RNA-TARGETING KNOCKDOWN AND REPLACEMENT COMPOSITIONS AND METHODS FOR USE

FIELD OF THE DISCLOSURE

[01] The disclosure is directed to molecular biology, gene therapy, and compositions and methods for modifying expression and activity of RNA molecules.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[02] The contents of the text file named "LOCN_005_001WO_SeqList_ST25", which was created on July 10, 2020 and is 6.07 MB in size, are hereby incorporated by reference in their entirety.

CROSS-REFERENCE TO RELATED APPLICATIONS

[03] This application claims priority to, and the benefit of, U.S. provisional application Nos. 62/872,604, filed July 10, 2019 and 62/968,819 filed January 31, 2020, under 35 USC § 119(e). The contents of each of these applications are hereby incorporated by reference in their entireties.

BACKGROUND

[04] There has been a long-felt but unmet need in the art for providing effective gain-orloss-of-function gene replacement therapies. There is also a long-felt need in the art for providing effective methods of RNA-targeting systems. The disclosure, thus, provides a combination of RNA-targeting and gene replacement strategies. In particular, the disclosure provides compositions and methods for specifically targeting and knocking down pathogenic RNA molecules, which lead to toxic gain-or-loss-of-function mutations, in a sequence-specific manner while also replacing the targeted, and knocked down, gene with a therapeutic replacement gene.

SUMMARY

[05] The disclosure provides a composition comprising a nucleic acid sequence encoding an RNA-guided target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target RNA when guided by a gRNA sequence, wherein a pathogenic RNA comprises the target RNA, and wherein the therapeutic protein is a replacement of gain-or-loss-of-function mutations encoded by the pathogenic RNA.

[06] The disclosure provides a composition comprising a nucleic acid sequence encoding a target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target RNA or a protein encoded by the target RNA, wherein a pathogenic RNA encoding a pathogenic protein with one or more gain-or-loss-of-function mutations comprises the target RNA, and wherein the therapeutic protein is a replacement protein for the pathogenic protein.

[07] The disclosure also provides a composition comprising a nucleic acid sequence encoding a target RNA knockdown and replacement therapeutic for treating retinitis pigmentosa (RP) comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target rhodopsin RNA or a protein encoded by the target rhodopsin RNA, wherein a pathogenic rhodopsin RNA encoding a pathogenic rhodopsin protein with one or more gain-or-loss-of-function rhodopsin mutations comprises the target rhodopsin RNA, and wherein the therapeutic protein is a wild-type rhodopsin protein.

[08] In some embodiments, the RNA-binding polypeptide is a RNA-guided RNA-binding protein. In some embodiments, the RNA-guided RNA-binding protein is Cas13a, Cas13b, Cas13c, or Cas13d. In some embodiments, the RNA-binding polypeptide is a non-guided RNA-binding polypeptide. In some embodiments, the non-guided RNA-binding polypeptide is PUF, or PUMBY protein. In some embodiments, the non-guided RNA-binding polypeptide a PUF or PUMBY fusion protein. In one embodiment, a PUF or PUMBY-based first RNA-binding protein is fused to a second RNA-bindng protein which is an zinc-finger endonuclease known as ZC3H12A of SEQ ID NO: 358 (also termed herein E17).

[09] In some embodiments, the therapeutic replacement gene (corresponding disease) is selected from the group consisting of: rhodopsin (Retinitis Pigmentosa), PRPF3 (Retinitis Pigmentosa), PRPF31 (autosomal dominant Retinitis Pigmentosa), GRN (FTD), SOD1

(ALS), PMP22 (Charcot Marie Tooth Disease), PABPN1 (Oculopharangeal Muscular Dystrophy), KCNQ4 (Hearing Loss), CLRN1 (Usher Syndrome), APOE2 (Alzheimer's Disease), APOE4 (Alzheimer's Disease), BEST1 (Eye Disease), MYBPC3 (Familial Cardiomyopathy), TNNT2 (Familial Cardiomyopathy), and TNNI3 (Familial Cardiomyopathy).

[010] In some embodiments, the therapeutic protein is rhodopsin or wild-type rhodopsin. In some embodiments, the therapeutic protein is human rhodopsin. In some embodiments, the therapeutic protein is "hardened" rhodopsin.

[011] In some embodiments of the compositions of the disclosure, the pathogenic rhodopsin RNA comprises or encodes at least one gain-or-loss-of-function mutation.

[012] In some embodiments, the rhodopsin target RNA comprises GCCAGCGTGGCATTCTACATCTTC (SEQ ID NO: 406). In some embodiments, the rhodopsin target RNA comprises CAACGAGTCTTTTGTCATCTACATGT (SEQ ID NO: 462), CGCCAGCGTGGCATTCTACATCTTCA (SEQ ID NO: 463), or CATCTATATCATGATGAACAAGCAGT (SEQ ID NO: 464).

[013] In some embodiments, the target RNA encodes an amino acid sequence comprising ASVAFYIF (SEQ ID NO: 407) at positions 269 to 276. In some embodiments, the target RNA encodes an amino acid comprising YASVAFYIFT (SEQ ID NO: 486) at positions 268 to 277.

[014] In some embodiments, the "hardened" rhodopsin is encoded by a nucleic acid sequence which does not comprise the rhodopsin target RNA comprising GCCAGCGTGGCATTCTACATCTTC (SEQ ID NO: 406).

[015] In some embodiments, the "hardened" rhodopsin is encoded by a nucleic acid sequence comprising GCTTCCGTAGCTTTTTATATTTTT (SEQ ID NO: 408).

[016] In some embodiments, the nucleic acid sequence comprises at least one promoter. In some embodiments, the at least one promoter is a constitutive promoter or a tissue-specific promoter. In some embodiments, the at least one promoter is selected from the group consisting of an opsin promoter, an EFS promoter, and a combination thereof. In some embodiments, the nucleic acid sequence comprises two promoters. In one embodiment, the two promoters are an opsin promoter driving expression of the replacement rhodopsin protein and an EFS promoter driving expression of the PUF or PUMBY-based RNA-binding protein fused to a second RNA-binding protein which is an effector protein such as ZC3H12A.

[017] In some embodiments disclosed herein is a vector comprising the knockdown replacement compositions disclosed herein. In some embodiments, the vector is selected from the group consisting of: adeno-associated virus, retrovirus, lentivirus, adenovirus, nanoparticle, micelle, liposome, lipoplex, polymersome, polyplex, and dendrimer. In some embodiments disclosed herein is a cell comprising the vectors disclosed herein.

[018] In some embodiments of the compositions disclosed herein, the RNA-binding polypeptide is a first RNA-binding polypeptide, and the nucleic acid sequence encodes a second RNA-binding polypeptide which binds RNA in a manner in which it associates with RNA. In some embodiments, the second RNA-binding polypeptide associates with RNA in a manner in which it cleaves RNA. In some embodiments, the second RNA-binding polypeptide is selected from the group consisting of: RNAse1, RNAse4, RNAse6, RNAse7, RNAse8, RNAse2, RNAse6PL, RNAseL, RNAseT2, RNAse11, RNAseT2-like, NOB1, ENDOV, ENDOG, ENDOD1, hFEN1, hSLFN14, hLACTB2, APEX2, ANG, HRSP12, ZC3H12A, RIDA, PDL6, NTHL, KIAA0391, APEX1, AGO2, EXOG, ZC3H12D, ERN2, PELO, YBEY, CPSF4L, hCG_2002731, ERCC1, RAC1, RAA1, RAB1, DNA2, FLJ35220, FLJ13173, ERCC4, Rnase1(K41R), Rnase1(K41R, D121E), Rnase1(K41R, D121E, H119N), Rnase1(H119N), Rnase1(R39D, N67D, N88A, G89D, R91D, H119N), Rnase1(R39D, N67D, N88A, G89D, R91D, H119N, K41R, D121E), Rnase1(R39D, N67D, N88A, G89D, R91D), TENM1, TENM2, RNAseK, TALEN, ZNF638, and hSMG6. In one embodiment, the second RNA-binding polypeptide is ZC3H12A.

[019] 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.

[020] In some embodiments of the compositions of the disclosure, the gRNA comprises a spacer sequence comprising ACATGTAGATGACAAAAGACTCGTTG (SEQ ID NO: 465), TGAAGATGTAGAATGCCACGCTGGCG (SEQ ID NO: 409), or ACTGCTTGTTCATCATGATATAGATG (SEQ ID NO: 466).

[021] 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.

[022] 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 a Pol II promoter. 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.

[023] 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, 22 nucleotides, 23 nucleotides, 24 nucleotides, 25 nucleotides, 26 nucleotides, 27 nucleotides, 28 nucleotides, or 29 nucleotides. In some embodiments, the spacer sequence comprises or consists of 26 nucleotides. In some embodiments, the spacer sequence is non-processed and comprises or consists of 30 nucleotides. In some embodiments the non-processed spacer sequence comprises or consists of 30-36 nucleotides.

[024] 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.

[025] 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.

[026] 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.

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

[028] 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.

[029] In some embodiments, the pathogenic RNA comprises the target RNA, and/or the target RNA is associated with the pathogenic RNA. In some embodiments, the pathogenic RNA encodes gain-or-loss-of-function mutations.

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

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

[032] In some embodiments of the compositions of the disclosure, the RNA binding protein is a non-guided RNA binding protein. In some embodiments, the non-guided RNA binding protein comprises a Pumilio and FBF (PUF) protein or an RNA binding portion thereof. In some embodiments, the RNA binding protein comprises a Pumilio-based assembly (PUMBY) protein or an RNA binding portion thereof.

[033] In some embodiments of the compositions of the disclosure, the RNA binding protein does not require multimerization for RNA-binding activity. In some embodiments, the 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.

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

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

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

[037] In some embodiments of the compositions of the disclosure, the sequence encoding the 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 at the N-terminus of the sequence encoding the RNA binding protein. In some embodiments, the RNA binding protein comprises an NLS at a C-terminus of the protein.

[038] In some embodiments of the compositions of the disclosure, the sequence encoding the 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 at the N-terminus of the sequence encoding the RNA binding protein. In some embodiments, the RNA binding protein comprises the first NLS or the second NLS at a C-terminus of the protein.

[039] In some embodiments of the compositions of the disclosure, the composition further comprises a second RNA binding protein. In some embodiments, 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. In some embodiments of the compositions of the disclosure, the sequence encoding the second RNA binding protein comprises or consists of an RNAse.

[040] In some embodiments, the compositions of the disclosure are used in methods for treating a subject in need thereof, the methods comprising contacting a target RNA with a nucleic acid sequence encoding the knockdown RNA and replacement protein.

[041] In some embodiments of the compositions disclosed herein are used in a method for reducing the level of expression of a pathogenic target RNA molecule or a protein encoded by the pathogenic RNA molecule and replacing gain-or-loss-of-function mutations caused by the pathogenic target RNA with a therapeutic replacement protein, the method comprising contacting the compositions disclosed herein and the pathogenic target RNA molecule comprising a target RNA sequence under conditions suitable for binding of the RNA binding protein to the target RNA sequence, wherein the level of expression of the pathogenic target RNA is reduced, and wherein the expression of the pathogenic target RNA is replaced with expression of a therapeutic replacement protein.

BRIEF DESCRIPTION OF THE DRAWINGS

[042] 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.

[043] FIGS. 1A-1E are schematic diagrams of exemplary embodiments of compositions of the disclosure that depict nucleic acid sequence designs that promote simultaneous knockdown and replacement of pathogenic RNAs. Nucleic acid sequences A-E each describe exemplary vector sequences. In these embodiments, a polymerase II ("Pol II") promoter drives expression of the RNA-targeting protein and a polymerase III promoter ("Pol III") drives expression of the optional single guide RNA ("sgRNA") in vectors that also encode a CRISPR-associated (Cas) RNA-targeting protein. The replacement protein is provided either by a second polymerase II promoter or via the same promoter that drives the RNA-targeting protein. In the case of a single polymerase II promoter system, the replacement gene and the RNA knockdown system are separated by either a 2A site or an internal ribosome entry site (IRES).

[044] FIG. 2 is a schematic diagram of embodiments of therapeutic compositions and methods of the disclosure involving the knockdown and replace vector. Certain schematic vector designs are packaged in a delivery vehicle such as adeno-associated virus (AAV) and delivered to target tissue in a manner determined by AAV serotype and administration

method. Once present in the target tissue, the therapeutic simultaneously replaces the mutated RNA and encoded protein while destroying the mutated RNA.

[045] FIG. 3 is a plasmid map showing an exemplary configuration of pmirGlo designed for a luciferase reporter assay for detecting knockdown effect of the compositions disclosed herein.

[046] FIG. 4 is a plasmid map showing a PUMBY-based knockdown and replacement embodiment of the compositions disclosed herein.

[047] FIG. 5 is a plasmid map showing a PUF-based knockdown and replacement embodiment of the compositions disclosed herein

[048] FIG. 6A-6C show embodiments of the compositions disclosed herein. FIG. 6A shows a schematic diagram of exemplary embodiments of compositions of the disclosure that depict nucleic acid sequence designs encoding PUF or PUMBY-based RNA-binding - effector fusion proteins. FIGS. 6B-6C show knockdown of Rhodopsin target RNA and replacement of the target RNA with "hardened" rhodopsin.

[049] FIGS. 7A-7B show knockdown of Rhodopsin target RNA and replacement of the target RNA with "hardened" rhodopsin.

[050] FIG. 8 shows a luciferase assay PUF-targeting Rhodopsin knockdown screen compared to no targeting.

DETAILED DESCRIPTION

[051] The disclosure provides a therapeutic combination of RNA-targeting and gene replacement. In particular, the disclosure provides compositions and methods for specifically targeting and knocking down pathogenic RNA molecules which lead to toxic gain-or-loss-of-function mutations in a sequence-specific manner while also replacing the targeted, and knocked down, gene with the corresponding therapeutic gene. In one embodiment, the pathogenic RNA comprises a target RNA sequence. In one embodiment, the pathogenic RNA comprises a target RNA sequence but the target RNA sequence does not comprise the gain-or-loss-of-function mutations. In another embodiment, the target RNA is in non-coding RNA. In a further embodiment, the pathogenic RNA comprises one or more additional target RNAs. In particular, the disclosure provides a composition comprising a nucleic acid sequence encoding a target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target RNA, wherein a pathogenic RNA comprises

the target RNA, and wherein the therapeutic protein is a wild-type replacement of the pathogenic RNA or protein encoded by the pathogenic RNA. The disclosure provides vectors, compositions and cells comprising the knockdown and replacement compositions. The disclosure provides methods of using the knockdown and replacement systems, the RNA-guided (such as CRISPR/Cas-based) or non-RNA-guided (PUF or PUMBY-based) RNA-binding proteins fusions, guide RNAs (gRNAs) corresponding to RNA-guided CRISPR/Cas proteins, therapeutic replacement genes or portions thereof, vectors, compositions and cells of the disclosure to treat a disease or disorder. The compositions also provide particular target RNA sequences or particular targeting RNA sequences (e.g., a particular gRNA spacer sequence).

[052] The compositions and methods of the disclosure provide a combined knockdown and therapeutic effect. Accordingly, the compositions comprise a nucleic acid sequence encoding 1) an RNA-binding polypeptide (RBP) or RNA-binding domain (RBD), capable of cleavage of a pathogenic RNA comprising a target RNA sequence, and 2) a replacement therapeutic protein. In some embodiments, the replacement therapeutic protein is the wild-type protein of the pathogenic target RNA or protein. In some embodiments, the therapeutic (e.g., wild-type) replacement protein replaces gain-or-loss-of-function mutations encoded by the pathogenic target RNA.

[053] In some embodiments, the RNA-binding polypeptide is an RNA-guided RNA-binding polypeptide. In some embodiments, the RNA-guided RNA-binding polypeptide is a CRISPR/Cas protein and the nucleic acid sequence further comprises an gRNA sequence which corresponds to the target RNA and the CRISPR/Cas protein. In some embodiments, the RNA-binding polypeptide is not an RNA-guided RNA-binding polypeptide. In particular embodiments, the non-RNA-guided RNA-binding polypeptide is a PUF protein or a PUMBY protein or portion thereof. In some embodiments, the pathogenic RNA comprising the target RNA encodes gain-or-loss-of-function mutations.

[054] In some embodiments, the pathogenic RNA encodes gain-or-loss-of-functionmutations in the rhodopsin gene and the replacement gene encodes human rhodopsin. In some embodiments, the pathogenic rhodopsin RNA comprises a rhodopsin target RNA. In one embodiment, the rhodopsin target RNA sequence comprises GCCAGCGTGGCATTCTACATCTTC (SEQ ID NO: 406). In some embodiments, the rhodopsin target RNA comprises CAACGAGTCTTTTGTCATCTACATGT (SEQ ID NO:

462), CGCCAGCGTGGCATTCTACATCTTCA (SEQ ID NO: 463), or CATCTATATCATGATGAACAAGCAGT (SEQ ID NO: 464).

[055] In another embodiment, the rhodopsin target RNA encodes an amino acid comprising ASVAFYIF (SEQ ID NO: 407). In one embodiment, the rhodopsin target RNA encodes an amino acid comprising ASVAFYIF (SEQ ID NO: 407) at e.g., position 269 to 276. In another embodiment, the target RNA encodes an amino acid comprising YASVAFYIFT (SEQ ID NO: 486). In another embodiment, the target RNA encodes an amino acid comprising YASVAFYIFT (SEQ ID NO: 486) at e.g., positions 268 to 277.

[056] In some embodiments, the replacement gene encodes "hardened" rhodopsin. "Hardened" rhodopsin is an engineered wild-type rhodopsin the expression of which is engineered to be incapable of knockdown using the compositions disclosed herein. In one embodiment, a "hardened" rhodopsin nucleic acid sequence comprising at least one mismatch. In another embodiment, a "hardened" rhodopsin nucleic acid sequence comprising two or more mismatches. In one embodiment, the "hardened" rhodopsin is encoded by a nucleic acid sequence which does not comprise the rhodopsin target RNA comprising GCCAGCGTGGCATTCTACATCTTC SEQ ID NO: 406. In another embodiment, the "hardened" rhodopsin is encoded by a nucleic acid sequence comprising GCTTCCGTAGCTTTTTATATTTTT (SEQ ID NO: 408). In some embodiments, the spacer sequence of the gRNA is a sequence which is complementary to the rhodopsin target RNA. In one embodiment, the spacer sequence targeting the rhodopsin target RNA is ACATGTAGATGACAAAAGACTCGTTG (SEQ ID NO: 465), TGAAGATGTAGAATGCCACGCTGGCG (SEQ ID NO: 409), or ACTGCTTGTTCATCATGATATAGATG (SEQ ID NO 466).

Guide RNAs

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

[058] Guide RNAs (gRNAs) of the disclosure may comprise of a spacer sequence and a scaffolding and/or a "direct repeat" (DR) 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. In some embodiments, the gRNA comprises a 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 guide RNA comprises a direct repeat (DR) sequence and a spacer sequence. 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 and/or a DR 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. In some embodiments, the scaffold sequence is a Cas9 scaffold sequence. In some embodiments, the DR sequence is a Cas13d sequence.

[059] In one embodiment, the gRNA that hybridizes with the one or more target RNA molecules in a Cas l3d-mediated manner includes one or more direct repeat (DR) sequences, one or more spacer sequences, such as, e.g., one or more sequences comprising an array of DR-spacer-DR-spacer. In one embodiment, a plurality of gRNAs are generated from a single array, wherein each gRNA can be different, for example target different RNAs or target multiple regions of a single RNA, or combinations thereof. In some embodiments, an isolated gRNA includes one or more direct repeat (DR) sequences, such as an unprocessed (e.g., about 36 nt) or processed DR (e.g., about 30 nt). In some embodiments, a gRNA can further include one or more spacer sequences specific for (e.g., is complementary to) the target RNA. In certain such embodiments, multiple pol III promoters can be used to drive multiple gRNAs, spacers and/or DRs. In one embodiment, a guide array comprises a DR (about 36nt)-spacer (about 30nt)-DR (about 36nt)-spacer (about 30nt)-DR (about 36nt).

[060] 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.

[061] 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 or mutated (e.g., pathogenic) 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).

[062] 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).

[063] Spacer sequences of the disclosure bind to the target sequence of an RNA molecule. In some embodiments, spacer sequences of the disclosure bind to pathogenic target RNA.

[064] 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.

[065] 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).

[066] 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.

[067] 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.

[068] 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, the spacer sequence of the disclosure comprises or consists of 26 nucleotides.

[069] Guide molecules generally exist in various states of processing. In one example, an unprocessed guide RNA is 36nt of DR followed by 30-32 nt of spacer. The guide RNA is processed (truncated/modified) by Cas l3d itself or other RNases into the shorter "mature" form. In some embodiments, an unprocessed guide sequence is about, or at least about 30, 35, 40, 45, 50, 55, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, or more nucleotides (nt) in length. In some embodiments, a processed guide sequence is about 44 to 60 nt (such as 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, or 70 nt). In some embodiments, an unprocessed spacer is about 28-32 nt long (such as 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 nt) while the mature (processed) spacer can be about 10 to 30 nt, 10 to 25 nt, 14 to 25 nt, 20 to 22 nt, or 14-30 nt (such as 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 nt). In some embodiments, an unprocessed DR is about 36 nt (such as 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 or 41 nt), while the processed DR is about 30 nt (such as 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 nt). In some embodiments, a DR sequence is truncated by 1-10 nucleotides (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, ot 10 nucleotides at e.g., the 5' end in order to be expressed as mature pre-processed guide RNAs.

[070] In some embodiments, a scaffold sequence, such as e.g., a Cas9 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. 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 GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 403). In some embodiments, the scaffold sequence comprises or consists of the sequence GGACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUUU (SEQ ID NO: 404). In some embodiments, the scaffold sequence comprises or consists of the sequence GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUU GAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 405).

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

[072] 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).

[073] Therapeutic or pharmaceutical compositions of the disclosure do not comprise a PAMmer oligonucleotide. In other embodiments, optionally, non-therapeutic or non-pharmaceutical compositions may comprise a PAMmer oligonucleotide. The term "PAMmer" refers to an oligonucleotide comprising a PAM sequence that is capable of interacting with a guide nucleotide sequence-programmable RNA binding protein. Non-limiting examples of PAMmers are described in O'Connell *et al. Nature* 516, pages 263–266 (2014), incorporated herein by reference. A PAM sequence refers to a protospacer adjacent motif comprising about 2 to about 10 nucleotides. PAM sequences are specific to the guide nucleotide sequence-programmable RNA binding protein with which they interact and are known in the art. For example, *Streptococcus pyogenes* PAM has the sequence 5'-NGG-3', where "N" is any nucleobase followed by two guanine ("G") nucleobases. Cas9 of *Francisella novicida* recognizes the canonical PAM sequence 5'-NGG-3', but has been engineered to recognize the PAM 5'-YG-3' (where "Y" is a pyrimidine), thus adding to the range of possible Cas9 targets. The Cpf1 nuclease of *Francisella novicida* recognizes the PAM 5'-TTTN-3' or 5'-YTN-3'.

[074] 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.

[075] 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.

[076] 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.

[077] 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.

[078] In some embodiments of the compositions of the disclosure, gRNAs correspond to target RNA molecules and an RNA-guided RNA binding protein. In some embodiments, the gRNAs correspond to an RNA-guided RNA binding fusion protein, wherein the fusion protein comprises first and second RNA binding proteins. In some embodiments, along a sequence encoding the RNA-binding 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.

[079] 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.

[080] 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.

[081] 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.

[082] 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.

[083] In some embodiments of the compositions of the disclosure, the sequence encoding the RNA-guided RNA binding 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*.

[084] Exemplary wild type *S. pyogenes* Cas9 proteins of the disclosure may comprise or consist of the amino acid sequence of
SEQ ID NO: 416.

[085] 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) of (EQ ID NO: 417.

[086] 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.

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

[088] In some embodiments the Cas9 protein can be *S. pyogenes* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 418.

[089] In some embodiments the Cas9 protein can be *S. aureus* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 419.

[090] In some embodiments the Cas9 protein can be *S. thermophiles* CRISPR1 Cas9 and may comprise or consist of the amino acid sequence ofSEQ ID NO: 420.

[091] In some embodiments the Cas9 protein can be *N. meningitidis* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 421.

[092] In some embodiments the Cas9 protein can be *Parvibaculum. lavamentivorans* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 422.

[093] In some embodiments the Cas9 protein can be *Corynebacter diphtheria* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 423.

[094] In some embodiments the Cas9 protein can be *Streptococcus pasteurianus* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 424.

[095] In some embodiments the Cas9 protein can be *Neisseria cinerea* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 425.

[096] In some embodiments the Cas9 protein can be *Campylobacter lari* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 426.

[097] In some embodiments the Cas9 protein can be *T. denticola* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 427.

[098] In some embodiments the Cas9 protein can be *S. mutans* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 428.

[099] In some embodiments the Cas9 protein can be *S. thermophilus* CRISPR 3 Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 429.

[0100] In some embodiments the Cas9 protein can be *C. jejuni* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 430.

[0101] In some embodiments the Cas9 protein can be *P. multocida* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 431.

[0102] In some embodiments the Cas9 protein can be *F. novicida* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 432.

[0103] In some embodiments the Cas9 protein can be *Lactobacillus buchneri* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 433.

[0104] In some embodiments the Cas9 protein can be *Listeria innocua* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 434.

[0105] In some embodiments the Cas9 protein can be *L. pneumophilia* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 435.

[0106] In some embodiments the Cas9 protein can be *N. lactamica* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 436.

[0107] In some embodiments the Cas9 protein can be *N. meningitides* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 437.

[0108] In some embodiments the Cas9 protein can be *B. longum* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 438.

[0109] In some embodiments the Cas9 protein can be *A. muciniphila* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 439.

[0110] In some embodiments the Cas9 protein can be *O. laneus* Cas9 and may comprise or consist of the amino acid sequence of SEQ ID NO: 440.

[0111] 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.

[0112] Exemplary wild type *Francisella tularensis subsp. Novicida* Cpf1 (FnCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence of SEQ ID NO: 441.

[0113] Exemplary wild type *Lachnospiraceae bacterium sp. ND2006* Cpf1 (LbCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence of SEQ ID NO: 442.

[0114] Exemplary wild type *Acidaminococcus sp. BV3L6* Cpf1 (AsCpf1) proteins of the disclosure may comprise or consist of the amino acid sequence of SEQ ID NO: 443.

[0115] In some embodiments of the compositions of the disclosure, the sequence encoding the 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.

[0116] 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	CCACCCCAATATCGAAGGGGACTAAAAC (SEQ ID NO: 444)
Cas13a2	LwaCas13a	Leptotrichia wadei	WP_021746774.1	GATTTAGACTACCCCAAAAACGAAGGGGACTAAAAC (SEQ ID NO: 445)
Cas13a3	LseCas13a	Listeria seeligeri	WP_012985477.1	GTAAGAGACTACCTCTATATGAAAGAGGACTAAAAC (SEQ ID NO: 446)
Cas13a4	LbmCas13a	Lachnospiraceae bacterium MA2020	WP_044921188.1	GTATTGAGAAAAGCCAGATATAGTTGGCAATAGAC (SEQ ID NO: 447)
Cas13a5	LbnCas13a	Lachnospiraceae bacterium NK4A179	WP_022785443.1	GTTGATGAGAAGAGCCCAAGATAGAGGGCAATAAC (SEQ ID NO: 448)
Cas13a6	CamCas13a	[Clostridium] aminophilum DSM 10710	WP_031473346.1	GTCTATTGCCCTCTATATCGGGCTGTTCTCCAAAC (SEQ ID NO: 449)
Cas13a7	CgaCas13a	Carnobacterium gallinarum DSM 4847	WP_034560163.1	ATTAAAGACTACCTCTAAATGTAAGAGGACTATAAC (SEQ ID NO: 450)
Cas13a8	Cga2Cas13a	Carnobacterium gallinarum DSM 4847	WP_034563842.1	AATATAAACTACCTCTAAATGTAAGAGGACTATAAC (SEQ ID NO: 451)
Cas13a9	Pprcas13a	Paludibacter propionicigenes WB4	WP_013443710.1	CTTGTGGATTATCCCAAAATTGAAGGGAACTACAAC (SEQ ID NO: 452)
Cas13a10	LweCas13a	Listeria weihenstephanensis FSL R9-0317	WP_036059185.1	GATTTAGAGTACCTCAAAATAGAAGAGGTCTAAAAC (SEQ ID NO: 453)

Cas13a11	LbfCas13a	Listeriaceae bacterium FSL M6-0635 (Listeria newyorkensis)	WP_036091002.1	GATTTAGAGTACCTCAAAACAAAAG AGGACTAAAAC (SEQ ID NO: 454)
Cas13a12	Lwa2cas13a	Leptotrichia wadei F0279	WP_021746774.1	GATATAGATAACCCCAAAAACGAA GGGATCTAAAAC (SEQ ID NO: 455)
Cas13a13	RcsCas13a	Rhodobacter capsulatus SB 1003	WP_013067728.1	GCCTCACATCACCGCCAAGACGACG GCGGACTGAAC(SEQ ID NO: 456)
Cas13a14	RcrCas13a	Rhodobacter capsulatus R121	WP_023911507.1	GCCTCACATCACCGCCAAGACGACG GCGGACTGAAC (SEQ ID NO: 457)
Cas13a15	RcdCas13a	Rhodobacter capsulatus DE442	WP_023911507.1	GCCTCACATCACCGCCAAGACGACG GCGGACTGAAC (SEQ ID NO: 458)

[0117] Exemplary wild type Cas13a proteins of the disclosure may comprise or consist of the amino acid sequence of SEQ ID NO: 459.

[0118] 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	EHO06562.1	1160
Myroides odoratimimus CCUG 3837	EKB06014.1	1158

Myroides odoratimimus CCUG 3837	WP_006265509.1	1158
Myroides odoratimimus CCUG 12901	WP_006261414.1	1158
Myroides odoratimimus CCUG 12901	EHO08761.1	1158
Myroides odoratimimus (NZ_CP013690.1)	WP_058700060.1	1160
Bergeyella zoohelcum ATCC 43767	EKB54193.1	1225
Capnocytophaga cynodegmi	WP_041989581.1	1219
Bergeyella zoohelcum ATCC 43767	WP_002664492.1	1225
Flavobacterium sp. 316	WP_045968377.1	1156
Psychroflexus torquis ATCC 700755	WP_015024765.1	1146
Flavobacterium columnare ATCC 49512	WP_014165541.1	1180
Flavobacterium columnare	WP_060381855.1	1214
Flavobacterium columnare	WP_063744070.1	1214
Flavobacterium columnare	WP_065213424.1	1215
Chryseobacterium sp. YR477	WP_047431796.1	1146
Riemerella anatipestifer ATCC 11845 = DSM 15868	WP_004919755.1	1096
Riemerella anatipestifer RA-CH-2	WP_015345620.1	949
Riemerella anatipestifer	WP_049354263.1	949
Riemerella anatipestifer	WP_061710138.1	951
Riemerella anatipestifer	WP_064970887.1	1096
Prevotella saccharolytica F0055	EKY00089.1	1151
Prevotella saccharolytica JCM 17484	WP_051522484.1	1152
Prevotella buccae ATCC 33574	EFU31981.1	1128
Prevotella buccae ATCC 33574	WP_004343973.1	1128
Prevotella buccae D17	WP_004343581.1	1128
Prevotella sp. MSX73	WP_007412163.1	1128
Prevotella pallens ATCC 700821	EGQ18444.1	1126
Prevotella pallens ATCC 700821	WP_006044833.1	1126
Prevotella intermedia ATCC 25611 = DSM 20706	WP_036860899.1	1127
Prevotella intermedia	WP_061868553.1	1121
Prevotella intermedia 17	AFJ07523.1	1135
Prevotella intermedia	WP_050955369.1	1133
Prevotella intermedia	BAU18623.1	1134
Prevotella intermedia ZT	KJJ86756.1	1126
Prevotella aurantiaca JCM 15754	WP_025000926.1	1125
Prevotella pleuritidis F0068	WP_021584635.1	1140
Prevotella pleuritidis JCM 14110	WP_036931485.1	1117
Prevotella falsenii DSM 22864 = JCM 15124	WP_036884929.1	1134
Porphyromonas gulae	WP_039418912.1	1176
Porphyromonas sp. COT-052 OH4946	WP_039428968.1	1176
Porphyromonas gulae	WP_039442171.1	1175
Porphyromonas gulae	WP_039431778.1	1176
Porphyromonas gulae	WP_046201018.1	1176
Porphyromonas gulae	WP_039434803.1	1176
Porphyromonas gulae	WP_039419792.1	1120
Porphyromonas gulae	WP_039426176.1	1120
Porphyromonas gulae	WP_039437199.1	1120
Porphyromonas gingivalis TDC60	WP_013816155.1	1120
Porphyromonas gingivalis ATCC 33277	WP_012458414.1	1120
Porphyromonas gingivalis A7A1-28	WP_058019250.1	1176
Porphyromonas gingivalis JCVI SC001	EOA10535.1	1176
Porphyromonas gingivalis W50	WP_005874195.1	1176

Porphyromonas gingivalis	WP_052912312.1	1176
Porphyromonas gingivalis AJW4	WP_053444417.1	1120
Porphyromonas gingivalis	WP_039417390.1	1120
Porphyromonas gingivalis	WP_061156470.1	1120

[0119] Exemplary wild type *Bergeyella zoohelcum* ATCC 43767 Cas13b (BzCas13b) proteins of the disclosure may comprise or consist of the amino acid sequence of SEQ ID NO: 460.

[0120] In some embodiments of the compositions of the disclosure, the sequence encoding the RNA binding protein comprises a sequence isolated or derived from a Cas13d protein. Cas13d is an effector of the type VI-D CRISPR-Cas systems. In some embodiments, the Cas13d protein is an RNA-guided RNA endonuclease enzyme that can cut or bind RNA. In some embodiments, the Cas13d protein can include one or more higher eukaryotes and prokaryotes nucleotide-binding (HEPN) domains. In some embodiments, the Cas13d protein can include either a wild-type or mutated HEPN domain. In some embodiments, the Cas13d protein includes a mutated HEPN domain that cannot cut RNA but can process guide RNA. In some embodiments, the 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 Cas13d protein, without limitation.

[0121] In some embodiments, Cas13d sequences of the disclosure include without limitation SEQ ID NOS: 1-296 of WO 2019/040664, so numbered herein and included herewith.

[0122] SEQ ID NO: 1 is an exemplary Cas13d sequence from Eubacterium siraeum containing a HEP site.

[0123] SEQ ID NO: 2 is an exemplary Cas13d sequence from Eubacterium siraeum containing a mutated HEPN site.

[0124] SEQ ID NO: 3 is an exemplary Cas13d sequence from uncultured Ruminococcus sp. containing a HEPN site.

[0125] SEQ ID NO: 4 is an exemplary Cas13d sequence from uncultured Ruminococcus sp. containing a mutated HEPN site.

[0126] SEQ ID NO: 5 is an exemplary Cas13d sequence from Gut_metagenome_contig2791000549.

[0127] SEQ ID NO: 6 is an exemplary Cas13d sequence from Gut_metagenome_contig855000317

[0128] SEQ ID NO: 7 is an exemplary Cas13d sequence from Gut_metagenome_contig3389000027.

[0129] SEQ ID NO: 8 is an exemplary Cas13d sequence from Gut_metagenome_contig8061000170.

[0130] SEQ ID NO: 9 is an exemplary Cas13d sequence from Gut_metagenome_contigl509000299.

[0131] SEQ ID NO: 10 is an exemplary Cas13d sequence from Gut_metagenome_contig9549000591.

[0132] SEQ ID NO: 11 is an exemplary Cas13d sequence from Gut_metagenome_contig71000500.

[0133] SEQ ID NO: 12 is an exemplary Cas13d sequence from human gut metagenome.

[0134] SEQ ID NO: 13 is an exemplary Cas13d sequence from Gut_metagenome_contig3915000357.

[0135] SEQ ID NO: 14 is an exemplary Cas13d sequence from Gut_metagenome_contig4719000173.

[0136] SEQ ID NO: 15 is an exemplary Cas13d sequence from Gut_metagenome_contig6929000468.

[0137] SEQ ID NO: 16 is an exemplary Cas13d sequence from Gut_metagenome_contig7367000486.

[0138] SEQ ID NO: 17 is an exemplary Cas13d sequence from Gut_metagenome_contig7930000403.

[0139] SEQ ID NO: 18 is an exemplary Cas13d sequence from Gut_metagenome_contig993000527.

[0140] SEQ ID NO: 19 is an exemplary Cas13d sequence from Gut_metagenome_contig6552000639.

[0141] SEQ ID NO: 20 is an exemplary Cas13d sequence from Gut_metagenome_contigll932000246.

[0142] SEQ ID NO: 21 is an exemplary Cas13d sequence from Gut_metagenome_contigl2963000286.

[0143] SEQ ID NO: 22 is an exemplary Cas13d sequence from Gut_metagenome_contig2952000470.

[0144] SEQ ID NO: 23 is an exemplary Cas13d sequence from Gut_metagenome_contig451000394.

[0145] SEQ ID NO: 24 is an exemplary Cas13d sequence from Eubacterium_siraeum_DSM_l5702.

[0146] SEQ ID NO: 25 is an exemplary Cas13d sequence from gut_metagenome_P19E0k2120140920,_c369000003.

[0147] SEQ ID NO: 26 is an exemplary Cas13d sequence from Gut_metagenome_contig7593000362.

[0148] SEQ ID NO: 27 is an exemplary Cas13d sequence from Gut_metagenome_contigl2619000055.

[0149] SEQ ID NO: 28 is an exemplary Cas13d sequence from Gut_metagenome_contigl405000151.

[0150] SEQ ID NO: 29 is an exemplary Cas13d sequence from Chicken_gut_metagenome_c298474.

[0151] SEQ ID NO: 30 is an exemplary Cas13d sequence from Gut_metagenome_contigl516000227.

[0152] SEQ ID NO: 31 is an exemplary Cas13d sequence from Gut_metagenome_contigl838000319.

[0153] SEQ ID NO: 32 is an exemplary Cas13d sequence from Gut_metagenome_contig13123000268.

[0154] SEQ ID NO: 33 is an exemplary Cas13d sequence from Gut_metagenome_contig5294000434.

[0155] SEQ ID NO: 34 is an exemplary Cas13d sequence from Gut_metagenome_contig6415000192.

[0156] SEQ ID NO: 35 is an exemplary Cas13d sequence from Gut_metagenome_contig6144000300.

[0157] SEQ ID NO: 36 is an exemplary Cas13d sequence from Gut_metagenome_contig9118000041.

[0158] SEQ ID NO: 37 is an exemplary Cas13d sequence from Activated_sludge_metagenome_transcript_124486.

[0159] SEQ ID NO: 38 is an exemplary Cas13d sequence from Gut_metagenome_contig1322000437.

[0160] SEQ ID NO: 39 is an exemplary Cas13d sequence from Gut_metagenome_contig4582000531.

[0161] SEQ ID NO: 40 is an exemplary Cas13d sequence from Gut_metagenome_contig9190000283.

[0162] SEQ ID NO: 41 is an exemplary Cas13d sequence from Gut_metagenome_contigl709000510.

[0163] SEQ ID NO: 42 is an exemplary Cas13d sequence from M24_(LSQX01212483_Anaerobic_digester_metagenome) with a HEPN domain.

[0164] SEQ ID NO: 43 is an exemplary Cas13d sequence from Gut_metagenome_contig3833000494.

[0165] SEQ ID NO: 44 is an exemplary Cas13d sequence from Activated_sludge_metagenome_transcript_117355.

[0166] SEQ ID NO: 45 is an exemplary Cas13d sequence from Gut_metagenome_contigll061000330.

[0167] SEQ ID NO: 46 is an exemplary Cas13d sequence from Gut_metagenome_contig338000322 from sheep gut metagenome.

[0168] SEQ ID NO: 47 is an exemplary Cas13d sequence from human gut metagenome.

[0169] SEQ ID NO: 48 is an exemplary Cas13d sequence from Gut_metagenome_contig9530000097.

[0170] SEQ ID NO: 49 is an exemplary Cas13d sequence from Gut_metagenome_contigl750000258.

[0171] SEQ ID NO: 50 is an exemplary Cas13d sequence from Gut_metagenome_contig5377000274.

[0172] SEQ ID NO: 51 is an exemplary Cas13d sequence from gut_metagenome_P19E0k2120140920_c248000089.

[0173] SEQ ID NO: 52 is an exemplary Cas13d sequence from Gut_metagenome_contigll400000031.

[0174] SEQ ID NO: 53 is an exemplary Cas13d sequence from Gut_metagenome_contig7940000191.

[0175] SEQ ID NO: 54 is an exemplary Cas13d sequence from Gut_metagenome_contig6049000251.

[0176] SEQ ID NO: 55 is an exemplary Cas13d sequence from Gut_metagenome_contigl137000500.

[0177] SEQ ID NO: 56 is an exemplary Cas13d sequence from Gut_metagenome_contig9368000105.

[0178] SEQ ID NO: 57 is an exemplary Cas13d sequence from Gut_metagenome_contig546000275.

[0179] SEQ ID NO: 58 is an exemplary Cas13d sequence from Gut_metagenome_contig7216000573.

[0180] SEQ ID NO: 59 is an exemplary Cas13d sequence from Gut_metagenome_contig4806000409.

[0181] SEQ ID NO: 60 is an exemplary Cas13d sequence from Gut_metagenome_contigl0762000480.

[0182] SEQ ID NO: 61 is an exemplary Cas13d sequence from Gut_metagenome_contig4114000374.

[0183] SEQ ID NO: 62 is an exemplary Cas13d sequence from Ruminococcus_flavefaciens_FD1.

[0184] SEQ ID NO: 63 is an exemplary Cas13d sequence from Gut_metagenome_contig7093000170.

[0185] SEQ ID NO: 64 is an exemplary Cas13d sequence from Gut_metagenome_contigl1113000384.

[0186] SEQ ID NO: 65 is an exemplary Cas13d sequence from Gut_metagenome_contig6403000259.

[0187] SEQ ID NO: 66 is an exemplary Cas13d sequence from Gut_metagenome_contig6193000124.

[0188] SEQ ID NO: 67 is an exemplary Cas13d sequence from Gut_metagenome_contig721000619.

[0189] SEQ ID NO: 68 is an exemplary Cas13d sequence from Gut_metagenome_contigl666000270.

[0190] SEQ ID NO: 69 is an exemplary Cas13d sequence from Gut_metagenome_contig2002000411.

[0191] SEQ ID NO: 70 is an exemplary Cas13d sequence from Ruminococcus_albus.

[0192] SEQ ID NO: 71 is an exemplary Cas13d sequence from Gut_metagenome_contig13552000311.

[0193] SEQ ID NO: 72 is an exemplary Cas13d sequence from Gut_metagenome_contigl0037000527.

[0194] SEQ ID NO: 73 is an exemplary Cas13d sequence from Gut_metagenome_contig238000329.

[0195] SEQ ID NO: 74 is an exemplary Cas13d sequence from Gut_metagenome_contig2643000492.

[0196] SEQ ID NO: 75 is an exemplary Cas13d sequence from Gut_metagenome_contig874000057.

[0197] SEQ ID NO: 76 is an exemplary Cas13d sequence from Gut_metagenome_contig4781000489.

[0198] SEQ ID NO: 77 is an exemplary Cas13d sequence from Gut_metagenome_contigl2144000352.

[0199] SEQ ID NO: 78 is an exemplary Cas13d sequence from Gut_metagenome_contig5590000448.

[0200] SEQ ID NO: 79 is an exemplary Cas13d sequence from Gut_metagenome_contig9269000031.

[0201] SEQ ID NO: 80 is an exemplary Cas13d sequence from Gut_metagenome_contig8537000520.

[0202] SEQ ID NO: 81 is an exemplary Cas13d sequence from Gut_metagenome_contigl845000130.

[0203] SEQ ID NO: 82 is an exemplary Cas13d sequence from gut_metagenome_P13E0k2l20140920_c3000072.

[0204] SEQ ID NO: 83 is an exemplary Cas13d sequence from gut_metagenome_P1 E0k2l20140920_cI000078.

[0205] SEQ ID NO: 84 is an exemplary Cas13d sequence from Gut_metagenome_contigl2990000099.

[0206] SEQ ID NO: 85 is an exemplary Cas13d sequence from Gut_metagenome_contig525000349.

[0207] SEQ ID NO: 86 is an exemplary Cas13d sequence from Gut_metagenome_contig7229000302.

[0208] SEQ ID NO: 87 is an exemplary Cas13d sequence from Gut_metagenome_contig3227000343.

[0209] SEQ ID NO: 88 is an exemplary Cas13d sequence from Gut_metagenome_contig7030000469.

[0210] SEQ ID NO: 89 is an exemplary Cas13d sequence from Gut_metagenome_contig5149000068.

[0211] SEQ ID NO: 90 is an exemplary Cas13d sequence from Gut_metagenome_contig400200045.

[0212] SEQ **ID NO: 91** is an exemplary Cas13d sequence from Gut_metagenome_contigl0420000446.

[0213] SEQ ID NO: 92 is an exemplary Cas13d sequence from new_flavefaciens_strain_XPD3002 (CasRx).

[0214] SEQ ID NO: 93 is an exemplary Cas13d sequence from M26_Gut_metagenome_contig698000307.

[0215] SEQ ID NO: 94 is an exemplary Cas13d sequence from M36_Uncultured_ Eubacterium_sp_TS28_c40956.

[0216] SEQ ID NO: 95 is an exemplary Cas13d sequence from **M**12_gut_metagenome_P25C0k2l20140920 _c134000066.

[0217] SEQ ID NO: 96 is an exemplary Cas13d sequence from human gut metagenome.

[0218] SEQ ID NO: 97 is an exemplary Cas13d sequence from MlO_gut_metagenome _P25C90k2120 l 40920_c2800004 l.

[0219] SEQ ID NO: 98 is an exemplary Cas13d sequence from 30 Ml I_gut_metagenome_P25C7k2120140920_c4078000105.

[0220] SEQ ID NO: 99 is an exemplary Cas13d sequence from gut_metagenome_P25C0k2120l40920_c32000045.

[0221] SEQ ID NO: 100 is an exemplary Cas13d sequence from M13_gut_metagenome _P23C7k2l20140920 _c3000067.

[0222] SEQ ID NO: 101 is an exemplary Cas13d sequence from M5_gut_metagenome_Pl8E90k2120140920.

[0223] SEQ ID NO: 102 is an exemplary Cas13d sequence from M2l_gut_metagenome_Pl8E0k2120140920.

[0224] SEQ ID NO: 103 is an exemplary Cas13d sequence from M7_gut_metagenome _P38C7k2120 l 40920_c484 l 000003.

[0225] SEQ ID NO: 104 is an exemplary Cas13d sequence from Ruminococcus_bicirculans.

[0226] SEQ ID NO: 105 is an exemplary Cas13d sequence.

[0227] SEQ ID NO: 106 is an exemplary Cas13d consensus sequence.

[0228] SEQ ID NO: 107 is an exemplary Cas13d sequence from M18_gut_metagenome _P22EOk2l20140920_c3395000078.

[0229] SEQ ID NO: 108 is an exemplary Cas13d sequence from M17_gut_metagenome_P22E90k2120140920_c114.

[0230] SEQ ID NO: 109 is an exemplary Cas13d sequence from Ruminococcus_sp_CAG57.

[0231] SEQ ID NO: 110 is an exemplary Cas13d sequence from gut_metagenome_Pl 1E90k2120 l 40920_c43000123.

[0232] SEQ ID NO: 111 is an exemplary Cas13d sequence from M6_gut_metagenome_P13E90k2120 l 40920_c7000009.

[0233] SEQ ID NO: 112 is an exemplary Cas13d sequence from Ml9_gut_metagenome_Pl 7E90k2120140920.

[0234] SEQ ID NO: 113 is an exemplary Cas13d sequence from gut_metagenome_Pl7E0k2120l40920,_c87000043.

[0235] SEQ ID NO: 114 is an exemplary human codon optimized Eubacterium siraeum Cas13d nucleic acid sequence.

[0236] SEQ ID NO: 115 is an exemplary human codon optimized Eubacterium siraeum Cas13d nucleic acid sequence with a mutant HEPN domain.

[0237] SEQ ID NO: 116 is an exemplary human codon-optimized Eubacterium siraeum Cas13d nucleic acid sequence with N-terminal **NLS.**

[0238] SEQ ID NO: 117 is an exemplary human codon-optimized Eubacterium siraeum Cas13d nucleic acid sequence with N- and C-terminal NLS tags.

[0239] SEQ ID NO: 118 is an exemplary human codon-optimized uncultured Ruminococcus sp. Cas13d 30 nucleic acid sequence.

[0240] SEQ ID NO: 119 is an exemplary human codon-optimized uncultured Ruminococcus sp. Cas13d nucleic acid sequence with a mutant HEPN domain.

[0241] SEQ ID NO: 120 is an exemplary human codon-optimized uncultured Ruminococcus sp. Cas13d nucleic acid sequence with N-terminal NLS.

[0242] SEQ ID NO: 121 is an exemplary human codon-optimized uncultured Ruminococcus sp. Cas13d nucleic acid sequence with N- and C-terminal NLS tags.

[0243] SEQ ID NO: 122 is an exemplary human codon-optimized uncultured Ruminococcus flavefaciens FDl Cas13d nucleic acid sequence.

[0244] SEQ ID NO: 123 is an exemplary human codon-optimized uncultured Ruminococcus flavefaciens FDl Casl3d nucleic acid sequence with mutated HEPN domain.

[0245] SEQ ID NO: 124 is an exemplary Cas13d nucleic acid sequence from Ruminococcus bicirculans.

[0246] SEQ ID NO: 125 is an exemplary Cas13d nucleic acid sequence from Eubacterium siraeum.

[0247] SEQ ID NO: 126 is an exemplary Cas13d nucleic acid sequence from Ruminococcus flavefaciens FD1.

[0248] SEQ ID NO: 127 is an exemplary Cas13d nucleic acid sequence from Ruminococcus albus.

[0249] SEQ ID NO: 128 is an exemplary Cas13d nucleic acid sequence from Ruminococcus flavefaciens XPD.

[0250] SEQ ID NO: 129 is an exemplary consensus **DR** nucleic acid sequence for *E. siraeum* Cas13d.

[0251] SEQ ID NO: 130 is an exemplary consensus **DR** nucleic acid sequence for *Rum. Sp.* Cas13d.

[0252] SEQ ID NO: 131 is an exemplary consensus **DR** nucleic acid sequence for *Rum. Flavefaciens strain XPD3002* Cas13d (CasRx).

[0253] SEQ ID NOS: 132-137 are exemplary consensus **DR** nucleic acid sequences.

[0254] SEQ ID NO: 138 is an exemplary 50% consensus sequence for seven full-length Cas13d orthologues.

[0255] SEQ ID NO: 139 is an exemplary Cas13d nucleic acid sequence from Gut metagenome PlEO.

[0256] SEQ ID NO: 140 is an exemplary Cas13d nucleic acid sequence from Anaerobic digester.

[0257] SEQ ID NO: 141 is an exemplary Cas13d nucleic acid sequence from Ruminococcus sp. CAG:57.

[0258] SEQ ID NO: 142 is an exemplary human codon-optimized uncultured Gut metagenome PlEO Cas13d nucleic acid sequence.

[0259] SEQ ID NO: 143 is an exemplary human codon-optimized Anaerobic Digester Cas13d nucleic acid sequence.

[0260] SEQ ID NO: 144 is an exemplary human codon-optimized Ruminococcus flavefaciens XPD Cas13d nucleic acid sequence.

[0261] SEQ ID NO: 145 is an exemplary human codon-optimized Ruminococcus albus Cas13d nucleic acid sequence.

[0262] SEQ ID NO: 146 is an exemplary processing of the *Ruminococcus* sp. CAG:57 CRISPR array.

[0263] SEQ ID NO: 147 is an exemplary Cas13d protein sequence from contig emb |OBVH01003037.l, human gut metagenome sequence (also found in WGS contigs emb |OBXZ01000094. 1 | and emb |OBJFO1000033.1.

[0264] SEQ ID NO: 148 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO:147).

[0265] SEQ ID NO: 149 is an exemplary Cas13d protein sequence from contig tpg |DBYI01000091.1 | (Uncultivated Ruminococcus flavefaciens UBA1190 assembled from bovine gut metagenome).

[0266] SEQ ID NOS: 150-152 are exemplary consensus DR nucleic acid sequences (goes with SEQ ID NO: 149).

[0267] SEQ ID NO: 153 is an exemplary Cas13d protein sequence from contig tpg |DJXD01000002.1 | (uncultivated Ruminococcus assembly, UBA7013, from sheep gutmetagenome).

[0268] SEQ ID NO: 154 is an exemplary consensus DRnucleic acid sequence (goes with SEQ ID NO: 153).

[0269] SEQ ID NO: 155 is an exemplary Cas13d protein sequence from contig OGZC01000639.1 (human gut metagenome assembly).

[0270] SEQ ID NOS: 156-177 are exemplary consensus DR nucleic acid sequences (goes with SEQID NO: 155).

[0271] SEQ ID NO: 158 is an exemplary Cas13d protein sequence from contig emb |OHBM01000764.1 (human gut metagenome assembly).

[0272] SEQ ID NO: 159 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO:158).

[0273] SEQ ID NO: 160 is an exemplary Cas13d protein sequence from contig emb |0HCP01000044.1 (human gut metagenome assembly).

[0274] SEQ ID NO: 161 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 160).

[0275] SEQ ID NO: 162 is an exemplary Cas13d protein sequence from contig embl0GDF01008514.1 | (human gut metagenome assembly).

[0276] SEQ ID NO: 163 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 162).

[0277] SEQ ID NO: 164 is an exemplary Cas13d protein sequence from contig emb |0GPN01002610.1 (human gut metagenome assembly).

[0278] SEQ ID NO: 165 is an exemplary consensus DRnucleic acid sequence (goes with SEQ ID NO: 164).

[0279] SEQ ID NO: 166 is an exemplary Cas13d protein sequence from contig NFIR01000008. l (Eubacterium sp. An3, from chicken gut metagenome).

[0280] SEQ ID NO: 167 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 166).

[0281] SEQ ID NO: 168 is an exemplary Cas13d protein sequence from contig NFLV01000009.l (Eubacterium sp. An11 from chicken gut metagenome).

[0282] SEQ ID NO: 169 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 168).

[0283] SEQ ID NOS: 171-174 are an exemplary Cas13d motif sequences.

[0284] SEQ ID NO: 175 is an exemplary Cas13d protein sequence from contig OJMM01002900 human gut metagenome sequence.

[0285] SEQ ID NO: 176 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 175).

[0286] SEQ ID NO: 177 is an exemplary Cas13d protein sequence from contig ODAI011611274.l gut metagenome sequence.

[0287] SEQ ID NO: 178 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 177).

[0288] SEQ ID NO: 179 is an exemplary Cas13d protein sequence from contig OIZX01000427.l.

[0289] SEQ ID NO: 180 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO:179).

[0290] SEQ ID NO: 181 is an exemplary Cas13d protein sequence from contig emb |OCVV012889144.l |.

[0291] SEQ ID NO: 182 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 181).

[0292] SEQ ID NO: 183 is an exemplary Cas13d protein sequence from contig OCTW011587266.l

[0293] SEQ ID NO: 184 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 183).

[0294] SEQ ID NO: 185 is an exemplary Cas13d protein sequence from contig emb |OGNFO 1009141.1.

[0295] SEQ ID NO: 186 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 185).

[0296] SEQ ID NO: 187 is an exemplary Cas13d protein sequence from contig emb |OIEN01002196.1.

[0297] SEQ ID NO: 188 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 187).

[0298] SEQ ID NO: 189 is an exemplary Cas13d protein sequence from contig e-k87_11092736.

[0299] SEQ ID NOS: 190-193 are exemplary consensus DR nucleic acid sequences (goes with SEQ ID NO: 189).

[0300] SEQ ID NO: 194 is an exemplary Cas13d sequence from Gut_metagenome_contig6893000291.

[0301] SEQ ID NOS: 195-197 are exemplary Cas13d motif sequences.

[0302] SEQ ID NO: 198 is an exemplary Cas13d protein sequence from Ga0224415_10007274.

[0303] SEQ ID NO: 199 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 198).

[0304] SEQ ID NO: 200 is an exemplary Cas13d protein sequence from EMG_l0003641.

[0305] SEQ ID NO: 202 is an exemplary Cas13d protein sequence from Ga0129306_1000735.

[0306] SEQ ID NO: 201 is an exemplary consensus **DR** nucleic acid sequence (goes with SEQ ID NO: 200).

[0307] SEQ ID NO: 202 is an exemplary Cas13d protein sequence from Ga0129306_1000735.

[0308] SEQ ID NO: 203 is an exemplary consensus **DR** nucleic acid sequence (goes with SEQ ID NO: 203

[0309] SEQ ID NO: 204 is an exemplary Cas13d protein sequence from GaO129317_1 008067.

[0310] SEQ ID NO: 205 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 204).

[0311] SEQ ID NO: 206 is an exemplary Cas13d protein sequence from Ga0224415_10048792.

[0312] SEQ ID NO: 207 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 206).

[0313] SEQ ID NO: 208 is an exemplary Cas13d protein sequence from 160582958 _gene49834.

[0314] SEQ ID NO: 209 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 208).

[0315] SEQ ID NO: 210 is an exemplary Cas13d protein sequence from 250twins_35838_GL0110300.

[0316] SEQ ID NO: 211 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 210).

[0317] SEQ ID NO: 212 is an exemplary Cas13d protein sequence from 250twins_36050_GLOI58985.

[0318] SEQ ID NO: 213 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 212).

[0319] SEQ ID NO: 214 is an exemplary Cas13d protein sequence from 31009_GL0034153.

[0320] SEQ ID NO: 215 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 214).

[0321] SEQ ID NO: 216 is an exemplary Cas13d protein sequence from 530373_GL0023589.

[0322] SEQ ID NO: 217 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 216).

[0323] SEQ ID NO: 218 is an exemplary Cas13d protein sequence from BMZ-l 1B_GL0037771.

[0324] SEQ ID NO: 219 is an exemplary consensus DR nucleic acid sequence (goes

with SEQ ID NO: 218).

[0325] SEQ ID NO: 220 is an exemplary Cas13d protein sequence from BMZ-l 1B_GL0037915.

[0326] SEQ ID NO: 221 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 220).

[0327] SEQ ID NO: 222 is an exemplary Cas13d protein sequence from BMZ-1 1B_GL00696 l 7.

[0328] SEQ ID NO: 223 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 222).

[0329] SEQ ID NO: 224 is an exemplary Cas13d protein sequence from DLF014_GL0011914.

[0330] SEQ ID NO: 225 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 224).

[0331] SEQ ID NO: 226 is an exemplary Cas13d protein sequence from EYZ-362B_GL0088915.

[0332] SEQ ID NO: 227-228 are exemplary consensus DR nucleic acid sequences (goes with SEQ ID NO: 226).

[0333] SEQ ID NO: 229 is an exemplary Cas13d protein sequence from Ga0099364 10024192.

[0334] SEQ ID NO: 230 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 229).

[0335] SEQ ID NO: 231 is an exemplary Cas13d protein sequence from Ga0187910_10006931.

[0336] SEQ ID NO: 232 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 231).

[0337] SEQ ID NO: 233 is an exemplary Cas13d protein sequence from Ga0187910_10015336.

[0338] SEQ ID NO: 234 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 233).

[0339] SEQ ID NO: 235 is an exemplary Cas13d protein sequence from Ga0187910_10040531.

[0340] SEQ ID NO: 236 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 23).

[0341] SEQ ID NO: 237 is an exemplary Cas13d protein sequence from Ga0187911_10069260.

[0342] SEQ ID NO: 238 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 237).

[0343] SEQ ID NO: 239 is an exemplary Cas13d protein sequence from MH0288_GL0082219.

[0344] SEQ ID NO: 240 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 239).

[0345] SEQ ID NO: 241 is an exemplary Cas13d protein sequence from O2.UC29-0_GL0096317.

[0346] SEQ ID NO: 242 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 241).

[0347] SEQ ID NO: 243 is an exemplary Cas13d protein sequence from PIG-014_GL0226364.

[0348] SEQ ID NO: 244 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 243).

[0349] SEQ ID NO: 245 is an exemplary Cas13d protein sequence from PIG-018_GL0023397.

[0350] SEQ ID NO: 246 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 245).

[0351] SEQ ID NO: 247 is an exemplary Cas13d protein sequence from PIG-025_GL0099734.

[0352] SEQ ID NO: 248 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 247).

[0353] SEQ ID NO: 249 is an exemplary Cas13d protein sequence from PIG-028_GL0185479.

[0354] SEQ ID NO: 250 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 249).

[0355] SEQ ID NO: 251 is an exemplary Cas13d protein sequence from - Ga0224422_10645759.

[0356] SEQ ID NO: 252 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 251).

[0357] SEQ ID NO: 253 is an exemplary Cas13d protein sequence from ODAI chimera.

[0358] SEQ ID NO: 254 is an exemplary consensus DR nucleic acid sequence (goes with SEQ ID NO: 253).

[0359] SEQ **ID NO:** 255 is an HEPN motif.

[0360] SEQ ID NOs: 256 and 257 are exemplary Cas13d nuclear localization signal amino acid and nucleic acid sequences, respectively.

[0361] SEQ ID NOs: 258 and 260 are exemplary SV40 large T antigen nuclear localization signal amino acid and nucleic acid sequences, respectively.

[0362] SEQ ID NO: 259 is a dCas9 target sequence.

[0363] SEQ ID NO: 261 is an artificial Eubacterium siraeum nCasl array targeting ccdB.

[0364] SEQ ID NO: 262 is a full 36 nt direct repeat.

[0365] SEQ ID NOs: 263-266 are spacer sequences.

[0366] SEQ ID NO: 267 is an artificial uncultured Ruminoccus sp. nCasl array targeting ccdB.

[0367] SEQ ID NO: 268 is a full 36 nt direct repeat.

[0368] SEQ ID NOs: 269-272 are spacer sequences.

[0369] SEQ ID NO: 273 is a ccdB target RNA sequence.

[0370] SEQ ID NOs: 274-277 are spacer sequences.

[0371] SEQ ID NO: 278 is a mutated Cas13d sequence, NLS-Ga_053l(trunc)-NLS-HA. This mutant has a deletion of the non-conserved N-terminus.

[0372] SEQ ID NO: 279 is a mutated Cas13d sequence, NES-Ga_053l(trunc)-NES-HA. This mutant has a deletion of the non-conserved N-terminus.

[0373] SEQ ID NO: 280 is a full-length Cas13d sequence, NLS-RfxCas13d-NLS-HA.

[0374] SEQ ID NO: 281 is a mutated Cas13d sequence, NLS-RfxCas13d(del5)-NLS-HA. This mutant has a deletion of amino acids 558-587.

[0375] SEQ ID NO: 282 is a mutated Cas13d sequence, NLS-RfxCas13d(del5.12)-NLS-HA. This mutant has a deletion of amino acids 558-587 and 953-966.

[0376] SEQ ID NO: 283 is a mutated Cas13d sequence, NLS-RfxCas13d(del5.13)-NLS-HA. This mutant has a deletion of amino acids 376-392 and 558-587.

[0377] SEQ ID NO: 284 is a mutated Cas13d sequence, NLS-RfxCas13d(del5.12+5.13)-NLS-HA. This mutant has a deletion of amino acids 376-392, 558-587, and 953-966.

[0378] SEQ ID NO: 285 is a mutated Cas13d sequence, NLS-RfxCas13d(dell3)-NLS-HA. This mutant has a deletion of amino acids 376-392.

[0379] SEQ ID NO: 286 is an effector sequence used to edit expression of ADAR2. Amino acids 1 to 969 are dRfxCas13, aa 970 to 991 are an NLS sequence, and amino acids 992 to 1378 are ADAR2DD.

[0380] SEQ ID NO: 287 is an exemplary HIV NES protein sequence.

[0381] SEQ ID NOS: 288-291 are exemplary Cas13d motif sequences.

[0382] SEQ ID NO: 292 is Cas13d ortholog sequence **MH**_4866.

[0383] SEQ ID NO: 293 is an exemplary Cas13d protein sequence from 037_-_emblOIZA01000315.ll

[0384] SEQ ID NO: 294 is an exemplary Cas13d protein sequence from PIG-022 GL002635 l.

[0385] SEQ ID NO: 295 is an exemplary Cas13d protein sequence from PIG-046_GL0077813.

[0386] SEQ ID NO: 296 is an exemplary Cas13d protein sequence from pig_chimera.

[0387] SEQ ID NO: 297 is an exemplary nuclease-inactive or dead Cas13d (dCas13d) protein sequence from Ruminococcus flavefaciens XPD3002 (CasRx)

[0388] SEQ ID NO: 298 is an exemplary Cas13d protein sequence.

[0389] SEQ ID NO: 299 is an exemplary Cas13d protein sequence from (contig tpg|DJXD01000002.1| ; uncultivated *Ruminococcus* assembly, UBA7013, from sheep gut metagenome)..

[0390] SEQ ID NO: 300 is an exemplary Cas13d direct repeat nucleotide sequence from Cas13d (contig tpg|DJXD01000002.1| ; uncultivated *Ruminococcus* assembly, UBA7013, from sheep gut metagenome (goes with SEQ ID NO: 299).

[0391] SEQ ID NO: 301 is an exemplary Cas13d protein contig emb|OBLI01020244.

[0392] 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 Cas13d proteins and both of which are incorporated by reference herein in their entireties. Also see WO Publication Nos. WO2018/183403 (CasM, which is Cas13d) and WO2019/006471 (Cas13d), which are incorporated herein by reference in their entirety.

[0393] SEQ ID NO: 467 is an exemplary CasM protein from Eubacterium siraeum.

[0394] SEQ ID NO: 468 is an exemplary CasM protein from Ruminococcus sp., isolate 2789STDY5834971.

[0395] SEQ ID NO: 469 is an exemplary CasM protein from Ruminococcus bicirculans.

[0396] SEQ ID NO: 470 is an exemplary CasM protein from Ruminococcus sp., isolate 2789STDY5608892.

[0397] SEQ ID NO: 471 is an exemplary CasM protein from Ruminococcus sp. CAG:57.

[0398] SEQ ID NO: 472 is an exemplary CasM protein from Ruminococcus flavefaciens FD-1.

[0399] SEQ ID NO: 473 is an exemplary CasM protein from Ruminococcus albus strain KH2T6.

[0400] SEQ ID NO: 474 is an exemplary CasM protein from Ruminococcus flavefaciens strain XPD3002.

[0401] SEQ ID NO: 475 is an exemplary CasM protein from Ruminococcus sp., isolate 2789STDY5834894.

[0402] SEQ ID NO: 476 is an exemplary RtcB homolog.

[0403] SEQ ID NO: 477 is an exemplary WYL from Eubacterium siraeum + C-terminal NLS.

[0404] SEQ ID NO: 478 is an exemplary WYL from Ruminococcus sp.isolate 2789STDY5834971 + C-term NLS.

[0405] SEQ ID NO: 479 is an exemplary WYL from Ruminococcus bicirculans + C-term NLS.

[0406] SEQ ID NO: 480 is an exemplary WYL from Ruminococcus sp. isolate 2789STDY5608892 + C-term NLS.

[0407] SEQ ID NO: 481 is an exemplary WYL from Ruminococcus sp. CAG:57 + C-term NLS.

[0408] SEQ ID NO: 482 is an exemplary WYL from Ruminococcus flavefaciens FD-1 + C-term NLS.

[0409] SEQ ID NO: 483 is an exemplary WYL from Ruminococcus albus strain KH2T6 + C-term NLS.

[0410] SEQ ID NO: 484 is an exemplary WYL from Ruminococcus flavefaciens strain XPD3002 + C-term NLS.

[0411] SEQ ID NO: 485 is an exemplary RtcB from Eubacterium siraeum + C-term NLS.

[0412] Exemplary wild type Cas13d proteins of the disclosure may comprise or consist of the amino acid sequence SEQ ID NO: 92 or SEQ ID NO: 298 (Cas13d protein also known as CasRx).

[0413] An exemplary direct repeat sequence of *Ruminococcus flavefaciens* XPD3002 Cas13d (CasRx) comprises the nucleic acid sequence: AACCCCTACCAACTGGTCGGGGTTTGAAAC (SEQ ID NO: 461).

Therapeutic Replacement Genes (Corresponding Disease/Disorder to be Treated)

[0414] Compositions comprising therapeutic replacement genes disclosed herein include any effective gain-or-loss-of-function gene replacement therapies. Exemplary therapeutic replacement genes (corresponding diseases) include, without limitation, genes (diseases/disorders) such as rhodopsin (Retinitis Pigmentosa), PRPF3 – Pre-mRNA Splicing Factor 3 (autosomal dominant Retinitis Pigmentosa), PRPF31 (autosomal dominant Retinitis Pigmentosa), GRN (Frontotemporal dementia (FTD)), SOD1 (ALS), PMP22 (Charcot Marie Tooth Disease), PABPN1 (Oculopharangeal Muscular Dystrophy), KCNQ4 (Hearing Loss), CLRN1 (Usher Syndrome), APOE2 (Alzheimer's Disease), APOE4 (Alzheimer's Disease), BEST1 (Eye Disease), MYBPC3 (Familial Cardiomyopathy), TNNT2 (Familial Cardiomyopathy), and TNNI3 (Familial Cardiomyopathy).

[0415] In some embodiments, therapeutic replacement genes are codon optimized. In some embodiments, the codons relevant to the target site are not codon optimized. In some embodiments, the RNA-targeting proteins of the disclosure ensure cleavage of the mutant allele but not cleavage of the transgene or therapeutic replacement gene.

[0416] Exemplary therapeutic replacement genes and corresponding sequences include, without limitation, the following:

Rhodopsin (Human RHO)

[0417] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Rhodopsin:

MNGTEGPNFYVPFSNATGVVRSPFEYPQYYLAEPWQFSMLAAYMFLLIVLGFPINFL TLYVTVQHKKLRTPLNYILLNLAVADLFMVLGGFTSTLYTSLHGYFVFGPTGCNLEG FFATLGGEIALWSLVVLAIERYVVVCKPMSNFRFGENHAIMGVAFTWVMALACAAP PLAGWSRYIPEGLQCSCGIDYYTLKPEVNNESFVIYMFVVHFTIPMIIIFFCYGQLVFT VKEAAAQQQESATTQKAEKEVTRMVIIMVIAFLICWVPYASVAFYIFTHQGSNFGPIF MTIPAFFAKSAAIYNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASATVSKTETSQ VAPA (SEQ ID NO: 302).

Super Oxide Dismutase 1 (SOD1)

[0418] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Super Oxide Dismutase 1:

MATKAVCVLKGDGPVQGIINFEQKESNGPVKVWGSIKGLTEGLHGFHVHEFGDNTA GCTSAGPHFNPLSRKHGGPKDEERHVGDLGNVTADKDGVADVSIEDSVISLSGDHCII GRTLVVHEKADDLGKGGNEESTKTGNAGSRLACGVIGIAQ (SEQ ID NO:303).

Peripheral Myelin Protein 22 (PMP22)

[0419] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Peripheral Myelin Protein 22:

MLLLLLSIIVLHVAVLVLLFVSTIVSQWIVGNGHATDLWQNCSTSSSGNVHHCFSSSP NEWLQSVQATMILSIIFSILSLFLFFCQLFTLTKGGRFYITGIFQILAGLCVMSAAAIYT VRHPEWHLNSDYSYGFAYILAWVAFPLALLSGVIYVILRKRE (SEQ ID NO: 304).

Poly(A) Binding Protein Nuclear 1 (PABPN1)

[0420] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Poly(A) Binding Protein Nuclear 1:

MAAAAAAAAAAGAAGGRGSGPGRRRHLVPGAGGEAGEGAPGGAGDYGNGLESEE LEPEELLLEPEPEPEPEEEPPRPRAPPGAPGPGPGSGAPGSQEEEEEPGLVEGDPGDGAI

EDPELEAIKARVREMEEEAEKLKELQNEVEKQMNMSPPPGNAGPVIMSIEEKMEAD
ARSIYVGNVDYGATAEELEAHFHGCGSVNRVTILCDKFSGHPKGFAYIEFSDKESVR
TSLALDESLFRGRQIKVIPKRTNRPGISTTDRGFPRARYRARTTNYNSSRSRFYSGFNS
RPRGRVYRGRARATSWYSPY (SEQ ID NO: 305).

Potassium Voltage-Gated Channel Subfamily Q Member 4 (KCNQ4)

[0421] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Potassium Voltage-Gated Channel Subfamily Q Member 4:

MAEAPPRRLGLGPPPGDAPRAELVALTAVQSEQGEAGGGGSPRRLGLLGSPLPPGAP
LPGPGSGSGSACGQRSSAAHKRYRRLQNWVYNVLERPRGWAFVYHVFIFLLVFSCL
VLSVLSTIQEHQELANECLLILEFVMIVVFGLEYIVRVWSAGCCCRYRGWQGRFRFA
RKPFCVIDFIVFVASVAVIAAGTQGNIFATSALRSMRFLQILRMVRMDRRGGTWKLL
GSVVYAHSKELITAWYIGFLVLIFASFLVYLAEKDANSDFSSYADSLWWGTITLTTIG
YGDKTPHTWLGRVLAAGFALLGISFFALPAGILGSGFALKVQEQHRQKHFEKRRMP
AANLIQAAWRLYSTDMSRAYLTATWYYYDSILPSFRELALLFEHVQRARNGGLRPL
EVRRAPVPDGAPSRYPPVATCHRPGSTSFCPGESSRMGIKDRIRMGSSQRRTGPSKQH
LAPPTMPTSPSSEQVGEATSPTKVQKSWSFNDRTRFRASLRLKPRTSAEDAPSEEVAE
EKSYQCELTVDDIMPAVKTVIRSIRILKFLVAKRKFKETLRPYDVKDVIEQYSAGHLD
MLGRIKSLQTRVDQIVGRGPGDRKAREKGDKGPSDAEVVDEISMMGRVVKVEKQV
QSIEHKLDLLLGFYSRCLRSGTSASLGAVQVPLFDPDITSDYHSPVDHEDISVSAQTLS
ISRSVSTNMD (SEQ ID NO: 306).

Clarin 1 (CLRN1)

[0422] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Clarin 1:

MPSQQKKIIFCMAGVFSFACALGVVTALGTPLWIKATVLCKTGALLVNASGQELDKF
MGEMQYGLFHGEGVRQCGLGARPFRFSFFPDLLKAIPVSIHVNVILFSAILIVLTMVG
TAFFMYNAFGKPFETLHGPLGLYLLSFISGSCGCLVMILFASEVKIHHLSEKIANYKEG
TYVYKTQSEKYTTSFWVIFFCFFVHFLNGLLIRLAGFQFPFAKSKDAETTNVAADLM
(SEQ ID NO: 307).

Apolipoprotein 2 (APOE2)

[0423] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Apolipoprotein 2:

MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDY
LRWVQTLSEQVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETRAR

LSKELQAAQARLGADMEDVCGRLVQYRGEVQAMLGQSTEELRVRLASHLRKLRKR
LLRDADDLQKCLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGSLAGQPL
QERAQAWGERLRARMEEMGSRTRDRLDEVKEQVAEVRAKLEEQAQQIRLQAEAFQ
ARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH (SEQ ID NO: 308).

Apolipoprotein 4 (APOE4)

[0424] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Apolipoprotein 4:

MKVLWAALLVTFLAGCQAKVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDY
LRWVQTLSEQVQEELLSSQVTQELRALMDETMKELKAYKSELEEQLTPVAEETRAR
LSKELQAAQARLGADMEDVRGRLVQYRGEVQAMLGQSTEELRVRLASHLRKLRKR
LLRDADDLQKRLAVYQAGAREGAERGLSAIRERLGPLVEQGRVRAATVGSLAGQPL
QERAQAWGERLRARMEEMGSRTRDRLDEVKEQVAEVRAKLEEQAQQIRLQAEAFQ
ARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH (SEQ ID NO: 309).

Bestrophin-1 (BEST1)

[0425] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Bestrophin-1:

MTITYTSQVANARLGSFSRLLLCWRGSIYKLLYGEFLIFLLCYYIIRFIYRLALTEEQQL
MFEKLTLYCDSYIQLIPISFVLGFYVTLVVTRWWNQYENLPWPDRLMSLVSGFVEGK
DEQGRLLRRTLIRYANLGNVLILRSVSTAVYKRFPSAQHLVQAGFMTPAEHKQLEKL
SLPHNMFWVPWVWFANLSMKAWLGGRIRDPILLQSLLNEMNTLRTQCGHLYAYD
WISIPLVYTQVVTVAVYSFFLTCLVGRQFLNPAKAYPGHELDLVVPVFTFLQFFYV
GWLKVAEQLINPFGEDDDDFETNWIVDRNLQVSLLAVDEMHQDLPRMEPDMYWN
KPEPQPPYTAASAQFRRASFMGSTFNISLNKEEMEFQPNQEDEEDAHAGIIGRFLGLQ
SHDHHPPRANSRTKLLWPKRESLLHEGLPKNHKAAKQNVRGQEDNKAWKLKAVD
AFKSAPLYQRPGYYSAPQTPLSPTPMFFPLEPSAPSKLHSVTGIDTKDKSLKTVSSGA
KKSFELLSESDGALMEHPEVSQVRRKTVEFNLTDMPEIPENHLKEPLEQSPTNIHTTL
KDHMDPYWALENRDEAHS (SEQ ID NO: 310).

Cardiac Myosin-Binding Protein-C (MYBPC3)

[0426] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Cardiac Myosin-Binding Protein-C:

MPEPGKKPVSAFSKKPRSVEVAAGSPAVFEAETERAGVKVRWQRGGSDISASNKYG
LATEGTRHTLTVREVGPADQGSYAVIAGSSKVKFDLKVIEAEKAEPMLAPAPAPAEA
TGAPGEAPAPAAELGESAPSPKGSSSAALNGPTPGAPDDPIGLFVMRPQDGEVTVGG

SITFSARVAGASLLKPPVVKWFKGKWVDLSSKVGQHLQLHDSYDRASKVYLFELHI
TDAQPAFTGSYRCEVSTKDKFDCSNFNLTVHEAMGTGDLDLLSAFRRTSLAGGGRRI
SDSHEDTGILDFSSLLKKRDSFRTPRDSKLEAPAEEDVWEILRQAPPSEYERIAFQYGV
TDLRGMLKRLKGMRRDEKKSTAFQKKLEPAYQVSKGHKIRLTVELADHDAEVKWL
KNGQEIQMSGSKYIFESIGAKRTLTISQCSLADDAAYQCVVGGEKCSTELFVKEPPVL
ITRPLEDQLVMVGQRVEFECEVSEEGAQVKWLKDGVELTREETFKYRFKKDGQRHH
LIINEAMLEDAGHYALCTSGGQALAELIVQEKKLEVYQSIADLMVGAKDQAVFKCE
VSDENVRGVWLKNGKELVPDSRIKVSHIGRVHKLTIDDVTPADEADYSFVPEGFACN
LSAKLHFMEVKIDFVPRQEPPKIHLDCPGRIPDTIVVVAGNKLRLDVPISGDPAPTVIW
QKAITQGNKAPARPAPDAPEDTGDSDEWVFDKKLLCETEGRVRVETTKDRSIFTVEG
AEKEDEGVYTVTVKNPVGEDQVNLTVKVIDVPDAPAAPKISNVGEDSCTVQWEPPA
YDGGQPILGYILERKKKKSYRWMRLNFDLIQELSHEARRMIEGVVYEMRVYAVNAI
GMSRPSPASQPFMPIGPPSEPTHLAVEDVSDTTVSLKWRPPERVGAGGLDGYSVEYC
PEGCSEWVAALQGLTEHTSILVKDLPTGARLLFRVRAHNMAGPGAPVTTTEPVTVQ
EILQRPRLQLPRHLRQTIQKKVGEPVNLLIPFQGKPRPQVTWTKEGQPLAGEEVSIRN
SPTDTILFIRAARRVHSGTYQVTVRIENMEDKATLVLQVVDKPSPPQDLRVTDAWGL
NVALEWKPPQDVGNTELWGYTVQKADKKTMEWFTVLEHYRRTHCVVPELIIGNGY
YFRVFSQNMVGFSDRAATTKEPVFIPRPGITYEPPNYKALDFSEAPSFTQPLVNRSVIA
GYTAMLCCAVRGSPKPKISWFKNGLDLGEDARFRMFSKQGVLTLEIRKPCPFDGGIY
VCRATNLQGEARCECRLEVRVPQ (SEQ ID NO: 311).

Cardiac Troponin T2 (TNNT2)

[0427] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Cardiac Troponin T2:

MSDEEVEQVEEQYEEEEEAQEEAAEVHEEVHEPEEVQEDTAEEDAEEEKPRPKLTAP
KIPEGEKVDFDDIQKKRQNKDLMELQALIDSHFEARKKEEEELVALKERIEKRRAER
AEQQRIRAEKERERQNRLAEEKARREEEDAKRRAEDDLKKKKALSSMGANYSSYLA
KADQKRGKKQTAREMKKKILAERRKPLNIDHLGEDKLRDKAKELWETLHQLEIDKF
EFGEKLKRQKYDITTLRSRIDQAQKHSKKAGTPAKGKVGGRWK (SEQ ID NO: 312).

Cardiac Troponin TI3 (TNNI3)

[0428] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Cardiac Troponin TI3:

MADGSSDAAREPRPAPAPIRRRSSNYRAYATEPHAKKKSKISASRKLQLKTLLLQIAK
QELEREAEERRGEKGRALSTRCQPLELAGLGFAELQDLCRQLHARVDKVDEERYDIE

AKVTKNITEIADLTQKIFDLRGKFKRPTLRRVRISADAMMQALLGARAKESLDLRAH LKQVKKEDTEKENREVGDWRKNIDALSGMEGRKKKFES (SEQ ID NO: 313).

Pre-mRNA processing factor 31 (PRPF31)

[0429] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of pre-mRNA processing factor 31 (PRPF31) (autosomal dominant Retinitis Pigmentosa):

MSLADELLADLEEAAEEEEGGSYGEEEEEPAIEDVQEETQLDLSGDSVKTIAKLWDS KMFAEIMMKIEEYISKQAKASEVMGPVEAAPEYRVIVDANNLTVEIENELNIIHKFIR DKYSKRFPELESLVPNALDYIRTVKELGNSLDKCKNNENLQQILTNATIMVVSVTAS TTQGQQLSEEELERLEEACDMALELNASKHRIYEYVESRMSFIAPNLSIIIGASTAAKI MGVAGGLTNLSKMPACNIMLLGAQRKTLSGFSSTSVLPHTGYIYHSDIVQSLPPDLR RKAARLVAAKCTLAARVDSFHESTEGKVGYELKDEIERKFDKWQEPPPVKQVKPLP APLDGQRKKRGGRRYRKMKERLGLTEIRKQANRMSFGEIEEDAYQEDLGFSLGHLG KSGSGRVRQTQVNEATKARISKTLQRTLQKQSVVYGGKSTIRDRSSGTASSVAFTPL QGLEIVNPQAAEKKVAEANQKYFSSMAEFLKVKGEKSGLMST (SEQ ID NO: 487).

Progranulin (GRN) (FTD)

[0430] Exemplary therapeutic replacement genes may comprise or consist of the amino acid sequence of Progranulin (GRN) (frontotemporal dementia (FTD)):

MWTLVSWVALTAGLVAGTRCPDGQFCPVACCLDPGGASYSCCRPLLDKWPTTLSR HLGGPCQVDAHCSAGHSCIFTVSGTSSCCPFPEAVACGDGHHCCPRGFHCSADGRSC FQRSGNNSVGAIQCPDSQFECPDFSTCCVMVDGSWGCCPMPQASCCEDRVHCCPHG AFCDLVHTRCITPTGTHPLAKKLPAQRTNRAVALSSSVMCPDARSRCPDGSTCCELP SGKYGCCPMPNATCCSDHLHCCPQDTVCDLIQSKCLSKENATTDLLTKLPAHTVGD VKCDMEVSCPDGYTCCRLQSGAWGCCPFTQAVCCEDHIHCCPAGFTCDTQKGTCE QGPHQVPWMEKAPAHLSLPDPQALKRDVPCDNVSSCPSSDTCCQLTSGEWGCCPIPE AVCCSDHQHCCPQGYTCVAEGQCQRGSEIVAGLEKMPARRASLSHPRDIGCDQHTS CPVGQTCCPSLGGSWACCQLPHAVCCEDRQHCCPAGYTCNVKARSCEKEVVSAQP ATFLARSPHVGVKDVECGEGHFCHDNQTCCRDNRQGWACCPYRQGVCCADRRHC CPAGFRCAARGTKCLRREAPRWDAPLRDPALRQLL (SEQ ID NO: 488).

gRNA Target Sequences

[0431] In some embodiments of the compositions of the disclosure, a target sequence of an RNA molecule comprises a pathogenic sequence. In some embodiments, the target RNA comprises a sequence motif corresponding to the spacer sequence of the guide RNA of the

RNA-guided RNA-binding protein. In some embodiments, one or more spacer sequences are used to target one or more target sequences. In some embodiments, multiple spacers are used to target multiple target RNAs. Such target RNAs can be different taget sites within the same RNA molecule or can be different target sites within different RNA molecules. Spacer sequences can also target non-coding RNA. In some embodiments, multiple promoters, e.g., pol III promoters) can be used to drive multiple spacers in a gRNA for targeting multiple target RNAs. In some embodiments, when the target RNA(s) or target sequence motif(s) is/are targeted and knocked down by the RNA-targeting compositions disclosed herein, then pathogenic or disease-causing gain-or-loss-of-function mutations are destroyed.

[0432] In some embodiments of the compositions and methods of the disclosure, the sequence motif of the target RNA is a signature of a disease or disorder.

[0433] 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.

[0434] A target sequence motif of the disclosure may comprise, consist of, be situated by, or be associated with 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.

[0435] A target 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.

[0436] A target 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.

[0437] A target 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.

[0438] 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. In some embodiments, the target sequence of an RNA molecule comprises or consists of between 20-30 nucleotides or nucleic acid bases, inclusive of the endpoints. In some embodiments, the target sequence of an RNA molecule comprises or consists of about 26 nucleotides or nucleic acid bases, inclusive of the endpoints.

[0439] 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.

[0440] 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.

[0441] 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. In some embodiments of the compositions and methods of the disclosure, one or more target sequences of an RNA molecule binds to one or more guide RNA spacer sequences of the disclosure.

[0442] 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.

[0443] 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.

[0444] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding Rhodospin protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
TGGGGTTTTT CCCATTCCCA GGACTGCCTC CTCCACCTCT AGCCCCAGGG GACTCTGTGC        60
TGCTTGGCTC TGCTCATTGC TCAATCCAGC CATCCCAGGG TCAGGGATCA GGTGGAAGCT       120
GGCAGTTTCA ATCTATCCTG TGGATAGAGT GTGAAAGCAA CAAAACCCAC CACCGTTAAT       180
ACCAACATAG GAGCTGAGCT TTTAATGGCC CAATTTGCCT TAGTCTCCAG GCAGAGCTGG       240
GTAAAGCTAG AGCTTCTGGC TTTGCTTATA TGGAGAAGGG GGAGCAGTTA CTGAGGCAGC       300
TTAATTCTGA CACCTCAGAG ATGTGGCCAG CTTTTTGGAG CAGATTCTCC AGAATGGAGA       360
ATGGACTAGC AACTGCTGAA GATGGGCTTG TCTGGCAAGG GAAACTGGAA ACTGGGGCCC       420
ATGAACATCC CCAAGGAAGG TAGGCCCAGT GGAATTTCCC ACTCTTTGTT TCTAAGCTCT       480
TCGAGATAAG GATGACATCA GGGACTCAGC TGTTAATTAA ATGTGGGCGG GTGAGCATGG       540
CTTCTAGAGG CTCCATGCTC TAGATGCTGG GACCCAGGTG CTAGAGCAAA AGAGCAGGTG       600
GCTTCCAGAG GCTGAGAGAA AGGCCTGTCT CTCCATAGGC CACATTGGGA AGGGGAGGCA       660
CGGGACCTGG GGCCCCACAC TAGGGGTGAG ACCCCAGGCC CAATCTCACC CTCATTGGGA       720
ACTTGGCCTT CACCGTCCCC CTCCCCCAGT GTTGTTTTTT CAGGTCTGAT GACTGCATTC       780
TGCATTCCTG TGACTGTCCC TGCCTACAGC CCAACCCCCA GCCCTGGTCT GGCCTTGATG       840
CCTAGCTAAT TTTTAAAAAC CTGCCCCAAG GTTGGGTGAA ACCCCATCAT CTGAATGCCC       900
AATCTCAAAA TGTTCACTAT CAGGAGGTGA TAATCATAGT AATTAACTAG TTACATTAAT       960
TGATGTTATT CACAACATTA ACTAGAATCT GTACAGCTTC TTGCTATTTA CAAAGTGCTG      1020
AAACACACAC ATAGACACAC ACACACCTCT TTTGGTCTTC TCAGTAGCTG CGTGTCGGCA      1080
GGACCAGGGA TCTGGGATTT CCATTTTATA GGAGAAGAAA GTGAGGCCCA GGGAGGGAAA      1140
AACAACTGCT CCATATCATT AGCCAAGTAT GAGTTGCTGC TGCTGCGAGG GTCTGAGAGG      1200
ATAGATATGT TCTCCCTTCC CATTCATTCC TCCATTCCTT CCTGCATCCA TCCAGCATTT      1260
ATTAAGCACC TACTGTGTGC CCCATTCTGT GCTAGACACT TATCCCTAAG CTGGGACACT      1320
TTTCCAGAAA GCAAGAATCC TCGTGTTCCT GAAAGATGAG TTGGGAGGAG GAGGGGCACA      1380
CATCCCGCTG GCCTTGGGGA ACGTGGGACT CCAGATCAGT AGGTCTTGGT GGATGTCCCT      1440
TCTCAGGCTG TCCCAGGTGA GTGAGGAGCC TCATTAATTA TTTCTTAAAA AAAAAAAAAA      1500
AATTAAGGAG CCTATGTGAC TTCGTTCATT CTGCACAGGC GCTGCTCCTG GTGGGATGGC      1560
TGTGGCTGGG GGAAGGTGTA GGGGATGGGA GACGCCTATA GTCGGCCACA GAGTCCTAGG      1620
CAGGTCTTAG GCCGGGGCCA CCTGGCTCGT CTCCGTCTTG GACACGGTAG CAGAGGCCTC      1680
ATCGTCACCC AGTGGGTTCT TGCCGCAGCA GATGGTGGTG AGCATGCAGT TCCGGAACTG      1740
CTTGTTCATC ATGATATAGA TGACAGGGTT GTAGATGGCG GCGCTCTTGG CAAAGAACGC      1800
TGGGATGGTC ATGAAGATGG GACCGAAGTT GGAGCCCTGG TGGGTGAAGA TGTAGAATGC      1860
CACGCTGGCG TAGGGCACCC AGCAGATCAG GAAAGCGATG ACCATGATGA TGACCATGCG      1920
GGTGACCTCC TTCTCTGCCT TCTGTGTGGT GGCTGACTCC TGCTGCTGGG CAGCGGCCTC      1980
CTTGACGGTG AAGACGAGCT GCCCATAGCA GAAAAAGATG ATAATCATGG GGATGGTGAA      2040
GTGGACCACG AACATGTAGA TGACAAAAGA CTCGTTGTTG ACCTCCGGCT TGAGCGTGTA      2100
GTAGTCGATT CCACACGAGC ACTGCAGGCC CTCGGGGATG TACCTGGACC AGCCGGCGAG      2160
TGGGGGTGCG GCGCAGGCCA GCGCCATGAC CCAGGTGAAG GCAACGCCCA TGATGGCATG      2220
GTTCTCCCCG AAGCGGAAGT TGCTCATGGG CTTACACACC ACCACGTACC GCTCGATGGC      2280
CAGGACCACC AAGGACCACA GGGCAATTTC ACCGCCCAGG GTGGCAAAGA AGCCCTCCAA      2340
ATTGCATCCT GTGGGCCCGA AGACGAAGTA TCCATGCAGA GAGGTGTAGA GGGTGCTGGT      2400
GAAGCCACCT AGGACCATGA AGAGGTCAGC CACGGCTAGG TTGAGCAGGA TGTAGTTGAG      2460
AGGCGTGCGC AGCTTCTTGT GCTGGACGGT GACGTAGAGC GTGAGGAAGT TGATGGGGAA      2520
GCCCAGCACG ATCAGCAGAA ACATGTAGGC GGCCAGCATG GAGAACTGCC ATGGCTCAGC      2580
CAGGTAGTAC TGTGGGTACT CGAAGGGGCT GCGTACCACA CCCGTCGCAT TGGAGAAGGG      2640
CACGTAGAAG TTAGGGCCTT CTGTGCCATT CATGGCTGTG GCCCTTGTGG CTGACCCGTG      2700
GCTGCTCCCA CCCAAGAATG CTGCGAAGGC CTGAGCTCAG CCACTCAGGG CTCCAGCTGG      2760
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ATGACTCT 2768

(SEQ ID NO: 314).

[0445] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a Rhodopsin protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 619 to SEQ ID NO: 3361.

[0446] In some embodiments, exemplary gRNA spacer sequences and corresponding Rho target sequences comprises or consists of the sequences as deailed in table 1.

[0447] Table 1: Spacer sequences and target sequences used for Rhodopsin targeting

Spacer	Spacer Sequences	Target Sequences
Rho guide 1	ACATGTAGATGACAAAAGACTCGTTG (SEQ ID NO: 465)	CAACGAGTCTTTTGTCATCTACATGT (SEQ ID NO: 462)
Rho guide 2	TGAAGATGTAGAATGCCACGCTGGCG (SEQ ID NO: 409)	CGCCAGCGTGGCATTCTACATCTTCA (SEQ ID NO: 463)
Rho guide 3	ACTGCTTGTTCATCATGATATAGATG (SEQ ID NO: 466)	CATCTATATCATGATGAACAAGCAGT (SEQ ID NO: 464)

[0448] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding SOD1 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
ttttttttttt ttttttttag tttgaatttg gattctttta atagcctcat aataagtgcc        60
atacagggtt tttattcaca ggcttgaatg acaaagaaat tctgacaagt ttaataccca       120
tctgtgattt aagtctggca aaatacaggt cattgaaaca gacattttaa ctgagtttta       180
taaaactata caaatcttcc aagtgatcat aaatcagttt ctcactacag gtactttaaa       240
gcaactctga aaaagtcaca caattacact tttaagatta cagtgtttaa tgtttatcag       300
gatacatttc tacagctagc aggataacag atgagttaag gggcctcaga ctacatccaa       360
gggaatgttt attgggcgat cccaattaca ccacaagcca aacgacttcc agcgtttcct       420
gtctttgtac tttcttcatt tccacctttg cccaagtcat ctgctttttc atggaccacc       480
agtgtgcggc caatgatgca atggtctcct gagagtgaga tcacagaatc ttcaatagac       540
acatcggcca caccatcttt gtcagcagtc acattgccca agtctccaac atgcctctct       600
tcatcctttg gcccaccgtg ttttctggat agaggattaa agtgaggacc tgcactggta       660
cagcctgctg tattatctcc aaactcatga acatggaatc catgcaggcc ttcagtcagt       720
cctttaatgc ttccccacac cttcactggt ccattacttt ccttctgctc gaaattgatg       780
atgccctgca ctgggccgtc gcccttcagc acgcacacgg ccttcgtcgc cataactcgc       840
taggccacgc cgaggtcctg gttccgagga ctgcaacgga aaccccagac gctgcaggag       900
actacgacgc aaaccagcac cccgtctccg cgactacttt ataggccaga cctccgcgcc       960
tcgcccactc tggccccaaa c                                                 981
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(SEQ ID NO: 315).

[0449] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a SOD1 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 3362 to SEQ ID NO: 4317.

[0450] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding PMP22 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
TGAGTTACTC TGATGTTTAT TTTAATGCAT CTTAGTCCAC ACAGTTGGTA TAAAATCAGA        60
AAATGCAAAG CAAAAACAAA AGGTCTGGAG TCTTAGCATC AGAAGGGCAC CATATATACA       120
TCTACAGTTG GTGGCCAATA CAAGTCATTG CCAGACAGTC CTTGGAGGCA CAGAACAGCC       180
TAGACCCAGC CAAGCTCTAG GAACTCACGG TCCCAAGGAG TCTAGACGCT TGTTCTGATG       240
CTCCGACCGT AAGAAAAATG TGGGAGTGAT GAAGGCTTTA TGATTTACTC ATTATAGTAA       300
TAATAGCAGC CTAGCTAGGT ACAAAAGCAG TTATAAACCA TTTATATTAC ACAGAATTAT       360
TCAGGTCTCC ATTCTATCTT ATGTTGTAAA ATTGTTAATT GGATTTCCAG TGAGTTGTTT       420
AGATGATTAG TGATAATAAG GAATGGTAAA TCCATAGCAC CATTTCAAAG ACTTGTTGTC       480
ACTGATTTCT CATTTAGATG TGTGACGAAG ATACTCCACC TGTAAGGGCA AGTATGCCAA       540
TGCCACAAGC CGTGTTTTTG CAAGGGCTCC AGTTTGGGCA TTTTGTCCGT GTGCGCGTAA       600
AGCTTCACAC AGAGGTTCGG GCAGCGGCTG TTTCTGTTGG ATGCACTGGG TCACCCACCA       660
GAAAAGGGCT TTTGGACATT TGGGGTTTCT ACCCACACTT TGGTTTTCTA AATGAGGTGG       720
ACTGGGAGGG AGGTATCTTC TTTCAGATGA AAGGGAAGGG GCGAGATGGA GTTATCTTAT       780
TTCTGGGTAA AACAAAACAA ACAAACAAAA AACAAAACAA AAATACTGAG CTGGATTATA       840
CTGTTAGGAT GTAAAGTTCC TTAGCTACTT CTTTAAGGCT CAACACGAGG CTGATGGTCA       900
ACATAAAAAG CAAACAATAC TATGTACATA TATGTAAAAA GTGTTATAAA TAGGTTTTAT       960
AAACCGGAGA TATTATATAC ATCTTCAATC AACAGCAACC CCCACCTCCA CTGCTTTCTG      1020
TTTGGTTTGG TTTGAGTTTG GGATTTTGGG CTAGCTCTTT TTTCTTTGTC TGCTTTCTGT      1080
TTTCCCTTCC TCCCTTCCCT ATGTACGCTC AGAGCCTCAG ACAGACCGTC TGGGCGCCTC      1140
ATTCGCGTTT CCGCAAGATC ACATAGATGA CACCGCTGAG AAGGGCCAGG GGGAAGGCCA      1200
CCCAGGCCAG GATGTAGGCG AAACCGTAGG AGTAATCCGA GTTGAGATGC CACTCCGGGT      1260
GCCTCACCGT GTAGATGGCC GCAGCACTCA TCACGCACAG ACCAGCAAGA ATTTGGAAGA      1320
TTCCAGTGAT GTAAAACCTG CCCCCCTTGG TGAGGGTGAA GAGTTGGCAG AAGAACAGGA      1380
ACAGAGACAG AATGCTGAAG ATGATCGACA GGATCATGGT GGCCTGGACA GACTGCAGCC      1440
ATTCGTTTGG TGATGATGAG AAACAGTGGT GGACATTTCC TGAGGAAGAG GTGCTACAGT      1500
TCTGCCAGAG ATCAGTTGCG TGTCCATTGC CCACGATCCA TTGGCTGACG ATCGTGGAGA      1560
CGAACAGCAG CACCAGCACC GCGACGTGGA GGACGATGAT ACTCAGCAAC AGGAGGAGCA      1620
TTCTGGCGGC AAGTTCTGCT CAGCGGAGTT TCTGCCCGGC CAAACAGCGT AACCCCTTCT      1680
TCCAAGCAGA TTTCTTTGCA GCCAAATGCA AGGGATGTTA AGGCAAGACC CTCCCCACAG      1740
GGCAGTCAGA GACCCGCAGC CGACAGACTA AGCCTGCAGC TTCCAACCAG GCTCCCCGAG      1800
ATGTTCCCTG GTGGTGCTCC CTGTAACT                                         1828
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(SEQ ID NO: 316).

[0451] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a PMP22 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 4318 to SEQ ID NO: 6120.

[0452] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding PABPN1 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
TTTTTTTTTT TTTTTTTTAC ACCCAAAAGG CCAAACAATT TTTATTTTCA AAAACAACTT        60
TATTCATGAC ACATATTAAA AAAAAATTCC CACCCCTGGA AATGAGCTAA AAAAATAAAC       120
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AAAATCCACC TCCCACCTCC CTGTTCCCAC TTCCTCCCAT TCCCTCCAAA TAAAAGGGAA       180
AAAAGGCAAA GGAAAAAAAA AAAAAACAAA AAAACAAAAC AACTGAAAAA CAAAAACACC       240
CCTAAACCCC CCAAAACAAG GTAGTGCATT TCCCCAGGGG GAAGGGGAAT TTACACTGGA       300
GCCGCTGGGA GCGGAACGGA GATCTTCCGG CTACAGAAAC CTGCAAAGAA AGACACTCAA       360
AACAGAAAAA GAAACACAAA AGGAAACAAA ATAGATCACC AGGCAATCTG GAGGGGCAGG       420
GAGCCGGAGA AGAGGGGTGG GGTGGGTGGT AGACCTGGCT GGACAGGAGC AGGCAGGAGG       480
GGACTGTGAA AGGCGAGGAG AAGACGGAGG GAAGGTAACA AGCAGAACAG TTTGGTGTCC       540
TTCCAGAGCC CTGGGTAAAA AAAAAAACCT CCTACCACCC ACGCCCACCT ACCCTTGAGC       600
AGCCCCCAAG GGGGTGAAGT GGGGCAGGGA AACATGGGCA GCAGCTTGCG CAGTTGAGAC       660
GTGTCCATGG CGAATCCCCA GAGTGAATAA GCAGCCCCCT GCCCCACTCC CTGGGCCTTC       720
CCCTACTCCC CAAAGCAGGT CCCTCCTCAG CAGTTAGTTA TGGGATTCTC CCCCCTTCCA       780
CAGTATATCT TTTTTTTAAA AAATATTTTT TTTCCATCAA GGTCATCTTC TGTTTTTCTT       840
TTTTTTTTTT TTAATTCTTT TTTTTTTCCT TCTTTCCTCT TTTTTTCCTC TCTCTCCTCC       900
TAATACACAC TTTTTTTAGT AAGGGGAATA CCATGATGTC GCTCTAGCCC GGCCCCTGTA       960
GACGCGACCC CGGGGCCTGC TGTTAAAACC ACTGTAGAAT CGAGAGCGGG AGCTGTTGTA      1020
GTTGGTGGTC CGGGCGCGGT AGCGGGCTCG TGGAAAACCC CGGTCTGTTG TGCTGATGCC      1080
TGGTCTGTTG GTTCGTTTTG GGATCACCTT GATTTGCCTT CCTCTAAATA GGGACTCATC      1140
TAAGGCCAAG GAAGTCCTCA CTGACTCTTT GTCTGAGAAC TCTATATACG CAAACCCTTT      1200
GGGATGGCCA CTAAATTTGT CACACAGTAT GGTAACACGG TTGACTGAAC CACAGCCATG      1260
AAAGTGAGCT TCCAGCTCTT CTGCTGTTGC ACCATAGTCC ACATTGCCAA CATAGATGGA      1320
ACGGGCATCA GCCTCCATCT TCTCCTCAAT GGACATGATC ACCGGGCCAG CATTGCCTGG      1380
AGGTGGACTC ATATTCATCT GCTTCTCTAC CTCGTTCTGT AGCTCCTTTA GCTTCTCAGC      1440
TTCTTCCTCC ATCTCCCTGA CTCGAGCTTT GATAGCTTCC AGCTCCGGGT CCTCAATGGC      1500
GCCGTCCCCC GGGTCACCCT CGACCAGTCC CGGCTCCTCC TCCTCCTCTT GGCTGCCGGG      1560
GGCTCCCGAA CCAGGCCCAG GGCCCGGAGC TCCCGGGGGG GCGCGGGGCC GGGGCGGCTC      1620
CTCTTCGGGC TCGGGCTCCG GCTCGGGCTC CAGCAGCAGC TCCTCAGGCT CCAGTTCCTC      1680
AGACTCCAGG CCGTTCCCGT AGTCCCCTGC GCCCCCCGGG GCCCCCTCCC CGGCCTCCCC      1740
ACCGGCCCCG GGCACAAGAT GGCGCCGCCG CCCCGGCCCG GAGCCCCGAC CGCCCGCAGC      1800
CCCCGCTGCT GCTGCCGCCG CCGCCGCCGC CGCCATCGCC GCTCAGACTG GGGCCCGCCG      1860
CCCGGCGATT GGAGAGCTGC GCCGGCCACG CCGAGGACTC ATTAGTCAAG CTGCCTGCCC      1920
GTCACCATGA GCTAGTACTC CATTGGGGAA TATTACTTGG CAATCAAATA AGGCCCCACC      1980
TCTAAGGCGG GGCACTGCGC CAAATTCTCA AATCCCGGTA GGGGAAATCT GCCTGTCAAT      2040
CAACACGCGT CCCACCTCCT ATCGAGTCCT TAGGTAATAA TACCGCCACG CTGTGACGAT      2100
ATTCCTGCTT CTCCCCGGCC TACGGGCGGG CCCGCGAAGT ATGGGACGCT CCGTGATTGG      2160
CCCTAGCTAG GCGACTGGAA AGGACCAATC TTCCGATCGC CTCACCGCAG TGGCCCAGTC      2220
TCAGATGCCG ATTGGCTTGC GAGAGTCGAA GGGGTGACAC TCGTTTCGTG ACAGGTGAAC      2280
CTTGCCCCCG AAAGGACTGC CGGGCTTCAA ACTTGGGAAA CCCGAGGTCA CATGACTAGC      2340
CAGTCCTAGG GGGCCGCCAT CTTGATACTA CTGCTTGCCA GCTAGTGAGC TGTTGGCCGG      2400
GTGAGGCCCA AAACAGAGCA GCAGTTTCAG GAAACTTGTA TCTCGACCAG GAAGCACCAG      2460
TAGATGGGAT GTTGCTGAAA ATGGAGGTTG TGAATGAAGC ATTCCAGGAG GGAGCTTACT      2520
TTCCCCATCC AGGTTATTGG CACCATCATC CACTAGCTCT CCCGCACCAG AAAGCAGGGA      2580
GGATTCCTCA GTCCAGAGCT ACTAGTCACA AGTCCTGTCT GTCCCGCCCT CTTGCGTAGG      2640
CCTTCTGCTC CCCAGTTCCA TTTTCTTTTT CCTGGACAGC TTCCAATGTC ACCCCTCCAA      2700
TCTGCACCGC TAACAGACTG GCCCCCCTTT GCTGGCGAGG TAAAGTCTCA AAACCGTAAA      2760
TCACGGCCTT CGATACGCCA GCATGTGGTT ACTTTGTGGA TGTTGTTTCC TTCCACTCTT      2820
CTCGTTCCTT TGGGTGTACC TGCACCCAGT CTGTGCCTCT AACATGTAGT CCCCCTTCAA      2880
TCAAACCACT GCAAACCCCA GCTTCCCCTC ATTTCCCAGG ACAAGTGGGC CTATCTCCAC      2940
GGCGCGCTTA ATTGTTTTAC TGTTTCCTAA CTAGGTTGTG AGCGCCTGCA GATGAGGGGC      3000
CGGTTCCTAT TTATATTCGC ATCTCCACGG CCTGGCAATA TGCCTACCAC ATAATGTCCT      3060
GTTAGATGTT TGTTGATTGA ACAGGCATTG ATTGGGGATT TGGGTGCCAC CCTTCATT        3118
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(SEQ ID NO: 317).

[0453] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a PABPN1 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 6121 to SEQ ID NO: 9213.

[0454] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding KCNQ4 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
tcctcttgaa aactttaatg aaaccaataa gttaataagt taacaaagtg aggtacttgc      60
atatccgaga accgagttga tggcggaacc tcgatcgtgg aaggaaggag ggcttcttcc     120
catgtctcct tggtcatagg gtaatcccat tctcctggcc ctctccacag ctgtctctga     180
gtgggtggag gggtggctct ctggggacag tgcagtgcag gtggggtgag ccaggagtgg     240
ctccatccag ggcatgacgg tcacatgcag agggcatgtt ggggcagagg ctgggcacca     300
tggactgagt aagatccagt ccaagccctg gtgctggcgc atgcaggtcc ccagcacgag     360
atgcaggcag cgtggcctcg gccccttgtt cccacagagg gttgccattg gtccactacc     420
cctcaccttc ctgccacctg cattggcttt gcagaagagg agcaaagggg tggggataag     480
aaagtgttcc tggagtgaat gggggctgac gcctggatgt ggcgctgcca attgaagtga     540
agaagtgggg tgtggaggga ggggagacag tgcatgtgaa agccggatgc gcggttggta     600
tagctattac atgcattctt attctctctg ggagttagag tgactaggaa ggcacaggcc     660
tggctagaag tgggcctggg gtctctccag tggtgtcagg cctgggcccg agttgtgctg     720
tggagacacg gaggcgggcc cttggggaca ggcagaggca gtgggttagg gctcctggac     780
cagagaggag gagatcttct tgaagaaggg gagcgggtaa aggtctgggt ggggcctgga     840
gagctctcgg gttacttcaa agctccttct cctgctcaga tggagttggg ggtcttcctc     900
ttagtggtcg tcaccctgca aggccagtcc actggcatct gctgcagagg cccaggagcg     960
gctgagtggc tctgcactca tgtctccaag tgtgtcacgt cattgcatgt ttaagtgtga    1020
gaggctttct tcctgtctgg agaaactaaa aggctatgtt tgtgcgatcg tccacgaagc    1080
actgtctggg agttttgggg aggatacaga aacctgtgct gactatgagt ggtgtctgga    1140
gggccaagga ggggagtggc cacccagcag gaggagggag cccctgggag ctgacacttg    1200
caaatgggaa gaaaggccca ggcattgccc ttaggagagg gcaggggaga aacaggcccc    1260
atgctgggag gggaaggagt gataagccaa agaataggga cagggtggtg gccaagcagc    1320
ctgagaccag ctactgctgg gggcccagct cccaatgggg gagggacaga gagaccccat    1380
gggggtcccc aaagttgcat atttaacatg gtttgcatat atggtgtcca cctgccttgt    1440
ggcagggtcc tgtgctttgt gggtacctgt gggggcacct tggcccaccc ccaagtgggg    1500
cggggacttg gcgggagggc ttccctgctg tgtccgaagg gtgggccaga gacctcacct    1560
tgcatagctg gaagctgggt cagggcccag ggccccagcg cccgctccca ctcccggggc    1620
tgtgcccctg ccaggtgggc cacatagggc cttgatggag tgggcagggc actgggggggc    1680
ccagaggccg cgggacggcc gctgctgctg ccctctgtgc gacctggcat ctcacgctcc    1740
tgaggtgggg aaggggcgct ggcacccaca gcaggtacca cgcccactca ggcagctcaa    1800
tactgcgtgt ggtctctctc cccgtgaggg agtgagttca agtacgagag gagtccggag    1860
gcctcagagg gcagtcggag cgccaggccg cggggctggc cgtgtgctgc cctgcctctg    1920
agaagtccct cagtccatgt tggtgctgac cgagcgggag atgctgagcg tctgtgcgga    1980
gacggagatg tcctcgtggt ccacagggct gtggtagtcg gaggtgatgt cggggtcgaa    2040
cagcggcact tgcacggcgc ccaggctggc cgaggtgcca gagcgcaggc agcgcgaata    2100
gaagcccaac agcaggtcca gcttgtgctc gatggactgc acctgcttct ccaccttgac    2160
cacgcgtccc atcatgctga tttcatccac cacctccgcg tcggagggcc ccttgtcgcc    2220
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cttctcccgg gccttcctgt ccccgggccc ccgacccaca atttggtcca cccgagtttg      2280
caggctcttg atccggccca gcatgtccag gtggcctgct gagtactgct caatgacgtc      2340
cttcacgtcg tacggtcgca gtgtctcctt gaatttcctt ttggccacca ggaacttgag      2400
aatcctgatg gagcggatga ctgtcttcac agcaggcatg atgtcgtcca ccgtgagctc      2460
acactggtag ctcttctcct ctgctacttc ctctgagggg gcatcctcag cagaggtgcg      2520
gggtttgagt ctcagagatg cccggaagcg ggtgcggtca ttgaagctcc agctcttttg      2580
caccttggtg gggctggtgg cctcacccac ctgctcgctg cttggggagg tgggcattgt      2640
tggaggtgcc agatgctgct tggaaggacc cgtccgccgc tgggagctgc ccatgcggat      2700
gcggtctttg atgcccatcc ggctgctttc cccagggcag aaggaggtgc tgcccggccg      2760
gtggcaggtg gcaacgggcg ggtaacggga gggtgctccg tcgggtaccg gcgcccgccg      2820
cacctccagg ggccgtaggc ccccattgcg ggcccgttgc acgtgctcaa acaagagggc      2880
cagctctctg aaggatggga ggatactgtc atagtagtac caggtggctg tcaggtaggc      2940
ccggctcata tcggtggagt acaggcgcca ggcagcctgg atgaggttgg ctgccggcat      3000
cctccgcttc tcgaagtgct tctgccggtg ctgctcctgg accttcaggg caaagccgga      3060
gcctaggatg ccggcaggca gggcaaagaa agagatgccc agtaaggcga agccagcagc      3120
caggaccctg cccagccatg tgtgcggtgt cttgtcacca tagccgatgg ttgtcaatgt      3180
aatcgtcccc caccagagcg agtcggcgta ggaggagaag tcggagttgg cgtccttctc      3240
agccaggtag accaggaagg aggcgaagat gagcaccagg aacccgatgt accaggcggt      3300
gatcagctcc ttgctatgcg cgtagaccac tgagcccagc agcttccagg tgccgccgcg      3360
gcggtccatg cgcaccatgc gcaggatctg caggaagcgc atgctgcgca gcgcggacgt      3420
ggcgaagatg ttgccctggg tacccgcggc gatgacggcc accgaggcca cgaacacgat      3480
gaagtcgatg acacagaagg gctttctggc aaagcggaag cgaccctgcc atcctcggta      3540
gcggcagcag catccggcgg accagacccg gacgatgtac tccaagccga aaaccacgat      3600
catcacgaat tccaagatga ggagacactc gttggcaagt tcctggtgct cctggatagt      3660
ggacagcaca gacagcacca ggcagctgaa gaccagcaaa aatatgaaga cgtggtagac      3720
gaaggcccag ccgcggggcc gctccagcac gttgtagacc cagttctgca ggcggcggta      3780
gcgcttgtgc gcggccgagg agcgctggcc gcaggcggag cccgagccgg agcccggccc      3840
agggaggggc gcgcccggcg gcaggggggct gcccaggagg ccgaggcggc gcggggagcc      3900
gcccccgccc gcctcgccct gttcgctctg cacggccgtg agcgccacta gctccgcgcg      3960
gggggcgtcc ccgggcgggg gacccaggcc gaggcggcgc gggggggcct cggccatggg      4020
cggcgccggg ctgggggcgc cggggcccgg gcacggtccg gggcgggggc gcgctcgggg      4080
cgctcagaga cgcatggctc ggacccgggg ccagaggggc gacccggggc gggcgcgggc      4140
ggcgggggct aggggccggg ccggggccgc gggcgggcgc tcggagcctg ggggccgccg      4200
gagcccgcac tgacctcccg cttccccggc gactggggct gctctttccg actccaactc      4260
tcttattacg cgctccatgc cgctcgcctt tccacctgcc accggcgcgc gccgctcaca      4320
tgtc                                                                  4324
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4324 (SEQ ID NO: 318).

[0455] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a KCNQ4 protein of the disclosure may

comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 9214 to SEQ ID NO: 13512.

[0456] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding CLRN1 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
agcatctgga aactcggtgt gttctgatgt ctgctggcga atagcgaatt gacaccagag        60
caagttattt ctcaggtata cggttgtttc atccttgtaa atagttccaa agggaaacag       120
tgttttattt taaggagtac tttcaaacct attatatgag ggctgctgag tactcagcac       180
ctgtggtcag aggcctagtg atctgtttgc tgtcattctc tgctttttcc ttggtgcttt       240
ctggaggaca gcaggttgag gatgaaggaa gggtcagttc caggctcagc tgtggccttt       300
agtcagctgc agatcaattt gatgggtaat tcaggggaaa aaaaaagttg acctgggtca       360
tgcttggtga cagccagaac aagaccaaga tgatacagtg ataccgtcat aatcccagat       420
ttaatataat tttcataatt gcatattagt actcgagaca ctatagctag aaaaacagcc       480
cctaataagt cattttgcat caaatgtact aagcagagat cattttttcat gattcctcag      540
tggtcctaac aattatgttc attgaaagta ctgtcgtgaa tgtaattggg actcaggcac       600
gggaggaaaa ataccctaag cttggttttt tcttcttttc ttcttttaga gtttgcagat       660
tttgaccaac agacatggtt aataagacta tgctttttta aagcctatat tttatattta       720
ttttattttt taattttgtt agtgacaggg tctcacttta ttgcccaggc tgtaactcga       780
actcctgaac tcaaatgatc ttcccacctt ggcctcctga agtgctggaa ttacaggtgt       840
gagtcaccac gcctggccta agagtatact ttaaacaaat tttttaaaat gtgtgttgat       900
acattttata gatgttcatt taatacacta ctgttttagg aaagcgattg cagctcagtt       960
ttctgaaatc tggcaacaaa tgtgtggata tattagagat attatttgtt tttattaaaa      1020
tatattccat gtgcctttga tatctttttg ataggaagac atcttacaca cacacacaca      1080
cacacacaca cacatatata tatatggagt aacaatttgt cgattctagt caactgcctt      1140
tgactacctg ggtcaagcaa tttcccacca gataaaacaa cttttcaaag ccttccttct      1200
gcttccctta ctttccagcc tgtatcctta gtacgtaatt tgtaaacatt gtcacgaagg      1260
gtcctgatgc tttaatatat gcagactaaa aggatatgca aaattaacca catctaaaag      1320
tgaccaaagc aagtctactc ccttgtaaaa ttatagaaag gtttgccttt cagtacatta      1380
gatctgcagc tacattagtt gtttctgcgt ctttagattt tgcaaaaggg aactgaaatc      1440
cagcaagtcg tattaggagc ccattcagaa aatgaacaaa aaagcaaaag aaaatgaccc      1500
agaatgaggt ggtatatttt tcactttgcg ttttgtagac ataagtccct tctttataat      1560
ttgcaatttt ttctgagagg tgatggattt tcacttcaga ggcaaacaat atcatgacaa      1620
gacagccaca ggagcctgaa atgaagctca aaaggtacag ccctagggga ccatgcagag      1680
tttcaaaagg ttttccaaaa gcattgtaca tgaagaaggc tgtccccacc atggttaaca      1740
caataaggat ggcagagaag agaatgacat tgacgtggat gctcactggg attgctttga      1800
gcaaatctgg aaaaaatgag aaccgaaagg gccttgctcc caacccacac tgcctcacac      1860
cctctccgtg gaaaagcccg tactgcattt cacccataaa cttgtccagc tcctgccctg      1920
aggcattgac gagcagagct cccgttttgc agaggacagt ggctttgatc cacaacggtg      1980
tccccaaggc tgtcacaact ccgagggcac atgcaaaact gaacactccg gccatgcaaa      2040
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aaatgatttt cttctgttgg cttggcatga tgagaaacgg cttctgt 2087

(SEQ ID NO: 319).

[0457] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a CLRN1 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 13513 to SEQ ID NO: 15574.

[0458] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding APOE2 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

tgcgtgaaac ttggtgaatc tttattaaac tagggtccac cccaggagga cggctggggc 60
ggggacaggg tctcccgctg caggctgcgc ggaggcagga ggcacggggt ggcgtggggt 120
cgcatggctg caggcttcgg cgttcagtga ttgtcgctgg gcacaggggc ggcgctggtg 180
cccacggcag cctgcacctt ctccaccagc ccggcccact ggcgctgcat gtcttccacc 240
aggggctcga accagctctt gaggcgggcc tggaaggcct cggcctgcag gcgtatctgc 300
tgggcctgct cctccagctt ggcgcgcacc tccgccacct gctccttcac ctcgtccagg 360
cggtcgcggg tccggctgcc catctcctcc atccgcgcgc gcagccgctc gccccaggcc 420
tgggcccgct cctgtagcgg ctggccggcc agggagccca cagtggcggc ccgcacgcgg 480
ccctgttcca ccaggggccc caggcgctcg cggatggcgc tgaggccgcg ctcggcgccc 540
tcgcgggccc cggcctggta cactgccagg cgcttctgca ggtcatcggc atcgcggagg 600
agccgcttac gcagcttgcg caggtgggag gcgaggcgca cccgcagctc ctcggtgctc 660
tggccgagca tggcctgcac ctcgccgcgg tactgcacca ggcggccgca cacgtcctcc 720
atgtccgcgc ccagccgggc ctgcgccgcc tgcagctcct tggacagccg tgcccgcgtc 780
tcctccgcca ccggggtcag ttgttcctcc agttccgatt tgtaggcctt caactccttc 840
atggtctcgt ccatcagcgc cctcagttcc tgggtgacct gggagctgag cagctcctcc 900
tgcacctgct cagacagtgt ctgcacccag cgcaggtaat cccaaaagcg acccagtgcc 960
agttcccagc gctggccgct ctgccactcg gtctgctggc gcagctcggg ctccggctct 1020
gtctccaccg cttgctccac cttggcctgg catcctgcca ggaatgtgac cagcaacgca 1080
gcccacagaa ccttcatctt cctgcctgtg attggccagt ctggaggcca ggggttccca 1140
gggtcccagc tctttctaga ggcccctgag ctcatccccg tgcccccgac tgcgcttctc 1200
accggctcct ggggaaggac gtccttcacc tccgctgggg ctgagtag 1248
SEQ ID NO: 320).

[0459] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a APOE2 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 15575 to SEQ ID NO: 16797.

[0460] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding TNNI3 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

tttcagctca gagagaagct ttattcctca gggccctcct cagggcaggg gcagtaggca 60
ggaaggctca gctctcaaac tttttcttgc ggccctccat tccactcagt gcatcgatgt 120
tcttgcgcca gtctcccacc tcccggtttt ccttctcggt gtcctccttc ttcacctgct 180
tgaggtgggc ccgcaggtcc agggactcct tagcccgggc ccccagcagc gcctgcatca 240
tggcatctgc agagatcctc actctccgca gggtgggccg cttaaacttg cctcgaaggt 300
caaagatctt ctgagtcaga tctgcaatct ccgtgatgtt cttggtgact tttgcctcta 360
tgtcgtatct ctcttcatcc accttgtcca cacgggcgtg gagctgtcgg cacaagtcct 420
gcagctccgc gaagcccagc ccggccaact ccagcggctg gcagcgggtg ctcagagcgc 480
gccccttctc tccgcgccgc tcctccgcct ctcgctccag ctcttgcttt gcaatctgca 540

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gcagcagagt cttcagctgc aattttctcg aggcggagat cttagatttt ttcttggcgt       600
gcggctccgt ggcataagcg cggtagttgg aggagcggcg tctgattggg gctggtgcag       660
ggcgaggttc cctagccgca tcgctgctcc catccgccat gctgagactc aggccgggaa       720
tggcaggagg cagggcgagg acaggggcgt ttggagggtc agtgaggggg ccgcccgggt       780
gaccttcagg gtcccaggga ccgtcagtct cctccgggct gcttgagact ccccgaggac       840
act                                                                    843
 (SEQ ID NO: 321).
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[0461] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a TNNI3 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 16798 to SEQ ID NO: 17615.

[0462] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding BEST1 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
aacgagtatt tgtatttatt aaactcatta gtttgggcag tatactaagg tgtggctgtc        60
ttggattcag atagaactaa gggttcccga ctctgaatcc agagtctgag ttaaatgttt       120
ccaatggttc agtctagctt tcacagtttt tatgaataaa aggcattaaa ggctgaagta       180
gtctgggatt tttatctatt aagctaacca tttgattcag gctgttgtag gacatgttct       240
tcagtgtgga cagctgtatg gctgtgactg gatcagtgtc ctgctggtgt acacacaggt       300
gaggacctgg ctggcgaagc atccccatta ggaagcaggt taggaatgtg cttcatccct       360
gttttccaag gcccaataag gatccatgtg atctttgagt gtagtgtgta tgttggttgg       420
tgattgttcc aaaggttctt tgaggtgatt ttcggggatc tctggcatat ccgtcaggtt       480
aaactccaca gttttcctcc tcacttgaga tacttctggg tgctccatca aggccccatc       540
gctctctgag agcaattcaa aacttttctt ggccccagaa ctcacagtct ttaagctttt       600
gtctttggtg tctatgcctg tgacactgtg aagctttgac ggcgctgatg gttctagggg       660
gaagaacatg ggagtggggc tgaggggcgt ctgtggggca ctgtagtagc ctggcctctg       720
atacagtggg gcagacttga aggcgtccac agccttaagc ttccaggcct tgttgtcttc       780
ctggccccta acgttctgtt tggctgcctt gtggtttttg ggcaggccct cgtggagaag       840
ggattccctc ttgggccaca gtagtttggt ccttgagttt gccctgggag gatggtgatc       900
atgggactgc aggcctagga agcggccaat gatgccagcg tgagcatcct cctcgtcctc       960
ctgattgggc tggaactcca tctcctcttt gttcaggctg atgttgaagg tggagcccat      1020
aaaggaggct cgacggaact gggcggaagc agctgtgtag gggggctgtg gctcgggctt      1080
attccagtac atgtccggct ccatccgagg caggtcctgg tgcatctcat ccacagccaa      1140
cagggacacc tgcaaattcc tgtcgacaat ccagttggtc tcaaaatcat catcatcctc      1200
tccaaagggg ttgatgagct gctctgccac cttcagccag ccaacataga agaagaactg      1260
caggaacgtg aagacgggca caacgaggtc cagctcatgg ccagggtagg ccttggctgg      1320
gttcagaaac tgccgcccaa ctagacaagt caggaagaag ctgtacaccg ccacagtcac      1380
cacctgtgta tacaccagtg ggatactaat ccagtcgtag gcatacaggt gtccacactg      1440
agtacgcaag gtgttcatct cgttcagcag gctctggagc aggatagggt cccggattcg      1500
acctccaagc cacgccttca ttgacaggtt ggcaaaccac acccagggca cccagaacat      1560
gttgtgtggt aggctcagtt tctccaactg cttgtgttct gccggagtca taaagcctgc      1620
ttgcaccagg tgctgggcgc tggggaagcg cttgtagact gcggtgctga cgctgcgcag      1680
gatgagcacg ttgcccaggt tggcgtagcg gatgagcgtg cgccgcagca gccggccttg      1740
ctcgtccttg ccttcgacga agcccgacac caggctcatg aggcggtcgg gccacggcag      1800
gttctcgtac tggttccacc agcgggtcac gaccagcgtc acgtagaagc ccagcacgaa      1860
ggaaatgggg atgagctgga tgtagctgtc gcaatacaga gtcagtttct caaacatcag      1920
ctgttgttct tccgtgaggg ccagcctata aataaagcgg atgatgtagt agcagagcag      1980
gaagattaag aactcgccat atagcagctt gtagatgctg ccccgccagc acagcagcag      2040
gcgggagaag gagcctaagc gggcattagc cacttggctt gtgtaagtga tggtcatggc      2100
caggcagtgg gctgcagcag gtgggcttgg gtcaggtggg gttccaggtg ggtccgatga      2160
tcccacagaa ggtctggcga ctaggctggt gggactccct gggactctgt                 2210
 (SEQ ID NO: 322).
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[0463] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a BEST1 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 17616 to SEQ ID NO: 19800.

[0464] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding MYBPC3 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
tgagttctct gtgactgcac ttatctttta ttgcccaata aacattggga agacatagca        60
ggccagaaag gcctgtcccc agacattgtt tcttgaggcc accctccttt taccccaaag       120
atccaggggc ttccttcagg agccctgtgg accagtctgt gcaacaccca ctcaggactg       180
cccgacaact gccctgctga tcccccatcg cagcacagga gacacacttg tcacacatac       240
atccaacagt agggaggggt ttccccaact tccctccagg ctcctggcac ggggctggca       300
tccggttgta cctggccatc cccaggagcc agcctggtca ctgaggcact cgcacctcca       360
ggcggcactc acaccgtgcc tcgccctgta agttggtggc cctgcagaca tagatgcccc       420
cgtcaaaggg gcagggcttt ctaatctcca gagtcaacac tccctgcttg ctgaacatgc       480
ggaagcgggc gtcttctccc aggtccaggc cattcttgaa ccaggaaatc ttgggcttgg       540
ggctaccccg gacagcacag cagagcatag cagtgtagcc cgcgatgacc gagcggttca       600
ccaggggctg ggtgaagctt ggggcctcgg agaagtccag ggccttatag ttgggtggct       660
cataggtgat gcctggtctg gggataaaga cgggctcctt ggtggtggcc gctctgtcac       720
taaagccaac catattctgg ctgaagacgc ggaagtagta gccattgcca atgatgagct       780
ctggcaccac gcagtgggtg cggcggtaat gctccaagac ggtgaaccac tccatggtct       840
tcttgtcggc tttctgcact gtgtaccccc agagctccgt gttgccgaca tcctggggtg       900
gcttccactc cagagccaca ttaagacccc aggcgtcagt cacccggaga tcctggggag       960
gacttggctt gtcaacaacc tgcagcacca gcgtggcctt gtcctccatg ttctcaatgc      1020
gcaccgtcac ctggtaagtg cctgaatgca cgcggcgagc ggcccggatg aacaggatgg      1080
tgtctgtggg gctgttgcgg atgctcacct cctcgcctgc caggggctgc ccctctttgg      1140
tccaggtcac ctgaggccgg ggcttgccct ggaaagggat gagaaggttc acaggctccc      1200
cgaccttctt ctgaatggtc tggcgcaggt gcctgggcag ctgaagccgt ggccgttgca      1260
ggatctcctg cactgtcacc ggctccgtgg tggtaacagg ggctccaggc cctgccatat      1320
tgtgtgcccg cactcggaaa agcagccggg cccccgtggg caggtccttc accagtatcg      1380
atgtgtgctc tgtcagcccc tgcagggcag ccacccactc tgagcagccc tctgggcagt      1440
actccacgct gtagccatcc aggcctcctg ctcccacgcg ctctgggggc cgccacttga      1500
gggagaccgt ggtgtcagag acgtcctcta ctgccaggtg ggtgggttcg ctgggggggac      1560
cgataggcat gaagggctgg gaggcagggc tgggcctgga catgccgatg gcgttgaccg      1620
cgtagacgcg catctcgtac accacgccct cgatcatgcg ccgcgcttca tgactcagct      1680
cctgaatcag gtcgaagttc agccgcatcc accggtagct cttcttcttc ttgcgctcca      1740
ggatgtagcc caggatgggc tgcccgccat cgtaggcagg cggctcccac tgtactgtgc      1800
aggagtcctc tcccacgttg ctgatcttgg gggccgcagg tgcgtctggc acgtcgatga      1860
ccttgactgt gaggttgacc tggtcctcgc ccacagggtt cttcactgtg accgtgtaga      1920
cgccctcatc ttccttctct gccccctcga ccgtgaagat gctgcggtcc ttggtggtct      1980
ccacgcggac ccggccctcg gtctcacaca gcagcttctt gtcaaacacc cactcatcgc      2040
tgtcacctgt gtcctctggg gcatctgggg ctggcctggc tggggcctta ttcccctgcg      2100
tgatagcctt ctgccagatc acagtgggag cagggtcccc agagataggg acgtccagac      2160
gtagcttatt tccagctaca accacaatgg tgtctggtat gcggcctggg cagtccaggt      2220
ggatcttggg aggttcctgc ctgggtacga agtcaatctt gacctccatg aagtggagct      2280
tggctgacag gttgcaggcg aagccctcgg gcacaaagct gtagtcagcc tcgtcggcag      2340
gtgtgacgtc gtcaatggtc agtttgtgga cccgcccgat gtgggacacc tttatgcggc      2400
tgtcgggcac cagctccttc ccattcttca gccacacacc ccgaacattc tcatctgaga      2460
cctcacattt gaacaccgcc tggtcctttg cgcccaccat caggtctgcg atgctctggt      2520
acacctccag cttcttttcc tgcacaatga gctcagccag cgcctggccc ccgctagtgc      2580
acagtgcata gtgccccgcg tcctccagca tggcctcgtt gatgatcagg tggtgtctct      2640
gcccgtcctt cttgaaccgg tatttgaagg tctcctcccg ggtcagctcc accccgtcct      2700
tcagccattt gacttgcgcc ccctcctccg atacttcaca ctcaaactcc acccgctgcc      2760
ccaccatcac cagctggtcc tccaaggggc gcgtgatgag cacaggggggc tctttcacaa      2820
agagctccgt gctacacttc tcgccaccca ccacgcactg gtaggctgcg tcgtccgcca      2880
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```
atgagcactg gctgatggtc agggtacgct tggcaccgat ggactcaaag atgtacttgc      2940
tgccgctcat ctggatctcc tggccattct tgagccattt gacctcagcg tcatggtcag      3000
ccagttccac ggtcagccgg atcttgtggc ctttgctcac ctggtaggcc ggctccagct      3060
tcttctgaaa ggctgtgctc ttcttctcat cgcgcctcat gcccttgagc ctctttagca      3120
tgccgcgcag gtcagtgacg ccgtactgga aggcgatgcg ctcgtactca gatgggggtg      3180
cctgccgtag gatctcccac acgtcctcct ctgctggtgc ctccagcttc gagtccctcg      3240
gggtccggaa actgtctctc ttttttcagca gtgagctgaa gtccagaatc ccagtgtcct      3300
catggctatc actgatccgc cgaccacctc cagccaggct cgtgcggcgg aaggctgata      3360
ggaggtccag gtctccggtg cccatggcct cgtggacagt gagattgaag ttggagcagt      3420
caaatttgtc cttggtggac acctcacagc ggtagctgcc agtgaaggca ggctgggcat      3480
cggtgatgtg cagctcgaac agatagacct tgctggcgcg gtcgtagctg tcgtgcagct      3540
gcaggtgctg gcccaccttg ctgctcaggt ccacccattt gcccttgaac cacttgacca      3600
caggcggctt caggaggctg gcgccggcca cgcgggctga gaaggtgatg ctgccaccca      3660
cggtcacctc gccatcctgt ggccgcatca cgaagaggcc aatggggtca tcgggggctc      3720
caggggtagg accattgaga gctgctgagc ttgacccttt gggacttggg gcactttctc      3780
ccagctcagc ggctggggcc ggggcttctc caggggctcc agtggcctca gcaggggcag      3840
gggcaggggc cagcatgggc tctgccttct ctgcctctat gaccttgagg tcgaacttga      3900
ccttggagga gccagcaatg actgcgtaag atccctggtc ggcagggccc acttcccgca      3960
ctgtcagcgt atgccgtgtg ccctctgtgg ccaggccgta cttgttgctg gcgctgatgt      4020
cactgcctcc gcgctgccag cgcaccttca ctcctgcccg ctctgtctcg gcctcgaaca      4080
cggcagggct gcctgcggcc acttccactg accgtggctt cttgctaaaa gctgagactg      4140
gcttcttccc cggctcaggc atcctgagag acgtcacacc aggcacgaag caggcacagg      4200
tcacccaaag agggact                                                     4217
 (SEQ ID NO: 323).
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[0465] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a MYBPC3 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 19801 to SEQ ID NO: 23992.

[0466] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding TNNT2 protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
tcagtgtgtg gtggcttttt attactggtg tggagtgggt gtgggggcag gcaggagtgg        60
tggctcccac ctaggccagc tccccatttc caaacaggag ctgcctgggg tgcccaggag       120
ggcccgggaa ctgggggagt gcaggccgga ggcaggtgcg agcgaggagc agatctttgg       180
tgaaggaggc caggctctat ttccagcgcc cggtgacttt agccttcccg cgggtcttgg       240
agactttctg gttatcgttg atcctgtttc ggagaacatt gatctcatat ttctgctgct       300
tgaacttctc ctgcaggtcg aacttctctg cctccaagtt atagatgctc tgccacagct       360
ccttggcctt ctccctcagc tgatcttcat tcaggtggtc aatggccagc accttcctcc       420
tctcagccag aatcttcttc ttctttttccc gctcagtctg cctcttccca cttttccgct       480
ctgtctgggc ctgcttctgg atgtaacccc caaaatgcat catgttggac aaagccttct       540
tcttccgggc ctcatcctca gccttcctcc tgttctcctc ctcctctcgt cgagccctct       600
cttcagccag gcggttctgc cgctccttct cccgctcatt ccggatgcgc tgctgctcgg       660
cccgctctgc ccgacgtctc tcgatcctgt ctttgagaga aacgagctcc tcctcctctt       720
tcttcctgtt ctcaaagtga gcctcgatca gcgcctgcaa ctcattcagg tccttctcca       780
tgcgcttccg gtggatgtca tcaaagtcca ctctctctcc atcggggatc ttgggaggca       840
ccaagttggg catgaacgac ctgggctttg gtttggactc ctccattggg ccatcttcag       900
cctcctttgc ttcctcttct tcttcatctt cttctgccct ggtctcctcg gtctcagcct       960
ctgcttcagc atcctcttcc gctgcctcct cctgctcgtc ttcgtcctct ctccagtcct      1020
cctcttcttc aacagctgct tcttcctgct cctcctcctc gtactcttcc accacctctt      1080
ctatgtcaga catggtctct gctctccctc caaaaggaga aaaaagtcag tgcaggtaca      1140
aagggaagcc tgccttcctc agaagagctc tggcccccgt tgtacagaga tcagcgaggc      1200
ctagggtgaa tctagttcca cccctcatga gctgtgtgac ctcagaacag cagctgccga      1260
cagatcctgg aggcgtctgc tcagtctcag cggggactgg gtgaggcaga ggatggagag      1320
ggctttaagc aggcatgtgg gctggggcct ggtgagccag cc                         1362
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SEQ ID NO: 324).

[0467] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a TNNT2 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 23993 to SEQ ID NO: 25329.

[0468] Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding pre-mRNA processing factor 31 (PRPF31) protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
tcttgacaat gtccttttaa ttgtactctt ttcaaaaaat ctccttttctc agttaaaaaa        60
gacaaggcat gatgaagacc tgctctagcc catactgggc ggtgatctcg gtcctggggg       120
aggccaggcc ggactcttcc aaggcctcct ccctgggcag tcccagcaat ggggccagtg       180
gcagggcagg ttctccctgc cagaacccga tcctagccct tcagaaggac tggacctctg       240
tgtcccttca gtgggaagcc accttggaca cacgcagtca ttcaggtgga cataaggcca       300
ctcttctcgc ccttgacctt gaggaactca gccatgctgg agaaatactt ctggttggcc       360
tcagccacct tcttctctgc cgcctgtggg ttcacaatct ccaggccctg gagtggggtg       420
aaggccacgc tggaggccgt gcccgaggag cggtcgcgga tggtggactt cccgccatat       480
acgacgctct gcttctgcag ggtccgctgc agcgtcttgg agatcctggc cttggtggcc       540
tcgtttacct gtgtctgccg cacacgccca ctgcccgact tgcccaggtg gcccaggctg       600
aatcccaggt cctcctggta ggcgtcctcc tcgatctctc cgaagctcat acggttggcc       660
tgcttccgga tctccgtcag ccccagccgc tccttcatct tgcggtacct gcggccgcct       720
cgcttcttcc gctgtccatc caggggcgca ggcagcggct tcacctgctt cacaggcggc       780
ggctcctgcc acttgtcgaa tttgcgctcg atctcatcct tcagttcgta gcccaccttc       840
ccttctgtgc tctcgtggaa actgtccaca cgggctgcca gtgtgcactt ggcggccacc       900
agccgggccg ctttccgccg cagatccggt ggcagggact gcacgatgtc actgtggtag       960
atgtagccgg tgtggggcag cactgaggta gacgagaagc ccgacagcgt cttgcgctgg      1020
gccccgagca gcatgatgtt gcaggcgggc atcttggaga ggttggtcag gccgccggcc      1080
acacccatga tcttggcggc cgtggatgcc ccgataatga tggacaggtt gggtgcgatg      1140
aaggacatcc gggactccac atactcgtag atgcggtgct tggaggcgtt cagctccagc      1200
gccatgtcgc aggcctcctc cagccgctcc agctcctcct ccgacagctg ctgcccctgg      1260
gtggtggagg cggtgacgct gacgaccatg atggtggcat tggtgaggat ctgctgcagg      1320
ttctcattgt tcttgcactt gtccaggctg ttgcccagct ccttgaccgt gcggatgtaa      1380
tccagtgcat tggggaccaa ggactccagt tcagggaatc tctttgagta cttatcccgg      1440
atgaacttat ggatgatgtt cagctcgttt tcgatctcca cggtcaggtt gttggcatcc      1500
acgatgacgc ggtattcagg cgcggcctcc actggtccca tcacttctga agctttggct      1560
tgcttgctga tatactcctc aatcttcatc ataatctcag caaacatctt actatcccat      1620
agcttggcga tggtcttgac tgaatccccg gaaagatcca gctgtgtctc ctcctgcaca      1680
tcctcgatcg ctggctcctc ttcttcctcc ccatagcttc ctccttcctc ctcttctgct      1740
gcctcttcga gatcagctaa gagctcatct gccagagaca tcccgaggcc tctcctctcc      1800
gcgcaccact gtttctagcg ttagtcgctc acc                                  1833
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(SEQ ID NO: 491).

[0469] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a PRPF31 protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 25330 to SEQ ID NO: 27137.

Compositions of the disclosure may comprise a gRNA comprising a spacer sequence that specifically binds to a target sequence of an RNA molecule encoding Progranulin (GRN) protein comprising or consisting of about 20-30 nucleotides of the sequence of:

```
ttaagaaagt gtacaaactt tattgaaacg cacacgcgca cacacacaaa cacccctgtg        60
gatagggaaa agcacctggc cacagggtcc actgaaacgg ggaggggatg gcagcttgta       120
atgtggcttt tgccacaacc cccttctgac agggaaggcc ttagattgag gccccacctc       180
ccatggtgat ggggagctca gaatggggtc cagggagaat ttggttaggg ggaggtgcta       240
gggaggcctg agcagagggc accctccgag tggggtcccg agggctgcag agtcttcagt       300
actgtccctc acagcagctg tctcaaggct gggtccctca aaggggcgtc ccagcgcggg       360
gcctccctgc gcaaacactt ggtacccctg gctgcgcagc ggaagccagc aggacagcag       420
tggcgccgat cagcacaaca gacgccctgg cggtagggac agcaggccca gccctgtcgg       480
ttgtctcggc agcaggtctg gttatcatgg cagaagtgtc cttccccaca ctccacgtcc       540
ttcacaccca cgtgagggct acgggccagg aaggtggcag gctgggcaga gaccacttcc       600
ttctcgcagg atcgagcctt cacgttgcag gtgtagccag ccgggcagca gtgctggcga       660
tcctcgcagc acacagcatg gggcaactgg cagcaggccc agctcccacc caggctcggg       720
cagcaggtct gccccaccgg gcagctggtg tgctggtcac agccgatgtc tctggggtgg       780
gataaggaag cccggcgggc aggcatcttc tccagtccag ccacgatctc gcttcctcgc       840
tgacactgcc cctcagctac acacgtgtag ccctgggggc agcagtgctg gtggtccgag       900
cagcagacag cctctgggat tggacagcag ccccactccc cagacgtgag ttggcagcag       960
gtatcggagg agggacagct gctgacatta tcacagggga catctctctt caaggcttgt      1020
gggtctggca ggctgaggtg agctggggcc ttctccatcc agggcacctg gtggggcccc      1080
tgttcacagg tacccttctg cgtgtcacac gtaaaccccg cgggacagca gtgtatgtgg      1140
tcctcacagc acacagcctg ggtaaaaggg cagcagcccc aggcccccga ctgtagacgg      1200
cagcaggtat agccatctgg gcagctcacc tccatgtcac atttcacatc ccccactgtg      1260
tgcgcaggca gcttagtgag gaggtccgtg gtagcgttct ccttggagag gcacttactc      1320
tggatcaggt cacacacagt gtcttggggg cagcagtgca ggtgatcgga gcagcaggtg      1380
gcgttgggca ttgggcagca gccatacttc ccactgggca gctcacagca ggtagaacca      1440
tcagggcacc gggaccgtgc gtccggacac atgaccgagc tggacaaggc cactgccctg      1500
ttagtcctct gggcagggag cttctttgcc agggggtggg tgcccgtggg tgtgatgcag      1560
cgggtgtgaa ccaggtcgca gaaggcaccg tgcggacagc agtgcaccct gtcttcacag      1620
caggaagcct ggggcatggg gcagcacccc caggagccat cgaccataac acagcacgtg      1680
gagaagtccg ggcattcgaa ctgactatca gggcactgga tggcacccac ggagttgtta      1740
cctgatcttt ggaagcagga tcgcccgtct gcactgcagt ggaagccccg tgggcagcag      1800
tgatggccat ccccgcatgc cacggcctct gggaaggggc agcaactgga agtccctgag      1860
acggtaaaga tgcaggagtg gccggcagag cagtgggcat caacctggca ggggccaccc      1920
agatgcctgc tcagtgttgt gggccatttg tccagaaggg gacggcagca gctgtagctg      1980
gctcctccgg ggtccaggca gcaggccaca gggcagaact gaccatctgg gcaccgcgtt      2040
ccagccacca gccctgctgt taaggccacc cagctcacca gggtccacat ggtctgcctg      2100
cgtccgactc cgcggtcctt gggcagcagc                                       2130
 (SEQ ID NO: 492).
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[0470] Exemplary gRNA spacer sequences of the disclosure that specifically bind to a target sequence of an RNA molecule encoding a Progranulin (GRN) protein of the disclosure may comprise or consist of a nucleic acid having a sequence selected from any one of any one of SEQ ID NO: 27138 to SEQ ID NO: 29242.

[0471]

RNA Molecules

[0472] In some embodiments of the compositions and methods of the disclosure, an RNA molecule of the disclosure comprises a target RNA sequence. In some embodiments, a pathogenic RNA comprises the target RNA sequence or the target sequence is associated with the pathogenic RNA. 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. In some embodiments the target RNA is non-coding RNA.

[0473] 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.

[0474] 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.

[0475] 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.

[0476] 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.

[0477] 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)).

[0478] 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).

[0479] 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.

[0480] 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.

[0481] 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

[0482] In some embodiments of the compositions of the disclosure, there may be an optional second RNA binding protein which comprises or consists of a nuclease or endonuclease domain. In some embodiments, the second RNA-binding protein is an effector protein. 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. In some embodiments, the second RNA-binding protein is fused to a first RNA-binding protein which is a PUF, PUMBY, or PPR-based protein.

[0483] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of an RNAse.

[0484] 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: 325.

[0485] 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: 326.

[0486] 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: 327.

[0487] 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: 328.

[0488] 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: 329.

[0489] 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: 330.

[0490] 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: 331.

[0491] 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: 332.

[0492] 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: 333.

[0493] 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: 334.

[0494] 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: 335.

[0495] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a mutated RNAse.

[0496] 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: 336.

[0497] 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: 337.

[0498] 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: 338.

[0499] 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: 339.

[0500] In some embodiments, the second RNA binding protein comprises or consists of a mutated Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide.

[0501] In some embodiments, the Rnase1 (Rnase1(R39D, N67D, N88A, G89D, R91D, H119N)) polypeptide comprises or consists of SEQ ID NO: 340.

[0502] 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: 341.

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: 342.

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: 343.

[0503] In some embodiments of the compositions of the disclosure, the second RNA binding protein comprises or consists of a NOB1 polypeptide. In some embodiments, the NOB1 polypeptide comprises or consists of SEQ ID NO: 344.

[0504] 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: 345.

[0505] 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: 346.

[0506] 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: 347.

[0507] 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: 348.

[0508] 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: 349.

[0509] 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: 340.

[0510] 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: 351.

[0511] 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: 352.

[0512] 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: 353.

[0513] In some embodiments, the APEX2 polypeptide comprises or consists of SEQ ID NO: 354.

[0514] 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: 355.

[0515] 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: 356.

[0516] 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: 357.

[0517] 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: 358.

[0518] In some embodiments, the ZC3H12A polypeptide comprises or consists of SEQ ID NO: 359.

[0519] 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: 360.

[0520] 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: 361.

[0521] 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: 362.

[0522] 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: 363.

[0523] 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: 364.

[0524] 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: 365.

[0525] 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: 366.

[0526] 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: 367.

[0527] 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: 368.

[0528] 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: 369.

[0529] 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: 370.

[0530] In some embodiments, the hCG_2002731 polypeptide comprises or consists of SEQ ID NO: 371.

[0531] 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: 372.

[0532] 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: 373.

[0533] 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: 374.

[0534] 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: 375.

[0535] 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: 376.

[0536] 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: 377.

[0537] 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: 378.

[0538] 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: 379.

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: 380.

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: 381.

[0539] 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: 382. In some embodiments, the TALEN polypeptide comprises or consists of SEQ ID NO: 383.

[0540] 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: 384.

[0541] 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.

[0542] 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

[0543] 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 optionally (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.

[0544] 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.

[0545] In some embodiments, a target RNA-binding fusion protein is not an RNA-guided target RNA-binding fusion protein and as such comprises at least one RNA-binding polypeptide which is capable of binding a target RNA without a corresponding gRNA sequence. Such non-guided RNA-binding polypeptides include, without limitation, at least one RNA-binding protein or RNA-binding portion thereof which is a PUF (Pumilio and FBF homology family). This type RNA-binding polypeptide can be used in place of a gRNA-guided RNA binding protein such as CRISPR/Cas. The unique RNA recognition mode of PUF proteins (named for Drosophila Pumilio and C. elegans fem-3 binding factor) that are involved in mediating mRNA stability and translation are well known in the art. The PUF domain of human Pumilio1, also known in the art, binds tightly to cognate RNA sequences and its specificity can be modified. It contains eight PUF repeats that recognize eight consecutive RNA bases with each repeat recognizing a single base. Since two amino acid side chains in each repeat recognize the Watson-Crick edge of the corresponding base and determine the specificity of that repeat, a PUF domain can be designed to specifically bind most 8-nt RNA. *Wang et al., Nat Methods. 2009; 6(11): 825-830.* See also WO2012/068627 which is incorporated by reference herein in its entirety.

[0546] The modular nature of the PUF-RNA interaction has been used to rationally engineer the binding specificity of PUF domains (Cheong, C. G. & Hall, T. M. (2006) PNAS 103: 13635-13639; Wang, X. et al (2002) Cell 110: 501-512). However, only the successful design of PUF domains with repeats that recognize adenine, guanine or uracil have been reported prior to the teachings of WO2012/06827 supra. While the wild-type PumHD does not bind C, molecular engineering has shown that some of the Pum units can be mutated to bind C with good yield and specificity. See e.g., Dong, S. et al. Specific and modular binding code for cytosine recognition in Pumilio/FBF (PUF) RNA-binding domains, The Journal of biological chemistry 286, 26732-26742 (2011). Accordingly, PumHD is a modified version of the WT Pumilio protein that exhibits programmable binding to arbitrary 8-base sequences of RNA. Each of the eight units of PumHD can bind to all four RNA bases, and the RNA bases flanking the target sequence do not affect binding. See also the following for art-recognized RNA-binding rules of PUF design: Filipovska A, Razif MF, Nygård KK, & Rackham O. A universal code for RNA recognition by PUF proteins. *Nature chemical biology*, *7*(7), 425-427 (2011); Filipovska A, & Rackham O. Modular recognition of nucleic

acids by PUF, TALE and PPR proteins. *Molecular BioSystems*, *8*(3), 699-708 (2012); Abil Z, Denard CA, & Zhao H. Modular assembly of designer PUF proteins for specific post-transcriptional regulation of endogenous RNA. *Journal of biological engineering*, *8*(1), 7 (2014); Zhao Y, Mao M, Zhang W, Wang J, Li H, Yang Y, Wang Z, & Wu J. Expanding RNA binding specificity and affinity of engineered PUF domains. *Nucleic Acids Research*, 46(9), 4771–4782 (2018); Shinoda K, Tsuji S, Futaki S, & Imanishi M. Nested PUF Proteins: Extending Target RNA Elements for Gene Regulation. *ChemBioChem*, *19*(2), 171-176 (2018); Koh YY, Wang Y, Qiu C, Opperman L, Gross L, Tanaka Hall TM, & Wickens M. Stacking Interactions in PUF-RNA Complexes. RNA, 17(4), 718-727 (2011).

[0547] As such, it is well known in the art that human PUM1 (1186 amino acids) contains an RNA-binding domain (RBD) in the C-terminus of the protein (also known as Pumilio homology domain PUM-HD amino acid 828-amino acid 1175) and that PUFs are based on the RBD of human PUM1. There are 8 structural repeat modules of 36 amino acids (except moldule 7 has 43 amino acids) for RNA binding and flanking N- and C- terminal regions important for protein structure and stability. Within each repeat module, amino acids 12, 13, and 16 are important for RNA binding with 12 and 16 controlling RNA base recognition. Amino acid 13 stacks with RNA bases and can be modified to tune specificity and affinity. Alternatively, the PUF design may maintain amino acid 13 as human PUM1's native residue. Recognition occurs in reverse orientation as N- and C-terminal PUF recognizes 3' to 5' RNA. Accordingly, PUF engineering of 8 modules (8PUF), as known in the art, mimics a human protein. An exemplary 8-mer RNA recognition (8PUF) would designed as follows: R1'-R1-R2-R3-R4-R5-R6-R7-R8-R8'. In one embodiment, an 8PUF is used as the RBD. In another embodiment, a variation of the 8PUF design is used to create a 12-mer RNA recognition (12PUF) RBD or a 16-mer RNA recognition (16PUF) RBD. Repeats 1-8 of wild type human PUM1 are provided herewith at SEQ ID NOS: 609-616, respectively. The nucleic acid sequence encoding the PUF domain from human PUM1 is SEQ ID NO: 617 and the amino acid sequence of the PUF domain from human PUM1 amino acids 828-176 is SEQ ID NO: 618. See also US Patent 9,580,714 which is incorporated herein in its entirety.

[0548] 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, which has been widely used in native and modified form for targeting RNA, has been engineered into a protein architecture designed 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) are concatenated in chains of varying composition and length, to bind desired target RNAs. In essence, PUMBY is a more simple and modular form of PumHD, in which a single protein unit of PumHD is concatenated into arrays of arbitrary size and binding sequence specificity. 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.

[0549] 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, the PUF or PUMBY RNA-binding proteins are fused with a nuclease domain such as E17.

[0550] Exemplary PUF RNA-binding protein used in the compositions and methods disclosed herein are as follows:

[0551] In some embodiments, a PUF26 protein (original sequence) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 393.

[0552] In some embodiments, a PUF26 protein of the disclosure is encoded by an optimized nucleic acid sequence comprising or consisting of of SEQ ID NO: 394.

[0553] In some embodiments, a PUF54 protein (original sequence) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 395.

[0554] In some embodiments, a PUF54 protein of the disclosure is encoded by an optimized nucleic acid sequence comprising or consisting of of SEQ ID NO: 396.

[0555] In some embodiments, a PUF60 protein (original sequence) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 397.

[0556] In some embodiments, a PUF60 protein of the disclosure is encoded by an optimized nucleic acid sequence comprising or consisting of of SEQ ID NO: 398.

[0557] In some embodiments, a PUF110 protein (original sequence) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 399.

[0558] In some embodiments, a PUF110 protein of the disclosure is encoded by an optimized nucleic acid sequence comprising or consisting of SEQ ID NO: 400.

[0559] Exemplary PUF RNA-binding proteins (targeting 8 Rho nucleotides) used in the compositions and methods disclosed herein are as follows:

[0560] In some embodiments, a PUF08 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 491.

[0561] In some embodiments, a PUF08 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 492.

[0562] In some embodiments, a PUF16 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 493.

[0563] In some embodiments, a PUF16 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 494.

[0564] In some embodiments, a PUF22 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 495.

[0565] In some embodiments, a PUF22 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 496.

[0566] In some embodiments, a PUF34 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 497.

[0567] In some embodiments, a PUF34 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 498.

[0568] In some embodiments, a PUF56 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 499.

[0569] In some embodiments, a PUF56 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 500.

[0570] In some embodiments, a PUF64 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 501.

[0571] In some embodiments, a PUF64 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 502.

[0572] In some embodiments, a PUF66 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 503.

[0573] In some embodiments, a PUF66 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 504.

[0574] In some embodiments, a PUF90 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 505.

[0575] In some embodiments, a PUF90 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 506.

[0576] In some embodiments, a PUF102 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 507.

[0577] In some embodiments, a PUF102 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 508.

[0578] In some embodiments, a PUF112 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 509.

[0579] In some embodiments, a PUF112 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 510.

[0580] In some embodiments, a PUF122 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 511.

[0581] In some embodiments, a PUF122 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 512.

[0582] In some embodiments, a PUF128 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 513.

[0583] In some embodiments, a PUF128 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 514.

[0584] In some embodiments, a PUF130 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 515.

[0585] In some embodiments, a PUF130 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 516.

[0586] In some embodiments, a PUF154 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 517.

[0587] In some embodiments, a PUF154 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 518.

[0588] In some embodiments, a PUF166 (targeting 8 nucleotides) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 519.

[0589] In some embodiments, a PUF166 (targeting 8 nucleotides) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 520.

[0590] Exemplary PUF RNA-binding proteins (targeting 16 Rho nucleotides) are as follows:

[0591] In some embodiments, a PUF26 (Design 1-P001IS) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 521.

[0592] In some embodiments, a PUF26 (Design 1-P001IS) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 522.

[0593] In some embodiments, a PUF26 (Design 2-P001KZ) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 523.

[0594] In some embodiments, a PUF26 (Design 2-P001KZ) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 524.

[0595] In some embodiments, a PUF26 (Design 3-P001LE) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 525.

[0596] In some embodiments, a PUF26 (Design 3-P001LE) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 526.

[0597] In some embodiments, a PUF54 (Design 1-P001IT) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 527.

[0598] In some embodiments, a PUF54 (Design 1-P001IT) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 528.

[0599] In some embodiments, a PUF54 (Design 2-P001LA) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 529.

[0600] In some embodiments, a PUF54 (Design 2-P001LA) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 530.

[0601] In some embodiments, a PUF54 (Design 3-P001LF) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 531.

[0602] In some embodiments, a PUF54 (Design 3-P001LF) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 532.

[0603] In some embodiments, a PUF60 (Design 1-P001IU) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 533.

[0604] In some embodiments, a PUF60 (Design 1-P001IU) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 534.

[0605] In some embodiments, a PUF60 (Design 2-P001LB) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 535.

[0606] In some embodiments, a PUF60 (Design 2-P001LB) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 536.

[0607] In some embodiments, a PUF60 (Design 3-P001LG) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 537.

[0608] In some embodiments, a PUF60 (Design 3-P001LG) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 538.

[0609] In some embodiments, a PUF110 (Design 1-P001IV) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 539.

[0610] In some embodiments, a PUF110 (Design 1-P001IV) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 540.

[0611] In some embodiments, a PUF110 (Design 2-P001LC) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 541.

[0612] In some embodiments, a PUF110 (Design 2-P001LC) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 542.

[0613] In some embodiments, a PUF110 (Design 3-P001LH) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 543.

[0614] In some embodiments, a PUF110 (Design 3-P001LH) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of SEQ ID NO: 545.

[0615] Exemplary PUMBY RNA-binding proteins (targeting 8 Rho nucleotides) are as follows:

[0616] In some embodiments, a PUM14 protein of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 401.

[0617] In some embodiments, a PUM14 protein of the disclosure is encoded by a nucleic acid sequence comprising or consisting of of SEQ ID NO: 402.

[0618] Exemplary PUMBY RNA-binding proteins (targeting 16 Rho nucleotides) are as follows:

[0619] In some embodiments, a PUM14 protein (Design 1-P001JG) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 545.

[0620] In some embodiments, a PUM14 protein (Design 1-P001JG) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of of SEQ ID NO: 546.

[0621] In some embodiments, a PUM14 protein (Design 2-P001JB) of the disclosure comprises or consists of the amino acid sequence of SEQ ID NO: 547.

[0622] In some embodiments, a PUM14 protein (Design 2-P001JB) of the disclosure is encoded by a nucleic acid sequence comprising or consisting of of SEQ ID NO: 548.

[0623] 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.

[0624] 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.

[0625] 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.

[0626] 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 at the N-terminus of 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 at the N-terminus of 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.

[0627] 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.

[0628] 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. In another embodiment, an exemplary fusion protein is a PUF or PUMBY-based first RNA-binding protein fused to a second RNA-bindng protein which is an zinc-finger endonuclease known as ZC3H12A of SEQ ID NO: 358 (also termed E17).

Vectors

[0629] 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 one embodiment, the vector comprises three guide RNAs. In one embodiment, the vector comprises four guide RNAs. In some embodiments, the vector further comprises a guided or non-guided RNA-binding protein of the disclosure. In some embodiments, the vector further comprises a RNA-binding fusion protein 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 RNA-guided RNA-binding systems comprising a RNA-binding protein and a gRNA are in a single vector. In a particular embodiment, the single vector comprises the RNA-guided RNA-binding systems which are Cas13d RNA-guided RNA-binding systems. In one embodiment, the single vector comprises

the Cas13dRNA-guided RNA-binding systems which are CasRx RNA-guided RNA-binding systems. In another embodiment, the single vector comprises a non-guided RNA-binding system comprising a PUF or PUMBY-based protein fused with a nuclease domain such as ZC3H12A.

[0630] 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 an RNA-binding protein or RNA-binding 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 vectors or vector serotypes. In some embodiments, the first vector and the second vector are not identical vectors or vector serotypes.

[0631] In some embodiments of the compositions and methods of the disclosure, the vector is or comprises a component of a "2-component Cas9-based RNA targeting system" comprising (a) nucleic acid sequence encoding an RNA-binding protein or RNA-binding fusion protein and a therapeutic replacement 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 (e.g., a pathogenic RNA comprising a target RNA sequence); and on its 3' end, an RNA sequence (or scaffold sequence) capable of binding to or associating with the CRISPR/Cas9 protein of the fusion protein; and wherein the 2-component RNA targeting system recognizes and alters the target RNA (e.g., comprised within pathogenic 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 RNA comprising one or more gain-or-loss-of-function mutations.

[0632] One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be inserted, such as by standard molecular cloning techniques. Another type of vector is a viral vector, wherein virally -derived DNA or RNA sequences are present in the vector for packaging into a virus (e.g., retroviruses, replication defective retroviruses, adenoviruses, replication defective adenoviruses, and adeno-associated viruses). Viral vectors also include polynucleotides carried by a virus for

transfection into a host cell. In some embodiments, the vector is a lentivirus (such as an integration-deficient lentiviral vector) or adeno-associated viral (AAV) vector. Vectors are capable of autonomous replication in a host cell into which they are introduced such as e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors and other vectors such as, e.g., non-episomal mammalian vectors, are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome.

[0633] In some embodiments, vectors such as e.g., expression vectors, are capable of directing the expression of genes to which they are operatively-linked. Common expression vectors are often in the form of plasmids. In some embodiments, recombinant expression vectors comprise a nucleic acid provided herein such as e.g., a guide RNA which can be expressed from an RNA sequence or a RNA sequence, and a nucleic acid encoding a Cas 13d protein, in a form suitable for expression of the nucleic acid in a host cell. Recombinant expression vectors include one or more regulatory elements, which may be selected on the basis of the host cells to be used for expression, that is operatively-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory element(s) in a manner that allows for expression of the nucleotide sequence such as e.g., in an in vitro transcription/translation system or in a host cell when the vector is introduced into the host cell. Certain embodiments of a vector depend on factors such as the choice of the host cell to be transformed, and the level of expression desired. A vector can be introduced into host cells to thereby produce transcripts, proteins, or peptides, including fusion proteins or peptides, encoded by nucleic acids as described herein such as, e.g., CRISPR transcripts, proteins, enzymes, mutant forms thereof, fusion proteins thereof, etc..

[0634] 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.

[0635] 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 (AAVrh10), AAV11 or AAV12. In one embodiment, the AAV vector comprises a modified capsid. In one embodiment the AAV vector is an AAV2-Tyr mutant vector. In one embodiment the AAV vector comprises a capsid with a non-tyrosine amino acid at a position that corresponds to a surface-exposed tyrosine residue in position Tyr252, Tyr272, Tyr275, Tyr281, Tyr508, Tyr612, Tyr704, Tyr720, Tyr730 or Tyr673 of wild-type AAV2. See also WO 2008/124724 incorporated herein in its entirety. In some embodiments, the AAV vector comprises an engineered capsid. AAV vectors comprising engineered capsids include without limitation, AAV2.7m8, AAV9.7m8, AAV2 2tYF, and AAV8 Y733F). 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).

[0636] 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.

[0637] 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. In some embodiments, expression control by a promoter is constituitive or

ubiquitous. Non-limiting exemplary promoters include a pol III promoter such as, e.g., U6 and H1 promoters and/or a pol II promoter e.g., SV40, CMV (optionally including the CMV enhancer), RSV (Rous Sarcoma Virus LTR promoter (optionally including RSV enhancer), CBA (hybrid CMV enhancer/ chicken ß-actin), CAG (hybrid CMV enhancer fused to chicken ß-actin), truncated CAG, Cbh (hybrid CBA), EF-1a (human longation factor alpha-1) or EFS (short intron-less EF-1 alphs), PGK (phosphoglycerol kinase), CEF (chicken embryo fibroblasts), UBC (ubiquitinC), GUSB (lysosomal enzyme beta-glucuronidase), UCOE (ubiquitous chromatin opening element), hAAT (alpha-1 antitrypsin), TBG (thyroxine binding globulin), Desmin, MCK (muscle creatine kinase), C5-12 (synthetic muscle promoter), NSE (neuron-specific enolase), Synapsin, Synapsin-1 (SYN-1), opsin, PDGF (platelet-derived growth factor), PDGF-A, MecP2 (methyl CpG-binding protein 2), CaMKII (Calcium/ Calmodulin-dependent protein kinase II), mGluR2 (metabotropic glutamate receptor 2), NFL (neurofilament light), NFH (neurofilament heavy), nβ2, PPE (rat preproenkephalin), ENK (preproenkephalin), Preproenkephalin-neurofilament chimeric promoter, EAAT2 (glutamate transporter), GFAP (glial fibrillary acidic protein), MBP (myelin basic protein), human rhodopsin kinase promoter (hGRK1), ß-actin promoter, dihydrofolate reductase promoter, and combinations thereof. 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, MCK enhancer, R-U5' segment in LTR of HTLV-1, SV40 enhancer, the intron sequence between exons 2 and 3 of rabbit ß-globin, and WPRE.

[0638] 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.

[0639] In one embodiment, the vector configuration is shown in e.g., Figures 1, 2 or 6. In another embodiment, the vector configuration comprises a promoter or regulatory sequence driving the expression of the nucleic acid encoding the RNA-binding protein in operable linkage with a promoter or regulatory sequence driving the expression of the replacement gene. In another embodiment, a vector configuration comprises an promoter such as a rhodopsin kinase promoter driving expression of the nucleic acid encoding the PUF or PUMBY fusion protein in operable linkage with a promoter such as an opsin promoter driving expression of a nucleic acid sequence encoding the replacement or "hardened" rhodopsin protein. In another embodiment, a vector configuration comprises an promoter such as an opsin promoter driving expression of the nucleic acid encoding the PUF or PUMBY fusion protein in operable linkage with a promoter such as an rhodopsin kinase promoter driving expression of a nucleic acid sequence encoding the replacement or "hardened" rhodopsin protein. In another embodiment, the nucleic acid encoding the RNA-binding protein operably linked to the nucleic acid encoding the replacement protein via an IRES or a 2A peptide.

[0640] 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 AAV2-Tyr mutant 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, an AAV-Tyr mutant 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

[0641] Provided herein are the nucleic acid sequences encoding the knockdown and replacement therapeutics disclosed herein for use in gene transfer and expression techniques described herein. It should be understood, although not always explicitly stated that the

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

[0642] 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., a nucleic acid sequenc encoding SEQ ID NO: 92 (Cas13d known as CasRx) or the nucleic acid sequence encoding SEQ ID NO: 298 (Cas13d known as CasRx), 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).

[0643] "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.

[0644] Examples of stringent hybridization conditions include: incubation temperatures of about 25°C to about 37°C; hybridization buffer concentrations of about 6x SSC to about 10x SSC; formamide concentrations of about 0% to about 25%; and wash solutions from about 4x SSC to about 8x SSC. Examples of moderate hybridization conditions include: incubation temperatures of about 40°C to about 50°C; buffer concentrations of about 9x SSC to about 2x SSC; formamide concentrations of about 30% to about 50%; and wash solutions of about 5x SSC to about 2x SSC. Examples of high stringency conditions include: incubation temperatures of about 55°C to about 68°C; buffer concentrations of about lx SSC to about 0.1x SSC; formamide concentrations of about 55% to about 75%; and wash solutions of about lx SSC, 0.1x SSC, or deionized water. In general, hybridization incubation times are from 5 minutes to 24 hours, with 1, 2, or more washing steps, and wash incubation times are about 1, 2, or 15 minutes. SSC is 0.15 M NaCl and 15 mM citrate buffer. It is understood that equivalents of SSC using other buffer systems can be employed.

[0645] "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

[0646] In some embodiments of the compositions and methods of the disclosure, a cell of the disclosure is a prokaryotic cell.

[0647] 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.

[0648] 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.

[0649] 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).

[0650] In some embodiments of the disclosure, a somatic cell is an ocular cell. An ocular cell includes, without limitation, corneal epithelial cells, keratyocytes, retinal pigment epithelial (RPE) cells, lens epithelial cells, iris pigment epithelial cells, conjunctival fibroblasts, non-pigmented ciliary epithelial cells, trabecular meshwork cells, ocular choroid fibroblasts, conjunctival epithelial cells, In some embodiments, an ocular cell is a retinal cell or a corneal cell. In one embodiment, a retinal cell is a photoreceptor cell or a retinal pigment epithelial cell. In another embodiment, a retinal cell is a ganglion cell, an amacrine cell, a bipolar cell, a horizontal cell, a Müller glial cell, a rod cell, or a cone cell.

[0651] 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.

[0652] 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.

[0653] 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.

[0654] 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.

[0655] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a primary cell.

[0656] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is a cultured cell.

[0657] 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.

[0658] In some embodiments of the compositions and methods of the disclosure, a somatic cell of the disclosure is autologous or allogeneic.

Methods of Use

[0659] 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 of the disclosure and the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the RNA-binding protein or RNA-binding fusion protein (or a portion thereof) to the RNA molecule.

[0660] The disclosure provides a method of modifying an activity of a protein encoded by an RNA molecule comprising contacting the composition of the disclosure and the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the RNA-binding protein or the fusion protein (or a portion thereof) to the RNA molecule.

[0661] 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 of the disclosure and a cell comprising the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the RNA-binding protein or 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 of the disclosure comprises a vector comprising a guide RNA of the disclosure and an RNA-binding protein or fusion protein of the disclosure and the therapeutic replacement protein of the disclosure. In some embodiments, the vector is an AAV.

[0662] The disclosure provides a method of modifying an activity of a protein encoded by an RNA molecule comprising contacting the composition of the disclosure and a cell comprising the RNA molecule under conditions suitable for binding of one or more of the guide RNA or the RNA-binding protein or 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 of the disclosure comprises a vector comprising a guide RNA or a single guide RNA sequence of the disclosure and a nucleic acid sequence encoding the RNA-binding protein or fusion protein of the disclosure and the therapeutic replacement protein of the disclosure. In some embodiments, the vector is an AAV.

[0663] The disclosure provides a method of modifying the level of expression of an RNA molecule of the disclosure or a protein encoded by the RNA molecule comprising contacting the composition of the disclosure and the RNA molecule under conditions suitable for RNA

nuclease activity wherein the RNA-binding protein or fusion protein induces a break in the RNA molecule.

[0664] The disclosure provides a method of modifying an activity of a protein encoded by an RNA molecule comprising contacting the composition of the disclosure and the RNA molecule under conditions suitable for RNA nuclease activity wherein the RNA-binding protein or fusion protein induces a break in the RNA molecule.

[0665] 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 of the disclosure and a cell comprising the RNA molecule under conditions suitable for RNA nuclease activity wherein the RNA-binding protein or fusion protein induces a break in the RNA molecule. In some embodiments, the composition of the disclosure additionally provides a replacement therapeutic protein which corresponds to a pathogenic RNA comprising a target RNA. 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, an RNA-binding fusion protein of the disclosure, and a therapeutic replacement protein of the disclosure. In some embodiments, the vector is an AAV.

[0666] 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 RNA-binding protein or 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 nucleic acid sequence encoding an RNA-binding protein or fusion protein of the disclosure and a therapeutic replacement protein. In some embodiments, the vector is an AAV.

[0667] 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.

[0668] 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 nucleic acid sequence encoding an RNA-binding protein

or fusion protein of the disclosure and a therapeutic replacement protein of the disclosure, wherein the composition modifies, reduces or ablates a level of expression of a pathogenic target RNA of an RNA molecule of the disclosure or a protein encoded by the RNA molecule (compared to the level of expression of a corresponding wild-type protein), and wherein the therapeutic protein replaces gain-or-loss-of-function mutations encoded by the pathogenic RNA.

[0669] 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 nucleic acid sequence encoding an RNA-binding protein or fusion protein of the disclosure and a therapeutic replacement protein of the disclosure, wherein the composition modifies, reduces or ablates a level of expression of a pathogenic target RNA of an RNA molecule of the disclosure or a protein encoded by the RNA molecule (compared to the level of expression of a corresponding wild-type protein), and wherein the therapeutic protein replaces gain-or-loss-of-function mutations encoded by the pathogenic RNA.

[0670] In some embodiments of the compositions and methods of the disclosure, a disease or disorder includes, without limitation, a disease or disorder related to rhodopsin expression or lack thereof. In some embodiments, the disease or disorder is a retinal degenerative disorder or retinopathy. In some embodiments, the retinal degenerative disorder is retinitis pigmentosa.

[0671] Retinitis pigmentosa is an autosomal dominant disorder caused by gain-or-loss-of-function mutations in the rhodopsin gene. Loss of rod photoreceptor cells which express rhodopsin leads to loss of cone photoreceptor cells which causes a degenerative loss of vision. Mutations in the human rhodopsin gene affect the protein's folding, trafficking and activity which most often triggers retinal degeneration in afflicted patients. A single base-substitution at codon position 23 in the human opsin gene (P23H) is also a common cause of retinitis pigmentosa. Retinitis pigmentosa is one of the most common forms of inherited retinal degeneration with a prevalence of 1 in 4000. The disease is the result of varying inheritance patterns (autosomal dominant, autosomal recessive, and X-linked) depending on the mutated gene.

[0672] 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.

[0673] 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, AAT (alpha 1 anti-trypsin deficiency), Wegener's granulomatosis, Wilson disease, Hereditary Hemochromatosis Types 1-5, Type I tyrosinemia, Argininosuccinate Lyase Deficiency, Glycogen storage disease type I-VIII, Citrin deficiency, Cholesteryl ester storage disease, progressive familial intrahepatic cholestasis type 3, polycystic kidney disease, Alström syndrome, and Congenital hepatic fibrosis.

[0674] 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.

[0675] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a metabolic disease or disorder. In some embodiments, the metabolic disease or disorder is related to inborn errors of the metabolism. In some embodiments, the metabolic disease or disorder related to inborn errors of the metabolism include, without limitation, disorders of amino acid metabolism, disorders of carbohydrate metabolism, disorder or defects of urea cycle, disorders of organic acid metabolism (e.g., organic acidurias), disorders of fatty acid oxidation and mitochondrial metabolism, disorders of porphyrin metabolism, disorders of purine or pyrimidine metabolism, disorders of steroid metabolism, disorders of peroxisomal function, lysosomal storage disorders, and cholestatic diseases.

[0676] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, mitochondrial diseases. In some embodiments, the mitochondrial disease includes, but is not limited to, Leber's hereditary optic neuropathy (LHON), Leigh's disease or syndrome, Neuropathy, Ataxia, and Retinitis Pigmentosa (NARP), Kearns-Sayre syndrome (KSS), Pearson syndrome, Chronic Progressive External Opthalmoplegia (CPEO), Mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE), Mitochondrial Encephalomyopathy Lactic Acidosis and Strokelike Episodes (MELAS), and Mitochondrial Enoyl CoA Reductase Protein Associated Neurodegeneration (MEPAN).

[0677] 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.

[0678] 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.

[0679] 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.

[0680] 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.

[0681] In some embodiments of the compositions and methods of the disclosure, a disease or disorder of the disclosure includes, but is not limited to, a proliferative disease or disorder. In some embodiments, the proliferative disease or disorder is a cancer. In some embodiments, the cancer includes, but is not limited to, Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Adrenocortical Carcinoma, AIDS-Related Cancers, Kaposi Sarcoma (Soft Tissue Sarcoma), AIDS-Related Lymphoma (Lymphoma), Primary CNS Lymphoma (Lymphoma), Anal Cancer, Appendix Cancer, Gastrointestinal Carcinoid Tumors, Astrocytomas, Atypical Teratoid/Rhabdoid Tumor, Central Nervous System (Brain Cancer), Basal Cell Carcinoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Ewing Sarcoma, Osteosarcoma, Malignant Fibrous Histiocytoma, Brain Tumors, Breast Cancer, Burkitt Lymphoma, Carcinoid Tumor, Carcinoma, Cardiac (Heart) Tumors, Embryonal Tumors, Germ Cell Tumor, Primary CNS Lymphoma, Cervical Cancer, Cholangiocarcinoma, Chordoma, Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Chronic Myeloproliferative Neoplasms, Colorectal Cancer , Craniopharyngioma, Cutaneous T-Cell Lymphoma, Ductal Carcinoma In Situ, Embryonal Tumors, Endometrial Cancer (Uterine Cancer), Ependymoma, Esophageal Cancer, Esthesioneuroblastoma (Head and Neck Cancer), Ewing Sarcoma (Bone Cancer), Extracranial Germ Cell Tumor, Extragonadal Germ Cell Tumor, Eye Cancer, Childhood Intraocular Melanoma, Intraocular Melanoma, Retinoblastoma, Fallopian Tube Cancer, Fibrous Histiocytoma of Bone, Malignant, and Osteosarcoma, Gallbladder Cancer, Gastric

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

Cancer, Oropharyngeal Cancer, Hypopharyngeal Cancer, Thymoma and Thymic Carcinoma , Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Renal Cell Cancer, Urethral Cancer, Uterine Sarcoma, Vaginal Cancer, Vascular Tumors (Soft Tissue Sarcoma), Vulvar Cancer, Wilms Tumor and Other Childhood Kidney Tumors.

[0682] 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.

[0683] 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.

[0684] 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.

[0685] 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.

[0686] In some embodiments of the methods of the disclosure, a subject of the disclosure is a human.

[0687] 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.

[0688] 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.

[0689] In some embodiments of the methods of the disclosure, a therapeutically effective amount eliminates the disease or disorder.

[0690] 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.

[0691] 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.

[0692] 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.

[0693] In some embodiments, the compositions disclosed herein are formulated as pharmaceutical compositions. Briefly, pharmaceutical compositions for use as disclosed herein may comprise a protein(s) or a polynucleotide encoding the 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 routes of administration, such as e.g., oral, enteral, topical, transdermal, intranasal, and/or inhalation; and for routes of administration via injection or infusion such as, e.g., intravenous, intramuscular, subpial, intrathecal, intrastriatal, subcutaneous, intradermal, intraperitoneal, intratumoral, intravenous, intraocular, and/or parenteral administration. In some embodiments, intraocular administration includes, without limitation, subretinal, intravitreal, deep intravitreal, or topical (via eye drops) administration. In one embodiment, subretinal injection targets photoreceptors and RPE (retinal pigment epithelium) cells. In certain embodiments, the compositions of the present disclosure are formulated for intravenous administration.

Example Embodiments:

[0694] Embodiment 1. A composition comprising a nucleic acid sequence encoding an RNA-guided target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target RNA when guided by a gRNA sequence, wherein a pathogenic RNA comprises the target RNA, and wherein the therapeutic protein is a replacement of gain-or-loss-of-function mutations encoded by the pathogenic RNA.

[0695] Or

[0696] A composition comprising a nucleic acid sequence encoding a target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target RNA, wherein a pathogenic RNA comprises the target RNA, and wherein the therapeutic protein is a replacement of gain-or-loss-of-function mutations encoded by the pathogenic RNA.

[0697] Or

[0698] A composition comprising a nucleic acid sequence encoding a target RNA knockdown and replacement therapeutic comprising (a) an RNA-binding polypeptide or portion thereof; and (b) a therapeutic protein, wherein the RNA-binding polypeptide binds and cleaves a target RNA, wherein a pathogenic RNA comprises the target RNA, and wherein the pathogenic RNA encodes one or more gain-of-function rhodopsin mutations, and wherein the therapeutic protein is wild-type rhodopsin or “hardened” rhodopsin which replaces the gain-or-loss-of-function rhodopsin mutations.

[0699] Embodiment 2. The composition of embodiment 1, wherein the therapeutic protein is selected from the group consisting of rhodopsin (Retinitis Pigmentosa), PRPF3 (Retinitis Pigmentosa), PRPF31 (autosomal dominant Retinitis Pigmentosa), GRN (FTD), SOD1 (ALS), PMP22 (Charcot Marie Tooth Disease), PABPN1 (Oculopharangeal Muscular Dystrophy), KCNQ4 (Hearing Loss), CLRN1 (Usher Syndrome), APOE2 (Alzheimer's Disease), APOE4 (Alzheimer's Disease), BEST1 (Eye Disease), MYBPC3 (Familial Cardiomyopathy), TNNT2 (Familial Cardiomyopathy), and TNNI3 (Familial Cardiomyopathy).

[0700] Embodiment 3. The composition of embodiment 1 or 2, wherein the pathogenic target sequence comprises or encodes at least one gain-or-loss-of-function mutation.

[0701] Embodiment 4. The composition of embodiment 1, wherein the sequence comprising the gRNA comprises a promoter capable of expressing the gRNA in a eukaryotic cell.

[0702] Embodiment 5. The composition of embodiment 4, wherein the eukaryotic cell is an animal cell.

[0703] Embodiment 6. The composition of embodiment 4, wherein the animal cell is a mammalian cell.

[0704] Embodiment 7. The composition of embodiment 5, wherein the animal cell is a human cell.

[0705] Embodiment 8. The composition of any one of embodiments 1-7, wherein the promoter is a constitutively active promoter.

[0706] Embodiment 9. 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.

[0707] Embodiment 9. The composition of embodiment 9, wherein the promoter is isolated or derived from a U6 promoter.

[0708] Embodiment 10. The composition of any one of embodiments 1-9, wherein the promoter is isolated or derived from a promoter capable of driving expression of a transfer RNA (tRNA).

[0709] Embodiment 11. 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.

[0710] Embodiment 12. The composition of embodiment 11, wherein the promoter is isolated or derived from a valine tRNA promoter.

[0711] Embodiment 13. 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.

[0712] Embodiment 14. 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.

[0713] Embodiment 15. The composition of embodiment 14, wherein the spacer sequence has 100% complementarity to the target RNA sequence.

[0714] Embodiment 16. The composition of any one of embodiments 13-15, wherein the spacer sequence comprises or consists of 20 nucleotides.

[0715] Embodiment 17. The composition of any one of embodiments 13-15, wherein the spacer sequence comprises or consists of 26 nucleotides.

[0716] Embodiment 18. The composition of any one of embodiments 1-17, wherein the sequence comprising the gRNA comprises a direct repeat (DR) or scaffold sequence that specifically binds to the first RNA binding protein.

[0717] Embodiment 20. The composition of embodiment 18, wherein the scaffold sequence comprises a stem-loop structure.

[0718] Embodiment 21. The composition of embodiment 19 or 20, wherein the scaffold sequence comprises or consists of 90 nucleotides.

[0719] Embodiment 22. The composition of embodiment 19 or 20, wherein the scaffold sequence comprises or consists of 93 nucleotides.

[0720] Embodiment 23. The composition of embodiment 22, wherein the scaffold sequence comprises the sequence GUUUAAGAGCUAUGCUGGAAACAGCAUAGCAAGUUUAAAUAAGGCUAGUCCG UUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU (SEQ ID NO: 403).

[0721] Embodiment 24. The composition of embodiment 19, wherein the scaffold sequence comprises a step-loop structure.

[0722] Embodiment 25. The composition of embodiment 19, wherein the scaffold sequence comprises or consists of 85 nucleotides.

[0723] Embodiment 26. The composition of embodiment 25, wherein the scaffold sequence comprises the sequence GGACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGAAAAAGU GGCACCGAGUCGGUGCUUUUU (SEQ ID NO: 404).

[0724] Embodiment 27. The composition of embodiment 19, wherein the sequence comprising the gRNA comprises a DR sequence that specifically binds to the first RNA binding protein.

[0725] Embodiment 28. The composition of embodiment 27, wherein the DR sequence comprises a stem-loop structure.

[0726] Embodiment 29. The composition of embodiment 27, wherein the DR sequence comprises or consists of about 20-36 nucleotides.

[0727] Embodiment 30. The composition of embodiment 27, wherein the scaffold sequence comprises or consists of 30-32 nucleotides.

[0728] Embodiment 31. The composition of embodiment 27, wherein the DR sequence comprises the nucleotide sequence comprising AACCCCTACCAACTGGTCGGGGTTTGAAAC (SEQ ID NO: 461).

[0729] Embodiment 32. The composition of any one of embodiments 1-31, wherein the gRNA does not bind or does not selectively bind to a second sequence within the RNA molecule.

[0730] Embodiment 33. The composition of embodiment 32, wherein an RNA genome or an RNA transcriptome comprises the RNA molecule.

[0731] Embodiment 34. The composition of any one of embodiments 1-33, wherein the RNA binding protein comprises a CRISPR-Cas protein.

[0732] Embodiment 35. The composition of embodiment 34, wherein the CRISPR-Cas protein is a Type II CRISPR-Cas protein.

[0733] Embodiment 36. The composition of embodiment 35, wherein the RNA binding protein comprises a Cas9 polypeptide or an RNA-binding portion thereof.

[0734] Embodiment 37. The composition of embodiment 34, wherein the CRISPR-Cas protein is a Type V CRISPR-Cas protein.

[0735] Embodiment 38. The composition of embodiment 34, wherein the RNA binding protein comprises a Cpf1 polypeptide or an RNA-binding portion thereof.

[0736] Embodiment 39. The composition of embodiment 34, wherein the CRISPR-Cas protein is a Type VI CRISPR-Cas protein.

[0737] Embodiment 40. The composition of embodiment 39, wherein the RNA binding protein comprises a Cas13 polypeptide or an RNA-binding portion thereof.

[0738] Embodiment 41. The composition of any one of embodiments 34-40, wherein the CRISPR-Cas protein comprises a native RNA nuclease activity.

[0739] Embodiment 42. The composition of embodiment 41, wherein the native RNA nuclease activity is reduced or inhibited.

[0740] Embodiment 43. The composition of embodiment 41, wherein the native RNA nuclease activity is increased or induced.

[0741] Embodiment 44. The composition of any one of embodiments 34-43, wherein the CRISPR-Cas protein comprises a native DNA nuclease activity and wherein the native DNA nuclease activity is inhibited, inactive, and/or dead (e.g., dCas).

[0742] Embodiment 45. The composition of embodiment 34, wherein the CRISPR-Cas protein comprises a mutation.

[0743] Embodiment 46. The composition of embodiment 45, wherein a nuclease domain of the CRISPR-Cas protein comprises the mutation.

[0744] Embodiment 47. The composition of embodiment 45, wherein the mutation occurs in a nucleic acid encoding the CRISPR-Cas protein.

[0745] Embodiment 48. The composition of embodiment 45, wherein the mutation occurs in an amino acid encoding the CRISPR-Cas protein.

[0746] Embodiment 49. The composition of any one of embodiments 45-48, wherein the mutation comprises a substitution, an insertion, a deletion, a frameshift, an inversion, or a transposition.

[0747] Embodiment 50. The composition of any one of embodiments 45-49, 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.

[0748] Embodiment 51. The composition of any one of embodiments 2-3, wherein the RNA binding protein comprises a Pumilio and FBF (PUF) protein.

[0749] Embodiment 52. The composition of embodiment 51, wherein the RNA binding protein comprises a Pumilio-based assembly (PUMBY) protein.

[0750] Embodiment 53. The composition of any one of embodiments 51-52, wherein the RNA binding protein does not require multimerization for RNA-binding activity.

[0751] Embodiment 54. The composition of embodiment 53, wherein the RNA binding protein is not a monomer of a multimer complex

[0752] Embodiment 55. The composition of embodiment 54, wherein a multimer protein complex does not comprise the first RNA binding protein.

[0753] Embodiment 56. The composition of any one of embodiments 1-55, wherein the RNA binding protein selectively binds to a pathogenic target sequence within the RNA molecule.

[0754] Embodiment 57. The composition of embodiment 56, wherein the RNA binding protein does not comprise an affinity for a second sequence within the RNA molecule.

[0755] Embodiment 58. The composition of embodiment 56 or 57, wherein the RNA binding protein does not comprise a high affinity for or selectively bind a second sequence within the RNA molecule.

[0756] Embodiment 59. The composition of embodiment 58, wherein an RNA genome or an RNA transcriptome comprises the RNA molecule.

[0757] Embodiment 60. The composition of any one of embodiments 1-59, wherein the RNA binding protein comprises between 2 and 1300 amino acids, inclusive of the endpoints.

[0758] Embodiment 61. The composition of any one of embodiments 1-60, wherein the sequence encoding the RNA binding protein further comprises a sequence encoding a nuclear localization signal (NLS).

[0759] Embodiment 62. The composition of embodiment 61, wherein the sequence encoding a nuclear localization signal (NLS) is positioned 3' to the sequence encoding the first RNA binding protein.

[0760] Embodiment 63. The composition of embodiment 62, wherein the RNA binding protein comprises an NLS at a C-terminus of the protein.

[0761] Embodiment 64. The composition of any one of embodiments 1-63, wherein the sequence encoding the RNA binding protein further comprises a first sequence encoding a first NLS and a second sequence encoding a second NLS.

[0762] Embodiment 65. The composition of embodiment 64, wherein the sequence encoding the first NLS or the second NLS is positioned 3' to the sequence encoding the RNA binding protein.

[0763] Embodiment 66. The composition of embodiment 65, wherein the RNA binding protein comprises the first NLS or the second NLS at a C-terminus of the protein.

[0764] Embodiment 67. The composition of any one of embodiments 1-66, wherein the second RNA binding protein comprises or consists of a nuclease domain.

[0765] Embodiment 68. A composition comprising a sequence encoding 1) 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 pathogenic target RNA not guided by a gRNA sequence, and wherein the second RNA-binding polypeptide comprises RNA-nuclease activity; and 2) a therapeutic replacement protein, wherein the therapeutic replacement protein replaces a corresponding gene comprising at least one gain-or-loss-of-functionmutation encoded by the pathogenic target RNA.

Embodiment 69. The composition of embodiment 68, wherein the first RNA-binding polypeptide or portion thereof is a PUF, PUMBY, or PPR polypeptide or portion thereof.

Embodiment 70. A method for modifying the level of expression of a pathogenic RNA molecule or a protein encoded by the RNA molecule, the method comprising contacting the composition of embodiments 1, 2, 3 or 68 and the RNA molecule under conditions suitable for binding of the RNA-binding protein or a portion thereof to the RNA molecule.

Embodiment 71. A method of manufacturing the RNA-targeting knockdown and replacement compositions disclosed herein or the vectors comprising the RNA-targeting knockdown and replacement compositions disclosed herein.

EXAMPLES

Example 1: RNA-guided Cleavage of Target mRNAs

[0766] Various RNA-targeting proteins with and without an effector nuclease were constructed. The RNA-targeting proteins are either CRISPR-associated (Cas) proteins or engineered RNA binding proteins known as PUF or Pumby proteins (Fig. 1A-1E). Plasmids encoding the RNA-guided-targeting RNA-binding proteins are co-transfected with a plasmid encoding a corresponding guide RNA that targets a target RNA sequence, e.g., in genes encoding SOD1, human Rhodopsin, PRPF3, PMP22, PABPN1, KCNQ4, CLRN1, APOE2, APOE4, BEST1, MYBPC3, TNNT2, TNN13, or some other gene or mutated gene which causes a disease or leads to a disorder. Plasmids and vectors were designed using exemplary guide RNA spacer sequences which are specific to the target RNA. See SEQ ID NO: 250 to SEQ ID NO: 24960 for exemplary gRNA sequences targeting RHO, SOD1, PMP22, PABPN1, KCNQ4, CLRN1, APOE2, TNNI3, BEST1, MYBPC3, and TNNT2. A plasmid encoding a Cas13d RNA-guided-targeting RNA-binding protein was co-transfected with a plasmid encoding a corresponding guide RNA that targets a target RNA sequence. A Cas13d system based on CasRx sequences was used. Three gRNAs comprising the below spacer sequences targeting rhodopsin target RNA were constructed and used for knockdown of the rhodopsin target sequence below. The gRNAs comprised a CasRx DR sequence with the nucleic acid sequence AACCCCTACCAACTGGTCGGGGTTTGAAAC (SEQ ID NO: 461). The transfected cell line was co-transfected with a plasmid encoding the target RNA. In addition, a cell line which natively expressed the target RNA is used. The level of the target RNA was evaluated by RT-PCR. We observed knockdown of WT RHO containing mRNA.

[0767] Spacer sequences and target sequences used for Rho targeting are as detailed in table 2.

[0768] Table 2: Spacer sequences and target sequences used for Rho targeting

Spacer	Spacer Sequences	Target Sequences
Rho guide 1	ACATGTAGATGACAAAAGACTCGTTG (SEQ ID NO: 465)	CAACGAGTCTTTTGTCATCTACATGT (SEQ ID NO: 462)
Rho guide 2	TGAAGATGTAGAATGCCACGCTGGCG (SEQ ID NO: 409)	CGCCAGCGTGGCATTCTACATCTTCA (SEQ ID NO: 463)
Rho guide 3	ACTGCTTGTTCATCATGATATAGATG (SEQ ID NO: 466)	CATCTATATCATGATGAACAAGCAGT (SEQ ID NO: 464)

Example 2: Simultaneous Knockdown and Replacement of Target Genes

[0769] Vectors that carry an RNA-targeting system described in Example 1 with a codon-optimized version of the targeted gene, lacking the corresponding pathogenic mutation, were constructed (FIG. 2). The resulting vectors are capable of knocking down the endogenous, mutated gene and reconstituting expression of the same gene with a wild-type copy. Cells are transfected with the vectors. In addition, cells are infected with AAV vectors comprising the RNA-targeting systems (FIG. 2). We assess levels of both the mutated gene in cells and levels of the reconstituted, therapeutic replacement gene (FIG. 2).

Example 3: Simultaneous Knockdown and Replacement of Target Genes in a Model of disease

[0770] Vectors that carry an RNA-targeting system described in Example 1 with a codon-optimized version of the targeted gene, lacking the corresponding pathogenic mutation, were constructed. The resulting vectors are capable of knocking down the endogenous, mutated gene and reconstituting expression of the same gene with a wild-type copy. Mice harboring mutated copies of one of the following genes are treated with AAV vectors carrying the above systems (associated human disease in parentheses): rhodopsin (Retinitis Pigmentosa), PRPF3 (Retinitis Pigmentosa), PRPF31 (autosomal dominant Retinitis Pigmentosa), GRN (FTD), SOD1 (ALS), PMP22 (Charcot Marie Tooth Disease), PABPN1 (Oculopharangeal Muscular Dystrophy), KCNQ4 (Hearing Loss), CLRN1 (Usher Syndrome), APOE2 (Alzheimer's Disease), APOE4 (Alzheimer's Disease), BEST1 (Eye Disease), MYBPC3 (Familial Cardiomyopathy), TNNT2 (Familial Cardiomyopathy), and TNNI3 (Familial Cardiomyopathy). We assess levels of both the mutated gene in cells and levels of the reconstituted, unmutated therapeutic replacement gene in the target tissue. We also assess functional/behavioral/physiological changes in situations where these phenomena are modulated by the disease model.

Example 4: Rhodopsin Knockdown and Replacement

[0771] For rhodopsin (RHO) knockdown detection a luciferase reporter assay was designed using the pmirGlo plasmid (FIG. 3) by introducing the wild type (WT) RHO mRNA sequence in the 3'UTR of Firefly luciferase driven by the human phosphoglycerate kinase (hPGK). The reporter plasmid also expressed Renilla luciferase driven by the SV40 promoter for normalization purposes. For knockdown and replacement of RHO 500ng of the

'Knockdown and Replace' PUM and PUF constructs (1 PUMBY construct PUM14, 4 PUF constructs 26, 54, 60, 110 with different optimized PUF sequences – PUF sequences listed below) that express "hardened" Rhodopsin (RHO) open reading frame driven by the opsin promoter and EFS-promoter driven PUMBY or PUF protein linked to ZC3H12A, also termed E17 (FIG. 4, FIG. 5, FIG. 6A) targeting, for cleavage, a specific site on the WT RHO mRNA were transfected using Lipofectamine 3000 (Thermo) into CosM6 cells (according to the manufacturer's protocol) along with the 100ng of the pmirGlo reporter. Cells were washed and RNA was collected using the Qiagen RNeasy kit. RT-qPCR for normal and hardened Rhodopsin was performed using the Quantabio 1-step RT-qPCR kit, Biorad qPCR machine and the following primer sets: Firefly Luciferase – Forward: GTGGTGTGCAGCGAGAATAG (SEQ ID NO: 410) Reverse: CGCTCGTTGTAGATGTCGTTAG (SEQ ID NO: 411); Renilla Luciferase - Forward: TTCTGGATTCATCGACTGTG (SEQ ID NO: 412) Reverse: TTCAGCAATATCACGGGTAG (SEQ ID NO: 413); Hardened RHO - Forward: ACTGCATGCTCACCACCAT (SEQ ID NO: 414) Reverse: CGAAGAACTCCAGCATGAGA (SEQ ID NO: 415). Firefly luciferase expression was used as the measure of WT RHO mRNA knockdown normalized Renilla Luciferase mRNA expression used to control for transfection. Hardened Rhodopsin expression was normalized to GAPDH and was a measure of replacement. We observed that our knockdown and replace vectors were able to knockdown WT RHO containing mRNA and decrease Firefly Luciferase expression while simultaneously expressing hardened RHO levels of which were sustained. (FIGS. 6B-C and 7A-B).

[0772] Table 3: PUF and PUMBY Sequences used in the Knockdown and Replacement Studies

Construct	Target sequence 8 nucleotides	Target sequence 16 nucleotides	Hardened Sequence on replacement
PUF110 (A000YH)	UCAUCAUG (SEQ ID NO: 549)	GUCAUCAUCAUGGUCA (SEQ ID NO: 550)	GTGATTATTATGGTGA (SEQ ID NO: 551)
PUF54 (A000XL)	CCUGUGGU (SEQ ID NO: 552)	UUGCCCUGUGGUCCUU (SEQ ID NO: 553)	TCGCTCTCTGGTCTTT (SEQ ID NO: 554)
PUF60 (A000XM)	GGUGUGUA (SEQ ID NO: 555)	UGGUGGUGUGUAAGCC (SEQ ID NO: 556)	TCGTCGTCTGCAAACC (SEQ ID NO: 557)
PUF26 (A000XK)	UCUACGUC (SEQ ID NO: 558)	ACGCUCUACGUCACCG (SEQ ID NO: 559)	ACCCTGTATGTGACAG (SEQ ID NO: 560)
PUMBY14 (A000FS)	GUGGCAUUCUACAU (SEQ ID NO: 561)	CGUGGCAUUCUACAUC (SEQ ID NO: 562)	CGTAGCTTTTTATATT (SEQ ID NO: 563)

[0773] The following sequences are present at the Knockdown module for the above referenced plasmids.

[0774] Original PUF26 amino acid sequence:

[0775] MGRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAER QLVFNEILQAAYQLMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGS RVIEKALEFIPSDQQNEMVRELDGHVLKCVKDQNGSYVVRKCIECVQPQSLQFIIDAF KGQVFALSTHPYGCRVIQRILEHCLPDQTLPILEELHQHTEQLVQDQYGNYVIQHVLE HGRPEDKSKIVAEIRGNVLVLSQHKFASYVVRKCVTHASRTERAVLIDEVCTMNDGP HSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAK LEKYYMKNGVDLG (SEQ ID NO: 393

[0776] The Optimized (for Homo sapiens(Human)) sequence of PUF26

[0777] A 285 T 205 C 286 G 292 | GC%: 54.12% | Length: 1068

[0778] ATGGGAAGGAGCAGACTCCTCGAGGACTTTAGGAACAATAGATACCCCA ACCTCCAGCTGAGAGAAATCGCCGGCCACATCATGGAGTTCAGCCAAGACCAGC ACGGATCTAGATTCATTAGGCTGAAGCTCGAGAGAGCTACACCCGCCGAGAGGC AACTGGTGTTCAATGAGATTCTGCAAGCCGCCTACCAGCTCATGGTCGACGTCTT CGGAAACTACGTGATCCAGAAGTTCTTCGAGTTCGGATCTCTGGAGCAGAAACTC GCTCTGGCTGAGAGGATCAGAGGCCATGTGCTGTCTCTGGCTCTCCAGATGTACG GCTCTAGAGTGATCGAGAAAGCCCTCGAGTTCATCCCCTCCGACCAACAGAATG AGATGGTGAGGGAGCTGGACGGCCACGTGCTGAAATGTGTGAAGGACCAGAAC GGCTCCTACGTCGTGAGAAAGTGCATTGAGTGCGTGCAGCCCCAGAGCCTCCAG TTTATCATCGACGCCTTCAAGGGCCAAGTGTTCGCTCTCAGCACCCATCCTTACG GCTGTAGAGTCATCCAGAGAATTCTGGAGCATTGCCTCCCCGACCAGACACTGCC TATTCTCGAGGAGCTCCATCAGCATACCGAGCAACTCGTCCAAGACCAGTACGG CAACTACGTGATTCAGCATGTGCTGGAGCATGGCAGACCCGAGGACAAGAGCAA GATCGTGGCTGAGATCAGAGGCAATGTGCTGGTGCTGAGCCAGCACAAATTCGC CAGCTATGTGGTGAGGAAGTGTGTGACACACGCCTCTAGAACAGAGAGGGCTGT GCTCATCGATGAGGTGTGCACCATGAACGATGGCCCTCACAGCGCTCTGTACACC ATGATGAAGGACCAGTACGCCAACTACGTGGTGCAGAAAATGATCGACGTGGCT GAGCCCGGCCAGAGGAAAATCGTGATGCACAAGATCAGACCTCATATCGCCACC CTCAGAAAGTACACCTATGGCAAACACATTCTGGCCAAGCTCGAGAAGTACTAC ATGAAAAATGGCGTCGATCTGGGC (SEQ ID NO: 394)

[0779] The original sequence of PUF54

[0780] MGRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGNRFIQLKLERATPAER QLVFNEILQAAYQLMVDVFGSYVIEKFFEFGSLEQKLALAERIRGHVLSLALQMYGS RVIEKALEFIPSDQQNEMVRELDGHVLKCVKDQNGNYVVQKCIECVQPQSLQFIIDA FKGQVFALSTHPYGSRVIERILEHCLPDQTLPILEELHQHTEQLVQDQYGNYVIQHVL EHGRPEDKSKIVAEIRGNVLVLSQHKFASYVVRKCVTHASRTERAVLIDEVCTMNDG PHSALYTMMKDQYASYVVRKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAK LEKYYMKNGVDLG (SEQ ID NO: 395)

[0781] The Optimized (for Homo sapiens(Human)) sequence of PUF54

[0782] A 290 T 194 C 285 G 299 | GC%: 54.68% | Length: 1068

[0783] ATGGGAAGATCCAGACTGCTGGAGGACTTTAGAAACAATAGGTACCCCA ATCTGCAGCTGAGAGAGATCGCCGGCCACATCATGGAATTCAGCCAAGACCAGC ACGGCAATAGATTCATCCAGCTGAAGCTCGAGAGGGCTACACCCGCTGAGAGGC AGCTGGTCTTCAACGAGATTCTGCAAGCCGCCTATCAACTGATGGTGGACGTGTT CGGCAGCTATGTGATCGAGAAGTTCTTCGAATTCGGCTCTCTGGAACAGAAGCTG GCTCTGGCCGAGAGGATCAGAGGCCATGTGCTGTCTCTGGCTCTGCAGATGTACG GCTCTAGAGTCATCGAGAAGGCCCTCGAGTTCATCCCCTCCGACCAACAGAACG AGATGGTGAGGGAGCTGGACGGACACGTGCTGAAGTGCGTGAAGGACCAGAAC GGAAACTACGTCGTCCAGAAGTGCATCGAATGCGTGCAGCCCCAGAGCCTCCAG TTCATTATCGACGCCTTCAAGGGCCAAGTGTTCGCCCTCAGCACACACCCTTACG GAAGCAGAGTGATCGAGAGGATTCTGGAGCACTGTCTGCCCGACCAGACACTGC CTATTCTGGAGGAGCTGCACCAACACACAGAGCAGCTGGTGCAAGACCAGTACG GCAACTATGTCATTCAGCACGTCCTCGAGCATGGCAGACCCGAGGACAAAAGCA AGATCGTCGCCGAAATCAGAGGCAATGTGCTGGTGCTCAGCCAACACAAGTTCG CTTCCTACGTCGTGAGGAAGTGCGTGACACACGCTTCCAGAACAGAGAGAGCCG TGCTCATCGATGAGGTGTGCACCATGAACGATGGCCCTCACAGCGCTCTGTATAC CATGATGAAGGACCAATACGCCAGCTATGTGGTGAGAAAGATGATCGACGTGGC TGAACCCGGCCAGAGAAAGATCGTGATGCACAAGATCAGACCCCACATTGCCAC ACTGAGGAAGTATACCTACGGCAAGCACATTCTGGCCAAGCTCGAGAAGTACTA CATGAAGAACGGAGTGGATCTGGGC (SEQ ID NO: 396)

[0784] The original sequence of PUF60

[0785] MGRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAER QLVFNEILQAAYQLMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGS RVIEKALEFIPSDQQNEMVRELDGHVLKCVKDQNGNYVVQKCIECVQPQSLQFIIDA

FKGQVFALSTHPYGSRVIERILEHCLPDQTLPILEELHQHTEQLVQDQYGNYVIQHVL
EHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDG
PHSALYTMMKDQYASYVVEKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAK
LEKYYMKNGVDLG (SEQ ID NO: 397)

[0786] The Optimized (for Homo sapiens(Human)) sequence of PUF60

[0787] A 288 T 201 C 281 G 298 | GC%: 54.21% | Length: 1068

[0788] ATGGGAAGATCCAGACTGCTGGAGGACTTTAGAAATAATAGATACCCCA
ATCTGCAGCTGAGGGAAATCGCTGGCCACATCATGGAGTTCTCCCAAGACCAGC
ATGGATCTAGATTCATCCAGCTGAAGCTCGAGAGAGCCACCCCCGCCGAAAGGC
AGCTCGTCTTCAACGAAATTCTGCAAGCCGCCTACCAACTGATGGTGGATGTGTT
TGGCAACTACGTGATCCAGAAGTTCTTCGAATTTGGCAGCCTCGAGCAGAAGCTG
GCTCTGGCCGAAAGAATTAGAGGCCATGTGCTGTCTCTGGCCCTCCAGATGTATG
GCTCTAGAGTCATCGAAAAGGCTCTGGAGTTCATCCCCTCCGACCAGCAGAACG
AGATGGTGAGAGAGCTCGACGGACATGTGCTGAAGTGTGTGAAGGACCAGAACG
GCAATTACGTCGTCCAGAAGTGCATCGAGTGCGTGCAGCCCCAGTCTCTGCAGTT
TATCATCGACGCCTTCAAGGGCCAAGTGTTCGCTCTGAGCACACACCCTTACGGC
AGCAGAGTGATCGAGAGGATTCTGGAACACTGTCTGCCCGACCAGACACTGCCT
ATTCTGGAGGAGCTGCACCAGCACACAGAGCAGCTGGTGCAAGACCAGTACGGC
AACTATGTGATCCAGCATGTGCTGGAGCATGGCAGACCCGAGGACAAGAGCAAG
ATCGTGGCCGAAATCAGAGGCAACGTGCTGGTGCTGAGCCAGCACAAGTTCGCC
TCCAACGTGGTGGAAAAGTGCGTGACCCACGCTTCTAGAACAGAAAGGGCTGTG
CTCATCGATGAGGTGTGTACCATGAACGATGGCCCTCACAGCGCTCTGTACACCA
TGATGAAAGACCAGTACGCCAGCTACGTGGTGGAGAAAATGATCGACGTCGCTG
AGCCCGGCCAGAGGAAGATCGTGATGCACAAGATCAGACCCCACATTGCCACAC
TGAGGAAGTACACCTATGGCAAACACATTCTGGCCAAGCTCGAGAAGTACTACA
TGAAGAACGGAGTGGATCTGGGC (SEQ ID NO: 398)

[0789] The original sequence of PUF110

[0790] MGRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIELKLERATPAER
QLVFNEILQAAYQLMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGC
RVIQKALEFIPSDQQNEMVRELDGHVLKCVKDQNGSYVVRKCIECVQPQSLQFIIDAF
KGQVFALSTHPYGNRVIQRILEHCLPDQTLPILEELHQHTEQLVQDQYGCYVIQHVLE
HGRPEDKSKIVAEIRGNVLVLSQHKFASYVVRKCVTHASRTERAVLIDEVCTMNDGP

HSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAK
LEKYYMKNGVDLG (SEQ ID NO: 399)

[0791] The Optimized (for Homo sapiens(Human)) sequence of PUF110

[0792] A 292 T 196 C 293 G 287 | GC%: 54.31% | Length: 1068

[0793] ATGGGAAGATCCAGACTGCTGGAGGACTTTAGAAACAATAGGTACCCCA
ACCTCCAGCTGAGAGAAATCGCCGGCCACATCATGGAGTTCAGCCAAGACCAGC
ACGGCTCTAGATTTATTGAGCTGAAGCTCGAGAGAGCCACCCCCGCCGAGAGGC
AACTGGTGTTCAATGAGATTCTGCAAGCCGCCTACCAGCTCATGGTCGACGTCTT
CGGCAACTACGTCATCCAGAAGTTCTTCGAGTTCGGCTCTCTGGAACAGAAGCTG
GCTCTGGCCGAGAGGATCAGAGGCCACGTGCTGTCCCTCGCTCTGCAGATGTACG
GCTGTAGGGTGATCCAGAAGGCTCTGGAGTTCATCCCTTCCGACCAGCAGAACG
AGATGGTGAGAGAGCTGGATGGACACGTGCTGAAATGCGTCAAGGACCAGAAC
GGCTCCTATGTGGTGAGAAAGTGCATCGAGTGCGTGCAGCCCCAGTCTCTGCAGT
TCATCATCGACGCCTTCAAGGGCCAAGTCTTCGCCCTCAGCACACACCCTTACGG
AAATAGAGTCATCCAGAGGATTCTGGAACACTGTCTGCCCGACCAGACACTGCC
TATTCTGGAGGAGCTGCACCAACACACAGAGCAGCTGGTCCAAGACCAGTATGG
CTGCTACGTGATCCAGCACGTGCTGGAGCATGGAAGACCCGAGGATAAGAGCAA
GATCGTCGCCGAAATCAGAGGCAATGTGCTGGTGCTCAGCCAACACAAGTTCGC
TTCCTACGTCGTGAGGAAATGCGTGACACACGCTTCTAGAACAGAAAGGGCCGT
GCTCATCGATGAGGTGTGCACCATGAACGATGGCCCCCACAGCGCTCTGTATACC
ATGATGAAGGACCAGTACGCCAACTACGTGGTGCAGAAGATGATCGACGTGGCT
GAGCCCGGCCAGAGGAAGATTGTGATGCACAAGATTAGGCCCCATATCGCCACA
CTGAGAAAGTACACCTACGGAAAGCATATCCTCGCCAAGCTCGAGAAGTACTAC
ATGAAGAACGGCGTCGACCTCGGC (SEQ ID NO: 400)

[0794] The PUMBY (PUM14) targeting rhodopsin comprises the amino acid sequence:
MGRSRLLEDFRNNRYPNLQLREIAHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAE
IRGHTEQLVQDQYGCYVIQHVLEHGRPEDKSKIVAEIRGHTEQLVQDQYGSYVIRHV
LEHGRPEDKSKIVAEIRGHTEQLVQDQYGCYVIQHVLEHGRPEDKSKIVAEIRGHTEQ
LVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGHTEQLVQDQYGSYVIRHVLEHGRPE
DKSKIVAEIRGHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGHTEQLVQDQY
GNYVIQHVLEHGRPEDKSKIVAEIRGHTEQLVQDQYGCYVIQHVLEHGRPEDKSKIV
AEIRGHTEQLVQDQYGSYVIRHVLEHGRPEDKSKIVAEIRGHTEQLVQDQYGSYVIE
HVLEHGRPEDKSKIVAEIRGHTEQLVQDQYGSYVIEHVLEHGRPEDKSKIVAEIRGHT

EQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGHTEQLVQDQYGSYVIEHVLEHG
RPEDKSKIVAEIRGPHIATLRKYTYGKHILAKLEKYYMKNGVDLGGR (SEQ ID NO:
401).

[0795] The PUMBY (PUM14) targeting rhodopsin comprises the nucleic acid sequence:
ATGGGCAGAAGCCGGCTGCTGGAAGATTTCCGGAACAACAGATACCCCAACCTG
CAGCTGAGAGAGATCGCCCACACAGAGCAGCTGGTGCAGGACCAGTACGGCAAC
TACGTGATCCAGCATGTGCTGGAACACGGCAGACCCGAGGACAAGTCTAAGATC
GTGGCCGAGATCAGAGGCCACACCGAACAGCTCGTCCAGGATCAATACGGCTGT
TATGTGATTCAGCACGTCCTCGAGCACGGACGGCCTGAGGATAAGAGCAAAATT
GTGGCCGAAATCCGGGGCCATACTGAACAACTGGTTCAGGATCAGTATGGGTCC
TATGTGATCCGCCACGTCCTGGAACATGGACGCCCAGAGGACAAAAGCAAGATC
GTCGCTGAGATTCGGGGACATACCGAGCAACTCGTCCAAGACCAGTACGGCTGT
TACGTGATCCAGCATGTGCTGGAACACGGCAGACCCGAGGACAAGTCTAAGATC
GTGGCCGAGATCAGAGGCCACACCGAACAGCTGGTGCAGGACCAGTACGGCAAC
TATGTGATTCAGCACGTCCTCGAGCACGGACGGCCTGAGGATAAGAGCAAAATT
GTGGCCGAAATCCGGGGACACACAGAGCAGCTCGTCCAGGATCAGTATGGCTCC
TACGTGATCAGACACGTTTTGGAGCACGGCAGGCCAGAAGATAAGTCCAAGATT
GTCGCTGAGATTCGCGGGCATACTGAGCAACTGGTGCAAGATCAATACGGGAAT
TACGTCATCCAACACGTTCTCGAACATGGAAGGCCAGAGGACAAAAGCAAGATC
GTCGCAGAAATTAGGGGCCATACAGAACAACTGGTCCAGGACCAGTACGGCAAC
TACGTGATCCAGCATGTGCTGGAACACGGCAGACCCGAGGACAAGTCTAAGATC
GTGGCCGAGATCAGAGGCCACACCGAACAGCTGGTGCAGGATCAGTACGGCTGT
TATGTGATTCAGCACGTCCTCGAGCACGGACGGCCTGAGGATAAGAGCAAAATT
GTGGCCGAAATCCGGGGACACACAGAGCAGCTGGTCCAAGACCAGTATGGAAGC
TATGTCATCAGGCACGTCCTGGAACATGGACGCCCAGAGGACAAAAGCAAGATC
GTCGCTGAGATTCGGGGCCATACTGAGCAGCTCGTTCAGGACCAATACGGGTCTT
ACGTGATCGAACACGTGTTGGAGCATGGCAGGCCCGAAGATAAGTCCAAAATTG
TCGCAGAGATACGCGGCCACACCGAACAGCTGGTGCAGGATCAGTACGGCAGCT
ACGTGATCGAGCATGTGCTGGAACACGGCAGACCCGAGGACAAGTCTAAGATCG
TGGCCGAGATCAGAGGCCACACCGAGCAGCTCGTTCAGGACCAGTATGGCAATT
ATGTGATCCAGCACGTCCTCGAGCACGGACGGCCTGAGGATAAGAGCAAAATTG
TGGCCGAAATCCGGGGACACACAGAGCAACTGGTCCAAGACCAGTACGGCTCCT
ATGTGATTGAACACGTTCTGGAACATGGACGCCCAGAGGACAAAAGCAAGATCG

TCGCTGAGATTCGGGGCCCTCACATTGCCACACTGCGGAAGTACACCTACGGCA AGCACATCCTGGCCAAGCTGGAAAAGTACTACATGAAGAACGGCGTGGACCTCG GCGGCAGA (SEQ ID NO: 402).

Example 5: Knockdown Replacement Screening of Additional Candidates

[0796] A Rhodopsin (RHO) knockdown detection luciferase reporter assay was described and carried out as in previous Example 4.

[0797] Additional PUF candidates were detailed as depicted in Table 4.

[0798] Table 4: Additional PUF candidates for Knockdown Replacement

Construct	Target sequence 8 nucleotides	Target sequence 16 nucleotides	Hardened Sequence on replacement
PUF08 (P001MC)	CGGGUGUG (SEQ ID NO: 564)	GCGACGGGUGUGGUAC (SEQ ID NO: 579)	GCCACCGGCGTCGTGC (SEQ ID NO: 594)
PUF16 (P001MD)	CAGUUCUC (SEQ ID NO: 565)	AUGGCAGUUCUCCAUG (SEQ ID NO: 580)	CTGGCAATTTTCTATG (SEQ ID NO: 595)
PUF22 (P001ME)	CUGGGCUU (SEQ ID NO: 566)	CGUGCUGGGCUUCCCC (SEQ ID NO: 581)	TGTCCTCGGATTTCCT (SEQ ID NO: 596)
PUF34 (P001MG)	AACCUAGC (SEQ ID NO: 567)	GCUCAACCUAGCCGUG (SEQ ID NO: 582)	CCTGAATCTGGCTGTC (SEQ ID NO: 597)
PUF56 (P001MI)	UGGUCCUG (SEQ ID NO: 568)	UUGGUGGUCCUGGCCA (SEQ ID NO: 583)	TTAGTCGTGCTCGCTA (SEQ ID NO: 598)
PUF64 (P00005)	UUCGGGGA (SEQ ID NO: 569)	CCGCUUCGGGGAGAAC (SEQ ID NO: 584)	TCGGTTTGGCGAAAAT (SEQ ID NO: 599)
PUF66 (P001MK)	UGCCAUCA (SEQ ID NO: 570)	ACCAUGCCAUCAUGGG (SEQ ID NO: 585)	ATCACGCTATTATGGG (SEQ ID NO: 600)
PUF90 (P001MM)	CGUGGUCC (SEQ ID NO: 571)	UGUUCGUGGUCCACUU (SEQ ID NO: 586)	TGTTTGTCGTGCATTT (SEQ ID NO: 601)
PUF102 (P001MN)	GCAGCAGG (SEQ ID NO: 572)	CCCAGCAGCAGGAGUC (SEQ ID NO: 587)	CTCAACAACAAGAATC (SEQ ID NO: 602)
PUF112 (P001MP)	GCUUUCCU (SEQ ID NO: 573)	CAUCGCUUUCCUGAUC (SEQ ID NO: 588)	GATTGCATTTCTCATT (SEQ ID NO: 603)
PUF122 (P001MQ)	UCGGUCCC (SEQ ID NO: 574)	AACUUCGGUCCCAUCU (SEQ ID NO: 589)	AATTTTGGCCCTATTT (SEQ ID NO: 604)
PUF128 (P001MR)	GCGCCGCC (SEQ ID NO: 575)	AAGAGCGCCGCCAUCU (SEQ ID NO: 590)	AAAAGTGCTGCTATTT (SEQ ID NO: 605)
PUF130 (P00006)	AACCCUGU (SEQ ID NO: 576)	CUACAACCCUGUCAUC (SEQ ID NO: 591)	TTATAATCCAGTGATT (SEQ ID NO: 606)
PUF154 (P001MS)	ACUAUAGG (SEQ ID NO: 577)	GCCGACUAUAGGCGUC (SEQ ID NO: 592)	GCAGATTAGAGCCGAC (SEQ ID NO: 607)
PUF166 (P001MT)	CACAUAGG (SEQ ID NO: 578)	AAGUCACAUAGGCUCC (SEQ ID NO: 593)	AACTCTCAGAGCCTCT (SEQ ID NO: 608)

INCORPORATION BY REFERENCE

[0799] 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

[0800] 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.