P. multocida* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 162)

MQTTNLSYILGLDLGIASVGWAVVEINENEDPIGLIDVGVRIFERAEVPK

TGESLALSRRLARSTRRLIRRRAHRLLLAKRFLKREGILSTIDLEKGLPN

QAWELRVAGLERRLSAIEWGAVLLHLIKHRGYLSKRKNESQTNNKELGAL

LSGVAQNHQLLQSDDYRTPAELALKKFAKEEGHIRNQRGAYTHTFNRLDL

LAELNLLFAQQHQFGNPHCKEHIQQYMTELLMWQKPALSGEAILKMLGKC

THEKNEFKAAKHTYSAERFVWLTKLNNLRILEDGAERALNEEERQLLINH

PYEKSKLTYAQVRKLLGLSEQAIFKHLRYSKENAESATFMELKAWHAIRK

ALENQGLKDTWQDLAKKPDLLDEIGTAFSLYKTDEDIQQYLTNKVPNSVI

NALLVSLNFDKFIELSLKSLRKILPLMEQGKRYDQACREIYGHHYGEANQ

KTSQLLPAIPAQEIRNPVVLRTLSQARKVINAIIRQYGSPARVHIETGRE

LGKSFKERREIQKQQEDNRTKRESAVQKFKELFSDFSSEPKSKDILKFRL

YEQQHGKCLYSGKEINIHRLNEKGYVEIDHALPFSRTWDDSFNNKVLVLA

SENQNKGNQTPYEWLQGKINSERWKNFVALVLGSQCSAAKKQRLLTQVID

DNKFIDRNLNDTRYIARFLSNYIQENLLLVGKNKKNVFTPNGQITALLRS

RWGLIKARENNNRHHALDAIVVACATPSMQQKITRFIRFKEVHPYKIENR

-continued

YEMVDQESGEIISPHFPEPWAYFRQEVNIRVFDNHPDTVLKEMLPDRPQA

NHQFVQPLFVSRAPTRKMSGQGHMETIKSAKRLAEGISVLRIPLTQLKPN

LLENMVNKEREPALYAGLKARLAEFNQDPAKAFATPFYKQGGQQVKAIRV

EQVQKSGVLVRENNGVADNASIVRTDVFIKNNKFFLVPIYTWQVAKGILP

NKAIVAHKNEDEWEEMDEGAKFKFSLFPNDLVELKTKKEYFFGYYIGLDR

ATGNISLKEHDGEISKGKDGVYRVGVKLALSFEKYQVDELGKNRQICRPQ

QRQPVR

[0120] In some embodiments the Cas9 protein can be *F. novicida* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 163)

MNFKILPIAIDLGVKNTGVFSAFYQKGTSLERLDNKNGKVYELSKDSYTL

LMNNRTARRHQRRGIDRKQLVKRLFKLIWTEQLNLEWDKDTQQAISFLFN

RRGFSFITDGYSPEYLNIVPEQVKAILMDIFDDYNGEDDLDSYLKLATEQ

ESKISEIYNKLMQKILEFKLMKLCTDIKDDKVSTKTLKEITSYEFELLAD

YLANYSESLKTQKFSYTDKQGNLKELSYYHHDKYNIQEFLKRHATINDRI

LDTLLTDDLDIWNFNFEKFDFDKNEEKLQNQEDKDHIQAHLHHFVFAVNK

IKSEMASGGRHRSQYFQEITNVLDENNHQEGYLKNFCENLHNKKYSNLSV

KNLVNLIGNLSNLELKPLRKYFNDKIHAKADHWDEQKFIETYCHWILGEW

RVGVKDQDKKDGAKYSYKDLCNELKQKVTKAGLVDFLLELDPCRTIPPYL

DNNNRKPPKCQSLILNPKFLDNQYPNWQQYLQELKKLQSIQNYLDSFETD

LKVLKSSKDQPYFVEYKSSNQQIASGQRDYKDLDARILQFIFDRVKASDE

LLLNEIYFQAKKLKQKASSELEKLESSKKLDEVIANSQLSQILKSQHTNG

IFEQGTFLHLVCKYYKQRQRARDSRLYIMPEYRYDKKLHKYNNTGRFDDD

NQLLTYCNHKPRQKRYQLLNDLAGVLQVSPNFLKDKIGSDDDLFISKWLV

EHIRGFKKACEDSLKIQKDNRGLLNHKINIARNTKGKCEKEIFNLICKIE

GSEDKKGNYKHGLAYELGVLLFGEPNEASKPEFDRKIKKFNSIYSFAQIQ

QIAFAERKGNANTCAVCSADNAHRMQQIKIIEPVEDNKDKIILSAKAQRL

PAIPTRIVDGAVKKMATILAKNIVDDNWQNIKQVLSAKHQLHIPIIIESN

AFEFEPALADVKGKSLKDRRKKALERISPENIFKDKNNRIKEFAKGISAY

SGANLTDGDFDGAKEELDHIIPRSHKKYGTLNDEANLICVTRGDNKNKGN

RIFCLRDLADNYKLKQFETTDDLEIEKKIADTIWDANKKDFKFGNYRSFI

NLTPQEQKAFRHALFLADENPIKQAVIRAINNRNRTFVNGTQRYFAEVLA

NNIYLRAKKENLNTDKISFDYFGIPTIGNGRGIAEIRQLYEKVDSDIQAY

AKGDKPQASYSHLIDAMLAFCIAADEHRNDGSIGLEIDKNYSLYPLDKNT

GEVFTKDIFSQIKITDNEFSDKKLVRKKAIEGFNTHRQMTRDGIYAENYL

PILIHKELNEVRKGYTWKNSEEIKIFKGKKYDIQQLNNLVYCLKFVDKPI

SIDIQISTLEELRNILTTNNIAATAEYYYINLKTQKLHEYYIENYNTALG

YKKYSKEMEFLRSLAYRSERVKIKSIDDVKQVLDKDSNFIIGKITLPFKK

EWQRLYREWQNTTIKDDYEFLKSFFNVKSITKLHKKVRKDFSLPISTNEG

-continued

KFLVKRKTWDNNFIYQILNDSDSRADGTKPFIPAFDISKNEIVEAIIDSF

TSKNIFWLPKNIELQKVDNKNIFAIDTSKWFEVETPSDLRDIGIATIQYK

IDNNSRPKVRVKLDYVIDDDSKINYFMNHSLLKSRYPDKVLEILKQSTII

EFESSGFNKTIKEMLGMKLAGIYNETSNN

[0121] In some embodiments the Cas9 protein can be *Lactobacillus buchneri* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 164)

MKVNNYHIGLDIGTSSIGWVAIGKDGKPLRVKGKTAIGARLFQEGNPAAD

RRMFRTTRRRLSRRKWRLKLLEEIFDPYITPVDSTFFARLKQSNLSPKDS

RKEFKGSMLFPDLTDMQYHKNYPTIYHLRHALMTQDKKFDIRMVYLAIHH

IVKYRGNFLNSTPVDSFKASKVDFVDQFKKLNELYAAINPEESFKINLAN

SEDIGHQFLDPSIRKFDKKKQIPKIVPVMMNDKVTDRLNGKIASEIIHAI

LGYKAKLDVVLQCTPVDSKPWALKFDDEDIDAKLEKILPEMDENQQSIVA

ILQNLYSQVTLNQIVPNGMSLSESMIEKYNDHHDHLKLYKKLIDQLADPK

KKAVLKKAYSQYVGDDGKVIEQAEFWSSVKKNLDDSELSKQIMDLIDAEK

FMPKQRTSQNGVIPHQLHQRELDEIIEHQSKYYPWLVEINPNKHDLHLAK

YKIEQLVAFRVPYYVGPMITPKDQAESAETVFSWMERKGTETGQITPWNF

DEKVDRKASANRFIKRMTTKDTYLIGEDVLPDESLLYEKFKVLNELNMVR

VNGKLLKVADKQAIFQDLFENYKHVSVKKLQNYIKAKTGLPSDPEISGLS

DPEHFNNSLGTYNDFKKLFGSKVDEPDLQDDFEKIVEWSTVFEDKKILRE

KLNEITWLSDQQKDVLESSRYQGWGRLSKKLLTGIVNDQGERIIDKLWNT

NKNFMQIQSDDDFAKRIHEANADQMQAVDVEDVLADAYTSPQNKKAIRQV

VKVVDDIQKAMGGVAPKYISIEFTRSEDRNPRRTISRQRQLENTLKDTAK

SLAKSINPELLSELDNAAKSKKGLTDRLYLYFTQLGKDIYTGEPINIDEL

NKYDIDHILPQAFIKDNSLDNRVLVLTAVNNGKSDNVPLRMFGAKMGHFW

KQLAEAGLISKRKLKNLQTDPDTISKYAMHGFIRRQLVETSQVIKLVANI

LGDKYRNDDTKIIEITARMNHQMRDEFGFIKNREINDYHHAFDAYLTAFL

GRYLYHRYIKLRPYFVYGDFKKFREDKVTMRNFNFLHDLTDDTQEKIADA

ETGEVIWDRENSIQQLKDVYHYKFMLISHEVYTLRGAMFNQTVYPASDAG

KRKLIPVKADRPVNVYGGYSGSADAYMAIVRIHNKKGDKYRVVGVPMRAL

DRLDAAKNVSDADFDRALKDVLAPQLTKTKKSRKTGEITQVIEDFEIVLG

KVMYRQLMIDGDKKFMLGSSTYQYNAKQLVLSDQSVKTLASKGRLDPLQE

SMDYNNVYlEILDKVNQYFSLYDMNKFRHKLNLGFSKFISFPNHNVLDGN

TKVSSGKREILQEILNGLHANPTFGNLKDVGITTPFGQLQQPNGILLSDE

TKIRYQSPTGLFERTVSLKDL

[0122] In some embodiments the Cas9 protein can be *Listeria innocua* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 165)

MKKPYTIGLDIGTNSVGWAVLTDQYDLVKRKMKIAGDSEKKQIKKNFWGV

RLFDEGQTAADRRMARTARRRIERRRNRISYLQGIFAEEMSKTDANFFCR

LSDSFYVDNEKRNSRHPFFATIEEEVEYHKNYPTIYHLREELVNSSEKAD

LRLVYLALAHIIKYRGNFLIEGALDTQNTSVDGIYKQFIQTYNQVFASGI

EDGSLKKLEDNKDVAKILVEKVTRKEKLERILKLYPGEKSAGMFAQFISL

IVGSKGNFQKPFDLIEKSDIECAKDSYEEDLESLLALIGDEYAELFVAAK

NAYSAVVLSSIITVAETETNAKLSASMIERFDTHEEDLGELKAFIKLHLP

KHYEEIFSNTEKHGYAGYIDGKTKQADFYKYMKMTLENIEGADYFIAKIE

KENFLRKQRTFDNGAIPHQLHLEELEAILHQQAKYYPFLKENYDKIKSLV

TFRIPYFVGPLANGQSEFAWLTRKADGEIRPWNIEEKVDFGKSAVDFIEK

MTNKDTYLPKENVLPKHSLCYQKYLVYNELTKVRYINDQGKTSYFSGQEK

EQIFNDLFKQKRKVKKKDLELFLRNMSHVESPTIEGLEDSFNSSYSTYHD

LLKVGIKQEILDNPVNTEMLENIVKILTVFEDKRMIKEQLQQFSDVLDGV

VLKKLERRHYTGWGRLSAKLLMGIRDKQSHLTILDYLMNDDGLNRNLMQL

INDSNLSFKSIIEKEQVTTADKDIQSIVADLAGSPAIKKGILQSLKIVDE

LVSVMGYPPQTIVVEMARENQTTGKGKNNSRPRYKSLEKAIKEFGSQILK

EHPTDNQELRNNRLYLYYLQNGKDMYTGQDLDIHNLSNYDIDHIVPQSFI

TDNSIDNLVLTSSAGNREKGDDVPPLEIVRKRKVFWEKLYQGNLMSKRKF

DYLTKAERGGLTEADKARFIHRQLVETRQITKNVANILHQRFNYEKDDHG

NTMKQVRIVTLKSALVSQFRKQFQLYKVRDVNDYHHAHDAYLNGVVANTL

LKVYPQLEPEFVYGDYHQFDWFKANKATAKKQFYTNIMLFFAQKDRIIDE

NGEILWDKKYLDTVKKVMSYRQMNIVKKTEIQKGEFSKATIKPKGNSSKL

IPRKTNWDPMKYGGLDSPNMAYAVVIEYAKGKNKLVFEKKIIRVTIMERK

AFEKDEKAFLEEQGYRQPKVLAKLPKYTLYECEEGRRRMLASANEAQKGN

QQVLPNHLVTLLHHAANCEVSDGKSLDYIESNREMFAELLAHVSEFAKRY

TLAEANLNKINQLFEQNKEGDIKAIAQSFVDLMAFNAMGAPASFKFFETT

IERKRYNNLKELLNSTIIYQSITGLYESRKRLDD

[0123] In some embodiments the Cas9 protein can be *L. pneumophilia* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 166)

MESSQILSPIGIDLGGKFTGVCLSHLEAFAELPNHANTKYSVILIDHNNF

QLSQAQRRATRHRVRNKKRNQFVKRVALQLFQHILSRDLNAKEETALCHY

LNNRGYTYVDTDLDEYIKDETTINLLKELLPSESEHNFIDWFLQKMQSSE

FRKILVSKVEEKKDDKELKNAVKNIKNFITGFEKNSVEGHRHRKVYFENI

KSDITKDNQLDSIKKKIPSVCLSNLLGHLSNLQWKNLHRYLAKNPKQFDE

QTFGNEFLRMLKNFRHLKGSQESLAVRNLIQQLEQSQDYISILEKTPPEI

TIPPYEARTNTGMEKDQSLLLNPEKLNNLYPNWRNLIPGIIDAHPFLEKD

LEHTKLRDRKRIISPSKQDEKRDSYILQRYLDLNKKIDKFKIKKQLSFLG

-continued

QGKQLPANLIETQKEMETHFNSSLVSVLIQIASAYNKEREDAAQGIWFDN

AFSLCELSNINPPRKQKILPLLVGAILSEDFINNKDKWAKFKIFWNTHKI

GRTSLKSKCKEIEEARKNSGNAFKIDYEEALNHPEHSNNKALIKIIQTIP

DIIQAIQSHLGHNDSQALIYHNPFSLSQLYTILETKRDGFHKNCVAVTCE

NYWRSQKTEIDPEISYASRLPADSVRPFDGVLARMMQRLAYEIAMAKWEQ

IKHIPDNSSLLIPIYLEQNRFEFEESFKKIKGSSSDKTLEQAIEKQNIQW

EEKFQRIINASMNICPYKGASIGGQGEIDHIYPRSLSKKHFGVIFNSEVN

LIYCSSQGNREKKEEHYLLEHLSPLYLKHQFGTDNVSDIKNFISQNVANI

KKYISFHLLTPEQQKAARHALFLDYDDEAFKTITKFLMSQQKARVNGTQK

FLGKQIMEFLSTLADSKQLQLEFSIKQITAEEVHDHRELLSKQEPKLVKS

RQQSFPSHAIDATLTMSIGLKEFPQFSQELDNSWFINHLMPDEVHLNPVR

SKEKYNKPNISSTPLFKDSLYAERFIPVWVKGETFAIGFSEKDLFEIKPS

NKEKLFTLLKTYSTKNPGESLQELQAKSKAKWLYFPINKTLALEFLHHYF

HKEIVTPDDTTVCHFINSLRYYTKKESITVKILKEPMPVLSVKFESSKKN

VLGSFKHTIALPATKDWERLFNHPNFLALKANPAPNPKEFNEFIRKYFLS

DNNPNSDIPNNGHNIKPQKHKAVRKVFSLPVIPGNAGTMMRIRRKDNKGQ

PLYQLQTIDDTPSMGIQINEDRLVKQEVLMDAYKTRNLSTIDGINNSEGQ

AYATFDNWLTLPVSTFKPEIIKLEMKPHSKTRRYIRITQSLADFIKTIDE

ALMIKPSDSIDDPLNMPNEIVCKNKLFGNELKPRDGKMKIVSTGKIVTYE

FESDSTPQWIQTLYVTQLKKQP

[0124] In some embodiments the Cas9 protein can be *N. lactamica* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 167)

MAAFKPNPMNYILGLDIGIASVGWAMVEVDEEENPIRLIDLGVRVFERAE

VPKTGDSLAMARRLARSVRRLTRRRAHRLLRARRLLKREGVLQDADFDEN

GLVKSLPNTPWQLRAAALDRKLTCLEWSAVLLHLVKHRGYLSQRKNEGET

ADKELGALLKGVADNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYS

HTFSRKDLQAELNLLFEKQKEFGNPHVSDGLKEDIETLLMAQRPALSGDA

VQKMLGHCTFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDT

ERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEM

KAYHAISRALEKEGLKDKKSPLNLSTELQDEIGTAFSLFKTDKDITGRLK

DRVQPEILEALLKHISFDKFVQISLKALRRIVPLMEQGKRYDEACAEIYG

DHYCKKNAEEKIYLPPIPADEIRNPVVLRALSQARKVINCVVRRYGSPAR

IHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKS

KDILKLRLYEQQHGKCLYSGKEINLVRLNEKGYVEIDHALPFSRTWDDSF

NNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQ

RILLQKFDEEGFKERNLNDTRYVNRFLCQFVADHILLTGKGKRRVFASNG

QITNLLRGFWGLRKVRIENDRHHALDAVVVACSTVAMQQKITRFVRYKEM

NAFDGKTIDKETGEVLHQKAHFPQPWEFFAQEVMIRVFGKPDGKPEFEEA

-continued

DTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSA

KRLDEGISVLRVPLTQLKLKGLEKMVNREREPKLYDALKAQLETHKDDPA

KAFAEPFYKYDKAGSRTQQVKAVRIEQVQKTGVWVRNHNGIADNATMVRV

DVFEKGGKYYLVPIYSWQVAKGILPDRAVVAFKDEEDWTVMDDSFEFRFV

LYANDLIKLTAKKNEFLGYFVSLNRATGAIDIRTHDTDSTKGKNGIFQSV

GVKTALSFQKNQIDELGKEIRPCRLKKRPPVR

[0125] In some embodiments the Cas9 protein can be *N. meningitides* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 168)

MAAFKPNPINYILGLDIGIASVGWAMVEIDEDENPICLIDLGVRVFERAE

VPKTGDSLAMARRLARSVRRLTRRRAHRLLRARRLLKREGVLQAADFDEN

GLIKSLPNTPWQLRAAALDRKLTPLEWSAVLLHLIKHRGYLSQRKNEGET

ADKELGALLKGVADNAHALQTGDFRTPAELALNKFEKESGHIRNQRGDYS

HTFSRKDLQAELILLFEKQKEFGNPHVSGGLKEGIETLLMTQRPALSGDA

VQKMLGHCTFEPAEPKAAKNTYTAERFIWLTKLNNLRILEQGSERPLTDT

ERATLMDEPYRKSKLTYAQARKLLGLEDTAFFKGLRYGKDNAEASTLMEM

KAYHAISRALEKEGLKDKKSPLNLSPELQDEIGTAFSLFKTDEDITGRLK

DRIQPEILEALLKHISFDKFVQISLKALRRIVPLMEQGKRYDEACAEIYG

DHYGKKNTEEKIYLPPIPADEIRNPVVLRALSQARKVINGVVRRYGSPAR

IHIETAREVGKSFKDRKEIEKRQEENRKDREKAAAKFREYFPNFVGEPKS

KDILKLRLYEQQHGKCLYSGKEINLGRLNEKGYVEIDHALPFSRTWDDSF

NNKVLVLGSENQNKGNQTPYEYFNGKDNSREWQEFKARVETSRFPRSKKQ

RILLQKFDEDGFKERNLNDTRYVNRFLCQFVADRMRLTGKGKKRVFASNG

QITNLLRGFWGLRKVRAENDRHHALDAVVVACSTVAMQQKITRFVRYKEM

NAFDGKTIDKETGEVLHQKTHFPQPWEFFAQEVMIRVFGKPDGKPEFEEA

DTPEKLRTLLAEKLSSRPEAVHEYVTPLFVSRAPNRKMSGQGHMETVKSA

KRLDEGVSVLRVPLTQLKLKDLEKMVNREREPKLYEALKARLEAHKDDPA

KAFAEPFYKYDKAGNRTQQVKAVRVEQVQKTGVWVRNHNGIADNATMVRV

DVFEKGDKYYLVPIYSWQVAKGILPDRAVVQGKDEEDWQLIDDSFNFKFS

LHPNDLVEVITKKARMFGYFASCHRGTGNINIRIHDLDHKIGKNGILEGI

GVKTALSFQKYQIDELGKEIRPCRLKKRPPVR

[0126] In some embodiments the Cas9 protein can be *B. longum* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 169)

MLSRQLLGASHLARPVSYSYNVQDNDVHCSYGERCFMRGKRYRIGIDVGL

NSVGLAAVEVSDENSPVRLLNAQSVIHDGGVDPQKNKEAITRKNMSGVAR

RTRRMRRRKRERLHKLDMLLGKFGYPVIEPESLDKPFEEWHVRAELATRY

IEDDELRRESISIALRHMARHRGWRNPYRQVDSLISDNPYSKQYGELKEK

-continued

AKAYNDDATAAEEESTPAQLVVAMLDAGYAEAPRLRWRTGSKKPDAEGYL

PVRLMQEDNANELKQIFRVQRVPADEWKPLFRSVFYAVSPKGSAEQRVGQ

DPLAPEQARALKASLAFQEYRIANVITNLRIKDASAELRKLTVDEKQSIY

DQLVSPSSEDITWSDLCDFLGFKRSQLKGVGSLTEDGEERISSRPPRLTS

VQRIYESDNKIRKPLVAWWKSASDNEHEAMIRLLSNTVDIDKVREDVAYA

SAIEFIDGLDDDALTKLDSVDLPSGRAAYSVETLQKLTRQMLTTDDDLHE

ARKTLFNVTDSWRPPADPIGEPLGNPSVDRVLKNVNRYLMNCQQRWGNPV

SVNIEHVRSSFSSVAFARKDKREYEKNNEKRSIFRSSLSEQLRADEQMEK

VRESDLRRLEAIQRQNGQCLYCGRTITFRTCEMDHIVPRKGVGSTNTRTN

FAAVCAECNRMKSNTPFAIWARSEDAQTRGVSLAEAKKRVTMFTFNPKSY

APREVKAFKQAVIARLQQTEDDAAIDNRSIESVAWMADELHRRIDWYFNA

KQYVNSASIDDAEAETMKTTVSVFQGRVTASARRAAGIEGKIHFIGQQSK

TRLDRRHHAVDASVIAMMNTAAAQTLMERESLRESQRLIGLMPGERSWKE

YPYEGTSRYESFHLWLDNMDVLLELLNDALDNDRIAVMQSQRYVLGNSIA

HDATIHPLEKVPLGSAMSADLIRRASTPALWCALTRLPDYDEKEGLPEDS

HREIRVHDTRYSADDEMGFFASQAAQIAVQEGSADIGSAIHHARVYRCWK

TNAKGVRKYFYGMIRVFQTDLLRACHDDLFTVPLPPQSISMRYGEPRVVQ

ALQSGNAQYLGSLVVGDEIEMDFSSLDVDGQIGEYLQFFSQFSGGNLAWK

HWVVDGFFNQTQLRIRPRYLAAEGLAKAFSDDVVPDGVQKIVTKQGWLPP

VNTASKTAVRIVRRNAFGEPRLSSAHHMPCSWQWRHE

[0127] In some embodiments the Cas9 protein can be *A. muciniphila* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 170)

MSRSLTFSFDIGYASIGWAVIASASHDDADPSVCGCGTVLFPKDDCQAFK

RREYRRLRRNIRSRRVRIERIGRLLVQAQIITPEMKETSGHPAPFYLASE

ALKGHRTLAPIELWHVLRWYAHNRGYDNNASWSNSLSEDGGNGEDTERVK

HAQDLMDKHGTATMAETICRELKLEEGKADAPMEVSTPAYKNLNTAFPRL

IVEKEVRRILELSAPLIPGLTAEIIELIAQHHPLTTEQRGVLLQHGIKLA

RRYRGSLLFGQLIPRFDNRIISRCPVTWAQVYEAELKKGNSEQSARERAE

KLSKVPTANCPEFYEYRMARILCNIRADGEPLSAEIRRELMNQARQEGKL

TKASLEKAISSRLGKETETNVSNYFTLHPDSEEALYLNPAVEVLQRSGIG

QILSPSVYRIAANRLRRGKSVTPNYLLNLLKSRGESGEALEKKIEKESKK

KEADYADTPLKPKYATGRAPYARTVLKKVVEEILDGEDPTRPARGEAHPD

GELKAHDGCLYCLLDTDSSVNQHQKERRLDTMTNNHLVRHRMLILDRLLK

DLIQDFADGQKDRISRVCVEVGKELTTFSAMDSKKIQRELTLRQKSHTDA

VNRLKRKLPGKALSANLIRKCRIAMDMNWTCPFTGATYGDHELENLELEH

IVPHSFRQSNALSSLVLTWPGVNRMKGQRTGYDFVEQEQENPVPDKPNLH

ICSLNNYRELVEKLDDKKGHEDDRRRKKKRKALLMVRGLSHKHQSQNHEA

-continued

MKEIGMTEGMMTQSSHLMKLACKSIKTSLPDAHIDMIPGAVTAEVRKAWD

VFGVFKELCPEAADPDSGKILKENLRSLTHLHHALDACVLGLIPYIIPAH

HNGLLRRVLAMRRIPEKLIPQVRPVANQRHYVLNDDGRMMLRDLSASLKE

NIREQLMEQRVIQHVPADMGGALLKETMQRVLSVDGSGEDAMVSLSKKKD

GKKEKNQVKASKLVGVFPEGPSKLKALKAAIEIDGNYGVALDPKPVVIRH

IKVFKRIMALKEQNGGKPVRILKKGMLIHLTSSKDPKHAGVWRIESIQDS

KGGVKLDLQRAHCAVPKNKTHECNWREVDLISLLKKYQMKRYPTSYTGTP

R

[0128] In some embodiments the Cas9 protein can be *O. laneus* Cas9 and may comprise or consist of the amino acid sequence:

(SEQ ID NO: 171)

METTLGIDLGTNSIGLALVDQEEHQILYSGVRIFPEGINKDTIGLGEKEE

SRNATRRAKRQMRRQYFRKKLRKAKLLELLIAYDMCPLKPEDVRRWKNWD

KQQKSTVRQFPDTPAFREWLKQNPYELRKQAVTEDVTRPELGRILYQMIQ

RRGFLSSRKGKEEGKIFTGKDRMVGIDETRKNLQKQTLGAYLYDIAPKNG

EKYRFRTERVRARYTLRDMYIREFEIIWQRQAGHLGLAHEQATRKKNIFL

EGSATNVRNSKLITHLQAKYGRGHVLIEDTRITVTFQLPLKEVLGGKIEI

EEEQLKFKSNESVLFWQRPLRSQKSLLSKCVFEGRNFYDPVHQKWIIAGP

TPAPLSHPEFEEFRAYQFINNIIYGKNEHLTAIQREAVFELMCTESKDFN

FEKIPKHLKLFEKFNFDDTTKVPACTTISQLRKLFPHPVWEEKREEIWHC

FYFYDDNTLLFEKLQKDYALQTNDLEKIKKIRLSESYGNVSLKAIRRINP

YLKKGYAYSTAVLLGGIRNSFGKRFEYFKEYEPEIEKAVCRILKEKNAEG

EVIRKIKDYLVHNRFGFAKNDRAFQKLYHHSQAITTQAQKERLPETGNLR

NPIVQQGLNELRRTVNKLLATCREKYGPSFKFDHIHVEMGRELRSSKTER

EKQSRQIRENEKKNEAAKVKLAEYGLKAYRDNIQKYLLYKEIEEKGGTVC

CPYTGKTLNISHTLGSDNSVQIEHIIPYSISLDDSLANKTLCDATFNREK

GELTPYDFYQKDPSPEKWGASSWEEIEDRAFRLLPYAKAQRFIRRKPQES

NEFISRQLNDTRYISKKAVEYLSAICSDVKAFPGQLTAELRHLWGLNNIL

QSAPDITFPLPVSATENHREYYVITNEQNEVIRLFPKQGETPRIEKGELL

LTGEVERKVFRCKGMQEFQTDVSDGKYWRRIKLSSSVTWSPLFAPKPISA

DGQIVLKGRIEKGVFVCNQLKQKLKTGLPDGSYWISLPVISQTFKEGESV

NNSKLTSQQVQLFGRVREGIFRCHNYQCPASGADGNFWCTLDTDTAQPAF

TPIKNAPPGVGGGQIILTGDVDDKGIFHADDDLHYELPASLPKGKYYGIF

TVESCDPTLIPIELSAPKTSKGENLIEGNIWVDEHTGEVRFDPKKNREDQ

RHHAIDAIVIALSSQSLFQRLSTYNARRENKKRGLDSTEHFPSPWPGFAQ

DVRQSVVPLLVSYKQNPKTLCKISKTLYKDGKKIHSCGNAVRGQLHKETV

YGQRTAPGATEKSYHIRKDIRELKTSKHIGKVVDITIRQMLLKHLQENYH

IDITQEFNIPSNAFFKEGVYRIFLPNKHGEPVPIKKIRMKEELGNAERLK

DNINQYVNPRNNHHVMIYQDADGNLKEEIVSFWSVIERQNQGQPIYQLPR

-continued

EGRNIVSILQINDTFLIGLKEEEPEVYRNDLSTLSKHLYRVQKLSGMYYT

FRHHLASTLNNEREEFRIQSLEAWKRANPVKVQIDEIGRITFLNGPLC.

[0129] 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 or portion thereof. 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.

[0130] Exemplary wild type *Francisella tularensis* subsp. *Novicida* Cpf1 (FnCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence:

(SEQ ID NO: 172)

```
   1 MSIYQEFVNK YSLSKTLRFE LIPQGKTLEN IKARGLILDD EKRAKDYKKA KQIIDKYHQF
  61 FIEEILSSVC ISEDLLQNYS DVYFKLKKSD DDNLQKDFKS AKDTIKKQIS EYIKDSEKFK
 121 NLFNQNLIDA KKGQESDLIL WLKQSKDNGI ELFKANSDIT 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 EIAQNKDNLA QISIKYQNQG KKDLLQASAE DDVKAIKDLL DQTNNLLHKL
 541 KIFHISQSED KANILDKDEH FYLVFEECYF ELANIVPLYN KIRNYITQKP YSDEKFKLNF
 601 ENSTLANGWD KNKEPDNTAI LFIKDDKYYL GVMNKKNNKI FDDKAIKENK GEGYKKIVYK
 661 LLPGANKMLP KVFFSAKSIK FYNPSEDILR IRNHSTHTKN GSPQKGYEKF EFNIEDCRKF
 721 IDFYKQSISK HPEWKDFGFR FSDTQRYNSI DEFYREVENQ GYKLTFENIS ESYIDSVVNQ
 781 GKLYLFQIYN KDFSAYSKGR PNLHTLYWKA LFDERNLQDV VYKLNGEAEL FYRKQSIPKK
 841 ITHPAKEAIA NKNKDNPKKE SVFEYDLIKD KRFTEDKFFF HCPITINFKS SGANKFNDEI
 901 NLLLKEKAND VHILSIDRGE RHLAYYTLVD GKGNIIKQDT FNIIGNDRMK TNYHDKLAAI
 961 EKDRDSARKD WKKINNIKEM KEGYLSQVVH EIAKLVIEYN AIVVFEDLNF GFKRGRFKVE
1021 KQVYQKLEKM LIEKLNYLVF KDNEFDKTGG VLRAYQLTAP FETFKKMGKQ TGIIYYVPAG
1081 FTSKICPVTG FVNQLYPKYE SVSKSQEFFS KFDKICYNLD KGYFEFSFDY KNFGDKAAKG
1141 KWTIASFGSR LINFRNSDKN HNWDTREVYP TKELEKLLKD YSIEYGHGEC IKAAICGESD
1201 KKFFAKLTSV LNTILQMRNS KTGTELDYLI SPVADVNGNF FDSRQAPKNM PQDADANGAY
1261 HIGLKGLMLL GRIKNNQEGK KLNLVIKNEE YFEFVQNRNN.
```

[0131] Exemplary wild type *Lachnospiraceae bacterium* sp. ND2006 Cpf1 (LbCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence:

(SEQ ID NO: 173)

```
   1 AASKLEKFTN CYSLSKTLRF KAIPVGKTQE NIDNKRLLVE DEKRAEDYKG VKKLLDRYYL
  61 SFINDVLHSI KLKNLNNYIS LFRKKTRTEK ENKELENLEI NLRKEIAKAF KGAAGYKSLF
 121 KKDIIETILP EAADDKDEIA LVNSFNGFTT AFTGFFDNRE NMFSEEAKST SIAFRCINEN
 181 LTRYISNMDI FEKVDAIFDK HEVQEIKEKI LNSDYDVEDF FEGEFFNFVL TQEGIDVYNA
 241 IIGGFVTESG EKIKGLNEYI NLYNAKTKQA LPKFKPLYKQ VLSDRESLSF YGEGYTSDEE
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 301 VLEVFRNTLN KNSEIFSSIK KLEKLFKNFD EYSSAGIFVK NGPAISTISK DIFGEWNLIR

 361 DKWNAEYDDI HLKKKAVVTE KYEDDRRKSF KKIGSFSLEQ LQEYADADLS VVEKLKEIII

 421 QKVDEIYKVY GSSEKLFDAD FVLEKSLKKN DAVVAIMKDL LDSVKSFENY IKAFFGEGKE

 481 TNRDESFYGD FVLAYDILLK VDHIYDAIRN YVTQKPYSKD KFKLYFQNPQ FMGGWDKDKE

 541 TDYRATILRY GSKYYLAIMD KKYAKCLQKI DKDDVNGNYE KINYKLLPGP NKMLPKVFFS

 601 KKWMAYYNPS EDIQKIYKNG TFKKGDMFNL NDCHKLIDFF KDSISRYPKW SNAYDFNFSE

 661 TEKYKDIAGF YREVEEQGYK VSFESASKKE VDKLVEEGKL YMFQIYNKDF SDKSHGTPNL

 721 HTMYFKLLFD ENNHGQIRLS GGAELFMRRA SLKKEELVVH PANSPIANKN PDNPKKTTTL

 781 SYDVYKDKRF SEDQYELHIP IAINKCPKNI FKINTEVRVL LKHDDNPYVI GIDRGERNLL

 841 YIVVVDGKGN IVEQYSLNEI INNFNGIRIK TDYHSLLDKK EKERFEARQN WTSIENIKEL

 901 KAGYISQVVH KICELVEKYD AVIALEDLNS GFKNSRVKVE KQVYQKFEKM LIDKLNYMVD

 961 KKSNPCATGG ALKGYQITNK FESFKSMSTQ NGFIFYIPAW LTSKIDPSTG FVNLLKTKYT

1021 SIADSKKFIS SFDRIMYVPE EDLFEFALDY KNFSRTDADY IKKWKLYSYG NRIRIFAAAK

1081 KNNVFAWEEV CLTSAYKELF NKYGINYQQG DIRALLCEQS DKAFYSSFMA LMSLMLQMRN

1141 SITGRTDVDF LISPVKNSDG IFYDSRNYEA QENAILPKNA DANGAYNIAR KVLWAIGQFK

1201 KAEDEKLDKV KIAISNKEWL EYAQTSVK.
```

[0132] Exemplary wild type *Acidaminococcus* sp. BV3L6 Cpf1 (AsCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence:

(SEQ ID NO: 174)

```
   1 MTQFEGFTNL YQVSKTLRFE LIPQGKTLKH IQEQGFIEED KARNDHYKEL KPIIDRIYKT

  61 YADQCLQLVQ LDWENLSAAI DSYRKEKTEE TRNALIEEQA TYRNAIHDYF IGRTDNLTDA

 121 INKRHAEIYK GLFKAELFNG KVLKQLGTVT TTEHENALLR SFDKFTTYFS GFYENRKNVF

 181 SAEDISTAIP HRIVQDNFPK FKENCHIFTR LITAVPSLRE HFENVKKAIG IFVSTSIEEV

 241 FSFPFYNQLL TQTQIDLYNQ LLGGISREAG TEKIKGLNEV LNLAIQKNDE TAHIIASLPH

 301 RFIPLFKQIL SDRNTLSFIL EEFKSDEEVI QSFCKYKTLL RNENVLETAE ALFNELNSID

 361 LTHIFISHKK LETISSALCD HWDTLRNALY ERRISELTGK ITKSAKEKVQ RSLKHEDINL

 421 QEIISAAGKE LSEAFKQKTS EILSHAHAAL DQPLPTTLKK QEEKEILKSQ LDSLLGLYHL

 481 LDWFAVDESN EVDPEFSARL TGIKLEMEPS LSFYNKARNY ATKKPYSVEK FKLNFQMPTL

 541 ASGWDVNKEK NNGAILFVKN GLYYLGIMPK QKGRYKALSF EPTEKTSEGF DKMYYDYFPD

 601 AAKMIPKCST QLKAVTAHFQ THTTPILLSN NFIEPLEITK EIYDLNNPEK EPKKFQTAYA

 661 KKTGDQKGYR EALCKWIDFT RDFLSKYTKT TSIDLSSLRP SSQYKDLGEY YAELNPLLYH

 721 ISFQRIAEKE IMDAVETGKL YLFQIYNKDF AKGHHGKPNL HTLYWTGLFS PENLAKTSIK

 781 LNGQAELFYR PKSRMKRMAH RLGEKMLNKK LKDQKTPIPD TLYQELYDYV NHRLSHDLSD

 841 EARALLPNVI TKEVSHEIIK DRRFTSDKFF FHVPITLNYQ AANSPSKFNQ RVNAYLKEHP

 901 ETPIIGIDRG ERNLIYITVI DSTGKILEQR SLNTIQQFDY QKKLDNREKE RVAARQAWSV

 961 VGTIKDLKQG YLSQVIHEIV DLMIHYQAVV VLENLNFGFK SKRTGIAEKA VYQQFEKMLI

1021 DKLNCLVLKD YPAEKVGGVL NPYQLTDQFT SFAKMGTQSG FLFYVPAPYT SKIDPLTGFV

1081 DPFVWKTIKN HESRKHFLEG FDFLHYDVKT GDFILHFKMN RNLSFQRGLP GFMPAWDIVF
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1141 EKNETQFDAK GTPFIAGKRI VPVIENHRFT GRYRDLYPAN ELIALLEEKG IVFRDGSNIL

1201 PKLLENDDSH AIDTMVALIR SVLQMRNSNA ATGEDYINSP VRDLNGVCFD SRFQNPEWPM

1261 DADANGAYHI ALKGQLLLNH LKESKDLKLQ NGISNQDWLA YIQELRN.

[0133] 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 propionicigenes* 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.

[0134] 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 propionicigenes* WB4	WP_013443710.1	CTTGTGGATTATCCCAAAATTGAAG GGAACTACAAC (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)

-continued

Cas13a number	Cas13a abbreviation	Organism name	Accession number	Direct Repeat sequence
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)

[0135] Exemplary wild type Cas13a proteins of the disclosure may comprise or consist of the amino acid sequence:

(SEQ ID NO: 190)

```
   1 MGNLFGHKRW YEVRDKKDFK IKRKVKVKRN YDGNKYILNI NENNNKEKID NNKFIRKYIN

  61 YKKNDNILKE FTRKFHAGNI LFKLKGKEGI IRIENNDDFL ETEEVVLYIE AYGKSEKLKA

 121 LGITKKKIID EAIRQGITKD DKKIEIKRQE NEEEIEIDIR DEYTNKTLND CSIILRIIEN

 181 DELETKKSIY EIFKNINMSL YKIIEKIIEN ETEKVFENRY YEEHLREKLL KDDKIDVILT

 241 NFMEIREKIK SNLEILGFVK FYLNVGGDKK KSKNKKMLVE KILNINVDLT VEDIADFVIK

 301 ELEFWNITKR IEKVKKVNNE FLEKRRNRTY IKSYVLLDKH EKFKIERENK KDKIVKFFVE

 361 NIKNNSIKEK IEKILAEFKI DELIKKLEKE LKKGNCDTEI FGIFKKHYKV NFDSKKFSKK

 421 SDEEKELYKI IYRYLKGRIE KILVNEQKVR LKKMEKIEIE KILNESILSE KILKRVKQYT

 481 LEHIMYLGKL RHNDIDMTTV NTDDFSRLHA KEELDLELIT FFASTNMELN KIFSRENINN

 541 DENIDFFGGD REKNYVLDKK ILNSKIKIIR DLDFIDNKNN ITNNFIRKFT KIGTNERNRI

 601 LHAISKERDL QGTQDDYNKV INIIQNLKIS DEEVSKALNL DVVFKDKKNI ITKINDIKIS

 661 EENNNDIKYL PSFSKVLPEI LNLYRNNPKN EPFDTIETEK IVLNALIYVN KELYKKLILE

 721 DDLEENESKN IFLQELKKTL GNIDEIDENI IENYYKNAQI SASKGNNKAI KKYQKKVIEC

 781 YIGYLRKNYE ELFDFSDFKM NIQEIKKQIK DINDNKTYER ITVKTSDKTI VINDDFEYII

 841 SIFALLNSNA VINKIRNRFF ATSVWLNTSE YQNIIDILDE IMQLNTLRNE CITENWNLNL

 901 EEFIQKMKEI EKDFDDFKIQ TKKEIFNNYY EDIKNNILTE FKDDINGCDV LEKKLEKIVI

 961 FDDETKFEID KKSNILQDEQ RKLSNINKKD LKKKVDQYIK DKDQEIKSKI LCRIIFNSDF

1021 LKKYKKEIDN LIEDMESENE NKFQEIYYPK ERKNELYIYK KNLFLNIGNP NFDKIYGLIS

1081 NDIKMADAKF LFNIDGKNIR KNKISEIDAI LKNLNDKLNG YSKEYKEKYI KKLKENDDFF

1141 AKNIQNKNYK SFEKDYNRVS 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.
```

[0136] Exemplary Cas13b proteins include, but are not limited to:

Species	Cas13b Accession	Cas13b Size (aa)
Paludibacter propionicigenes 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	EH006562.1	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	E0A10535.1	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

[0137] Exemplary wild type Bergeyella zoohelcum ATCC 43767 Cas13b (BzCas13b) proteins of the disclosure may comprise or consist of the amino acid sequence:

(SEQ ID NO: 191)

```
  1 menktslgnn iyynpfkpqd ksyfagyfna amentdsvfr elgkrlkgke ytsenffdai

 61 fkenislvey eryvkllsdy fpmarlldkk evpikerken fkknfkgiik avrdlrnfyt

121 hkehgeveit deifgvldem lkstvltvkk kkvktdktke ilkksiekql dilcqkkley

181 lrdtarkiee krrnqrerge kelvapfkys dkrddliaai yndafdvyid kkkdslkess

241 kakyntksdp qqeegdlkip iskngvvfll slfltkqeih afkskiagfk atvideatvs

301 eatvshgkns icfmatheif shlaykklkr kvrtaeinyg eaenaeqlsv yaketlmmqm

361 ldelskvpdv vyqnlsedvq ktfiedwney lkenngdvgt meeeqvihpv irkryedkfn

421 yfairfldef aqfptlrfqv hlgnylhdsr pkenlisdrr ikekitvfgr lselehkkal

481 fikntetned rehyweifpn pnydfpkeni svndkdfpia gsildrekqp vagkigikvk

541 llnqqyvsev dkavkahqlk qrkaskpsiq niieeivpin esnpkeaivf ggqptaylsm

601 ndihsilyef fdkwekkkek lekkgekelr keigkelekk ivgkiqaqiq qiidkdtnak

661 ilkpyqdgns taidkeklik dlkqeqnilq klkdeqtvre keyndfiayq dknreinkvr
```

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```
 721 drnhkqylkd nlkrkypeap arkevlyyre kgkvavwlan dikrfmptdf knewkgeqhs

 781 llqkslayye qckeelknll pekvfqhlpf klggyfqqky lyqfytcyld krleyisglv

 841 qqaenfksen kvfkkvenec fkflkkqnyt hkeldarvqs ilgypifler gfmdekptii

 901 kgktfkgnea lfadwfryyk eyqnfqtfyd tenyplvele kkqadrkrkt kiyqqkkndv

 961 ftllmakhif ksvfkqdsid qfsledlyqs reerlgnqer arqtgerntn yiwnktvdlk

1021 lcdgkitven vklknvgdfi kyeydqrvqa flkyeeniew qaflikeske eenypyvver

1081 eieqyekvrr eellkevhli eeyilekvkd keilkkgdnq nfkyyilngl lkqlknedve

1141 sykvfnlnte pedvninqlk qeatdleqka fvltyirnkf ahnqlpkkef wdycqekygk

1201 ektyaey faevfkkeke alik.
```

[0138] 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 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. Also see WO Publication No. WO2019/040664 & US2019/0062724, which is incorporated herein by reference in its entirety, for further examples and sequences of CasRX/Cas13d protein, without limitation, specific reference is made to

[0139] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig6049000251:

(SEQ ID NO: 54)

```
LYLTSFGKGN AAVIEQKIEP ENGYRVTGMQ ITPSITVNKA TDESVRFRVK RKIAQKDEFI   60

ADNPMHEGRH RIEPSAGSDM LGLKTKLEKY YFGKEFDDNL HIQIIYNILD IEKILAVYST  120

NITA.                                                               124
```

[0140] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig546000275:

(SEQ ID NO: 57)

```
MDSYRPKLYK LIDFCIFKHY HEYTEISEKN VDTLRAAVSE EQKESFYADE AKRLWGIFDK    60

QFLGFCKKIN VWVNGSHEKE ILGYIDKDAY RKKSDVSYFS KFLYAMSFFL DGKEINDLLT   120

TLINKFDNIA SFISTAKELD AEIDRILEKK LDPVTGKPLK GKNSFRNFIA NNVIENKRFI   180

YVIKFCNPKN VLKLVKNTKV TEFVLKRMPE SQIDRYYSSC IDTEKNPSVD KKISDLAEMI   240

KKIAFDDFRN VRQKTRTREE SLEKERFKAV IGLYLTVVYL LIKNLVNVNS RYVMAFHCLE   300

RDAKLYGINI GKNYIELTED LCRENENSRS AYLARNKRLR DCVKQNIDNA KNMKSKEK.    358
```

[0141] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig4114000374:

(SEQ ID NO: 61)

```
DTKINPQTWL YQLENTPDLD NEYRDTLDHF FDERFNEINE HFVTQNATNL CIMKEVFPDE   60

DFKSIADLYY DFIVVKSYKN IGFSIKKLRE KMLELPEAKR VTSTEMDSVR SKLYKLIDFC  120

IFKHYHEKPE TVEMIVSMLR AYTSEDMKE.                                    149
```

[0142] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig721000619:

(SEQ ID NO: 67)

```
KEGSTMAKNE KKKSTAKALG LKSSFVVNND IYMTSFGKGN KAVLEKKITE NTIENKSDTT   60

YFDVINRDPK GFTLEGRRIA DMTAFSNDPK YHVNVVNGKF LEDQLGARSE LEKKVFGRTF  120

DDNVHIQLIH NILDIEKIMA QYVSDIVYLL HNTIKRDMND DIMGYISIRN SFDDFCHPER  180

IPDRKAKDNL QKQHDIFFDE ILKCGRLAYF GNAFFEDGSD NKEIAKLKRY KEIYHIIALM  240

GSLRQSYFHG ENSDKNFQGP TWAYTLESNL TGKYKEFKDT LDKTFDERYE MISKDFGSTN  300

MVNLQILEEL LKMLYGNVSP.                                             320
```

[0143] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig2002000411:

(SEQ ID NO: 69)

```
EKQNKAKYQA IISLYLMVMY QIVKNMIYVN SRYVIAFHCL ERDSNQLLGR FNSRDASMYN   60

KLTQKFITDK YLNDGAQGCS KKVGNYLSHN ITCCSDELRK EYRNQVDHFA VVRMIGKYAA  120

DIGKFSTWFE LYHYVMQRII FDKRNPLSET ERTYKQLIAK HHTYCKDLVK ALNTPFGYNL  180

ARYKNLSIGE LFDRNNYNAK TKET.                                        204
```

[0144] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contigl3552000311:

(SEQ ID NO: 71)

```
LIDFLIYDLY YNRKPARIEE IVDKLRESVN DEEKESIYSA ETKYVYEALG KVLVRSLKKY   60

LNGATIRDLK NRYDAKTANR IWDISEHSKS GHVNCFCKLI YMMTLMLDGK EINDLLTTLV  120

NKFDNIASFI DVMDELGLEH SFTDNYKMFA DSKAICLDLQ FINSFARMSK IDDEKSKRQL  180

FRDALVVLDI GDKNEDWIEK YLTSDIFKRD ENGNKIDGEK RDFRNFIANN VIKSARFKYL  240

VKYSSADGMI KLKKNEKLIS FVLEQLPETQ IDRYYESCGL DCAVADRKVR IEKLTGLIRD  300

MRFDNFRGVN YSNDACKKDK QAKAKYQAII SLYLMVLYQI VKNMIYVNSR YVIAFHCLER  360

DLLFFNIELD NSYQYSNCNE LTEKFIKDKY MKEGALGFNM KAGRYLTKNI GNCSNELRKI  420

YRNQVDHFAV VRKIGNYAAD IASVGSWFE.                                    449
```

[0145] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig10037000527:

(SEQ ID NO: 72)

```
YMDQNFANSD AWAIHVYRNK IQHLDAVRHA DMYIGDIREF HSWFELYHYI IQRRIIDQYA   60

YESTPGSSRD GSAIIDEERL NPATRRYFRL ITTYKT.                            96
```

[0146] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig238000329:

(SEQ ID NO: 73)

```
RYDKDRSKIY TMMDFVIYRY YIDNNNDSID FINKLRSSID EKSKEKLYNE EANRLWNKLK   60

EYMLYIKEFN GKLASRTPDR DGNISEFVES LPKIHRLLPR GQKISNFSKL MYLLTMFLDG  120
```

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```
KEINDLLTTL INKFENIQGF LDIMPEINVN AKFEPEYVFF NKSHEIAGEL KLIKGFAQMG  180

EPAATLKLEM TADAIKILGT EKEDAELIKL AESLFKDENG KLLGNKQHGM RNFIGNNVIK  240

SKRFHYLIRY GDPAHLHKIA TNKNVVRFVL GRIADMQKKQ GQKGKNQIDR YYEVCVGNKD  300

IKKTIEEKID ALTDIIVNMN YDQFEKKKAV IENQNRGKTF EEKNKYKRDN AEREKFKKII  360

SLYLTVIYHI LKNIVNVNSR YILGFHCLER DKQLYIEKYN KDKLDGFVAL TKFCLGDEER  420

YEDLKAKAQA SIQALETANP KLYAKYMNYS DEEKKEEFKK QLNRERVKNA RNAYLKNIKN  480

YIMIRLQLRD QTDSSGYLCG EFRDKVAHLE VARHAHEYI.                        519
```

[0147] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut meta enome conti 2643000492:

```
                                                          (SEQ ID NO: 84)
NGEIVSLAEK EAFSAKIADK NIGCKIENKQ FRHPKGYDVI ADNPIYKGSP RQDMLGLKET   60

LEKRYFSPSD SIDNVRVQVA HNILDIEKIL AEYITNAVYS FDNIAGFGKD IIGDDFSPVY  120

TYDKFEKSDR YEYFKNLLNN SRLGYYGQAF FECDDSKENK KKKDAIKCYN IIALLSGLRH  180

W.                                                                 181
```

[0148] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig874000057:

```
                                                          (SEQ ID NO: 85)
MSKNKESYAK GMGLKSALVS GSKVYMTSFE GGNDAKLEKV VENSEIVSLA EKESFSAEIF   60

KKNIGCKIEN KKFKHPKRYD VIADNPLYKG SVRQDMLGLK ETLEKRYFNS ADGTDNVCIQ  120

VIHNILDIEK ILAEYITNAV YSFDNIAGFG EDIIGMGGFK PIYTYKQFKE PDKYNKKFDD  180

ILNNSRLGYY GKAFFEKNDL KHNPNKKKRD KNPYILKYDN ECYYIIALLS GLRHWNIHSH  240

AKDDLVSYRW LYNLDSILNR EYISTLNYLY DDIADELTES FSKNSSANVN YIAETLNIDP  300

SEFAQQYFRF SIMKEQKNMG FNVSKLREIM LDRKELSDIR DNHRVFDSIR SKLYTMMDFV  360

IYRYYIEEAA KTEAENRNLP ENEKKISEKD FFVINLRGSF DENQKEKLYI EEAKRLWEKL  420

KDIMLKIKEF RGEKVKEYKK.                                             440
```

[0149] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig4781000489:

```
                                                          (SEQ ID NO: 86)
LDKQLDYEYI RTLNYMFNDI ADELTRTFSK NSAANVNYIA ETLNIDPNKF AEQYFRFSIM   60

KEQKNLGFNL TKLRESMLDR RELSDIRDNH NVFDSIRPKL YTMMDFVIYK HYIDEAKKTE  120

AENKSLPDDR KNLSEKD.                                                137
```

[0150] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig12144000352:

```
                                                          (SEQ ID NO: 87)
RMGEPVANTK RVMMIDAVKI LGTDLSDDEL KEMADSFFKD SDGNLLKKGK HGMRNFITNN   60

VIKNKRFHYL IRYGDPAHLH EIAKNEA.                                      87
```

[0151] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig5590000448:

(SEQ ID NO: 88)

```
VHNNEEKDLI KYTWLYNLDK YLDAEYITTL NYMYNDIGDE LTDSFSKNSA ANINYIAETL   60

GIDPKTFAEQ YFRFSIMKEQ KNLGFNLTKL REVMLDRKDM SEIRENHNDF DSIRAKVYTM  120

MDFVIYRYYI EEAAKVNAAN KSLPDNEKSL SEKDIFVISL RGSFNEDQKD RLYYDEAQRL  180

WSKVGKLMLK IKKFRGKDTR KYKNMGTPRI RRLIPEGRDI STFSKLMYAL TMFLDGKEIN  240

DLLTTLINKF DNIQSFLKVM PLIGVNAKFA EEYSFFNNSE KIADELRLIK SFARMGEPVA  300

DARRAMYIDA IRILGTDLSD DELKALADSF SLDENGNKLG KGKHGMRNFI INNVITNKRF  360

HYLIRYGNPV HLHEIAKNEA VVKFVLGRIA DIQKKQGQNG KNQIDRYYET CIGK.       414
```

[0152] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig525000349:

(SEQ ID NO: 89)

```
MSKKENRKSY VKGLGLKSTL VSDSKVYLTT FADGSNAKLE KCVENNKIIC ISNDKEAFAA   60

SIANKNVGYK IKNDEKFRHP KGYDIISNNP LLHNNSVQQD MLGLKNVLEK RYFGKSSGGD  120

NNLCIQIIHN IIDIEKILSE YIPNVVYAFN NIAGFKDEHN NIIDIIGTQT YNSSYTYADF  180

SKDKSDKKYI EFQKLLKNKR LGYWGKAFFT GQGNNAKVRQ ENQCFHIIAL LISLRNWATH  240

SNELDKHTKR TWLYKLDDTN ILNAEYVKTL NYLYDTIADE LTKSFSKNGA VNVNYLAKKY  300

NIKDDLPGFS EQYFRFSIMK EQKNLGFNIS KLRENMLDFK DMSVI.                  345
```

[0153] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig7229000302:

(SEQ ID NO: 90)

```
KKISSLTKFC LGESDEKKLK ALAKKSLEEL KTTNSKLYEN YIKYSDERKA EEAKRQINRE   60

RAKTAMNAHL RNTKWNDIMY GQLKDLADSK SRICSEFRNK AAHLEVARYA HMYINDISEV  120

KSYFRLYHYI MQRRIIDVIE NNPKAKYEGK VKVYFEDVKK NKKYNKNLLK LMCVPFGYCI  180

PRFKNLSIEQ MFDMNETDNS DKKKEK.                                       206
```

[0154] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig3227000343:

(SEQ ID NO: 91)

```
IGDISEVNSY FQLYHYIMQR ILIDKIGSKT TGKAKEYFDS VIVNKKYDDR LLKLLCSPLG   60

YCLTRYKDLS IEALFDMNEA AKYDKLNKER KNKKK.                             95
```

[0155] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Gut_metagenome_contig7030000469:

(SEQ ID NO: 92)

```
SIRSKLYTMM DFVIYRYYIE ESAKAAAENK PSESDSFVIR LRGSFNENQK EELYIEEAER   60

LWKKFGEIML KIKEFRGEKV KEYKKEVPRI ERILPHGKDI SAFSKLMYML SMFLD.      115
```

[0156] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d gut_metagenome_P17E0k2120140920, c87000043:

(SEQ ID NO: 93)

```
MYFSKMIYML TYFLDGKEIN DLLTTLISKF DNIKEFLKIM KSSAVDVECE LTAGYKLFND   60

SQRITNELFI VKNIASMRKP AASAKLTMFR DALTILGIDD KITDDRISEI LKLKEKGKGI  120

HGLRNFITNN VIESSRFVYL IKYANAQKIR EVAKNEKVVM FVLGGIPDTQ IERYYKSCVE  180

FPDMNSSLEA KRSELARMIK NISFDDFKNV KQQAKGRENV AKERAKAVIG LYLT.       234
```

[0157] 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|OBXZ01000094.11 and emb|OBJF01000033.11):

(SEQ ID NO: 94)

```
MAKKKRITAK ERKQNHRELL MKKADSNAEK EKAKKPVVEN KPDTAISKDN TPKPNKEIKK   60

SKAKLAGVKW VIKANDDVAY ISSFGKGNNS VLEKRIMGDV SSNVNKDSHM YVNPKYTKKN  120

YEIKNGFSSG SSLVTYPNKP DKNSGMDALC LKPYFEKDFF GHIFTDNMHI QAIYNIFDIE  180

KILAKHITNI IYTVNSFDRN YNQSGNDTIG FGLNYRVPYS EYGGGKDSNG EPKNQSKWEK  240

RDNFIKFYNE SKPHLGYYEN IFYDHGEPIS EEKFYNYLNI LNFIRNNTFH YKDDDIELYS  300

ENYSEEFVFI NCLNKFVKNK FKNVNKNFIS NEKNNLYIIL NAYGKDTENV EVVKKYSKEL  360

YKLSVLKTNK NLGVNVKKLR ESAIEYGYCP LPYDKEKEVA KLSSVKHKLY KTYDFVITHY  420

LNSNDKLLLE IVETLRLSKN DDEKENVYKK YAEKLFKADD VINPIKAISK LFARKGNKLF  480

KEKIIIKKEY IEDVSIDKNI YDFTKVIFFM TCFLDGKEIN DLLTNIISKL QVIEDHNNVI  540

KFISNNKDAV YKDYSDKYAI FRNAGKIATE LEAIKSIARM ENKIENAPQE PLLKDALLSL  600

GVSDDTKVLE NTYNKYFDSK EKTDKQSQKV STFLMNNVIN NNRFKYVIKY INPADINGLA  660

KNRYLVKFVL SKIPEEQIDS YYKLFSNEEE PGCEEKIKLL TKKISKLNFQ TLFENNKIPN  720

VEKEKKKAII TLYFTIVYIL VKNLVNINGL YTLALYFVER DGYFYKDICG KKDKKKSYND  780

VDYLLLPEIF SGSKYREETK NLKLPKEKDR DIMKKYLPND KDREKYNKFF TAYRNNIVHL  840

NIIAKLSELT KNIDKDINSY FDIYHYCTQR VMFNYCKEKN DVVLAKMKDL AHIKSDCNEF  900

SSKHTYPFSS AVLRFMNLPF AYNVPRFKNL SYKKFFDKQ.                        939
```

[0158] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): contig tpg|DJXD01000002.11 (uncultivated Ruminococcus assembly, UBA7013, from sheep gut metagenome):

(SEQ ID NO: 95)

```
MKKQKSKKTV SKTSGLKEAL SVQGTVIMTS FGKGNMANLS YKIPSSQKPQ NLNSSAGLKN   60

VEVSGKKIKF QGRHPKIATT DNPLFKPQPG MDLLCLKDKL EMHYFGKTFD DNIHIQLIYQ  120

ILDIEKILAV HVNNIVFTLD NVLHPQKEEL TEDFIGAGGW RINLDYQTLR GQTNKYDRFK  180

NYIKRKELLY FGEAFYHENE RRYEEDIFAI LTLLSALRQF CFHSDLSSDE SDHVNSFWLY  240

QLEDQLSDEF KETLSILWEE VTERIDSEFL KTNTVNLHIL CHVFPKESKE TIVRAYYEFL  300

IKKSFKNMGF SIKKLREIML EQSDLKSFKE DKYNSVRAKL YKLFDFIITY YYDHHAFEKE  360

ALVSSLRSSL TEENKEEIYI KTARTLASAL GADFKKAAAD VNAKNIRDYQ KKANDYRISF  420

EDIKIGNTGI GYFSELIYML TLLLDGKEIN DLLTTLINKF DNIISFIDIL KKLNLEFKFK  480
```

-continued

PEYADFFNMT NCRYTLEELR VINSIARMQK PSADARKIMY RDALRILGMD NRPDEEIDRE 540

LERTMPVGAD GKFIKGKQGF RNFIASNVIE SSRFHYLVRY NNPHKTRTLV KNPNVVKFVL 600

EGIPETQIKR YFDVCKGQEI PPTSDKSAQI DVLARIISSV DYKIFEDVPQ SAKINKDDPS 660

RNFSDALKKQ RYQAIVSLYL TVMYLITKNL VYVNSRYVIA FHCLERDAFL HGVTLPKMNK 720

KIVYSQLTTH LLTDKNYTTY GHLKNQKGHR KWYVLVKNNL QNSDITAVSS FRNIVAHISV 780

VRNSNEYISG IGELHSYFEL YHYLVQSMIA KNNWYDTSHQ PKTAEYLNNL KKHHTYCKDF 840

VKAYCIPFGY VVPRYKNLTI NELFDRNNPN PEPKEEV. 877

[0159] An exemplary direct repeat sequence of CasRX/Cas13d Metagenomic hit (no protein accession): contig tpg|DJXD01000002.11 (uncultivated Ruminococcus assembly, UBA7013, from sheep gut metagenome) (SEQ ID NO: 95) comprises or consists of the nucleic acid sequence:

CasRX/Cas13d DR:

[0160]

(SEQ ID NO: 96)

caactacaac cccgtaaaaa tacggggttc tgaaac. 36

[0161] 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):

(SEQ ID NO: 97)

MKKKNIRATR EALKAQKIKK SQENEALKKQ KLAEEAAQKR REELEKKNLA QWEETSAEGR 60

RSRVKAVGVK SVFVVGDDLY LATFGNGNET VLEKKITPDG KITTFPEEET FTAKLKFAQT 120

EPTVATSIGI SNGRIVLPEI SVDNPLHTTM QKNTIKRSAG EDILQLKDVL ENRYFDRSFN 180

DDLHIRLIYN ILDIEKILAE YTTNAVFAID NVSGCSDDFL SNFSTRNQWD EFQNPEQHRE 240

HFGNKDNVIC SVKKQQDLFF NFFKNNRIGY FGKAFFHAES ERKIVKKTEK EVYHILTLIG 300

SLRQWITHST EGGISRLWLY QLEDALSREY QETMNNCYNS TIYGLQKDFE KTNAPNLNFL 360

AEILGKNASE LAEPYFRFII TKEYKNLGFS IKTLREMLLD QPDLQEIREN HNVYDSIRSK 420

LYKMIDFVLV YAYSNERKSK ADALASNLRS AITEDAKKRI YQNEADQLWT SYQELFKRIR 480

GFKGAQVKEY SSKNMPIPIQ KQIQNILKPA EQVTYFTKLM YLLTMFLDGK EINDLLTTLI 540

NKFDNISSLL KTMEQLELQT TFKEDYTFFQ QSSRLCKEIT QLKSFARMGN PISNLKEVMM 600

VDAIQILGTE KSEQELQSMA CFFFRDKNGK KLNTGEHGMR NFIGNNVISN TRFQYLIRYG 660

NPQKLHTLSQ NETVVRFVLS RIAKNQRVQG MNGKNQIDRY YETCGGTNSW SVSEEEKINF 720

LCKILTNMSY DQFQDVKQSG AEITAEEKRK KERYKAIISL YLTVLYQLIK NLVNINARYI 780

IAFHCLERDA ILYSSKFNTS INLKKRYTAL TEMILGYETD EKARRKDTRT VYEKAEAAKN 840

RHLKNVKWNC KTRENLENAD KNAIVAFRNI VAHLWIIRDA DRFITGMGAM KRYFDCYHYL 900

LQRELGYILE KSNQGSEYTK KSLEKVQQYH SYCKDFLHML CLPFAYCIPR YKNLSIAELF 960

DRHEPEAEPK EEASSVNNSQ FITT. 984

[0162] 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):

(SEQ ID NO: 98)

```
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 EEIYYTFMLL  300

STARNFSAHY LDRNRAKSSD AEDFDGTSVI MYNLDNEELY KKLYNKKVHM ALTGMKKVLD  360

ANFNKKVEHL NNSFIKNSAK DFVILCEVLG IKSRDEKTKF VKDYYDFVVR KNYKHLGFSV  420

KELRELLFAN HDSNKYIKEF DKISNKKFDS VRSRLNRLAD YIIYDYYNKN NAKVSDLVKY  480

LRAAADDEQK KKIYLNESIN LVKSGILERI KKILPKLNGK IIGNMQPDST ITASMLHNTG  540

KDWHPISENA HYFTKWIYTL TLFMDGKEIN DLVTTLINKF DNIASFIEVL KSQSVCTHFS  600

EERKMFIDSA EICSELSAMN SFARMEAPGA SSKRAMFVEA ARILGDNRSK EELEEYFDTL  660

FDKSASKKEK GFRNFIRNNV VDSNRFKYLT RYTDTSSVKA FSNNKALVKF AIKDIPQEQI  720

LRYYNSCFGA SERYYNDGMS DKLVEAIGKI NLMQFNGVIQ QADRNMLPEE KKKANAQKEK  780

YKSIIRLYLT VCYLFFKNLV YVNSRYYSAF YNLEKDRSLF EINGELKPTG KFDEGHYTGL  840

VKLFIDNGWI NPRASAYLTV NLANSDETAI RTFRNTAEHL EALRNADKYL NDLKQFDSYF  900

EIYHYITQRN IKEKCEMLKE QTVKYNNDLL KYHGYSKDFV KALCVPFGYN LPRFKNLSID  960

ALFDKNDKRE KLKKGFED.                                               978
```

[0163] 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):

(SEQ ID NO: 99)

```
MAKKITAKQK REEKERLNKQ KWAKNDSVII VPETKEEIKT GEIQDNNRKR SRQKSQAKAM    60

GLKAVLSFDN KIAIASFVSS KNAKSSHIER ITDKEGTTIS VNSKMFESSV NKRDINIEKR   120

ITIEEPQQDG TIKKEEKGVK STTCNPYFKV GGKDYIGIKE IAEEHFFGRA FPNENLRVQI   180

AYNIFDVQKI LGTFVNNIIY SFYNLSRDEV QSDNDVIGML YSISDYDRQK ETETFLQAKS   240

LLKQTEAYYA YFDDVFKKNK KPDKNKEGDN SKQYQENLRH NFNILRVLSF LRQICMHAEV   300

HVSDDEGCTR TQNYTDSLEA LFNISKAFGK KMPELKTLID NIYSKGINAI NDEFVKNGKN   360

NLYILSKVYP NEKREVLLRE YYNFVVCKEG SNIGISTRKL KETMIAQNMP SLKEENTYRN   420

KLYTVMNFIL VRELKNCATI REQMIKELRA NMDEEEGRDR IYSKYAKEIY LYVKDKLKLM   480

LNVPKEEAEG IIIPGKEDPV KFSHGKLDKK EIESFCLTTK NTEDITKVIY FLCKFLDGKE   540

INELCCAMMN KLDGISDLIE TAKQCGEDVE FVDQFKCLSK CATMSNQIRI VKNISRMKKE   600

MTIDNDTIFL DALELLGRKI EKYQKDKNGD YVKDEKGKKV YTKDYNNFQD MFFEGKNHRV   660

RNFVSNNVIK SKWFSYVVRY NKPAECQALM RNSKLVKFAL DELPDSQIEK YYISVFGEKS   720

SSSNEEMRRE LLKKLCDFSV RGFLDEIVLL SEDEMKQKDK FSEKEKKKSL IRLYLTIVYL   780

ITKSMVKINT RFSIACATYE RDYILLCQSE KAERAWEKGA TAFALTRKFL NHDKPTFEQY   840

YTREREISAM PQEKRKELRK ENDQLLKKTH YSKHAYCYIV DNVNNLTGAV ANDNGRGLPC   900
```

-continued

```
LSEKNDNANL FLEMRNKIVH LNVVHDMVKY INEIKNITSY YAFFCYVLQR MIIGNNSNEQ   960

NKFKAKYSKT LQEFGTYSKD LMWVLNLPFA YNLPRYKNLS NEQLFYDEEE RMEKIVGRKN  1020

DSR.                                                               1023
```

[0164] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OGDF01008514.11 (human gut metagenome assembly):

(SEQ ID NO: 100)

```
MTETKPKRED IAKTPAAKSR SKAAGLKSTF AVNGSVLLTS FGRGNDAVPE KLITEKAVSE   60

INTVKPRFSV EKPATSYSSS FGIKSHISAT ADNPLAGRAP VGEDAIHAKE VLEQRVFGKT  120

FSDDNIHIQL IYNILDIRKI LSTYANNVVF TINSMRRLDE YDREQDYLGY LYTGNSYERL  180

LDIADKYAVD GEDWRNTAAG ISNDFEKKQF QTINGFWDLL DMIEPYMCYF SEAFFCETTV  240

KDPDSGRIVP CLEQRSDGDI YNILRILSIV RQTCMHDNAS MRTVMFTLGQ NSVRDRKNGF  300

DELAELLDYL YDEKIDIVNR DFLRNQKNNI ELLSRIYGSS ADSPERDRLV QNFYDFRVLS  360

QDKNLGFSIK KLREKLLDSP ALSVVRSKKY DTMRSKIYSL IDFMIYRKFS ENHVAVDDFV  420

EELRSLLTED EKESAYSRWA ETLINDGFAQ EILVKLLPQT DPAVIGKIKG KKLLNDSIAG  480

IKLKKDASFF TKIINVLCMF QDGKEINELV SSLVNKFANI QSFVDVMRSQ GIDSGFTADY  540

AMFAESGRIS RELHILKGIA RMQHSIAGLG DVKIYGSDDK FHGVSRRVYT DAAYILGFGE  600

RSEDNDGYVD DYVSSKLLGG ADKNLRNFIT NNVIKNRRFL YTVRYMNPKR AKKLVQNDAL  660

VVLALSGIPE TQIDRYYKSC IEKRSFNPDL NEKIAALSEM ITTLKIDDFE DVKQNPEKNA  720

NYEAKKNQRI SKERYKACIG LYLTVLYLIC KNLVKINARY SIAIGCLERD TQLHGVDFKG  780

AAYMTRDVFI AKGWINPKKP TVKSIKEQYA FLTPYIFTTY RNMIAHLAAV TNAYKYIPQM  840

DRFKSWFHLY HTVIQHSLIQ QYEYDRDYGR KGAPVVSERV LQLLEQCREH SNYSRDLLHI  900

LNLPFGYNLP RYLNLSSEKY FDANAI.                                      926
```

[0165] 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):

(SEQ ID NO: 101)

```
MAKKITAKQK REEKERLNKQ KWAKQDTPVV PKSKTEEKPV AASDDKLLKT TQVKKVQTKS    60

KAKAMGLKTV LSFDDKIAIA SFVNDKKTKL PHIERITDKS GTTIHENARM FDSSVDEQNV   120

NIEKRMTIEE KQNDGTFKKD EKDVKATICN PYFKTCGKDY IGIKDVAEKY FFGKTFPNEN   180

LRVQIAYNVF DIQKILGTYV NNIIYSFYNL RRDGKSDVDI IGSLYAFADF DNQLKDKPAF   240

REAKDLLKNT EAYFSYFGDV FKKSKKGKKD ENNEDYEKNL RHNFNVLRVL SFLRQICTHA   300

YVKCTGGAKN NGDSTKVEAE SLDALFNITE YFAKTAPELS KTINEIYKEG IDRINNDFVT   360

NGKNNLYILS KVYPDMQRNE LVKKYYQFVV CKEGNNVGIN TRKLKESIIS QHPWITTPQD   420

NNKANDYESC RHKLYTIMCF ILVAELDAHE SIRDNMVAEL RANMDGDDGR DAIYEKYAKD   480

IYHIVKDKLL AMQKVFDEEL VPVKVEGKND PQQFTHGKLG KKEIESFCLS DKNTSDIAKV   540

VYFLCNFLDG KEINELCCAM MNKFDGIGDL IDTAKQCGEE VKFIEEFACL SNCRKITNDI   600

RVAKSISKMK NKVNIDNDII YLDAIELLGR KIEKYQKDEN GKILLGTDGK RLYTQEYKYF   660

NDMFFNAGNH KVRNFIANNV MQSKWFFYVV RYNKPAECQI IMRNKTLVKF TLDDLPDMQI   720
```

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QRYYSSVFGD NNMPAVDEMR KRLLDKINQF SVRGFLDELD EIVLMSDEES KRNKSSEKEQ 780

KKSLIRLYLT IAYLITKSMV KINTRFSIAC AMYERDYALL CQSEMKGGPW DGGAQALAVT 840

RKFLNHDREV FDRYCAREAE IARLPSEERK PLRKANDKLL KQTHYTNHSY TYIVNNLNSF 900

TDIDYCAKDV GLPAPNDKND NASILGEMRN DIAHLNIVHD MVKYIEELKD ISSYYAFYCY 960

VLQRRLVGKD PNCQNKFKAK YAKELNDYGT YNKNLMWMLN LPFAYNLPRY KNLSSEFLFY 1020

DMEYNKKDDE. 1030

[0166] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): from contig emb|OBLI01020244 and emb OBLIO1038679 (from pig gut metagenome):

(SEQ ID NO: 102)

MAKKITAKQR REERERQNKQ KWAKKQADAT AVFECEADIK PADSKDEDCT NIYIKREKKK 60

TQAKAMGLKT VLGFDNKIAI ASFMSSKDSK SSHIERITDP NGKTIREDVR MFDSNVDECS 120

INLEKRMTVE ERQKDGTIKK DEKDVKSTIC NPYSNECGKD YIGIKSVAEE LFFGRTFPND 180

NLRVQIAYNI FDIQKILGTY INNIIYSFYN LSRDESQSDN DVIGTLYMLK DFDGQKETDT 240

FRQARALLER TEAYYSYFDN VFKKIDKNKK KSDDCKRERN EILRYNFNVL RVLSFLRQIC 300

AHAQVKISNE HDREKGGGLV DSLDALFNIS RFFDAVAPEL NEVINSVYSK GIDDINDNFV 360

KNGKNNFYIL SKIYPEVARE DLLREYYYFV VSKEGNNIGI STKKLKEAII VQDMSYIKSE 420

DYDTYRNKLY TVLCFILVKE LNERTTIREQ MVADLRANMN GDIGREDIYS KYAKIIYAQV 480

KPRFDTMKSA FEEEAKDVIV PDKKKPVKFS HGKLDKNEIE RFCITSANTD SVAKIIYFLC 540

KFLDGKEINE LCCAMMNKLD GINDLIETAE QCGAKVEFVD KFSVLSNCET ISDQIRIVKS 600

ISKMKKEIAI DNDTIFLDAL ELLGRKIDKY KKDATGKYLK DENGKYLYSK EYDDFQYMFF 660

KDSHRVRNFI SNSVIKSKWF SYIVRYNQPS ECRAIMKNKT LVKFALDELP DLQIQRYFVA 720

LYGDEDLPSY GEMRKILLKK LHDFSIKGFL DEIVLLSDLD MESQDKYCEK EQKKSLFRLY 780

LTIAYLITKS MVKINTRFSI ACATYERDYA LLCASNKQER AWSSGATALA LTRRFLNQDK 840

LIFEKHYARE GEISKLPKEE RKAMRKVNDQ LLKRTHFSKH SYCYIVDNVN RLTGGECRTD 900

KRVLPVLNEK NDNAGILLDF RKTIAHLNVV HKMVDYVDEI KGITSYYAFF CYVLQRMLVG 960

NNLNEKNAIK EKYSATVKSF GTYSKDFMWL INLPFAYNLP RYKNLSNEQL FYDEEERNET 1020

EEQIDRL. 1027

[0167] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): contig OIZX01000427.1:

(SEQ ID NO: 103)

MAKKKKTARQ LREEMQQQRK QAIQKQQEQR QEKAAAARET AAPEQPAAAP VPKRQRKSLA 60

KAAGLKSNFI LDPQRRTTVM TAFGQGSTAI LEKQIVDRAI SDLQPVQQFQ VEPASAAKYR 120

LKNSRVRFPN VTADDPLYRR KDGGFVPGMD ALRRKNVLEQ RFFGKSFADN IHIQMIYSIL 180

DIHKILAAAS GHIVHLLNIV NGSKDRDFIG MLAAHVLYNE LNEEAKRSIA DFCKSPRLIY 240

YSAAFYETLD NGKSERRSNE DIFNILALMT CLRNFSSHHS IAIKVKDYSA AGLYNLRRLG 300

PDMKKMLDTF YTEAFIQLNQ SFQDHNTTNL TCLFDILNIS DSARQKQLAE EFYRYVVFKE 360

QKNLGFSVRK LREEMLLLPD AAVIADKRYD TCRSKLYNLM DFLILRVYRT GRADRCDKLP 420

EALRAALTDE EKAVVYHKEA LSLWNEMRTL ILDGLLPQMT PENLSRLSGQ KRKGELSLDD 480

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```
AMLKECLYEP GPVPEDAAPE EANAEYFCRM IYLATLFMDG KEINTLLTTL ISKFENIAAF  540

LQTMEQLNIE AELGPEYAMF TRSRAVAEQL RVINSFALMK KPQVNAKQQL YRAAVTLLGT  600

EDPDGVTDEM LCIDPVTGKM LPPNQRHHGD TGLRNFIANN VVESRRFQYL IRYSDPAQLH  660

QLASNKKLVR FVLSSIPDTQ INRYYETCGQ TRLAGRAAKV EFLTDMIAAI RFDQFRDVNQ  720

KERGANTQKE RYKAMLGLYQ TVLYLAVKNL VNINARYVMA FHCVERDMFL YDGELTDPKG  780

ESVSAFLAVN GKKGVQPQYL LLTQLFIRRD YLKRSACEQI QHNMENISDR LLREYRNAVA  840

HLNVIAHLAD YSADMREITS YYGLYHYLMQ RHLFKRHAWQ IRQPERPTEE EQKLIEQEQK  900

QLAWEKALFD KTLQYHSYNK DLVKALNAPF GYNLARYKNL SIEPLFSKEA APAAEIKATH  960

A.                                                                  961
```

[0168] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): contig OCTW011587266.1:

(SEQ ID NO: 104)

```
MKQNDRENNN KIKKSAAKAV GVKSLARLSD GSTVVSSFGK GAAAELESLI TGGEIRKLSD   60

KAILEITDDT QNKNAYNVKS SRIPNLTART DKLSDKSGMD DLGFKRELEL EVFGQCFDDS  120

IHIQIAHAVF DIQKSLAAVI PNVLYTLNNL DRSYSTDNTS DKKDIIGNTL NYQHSYESFN  180

VEKRGEFTEY YNAAKDRFSY FPDILCVLEK VNGKDRYQPK SEKDAFNVLS SVNMLRNSLF  240

HFAPKSNDGK ARIAVFKNQF DSDFSHITST VNKIYSAKIA GVNENFLNNE GNNLYIILKA  300

TNWDIKKIVP QLYRFSVLKS 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 GATDIQAQAK ALLDCITGIS FDAIKDDKHL  660

HKSKEKSPQR SADRERKKAM LTLYYTIVYI FVKQMLHINS LYTIGFFYLE RDQRFIYSRA  720

KKENKNPSKN SYLNDFRSVT AYFIPSEIMK RIEKNENKGF LEDFEALWNS CGKTSRLRKE  780

DVLLYARYIS PDHALKNYKM ILNSYRNKIA HINVIMSAGK YTGGIKRMDS YFSVFQHLVQ  840

CDILSNPNNK GKCFESESLK PLLLDMKFDG TDEKLYSKRL TRALNIPFGY NVPRYKNLTF  900

EKIYLKSSIN E.                                                      911
```

[0169] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OGNF01009141.1:

(SEQ ID NO: 105)

```
MADIDKKKSS AKAAGLKSTF VLENNKLLMT SFGNGNKAVI EKIIDEKVDS INEPEVFSVT   60

PCDKKFELQP AKRGLAADSL VDNPLKSKKT AGDDAIHSRK FLERQFFDGN TFNDNIHIQL  120

IYNILDIEKI LSVHVNDIVY SVNNILSRGE GMEYNDYIGT LNLKSFETYK NNLVNKKKFD  180

LDRVKKIPQL AYFGSAFYNT PEDTSAKITK TKIKSNEEIY YTFMLLSTAR NFSAHYLDRN  240

RAKSSDAEDF DGTSVIMYNL DNEELYKKLY NKKVHMALTG MKKVLDANFN KKVEHLNNSF  300

IKNSAKDFVI LCEVLGIKSR DEKTKFVKDY YDFVVRKNYK HLGFSVKELR ELLFANHDSN  360

KYIKEFDKIS NKKFDSVRSR LNRLADYIIY DYYNKNNAKV SDLVKYLRAA ADDEQKKKIY  420
```

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```
LNESINLVKS GILERIKKIL PKLNGKIIGN MQPDSTITAS MLHNTGKDWH PISENAHYFT  480

KWIYTLTLFM DGKEINDLVT TLINKFDNIA SFIEVLKSQS VCTHFSEERK MFIDSAEICS  540

ELSAMNSFAR MEAPGASSKR AMFVEAARIL GDNRSKEELE EYFDTLFDKS ASKKEKGFRN  600

FIRNNVVDSN RFKYLTRYTD TSSVKAFSNN KALVKFAIKD IPQEQILRYY NSCFGASERY  660

YNDGMSDKLV EAIGKINLMQ FNGVIQQADR NMLPEEKKKA NAQKEKYKSI IRLYLTVCYL  720

FFKNLVYVNS RYYSAFYNLE KDRSLFEING ELKPTGKFDE GHYTGLVKLF IDNGWINPRA  780

SAYLTVNLAN SDETAIRTFR NTAEHLEALR NADKYLNDLK QFDSYFEIYH YITQRNIKEK  840

CEMLKEQTVK YNNDLLKYHG YSKDFVKALC VPFGYNLPRF KNLSIDALFD KNDKREKLKK  900

GFED.                                                              904
```

[0170] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): contig emb|OIEN01002196.1:

(SEQ ID NO: 106)

```
MERQKRKMKS KSKMAGVKSV FVIGDELLMT SFGDGDDAVL EKDIDENGVV NDCRNPAAYD   60

AVYGTDSIRV KKTNNNIRAK VNNPLAKSNI RSEESALFRT RVNEYKREQK DKYETLFFGK  120

TFDDNIHIQL ISKILDIEKT FSVVIGNIVY AINNLSLEQS IDRPIDIFGD KNTQGISLRE  180

DNDYLKTMLP RCEYLFHNIL NSDSDNNSKM NYNKVNKGKE EKDNRNNENI EKLKKALEVI  240

KIIRVDSFHG VDGIKGDQKF PRSKYNLAVN YNEEIQKTIS EPFNRKVEEV QQDFYRNSCV  300

NIDFLKEIMY GSNYTDRGSD SLECSYFNFA ILKQNKNMGF SITSIRECLL DLYELNFESM  360

QNLRPRANSF CDFLIYDYYC KNESERANLV DCLRSAASEE EKKNIYFQTA ERVKEKFRNA  420

FNRISRFDAS YIKNSREKNL SGGSSLPKYS FIEGFTKRSK KINDNDEKNA DLFCNMLYYL  480

AQFLDGKEIN IFLTSIHNIF QNIDSFLKVM KEKGMECKFQ KDFKMFSHAG HVAKKIEIVI  540

SLAKMKKTLD FYNAQALKDA VTILGVSKKH QYLDMNSYLD FYMFDNRSGA TGKNAGKDHN  600

LRNFLVSNVI RSRKFNYLSR YSNLAEVKKL AQNPSLVQFV LSRIEPSLIC RYYESSQGIS  660

SEGITIDEQI KKLTGIIVDM NIDSFENINN GEIGMRYSKA TPQSIERRNQ MRVCVGLYLN  720

VLYQIEKNLM NVNARYVLAF AFAERDALML NFTLEECKKN KKRSSGGFSF IEMTQFFIDK  780

KLFKVATEAI KKNVLKYNGN PESLNHIPGE YICKNMEGYH ENTVRNFRNM VAHLTAVARV  840

PLYISEVTQI DSYYALYHYC MQMNILQGIE QSGKILDNIK LKNALENARV HRTYSKDAVK  900

YLCLPFAYNI SRYKALTIKD LFDWTEYSCK KDE.                              933
```

[0171] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Metagenomic hit (no protein accession): contig e-k87_11092736:

(SEQ ID NO: 107)

```
MKRQKTFAKR IGIKSTVAYG QGKYAITTFG KGSKAEIAVR 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
```

-continued

```
IYEASVSVAN SGQMGKVAPW KGAIDNALVK LREAPDEEAK EKIYNVLAAS IRNDSLFLRL   420

KSACDKFGAE QNRPVFPNEL RNNRDIRNVR SEWLEATQDV DAAAFVQLIA FLCNFLEGKE   480

INELVTALIK KFEGIQALID LLRNLEGVDS IRFENEFALF NDDKGNMAGR IARQLRLLAS   540

VGKMKPDMTD AKRVLYKSAL EILGAPPDEV SDEWLAENIL LDKSNNDYQK AKKTVNPFRN   600

YIAKNVITSR SFYYLVRYAK PTAVRKLMSN PKIVRYVLKR LPEKQVASYY SAIWTQSESN   660

SNEMVKLIEM IDRLTTEIAG FSFAVLKDKK DSIVSASRES RAVNLEVERL KKLTTLYMSI   720

AYIAVKSLVK VNARYFIAYS ALERDLYFFN EKYGEEFRLH FIPYELNGKT CQFEYLAILK   780

YYLARDEETL KRKCEICEEI KVGCEKHKKN ANPPYEYDQE WIDKKKALNS ERKACERRLH   840

FSTHWAQYAT KRDENMAKHP QKWYDILASH YDELLALQAT GWLATQARND AEHLNPVNEF   900

DVYIEDLRRY PEGTPKNKDY HIGSYFEIYH YIRQRAYLEE VLAKRKEYRD SGSFTDEQLD   960

KLQKILDDIR ARGSYDKNLL KLEYLPFAYN LPRYKNLTTE ALFDDDSVSG KKRVAEWRER  1020

EKTREAEREQ RRQR.                                                    1034
```

[0172] 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:
                                (SEQ ID NO: 108)
gtgagaagtc tccttatggg gagatgctac.
```

[0173] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Ga0129306_1000735:

```
                                                          (SEQ ID NO: 109)
MQKQREQQTV TDESERKKKP LKSGAKAAGL KSVFVLSEGK ELLTSFGRGN EAVPEKRVTG   60

GTIANARTDN KEAFSAALQN KRFEVFGRTA GSSDDPLAVS RAPGQDLIGA KTALEERYFG  120

RAFADNIHMQ VIYAIQDINK ILAVHANNIV YTLNNLDREA DPETDDFIGS GYLTLKNTFE  180

TYCDPAALNE REREKVTVSK QHFDAFMQNP RLAYYGNAFF RKLSKAERLA RGREIFDKES  240

PERRQEILGS RGKNKSVDDE IRALAPEWVK REERDVYSEL VLMSELRQSC FHGQQKNSAR  300

IFRLDNDLGP GVDGARELLD RLYAEKINDL RSFDKTSASS NFRLLFNAYH ADNEKKKELA  360

QEFYRFSVLK 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 SLYLNVAYQI VKNLVYVNAR YTMAYHCAER DTELLLNAAG  780

EGNLLRRDRS WPARLHLPRR ALARRRDRVE VMERDVARGP EAYNRDEWLG LVRTLRREKR  840

VCDNLHNNYA YLCGADAEPG DASLSLLFVY RNKAAHLSVL NKGGRLSGDL KEAKSWFYVY  900

HFLMQRVLEE EFRNTQALPE RLRELLMMAE RYRGCSKDLI KVLNLTFAYN LPRYKNLSID  960

GRFDKNHPDP SDE.                                                     973
```

[0174] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Ga0129317_1008067:

(SEQ ID NO: 110)

```
MKKQKKSLVK AAGLKSAFVV GDSVYLTSFG KGNAARLDTK INPDNSTERY VSDSEKHTLK   60

INSITDTELR LSGPFPKQAE AKNPTHKKDN EQKNTRQDML GLKSTLEKFY FGSTFDDNIH  120

IQIIHNIQDI AKILAAHSNN AGYALDNMLA YQGVEFSDMI GYMGTSRTFD NYDPNHKNNK  180

DFFRFLKLPR LGYFGSAFYS QKGKDFEKRS DEEVYNICAL MGQIRQCCFH GKQEKYQLKW  240

LYNFHNFKSN KPFLDTLDKH FDEMIDRINK NFIKNNTPDL IILSGLYPDM AKKELVRLFY  300

DFTTVKEYKN MGFSVKKLRE KMLESEEASD FRDKDYDSVR RKLYKLMDFC IYYLYYSDSE  360

RNENLVSRLR ESLTDENKDI IYSKEAKIVW NELRKKFSTI LDNVKGSNIK KLENVKEKFI  420

SEDEFDDIKL DIDISYFSKL MYVMCYFLDG KEINDLLTTL VSKFDNIGSI IEAATQIGIN  480

IEFIDDFKFF DRSKDISVEL NIIRNFARMQ APVPNAKRAM QEDAIRILGG SEEDIFSILD  540

DMTGYDKSGK KLAQSKKGFR NFIINNVVES SRFKYIVRYS NPQKIRKLAN NSVVVGFVLG  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 PFGYNLPRFK  840

NLSIEGKFDM HESK.                                                   854
```

[0175] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d Ga0224415_10048792:

(SEQ ID NO: 111)

```
MSKKENRKSY VKGLGLKSTL VSDSKVYLTT FADGSNAKLE KCVENNKIIC ISNDKEAFAA   60

SIANKNVGYK IKNDEKFRHP KGYDIISNNP LLHNNSVQQD MLGLKNVLEK RYFGKSSGGD  120

NNLCIQIIHN IIDIEKILSE YIPNVVYAFN NIAGFKDEHN NIIDIIGTQT YNSSYTYADF  180

SKDKSDKKYI EFQKLLKNKR LGYWGKAFFT GQGNNAKVRQ ENQCFHIIAL LISLRNWATH  240

SNELDKHTKR TWLYKLDDTN ILNAEYVKTL NYLYDTIADE LTKSFSKNGA VNVNYLAKKY  300

NIKDDLPGFS EQYFRFSIMK EQKNLGFNIS KLRENMLDFK DMSVIRDDHN RYDKDRSKIY  360

TMMDFVIYRY YIDNNNDSID FINKLRSSID EKSKEKLYNE EANRLWNKLK EYMLYIKEFN  420

GKLASRTPDR DGNISEFVES LPKIHRLLPR GQKISNFSKL MYLLTMFLDG KEINDLLTTL  480

INKFENIQGF LDIMPEINVN AKFEPEYVFF NKSHEIAGEL KLIKGFAQMG EPAATLKLEM  540

TADAIKILGT EKEDAELIKL AESLFKDENG KLLGNKQHGM RNFIGNNVIK SKRFHYLIRY  600

GDPAHLHKIA TNKNVVRFVL GRIADMQKKQ GQKGKNQIDR YYEVCVGNKD IKKTIEEKID  660

ALTDIIVNMN YDQFEKKKAV IENQNRGKTF EEKNKYKRDN AEREKFKKII SLYLTVIYHI  720

LKNIVNVNSR YILGFHCLER DKQLYIEKYN KDKLDGFVAL TKFCLGDEER FEDLKAKAQA  780

SIQALETANP KLYAKYMNYS DEEKKEEFKK QLNRERVKNA RNAYLKNIKN YIMIRLQLRD  840

QTDSSGYLCG EFRDKVAHLE VARHAHEYIG NIKEVNSYFQ LYHYIMQCRL YDVLKNNTKA  900

EAMVKGKAKE YFEALEKEGT YNDKLLKIAC VPFGYCIPRY KNLSMEELFD MNEEKKFKKK  960

APENT.                                                             965
```

[0176] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence CasRX/Cas13d 160582958 gene49834:

(SEQ ID NO: 112)

```
MKNSVTFKLI QAQENKEAAR KKAKDIAEQA RIAKRNGVVK KEENRINRIQ IEIQTQKKSN    60

TQNAYHLKSL AKAAGVKSVF AIGNDLLMTG FGPGNDATIE KRVFQNRAIE TLSSPEQYSA   120

EFQNKQFKIK GNIKVLNHST QKMEEIQTEL QDNYNRPHFD LLGCKNVLEQ KYFGRTFSDN   180

IHVQIAYNIM DIEKLLTPYI NNIIYTLNEL MRDNSKDDFF GCDSHFSVAY LYDELKAGYS   240

DRLKTKPNLS KNIDRIWNNF CNYMNSDSGN TEARLAYFGE LFYKPKETGD AKSDYKTHLS   300

NNQKEEWELK SDKEVYNIFA ILCDLRHFCT HGESITPSGK PFPYNLEKNL FPEAKQVLNS   360

LFEEKAESLG AEAFGKTAGK TDVSILLKVF EKEQASQKEQ QALLKEYYDF KVQKTYKNMG   420

FSIKKLREAI MEIPDAAKFK DDLYSSLRHK LYGLFDFILV KHFLDTSDSE NLQNNDIFRQ   480

LRACRCEEEK DQVYRSIAVK VWEKVKKKEL NMFKQVVVIP SLSKDELKQM EMTKNTELLS   540

SIETISTQAS LFSEMIFMMT YLLDGKEINL LCTSLIEKFE NIASFNEVLK SPQIGYETKY   600

TEGYAFFKNA DKTAKELRQV NNMARMTKPL GGVNTKCVMY NEAAKILGAK PMSKAELESV   660

FNLDNHDYTY SPSGKKIPNK NFRNFIINNV ITSRRFLYLI RYGNPEKIRK IAINPSIISF   720

VLKQIPDEQI KRYYPPCIGK RTDDVTLMRD ELGKMLQSVN FEQFSRVNNK QNAKQNPNGE   780

KARLQACVRL YLTVPYLFIK NMVNINARYV LAFHCLERDH ALCFNSRKLN DDSYNEMANK   840

FQMVRKAKKE QYEKEYKCKK QETGTAHTKK IEKLNQQIAY IDKDIKNMHS YTCRNYRNLV   900

AHLNVVSKLQ NYVSELPNDY QITSYFSFYH YCMQLGLMEK VSSKNIPLVE SLKNEANDAQ   960

SYSAKKTLEY FDLIEKNRTY CKDFLKALNA PFSYNLPRFK NLSIEALFDK NIVYEQADLK  1020

KE.                                                                 1022
```

[0177] An exemplary direct repeat sequence of CasRX/Cas13d proteins may comprise or consist of the sequence CasRX/Cas13d 160582958_gene49834 (SEQ ID NO: 112) comprises or consists of the nucleic acid sequence: CasRX/Cas13d DR:

(SEQ ID NO: 113)

```
gaactacacc cctctgttct tgtaggggtc taacac.    36
```

[0178] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d 250twins_35838_GL0110300:

(SEQ ID NO: 114)

```
MGNKQRVSAQ KRRENAKLCN QQKARQAESQ RDKIKNMNVE KMKNINTNDI KHTKTTAKKL   60

GLKSTIIADK KIILTSFINE QSSKTANIEK VAGFKGDTID TISYTPRMFR SEINPGEIVI  120

SKGDDLSEFA NPANFPIGRD YVKIRSALEK QYFGKEFPED NLHVQIAYNV ADIKKILSVY  180

INNIIYMFYN LARSEEYDIF YNSQSENSGR DCDVIGSLYY QASYRNQDAN RFEKDGKKKA  240

IDSLLDDTRA YYTYFDGLFS VPKREDDGKI KESEKEKAKD QNFDVLRLLS VGRQLTFHSD  300

KSNNEAYLFD LSKLTRAAQD ENRRQDIQSL LNILNSTCRS NLEGVNGDFV KHAKNNLYVL  360

NQLYPSLKAN DLIGEYYNFI VKKENRNIGI RLITVRELII EHNYTNLKDS KYDTYRNKIY  420

TVLNFILFRE IQENSIAIKN FREKLRSTEK 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

FRNFLINNVI KSKWFFYIAK YVKPADCAKM MSNKKMIEFA LRDLPETQIK RYYYTITGNE  720
```

-continued

```
ALGDAESLKG VIIEQLHAFS IKNTLLSIKN MGEGEYKIQQ IGSSKEKLKA IVNLYLTVAY  780

LLTKSLVKVN IRFSIAFGCL ERDLVLQKKS EKKFDAIINE ILLEDDKIRK ECDKERAQAK  840

TLPRELAQER FAQIKRRESG CYFKSYHVYD YLSKNSNEFK QNHIDFAVTS YRNNVEHLNV  900

VHCMTKYFSE VKDVKSYYGV YCYIMQRMLC DELIIKNQDK PDVRQTFEEY NRLLKDHGTY  960

SKNLMWLLNF PFAYNLARYK NLSNEDLFNA KNNDQKSK.                          998
```

[0179] Exemplary CasRX/Cas13d proteins may comprise or consist of the sequence: CasRX/Cas13d 250twins_36050_GL0158985:

(SEQ ID NO: 115)

```
MKKKHQSAAE KRQVKKLKNQ EKAQKYASEP SPLQSDTAGV ECSQKKTVVS HIASSKTLAK     60

AMGLKSTLVM GDKLVITSFA ASKAVGGAGY KSANIEKITD LQGRVIEEHE RMFSADVGEK    120

NIELSKNDCH TNVNNPVVTN IGKDYIGLKS RLEQEFFGKT FENDNLHVQL AYNILDIKKI    180

LGTYVNNIIY IFYNLNRAGT GRDERMYDDL IGTLYAYKPM EAQQTYLLKG DKDMRRFEEV    240

KQLLQNTSAY YVYYGTLFEK VKAKSKKEQR AKEAEIDACT AHNYDVLRLL SLMRQLCMHS    300

VAGTAFKLAE SALFNIEDVL SADLKEILDE AFSGAVNKLN DGFVQHSGNN LYVLQQLYPN    360

ETIERIAEKY YRLTVRKEDL NMGVNIKKLR ELIVGQYFPE VLDKEYDLSK NGDSVVTYRS    420

KIYTVMNYIL LYYLEDHDSS RESMVEALRQ NREGDEGKEE IYRQFAKKVW NGVSGLFGVC    480

LNLFKTEKRN KFRSKVALPD VSGAAYMLSS ENIDYFVKML FFVCKFLDGK EINELLCALI    540

NKFDNIADIL DAAAQCGSSV WFVDSYRFFE RSRRISAQIR IVKNIASKDF KKSKKDSDES    600

YPEQLYLDAL ALLGDVISKY KQNRDGSVVI DDQGNAVLTE QYKRFRYEFF EEIKRDESGG    660

IKYKKSGKPE YNHQRRNFIL NNVLKSKWFF YVVKYNRPSS CRELMKNKEI LRFVLRDIPD    720

SQVRRYFKAV QGEEAYASAE AMRTRLVDAL SQFSVTACLD EVGGMTDKEF ASQRAVDSKE    780

KLRAIIRLYL TVAYLITKSM VKVNTRFSIA FSVLERDYYL LIDGKKKSSD YTGEDMLALT    840

RKFVGEDAGL YREWKEKNAE AKDKYFDKAE RKKVLRQNDK MIRKMHFTPH SLNYVQKNLE    900

SVQSNGLAAV IKEYRNAVAH LNIINRLDEY IGSARADSYY SLYCYCLQMY LSKNFSVGYL    960

INVQKQLEEH HTYMKDLMWL LNIPFAYNLA RYKNLSNEKL FYDEEAAAEK ADKAENERGE.   1020
```

[0180] 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.

[0181] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

[0182] Cas13d (*Ruminococcus flavefaciens* XPD3002) Sequence:

(SEQ ID NO: 45)

```
   1 IEKKKSFAKG MGVKSTLVSG SKVYMTTFAE GSDARLEKIV EGDSIRSVNE GEAFSAEMAD

  61 KNAGYKIGNA KFSHPKGYAV VANNPLYTGP VQQDMLGLKE TLEKRYFGES ADGNDNICIQ

 121 VIHNILDIEK ILAEYITNAA YAVNNISGLD KDIIGFGKFS TVYTYDEFKD PEHHRAAFNN

 181 NDKLINAIKA QYDEFDNFLD NPRLGYFGQA FFSKEGRNYI INYGNECYDI LALLSGLAHW

 241 VVANNEEESR ISRTWLYNLD KNLDNEYIST LNYLYDRITN ELTNSFSKNS AANVNYIAET

 301 LGINPAEFAE QYFRFSIMKE QKNLGFNITK LREVMLDRKD MSEIRKNHKV FDSIRTKVYT

 361 MMDFVIYRYY IEEDAKVAAA NKSLPDNEKS LSEKDIFVIN LRGSFNDDQK DALYYDEANR

 421 IWRKLENIMH NIKEFRGNKT REYKKKDAPR LPRILPAGRD VSAFSKLMYA LTMFLDGKEI
```

-continued

481NDLLTTLINK FDNIQSFLKV MPLIGVNAKF VEEYAFFKDS AKIADELRLI KSFARMGEPI

541ADARRAMYID AIRILGTNLS YDELKALADT FSLDENGNKL KKGKHGMRNF IINNVISNKR

601FHYLIRYGDP AHLHEIAKNE AVVKFVLGRI ADIQKKQGQN GKNQIDRYYE TCIGKDKGKS

661VSEKVDALTK IITGMNYDQF DKKRSVIEDT GRENAEREKF KKIISLYLTV IYHILKNIVN

721INARYVIGFH CVERDAQLYK EKGYDINLKK LEEKGFSSVT KLCAGIDETA PDKRKDVEKE

781MAERAKESID SLESANPKLY ANYIKYSDEK KAEEFTRQIN REKAKTALNA YLRNTKWNVI

841IREDLLRIDN KTCTLFANKA VALEVARYVH AYINDIAEVN SYFQLYHYIM QRIIMNERYE

901KSSGKVSEYF DAVNDEKKYN DRLLKLLCVP FGYCIPRFKN LSIEALFDRN EAAKFDKEKK

961KVSGNS.

[0183] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

[0184] Cas13d (contig e-k87_11092736):

(SEQ ID NO: 46)

MKRQKTFAKRIGIKSTVAYGQGKYAITTFGKGSKAEIAVRSADPPEETLP

TESDATLSIHAKFAKAGRDGREFKCGDVDETRIHTSRSEYESLISNPAES

PREDYLGLKGTLERKFFGDEYPKDNLRIQIIYSILDIQKILGLYVEDILH

FVDGLQDEPEDLVGLGLGDEKMQKLLSKALPYMGFFGSTDVFKVTKKREE

RAAADEHNAKVFRALGAIRQKLAHFKWKESLAIFGANANMPIRFFQGATG

GRQLWNDVIAPLWKKRIERVRKSFLSNSAKNLWVLYQVFKDDTDEKKKAR

ARQYYHFSVLKEGKNLGFNLTKTREYFLDKFFPIFHSSAPDVKRKVDTFR

SKFYAILDFIIYEASVSVANSGQMGKVAPWKGAIDNALVKLREAPDEEAK

EKIYNVLAASIRNDSLFLRLKSACDKFGAEQNRPVFPNELRNNRDIRNVR

SEWLEATQDVDAAAFVQLIAFLCNFLEGKEINELVTALIKKFEGIQALID

LLRNLEGVDSIRFENEFALFNDDKGNMAGRIARQLRLLASVGKMKPDMTD

AKRVLYKSALEILGAPPDEVSDEWLAENILLDKSNNDYQKAKKTVNPFRN

YIAKNVITSRSFYYLVRYAKPTAVRKLMSNPKIVRYVLKRLPEKQVASYY

SAIWTQSESNSNEMVKLIEMIDRLTTEIAGFSFAVLKDKKDSIVSASRES

RAVNLEVERLKKLTTLYMSIAYIAVKSLVKVNARYFIAYSALERDLYFFN

EKYGEEFRLHFIPYELNGKTCQFEYLAILKYYLARDEETLKRKCEICEEI

KVGCEKHKKNANPPYEYDQEWIDKKKALNSERKACERRLHFSTHWAQYAT

KRDENMAKHPQKWYDILASHYDELLALQATGWLATQARNDAEHLNPVNEF

DVYIEDLRRYPEGTPKNKDYHIGSYFEIYHYIRQRAYLEEVLAKRKEYRD

SGSFTDEQLDKLQKILDDIRARGSYDKNLLKLEYLPFAYNLPRYKNLTTE

ALFDDDSVSGKKRVAEWRREREKTREAEREQRRQR.

[0185] An exemplary direct repeat sequence of Cas13d (contig e-k87_11092736) (SEQ ID NO: 46) comprises or consists of the nucleic acid sequence:Cas13d (contig e-k87_11092736) Direct Repeat Sequence): GTGAGAAGTCTC-CTTATGGGGAGATGCTAC (SEQ ID NO: 47).

[0186] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

[0187] Cas13d (160582958_gene49834):

(SEQ ID NO: 48)

MKNSVTFKLIQAQENKEAARKKAKDIAEQARIAKRNGVVKKEENRINRIQ

IEIQTQKKSNTQNAYHLKSLAKAAGVKSVFAIGNDLLMTGFGPGNDATIE

KRVFQNRAIETLSSPEQYSAEFQNKQFKIKGNIKVLNHSTQKMEEIQTEL

QDNYNRPHFDLLGCKNVLEQKYFGRTFSDNIHVQIAYNIMDIEKLLTPYI

NNIIYTLNELMRDNSKDDFFGCDSHFSVAYLYDELKAGYSDRLKTKPNLS

KNIDRIWNNFCNYMNSDSGNTEARLAYFGELFYKPKETGDAKSDYKTHLS

NNQKEEWELKSDKEVYNIFAILCDLRHFCTHGESITPSGKPFPYNLEKNL

FPEAKQVLNSLFEEKAESLGAEAFGKTAGKTDVSILLKVFEKEQASQKEQ

QALLKEYYDFKVQKTYKNMGFSIKKLREAIMEIPDAAKFKDDLYSSLRHK

LYGLFDFILVKHFLDTSDSENLQNNDIFRQLRACRCEEEKDQVYRSIAVK

VWEKVKKKELNMFKQVVVIPSLSKDELKQMEMTKNTELLSSIETISTQAS

LFSEMIFMMTYLLDGKEINLLCTSLIEKFENIASFNEVLKSPQIGYETKY

TEGYAFFKNADKTAKELRQVNNMARMTKPLGGVNTKCVMYNEAAKILGAK

PMSKAELESVFNLDNHDYTYSPSGKKIPNKNFRNFIINNVITSRRFLYLI

RYGNPEKIRKIAINPSIISFVLKQIPDEQIKRYYPPCIGKRTDDVTLMRD

ELGKMLQSVNFEQFSRVNNKQNAKQNPNGEKARLQACVRLYLTVPYLFIK

NMVNINARYVLAFHCLERDHALCFNSRKLNDDSYNEMANKFQMVRKAKKE

QYEKEYKCKKQETGTAHTKKIEKLNQQIAYIDKDIKNMHSYTCRNYRNLV

AHLNVVSKLQNYVSELPNDYQITSYFSFYHYCMQLGLMEKVSSKNIPLVE

SLKNEANDAQSYSAKKTLEYFDLIEKNRTYCKDFLKALNAPFSYNLPRFK

NLSIEALFDKNIVYEQADLKKE.

[0188] An exemplary direct repeat sequence of Cas13d (160582958_gene49834) (SEQ ID NO: 48) comprises or consists of the nucleic acid sequence:

[0189] Cas13d (160582958_gene49834) Direct Repeat Sequence:

(SEQ ID NO: 49)

GAACTACACCCCTCTGTTCTTGTAGGGGTCTAACAC.

[0190] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence:

[0191] Cas13d (contig tpg|DJXD01000002.1|; uncultivated Ruminococcus assembly, UBA7013, from sheep gut metagenome):

(SEQ ID NO: 50)

MKKQKSKKTVSKTSGLKEALSVQGTVIMTSFGKGNMANLSYKIPSSQKPQ

NLNSSAGLKNVEVSGKKIKFQGRHPKIATTDNPLFKPQPGMDLLCLKDKL

EMHYFGKTFDDNIHIQLIYQILDIEKILAVHVNNIVFTLDNVLHPQKEEL

TEDFIGAGGWRINLDYQTLRGQTNKYDRFKNYIKRKELLYFGEAFYHENE

RRYEEDIFAILTLLSALRQFCFHSDLSSDESDHVNSFWLYQLEDQLSDEF

KETLSILWEEVTERIDSEFLKTNTVNLHILCHVFPKESKETIVRAYYEFL

IKKSFKNMGFSIKKLREIMLEQSDLKSFKEDKYNSVRAKLYKLFDFIITY

YYDHHAFEKEALVSSLRSSLTEENKEEIYIKTARTLASALGADFKKAAAD

VNAKNIRDYQKKANDYRISFEDIKIGNTGIGYFSELIYMLTLLLDGKEIN

DLLTTLINKFDNIISFIDILKKLNLEFKFKPEYADFFNMTNCRYTLEELR

VINSIARMQKPSADARKIMYRDALRILGMDNRPDEEIDRELERTMPVGAD

GKFIKGKQGFRNFIASNVIESSRFHYLVRYNNPHKTRTLVKNPNVVKFVL

EGIPETQIKRYFDVCKGQEIPPTSDKSAQIDVLARIISSVDYKIFEDVPQ

SAKINKDDPSRNFSDALKKQRYQAIVSLYLTVMYLITKNLVYVNSRYVIA

FHCLERDAFLHGVTLPKMNKKIVYSQLTTHLLTDKNYTTYGHLKNQKGHR

KWYVLVKNNLQNSDITAVSSFRNIVAHISVVRNSNEYISGIGELHSYFEL

YHYLVQSMIAKNNWYDTSHQPKTAEYLNNLKKHHTYCKDFVKAYCIPFGY

VVPRYKNLTINELFDRNNPNPEPKEEV.

[0192] 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: Cas13d (contig tpg|DJXD01000002.1|; uncultivated *Ruminococcus* assembly, UBA7013, from sheep gut metagenome) Direct Repeat Sequence: CAACTACAACCCCGTAAAAATACGGGGTTCT-GAAAC (SEQ ID NO: 51).

gRNA Target Sequences

[0193] 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.

[0194] In some embodiments of the compositions and methods of the disclosure, the sequence motif is a signature of a disease or disorder.

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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.

[0199] 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.

[0200] 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.

[0201] 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.

[0202] 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.

[0203] 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.

[0204] 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.

[0205] 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

[0206] 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.

[0207] 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.

[0208] 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.

[0209] 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.

[0210] 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.

[0211] 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)).

[0212] 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).

[0213] 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.

[0214] 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.

[0215] 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.

RNA-Binding Endonucleases

[0216] 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.

[0217] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an RNAse.

[0218] 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)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGLCKPVNTFVHEP

LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTS

PKERHIIVACEGSPYVPVHFDASVEDST.

[0219] 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)
QDGMYQRFLRQHVHPEETGGSDRYCDLMMQRRKMTLYHCKRFNTFIHEDI

WNIRSICSTTNIQCKNGKMNCHEGVVKVTDCRDTGSSRAPNCRYRAIAST

RRVVIACEGNPQVPVHFDG.

[0220] 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)
WPKRLTKAHWFEIQHIQPSPLQCNRAMSGINNYTQHCKHQNTFLHDSFQN

VAAVCDLLSIVCKNRRHNCHQSSKPVNMTDCRLTSGKYPQCRYSAAAQYK

FFIVACDPPQKSDPPYKLVPVHLDSIL.

[0221] 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)
APARAGFCPLLLLLLLGLWVAEIPVSAKPKGMTSSQWFKIQHMQPSPQAC

NSAMKNINKHTKRCKDLNTFLHEPFSSVAATCQTPKIACKNGDKNCHQSH

GPVSLTMCKLTSGKYPNCRYKEKRQNKSYVVACKPPQKKDSQQFHLVPVH

LDRVL.

[0222] 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)
TSSQWFKTQHVQPSPQACNSAMSIINKYTERCKDLNTFLHEPFSSVAITC

QTPNIACKNSCKNCHQSHGPMSLTMGELTSGKYPNCRYKEKHLNTPYIVA

CDPPQQGDPGYPLVPVHLDKVV.

[0223] In some embodiments, the second RNA binding protein comprises or consists of an RNAse2. In some embodiments, the RNAse2 protein comprises or consists of:

(SEQ ID NO: 25)
KPPQFTWAQWFETQHINMTSQQCTNAMQVINNYQRRCKNQNTFLLTTFAN

VVNVCGNPNMTCPSNKTRKNCHHSGSQVPLIHCNLTTPSPQNISNCRYAQ

TPANMFYIVACDNRDQRRDPPQYPVVPVHLDRII.

[0224] 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)
DKRLRDNHEWKKLIMVQHWPETVCEKIQNDCRDPPDYWTIHGLWPDKSEG

CNRSWPFNLEEIKKNWMEITDSSLPSPSMGPAPPRWMRSTPRRSTLAEAW

NSTGSWTSTGGCALPPAALPSGDLCCRPSLTAGSRGVGVDLTALHQLLHV

HYSATGIIPEECSEPTKPFQIILHHDHTEWVQSIGMPIWGTISSSESAIG

KNEESQPACAVLSHDS.

[0225] 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)
AAVEDNHLLIKAVQNEDVDLVQQLLEGGANVNFQEEEGGWTPLHNAVQMS

REDIVELLLRHGADPVLRKKNGATPFILAAIAGSVKdLLKLFLSKGADVN

ECDFYGFTAFMEAAVYGKVKALKFLYKRGANVNLRRKTKEDQERLRKGGA

TALMDAAEKGHVEVLKILLDEMGADVNACDNMGRNALIHALLSSDDSDVE

AITHLLLDHGADVNVRGERGKTPLILAVEKKHLGLVQRLLEQEHIEINDT

DSDGKTALLLAVELKLKKIAELLCKRGASTDCGDLVMTARRNYDHSLVKV

LLSHGAKEDFHPPAEDWKPQSSHWGAALKDLHRIYRPMIGKLKFFIDEKY

KIADTSEGGIYLGFYEKQEVAVKTFCEGSPRAQREVSCLQSSRENSHLVT

FYGSESHRGHLFVCVTLCEQTLEACLDVHRGEDVENEEDEFARNVLSSIF

KAVQELHLSCGYTHQDLQPQNILIDSKKAAHLADFDKSIKWAGDPQEVKR

DLEDLGRLVLYVVKKGSISFEDLKAQSNEEVVQLSPDEETKDLIHRLFHP

GEHVRDCLSDLLGHPFFWTWESRYRTLRNVGNESDIKTRKSESEILRLLQ

PGPSEHSKSFDKWTTKINECVMKKMNKFYEKRGNFYQNTVGDLLKFIRNL

GEHIDEEKHKKMKLKIGDPSLYFQKTFPDLVIYVYTKLQNTEYRKHFPQT

HSPNKPQCDGAGGASGLASPGC.

[0226] 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)
VQHWPETVCEKIQNDCRDPPDYWTIHGLWPDKSEGCNRSWPFNLEEIKDL

LPEMRAYWPDVIHSFPNRSRFWKHEWEKHGTCAAQVDALNSQKKYFGRSL

ELYRELDLNSVLLKLGIKPSINYYQVADFKDALARVYGVIPKIQCLPPSQ

DEEVQTIGQIELCLTKQDQQLQNCTEPGEQPSPKQEVWLANGAAESRGLR

VCEDGPVFYPPPKKTKH.

[0227] In some embodiments, the second RNA binding protein comprises or consists of an RNAse11. In some embodiments, the RNAse1111 protein comprises or consists of:

(SEQ ID NO: 29)
EASESTMKIIKEEFTDEEMQYDMAKSGQEKQTIEILMNPILLVKNTSLSM

SKDDMSSTLLTFRSLHYNDPKGNSSGNDKECCNDMTVWRKVSEANGSCKW

SNNFIRSSTEVMRRVHRAPSCKFVQNPGISCCESLELENTVCQFTTGKQF

PRCQYHSVTSLEKILTVLTGHSLMSWLVCGSKL.

[0228] 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)
XLGGADKRLRDNHEWKKLIMVQHWPETVCEKIQNDCRDPPDYWTIHGLWP

DKSEGCNRSWPFNLEEIKDLLPEMRAYWPDVIHSFPNRSRFWKHEWEKHG

TCAAQVDALNSQKKYFGRSLELYRELDLNSVLLKLGIKPSINYYQTTEED

LNLDVEPTTEDTAEEVTIHVLLHSALFGEIGPRRW.

[0229] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a mutated RNAse.

[0230] 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:

(SEQ ID NO: 116)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCRPVNTFVHEP

LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTS

PKERHIIVACEGSPYVPVHFDASVEDST.

[0231] 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)) polypeptide comprises or consists of:

(SEQ ID NO: 117)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCRPVNTFVHEP

LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTS

PKERHIIVACEGSPYVPVHFEASVEDST.

[0232] 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:

(SEQ ID NO: 118)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCRPVNTFVHEP

LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTS

PKERHIIVACEGSPYVPVNFEASVEDST.

[0233] 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:

(SEQ ID NO: 119)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEP

LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTS

PKERHIIVACEGSPYVPVNFDASVEDST.

[0234] 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:

KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRN-
MTQGDCKPVNTFVHEPLVDVQNV

CFQEKVTCKDGQGNCYKSNSSMHITDCRLTADS-
DYPNCAYRTSPKERHIIVACEGSPYV

[0235] 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:

(SEQ ID NO: 121)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGDCRPVNTFVHEP

LVDVQNVCFQEKVTCKDGQGNCYKSNSSMHITDCRLTADSDYPNCAYRTS

PKERHIIVACEGSPYVPVNFEASVEDST.

[0236] 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:

(SEQ ID NO: 122)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGDCKPVNTFVHEP

LVDVQNVCFQEKVTCKDGQGNCYKSNSSMHITDCRLTADSDYPNCAYRTS

PKERHIIVACEGSPYVPVHFDASVEDST.

[0237] 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 that comprises or consists of:

(SEQ ID NO: 208)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGDCRPVNTFVHEP

LVDVQNVCFQEKVTCKDGQGNCYKSNSSMHITDCRLTADSDYPNCAYRTS

PKERHIIVACEGSPYVPVNFEASVEDST.

[0238] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a NOB 1 polypeptide. In some embodiments, the NOB 1 polypeptide comprises or consists of:

(SEQ ID NO: 31)
APVEHVVADAGAFLRHAALQDIGKNIYTIREVVTEIRDKATRRRLAVLPY
ELRFKEPLPEYVRLVTEFSKKTGDYPSLSATDIQVLALTYQLEAEFVGVS
HLKQEPQKVKVSSSIQHPETPLHISGFHLPYKPKPPQETEKGHSACEPEN
LEFSSFMFWRNPLPNIDHELQELLIDRGEDVPSEEEEEEENGFEDRKDDS
DDDGGGWITPSNIKQIQQELEQCDVPEDVRVGCLTTDFAMQNVLLQMGLH
VLAVNGMLIREARSYILRCHGCFKTTSDMSRVFCSHCGNKTLKKVSVTV.

[0239] 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 protein comprises or consists of:

(SEQ ID NO: 32)
APSGLQRVGGVDVSFVKGDSVRACASLVVLSFPELEVVYEESRMVSLTAP
YVSGFLAFREVPFLLELVQQLREKEPGLMPQVLLVDGNGVLHHRGFGVAC
HLGVLTDLPCVGVAKKLLQVDGLENNALHKEKIRLLQTRGDSFPLLGDSG
TVLGMALRSHDRSTRPLYISVGHRMSLEAAVRLTCCCCRFRIPEPVRQAD
ICSREHIRKS.

[0240] In some embodiments, the second RNA binding protein comprises or consists of an endonuclease G (ENDOG). In some embodiments, the ENDOG protein comprises or consists of:

(SEQ ID NO: 33)
AELPPVPGGPRGPGELAKYGLPGLAQLKSRESYVLCYDPRTRGALWVVEQ
LRPERLRGDGDRRECDFREDDSVHAYHRATNADYRGSGFDRGHLAAAANH
RWSQKAMDDTFYLSNVAPQVPHLNQNAWNNLEKYSRSLTRSYQNVYVCTG
PLFLPRTEADGKSYVKYQVIGKNHVAVPTHFFKVLILEAAGGQIELRTYV
MPNAPVDEAIPLERFLVPIESIERASGLLFVPNILARAGSLKAITAGSK.

[0241] In some embodiments, the second RNA binding protein comprises or consists of an endonuclease D1 (ENDOD1). In some embodiments, the ENDOD1 protein comprises or consists of:

(SEQ ID NO: 34)
RLVGEEEAGFGECDKFFYAGTPPAGLAADSHVKICQRAEGAERFATLYST
RDRIPVYSAFRAPRPAPGGAEQRWLVEPQIDDPNSNLEEAINEAEAITSV
NSLGSKQALNTDYLDSDYQRGQLYPFSLSSDVQVATFTLTNSAPMTQSFQ
ERWYVNLHSLMDRALTPQCGSGEDLYILTGTVPSDYRVKDKVAVPEFVWL
AACCAVPGGGWAMGFVKHTRDSDIIEDVMVKDLQKLLPFNPQLFQNNCGE
TEQDTEKMKKILEVVNQIQDEERMVQSQKSSSPLSSTRSKRSTLLPPEAS

-continued

EGSSSFLGKLMGFIATPFIKLFQLIYYLVVAILKNIVYFLWCVTKQVING
IESCLYRLGSATISYFMAIGEELVSIPWKVLKVVAKVIRALLRILCCLLK
AICRVLSIPVRVLVDVATFPVYTMGAIPIVCKDIALGLGGTVSLLFDTAF
GTLGGLFQVVFSVCKRIGYKVTFDNSGEL.

[0242] In some embodiments, the second RNA binding protein comprises or consists of a Human flap endonuclease-1 (hFEN1). In some embodiments, the hFEN1 polypeptide comprises or consists of:

(SEQ ID NO: 35)
MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAVRQGG
DVLQNEEGETTSHLMGMFYRTIRMMENGIKPVYVFDGKPPQLKSGELAKR
SERRAEAEKQLQQAQAAGAEQEVEKFTKRLVKVTKQHNDECKHLLSLMGI
PYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGSPVLMRHLTASEAK
KLPIQEFHLSRILQELGLNQEQFVDLCILLGSDYCESIRGIGPKRAVDLI
QKHKSIEEIVRRLDPNKYPVPENWLHKEAHQLFLEPEVLDPESVELKWSE
PNEEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLDDFFKVTGSL
SSAKRKEPEPKGSTKKKAKTGAAGKFKRGK.

[0243] 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:

(SEQ ID NO: 124)
MESGQPARRIAMAPLLEYERQLVLELLDTDGLVVCARGLGADRLLYHFLQ
LHCHPACLVLVLNTQPAEEEYFINQLKIEGVEHLPRRVTNEITSNSRYEV
YTQGGVIFATSRILVVDFLTDRIPSDLITGILVYRAHRIIESCQEAFILR
LFRQKNKRGFIKAFTDNAVAFDTGFCHVERVMRNLFVRKLYLWPRFHVAV
NSFLEQHKPEVVEIHVSMTPTMLAIQTAILDILNACLKELKCHNPSLEVE
DLSLENAIGKPFDKTIRHYLDPLWHQLGAKTKSLVQDLKILRTLLQYLSQ
YDCVTFLNLLESLRATEKAFGQNSGWLFLDSSTSMFINARARVYHLPDAK
MSKKEKISEKMEIKEGEGILWG.

[0244] 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:

(SEQ ID NO: 123)
CSPQESGMTALSARMLTRSRSLGPGAGPRGCREEPGPLRRREAAAEARKS
HSPVKRPRKAQRLRVAYEGSDSEKGEGAEPLKVPVWEPQDWQQQLVNIRA
MRNKKDAPVDHLGTEHCYDSSAPPKVRRYQVLLSLMLSSQTKDQVTAGAM
QRLRARGLTVDSILQTDDATLGKLIYPVGFWRSKVKYIKQTSAILQQHYG
GDIPASVAELVALPGVGPKMAHLAMAVAWGTVSGIAVDTHVHRIANRLRW
TKKATKSPEETRAALEEWLPRELWHEINGLLVGFGQQTCLPVHPRCHACL
NQALCPAAQGL.

[0245] 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:

(SEQ ID NO: 36)
ESTHVEFKRFTTKKVIPRIKEMLPHYVSAFANTQGGYVLIGVDDKSKEVV

GCKWEKVNPDLLKKEIENCIEKLPTFHFCCEKPKVNFTTKILNVYQKDVL

DGYVCVIQVEPFCCVVFAEAPDSWIMKDNSVTRLTAEQWVVMMLDTQSAP

PSLVTDYNSCLISSASSARKSPGYPIKVHKFKEALQ.

[0246] 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.

[0247] In some embodiments, the hLACTB2 polypeptide comprises or consists of:

(SEQ ID NO: 37)
TLQGTNTYLVGTGPRRILIDTGEPAIPEYISCLKQALTEFNTAIQEIVVT

HWHRDHSGGIGDICKSINNDTTYCIKKLPRNPQREEIIGNGEQQYVYLKD

GDVIKTEGATLRVLYTPGHTDDHMALLLEEENAIFSGDCILGEGTTVFED

LYDYMNSLKELLKIKADIIYPGHGPVIHNAEAKIQQYISHRNIREQQILT

LFRENFEKSFTVMELVKIIYKNTPENLHEMAKHNLLLHLKKLEKEGKIFS

NTDPDKKWKAHL.

[0248] 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:

(SEQ ID NO: 38)
MLRVVSWNINGIRRPLQGVANQEPSNCAAVAVGRILDELDADIVCLQETK

VTRDALTEPLAIVEGYNSYFSFSRNRSGYSGVATFCKDNATPVAAEEGLS

GLFATQNGDVGCYGNMDEFTQEELRALDSEGRALLTQHKIRTWEGKEKTL

TLINVYCPHADPGRPERLVFKMRFYRLLQIRAEALLAAGSHVIILGDLNT

AHRPIDHWDAVNLECFEEDPGRKWMDSLLSNLGCQSASHVGPFIDSYRCF

QPKQEGAFTCWSAVTGARHLNYGSRLDYVLGDRTLVIDTFQASFLLPEVM

GSDHCPVGAVLSVSSVPAKQCPPLCTRFLPEFAGTQLKILRFLVPLEQSP

VLEQSTLQHNNQTRVQTCQNKAQVRSTRPQPSQVGSSRGQKNLKSYFQPS

PSCPQASPDIELPSLPLMSALMTPKTPEEKAVAKVVKGQAKTSEAKDEKE

LRTSFWKSVLAGPLRTPLCGGHREPCVMRTVKKPGPNLGRRFYMCARPRG

PPTDPSSRCNFFLWSRPS.

[0249] In some embodiments, the APEX2 polypeptide comprises or consists of:

(SEQ ID NO: 39)
MLRVVSWNINGIRRPLQGVANQEPSNCAAVAVGRILDELDADIVCLQETK

VTRDALTEPLAIVEGYNSYFSFSRNRSGYSGVATFCKDNATPVAAEEGLS

GLFATQNGDVGCYGNMDEFTQEELRALDSEGRALLTQHKIRTWEGKEKTL

TLINVYCPHADPGRPERLVFKMRFYRLLQIRAEALLAAGSHVIILGDLNT

AHRPIDHWDAVNLECFEEDPGRKWMDSLLSNLGCQSASHVGPFIDSYRCF

QPKQEGAFTCWSAVTGARHLNYGSRLDYVLGDRTLVIDTFQASFLLPEVM

GSDHCPVGAVLSVSSVPAKQCPPLCTRFLPEFAGTQLKILRFLVPLEQS

P.

[0250] 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:

(SEQ ID NO: 125)
PKRGKKGAVAEDGDELRTEPEAKKSKTAAKKNDKEAAGEGPALYEDPPDQ

KTSPSGKPATLKICSWNVDGLRAWIKKKGLDWVKEEAPDILCLQETKCSE

NKLPAELQELPGLSHQYWSAPSDKEGYSGVGLLSRQCPLKVSYGIGDEEH

DQEGRVIVAEFDSFVLVTAYVPNAGRGLVRLEYRQRWDEAFRKFLKGLAS

RKPLVLCGDLNVAHEEIDLRNPKGNKKNAGFTPQERQGFGELLQAVPLAD

SFRHLYPNTPYAYTFWTYMMNARSKNVGWRLDYFLLS.

[0251] 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:

(SEQ ID NO: 40)
QDNSRYTHFLTQHYDAKPQGRDDRYCESIMRRRGLTSPCKDINTFIHGNK

RSIKAICENKNGNPHRENLRISKSSFQVTTCKLHGGSPWPPCQYRATAGF

RNVVVACENGLPVHLDQSIFRRP.

[0252] 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:

(SEQ ID NO: 41)
SSLIRRVISTAKAPGAIGPYSQAVLVDRTIYISGQIGMDPSSGQLVSGGV

AEEAKQALKNMGEILKAAGCDFTNVVKTTVLLADINDFNTVNEIYKQYFK

SNFPARAAYQVAALPKGSRIEIEAVAIQGPLTTASL.

[0253] 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:

(SEQ ID NO: 42)
GGGTPKAPNLEPPLPEEEKEGSDLRPVVIDGSNVAMSHGNKEVFSCRGIL

LAVNWFLERGHTDITVFVPSWRKEQPRPDVPITDQHILRELEKKKILVFT

PSRRVGGKRVVCYDDRFIVKLAYESDGIVVSNDTYRDLQGERQEWKRFIE

ERLLMYSFVNDKFMPPDDPLGRHGPSLDNFLRKKPLTLE.

[0254] In some embodiments, the ZC3H12A polypeptide comprises or consists of:

(SEQ ID NO: 43)
SGPCGEKPVLEASPTMSLWEFEDSHSRQGTPRPGQELAAEEASALELQMK

VDFFRKLGYSSTEIHSVLQKLGVQADTNTVLGELVKHGTATERERQTSPD

PCPQLPLVPRGGGTPKAPNLEPPLPEEEKEGSDLRPVVIDGSNVAMSHGN

KEVFSCRGILLAVNWFLERGHTDITVFVPSWRKEQPRPDVPITDQHILRE

LEKKKILVFTPSRRVGGKRVVCYDDRFIVKLAYESDGIVVSNDTYRDLQG

ERQEWKRFIEERLLMYSFVNDKFMPPDDPLGRHGPSLDNFLRKKPLTLEH

RKQPCPYGRKCTYGIKCRFFHPERPSCPQRSVADELRANALLSPPRAPSK

DKNGRRPSPSSQSSSLLTESEQCSLDGKKLGAQASPGSRQEGLTQTYAPS

GRSLAPSGGSGSSFGPTDWLPQTLDSLPYVSQDCLDSGIGSLESQMSELW

GVRGGGPGEPGPPRAPYTGYSPYGSELPATAAFSAFGRAMGAGHFSVPAD

YPPAPPAFPPREYWSEPYPLPPPTSVLQEPPVQSPGAGRSPWGRAGSLAK

EQASVYTKLCGVFPPHLVEAVMGRFPQLLDPQQLAAEILSYKSQHPSE.

[0255] 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 polypeptidecomprises or consists of:

(SEQ ID NO: 44)
SSLIRRVISTAKAPGAIGPYSQAVLVDRTIYISGQIGMDPSSGQLVSGGV

AEEAKQALKNMGEILKAAGCDFTNVVKTTVLLADINDFNTVNEIYKQYFK

SNFPARAAYQVAALPKGSRIEIEAVAIQGPLTTASL.

[0256] 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:

(SEQ ID NO: 126)
EALFFPSQVTCTEALLRAPGAELAELPEGCPCGLPHGESALSRLLRALLA

ARASLDLCLFAFSSPQLGRAVQLLHQRGVRVRVVTDCDYMALNGSQIGLL

RKAGIQVRHDQDPGYMHHKFAIVDKRVLITGSLNWTTQAIQNNRENVLIT

EDDEYVRLFLEEFERIWEQFNPTKYTFFPPKKSHGSCAPPVSRAGGRLLS

WHRTCGTSSESQT.

[0257] 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:

(SEQ ID NO: 127)
KARYKTLEPRGYSLLIRGLIHSDRWREALLLLEDIKKVITPSKKNYNDCI

QGALLHQDVNTAWNLYQELLGHDIVPMLETLKAFFDFGKDIKDDNYSNKL

LDILSYLRNNQLYPGESFAHSIKTWFESVPGKQWKGQFTTVRKSGQCSGC

GKTIESIQLSPEEYECLKGKIMRDVIDGGDQYRKTTPQELKRFENFIKSR

PPFDVVIDGLNVAKMFPKVRESQLLLNVVSQLAKRNLRLLVLGRKHMLRR

SSQWSRDEMEEVQKQASCFFADDISEDDPFLLYATLHSGNHCRFITRDLM

RDHKACLPDAKTQRLFFKWQQGHQLAIVNRFPGSKLTFQRILSYDTVVQT

TGDSWHIPYDEDLVERCSCEVPTKWLCLHQKT.

[0258] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an argonaute 2 (AGO2) polypeptide.

[0259] In some embodiments of the compositions of the disclosure, the AGO2 polypeptide comprises or consists of:

(SEQ ID NO: 128)
SVEPMFRHLKNTYAGLQLVVVILPGKTPVYAEVKRVGDTVLGMATQCVQM

KNVQRTTPQTLSNLCLKINVKLGGVNNILLPQGRPPVFQQPVIFLGADVT

HPPAGDGKKPSIAAVVGSMDAHPNRYCATVRVQQHRQEIIQDLAAMVREL

LIQFYKSTRFKPTRIIFYRDGVSEGQFQQVLHHELLAIREACIKLEKDYQ

PGITFIVVQKRHHTRLFCTDKNERVGKSGNIPAGTTVDTKITHPTEFDFY

LCSHAGIQGTSRPSHYHVLWDDNRFSSDELQILTYQLCHTYVRCTRSVSI

PAPAYYAHLVAFRARYHLVDKEHDSAEGSHTSGQSNGRDHQALAKAVQVH

QDTLRTMYFA.

[0260] 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:

(SEQ ID NO: 129)
QGAEGALTGKQPDGSAEKAVLEQFGFPLTGTEARCYTNHALSYDQAKRVP

RWVLEHISKSKIMGDADRKHCKFKPDPNIPPTFSAFNEDYVGSGWSRGHM

APAGNNKFSSKAMAETFYLSNIVPQDFDNNSGYWNRIEMYCRELTERFED

VWVVSGPLTLPQTRGDGKKIVSYQVIGEDNVAVPSHLYKVILARRSSVST

EPLALGAFVVPNEAIGFQPQLTEFQVSLQDLEKLSGLVFFPHLDRTSDIR

NICSVDTCKLLDFQEFTLYLSTRKIEGARSVLRLEKIMENLKNAEIEPDD

YFMSRYEKKLEELKAKEQSGTQIRKPS.

[0261] 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:

(SEQ ID NO: 130)
EHPSKMEFFQKLGYDREDVLRVLGKLGEGALVNDVLQELIRTGSRPGALE

HPAAPRLVPRGSCGVPDSAQRGPGTALEEDFRTLASSLRPIVIDGSNVAM

SHGNKETFSCRGIKLAVDWFRDRGHTYIKVFVPSWRKDPPRADTPIREQH

VLAELERQAVLVYTPSRKVHGKRLVCYDDRYIVKVAYEQDGVIVSNDNYR

DLQSENPEWKWFIEQRLLMFSFVNDRFMPPDDPLGRHGPSLSNFLSRKPK

PPEPSWQHCPYGKKCTYGIKCKFYHPERPHHAQLAVADELRAKTGARPGA

GAEEQRPPRAPGGSAGARAAPREPFAHSLPPARGSPDLAALRGSFSRLAF

SDDLGPLGPPLPVPACSLTPRLGGPDWVSAGGRVPGPLSLPSPESQFSPG

DLPPPPGLQLQPRGEHRPRDLHGDLLSPRRPPDDPWARPPRSDRFPGRSV

WAEPAWGDGATGGLSVYATEDDEGDARARARIALYSVFPRDQVDRVMAAF

PELSDLARLILLVQRCQSAGAPLGKP.

[0262] 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:

(SEQ ID NO: 131)
RQQQPQVVEKQQETPLAPADFAHISQDAQSLHSGASRRSQKRLQSPSKQA

QPLDDPEAEQLTVVGKISFNPKDVLGRGAGGTFVFRGQFEGRAVAVKRLL

RECFGLVRREVQLLQESDRHPNVLRYFCTERGPQFHYIALELCRASLQEY

VENPDLDRGGLEPEVVLQQLMSGLAHLHSLHIVHRDLKPGNILITGPDSQ

GLGRVVLSDFGLCKKLPAGRCSFSLHSGIPGTEGWMAPELLQLLPPDSPT

SAVDIFSAGCVFYYVLSGGSHPFGDSLYRQANILTGAPCLAHLEEEVHDK

VVARDLVGAMLSPLPQPRPSAPQVLAHPFFWSRAKQLQFFQDVSDWLEKE

SEQEPLVRALEAGGCAVVRDNWHEHISMPLQTDLRKFRSYKGTSVRDLLR

AVRNKKHHYRELPVEVRQALGQVPDGFVQYFTNRFPRLLLHTHRAMRSCA

SESLFLPYYPPDSEARRPCPGATGR.

[0263] 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:

(SEQ ID NO: 132)
KLVRKNIEKDNAGQVTLVPEEPEDMWHTYNLVQVGDSLRASTIRKVQTES

STGSVGSNRVRTTLTLCVEAIDFDSQACQLRVKGTNIQENEYVKMGAYHT

IELEPNRQFTLAKKQWDSVVLERIEQACDPAWSADVAAVVMQEGLAHICL

VTPSMTLTRAKVEVNIPRKRKGNCSQHDRALERFYEQVVQAIQRHIHFDV

VKCILVASPGFVREQFCDYLFQQAVKTDNKLLLENRSKFLQVHASSGHKY

SLKEALCDPTVASRLSDTKAAGEVKALDDFYKMLQHEPDRAFYGLKQVEK

ANEAMAIDTLLISDELFRHQDVATRSRYVRLVDSVKENAGTVRIFSSLHV

SGEQLSQLTGVAAILRFPVPELSDQEGDSSSEED.

[0264] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a YBEY metallopeptidase (YBEY) polypeptide. In some embodiments, the YBEY polypeptide comprises or consists of:

(SEQ ID NO: 133)
SLVIRNLQRVIPIRRAPLRSKIEIVRRILGVQKFDLGIICVDNKNIQHIN

RIYRDRNVPTDVLSFPFHEHLKAGEFPQPDFPDDYNLGDIFLGVEYIFHQ

CKENEDYNDVLTVTATHGLCHLLGFTHGTEAEWQQMFQKEKAVLDELGRR

TGTRLQPLTRGLFGGS.

[0265] 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 polypeptide comprises or consists of:

(SEQ ID NO: 134)
QEVIAGLERFTFAFEKDVEMQKGTGLLPFQGMDKSASAVCNFFTKGLCEK

GKLCPFRHDRGEKMVVCKHWLRGLCKKGDHCKFLHQYDLTRMPECYFYSK

FGDCSNKECSFLHVKPAFKSQDCPWYDQGFCKDGPLCKYRHVPRIMCLNY

LVGFCPEGPKCQFAQKIREFKLLPGSKI.

[0266] 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:

(SEQ ID NO: 135)
KLVRKNIEKDNAGQVTLVPEEPEDMWHTYNLVQVGDSLRASTIRKVQTES

STGSVGSNRVRTTLTLCVEAIDFDSQACQLRVKGTNIQENEYVKMGAYHT

IELEPNRQFTLAKKQWDSVVLERIEQACDPAWSADVAAVVMQEGLAHICL

VTPSMTLTRAKVEVNIPRKRKGNCSQHDRALERFYEQVVQAIQRHIHFDV

VKCILVASPGFVREQFCDYMFQQAVKTDNKLLLENRSKFLQVHASSGHKY

SLKEALCDPTVASRLSDTKAAGEVKALDDFYKMLQHEPDRAFYGLKQVEK

ANEAMAIDTLLISDELFRHQDVATRSRYVRLVDSVKENAGTVRIFSSLHV

SGEQLSQLTGVAAILRFPVPELSDQEGDSSSEED.

[0267] In some embodiments, the hCG_2002731 polypeptide comprises or consists of:

(SEQ ID NO: 136)
DPAWSADVAAVVMQEGLAHICLVTPSMTLTRAKVEVNIPRKRKGNCSQHD

RALERFYEQVVQAIQRHIHFDVVKCILVASPGFVREQFCDYMFQQAVKTD

NKLLLENRSKFLQVHASSGHKYSLKEALCDPTVASRLSDTKAAGEVKALD

DFYKMLQHEPDRAFYGLKQVEKANEAMAIDTLLISDELFRHQDVATRSRY

VRLVDSVKENAGTVRIFSSLHVSGEQLSQLTGVAAILRFPVPELSDQEGD

SSSEED.

[0268] 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:

(SEQ ID NO: 137)
MDPGKDKEGVPQPSGPPARKKFVIPLDEDEVPPGVRGNPVLKFVRNVPWE

FGDVIPDYVLGQSTCALFLSLRYHNLHPDYIHGRLQSLGKNFALRVLLVQ

VDVKDPQQALKELAKMCILADCTLILAWSPEEAGRYLETYKAYEQKPADL

LMEKLEQDFVSRVTECLTTVKSVNKTDSQTLLTTFGSLEQLIAASREDLA

LCPGLGPQK.

[0269] 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:

(SEQ ID NO: 138)
KESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCKPVNTFVHEP

LVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTS

PKERHIIVACEGSPYVPVHFDASVEDST.

[0270] 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:

(SEQ ID NO: 139)
QDNSRYTHFLTQHYDAKPQGRDDRYCESIMRRRGLTSPCKDINTFIHGNK

RSIKAICENKNGNPHRENLRISKSSFQVTTCKLHGGSPWPPCQYRATAGF

RNVVVACENGLPVHLDQSIFRRP.

[0271] 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:

(SEQ ID NO: 140)
GLGLVQPSYGQDGMYQRFLRQHVHPEETGGSDRYCNLMMQRRKMTLYHCK

RFNTFIHEDIWNIRSICSTTNIQCKNGKMNCHEGVVKVTDCRDTGSSRAP

NCRYRAIASTRRVVIACEGNPQVPVHFDG.

[0272] 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:

(SEQ ID NO: 141)
XSAVDNILLKLAKFKIGFLRLGQIQKVHPAIQQFTEQEICRSKSIKSLAL

LEELYNSQLIVATTCMGINHPIFSRKIFDFCIVDEASQISQPICLGPLFF

SRRFVLVGDHQQLPPLVLNREARALGMSESLFKRLEQNKSAVVQLTVQYR

-continued
MNSKIMSLSNKLTYEGKLECGSDKVANAVINLRHFKDVKLELEFYADYSD

NPWLMGVFEPNNPVCFLNTDKVPAPEQVEKGGVSNVTEAKLIVFLTSIFV

KAGCSPSDIGIIAPYRQQLKIINDLLARSIGMVEVNTVDKYQGRDKSIVL

VSFVRSNKDGTVGELLKDWRRLNVAITRAKHKLILLGCVPSLNCYPPLEK

LLNHLNSEKLISFFFCIWSHLIALL.

[0273] 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:

(SEQ ID NO: 142)
MALRSHDRSTRPLYISVGHRMSLEAAVRLTCCCCRFRIPEPVRQADICSR

EHIRKSLGLPGPPTPRSPKAQRPVACPKGDSGESSALC.

[0274] 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:

(SEQ ID NO: 143)
CYTNHALSYDQAKRVPRWVLEHISKSKIMGDADRKHCKFKPDPNIPPTFS

AFNEDYVGSGWSRGHMAPAGNNKFSSKAMAETFYLSNIVPQDFDNNSGYW

NRIEMYCRELTERFEDVWVVSGPLTLPQTRGDGKKIVSYQVIGEDNVAVP

SHLYKVILARRSSVSTEPLALGAFVVPNEAIGFQPQLTEFQVSLQDLEKL

SGLVFFPHLDRT.

[0275] 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:

(SEQ ID NO: 144)
VTVSQMTSVLNGKTRRFADIQLQHGALCFNIRYGTTVEEEKNHVLEIARQ

RAVAQAWTKEQRRLQEGEEGIRAWTEGEKQQLLSTGRVQGYDGYFVLSVE

QYLELSDSANNIHFMRQSEIGRR.

[0276] 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:

(SEQ ID NO: 145)
TVSQPTLLVNGKTRRFTNIEFQYSTLLLSIRYGLTPDTLDEEKARVLDQA

RQRALGTAWAKEQQKARDGREGSRLWTEGEKQQLLSTGRVQGYEGYYVLP

VEQYPELADSSSNIQFLRQNEMGKR.

[0277] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a Ribonuclease Kappa (RNAseK) polypeptide. In some embodiments, the RNAseK polypeptide comprises or consists of:

(SEQ ID NO: 204)

MGWLRPGPRPLCPPARASWAFSHRFPSPLAPRRSPTPFFMASLLCCGPKL

AACGIVLSAWGVIMLIMLGIFFNVHSAVLIEDVPFTEKDFENGPQNIYNL

YEQVSYNCFIAAGLYLLLGGFSFCQVRLNKRKEYMVR.

[0278] 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, the TALEN polypeptide comprises or consists of:

(SEQ ID NO: 205)

1 MRIGKSSGWL NESVSLEYEH VSPPTRPRDT RRRPRAAGDG GLAHLHRRLA VGYAEDTPRT

61 EARSPAPRRP LPVAPASAPP APSLVPEPPM PVSLPAVSSP RFSAGSSAAI TDPFPSLPPT

121 PVLYAMAREL EALSDATWQP AVPLPAEPPT DARRGNTVFD EASASSPVIA SACPQAFASP

181 PRAPRSARAR RARTGGDAWP APTFLSRPSS SRIGRDVFGK LVALGYSREQ IRKLKQESLS

241 EIAKYHTTLT GQGFTHADIC RISRRRQSLR VVARNYPELA AALPELTRAH IVDIARQRSG

301 DLALQALLPV ATALTAAPLR LSASQIATVA QYGERPAIQA LYRLRRKLTR APLHLTPQQV

361 VAIASNTGGK RALEAVCVQL PVLRAAPYRL STEQVVAIAS NKGGKQALEA VKAHLLDLLG

421 APYVLDTEQV VAIASHNGGK QALEAVKADL LDLRGAPYAL STEQVVAIAS HNGGKQALEA

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 PVLRATPYDL NTAQIVAIAS HDGGKPALEA

901 VWAKLPVLRG APYALSTAQV VAIACISGQQ ALEAIEAHMP TLRQASHSLS PERVAAIACI

961 GGRSAVEAVR QGLPVKAIRR IRREKAPVAG PPPASLGPTP QELVAVLHFF RAHQQPRQAF

1021 VDALAAFQAT RPALLRLLSS VGVTEIEALG GTIPDATERW QRLLGRLGFR PATGAAAPSP

1081 DSLQGFAQSL ERTLGSPGMA GQSACSPHRK RPAETAIAPR SIRRSPNNAG QPSEPWPDQL

1141 AWLQRRKRTA RSHIRADSAA SVPANLHLGT RAQFTPDRLR AEPGPIMQAH TSPASVSFGS

1201 HVAFEPGLPD PGTPTSADLA SFEAEPFGVG PLDFHLDWLL QILET.

[0279] In some embodiments, the TALEN polypeptide comprises or consists of:

(SEQ ID NO: 206)

1 mdpirsrtps parellpgpq pdrvqptadr ggappaggpl dglparrtms rtrlpsppap

61 spafsagsfs dllrqfdpsl ldtslldsmp avgtphtaaa paecdevqsg lraaddpppt

121 vrvavtaarp prakpaprrr aaqpsdaspa aqvdlrtlgy sqqqqekikp kvgstvaqhh

181 ealvghgfth ahivalsrhp aalgtvavky qdmiaalpea thedivgvgk qwsgaralea

241 lltvagelrg pplqldtgql vkiakrggvt aveavhasrn altgaplnlt paqvvaiasn

301 nggkqaletv qrllpvlcqa hgltpaqvva iashdggkqa letmqrllpv lcqahglppd

361 qvvaiasnig gkqaletvqr llpvlcqahg ltpdqvvaia shgggkqale tvqrllpvlc

421 qahgltpdqv vaiashdggk qaletvqrll pvlcqahglt pdqvvaiasn gggkqaletv

481 qrllpvlcqa hgltpdqvva iasnggkqal etvqrllpvl cqahgltpdq vvaiashdgg

541 kqaletvqrl lpvlcqthgl tpaqvvaias hdggkqalet vqqllpvlcq ahgltpdqvv

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 601 aiasniggkq alatvqrllp vlcqahgltp dqvvaiasng ggkqaletvq rllpvlcqah
 661 gltpdqvvai asngggkqal etvqrllpvl cqahgltqvq vvaiasnigg kqaletvqrl
 721 lpvlcqahgl tpaqvvaias hdggkqalet vqrllpvlcq ahgltpdqvv aiasngggkq
 781 aletvqrllp vlcqahgltq eqvvaiasnn ggkqaletvq rllpvlcqah gltpdqvvai
 841 asngggkqal etvqrllpvl cqahgltpaq vvaiasnigg kqaletvqrl lpvlcqdhgl
 901 tlaqvvaias niggkqalet vqrllpvlcq ahgltqdqvv aiasniggkq aletvqrllp
 961 vlcqdhgltp dqvvaiasni ggkqaletvq rllpvlcqdh gltldqvvai asnggkqale
1021 tvqrllpvlc qdhgltpdqv vaiasnsggk qaletvqrll pvlcqdhglt pnqvvaiasn
1081 ggkqalesiv aqlsrpdpal aaltndhlva laclggrpam davkkglpha pelirrvnrr
1141 igertshrva dyaqvvrvle ffqchshpay afdeamtqfg msrnglvqlf rrvgvtelea
1201 rggtlppasq rwdrilqasg mkrakpspts aqtpdqaslh afadslerdl dapspmhegd
1261 qtgassrkrs rsdravtgps aqhsfevrvp eqrdalhlpl swrvkrprtr iggglpdpgt
1321 piaadlaass tvmweqdaap fagaaddfpa fneeelawlm ellpqsgsvg gti.
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[0280] 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. In some embodiments, the ZNF638 polypeptide polypeptide comprises or consists of:

(SEQ ID NO: 207)

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   1 MSRPRFNPRG DFPLQRPRAP NPSGMRPPGP FMRPGSMGLP RFYPAGRARG IPHRFAGHES
  61 YQNMGPQRMN VQVTQHRTDP RLTKEKLDFH EAQQKKGKPH GSRWDDEPHI SASVAVKQSS
 121 VTQVTEQSPK VQSRYTKESA SSILASFGLS NEDLEELSRY PDEQLTPENM PLILRDIRMR
 181 KMGRRLPNLP SQSRNKETLG SEAVSSNVID YGHASKYGYT EDPLEVRIYD PEIPTDEVEN
 241 EFQSQQNISA SVPNPNVICN SMFPVEDVFR QMDFPGESSN NRSFFSVESG TKMSGLHISG
 301 GQSVLEPIKS VNQSINQTVS QTMSQSLIPP SMNQQPFSSE LISSVSQQER IPHEPVINSS
 361 NVHVGSRGSK KNYQSQADIP IRSPFGIVKA SWLPKFSHAD AQKMKRLPTP SMMNDYYAAS
 421 PRIFPHLCSL CNVECSHLKD WIQHQNTSTH IESCRQLRQQ YPDWNPEILP SRRNEGNRKE
 481 NETPRRRSHS PSPRRSRRSS SSHRFRRSRS PMHYMYRPRS RSPRICHRFI SRYRSRSRSR
 541 SPYRIRNPFR GSPKCFRSVS PERMSRRSVR SSDRKKALED VVQRSGHGTE FNKQKHLEAA
 601 DKGHSPAQKP KTSSGTKPSV KPTSATKSDS NLGGHSIRCK SKNLEDDTLS ECKQVSDKAV
 661 SLQRKLRKEQ SLHYGSVLLI TELPEDGCTE EDVRKLFQPF GKVNDVLIVP YRKEAYLEME
 721 FKEAITAIMK YIETTPLTIK GKSVKICVPG KKKAQNKEVK KKTLESKKVS ASTLKRDADA
 781 SKAVEIVTST SAAKTGQAKA SVAKVNKSTG KSASSVKSVV TVAVKGNKAS IKTAKSGGKK
 841 SLEAKKTGNV KNKDSNKPVT IPENSEIKTS IEVKATENCA KEAISDAALE ATENEPLNKE
 901 TEEMCVMLVS NLPNKGYSVE EVYDLAKPFG GLKDILILSS HKKAYIEINR KAAESMVKFY
 961 TCFPVLMDGN QLSISMAPEN MNIKDEEAIF ITLVKENDPE ANIDTIYDRF VHLDNLPEDG
1021 LQCVLCVGLQ FGKVDHHVFI SNRNKAILQL DSPESAQSMY SFLKQNPQNI GDHMLTCSLS
1081 PKIDLPEVQI EHDPELEKES PGLKNSPIDE SEVQTATDSP SVKPNELEEE STPSIQTETL
1141 VQQEEPCEEE AEKATCDSDF AVETLELETQ GEEVKEEIPL VASASVSIEQ FTENAEECAL
1201 NQQMFNSDLE KKGAEIINPK TALLPSDSVF AEERNLKGIL EESPSEAEDF ISGITQTMVE
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1261 AVAEVEKNET VSEILPSTCI VTLVPGIPTG DEKTVDKKNI SEKKGNMDEK EEKEFNTKET

1321 RMDLQIGTEK AEKNEGRMDA EKVEKMAAMK EKPAENTLFK 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 GVEGELSFVT LDEIGEEEDA AAHLAQALVT

1621 VDEVIDEEEL 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 TPTKRVRIGK

1861 TLPSEKAVVT EPAKGEEAFQ MSEVDEESGL KDSEPERKRK KTEDSSSGKS VASDVPEELD

1921 FLVPKAGFFC PICSLFYSGE KAMTNHCKST RHKQNTEKFM AKQRKEKEQN EAEERSSR.

[0281] 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, for example, and without limitation, see FIG. **9**, which shows PIN-dSauCas9, PIN-dSauCas9dHNH, PIN-dSPCas9, and dcjeCas9-PIN.

[0282] 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, 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, a nuclease comprises a sequence isolated or derived from a TALEN or a nuclease domain thereof. In some embodiments, a nuclease comprises a sequence isolated or derived from a zinc finger nuclease or a nuclease domain thereof.

Fusion Proteins

[0283] 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-biding polypeptide binds a target RNA, and wherein the second RNA-binding polypeptide comprises RNA-nuclease activity.

[0284] 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.

[0285] 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 Pumiliol, 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.

[0286] 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.

[0287] 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

(SEQ ID NO: 209)

```
MDKSKQMNIN NLSNIPEVID PGITIPIYEE EYENNGESNS QLQQQPQKLG SYRSRAGKFS    60

NTLSNLLPSI SAKLHHSKKN SHGKNGAEFS SSNNSSQSTV ASKTPRASPS RSKMMESSID   120

GVTMDRPGSL TPPQDMEKLV HFPDSSNNFL IPAPRGSSDS FNLPHQISRT RNNTMSSQIT   180

SISSIAPKPR TSSGIWSSNA SANDPMQQHL LQQLQPTTSN NTTNSNTLND YSTKTAYFDN   240

MVSTSGSQMA DNKMNTNNLA IPNSVWSNTR QRSQSNASSI YTDAPLYEQP ARASISSHYT   300

IPTQESPLIA DEIDPQSINW VTMDPTVPSI NQISNLLPTN TISISNVFPL QHQQPQLNNA   360

INLTSTSLAT LCSKYGEVIS ARTLRNLNMA LVEFSSVESA VKALDSLQGK EVSMIGAPSK   420

ISFAKILPMH QQPPQFLLNS QGLPLGLENN NLQPQPLLQE QLFNGAVTFQ QQGNVSIPVF   480

NQQSQQSQHQ NHSSGSAGFS NVLHGYNNNN SMHGNNNNSA NEKEQCPFPL PPPNVNEKED   540

LLREIIELFE ANSDEYQINS LIKKSLNHKG TSDTQNFGPL PEPLSGREFD PPKLRELRKS   600

IDSNAFSDLE IEQLAIAMLD ELPELSSDYL GNTIVQKLFE HSSDIIKDIM LRKTSKYLTS   660

MGVHKNGTWA CQKMITMAHT PRQIMQVTQG VKDYCTPLIN DQFGNYVIQC VLKFGFPWNQ   720

FIFESIIANF WVIVQNRYGA RAVRACLEAH DIVTPEQSIV LSAMIVTYAE YLSTNSNGAL   780

LVTWFLDTSV LPNRHSILAP RLTKRIVELC GHRLASLTIL KVLNYRGDDN ARKIILDSLF   840

GNVNAHDSSP PKELTKLLCE TNYGPTFVHK VLAMPLLEDD LRAHIIKQVR KVLTDSTQIQ   900

PSRRLLEEVG LASPSSTHNK TKQQQQQHHN SSISHMFATP DTSGQHMRGL SVSSVKSGGS   960

KHTTMNTTTT NGSSASTLSP GQPLNANSNS SMGYFSYPGV FPVSGFSGNA SNGYAMNNDD  1020

LSSQFDMLNF NNGTRLSLPQ LSLTNHNNTT MELVNNVGSS QPHTNNNNNN NNTNYNDDNT  1080

VFETLTLHSA N.                                                      1091
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[0288] In some embodiments, a PUF3 protein of the disclosure comprises or consists of the amino acid sequence of

[0289] 1 MEMNMDMDMD MELASIVSSL SALSHSNNNG GQAAAAGIVi GGAAGSQQIG GFRRSSFTTA

[0290] 61 NEVDSEILLL HGSSESSPIF KKTALSVGTA PPFSTNSKKF FGNGGNYYQY RSTDTASLSS

[0291] 121 ASYNNYHTHH TAANLGKNNK VNHLLGQYSA SIAGPVYYNG NDNNNSGGEG FFEKFGKSLI

[0292] 181 DGTRELESQD RPDANTQSQ FITKSVSNAS LDTQNTFEQN VESDKNFNKL NRNTTNSGSL

[0293] 241 YHSSSNSGSS ASLESENAHY PKRNIWNVAN TPVFRPSNNP AAVGATNVAL PNQQDGPANN

[0294] 301 NFPPYMNGFP PNQFHQGPHY QNFPNYLIGS PSNFISQMIS VQIPANEDTE DSNGKKKKKA

[0295] 361 NRPSSVSSPS SPPNNSPFPF AYPNPMMFMP PPPLSAPQQQ QQQQQQQQQE DQQQQQQQEN

[0296] 421 PYTYYPTPNP IPVKMPKDEK TFKKRNNKNH PANNNNANK QANPIYLENSI PTKNTSKKNA

[0297] 481 SSKSNESTAN NHKSHSHSHP HSQSLQQQQQ TYHRSPLLEQ LRNSSSDKNS NSNMSLKDIF

[0298] 541 GHSLEFCKDQ HGSRFIQREL ATSPASEKEV I FNEIRDDAI ELSNDVFGNY VIQKFFEFGS

[0299] 601 KIQKNTLVDQ FKGNMKIQLSL QMYACRVQK ALEYIDSNQR IELVLELSDS VLQMIKDQNG

[0300] 661 NHVIQKAIE IPiEKLPFIL SSLTGHIYHL STHSYGCRVI QRLLEFGSSE DQESILNELK

[0301] 721 DFIPYLIQDQ YGNYVIQYVL QQDQFTNKEM VDIKQEIIET VANNVVEYSK HKEASNVVEK

[0302] 781 SILYGSKNQK DLIISKILPR DKNHALNLED DSPMILMIKD QFANYVIQKL VNVSEGEGKK

[0303] 841 LIVIAIRAYL DKLNKSNSLG NRHLASVEKL AALVENAEV (SEQ ID NO: 210). In some embodiments, a PUF4 protein of the disclosure comprises or consists of the amino acid sequence of

(SEQ ID NO: 211)

```
  1MSTKGLKEEI DDVPSVDPVV SETVNSALEQ LQLDDPEENA TSNAFANKVS QDSQFANGPP

 61SQMFPHPQMM GGMGFMPYSQ MMQVPHNPCP FFPPPDFNDP TAPLSSSPLN AGGPPMLFKN

121DSLPFQMLSS GAAVATQGGQ NLNPLINDNS MKVLPIASAD PLWTHSNVPG SASVAIEETT

181ATLQESLPSK GRESNNKASS FRRQTFHALS PTDLINAANN VTLSKDFQSD MQNFSKAKKP

241SVGANNTAKT RTQSISFDNT PSSTSFIPPT NSVSEKLSDF KIETSKEDLI NKTAPAKKES

301PTTYGAAYPY GGPLLQPNPI MPGHPHNISS PIYGIRSPFP NSYEMGAQFQ PFSPILNPTS

361HSLNANSPIP LTQSPIHLAP VLNPSSNSVA FSDMKNDGGK PTTDNDKAGP NVRMDLINPN

421LGPSMQPFHI LPPQQNTPPP PWLYSTPPPF NAMVPPHLLA QNHMPLMNSA NNKHHGRNNN

481SMSSHNDNDN IGNSNYNNKD TGRSNVGKMK NMKNSYHGYY NNNNNNNNNN NNNNNSNATN

541SNSAEKQRKI EESSRFADAV LDQYIGSIHS LCKDQHGCRF LQKQLDILGS KAADAIFEET

601KDYTVELMTD SFGNYLIQKL LEEVTTEQRI VLTKISSPHF VEISLNPHGT RALQKLIECI

661KTDEEAQIVV DSLRPYTVQL SKDLNGNHVI QKCLQRLKPE NFQFIFDAIS DSCIDIATHR

721HGCCVLQRCL DHGTTEQCDN LCDKLLALVD KLTLDPFGNY VVQYIITKEA EKNKYDYTHK

781IVHLLKPRAI ELSIHKFGSN VIEKILKTAI VSEPMILEIL NNGGETGIQS LLNDSYGNYV

841LQTALDISHK QNDYLYKRLS EIVAPLLVGP IRNTPHGKRI IGMLHLDS.
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[0304] In some embodiments, a PUF5 protein of the disclosure comprises or consists of the amino acid sequence of

[0305] 1 MSDSTGRINS KASDSSSISD HQTADL-SIFN GSFDGGAFSS SNIPLFNFMG TGNQRFQYSP

[0306] 61 HPFAKSSDPC RLAALTPSTP KGPLNLT-PAD FGLADFSVGN ESFADFTANN TSFVGN-VQSN

[0307] 121 VRSTRLLPAW AVDNSGNIRD DLTLQDVVSN GSLIDFAMDR TGVKFLERHF PEDHDNEMHF

[0308] 181 VLFDKLTEQG AVFTSLCRSA AGNFI-IQKFV EHATLDEQER LVRKMCDNGL IEMCLDK-FAC

[0309] 241 RVVQMSIQKF DVSIAMKLVE KISSLD-FLPL CTDQCAIHVL QKVVKLLPIS AWSFFVK-FLC

[0310] 301 RDDNLMTVCQ DKYGCRLVQQ TID-KLSDNPK LHCFNTRLQL LHGLMTSVAR NCFRLSSNEF

[0311] 361 ANYVVQYVIK SSGVMEMYRD TIIEK-CLLRN ILSMSQDKYA SHVVEGAFLF APPLLLSEMM

[0312] 421 DEIFDGYVKD QETNRDALDI LLF-HQYGNYV VQQMISICIS ALLGKEERKM VASEM-RLYAK

[0313] 481 WFDRIKNRVN RHSGRLERFS SGKKI-TIESLQ KLNVPMTMTN EPMPYWAMPT PLMDI-SAHFM

[0314] 541 NKLNFQKNSV FDE (SEQ ID NO: 212). In some embodiments, a PUF6 protein of the disclosure comprises or consists of the amino acid sequence of

[0315] 1 MTPNRRSTDS YNMLGASFDF DPDFSLL-SNK THKNKNPKPP VKLLPYRHGS NTTSS-DLDNY

[0316] 61 IFNSGSGSSD DETPPPAAPI FISLEEV-LLN GLLIDFAIDP SGVKFLEANY PLDSEDQIRPK

[0317] 121 AVFEKLTEST TLFVGLCHSR NGN-FIVQKLV ELATPAEQRE LLRQMIDGGL LVMCK-DKFAC

[0318] 181 RVVQLALQKF DHSNVFQLIQ ELSTFD-LAAM CTDQISIHVI QRVVKQLPVD MWTFFVH-FLS

[0319] 241 SGDSLMAVCQ DKYGC:LVQQ VIDR-LAENPK LPCFKFRIQL LHSLMTCIVR NCYRLSS-NEF

[0320] 301 ANYVIQYVIK SSGIMEMYRD TIIDK-CLLRN LLSMSQDKYA SHVIEGAFLF APPALL-HEMM

[0321] 361 EEIFSGYVKD VELNRDALDI LLF-HQYGNYV VQQMISICTA ALIGKEEPQL PPAILLLYSG

[0322] 421 WYEKMKQRVL QHASRLERFS SGKKI-IDSVM RHGVPTAAAI NAQAAPSLME LTAQFDAFP

[0323] 4181 SFLAR (SEQ ID NO: 213). In some embodiments, a PUF7 protein of the disclosure comprises or consists of the amino acid sequence of

[0324] 1 MTPNRRSTDS YNMLGASFDF DPDFSLL-SNK THKNKNPKPP VKLLPYRIGS NTTSSDSDSY

[0325] 61 IFNSGSGSSD AETPAPVAPI FISLEDV-LLN GQLIDFAIDP SGVKFLEANY PLDSEDQIRK

[0326] 121 AVFEKFTEST TLFVGLCHSR NGN-FIVQKLV ELATPAEQRE LLRQMIDGGL LAMCK-DKFAC

[0327] 181 RVVQLALQKF DHSNVFQLIQ ELSTFD-LAAM CTDQISIHVI QRVVKQLPVD MWTFFVH-FLS

[0328] 241 SGDSLMAVCQ DKYGCRLVQQ VIDR-LAENPK LPCFKFEPRIQL LHSLMTCIVR NCYRLSSNEF

[0329] 301 ANYVIQYVIK SSGIMEMYRD TIIDK-CLLRN LLSMSQDKYA SHVIEGAFLF APPALL-HEMM

[0330] 361 EEIFSGYVKD VESNRDALDI LLFHQYGNYV VQQMISICTA ALIGKEEREL PPAILLLYSG

[0331] 421 WYEKMKQRVL QHASRLERFS SGKKIIDSVM RHGVPTAAAV NAQAAPSLME LTAQFDAMFP

[0332] 481 SFLAR (SEQ ID NO: 214). In some embodiments, a PUF8 protein of the disclosure comprises or consists of the amino acid sequence of

[0333] 1 MSRPISIGNT CTFDPSASPI ESLGRSIGAQ KIVDSVCGSP IRSYGRHIST NPKNERLPDT

[0334] 61 PEFQFATYMH QGGKVIGQNT LHMFGTPPSC YCAQENIPIS SNVGHVLSTI NNNYMNHQYN

[0335] 121 GSNMFSNQMT QMLQAQAYND LQMHQAIHSQS IRVPVQPSAT GIFSNPYPREP TTTDDLLTRY

[0336] 181 RANPAMMKNL KLSDIRGALL KFAKDQVGSR FIQQELASSK DRFEKDSIFD EVVSNADELV

[0337] 241 DDIFGNYVVQ KFFEYGEERH WARLVDAIID RVPEYAFQMY ACRVLQKALE KINEPLQIKI

[0338] 301 LSQIRHVIHR CMKDCNGNHV VOKAIEKVSP QYVQFIVDTL LESSNTIYEM SVDPYGCRVV

[0339] 361 QRCLEHCSPS QTKPVIGQIH KRFDEIANNQ YGNYVQIIHVI EHGSEEDRMV IVTRVSNNLF

[0340] 421 EFATHKYSSN VIEKCLEQGA VYHKSMIVGA ACHHQEGSVP IVVQMMKDQY ANYVVQKMFD

[0341] 481 QVTSEQRREL ILTVNRPHPV LRQFPHGKHI LAKLEKYFQK PANMSYPYQD MQGSH (SEQ ID NO: 215). In some embodiments, a PUF9 protein of the disclosure comprises or consists of the amino acid sequence of

(SEQ ID NO: 216)

```
  1MADPNWAYAP PTNYYADHSI AKPIMISGGH PSQDQGHSPK SESFGQSVTT AFNGMVDNLV

 61GSPSSSVQQR NYFTTTPFPI SRSPNDRNDD KIMGNGSYGV PIPIPQDGVP QGTPDFQMTP

121FLQQGGHLIG GSPNGPVQVS GNWYSGGAGI FSTMQQADPS NGMPGMAAEF VNNENGMPGP

181NGMHQQAMIS GSPPFPYQNM MNLTTSFGAM GLGPQQIQQR DPQMFQQPIL HEPIQGMAQN

241GFGQQVFFTQ MQNQQHPQGQ AQQQLQQLAQ QHQQQQNSQQ FFGQGPNGMG NGGVMNDWSQ

301RSFGMPQQQA QQNGLPPNFS QNPPRRRGPE DPNGQTPKTL QDIKNNVIEF AKDQHGSRFI

361QQKLERASLR DKAAIFTPVL ENAEELMTDV FGNYVIQKFF EFGNNEQRNQ LVGTIRGNVM

421KLALQMYGCR VIQKALEYVE EKYQHEILGE MEGQVLKCVK DQNGNHVIQK VIERVEPERL

481QFIIDAFTKN NSDNVYTLSV HPYGCRVIQR VLEYCNEEQK QPVLDALQIH LKQLVLDQYG

541NYVIQHVIEH GSPSDKEQIV QDVISDDLLK FAQHKFASNV IEKCLTFGGH AERNLIIDKV

601CGDPNDPSPP LLQMMKDPFA NYVVQKMLDV ADPQHRKKIT LTIKPHIATL RKYNFGKHIL

661LKLEKYFAKQ APANSSNSSS NDQIYEHSPF DIPLGADFSN HPF.
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[0342] 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.

[0343] 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.

[0344] 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.

[0345] In some embodiments, 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, a nuclear localization signal (NLS) is positioned 3' to 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 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 first NLS or the second NLS is positioned 3' to 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.

[0346] 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.

[0347] 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. For example, one such exemplary fusion protein is E99 which is configured so that RNAse1 (R39D, N67D, N88A, G89D, R19D, H119N, K41R) is located at the N-terminus of SpyCas9 whereas another exemplary fusion protein, E100, is configured so that RNAse1(R39D, N67D, N88A, G89D, R19D, H119N, K41R) is located at the C-terminus of SpyCas9. See FIG. **6**.

Vectors

[0348] 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.

[0349] 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.

[0350] 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 (or spacer sequence) that hybridizes to or binds to a target RNA sequence; and on its 3' end, an RNA sequence (or scaffold sequence) capable of 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 in a single 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.

[0351] 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.

[0352] 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).

[0353] 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.

[0354] 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.

[0355] 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.

[0356] 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 of total 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: Spring-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

[0357] 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 condi-

tions or its complementary strand. Alternatively, an equivalent polypeptide or protein is one that is expressed from an equivalent polynucleotide.

[0358] 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, an 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, 3.sup.rd Edition, W.H. 5 Freeman and Co., NY).

[0359] "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, Hoogstein 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.

[0360] Examples of stringent hybridization conditions include: incubation temperatures of about 25° C. to about 37° C.; hybridization buffer concentrations of about 6×SSC to about 10×SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4×SSC to about 8×SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40° C. to about 50° C.; buffer concentrations of about 9×SSC to about 2×SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5×SSC to about 2×SSC. Examples of high stringency conditions include: incubation temperatures of about 55° C. to about 68° C.; buffer concentrations of about 1×SSC to about 0.1×SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about 1×SSC, 0.1×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.

[0361] "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

[0362] In some embodiments of the compositions and methods of the disclosure, a cell of the disclosure is a prokaryotic cell.

[0363] 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.

[0364] 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.

[0365] 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).

[0366] 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.

[0367] 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.

[0368] 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.

[0369] 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.

[0370] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a primary cell.

[0371] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a cultured cell.

[0372] 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.

[0373] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is autologous or allogeneic.

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 or a single 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 con-

ditions 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 or a single 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), Hypogammalglobulinemia, 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.

[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.

[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, 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.

[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, an infectious 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 pediatric or a developmental 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 cardiovascular disease or disorder.

[0393] 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 Can-

cer, 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, Sezary 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.

[0394] 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.

[0395] 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.

[0396] 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.

[0397] 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.

[0398] In some embodiments of the methods of the disclosure, a subject of the disclosure is a human.

[0399] 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.

[0400] 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.

[0401] In some embodiments of the methods of the disclosure, a therapeutically effective amount eliminates the disease or disorder.

[0402] 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.

[0403] 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.

[0404] 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.

[0405] 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.

EXAMPLE EMBODIMENTS

Embodiment 1

[0406] A composition comprising:

[0407] (a) a sequence comprising a guide RNA (gRNA) that specifically binds a target sequence within an RNA molecule and

[0408] (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,

[0409] wherein neither the first RNA-binding polypeptide nor the second RNA-binding polypeptide comprises a significant DNA-nuclease activity,

[0410] wherein the first RNA-binding polypeptide and the second RNA-binding polypeptide are not identical, and

[0411] wherein the second RNA-binding polypeptide comprises an RNA-nuclease activity; or

[0412] a composition comprising nucleic acid sequence encoding a fusion protein, the fusion protein comprising a first RNA-binding polypeptide and a second RNA-binding polypeptide, wherein the first RNA-binding polypeptide is not a guided RNA-binding polypeptide, 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

[0413] The composition of embodiment 1, wherein the target sequence comprises at least one repeated sequence.

Embodiment 3

[0414] The composition of embodiment 1 or 2, wherein the sequence comprising the gRNA comprises a promoter capable of expressing the gRNA in a eukaryotic cell.

Embodiment 4

[0415] The composition of embodiment 3, wherein the eukaryotic cell is an animal cell.

Embodiment 5

[0416] The composition of embodiment 4, wherein the animal cell is a mammalian cell.

Embodiment 6

[0417] The composition of embodiment 5, wherein the animal cell is a human cell.

Embodiment 7

[0418] The composition of any one of embodiments 1-6, wherein the promoter is a constitutively active promoter.

Embodiment 8

[0419] The composition of any one of embodiments 1-7, wherein the promoter is isolated or derived from a promoter capable of driving expression of an RNA polymerase.

Embodiment 9

[0420] The composition of embodiment 8, wherein the promoter is isolated or derived from a U6 promoter.

Embodiment 10

[0421] The composition of any one of embodiments 1-7, wherein the promoter is isolated or derived from a promoter capable of driving expression of a transfer RNA (tRNA).

Embodiment 11

[0422] The composition of embodiment 10, wherein the promoter 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.

Embodiment 12

[0423] The composition of embodiment 10, wherein the promoter is isolated or derived from a valine tRNA promoter.

Embodiment 13

[0424] The composition of any one of embodiments 1-12, wherein the sequence comprising the gRNA comprises a spacer sequence that specifically binds to the target RNA sequence.

Embodiment 14

[0425] The composition of embodiment 13, wherein 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.

Embodiment 15

[0426] The composition of embodiment 13, wherein the spacer sequence has 100% complementarity to the target RNA sequence.

Embodiment 16

[0427] The composition of any one of embodiments 13-15, wherein the spacer sequence comprises or consists of 20 nucleotides.

Embodiment 17

[0428] The composition of any one of embodiments 13-15, wherein the spacer sequence comprises or consists of 21 nucleotides.

Embodiment 18

[0429] The composition of embodiment 17, wherein the spacer sequence comprises the sequence UGGAGCGAGCAUCCCCCAAA (SEQ ID NO: 1), GUUUGGGGGAUGCUCGCUCCA (SEQ ID NO: 2), CCCUCACUGCUGGGGAGUCC (SEQ ID NO: 3), GGACUCCCCAGCAGUGAGGG (SEQ ID NO: 4), GCAACUGGAUCAAUUUGCUG (SEQ ID NO: 5), GCAGCAAAUUGAUCCAGUUGC (SEQ ID NO: 6),

GCAUUCUUAUCUGGUCAGUGC (SEQ ID NO: 7), GCACUGACCAGAUAAGAAUG (SEQ ID NO: 8), GAGCAGCAGCAGCAGCAGCAG (SEQ ID NO: 9), GCAGGCAGGCAGGCAGGCAGG (SEQ ID NO: 10), GCCCCGGCCCCGGCCCCGGC (SEQ ID NO: 11), or GCTGCTGCTGCTGCTGCTGC (SEQ ID NO: 12), GGGGCCGGGGCCGGGGCCGG (SEQ ID NO: 74), GGGCCGGGGCCGGGGCCGGG (SEQ ID NO: 75), GGCCGGGGCCGGGGCCGGGG (SEQ ID NO: 76), GCCGGGGCCGGGGCCGGGGC (SEQ ID NO: 77), CCGGGGCCGGGGCCGGGGCC (SEQ ID NO: 78), CGGGGCCGGGGCCGGGGCCG (SEQ ID NO: 79).

Embodiment 19

[0430] The composition of any one of embodiments 1-18, wherein the sequence comprising the gRNA comprises a scaffold sequence that specifically binds to the first RNA binding protein.

Embodiment 20

[0431] The composition of embodiment 19, wherein the scaffold sequence comprises a stem-loop structure.

Embodiment 21

[0432] The composition of embodiment 19 or 20, wherein the scaffold sequence comprises or consists of 90 nucleotides.

Embodiment 22

[0433] The composition of embodiment 19 or 20, wherein the scaffold sequence comprises or consists of 93 nucleotides.

Embodiment 23

[0434] The composition of embodiment 22, wherein the scaffold sequence comprises the sequence

(SEQ ID NO: 13)
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUC

CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU.

Embodiment 24

[0435] The composition of embodiment 16, wherein the spacer sequence comprises the sequence GUGAUAAGUGGAAUGCCAUG (SEQ ID NO: 14), CUGGUGAACUUCCGAUAGUG (SEQ ID NO: 15), or GAGATATAGCCTGGTGGTTC (SEQ ID NO: 16).

Embodiment 25

[0436] The composition of embodiment 19 or 24, wherein the scaffold sequence comprises a step-loop structure.

Embodiment 26

[0437] The composition of embodiment 25, wherein the scaffold sequence comprises or consists of 85 nucleotides.

Embodiment 27

[0438] The composition of embodiment 26, wherein the scaffold sequence comprises the sequence

(SEQ ID NO: 17)
GGACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAA

GUGGCACCGAGUCGGUGCUUUUU.

Embodiment 28

[0439] The composition of embodiment 16, wherein the spacer sequence comprises the sequence 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 29

[0440] The composition of embodiment 28, wherein the sequence comprising the gRNA comprises a scaffold sequence that specifically binds to the first RNA binding protein.

Embodiment 30

[0441] The composition of embodiment 29, wherein the scaffold sequence comprises a stem-loop structure.

Embodiment 31

[0442] The composition of embodiment 29 or 30, wherein the scaffold sequence comprises or consists of 90 nucleotides.

Embodiment 32

[0443] The composition of embodiment 30 or 31, wherein the scaffold sequence comprises or consists of 93 nucleotides.

Embodiment 33

[0444] The composition of embodiment 32, wherein the scaffold sequence comprises the sequence

(SEQ ID NO: 82)
GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUC

CGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU
or

(SEQ ID NO: 83)
GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAC

UUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU.

Embodiment 34

[0445] The composition of any one of embodiments 1-33, wherein the gRNA does not bind or does not selectively bind to a second sequence within the RNA molecule.

Embodiment 35

[0446] The composition of embodiment 34, wherein an RNA genome or an RNA transcriptome comprises the RNA molecule.

Embodiment 36

[0447] The composition of any one of embodiments 1-35, wherein the first RNA binding protein comprises a CRISPR-Cas protein.

Embodiment 37

[0448] The composition of embodiment 36, wherein the CRISPR-Cas protein is a Type II CRISPR-Cas protein.

Embodiment 38

[0449] The composition of embodiment 37, wherein the first RNA binding protein comprises a Cas9 polypeptide or an RNA-binding portion thereof.

Embodiment 39

[0450] The composition of embodiment 36, wherein the CRISPR-Cas protein is a Type V CRISPR-Cas protein.

Embodiment 40

[0451] The composition of embodiment 39, wherein the first RNA binding protein comprises a Cpf1 polypeptide or an RNA-binding portion thereof.

Embodiment 41

[0452] The composition of embodiment 36, wherein the CRISPR-Cas protein is a Type VI CRISPR-Cas protein.

Embodiment 42

[0453] The composition of embodiment 41, wherein the first RNA binding protein comprises a Cas13 polypeptide or an RNA-binding portion thereof.

Embodiment 43

[0454] The composition of any one of embodiments 36-42, wherein the CRISPR-Cas protein comprises a native RNA nuclease activity.

Embodiment 44

[0455] The composition of embodiment 43, wherein the native RNA nuclease activity is reduced or inhibited.

Embodiment 45

[0456] The composition of embodiment 43, wherein the native RNA nuclease activity is increased or induced.

Embodiment 46

[0457] The composition of any one of embodiments 36-45, wherein the CRISPR-Cas protein comprises a native DNA nuclease activity and wherein the native DNA nuclease activity is inhibited.

Embodiment 47

[0458] The composition of embodiment 46, wherein the CRISPR-Cas protein comprises a mutation.

Embodiment 48

[0459] The composition of embodiment 47, wherein a nuclease domain of the CRISPR-Cas protein comprises the mutation.

Embodiment 49

[0460] The composition of embodiment 47, wherein the mutation occurs in a nucleic acid encoding the CRISPR-Cas protein.

Embodiment 50

[0461] The composition of embodiment 47, wherein the mutation occurs in an amino acid encoding the CRISPR-Cas protein.

Embodiment 51

[0462] The composition of any one of embodiments 47-50, wherein the mutation comprises a substitution, an insertion, a deletion, a frameshift, an inversion, or a transposition.

Embodiment 52

[0463] The composition of any one of embodiments 47-50, 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 53

[0464] The composition of any one of embodiments 1-35, wherein the first RNA binding protein comprises a *Pumilio* and FBF (PUF) protein.

Embodiment 54

[0465] The composition of embodiment 53, wherein the first RNA binding protein comprises a *Pumilio*-based assembly (PUMBY) protein.

Embodiment 55

[0466] The composition of any one of embodiments 1-54, wherein the first RNA binding protein does not require multimerization for RNA-binding activity.

Embodiment 56

[0467] The composition of embodiment 55, wherein the first RNA binding protein is not a monomer of a multimer complex Embodiment 57. The composition of embodiment 55, wherein a multimer protein complex does not comprise the first RNA binding protein.

Embodiment 58

[0468] The composition of any one of embodiments 1-57, wherein the first RNA binding protein selectively binds to a target sequence within the RNA molecule.

Embodiment 59

[0469] The composition of embodiment 58, wherein the first RNA binding protein does not comprise an affinity for a second sequence within the RNA molecule.

Embodiment 60

[0470] The composition of embodiment 58 or 59, wherein the first RNA binding protein does not comprise a high affinity for or selectively bind a second sequence within the RNA molecule.

Embodiment 61

[0471] The composition of embodiment 60, wherein an RNA genome or an RNA transcriptome comprises the RNA molecule.

Embodiment 62

[0472] The composition of any one of embodiments 1-61, wherein the first RNA binding protein comprises between 2 and 1300 amino acids, inclusive of the endpoints.

Embodiment 63

[0473] The composition of any one of embodiments 1-62, wherein the sequence encoding the first RNA binding protein further comprises a sequence encoding a nuclear localization signal (NLS).

Embodiment 64

[0474] The composition of embodiment 63, wherein the sequence encoding a nuclear localization signal (NLS) is positioned 3' to the sequence encoding the first RNA binding protein.

Embodiment 65

[0475] The composition of embodiment 64, wherein the first RNA binding protein comprises an NLS at a C-terminus of the protein.

Embodiment 66

[0476] The composition of any one of embodiments 1-62, 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 67

[0477] The composition of embodiment 66, wherein the sequence encoding the first NLS or the second NLS is positioned 3' to the sequence encoding the first RNA binding protein.

Embodiment 68

[0478] The composition of embodiment 67, wherein the first RNA binding protein comprises the first NLS or the second NLS at a C-terminus of the protein.

Embodiment 69

[0479] The composition of any one of embodiments 1-68, wherein the second RNA binding protein comprises or consists of a nuclease domain.

Embodiment 70

[0480] The composition of embodiment 69, wherein the sequence encoding the second RNA binding protein comprises or consists of an RNAse.

Embodiment 71

[0481] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAse1.

Embodiment 72

[0482] The composition of embodiment 71, wherein the RNAse1 protein comprises or consists of SEQ ID NO: 20.

Embodiment 73

[0483] The composition of embodiment 72, wherein the second RNA binding protein comprises or consists of an RNAse4.

Embodiment 74

[0484] The composition of embodiment 73, wherein the RNAse4 protein comprises or consists of: (SEQ ID NO: 21.

Embodiment 75

[0485] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAse6.

Embodiment 76

[0486] The composition of embodiment 75, wherein the RNAse6 protein comprises or consists of SEQ ID NO: 22.

Embodiment 77

[0487] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAse7.

Embodiment 78

[0488] The composition of embodiment 77, wherein the RNAse7 protein comprises or consists of SEQ ID NO: 23.

Embodiment 79

[0489] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAse8.

Embodiment 80

[0490] The composition of embodiment 79, wherein the RNAse8 protein comprises or consists of SEQ ID NO: 24.

Embodiment 81

[0491] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAse2.

Embodiment 82

[0492] The composition of embodiment 81, wherein the RNAse2 protein comprises or consists of SEQ ID NO: 25.

Embodiment 83

[0493] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAse6PL.

Embodiment 84

[0494] The composition of embodiment 83, wherein the RNAse6PL protein comprises or consists of SEQ ID NO: 26.

Embodiment 85

[0495] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAseL.

Embodiment 86

[0496] The composition of embodiment 85, wherein the RNAseL protein comprises or consists of SEQ ID NO: 27.

Embodiment 87

[0497] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAseT2.

Embodiment 88

[0498] The composition of embodiment 87, wherein the RNAseT2 protein comprises or consists of SEQ ID NO: 28.

Embodiment 89

[0499] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAse11.

Embodiment 90

[0500] The composition of embodiment 89, wherein the RNAse11 comprises or consists of SEQ ID NO: 29.

Embodiment 91

[0501] The composition of embodiment 70, wherein the second RNA binding protein comprises or consists of an RNAseT2-like.

Embodiment 92

[0502] The composition of embodiment 91, wherein the RNAseT2-like protein comprises or consists of SEQ ID NO: 30.

Embodiment 93

[0503] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a NOB 1 polypeptide.

Embodiment 94

[0504] The composition of embodiment 93, wherein the NOB 1 polypeptide comprises or consists of SEQ ID NO: 31.

Embodiment 95

[0505] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an endonuclease.

Embodiment 96

[0506] The composition of embodiment 95, wherein the second RNA binding protein comprises or consists of an endonuclease V (ENDOV).

Embodiment 97

[0507] The composition of embodiment 96, wherein the ENDOV protein comprises or consists of SEQ ID NO: 32.

Embodiment 98

[0508] The composition of embodiment 95, wherein the second RNA binding protein comprises or consists of an endonuclease G (ENDOG).

Embodiment 99

[0509] The composition of embodiment 98, wherein the ENDOG protein comprises or consists of SEQ ID NO: 33.

Embodiment 100

[0510] The composition of embodiment 95, wherein the second RNA binding protein comprises or consists of an endonuclease D1 (ENDOD1).

Embodiment 101

[0511] The composition of embodiment 100, wherein the ENDOD1 protein comprises or consists of SEQ ID NO: 34.

Embodiment 102

[0512] The composition of embodiment 95, wherein the second RNA binding protein comprises or consists of a Human flap endonuclease-1 (hFEN1).

Embodiment 103

[0513] The composition of embodiment 102, wherein the hFEN1 protein comprises or consists of SEQ ID NO: 35.

Embodiment 104

[0514] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a human Schlafen 14 (hSLFN14) polypeptide.

Embodiment 105

[0515] The composition of embodiment 104, wherein the hSLFN14 polypeptide comprises or consists of SEQ ID NO: 36.

Embodiment 106

[0516] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a human beta-lactamase-like protein 2 (hLACTB2) polypeptide.

Embodiment 107

[0517] The composition of embodiment 106, wherein the hLACTB2 polypeptide comprises or consists of SEQ ID NO: 37.

Embodiment 108

[0518] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an apurinic/apyrimidinic (AP) endodeoxyribonuclease (APEX2) polypeptide.

Embodiment 109

[0519] The composition of embodiment 108, wherein the APEX2 polypeptide comprises or consists of SEQ ID NO: 38.

Embodiment 110

[0520] The composition of embodiment 108, wherein the APEX2 polypeptide comprises or consists of: SEQ ID NO: 39.

Embodiment 111

[0521] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an angiogenin (ANG) polypeptide.

Embodiment 112

[0522] The composition of embodiment 111, wherein the ANG polypeptide comprises or consists of SEQ ID NO: 40.

Embodiment 113

[0523] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a heat responsive protein 12 (HRSP12) polypeptide.

Embodiment 114

[0524] The composition of embodiment 113, wherein the HRSP12 polypeptide comprises or consists of SEQ ID NO: 41.

Embodiment 115

[0525] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12A (ZC3H12A) polypeptide.

Embodiment 116

[0526] The composition of embodiment 115, wherein the ZC3H12A polypeptide comprises or consists of SEQ ID NO: 42.

Embodiment 117

[0527] The composition of embodiment 115, wherein the ZC3H12A polypeptide comprises or consists of SEQ ID NO: 43.

Embodiment 118

[0528] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Reactive Intermediate Imine Deaminase A (RIDA) polypeptide.

Embodiment 119

[0529] The composition of embodiment 118, wherein the RIDA polypeptide comprises or consists of SEQ ID NO: 44.

Embodiment 120

[0530] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Phospholipase D Family Member 6 (PDL6) polypeptide.

Embodiment 121

[0531] The composition of embodiment 120, wherein the PDL6 polypeptide comprises or consists of: (SEQ ID NO: 126.

Embodiment 122

[0532] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Endonuclease III-like protein 1 (NTHL) polypeptide.

Embodiment 123

[0533] The composition of embodiment 122, wherein the NTHL polypeptide comprises or consists of SEQ ID NO: 123.

Embodiment 124

[0534] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Mitochondrial ribonuclease P catalytic subunit (KIAA0391) polypeptide.

Embodiment 125

[0535] The composition of embodiment 124, wherein the KIAA0391 polypeptide comprises or consists of SEQ ID NO: 127.

Embodiment 126

[0536] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an apurinic or apyrimidinic site lyase (APEX1) polypeptide.

Embodiment 127

[0537] The composition of embodiment 126, wherein the APEX1 polypeptide comprises or consists of SEQ ID NO: 125.

Embodiment 128

[0538] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an argonaute 2 (AGO2) polypeptide.

Embodiment 129

[0539] The composition of embodiment 128, wherein the AGO2 polypeptide comprises or consists of SEQ ID NO: 128.

Embodiment 130

[0540] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mitochondrial nuclease EXOG (EXOG) polypeptide.

Embodiment 131

[0541] The composition of embodiment 130, wherein the EXOG polypeptide comprises or consists of SEQ ID NO: 129.

Embodiment 132

[0542] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Zinc Finger CCCH-Type Containing 12D (ZC3H12D) polypeptide.

Embodiment 133

[0543] The composition of embodiment 132, wherein the ZC3H12D polypeptide comprises or consists of SEQ ID NO: 130.

Embodiment 134

[0544] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an endoplasmic reticulum to nucleus signaling 2 (ERN2) polypeptide.

Embodiment 135

[0545] The composition of embodiment 134, wherein the ERN2 polypeptide comprises or consists of SEQ ID NO: 131.

Embodiment 136

[0546] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a pelota mRNA surveillance and ribosome rescue factor (PELO) polypeptide.

Embodiment 137

[0547] The composition of embodiment 136, wherein the PELO polypeptide comprises or consists of SEQ ID NO: 132.

Embodiment 138

[0548] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a YBEY metallopeptidase (YBEY) polypeptide.

Embodiment 139

[0549] The composition of embodiment 138, wherein the YBEY polypeptide comprises or consists of SEQ ID NO: 133.

Embodiment 140

[0550] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a cleavage and polyadenylation specific factor 4 like (CPSF4L) polypeptide.

Embodiment 141

[0551] The composition of embodiment 140, wherein the CPSF4L comprises or consists of SEQ ID NO: 134.

Embodiment 142

[0552] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an hCG_200273 ipolypeptide.

Embodiment 143

[0553] The composition of embodiment 142, wherein the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 135.

Embodiment 144

[0554] The composition of embodiment 142, wherein the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 136.

Embodiment 145

[0555] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of an Excision Repair Cross-Complementation Group 1 (ERCC1) polypeptide.

Embodiment 146

[0556] The composition of embodiment 145, wherein the ERCC1 polypeptide comprises or consists of SEQ ID NO: 137.

Embodiment 147

[0557] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a ras-related C3 botulinum toxin substrate 1 isoform (RAC1) polypeptide.

Embodiment 148

[0558] The composition of embodiment 147, wherein the RAC1 polypeptide comprises or consists of SEQ ID NO: 138.

Embodiment 149

[0559] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Ribonuclease A A1 (RAA1) polypeptide.

Embodiment 150

[0560] The composition of embodiment 149, wherein the RAA1 polypeptide comprises or consists of SEQ ID NO: 139.

Embodiment 151

[0561] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a Ras Related Protein (RAB1) polypeptide.

Embodiment 152

[0562] The composition of embodiment 151, wherein the RAB1 polypeptide comprises or consists of SEQ ID NO: 140.

Embodiment 153

[0563] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a DNA Replication Helicase/Nuclease 2 (DNA2) polypeptide.

Embodiment 154

[0564] The composition of embodiment 153, wherein the DNA2 polypeptide comprises or consists of SEQ ID NO: 141.

Embodiment 155

[0565] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a FLJ35220 polypeptide.

Embodiment 156

[0566] The composition of embodiment 155, wherein the FLJ35220 polypeptide comprises or consists of SEQ ID NO: 142.

Embodiment 157

[0567] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a FLJ13173 polypeptide.

Embodiment 158

[0568] The composition of embodiment 157, wherein the FLJ13173 polypeptide comprises or consists of: (SEQ ID NO: 143.

Embodiment 159

[0569] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a DNA repair endonuclease XPF (ERCC4) polypeptide.

Embodiment 160

[0570] The composition of embodiment 159, wherein the ERCC4 polypeptide comprises or consists of SEQ ID NO: 64.

Embodiment 161

[0571] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R)) polypeptide.

Embodiment 162

[0572] The composition of embodiment 161, wherein the Rnase1(K41R) polypeptide comprises or consists of SEQ ID NO: 116.

Embodiment 163

[0573] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E)) polypeptide.

Embodiment 164

[0574] The composition of embodiment 163, wherein the Rnase1 (Rnase1(K41R, D121E)) polypeptide comprises or consists of SEQ ID NO: 117.

Embodiment 165

[0575] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide.

Embodiment 166

[0576] The composition of embodiment 165, wherein the Rnase1 (Rnase1(K41R, D121E, H119N)) polypeptide comprises or consists of SEQ ID NO: 118.

Embodiment 167

[0577] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(H119N)) polypeptide.

Embodiment 168

[0578] The composition of embodiment 167, wherein the Rnase1 (Rnase1(H119N)) polypeptide comprises or consists of SEQ ID NO: 119.

Embodiment 169

[0579] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide.

Embodiment 170

[0580] The composition of embodiment 169, wherein the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide comprises or consists of SEQ ID NO: 120.

Embodiment 171

[0581] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide.

Embodiment 172

[0582] The composition of embodiment 171, wherein the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E)) polypeptide comprises or consists of SEQ ID NO: 121.

Embodiment 173

[0583] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide.

Embodiment 174

[0584] The composition of embodiment 173, wherein the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D)) polypeptide comprises or consists of SEQ ID NO: 122.

Embodiment 175

[0585] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of Teneurin Transmembrane Protein 1 (TENM1) polypeptide.

Embodiment 176

[0586] The composition of embodiment 175, wherein the TENM1 polypeptide comprises or consists of SEQ ID NO: 144.

Embodiment 177

[0587] The composition of embodiment 69, wherein the second RNA binding protein comprises or consists of Teneurin Transmembrane Protein 2 (TENM2) polypeptide.

Embodiment 178

[0588] The composition of embodiment 177, wherein the TENM2 polypeptide comprises or consists of SEQ ID NO: 145.

Embodiment 179

[0589] A composition comprising 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-biding polypeptide binds a target RNA not guided by a gRNA sequence, and wherein the second RNA-binding polypeptide comprises RNA-nuclease activity.

Embodiment 180

[0590] The composition of embodiment 179, wherein the first RNA-binding polypeptide or portion thereof is a PUF, PUMBY, or PPR polypeptide or portion thereof.

Embodiment 181

[0591] A method for modifying the level of expression of an RNA molecule or a protein encoded by the RNA molecule, the method comprising contacting the composition of embodiments 1 or 179 and the RNA molecule under conditions suitable for binding of the fusion protein or a portion thereof to the RNA molecule.

EXAMPLES

Example 1: Methods

[0592] HEK-293 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 0.5×10^5 cells per well of a 96-well plate for luciferase assays. 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 primers in a sequence adjacent to the CTG repeat in the reporter plasmid using the following primers:

Forward Primer
TetCTG_DMPK_E15_F
SEQ ID NO: 83
TCGGAGCGGTTGTGAACT

-continued
Reverse Primer
TetCTG_DMPK_E15_R
SEQ ID NO: 84
GTTCGCCGTTGTTCTGTC

[0593] Relative abundance of the CTG repeat reporter was determined by normalization to GAPDH. Next, levels of the CTG-targeting sgRNA were normalized to a non-targeting sgRNA to generate a final value reported in the associated data package.

CTG-targeting spacer
SEQ ID NO: 85
AGCAGCAGCAGCAGCAG

Non-targeting control spacer (λ2)
SEQ ID NO: 86
GTGATAAGTGGAATGCCATG

sgRNA scaffold (N's indicate spacer)
SEQ ID NO: 87
GNNNNNNNNNNNNNNNNNNNGUUUAAGAGCUAUGCUGGAAACAGCAUAG

CAAGUUUAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAG

UCGGUGCUUUUUUU

[0594] Luciferase assays were conducted with the Promega Dual Luciferase kit according to manufacturer's directions. Reported values are a ratio of firefly and *renilla* luciferase luminescence readings.

Example 2: RNA-Guided Cleavage of Repetitive RNA Molecules and mRNA Molecules

[0595] Experimental Design: Various fusions of human proteins with annotated RNA endonuclease activity and Cas9 (*Streptococcus pyogenes* or *Campylobacter jejuni*) were constructed. Plasmids encoding the above fusions were co-transfected with either a repeat-containing plasmid or a luciferase assay plasmid (comprising an mRNA sequence encoding a luciferase protein). A level of CTG repeat-containing RNA was measured with qPCR in the condition in which an RNA endonuclease/Cas9 fusion was co-transfected with a repetitive RNA. A level of luciferase protein was measured using a luminescence assay in the condition in which an RNA endonuclease/Cas9 fusion was co-transfected with a luciferase assay plasmid. All measurements were normalized to a non-targeting sgRNA control construct (FIGS. **3**A-**5** and FIG. **9**).

Example 3: RNA-Guided Cleavage of Viral RNA Molecules

[0596] 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 0.5×10^5 cells per well. Cells were transfected with plasmids encoding *Campylobacter jejuni* Cas9 (CjeCas9) fused to the gene NTHL 1 (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 1. The sequences of the constructs used in this study are presented below.

[0597] 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 2.

[0598] FIG. **7** 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 2. Zika NS5 expression is displayed as fold change relative to the endonuclease loaded with an sgRNA containing a control (Lambda) spacer sequence.

[0599] Immunofluorescence microscopy was used to visualize Zika NS5 expression in the presence of E43 or E67 endonucleases fused to CjeCas9. FIG. **8**A 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 CjeCas9-endonuclease fusions loaded with a non-Zika NS5 targeting sgRNA (FIGS. **8**A and **8**B). FIG. **6** is a list of exemplary endonucleases for use in the compositions of the disclosure.

TABLE 1

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)

TABLE 2

sgRNA sequences		
NS5-targeting spacer 1	gcaatgatcttcatgttgggagc	(SEQ ID NO: 196)
NS5-targeting spacer 2	gaaccttgttgatgaactcttc	(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)	GNNNNNNNNNNNNNNNNNNNGUUUAAGAGCUAUG CUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGU CCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGU GCUUUUUUU	(SEQ ID NO: 201)

-continued

tttcgatttcttggctttatatatcttGTGGAAAGGACGAAACACCNNNN

NNNNNNNNNNNNNNNGTTTTAGTCCCTGAAGGGACTAAAATAAAGAGTTT

GCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTTTTCCTGCAG

CCCGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCA

GCTTTTGTTCCCTTTAGTGAGGGTTAATTGCGCGAATTCGCTAGCTAGGT

CTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCA

CATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGATCC

GGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA

CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAG

TAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAG

GACCGGTTCTAGAGCGCTATTTAGAACCatg**TGTTCTCCCCAAGAATCTG**

GCATGACCGCTCTTTCAGCGAGGATGTTGACGCGAAGCAGATCCCTGGGA

CCTGGGGCCGGGCCACGAGGGTGTCGGGAAGAACCAGGACCGTTGCGACG

GAGGGAAGCAGCAGCGGAAGCTCGGAAATCCCATTCTCCGGTTAAACGAC

CCCGCAAGGCACAACGGCTCAGGGTTGCTTACGAGGGGAGCGATTCCGAA

AAGGGTGAAGGAGCAGAGCCCTTGAAGGTTCCAGTATGGGAACCCCAGGA

TTGGCAGCAGCAGCTTGTAAACATCCGAGCAATGAGGAACAAAAAAGATG

CACCTGTTGATCACCTCGGAACCGAACATTGTTATGATTCTAGTGCGCCG

CCAAAAGTCCGCCGGTATCAGGTTCTGTTGAGTTTGATGCTGAGTAGTCA

GACTAAGGACCAGGTTACGGCCGGAGCAATGCAACGGCTTCGGGCACGGG

(SEQ ID NO: 202)

gtttattacagggacagcagagatccagtttggttaattaaggtaccgag

ggcctatttcccatgattccttcatatttgcatatacgatacaaggctgt

tagagagataattagaattaatttgactgtaaacacaaagatattagtac

aaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagtttta

aaattatgttttaaaatggactatcatatgcttaccgtaacttgaaagta

-continued

GACTCACGGTCGATAGCATTTTGCAGACCGATGACGCAACATTGGGTAAA

CTCATATATCCAGTTGGCTTCTGGCGGAGCAAAGTGAAGTACATCAAGCA

GACCTCAGCCATTCTCCAACAACATTACGGAGGTGATATACCCGCAAGCG

TAGCTGAACTGGTAGCACTGCCGGGCGTCGGTCCCAAAATGGCACATCTG

GCTATGGCGGTTGCTTGGGGAACGGTGTCTGGTATCGCAGTTGATACGCA

TGTCCACCGCATCGCCAATCGGCTGAGGTGGACTAAAAAAGCCACTAAGT

-continued

CTCCTGAAGAAACACGGGCTGCTCTGGAAGAGTGGCTTCCACGAGAGCTG

TGGCATGAAATCAATGGATTGCTGGTTGGTTTCGGGCAGCAGACATGCTT

GCCCGTGCACCCCCGGTGTCATGCTTGCTTGAACCAGGCTTTGTGCCCAG

CTGCCCAGGGCCTGAGTGGAAGTGAGACACCGGGAACATCTGAGTCTGCG

ACCCCGGAGAGCacaaacGCGCGAATCCTGGCCTTCGcgATTGGCATTAG

CAGCATCGGCTGGGCATTCTCTGAAAACGACGAACTGAAGGATTGCGGCG

TGCGAATTTTCACTAAGGTCGAAAATCCCAAAACTGGTGAATCACTCGCT

CTCCCTAGACGACTGGCACGCTCCGCACGAAAGAGGCTTGCCCGCCGCAA

GGCACGCTTGAACCATCTTAAACACCTTATTGCAAATGAGTTTAAACTGA

ATTATGAGGACTACCAATCCTTTGACGAGTCTCTTGCTAAAGCCTACAAA

GGGAGCCTTATATCCCCGTATGAGCTCCGGTTCAGAGCACTCAACGAACT

GCTGTCCAAACAGGATTTTGCTCGCGTGATTCTCCACATAGCGAAGAGGC

GAGGATACGATGACATTAAAAACAGTGATGATAAGGAAAAAGGGGCCATA

CTCAAAGCGATTAAGCAAAATGAAGAGAAGCTCGCTAACTATCAATCAGT

AGGGGAGTATCTCTATAAAGAGTACTTCCAGAAGTTCAAAGAAAATAGCA

AGGAATTTACTAATGTCCGGAATAAAAAGGAGTCTTACGAAAGATGTATT

GCGCAATCTTTCCTCAAGGACGAGCTCAAATTGATTTTCAAGAAACAAAG

GGAATTTGGGTTCAGCTTCTCAAAAAAATTTGAGGAAGAGGTTCTGAGCG

TTGCCTTTTACAAACGCGCCCTTAAGGACTTCTCACATCTCGTAGGGAAT

TGTAGTTTCTTCACCGATGAAAAACGGGCGCCAAAAAATAGCCCTTTGGC

TTTTATGTTTGTCGCTCTGACTCGCATCATTAATCTGCTCAACAACCTTA

AAAACACGGAAGGGATTCTGTACACAAAGGATGATCTGAACGCTCTGCTT

AACGAAGTTTTGAAGAACGGGACTTTGACCTACAAACAAACCAAAAAGCT

TCTTGGTCTCAGTGATGACTACGAATTCAAGGGAGAAAAAGGGACATATT

TCATCGAATTCAAGAAGTATAAGGAGTTCATCAAAGCCTTGGGCGAGCAC

AACTTGTCTCAAGATGATCTCAACGAAATTGCTAAGGATATCACTCTGAT

TAAAGACGAGATCAAGCTCAAAAAGGCGTTGGCGAAGTATGACCTTAACC

AAAACCAAATAGATAGCCTCAGCAAGTTGGAATTTAAAGATCACTTGAAT

ATAAGTTTCAAGGCCCTTAAGTTGGTCACCCCCTTGATGCTTGAAGGAAA

GAAATATGATGAGGCATGTAATGAGCTGAATCTCAAGGTTGCTATTAACG

AAGACAAAAAAGATTTCCTCCCAGCTTTCAATGAGACTTACTATAAGGAC

GAGGTTACCAATCCTGTGGTGCTCCGAGCCATCAAAGAGTATCGAAAGGT

CCTGAATGCTTTGCTCAAAAAATACGGTAAGGTACACAAAATAAATATTG

AGCTCGCAAGGGAGGTCGGTAAGAACCACTCCCAGCGCGCCAAAATAGAA

AAGGAACAGAATGAAAATTACAAAGCGAAAAAGGACGCCGAGCTCGAGTG

CGAAAAGCTGGGCCTGAAAATAAACAGCAAGAACATTCTCAAACTCCGCC

TCTTCAAAGAACAAAAAGAATTTTGTGCTTATAGTGGTGAGAAAATAAAA

ATCTCCGATCTTCAAGACGAGAAGATGCTCGAAATAGACgcgATATATCC

ATATAGCAGGTCTTTTGACGATTCTTACATGAATAAAGTGCTTGTTTTCA

-continued

CTAAGCAGAATCAGGAAAAGTTGAATCAGACCCCCTTTGAGGCCTTTGGC

AACGACTCAGCAAAGTGGCAGAAGATCGAGGTCTTGGCTAAGAATCTTCC

TACTAAGAAACAGAAAAGGATATTGGATAAGAACTATAAAGACAAAGAAC

AAAAGAACTTTAAAGACCGCAACCTCAATGACACCAGATACATAGCAAGA

TTGGTTCTGAACTACACAAAAGATTATTTGGACTTCTTGCCGCTGTCTGA

TGATGAGAACACGAAACTCAACGACACGCAAAAGGGGTCTAAAGTCCACG

TCGAAGCTAAATCTGGGATGCTCACCTCAGCATTGAGGCATACGTGGGGA

TTCTCAGCAAAGGACCGAAACAATCACCTGCACCATGCCATTGACGCAGT

TATCATAGCGTATGCCAATAATTCAATAGTAAAAGCGTTTAGCGACTTCA

AGAAGGAACAAGAGTCCAACAGCGCCGAGCTCTACGCAAAAAAGATTAGT

GAACTCGACTACAAAAACAAAAGAAAATTCTTTGAGCCGTTCAGCGGATT

TCGACAGAAGGTATTGGATAAAATAGATGAAATTTTCGTGAGCAAACCCG

AAAGGAAAAAGCCCTCAGGCGCCTTGCACGAAGAGACTTTCAGGAAGGAA

GAGGAATTCTACCAAAGCTACGGCGGAAAAGAGGGAGTTTTGAAGGCTCT

CGAACTTGGAAAGATTAGGAAGGTGAACGGCAAGATAGTGAAAAACGGCG

ATATGTTCCGGGTTGATATCTTCAAACATAAAAAAACGAATAAATTTTAT

GCTGTGCCTATATACACTATGGACTTCGCACTTAAGGTCCTGCCGAATAA

GGCGGTAGCCCGATCTAAAAAAGGCGAAATTAAGGACTGGATTTTGATGG

ATGAAAATTACGAGTTCTGCTTTTCTCTCTACAAGGATTCCCTTATATTG

ATACAGACGAAAGATATGCAGGAACCGGAATTCGTGTATTACAACGCTTT

TACTTCCTCTACGGTATCTTTGATTGTCTCCAAACATGACAACAAATTCG

AAACACTCAGTAAAAACCAAAAGATTCTCTTTAAAAATGCGAACGAGAAA

GAAGTAATTGCAAAATCAATTGGCATCCAAAATTTGAAAGTTTTTGAAAA

ATATATAGTATCTGCCCTCGGAGAGGTTACTAAAGCGGAATTTAGACAGC

GAGAGGACTTCAAAAAATCAGGTCCACCCAAGAAAAAACGCAAGGTGGAA

GATCCGAAGAAAAAGCGAAAAGTGGATGTGtaaCGTTTTCCGGGACGCCG

GCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCAC

CCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAGCAT

CACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTT

TGTCCAAACTCATCAATGTATCTTATCATGTCTGTATACCG.

[0600] A E67-CjeCas9 and sgRNA plasmid may comprise or consist of the sequence (U6: N's=sgRNA spacer, E67, CieCas9):

(SEQ ID NO: 203)

gtttattacagggacagcagagatccagtttggttaattaaggtaccgag

ggcctatttcccatgattccttcatatttgcatatacgatacaaggctgt

tagagagataattagaattaatttgactgtaaacacaaagatattagtac

aaaatacgtgacgtagaaagtaataatttcttgggtagtttgcagtttta

aaattatgttttaaaatggactatcatatgcttaccgtaacttgaaagta

-continued

tttcgatttcttggctttatatatcttGTGGAAAGGACGAAACACCNNNN

NNNNNNNNNNNNNNNGTTTTAGTCCCTGAAGGGACTAAAATAAAGAGTTT

GCGGGACTCTGCGGGGTTACAATCCCCTAAAACCGCTTTTTTTCCTGCAG

CCCGGGGGGATCCACTAGTTCTAGAGCGGCCGCCACCGCGGTGGAGCTCCA

GCTTTTGTTCCCTTTAGTGAGGGTTAATTGCGCGAATTCGCTAGCTAGGT

CTTGAAAGGAGTGGGAATTGGCTCCGGTGCCCGTCAGTGGGCAGAGCGCA

CATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGATCC

GGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTA

CTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAG

TAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAG

GACCGGTTCTAGAGCGCTATTTAGAACCatg**CAGGAGGTAATAGCGGGGC**

TTGAGCGATTTACCTTTGCCTTCGAAAAAGACGTAGAGATGCAGAAGGGA

ACCGGCCTGCTCCCATTTCAAGGTATGGACAAATCAGCATCTGCCGTGTG

CAATTTTTTCACCAAGGGTCTGTGTGAAAAGGGGAAGCTCTGTCCATTTC

GCCATGATCGCGGAGAGAAGATGGTGGTGTGTAAGCACTGGCTGAGAGGG

CTTTGCAAAAAAGGCGACCACTGCAAATTTCTTCACCAATATGACCTGAC

TCGAATGCCTGAGTGTTATTTTTACAGTAAGTTCGGTGACTGTAGCAACA

AAGAATGCAGCTTCTTGCATGTCAAACCAGCATTCAAGTCACAGGATTGC

CCGTGGTACGATCAGGGTTTTTGCAAGGACGGTCCCCTCTGCAAATATCG

ACACGTACCCAGAATTATGTGCCTTAATTACCTGGTCGGCTTCTGTCCTG

AAGGGCCAAAATGTCAGTTTGCTCAAAAAATTCGCGAGTTCAAATTGCTC

CCTGGGTCTAAAATTTGGGAACCCCAGGATTGGCAGCAGCAGCTTGTAAA

CATCCGAGCAATGAGGAACAAAAAAGATGCACCTGTTGATCACCTCGGAA

CCGAACATTGTTATGATTCTAGTGCGCCGCCAAAAGTCCGCCGGTATCAG

GTTCTGTTGAGTTTGATGCTGAGTAGTCAGACTAAGGACCAGGTTACGGC

CGGAGCAATGCAACGGCTTCGGGCACGGGGACTCACGGTCGATAGCATTT

TGCAGACCGATGACGCAACATTGGGTAAACTCATATATCCAGTTGGCTTC

TGGCGGAGCAAAGTGAAGTACATCAAGCAGACCTCAGCCATTCTCCAACA

ACATTACGGAGGTGATATACCCGCAAGCGTAGCTGAACTGGTAGCACTGC

CGGGCGTCGGTCCCAAAATGGCACATCTGGCTATGGCGGTTGCTTGGGGA

ACGGTGTCTGGTATCGCAGTTGATACGCATGTCCACCGCATCGCCAATCG

GCTGAGGTGGACTAAAAAAGCCACTAAGTCTCCTGAAGAAACACGGGCTG

CTCTGGAAGAGTGGCTTCCACGAGAGCTGTGGCATGAAATCAATGGATTG

CTGGTTGGTTTCGGGCAGCAGACATGCTTGCCCGTGCACCCCCGGTGTCA

TGCTTGCTTGAACCAGGCTTTGTGCCCAGCTGCCCAGGGCCTGAGTGGAA

GTGAGACACCGGGAACATCTGAGTCTGCGACCCCGGAGAGCacaaac**GCG**

CGAATCCTGGCCTTCGcgATTGGCATTAGCAGCATCGGCTGGGCATTCTC

TGAAAACGACGAACTGAAGGATTGCGGCGTGCGAATTTTCACTAAGGTCG

AAAATCCCAAAACTGGTGAATCACTCGCTCTCCCTAGACGACTGGCACGC

-continued

TCCGCACGAAAGAGGCTTGCCCGCCGCAAGGCACGCTTGAACCATCTTAA

ACACCTTATTGCAAATGAGTTTAAACTGAATTATGAGGACTACCAATCCT

TTGACGAGTCTCTTGCTAAAGCCTACAAAGGGAGCCTTATATCCCCGTAT

GAGCTCCGGTTCAGAGCACTCAACGAACTGCTGTCCAAACAGGATTTTGC

TCGCGTGATTCTCCACATAGCGAAGAGGCGAGGATACGATGACATTAAAA

ACAGTGATGATAAGGAAAAAGGGGCCATACTCAAAGCGATTAAGCAAAAT

GAAGAGAAGCTCGCTAACTATCAATCAGTAGGGGAGTATCTCTATAAAGA

GTACTTCCAGAAGTTCAAAGAAAATAGCAAGGAATTTACTAATGTCCGGA

ATAAAAAGGAGTCTTACGAAAGATGTATTGCGCAATCTTTCCTCAAGGAC

GAGCTCAAATTGATTTTCAAGAAACAAAGGGAATTTGGGTTCAGCTTCTC

AAAAAAATTTGAGGAAGAGGTTCTGAGCGTTGCCTTTTACAAACGCGCCC

TTAAGGACTTCTCACATCTCGTAGGGAATTGTAGTTTCTTCACCGATGAA

AAACGGGCGCCAAAAAATAGCCCTTTGGCTTTTATGTTTGTCGCTCTGAC

TCGCATCATTAATCTGCTCAACAACCTTAAAAACACGGAAGGGATTCTGT

ACACAAAGGATGATCTGAACGCTCTGCTTAACGAAGTTTTGAAGAACGGG

ACTTTGACCTACAAACAAACCAAAAAGCTTCTTGGTCTCAGTGATGACTA

CGAATTCAAGGGAGAAAAAGGGACATATTTCATCGAATTCAAGAAGTATA

AGGAGTTCATCAAAGCCTTGGGCGAGCACAACTTGTCTCAAGATGATCTC

AACGAAATTGCTAAGGATATCACTCTGATTAAAGACGAGATCAAGCTCAA

AAAGGCGTTGGCGAAGTATGACCTTAACCAAAACCAAATAGATAGCCTCA

GCAAGTTGGAATTTAAAGATCACTTGAATATAAGTTTCAAGGCCCTTAAG

TTGGTCACCCCCTTGATGCTTGAAGGAAAGAAATATGATGAGGCATGTAA

TGAGCTGAATCTCAAGGTTGCTATTAACGAAGACAAAAAAGATTTCCTCC

CAGCTTTCAATGAGACTTACTATAAGGACGAGGTTACCAATCCTGTGGTG

CTCCGAGCCATCAAAGAGTATCGAAAGGTCCTGAATGCTTTGCTCAAAAA

ATACGGTAAGGTACACAAAATAAATATTGAGCTCGCAAGGGAGGTCGGTA

AGAACCACTCCCAGCGCGCCAAAATAGAAAAGGAACAGAATGAAAATTAC

AAAGCGAAAAAGGACGCCGAGCTCGAGTGCGAAAAGCTGGGCCTGAAAAT

AAACAGCAAGAACATTCTCAAACTCCGCCTCTTCAAAGAACAAAAAGAAT

TTTGTGCTTATAGTGGTGAGAAAATAAAAATCTCCGATCTTCAAGACGAG

AAGATGCTCGAAATAGACgcgATATATCCATATAGCAGGTCTTTTGACGA

TTCTTACATGAATAAAGTGCTTGTTTTCACTAAGCAGAATCAGGAAAAGT

TGAATCAGACCCCCTTTGAGGCCTTTGGCAACGACTCAGCAAAGTGGCAG

AAGATCGAGGTCTTGGCTAAGAATCTTCCTACTAAGAAACAGAAAAGGAT

ATTGGATAAGAACTATAAAGACAAAGAACAAAAGAACTTTAAAGACCGCA

ACCTCAATGACACCAGATACATAGCAAGATTGGTTCTGAACTACACAAAA

GATTATTTGGACTTCTTGCCGCTGTCTGATGATGAGAACACGAAACTCAA

CGACACGCAAAAGGGGTCTAAAGTCCACGTCGAAGCTAAATCTGGGATGC

TCACCTCAGCATTGAGGCATACGTGGGGATTCTCAGCAAAGGACCGAAAC

-continued

AATCACCTGCACCATGCCATTGACGCAGTTATCATAGCGTATGCCAATAA

TTCAATAGTAAAAGCGTTTAGCGACTTCAAGAAGGAACAAGAGTCCAACA

GCGCCGAGCTCTACGCAAAAAAGATTAGTGAACTCGACTACAAAAACAAA

AGAAAATTCTTTGAGCCGTTCAGCGGATTTCGACAGAAGGTATTGGATAA

AATAGATGAAATTTTCGTGAGCAAACCCGAAAGGAAAAAGCCCTCAGGCG

CCTTGCACGAAGAGACTTTCAGGAAGGAAGAGGAATTCTACCAAAGCTAC

GGCGGAAAAGAGGGAGTTTTGAAGGCTCTCGAACTTGGAAAGATTAGGAA

GGTGAACGGCAAGATAGTGAAAAACGGCGATATGTTCCGGGTTGATATCT

TCAAACATAAAAAAACGAATAAATTTTATGCTGTGCCTATATACACTATG

GACTTCGCACTTAAGGTCCTGCCGAATAAGGCGGTAGCCCGATCTAAAAA

AGGCGAAATTAAGGACTGGATTTTGATGGATGAAAATTACGAGTTCTGCT

TTTCTCTCTACAAGGATTCCCTTATATTGATACAGACGAAAGATATGCAG

GAACCGGAATTCGTGTATTACAACGCTTTTACTTCCTCTACGGTATCTTT

GATTGTCTCCAAACATGACAACAAATTCGAAACACTCAGTAAAAACCAAA

AGATTCTCTTTAAAAATGCGAACGAGAAAGAAGTAATTGCAAAATCAATT

GGCATCCAAAATTTGAAAGTTTTTGAAAAATATATAGTATCTGCCCTCGG

AGAGGTTACTAAAGCGGAATTTAGACAGCGAGAGGACTTCAAAAAATCAG

GTCCACCCAAGAAAAAACGCAAGGTGGAAGATCCGAAGAAAAAGCGAAAA

-continued

GTGGATGTGtaaCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCG

GGGATCTCATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCT

TATAATGGTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGC

ATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTAT

CTTATCATGTCTGTATACCG.

INCORPORATION BY REFERENCE

[0601] 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 embodimented 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

[0602] 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.

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20200071718A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

1. A composition comprising a nucleic acid sequence encoding an RNA-guided target RNA-binding fusion protein comprising (a) a first RNA-binding polypeptide or portion thereof, and (b) a second RNA-binding polypeptide, wherein the first RNA-binding polypeptide binds a target RNA when guided by a gRNA sequence, and wherein the second RNA-binding polypeptide comprises RNA-nuclease activity.

2. The composition of claim **1**, wherein the first RNA-binding polypeptide or portion thereof is a CRISPR/Cas polypeptide or portion thereof.

3. The composition of claim **2**, 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.

4. The composition of claim **1**, wherein the second RNA-binding polypeptide binds RNA in a manner in which it associates with RNA.

5. The composition of claim **4**, wherein the second RNA-binding polypeptide associates with RNA in a manner in which it cleaves RNA.

6. The composition of claim **1**, wherein the nucleic acid sequence comprises a promoter.

7. The composition of claim **6**, wherein the promoter is a constitutive promoter or a tissue-specific promoter.

8. The composition of claim **1**, wherein the nucleic acid sequence further comprises a gRNA sequence, wherein the gRNA sequence comprises a spacer sequence that specifically binds a target sequence within an RNA molecule and a scaffold sequence that specifically binds to the first RNA-binding polypeptide.

9. The composition of claim **8**, wherein the spacer sequence comprises a 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.

10. The composition of claim **8**, wherein the nucleic acid sequence comprises a promoter which drives expression of the gRNA sequence.

11. The composition of claim **9**, wherein the promoter is a polymerase III promoter.

12. The composition of claim **10**, wherein the polymerase III promoter is a U6 promoter.

13. The composition of claim **1**, wherein the promoter is a tRNA promoter.

14. The composition of claim **1**, wherein the fusion protein comprises an NLS, NES or tag.

15. A vector comprising the composition of claim **1**.

16. The vector of claim **15**, wherein the vector is selected from the group consisting of: adeno-associated virus, retrovirus, lentivirus, adenovirus, nanoparticle, micelle, liposome, lipoplex, polymersome, polyplex, and dendrimer.

17. A cell comprising the vector of claim **15**.

18. The composition of claim **1**, 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, NOB 1, 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, and ZNF638.

19. A composition comprising:

(a) a guide RNA (gRNA) sequence comprising a spacer sequence that specifically binds a target sequence within an RNA molecule and a scaffold sequence that specifically binds to the first RNA-binding polypeptide;

(b) a nucleic acid sequence encoding a fusion protein, the fusion protein comprising 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.

20. A method for modifying the level of expression of a target RNA molecule or a protein encoded by the RNA molecule, the method comprising contacting the composition of claim **19** and the RNA molecule under conditions suitable for binding of the fusion protein or a portion thereof to the RNA molecule.

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