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 DISCLOSURE

[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 or 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.

[0007a] In one aspect, provided herein is 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 wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

[0007b] In one aspect, provided herein is a system comprising one or more nucleic acids including: i) a nucleic acid that encodes a polypeptide comprising a RNA hairpin binding domain; ii) a nucleic acid that is or encodes 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 nucleic acid that encodes a polypeptide comprising a RNA regulatory domain; wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

[0007c] In one aspect, provided herein is a fusion protein comprising: a) a RNA hairpin binding domain and a RNA regulatory domain; or b) a RNA regulatory domain and a first dimerization domain; or c) a RNA hairpin binding domain and a second dimerization domain, wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

[0007d] In one aspect, provided herein is a nucleic acid encoding the fusion protein as disclosed herein.

[0007e] In one aspect, provided herein is a delivery vehicle comprising the system as disclosed herein, the conjugate as disclosed herein, or the fusion protein as disclosed herein, and optionally wherein the delivery vehicle comprises liposome(s), particle(s), exosome(s), microvesicle(s), a gene-gun or one or more nucleic acid vector(s).

[0007f] In one aspect, provided herein is a composition comprising the system, the conjugate, the fusion protein, or the delivery vehicle as disclsoed herein.

[0007g] In one aspect, provided herein is a cell comprising the system, the conjugate, the fusion protein, the delivery vehicle, or the composition as disclosed herein.

[0007g] In one aspect, provided herein is a method of modulating at least one target RNA comprising contacting the target RNA with the system, the conjugate, the fusion protein, the delivery vehicle, or the composition as disclosed herein, wherein the method is not an *in vivo* method.

[0007h] In one aspect, provided herein is a kit comprising the system, the conjugate, the fusion protein, the delivery vehicle, or the composition as disclosed herein.

[0007i] In one aspect, provided herein is a use of the system, the conjugate, the fusion protein, the delivery vehicle, or the composition in the manufacture of a medicament for modulating a target RNA in a subject.

[0007j] In one aspect, provided herein is the system, the conjugate, the fusion protein, the delivery vehicle, or the composition for use in therapy.

[0008] Examples 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 examples, the following are included: i) and ii), i) and iii), ii) and iii), or i), ii), and iii). Any example disclosed herein can contain any of these combinations.

[0009] Further examples 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 examples 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 examples, the RNA regulatory domain and the RNA targeting molecule are operably linked through a peptide bond. In some examples, the polypeptide further comprises one or more linkers. In some examples, the RNA regulatory domain and the RNA targeting molecule are operably linked through non-covalent interactions. In some examples, 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 examples, the conjugate comprises one or more nuclear localization signals (NLS)s.

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

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

[0014] Further examples 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 examples, modulating at least one target RNA comprises cleaving, demethylating, methylating, activating translation, repressing translation, promoting degradation, or binding to the RNA. In some examples, at least two target RNAs are modulating. In some examples, 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 examples, the multiple RNAs are modulated by the same RNA regulatory domain or by a regulatory domain with the same activity. In some examples, 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 examples, the RNA regulatory domain does not bind to RNA. In some examples, the RNA regulatory domain comprises a polypeptide that does not have RNA binding activity. In some examples, the RNA regulatory domain does not bind to modified RNA. In some examples, 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. 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 examples 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.

[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 examples, 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 examples, 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 examples, 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 examples, 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 examples, the dimerization is inducible. In some examples, 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 examples of the disclosure. In some examples, 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 examples, the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof. In some examples, 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 modifiied 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 examples, the dimerization domain may be a modified fragment.

[0026] In some examples, i, ii, and/or iii are human or are human-derived. In some examples, 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 examples, 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 examples, the nucleic acids or polypeptides of the disclosure are synthetic, are non-natural, and/or do not occur naturally in nature.

[0028] In some examples, the system further comprises a stabilizer polypeptide; wherein the stabilizer polypeptide comprises a cationic polypeptide that binds non-specifically to nucleic acids. In some examples, the stabilizer polypeptide is human-derived. In some examples, the stabilizer polypeptide is operably linked to the RNA regulatory domain and/or RNA hairpin binding domain. In some examples, the stabilizer polypeptide comprises ORF5 or a fragment thereof. In some examples, 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 examples, the stabilizer polypeptide comprises HEBGF or a fragment thereof. In some examples, 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 examples, the stabilizer polypeptide comprises β-defensin 3 or a fragment thereof. In some examples, 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 examples, 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 examples, 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 examples, 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 examples, the RNA hairpin binding domain comprises a RNA hairpin binding domain from U1A (TBP6.7), SLBP, or variants thereof. In some examples, the RNA hairpin binding domain comprises a RNA hairpin binding domain from U1A (TBP6.7), SLBP, Ku70, nucleolin, or variants thereof. In some examples, 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.

[0031] In some examples, the RNA targeting molecule comprises a TAR hairpin scaffold. In some examples, 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 examples, the RNA targeting molecule comprises a SLBP hairpin scaffold. In some examples, 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 examples, the RNA targeting molecule comprises exactly one hairpin. In some examples, the RNA targeting molecule comprises at least one hairpin. In some examples, the RNA targeting molecule comprises exactly two hairpins. In some examples, the RNA targeting molecule comprises at least two hairpins. In some examples, the RNA targeting molecule comprises exactly three hairpins. In some examples, the RNA targeting molecule comprises at least three hairpins. In some examples, the RNA targeting molecule comprises exactly four hairpins. In some examples, the RNA targeting molecule comprises at least four hairpins. In some examples, the RNA targeting molecule comprises exactly five hairpins. In some examples, the RNA targeting molecule comprises at least five hairpins. In some examples, the RNA targeting molecule comprises 1-4 hairpins. In some examples, the RNA targeting molecule comprises 1-3 hairpins. In some examples, the RNA targeting molecule comprises 1-2 hairpins. In some examples,the RNA targeting molecule comprises 2-4 hairpins. In some examples,the RNA targeting molecule comprises 2-3 hairpins. In some examples, 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 examples, the RNA targeting 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 examples, the RNA targeting molecule binds to more than one RNA binding protein. In some examples, the RNA targeting molecule comprises two, three, or four hairpin structures and binds to at least two RNA binding proteins. In some examples, the RNA regulatory system comprises at least two regulatory domains, wherein each regulatory domain binds to a different RNA binding molecule.

[0033] In some examples, the RNA targeting molecule comprises one or more modified nucleotides. In some examples, 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 examples, the modification includes one described herein.

[0034] In some examples, 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.

[0035] In some examples, 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, 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 examples, 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, 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 examples, 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, 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 examples, the RNA hairpin binding domain, stabilizer polypeptide, or RNA hairpin binding domain comprises a linker. In some examples, 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 examples, the linker is a rigid linker. In some examples, the linker is a flexible linker. In some examples, the linker comprises glycine and serine residues. In some examples, the linker is at least 4 amino acids. In some examples, 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 examples, the stabilizer polypeptide comprises a polypeptide, such as a RNA-binding polypeptide, from cJun, HBEGF, HRX, NDEK, NHGF, beta-defensin3, or scGFP. In some examples, the RNA regulatory domain is operably linked to the stabilizer polypeptide at the carboxy terminus of the RNA regulatory domain. In some examples, the RNA regulatory domain is operably linked to the stabilizer polypeptide at the amino terminus of the RNA regulatory domain. In some examples, 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 examples, 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 examples, 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 examples, 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 examples, the RNA targeting region comprises at least 12 nucleotides. In some examples, 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 examples, 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 examples, the RNA regulatory domain comprises an activity described herein.

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

ADAR protein. In some examples, 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 examples, the RNA regulatory domain further comprises a helical region. In some examples, 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 examples, the RNA regulatory domain increases translation of a target RNA. In some examples, the RNA regulatory domain increases degradation of a target RNA. In some examples, the RNA regulatory domain modifies the localization of a target RNA. In some examples, the RNA regulatory domain modifies the processing of the target RNA.

[0044] In some examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, the NES is at the carboxy terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some examples, the NES is at the amino terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some examples, 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 examples, 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 examples, the NLS is at the carboxy terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some examples, the NLS is at the amino terminus of the RNA regulatory domain, the RNA hairpin binding domain, and/or the stabilizing polypeptide. In some examples, 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 examples, the RNA targeting region of ii hybridizes to a target RNA in a prokaryotic or eukaryotic cell. In some examples, the target RNA is in a human cell. In some examples, the target RNA is in vitro or in vivo.

[0048] In some examples, the system comprises at least two of each i, ii, and iii. In some examples, the at least two of i, ii, and iii are expressed in the same cell. In some examples, the method comprises modulating at least two target RNAs. In some examples, 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 examples, 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 examples, the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.

[0050] In some examples, 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 examples of the disclosure. In some examples, the one or more nucleic acid vectors are optimized for expression in an eukaryotic cell. In some examples, the expression of the domains, RNA, or polypeptides in the cell or from a vector is constitutive. In some examples, the expression of the domains, RNA, or polypeptides in the cell or from a vector is conditional. In some examples, i, ii, and iii are on a single vector. In some examples, i, ii, iii, and the stabilizer polypeptide are encoded on a single vector. In some examples, i, iii, and the stabilizer polypeptide are encoded on a single vector. In some examples, one or more

of the vectors are viral vectors. In some examples, the one or more vectors comprise one or more retroviral, lentiviral, adenoviral, adeno-associated or herpes simplex viral vectors. In some examples, one or more of the vectors are non-viral vectors. In some examples, the system or composition is non-viral, which denotes that it does not contain any viral components.

[0051] In some examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples of the disclosure may be useful for the treatment of a disease or condition, such as cancer or autoimmunity. In some examples, the methods and compositions are for the acute treatment of a disease or condition. In some examples, the methods and compositions are useful for the temporary modulation of RNA. In some examples exclude permanent modification of gene activity. In some examples, 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.

[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 example.

[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.

[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 examples described in the context of the term "comprising" may also be implemented in the context of the term "consisting of" or "consisting essentially of."

[0058] It is specifically contemplated that any limitation discussed with respect to one example of the invention may apply to any other example of the invention. 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. Examples of an example set forth in the Examples are also examples that may be implemented in the context of examples discussed elsewhere in a different Example or elsewhere in the application, such as in the Summary of Invention, Detailed Description of the Examples, Claims, and Description of Figure Legends.

[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 examples of the invention, are given by way of illustration only, since various changes and modifications within

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,

wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

2. The system of claim 1, wherein:

A) the RNA hairpin binding domain and the RNA regulatory domain are operably linked; and/or

B) i) and iii) are operably linked through a peptide bond, or

wherein i) and iii) are operably linked through non-covalent interactions; and/or

C) 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, optionally wherein the dimerization is inducible, further optionally wherein the dimerization comprises ligand-induced dimerization, and further optionally wherein:

- one of the first or second dimerization domain comprises PYR/PYR1-like (PYL1), the other of the first or second dimerization domain comprises ABA insensitive 1 (ABI1), and the ligand comprises abscisis acid (ABA) or derivatives or fragments thereof; or

- the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof; or

- one of the first or second dimerization domains comprises FK506 binding protein (FKBP), the other of the first or second dimerization domain comprises FKBP-Rap binding domain of mammalian target of Rap mTOR (Frb), and the ligand comprises rapamycin (Rap) or derivatives or fragments thereof; and/or

D) ii) comprises at least two hairpin structures; and/or

E) ii) comprises one or more modified nucleotides; and/or

F) the system further comprises a stabilizer polypeptide; wherein the stabilizer polypeptide comprises a cationic polypeptide that binds non-specifically to nucleic acids, optionally wherein the stabilizer polypeptide comprises HBEGF, beta-defensin, or variants or portions thereof, and further optionally wherein the stabilizer polypeptide is human-derived; and/or

G) the total size of the system is less than 150 kDa; and/or

H) i) comprises U1A, SLBP, or variants thereof; and/or

I) ii) comprises a TAR hairpin scaffold of SEQ ID NO:1; and/or

J) ii) comprises a SLBP hairpin scaffold of SEQ ID NO:2; and/or

K) ii) comprises a linker, optionally wherein the linker is at least 5 amino acids; and/or

L) the RNA targeting region comprises at least 12 nucleotides; and/or

M) iii) comprises a nuclease, methylase, demethylase, translational activator, translational repressor, single-stranded RNA cleavage activity, double-stranded RNA cleavage activity, or RNA binding activity; and/or

N) iii) comprises a Pin nuclease domain or a m6A reader protein or portion thereof, optionally wherein iii) comprises YTHDF1, YTHDF2, or ADAR; and/or

O) the RNA targeting region of ii) hybridizes to a target RNA in a prokaryotic or eukaryotic cell; and/or

P) i) and/or iii) comprises one or more nuclear localization signals (NLS)s and/or

Q) the system comprises at least two of each i, ii, and iii; and/or

R) the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.

3. A system comprising one or more nucleic acids including: i) a nucleic acid that encodes a polypeptide comprising a RNA hairpin binding domain; ii) a nucleic acid that is or encodes 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 nucleic acid that encodes a polypeptide comprising a RNA regulatory domain; wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

4. The system of claim 3, wherein:

A) the RNA hairpin binding domain and the RNA regulatory domain are operably linked; and/or

B) the system further comprises a regulatory element operably linked to i, ii, and/or iii; and/or

C) the one or more nucleic acids are optimized for expression in an eukaryotic cell, optionally wherein the expression is constitutive or conditional; and/or

D) i, ii, and iii are on a single nucleic acid; and/or

E) wherein:

a) one or more of the nucleic acids are viral vectors, optionally wherein the one or more nucleic acids comprise one or more retroviral, lentiviral, adenoviral, adeno-associated or herpes simplex viral vectors; or

b) one or more of the nucleic acids are non-viral vectors.

5. 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.

6. The conjugate of claim 5, wherein:

A) the RNA regulatory domain is human derived; and/or

B) the RNA regulatory domain and the RNA targeting molecule are operably linked through a peptide bond, or the RNA regulatory domain and the RNA targeting molecule are operably linked through non-covalent interactions; and/or

C) the polypeptide further comprises one or more linkers; and/or

D) 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, optionally wherein the dimerization is inducible, further optionally wherein the dimerization comprises ligand-induced dimerization, and further optionally:

a) one of the first or second dimerization domains comprises PYR/PYR1-like (PYL1), the other of the first or second dimerization domain comprises ABA insensitive 1 (ABI1), and the ligand comprises abscisis acid (ABA) or derivatives or fragments thereof; or

b) the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof; or

c) one of the first or second dimerization domains comprises FK506 binding protein (FKBP), the other of the first or second dimerization domain comprises FKBP-Rap binding domain of mammalian target of Rap mTOR (Frb), and the ligand comprises rapamycin (Rap) or derivatives or fragments thereof; and/or

E) the RNA targeting molecule comprises at least two hairpin structures; and/or

F) the RNA targeting molecule comprises one or more modified nucleotides and/or

G) the RNA targeting molecule comprises a TAR hairpin scaffold of SEQ ID NO:1 and/or

H) the RNA targeting molecule comprises a SLBP hairpin scaffold of SEQ ID NO:2; and/or

I) the RNA targeting molecule comprises a linker, optionally wherein the linker comprises at least 5 amino acids; and/or

J) the RNA targeting region comprises at least 12 nucleotides; and/or

K) 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; or

L) the RNA regulatory domain comprises a Pin nuclease domain or a m6A reader protein or portion thereof, optionally wherein the RNA regulatory domain comprises YTHDF1, YTHDF2, or ADAR; and/or

M) the RNA targeting region of the RNA targeting molecule hybridizes to a target RNA in a prokaryotic or eukaryotic cell; and/or

N) the conjugate comprise one or more nuclear localization signals (NLS)s; and/or

O) the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.

7. A fusion protein comprising:

a) a RNA hairpin binding domain and a RNA regulatory domain; or

b) a RNA regulatory domain and a first dimerization domain; or

c) a RNA hairpin binding domain and a second dimerization domain,

wherein the RNA hairpin binding domain and/or the hairpin structure are human or are human-derived.

8. The fusion protein of claim 7(b) or 7(c), wherein dimerization of the first and/or second dimerization domain is inducible, optionally wherein the dimerization comprises ligand-induced dimerization, and further optionally wherein:

i) the first and second dimerization domains are selected from PYL1 and ABI1 and the ligand comprises ABA or derivatives or fragments thereof; or

ii) the first and/or second dimerization domain comprises FKBP12 and the ligand comprises FK1012 or derivatives or fragments thereof; or

iii) first and second dimerization domains are selected from FKBP and Frb, and the ligand comprises rapamycin or derivatives or fragments thereof.

9. The fusion protein of any one of claim 7 or 8, wherein:

A) the RNA hairpin binding domain and/or RNA regulatory domain are human-derived; and/or

B) the fusion protein is less than 150 kDa; and/or

C) the RNA hairpin binding domain comprises U1A, SLBP, or variants or fragments thereof and/or

D) 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; and/or

E) the RNA regulatory domain comprises a Pin nuclease domain or a m6A reader protein or portion thereof, optionally wherein the RNA regulatory domain comprises YTHDF1 or YTHDF2; and/or

F) the fusion protein further comprises one or more nuclear localization signals (NLS)s; and/or

G) the RNA regulatory domain cleaves RNA, promotes RNA translation, inhibits RNA translation, or modifies the base sequence of RNA.

10. A nucleic acid encoding the fusion protein of any one of claims 7-9.

11. A delivery vehicle comprising the system of any one of claims 1-4, the conjugate of claim 5 or claim 6, or the fusion protein of any one of claims 7-9, and optionally wherein the delivery vehicle comprises liposome(s), particle(s), exosome(s), microvesicle(s), a gene-gun or one or more nucleic acid vector(s).

12. A composition comprising the system of any one of claims 1-4, the conjugate of claim 5 or claim 6, the fusion protein of any one of claims 7-9, or the delivery vehicle of claim 11.

13. A cell comprising the system of any one of claims 1-4, the conjugate of claim 5 or claim 6, the fusion protein of any one of claims 7-9, the delivery vehicle of claim 11, or the composition of claim 12.

14. A method of modulating at least one target RNA comprising contacting the target RNA with the system of any one of claims 1-4, the conjugate of claim 5 or claim 6, the fusion protein of any one of claims 7-9, the delivery vehicle of claim 11, or the composition of claim 12, wherein the method is not an *in vivo* method.

15. The method of claim 14, wherein:

a) modulating the at least one target RNA comprises cleaving, demethylating, methylating, activating translation, repressing translation, promoting degradation, and/or binding to the RNA; and/or

b) the target RNA is in a prokaryotic or eukaryotic cell, and optionally wherein:

i) the target RNA is in a human cell; and/or

ii) the target RNA is in vitro.

16. A cell or progeny thereof comprising modulated target RNA, wherein the target RNA has been modulated according to the method of claim 14 or claim 15.

17. A multicellular organism comprising one or more cells according to claim 16.

18. A plant or animal comprising one or more cells according to claim 17.

19. A kit comprising the system of any one of claims 1-4, the conjugate of claim 5 or claim 6, the fusion protein of any one of claims 7-9, the delivery vehicle of claim 11, or the composition of claim 12.

20. Use of the system of any one of claims 1-4, the conjugate of claim 5 or claim 6, the fusion protein of any one of claims 7-9, the delivery vehicle of claim 11, or the

composition of claim 12 in the manufacture of a medicament for modulating a target RNA in a subject.

21. The system of any one of claims 1-4, the conjugate of claim 5 or claim 6, the fusion protein of any one of claims 7-9, the delivery vehicle of claim 11, or the composition of claim 12 for use in therapy.