

195. The method of Claim 96, comprising introducing a nucleic acid of Claim 180 or a vector of Claim 181 into said sample under conditions in which said Cas9 polypeptide is expressed.

196. The method of Claim 194 or 195, further comprising introducing a single guide RNA.

197. The method of any one of Claims 194-196, further comprising introducing an antisense oligonucleotide.

198. The method of any one of Claims 194-197, wherein said sample comprises a tissue.

199. The method of any one of Claims 194-198, wherein said sample comprises a plurality of cells.

200. The method of any one of Claims 194-199, comprising modifying a target RNA in said sample.

201. The method of Claim 200, wherein said target RNA comprises a repeat sequence.

202. The method of Claim 201, wherein said repeat sequence is selected from the group consisting of CTG, CCTG, CAG, GGGGCC, and any combination thereof.

203. The method of Claim 201 or 202, wherein said repeat sequence is associated with a disease.

204. The method of Claim 203, wherein said disease is selected from the group consisting of myotonic dystrophy, Huntington's disease, familial ALS, cancer, spinal muscular atrophy and Fragile X syndrome.

[0006a] In one aspect, the present disclosure refers to an engineered nucleoprotein complex comprising: (a) a nuclease-inactive RNA-targeted Cas9 (RCas9) polypeptide, wherein the nuclease-inactive RCas9 polypeptide: (1) lacks all or part of an HNH domain, all or part of at least one RuvC nuclease domain, all or part of a Cas9 polypeptide DNase active site, all or part of a $\beta\beta\alpha$ -metal fold comprising a Cas9 polypeptide active site, or a combination thereof as compared to a corresponding wild type (WT) Cas9 polypeptide; (2) lacks DNase, DNA cleaving capability or activity, or nickase activity; and (3) lacks all or part of: an HNH and/or RuvC nuclease domain of Cas9, a Cas9 polypeptide

Dnase active site, a $\beta\beta\alpha$ -metal fold that comprising a Cas9 polypeptide active site, or combinations thereof; further wherein the nuclease-inactive Rcas9 polypeptide has a reduced polypeptide size as compared to a corresponding wild type (WT) Cas9 polypeptide that permits packaging of the RCas9-coding nucleotide in a delivery vector; and (b) a recombinant or synthetic single guide RNA (sgRNA) comprising: (1) on its 5' end, an RNA sequence that hybridizes to or binds to a target RNA sequence having at least 60% complementarity to the sgRNA 5' end, wherein the target RNA sequence comprises a repeat sequence, wherein the RNA sequence that hybridizes to or binds to the target RNA sequence is capable of hybridizing or binding to the repeat sequence, and wherein the repeat sequence is CUG, CCUG, CAG, GGGGCC, or any combination thereof; and (2) on its 3' end: (i) an RNA sequence capable of binding to or associating with the nuclease-inactive RCas9 polypeptide, or (ii) a linker that covalently links or non-covalently links the 5' RNA sequence of the sgRNA with the nuclease-inactive RCas9 polypeptide, wherein the RNA sequence capable of binding to or associating with the nuclease-inactive RCas9 polypeptide comprises or is derived from the wild type guide RNA of an archaeal or a bacterial organism from which RCas9 is derived; and wherein optionally the nucleoprotein complex does not comprise a PAMmer oligonucleotide.

[0006b] In another aspect, the present disclosure refers to a first nucleic acid encoding the nuclease-inactive RCas9 polypeptide as disclosed herein and a second nucleic acid encoding the sgRNA as disclosed herein.

[0006c] In another aspect, the present disclosure refers to a chimeric nucleic acid encoding the engineered nucleoprotein complex as disclosed herein.

[0006d] In another aspect, the present disclosure refers to a cell comprising: (a) the engineered nucleoprotein complex as disclosed herein; or a nucleic acid as disclosed herein, or nucleic acid as disclosed herein, wherein the cell is a mammalian cell or a human cell.

[0006e] In another aspect, the present disclosure refers to a pharmaceutical composition comprising: (a) the engineered nucleoprotein complex as disclosed herein or (b) a nucleic acids as disclosed herein, or the nucleic acid as disclosed herein, wherein optionally the pharmaceutical compound is formulated for enteral or parenteral delivery, or for intravenous (IV) delivery.

[0006f] In another aspect, the present disclosure refers to a use of the engineered nucleoprotein complex of the disclosure, the nucleic acids of the disclosure or the nucleic acid of the disclosure, the cell of the disclosure, or the pharmaceutical composition of the disclosure in the manufacture of a medicament for treating, preventing, ameliorating or reducing a symptom of a disease or condition, or an infection, in a mammalian or a human subject.

[0006g] In another aspect, the present disclosure refers to a method of expressing the engineered nucleoprotein complex as disclosed herein, comprising introducing the nucleic acids of the disclosure or the nucleic acid of the disclosure into a cell *in vitro* under conditions in which said cell expresses said nucleoprotein complex.

[0006H] IN ANOTHER ASPECT, THE PRESENT DISCLOSURE REFERS TO A VECTOR COMPRISING THE NUCLEIC ACIDS OF THE DISCLOSURE OR THE NUCLEIC ACID OF THE DISCLOSURE. BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1. *S. pyogenes* Cas9 and single guide RNA (sgRNA) complexes bound to DNA or RNA. A: The Cas9:sgRNA complex may require a DNA NGG motif referred to as the protospacer adjacent motif (PAM). In the case of DNA binding, the PAM is supplied by the DNA target itself. The mechanism of DNA targeting by Cas9 is described extensively (Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol.* 2014;32(4):347-55; Sternberg SH, Redding S, Jinek M, Greene

WHAT IS CLAIMED IS:

1. An engineered nucleoprotein complex comprising:

(a) a nuclease-inactive RNA-targeted Cas9 (RCas9) polypeptide, wherein the nuclease-inactive RCas9 polypeptide:

(1) lacks all or part of an HNH domain, all or part of at least one RuvC nuclease domain, all or part of a Cas9 polypeptide DNase active site, all or part of a $\beta\beta\alpha$ -metal fold comprising a Cas9 polypeptide active site, or a combination thereof as compared to a corresponding wild type (WT) Cas9 polypeptide;

(2) lacks DNase, DNA cleaving capability or activity, or nickase activity; and

(3) lacks all or part of: an HNH and/or RuvC nuclease domain of Cas9, a Cas9 polypeptide Dnase active site, a $\beta\beta\alpha$ -metal fold that comprising a Cas9 polypeptide active site, or combinations thereof; further wherein the nuclease-inactive Rcas9 polypeptide has a reduced polypeptide size as compared to a corresponding wild type (WT) Cas9 polypeptide that permits packaging of the RCas9-coding nucleotide in a delivery vector;

and

(b) a recombinant or synthetic single guide RNA (sgRNA) comprising:

(1) on its 5' end, an RNA sequence that hybridizes to or binds to a target RNA sequence having at least 60% complementarity to the sgRNA 5' end, wherein the target RNA sequence comprises a repeat sequence, wherein the RNA sequence that hybridizes to or binds to the target RNA sequence is capable of hybridizing or binding to the repeat sequence, and wherein the repeat sequence is CUG, CCUG, CAG, GGGGCC, or any combination thereof;

and

(2) on its 3' end:

(i) an RNA sequence capable of binding to or associating with the nuclease-inactive RCas9 polypeptide, or

(ii) a linker that covalently links or non-covalently links the 5' RNA sequence of the sgRNA with the nuclease-inactive RCas9 polypeptide,

wherein the RNA sequence capable of binding to or associating with the nuclease-inactive RCas9 polypeptide comprises or is derived from the wild type guide RNA of an archaeal or a bacterial organism from which RCas9 is derived;

and wherein the nucleoprotein complex does not comprise a PAMmer oligonucleotide.

2. The engineered nucleoprotein complex of claim 1, wherein the nuclease-inactive RCas9 polypeptide or the RNA sequence capable of binding to or associating with the nuclease-inactive RCas9 polypeptide of the sgRNA comprises a sequence isolated or derived from an archaeal or bacterial Cas9 polypeptide isolated or derived from selected from the groups consisting of *Haloferax mediteranii*, *Mycobacterium tuberculosis*, *Francisella tularensis* subsp. *novicida*, *Pasteurella multocida*, *Neisseria meningitidis*, *Campylobacter jejune*, *Streptococcus thermophilus* LMD-9 CRISPR 3, *Campylobacter lari* CF89-12, *Mycoplasma gallisepticum* str. F, *Nitratifractor salmuginis* str DSM 16511, *Parvibaculum lavamentivorans*, *Roseburia intestinalis*, *Neisseria cinerea*, *Gluconacetobacter diazotrophicus*, *Azospirillum* B510, *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 diphtheriae*, *Streptococcus aureus* and *Francisella novicida*

3. The engineered nucleoprotein complex of claim 1 or 2, wherein the 5' end of the sgRNA is between about 15 to 25 nucleotides in length, and wherein the RNA sequence capable of binding to or associating with the nuclease-inactive RCas9 polypeptide is between about 85 and 100 nucleotides in length.

4. The engineered nucleoprotein complex of any one of claims 1-3, wherein the nuclease-inactive RCas9 polypeptide is covalently or non-covalently linked to an effector polypeptide, a targeting agent, an enzyme, a detectable moiety or an RNA modifying polypeptide.

5. The engineered nucleoprotein complex of claims 4, wherein said RNA modifying polypeptide comprises a splicing factor or an RNA splicing domain, a RBFOX2 domain-

containing protein, a protein known to influence RNA splicing, or an RNA cleaving domain and/or a PIN domain-containing protein.

6. The engineered nucleoprotein complex of claim 5, wherein the RNA cleaving domain comprises an endonuclease.

7. The engineered nucleoprotein complex of any one of claims 1-6, wherein the RNA sequence capable of binding to or associating with the nuclease-inactive RCas9 polypeptide of said sgRNA comprises a secondary RNA structure.

8. The engineered nucleoprotein complex of any one of claims 1-7, wherein said sgRNA comprises one or more point mutations that remove a transcription termination sequence, or an alteration, an additional nucleotide or chemical moiety at the 5' end which stabilizes said single guide RNA against degradation.

9. The engineered nucleoprotein complex of any one of claims 1-8, wherein said sgRNA comprises a sequence that is complementary to at least a portion of the target RNA, wherein said portion is between about 5% and 20% of the target RNA.

10. The engineered nucleoprotein complex of any one of claims 1-9, wherein said engineered nucleoprotein complex is adapted to be delivered to the nucleus of a cell, and wherein said nuclease-inactive RCas9 polypeptide is covalently or non-covalently linked to one or more of a nuclear localization signal, one or more linker peptide(s), one or more XTEN peptide(s), or an SV40 nuclear localization signal.

11. The engineered nucleoprotein complex of any of claims 1-10, wherein said nuclease-inactive RCas9 polypeptide is associated with an effector polypeptide, a targeting agent, an enzyme and/or a detectable agent or moiety, wherein the nuclease-inactive RCas9 polypeptide is a recombinant or synthetic polypeptide.

12. A first nucleic acid encoding the nuclease-inactive RCas9 polypeptide as recited in any one of claims 1, 2, 4, 10 or 11 and a second nucleic acid encoding the sgRNA as recited in any one of claims 1, 3, 8 or 9.

13. A chimeric nucleic acid encoding the engineered nucleoprotein complex of any one of claims 1-11.

14. A cell comprising:

(a) the engineered nucleoprotein complex of any of claims 1-11; or

(b) a nucleic acid of claim 12, or nucleic acid of claim 13,

wherein the cell is a mammalian cell or a human cell.

15. A pharmaceutical composition comprising

(a) the engineered nucleoprotein complex of any of claims 1-11; or

(b) a nucleic acids of claim 12, or the nucleic acid of claim 13,

wherein optionally the pharmaceutical compound is formulated for enteral or parenteral delivery, or for intravenous (IV) delivery.

16. The engineered nucleoprotein complex of any one of claims 1-11, the nucleic acids of claim 12 or the nucleic acid of claim 13, the cell of claim 14, or the pharmaceutical composition of claim 15 for use in therapy.

17. Use of the engineered nucleoprotein complex of any one of claims 1-11, the nucleic acids of claim 12 or the nucleic acid of claim 13, the cell of claim 14, or the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating, preventing, ameliorating or reducing a symptom of a disease or condition, or an infection, in a mammalian or a human subject.

18. The use of claim 16 or 17, wherein the disease or the condition is caused by a repeat sequence selected from the group consisting of CTG, CCTG, CAG, GGGGCC, and any combination thereof, and wherein the disease or the condition is caused by or associated with a RNA microsatellite repeat expansion.

19. The use according to any one of claims 16-18, wherein the engineered nucleoprotein complex, nucleic acid, cell or pharmaceutical composition is to be administered by a route selected from the group consisting of oral, pulmonary, intraperitoneal (ip), intravenous (iv), intramuscular (im), subcutaneous (sc), transdermal, buccal, nasal, sublingual, ocular, rectal and vaginal.

20. A method of expressing the engineered nucleoprotein complex of any one of claims 1-11, comprising introducing the nucleic acids of claim 12 or the nucleic acid of claim 13 into a cell *in vitro* under conditions in which said cell expresses said nucleoprotein complex.

21. A vector comprising the nucleic acids claim of claim 12 or the nucleic acid of claim 13.

22. The vector of claim 21, wherein the vector is a single vector.

23. The vector of claim 21 or 22, wherein the vector contains or carries the nucleic acid(s) encoding the engineered nucleoprotein complex or one or more components thereof; or wherein the vector contains or carries each component of the engineered nucleoprotein complex.

24. The vector of any one of claims 21-23, further comprising a regulatable or a constitutive promoter that regulates expression of one or more of the nuclease-inactive RCas9 polypeptide, the sgRNA, the effector polypeptide, or combinations thereof.

25. The vector of any one of claims 21-24 is, wherein the vector comprises or is derived from an adenovirus, an adeno-associated virus (AAV), a retrovirus, a herpes simplex virus, a human immunodeficiency virus (HIV), or a synthetic vector.