

SYSTEMS AND METHODS FOR MODULATING RNA**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of priority of U.S. Provisional Patent Application No. 62/788,571 filed January 4, 2019, U.S. Provisional Patent Application No. 62/831,342 filed April 9, 2019, U.S. Provisional Patent Application No. 62/903,080 filed September 20, 2019, and U.S. Provisional Patent Application No. 62/929,339 filed November 1, 2019, all of which are hereby incorporated by reference in their entirety.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under GM119840 and HG008935 awarded by The National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION**1. Field of the Invention**

[0003] The present invention relates generally to the field of chemistry and medicine. More particularly, it concerns the use of a system for modulating RNA.

2. Description of Related Art

[0004] Programmable nucleic acid-binding proteins have revolutionized genome studies and editing technologies (Chandrasegaran and Carroll, 2016; Filipovska et al., 2011; Gootenberg et al., 2018; Hilton et al., 2015; Joung and Sander, 2012; Kearns et al., 2015; Strutt et al., 2018) and are opening up new therapeutic opportunities to treat human diseases (Liao et al., 2017; Monteys et al., 2017). In particular, the CRISPR/Cas9 system, which evolved as a bacterial immune defense mechanism, has transformed the ability to study and manipulate cellular DNA site-specifically (Cong et al., 2013; Jiang et al., 2013; O'Connell et al., 2014; Wiedenheft et al., 2012). A key advantage of CRISPR/Cas systems compared to previous methods (Desjarlais and Berg, 1993; Hockemeyer et al., 2011; Joung and Sander, 2012; Schierling et al., 2012) is that they are easily programmable to target virtually any locus of interest. The CRISPR/Cas system is a ribonucleoprotein complex that uses base pair interactions of a displayed guide RNA (gRNA) to interact with a target nucleic acid sequence. The simple nature of base pair-guided targeting opens up the possibility to program systems to interact with a defined nucleic acid sequence by simply changing the nucleic acid sequence on the guiding strand.

[0005] While targeting DNA directly will have profound clinical ramifications, diseases that involve subtle alterations to many genes will be challenging to target using DNA editing

technologies (Fuxman Bass et al., 2015). Additionally, the potential side effects or risks of permanent genetic alteration might not be tolerated. For example, the genes one may want to target to activate an enhanced wound healing response are likely targets that could pose a risk for cancer development, making permanent DNA-based strategy risky. Targeting information flow at the RNA level presents several opportunities for therapeutic intervention, including but not limited to the ability to halt treatment if side effects emerge, the ability to target genes that would be too risky to alter at the DNA level, and the ability to manipulate gene expression without permanent alterations to the host genome. While inhibiting or enhancing transcription at the genome level provides one possibility for controlling gene expression (Du et al., 2017; Fuxman Bass et al., 2015; Qi et al., 2013), recently discovered RNA epitranscriptomic regulatory mechanisms offer a broad range of RNA regulatory processes to target, including editing, degradation, transport, and translation of RNA transcripts (Nishikura, 2010; Roundtree et al., 2017; Zhao et al., 2017). Although the mechanisms and consequences of this epitranscriptomic regulatory layer are just beginning to be uncovered, it is apparent that the information flow through RNA is tightly regulated, offering many new opportunities for both basic research discoveries as well as therapeutic development.

[0006] Programmable RNA-targeting tools analogous to the dCas9 DNA-targeting systems hold great promise for studying the mechanisms of epitranscriptomic regulation and for therapeutic applications. The current tools for RNA targeting involve the delivery of large complexes and pose immunogenicity issues. From a basic science perspective, the large size of the delivery vehicle could lead to potential perturbations to the RNA under interrogation, convoluting the study of RNA regulatory mechanisms. From a translational perspective, the large size presents challenges for viral packaging or direct protein delivery. Additionally, while DNA-editing therapies will likely consist of a one-time, irreversible treatment, RNA-targeting therapies will need to be continually administered, making delivery concerns especially important. Moreover, it was recently discovered that 85% of people already have circulating antibodies to CRISPR/Cas proteins (Kim et al., 2018; Wagner et al., 2018), suggesting immunogenicity issues may prove problematic in clinical applications. Therefore, there is a need in the art for improved systems that can target RNA and be delivered efficiently without activating an immune response.

SUMMARY OF THE INVENTION

[0007] To overcome the large size and microbial-derived nature of current RNA-targeting systems, the inventors present a CRISPR/Cas-inspired RNA targeting system (CIRTS), a

general method for engineering programmable RNA effector proteins. Similar to CRISPR/Cas-based systems, CIRTs is a ribonucleoprotein complex that uses Watson-Crick-Franklin base pair interactions to deliver protein cargo site-selectively in the transcriptome. The inventors show they can easily engineer CIRTs that deliver a range of regulatory proteins to transcripts, including nucleases for degradation, deadenylation regulatory machinery for degradation, or translational activation machinery for enhanced protein production. However, CIRTs are up to 5-fold smaller than the smallest current CRISPR/Cas systems and can be engineered entirely from human parts.

[0008] Aspects of the disclosure relate to a RNA regulatory system or method comprising at least one of each: i) a RNA hairpin binding domain; ii) a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i; and iii) a RNA regulatory domain. In some embodiments, the following are included: i) and ii), i) and iii), ii) and iii), or i), ii), and iii). Any embodiment disclosed herein can contain any of these combinations.

[0009] Further aspects relate to a vector system comprising one or more nucleic acid vectors comprising a nucleotide encoding: i) a RNA hairpin binding domain; ii) a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i), and iii) a RNA regulatory domain.

[0010] Further aspects relate to a fusion protein comprising a RNA hairpin binding protein and a RNA regulatory domain and nucleic acids encoding such fusion proteins.

[0011] Further aspects relate to a conjugate comprising a RNA regulatory domain operably linked to a RNA targeting molecule, wherein the RNA targeting molecule comprises a RNA targeting region and at least one hairpin structure. In some embodiments, the RNA regulatory domain and the RNA targeting molecule are operably linked through a peptide bond. In some embodiments, the polypeptide further comprises one or more linkers. In some embodiments, the RNA regulatory domain and the RNA targeting molecule are operably linked through non-covalent interactions. In some embodiments, the RNA regulatory domain is covalently linked to a first dimerization domain and the RNA targeting molecule is covalently linked to a second dimerization domain and wherein the first and second dimerization domain are capable of dimerizing to form a non-covalent or covalent linkage. In some embodiments, the conjugate comprises one or more nuclear localization signals (NLS)s.

[0012] Yet further aspects relate to a delivery vehicle comprising a system of the disclosure. In some embodiments, the delivery vehicle comprises liposome(s), particle(s), exosome(s), microvesicle(s), a gene-gun or one or more nucleic acid vector(s).

5 [0013] Further aspects relate to a composition or a cell comprising a system, delivery vehicle, or fusion protein of the disclosure.

[0014] Further aspects relate to a method of modulating at least one target RNA comprising contacting the target RNA with a system, composition, or fusion protein of the disclosure. In some embodiments, modulating at least one target RNA comprises cleaving, demethylating, methylating, activating translation, repressing translation, promoting degradation, or binding
10 to the RNA. In some embodiments, at least two target RNAs are modulating. In some embodiments, at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, or 25 (or any derivable range therein) target RNAs are modulated. In some embodiments, the multiple RNAs are modulated by the same RNA regulatory domain or by a regulatory domain with the same activity. In some
15 embodiments, the different target RNAs are modulated with a different activity, such as by cleaving, demethylating, methylating, activating translation, repressing translation, promoting degradation, or binding to the RNA.

[0015] In some embodiments, the RNA regulatory domain does not bind to RNA. In some
embodiments, the RNA regulatory domain comprises a polypeptide that does not have RNA
binding activity. In some embodiments, the RNA regulatory domain does not bind to modified
20 RNA. In some embodiments, the RNA regulatory domain does not bind to m6A modified RNA.

[0016] Further aspects relate to a cell or progeny thereof comprising modulated target RNA, wherein the target RNA has been modulated according to a method of the disclosure. Further aspects relate to a multicellular organism comprising one or more cells of the disclosure.
25 Further aspects relate to a plant or animal comprising one or more cells of the disclosure. Further aspects relate to a kit comprising a system, vector, delivery vehicle, or fusion protein of the disclosure.

[0017] Further aspects relate to a method for modulating a target RNA in a subject, the method comprising administering a system or composition of the disclosure to the subject.

30 [0018] The term “RNA hairpin” refers to a RNA molecule with stem-loop intramolecular base pairing. A hairpin can occur when two regions of the same strand, usually complementary in nucleotide sequence when read in opposite directions, base-pair to form a double helix that ends in an unpaired loop. The disclosure relates to engineered RNA targeting molecules

comprising a RNA targeting region and one or more hairpins. Accordingly, the engineered RNA molecules of the disclosure are chimeric molecules that are non-naturally occurring.

[0019] The term “RNA targeting region” refers to a region of the RNA that is capable of hybridizing to a target RNA. The target RNA may be a disease associated RNA or one that is a modulation target according to the current systems and methods.

[0020] The “RNA regulatory domain” refers to a peptide or polypeptide that has activity directed to RNA. Examples of activity include methylation activity, RNA-binding activity, nuclease activity, and translational activation or repression activity. Further examples of activities and proteins comprising RNA regulatory domains are described throughout the disclosure.

[0021] In some embodiments, the RNA hairpin binding domain and the RNA regulatory domain are operably linked. The term “operably linked” refers to two proteins that are linked through either covalent or non-covalent interactions. For example, the two proteins may be covalently linked through a peptide bond. In some embodiments, the proteins are non-covalently linked. One or more proteins of the disclosure may be operably linked to another protein through linkage to a pair of accessory proteins that have a strong affinity for each other. Such accessory proteins are known in the art. For example, the SunTag is one such system that includes an antibody with a strong affinity for a peptide. One protein, polypeptide, or domain of the disclosure may be linked to a SunTag peptide and another protein, polypeptide, or domain of the disclosure may be linked to an antibody to allow operable linkage of the two proteins, polypeptides, or domains through the interaction of the SunTag peptide and antibody. Further examples include biotin and avidin/streptavidin and spytag and spycatcher.

[0022] In some embodiments, the system is inducible by providing the RNA regulatory domain and the hairpin binding domain as two unlinked polypeptides that become linked upon the presence of a stimulant. The induction may be, for example, by light induction or by chemical induction. Such inducibility allows for activation of the RNA regulation at a desired moment in time. In some embodiments, the RNA regulatory domain is covalently linked to a first dimerization domain and the RNA hairpin binding domain is covalently linked to a second dimerization domain and wherein the first and second dimerization domain are capable of dimerizing to form a non-covalent or covalent linkage. In some embodiments, the dimerization is inducible. In some aspects, the dimerization is induced through binding of the dimerization domains to a ligand. The term inducible refers to dimerization that is formed in response to a stimulus, such as a ligand, a chemical, a temperature change, or light, for example.

[0023] Light inducibility is for instance achieved by designing a fusion complex wherein the first and second dimerization domains comprise CRY2PHR and CIBN. This system is particularly useful for light induction of protein interactions in living cells and is further described in Konermann S, et al. Nature. 2013;500:472-476, which is herein incorporated by reference.

[0024] Suitable dimerization domains and corresponding ligands are known in the art. For example, Liang, F.S., Ho, W.Q., and Crabtree, G.R. (2011). Engineering the ABA plant stress pathway for regulation of induced proximity. Sci. Signal. 4, rs2, which is incorporated by reference, describes suitable dimerization/ligand systems that are useful in embodiments of the disclosure. In some embodiments, one of the first or second dimerization domain comprises PYR/PYR1-like (PYL1), the other of the first or second domain comprises ABA insensitive 1 (ABI1), and the ligand comprises abscisic acid (ABA) or derivatives or fragments thereof. The dimerization domain may be a fragment or portion of the whole protein and may be a substituted or modified. In some embodiments, the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof. In some embodiments, one of the first or second dimerization domain comprises FK506 binding protein (FKBP), the other of the first or second domain comprises FKBP-Rap binding domain of mammalian target of Rap mTOR (Frb), and the ligand comprises rapamycin (Rap) or derivatives or fragments thereof.

[0025] Derivatives refer to modified ligands and domains that retain binding or have enhanced binding to their dimerization domain or ligand, respectively. Fragments refer to contiguous portions of the dimerization domains that retain binding to the ligand. In some embodiments, the dimerization domain may be a modified fragment.

[0026] In some embodiments, i, ii, and/or iii are human or are human-derived. In some embodiments, the system, conjugate, and/or fusion protein is non-immunogenic. A human protein, polypeptide, domain, or nucleic acid refers to a protein, polypeptide, domain, or nucleic acid that is from the human genome, although it may be produced recombinantly in non-human systems. The term "human-derived" refers to a protein, polypeptide, domain, or nucleic acid that is a variant or fragment of a protein, polypeptide, domain, or nucleic acid from the human genome, although it may be produced recombinantly in non-human systems. In some embodiments, the fusion protein, conjugate, system, or parts thereof, such as parts i, ii, and/or iii are non-immunogenic and/or non-toxic when expressed in or administered to humans.

[0027] In some embodiments, the nucleic acids or polypeptides of the disclosure are synthetic, are non-natural, and/or do not occur naturally in nature.

[0028] In some embodiments, the system further comprises a stabilizer polypeptide; wherein the stabilizer polypeptide comprises a cationic polypeptide that binds non-specifically to nucleic acids. In some embodiments, the stabilizer polypeptide is human-derived. In some embodiments, the stabilizer polypeptide is operably linked to the RNA regulatory domain and/or RNA hairpin binding domain. In some embodiments, the stabilizer polypeptide comprises ORF5 or a fragment thereof. In some embodiments, the stabilizer polypeptide comprises SEQ ID NO:5, a variant thereof, or a polypeptide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity or homology to SEQ ID NO:5. In some embodiments, the stabilizer polypeptide comprises HEBGF or a fragment thereof. In some embodiments, the stabilizer polypeptide comprises SEQ ID NO:19, a variant thereof, or a polypeptide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity or homology to SEQ ID NO:19. In some embodiments, the stabilizer polypeptide comprises β -defensin 3 or a fragment thereof. In some embodiments, the stabilizer polypeptide comprises SEQ ID NO:20, a variant thereof, or a polypeptide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity or homology to SEQ ID NO:20.

[0029] In some embodiments, the stabilizer polypeptide, conjugate, fusion protein, conjugate, RNA regulatory domain, and/or RNA hairpin binding domain are less than, more than, or are at most or at least 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 125, 120, 115, 110, 105, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 kDa (or any derivable range therein). In some embodiments, the total complex comprising the RNA regulatory domain and hairpin binding domain is less than, more than, or is at most or at least 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 125, 120, 115, 110, 105, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 kDa (or any derivable range therein). In some embodiments, the total complex comprising the stabilizer polypeptide, RNA regulatory domain and hairpin binding domain is less than, more than, or is at most or at least 175, 170, 165, 160, 155, 150, 145, 140, 135, 130, 125, 120, 115, 110, 105, 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, or 5 kDa (or any derivable range therein).

[0030] In some embodiments, the RNA hairpin binding domain comprises a RNA hairpin binding domain from U1A (TBP6.7), SLBP, or variants thereof. In some embodiments, the RNA hairpin binding domain comprises a RNA hairpin binding domain from U1A (TBP6.7), SLBP, Ku70, nucleolin, or variants thereof. In some embodiments, the RNA hairpin binding

domain comprises SEQ ID NO:7 or 18, a variant thereof, or a polypeptide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity or homology to SEQ ID NO:7 or 18.

- 5 **[0031]** In some embodiments, the RNA targeting molecule comprises a TAR hairpin scaffold. In some embodiments, the RNA targeting molecule comprises the TAR hairpin scaffold of SEQ ID NO:1 or a nucleotide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity to SEQ ID NO:1. In some embodiments, the RNA targeting
- 10 molecule comprises a SLBP hairpin scaffold. In some embodiments, the RNA targeting molecule comprises the SLBP hairpin scaffold of SEQ ID NO:2 or a nucleotide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity to SEQ ID NO:2.

- [0032]** In some embodiments, the RNA targeting molecule comprises exactly one hairpin.
- 15 In some embodiments, the RNA targeting molecule comprises at least one hairpin. In some embodiments, the RNA targeting molecule comprises exactly two hairpins. In some embodiments, the RNA targeting molecule comprises at least two hairpins. In some embodiments, the RNA targeting molecule comprises exactly three hairpins. In some embodiments, the RNA targeting molecule comprises at least three hairpins. In some
- 20 embodiments, the RNA targeting molecule comprises exactly four hairpins. In some embodiments, the RNA targeting molecule comprises at least four hairpins. In some embodiments, the RNA targeting molecule comprises exactly five hairpins. In some embodiments, the RNA targeting molecule comprises at least five hairpins. In some embodiments, the RNA targeting molecule comprises 1-4 hairpins. In some embodiments, the
- 25 RNA targeting molecule comprises 1-3 hairpins. In some embodiments, the RNA targeting molecule comprises 1-2 hairpins. In some embodiments, the RNA targeting molecule comprises 2-4 hairpins. In some embodiments, the RNA targeting molecule comprises 2-3 hairpins. In some embodiments, the RNA targeting molecule comprises at least, at most, or exactly 1, 2, 3, 4, 5, or 6 hairpins (or any range derivable therein). In some embodiments, the RNA targeting
- 30 molecule comprises at least one hairpin that does not bind to the RNA hairpin binding protein and at least one hairpin that binds to the RNA hairpin binding protein. In some embodiments, the RNA targeting molecule binds to more than one RNA binding protein. In some embodiments, the RNA targeting molecule comprises two, three, or four hairpin structures and binds to at least two RNA binding proteins. In some embodiments, the RNA regulatory system

comprises at least two regulatory domains, wherein each regulatory domain binds to a different RNA binding molecule.

5 [0033] In some embodiments, the RNA targeting molecule comprises one or more modified nucleotides. In some embodiments, the modified nucleotides comprise a modification such as a phosphorothioate, locked nucleotides, ethylene bridged nucleotides, peptide nucleic acids, 5'E-VP, or is modified to a morpholino. In some embodiments, the modification includes one described herein.

10 [0034] In some embodiments, the RNA hairpin binding domain comprises the RNA hairpin binding domain of U1A, a variant thereof, or a polypeptide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity or homology to SEQ ID NO:7 and the RNA targeting molecule comprises a TAR hairpin scaffold or a nucleotide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity to SEQ ID NO:1.

15 [0035] In some embodiments, the RNA hairpin binding domain comprises the RNA hairpin binding domain of SLBP, a variant thereof, or a polypeptide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity or homology to SEQ ID NO:18 and the RNA targeting molecule comprises a SLBP hairpin scaffold or a nucleotide with at least 60, 20 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity to SEQ ID NO:2.

[0036] In some embodiments, the RNA hairpin binding domain comprises the RNA hairpin binding domain of ku70 or a variant thereof, and the RNA targeting molecule comprises a hairpin scaffold or a nucleotide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 25 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity to SEQ ID NO:83.

[0037] In some embodiments, the RNA hairpin binding domain comprises the RNA hairpin binding domain of nucleolin or a variant thereof, and the RNA targeting molecule comprises a hairpin scaffold or a nucleotide with at least 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 30 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity to one of SEQ ID NO:84-86.

[0038] In some embodiments, the RNA hairpin binding domain, stabilizer polypeptide, or RNA hairpin binding domain comprises a linker. In some embodiments, the linker comprises a polypeptide comprising SEQ ID NO:6, 21, 22, 23, or 25 or a polypeptide with at least 60, 61,

62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% (or any derivable range therein) identity or homology to SEQ ID NO:6, 21, 22, 23, or 25. In some embodiments, the linker is a rigid linker. In some embodiments, the linker is a flexible linker. In some embodiments, the linker comprises glycine and serine residues. In some embodiments, the linker is at least 4 amino acids. In some embodiments, the linker is at least or at most or exactly 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids (or any derivable range therein).

[0039] In some embodiments, the stabilizer polypeptide comprises a polypeptide, such as a RNA-binding polypeptide, from cJun, HBEGF, HRX, NDEK, NHGF, beta-defensin3, or scGFP. In some embodiments, the RNA regulatory domain is operably linked to the stabilizer polypeptide at the carboxy terminus of the RNA regulatory domain. In some embodiments, the RNA regulatory domain is operably linked to the stabilizer polypeptide at the amino terminus of the RNA regulatory domain. In some embodiments, the RNA regulatory domain is operably linked to the RNA hairpin binding domain polypeptide at the carboxy terminus of the RNA regulatory domain. In some embodiments, the RNA regulatory domain is operably linked to the RNA hairpin binding domain polypeptide at the amino terminus of the RNA regulatory domain. In some embodiments, the RNA hairpin binding domain polypeptide is operably linked to the stabilizer polypeptide at the carboxy terminus of the RNA hairpin binding domain polypeptide. In some embodiments, the RNA hairpin binding domain polypeptide is operably linked to the stabilizer polypeptide at the amino terminus of the RNA hairpin binding domain polypeptide.

[0040] In some embodiments, the RNA targeting region comprises at least 12 nucleotides. In some embodiments, the RNA targeting region comprises at least, at most, or exactly 8, 9, 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, 35, 36, 37, 38, 39, 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 79 nucleotides (or any derivable range therein).

[0041] In some embodiments, the RNA regulatory domain comprises a nuclease, methylase, demethylase, translational activator, translational repressor, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, or RNA binding activity. In some embodiments, the RNA regulatory domain comprises an activity described herein.

[0042] In some embodiments, the RNA regulatory domain comprises a Pin nuclease domain or a m6A reader protein or portion thereof. In some embodiments, the RNA regulatory domain comprises a domain or polypeptide from SMG6, YTHDF1, or YTHDF2. In some

embodiments, the RNA regulatory domain comprises a domain or polypeptide from an ADAR protein. In some embodiments, the RNA regulatory domain comprises a domain or polypeptide from a human ADAR protein. In some embodiments, the RNA regulatory domain comprises a polypeptide that has at least, at most, or exactly 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity or homology to SEQ ID NO:9, 11, 15, 16, 17, or 123-125. In some embodiments, the RNA regulatory domain further comprises a helical region. In some embodiments, the helical region comprises a polypeptide that has at least, at most, or exactly 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity or homology to SEQ ID NO:24.

[0043] In some embodiments, the RNA regulatory domain increases translation of a target RNA. In some embodiments, the RNA regulatory domain increases degradation of a target RNA. In some embodiments, the RNA regulatory domain modifies the localization of a target RNA. In some embodiments, the RNA regulatory domain modifies the processing of the target RNA.

[0044] In some embodiments, the RNA regulatory domain comprises a polypeptide, such as a polypeptide having RNA regulatory activity from IFIT2, eIF4a, eIF4e, PABP, PAIP, SLBP, BOLL, ICP27, YTHDF1, YTHDF2, or YTHDF3. In some embodiments, the RNA regulatory domain comprises a polypeptide, such as a polypeptide having RNA regulatory activity from YTHDF2, TOB2, ZFP36, CNOT7, RNaseA, RNaseL, RNaseP, RNase4, RNase1, RNaseU2, or HRSP12. In some embodiments, the RNA regulatory domain increases the expression of a polypeptide encoded by the target RNA and wherein the RNA regulatory domain comprises IFIT2, eIF4a, eIF4e, PABP, PAIP, SLBP, BOLL, ICP27, YTHDF1, or YTHDF3. In some embodiments, the RNA regulatory domain comprises a polypeptide, such as a polypeptide having RNA regulatory activity from YTHDF2, TOB2, ZFP36, CNOT7, RNaseA, RNaseL, RNaseP, RNase4, RNase1, RNaseU2, or HRSP12. In some embodiments, the RNA regulatory domain decreases the expression of a polypeptide encoded by the target RNA and wherein the RNA regulatory domain comprises YTHDF2, TOB2, ZFP36, CNOT7, RNaseA, RNaseL, RNaseP, RNase4, RNase1, RNaseU2, or HRSP12.

[0045] In some embodiments, one or more nuclear export signals (NES) are fused to the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some embodiments, the NES is at the carboxy terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some embodiments, the

NES is at the amino terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some embodiments, the NES comprises a polypeptide that has at least, at most, or exactly 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity or homology to SEQ ID NO:8.

[0046] In some embodiments, one or more nuclear localization signals (NLS) are fused to the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some embodiments, the NLS is at the carboxy terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some embodiments, the NLS is at the amino terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some embodiments, the NES comprises a polypeptide that has at least, at most, or exactly 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 75, 76, 77, 78, 79, 80, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% (or any derivable range therein) identity or homology to SEQ ID NO:13.

[0047] In some embodiments, the RNA targeting region of ii hybridizes to a target RNA in a prokaryotic or eukaryotic cell. In some embodiments, the target RNA is in a human cell. In some embodiments, the target RNA is in vitro or in vivo.

[0048] In some embodiments, the system comprises at least two of each i, ii, and iii. In some embodiments, the at least two of i, ii, and iii are expressed in the same cell. In some embodiments, the method comprises modulating at least two target RNAs. In some embodiments, the system comprises at least, at most, or exactly 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, or 50 or more (or any derivable range therein) of i, ii, and iii. In some embodiments, at least, at most, or exactly 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, or 50 or more (or any derivable range therein) target RNAs are modulated in a cell.

[0049] In some embodiments, the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.

[0050] In some embodiments, the vectors of the disclosure further comprise a regulatory element operably linked to the nucleotide encoding i, ii, and/or iii. Regulatory elements, in addition to a NLS and NES, as previously described, also include promoters, polyadenylation signals, enhancers, etc. Other regulatory elements are known in the art and described herein and may be used in the embodiments of the disclosure. In some embodiments, the one or more nucleic acid vectors are optimized for expression in an eukaryotic cell. In some embodiments, the expression of the domains, RNA, or polypeptides in the cell or from a vector is constitutive. In some embodiments, the expression of the domains, RNA, or polypeptides in the cell or from

a vector is conditional. In some embodiments, i, ii, and iii are on a single vector. In some embodiments, i, ii, iii, and the stabilizer polypeptide are encoded on a single vector. In some embodiments, i, iii, and the stabilizer polypeptide are encoded on a single vector. In some embodiments, one or more of the vectors are viral vectors. In some embodiments, the one or more vectors comprise one or more retroviral, lentiviral, adenoviral, adeno-associated or herpes simplex viral vectors. In some embodiments, one or more of the vectors are non-viral vectors. In some embodiments, the system or composition is non-viral, which denotes that it does not contain any viral components.

[0051] In some embodiments, there is a system or kit comprising one or more of the following components: a polypeptide comprising a RNA regulatory domain, a polypeptide comprising a RNA binding domain, a polypeptide comprising a stabilizer, a nucleic acid encoding for a RNA regulatory domain, a nucleic acid encoding for a RNA binding domain, a nucleic acid encoding a stabilizer, a nucleic acid encoding a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure; a conjugate of the disclosure; a vector of the disclosure, a fusion protein of the disclosure, a recombinant host cell, an expression construct, an engineered viral vector, or an engineered attenuated virus. In certain embodiments, a polypeptide of the disclosure is under the control of a heterologous promoter. It is specifically contemplated that any protein or polypeptide function that are used in embodiments, may be used a nucleic acid encoding that protein or polypeptide function. Also, any and all polypeptides, proteins, nucleic acid molecules may be contained within a cell or other living organism, such as a virus (for instance, a phage).

[0052] A kit may include one or more components that are separate or together in a suitable container means, such as a sterile, non-reactive container. In some embodiments, cells or viruses are provided that contain one or more nucleic acid constructs that encode the polypeptides of the disclosure. The term “promoter” is used according to its ordinary meaning to those in the field of molecular biology; it generally refers to a site on a nucleic acid in which a polymerase can bind to initiate transcription. In specific embodiments, the promoter is recognized by a T7 RNA polymerase.

[0053] The compositions, vectors, systems, methods, and proteins of the disclosure are useful for a variety of clinical and research-related applications. The embodiments of the disclosure may be useful for the treatment of a disease or condition, such as cancer or autoimmunity. In some embodiments, the methods and compositions are for the acute treatment of a disease or condition. In some embodiments, the methods and compositions are useful for the temporary modulation of RNA. In some embodiments exclude permanent

modification of gene activity. In some embodiments, the methods and compositions are safer due to the acute modulation of RNA and/or due to the ability to control the expression of the system in vivo.

5 [0054] As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an” may mean one or more than one.

[0055] As used herein, the terms “or” and “and/or” are utilized to describe multiple components in combination or exclusive of one another. For example, “x, y, and/or z” can refer to “x” alone, “y” alone, “z” alone, “x, y, and z,” “(x and y) or z,” “x or (y and z),” or “x or y or z.” It is specifically contemplated that x, y, or z may be specifically excluded from an
10 embodiment.

[0056] Throughout this application, the term “about” is used according to its plain and ordinary meaning in the area of cell biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

15 [0057] The term “comprising,” which is synonymous with “including,” “containing,” or “characterized by,” is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. The phrase “consisting of” excludes any element, step, or ingredient not specified. The phrase “consisting essentially of” limits the scope of described subject matter to the specified materials or steps and those that do not materially affect its basic and novel characteristics. It is contemplated that embodiments described in the context of the term “comprising” may also be implemented in the context of the term “consisting of” or “consisting
20 essentially of.”

[0058] It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention.
25 Furthermore, any composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any composition of the invention. Aspects of an embodiment set forth in the Examples are also embodiments that may be implemented in the context of embodiments discussed elsewhere in a different Example or elsewhere in the application, such as in the Summary of Invention, Detailed Description of the Embodiments, Claims, and Description of Figure Legends.
30

[0059] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within

the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0060] The following drawings form part of the present specification and are included to
5 further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0061] **FIG. 1A-D. Design of CRISPR/Cas-inspired RNA targeting system (CIRTS)**
(A) Schematic overview of the design strategy. CIRTS is composed of a ssRNA binding protein
10 an RNA hairpin binding protein, an effector protein and a guiding RNA. (B) List of modular CIRTS constructs used in this work. (C) Design of the guiding RNA for TBP6.7. The HIV TAR hairpin was fused to a nucleotide linker (L) and a guide sequence. (D) Design of the guiding RNA for the RRM of SLBP. The human histone mRNA hairpin was fused to a flexible five nucleotide linker and a guide sequence.

15 [0062] **FIG. 2A-C. CIRTS-1 in vitro binding and RNA cleavage assays** (A) Electrophoretic mobility assay (EMSA) evaluating the binding affinity of MBP-CIRTS-1: on-target gRNA (R3) complex to a labeled RNA substrate (R1). EDTA was supplemented to the reaction buffer to avoid any cleavage. (B) Calculation of the binding affinity by fitting the fraction of TBP6.7:gRNA bound to substrate to a quadratic binding equation. (C) Cleavage
20 assay run on a denaturing gel after 2h of incubation. A labeled RNA substrate (R2) is cleavage in a gRNA-dependent manner.

[0063] **FIG. 3A-G. CIRTS mammalian cell reporter assays** (A) General overview of the dual luciferase assay. A reporter construct that contains both firefly luciferase and Renilla luciferase is used in all assays. The inventors targeted their CIRTS to the firefly luciferase
25 transcript, while keeping Renilla luciferase constant to use as a transfection control. For all subsequent assays, HEK293T cells were transfected with the reporter vector, the CIRTS vector, and a gRNA vector. (B) Catalytically-inactive CIRTS-0 was used as a control. After 48h of incubation, the inventors observed no decrease in protein readout. (C) Comparison of CIRTS-1 with Cas13b nuclease. Cells transfected with either CIRTS-1 or Cas13 and the corresponding
30 gRNA targeting show reduced protein levels after incubation. (D) HEK293T cells transfected with CIRTS-2 show an increase in protein level after 48h, (E) cells transfected with CIRTS-3, however, show the anticipated decrease in protein level. (F) Switching the hairpin-binding protein to SLBP still results in decrease protein levels after 48h. (G) Cells transfected with a

fully humanized CIRT5 (CIRT5-5 and CIRT5-6) system and an on-target gRNA for firefly luciferase result again in decreased protein levels.

[0064] **FIG. 4A-C. Targeting endogenous transcripts with CIRT5** (A) Nuclease-mediated knockdown of five endogenous transcripts upon transfection of cells with CIRT5-1 as assayed using qPCR. CIRT5-1 can be used to target endogenous transcripts of interest by co-transfecting a gRNA the corresponding on-target guiding sequence. (B) qPCR analysis of non-nuclease-mediated knockdown of endogenous transcripts with CIRT5-3. Cells transfected with CIRT5-3 show gRNA-dependent decreases in RNA level for all five transcripts tested. (C) Analysis of protein levels after transfection with CIRT5-2 or CIRT5-3 using Western blot. CIRT5-2 can induce an increase in protein levels, whereas CIRT5-3 shows the expected decrease in protein levels as a control.

[0065] **FIG. 5A-B. Multidimensional targeting with CIRT5.** (A) Schematic of vectors used for multiplexed targeting. CIRT5-6 was co-transfected with its gRNA construct targeting PPIB, while CIRT5-7 was co-transfected with the corresponding gRNA construct targeting SMARCA4. (B) Heat map showing knockdown of multiplexed targeting. When both CIRT5-6 and CIRT5-7 are present in cells, co-transfection of either on-target or off-target control gRNA can guide the CIRT5 to decrease endogenous transcripts. When both CIRT5 have an on-target gRNA for PPIB or SMARCA4 present, both transcripts can be knocked down in the same samples.

[0066] **FIG. 6. Comparing CIRT5 to other DNA and RNA-targeting CRISPR/Cas systems.** Schematic size comparison of currently used Cas9, Cas13, and fusion protein systems. Cas9 and Cas13-based delivery systems are substantially larger than engineered CIRT5 systems.

[0067] **FIG. 7A-C. Controls EMSA and Cleavage Assay.** (A) EMSA evaluating binding shifts dependent on MBP-CIRT5-1 in the absence of any gRNA. (B) EMSA assaying binding shifts in the presence of only labeled substrate (R1) and on-target gRNA (R3). (C) Full cleavage gel shown in main text FIG. 2C.

[0068] **FIG. 8A-D. CIRT5 linker and gRNA optimization.** (A) Luciferase assay with the CIRT5 nuclease system using different linkers between the hairpin-binding protein and the effector protein. (B) Luciferase assay with CIRT5-YTHDF2-mediated decay using different linkers between the hairpin-binding protein and the effector protein. (C) Different engineered gRNA for TBP6.7 based on the design shown in FIG. 1C. Two different targeting lengths of 20 and 40 nucleotides were used in combination with different numbers of linking nucleotides (L) between the hairpin and the guiding sequence. The dual luciferase assay was used to assess

nuclease-mediated decay. (D) The same engineered gRNAs as in FIG. 8C were used with CIRT5-3 to induce epitranscriptome-induced RNA decay. n = 3 biological replicates

[0069] **FIG. 9A-H. Control luciferase assays and RT-qPCRs.** (A) RT-qPCR analysis of RNA levels with the 'dead' Pin nuclease domain CIRT5 (CIRT5-0). (B) Luciferase assay comparing the nuclease-mediated decay of TBP6.7-Pin nuclease domain without (CIRT5-8) and with (CIRT5-9) the additional ssRNA binding protein ORF5. (C) CIRT5 nuclease can mediate decreases in RNA and therefore protein level in both the nucleus and the cytoplasm (n=6). (D) Comparison of RNA levels when cells were transfected with CIRT5-1 and active Cas13b nuclease. CIRT5-1-Pin mediated RNA cleavage showed substantially less RNA degradation compared to the Cas13b system. (E-H) All engineered CIRT5 system the inventors tested in the dual luciferase assay were also subjected to RT-qPCR analysis to assess changes in RNA levels. CIRT5-2, which contain the YTHDF1 effector domain inducing translation activation showed no significant changes in RNA level while all YTHDF2-containing CIRT5 show the expected decrease in RNA levels (S3F and S3H: n = 2 or 3). n = 3 biological replicates unless otherwise noted. Student t-test: *P < 0.05, **P < 0.01, ***P < 0.001.

[0070] **FIG. 10A-C. Immunoprecipitation, Control qPCR Western Blot, Y2 truncations.** (A) Cells were transfected with CIRT5-0-3xFLAG and a gRNA for either PPIB, B4GALTN1, or NT. After crosslinking and FLAG IP, pulled down RNA was quantified using RT-qPCR. Reactions containing on-target gRNA for either transcript showed 3.5 to 5-fold enrichment for these transcripts, indicating guided RNA targeting (n = 2 or 3). (B) RT-qPCR analysis of RNA level when cells were transfected with CIRT5-2. As anticipated, no significant changes in RNA level were observed when a YTHDF1-containing protein was used. (C) Different truncations of YTHDF2 were assayed to determine which would be more efficient. The inventors compared luciferase data (left) with qPCR data (right) and concluded to use the Y2(100-200) construct for luciferase analysis and the Y2(1-200) construct for endogenous targeting to enable the best possible quantifications of the tools. n = 3 biological replicates unless otherwise noted. Student t-test: *P < 0.05, **P < 0.01.

[0071] **FIG. 11A-C. Endogenous targeting with CIRT5.** (A) Changes in RNA levels assessed after transfection of CIRT55-7 alone for PPIB. (B) Similar to FIG. 11A, SMARCA4 levels were assayed when cells were transfected with CIRT55-7. (C) gRNA screen along SMARCA4 using CIRT5-3 to induce gRNA-dependent RNA decay. The inventors see significant changes in the amount of induce decay dependent on where the transcript is targeted (n = 2 or 3). n = 3 biological replicates unless otherwise noted. Student t-test: *P < 0.05, **P < 0.01, ***P < 0.001.

[0072] FIG. 12A-D. Design of CRISPR/Cas-inspired RNA targeting system (CIRTS)

(A) Schematic overview of the design strategy. CIRTS is composed of a ssRNA binding protein, an RNA hairpin binding protein, an effector protein, and a guiding RNA. (B) List of key CIRTS used in this work. (C) Design of the guiding RNA for TBP6.7. The HIV TAR hairpin was fused to a nucleotide linker (L) and a guide sequence. The nucleotide linker was altered during optimization (as described in the supporting information) but L = UUAAUU was used for all work thereafter. (D) Design of the guiding RNA for the RNA recognition motif (RRM) of SLBP. The human histone mRNA hairpin was fused to a flexible five nucleotide linker and a guide sequence.

[0073] FIG. 13A-B. CIRTS-1 *in vitro* binding and RNA cleavage assays.

(A) Filter binding assay evaluating the binding affinity of MBP-CIRTS-1 with on-target gRNA and non-targeting RNA complex to a labeled RNA substrate. Fitting the data to a quadratic binding equation revealed an apparent K_D of 22 ± 7 nM for the on-target:protein complex and an apparent K_D around 500 nM for the non-targeting:protein interaction. (B) Cleavage assay run on a 10% denaturing Urea PAGE gel in presence of 0.5 mM $MnCl_2$. An IR800-labeled RNA substrate is cleaved in a gRNA-dependent manner.

[0074] FIG. 14A-G. CIRTS mammalian cell reporter assays.

(A) General overview of the dual luciferase assay. A reporter construct that contains both firefly luciferase and Renilla luciferase is used in all assays. The inventors targeted CIRTS to the firefly luciferase transcript, while using Renilla luciferase as an internal control. For all subsequent assays, HEK293T cells were transfected with the reporter vector, a CIRTS vector, and a gRNA vector. (B) Catalytically-inactive CIRTS-0 was used as a control. After 48 h of incubation, the inventors observed no decrease in protein readout. Values shown as mean $\pm$ SEM with $n = 3$ biological replicates. (C) Comparison of CIRTS-1 with Cas13b nuclease. Cells transfected with either CIRTS-1 or Cas13 and the corresponding gRNA targeting Fluc show reduced protein levels after incubation. Values shown as mean $\pm$ SEM with $n = 3$ biological replicates. Student t-test: $*P < 0.05$, $**P < 0.01$. (D) HEK293T cells transfected with CIRTS-2 show an increase in protein level after 48 h. Values shown as mean $\pm$ SEM with $n = 3$ biological replicates. Student t-test: $**P < 0.01$. (E) HEK293T cells transfected with CIRTS-3 show the anticipated decrease in protein level. Values shown as mean $\pm$ SEM with $n = 3$ biological replicates. Student t-test: $*P < 0.05$. (F) Switching the hairpin-binding protein to SLBP still results in decrease protein levels after 48 h. Values shown as mean $\pm$ SEM with $n = 3$ biological replicates. Student t-test: $*P < 0.05$. (G) Cells transfected with a fully humanized CIRTS (CIRTS-5 and CIRTS-6)

system and an on-target gRNA for firefly luciferase result again in decreased protein levels. Values shown as mean $\pm$ SEM with n = 3 biological replicates. Student t-test: *P < 0.05.

[0075] **FIG. 15A-B. CIRTSS for RNA editing.** (A) Schematic overview of the RNA editing reporter assay used. A single G-to-A mutation was introduced in the coding sequence of firefly luciferase resulting in a W417X (X = STOP) codon switch and no measurable firefly luciferase signal (FIG. 22J). (B) Delivery of CIRTSS-7 (hADAR2 wt) and CIRTSS-8 (hADAR E488Q) with an on-target gRNA shows significant RNA editing that results in measurable firefly luciferase signal. Both the background and the editing efficiency of CIRTSS-8, the hyperactive hADAR2 mutant, are found to be higher compared to wildtype. Values shown as mean $\pm$ SEM with n = 3 biological replicates. Student t-test: ***P < 0.001.

[0076] **FIG. 16A-C. Targeting endogenous transcripts with CIRTSS.** (A) Nuclease-mediated knockdown of five endogenous transcripts upon transfection of cells with CIRTSS-1 as assayed using qPCR. CIRTSS-1 can be used to target endogenous transcripts of interest by co-transfecting a gRNA with the corresponding on-target guiding sequence. Values shown as mean $\pm$ SEM with n = 3 biological replicates. Student t-test: *P < 0.05, **P < 0.01, ***P < 0.001. (B) qPCR analysis of YTHDF2-mediated knockdown of endogenous transcripts with CIRTSS-3. Cells transfected with CIRTSS-3 show gRNA-dependent decreases in RNA level for all five transcripts tested. Values shown as mean $\pm$ SEM with n = 3 biological replicates. Student t-test: *P < 0.05. (C) Analysis of protein levels after transfection with CIRTSS-2 or CIRTSS-3 by Western blot. CIRTSS-2 induces an increase in protein levels, whereas CIRTSS-3 shows the expected decrease in protein levels, both in a gRNA-dependent manner.

[0077] **FIG. 17. Targeting accessibility determines knockdown efficiency of CIRTSS.** gRNA screen along SMARCA4 using CIRTSS-3 to induce gRNA-dependent RNA decay. The inventors observe significant changes in the amount of induced decay dependent on where the transcript is targeted (n = 2 or 3).

[0078] **FIG. 18A-D. Multidimensional targeting with CIRTSS.** (A) Schematic of delivery of CIRTSS-6 and three gRNAs. (B) CIRTSS-6 can be delivered with three distinct gRNAs for PPIB, SMARCA4, and NRAS and simultaneously cause knockdown of all three transcripts. n = 5 biological replicates. Student t-test: **P < 0.05, ***P < 0.001. (C) Schematic of simultaneous CIRTSS delivery with different effector proteins. (D) Changes in luciferase protein levels and PPIB transcript levels when cells were transfected with both CIRTSS-9 (YTHDF1) and CIRTSS-10 (YTHDF2) and gRNAs for Fluc and PPIB respectively. Both orthogonal CIRTSS retain their individual functions and act simultaneously in cells. n = 5 biological replicates. Student t-test: *P < 0.1, **P < 0.05.

[0079] FIG. 19A-C. AAV Delivery of CIRTS. (A) Transfer plasmid for AAV delivery containing both the CIRTS-6 (YTHDF2) as well as the gRNA component of the system. The total insert size between the two inverted terminal repeats (ITR) was 2.7 kb. (B) AAV-packaged CIRTS-6 and a gRNA targeting luciferase was delivered to HEK293T cells to knockdown firefly luciferase in the dual luciferase reporter assay. (C) AAV-packaged CIRTS-6 and a gRNA targeting SMARCA4 was delivered to HEK293T cells to knockdown the endogenous gene, which revealed efficiency comparable to that achieved by transient transfection. Values shown as mean $\pm$ SEM with $n = 3$ biological replicates. Student t-test: * $P < 0.05$.

[0080] FIG. 20. Comparing CIRTS to other DNA and RNA-targeting CRISPR/Cas systems. Schematic size comparison of commonly used Cas9, Cas12, Cas13, and fusion protein systems.

[0081] FIG. 21. CIRTS List continued from FIG. 12B. Reference list of all remaining CIRTS used in this work.

[0082] FIG. 22A-J. Control luciferase assays and RT-qPCRs. (A) Luciferase assay comparing the nuclease-mediated decay of TBP6.7-Pin nuclease domain without (CIRTS-11) and with (CIRTS-12) the additional ssRNA binding protein ORF5. (B) CIRTS nuclease can mediate decreases in RNA and therefore protein level in both the nucleus and the cytoplasm ($n=6$). (C) RT-qPCR analysis of RNA levels with the 'dead' Pin nuclease domain CIRTS (CIRTS-0). (D) Comparison of RNA levels when cells were transfected with CIRTS-1 and active Cas13b nuclease. CIRTS-1-Pin mediated RNA cleavage showed substantially less RNA degradation compared to the Cas13b system. (E-H) All engineered CIRTS system tested in the dual luciferase assay were also subjected to RT-qPCR analysis to assess changes in RNA levels. CIRTS-2, which contain the YTHDF1 effector domain inducing translation activation showed no significant changes in RNA level while all YTHDF2-containing CIRTS show the expected decrease in RNA levels. (I) Engineered CIRTS-18 containing the PP7 dimer as the hairpin binding protein. Knockdown of PPIB after transfection with CIRTS-18 as measured by qPCR. (J) Comparison of reporter only and reporter with CIRTS-7 (hADAR wt) with non-targeting or targeting gRNA (S3F and S3H: $n = 2$ or 3). $n = 3$ biological replicates unless otherwise noted. Student t-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0083] FIG. 23A-D. CIRTS linker and gRNA optimization. (A) Luciferase assay with the CIRTS nuclease system using different linkers between the hairpin-binding protein and the effector protein. Previously published L8 = SGSETPGTSESATPES (SEQ ID NO:133) (Guilinger et al., 2014), 10 nm helical linker =

EEEEKKKQEEEEAEERLRRIQEEMEKERKRREEDEKRRRKEEEEERRMKLEMEAKRKQ
 EEEERKKREDDEKRKKK (SEQ ID NO:134). (B) Luciferase assay with CIRT5-YTHDF2-
 mediated decay using different linkers between the hairpin-binding protein and the effector
 protein. (C) Different engineered gRNA for TBP6.7 based on the design shown in Figure 1C.
 5 Two different targeting lengths of 20 and 40 nucleotides were used in combination with
 different numbers of linking nucleotides (L) between the hairpin and the guiding sequence. The
 dual luciferase assay was used to assess nuclease-mediated decay. NT = non-targeting, Fluc
 gRNA containing different linker nuclease (Figure 1), L2 = UU, L3 = UUU, L5 = UUAUU.
 (D) The same engineered gRNAs as in Figure S3C were used with CIRT5-3 to induce
 10 epitranscriptome-induced RNA decay. NT = non-targeting, Fluc gRNA containing different
 linker nuclease (Figure 1), L2 = UU, L3 = UUU, L5 = UUAUU. n = 3 biological replicates.

[0084] FIG. 24A-D. Control qPCR, Western Blot, YTHDF2 truncations. (A) CIRT5-
 1 can be delivered to RNA species other than mRNA. As a proof-of-principle, the inventors
 transfected cells with CIRT5-1 (Pin nuclease) and two different gRNAs for the lncRNA
 15 MALAT1 and assessed RNA levels by RT-qPCR. (B) RT-qPCR analysis of RNA level when
 cells were transfected with CIRT5-2. As anticipated, no significant changes in RNA level were
 observed when a YTHDF1-containing protein was used. (C) Quantification of protein levels
 as measured by Western blot when cells were transfected with CIRT5-2 or CIRT5-3 and
 targeted to PPIB (n=3). (D) Different truncations of YTHDF2 were assayed to determine
 20 which would be more efficient. The inventors compared luciferase data (left) with qPCR data
 (right) and concluded to use the Y2(100-200) construct for luciferase analysis and the Y2(1-
 200) construct for endogenous targeting to enable the best possible quantifications of the tools.
 n = 3 biological replicates unless otherwise noted. Student t-test: *P < 0.05, **P < 0.01.

[0085] FIG. 25A-H. Targeting Specificity of CIRT5. (A) Schematic of the KRAS4b-
 25 luciferase mismatch reporter assay. The inventors chose four KRAS4b variants that have an
 increasing number of mismatches to the designed 20 nt length gRNA and fused it N-terminal
 to the dual luciferase reporter. (B) CIRT5-mediated knockdown of KRAS4b-Fluc with
 different numbers of mismatches between the gRNA and target RNA as described in Figure
 S5A. CIRT5 was found to be most sensitive to mismatches in the middle of its guiding
 30 sequence. (C) Cas13b-mediated knockdown in the same KRAS4b-Fluc reporter assay as
 described above. Cas13b shows a higher knockdown efficiency but is also less sensitive to
 mismatches introduced. Similar to CIRT5, Cas13b is knockdown is most affected by
 mismatches at the center of the guiding target duplex region. (D) Knockdown efficiency of
 CIRT5 on the KRAS4b-luciferase mismatch reporter when using a 40 nt gRNA length. A

longer guiding sequence in the gRNA can rescue some of the loss in knockdown efficiency. (E-F) Mean expression levels of the transcriptome in $\log_2(\text{transcript per million (TPM)} + 1)$ when CIRT5 Pin nuclease (E) or CIRT5 YTHDF2 (F) are deployed to SMARCA4 in cells (n=3). (G) Knockdown levels of SMARCA4 as determined by RNA sequencing. (H) Cells were transfected with CIRT5-0-3xFLAG and a gRNA for either PPIB, B4GALNT1, or NT. After crosslinking and FLAG IP, pulled down RNA was quantified using RT-qPCR. Reactions containing on-target gRNA for either transcript showed 3.5 to 5-fold enrichment for these transcripts, indicating guided RNA targeting (n = 2 or 3).

[0086] FIG. 26A-C. Endogenous targeting with CIRT5. (A) Changes in RNA levels as assessed by RT-qPCR after transfection of CIRT5-7 alone for PPIB. (B) Similar to Figure S6A, SMARCA4 levels were assayed when cells were transfected with CIRT5-7. (C) Comparison of knockdown levels of PPIB after delivery of active Cas13b nuclease or an engineered dCas13b-YTHDF2(1-200) construct to PPIB (n = 2 or 3). n = 3 biological replicates unless otherwise noted. Student t-test: *P < 0.01, **P < 0.05, ***P < 0.01, ****P < 0.001.

[0087] FIG. 27A-C. Multiplexed targeting with CIRT5. (A) Schematic of vectors used for multiplexed targeting. Cells were transfected with an expression vector for CIRT5-6, and an expression vector for CIRT5-10, an expression vector for a CIRT5-6 gRNA construct targeting PPIB or a non-targeting control, and an expression vector for a CIRT5-9 gRNA targeting SMARCA4 or a non-targeting control. (B) Heat map showing knockdown of multiplexed targeting described in (A). When both CIRT5 have an on-target gRNA for PPIB or SMARCA4 present, both transcripts can be knocked down in the same samples. Values shown as mean expression level of each target transcript relative to GAPDH, with n = 8 biological replicates. (C) Computational prediction of immunogenicity. The inventors first predicted 9-mer peptides that are MHC I binders using the IEDB database and subjected the top one percentile of binders to immunogenicity predictions using the IEDB immunogenicity predictor.

[0088] FIG. 28. gRNA screen with ADAR. Testing whether guide RNA designs that feature multiple hairpins increase the potency of CIRT5. Left panel shows gRNA designs feature either the origins design, or guides with one TAR hairpin on either end of the guide, or two hairpins on either end. The additional hairpin guides increase the potency of CIRT5 in an ADAR activity assay in cells. Right panel is testing whether the second hairpin needs to be a TAR hairpin, or if just a "stabilizing hairpin" can function - meaning a hairpin that does not directly interact with the CIRT5 protein but slows degradation. As seen in the data, the second hairpin increase potency compared to one hairpin gRNA design.

[0089] **FIG. 29A-B.** (A) C-to-U Editor: The data in A demonstrates that a CIRTS based on a C-to-U base editor is also functional using a mammalian cell reporter assay. (B) ssRNA binding proteins. The data in B demonstrates that other ssRNA binding proteins can function as a RNA binding proteins in the systems and methods of the disclosure.

5 [0090] **FIG. 30A-C.** (A): RNA regulatory domain-containing proteins that may activate translation, arraying gRNAs at various locales on a reporter RNA and measuring translational activation of each. (B-C). RNA regulatory domain-containing proteins that potentially degrade or destabilize an RNA, arraying gRNAs at various locales on a reporter RNA and measuring RNA degradation of each.

10 [0091] **FIG. 31A-B.** (A) Embodiments demonstrating the different orientations of the elements of the systems of the disclosure. (B) Data using CNOT7 in different orientations (as shown in A) and on two RNA targets: a luciferase reporter (left) and an endogenous RNA (right). Several different orientations of the proteins still function, indicating the proteins can be engineered in differed orders depending on the needs of the effector.

15 [0092] **FIG. 32A-D.** A CIRTS biosensor for inducible RNA targeting. (A) Schematic overview of the abscisic acid (ABA) CIRTS biosensor design. The gRNA-mediated targeting component of CIRTS is fused to one of the ABA heterodimerization domains (ABI) while the effector component of CIRTS is fused to its binding partner (PYL). Upon addition of the small molecule ABA, the two CIRTS components dimerize and bring the effector in proximity of the
20 targeted transcript. (B) ABA-inducible RNA degradation of an red-fluorescent protein (RFP) reporter transcript 48h after transfection can be mediated by the Pin nuclease domain or YTHDF2. (C) Translation activation of RFP by ABA-induced CIRTS-YTHDF1 48h after transfection. (D) Delivery of CIRTS-hADAR with on-target gRNA in the presence of ABA to cells transfected with a mutation-deactivated luciferase reporter (FlucW417X for A-to-I editing or the GlucC82R for C-to-U editing) induces ABA-dependent RNA editing.
25

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0093] Epitranscriptomic regulation controls information flow through the central dogma and provides unique opportunities for manipulating cell state at the RNA level. However, both
30 fundamental mechanistic studies and potential translational applications are impeded by a lack of effective methods to target specific RNAs with effector proteins. Here, the inventors present the design and validation of a CRISPR/Cas-inspired RNA targeting system (CIRTS), a new protein engineering strategy for constructing programmable RNA regulatory systems. The inventors show that CIRTS is a simple and generalizable approach to deliver a range of effector proteins, including nucleases, degradation machinery, and translational activators, to target

transcripts. CIRTS are not only smaller than naturally-occurring CRISPR/Cas programmable RNA binding systems, but can be built entirely from human protein parts. The small size and human-derived nature of CIRTS provides a less perturbative method for fundamental RNA regulatory studies as well as a potential strategy to avoid immune issues when applied to

5 epitranscriptome-modulating therapies.

I. RNA REGULATORY DOMAIN

[0094] It is contemplated that any RNA regulatory domain may be used in the methods and systems of the current disclosure. For example, a RNA regulatory domain with one or more of the following activities may be used: methylation, 5'-3' guanylation, phosphoribosylation,

10 deamination, carbamoylation, isopentenylolation, agmatinylation, acetylation, lysylation, O/S exchange, galactosylation, glutamylation, mannosylation, hydrogenation, pseudouridine formation, carboxymethylaminomethylation, aminomethylation, decarboxymethylation, dehydrogenation, carboxymethylation, hydroxylation, methylthiolation, 3-amino-3-carboxypropylation, demethylation, 5'-5' guanylation, and dephosphorylation.

15 [0095] Exemplary RNA regulatory domains include domains from the following proteins of Table 1 (or functional fragments thereof):

Table 1

Acronym	Full Name	Organism	Activity
BCDIN3D	BCDIN3 domain containing	Homo sapiens	methylation
Nop1	rRNA 2'-O-methyltransferase fibrillarin	Saccharomyces cerevisiae	methylation
Nsr	rRNA (adenosine-2'-O-)-methyltransferase	Streptomyces actuosus	methylation
Trm13	tRNA-2'-O-methyltransferase TRM13	Saccharomyces cerevisiae	methylation
Tsr	rRNA (adenosine-2'-O-)-methyltransferase	Streptomyces cyaneus	methylation
KamB	rRNA adenine N-1-methyltransferase	Streptomyces sp. DSM 40477	methylation
NpmA	rRNA adenine N-1-methyltransferase	Escherichia coli	methylation
Rrp8	Ribosomal RNA-processing protein 8	Saccharomyces cerevisiae	methylation
TRMT10C	tRNA methyltransferase 10 C, mitochondrial	Homo sapiens	methylation

Trm61	tRNA (adenine(58)-N(1))-methyltransferase catalytic subunit TRM61	Saccharomyces cerevisiae	methylation
TrmI	tRNA (adenine(57)-N(1)/adenine(58)-N(1))-methyltransferase TrmI	Pyrococcus abyssi	methylation
TrmI	tRNA (adenine(58)-N(1))-methyltransferase TrmI	Thermus thermophilus	methylation
TrmI	tRNA (adenine(58)-N(1))-methyltransferase TrmI	Mycobacterium tuberculosis	methylation
TrmK	tRNA (adenine(22)-N(1))-methyltransferase	Bacillus subtilis	methylation
Trmt61A	tRNA (adenine(58)-N(1))-methyltransferase catalytic subunit TRMT61A	Homo sapiens	methylation
Trmt61B	tRNA (adenine(58)-N(1))-methyltransferase, mitochondrial	Homo sapiens	methylation
METTL14		Homo sapiens	methylation
METTL3	N6-adenosine-methyltransferase 70 kDa subunit	Homo sapiens	methylation
RsmA	Dimethyladenosine transferase	Saccharomyces cerevisiae	methylation
ErmAM	rRNA adenine N-6-methyltransferase	Streptococcus pneumoniae	methylation
Cfr	Ribosomal RNA large subunit methyltransferase Cfr	Staphylococcus sciuri	methylation
Nop1	rRNA 2'-O-methyltransferase fibrillarin	Saccharomyces cerevisiae	methylation
Dnmt2	tRNA (cytosine(38)-C(5))-methyltransferase	Homo sapiens	methylation
Trm4	tRNA (cytosine(34)-C(5))-methyltransferase	Homo sapiens	methylation
MRM1	rRNA methyltransferase 1, mitochondrial	Homo sapiens	methylation
RNMTL1	RNA methyltransferase-like protein 1	Homo sapiens	methylation
TRM5	tRNA (guanine(37)-N1)-methyltransferase	Homo sapiens	methylation
TRMT10A	tRNA methyltransferase 10 homolog A	Homo sapiens	methylation
TRMT10B	tRNA methyltransferase 10 homolog B	Homo sapiens	methylation

TRMT10C	tRNA methyltransferase 10 C, mitochondrial	Homo sapiens	methylation
TRMB	tRNA (guanine-N(7))-methyltransferase	Homo sapiens	methylation
Hcm1	RNA cap guanine-N7 methyltransferase	Homo sapiens	methylation
MRM2	rRNA methyltransferase 2, mitochondrial	Homo sapiens	methylation
ALKBH8	Alkylated DNA repair protein alkB homolog 8	Homo sapiens	methylation
BCDIN3D	BCDIN3 domain containing	Homo sapiens	methylation
Tgs1	trimethylguanosinesynthase 1	Homo sapiens	methylation
Trm1	tRNA (guanine(26)-N(2))-dimethyltransferase	Homo sapiens	methylation
Thg1	tRNA(His) guanylyltransferase	Saccharomyces cerevisiae	5'-3' guanylylation
Rit1	tRNA A64-2'-O-ribosylphosphate transferase	Saccharomyces cerevisiae	phosphoribosylation
Tad1	tRNA-specific adenosine deaminase 1	Saccharomyces cerevisiae	deamination
Tad1	tRNA-specific adenosine deaminase 1	Homo sapiens	deamination
Tad2	tRNA-specific adenosine deaminase subunit TAD2	Saccharomyces cerevisiae	deamination
Tad3	tRNA-specific adenosine deaminase subunit TAD3	Saccharomyces cerevisiae	deamination
TadA	tRNA-specific adenosine deaminase	Escherichia coli	deamination
TadA	tRNA-specific adenosine deaminase, chloroplastic	Arabidopsis thaliana	deamination
CDAT8	tRNA-specific cytidine deaminase	Methanopyrus kandleri	deamination
MiaA	tRNA (adenosine(37)-N6)-dimethylallyltransferase	Escherichia coli	isopentenylolation
Mod5	tRNA dimethylallyltransferase, mitochondrial	Saccharomyces cerevisiae	isopentenylolation
Mod5	tRNA dimethylallyltransferase, mitochondrial	Homo sapiens	isopentenylolation
Sua5	tRNA threonylcarbamoyladenosine biosynthesis protein SUA5	Saccharomyces cerevisiae	carbamoylation

TsaD	tRNA N6-threonylcarbamoyladenosine(37) synthesis protein	Escherichia coli	carbamoylation
TiaS	tRNA(Ile2) 2-agmatinylcytidine synthase TiaS	Archaeoglobus fulgidus	agmatinylation
NAT10	N-acetyltransferase 10	Homo sapiens	acetylation
TilS	tRNA lysidine(34) synthetase	Escherichia coli	lysylation
TtcA	tRNA 2-thiocytidine(32) synthetase	Escherichia coli	O/S exchange
TrmU	Mitochondrial tRNA-specific 2-thiouridylase 1	Homo sapiens	O/S exchange
Man/Gal-Q-transferase	tRNA-queueine glycosyltransferase	various eukaryotes	galactosylation
GluQRS	tRNA glutamyl-Q(34) synthetase	Escherichia coli	glutamylation
Man/Gal-Q-transferase	tRNA-queueine glycosyltransferase	various eukaryotes	mannosylation
DusI	tRNA-dihydrouridine synthase 1	Saccharomyces cerevisiae	hydrogenation
DusA	tRNA dihydrouridine synthase A	Escherichia coli	hydrogenation
PusI	tRNA pseudouridine synthase 1, mitochondrial	Homo sapiens	pseudouridine formation
PusI	tRNA pseudouridine synthase 1	Saccharomyces cerevisiae	pseudouridine formation
RluA	tRNA pseudouridine(32) synthase / Ribosomal large subunit pseudouridine synthase A	Escherichia coli	pseudouridine formation
MnmE	tRNA uridine(34) 5-carboxymethylaminomethyl synthesis GTPase	Escherichia coli	carboxymethylaminomethylation
MnmE	tRNA uridine(34) 5-carboxymethylaminomethyl synthesis GTPase	Escherichia coli	aminomethylation
MnmCD	tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase / FAD-dependent cmnm(5)s(2)U34 oxidoreductase	Escherichia coli	decarboxymethylation
FTO	Alpha-ketoglutarate-dependent dioxygenase FTO	Homo sapiens	dehydrogenation
CmoB	tRNA 5-methoxyuridine(34) synthase	Escherichia coli	carboxymethylation

MiaE	tRNA 2-methylthio-N6-isopentenyl adenosine(37) hydroxylase	Salmonella typhimurium	hydroxylation
FTO	Alpha-ketoglutarate-dependent dioxygenase FTO	Homo sapiens	hydroxylation
ALKBH8	Alkylated DNA repair protein alkB homolog 8	Homo sapiens	hydroxylation
MiaB	tRNA (N6-isopentenyl adenosine(37)-C2)-methylthiotransferase	Escherichia coli	methylthiolation
TYW2	tRNA wybutosine-synthesizing protein 2	Saccharomyces cerevisiae	3-amino-3-carboxypropylation
ALKBH5		Homo sapiens	demethylation
FTO	Alpha-ketoglutarate-dependent dioxygenase FTO	Homo sapiens	demethylation
HCAP1	RNA-capping enzyme	Homo sapiens	5'-5' guanylation
HCAP1	RNA-capping enzyme	Homo sapiens	dephosphorylation

[0096] Further RNA regulatory domains include functional domains from the following human proteins of Table 2:

Table 2

Gene Symbol	Synonyms	Gene Description
RBM5	LUCA15; H37	RNA binding motif protein 5 [Source:HGNC Symbol;Acc:9902]
RBM6	DEF-3; 3G2; NY-LU-12; g16; DEF3	RNA binding motif protein 6 [Source:HGNC Symbol;Acc:9903]
		Putative RNA exonuclease NEF-sp (EC 3.1.-.-) [Source:UniProtKB/Swiss-Prot;Acc:Q96IC2]
YBX2	MSY2; CSDA3	Y box binding protein 2 [Source:HGNC Symbol;Acc:17948]
CSDE1	D1S155E; UNR	cold shock domain containing E1, RNA-binding [Source:HGNC Symbol;Acc:29905]
PTBP1	HNRPI; HNRNP-I; PTB2; PTB3; PTB-1; PTB4; pPTB	polypyrimidine tract binding protein 1 [Source:HGNC Symbol;Acc:9583]
ZC3H3	KIAA0150	zinc finger CCCH-type containing 3 [Source:HGNC Symbol;Acc:28972]
MATR3	KIAA0723; MGC9105	matrin 3 [Source:HGNC Symbol;Acc:6912]
SAMD4A	KIAA1053; DKFZP434H0350;	sterile alpha motif domain containing 4A [Source:HGNC Symbol;Acc:23023]

	Smaug; SMG; SMGA; hSmaug1	
YTHDC2	FLJ2194; FLJ10053; DKFZp564A186	YTH domain containing 2 [Source:HGNC Symbol;Acc:24721]
CUGBP2		CUG triplet repeat, RNA binding protein 2 [Source:HGNC Symbol;Acc:2550]
PUM2	PUMH2; KIAA0235	pumilio homolog 2 (Drosophila) [Source:HGNC Symbol;Acc:14958]
RC3H2	FLJ20301; FLJ20713; RNF164	ring finger and CCCH-type zinc finger domains 2 [Source:HGNC Symbol;Acc:21461]
ZC3H11A	KIAA0663	zinc finger CCCH-type containing 11A [Source:HGNC Symbol;Acc:29093]
PARP12	FLJ22693; PARP- 12; ZC3H1	poly (ADP-ribose) polymerase family, member 12 [Source:HGNC Symbol;Acc:21919]
CSDA	dbpA; ZONAB; CSDA1	cold shock domain protein A pseudogene 1 [Source:HGNC Symbol;Acc:2429]
SFRS8	SWAP	splicing factor, arginine/serine-rich 8 (suppressor-of- white-apricot homolog, Drosophila) [Source:HGNC Symbol;Acc:10790]
EIF4B		eukaryotic translation initiation factor 4B [Source:HGNC Symbol;Acc:3285]
U2AF2	U2AF65	U2 small nuclear RNA auxiliary factor 2 [Source:HGNC Symbol;Acc:23156]
SFRS14	KIAA0365	splicing factor, arginine/serine-rich 14 [Source:HGNC Symbol;Acc:18641]
SPEN	KIAA0929; MINT; SHARP; RBM15C	spen homolog, transcriptional regulator (Drosophila) [Source:HGNC Symbol;Acc:17575]
ZC3H15	LEREPO4	zinc finger CCCH-type containing 15 [Source:HGNC Symbol;Acc:29528]
YBX1	YB-1; YB1; DBPB; NSEP-1; MDR-NF1; BP-8; CSDB; CSDA2	Y box binding protein 1 [Source:HGNC Symbol;Acc:8014]
ELAVL1	HuR; Hua; MelG	ELAV (embryonic lethal, abnormal vision, Drosophila)- like 1 (Hu antigen R) [Source:HGNC Symbol;Acc:3312]
THUMPD1	FLJ20274	THUMP domain containing 1 [Source:HGNC Symbol;Acc:23807]
DHX8	HRH1; PRP22; PRPF22	DEAH (Asp-Glu-Ala-His) box polypeptide 8 [Source:HGNC Symbol;Acc:2749]
SRBD1	FLJ10379	S1 RNA binding domain 1 [Source:HGNC Symbol;Acc:25521]
PABPC1	PABP1; PABPL1	poly(A) binding protein, cytoplasmic 1 [Source:HGNC Symbol;Acc:8554]

DAZAP1	MGC19907	DAZ associated protein 1 [Source:HGNC Symbol;Acc:2683]
IGF2BP2	IMP-2	insulin-like growth factor 2 mRNA binding protein 2 [Source:HGNC Symbol;Acc:28867]
ZNF638	NP220; MGC26130; Zfp638	zinc finger protein 638 [Source:HGNC Symbol;Acc:17894]
SART3	KIAA0156; RP11-13G14	squamous cell carcinoma antigen recognized by T cells 3 [Source:HGNC Symbol;Acc:16860]
MKRN2	RNF62; HSPC070	makorin ring finger protein 2 [Source:HGNC Symbol;Acc:7113]
RBM7		RNA binding motif protein 7 [Source:HGNC Symbol;Acc:9904]
RBMS2	SCR3	RNA binding motif, single stranded interacting protein 2 [Source:HGNC Symbol;Acc:9909]
MBNL3	CHCR; FLJ11316; MBLX39; MBXL	muscleblind-like 3 (Drosophila) [Source:HGNC Symbol;Acc:20564]
SNRPA	U1A; U1-A; Mud1	small nuclear ribonucleoprotein polypeptide A [Source:HGNC Symbol;Acc:11151]
SYNJ2	INPP5H	synaptojanin 2 [Source:HGNC Symbol;Acc:11504]
A2BP1		Fox-1 homolog A (Ataxin-2-binding protein 1)(Hexaribonucleotide-binding protein 1) [Source:UniProtKB/Swiss-Prot;Acc:Q9NWB1]
		Trinucleotide repeat-containing gene 6C protein [Source:UniProtKB/Swiss-Prot;Acc:Q9HCJ0]
DDX43	HAGE; DKFZp434H2114; CT13	DEAD (Asp-Glu-Ala-Asp) box polypeptide 43 [Source:HGNC Symbol;Acc:18677]
KIAA0020	XTP5; PEN; PUF6	KIAA0020 [Source:HGNC Symbol;Acc:29676]
CNOT4	CLONE243; NOT4H	CCR4-NOT transcription complex, subunit 4 [Source:HGNC Symbol;Acc:7880]
YTHDC1	YT521; KIAA1966; YT521-B	YTH domain containing 1 [Source:HGNC Symbol;Acc:30626]
PPIE	CyP-33; MGC3736; MGC111222	peptidylprolyl isomerase E (cyclophilin E) [Source:HGNC Symbol;Acc:9258]
CHERP	ERPROT213-21; DAN16	calcium homeostasis endoplasmic reticulum protein [Source:HGNC Symbol;Acc:16930]
RBM22	FLJ10290; ZC3H16; fSAP47; Cwc2	RNA binding motif protein 22 [Source:HGNC Symbol;Acc:25503]
KHSRP	KSRP; FBP2; FUBP2	KH-type splicing regulatory protein [Source:HGNC Symbol;Acc:6316]

FUS	TLS; FUS1; hnRNP-P2	fused in sarcoma [Source:HGNC Symbol;Acc:4010]
RBM41	FLJ11016	RNA binding motif protein 41 [Source:HGNC Symbol;Acc:25617]
PCBP4	MCG10; LIP4	poly(rC) binding protein 4 [Source:HGNC Symbol;Acc:8652]
PABPC4	iPABP; APP-1	poly(A) binding protein, cytoplasmic 4 (inducible form) [Source:HGNC Symbol;Acc:8557]
TNRC6A	CAGH26; KIAA1460; GW182	trinucleotide repeat containing 6A [Source:HGNC Symbol;Acc:11969]
RBM27	KIAA1311; ARRS1; Psc1	RNA binding motif protein 27 [Source:HGNC Symbol;Acc:29243]
HNRNPC	hnRNP C	heterogeneous nuclear ribonucleoprotein C (C1/C2) [Source:HGNC Symbol;Acc:5035]
DAZL	DAZH; SPGYLA; MGC26406; DAZL1	deleted in azoospermia-like [Source:HGNC Symbol;Acc:2685]
HNRNPH3	2H9	heterogeneous nuclear ribonucleoprotein H3 (2H9) [Source:HGNC Symbol;Acc:5043]
SETD1A	KIAA0339; Set1; KMT2F	SET domain containing 1A [Source:HGNC Symbol;Acc:29010]
CIRBP	CIRP	cold inducible RNA binding protein [Source:HGNC Symbol;Acc:1982]
HNRNPM	HTGR1; HNRNPM4; HNRPM4	heterogeneous nuclear ribonucleoprotein M [Source:HGNC Symbol;Acc:5046]
TRMT2A	HTF9C	TRM2 tRNA methyltransferase 2 homolog A (S. cerevisiae) [Source:HGNC Symbol;Acc:24974]
SF3A1	SF3a120; SAP114; PRPF21; Prp21	splicing factor 3a, subunit 1, 120kDa [Source:HGNC Symbol;Acc:10765]
SNRPD3	SMD3; Sm-D3	small nuclear ribonucleoprotein D3 polypeptide 18kDa [Source:HGNC Symbol;Acc:11160]
POLDIP3	PDIP46; KIAA1649	polymerase (DNA-directed), delta interacting protein 3 [Source:HGNC Symbol;Acc:23782]
ZMAT5		zinc finger, matrin type 5 [Source:HGNC Symbol;Acc:28046]
RBM9	HNRBP2; FOX-2	RNA binding motif protein 9 [Source:HGNC Symbol;Acc:9906]
ZC3H7B	RoXaN; FLJ13787; DKFZp434K0920; KIAA1031	zinc finger CCCH-type containing 7B [Source:HGNC Symbol;Acc:30869]
RBM23	FLJ10482	RNA binding motif protein 23 [Source:HGNC Symbol;Acc:20155]

SFRS5	SRP40; HRS	splicing factor, arginine/serine-rich 5 [Source:HGNC Symbol;Acc:10787]
ZC3H14	FLJ11806; UKp68; NY-REN-37	zinc finger CCCH-type containing 14 [Source:HGNC Symbol;Acc:20509]
ACIN1	KIAA0670; fSAP152	apoptotic chromatin condensation inducer 1 [Source:HGNC Symbol;Acc:17066]
PABPN1	PAB2	poly(A) binding protein, nuclear 1 [Source:HGNC Symbol;Acc:8565]
PABPC1L	dJ1069P2.3; PABPC1L1; ePAB	poly(A) binding protein, cytoplasmic 1-like [Source:HGNC Symbol;Acc:15797]
BRUNOL4		bruno-like 4, RNA binding protein (Drosophila) [Source:HGNC Symbol;Acc:14015]
CSTF2		cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa [Source:HGNC Symbol;Acc:2484]
ZC3H12B	MCPIP2	zinc finger CCCH-type containing 12B [Source:HGNC Symbol;Acc:17407]
FMR1	FMRP; FRAXA; MGC87458	fragile X mental retardation 1 [Source:HGNC Symbol;Acc:3775]
HTATSF1	TAT-SF1	HIV-1 Tat specific factor 1 [Source:HGNC Symbol;Acc:5276]
RBM3	IS1-RNPL	RNA binding motif (RNP1, RRM) protein 3 [Source:HGNC Symbol;Acc:9900]
ESRP2	FLJ21918	epithelial splicing regulatory protein 2 [Source:HGNC Symbol;Acc:26152]
MTHFSD	FLJ12998	methenyltetrahydrofolate synthetase domain containing [Source:HGNC Symbol;Acc:25778]
DNAJC17	FLJ10634	DnaJ (Hsp40) homolog, subfamily C, member 17 [Source:HGNC Symbol;Acc:25556]
MYEF2	MEF-2; FLJ11213; KIAA1341; HsT18564	myelin expression factor 2 [Source:HGNC Symbol;Acc:17940]
ESRP1	FLJ20171	epithelial splicing regulatory protein 1 [Source:HGNC Symbol;Acc:25966]
HNRNPL		heterogeneous nuclear ribonucleoprotein L [Source:HGNC Symbol;Acc:5045]
SNRNP70	U1-70K; Snp1	small nuclear ribonucleoprotein 70kDa (U1) [Source:HGNC Symbol;Acc:11150]
SFRS16	SWAP2; CLASP	splicing factor, arginine/serine-rich 16 [Source:HGNC Symbol;Acc:17731]
TRMT1	FLJ20244	TRM1 tRNA methyltransferase 1 homolog (S. cerevisiae) [Source:HGNC Symbol;Acc:25980]
NOVA2	ANOVA	neuro-oncological ventral antigen 2 [Source:HGNC Symbol;Acc:7887]

SF4	F23858; DKFZp434E2216; RBP	splicing factor 4 [Source:HGNC Symbol;Acc:18643]
ZC3HAV1	ZAP; FLB6421; FLJ13288; MGC48898; ZC3HDC2; ZC3H2; PARP13	zinc finger CCCH-type, antiviral 1 [Source:HGNC Symbol;Acc:23721]
EIF3B	PRT1; eIF3b	eukaryotic translation initiation factor 3, subunit B [Source:HGNC Symbol;Acc:3280]
RBM28	FLJ10377	RNA binding motif protein 28 [Source:HGNC Symbol;Acc:21863]
LSM5	YER146W	LSM5 homolog, U6 small nuclear RNA associated (S. cerevisiae) [Source:HGNC Symbol;Acc:17162]
EIF4H	WSCR1; KIAA0038	eukaryotic translation initiation factor 4H [Source:HGNC Symbol;Acc:12741]
ELAVL2	HuB; HEL-N1	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B) [Source:HGNC Symbol;Acc:3313]
FUBP3		far upstream element (FUSE) binding protein 3 [Source:HGNC Symbol;Acc:4005]
CPEB3	KIAA0940	cytoplasmic polyadenylation element binding protein 3 [Source:HGNC Symbol;Acc:21746]
LARP4B		La ribonucleoprotein domain family, member 4B [Source:HGNC Symbol;Acc:28987]
SUPT6H	KIAA0162; SPT6H	suppressor of Ty 6 homolog (S. cerevisiae) [Source:HGNC Symbol;Acc:11470]
PPARGC1A	PGC1; PGC1A	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha [Source:HGNC Symbol;Acc:9237]
CPSF6	CFIM; HPBR11-4; HPBR11-7; CFIM68	microRNA 1279 [Source:HGNC Symbol;Acc:35357]
KRR1	RIP-1	KRR1, small subunit (SSU) processome component, homolog (yeast) [Source:HGNC Symbol;Acc:5176]
SFRS9	SRp30c	splicing factor, arginine/serine-rich 9 [Source:HGNC Symbol;Acc:10791]
SFRS3	SRp20	splicing factor, arginine/serine-rich 3 [Source:HGNC Symbol;Acc:10785]
RBM24	FLJ30829; dJ259A10.1	RNA binding motif protein 24 [Source:HGNC Symbol;Acc:21539]
KHDRBS2	SLM1; SLM-1; MGC26664	KH domain containing, RNA binding, signal transduction associated 2 [Source:HGNC Symbol;Acc:18114]
QKI	QK3	quaking homolog, KH domain RNA binding (mouse) [Source:HGNC Symbol;Acc:21100]

CPEB4	KIAA1673	cytoplasmic polyadenylation element binding protein 4 [Source:HGNC Symbol;Acc:21747]
FXR1		fragile X mental retardation, autosomal homolog 1 [Source:HGNC Symbol;Acc:4023]
NCL	C23	nucleolin [Source:HGNC Symbol;Acc:7667]
		Pre-mRNA branch site protein p14 (SF3B 14 kDa subunit) [Source:UniProtKB/Swiss-Prot;Acc:Q9Y3B4]
HDLBP	HBP	high density lipoprotein binding protein [Source:HGNC Symbol;Acc:4857]
SFRS7	9G8; ZCRB2; HSSG1; AAG3; RBM37; ZCCHC20	splicing factor, arginine/serine-rich 7, 35kDa [Source:HGNC Symbol;Acc:10789]
PNO1		partner of NOB1 homolog (S. cerevisiae) [Source:HGNC Symbol;Acc:32790]
TIA1		TIA1 cytotoxic granule-associated RNA binding protein [Source:HGNC Symbol;Acc:11802]
SFRS4	SRP75	splicing factor, arginine/serine-rich 4 [Source:HGNC Symbol;Acc:10786]
SFPQ	PSF	splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated) [Source:HGNC Symbol;Acc:10774]
SFRS11	p54; NET2	splicing factor, arginine/serine-rich 11 [Source:HGNC Symbol;Acc:10782]
PRPF3	Prp3; HPRP3	PRP3 pre-mRNA processing factor 3 homolog (S. cerevisiae) [Source:HGNC Symbol;Acc:17348]
PTBP2	brPTB; nPTB; PTB; PTBLP	polypyrimidine tract binding protein 2 [Source:HGNC Symbol;Acc:17662]
ROD1	PTBP3	ROD1 regulator of differentiation 1 (S. pombe) [Source:HGNC Symbol;Acc:10253]
RBM18	MGC2734	RNA binding motif protein 18 [Source:HGNC Symbol;Acc:28413]
C14orf156	DC50	SRA stem-loop-interacting RNA-binding protein, mitochondrial Precursor [Source:UniProtKB/Swiss-Prot;Acc:Q9GZT3]
RBM25	S164; fSAP94; NET52; Snu71	RNA binding motif protein 25 [Source:HGNC Symbol;Acc:23244]
ENOX1	FLJ10094; PIG38; CNOX; cCNOX	ecto-NOX disulfide-thiol exchanger 1 [Source:HGNC Symbol;Acc:25474]
TARDBP	TDP-43; ALS10	TAR DNA binding protein [Source:HGNC Symbol;Acc:11571]
AKAP1	AKAP121; AKAP149; SAKAP84; S-	A kinase (PRKA) anchor protein 1 [Source:HGNC Symbol;Acc:367]

	AKAP84; AKAP84	
PSPC1	PSP1; FLJ10955	paraspeckle component 1 [Source:HGNC Symbol;Acc:20320]
ZCCHC17	PS1D; HSPC251; pNO40	zinc finger, CCHC domain containing 17 [Source:HGNC Symbol;Acc:30246]
KHDRBS1	Sam68; p62; FLJ34027	KH domain containing, RNA binding, signal transduction associated 1 [Source:HGNC Symbol;Acc:18116]
ZC3H7A	HSPC055; FLJ20318	zinc finger CCCH-type containing 7A [Source:HGNC Symbol;Acc:30959]
HNRNPA2B1		heterogeneous nuclear ribonucleoprotein A2/B1 [Source:HGNC Symbol;Acc:5033]
BICC1		bicaudal C homolog 1 (Drosophila) [Source:HGNC Symbol;Acc:19351]
RBM19	DKFZp586F1023; KIAA0682	RNA binding motif protein 19 [Source:HGNC Symbol;Acc:29098]
ZC3H13	DKFZp434D1812	zinc finger CCCH-type containing 13 [Source:HGNC Symbol;Acc:20368]
SFRS6	SRP55; B52	splicing factor, arginine/serine-rich 6 [Source:HGNC Symbol;Acc:10788]
RNF113A	RNF113; Cwc24	ring finger protein 113A [Source:HGNC Symbol;Acc:12974]
SNRPD2	Sm-D2	small nuclear ribonucleoprotein D2 polypeptide 16.5kDa [Source:HGNC Symbol;Acc:11159]
SNRPB	COD; SmB/SmB'; Sm-B/B'; snRNP-B	small nuclear ribonucleoprotein polypeptides B and B1 [Source:HGNC Symbol;Acc:11153]
SNRPB2	Msl1	small nuclear ribonucleoprotein polypeptide B" [Source:HGNC Symbol;Acc:11155]
HNRNPR	hnRNP-R	heterogeneous nuclear ribonucleoprotein R [Source:HGNC Symbol;Acc:5047]
RALY	P542; HNRPCL2	RNA binding protein, autoantigenic (hnRNP-associated with lethal yellow homolog (mouse)) [Source:HGNC Symbol;Acc:15921]
RBM42	MGC10433	RNA binding motif protein 42 [Source:HGNC Symbol;Acc:28117]
HNRNPH2	hnRNPH'; FTP3; HNRPH'	heterogeneous nuclear ribonucleoprotein H2 (H') [Source:HGNC Symbol;Acc:5042]
ZFP36	RNF162A; TIS11; G0S24; TTP; NUP475	zinc finger protein 36, C3H type, homolog (mouse) [Source:HGNC Symbol;Acc:12862]
NAA38	YJR022W	N(alpha)-acetyltransferase 38, NatC auxiliary subunit [Source:HGNC Symbol;Acc:20471]
SNRPN	SMN; SM-D; HCERN3;	small nuclear ribonucleoprotein polypeptide N [Source:HGNC Symbol;Acc:11164]

	SNRNP-N; SNURF-SNRPN; RT-LI	
FXR2		fragile X mental retardation, autosomal homolog 2 [Source:HGNC Symbol;Acc:4024]
SAFB2	KIAA0138	scaffold attachment factor B2 [Source:HGNC Symbol;Acc:21605]
LSM7	YNL147W	LSM7 homolog, U6 small nuclear RNA associated (S. cerevisiae) [Source:HGNC Symbol;Acc:20470]
LSM4	YER112W	LSM4 homolog, U6 small nuclear RNA associated (S. cerevisiae) [Source:HGNC Symbol;Acc:17259]
ZC3H4	KIAA1064	zinc finger CCCH-type containing 4 [Source:HGNC Symbol;Acc:17808]
EIF3G	eIF3-delta; eIF3-p44; eIF3g	eukaryotic translation initiation factor 3, subunit G [Source:HGNC Symbol;Acc:3274]
DKC1	XAP101; dyskerin; NAP57; NOLA4	small nucleolar RNA, H/ACA box 56 [Source:HGNC Symbol;Acc:32650]
PPIL4		peptidylprolyl isomerase (cyclophilin)-like 4 [Source:HGNC Symbol;Acc:15702]
RBM39	CC1.3; HCC1; CAPER; fSAP59	RNA binding motif protein 39 [Source:HGNC Symbol;Acc:15923]
ANKHD1	MASK; FLJ20288; FLJ11979; FLJ10042; FLJ14127; KIAA1085	ankyrin repeat and KH domain containing 1 [Source:HGNC Symbol;Acc:24714]
KHDRBS3	T-STAR; Etle; etoile; SALP; SLM2; SLM-2	KH domain containing, RNA binding, signal transduction associated 3 [Source:HGNC Symbol;Acc:18117]
RBM8A	ZNRP; BOV-1A; BOV-1B; BOV-1C; RBM8B; Y14	RNA binding motif protein 8A [Source:HGNC Symbol;Acc:9905]
LIN28		lin-28 homolog (C. elegans) [Source:HGNC Symbol;Acc:15986]
GRSF1		G-rich RNA sequence binding factor 1 [Source:HGNC Symbol;Acc:4610]
ANKRD17	GTAR; KIAA0697; FLJ22206; NY-BR-16	ankyrin repeat domain 17 [Source:HGNC Symbol;Acc:23575]
UNK	KIAA1753	unkempt homolog (Drosophila) [Source:HGNC Symbol;Acc:29369]
NIP7	CGI-37; FLJ10296; HSPC031; KD93	nuclear import 7 homolog (S. cerevisiae) [Source:HGNC Symbol;Acc:24328]

TOE1		target of EGR1, member 1 (nuclear) [Source:HGNC Symbol;Acc:15954]
RBM38	HSRNASEB; SEB4D; seb4B; dJ800J21.2	RNA binding motif protein 38 [Source:HGNC Symbol;Acc:15818]
SRRM1	SRM160; POP101; MGC39488	serine/arginine repetitive matrix 1 [Source:HGNC Symbol;Acc:16638]
MKRN1	RNF61	makorin ring finger protein 1 [Source:HGNC Symbol;Acc:7112]
EIF2S1	EIF-2alpha; EIF2A	eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa [Source:HGNC Symbol;Acc:3265]
THUMPD3	DKFZP434F091	THUMP domain containing 3 [Source:HGNC Symbol;Acc:24493]
RBMX2	CGI-79	RNA binding motif protein, X-linked 2 [Source:HGNC Symbol;Acc:24282]
PUM1	PUMH1; KIAA0099	pumilio homolog 1 (Drosophila) [Source:HGNC Symbol;Acc:14957]
MSI1		musashi homolog 1 (Drosophila) [Source:HGNC Symbol;Acc:7330]
SYNCRIP	NSAP1; GRY- RBP; dJ3J17.2; HNRPQ1; hnRNP- Q	synaptotagmin binding, cytoplasmic RNA interacting protein [Source:HGNC Symbol;Acc:16918]
ZC3H10	FLJ14451	zinc finger CCCH-type containing 10 [Source:HGNC Symbol;Acc:25893]
HNRNPA1	hnRNPA1; hnRNP-A1	heterogeneous nuclear ribonucleoprotein A1 [Source:HGNC Symbol;Acc:5031]
RC3H1	KIAA2025; roquin; RP5- 1198E17.5; RNF198	ring finger and CCCH-type zinc finger domains 1 [Source:HGNC Symbol;Acc:29434]
IGF2BP3	IMP-3; CT98	insulin-like growth factor 2 mRNA binding protein 3 [Source:HGNC Symbol;Acc:28868]
NUPL2	NLP_1; CG1; hCG1; H RG271G13.9	nucleoporin like 2 [Source:HGNC Symbol;Acc:17010]
SFRS1	ASF; SF2; SRp30a; SF2p33; MGC5228	splicing factor, arginine/serine-rich 1 [Source:HGNC Symbol;Acc:10780]
TRA2B	Htra2-beta	transformer 2 beta homolog (Drosophila) [Source:HGNC Symbol;Acc:10781]
CPEB2		cytoplasmic polyadenylation element binding protein 2 [Source:HGNC Symbol;Acc:21745]
ALKBH8	MGC10235	alkB, alkylation repair homolog 8 (E. coli) [Source:HGNC Symbol;Acc:25189]

SLTM	Met; FLJ13213	SAFB-like, transcription modulator [Source:HGNC Symbol;Acc:20709]
PNPT1	PNPase; OLD35; old-35	polyribonucleotide nucleotidyltransferase 1 [Source:HGNC Symbol;Acc:23166]
THUMPD2	MGC2454	THUMP domain containing 2 [Source:HGNC Symbol;Acc:14890]
ASCC1	CGI-18; ASC1p50; Em:AC022392.3	activating signal cointegrator 1 complex subunit 1 [Source:HGNC Symbol;Acc:24268]
SSB	LARP3; La	Sjogren syndrome antigen B (autoantigen La) [Source:HGNC Symbol;Acc:11316]
HNRNPD		heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa) [Source:HGNC Symbol;Acc:5036]
LARP1B	FLJ10378; DKFZp434K245; DKFZp686E0316	La ribonucleoprotein domain family, member 1B [Source:HGNC Symbol;Acc:24704]
G3BP2	KIAA0660	GTPase activating protein (SH3 domain) binding protein 2 [Source:HGNC Symbol;Acc:30291]
ZCRB1	MADP-1; MADP1; RBM36; ZCCHC19	zinc finger CCHC-type and RNA binding motif 1 [Source:HGNC Symbol;Acc:29620]
SNRPF	Sm-F	small nuclear ribonucleoprotein polypeptide F [Source:HGNC Symbol;Acc:11162]
		Heterogeneous nuclear ribonucleoprotein A1-like protein 2 (hnRNP core protein A1-like protein 2) [Source:UniProtKB/Swiss-Prot;Acc:Q32P51]
SETD1B	KIAA1076; Set1B; KMT2G	SET domain containing 1B [Source:HGNC Symbol;Acc:29187]
RBM26	PRO1777; SE70-2; FLJ20957; ZC3H17; ARRS2	RNA binding motif protein 26 [Source:HGNC Symbol;Acc:20327]
MBNL2	MBLL; MBLL39	muscleblind-like 2 (Drosophila) [Source:HGNC Symbol;Acc:16746]
RNF113B	RNF161	ring finger protein 113B [Source:HGNC Symbol;Acc:17267]
NOVA1		neuro-oncological ventral antigen 1 [Source:HGNC Symbol;Acc:7886]
BRUNOL6		bruno-like 6, RNA binding protein (Drosophila) [Source:HGNC Symbol;Acc:14059]
DUS3L	DUS3; FLJ13896	dihydrouridine synthase 3-like (S. cerevisiae) [Source:HGNC Symbol;Acc:26920]
SF3B4	SAP49; SF3b49; Hsh49	splicing factor 3b, subunit 4, 49kDa [Source:HGNC Symbol;Acc:10771]
LGTN		ligatin [Source:HGNC Symbol;Acc:6583]

HNRPLL		heterogeneous nuclear ribonucleoprotein L-like [Source:HGNC Symbol;Acc:25127]
SNRPG	Sm-G	small nuclear ribonucleoprotein polypeptide G [Source:HGNC Symbol;Acc:11163]
ZC3H8	Fliz1	zinc finger CCCH-type containing 8 [Source:HGNC Symbol;Acc:30941]
RBMS3		RNA binding motif, single stranded interacting protein [Source:HGNC Symbol;Acc:13427]
G3BP1	HDH-VIII; G3BP	GTPase activating protein (SH3 domain) binding protein 1 [Source:HGNC Symbol;Acc:30292]
NONO	NRB54; NMT55; P54NRB; P54	non-POU domain containing, octamer-binding [Source:HGNC Symbol;Acc:7871]
RBMX	RNMX; hnRNP-G	RNA binding motif protein, X-linked [Source:HGNC Symbol;Acc:9910]
A1CF	ACF; ASP; ACF64; ACF65; APOBEC1CF	APOBEC1 complementation factor [Source:HGNC Symbol;Acc:24086]
PPRC1	PRC; KIAA0595; MGC74642	peroxisome proliferator-activated receptor gamma, coactivator-related 1 [Source:HGNC Symbol;Acc:30025]
PDCD11	KIAA0185; ALG-4	programmed cell death 11 [Source:HGNC Symbol;Acc:13408]
TUT1	FLJ22347; FLJ22267; FLJ21850; PAPD2; TUTase	terminal uridylyl transferase 1, U6 snRNA-specific [Source:HGNC Symbol;Acc:26184]
CUGBP1		CUG triplet repeat, RNA binding protein 1 [Source:HGNC Symbol;Acc:2549]
RPS3	FLJ26283; FLJ27450; MGC87870	ribosomal protein S3 [Source:HGNC Symbol;Acc:10420]
ZC3H12C	KIAA1726; MCP1P3	zinc finger CCCH-type containing 12C [Source:HGNC Symbol;Acc:29362]
CPSF7	FLJ12529	cleavage and polyadenylation specific factor 7, 59kDa [Source:HGNC Symbol;Acc:30098]
YTHDF1	FLJ20391	YTH domain family, member 1 [Source:HGNC Symbol;Acc:15867]
PABPC3	PABP3; tPABP	poly(A) binding protein, cytoplasmic 3 [Source:HGNC Symbol;Acc:8556]
TIAL1	TIAR	TIA1 cytotoxic granule-associated RNA binding protein-like 1 [Source:HGNC Symbol;Acc:11804]
RBM46	MGC27016; CT68	RNA binding motif protein 46 [Source:HGNC Symbol;Acc:28401]
UHMK1	KIS; Kist	U2AF homology motif (UHM) kinase 1 [Source:HGNC Symbol;Acc:19683]

BOLL	BOULE	bol, boule-like (Drosophila) [Source:HGNC Symbol;Acc:14273]
ZFP36L2	ERF2; RNF162C; TIS11D	zinc finger protein 36, C3H type-like 2 [Source:HGNC Symbol;Acc:1108]
PAN3		PAN3 poly(A) specific ribonuclease subunit homolog (S. cerevisiae) [Source:HGNC Symbol;Acc:29991]
MBNL1	KIAA0428; EXP42; EXP40; EXP35; EXP	muscleblind-like (Drosophila) [Source:HGNC Symbol;Acc:6923]
HNRPDL	JKTBP; laAUF1	heterogeneous nuclear ribonucleoprotein D-like [Source:HGNC Symbol;Acc:5037]
CARHSP1	CRHSP-24; CSDC1	calcium regulated heat stable protein 1, 24kDa [Source:HGNC Symbol;Acc:17150]
RBMS1	SCR2; MSSP-1; MSSP-2; MSSP-3; YC1; HCC-4; DKFZp564H0764	RNA binding motif, single stranded interacting protein 1 [Source:HGNC Symbol;Acc:9907]
SFRS12	DKFZp564B176; SRrp86; SRrp508	splicing factor, arginine/serine-rich 12 [Source:HGNC Symbol;Acc:17882]
MSI2		musashi homolog 2 (Drosophila) [Source:HGNC Symbol;Acc:18585]
SFRS13B	SRrp35; SFRS19	splicing factor, arginine/serine-rich 13B [Source:HGNC Symbol;Acc:21220]
C4orf23	FLJ35725	Probable tRNA (uracil-O(2)-)-methyltransferase (EC 2.1.1.n2) [Source:UniProtKB/Swiss-Prot;Acc:Q8IYL2]
MKI67IP	Nopp34; NIFK	MKI67 (FHA domain) interacting nucleolar phosphoprotein [Source:HGNC Symbol;Acc:17838]
LARP1	LARP; KIAA0731; MGC19556	La ribonucleoprotein domain family, member 1 [Source:HGNC Symbol;Acc:29531]
RBM45	DRB1; FLJ44612	RNA binding motif protein 45 [Source:HGNC Symbol;Acc:24468]
PPARGC1B	PERC; PGC1B	peroxisome proliferator-activated receptor gamma, coactivator 1 beta [Source:HGNC Symbol;Acc:30022]
LSM11	FLJ38273	LSM11, U7 small nuclear RNA associated [Source:HGNC Symbol;Acc:30860]
SFRS15	KIAA1172; DKFZp434E098; SRA4; SCAF4	splicing factor, arginine/serine-rich 15 [Source:HGNC Symbol;Acc:19304]
RBPMS	HERMES	RNA binding protein with multiple splicing [Source:HGNC Symbol;Acc:19097]
ZC3H18	NHN1	zinc finger CCCH-type containing 18 [Source:HGNC Symbol;Acc:25091]
SYNJ1	INPP5G	synaptojanin 1 [Source:HGNC Symbol;Acc:11503]
IGF2BP1	IMP-1; ZBP1	insulin-like growth factor 2 mRNA binding protein 1 [Source:HGNC Symbol;Acc:28866]

TNRC4		trinucleotide repeat containing 4 [Source:HGNC Symbol;Acc:11967]
U2AF1	U2AF35; RNU2AF1; RN	U2 small nuclear RNA auxiliary factor 1 [Source:HGNC Symbol;Acc:12453]
SAFB	HET; SAFB1	scaffold attachment factor B [Source:HGNC Symbol;Acc:10520]
CPSF4	NAR; CPSF30	cleavage and polyadenylation specific factor 4, 30kDa [Source:HGNC Symbol;Acc:2327]
BRUNOL5		bruno-like 5, RNA binding protein (Drosophila) [Source:HGNC Symbol;Acc:14058]
U2AF1L4	MGC33901; U2af26	U2 small nuclear RNA auxiliary factor 1-like 4 [Source:HGNC Symbol;Acc:23020]
SFRS2	SC-35; SC35; PR264; SFRS2A	splicing factor, arginine/serine-rich 2 [Source:HGNC Symbol;Acc:10783]
LARP4	PP13296	La ribonucleoprotein domain family, member 4 [Source:HGNC Symbol;Acc:24320]
RAVER1	KIAA1978	ribonucleoprotein, PTB-binding 1 [Source:HGNC Symbol;Acc:30296]
ELAVL4	PNEM	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D) [Source:HGNC Symbol;Acc:3315]
RAVER2	KIAA1579; FLJ10770	ribonucleoprotein, PTB-binding 2 [Source:HGNC Symbol;Acc:25577]
FUBP1	FBP	far upstream element (FUSE) binding protein 1 [Source:HGNC Symbol;Acc:4004]
RBM15	OTT; OTT1	RNA binding motif protein 15 [Source:HGNC Symbol;Acc:14959]
DHX57	DDX57	DEAH (Asp-Glu-Ala-Asp/His) box polypeptide 57 [Source:HGNC Symbol;Acc:20086]
TDRD10	DKFZp434M202	tudor domain containing 10 [Source:HGNC Symbol;Acc:25316]
TIPARP	DKFZP434J214; DKFZp686N0351; DDF1; PARP7; PARP-7; PARP-1; pART14; RM1	TCDD-inducible poly(ADP-ribose) polymerase [Source:HGNC Symbol;Acc:23696]
RBM47	FLJ20273; NET18	RNA binding motif protein 47 [Source:HGNC Symbol;Acc:30358]
SR140		U2-associated protein SR140 (140 kDa Ser/Arg-rich domain protein) [Source:UniProtKB/Swiss-Prot;Acc:O15042]
TTC14	FLJ00166; KIAA1980	tetratricopeptide repeat domain 14 [Source:HGNC Symbol;Acc:24697]
LSM6	YDR378C	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae) [Source:HGNC Symbol;Acc:17017]

TRA2A	htra-2-alpha; tra2a	transformer 2 alpha homolog (Drosophila) [Source:HGNC Symbol;Acc:16645]
HNRNPK	CSBP; TUNP	microRNA 7-1 [Source:HGNC Symbol;Acc:31638]
ENOX2	APK1; tNOX	ecto-NOX disulfide-thiol exchanger 2 [Source:HGNC Symbol;Acc:2259]
LARP6	acheron; FLJ11196	La ribonucleoprotein domain family, member 6 [Source:HGNC Symbol;Acc:24012]
KIAA0430	LKAP	KIAA0430 [Source:HGNC Symbol;Acc:29562]
RBPMS2		RNA binding protein with multiple splicing 2 [Source:HGNC Symbol;Acc:19098]
SNRPD1	HsT2456; Sm-D1	small nuclear ribonucleoprotein D1 polypeptide 16kDa [Source:HGNC Symbol;Acc:11158]
SAMD14	FLJ36890	sterile alpha motif domain containing 14 [Source:HGNC Symbol;Acc:27312]
		Fox-1 homolog C [Source:UniProtKB/Swiss-Prot;Acc:A6NFN3]
POLR2G	hRPB19; hsRPB7; RPB7	polymerase (RNA) II (DNA directed) polypeptide G [Source:HGNC Symbol;Acc:9194]
SF1	ZFM1	splicing factor 1 [Source:HGNC Symbol;Acc:12950]
HNRNPH1	hnRNPH	heterogeneous nuclear ribonucleoprotein H1 (H) [Source:HGNC Symbol;Acc:5041]
ZRSR2	U2AF1-RS2; URP	zinc finger (CCCH type), RNA-binding motif and serine/arginine rich 2 [Source:HGNC Symbol;Acc:23019]
PCBP1	HNRPE1; hnRNP-E1; HNRPX; hnRNP-X	poly(rC) binding protein 1 [Source:HGNC Symbol;Acc:8647]
RBM1Y1F	MGC33094	RNA binding motif protein, Y-linked, family 1, member F [Source:HGNC Symbol;Acc:23974]
HNRNPF		heterogeneous nuclear ribonucleoprotein F [Source:HGNC Symbol;Acc:5039]
HNRNPA3		heterogeneous nuclear ribonucleoprotein A3 [Source:HGNC Symbol;Acc:24941]
RBMXL2	HNRNPG-T; HNRPGT	RNA binding motif protein, X-linked-like 2 [Source:HGNC Symbol;Acc:17886]
LSM3	YLR438C; SMX4; USS2	LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae) [Source:HGNC Symbol;Acc:17874]
NCBP2L		nuclear cap binding protein subunit 2-like [Source:HGNC Symbol;Acc:31795]
CSDC2	PIPPin	cold shock domain containing C2, RNA binding [Source:HGNC Symbol;Acc:30359]
TAF15	hTAFII68; RBP56; Npl3	TAF15 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 68kDa [Source:HGNC Symbol;Acc:11547]

RBM4B	MGC10871; ZCCHC15; RBM4L; ZCRB3B	RNA binding motif protein 4B [Source:HGNC Symbol;Acc:28842]
RBM4	LARK; RBM4A; ZCRB3A; ZCCHC21	RNA binding motif protein 4 [Source:HGNC Symbol;Acc:9901]
LARP7	HDCMA18P; PIP7S; DKFZP564K112	La ribonucleoprotein domain family, member 7 [Source:HGNC Symbol;Acc:24912]
PABPC5	PABP5	poly(A) binding protein, cytoplasmic 5 [Source:HGNC Symbol;Acc:13629]
LSM1	CASM; YJL124C	LSM1 homolog, U6 small nuclear RNA associated (S. cerevisiae) [Source:HGNC Symbol;Acc:20472]
RBMXL3	FLJ40249	RNA binding motif protein, X-linked-like 3 [Source:HGNC Symbol;Acc:26859]
MEX3C	FLJ38871; RNF194	mex-3 homolog C (C. elegans) [Source:HGNC Symbol;Acc:28040]
RBM44	FLJ40411	RNA binding motif protein 44 [Source:HGNC Symbol;Acc:24756]
CSTF2T	DKFZp434C1013; KIAA0689; CstF- 64T	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kDa, tau variant [Source:HGNC Symbol;Acc:17086]
HNRNPA0	hnRNPA0	heterogeneous nuclear ribonucleoprotein A0 [Source:HGNC Symbol;Acc:5030]
ZC3H12D	dJ281H8.1; MCPIP4	zinc finger CCCH-type containing 12D [Source:HGNC Symbol;Acc:21175]
SAMD4B	FLJ10211; MGC99832; SMGB; hSmaug2	sterile alpha motif domain containing 4B [Source:HGNC Symbol;Acc:25492]
HNRNPCL1		heterogeneous nuclear ribonucleoprotein C-like 1 [Source:HGNC Symbol;Acc:29295]
MKRN3	RNF63; ZFP127; MGC88288	makorin ring finger protein 3 [Source:HGNC Symbol;Acc:7114]
RBM15B	HUMAGCGB; OTT3	RNA binding motif protein 15B [Source:HGNC Symbol;Acc:24303]
PUF60	FIR; SIAHBP1; RoBPI	poly-U binding splicing factor 60KDa [Source:HGNC Symbol;Acc:17042]
TRNAU1AP	SECP43; FLJ20503	tRNA selenocysteine 1 associated protein 1 [Source:HGNC Symbol;Acc:30813]
SFRS2B	SRP46	splicing factor, arginine/serine-rich 2B [Source:HGNC Symbol;Acc:16988]
MEX3D	Tino; KIAA2031; OK/SW-cl.4; RNF193	mex-3 homolog D (C. elegans) [Source:HGNC Symbol;Acc:16734]

LSM10	MGC15749	LSM10, U7 small nuclear RNA associated [Source:HGNC Symbol;Acc:17562]
SNRPE	Sm-E	small nuclear ribonucleoprotein polypeptide E [Source:HGNC Symbol;Acc:11161]
TDRKH	TDRD2	tudor and KH domain containing [Source:HGNC Symbol;Acc:11713]
RBM10	DXS8237E; KIAA0122; GPATC9; ZRANB5; GPATCH9	RNA binding motif protein 10 [Source:HGNC Symbol;Acc:9896]
LENG9		leukocyte receptor cluster (LRC) member 9 [Source:HGNC Symbol;Acc:16306]
EWSR1	EWS	Ewing sarcoma breakpoint region 1 [Source:HGNC Symbol;Acc:3508]
LSMD1	MGC14151; PFAAP2	LSM domain containing 1 [Source:HGNC Symbol;Acc:28212]
DND1	MGC34750; RBMS4	dead end homolog 1 (zebrafish) [Source:HGNC Symbol;Acc:23799]
MEX3B	DKFZp434J0617; RNF195	mex-3 homolog B (C. elegans) [Source:HGNC Symbol;Acc:25297]
PCBP3		poly(rC) binding protein 3 [Source:HGNC Symbol;Acc:8651]
THOC4	ALY; BEF	THO complex 4 [Source:HGNC Symbol;Acc:19071]
RBM12B		RNA binding motif protein 12B [Source:HGNC Symbol;Acc:32310]
SNRNP35	U1SNRNPBP	small nuclear ribonucleoprotein 35kDa (U11/U12) [Source:HGNC Symbol;Acc:30852]
PABPC1L2B		poly(A) binding protein, cytoplasmic 1-like 2B [Source:HGNC Symbol;Acc:31852]
RALYL	HNRPCL3	RALY RNA binding protein-like [Source:HGNC Symbol;Acc:27036]
DDX53	CAGE; CT26	DEAD (Asp-Glu-Ala-Asp) box polypeptide 53 [Source:HGNC Symbol;Acc:20083]
RBM33	DKFZp686F102; MGC20460; DKFZp434D1319	RNA binding motif protein 33 [Source:HGNC Symbol;Acc:27223]
RBM43	FLJ45645	RNA binding motif protein 43 [Source:HGNC Symbol;Acc:24790]
RBM11		RNA binding motif protein 11 [Source:HGNC Symbol;Acc:9897]
ZFP36L1	RNF162B; Berg36; ERF1; TIS11B; cMG1	zinc finger protein 36, C3H type-like 1 [Source:HGNC Symbol;Acc:1107]

YTHDF3	FLJ31657	YTH domain family, member 3 [Source:HGNC Symbol;Acc:26465]
RNPC3	KIAA1839; FLJ20008; RBM40	RNA-binding region (RNP1, RRM) containing 3 [Source:HGNC Symbol;Acc:18666]
PABPC1L2A		poly(A) binding protein, cytoplasmic 1-like 2A [Source:HGNC Symbol;Acc:27989]
DAZ3		deleted in azoospermia 3 [Source:HGNC Symbol;Acc:15965]
RDM1	MGC33977	RAD52 motif 1 [Source:HGNC Symbol;Acc:19950]
LIN28B	FLJ16517; CSDD2	lin-28 homolog B (C. elegans) [Source:HGNC Symbol;Acc:32207]
CPSF4L		cleavage and polyadenylation specific factor 4-like [Source:HGNC Symbol;Acc:33632]
DAZ1	SPGY	deleted in azoospermia 1 [Source:HGNC Symbol;Acc:2682]
ZC3H6	FLJ41410; FLJ45877; KIAA2035	zinc finger CCCH-type containing 6 [Source:HGNC Symbol;Acc:24762]
SFRS13A	TASR1; TASR2; SRp38; SRp40; SFRS13; FUSIP1; FUSIP2	splicing factor, arginine/serine-rich 13A [Source:HGNC Symbol;Acc:16713]
RBM34	KIAA0117	RNA binding motif protein 34 [Source:HGNC Symbol;Acc:28965]
RRP7A	CGI-96	ribosomal RNA processing 7 homolog A (S. cerevisiae) [Source:HGNC Symbol;Acc:24286]
ELAVL3	HUC; PLE21; DKFZp547J036; HUCL; MGC20653	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 3 (Hu antigen C) [Source:HGNC Symbol;Acc:3314]
C14orf21		Pumilio domain-containing protein C14orf21 [Source:UniProtKB/Swiss-Prot;Acc:Q86U38]
PCBP2	HNRPE2; hnRNP-E2	poly(rC) binding protein 2 [Source:HGNC Symbol;Acc:8648]
ZGPAT	dJ583P15.3; MGC44880; FLJ14972; ZC3HDC9; ZC3H9; GPATC6; GPATCH6; ZIP	zinc finger, CCCH-type with G patch domain [Source:HGNC Symbol;Acc:15948]
HNRNPAB	ABBP1; FLJ40338	heterogeneous nuclear ribonucleoprotein A/B [Source:HGNC Symbol;Acc:5034]
NOL8	FLJ20736; Nop132	nucleolar protein 8 [Source:HGNC Symbol;Acc:23387]

HELZ	KIAA0054; HUMORF5; DHRC	helicase with zinc finger [Source:HGNC Symbol;Acc:16878]
YTHDF2	HGRG8; NY- REN-2	YTH domain family, member 2 [Source:HGNC Symbol;Acc:31675]
SFMBT2	KIAA1617	Scm-like with four mbt domains 2 [Source:HGNC Symbol;Acc:20256]
L3MBTL3	KIAA1798	l(3)mbt-like 3 (Drosophila) [Source:HGNC Symbol;Acc:23035]
MEX3A		mex-3 homolog A (C. elegans) [Source:HGNC Symbol;Acc:33482]
RBM20		RNA binding motif protein 20 [Source:HGNC Symbol;Acc:27424]
DPPA5	Esg1	developmental pluripotency associated 5 [Source:HGNC Symbol;Acc:19201]
MIR1236		microRNA 1236 [Source:HGNC Symbol;Acc:33925]
		U6 snRNA-associated Sm-like protein LSm2 (snRNP core Sm-like protein Sm-x5)(Small nuclear ribonuclear protein D homolog)(Protein G7b) [Source:UniProtKB/Swiss-Prot;Acc:Q9Y333]
PPP1R10		Serine/threonine-protein phosphatase 1 regulatory subunit 10 (Phosphatase 1 nuclear targeting subunit)(MHC class I region proline-rich protein CAT53)(FB19 protein)(PP1-binding protein of 114 kDa)(p99) [Source:UniProtKB/Swiss- Prot;Acc:Q96QC0]
PRR3		Proline-rich protein 3 (MHC class I region proline-rich protein CAT56) [Source:UniProtKB/Swiss- Prot;Acc:P79522]
PABPN1L	ePABP2	poly(A) binding protein, nuclear 1-like (cytoplasmic) [Source:HGNC Symbol;Acc:37237]
RNPS1		RNA binding protein S1, serine-rich domain [Source:HGNC Symbol;Acc:10080]
DAZ2	pDP1678; MGC126442	deleted in azoospermia 2 [Source:HGNC Symbol;Acc:15964]
ZRSR1		U2 small nuclear ribonucleoprotein auxiliary factor 35 kDa subunit-related protein 1 (U2(RNU2) small nuclear RNA auxiliary factor 1-like 1)(CCCH type zinc finger, RNA-binding motif and serine/arginine rich protein 1) [Source:UniProtKB/Swiss-Prot;Acc:Q15695]
RBM16	KIAA1116	RNA binding motif protein 16 [Source:HGNC Symbol;Acc:20959]
		Putative uncharacterized protein ENSP00000395989 [Source:UniProtKB/TrEMBL;Acc:C9JFH9]

RBMXL1	KAT3	RNA binding motif protein, X-linked-like 1 [Source:HGNC Symbol;Acc:25073]
CPEB1	FLJ13203; CPEB	cytoplasmic polyadenylation element binding protein 1 [Source:HGNC Symbol;Acc:21744]
		Putative uncharacterized protein ENSP00000388079 Fragment [Source:UniProtKB/TrEMBL;Acc:C9JXI7]
		Putative uncharacterized protein ENSP00000383298 [Source:UniProtKB/TrEMBL;Acc:C9JCD7]
RBMX1J		RNA binding motif protein, Y-linked, family 1, member J [Source:HGNC Symbol;Acc:23917]
SNRPEL1	bA390F4.4	small nuclear ribonucleoprotein polypeptide E-like 1 [Source:HGNC Symbol;Acc:20733]
		Putative uncharacterized protein ENSP00000394145 Fragment [Source:UniProtKB/TrEMBL;Acc:C9JK19]
MCTS1	MCT-1	malignant T cell amplified sequence 1 [Source:HGNC Symbol;Acc:23357]
PABPC4L		poly(A) binding protein, cytoplasmic 4-like [Source:HGNC Symbol;Acc:31955]
RBMX1A1	YRRM1; YRRM2	RNA binding motif protein, Y-linked, family 1, member A1 [Source:HGNC Symbol;Acc:9912]
MKRNP5		makorin ring finger protein pseudogene 5 [Source:HGNC Symbol;Acc:7115]
RBM14	SIP; SYTIP1; COAA; DKFZp779J0927	RNA binding motif protein 14 [Source:HGNC Symbol;Acc:14219]
NSUN6	FLJ23743	NOP2/Sun domain family, member 6 [Source:HGNC Symbol;Acc:23529]
UNKL	ZC3HDC5L; ZC3H5L; FLJ23360	unkempt homolog (Drosophila)-like [Source:HGNC Symbol;Acc:14184]
RBMX1E		RNA binding motif protein, Y-linked, family 1, member E [Source:HGNC Symbol;Acc:23916]
HNRNPA1L2	LOC144983	heterogeneous nuclear ribonucleoprotein A1-like 2 [Source:HGNC Symbol;Acc:27067]
RBMX1B		RNA binding motif protein, Y-linked, family 1, member B [Source:HGNC Symbol;Acc:23914]
RBMX1D		RNA binding motif protein, Y-linked, family 1, member D [Source:HGNC Symbol;Acc:23915]
RBM12	HRIHFB2091; KIAA0765; SWAN	RNA binding motif protein 12 [Source:HGNC Symbol;Acc:9898]
DAZ4		deleted in azoospermia 4 [Source:HGNC Symbol;Acc:15966]
DGKQ	DAGK; DAGK7	diacylglycerol kinase, theta 110kDa [Source:HGNC Symbol;Acc:2856]

RBM17	SPF45; MGC14439	RNA binding motif protein 17 [Source:HGNC Symbol;Acc:16944]
MCM3AP	Map80; KIAA0572; GANP	minichromosome maintenance complex component 3 associated protein [Source:HGNC Symbol;Acc:6946]
PARN	DAN	poly(A)-specific ribonuclease (deadenylation nuclease) [Source:HGNC Symbol;Acc:8609]
TLR2	TIL4; CD282	toll-like receptor 2 [Source:HGNC Symbol;Acc:11848]
PARP10	FLJ14464	poly (ADP-ribose) polymerase family, member 10 [Source:HGNC Symbol;Acc:25895]
MOV10L1	DJ402G11.8; DKFZp434B0717	Mov10l1, Moloney leukemia virus 10-like 1, homolog (mouse) [Source:HGNC Symbol;Acc:7201]
CCAR1	FLJ10590; CARP-1; CARP1	cell division cycle and apoptosis regulator 1 [Source:HGNC Symbol;Acc:24236]
LEMD3	MAN1	LEM domain containing 3 [Source:HGNC Symbol;Acc:28887]
UPF3B	RENT3B; UPF3X; HUPF3B	UPF3 regulator of nonsense transcripts homolog B (yeast) [Source:HGNC Symbol;Acc:20439]
RCAN2	ZAKI-4	regulator of calcineurin 2 [Source:HGNC Symbol;Acc:3041]
SRRT	Asr2; serrate; ARS2	serrate RNA effector molecule homolog (Arabidopsis) [Source:HGNC Symbol;Acc:24101]
ZC3H12A	FLJ23231; MCP1P1	zinc finger CCCH-type containing 12A [Source:HGNC Symbol;Acc:26259]
ZC3HAV1L	MGC14289	zinc finger CCCH-type, antiviral 1-like [Source:HGNC Symbol;Acc:22423]
PARP14	KIAA1268; pART8	poly (ADP-ribose) polymerase family, member 14 [Source:HGNC Symbol;Acc:29232]
TMEM63A		transmembrane protein 63A [Source:HGNC Symbol;Acc:29118]
SLBP	HBP	stem-loop binding protein [Source:HGNC Symbol;Acc:10904]
GTF3A		general transcription factor IIIA [Source:HGNC Symbol;Acc:4662]
NUFIP1		nuclear fragile X mental retardation protein interacting protein 1 [Source:HGNC Symbol;Acc:8057]
SRRM2		serine/arginine repetitive matrix 2 [Source:HGNC Symbol;Acc:16639]
APTX		aprtaxin [Source:HGNC Symbol;Acc:15984]
ZFR		zinc finger RNA binding protein [Source:HGNC Symbol;Acc:17277]
UPF1		UPF1 regulator of nonsense transcripts homolog (yeast) [Source:HGNC Symbol;Acc:9962]
KIN		KIN, antigenic determinant of recA protein homolog (mouse) [Source:HGNC Symbol;Acc:6327]

ZNF239		zinc finger protein 239 [Source:HGNC Symbol;Acc:13031]
ZNF74		zinc finger protein 74 [Source:HGNC Symbol;Acc:13144]
SNRPC		small nuclear ribonucleoprotein polypeptide C [Source:HGNC Symbol;Acc:11157]
C1orf25		TRM1-like protein [Source:UniProtKB/Swiss-Prot;Acc:Q7Z2T5]
ZFR2		zinc finger RNA binding protein 2 [Source:HGNC Symbol;Acc:29189]
IREB2	ACO3; IRP2; IRP2AD; FLJ23381; IREB2	iron-responsive element binding protein 2 [Source:HGNC Symbol;Acc:6115]
ACO1	IRP1; ACONS; IREB1; IREBP; IREBP1; ACO1	aconitase 1, soluble [Source:HGNC Symbol;Acc:117]
TROVE2	RO60; SSA2; RORNP;	TROVE domain family, member 2 [Source:HGNC Symbol;Acc:11313]
TEP1	p240; TLP1; TP1; TROVE1; VAULT2	telomerase-associated protein 1 [Source:HGNC Symbol;Acc:11726]
GAPDH	GAPD	glyceraldehyde-3-phosphate dehydrogenase [Source:HGNC Symbol;Acc:4141]
ZRANB2		zinc finger, RAN-binding domain containing 2 [Source:HGNC Symbol;Acc:13058]

- [0097]** The RNA regulatory domain may be a protein selected from Table 1 or Table 2 or a functional domain from a protein selected from the list of proteins in Table 1 or 2. In some embodiments, the RNA regulatory domain comprises a fragment from a protein selected from the list of proteins in Table 1 or 2. In some embodiments, the RNA regulatory domain comprises a protein having at least, at most, or exactly 100, 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 89, 88, 87, 86, 85, 84, 83, 82, 81, 80, 79, 78, 77, 76, 75, 74, 73, 72, 71, 70, 69, 68, 67, 66, 65, 64, 63, 62, 61, or 60% (or any derivable range therein) homology or sequence identity to a protein of Table 1 or 2 or a fragment of a protein from Table 1 or 2.
- [0098]** In some embodiments, the RNA regulatory domain comprises at least, at most, or exactly 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, or 2000 contiguous amino acids (or any derivable range therein) of a protein from Table 1 or Table 2.

[0099] In some embodiments, the RNA regulatory domain comprises a fragment of a protein from Table 1 or 2, wherein the fragment has one or more of the following activities: methylation, 5'-3' guanylation, phosphoribosylation, deamination, carbamoylation, isopentenylation, agmatinylation, acetylation, lysylation, O/S exchange, galactosylation, glutamylation, mannosylation, hydrogenation, pseudouridine formation, carboxymethylaminomethylation, aminomethylation, decarboxymethylation, dehydrogenation, carboxymethylation, hydroxylation, methylthiolation, 3-amino-3-carboxypropylation, demethylation, 5'-5' guanylation, dephosphorylation, nuclease, editing, RNA transport, translational activation, translational repression, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, and RNA binding activity.

[0100] In some embodiments, the RNA regulatory domain is at or near the carboxy-terminus of the RNA hairpin binding protein. In some embodiments, the RNA regulatory domain is at or near the amino-terminus of the RNA hairpin binding protein. In some embodiments, the RNA regulatory domain is fused by way of a peptide bond to the RNA hairpin binding protein. In some embodiments, the RNA regulatory domain is linked to the RNA hairpin binding protein by a linker moiety.

II. RNA HAIRPIN BINDING DOMAINS AND HAIRPIN STRUCTURES

[0101] Other RNA hairpin binding domains and hairpin structures that they bind are known in the art and can be used in the systems, compositions, fusion proteins, kits, vectors, and methods of the disclosure. For example, embodiments include a RNA hairpin binding domain and hairpin structure according to the following table (Table 3), which lists proteins comprising RNA hairpin binding domains and the hairpin structure that they specifically bind to:

Table 3: RNA hairpin binding domain

RNA hairpin binding domain	Hairpin structure
MS2 coat protein (MCP) (bacteriophage)	AAACAUGAGGAUUACCCAUGU (SEQ ID NO:107) AAACAUGAGGAUCACCCAUGU (SEQ ID NO:108)
λ N (bacteriophage)	GCCCUGAAGAAGGGC (SEQ ID NO:109); GCCUGAAAAAGGGC (SEQ ID NO:110)
PP7 (Pseudomonas aeruginosa)	UAAGGAGUUUAUAUGGAAACCCUUA (SEQ ID NO:111)
Iron Responsive Protein (IRP)	CAGWGH wherein W is A or U and H is A, C, or U; NNNNNUGCNNNNNCAGUGNNNNNNNCNNNNN, wherein n is any nucleotide

Q β	AAACAUGAGGAUUACCCAUGU (SEQ ID NO:107); AAACAUGAGGAUCACCCAUGU (SEQ ID NO:108); AUGCAUGUCUAAGACAGCAU (SEQ ID NO:114)
GA	AAACAUGAGGAUUACCCAUGU (SEQ ID NO:107); AAACAUGAGGAUCACCCAUGU (SEQ ID NO:108); AAAACAUAAGGAAAACCUAUGUU (SEQ ID NO:115)
Bovine immunodeficiency virus (BIV) transactivator of transcription (tat)	GGCUCGUGUAGCUCAUUAGCUCCGAGCC (SEQ ID NO:112)
U1A (HUMAN)	AUUGCAC; GGAAUCCAUUGCACUCCGGAUUUUCTAG (SEQ ID NO:113); GGCCAGAUCUGAGCCUGGGAGCUCUCUGGCC (SEQ ID NO:1)
SLBP (HUMAN)	CCAAAGGCUCUUCUCAGAGCCACCCA (SEQ ID NO:2)
KU70 (HUMAN)	GGCGUCCCUCCCGAAGCUGCGCGCUCGGUCGAACAGGACGACC (SEQ ID NO:83)
NUCLEOLIN (HUMAN)	UCCCGA (SEQ ID NO:84); GGCCGAAAUCCCGAAUGAGGCC (SEQ ID NO:85); GGAUGCCUCCCGAGUGCAUCC (SEQ ID NO:86).

[0102] It is contemplated that multiple RNA hairpin binding domains and/or the RNA regulatory domain may be used in a multiplexed fashion by using RNA hairpin binding domains that bind to different hairpin structures to target multiple different RNAs in the same cell. The different RNAs may be modulated in the same or in different ways. For example, one RNA may be modulation with translational activation, while a second RNA may be modulated with translational repression in the same cell. Therefore, the systems of the disclosure can be used in a multiplexed fashion for the modulation of at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more RNAs in one cell, tissue, or organisms.

10 III. NUCLEIC ACIDS

[0103] In certain embodiments, there are recombinant nucleic acids encoding the proteins, polypeptides, regulatory domains, or RNA targeting molecules described herein.

[0104] As used in this application, the term “polynucleotide” refers to a nucleic acid molecule that either is recombinant or has been isolated free of total genomic nucleic acid.

- 15 Included within the term “polynucleotide” are oligonucleotides (nucleic acids 100 residues or fewer in length), recombinant vectors, including, for example, plasmids, cosmids, phage, viruses, and the like. Polynucleotides include, in certain aspects, regulatory sequences, isolated substantially away from their naturally occurring genes or protein encoding sequences. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be

RNA, DNA (genomic, cDNA or synthetic), analogs thereof, or a combination thereof. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide.

5 [0105] In this respect, the term “gene,” “polynucleotide,” or “nucleic acid” is used to refer to a nucleic acid that encodes a protein, polypeptide, or peptide (including any sequences required for proper transcription, post-translational modification, or localization). As will be understood by those in the art, this term encompasses genomic sequences, expression cassettes, cDNA sequences, and smaller engineered nucleic acid segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. A
10 nucleic acid encoding all or part of a polypeptide may contain a contiguous nucleic acid sequence encoding all or a portion of such a polypeptide. It also is contemplated that a particular polypeptide may be encoded by nucleic acids containing variations having slightly different nucleic acid sequences but, nonetheless, encode the same or substantially similar protein (see above).

15 [0106] In particular embodiments, there are isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a polypeptides (e.g., a polymerase, RNA polymerase, one or more truncated polymerase domains or interaction components that are polypeptides) that drive gene transcription dependent on polymerase activity from the polymerase domains when the interaction components interact. The term “recombinant” may
20 be used in conjunction with a polypeptide or the name of a specific polypeptide, and this generally refers to a polypeptide produced from a nucleic acid molecule that has been manipulated *in vitro* or that is a replication product of such a molecule.

[0107] The nucleic acid segments, regardless of the length of the coding sequence itself, may be combined with other nucleic acid sequences, such as promoters, polyadenylation
25 signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant nucleic acid protocol. In some cases, a nucleic acid sequence may encode a polypeptide sequence with
30 additional heterologous coding sequences, for example to allow for purification of the polypeptide, transport, secretion, post-translational modification, or for therapeutic benefits such as targeting or efficacy. As discussed above, a tag or other heterologous polypeptide may be added to the modified polypeptide-encoding sequence, wherein “heterologous” refers to a polypeptide that is not the same as the modified polypeptide.

[0108] In certain embodiments, there are polynucleotide variants having substantial identity to the sequences disclosed herein; those comprising at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher sequence identity, including all values and ranges there between, compared to a polynucleotide sequence provided herein using the methods described
5 herein (e.g., BLAST analysis using standard parameters). In certain aspects, the isolated polynucleotide will comprise a nucleotide sequence encoding a polypeptide that has at least 90%, preferably 95% and above, identity to an amino acid sequence described herein, over the entire length of the sequence; or a nucleotide sequence complementary to said isolated polynucleotide.

10 **A. Vectors**

[0109] Polypeptides may be encoded by a nucleic acid molecule. The nucleic acid molecule can be in the form of a nucleic acid vector. The term “vector” is used to refer to a carrier nucleic acid molecule into which a heterologous nucleic acid sequence can be inserted for introduction into a cell where it can be replicated and expressed. A nucleic acid sequence can
15 be “heterologous,” which means that it is in a context foreign to the cell in which the vector is being introduced or to the nucleic acid in which is incorporated, which includes a sequence homologous to a sequence in the cell or nucleic acid but in a position within the host cell or nucleic acid where it is ordinarily not found. Vectors include DNAs, RNAs, plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g.,
20 YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (for example Sambrook et al., 2001; Ausubel et al., 1996, both incorporated herein by reference). Vectors may be used in a host cell to produce a polymerase, RNA polymerase, one or more truncated polymerase domains or interaction components that are fused, attached or linked to the one or more truncated RNA polymerase domains.

[0110] The term “expression vector” refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. Expression vectors can contain a variety of “control sequences,” which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular
25 host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described herein.
30

B. Cells

[0111] The disclosure provides methods for modifying a target RNA of interest, in particular in prokaryotic cells, eukaryotic cells, tissues, organs, or organisms, more in particular in mammalian cells, tissues, organs, or organisms. The target RNA may be comprised in a nucleic acid molecule within a cell. In some embodiments, the target RNA is in a eukaryotic cell, such as a mammalian cell or a plant cell. The mammalian cell may be a human, non-human primate, bovine, porcine, rodent or mouse cell. The cell may be a non-mammalian eukaryotic cell such as poultry, fish or shrimp. The plant cell may be of a crop plant such as cassava, corn, sorghum, wheat, or rice. The plant cell may also be of an algae, tree or vegetable. The modulation of the RNA induced in the cell by the methods, systems, and compositions of the disclosure may be such that the cell and progeny of the cell are altered for improved production of biologic products such as an antibody, starch, alcohol or other desired cellular output. The modulation of the RNA induced in the cell may be such that the cell and progeny of the cell include an alteration that changes the biologic product produced.

[0112] The mammalian cell may be a human or non-human mammal, e.g., primate, bovine, ovine, porcine, canine, rodent, Leporidae such as monkey, cow, sheep, pig, dog, rabbit, rat or mouse cell. The cell may be a non-mammalian eukaryotic cell such as poultry bird (e.g., chicken), vertebrate fish (e.g., salmon) or shellfish (e.g., oyster, clam, lobster, shrimp) cell. The cell may also be a plant cell. The plant cell may be of a monocot or dicot or of a crop or grain plant such as cassava, corn, sorghum, soybean, wheat, oat or rice. The plant cell may also be of an algae, tree or production plant, fruit or vegetable (e.g., trees such as citrus trees, e.g., orange, grapefruit or lemon trees; peach or nectarine trees; apple or pear trees; nut trees such as almond or walnut or pistachio trees; nightshade plants; plants of the genus Brassica; plants of the genus Lactuca; plants of the genus Spinacia; plants of the genus Capsicum; cotton, tobacco, asparagus, carrot, cabbage, broccoli, cauliflower, tomato, eggplant, pepper, lettuce, spinach, strawberry, blueberry, raspberry, blackberry, grape, coffee, cocoa, etc.).

[0113] As used herein, the terms “cell,” “cell line,” and “cell culture” may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, “host cell” refers to a prokaryotic or eukaryotic cell, and it includes any transformable organism that is capable of replicating a vector or expressing a heterologous gene encoded by a vector. A host cell can, and has been, used as a recipient for vectors or viruses. A host cell may be

“transfected” or “transformed,” which refers to a process by which exogenous nucleic acid, such as a recombinant protein-encoding sequence, is transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny.

[0114] Some vectors may employ control sequences that allow it to be replicated and/or expressed in both prokaryotic and eukaryotic cells. One of skill in the art would further understand the conditions under which to incubate all of the above described host cells to maintain them and to permit replication of a vector. Also understood and known are techniques and conditions that would allow large-scale production of vectors, as well as production of the nucleic acids encoded by vectors and their cognate polypeptides, proteins, or peptides.

C. Expression Systems

[0115] Numerous expression systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with an embodiment to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. For example, the vectors, fusion proteins, RNA hairpin binding proteins, RNA targeting molecules, RNA regulatory domain, and accessory proteins of the disclosure may utilize an expression system, such as an inducible or constitutive expression system. Many such systems are commercially and widely available.

[0116] The insect cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Patents 5,871,986, 4,879,236, both herein incorporated by reference, and which can be bought, for example, under the name MAXBAC® 2.0 from INVITROGEN® and BACPACK™ BACULOVIRUS EXPRESSION SYSTEM FROM CLONTECH®.

[0117] In addition to the disclosed expression systems, other examples of expression systems include STRATAGENE®'s COMPLETE CONTROL Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible receptor, or its pET Expression System, an E. coli expression system. Another example of an inducible expression system is available from INVITROGEN®, which carries the T-REX™ (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. INVITROGEN® also provides a yeast expression system called the *Pichia methanolica* Expression System, which is designed for high-level production of recombinant proteins in the methylotrophic yeast *Pichia methanolica*. One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

D. Conjugation of Nucleic Acids and Polypeptides

[0118] Embodiments of the disclosure relate to the conjugation of nucleic acids to polypeptides. Methods of conjugation of nucleic acids to polypeptides are known in the art and include those described below. Embodiments of the disclosure relate to methods of making
5 nucleic acid-polypeptide molecules and the molecules themselves wherein the nucleic acid has been conjugated to the polypeptide by way of a method described herein. One such example includes click chemistry. The "click reaction", also known as "click chemistry" is a name often used to describe a stepwise variant of the Huisgen 1,3-dipolar cycloaddition of azides and alkynes to yield 1,2,3-triazole. This reaction is carried out under ambient conditions, or under
10 mild microwave irradiation, typically in the presence of a Cu(I) catalyst, and with exclusive regioselectivity for the 1,4-disubstituted triazole product when mediated by catalytic amounts of Cu(I) salts [V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed.* 2002, 41, 2596; H. C. Kolb, M. Finn, K. B. Sharpless, *Angew Chem., Int. Ed.* 2001, 40, 2004].

15 [0119] In other conjugation methods, a mutant form of the human DNA repair protein O6-alkylguanine-DNA alkyltransferase reacts rapidly and specifically with O6-benzylguanine (BG) and also with derivatives that carry a large moiety linked to the benzyl group. With guanine as the leaving group, the benzyl moiety becomes covalently attached to a cysteine in the active site of the enzyme. The enzyme has also been mutagenized to become specific for O6-benzylcytosine (BC) in a similar manner. These enzyme domains (about 20 kDa) are
20 commercially available as SNAP and CLIP tags, respectively.

[0120] A further conjugation method utilizes the Halo tag. The Halo tag makes use of a chemical reaction orthogonal to eukaryotes, i.e. the dehalogenation of haloalkane ligands, thus, leading to highly specific covalent labelling of the tag, and therefore protein, in both live and
25 fixed cells.

E. Nucleic Acid Modifications

[0121] The oligonucleotides of the disclosure, such as the RNA targeting molecules and other nucleic acids described herein may have modifications that increase the stability of the nucleic acid. In some embodiments, the RNA targeting molecule is an oligonucleotide analog.
30 The term "oligonucleotide analog" refers to compounds which function like oligonucleotides but which have non-naturally occurring portions. Oligonucleotide analogs can have altered sugar moieties, altered base moieties or altered inter-sugar linkages. The term "oligomers" is intended to encompass oligonucleotides, oligonucleotide analogs or oligonucleosides. Thus,

in speaking of “oligomers” reference is made to a series of nucleosides or nucleoside analogs that are joined via either natural phosphodiester bonds or other linkages, including the four atom linkers. Although the linkage generally is from the 3' carbon of one nucleoside to the 5' carbon of a second nucleoside, the term “oligomer” can also include other linkages such as 2'-5' linkages.

[0122] Oligonucleotide analogs also can include other modifications, particularly modifications that increase nuclease resistance, improve binding affinity, and/or improve binding specificity. For example, when the sugar portion of a nucleoside or nucleotide is replaced by a carbocyclic moiety, it is no longer a sugar. Moreover, when other substitutions, such a substitution for the inter-sugar phosphodiester linkage are made, the resulting material is no longer a true nucleic acid species. All such compounds are considered to be analogs. Throughout this specification, reference to the sugar portion of a nucleic acid species shall be understood to refer to either a true sugar or to a species taking the structural place of the sugar of wild type nucleic acids. Moreover, reference to inter-sugar linkages shall be taken to include moieties serving to join the sugar or sugar analog portions in the fashion of wild type nucleic acids.

[0123] The present disclosure concerns modified oligonucleotides, i.e., oligonucleotide analogs or oligonucleosides, and methods for effecting the modifications. These modified oligonucleotides and oligonucleotide analogs may exhibit increased chemical and/or enzymatic stability relative to their naturally occurring counterparts. Extracellular and intracellular nucleases generally do not recognize and therefore do not bind to the backbone-modified compounds. When present as the protonated acid form, the lack of a negatively charged backbone may facilitate cellular penetration.

[0124] The modified internucleoside linkages are intended to replace naturally-occurring phosphodiester-5'-methylene linkages with four atom linking groups to confer nuclease resistance and enhanced cellular uptake to the resulting compound. Preferred linkages have structure $\text{CH}_2\text{--RA--NR1--CH}_2$, $\text{CH}_2\text{--NR1--RA--CH}_2$, $\text{RA--NR1--CH}_2\text{--CH}_2$, $\text{CH}_2\text{--CH}_2\text{--NR1--RA}$, $\text{CH}_2\text{--CH}_2\text{--RA--NR1}$, or $\text{NR1--RA--CH}_2\text{--CH}_2$ where RA is O or NR2.

[0125] Modifications may be achieved using solid supports which may be manually manipulated or used in conjunction with a DNA synthesizer using methodology commonly known to those skilled in DNA synthesizer art. Generally, the procedure involves functionalizing the sugar moieties of two nucleosides which will be adjacent to one another in the selected sequence. In a 5' to 3' sense, an “upstream” synthon is modified at its terminal 3' site, while a “downstream” synthon is modified at its terminal 5' site.

[0126] Oligonucleosides linked by hydrazines, hydroxylamines, and other linking groups can be protected by a dimethoxytrityl group at the 5'-hydroxyl and activated for coupling at the 3'-hydroxyl with cyanoethyldiisopropyl-phosphite moieties. These compounds can be inserted into any desired sequence by standard, solid phase, automated DNA synthesis techniques. One of the most popular processes is the phosphoramidite technique. Oligonucleotides containing a uniform backbone linkage can be synthesized by use of CPG-solid support and standard nucleic acid synthesizing machines such as Applied Biosystems Inc. 380B and 394 and Milligen/Biosearch 7500 and 8800s. The initial nucleotide (number 1 at the 3'-terminus) is attached to a solid support such as controlled pore glass. In sequence specific order, each new nucleotide is attached either by manual manipulation or by the automated synthesizer system.

[0127] Free amino groups can be alkylated with, for example, acetone and sodium cyanoboro hydride in acetic acid. The alkylation step can be used to introduce other, useful, functional molecules on the macromolecule. Such useful functional molecules include but are not limited to reporter molecules, RNA cleaving groups, groups for improving the pharmacokinetic properties of an oligonucleotide, and groups for improving the pharmacodynamic properties of an oligonucleotide. Such molecules can be attached to or conjugated to the macromolecule via attachment to the nitrogen atom in the backbone linkage. Alternatively, such molecules can be attached to pendent groups extending from a hydroxyl group of the sugar moiety of one or more of the nucleotides. Examples of such other useful functional groups are provided by WO1993007883, which is herein incorporated by reference, and in other of the above-referenced patent applications.

[0128] Solid supports may include any of those known in the art for polynucleotide synthesis, including controlled pore glass (CPG), oxalyl controlled pore glass [53], TentaGel Support—an aminopolyethyleneglycol derivatized support [54] or Poros—a copolymer of polystyrene/divinylbenzene. Attachment and cleavage of nucleotides and oligonucleotides can be effected via standard procedures [55]. As used herein, the term solid support further includes any linkers (e.g., long chain alkyl amines and succinyl residues) used to bind a growing oligonucleoside to a stationary phase such as CPG.

1. Locked Nucleotides

[0129] In some embodiments, the nucleic acid of the disclosure, such as the RNA targeting molecule comprises a locked nucleic acid. A locked nucleic acid (LNA or Ln), also referred to as inaccessible RNA, is a modified RNA nucleotide. The ribose moiety of an LNA

nucleotide is modified with an extra bridge connecting the 2' oxygen and 4' carbon. The bridge "locks" the ribose in the 3'-endo (North) conformation, which is often found in the A-form duplexes. LNA nucleotides can be mixed with DNA or RNA residues in the oligonucleotide whenever desired and hybridize with DNA or RNA according to Watson-Crick base-pairing rules. Such oligomers are synthesized chemically and are commercially available. The locked ribose conformation enhances base stacking and backbone pre-organization. This significantly increases the hybridization properties (melting temperature) of oligonucleotides.

2. Ethylene Bridged Nucleotides

[0130] In some embodiments, the nucleic acid of the disclosure, such as the RNA targeting molecule comprises one or more ethylene bridged nucleotides. Ethylene-bridged nucleic acids (ENA or En) are modified nucleotides with a 2'-O, 4'C ethylene linkage. Like locked nucleotides, these nucleotides also restrict the sugar puckering to the N-conformation of RNA.

3. Peptide Nucleic Acids

[0131] In some embodiments, the nucleic acid of the disclosure, such as the RNA targeting molecule comprises one or more peptide nucleic acids. Peptide nucleic acids (PNA or Pn) mimic the behavior of DNA and binds complementary nucleic acid strands. The term, "peptide," when used herein may also refer to a peptide nucleic acid. PNA is an artificially synthesized polymer similar to DNA or RNA. DNA and RNA have a deoxyribose and ribose sugar backbone, respectively, whereas PNA's backbone is composed of repeating N-(2-aminoethyl)-glycine units linked by peptide bonds. The various purine and pyrimidine bases are linked to the backbone by a methylene bridge (-CH₂-) and a carbonyl group (-(C=O)-). PNAs are depicted like peptides, with the N-terminus at the first (left) position and the C-terminus at the last (right) position.

[0132] Since the backbone of PNAs contains no charged phosphate groups, the binding between PNA/DNA strands is stronger than between DNA/DNA strands due to the lack of electrostatic repulsion. PNAs are not easily recognized by either nucleases or proteases, making them resistant to degradation by enzymes. PNAs are also stable over a wide pH range. In some aspects, the PNAs described herein have improved cytosolic delivery over other PNAs.

4. 5'(E)-vinyl-phosphonate (VP) modification

[0133] In some embodiments, the nucleic acid of the disclosure, such as the RNA targeting molecule comprises one or more 5'(E)-vinyl-phosphonate (VP) modifications. 5'-Vinyl-phosphonate modifications (metabolically stable phosphate mimics) have been reported to enhance the metabolic stability and the potency of oligonucleotides.

5. Morpholinos

[0134] In some embodiments, the nucleic acid of the disclosure, such as the RNA targeting molecule comprises a morpholino. Morpholinos are synthetic molecules that are the product of a redesign of natural nucleic acid structure. Usually 25 bases in length, they bind to complementary sequences of RNA or single-stranded DNA by standard nucleic acid base-pairing. In terms of structure, the difference between Morpholinos and DNA is that, while Morpholinos have standard nucleic acid bases, those bases are bound to methylenemorpholine rings linked through phosphorodiamidate groups instead of phosphates. The figure compares the structures of the two strands depicted there, one of RNA and the other of a Morpholino. Replacement of anionic phosphates with the uncharged phosphorodiamidate groups eliminates ionization in the usual physiological pH range, so Morpholinos in organisms or cells are uncharged molecules. The entire backbone of a Morpholino is made from these modified subunits.

IV. Delivery Vehicles

[0135] The current disclosure contemplates several delivery systems compatible with nucleic acids that provide for roughly uniform distribution and have controllable rates of release. A variety of different media are described below that are useful in creating nucleic acid delivery systems. It is not intended that any one medium or carrier is limiting to the present invention. Note that any medium or carrier may be combined with another medium or carrier; for example, in one embodiment a polymer microparticle carrier attached to a compound may be combined with a gel medium.

[0136] Carriers or mediums contemplated by this disclosure comprise a material selected from the group comprising gelatin, collagen, cellulose esters, dextran sulfate, pentosan polysulfate, chitin, saccharides, albumin, fibrin sealants, synthetic polyvinyl pyrrolidone, polyethylene oxide, polypropylene oxide, block polymers of polyethylene oxide and polypropylene oxide, polyethylene glycol, acrylates, acrylamides, methacrylates including, but not limited to, 2-hydroxyethyl methacrylate, poly(ortho esters), cyanoacrylates, gelatin-resorcinol-aldehyde type bioadhesives, polyacrylic acid and copolymers and block copolymers thereof.

30 A. Microparticles

[0137] Some embodiments of the present disclosure contemplate a delivery system comprising a microparticle. Preferably, microparticles comprise liposomes, nanoparticles, microspheres, nanospheres, microcapsules, and nanocapsules. Preferably, some microparticles

contemplated by the present invention comprise poly(lactide-co-glycolide), aliphatic polyesters including, but not limited to, poly-glycolic acid and poly-lactic acid, hyaluronic acid, modified polysaccharides, chitosan, cellulose, dextran, polyurethanes, polyacrylic acids, pseudo-poly(amino acids), polyhydroxybutyrate-related copolymers, polyanhydrides, 5 polymethylmethacrylate, poly(ethylene oxide), lecithin and phospholipids.

B. Liposomes

[0138] One embodiment of the disclosure contemplates liposomes capable of attaching and releasing nucleic acids conjugates, polypeptides, and fusion proteins as described herein. Liposomes are microscopic spherical lipid bilayers surrounding an aqueous core that are made 10 from amphiphilic molecules such as phospholipids. For example, a liposome may trap a nucleic acid between the hydrophobic tails of the phospholipid micelle. Water soluble agents can be entrapped in the core and lipid-soluble agents can be dissolved in the shell-like bilayer. Liposomes have a special characteristic in that they enable water soluble and water insoluble chemicals to be used together in a medium without the use of surfactants or other emulsifiers. 15 Liposomes can form spontaneously by forcefully mixing phospholipids in aqueous media. Water soluble compounds are dissolved in an aqueous solution capable of hydrating phospholipids. Upon formation of the liposomes, therefore, these compounds are trapped within the aqueous liposomal center. The liposome wall, being a phospholipid membrane, holds fat soluble materials such as oils. Liposomes provide controlled release of incorporated 20 compounds. In addition, liposomes can be coated with water soluble polymers, such as polyethylene glycol to increase the pharmacokinetic half-life. One embodiment of the present invention contemplates an ultra high-shear technology to refine liposome production, resulting in stable, unilamellar (single layer) liposomes having specifically designed structural characteristics. These unique properties of liposomes allow the simultaneous storage of 25 normally immiscible compounds and the capability of their controlled release.

[0139] In some embodiments, the disclosure contemplates cationic and anionic liposomes, as well as liposomes having neutral lipids. Preferably, cationic liposomes comprise negatively-charged materials by mixing the materials and fatty acid liposomal components and allowing them to charge-associate. Clearly, the choice of a cationic or anionic liposome depends upon 30 the desired pH of the final liposome mixture. Examples of cationic liposomes include lipofectin, lipofectamine, and lipofectace.

[0140] One embodiment of the present disclosure contemplates a delivery system comprising liposomes that provides controlled release of at least one molecule described

herein. Preferably, liposomes that are capable of controlled release: i) are biodegradable and non-toxic; ii) carry both water and oil soluble compounds; iii) solubilize recalcitrant compounds; iv) prevent compound oxidation; v) promote protein stabilization; vi) control hydration; vii) control compound release by variations in bilayer composition such as, but not limited to, fatty acid chain length, fatty acid lipid composition, relative amounts of saturated and unsaturated fatty acids, and physical configuration; viii) have solvent dependency; ix) have pH-dependency and x) have temperature dependency.

[0141] The compositions of liposomes are broadly categorized into two classifications. Conventional liposomes are generally mixtures of stabilized natural lecithin (PC) that may comprise synthetic identical-chain phospholipids that may or may not contain glycolipids. Special liposomes may comprise: i) bipolar fatty acids; ii) the ability to attach antibodies for tissue-targeted therapies; iii) coated with materials such as, but not limited to lipoprotein and carbohydrate; iv) multiple encapsulation and v) emulsion compatibility.

[0142] Liposomes may be easily made in the laboratory by methods such as, but not limited to, sonication and vibration. Alternatively, compound-delivery liposomes are commercially available. For example, Collaborative Laboratories, Inc. are known to manufacture custom designed liposomes for specific delivery requirements.

C. Microspheres, Microparticles and Microcapsules

[0143] Microspheres and microcapsules are useful due to their ability to maintain a generally uniform distribution, provide stable controlled compound release and are economical to produce and dispense. Preferably, an associated delivery gel or the compound-impregnated gel is clear or, alternatively, said gel is colored for easy visualization by medical personnel.

[0144] Microspheres are obtainable commercially (Prolease™, Alkermes: Cambridge, Mass.). For example, a freeze dried medium comprising at least one therapeutic agent is homogenized in a suitable solvent and sprayed to manufacture microspheres in the range of 20 to 90 μm. Techniques are then followed that maintain sustained release integrity during phases of purification, encapsulation and storage. Scott et al., Improving Protein Therapeutics With Sustained Release Formulations, Nature Biotechnology, Volume 16:153-157 (1998). Modification of the microsphere composition by the use of biodegradable polymers can provide an ability to control the rate of nucleic acid release. Miller et al., Degradation Rates of Oral Resorbable Implants {Polylactates and Polyglycolates: Rate Modification and Changes in PLA/PGA Copolymer Ratios, J. Biomed. Mater. Res., Vol. 11:711-719 (1977).

[0145] Alternatively, a sustained or controlled release microsphere preparation is prepared using an in-water drying method, where an organic solvent solution of a biodegradable polymer metal salt is first prepared. Subsequently, a dissolved or dispersed medium of a nucleic acid is added to the biodegradable polymer metal salt solution. The weight ratio of a nucleic acid to the biodegradable polymer metal salt may for example be about 1:100000 to about 1:1, preferably about 1:20000 to about 1:500 and more preferably about 1:10000 to about 1:500. Next, the organic solvent solution containing the biodegradable polymer metal salt and nucleic acid is poured into an aqueous phase to prepare an oil/water emulsion. The solvent in the oil phase is then evaporated off to provide microspheres. Finally, these microspheres are then recovered, washed and lyophilized. Thereafter, the microspheres may be heated under reduced pressure to remove the residual water and organic solvent.

[0146] Other methods useful in producing microspheres that are compatible with a biodegradable polymer metal salt and nucleic acid mixture are: i) phase separation during a gradual addition of a coacervating agent; ii) an in-water drying method or phase separation method, where an antiflocculant is added to prevent particle agglomeration and iii) by a spray-drying method.

[0147] In one embodiment, the present invention contemplates a medium comprising a microsphere or microcapsule capable of delivering a controlled release of a nucleic acid for a duration of approximately between 1 day and 6 months. In one embodiment, the microsphere or microparticle may be colored to allow the medical practitioner the ability to see the medium clearly as it is dispensed. In another embodiment, the microsphere or microcapsule may be clear. In another embodiment, the microsphere or microparticle is impregnated with a radio-opaque fluoroscopic dye.

[0148] Controlled release microcapsules may be produced by using known encapsulation techniques such as centrifugal extrusion, pan coating and air suspension. Such microspheres and/or microcapsules can be engineered to achieve desired release rates. For example, Oliosphere™ (Macromed) is a controlled release microsphere system. These particular microsphere's are available in uniform sizes ranging between 5-500 µm and composed of biocompatible and biodegradable polymers. Specific polymer compositions of a microsphere can control the nucleic acid release rate such that custom-designed microspheres are possible, including effective management of the burst effect. ProMaxx™ (Epic Therapeutics, Inc.) is a protein-matrix delivery system. The system is aqueous in nature and is adaptable to standard pharmaceutical delivery models. In particular, ProMaxx™ are bioerodible protein

microspheres that deliver both small and macromolecular drugs, and may be customized regarding both microsphere size and desired release characteristics.

[0149] In one embodiment, a microsphere or microparticle comprises a pH sensitive encapsulation material that is stable at a pH less than the pH of the internal mesentery. The typical range in the internal mesentery is pH 7.6 to pH 7.2. Consequently, the microcapsules should be maintained at a pH of less than 7. However, if pH variability is expected, the pH sensitive material can be selected based on the different pH criteria needed for the dissolution of the microcapsules. The encapsulated nucleic acid, therefore, will be selected for the pH environment in which dissolution is desired and stored in a pH preselected to maintain stability. Examples of pH sensitive material useful as encapsulants are Eudragit™ L-100 or S-100 (Rohm GMBH), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, polyvinyl acetate phthalate, cellulose acetate phthalate, and cellulose acetate trimellitate. In one embodiment, lipids comprise the inner coating of the microcapsules. In these compositions, these lipids may be, but are not limited to, partial esters of fatty acids and hexitol anhydrides, and edible fats such as triglycerides. Lew C. W., Controlled-Release pH Sensitive Capsule And Adhesive System And Method. U.S. Pat. No. 5,364,634 (herein incorporated by reference).

[0150] In one embodiment, the present invention contemplates a microparticle comprising a gelatin, or other polymeric cation having a similar charge density to gelatin (i.e., poly-L-lysine) and is used as a complex to form a primary microparticle. A primary microparticle is produced as a mixture of the following composition: i) Gelatin (60 bloom, type A from porcine skin), ii) chondroitin 4-sulfate (0.005%-0.1%), iii) glutaraldehyde (25%, grade 1), and iv) 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC hydrochloride), and ultra-pure sucrose (Sigma Chemical Co., St. Louis, Mo.). The source of gelatin is not thought to be critical; it can be from bovine, porcine, human, or other animal source. Typically, the polymeric cation is between 19,000-30,000 daltons. Chondroitin sulfate is then added to the complex with sodium sulfate, or ethanol as a coacervation agent.

[0151] Following the formation of a microparticle, a nucleic acid is directly bound to the surface of the microparticle or is indirectly attached using a "bridge" or "spacer". The amino groups of the gelatin lysine groups are easily derivatized to provide sites for direct coupling of a compound. Alternatively, spacers (i.e., linking molecules and derivatizing moieties on targeting ligands) such as avidin-biotin are also useful to indirectly couple targeting ligands to the microparticles. Stability of the microparticle is controlled by the amount of glutaraldehyde-

spacer crosslinking induced by the EDC hydrochloride. A controlled release medium is also empirically determined by the final density of glutaraldehyde-spacer crosslinks.

[0152] In one embodiment, the present invention contemplates microparticles formed by spray-drying a composition comprising fibrinogen or thrombin with a nucleic acid. Preferably, these microparticles are soluble and the selected protein (i.e., fibrinogen or thrombin) creates the walls of the microparticles. Consequently, the nucleic acids are incorporated within, and between, the protein walls of the microparticle. Heath et al., Microparticles And Their Use In Wound Therapy. U.S. Pat. No. 6,113,948 (herein incorporated by reference). Following the application of the microparticles to living tissue, the subsequent reaction between the fibrinogen and thrombin creates a tissue sealant thereby releasing the incorporated compound into the immediate surrounding area.

[0153] One having skill in the art will understand that the shape of the microspheres need not be exactly spherical; only as very small particles capable of being sprayed or spread into or onto a surgical site (i.e., either open or closed). In one embodiment, microparticles are comprised of a biocompatible and/or biodegradable material selected from the group consisting of polylactide, polyglycolide and copolymers of lactide/glycolide (PLGA), hyaluronic acid, modified polysaccharides and any other well known material.

V. PROTEINACEOUS COMPOSITIONS

[0154] The polypeptides or polynucleotides of the disclosure, such as the CIRT fusion proteins, stabilizer polypeptide, linker, RNA hairpin binding domain, NES, RNA regulatory domain, tag, NLS, RNA targeting molecule, hairpin region of the RNA targeting molecule, helical region, or targeting region of the RNA targeting molecule may include 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 or more variant amino acids or nucleic acid substitutions or be at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% similar, identical, or homologous with at least, or at most 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128,

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[0155] The polypeptides or polynucleotides of the disclosure, such as the CIRT fusion proteins, stabilizer polypeptide, linker, RNA hairpin binding domain, NES, RNA regulatory domain, tag, NLS, RNA targeting molecule, hairpin region of the RNA targeting molecule, helical region, or targeting region of the RNA targeting molecule may include 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of SEQ ID NO:1-132.

[0156] In some embodiments, the fusion protein may comprise amino acids 1 to 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180,

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[0157] In some embodiments, the fusion protein may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202,

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[0158] In some embodiments, the fusion protein may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221,

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[0159] In other embodiments, the stabilizer polypeptide may comprise amino acids 1 to 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90, or any derivable range therein of SEQ ID NO:5, 19, or 20.

[0160] In some embodiments, the stabilizer polypeptide may comprise 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, 31, 32, 33,

34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 (or any derivable range therein) contiguous amino acids of SEQ ID NO:5, 19, or 20.

5 **[0161]** In some embodiments, the stabilizer polypeptide may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 (or any derivable range therein) contiguous amino acids of SEQ
10 ID NO:5, 19, or 20 that are at least, at most, or exactly 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% similar, identical, or homologous with one of SEQ ID NO:5, 19, or 20.

[0162] In some embodiments, the RNA hairpin binding domain may comprise amino acids
15 1 to 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, or 102, (or any derivable range therein) of SEQ ID NOs:7 or 18.

20 **[0163]** In some embodiments, the RNA hairpin binding domain may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, or 102 (or any
25 derivable range therein) contiguous amino acids of SEQ ID NOs:7 or 18.

[0164] In some embodiments, the RNA hairpin binding domain may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81,
30 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, or 102 (or any derivable range therein) contiguous amino acids of SEQ ID NOs:7 or 18 that are at least, at most, or exactly 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%,

89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% similar, identical, or homologous with one of SEQ ID NOS:7 or 18.

[0165] In some embodiments, the RNA regulatory domain may comprise amino acids 1 to 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, or 364 (or any derivable range therein) of SEQ ID NOs:9, 11, 15-17, or 123-125.

[0166] In some embodiments, the RNA regulatory domain may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239,

240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 5 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, or 364 (or any derivable range therein) contiguous amino acids of SEQ ID NOs:9, 11, 15-17, or 123-125.

[0167] In some aspects there is a nucleic acid molecule or polypeptide starting at position 10 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 15 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 20 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 25 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 30 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501,

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5 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596,
597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, or
615 of any of SEQ ID NOS:1-132 and comprising 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40,
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10 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90,
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112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130,
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15 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187,
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20 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282,
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25 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377,
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5 any of SEQ ID NOS:1-132.

[0168] In some embodiments, the RNA regulatory domain may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83,
10 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182,
15 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277,
20 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, or 364 (or any derivable range therein)
25 contiguous amino acids of SEQ ID NOS:9, 11, 15-17, or 123-125 that are at least, at most, or exactly 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% similar, identical, or homologous with one of SEQ ID NOS:9, 11, 15-17, or 123-125.

30 **[0169]** In some embodiments, the hairpin structure, such as the stem, loop, or both stem and loop, of the RNA targeting molecule may comprise nucleic acids 1 to 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43 (or any derivable range therein) of SEQ ID NOS:1, 2, 26, 28, or 83-86.

[0170] In some embodiments, the hairpin structure, such as the stem, loop, or both stem and loop, of the RNA targeting molecule may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43 (or any derivable range therein) contiguous nucleic acids of SEQ ID NOs:1, 2, 26, 28, or 83-86.

[0171] In some embodiments, the hairpin structure, such as the stem, loop, or both stem and loop, of the RNA targeting molecule may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, or 43 (or any derivable range therein) contiguous nucleic acids of SEQ ID NOs:1, 2, 26, 28, or 83-86 that are at least, at most, or exactly 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% similar or identical with one of SEQ ID NOs:1, 2, 26, 28, or 83-86.

[0172] In some embodiments, the RNA targeting region or the RNA targeting molecule may comprise nucleic acids 1 to 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 (or any derivable range therein) of SEQ ID NOs:30-62.

[0173] In some embodiments, the RNA targeting region or the RNA targeting molecule may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 (or any derivable range therein) contiguous nucleic acids of SEQ ID NOs:30-62.

[0174] In some embodiments, the RNA targeting region or the RNA targeting molecule may comprise 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 (or any derivable range therein) contiguous nucleic acids of SEQ ID NOs:30-62 that are at least, at most, or exactly 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% similar or identical with one of SEQ ID NOs:30-62.

[0175] In some aspects, the stabilizer polypeptide may have one or more substitutions that reduce or eliminate binding to endogenous proteins. In some aspects, the stabilizer polypeptide may have one or more substitutions that reduce or eliminate an activity directed to an endogenous protein.

[0176] The polypeptides and nucleic acids of the disclosure, such as the CIRT fusion proteins, stabilizer polypeptide, linker, RNA hairpin binding domain, NES, RNA regulatory domain, tag, NLS, RNA targeting molecule, hairpin region of the RNA targeting molecule, helical region, or targeting region of the RNA targeting molecule may include at least, at most, or exactly 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594,

595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, or 615 substitutions.

[0177] The substitution may be at amino acid position or nucleic acid position 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, 31, 32, 33, 34, 35, 36, 37, 38, 39, 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, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598,

599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, or 615 of one of SEQ ID NO:1-132.

[0178] Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Alternatively, substitutions may be non-conservative such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting a residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa.

[0179] Proteins may be recombinant or synthesized *in vitro*. Alternatively, a non-recombinant or recombinant protein may be isolated from bacteria. It is also contemplated that bacteria containing such a variant may be implemented in compositions and methods. Consequently, a protein need not be isolated.

[0180] The term “functionally equivalent codon” is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids.

[0181] It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids, or 5' or 3' sequences, respectively, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region.

[0182] The following is a discussion based upon changing of the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. For example, certain amino acids may be substituted for other amino acids in a protein structure without

appreciable loss of interactive binding capacity. Structures such as, for example, an enzymatic catalytic domain or interaction components may have amino acid substituted to maintain such function. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and in its underlying DNA coding sequence, and nevertheless produce a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the DNA sequences of genes without appreciable loss of their biological utility or activity.

[0183] In other embodiments, alteration of the function of a polypeptide is intended by introducing one or more substitutions. For example, certain amino acids may be substituted for other amino acids in a protein structure with the intent to modify the interactive binding capacity of interaction components. Structures such as, for example, protein interaction domains, nucleic acid interaction domains, and catalytic sites may have amino acids substituted to alter such function. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and in its underlying DNA coding sequence, and nevertheless produce a protein with different properties. It is thus contemplated by the inventors that various changes may be made in the DNA sequences of genes with appreciable alteration of their biological utility or activity.

[0184] In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

[0185] It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein. It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still produce a biologically equivalent and immunologically equivalent protein.

[0186] As outlined above, amino acid substitutions generally are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take into consideration the various foregoing characteristics are well known and include: arginine and lysine;

glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

[0187] In specific embodiments, all or part of proteins described herein can also be synthesized in solution or on a solid support in accordance with conventional techniques.

- 5 Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, (1984); Tam *et al.*, (1983); Merrifield, (1986); and Barany and Merrifield (1979), each incorporated herein by reference. Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence that encodes a peptide or polypeptide is inserted into an expression vector, transformed or
- 10 transfected into an appropriate host cell and cultivated under conditions suitable for expression.

[0188] One embodiment includes the use of gene transfer to cells, including microorganisms, for the production and/or presentation of proteins. The gene for the protein of interest may be transferred into appropriate host cells followed by culture of cells under the appropriate conditions. A nucleic acid encoding virtually any polypeptide may be employed.

- 15 The generation of recombinant expression vectors, and the elements included therein, are discussed herein. Alternatively, the protein to be produced may be an endogenous protein normally synthesized by the cell used for protein production.

VI. SEQUENCES

[0189] TAR hairpin Scaffold: ggccagaucugagccugggagcucucuggcc (SEQ ID NO:1)

- 20 [0190] Human SLBP hairpin scaffold: ccaaaggcucuucucagagccacca (SEQ ID NO:2)

Name:	Description:	
MBP-CIRTS-1	MBP signaling peptide	MKIEEGKLVINGDKGYNGLAEVGKKFEKDTGIKVTVEHP DKLEEKFPQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITP DKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLL PNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAA DGGYAFKYENGKYDIKDVGVNAGAKAGLTFLVDLIKXKH MNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYG VTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLL TDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQ KGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQ TNS (SEQ ID NO:3)
		ITSLYKKAGSETVRFQSHHHHHHSSGVDLGTENLYFQSNA (SEQ ID NO:4)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDDEE (SEQ ID NO:5)
	linker	GGSGSGSGSGSGSGSGS (SEQ ID NO:6)

	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	dead Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALTSGNELESIAFRSEITGQLGNNDDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:9)
		SSG
	6H tag	HHHHHH (SEQ ID NO:10)
CIRTS-0	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	dead Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALTSGNELESIAFRSEITGQLGNNADLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:11)
CIRTS-1		GS
	3xFLAG	DYKDHDGDYKDHDIDYKDDDDK (SEQ ID NO:12)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALTSGNELESIAFRSEITGQLGNNDDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:9)
CIRTS-2	NLS	KRPAATKKAGQAKKKK (SEQ ID NO:13)
		GS
	Tag	DYKDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)

	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	N terminal YTHDF1	MSATSVDTQRTKGQDNKVQNGSLHQKDTVHDNDFEPYLT GQSNQSNYSYPSMSDPYLSSYYPPSIGFPYSLNEAPWSTAGDP PIPYLTTYGQLSNGDHHFMHDAVFGQPGGLGNNIYQHRFNF FPENPAFSAWGTSGSQGQQTQSSAYGSSYTYPPSSLGGTVV DGQPGFHSDTLKAPGMNSLEQGMVGLKIGDVSSSAVKTV GSVVSSVALTGVLSGNGGTNVNMPVSKPTSWAAIASKPAK PQPKMKTKSGPVMGGGLPPPIKHNMIDGTWDNKGVPKA PVPQQAPSPQAAPQPQQAQPLPAQPPALAQPPYQSPQPP QTRWVAPRNRNAAFGQSGGAGSDSNSPGNVQPNAPSVES (SEQ ID NO:15)
		GS
CIRTS-3 _t	Tag	DYKDDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEYHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
CIRTS-3	YTHDF2 (101-200)	DAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGG STQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMN TIDQGMAA (SEQ ID NO:16)
		GS
	Tag	DYKDDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEYHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
CIRTS-3	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	N-terminal YTHDF2 (1-200)	MSASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPYLS QARPNNAYTAMSDSYLPSYSPSIGFSYSLGEAAWSTGGDT AMPYLTSYGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGF NFFPSGIDFSAWGNSSQGGSTQSSGYSSNYAYAPSSLGGA MIDGQSAFANETLNKAPGMNTIDQGMAA (SEQ ID NO:17)

CIRTS-4 _t		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	SLBP 124-198	ADFETDESVLMMRQKQINYGKNTIAYDRYIKEVPRHLRQPGI HPKTPNKFKKYSRSSWDQQIKLWKVALHFWD (SEQ ID NO:18)
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	YTHDF2 (101-200)	DAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGG STQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMN TIDQGMAA (SEQ ID NO:16)
CIRTS-4		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	SLBP 124-198	ADFETDESVLMMRQKQINYGKNTIAYDRYIKEVPRHLRQPGI HPKTPNKFKKYSRSSWDQQIKLWKVALHFWD (SEQ ID NO:18)
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	YTHDF2 (1-200)	MSASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPYLS QARPNNAYTAMSDSYLPSYSPSIGFSYSLGEAAWSTGGDT AMPYLTSYGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGF NFFPSGIDFSAWGNSSQGGSTQSSGYSSNYAYAPSSLGGA MIDGQSAFANETLNKAPGMNTIDQGMAA (SEQ ID NO:17)
CIRTS-5 _t		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	HBEGF	MRVTLSSKPQALATPNKEEHGKRKKKGKGLGKKRDPCLRK YKDFCIHGECKYVKELRAPSCICHPGYHGERCHGLS (SEQ ID NO:19)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLNSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFYDKPMRI QYAKTDKRIPAKMKGTFFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
CIRTS-5 _t	YTHDF2 (101-200)	DAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGG STQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMN TIDQGMAA (SEQ ID NO:16)
		GS
	tag	DYKDDDDK (SEQ ID NO:14)

CIRTS-5	HBEGF	MRVTLSSKPQALATPNKEEHGKRKKKGKGLGKKRDPCLRK YKDFCIHGECKYVKELRAPSCICHPGYHGERCHGLS (SEQ ID NO:19)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPPYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	YTHDF2 (1-200)	MSASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPYLS QARPNNAYTAMSDSYLPSYYSPSIGFSYSLGEAAWSTGGDT AMPYLTSYGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGF NFFPSGIDFSAWGNSSQGGSTQSSGYSSNYAYAPSSLGGA MIDGQSAFANETLNKAPGMNTIDQGMAA (SEQ ID NO:17)
	tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-6 _t	β-defensin	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKK (SEQ ID NO:20)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPPYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	YTHDF2 (101-200)	DAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGG STQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMN TIDQGMAA (SEQ ID NO:16)
	tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-6	β-defensin	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKK (SEQ ID NO:20)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPPYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	YTHDF2 (1-200)	MSASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPYLS QARPNNAYTAMSDSYLPSYYSPSIGFSYSLGEAAWSTGGDT AMPYLTSYGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGF NFFPSGIDFSAWGNSSQGGSTQSSGYSSNYAYAPSSLGGA MIDGQSAFANETLNKAPGMNTIDQGMAA (SEQ ID NO:17)
	tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-7	β-defensin	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKK (SEQ ID NO:20)

	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	SLBP 124-198	ADFETDESVLMMRRQKQINYGKNTIAYDRYIKEVPRHLRQPGIHPKTPNKFKKYSRRSWDQQIKLWKVALHFWD (SEQ ID NO:18)
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	YTHDF2 (1-200)	MSASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPYLSPQARPNNAYTAMSDSYLPSYSPSIGFSYSLGEAAWSTGGDTAMPYLTSYGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGQSTQSSGYSSNYAYAPSSLGGA MIDGQSAFANETLNKAPGMNTIDQGMAA (SEQ ID NO:17)
		GS
	tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-8	TBP6.7	MAVPETRPNHTIYINNLNSKIKKDELKKSLEYAIFSQFGQILDLVPRQRTPRGQAFVIFKEVSSATNALRSMQGFYDKPMRIQYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYL VVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALTSRGNELESIAFRSEDTGQLGNNDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:9)
		GS
	tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-9	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLNSKIKKDELKKSLEYAIFSQFGQILDLVPRQRTPRGQAFVIFKEVSSATNALRSMQGFYDKPMRIQYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYL VVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALTSRGNELESIAFRSEDTGQLGNNDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:9)
CIRTS-10		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)

	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSGGSGGS (SEQ ID NO:21)
	Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALT SRGNELESIAFRSEIDITGQLGNNDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:9)
		GS
	tag	DYKDDDDK (SEQ ID NO:14)
CIRTS- 11	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEYHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGGSGSGSGSGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	L8 linker	SGSETPGTSESATPES (SEQ ID NO:22)
	Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALT SRGNELESIAFRSEIDITGQLGNNDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:9)
CIRTS- 12		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEYHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGGSGSGSGSGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSG (SEQ ID NO:23)
	ER/K 10 nm	EEEEKKKQEEEEAERLRRIQEEMEKKRREEDEQRRRKEE EERRMKLEMEAKRKQEEEEERKKREDDEKRKKK (SEQ ID NO:24)
	linker	GSGGS (SEQ ID NO:25)
	Pin domain	MELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINE LDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDS CLRALT SRGNELESIAFRSEIDITGQLGNNDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPV RDIPAFLTWAQVG (SEQ ID NO:9)

CIRTS-13		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPPYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	L8 linker	SGSETPGTSESATPES (SEQ ID NO:22)
	Y2(101-200)	DAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGG STQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMN TIDQGMAA (SEQ ID NO:16)
CIRTS-14		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDEE (SEQ ID NO:5)
	linker	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPPYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
		GS
	HIV NES	LQLPPLERLTL (SEQ ID NO:8)
	linker	GGSG (SEQ ID NO:23)
	ER/K 10 nm	EEEEKKKQEEEEAERLRRIQEEMEKEKRREEDEQRRRKEE EERRMKLEMEAKRKQEEEEERKKREDDEKRRKKK (SEQ ID NO:24)
CIRTS-7X	linker	GSGGS (SEQ ID NO:25)
	Y2(101-200)	DAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGG STQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMN TIDQGMAA (SEQ ID NO:16)
		GS
	tag	DYKDDDDK (SEQ ID NO:14)
	β-defensin 3	MGIINTLQKYCYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKK (SEQ ID NO:20)
	GGSG ₆	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
CIRTS-7X	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKSLEYAIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPPYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
	GGSG ₆	GGSGSGSGSGSGSGSGGS (SEQ ID NO:6)
	hADAR(299-701)	LHLDQTPSRQPIPSEGLQLHLPQVLADAVSRLVLGKFGDLTD NFSSPHARRKVLAVVMTTGTVDKDAKVISVSTGTCINGE YMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQK RSIFQKSERGGFRLKENVQFHL YISTSPCGDARIFSPHEPILEE PADRHPNRKARGQLRTKIESGEGTIPVRSNASIQTWGVLQ GERLLTMSKSDKIARWNVVGIQGSLLSIFVEPIYFSSILGSLY

		HGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPG KAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCKHALYC RWMRVHGKVPSHLLRSKITKPNVYHESKLAKEYQAAKAR LFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID NO:123)
	NLS	KRPAATKKAGQAKKKK (SEQ ID NO:13)
		GS
	Flag tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-8X	β-defensin 3	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKK (SEQ ID NO:20)
	GG ₆	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKS L Y AIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGF PFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
	GG ₆	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	hADAR (299-701) E488Q	LHLDQTPSRQPIPSEGLQLHLPQVLADAVSRLVLGKFGDLTD NFSSPHARRKVLAVVMTTGTVDKDAKVISVSTGTCINGE YMSDRGLALNDCHAEIISRRSLLRFLYTQLELYLNNKDDQK RSIFQKSERGGFRLKENVQFHL YISTSPCGDARIFSPHEPILEE PADRHPNRKARGQLRTKIESGQGTIPVRSNASIQTWGVLQ GERLLTMS CSDKIARWNVVGIQGSLLSIFVEPIYFSSILGSLY HGDHLSRAMYQRISNIEDLPPLYTLNKPLLSGISNAEARQPG KAPNFSVNWTVGDSAIEVINATTGKDELGRASRLCKHALYC RWMRVHGKVPSHLLRSKITKPNVYHESKLAKEYQAAKAR LFTAFIKAGLGAWVEKPTEQDQFSLTP (SEQ ID NO:124)
	NLS	KRPAATKKAGQAKKKK (SEQ ID NO:13)
		GS
	Flag tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-9X	β-defensin 3	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKK (SEQ ID NO:20)
	GG ₆	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	TBP6.7	MAVPETRPNHTIYINNLSKIKKDELKKS L Y AIFSQFGQILDI LVPRQRTPRGQAFVIFKEVSSATNALRSMQGF PFYDKPMRI QYAKTDKRIPAKMKGTFV (SEQ ID NO:7)
	NES	GSLQLPPLERLTL (SEQ ID NO:124)
	GG ₆	GGSGGSGGSGGSGGSGGS (SEQ ID NO:6)
	NYTHDF1	MSATSVDTQRTKGQDNKVQNGSLHQKDTVHDNDFEPYLT GQSNQSNYSMSDPYLSSYPPSIGFPYSLNEAPWSTAGDP PIPYLT TYGQLSNGDHHFMHDAVFGQPGGLGNNIYQHRFNF FPENPAFSAWGTSGSQGQQTQSSAYGSSYTYPPSSLGGTVV DGQPGFHSDTL SKAPGMNSLEQGMVGLKIGDVSSSAVKTV GSVVSSVALTGVL SGNGGTNVNMPVSKPTSWAAIASKPAK PQPKMKT KSGPVMGGGLPPPPIKHNDIGTWDNKGVPKA PVPQQAPSPQAAPQPQQAQPLPAQPPALAQPYQSPQPPP QTRWVAPRNRNAAFGQSGGAGSDSNPGNVQPNSAPSVES (SEQ ID NO:15)
		GS
	Flag tag	DYKDDDDK (SEQ ID NO:14)
CIRTS-10X	β-defensin 3	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKK (SEQ ID NO:20)

	GGG ₆	GGSGGSGGSGGSGGSGGSGG (SEQ ID NO:6)
	SLBP	ADFETDESVLMMRRQKQINYGKNTIAYDRIKEVPRHLRQPGI HPKTPNKFKKYSRRSWDQQIKLWKVALHFWD (SEQ ID NO:18)
	NES	LQLPPLERLTL (SEQ ID NO:8)
	GGG ₆	GGSGGSGGSGGSGGSGGSGG (SEQ ID NO:6)
	YTHDF2(1 01-200)	MSASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPYLS QARPNNAYTAMSDSYLPSYSPSIGFSYSLGEAAWSTGGDT AMPYLTSYGQLSNGEPHFLPDAMFGQPALGSTPFLGQHGF NFFPSGIDFSAWGNSSQGGSTQSSGYSSNYAYAPSSLGGA MIDGQSAFANETLNKAPGMNTIDQGMAA (SEQ ID NO:17)
		GS
	Flag tag	DYKDDDDK (SEQ ID NO:14)
CIRTS- 18X	ORF5	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHIN ILETSDDDEE (SEQ ID NO:5)
	GGG ₆	GGSGGSGGSGGSGGSGGSGG (SEQ ID NO:6)
	PP7	MAKTIVLAVGEATRTLTEIQSTADRQIFEEKVGPLVGRRLT ASLRQNGAKTAYRVNLKLDQADVVDASTSVAGELPKVRYT QVWSDVTIVANSTEASRKSLEYDLTKSLVATSQVEDLVVNL VPLGRSLEGGSGGMAKTIVLAVGEATRTLTEIQSTADRQIFE EKVGPLVGRRLTASLRQNGAKTAYRVNLKLDQADVVDAS TSVAGELPKVRYTQVWSDVTIVANSTEASRKSLEYDLTKSL VATSQVEDLVVNLVPLGRSLE (SEQ ID NO:125)
	NES	LQLPPLERLTL (SEQ ID NO:8)
	GGG ₆	GGSGGSGGSGGSGGSGGSGG (SEQ ID NO:6)
	Y2(100- 200)	DAMFGQPALGSTPFLGQHGFNFFPSGIDFSAWGNSSQGG STQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMN TIDQGMAA (SEQ ID NO:16)
		GS
	Flag tag	DYKDDDDK (SEQ ID NO:14)

gRNA sequences used in this study (all gRNAs are expressed from a hU6 promoter)

Name:		Description:	DNA sequence
TBP- OT	26- 51	TBP6,7 gRNA (TAR)	GGCCAGATCTGAGCCTGGGAGCTCTCTGGCC (SEQ ID NO:26)
			TTATT
		off-target λ phage	Tgacagcccatgagcattccacttatcactggcatcctt (SEQ ID NO:27)
SLBP- OT	24- 18	SLBP gRNA	CCAAAGGCTCTTCTCAGAGCCACCCA (SEQ ID NO:28)
			TTATT
		λ target	GTGATAAGTGGAATGCCATG (SEQ ID NO:29)
PP7-NT		PP7 non- targeting gRNA λ phage	TAAGGAGTTTATATGGAAACCCTTA (SEQ ID NO:121)
			TTATT

	λ phage	GTGATAAGTGGAATGCCATG (SEQ ID NO:122)
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gRNA guiding sequencing; all expressed by the same hU6 promoter as TBP-OT

Target name:		Guiding Sequence:	Fig:
Fluciferase-40	18-20	CAGGTCGACTCTAGACTCGAGGCTAGCGAGCTCGTT TAAA (SEQ ID NO:30)	3
PPIB	23-26	CTTGGTGCTCTCCACCTTCCGCACCACCTCCATGCC CTCT (SEQ ID NO:31)	4
SMARCA4	23-25	CCGATGCGGTGGGCTCGGTCCTGCGCTTGCAGGTCC TGGT (SEQ ID NO:32)	4
NFKB1	22-67	GCCTCCACCAGCTCTCTGACTGTACCCCCAGAGACC TCAT (SEQ ID NO:33)	4
NRAS	25-30	AAGCATCTTCAACACCCTGTCTGGTCTTGGCTGAGG TTTC (SEQ ID NO:34)	4
B4GALTN1	24-50	CTTCGCACCGCAGCGCAGCGCGGCTCAGCTCCCGG CTCGT (SEQ ID NO:35)	4
Fluciferase-20		CTAGACTCGAGGCTAGCGAG (SEQ ID NO:36)	8
Fluciferase-30		CGACTCTAGACTCGAGGCTAGCGAGCTCGT (SEQ ID NO:37)	8
KRAS-2	22-45	CTTGTGGTAGTTGGAGCTGA (SEQ ID NO:116)	25B
KRAS-3	22-53	AGTTGGAGCTGATGGCGTAG (SEQ ID NO:117)	25B
KRAS-4	22-54	GTTGGAGCTGATGGCGTAGG (SEQ ID NO:118)	25B
KRAS-5	22-65	TGGCGTAGGCAAGAGTGCCT (SEQ ID NO:119)	25B
KRAS-6	22-66	GGCGTAGGCAAGAGTGCCTT (SEQ ID NO:120)	25B
SMARCA4-1	27-67	ggccctggccctcccctggagccatgctggccctagcc (SEQ ID NO:38)	11C
SMARCA4-2	27-71	gcccacccatttaaccagaaccagctgcaccagctcag (SEQ ID NO:39)	11C
SMARCA4-3	27-74	cctccaagccctggcctgaaggacccatggcgaatgctg (SEQ ID NO:40)	11C
SMARCA4-4	27-75	cgtccacccgcccctgcccgtgatgccaccgcagacc (SEQ ID NO:41)	11C
SMARCA4-5	27-78	ggaggtggtggtgtgcatgcccggaggacacagcgtggag (SEQ ID NO:42)	11C
SMARCA4-6	27-80	Tcacaggcaaaatccagaagctgaccaaggcagtgccac (SEQ ID NO:43)	11C
SMARCA4-7	27-81	catggctgaagatgaggaggggtaccgcaagctcatcgac (SEQ ID NO:44)	11C
SMARCA4-8	28-02	ctctggacgagaccagccagatgagcgacctccggtgaa (SEQ ID NO:45)	11C
SMARCA4-9	28-04	cccaccctgcccgtggaggagaagaagaagattccagatc (SEQ ID NO:46)	11C
SMARCA4-10	28-05	aatatggcgtgtcccaggcccttgacgtggcctgcagtc (SEQ ID NO:47)	11C
SMARCA4-11	28-07	ctcatcacgtacctcatggagcacaacgcacatcaatgggc (SEQ ID NO:48)	11C
SMARCA4-12	28-08	tggtgaaggtgtcttacaaggatccccagcagaagacg (SEQ ID NO:49)	11C
SMARCA4-13	28-10	accccgccgctgctgctgacgggcacaccgctgcagaac (SEQ ID NO:50)	11C

SMARCA4-14	28-11	cagtgggttaacgcaccctttgccatgaccggggaaaagg (SEQ ID NO:51)	11C
SMARCA4-15	28-12	acgactcaagaaggaagtcgaggccagttgccgaaaag (SEQ ID NO:52)	11C
SMARCA4-16	28-13	gtgctgctgactgatggctccgagaaggacaagaaggga (SEQ ID NO:53)	11C
SMARCA4-17	28-16	ggaaccacgaaggcggaggaccgggcatgctgctgaaaa (SEQ ID NO:54)	11C
SMARCA4-18	28-17	tccagtgcgcagacactgtgatcattttgacagcgactg (SEQ ID NO:55)	11C
SMARCA4-19	28-20	cgccagcgggcgtcaaccccgacttgaggagccacctct (SEQ ID NO:56)	11C
SMARCA4-20	28-22	gcggaggtggagcggctgacctgtgaggaggaggaggaga (SEQ ID NO:57)	11C
SMARCA4-21	28-26	tgatcaagtacaaggacagcagcagtgaggcgtcagctcag (SEQ ID NO:58)	11C
SMARCA4-22	28-27	cttcaagaagataaaggagcgcattcgcaaccacaagtac (SEQ ID NO:59)	11C
SMARCA4-23	28-30	agggtcccgagccaagccggctgtagtgacgatgacagt (SEQ ID NO:60)	11C
SMARCA4-24	28-32	tattatacagcagagaagctgtaggactgtttgtgactg (SEQ ID NO:61)	11C
SMARCA4-25	28-33	ggggaacacacgatacctgttttctttccgttgctggc (SEQ ID NO:62)	11C

RNA oligonucleotides used for *in vitro* characterization:

Gene Name:	Oligo Name:	Forward:	Figure:
R1	ssRNA target for EMSA	ATAGGCCAGTGAATTCGAGCTCGAATATGGATTACTTGGTAGAACAGCAATCTACGCCGGAAGCATAAAG (SEQ ID NO:63)	2A
R2	ssRNA target for cleavage assay	GGCCAGTGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAAATATGGATTACTTGGTAGAACAGCAATCTACTCGACCTGCAGGCATGCAAGCTTGGCGTAATCATGGTCA TAGCTGTTTCCTGTGTTTATCCGCTCACAATCCACAC AACATACGAGCCGGAAGCATAAAG (SEQ ID NO:64)	2C
R3	gRNA for EMSA	GGCCAGATCTGAGCCTGGGAGCTCTCTGGCCAgccacca cccacagagccgccaccaga (SEQ ID NO:65)	2A
R4	gRNA for cleavage assay	GGCCAGATCTGAGCCTGGGAGCTCTCTGGCCCTAGAT TGCTGTTCTACCAAGTAATCCAT (SEQ ID NO:66)	2C

5 qPCR Primers:

Gene Name:	Forward:	SEQ ID NO:	Reverse:	SEQ ID NO:
<i>GAPDH</i>	GTCTCCTCTGACTTCAACAGCG	67	ACCACCCTGTTGCTGTAGCCAA	68
<i>PPIB</i>	AACGCAGGCAAAGACACCAACG	69	TCTGTCTTGGTGCTCTCCACCT	70
<i>Fluciferase</i>	AGGTTACAACCGCCAA GAAGC	71	ATGAGAATCTCGCGGATCTTG	72
<i>PPIB</i>	AACGCAGGCAAAGACACCAACG	73	TCTGTCTTGGTGCTCTCCACCT	74
<i>NFKB1</i>	GCAGCACTACTTCTTG ACCACC	75	TCTGCTCCTGAGCATTGACGTC	76
<i>NRAS</i>	GAAACCTCAGCCAAG ACCAGAC	77	GGCAATCCCATAACAACCCTGAG	78
<i>B4GALTNI</i>	TGAGGCTGCTTTCACATCCGC	79	GAGGAAGGTCTTGGTGGCAATC	80
<i>SMARCA4</i>	CAAAGACAAGCACATCCTCGCC	81	GCCACATAGTGCGTGTTGAGCA	82
<i>MALAT1</i>	GAAGAAGGAAGGAGCGCTAA	126	CCTGCTACCTTCATCACCAA	127

[0191] RNA hairpin structure:

GGCGUCCCUCCCGAAGCUGCGCGCUCGGUCGAACAGGACGACC (SEQ ID NO:83)

[0192] Nucleolin recognition sequence: UCCCGA (SEQ ID NO:84).

- 5 [0193] RNA hairpin structure: GGCCGAAUCCCGAAUGAGGCC (SEQ ID NO:85) and
GGAUGCCUCCCGAGUGCAUCC (SEQ ID NO:86).

Name:	Description:	Polypeptide sequence
CIRTS-0	ORF5-TBP6.7-GGS ₆ -dead Pin domain	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSGSMAMPETRPNHTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTGGSGSGSGSGSGSGSGSGSMELEI RPLFLVPDTNGFIDHLASLARLLESRKYL VVPLIVINELDGL AKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDSCLR ALTSRGNELESIAFRSEDTGQLGNNADLILSCCLHYCKDKA KDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPVRDI PAFLTWAQVGGSDYKDHGDYKDHIDYKDDDDK (SEQ ID NO:87)
CIRTS-1	ORF5-TBP6.7-GGS ₆ -Pin domain-NLS	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSGSMAMPETRPNHTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFYDKPMRIQYAKTDKRIPAKM KGTFFVGGSGSGSGSGSGSGSGSGSMELEIRPLFLVPDTNGFI DHLASLARLLESRKYL VVPLIVINELDGLAKGQETDHRAG

		GYARVVQEKARKSIEFLEQRFESRDSCLRALTSRGNELESIA FRSEDITGQLGNNDLILSCCLHYCKDKAKDFMPASKEEPI RLLEVVLLTDDRNLRVKALTRNVPVRDIPAFLTWAQVGK RPAATKKAGQAKKKKGSDYKDDDDK (SEQ ID NO:88)
CIRTS-2	ORF5- TBP6.7- GGS ₆ - NYTHDF1	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSMAMPETRPNTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTLGGSGSGSGSGSGSGSGSMSATS VDTQRTKGQDNKVQNGSLHQKDTVHDNDFEPLYLTGQSNQ SNSYPSMSDPYLSYYPSSIGFPYSLNEAPWSTAGDPPIPYLT TYGQLSNGDHHFMHDAVFGQPGGLGNNIYQHRFNFFPENP AFSAWGTSGSQGQQTQSSAYGSSYTPPSSLGTVVDGQP GFHSDTLKAPGMNSLEQGMVGLKIGDVSSSAVKTVGSVV SSVALTGVLSGNGGTNVNMPVSKPTSWAAIASKPAKPQPK MKTSGPVMGGGLPPPIKHNMIDGTWDNKGVPVKAPVPQ QAPSPQAAPQPPQVAQPLPAQPPALAQPPYQSPQPPQTR WVAPRNRNAAFGQSGGAGSDSNSPGNVQPNAPSVEGSD YKDDDDK (SEQ ID NO:89)
CIRTS-3 _t	ORF5- TBP6.7- GGS ₆ - YTHDF2 (100-200)	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSMAMPETRPNTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTLGGSGSGSGSGSGSGSGSDAMF GQPGALGSTPFLGQHGFNFFPSGIDFAWGNSSQGQSTQS SGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMNTID QGMAAGSDYKDDDDK (SEQ ID NO:90)
CIRTS-3	ORF5- TBP6.7- GGS ₆ - YTHDF2 (1- 200)	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSMAMPETRPNTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTLGGSGSGSGSGSGSGSGSMSASS LLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPLYLSPQARP NNAYTAMSDSYLPSYSPSIGFSYSLGEAAWSTGGDTAMP YLTSYGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGFNFF PSGIDFAWGNSSQGQSTQSSGYSSNYAYAPSSLGGAMID GQSAFANETLNKAPGMNTIDQGMAAGSDYKDDDDK (SEQ ID NO:91)
CIRTS-4 _t	ORF5-SLBP- GGS ₆ - YTHDF2 (100-200)	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSADFETDESVMRR QKQINYGKNTIAYDRIKEVPRHLRQPGIHPKTPNFKKYS RRSWDQQIKLWKVALHFWDLQLPPLERLTLGGSGSGSGSG SGSGSGSDAMFGQPGALGSTPFLGQHGFNFFPSGIDFAW GNSSQGQSTQSSGYSSNYAYAPSSLGGAMIDGQSAFANE TLNKAPGMNTIDQGMAAGSDYKDDDDK (SEQ ID NO:92)

CIRTS-4	ORF5-SLBP-GGS ₆ -YTHDF2 (1-200)	MDDPSFLTGRSTYAKRRRARARMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETHEYHI NILETSDDEEGSGSGSGSGSGSGSGSADFETDESVLMMR QKQINYGKNTIAYDRIYKEVPRHLRQPGIHPKTPNKFYKYS RRSWDQQIKLWKVALHFWDLQLPPLERLTLGGSGSGSGSG GSGSGSGSMSASSLLEQRPKGQGNKVQNGSVHQDGLND DDFEPYLSQPARNNAYTAMSDSYLPSYYSPSIGFSYSLGE AAWSTGGDTAMPYLTSYGQLSNGEPHFLPDAMFGQPGAL GSTPFLGQHGFNFFPSGIDFSAWGNNSSQGQSTQSSGYSSN YAYAPSSLGGAMIDGQSAFANETLNKAPGMNTIDQGMAA GSDYKDDDDDK (SEQ ID NO:93)
CIRTS-5 _t	HBEGF-TBP6.7-GGS ₆ -YTHDF2 (100-200)	MRVTLSSKPQALATPNKEEHGKRKKKGKGLGKKRDPCLR KYKDFCIHGECKYVKELRAPSCICHPGYHGERCHGLS GSGSGSGSGSGSGSGSMAMPETRPNHTIYINNLNSKIKKDEL KKSLYAIQSFGQILDILVPRQRTPRGQAFVIFKEVSSATNA LRSMQGFPPFYDKPMRIQYAKTDKRIPAKMKGT FVGS LQLPLERLTLGGSGSGSGSGSGSGSGSDAMFGQPGAL GSTPFLGQHGFNFFPSGIDFSAWGNNSSQGQSTQSSGYSSN YAYAPSSLGGAMIDGQSAFANETLNKAPGMNTIDQGMAA GSDYKDDDDDK (SEQ ID NO:94)
CIRTS-5	HBEGF-TBP6.7-GGS ₆ -YTHDF2 (1-200)	MRVTLSSKPQALATPNKEEHGKRKKKGKGLGKKRDPCLR KYKDFCIHGECKYVKELRAPSCICHPGYHGERCHGLS GSGSGSGSGSGSGSGSMAMPETRPNHTIYINNLNSKIKKDEL KKSLYAIQSFGQILDILVPRQRTPRGQAFVIFKEVSSATNA LRSMQGFPPFYDKPMRIQYAKTDKRIPAKMKGT FVGS LQLPLERLTLGGSGSGSGSGSGSGSGSMSASSLLEQRPKGQ GNKVQNGSVHQDGLNDDDFEPYLSQPARNNAYTAMSDS YLPSYYSPSIGFSYSLGEAAWSTGGDTAMPYLTSYGQLS NGEPHFLPDAMFGQPGALGSTPFLGQHGFNFFPSGIDFSA WGNNSQGQSTQSSGYSSNYAYAPSSLGGAMIDGQSAFAN ETLNKAPGMNTIDQGMAAGSDYKDDDDDK (SEQ ID NO:95)
CIRTS-6 _t	β -defensin-TBP6.7-GGS ₆ -YTHDF2 (100-200)	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKKGGSGSGSGSGSGSGSGSGSMAMPETRPNHTIYINNL NSKIKKDELKKSLYAIQSFGQILDILVPRQRTPRGQAFVIFK EVSSATNALRSMQGFPPFYDKPMRIQYAKTDKRIPAKMKGT FVGS LQLPLERLTLGGSGSGSGSGSGSGSGSDAMFGQ P GALGSTPFLGQHGFNFFPSGIDFSAWGNNSSQGQSTQSSGY SSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMNTIDQGM AAGSDYKDDDDDK (SEQ ID NO:96)
CIRTS-6	β -defensin-TBP6.7-GGS ₆ -YTHDF2 (1-200)	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKKGGSGSGSGSGSGSGSGSGSMAMPETRPNHTIYINNL NSKIKKDELKKSLYAIQSFGQILDILVPRQRTPRGQAFVIFK EVSSATNALRSMQGFPPFYDKPMRIQYAKTDKRIPAKMKGT FVGS LQLPLERLTLGGSGSGSGSGSGSGSGSMSASSLLE QRPKGQGNKVQNGSVHQDGLNDDDFEPYLSQPARNNA YTAMSDSYLPSYYSPSIGFSYSLGEAAWSTGGDTAMPYLTS YGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGFNFFPSGI DFSAWGNNSSQGQSTQSSGYSSNYAYAPSSLGGAMIDGQS AFANETLNKAPGMNTIDQGMAAGSDYKDDDDDK (SEQ ID NO:97)

CIRTS-7	β -defensin-SLBP-GGS ₆ -YTHDF2 (1-200)	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGK CSTRGRKC CRRKKGGSGGSGGSGGSGGSGGSGGSA DFETDES VLMRRQKQI NYGKNTIA YDRYIKEVPRHLRQPGIHPKTPNFKKYSRRSW DQQIKLWKVALHFWDLQLPPLERLTLGGSGGSGGSGGSGG SGGSMASLLEQRPKGQGNKVQNGSVHQKDGLNDDDFE PYLSPQARPNNAYTAMSDSYLPSYYSPSIGFSYSLGEAAWS TGGDTAMPYLT SYGQLSNGEPHFLPDAMFGQP GALGSTPF LGQHGFNFFPSGIDFSAWGNSSQGGSTQSSGYSSNYAYAP SSLGGAMIDGQSAFANETLNKAPGMNTIDQGMAAGSDYK DDDDK (SEQ ID NO:98)
CIRTS-8	TBP6.7-GGS ₆ -Pin domain-NES	MAVPETRPNHTIYINNLNSKIKKDELKKSLY AIFSQFGQILD LVPRQRTPRGQAFVIFKEVSSATNALRSMQGFPFYDKPMRI QYAKTDKRIPAKMKGTFVGS LQLPPLERLTLGGSGGSGGS GGSGGSGGSMELEIRPLFLVPDTNGFIDHLASLARLLESRK YILVVPLIVINELDGLAKGQETDHRAGGYARVVQEKARKSIE FLEQRFESRDSCLRALTSRGNELESIAFRSEDTGQLGNND DLILSCCLHYCKDKAKDFMPASKEEPIRLLREVLLTDDRNL RVKALTRNVPVRDIPAFLTWAQVGGSDYKDDDDK (SEQ ID NO:99)
CIRTS-9	ORF5-TBP6.7-GGS ₆ -Pin domain-NES	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVET EYHI NILETSDDEEGSGGSGGSGGSGGSGGSGGSM AVPETRPNHTIY INNLNSKIKKDELKKSLY AIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPFYDKPMRIQYAKTDKRIPAKM KGTFVGS LQLPPLERLTLGGSGGSGGSGGSGGSGGSM E LEIRPLFLVPDTNGFIDHLASLARLLESRK YILVVPLIVINEL DGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDSCL RALTSRGNELESIAFRSEDTGQLGNND DLILSCCLHYCKDKA KDFMPASKEEPIRLLREVLLTDDRNL RVKALTRNVPVRDI PAFLTWAQVGGSDYKDDDDK (SEQ ID NO:100)
CIRTS-10	ORF5-TBP6.7-GGS ₃ -Pin domain	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVET EYHI NILETSDDEEGSGGSGGSGGSGGSGGSGGSM AVPETRPNHTIY INNLNSKIKKDELKKSLY AIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPFYDKPMRIQYAKTDKRIPAKM KGTFVGS LQLPPLERLTLGGSGGSGGSM E LEIRPLFLVPDTN GFIDHLASLARLLESRK YILVVPLIVINELDGLAKGQETDHR AGGYARVVQEKARKSIEFLEQRFESRDSCLRALTSRGNELE SIAFRSEDTGQLGNND DLILSCCLHYCKDKAKDFMPASKE EPIRLLREVLLTDDRNL RVKALTRNVPVRDIPAFLTWAQV GGSDYKDDDDK (SEQ ID NO:101)
CIRTS-11	ORF5-TBP6.7-L8-Pin domain	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVET EYHI NILETSDDEEGSGGSGGSGGSGGSGGSGGSM AVPETRPNHTIY INNLNSKIKKDELKKSLY AIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPFYDKPMRIQYAKTDKRIPAKM KGTFVGS LQLPPLERLTLSGSETPGTSESATPESMELEIRPLF LVPDTNGFIDHLASLARLLESRK YILVVPLIVINELDGLAKG QETDHRAGGYARVVQEKARKSIEFLEQRFESRDSCLRALTS RGNELESIAFRSEDTGQLGNND DLILSCCLHYCKDKAKDF

		MPASKEEPIRLLREVLLTDDRNLRVKALTRNVPVRDIPAF LTWAQVGGSDYKDDDDK (SEQ ID NO:102)
CIRTS-12	ORF5-TBP6.7-helical-Pin domain-NES	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSMAMPETRPNHTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTLGGSGEEEEKKKQEEEEAERLRRI QEEMEKERKRREDEQRRRKEEEEERRMKLEMEAKRKQEE EERKKREDDEKRRKKKSGSGSMELEIRPLFLVPDNGFIDHL ASLARLLESRKYILVVPLIVINELDGLAKGQETDHRAGGYA RVVQEKARKSIEFLEQRFESRDSCLRALTSRGNELESIAFRS EDITGQLGNNDLILSCCLHYCKDKAKDFMPASKEEPIRLL REVLLTDDRNLRVKALTRNVPVRDIPAFLTWAQVGGSDY KDDDDK (SEQ ID NO:103)
CIRTS-13	ORF5-TBP6.7-L8-Y2(100-200)	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSMAMPETRPNHTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTLGGSETPGTSESATPESDAMFGQPG ALGSTPFLGQHGFNFFPSGIDFSAWGNSSSQGSTQSSGYS SNYAYAPSSLGAMIDGQSAFANETLNKAPGMNTIDQGM AAGSDYKDDDDK (SEQ ID NO:104)
CIRTS-14	ORF5-TBP6.7-helical-Y2(100-200)	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSMAMPETRPNHTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTLGGSGEEEEKKKQEEEEAERLRRI QEEMEKERKRREDEQRRRKEEEEERRMKLEMEAKRKQEE EERKKREDDEKRRKKKSGSGSDAMFGQPGALGSTPFLGQH GFNFFPSGIDFSAWGNSSSQGSTQSSGYSSNYAYAPSSLG GAMIDGQSAFANETLNKAPGMNTIDQGMAGSDYKDDDD K (SEQ ID NO:105)
MBP-CIRTS-1	MBP-ORF5-TBP6.7-GGS ₆ -dead Pin domain	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETEHYI NILETSDDEEGSGSGSGSGSGSGSGSMAMPETRPNHTIY INNLNSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAF VIFKEVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKM KGTFFVGSLLPPLERLTLGGSGSGSGSGSGSGSGSMELEI RPLFLVPDNGFIDHLASLARLLESRKYILVVPLIVINELDGL AKGQETDHRAGGYARVVQEKARKSIEFLEQRFESRDSCLR ALTSRGNELESIAFRSEEDITGQLGNNDLILSCCLHYCKDKA KDFMPASKEEPIRLLREVLLTDDRNLRVKALTRNVPVRDI PAFLTWAQVGSSGHHHHHH (SEQ ID NO:106)
CIRTS-7X	β -defensin 3-TBP6.7-GGS ₆ -	MGIINTLQKYCYCRVGGRCVLSCLPKEEQIGKCKSTRGRKC CRRKKGGSGSGSGSGSGSGSGSGSMAMPETRPNHTIYINNL NSKIKKDELKKSLEYAIFSQFGQILDILVPRQRTPRGQAFVIFK EVSSATNALRSMQGFPHYDKPMRIQYAKTDKRIPAKMKG

	hADAR(299-701)	FVGGSGGSGGSGGSGGSLHLDQTPSRQPIPSEGLQLHL PQVLADAVSRLVLGKFGDLTDFSSPHARRKVLAVVMTT GTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCHAEIISR RSLLRFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQ FHL YISTSPCGDARIFSPHEPILEEPADRHPNRKARGQLRTKI ESGEGTIPVRSNASIQTWGVLQGERLLTMSCDKIARWNV VGIQGSLLSIFVEPIYFSSILGSLYHGDHL SRAMYQRISNIED LPPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVGDSAIE VINATTGKDELGRASRLCKHALYCRWMRVHGKVP SHLLR SKITKPNVYHESKLA AKEYQA AKARLFTAFIKAGLGAWVE KPTEQDQFSLTPKRPAATKKAGQAKKKKGS DYKDDDDK (SEQ ID NO:128)
CIRTS-8X	β -defensin 3-TBP6.7-GGS ₆ -hADAR (299-701) E488Q	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKKGGSGGSGGSGGSGGSGGSGGSM A VPETRPNHTIYINNL NSKIKKDELKKS L Y A I F S Q F G Q I L D I L V P R Q R T P R G Q A F V I F K EVSSATNALRSMQGF PFYDKPMRIQYAKTDKRIPAKMKGT FVGGSGGSGGSGGSGGSGGSLHLDQTPSRQPIPSEGLQLHL PQVLADAVSRLVLGKFGDLTDFSSPHARRKVLAVVMTT GTDVKDAKVISVSTGTKCINGEYMSDRGLALNDCHAEIISR RSLLRFLYTQLELYLNNKDDQKRSIFQKSERGGFRLKENVQ FHL YISTSPCGDARIFSPHEPILEEPADRHPNRKARGQLRTKI ESGQGTIPVRSNASIQTWGVLQGERLLTMSCDKIARWN VVIQGSLLSIFVEPIYFSSILGSLYHGDHL SRAMYQRISNIE DLPLYTLNKPLLSGISNAEARQPGKAPNFSVNWTVGDSAI EVINATTGKDELGRASRLCKHALYCRWMRVHGKVP SHLL RSKITKPNVYHESKLA AKEYQA AKARLFTAFIKAGLGAWV EKPTEQDQFSLTPKRPAATKKAGQAKKKKGS DYKDDDDK (SEQ ID NO:129)
CIRTS-9X	β -defensin 3-SLBP-GGS ₆ -NYTHDF1	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKKGGSGGSGGSGGSGGSGGSGGSM A VPETRPNHTIYINNL NSKIKKDELKKS L Y A I F S Q F G Q I L D I L V P R Q R T P R G Q A F V I F K EVSSATNALRSMQGF PFYDKPMRIQYAKTDKRIPAKMKGT FVGSLLPPLERLTLGGSGGSGGSGGSGGSGGSM SATSVDT QRTKGQDNKVQNGSLHQKDTVHDNDFEPYLTGQSNQSNS YPSMSDPYLSSYPPSIGFPYSLNEAPWSTAGDPPIPYLTTY GQLSNGDHHFMHDAVFGQPGGLGNNIYQHRFNFFPENPAF SAWGTSGSQGQQTQSSAYGSSYTYPPSSLGGTVVDGQPGF HSDTL SKAPGMNSLEQGMVGLKIGDVSSSAVKTVGSVVSS VALTGVL SGNNGT NVNMPVSKPTSWAAIASKPAKPQPKM KTKSGPVMGGGLPPPPIKHNM DIGTWDNKGPVPKAPVPQQ APSPQAAPQPQQVAQPLPAQPPALAQPPYQSPQPPQTRW VAPRNRNAAFGQSGGAGSDSN SPGNVQPN SAPSVESGSDY KDDDDK (SEQ ID NO:130)
CIRTS-10X	β -defensin 3-SLBP-GGS ₆ -YTHDF2 (1-200)	MGIINTLQKYYCRVRGGRC AVL SCLPKEEQIGKCSTRGRKC CRRKKGGSGGSGGSGGSGGSGGSGGSA DFETDES VLMRRQKQI NYGKNTIAYDRYIKEVPRHLRQPGIHPKTPNFKKYSRRSW DQQIKLWKVALHFWDLQLPPLERLTLGGSGGSGGSGGSGG SGGSM SASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFE PYLSPQARPNNAYTAMSDSYLPSYSPSIGFSYSLGEAAWS TGGDTAMPYLTSYGQLSNGEPHFLPDAMFGQPGALGSTPF

		LGQHGFNFFPSGIDFSAWGNSSQGQSTQSSGYSSNYAYAP SSLGGAMIDGQSAFANETLNKAPGMNTIDQMAAGSDYK DDDDK (SEQ ID NO:131)
CIRTS- 18X	ORF5-PP7- Y2(100-200)	MDDPSFLTGRSTYAKRRRARRMNVCKCGAILHNNKDCRSS TISGHKLDRLRFVKEGRVALEGETPVYRTWVKWVETHEYHI NILETSDDEEGSGSGSGSGSGSGSGSGMAKTIVLAVGEAT RTLTEIQSTADRQIFEEKVGPLVGRLRLTASLRQNGAKTAY RVNLKLDQADVVDASTSVAGELPKVRYTQVWVSHDVTIVA NSTEASRKSLYDLTKSLVATSQVEDLVVNLVPLGRSLEGG GGMAKTIVLAVGEATRTLTEIQSTADRQIFEEKVGPLVGRL RLTASLRQNGAKTAYRVNLKLDQADVVDASTSVAGELPK VRYTQVWVSHDVTIVANSTEASRKSLYDLTKSLVATSQVED LVVNLVPLGRSLELQLPPLERLTLGGSGSGSGSGSGSGSGG SDAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNSSQG QSTQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPG MNTIDQMAAGSDYKDDDDK (SEQ ID NO:132)

VII. EXAMPLES

[0194] The following examples are included to demonstrate preferred embodiments of the disclosure. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the disclosure, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the disclosure.

Example 1 - Programmable RNA-guided RNA effector proteins built from human parts

A. Introduction

[0195] To overcome the large size and microbial-derived nature of current RNA-targeting systems, the inventors present a CRISPR/Cas-inspired RNA targeting system (CIRTS), a general method for engineering programmable RNA effector proteins. The inventors show that CIRTS permits mining the human proteome for functional parts to build programmable RNA regulatory proteins. CIRTS is a ribonucleoprotein complex that uses Watson-Crick-Franklin base pair interactions to deliver protein cargo site-selectively in the transcriptome. The inventors show they can easily engineer CIRTS that deliver a range of regulatory proteins to transcripts, including nucleases for degradation, deadenylation regulatory machinery for degradation, or translational activation machinery for enhanced protein production. However,

CIRTS are up to 5-fold smaller than the smallest current CRISPR/Cas systems and can be engineered entirely from human parts.

[0196] The inventors reasoned that a minimal programmable RNA-targeting system will need the following components: (1) an RNA hairpin-binding protein that serves as the core of the system and is a selective, high affinity binder to a specific RNA structure displayed on an engineered gRNA, (2) a gRNA that features both the structure that interacts with the engineered hairpin-binding protein and a sequence with complementarity to the target RNA of interest, and (3) an effector protein, such as a nuclease or epitranscriptomic regulator, that acts on the targeted RNA in a proximity-dependent manner. In some embodiments, a charged protein that binds to the displayed gRNA sequence non-specifically is used to stabilize and protect the guiding RNA prior to target engagement. (**FIG. 1A**). CIRTS combines multiple protein domains performing these functions in an engineered system.

[0197] Here, the inventors present the design and validation of CIRTS. First, the inventors engineered a programmable CIRTS ribonuclease, which they used for both *in vitro* and mammalian cell reporter assay optimization and validation. The inventors then demonstrate the versatility of CIRTS by showing that all four of the constituent parts, including the gRNA, the hairpin binding protein, the ssRNA binding protein, and the effector domain of CIRTS-1 can be substituted for other parts and developed five additional CIRTS (**FIG. 1B**). The inventors are able to target endogenous epitranscriptomic regulatory pathways, including degradation machinery and translational activation, using CIRTS engineered as programmable m⁶A reader proteins. Additionally, the inventors show that they can target endogenous transcripts for proximity-dependent nuclease-mediated decay, non-nuclease-mediated decay, or translation activation, using CIRTS. Finally, the inventors show they can target multiple genes simultaneously using orthogonal CIRTS. Taken together, this work validates the CIRTS strategy as a viable new approach to engineer RNA effector proteins that are small and assembled from human parts.

B. Results

1. Development and *in vitro* validation of CIRTS-1

[0198] For the inventor's first-generation system, CIRTS-1, the inventors used an evolved version of the human hairpin-binding protein U1A protein (TBP6.7), which was previously engineered to bind the HIV TAR hairpin and has no endogenous human RNA hairpin targets (Blakeley and McNaughton, 2014; Crawford et al., 2016) (**FIG. 1B**). the inventors designed a gRNA that includes the TAR hairpin structure, a nucleotide linker sequence (L), and then a

guiding sequence (**FIG. 1C**). To develop and validate the system, the inventors first engineered a programmable nuclease by fusing TBP6.7 to the Pin nuclease domain of human SMG6, which has been previously used as a non-specific proximity-dependent RNA endonuclease (Batra et al., 2017; Choudhury et al., 2012). Although this simplest design already displayed gRNA-mediated transcript degradation in cell-based assays (**FIG. 9B, left**), the performance was quite poor, which the inventors attributed to the potential degradation of the displayed targeting sequence. The protein surface and hairpin channel of Cas13 systems tend to be highly charged, likely to non-specifically bind and stabilize the guiding RNA sequence (Liu et al., 2017). To engineer this RNA protection function into the inventor's system, the inventors aimed to add in an RNA binding protein with non-specific, low affinity for single-stranded RNAs. However, the human protein toolbox did not readily contain an annotated small, non-specific single-stranded RNA binding protein. Therefore, the inventors developed CIRTS-1 using a small viral ssRNA binding protein, ORF5 (Zhou et al., 2006). Altogether, CIRTS-1 is composed of a protein fusion complex of ORF5-TBP6.7-Pin nuclease domain along with a corresponding gRNA (**FIG. 1A**).

[0199] The inventors first characterized the programmable RNA binding and RNA nuclease activity of CIRTS-1 *in vitro* on model RNA target substrates. Using purified MBP-CIRTS-1 protein and gRNA in electrophoretic mobility shift assays (EMSA) run in the presence of EDTA to inactivate the nuclease, the inventors found MBP-CIRTS-1 binds a target RNA in a gRNA-dependent manner with an apparent K_D of 105 nM (**FIG. 2A and 2B**). If either the gRNA (**FIG. 7A**) or CIRTS-1 protein (**FIG. 7B**) are omitted from the assay, no measurable binding is detected, confirming the gRNA-dependent binding mode as designed in the system. Moreover, in a cleavage assay, the inventors found that CIRTS-1 degrades an RNA substrate in a gRNA- and Mn^{2+} -dependent manner (**FIG. 2C**). Collectively, these *in vitro* results validate the design principles behind CIRTS-1 and motivated the inventors to attempt to optimize the system for use in live cells.

2. Optimization of CIRTS-1 in live cells

[0200] To test the target nuclease activity of CIRTS-1 in live mammalian cells, the inventors established a dual luciferase reporter assay that reports on gRNA-dependent transcriptional changes on a target RNA (**FIG. 3A**). Using this system, the inventors optimized the deployment of CIRTS-1 by assaying different protein linker types (**FIG. 8A**), gRNA structures (**FIG. 8C**), and CIRTS-1 cellular localization (**FIG. 9C**). After optimization, the inventors compared the ability of optimized CIRTS-1 system to degrade the target reporter RNA to the current state-

of-the-art Cas13 system. The inventors designed gRNAs that target firefly luciferase mRNAs in the dual-luciferase reporter assay for both CIRT-1 and Cas13b, as well as control, off-target gRNAs for each programmable nuclease. To test whether just binding to the transcript alters transcript levels, the inventors engineered CIRT-0, which contains a previously reported
5 'dead' mutation in the nuclease domain of CIRT-1 (Eberle et al., 2009), serving as a negative control. The inventors found that CIRT-0 has no significant effect on the target transcript (**FIG. 3B and FIG. 9A**), indicating the CIRT ribonucleoprotein target RNA complex is minimally perturbative to the RNA of interest. Next, the inventors tested whether CIRT-1, with an active nuclease, could mediate degradation of the target. Indeed, the inventors found
10 gRNA-dependent degradation of the target gene, measured at both the protein level as monitored by luciferase activity (**FIG. 3C**) and the mRNA levels as monitored by RT-qPCR (**FIG 9D**). Encouragingly, the inventors found that CIRT-1 is only slightly less efficient at targeting the luciferase reporter gene as compared to targeted nuclease degradation by Cas13b, indicating the CIRT strategy is a viable method in live cells. Encouraged by the performance
15 of CIRT-1, the inventors next sought to assess the versatility of the design by testing whether the varied system components could be swapped for other parts and maintain CIRT function.

3. Modularity of CIRT

[0201] To explore the versatility of the CIRT design, the inventors next tested different protein domains for each component of the CIRT protein delivery system. First, the inventors
20 assayed whether CIRT could deliver RNA epitranscriptomic regulatory "reader" proteins, which they previously delivered using the dCas13b system (Rauch et al., 2018). The inventors swapped the Pin nuclease effector protein of CIRT-1 for the N-terminal domain of YTHDF1, a cytoplasmic *N*⁶-methyladenosine (m⁶A) reader protein that recruits the translation machinery, to generate CIRT-2. When CIRT-2 is delivered to the same target sequence as the CIRT-1
25 experiments, the RNA levels are relatively unchanged (**FIG. 9E**), but a significant increase in protein levels from the RNA are generated (**FIG. 3D**). The inventors then exchanged the YTHDF1 fragment for a fragment of YTHDF2, an m⁶A reader protein that recruits the RNA deadenylation machinery and induces RNA degradation, to generate CIRT-3. Delivery of
30 CIRT-3 to the reporter mRNA induces degradation of the target transcript as measured by both RNA (**FIG. 9F**) and protein levels (**FIG. 3E**). CIRT-1 through -3 demonstrate the versatility of the design strategy to deliver a range of effector protein cargoes to target RNA in live cells.

[0202] After demonstrating the modularity of the effector domain, the inventors set out to assess if other human parts could also be used for the RNA hairpin binding domain and non-specific ssRNA binding protein. The inventors replaced TBP6.7 in CIRT-3 with the RNA hairpin binding domain of the human histone stem loop binding protein (SLBP) to generate CIRT-4. Additionally, the inventors designed a gRNA based on the histone mRNA stem loop structure (FIG. 1D). Assaying CIRT-4 in the reporter assay (FIG. 3F) and by RT-qPCR to assess RNA levels (FIG. 9G) revealed similar performance as CIRT-3. Finally, the inventors sought to engineer entirely humanized versions of the CIRT system. As stated earlier, the inventors designed the first proof-of-concept systems based on the viral non-specific, single-stranded RNA binding protein, ORF5. Although there are no annotated human single-stranded, non-specific RNA binding proteins, the inventors reasoned highly charged, cationic human proteins could fulfill the role of ORF5 in the CIRT system (Cronican et al., 2011). The inventors therefore engineered HBEGF and β -defensin 3, two cationic human proteins, in the place of ORF5 in CIRT-3 to generate CIRT-5 and CIRT-6, respectively. Again, deploying these programmable effectors in the luciferase reporter assay revealed gRNA-dependent degradation of the target gene (FIG. 3G). The inventors also assayed changes of RNA levels by qPCR and found a decrease for both CIRT-5 and CIRT-6 (FIG. 9H). Collectively, the performance of CIRT-1 through CIRT-6 in the reporter assay demonstrates the modularity of the CIRT design in all dimensions, including the hairpin-binding domain and corresponding gRNA, the single-stranded RNA binding protein, and the effector protein. Next, the inventors sought to test whether the CIRT can also be used to target endogenous transcripts.

4. Targeting endogenous mRNAs with CIRT

[0203] As a first step toward selectively targeting endogenous transcripts, the inventors verified that CIRT-0 could bind a target endogenous transcript by analysis by RNA immunoprecipitation followed by RT-qPCR. The inventors designed gRNAs to target two endogenous transcripts that were previously targeted by Cas13 systems, PPIB, and SMARCA4 (Cox et al., 2017; Konermann et al., 2018). The inventors separately delivered each gRNA along with CIRT-0 fused to a 3x FLAG-tag. The inventors then isolated total RNA, immunoprecipitated with an anti-Flag antibody, and quantified the relative amounts of each target RNA bound to the protein. Indeed, both endogenous transcripts were enriched between 2.5- and 5-fold in a gRNA-dependent manner (FIG. 10A), validating CIRT can function as a programmable RNA-guided RNA binding protein on endogenous transcripts.

[0204] The inventors next sought to assess whether the CIRTS system could deliver an effector protein to a target endogenous transcript, using the CIRTS-1 programmable nuclease and CIRTS-3 programmable YTHDF2-mediated decay systems as exemplars. The inventors selected five RNA transcripts that have been previously validated as Cas13 targets, reasoning that these are accessible for RNA targeting by programmable RNA-binding systems. The inventors then designed gRNAs for each target, using the same binding sites on the targets that were previously used in Cas13 experiments, postulating these sites would also be accessible to CIRTS targeting (Konermann et al., 2018). The inventors assayed the effects of the CIRT system on RNA levels of each target by RT-qPCR. When cells were transfected with either CIRTS-1 or CIRTS-3, along with a specific gRNA expressing vector, the inventors saw a significant decrease in RNA level by RT-qPCR for each of the five endogenous transcripts: PPIB, NFKB1, NRAS, B4GALTN1, and SMARCA4 (**FIG. 4A and 4B**). The relative knockdown efficiency varied for each gene, which is also observed in other RNA-targeting systems and is potentially mediated by accessibility or other regulatory pathways specific to each gene. None-the-less, these results confirm that CIRTS can target endogenous transcripts and mediate decay through either active nuclease activity on the target or by triggering endogenous epitranscriptomic regulatory pathways.

[0205] Next, the inventors sought to assess whether CIRTS-2 could trigger protein production of endogenous transcripts through a YTHDF1-mediated epitranscriptomic pathways. The inventors selected an abundant transcript (CypB, the protein product of PPIB) with a reported, reliable antibody for analysis of protein production by Western blotting. Indeed, cells transfected with CIRTS-2 and an on-target gRNA showed an increase in protein level (**FIG. 4C**) without a change in RNA levels (**FIG. 10B**), confirming the YTHDF1 effect on the transcript. As a control, the same experiment performed with CIRTS-3, which delivers YTHDF2, results in decreased protein levels, which correlates with the decrease in mRNA levels (**FIG. 3B**). Taken together, these experiments show that the CIRTS platform is functional on endogenous transcripts in a gRNA-dependent manner, and can actively degrade a target transcript, trigger degradation machinery to act on the target transcript, or to active translation and increased protein production from the target transcript. The versatility of the CIRTS inspired the inventors to deploy them simultaneously to target more than one transcript.

5. Multiplexed targeting of multiple endogenous RNAs with CIRTs

[0206] Finally, the inventors aimed to assess whether CIRTS engineered with different hairpin binding domains could functionally orthogonally in live cells to selectively target

different transcripts. In principle, the CIRTS built from the TBP hairpin binding domain and the CIRTS built from the SLBP hairpin binding domain, which each use separately engineered gRNAs (FIG. 1C and 1D), should be orthogonal to one another. For these orthogonality tests of multi-targeting CIRTS, the inventors deployed two fully-humanized CIRTS that each deliver YTHDF2, CIRTS-6 and CIRTS-7, which each bind different hairpin structures and should have minimal crosstalk between one another. The inventors aimed to use CIRTS-6 to target PPIB and CIRTS-7 to target SMARCA4 (FIG. 5A). The inventors co-transfected cells with expression vectors for both CIRTS proteins, along with expression vectors for gRNAs for each CIRTS displaying either a control, non-targeted gRNA sequence, or a gRNA sequence targeting the respective endogenous gene. The inventors then assessed changes in relative RNA levels of the two target genes by RT-qPCR. Indeed, each CIRTS system degraded the target transcript in an on-target gRNA-dependent manner, without any crosstalk between the two systems (FIG. 5B). Critically, the inventors were able to selectively degrade one transcript or the other in the presence of both CIRTS proteins and were also able to simultaneously degrade both transcripts.

[0207] RNA degradation of CIRTS-6 showed levels comparable to when only CIRTS-6 is delivered to cells (FIG. 11A), targeting with CIRTS-7, however, resulted in slightly reduced RNA degradation as compared with individual transfection (FIG. 11B). For the multiplexed targeting experiment, the inventors transfected cells sequentially on two consecutive days with a CIRTS and a gRNA vector each day. The inventors attribute this decreased activity to decreased transfection efficiency on the second day of transfection with CIRTS-7. Additionally, while the TBP6.7-based CIRTS have RNA hairpins orthogonal to the endogenous mammalian cell machinery, the SLBP-based constructs could theoretically still bind to cellular histone mRNA, which could lead to a decrease in overall activity. Intriguingly, when both CIRTS were delivered with on-target gRNA, they performed at their best in this assay. At this point, the inventors conclude that the two orthogonal CIRTS systems can each be orthogonal to one another and can each simultaneously target endogenous transcripts in a gRNA-dependent manner. the inventors are currently working on engineering orthogonal CIRTS that have no more endogenous binding partners and can act without potentially perturbing cellular events.

C. Discussion

[0208] In summary, here the inventors presented CIRTS, a new strategy for engineering programmable RNA effector proteins. CIRTS are small, can be fully humanized, can target

endogenous RNAs in live cells, and can work orthogonally and synergistically together for multidimensional control. As research tools, CIRTS should provide advantages to previous methods because of their smaller size. For example, CIRTS-2 and CIRTS-3 are 65 and 36 kDa respectively, while the comparable Cas13b-based programmable YTHDF1 and YTHDF2 systems are 155 and 126 kDa, respectively (**FIG. 6**). CIRTS-1 is even smaller than the smallest DNA-targeting Cas protein found to date, Cas14a (Harrington et al., 2018). The smaller size of the delivery system should be less perturbative to the endogenous transcript under study presenting opportunities for uncovering the roles of RNA regulatory proteins in live cells.

[0209] From a translational perspective, the CIRTS should offer several key advantages and opportunities. The humanized nature of the CIRTS will provide a pathway toward avoiding immune responses, opening up the potential for continuously-delivered therapies. With respect to using accessory proteins such as human β -defensin 3, this protein has been extensively studied and the structural bases for its function has been elucidated (Dhople et al., 2006; Kluver et al., 2005). This knowledge will enable one skilled in the art to engineer a β -defensin 3 peptide that retains its charged nature but abolishes its endogenous functions. Currently, the small size of the CIRTS will still allow for multiple regulatory proteins to be simultaneously delivered in a viral delivery system, for example to target one transcript for degradation and another for translational activation. The multiplexable capacity of the CIRTS couple with the small size and diversity of effector proteins that can be delivered opens up possibilities for cell reprogramming by targeting multiple genes at once in multiple dimensions.

[0210] From a broader perspective, the CIRTS platform demonstrates the potential of combining parts contained within the human protein toolbox to engineer proteins with new properties. The CIRTS system provides a new approach for studying and exploiting RNA regulation and will open up many future opportunities to intervene in cell regulation for disease treatment.

Example 2 - Programmable RNA-guided RNA effector proteins built from human parts

A. RESULTS

1. Design of CIRTS

[0211] While DNA-targeting Cas9-based systems employ complex biophysical mechanisms to unwind DNA and anneal to a target sequence (Rutkauskas et al., 2015; Sternberg et al., 2014; Szczelkun et al., 2014), mechanistic studies of Cas13 showed that RNA targeting is initiated by a central seed region in the gRNA (Liu et al., 2017). Additionally, Cas13 systems display substantial variability in sequence context targetability on individual

transcripts (Abudayyeh et al., 2017; Cox et al., 2017; Konermann et al., 2018). Together these findings suggest that sequence complementarity between the gRNA and targeted transcript, as well as the accessibility of a given site, are key requirements for RNA targeting. The inventors sought to engineer a Cas13-inspired system that uses a defined protein-RNA interaction to display a gRNA sequence to deliver protein cargoes to a target RNA, similar to previous RNA tethering assays with overexpressed reporter constructs (Coller and Wickens, 2007). Indeed, hairpin-binding proteins and covalent RNA fusions have been used to deliver RNA editing machinery to transcripts (Montiel-Gonzalez et al., 2016; Sinnamon et al., 2017; Vogel et al., 2018).

[0212] Based on the current characterization of Cas13 (Abudayyeh et al., 2017; Cox et al., 2017; Gootenberg et al., 2018; Konermann et al., 2018; Liu et al., 2017), the inventors reasoned that a minimal programmable RNA-targeting system may have four components: (1) an RNA hairpin-binding protein that serves as the core of the system and is a selective, high affinity binder to a specific RNA structure displayed on an engineered gRNA, (2) a gRNA that features both the structure that interacts with the engineered hairpin-binding protein and a sequence with complementarity to the target RNA of interest, (3) a charged protein that could bind to the displayed gRNA sequence non-specifically to stabilize and protect the guiding RNA prior to target engagement, and (4) an effector protein, such as a ribonuclease or epitranscriptomic regulator, that acts on the targeted RNA in a proximity-dependent manner (FIG. 12A). While Cas13 houses all of these functional components in a single protein domain (Liu et al., 2017; Tambe et al., 2018), the inventors envisioned engineering a system that combines multiple protein domains that each perform one of these functions, which the inventors termed CRISPR/Cas-inspired RNA targeting system (CIRTS). CIRTS vary in their module composition and are uniquely numbered as listed in FIG. 12A and FIG. 21.

2. Development and in vitro validation of CIRTS-1

[0213] For the first-generation system, CIRTS-1, the inventors used an evolved version of the human hairpin-binding protein U1A protein (TBP6.7), which was previously engineered to bind the HIV trans-activation response (TAR) hairpin and has no endogenous human RNA hairpin targets (Blakeley and McNaughton, 2014; Crawford et al., 2016) (FIG. 12B). The inventors designed a gRNA that includes the TAR hairpin, a nucleotide linker sequence (L), and then a guiding sequence (FIG. 12C). To develop and validate the system, the inventors first engineered a programmable ribonuclease by fusing TBP6.7 to the Pin nuclease domain of human nonsense-mediated mRNA decay factor SMG6, which has been previously used as a

non-specific proximity-dependent RNA endonuclease (Batra et al., 2017; Choudhury et al., 2012). Although this simplest design already displayed promising gRNA-mediated transcript degradation in cell-based luciferase assays (FIG. 22A, left), the performance was quite poor, which the inventors attributed to the degradation of the displayed guiding sequence. The protein surface and hairpin channel of Cas13 systems tend to be highly charged, likely to non-specifically bind and stabilize the guiding RNA sequence (Liu et al., 2017). To engineer this RNA protection function into the system, the inventors aimed to add a non-specific, low affinity single-stranded RNA binding protein (ssRNA binding protein). However, the human proteome did not readily contain an annotated small, non-specific single-stranded RNA binding protein to the best of the knowledge of the inventors. Therefore, the inventors developed CIRTS-1 using a small viral ssRNA binding protein, ORF5 (Zhou et al., 2006). Altogether, CIRTS-1 is composed of a protein fusion complex of ORF5-TBP6.7-Pin nuclease domain along with a corresponding gRNA.

[0214] The inventors first characterized the programmable RNA binding and RNA nuclease activity of CIRTS-1 in vitro on model RNA target substrates. The Pin nuclease domain was previously shown to be active in the presence of Mn^{2+} and activity could be quenched by the addition of EDTA (Choudhury et al., 2012). Directly overexpressing CIRTS-1 led to insoluble protein in the cell pellet, which the inventors resolved by fusing an N-terminal MBP tag to CIRTS-1 (MBP-CIRTS-1). Using purified MBP-CIRTS-1 protein and gRNA in filter binding assays, the inventors found MBP-CIRTS-1 binds a target RNA in a gRNA-dependent manner with an apparent binding dissociation constant (KD) of 22 nM (FIG. 13A). Critically, if the inventors provide the system with a non-targeting gRNA, the inventors see ~ 50-fold weaker binding. Moreover, in a cleavage assay, the inventors found that MBP-CIRTS-1 degrades an RNA substrate in a gRNA- and Mn^{2+} -dependent manner, confirming the activity of the Pin ribonuclease domain in the fusion context (FIG. 13B). Collectively, these in vitro results validate the design principles behind CIRTS-1 and motivated the inventors to optimize the system for use in live cells.

3. Optimization of CIRTS-1 in live cells

[0215] To test the target nuclease activity of CIRTS-1 in live mammalian cells, the inventors established a dual luciferase reporter assay that reports on gRNA-dependent transcriptional changes on a target firefly luciferase (Fluc) RNA (FIG. 14A). Using this system, the inventors optimized the deployment of the Pin nuclease CIRTS by assaying different protein linker types (FIG. 23A), gRNA structures and lengths (FIG. 23C), and CIRTS nuclease cellular localization

(FIG. 22B). The inventors found that a long flexible linker between the hairpin-binding protein and 40 nucleotide long gRNA resulted in the best knockdown efficiency. After optimization, the inventors compared the ability of optimized CIRT-1 (Pin nuclease) system to degrade the target reporter RNA to the Cas13b system (Cox et al., 2017). The inventors designed gRNAs that target the firefly luciferase mRNA in the dual-luciferase reporter assay for both CIRT-1 and Cas13b, as well as control, non-targeting gRNAs (targeting a lambda phage sequence) for each programmable nuclease. To test whether binding to the transcript alters protein levels, the inventors engineered CIRT-0, which contains a previously reported ‘dead’ mutation in the Pin nuclease domain of CIRT-1 (Eberle et al., 2009), serving as a negative control. The inventors found that CIRT-0 has no significant effect on the expression level of the target transcript (FIG. 14B and FIG. 22C), indicating binding by the CIRT ribonucleoprotein to a target RNA is minimally perturbative to the targeted transcript. Next, the inventors tested whether CIRT-1, containing an active nuclease, could mediate degradation of the target. Indeed, the inventors found gRNA-dependent degradation of the target Fluc mRNA, measured at both the protein level as monitored by luciferase activity (FIG. 14C) and the mRNA levels as monitored by RT-qPCR (FIG. 22D). Both results suggest the CIRT strategy is a viable method in live cells. Although CIRT-1 is less efficient at targeting the reporter gene as compared to Cas13b, the performance was not dramatically different, especially considering that Cas13b systems have evolved to perform this knockdown function. Encouraged by the performance of CIRT-1 (Pin nuclease), the inventors next sought to assess the versatility of the design by testing whether each component of the system, including the gRNA, hairpin binding domain, non-specific RNA binding domain, and effector protein, could be swapped for other parts to achieve CIRT with diverse functions.

4. Modularity of CIRT on Reporter Transcripts

[0216] To explore the versatility of the CIRT design, the inventors next tested different protein domains for each component of the CIRT protein delivery system. First, the inventors assayed whether CIRT could deliver RNA epitranscriptomic regulatory “reader” proteins, which the inventors previously delivered using the dCas13b system (Rauch et al., 2018). For the study, the inventors chose to focus on regulatory proteins of N6-methyladenosine, the most prevalent mRNA modification. On average each transcript contains three modifications sites with high m6A abundance detected in the 3’UTR, and m6A has been shown to have regulatory roles in splicing (Xiao et al., 2016), translation (Meyer et al., 2015; Wang et al., 2015), and stability (Du et al., 2016; Wang et al., 2013). The inventors exchanged the Pin nuclease effector

protein of CIRTS-1 for the N-terminal domain of the YT521-B homology domain family protein 1 (YTHDF1), a cytoplasmic m6A reader protein that recruits the translation machinery (Wang et al., 2015), to generate CIRTS-2. Note that the inventors' current design does not include the C-terminal YTH domain of YTHDF1 that recognizes m6A to simplify studies of reader proteins in an m6A-independent manner and to avoid complications from varying m6A levels in cellular mRNAs when validating CIRTS. When CIRTS-2 (YTHDF1) is delivered to the same target sequence as the CIRTS-1 experiments, the RNA levels are relatively unchanged (FIG. 22E), but a significant increase in protein levels from the RNA is generated (FIG. 14D), as expected based on the known translation-activating role of YTHDF1 (Wang et al., 2015). The inventors then exchanged the YTHDF1 fragment for a fragment of YTHDF2, a different m6A reader protein that recruits the RNA deadenylation machinery and induces RNA degradation (Du et al., 2016; Wang et al., 2013), to generate CIRTS-3. Delivery of CIRTS-3 (YTHDF2) to the reporter mRNA induces degradation of the target transcript as measured by both RNA (FIG. 22F) and protein levels (FIG. 14E). CIRTS-1 through -3 demonstrate the versatility of the design strategy to deliver a range of effector protein cargoes to target RNA in live cells.

[0217] After demonstrating the modularity of the effector domain, the inventors set out to assess if other human parts could also be used for the RNA hairpin binding domain and non-specific ssRNA binding protein. The inventors replaced TBP6.7 in CIRTS-3 (YTHDF2) with the RNA hairpin binding domain of the human histone stem loop binding protein (SLBP) to generate CIRTS-4 (YTHDF2). Concurrently, the inventors designed a gRNA based on the histone mRNA stem loop structure (FIG. 12D). Assaying CIRTS-4 (YTHDF2) in the reporter assay (FIG. 14F) and by RT-qPCR to assess RNA levels (FIG. 22G) revealed similar performance as CIRTS-3, confirming other hairpin binding domains can be used as the core of the CIRTS.

[0218] Next, the inventors sought to engineer entirely humanized versions of the CIRTS system. As stated earlier, the inventors designed the initial proof-of-concept systems based on the viral non-specific, single-stranded RNA binding protein, ORF5. Although there are no annotated human single-stranded, non-specific RNA binding proteins, the inventors reasoned highly charged, cationic human proteins could fulfill the role of ORF5 in the CIRTS system (Cronican et al., 2011). The inventors therefore engineered HBEGF and β -defensin 3, two cationic human proteins, in the place of ORF5 in CIRTS-3 to generate CIRTS-5 and CIRTS-6, respectively. Again, deploying these programmable effectors in the luciferase reporter assay

revealed gRNA-dependent degradation of the target gene (FIG. 14G and S2H) mediated by the YTHDF2 epitranscriptomic regulation.

[0219] Finally, the inventors used CIRT5 to deliver the catalytic domain of human ADAR2 (hADAR2) to RNA transcripts to confirm CIRT5' versatility in scope of functions with an additional effector protein. The inventors designed a dual luciferase reporter that contains a G-to-A mutation in the coding region of firefly luciferase resulting in a premature stop of translation and no measurable firefly luciferase activity (FIG. 15A and 22J). The inventors then deployed CIRT5 to deliver wt ADAR2 (CIRT5-7) or hADAR 2 E488Q (CIRT5-8), a known hyperactive mutant of hADAR2 (Kuttan and Bass, 2012), to the mutated position, which resulted in gRNA-dependent rescue of luciferase activity in both cases (FIG. 15B). The hyperactive hADAR2 mutant showed higher editing efficiency and a higher background in the absence of an on-target gRNA based on luciferase assay. However, using the hyperactive mutant could be beneficial to allow targeting of a wider substrate scope as it has relaxed sequence constraints (Kuttan and Bass, 2012). Collectively, the performance of these various CIRT5 in the reporter assays demonstrates the modularity of the CIRT5 design, including the hairpin-binding domain and corresponding gRNA, the single-stranded RNA binding protein, and the effector protein.

5. Targeting endogenous mRNAs with CIRT5

[0220] The inventors next sought to assess whether the CIRT5 system could deliver an effector protein to a target endogenous transcript, using the CIRT5-1 programmable nuclease and CIRT5-3 programmable YTHDF2-mediated decay systems as exemplars. The inventors selected five RNA transcripts that have been previously validated as Cas13 targets, reasoning that these are accessible for RNA targeting by programmable RNA-binding systems. The inventors then designed gRNAs for each target, using the same binding sites on the targets that were previously used in Cas13 experiments (Abudayyeh et al., 2017; Konermann et al., 2018). The inventors assayed the effects of the CIRT system on RNA levels of each target by RT-qPCR. When cells were transfected with either CIRT5-1 or CIRT5-3, along with a specific gRNA expressing vector, the inventors observed a significant decrease in RNA level by RT-qPCR for each of the five endogenous mRNA transcripts: PPIB, NFKB1, NRAS, B4GALNT1, and SMARCA4 (FIG. 16A and 16B). In addition to targeting mRNA, the inventors also verified that the inventors can target other RNA species such as lncRNA by targeting CIRT5-1 (Pin nuclease) to MALAT1 (FIG. 24A). The relative knockdown efficiency varied for each gene, which is also observed in other RNA-targeting systems and is potentially mediated by

accessibility or other regulatory pathways specific to each gene. Nonetheless, these results confirm that CIRT5 can target endogenous transcripts and mediate decay through either active nuclease activity on the target or by triggering endogenous epitranscriptomic regulatory pathways.

5 [0221] Next, the inventors set out to assess whether CIRT5-2 could trigger protein production of an endogenous transcript through a YTHDF1-mediated epitranscriptomic pathways. The inventors selected an abundant transcript PPIB with a reported, reliable antibody for analysis of CypB (the protein product of PPIB) protein production by Western blotting. Indeed, cells transfected with CIRT5-2 and an on-target gRNA showed an increase in protein
10 level (FIG. 16C and FIG. 24C) without a change in RNA levels (FIG. 24B), consistent with prior reported YTHDF1 effects on the transcript (Wang et al., 2015). As a control, the same experiment performed with CIRT5-3, which delivers YTHDF2, results in slight decrease in protein levels, which correlates with the decrease in mRNA levels (FIG. 16B and 24C).

[0222] Finally, as a first test of transcript position-specific effects, the inventors tiled
15 gRNAs along the SMARCA4 mRNA and tested YTHDF2-mediated decay by CIRT5-3. The inventors found dramatically different performance of the system depending on where the gRNA lands on the targeted mRNA (FIG. 17), which is likely the result of both CIRT5 binding accessibility and the regulatory protein sequence requirements. Taken together, these experiments show that the CIRT5 platform is functional on endogenous transcripts in a gRNA-
20 dependent manner, and can actively degrade a target transcript, trigger degradation machinery to act on the target transcript, or activate translation and increase protein production from the target transcript.

6. Targeting Specificity of CIRT5

[0223] To gain insights into how specific CIRT5 is at targeting RNA substrates, the
25 inventors designed a series of experiments that address the sensitivity of the gRNA to mismatches, transcriptome-wide off-targets, and endogenous substrate targeting. To assess mismatch tolerance, the inventors designed a luciferase-based mismatch experiment that allows the inventors to assay targeting effects when introducing one, two, or three mismatches into the duplex formed between gRNA and target RNA. The inventors chose to fuse the disease-
30 relevant KRAS4b transcript to the luciferase reporter and asked whether the engineered system can differentiate between the cancer-associated G12D (target 1), the wild type (target 2), the G12C (target 3), and a G12W (target 4) KRAS4b variants (FIG. 25A). The inventors found that CIRT5 yields comparable knockdown of the G12D and wild type variants indicating that

one mismatch does not cause large changes in targeting specificity (FIG. 25B). However, when the inventors targeted the system to the G12C and G12W reporters, which contain two and three mismatches respectively, CIRT5 knockdown efficiency decreased.

[0224] The inventors next assessed whether increasing the gRNA length could affect the mismatch tolerance of CIRT5, focusing on mismatches in the center region of the duplex formed between gRNA and target RNA as they showed the largest effect on knockdown efficiency in the assay. As observed with the shorter 20 nt gRNA, the inventors see no difference in knockdown efficiency when the inventors target a reporter with no or one mismatches. However, a longer 40 nt gRNA can rescue some of the effects when the two-mismatch variant was targeted, indicating that the gRNA length contributes to the specificity of the system (FIG. 25D). As a comparison to existing technology, the inventors subjected Cas13b to the same mismatch assay, which showed that Cas13b is less sensitive to mismatches in general. Targeting Cas13b to reporters with one and two mismatches yielded little change in knockdown efficiency, while three mismatches led to a substantial decrease in knockdown efficiency (FIG. 25C). Both Cas13b and CIRT5 are most sensitive to mismatched base-pairing in the center of the duplex formed between gRNA and target RNA, a finding that agrees well with previous studies of Cas13b (Abudayyeh et al., 2017).

[0225] To assess transcriptome-wide off-targets, the inventors subjected the system to RNA sequencing. The inventors assayed effects of the CIRT5 Pin nuclease and CIRT5 YTHDF2 targeting the endogenous transcript SMARCA4 (FIG. 25E and 25F). In both cases, the inventors find no statistically significant off-targets. However, while the inventors see knockdown of the targeted transcript and even statistically significant knockdown by CIRT5-3 (YTHDF2) when the inventors look at the target transcript only ($p\text{val} < 0.1$), the knockdown levels do not fall into a statistically significant region when evaluated in a transcriptome-wide manner ($q\text{val} < 0.1$). At this point the inventors conclude that overexpression of the system does not introduce large perturbations to the transcriptome, and taken together with the inventors' mismatch studies, indicates that the targeting specificity and therefore knockdown efficiency can be further optimized by further optimization in future studies.

[0226] To verify CIRT5 bind the transcript of interest, the inventors furthermore performed RNA immunoprecipitation followed by RT-qPCR. The inventors designed gRNAs to target two endogenous transcripts that were previously targeted by Cas13 systems, PPIB, and B4GALNT1 (Cox et al., 2017; Konermann et al., 2018). The inventors separately delivered each gRNA along with CIRT5-0 (dead nuclease CIRT5) fused to a 3x FLAG-tag. The inventors then subjected lysates to immunoprecipitation with an anti-Flag antibody, and

quantified the relative amounts of each target RNA bound to the protein. Indeed, both endogenous transcripts were enriched between 2.5- and 5-fold in a gRNA-dependent manner (FIG. 25H), confirming CIRTS function as a programmable RNA-guided RNA binding protein on endogenous transcripts.

5 **7. Multiplexed targeting of multiple endogenous RNAs with CIRTS**

[0227] Together the targeting specificity and the modularity of CIRTS inspired the inventors to extend the application of CIRTS in a multiplexed targeting manner. Rather than delivering a single effector protein and targeting a single transcript at a time, the inventors set out to test whether CIRTS can target more than one transcript, or deliver more than one effector protein in the same sample. In principle, CIRTS built from the TBP hairpin binding domain and CIRTS built from the SLBP hairpin binding domain, which each use separately engineered gRNAs (FIG. 12C and 12D), should be orthogonal to one another, permitting selective targeting of multiple transcripts with either the same or even different CIRTS.

[0228] First, the inventors tested whether a single CIRTS can be used to simultaneously target multiple transcripts. The inventors co-transfected cells with CIRTS-6 along with three gRNAs targeting PPIB, SMARCA4, and NRAS and assessed changes in RNA level by RT-qPCR. As expected, the inventors observed a decrease in RNA levels for all three targeted transcripts (FIG. 18A and 18B). However, the inventors observed a slight decrease in efficiency when the inventors deploy several gRNAs or CIRTS in the same cells. The inventors attribute this decrease to simultaneous transfection of cells with four plasmids. Further optimization of the protein and gRNA levels and vectors are likely required to regain the performance of the single CIRTS/gRNA experiments.

[0229] To test whether two different types of effectors can be used simultaneously, the inventors next deployed both CIRTS-9, a fully humanized version of the YTHDF1 construct, to target firefly luciferase and CIRTS-10 (YTHDF2) to target SMARCA4 (FIG. 18C and 18D). The inventors find that both proteins are active and induce the anticipated increase in luciferase protein and decrease in RNA levels respectively. Moreover, to further corroborate the orthogonality of multiple-targeting CIRTS, the inventors deployed two CIRTS, CIRTS-6 (TBP6.7) and CIRTS-10 (SLBP) (FIG. 26A and 26B), but use different hairpin-binding modules, to deliver YTHDF2 to two different endogenous target mRNAs (FIG. 27A). Each CIRT system degraded the target transcript in an on-target gRNA-dependent manner, with minimal crosstalk between the two systems (FIG. 27B).

[0230] At this point, the inventors conclude that the TBP6.7 and SLBP-based CIRTSS can each simultaneously target endogenous transcripts in a gRNA-dependent manner. The inventors are currently working on engineering orthogonal CIRTSS that have no endogenous binding partners by evolving human proteins toward new specificities, as was done with TBP6.7. Although not human-derived, the inventors found that other hairpin-binding systems, such as PP7, can also be used to generate CIRTSS, suggesting it is possible to generate a range of selective and orthogonal systems (FIG. 22I). CIRTSS allow for multiple regulatory proteins to be simultaneously delivered, for example to target one transcript for degradation and another for translational activation, opening up possibilities for cell reprogramming by targeting multiple genes at once in multiple dimensions (Bao et al., 2017; Gao et al., 2016).

8. Viral Delivery of CIRTSS by AAV

[0231] Aside from the human-derived nature of CIRTSS, another core advantage is the small size of CIRTSS, which should permit more efficient viral packaging and delivery. Adenovirus-associated virus (AAV) is a versatile delivery vehicle to deliver transgenes and gene therapies to different cell types due to wide range of serotypes available (Gao et al., 2005), low immune response stimulation (Vasileva and Jessberger, 2005), and low risk of genome insertion (Gao et al., 2005; Naso et al., 2017). However, it has been challenging to package and deliver many Cas13 proteins due to a limited packaging capacity of about 4.7 kb (Wu et al., 2010). To showcase the possibility of CIRTSS to be delivered by AAV, the inventors designed a dual CIRTSS-6/gRNA transfer plasmid and packaged it in the AAV delivery vehicle. The total insert, including the CIRTSS protein and gRNA, is only 2.7 kb (FIG. 19A). The inventors found that transduction of HEK293T cells with the generated virus recapitulates the knockdown efficiency of CIRTSS-6 on both the luciferase reporter as well as an endogenous target (FIG. 19B and 19C), confirming viral-delivered CIRTSS are still functional and providing a pathway toward clinical deployment. In future applications, one could imagine packing more than one CIRT system into the AAV delivery vehicle to simultaneously target one transcript for upregulation and one transcript for degradation as previously shown by transient transfection.

B. DISCUSSION

[0232] In summary, here the inventors presented CIRTSS, a versatile strategy for engineering programmable RNA effector proteins. CIRTSS are small, can be fully humanized, can target endogenous RNAs in live cells, and can work for multidimensional transcriptome control. As research tools, CIRTSS should provide advantages to previous methods because of their smaller size. For example, CIRTSS-2 and CIRTSS-3 are 65 and 36 kDa respectively, while the

comparable Cas13b-based programmable YTHDF1 and YTHDF2 systems are 155 and 126 kDa, respectively (FIG. 20). CIRT-1 is even smaller than the smallest DNA-targeting Cas protein found to date, Cas14a and the smallest Cas12g RNA-targeting protein (Harrington et al., 2018; Yan et al., 2019).

5 **[0233]** From a translational perspective, CIRT should offer several key advantages and opportunities. The humanized nature of the CIRT will provide a pathway toward avoiding immune responses, opening up the potential for continuously-delivered therapies. While the fusions between the human proteins in the CIRT present potential limitations in the design where the immune system could respond to (Glaesner et al., 2010), this is a problem that can
10 in principle be engineered around. When the inventors computationally predicted the immunogenicity of the highest likelihood MHC I binding peptides in the inventors' engineered constructs, the inventors find that the fully humanized CIRT shows lower propensity to cause immune reactions (FIG. 27C), but further experimental testing is needed to discover where the limitations in the design emerge.

15 **[0234]** Several known challenges remain with the current CIRT system. First, the alternative hairpin binding protein SLBP in its current form has an endogenous RNA hairpin binding partner, which could influence stem loop RNA trafficking. To minimize endogenous effects of the inventors' fusion constructs, the inventors only included the minimal RNA recognition motif (RRM) necessary for hairpin recognition in the system and omitted regions
20 of potential interactions with other proteins or nucleic acids. Likewise, the inventors tried to keep the required RNA hairpin as small as possible to avoid potential endogenous interactions. The stem loop hairpin was already very short and could not be further truncated but the inventors chose to only use the minimally required region necessary for TBP6.7 binding to the TAR hairpin, resulting in a gRNA with less than half the original hairpin length. Second, the
25 cationic peptide, β -defensin 3, in its current form can theoretically still interact with its intracellular binding partners and elicit unwanted biological responses. However, human β -defensin 3 has been extensively studied and the structural bases for its function has been elucidated (Dhople et al., 2006; Kluver et al., 2005). The inventors can leverage this knowledge as the basis to engineer β -defensin 3 mutants that retain the highly charged nature required for
30 CIRT, but abolish endogenous functions, in order to engineer a human part-based, orthogonal RNA targeting system.

[0235] From a broader perspective, the CIRT platform demonstrates the potential of combining parts contained within the human protein toolbox to engineer proteins with new properties. The presented CIRT were created through minimal protein engineering and

optimization efforts, but function nearly as well as the naturally-evolved CRISPR/Cas systems. In particular, when the inventors compared the Cas13b-based knockdown by its endogenous nuclease and by delivering YTHDF2 (FIG. 26C) to CIRT5-mediated knockdown, the inventors find that while the Cas13b nuclease performs substantially better than CIRT5 nuclease, the
5 inventors see no difference in knockdown mediated by YTHDF2, suggesting CIRT5-3 (YTHDF2) in its current state can already be used to study the epitranscriptome effectively. Further work optimizing the CIRT5 using directed evolution will likely yield variants that have improved performance in mammalian systems (Hu et al., 2018). Understanding target site design and effector protein contextual requirements will also improve CIRT5 system
10 performance, and a better understanding of the epitranscriptomic pathways being exploited will allow the inventors to design better systems. Additionally, there are a range of other regulatory proteins contained within the human proteome that likely house unique RNA control properties (Dominguez et al., 2018), which can be coupled with CIRT5 to create programmable versions of each protein for both functional characterization and potential translational applications. The
15 CIRT5 system provides a new approach for studying and exploiting RNA regulation and will open up future opportunities to intervene in cell regulation for disease treatment.

* * *

[0236] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods
20 of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein
25 while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

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30 details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS

1. A RNA regulatory system comprising at least one of each:
 - i) a RNA hairpin binding domain;
 - ii) a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i; and
 - iii) a RNA regulatory domain.
2. The system of claim 1, wherein the RNA hairpin binding domain and the RNA regulatory domain are operably linked.
3. The system of claim 1, wherein parts i), ii), and/or iii) are human or are human-derived.
4. The system of claim 2 or 3, wherein i) and iii) are operably linked through a peptide bond.
5. The system of claim 2 or 3, wherein i) and iii) are operably linked through non-covalent interactions.
6. The system of any one of claims 1-5, wherein the RNA regulatory domain is covalently linked to a first dimerization domain and the RNA hairpin binding domain is covalently linked to a second dimerization domain and wherein the first and second dimerization domain are capable of dimerizing to form a non-covalent or covalent linkage.
7. The system of claim 6, wherein the dimerization is inducible.
8. The system of claim 7, wherein the dimerization comprises ligand-induced dimerization.
9. The system of claim 8, wherein one of the first or second dimerization domain comprises PYR/PYR1-like (PYL1), the other of the first or second domain comprises ABA insensitive 1 (ABI1), and the ligand comprises abscisic acid (ABA) or derivatives or fragments thereof.
10. The system of claim 8, wherein the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof.

11. The system of claim 8, wherein one of the first or second dimerization domains comprises FK506 binding protein (FKBP), the other of the first or second domain comprises FKBP-Rap binding domain of mammalian target of Rap mTOR (Frb), and the ligand comprises rapamycin (Rap) or derivatives or fragments thereof.
12. The system of any one of claims 1-11, wherein ii) comprises at least two hairpin structures.
13. The system of any one of claims 1-12, wherein ii) comprises one or more modified nucleotides.
14. The system of any one of claims 1-13, wherein the system further comprises a stabilizer polypeptide; wherein the stabilizer polypeptide comprises a cationic polypeptide that binds non-specifically to nucleic acids.
15. The system of claim 14, wherein the stabilizer polypeptide is human-derived.
16. The system of any one of claims 1-15, wherein the total size of the system is less than 150 kDa.
17. The system of any one of claims 1-16, wherein i) comprises U1A, SLBP, or variants thereof.
18. The system of any one of claims 1-17, wherein ii) comprises a TAR hairpin scaffold of SEQ ID NO:1.
19. The system of any one of claims 1-18, wherein ii) comprises a SLBP hairpin scaffold of SEQ ID NO:2.
20. The system of any one of claims 1-19, wherein ii) comprises a linker.
21. The system of claim 20, wherein the linker is at least 5 amino acids.
22. The system of any one of claims 1-21, wherein the RNA targeting region comprises at least 12 nucleotides.
23. The system of any one of claims 1-22, wherein iii) comprises a nuclease, methylase, demethylase, translational activator, translational repressor, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, or RNA binding activity.

24. The system of any one of claims 1-23, wherein iii) comprises a Pin nuclease domain or a m6A reader protein or portion thereof.
25. The system of claim 24, wherein iii) comprises YTHDF1, YTHDF2, or ADAR.
26. The system of any one of claims 14-25, wherein the stabilizer protein comprises HBEGF, beta-defensin, or variants or portions thereof.
27. The system of any one of claims 1-26, wherein the RNA targeting region of ii) hybridizes to a target RNA in a prokaryotic or eukaryotic cell.
28. The system of any one of claims 1-27, wherein i) and/or iii) comprises one or more nuclear localization signals (NLS)s.
29. The system of any one of claims 1-28, wherein the system comprises at least two of each i, ii, and iii.
30. The system of any one of claims 1-29, wherein the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.
31. A vector system comprising one or more nucleic acid vectors comprising a nucleotide encoding: i) a RNA hairpin binding domain; ii) a RNA targeting molecule comprising a RNA targeting region and at least one hairpin structure, wherein the hairpin structure of the RNA targeting molecule specifically binds to i), and iii) a RNA regulatory domain.
32. The vector system of claim 31, wherein the RNA hairpin binding domain and the RNA regulatory domain are operably linked.
33. The vector system of claim 31 further comprising a regulatory element operably linked to the nucleotide encoding i, ii, and/or iii.
34. The vector system of claim 31 or 33, wherein the one or more nucleic acid vectors are optimized for expression in an eukaryotic cell.
35. The vector system of any one of claims 31-34, wherein the expression is constitutive or conditional.

36. The vector system of any one of claims 31-35, wherein i, ii, and iii are on a single vector.
37. The vector system of any one of claims 31-36, wherein one or more of the vectors are viral vectors.
38. The vector system of claim 31-37, wherein the one or more vectors comprise one or more retroviral, lentiviral, adenoviral, adeno-associated or herpes simplex viral vectors.
39. The vector system of any one of claims 31-36, wherein one or more of the vectors are non-viral vectors.
40. A conjugate comprising a RNA regulatory domain operably linked to a RNA targeting molecule, wherein the RNA targeting molecule comprises a RNA targeting region and at least one hairpin structure.
41. The conjugate of claim 40, wherein the RNA regulatory domain is human derived.
42. The conjugate of claim 40 or 41, wherein the RNA regulatory domain and the RNA targeting molecule are operably linked through a peptide bond.
43. The conjugate of any one of claims 40-42, wherein the polypeptide further comprises one or more linkers.
44. The conjugate of claim 40 or 41, wherein the RNA regulatory domain and the RNA targeting molecule are operably linked through non-covalent interactions.
45. The conjugate of any one of claims 40-44, wherein the RNA regulatory domain is covalently linked to a first dimerization domain and the RNA targeting molecule is covalently linked to a second dimerization domain and wherein the first and second dimerization domain are capable of dimerizing to form a non-covalent or covalent linkage.
46. The conjugate of claim 45, wherein the dimerization is inducible.
47. The conjugate of claim 46, wherein the dimerization comprises ligand-induced dimerization.
48. The conjugate of claim 47, wherein one of the first or second dimerization domains comprises PYR/PYR1-like (PYL1), the other of the first or second domain comprises ABA

insensitive 1 (ABI1), and the ligand comprises abscisic acid (ABA) or derivatives or fragments thereof.

49. The conjugate of claim 47, wherein the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof.

50. The conjugate of claim 47, wherein one of the first or second dimerization domains comprises FK506 binding protein (FKBP), the other of the first or second domain comprises FKBP-Rap binding domain of mammalian target of Rap mTOR (Frb), and the ligand comprises rapamycin (Rap) or derivatives or fragments thereof.

51. The conjugate of any one of claims 40-50, wherein the RNA targeting molecule comprises at least two hairpin structures.

52. The conjugate of any one of claims 40-51, wherein the RNA targeting molecule comprises one or more modified nucleotides.

53. The conjugate of any one of claims 40-52, wherein the RNA targeting molecule comprises a TAR hairpin scaffold of SEQ ID NO:1.

54. The conjugate of any one of claims 40-53, wherein the RNA targeting molecule comprises a SLBP hairpin scaffold of SEQ ID NO:2.

55. The conjugate of any one of claims 40-54, wherein the RNA targeting molecule comprises a linker.

56. The conjugate of claim 55, wherein the linker comprises at least 5 amino acids.

57. The conjugate of any one of claims 40-56, wherein the RNA targeting region comprises at least 12 nucleotides.

58. The conjugate of any one of claims 40-57, wherein the RNA regulatory domain comprises a nuclease, methylase, demethylase, translational activator, translational repressor, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, or RNA binding activity.

59. The conjugate of any one of claims 40-57, wherein the RNA regulatory domain comprises a Pin nuclease domain or a m6A reader protein or portion thereof.

60. The conjugate of any one of claims 40-59, wherein the RNA regulatory domain comprises YTHDF1, YTHDF2, or ADAR.
61. The conjugate of any one of claims 40-60, wherein the RNA targeting region of the RNA targeting molecule hybridizes to a target RNA in a prokaryotic or eukaryotic cell.
62. The conjugate of any one of claims 40-61, wherein the conjugate comprise one or more nuclear localization signals (NLS)s.
63. The conjugate of any one of claims 40-62, wherein the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.
64. A fusion protein comprising a RNA hairpin binding domain and a RNA regulatory domain.
65. A fusion protein comprising a RNA regulatory domain and a first dimerization domain.
66. A fusion protein comprising a RNA hairpin binding domain and a second dimerization domain.
67. The fusion protein of claim 65 or 66, wherein dimerization of the first and/or second dimerization domain is inducible.
68. The fusion protein of claim 67, wherein the dimerization comprises ligand-induced dimerization.
69. The fusion protein of claim 68, wherein the first and second dimerization domains are selected from PYL1 and ABI1 and the ligand comprises ABA or derivatives or fragments thereof.
70. The fusion protein of claim 68, wherein the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof.
71. The fusion protein of claim 68, first and second dimerization domains are selected from FKBP and Frb, and the ligand comprises rapamycin or derivatives or fragments thereof.
72. The fusion protein of any one of claims 64-71, wherein the RNA hairpin binding domain and/or RNA regulatory domain are human-derived.

73. The fusion protein of any one of claims claim 64-72, wherein the fusion protein is less than 150 kDa.
74. The fusion protein of any one of claims 64-73, wherein the RNA hairpin binding domain comprises U1A, SLBP, or variants or fragments thereof.
75. The fusion protein of any one of claims 64-74, wherein the RNA regulatory domain comprises a nuclease, methylase, demethylase, translational activator, translational repressor, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, or RNA binding activity.
76. The fusion protein of claim 75, wherein the RNA regulatory domain comprises a Pin nuclease domain or a m6A reader protein or portion thereof.
77. The fusion protein of claim 76, wherein the RNA regulatory domain comprises YTHDF1 or YTHDF2.
78. The fusion protein of any one of claims 64-77 further comprising one or more nuclear localization signals (NLS)s.
79. The fusion protein of any one of claims 64-77, wherein the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.
80. A nucleic acid encoding the fusion protein of any one of claims 64-79.
81. A delivery vehicle comprising the system of any one of claims 1-39, the conjugate of any one of claims 40-63, or the fusion protein of any one of claims 64-79.
82. The delivery vehicle of claim 81, wherein the delivery vehicle comprises liposome(s), particle(s), exosome(s), microvesicle(s), a gene-gun or one or more nucleic acid vector(s).
83. A composition comprising the system of any one of claims 1-39, the conjugate of any one of claims 40-63, the fusion protein of any one of claims 64-79, or the delivery vehicle of any one of claims 81-82.

84. A cell comprising the system of any one of claims 1-39, the conjugate of any one of claims 40-63, the fusion protein of any one of claims 64-79, the delivery vehicle of any one of claims 81-82, or the composition of claim 83.
85. A method of modulating at least one target RNA comprising contacting the target RNA with the system of any one of claims 1-39, the conjugate of any one of claims 40-63, the fusion protein of any one of claims 64-79, the delivery vehicle of any one of claims 81-82, or the composition of claim 83.
86. The method of claim 85, wherein modulating the at least one target RNA comprises cleaving, demethylating, methylating, activating translation, repressing translation, promoting degradation, and/or binding to the RNA.
87. The method of claim 85 or 86, wherein the target RNA is in a prokaryotic or eukaryotic cell.
88. The method of claim 87, wherein the target RNA is in a human cell.
89. The method of claim 87 or 88, wherein the target RNA is in vitro or in vivo.
90. A cell or progeny thereof comprising modulated target RNA, wherein the target RNA has been modulated according to any one of claims 85-89.
91. A multicellular organism comprising one or more cells according to claim 90.
92. A plant or animal comprising one or more cells according to claim 91.
93. A kit comprising the system of any one of claims 1-39, the conjugate of any one of claims 40-63, the fusion protein of any one of claims 64-79, the delivery vehicle of any one of claims 81-82, or the composition of claim 83.
94. A method for modulating a target RNA in a subject, the method comprising administering the conjugate of any one of claims 40-63, the fusion protein of any one of claims 64-79, the delivery vehicle of any one of claims 81-82, or the composition of claim 83.