HUMAN-DERIVED SYNTHETIC REGULATORS AND USES THEREOF

CROSS REFERENCE TO EARLIER FILED APPLICATIONS

[0001] This PCT application claims the priority benefit of U.S. Provisional Application No. 63/633,522, filed April 12, 2024, and U.S. Provisional Application No. 63/679,518, filed August 5, 2024, each of which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

[0002] The content of the sequence listing is submitted electronically (Name: 4597_018PC02_Sequencelisting_ST26.xml; Size: 137,040 bytes; and Date of Creation: April 3, 2025) and is filed with the application is herein incorporated by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0003] While mRNA therapeutics hold tremendous potential for the treatment of diseases, there are still limitations to their use. For example, controlling expression of payload proteins from mRNA therapeutics in a tissue-specific manner remains a challenge. Further, immunogenicity of gene mRNA therapeutics can limit effectiveness and cause immune related toxicities. Given the strong potential of mRNA therapeutics, there is an immediate need for an mRNA therapeutic that is more suitable for human gene therapy.

BRIEF SUMMARY OF THE DISCLOSURE

[0004] Provided herein is a synthetic circuit comprising: (a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain; and (b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises a first sensor ("first type P sensor") comprising a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TS"); and wherein upon binding of the PUF RBD to the PUF TS, the effector domain is capable of downregulating the payload sequence.

[0005] In some aspects, the first type P sensor comprises a PUF TS comprising one or more nucleic acid sequences as set forth in 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3'

(TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), or 5' -UGUAGCUA -3' (TS #9).

[0006] In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5'-UGUAUAUA -3' (WT TS). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5'- GGAUGAA - 3' (TS #1). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5'-UGUACGUC -3' (TS #2). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5' - UCUACGUC -3' (TS #3). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGUACGAC –3' (TS #4). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGUCCGUC -3' (TS #5). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5' - UGUACGUG –3' (TS #6). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGGAAGUC -3' (TS #7). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5' - UGUGCCUC -3' (TS #8). In some aspects, the first type P sensor comprises the nucleic acid sequence as set forth in 5' - UGUAGCUA -3' (TS #9). In some aspects, the first type P sensor comprises at least 8 nucleotides in length.

[0007] In some aspects, the payload sequence comprises at least one first type P sensor, at least two first type P sensors, at least three first type P sensors, at least four first type P sensors, at least five first type P sensors, at least six first type P sensors, at least seven first type P sensors, at least eight first type P sensors, at least nine first type P sensors, or at least ten first type P sensors.

[0008] In some aspects, the payload sequence comprises at least two first type P sensors. In some aspects, the payload sequence comprises at least three first type P sensors. In some aspects, the payload sequence comprises at least four first type P sensors. In some aspects, the payload sequence comprises at least five first type P sensors. In some aspects, the payload sequence comprises at least six first type P sensors. In some aspects, the payload sequence comprises at least seven first type P sensors. In some aspects, the payload sequence comprises at least eight first type P sensors. In some aspects, the payload sequence comprises at least nine first type P sensors. In some aspects, the payload sequence comprises at least ten first type P sensors.

[0009] In some aspects, the PUF RBD comprises one or more amino acid substitutions compared to the wild type PUF RBD as set forth in SEQ ID NO: 1. In some aspects, the PUF

RBD comprises an amino acid sequence having at least about 70%, at about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the wild type PUF RBD as set forth in SEQ ID NO: 1, wherein the PUF RBD is capable of binding to the PUF TS. In some aspects, the amino acid substitution in the PUF RBD comprises a substitution corresponding to an amino acid position in R1, R2, R3, R4, R5, R6, R7, or R8 of the wild type PUF RBD as set forth in SEQ ID NO: 1, or any combination thereof. In some aspects, the PUF RBD comprises one or more sequences listed in Table 3.

[0010] In some aspects, the PUF RBD having the amino acid substitution is capable of binding to the PUF TS site with greater affinity than the wild type PUF RBD as set forth in SEQ ID NO: 1.

[0011] In some aspects, the PUF RBD having the amino acid substitution has less off-target binding than the wild type PUF RBD as set forth in SEQ ID NO: 1.

[0012] In some aspects, the effector domain comprises a degradation domain, a translation inhibition domain, a protein recruiting domain, or any combination thereof. In some aspects, the effector domain comprises a he degradation domain.

[0013] In some aspects, the effector domain is derived from one or more sequences listed in Table 4a. In some aspects, the degradation domain comprises one or more sequences listed in Table 4a. In some aspects, the effector domain comprises a degradation domain that is derived from cNOT7, TTP, MCPIP1PIN, DDX6, Dis3PIN, SMG6PIN, or any combination thereof.

[0014] In some aspects, the effector domain is derived from cNOT7. In some aspects, the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 34.

[0015] In some aspects, the effector domain is derived from TTP. In some aspects, the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 36.

[0016] In some aspects, the effector domain is derived from MCPIP1$_{PIN}$. In some aspects, the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least

about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 35.

[0017] In some aspects, the degradation domain is derived from DDX6. In some aspects, the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 31.

[0018] In some aspects, the degradation domain is derived from Dis3PIN. In some aspects, the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 32.

[0019] In some aspects, the degradation domain is derived from SMG6PIN. In some aspects, the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 33.

[0020] In some aspects, the payload sequence comprises a spacer sequence (type P spacer). In some aspects, the type P spacer sequence is located within the first type P sensor. In some aspects, the type P spacer sequence is located at the 5' of the first type P sensor. In some aspects, the type P spacer sequence is located at the 3' of the first type P sensor.

[0021] In some aspects, the regulator sequence is human-derived.

[0022] In some aspects, the payload sequence comprises a therapeutic protein.

[0023] In some aspects, the payload sequence comprises a plurality of first type P sensor.

[0024] In some aspects, the payload sequence comprises two first type P sensors, three first type P sensors, four first type P sensors, five first type P sensors, six first type P sensors, seven first type P sensors, eight first type P sensors, nine first type P sensors, or ten or more first type P sensors. In some aspects, each of the first type P sensors is the same. In some aspects, one or more of the first type P sensors are different.

[0025] In some aspects, the payload sequence comprises a plurality of the type P spacer. In some aspects, the type P spacer is located before the first type P sensor, between two first type P sensors, or after the final first type P sensor. In some aspects, the type P spacer is located between every first type P sensor.

[0026] In some aspects, the payload sequence comprises a replicon RNA.

[0027] In some aspects, the payload sequence comprises a modified RNA.

[0028] In some aspects, the payload sequence comprises a circular RNA.

[0029] In some aspects, the regulator sequence comprises a linear non-replicating RNA.

[0030] In some aspects, the regulator sequence comprises a circular RNA.

[0031] In some aspects, the payload sequence comprises an additional sensor that is capable of specifically recognizing a marker (second type P sensor). In some aspects, the regulator sequence comprises a sensor that is capable of specifically recognizing a marker (type R sensor). In some aspects, the regulator, the marker recognized by the second type P sensor (second type P marker), and/or the marker recognized by the type R sensor (type R marker) are not the same.

[0032] In some aspects, when the payload sequence and the regulator sequence are present in a target cell, the payload is expressed in the target cell for a first expression and the regulator is expressed in the target cell for a second expression, and wherein the first expression is greater than the second expression. In some aspects, the recognition of the type P marker by the second type P sensor inhibits the expression of the payload. In some aspects, the recognition of the type R marker by the type R sensor inhibits the expression of the regulator.

[0033] In some aspects, (a) a target cell does not express sufficient levels of the type P marker to turn on the second type P sensor, and (b) the target cell expresses sufficient levels of the type R marker to turn on the type R sensor.

[0034] In some aspects, (a) a non-target cell expresses sufficient levels of the type P marker to turn on the second type P sensor, (b) the non-target cell does not express sufficient levels of the type R marker to turn on the type R sensor, or (c) both (a) and (b).

[0035] In some aspects, the type P marker, type R marker, or both comprise a microRNA, a protein, a metabolite, or combinations thereof.

[0036] In some aspects, the regulator sequence comprises a plurality of the type R sensor. In some aspects, the plurality of the type R sensor comprises two type R sensors, three type R sensors, four type R sensors, five type R sensors, six type R sensors, seven type R sensors, or eight or more type R sensors. In some aspects, each of the type R sensors is the same. In some aspects, one or more of the type R sensors are different.

[0037] In some aspects, the regulator sequence comprises a spacer sequence (type R spacer). In some aspects, the regulator sequence comprises a plurality of type R spacer. In some aspects, each of the type R spacer is the same. In some aspects, one or more of the type R spacers are different.

[0038] In some aspects, the synthetic circuit comprises the plurality of the type R sensor, wherein two or more of the type R sensors are separated by a type R spacer. In some aspects, each of the type R sensors are separated by a type R spacer. In some aspects, at least one type R spacer is upstream of at least one type R sensor. In some aspects, the type R spacer is between about 1 to about 50 nucleotides in length.

[0039] The present disclosure also provides a synthetic circuit comprising: (a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain which comprises a cNOT7 domain; and (b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises four to eight first type P sensors, each of which comprises a target site ("TS") that is capable of being specifically bound by the PUF domain ("PUF TSs"), wherein the TS comprises one or more nucleic acids selected from 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), or 5' -UGUAGCUA -3' (TS #9), wherein each of the TSs is linked to each other by a type P spacer; and wherein upon binding of the PUF RBD to the PUF TSs, the effector domain is capable of downregulating the payload sequence.

[0040] In some aspects, a synthetic circuit of the present disclosure comprises: (a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain which comprises a TTP domain; and (b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises four to eight first type P sensors, each of which comprises a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TSs"), wherein the TS comprises one or more nucleic acids selected from 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), or 5' -UGUAGCUA -3' (TS #9), wherein each of the TSs is linked to each other by a type P spacer; and wherein upon binding of the PUF RBD to the PUF TSs, the effector domain is capable of downregulating the payload sequence.

[0041] Disclosure herein provides a synthetic circuit comprising: (a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain which comprises a $MCPIP1_{PIN}$ domain; and (b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises four to eight first type P sensors, each of which comprises a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TSs"), wherein the TS comprises one or more nucleic acids selected from 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), or 5' -UGUAGCUA -3' (TS #9), wherein each of the TSs is linked to each other by a type P spacer; and wherein upon binding of the PUF RBD to the PUF TSs, the effector domain is capable of downregulating the payload sequence.

[0042] In some aspects, the disclosure provides a nucleotide sequence comprising a target site ("PUF TS"), wherein the TS is capable of being specifically bound by an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD"), wherein the nucleotide sequence comprises 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), 5' -UGUAGCUA -3' (TS #9), or any combination thereof.

[0043] In some aspects, the disclosure provides a nucleotide sequence comprising a target site ("PUF TS") that is bound by an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD"), wherein the nucleotide sequence comprises at least two sites, at least three sites, at least four sites, at least five sites, at least six sites, at least seven sites, at least eight sites, at least nine sites, or at least ten sites, and wherein at least one of the sites comprises 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), 5' -UGUAGCUA -3' (TS #9), or any combination thereof.

[0044] In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5'- UGGAUGAA - 3' (TS #1). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight

PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5'- UGUACGUC -3' (TS #2). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UCUACGUC -3' (TS #3). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUACGAC – 3' (TS #4). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUCCGUC -3' (TS #5). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUACGUG – 3' (TS #6). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' -UGGAAGUC -3' (TS #7). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUGCCUC -3' (TS #8). In some aspects, the disclosure provides a nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' -UGUAGCUA -3' (TS #9).

[0045] In some aspects, a spacer sequence is located before the first PUF TS, between every PUF TS, and after the final PUF TS. In some aspects, a spacer sequence comprises at least about 5 bp, at least about 6 bp, at least about 7 bp, at least about 8 bp, at least about 9 bp, at least about 10 bp, at least about 11 bp, at least about 12 bp, at least about 13 bp, at least about 14 bp, or at least about 15 bp. In some aspects, the spacer sequence comprises between 5 bp and 15 bp, 6 bp and 15 bp, 7 bp and 15 bp, 8 bp and 15 bp, 9 bp and 15 bp, 10 bp and 15 bp, 11 bp and 15 bp, 12 bp and 15 bp, 13 bp and 15 bp, 14 bp and 15 bp, 5bp and 14 bp, 6 bp and 14 bp, 7 bp and 14 bp, 8 bp and 14 bp, 9 bp and 14 bp, 10 bp and 14 bp, 11 bp and 14 bp, 12 bp and 14 bp, 13 bp and 14 bp, 5 bp and 13 bp, 6 bp and 13 bp, 7 bp and 13 bp, 8 bp and 13 bp, 9 bp and 13 bp, 10 bp and 13 bp, 11 bp and 13 bp, 12 bp and 13 bp, 5 bp and 12 bp, 6 bp and 12 bp, 7 bp and 12 bp, 8 bp and 12 bp, 9 bp and 12 bp, 10 bp and 12 bp, 11 bp and 12 bp, 5 bp and 11 bp, 6 bp and 11 bp, 7 bp and 11 bp, 8 bp and 11 bp, 9 bp and 11 bp, 10 bp and 11 bp, 5 bp and 10 bp, 6 bp and 10 bp, 7 bp and 10 bp, 8 bp and 10 bp, 9 bp and 10 bp, 5 bp and

9 bp, 6 bp and 9 bp, 7 bp and 9 bp, 8 bp and 9 bp, 5 bp and 8 bp, 6 bp and 8 bp, 7 bp and 8 bp, 5 bp and 7 bp, 6 bp and 7 bp, or 5bp and 6bp.

[0046] In some aspects, the spacer comprises the nucleic acid sequence set forth in SEQ ID NO: 110 (GAACGGGTTTGA).

[0047] In some aspects, the disclosure provides a method of improving binding of a PUF RBD to a PUF TS in the synthetic circuit described herein, comprising adding a spacer sequence in the nucleotide sequence disclosed herein, wherein the spacer sequence improves binding of the PUF RBD to the PUF TS.

[0048] In some aspects, the disclosure provides a method of improving downregulation of a payload sequence in the synthetic circuit described herein, comprising adding a spacer sequence in the nucleotide sequence disclosed herein, wherein the spacer sequence to improve downregulation of the payload sequence.

[0049] In some aspects, the disclosure provides a reprogrammed human Pum1 (PUF) RNA binding domain (RBD) protein ("PUF RBD"), wherein there are one or more amino acid substitutions in the wild type PUF domain, and wherein the PUF RBD is capable of binding a target site ("PUF TS"), wherein the reprogrammed PUF RBD comprises the amino acid sequence as set forth in any one of SEQ ID NO: 2 - SEQ ID NO: 30. In some aspects, the reprogrammed PUF domain protein binds to the PUF TS with greater affinity than the wild type PUF domain. In some aspects, the reprogrammed PUF domain protein binds to the PUF TS at least about 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 3.5 fold, at least 4 fold, at least 4.5 fold, at least 5 fold, at least 5.5 fold, or at least 6 fold higher than the wild type PUF RBD domain as set forth in SEQ ID NO: 1. In some aspects, the reprogrammed PUF domain has reduced off-target binding than the wild type PUF RBD.

[0050] In some aspects, the disclosure provides a pharmaceutical composition comprising the synthetic circuit described herein and a pharmaceutically acceptable carrier. In some aspects, the pharmaceutical composition is formulated for intratumoral, intrathecal, intramuscular, intravenous, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

[0051] In some aspects, the disclosure provides a method of treating a disease or disorder in a subject in need thereof comprising administering the synthetic circuit described herein or the pharmaceutical composition disclosed herein to the subject.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0052] **FIG. 1** is a schematic of an RNA regulator and payload.

[0053] **FIG. 2** is a schematic of the RNA regulator and modified mRNA (modRNA) payload tested in Example 1.

[0054] **FIG. 3** shows the effectiveness of the following effectors cNOT7, TTP, DDX6, MCPIP1$_{PIN}$, Dis3$_{PIN}$ (isoform 2), and SMG6$_{PIN}$ at inhibiting expression of a modRNA payload (i.e., mVenus, a fluorescent reporter) 6 hours after cells were electroporated with the mRNA circuitry. The payload sequence contained 1, 4, 8, or no PUF$_{UGG}$ target sites (TS). An electroporation (EP) only control was also used. Fold change in mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group (no regulator, PUF$_{UGG}$-CNOT7, PUF$_{UGG}$-TTP, PUF$_{UGG}$-DDX6, PUF$_{UGG}$-MCPIP1$_{PIN}$, PUF$_{UGG}$-DIS3$_{PIN}$ 2, PUF$_{UGG}$-SMG6$_{PIN}$, and EP control).

[0055] **FIG. 4** shows the effectiveness of the following effectors TTP, cNOT7, and MCPIP1$_{PIN}$, at inhibiting expression of a modRNA payload (i.e., mVenus, a fluorescent reporter) 0-30 hours after cells were electroporated with the mRNA circuitry. The payload sequence contained 4x PUF$_{UGG}$ TS. An EP only control and a no regulator control were also used. mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group (PUF$_{UGG}$-TTP, PUF$_{UGG}$-cNOT7, PUF$_{UGG}$-MCPIP1$_{PIN}$, EP control, and no regulator).

[0056] **FIG. 5** shows the effectiveness of the following effectors TTP, cNOT7, and MCPIP1$_{PIN}$, at inhibiting expression of a modRNA payload (i.e., mVenus, a fluorescent reporter) 0-30 hours after cells were electroporated with the mRNA circuitry. The payload sequence contained 8x PUF$_{UGG}$ TS. An EP only control and a no regulator control were also used. mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group (PUF$_{UGG}$-TTP, PUF$_{UGG}$-CNOT7, PUF$_{UGG}$-MCPIP1$_{PIN}$, EP control, and no regulator).

[0057] **FIG. 6** shows the effectiveness of the following effectors TTP, cNOT7, and MCPIP1$_{PIN}$, at inhibiting expression of a modRNA payload (i.e., mVenus, a fluorescent reporter) 0-30 hours after cells were electroporated with the mRNA circuitry. The payload sequence contained 1x PUF$_{UGG}$ TS. An EP only control and a no regulator control were also used. mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group (PUF$_{UGG}$-TTP, PUF$_{UGG}$-CNOT7, PUF$_{UGG}$-MCPIP1$_{PIN}$, EP control, and no regulator).

[0058] **FIG. 7** is a schematic of the RNA regulator and self-amplifying replicon RNA (repRNA) payload (i.e., mVenus, a fluorescent reporter) tested in Example 2.

[0059] **FIG. 8** shows the effectiveness of the following effectors TTP, cNOT7, MCPIP1$_{PIN}$, and DDX6 at inhibiting expression of a repRNA payload (i.e., mVenus, a fluorescent reporter) 20 hours after cells were electroporated with the mRNA circuitry. The payload sequence contained 1, 4, 8, or no PUF$_{UGG}$ TS. An EP only control and a no regulator control were also used. Fold change in mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group (PUF$_{UGG}$-TTP, PUF$_{UGG}$-CNOT7, PUF$_{UGG}$-MCPIP1$_{PIN}$, PUF$_{UGG}$-DDX6, no regulator, and EP control). Baseline payload expression is shown by the dotted line.

[0060] **FIG. 9** shows the effectiveness of the following effectors TTP, cNOT7, MCPIP1$_{PIN}$, and DDX6 at inhibiting expression of a repRNA payload (i.e., mVenus, a fluorescent reporter) 0-200 hours after cells were electroporated with the mRNA circuitry. The payload sequence contained 8x PUF$_{UGG}$ TS. An EP only control and a no regulator control were also used. mVenus Mean Intensity Object Average, reflecting the amount of payload expressed, was measured for each group (PUF$_{UGG}$-TTP, PUF$_{UGG}$-CNOT7, PUF$_{UGG}$-MCPIP1$_{PIN}$, PUF$_{UGG}$-DDX6, no regulator, and EP control).

[0061] **FIG. 10** shows the effectiveness of the following PUF RNA binding domains (RBDs): PUF$_{UGG}$ cNOT7, PUF1.1-cNOT7, PUF1.2-cNOT7, PUF1.3-cNOT7, PUF3.1-cNOT7, PUF3.2-cNOT7, PUF3.3-cNOT7, PUF6.1-cNOT7, PUF6.2-cNOT7, and PUF6.3-cNOT7 at inhibiting expression of a payload (i.e., mVenus, a fluorescent reporter) 6 hours post electroporation. The payload sequences contained 8x TS. An EP only control and a no regulator control were also used. Fold change in mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group (EP, no regulator, PUF$_{UGG}$ cNOT7, PUF1.1-cNOT7, PUF1.2-cNOT7, PUF1.3-cNOT7, PUF3.1-cNOT7, PUF3.2-cNOT7, PUF3.3-cNOT7, PUF6.1-cNOT7, PUF6.2-cNOT7, and PUF6.3-cNOT7).

[0062] **FIGs. 11A-11E** show the effectiveness of reprogrammed PUF RBDs at inhibiting expression of a payload (i.e., mVenus, a fluorescent reporter) expressed by sequences containing 8x TS (1, 2, 3, 4, 5, 6, 7, or 8) 20 hours (**FIG. 11A**), 48 hours (**FIG. 11B**), 72 hours (**FIG. 11C**), 96 hours (**FIG. 11D**), and 120 hours (**FIG. 11E**) post EP. An EP only, no regulator, and a nonspecific regulator (PUF$_{UGG}$) controls were also used. Fold change in mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group [EP, no regulator, reprogrammed regulator, and nonspecific regulator (PUF$_{UGG}$)].

[0063] **FIGs. 12A-12D** show the effectiveness of revised PUF RBDs in inhibiting expression of a payload (i.e., mVenus, a fluorescent reporter) expressed by sequences containing 8x of the following TS: PUF2 (**FIG. 12A**), PUF7 (**FIG. 12B**), PUF1 (**FIG. 12C**), and PUF4 (**FIG. 12D**). An EP only, no EP, no regulator, and a nonspecific regulator (PUF_{UGG}) were used as controls. mVenus Mean Object Average Intensity, reflecting the amount of payload expressed, was measured for each group.

[0064] **FIG. 13** is a schematic showing the testing of TS placement, spacing, and number on downregulation of payload (i.e., mVenus, a fluorescent reporter) by cNOT7, TTP, and $MCPIP1_{PIN}$ as assessed in Example 5.

[0065] **FIG. 14** shows the effectiveness of the following effectors cNOT7, TTP, and $MCPIP1_{PIN}$ at inhibiting expression of a modRNA payload (i.e., mVenus, a fluorescent reporter) 6 hours after cells were electroporated with the mRNA circuitry. The payload sequence contained either 8 PUF_{UGG} TS with spacers or 7 PUF_{UGG} binding sites TS with no spacers. An EP only control and a no regulator control was also used. Fold change in mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group (no regulator, PUF_{UGG}-cNOT7, PUF_{UGG}-TTP, PUF_{UGG}-$MCPIP1_{PIN}$, and EP control).

[0066] **FIG. 15** is a schematic showing HEK293T and HUH7 cells containing a regulator and payload sequence. The payload sequence contained regulator and a miRNA target sites. The upper and lower panels show the target sites in either orientation. An expected full knockdown of the payload (i.e., mVenus, a fluorescent reporter) RNA when both regulator and miR target sites are present on the payload sequence in HEK293T cells is shown. An expected partial knockdown of the payload when both regulator and miR target sites are present on the payload sequence in HUH7 cells is also depicted.

[0067] **FIG. 16** shows the effectiveness of the following effectors: $Reporter^{4x\ PUF}$; $Reporter^{4x\ PUF}$, $PUF\text{-}TTP^{Low}$; $Reporter^{4x\ PUF}$, $PUF\text{-}TTP^{High}$; $Reporter^{4x\ PUF\ -\ 4x\ miRNA}$; $Reporter^{4x\ PUF\ -\ 4x\ miRNA}$, $PUF\text{-}TTP^{Low}$; $Reporter^{4x\ PUF\ -\ 4x\ miRNA}$, $PUF\text{-}TTP^{High}$; $Reporter^{4x\ miRNA\ -\ 4x\ PUF}$; $Reporter^{4x\ miRNA\ -\ 4x\ PUF}$, $PUF\text{-}TTP^{Low}$; $Reporter^{4x\ miRNA\ -\ 4x\ PUF}$, $PUF\text{-}TTP^{High}$; and EP control at inhibiting expression of a payload (i.e., mVenus, a fluorescent reporter) 24 hours after cells were electroporated. Fold change in mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group.

[0068] **FIG. 17A** is a schematic showing added synthetic repeats to a regulator sequence. **FIG. 17B** shows the effectiveness of added synthetic repeats to a regulator sequence in inhibiting expression of a repRNA payload sequence. Log2 fold change in mVenus Object

Average Intensity, reflecting the amount of payload expressed, was measured for each group. repRNA payload sequences were 8 nucleotides (nt), 10 nt, or 16 nt.

[0069] **FIG. 18A** is a schematic showing added synthetic repeats to a regulator sequence. **FIG. 18B** shows the effectiveness of added synthetic repeats to a regulator sequence in inhibiting expression a repRNA payload sequence. Log2 fold change in mVenus Object Average Intensity, reflecting the amount of payload expressed, was measured for each group. repRNA payload sequences were cognate or non-cognate 9 nt sequences.

[0070] **FIGs. 19A** and **19B** show the effectiveness of replicon spacers in between each target site (spacers throughout, ST, **FIG. 19A**) and after the stop codon (SASC **FIG. 19B**) for replicons on downregulation. Fold change in mVenus Object Average Intensity was assessed 24 hours post electroporation.

[0071] **FIG. 20** shows the effectiveness of spacer size length on downregulation. 2X TS of 12 bp or 20 bp in length were assessed in the presence and absence of a regulator. An electroporation only control was also used. mVenus Object Average Intensity was measured 18 hours post electroporation.

[0072] **FIG. 21** shows the effectiveness of 1-10X target sites on replicon with SASC (no spacers throughout). mVenus Object Average Intensity was measured 16 hours post electroporation.

[0073] **FIG. 22** shows the ability of effector domains to degrade linear N1-methyl-pseudouridine-modified mRNA payloads in HEK293T cells that were transfected with replicon fluorescent reporter mRNA containing 8x PUF_{UGG} target sites (TS) with either luciferase control mRNA (no regulator), or linear mRNA co-expressing PUF_{UGG} RBD and either TTP (C147R), cNOT7, or MCPIP1(PIN) effector domains. Fluorescent protein expression was quantified by live-cell imaging. The results are shown as fold change (expression relative to no regulator).

[0074] **FIG. 23** shows the ability of the TTP (C147R) effector domain to degrade linear N1-methyl-pseudouridine-modified mRNA payloads in HEK293T cells that were transfected with replicon fluorescent reporter mRNA containing 8x PUF_{UGG} target sites (TS) with either luciferase control mRNA (no regulator) or linear mRNA co-expressing PUF_{UGG} RBD and the TTP (C147R) effector domain at increasing doses (1000 fmols, 500 fmols, 250 fmols, 125 fmols, 75 fmols, 37.5 fmols, and 18.25 fmols). Fluorescent protein expression was quantified by live-cell imaging. The results are shown as fold change (expression relative to no regulator).

[0075] **FIGs. 24A-24C** are schematics of the engineered PUF and circuit. **FIG. 24A** is a shematic showing protein repeats engineered to enable the RNA-binding domain (RBD) of

PUF to bind specific RNA target sequences. **FIG. 24B** is a schematic showing the permutations applied to PUF_{UGG} positive control, generating PUF variants (PUFvarx) and their corresponding RNA target sequences. The substitutions of protein residues and RNA bases, compared to PUF_{UGG}, are highlighted. **FIG. 24C** is a schematic showing how PUF protein can bind a specific and programmable RNA target sequence through its RBD and repress payload protein expression through an effector domain. To enable control over payload expression, a circuit was constructed with a PUF target site cassette placed at the 3' UTR of the self-replicating (replicon) payload. Upon regulator expression, payload expression is inhibited.

[0076] **FIG. 25** shows the ability of reprogrammed RBDs to downregulate novel target site sequences. HEK293T cells were transfected with replicon fluorescent reporter mRNA containing either a positive control 8x PUF_{UGG} TS cassette, or a reprogrammed 8x PUFvar1 TS cassette, with or without their respective cognate PUF-TTP regulator, and quantified for fluorescent expression by live-cell imaging. The results are shown as absolute fluorescence expression.

[0077] **FIG. 26** shows HEK293T cells that were transfected with replicon fluorescent reporter mRNA containing either positive control 8x PUF_{UGG} TS cassette, or reprogrammed 8x PUFvarX TS cassette (i.e., PUFvar1, PUFvar2, PUFvar3, PUFvar4, or PUFvar5) with or without their respective cognate PUF-TTP regulator, and quantified for fluorescent expression by live-cell imaging. The results are shown as fold change (expression relative to no regulator).

[0078] **FIGs. 27A-27B** show the effectiveness of synthetic PUF domains on payload downregulation. **FIG. 27A** is a schematic showing PUF_{UGG} with 8 repeats (8R) was modified to incorporate two additional synthetic PUF repeats, forming PUF_{UGG}-10R. **FIG. 27B** shows the effectiveness of PUF_{UGG}-TTP with 8R or 10R with their respective cognate target RNA (e.g., 8R-8nt), or non-specific target RNA (e.g., 8R-10nt). Bold indicates cognate binding. The results are shown as regulator versus no regulator (log scale).

[0079] **FIGs. 28A-28D** show the effectiveness of a PUF-based regulator on multi-input miRNA sensing circuit and resulting cell-type specific payload expression in HEK.293T or Huh-7 cells transfected with a replicon mVenus reporter containing miR TS specific for miR-X and 8x TS for PUF_{UGG}10nt, with or without the addition of the cognate regulator PUF_{UGG}-TTP 10R. **FIGs. 28A** is a schematic of a multi-input circuit. Target sites on the 3' UTR of the mVenus payload facilitate the binding of either the PUF-based regulator (pink) or endogenous cell-specific miRNA (blue), enabling dual payload regulation (purple). In the two cell models used, HEK.293T cells exhibit high levels of miR-X activity, and Huh-7 cells exhibit moderate levels of miR-X activity. **FIG. 28B** shows the results of live-cell imaging for changes in

mVenus protein expression. **FIGs. 28C-28D** show the results of qPCR performed with primers and probe sets targeting genomic RNA of replicon encoding the non-structural proteins (NSP1, **FIG. 28C**) or its subgenomic RNA (**FIG. 28D**) encoding reporter mVenus. The results are shown as fold change.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0080] The present disclosure is generally directed to mRNA therapeutics employing human derived payload regulators that operate within genetic circuits. In some aspects, regulator proteins disclosed herein are comprised of human-derived PUF RNA binding domain ("PUF RBD"), which makes them more suitable for human gene therapy compared to proteins derived from prokaryotic origin (e.g., Cas9). As will be discussed in further detail herein, the disclosed PUF RBDs allow for the controlling of payload expression upon binding to its target site. The PUF RBD disclosed herein include substitutions having optimized target binding site sequences, which causes the reduction of off-target effects and increases therapeutic specificity.

Definitions

[0081] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present application, including the definitions, will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0082] Throughout this disclosure, the term "**a**" or "**an**" entity refers to one or more of that entity; for example, "a polynucleotide," is understood to represent one or more polynucleotides. As such, the terms "a" (or "an"), "**one or more**," and "**at least one**" can be used interchangeably herein.

[0083] Furthermore, "**and/or**" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0084] It is understood that wherever aspects are described herein with the language "**comprising**," otherwise analogous aspects described in terms of "**consisting of**" and/or "**consisting essentially of**" are also provided. As used herein, "**comprising**" is synonymous with "**including**," "**containing**," or "**characterized by**," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. As used herein, "**consisting of**" excludes any element, step, or ingredient not specified in the claim element. As used herein, "**consisting essentially of**" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim.

[0085] The term "**about**" is used herein to mean approximately, roughly, around, or in the regions of. When the term "about" is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term "about" is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower), unless indicated otherwise. Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification, including claims, are to be understood as being modified in all instances by the term "about." Accordingly, unless otherwise indicated to the contrary, the numerical parameters are approximations and can vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0086] The term "**at least**" prior to a number or series of numbers is understood to include the number adjacent to the term "at least," and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21-nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range. "At least" is also not limited to integers (*e.g.,* "at least 5%" includes 5.0%, 5.1%, 5.18% without consideration of the number of significant figures).

[0087] "**Nucleic acid**," "**nucleic acid molecule**," "**nucleotide sequence**," "**nucleic acid sequence**," "**polynucleotide**," and grammatical variants thereof are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine,

deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Single stranded nucleic acid sequences refer to single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA). Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.,* restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.,* the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA. A "nucleic acid composition" of the disclosure comprises one or more nucleic acids as described herein. As described herein, a polynucleotide of the present disclosure comprises DNA, RNA, or both. In some aspects, the term "**polynucleotide**" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, shRNA, siRNA, miRNA and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (*e.g.,* peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

[0088] As used herein, the term "**polypeptide**" encompasses both peptides and proteins, unless indicated otherwise.

[0089] The term "**coding region**" refers to a DNA or RNA region (the transcribed region) which "encodes" a particular protein, *e.g.,* such as a payload and/or regulator.

[0090] The term "**RNA**" is used herein to mean a molecule which comprises at least one ribonucleotide residue. "**Ribonucleotide**" relates to a nucleotide with a hydroxyl group at the 2′-position of a β-D-ribofuranosyl group. The term comprises double-stranded RNA, single-stranded RNA, isolated RNA such as partially or completely purified RNA, essentially pure RNA, synthetic RNA, recombinantly generated RNA such as modified RNA which differs from naturally occurring RNA by addition, deletion, substitution and/or alteration of one or

more nucleotides. The term "**mRNA**" means "**messenger-RNA**" and relates to a "**transcript**" which is generated by using a DNA template and encodes a peptide or protein. Typically, a mRNA comprises a 5′-UTR, a protein coding region and a 3′-UTR. mRNA only possesses limited half-life in cells and in vitro. In the context of the present disclosure, mRNA can be generated by *in vitro* transcription from a DNA template. The *in vitro* transcription methodology is known to the skilled person. For example, there is a variety of in vitro transcription kits commercially available. As further described herein, in some aspects, a RNA is a linear RNA. In some aspects, a RNA is a circular RNA. In some aspects, a RNA is a self-replicating RNA. In some aspects, a RNA is a non-replicating RNA.

[0091] As used herein, the term "**genetic circuit**" refers to a controllable gene expression system. As described herein, a genetic circuit useful for the present disclosure comprises a synthetic genetic circuit ("**synthetic circuit**"). As used herein, the term "**synthetic circuit**" refers to an engineered, non-natural genetic circuit. As is apparent from the present disclosure, synthetic circuits described herein have been specifically programmed to selectively express a payload in a cell of interest (*i.e.*, target cell).

[0092] As used herein, the term "**self-replicating RNA**" refers to a RNA (*e.g.*, mRNA) that is capable of directing its own amplification or replication within a cell (also referred to herein as "**repRNA**"). To direct its own amplification, the RNA molecule should encode the enzyme(s) necessary to catalyze RNA amplification (*e.g.*, alphavirus nonstructural proteins nsP1, nsP2, nsP3, nsP4) and also contain cis RNA sequences required for replication which are recognized and utilized by the encoded enzymes(s). An alphavirus RNA vector replicon should contain the following ordered elements: 5′ viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred to as 5′CSE, or 5′ cis replication sequence, or 5′ viral sequences required in cis for replication, or 5′ sequence which is capable of initiating transcription of an alphavirus), sequences which, when expressed, code for biologically active alphavirus nonstructural proteins (*e.g.*, nsP1, nsP2, nsP3, nsP4), and 3′ viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred as 3′CSE, or 3′ viral sequences required in cis for replication, or an alphavirus RNA polymerase recognition sequence). The alphavirus RNA vector replicon may contain a means to express one or more heterologous sequence(s), such as for example, an IRES or a viral (*e.g.*, alphaviral) subgenomic promoter (*e.g.*, junction region promoter) which may, in certain aspects, be modified in order to increase or reduce viral transcription of the subgenomic fragment, or to decrease homology with defective helper or structural protein expression cassettes, and one or more heterologous sequence(s) to be expressed. A replicon can also

contain additional sequences, for example, one or more heterologous sequence(s) encoding one or more polypeptides (*e.g.*, a protein-encoding gene or a 3′ proximal gene) and/or a polyadenylate tract. The replicon should not contain sequences encoding all of the alphavirus structural proteins (capsid, E1, E2). Non-limiting examples of heterologous sequences that can be expressed by replicon vectors are described, for example in U.S. Pat. No. 6,015,686, incorporated by reference in its entirety herein, and include, for example, antigens, lymphokines, cytokines, etc.

[0093] As used herein, the term "**circular RNA**" refers to a RNA (*e.g.*, mRNA) that forms a circular structure through covalent bonds. In the context of the present disclosure, circular RNA can be generated by methodology known to the skilled person (*e.g.*, Wesselhoeft, R. A. et al., 2018, Nature communications, 2018, 9(1), 1-10, herein incorporated by reference in its entirety). As is apparent from the present disclosure, any of the payload sequence and/or regulator sequence can be in the form of a circular RNA. Accordingly, in some aspects, a synthetic circuit provided herein comprises a payload sequence that is a circular RNA. In some aspects, a synthetic circuit provided herein comprises a regulator sequence that is a circular RNA. In some aspects, a synthetic circuit provided herein comprises a payload sequence and a regulator sequence, wherein the payload sequence is a circular RNA and the regulator sequence is a circular RNA. Unless indicated otherwise, a circular RNA is not self-replicating.

[0094] As used herein, the term "**payload sequence**" refers to a nucleotide sequence encoding a payload. As used herein, the term "**payload**" refers to any protein that can be encoded by the payload sequence. In some aspects, a payload comprises a therapeutic protein. As described herein, unless indicated otherwise, a payload does not comprise a regulator. Non-limiting examples of payloads are provided elsewhere in the present disclosure.

[0095] As used herein, the term "**regulator sequence**" refers to a nucleotide sequence encoding a regulator. As used herein, the term "**regulator**" comprises any agent that is capable of regulating the expression of the payload encoded by the payload sequence. Non-limiting examples of such regulators are provided elsewhere in the present disclosure. And, as described herein, a regulator useful for the present disclosure is capable of being specifically recognized by a type P sensor on the payload sequence. As also described herein, in some aspects, when the regulator is specifically recognized by a type P sensor, the expression of the payload (encoded by the payload sequence) is reduced or inhibited.

[0096] As used herein, the term "**sensor**" refers to any moiety that is capable of recognizing a marker and/or regulator described herein. As used herein, "**recognizing**" a marker (or

regulator) can comprise the physical interaction between the marker (or the regulator) and the sensor (*e.g.*, the marker binds to a specific marker recognition site within the sensor).

[0097] As used herein, the term "**type P sensor**" refers to a sensor that is present on the payload sequence. Accordingly, a payload sequence useful for the present disclosure comprises a coding region encoding a payload ("**payload coding region**") and a type P sensor. In some aspects, a payload sequence can comprise multiple type P sensors. As further described herein, in some aspects, a payload sequence can comprise: (a) payload coding region, (b) a first type P sensor that is capable of recognizing a regulator, and (c) a second type P sensor that is capable of recognizing a marker ("**type P marker**"). Unless indicated otherwise, the recognition of the type P marker by the second type P sensor activates the second type P sensor, such that the expression of the encoded payload is reduced or inhibited.

[0098] As used herein, the term "**type R sensor**" refers to a sensor that is present on the regulator sequence. In some aspects, a regulator sequence that can be used in constructing a synthetic circuit described herein comprises: (a) a coding region encoding a regulator ("**regulator coding region**") and a type R sensor, wherein the type R sensor is capable of recognizing a marker ("**type R marker**"). Unless indicated otherwise, the recognition of the type R marker by the type R sensor activates the type R sensor, such that the expression of the encoded regulator is reduced or inhibited.

[0099] The term "**sufficient level**" when used to describe a marker (*e.g.*, type P marker and/or type R marker) refers to the amount of the marker required to be recognized by a sensor (*e.g.*, type P sensor and/or type R sensor) and mediate downregulation of the sequence that comprises the sensor. As is apparent from the present disclosure, downregulation of the sequence can result in reduced or inhibition of the expression of any protein encoded by the sequence (*e.g.*, payload and/or regulator). Accordingly, in some aspects, a cell can express the marker and yet the sensor specific to the marker can remain inactive, where the cell does not express sufficient level of the marker.

[0100] The term "**sequence identity**" is used herein to mean a relationship between two or more amino acid (polypeptide or protein) sequences or two or more nucleic acid (polynucleotide) sequences, as determined by comparing the sequences. In certain aspects, sequence identity is calculated based on the full length of two given SEQ ID NO or on part thereof. Part thereof can mean at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of both SEQ ID NO, or any other specified percentage. The term "**identity**" can also mean the degree of sequence relatedness between amino acid or nucleic acid sequences, as the case can be, as determined by the match between strings of such sequences.

[0101] In some aspects, methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs.

[0102] As used herein, the terms "**effective amount**" or "**therapeutically effective amount**" of, *e.g.*, a synthetic circuit disclosed herein, refers to a quantity sufficient to, when administered to the subject, including a human, effect beneficial or desired results, including clinical results, and, as such, an "effective amount" or synonym thereto depends on the context in which it is being applied.

[0103] As used herein, the term "**target cell**" refers to a cell in which a payload (*e.g.*, encoded by the payload sequence) is desired to be expressed. As used herein, the term "**non-target cell**" refers to a cell in which a payload is not intended to be expressed.

[0104] As used herein, the term "**knockdown**" in connection with genetic control refers to stopping or decreasing the expression of one or more targeted genes. The knockdown can be temporary, or permanent. If the cells or model organisms survive a knockdown event, they can recover and eventually begin to express the gene as before.

[0105] As used herein, the term "**PUF**" refers to Pumilio/fem-3 mRNA binding factor (PUF) proteins. PUF proteins are exemplary hubs in mRNA control and are found throughout Eukarya. Porter et al., PNAS 112 (52) 15868-15873, Dec 14, 2015. A single PUF protein binds hundreds to thousands of mRNAs, in species from budding yeast to humans. In metazoans, PUF proteins support a broad range of processes, including the self-renewal of stem cells, tissue formation, learning, and memory. Most commonly, PUF proteins bind elements in 3′ untranslated regions (3′UTRs) and cause mRNA decay or translational repression, although other activities also have been reported. The PUF family has been divided into four clades, two of which include cytoplasmic proteins.

[0106] As used here, the term "**PUF RNA-binding domain**" refers to eight repeats of three α-helices, arranged in a ramped triangle that are capable of specifically binding to its target site (also referred to herein as "**PUF RBD**"). Each three α-helix unit is called a PUF repeat, eight of which are stacked on one another to form a crescent. RNAs bind to the inner face of the crescent, with one RNA base contacting one PUF repeat. In general, one helix in each repeat contacts an RNA. These "RNA-recognition helices" are distinguished by the presence of a particular pattern, characteristic of its RNA specificity: a small amino acid (often glycine) is followed by two variable residues, two hydrophobic residues, a variable residue, and a polar residue (often lysine or arginine). GX1X2VVX3K is typical. In this pattern, X1 and X3 make polar, base-specific contacts with the RNA base, whereas X2 stacks between bases. The X1,

X2, and X3 residues together play a large role in encoding for the recognition of a specific RNA bases. These three residues are termed a triplet or tripartite recognition motif (TRM).

[0107] As used herein, the term "**target site**" refers to a nucleic acid site that an RNA binding domain is capable of binding to (also referred to herein as "**TS**"). As used herein, the term "**PUF target sites**" refers to a nucleic acid sequence that a PUF RNA binding domain can bind to.

II. Synthetic Circuits

[0108] Provided herein is a synthetic circuit comprising a plurality of nucleotide sequences (*e.g.*, a first nucleotide sequence and a second nucleotide sequence), wherein one or more of the plurality of nucleotide sequences comprises a sensor, which is capable of regulating the activity and/or expression of one or more of the plurality of nucleotide sequences. In some aspects, the activity or expression of a payload can be controlled by direct or indirect interaction between the two nucleotide sequences. Accordingly, in some aspects, the present disclosure relates to a polynucleotide (*e.g.*, synthetic circuit) comprising: (a) a first nucleotide sequence (e.g., a regulator sequence) comprising a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain and (b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises a first sensor ("first type P sensor") comprising a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TS"). In some aspects, upon binding of the PUF RBD to the PUF TS, the effector domain is capable of downregulating the payload sequence. As used herein, regulating the "**expression of the regulator**" can comprise: (i) regulating the amount of regulator expressed in the cell, (ii) regulating the activity of the regulator, or (iii) both (i) and (ii). Similarly, regulating the "**expression of the payload**" can comprise: (i) regulating the amount of payload expressed in the cell, (ii) regulating the activity of the payload, or (iii) both (i) and (ii).

II.A. Regulator Sequence

[0109] As described herein, in some aspects, a synthetic circuit comprises a nucleotide sequence, which comprises or encodes a regulator ("**regulator sequence**"). Accordingly, in some aspects, a synthetic circuit of the present disclosure comprises a payload sequence (*e.g.*, any of the payload sequences described above) and a regulator sequence. In some aspects, the regulator sequence comprises a nucleotide sequence encoding PUF based engineered RNA binding domain and an effector domain. In some aspects, the effector domain is capable of

downregulating the payload sequence upon binding of the PUF RBD to a PUF TS (e.g., WT TS or TS #1-TS #9) located in the payload sequence.

[0110] In some aspects, the regulator sequence is linear (*e.g.*, linear RNA). In some aspects, the regulator sequence is circular (*e.g.*, circular RNA). In some aspects, the regulator sequence is non-replicating (*e.g.*, non-replicating RNA).

II.A.1. PUF-based engineered RNA Binding Domain (RBD)

[0111] In some aspects, the regulator sequence in the synthetic circuit comprises an RNA binding domain of PUF-based engineered RNA endonucleases. In some aspects, a synthetic circuit provided herein comprises a PUF-based engineered RNA endonucleases with reprogrammed PUF RNA binding domains. Pumilio/fem-3 mRNA binding factor (PUF) proteins are eukaryotic RNA-binding proteins (RBPs) that are involved in post-transcriptional gene regulation. The RNA-binding region of the human Pumilio 1 (PUM1) protein has 8 structural repeats (R1 – R8), which recognizes the 8nt target RNA sequence NRE: 5'-UGUAUAUA-3', which is also referred to herein as a Target Site (TS). The N-terminal repeat (R1) binds to the 3′-nucleotide residue (N8) of the target sequence, while the C-terminal repeat (R8) binds to the 5′-nucleotide residue (N1).

[0112] In some aspects, the PUF RBD useful for the synthetic circuit comprises one or more amino acid substitutions compared to the wild type PUF RBD as set forth in SEQ ID NO: 1. In some aspects, the PUF RBD useful for the synthetic circuit comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the wild type PUF RBD as set forth in SEQ ID NO: 1, wherein the PUF RBD is capable of binding to the PUF TS.

[0113] In some aspects, the amino acid substitution in the PUF RBD comprises a substitution corresponding to an amino acid position in R1, R2, R3, R4, R5, R6, R7, or R8 of the wild type PUF RBD as set forth in SEQ ID NO: 1, or any combination thereof. In some aspects, the amino acid substitution in the PUF RBD is in R1. In some aspects, the amino acid substitution in the PUF RBD is in R2. In some aspects, the amino acid substitution in the PUF RBD is in R3. In some aspects, the amino acid substitution in the PUF RBD is in R4. In some aspects, the amino acid substitution in the PUF RBD is in R5. In some aspects, the amino acid substitution in the PUF RBD is in R6. In some aspects, the amino acid substitution in the PUF RBD is in R7. In some aspects, the amino acid substitution in the PUF RBD is in R8. In some aspects, the amino acid substitution in the PUF RBD is capable of binding to the PUF TS site

with greater affinity than the wild type PUF RBD as set forth in SEQ ID NO: 1. In some aspects, the amino acid substitution in the PUF RBD has less off-target binding than the wild type PUF RBD as set forth in SEQ ID NO: 1.

[0114] In some aspects, the PUF RBD comprises additional synthetic PUF repeats. In some aspects, the PUF RBD comprises two, three, four, five, six, seven, or eight additional synthetic PUF repeats. In some aspects, the PUF RBD comprises two additional synthetic PUF repeats. In some aspects, the PUF RBD comprises three additional synthetic PUF repeats. In some aspects, the PUF RBD comprises four additional synthetic PUF repeats. In some aspects, the PUF RBD comprises five additional synthetic PUF repeats. In some aspects, the PUF RBD comprises six additional synthetic PUF repeats. In some aspects, the PUF RBD comprises seven additional synthetic PUF repeats. In some aspects, the PUF RBD comprises eight additional synthetic PUF repeats.

[0115] In some aspects, the PUF RBD comprises one or more sequences listed in Table 3. In some aspects, the PUF RBD comprises one or more sequences selected from SEQ ID Nos: 1-30.

[0116] In some aspects, the PUF RBD useful for the synthetic circuit comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the PUF RBD as set forth in any one of SEQ ID NOs: 1-30, wherein the PUF RBD is capable of binding to the PUF TS.

[0117] In some aspects, the PUF RBD useful for the synthetic circuit comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the PUF RBD as set forth in any one of SEQ ID NOs: 2-8, wherein the PUF RBD is capable of binding to the PUF TS.

[0118] In some aspects, the PUF RBD useful for the synthetic circuit comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the PUF RBD as set forth in any one of SEQ ID NOs: 9-30, wherein the PUF RBD is capable of binding to the PUF TS.

[0119] In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 2. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 3. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set

forth in SEQ ID NO: 4. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 5. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 6 In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 7. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 8. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 9. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 10. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 11. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 12. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 13. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 14. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 15. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 16. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 17. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 18. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 19. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 20. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 21. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 22. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 23. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 24. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 25. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 26. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 27. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 28. In some aspects, the PUF RBD useful for the

synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 29. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 30.

[0120] As described herein, in some aspects, synthetic circuits described herein comprise a payload sequence, wherein the payload sequence comprises a sensor (e.g., WT TS or TS#1-TS#9) that is capable of specifically recognizing a PUF RBD. Unless indicated otherwise, the specific binding of the PUF RBD to the PUF TS (i.e., first type P sensor) reduces or inhibits the expression of the payload encoded by the payload sequence. Accordingly, where a payload sequence comprises both a sensor (e.g., PUF TS) that is capable of specifically recognizing a PUF RBD (*e.g.*, first type P sensor) and a sensor that is capable of specifically recognizing a marker (*e.g.*, second type P sensor), the expression of the encoded payload can be regulated by at least two different manners. Not to be bound by any one theory, in some aspects, such a dual approach to regulation allows for greater selectivity in the expression of the payload.

[0121] In some aspects, when a synthetic circuit described herein is introduced into a non-target cell (*i.e.*, does not express sufficient level of a type R marker to be recognized by the type R sensor) such that the expression of the PUF RBD is increased, the PUF RBDs are capable of being specifically recognized by a type P sensor (*e.g.*, PUF TS), resulting in the activation of the type P sensor. As further described herein, activation of the type P sensor reduces or inhibits the expression of the payload encoded by the payload sequence. Accordingly, in some aspects, when a synthetic circuit provided herein is contacted with a population of cells comprising both target cells and non-target cells, the expression of the payload in the non-target cell (*i.e.*, has increased expression of the regulator) is decreased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the target cell (*i.e.*, has reduced expression of the regulator). In some aspects, when a synthetic circuit provided herein is contacted with a population of cells comprising both target cells and non-target cells, the expression of the payload in the target cell (*i.e.*, has reduced expression of the regulator) is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%, as compared to the corresponding expression in the non-target cell. In some aspects, as compared to the non-target cell, the expression of the payload in the target cell is increased by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-

fold, at least about 10-fold, at least about 12.5-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold.

II.A.2. Effector Domain

[0122] The present disclosure also provides an effector domain that is linked to the regulator (e.g., PUF RBD) such that upon binding of the PUF RBD to its target site, the effector domain is capable of downregulating the payload sequence. In some aspects, the effector domain is a degradation domain, a translation inhibitor domain, and/or a protein recruiting domain that is capable of recruiting another active protein to downregulate the payload expression. In some aspects, the effector domain comprises an endonuclease domain. In some aspects, the effector domain comprises an entire endonuclease. In some aspects, the effector domain comprise a fragment of an endonuclease that has an enzymatic activity. Non-limiting examples of the effector domain include cNOT7, TTP, DDX6, and MCPIP1.

[0123] In some aspects, the effector domain is a cNOT7. cNOT7 is known as CCR4-NOT transcription complex subunit 7. cNOT7 (uniprot no. Q9UIV1) is a deadenylase that has 3'-5' poly(A) exoribonuclease activity for synthetic poly(A) RNA substrate. Catalytic component of the CCR4-NOT complex which is one of the major cellular mRNA deadenylases and is linked to various cellular processes including bulk mRNA degradation, miRNA-mediated repression, translational repression during translational initiation and general transcription regulation. During miRNA-mediated repression the complex seems also to act as translational repressor during translational initiation. Additional complex functions may be a consequence of its influence on mRNA expression. cNOT7 associates with members of the BTG family such as TOB1 and BTG2 and is required for their anti-proliferative activity. In some aspects, cNOT comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 34. In some aspects, cNOT7 comprises the amino acid sequence as set forth in SEQ ID NO: 34.

[0124] In some aspects, the effector domain is TTP (ZFP36). TTP (uniprot no. P26651) is known as mRNA decay activator protein ZFP36. In some aspects, the effector domain is TTP mutant C147R. TTP is a zinc-finger RNA-binding protein that destabilizes several cytoplasmic AU-rich element (ARE)-containing mRNA transcripts by promoting their poly(A) tail removal

or deadenylation, and hence provide a mechanism for attenuating protein synthesis. TTP can also act as an 3'-untranslated region (UTR) ARE mRNA-binding adapter protein to communicate signaling events to the mRNA decay machinery. TTP recruits deadenylase CNOT7 (and probably the CCR4-NOT complex) via association with CNOT1, and hence promotes ARE-mediated mRNA deadenylation. TTP can also functions also by recruiting components of the cytoplasmic RNA decay machinery to the bound ARE-containing mRNAs, self regulates by destabilizing its own mRNA, binds to 3'-UTR ARE of numerous mRNAs and of its own mRNA, plays a role in anti-inflammatory responses; suppresses tumor necrosis factor (TNF)-alpha production by stimulating ARE-mediated TNF-alpha mRNA decay and several other inflammatory ARE-containing mRNAs in interferon (IFN)- and/or lipopolysaccharide (LPS)-induced macrophages (By similarity), plays a role in the regulation of dendritic cell maturation at the post-transcriptional level, and hence operates as part of a negative feedback loop to limit the inflammatory response, promotes ARE-mediated mRNA decay of hypoxia-inducible factor HIF1A mRNA during the response of endothelial cells to hypoxia, positively regulates early adipogenesis of preadipocytes by promoting ARE-mediated mRNA decay of immediate early genes (IEGs) (By similarity), negatively regulates hematopoietic/erythroid cell differentiation by promoting ARE-mediated mRNA decay of the transcription factor STAT5B mRNA, or plays a role in maintaining skeletal muscle satellite cell quiescence by promoting ARE-mediated mRNA decay of the myogenic determination factor MYOD1 mRNA (By similarity).

[0125] In some aspects, TTP comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 36. In some aspects, TTP comprises the amino acid sequence as set forth in SEQ ID NO: 36.

[0126] In some aspects, the effector domain is DDX6 (uniprot no P26196) is known as Probable ATP-dependent RNA helicase DDX6. DDX6 is a helicase involved in decapping and deadenylation. DDX6 is essential for the formation of P-bodies, cytosolic membrane-less ribonucleoprotein granules involved in RNA metabolism through the coordinated storage of mRNAs encoding regulatory functions, plays a role in P-bodies to coordinate the storage of translationally inactive mRNAs in the cytoplasm and prevent their degradation, in the process of mRNA degradation, plays a role in mRNA decapping, and blocks autophagy in nutrient-rich conditions by repressing the expression of ATG-related genes through degradation of their transcripts.

[0127] In some aspects, DDX6 comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 31. In some aspects, DDX6 comprises the amino acid sequence as set forth in SEQ ID NO: 31.

[0128] In some aspects, the effector domain is an endonuclease domain of MCPIP1 ($MCPIP1_{PIN}$). MCPIP is known as Endoribonuclease ZC3H12A (uniprot no. Q5D1E8). In some aspects, $MCPIP1_{PIN}$ comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 35. In some aspects, $MCPIP1_{PIN}$ comprises the amino acid sequence as set forth in SEQ ID NO: 35.

[0129] In some aspects, the effector domain is an endonuclease domain of Dis3 isoform 2 (Dis3 iso2 (PIN)). Dis3 is also known as exosome complex exonuclease RRP44 (uniprot no. Q9Y2L1). In some aspects, Dis3 iso2 (PIN) comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 32. In some aspects, Dis3 iso2 (PIN) comprises the amino acid sequence as set forth in SEQ ID NO: 32.

[0130] In some aspects, the effector domain is an endonuclease domain of Smg6 (PIN). Smg6 is also known as telomerase-binding protein EST1A (uniprot no Q86US8). In some aspects, Smg6 (PIN) comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the amino acid sequence as set forth in SEQ ID NO: 33. In some aspects, Smg6 (PIN) comprises the amino acid sequence as set forth in SEQ ID NO: 33.

[0131] In some aspects, the effector domain comprises an endonuclease domain of Ago2 (e.g., SEQ ID NO: 37), angiogenin precursor (e.g., SEQ ID NO: 38), APEX1 (e.g., SEQ ID NO: 39), APEX2 (e.g., SEQ ID NO: 40), BTG1 (e.g., SEQ ID NO: 41), CHTOP (e.g., SEQ ID NO: 42), CNOT2 (e.g., SEQ ID NO: 43), CNOT4 (e.g., SEQ ID NO: 44), CPEB2 (e.g., SEQ ID NO: 45), CPEB4 (e.g., SEQ ID NO: 46), CPSF4L (e.g., SEQ ID NO: 47), CPSF5 (e.g., SEQ ID NO: 48), Dis3 iso1 (PIN) (e.g., SEQ ID NO: 49), DNA2 (nuclease domain) (e.g., SEQ ID NO: 50), ENDOD1 (e.g., SEQ ID NO: 51), ENDOG (e.g., SEQ ID NO: 52), EndoV (e.g.,

SEQ ID NO: 53), ERCC1 (e.g., SEQ ID NO: 54), ERN2 (e.g., SEQ ID NO: 55), EXOG (e.g., SEQ ID NO: 56), FEN1(e.g., SEQ ID NO: 57), FIP1L1 (e.g., SEQ ID NO: 58), FUBP1 (e.g., SEQ ID NO: 59), HNRNPCL1 (e.g., SEQ ID NO: 60), HRSP12 (e.g., SEQ ID NO: 61), KIAA0391 (e.g., SEQ ID NO: 62), LACTB2 (e.g., SEQ ID NO: 63), LSM4 (e.g., SEQ ID NO: 64), MCPIP1 (e.g., SEQ ID NO: 65), MCPIP1 PIN with NTR (e.g., SEQ ID NO: 66), MCPIP4 (e.g., SEQ ID NO: 67), MEX3C (e.g., SEQ ID NO: 68), MYEF2 (C-term isoformB) (e.g., SEQ ID NO: 69), MYEF2 (isoform a) (e.g., SEQ ID NO: 70), NANOS2 (e.g., SEQ ID NO: 71), NANOS3 (e.g., SEQ ID NO: 72), NOB1 (e.g., SEQ ID NO: 73), NOP56 (e.g., SEQ ID NO: 74), NTHL1 (e.g., SEQ ID NO: 75), PARN (e.g., SEQ ID NO: 76), PDL6 (e.g., SEQ ID NO: 77), Pelota homolog (e.g., SEQ ID NO: 78), PRR3 (e.g., SEQ ID NO: 79), QKI (e.g., SEQ ID NO: 80), RAB1 (e.g., SEQ ID NO: 81), RAC1 (e.g., SEQ ID NO: 82), RBM22 (e.g., SEQ ID NO: 83), RBM7 (e.g., SEQ ID NO: 84), RIDA (e.g., SEQ ID NO: 85), RNAse L (e.g., SEQ ID NO: 86), RNAse1 (e.g., SEQ ID NO: 87), RNAse11 (e.g., SEQ ID NO: 88), RNAse2 (e.g., SEQ ID NO: 89), RNAse4 (e.g., SEQ ID NO: 90), RNAse6 (e.g., SEQ ID NO: 91), RNAse6PL (e.g., SEQ ID NO: 92), RNAse7 (e.g., SEQ ID NO: 93), RNAse8 (e.g., SEQ ID NO: 94), RNAseK (e.g., SEQ ID NO: 95), RNAseT2 (e.g., SEQ ID NO: 96), SLFN14 (e.g., SEQ ID NO: 97), SNRPA (e.g., SEQ ID NO: 98), SRSF11 (e.g., SEQ ID NO: 99), THRAP3 (e.g., SEQ ID NO: 100), TOB1 (e.g., SEQ ID NO: 101), TOB2 (e.g., SEQ ID NO: 102), U2AF2 (e.g., SEQ ID NO: 103), UTP11L (e.g., SEQ ID NO: 104), YBEY (e.g., SEQ ID NO: 105), YTHDF2 (e.g., SEQ ID NO: 106), ZFC3H1 (e.g., SEQ ID NO: 107), ZFP36L1 (e.g., SEQ ID NO: 108), or ZFP36L2 (e.g., SEQ ID NO: 109).

[0132] In some aspects, the effector domain comprises Ago2 (e.g., SEQ ID NO: 37), angiogenin precursor (e.g., SEQ ID NO: 38), APEX1 (e.g., SEQ ID NO: 39), APEX2 (e.g., SEQ ID NO: 40), BTG1 (e.g., SEQ ID NO: 41), CHTOP (e.g., SEQ ID NO: 42), CNOT2 (e.g., SEQ ID NO: 43), CNOT4 (e.g., SEQ ID NO: 44), CPEB2 (e.g., SEQ ID NO: 45), CPEB4 (e.g., SEQ ID NO: 46), CPSF4L (e.g., SEQ ID NO: 47), CPSF5 (e.g., SEQ ID NO: 48), Dis3 iso1 (PIN) (e.g., SEQ ID NO: 49), DNA2 (nuclease domain) (e.g., SEQ ID NO: 50), ENDOD1 (e.g., SEQ ID NO: 51), ENDOG (e.g., SEQ ID NO: 52), EndoV (e.g., SEQ ID NO: 53), ERCC1 (e.g., SEQ ID NO: 54), ERN2 (e.g., SEQ ID NO: 55), EXOG (e.g., SEQ ID NO: 56), FEN1(e.g., SEQ ID NO: 57), FIP1L1 (e.g., SEQ ID NO: 58), FUBP1 (e.g., SEQ ID NO: 59), HNRNPCL1 (e.g., SEQ ID NO: 60), HRSP12 (e.g., SEQ ID NO: 61), KIAA0391 (e.g., SEQ ID NO: 62), LACTB2 (e.g., SEQ ID NO: 63), LSM4 (e.g., SEQ ID NO: 64), MCPIP1 (e.g., SEQ ID NO: 65), MCPIP1 PIN with NTR (e.g., SEQ ID NO: 66), MCPIP4 (e.g., SEQ ID NO: 67), MEX3C (e.g., SEQ ID NO: 68), MYEF2 (C-term isoformB) (e.g., SEQ ID NO: 69),

MYEF2 (isoform a) (e.g., SEQ ID NO: 70), NANOS2 (e.g., SEQ ID NO: 71), NANOS3 (e.g., SEQ ID NO: 72), NOB1 (e.g., SEQ ID NO: 73), NOP56 (e.g., SEQ ID NO: 74), NTHL1 (e.g., SEQ ID NO: 75), PARN (e.g., SEQ ID NO: 76), PDL6 (e.g., SEQ ID NO: 77), Pelota homolog (e.g., SEQ ID NO: 78), PRR3 (e.g., SEQ ID NO: 79), QKI (e.g., SEQ ID NO: 80), RAB1 (e.g., SEQ ID NO: 81), RAC1 (e.g., SEQ ID NO: 82), RBM22 (e.g., SEQ ID NO: 83), RBM7 (e.g., SEQ ID NO: 84), RIDA (e.g., SEQ ID NO: 85), RNAse L (e.g., SEQ ID NO: 86), RNAse1 (e.g., SEQ ID NO: 87), RNAse11 (e.g., SEQ ID NO: 88), RNAse2 (e.g., SEQ ID NO: 89), RNAse4 (e.g., SEQ ID NO: 90), RNAse6 (e.g., SEQ ID NO: 91), RNAse6PL (e.g., SEQ ID NO: 92), RNAse7 (e.g., SEQ ID NO: 93), RNAse8 (e.g., SEQ ID NO: 94), RNAseK (e.g., SEQ ID NO: 95), RNAseT2 (e.g., SEQ ID NO: 96), SLFN14 (e.g., SEQ ID NO: 97), SNRPA (e.g., SEQ ID NO: 98), SRSF11 (e.g., SEQ ID NO: 99), THRAP3 (e.g., SEQ ID NO: 100), TOB1 (e.g., SEQ ID NO: 101), TOB2 (e.g., SEQ ID NO: 102), U2AF2 (e.g., SEQ ID NO: 103), UTP11L (e.g., SEQ ID NO: 104), YBEY (e.g., SEQ ID NO: 105), YTHDF2 (e.g., SEQ ID NO: 106), ZFC3H1 (e.g., SEQ ID NO: 107), ZFP36L1 (e.g., SEQ ID NO: 108), or ZFP36L2 (e.g., SEQ ID NO: 109).

[0133] In some aspects, an effector domain useful for the present circuit comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to one or more amino acid sequences as set forth in any one of SEQ ID NOs: 37 to 109. In some aspects, an effector domain comprises one or more amino acid sequences as set forth in any one of SEQ ID NOs: 37-109.

II.A.3. Type R Sensors

[0134] In some aspects, the regulator sequence comprises a sensor that is capable of specifically recognizing a marker. Accordingly, some aspects of the present disclosure relate to a synthetic circuit comprising a payload sequence and a regulator sequence, wherein the payload sequence comprises a sensor (type P sensor), and wherein the regulator sequence comprises a sensor ("**type R sensor**"). In some aspects, present disclosure provides a synthetic circuit comprising a payload sequence and a regulator sequence, wherein the payload sequence comprises a first type P sensor (*e.g.*, specifically recognizes a regulator) and a second type P sensor (*e.g.*, specifically recognizes a marker), and wherein the regulator sequence comprises a type R sensor (*e.g.*, specifically recognizes a marker). Where a synthetic circuit described

herein comprises both a type P sensor and a type R sensor, in some aspects, the type P sensor and the type R sensor are not the same (*e.g.*, do not specifically recognize the same ligand).

[0135] In some aspects, a regulator sequence useful for the present disclosure comprises a plurality of sensors. For example, in some aspects, a regulator sequence comprises about two type R sensors, about three type R sensors, about four type R sensors, about five type R sensors, about six type R sensors, about seven type R sensors, about eight type R sensors, about nine type R sensors, or about 10 or more type R sensors. In some aspects, the regulator sequence comprises at least two type R sensors. In some aspects, the regulator sequence comprises at least three type R sensors. In some aspects, the regulator sequence comprises at least four type R sensors. In some aspects, the regulator sequence comprises at least five type R sensors. In some aspects, the regulator sequence comprises at least six type R sensors. In some aspects, the regulator sequence comprises at least seven type R sensors. In some aspects, the regulator sequence comprises at least eight type R sensors. In some aspects, the regulator sequence comprises at least nine type R sensors. In some aspects, the regulator sequence comprises at least 10 type R sensors.

[0136] In some aspects, each of the plurality of sensors on the regulator sequence is the same. For instance, in some aspects, a synthetic circuit described herein comprises a payload sequence and a regulator sequence, wherein the regulator sequence comprises a plurality of type R sensors, and wherein each of the plurality of type R sensors recognize the same marker (*e.g.*, each of the type R sensors comprise the same binding site for the marker). In some aspects, one or more of the plurality of sensors on the regulator sequence are different. For instance, in some aspects, one or more of the plurality of type R sensors recognize different markers. In some aspects, one or more of the plurality of type R sensors recognize a different binding site for the same marker.

II.A.4. Type R Spacers

[0137] In some aspects, a regulator sequence provided herein further comprises a spacer sequence ("**type R spacer**"). Accordingly, in some aspects, a synthetic circuit provided herein comprises a payload sequence and a regulator sequence, wherein the payload sequence comprises a type P sensor (*e.g.*, first type P sensor and/or second type P sensor) and a type P spacer, and wherein the regulator sequence comprises a type R sensor and a type R spacer. In some aspects, the type R spacer and the type P spacer are not the same. In some aspects, the type R spacer and the type P spacer are the same.

[0138] In some aspects, a regulator sequence comprises a plurality of type R spacers. In some aspects, the regulator sequence comprises about two type R spacers, about three type R spacers, about four type R spacers, about five type R spacers, about six type R spacers, about seven type R spacers, about eight type R spacers, about nine type R spacers, or about 10 or more type R spacers. In some aspects, the regulator sequence comprises at least two type R spacers. In some aspects, the regulator sequence comprises at least three type R spacers. In some aspects, the regulator sequence comprises at least four type R spacers. In some aspects, the regulator sequence comprises at least five type R spacers. In some aspects, the regulator sequence comprises at least six type R spacers. In some aspects, the regulator sequence comprises at least seven type R spacers. In some aspects, the regulator sequence comprises at least eight type R spacers. In some aspects, the regulator sequence comprises at least nine type R spacers. In some aspects, the regulator sequence comprises at least 10 type R spacers. In some aspects, each of the type R spacers are the same. In some aspects, one or more of the type R spacers are different.

[0139] In some aspects, the type R spacer is positioned upstream of the type R sensor within the regulator sequence. In some aspects, the type R spacer is positioned downstream of the type R sensor. In some aspects, where the regulator sequence encodes the regulator, the type R spacer is positioned between the coding region of the regulator sequence and the type R sensor (*e.g.*, after the stop codon of the coding region and before the beginning of the type R sensor). As used herein, the term "**coding region of the regulator sequence**" refers to the portion of the regulator sequence that specifically encodes for the regulator.

[0140] Where the regulator sequence comprises a plurality of type R sensors, in some aspects, the type R spacer is upstream of one or more of the plurality of type R sensors. In some aspects, the type R spacer is downstream of one or more of the plurality of type R sensors. In some aspects, the type R spacer is positioned in between at least two of the plurality of type R sensors. In some aspects, each of the plurality of type R sensors are separated by a type R spacer.

[0141] Where the regulator sequence comprises a plurality of type R spacers, in some aspects, each of the type R spacers are the same. In some aspects, one or more of the type R spacers are different. In some aspects, a type R spacer can be of any suitable lengths such that the type R spacer aids in the binding of the type R sensor to its ligand (*e.g.*, marker). In some aspects, the type R spacer is between about 1 to about 100 nucleotides in length. In some aspects, the type R spacer is about 1 nucleotide in length, about 5 nucleotides in length, about 10 nucleotides in length, about 15 nucleotides in length, about 20 nucleotides in length, about

25 nucleotides in length, about 30 nucleotides in length, about 35 nucleotides in length, about 40 nucleotides in length, about 45 nucleotides in length, about 50 nucleotides in length, about 55 nucleotides in length, about 60 nucleotides in length, about 65 nucleotides in length, about 70 nucleotides in length, about 75 nucleotides in length, about 80 nucleotides in length, about 85 nucleotides in length, about 90 nucleotides in length, about 95 nucleotides in length, or about 100 nucleotides in length. In some aspects, the type R spacer is between about 1 to about 50 nucleotides in length. In some aspects, the type R spacer is about 5 nucleotides in length. In some aspects, the type R spacer is about 10 nucleotides in length. In some aspects, the type R spacer is about 15 nucleotides in length. In some aspects, the type R spacer is about 20 nucleotides in length. In some aspects, the type R spacer is about 25 nucleotides in length. In some aspects, the type R spacer is about 30 nucleotides in length. In some aspects, the type R spacer is about 35 nucleotides in length. In some aspects, the type R spacer is about 40 nucleotides in length. In some aspects, the type R spacer is about 45 nucleotides in length. In some aspects, the type R spacer is about 50 nucleotides in length.

[0142] Unless indicated otherwise, type R spacers useful for the present disclosure are not limited to any specific nucleotide sequences, as long as the type R spacers are of sufficient length to carry out their intended function (*e.g.*, aid in the binding of the type R sensors to their ligands). Accordingly, in some aspects, a type R spacer useful for the present disclosure comprises a randomly generated nucleotide sequence. In some aspects, where a plurality of type R spacers are used in separating a plurality of type R sensors, one or more of the plurality of type R spacers have a difference sequence, such that the plurality of type R spacers do not include randomly generated nucleotide sequences that repeat. In some aspects, the type R spacer is different than the type P spacer.

II.A.5. Markers

[0143] As is apparent from the present disclosure, synthetic circuits described herein can be programmed to selectively regulate the expression of a particular gene (or a protein encoded thereof) in a target cell. Not to be bound by any one theory, in some aspects, because the payload sequence and/or the regulator sequence comprise a sensor (*e.g.*, type P sensor or type R sensor) that has been programmed to recognize a specific marker, the sensor is "**turned on**" (*i.e.*, in an active form) only in cells that comprise sufficient level of the marker to be recognized by the sensor. Where a cell does not comprise sufficient level of the marker, the sensor is "**turned off**" (*i.e.*, in an inactive form) as the sensor does not specifically recognize the marker. The below table summarizes the possible scenarios with regard to marker level and

payload expression. The status of regulator expression is also listed in the table. Unless indicated otherwise, a marker useful for the present disclosure does not comprise a regulator as described herein.

[0144] To help illustrate, in some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence comprises a type P sensor that is capable of specifically recognizing a marker expressed in a non-target cell, and wherein the recognition of the marker by the type P sensor reduces or inhibits the expression of the encoded payload in the non-target cell. For such a synthetic circuit, when introduced into the non-target cell (*i.e.*, expresses sufficient level of the marker to be recognized by the type P sensor), the type P sensor becomes active (*i.e.*, bound to the marker) and thereby, inhibits or reduces the expression of the encoded payload in the non-target cell. However, when introduced into a target cell (*i.e.*, does not express sufficient level of the marker to be recognized by the type P sensor), the type P sensor remains inactive (*i.e.*, not bound to a ligand) and therefore, the payload is expressed in the target cell. As is apparent from the present disclosure, payload can be selectively expressed when both of the following are true: (1) NONE of the second type P sensors are activated, and (2) one or more of the type R sensors is/are activated. When either or both of the following two conditions are not met, payload expression can be inhibited:

[0145] (1) ALL of the type P markers are low

[0146] (2) at least one of the type R markers is high.

[0147] As used herein, "**expression of the payload**" (or grammatical equivalent thereof) refers to any of the following: (a) amount of the payload expressed in the cell, (b) how quickly the payload is expressed in the cell, (c) duration of the payload expression, or (d) any combination of (a) to (c). Not to be bound by any one theory, in some aspects, a synthetic circuit provided herein allows for the selective expression of a payload in a target cell by modulating the expression of the payload and regulator sequences. For instance, as is apparent from the present disclosure, when introduced into a target cell (*i.e.*, does not express sufficient level of a type P marker to be recognized by the type P sensor and expresses sufficient level of a type R marker to be recognized by at least one type R sensor), the expression of the payload in the target cell is increased as compared to the expression of the regulator in the target cell. In some aspects, the expression of the payload in the target cell is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%, as compared to the expression of the regulator in the target cell. In some aspects, the expression of the payload in the target cell is increased by at least about 1.5-fold, at least about 2-fold, at

least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 12.5-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold, as compared to the expression of the regulator. In some aspects, the expression of the regulator in the target cell is decreased in the target cell as compared to the expression of the payload. In some aspects, the expression of the regulator in the target cell is decreased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the expression of the payload in the target cell.

[0148] Unless indicated otherwise, a marker comprises any molecule that is expressed in a cell and can be specifically recognized by a sensor provided herein (*e.g.*, type P sensor and/or type R sensor). As described herein, in some aspects, a marker is selectively expressed (or expressed to a sufficient level) in certain cells but not in other cells. For instance, in some aspects, a marker is expressed to a sufficient level to be recognized by a sensor (*e.g.*, type P sensor or type R sensor) in a first cell but not in a second cell. For such aspects, when a synthetic circuit described herein is introduced into the first cell, the sensor (*e.g.*, type P sensor and/or type R sensor) specifically recognizes the marker and becomes active. When such a synthetic circuit is introduced into the second cell, the sensor (*e.g.*, type P sensor and/or type R sensor) remains inactive (*i.e.*, not bound to a marker).

[0149] Non-limiting examples of markers that can be used with the present disclosure include a microRNA (miRNA), a protein, a metabolite, or combinations thereof. In some aspects, the marker comprises a miRNA. In some aspects, a marker comprises a protein. In some aspects, a marker comprises a metabolite.

[0150] As further explained elsewhere in the present disclosure, a synthetic circuit provided herein can comprise a plurality of sensors. For instance, in some aspects, a synthetic circuit comprises a payload sequence, wherein the payload sequence comprises a plurality of sensors (*e.g.*, plurality of first type P sensor and/or plurality of second type P sensor). In some aspects, a synthetic circuit comprises a regulator sequence, wherein the regulator sequence comprises a plurality of sensors. In some aspects, a synthetic circuit comprises a payload sequence and a regulator sequence, wherein the payload sequence comprises a plurality of sensors, and wherein the regulator sequence comprises a plurality of sensors. Where a synthetic circuit comprises a plurality of sensors, in some aspects, each of the plurality of sensors can specifically recognize the same marker. For example, in some aspects, the payload sequence

of a synthetic circuit provided herein comprises a plurality of sensors, wherein each of the plurality of sensors recognizes the same miRNA. Where a synthetic circuit comprises a plurality of sensors, in some aspects, one or more of the plurality of sensors specifically recognizes different markers. For example, in some aspects, a synthetic circuit comprises a payload sequence and a regulator sequence, wherein the payload sequence and the regulator sequence each comprise a sensor, wherein the sensor of the payload sequence specifically recognizes a first marker (*e.g.*, miRNA) and wherein the sensor of the regulator sequence specifically recognizes a second marker (*e.g.*, metabolite or a different miRNA).

II.B. Payload Sequence

[0151] As is apparent from the present disclosure, in some aspects, a synthetic circuit comprises a nucleotide sequence encoding a payload (payload sequence). In some aspects, the payload sequence can encode any suitable proteins known in the art. Non-limiting examples of suitable payloads include a therapeutic protein, reporter protein, immunomodulatory protein, chimeric antigen receptor (CAR), or combinations thereof.

[0152] In some aspects, the payload sequence is linear (*e.g.*, linear RNA). In some aspects, the payload sequence is circular (*e.g.*, circular RNA). In some aspects, the payload sequence is self-replicating (*e.g.*, self-replicating RNA). In some aspects, the payload sequence is non-replicating (*e.g.*, non-replicating RNA).

[0153] In some aspects, the payload sequence in the synthetic circuit comprises a first sensor ("first type P sensor") comprising a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TS"). In some aspects the payload sequence comprises a plurality of type P sensors. In some aspects the payload sequence further comprises type P spacers between two type P sensors.

II.B.1. Type P Sensors

[0154] In some aspects, the payload sequence comprises a sensor that is capable of being specifically bound by a regulator (*e.g.*, encoded by the regulator sequence, e.g., RNA binding domain, e.g., PUF RBD). In some aspects, the payload sequence comprises a sensor that is capable of recognizing a marker. In some aspects, the payload sequence comprises a first sensor comprising a target site that is capable of being specifically bound by a PUF RBD (first type P sensor) and a second sensor that is capable of recognizing a marker (second type P sensor).

[0155] In some aspects, the first type P sensor comprises a target site (TS) that is capable of being bound specifically by a PUF RNA binding domain, e.g., the wild type PUF RBD as set forth in SEQ ID NO: 1. In some aspects, the TS comprises 5'-UGUAUAUA -3' (WT TS), 5'-UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), 5' -UGUAGCUA -3' (TS #9), or 5' -UGGUGGAGAA -3' (TS #10). In some aspects, the TS comprises 5'-UGUAUAUA -3' (WT TS). In some aspects, the TS comprises 5'- UGGAUGAA - 3' (TS #1). In some aspects, the TS comprises 5'- UGUACGUC -3' (TS #2). In some aspects, the TS comprises 5' - UCUACGUC -3' (TS #3). In some aspects, the TS comprises 5' - UGUACGAC – 3' (TS #4). In some aspects, the TS comprises 5' - UGUCCGUC -3' (TS #5). In some aspects, the TS comprises 5' - UGUACGUG – 3' (TS #6). In some aspects, the TS comprises 5' -UGGAAGUC -3' (TS #7). In some aspects, the TS comprises 5' - UGUGCCUC -3' (TS #8). In some aspects, the TS comprises 5' -UGUAGCUA -3' (TS #9). In some aspects, the TS comprises 5' -UGGUGGAGAA -3' (TS #10).

[0156] In some aspects, the TS comprises two copies of 5'- UGGAUGAA - 3' (TS #1). In some aspects, the TS comprises three copies of 5'- UGGAUGAA - 3' (TS #1). In some aspects, the TS comprises four copies of 5'- UGGAUGAA - 3' (TS #1). In some aspects, the TS comprises five copies of 5'- UGGAUGAA - 3' (TS #1). In some aspects, the TS comprises six copies of 5'- UGGAUGAA - 3' (TS #1). In some aspects, the TS comprises seven copies of 5'- UGGAUGAA - 3' (TS #1). In some aspects, the TS comprises eight copies of 5'-UGGAUGAA - 3' (TS #1).

[0157] In some aspects, the TS comprises two copies of 5'- UGUACGUC -3' (TS #2). In some aspects, the TS comprises three copies of 5'- UGUACGUC -3' (TS #2). In some aspects, the TS comprises four copies of 5'- UGUACGUC -3' (TS #2). In some aspects, the TS comprises five copies of 5'- UGUACGUC -3' (TS #2). In some aspects, the TS comprises six copies of 5'- UGUACGUC -3' (TS #2). In some aspects, the TS comprises seven copies of 5'-UGUACGUC -3' (TS #2). In some aspects, the TS comprises eight copies of 5'- UGUACGUC -3' (TS #2).

[0158] In some aspects, the TS comprises two copies of 5' - UCUACGUC -3' (TS #3). In some aspects, the TS comprises three copies of 5' - UCUACGUC -3' (TS #3). In some aspects, the TS comprises four copies of 5' - UCUACGUC -3' (TS #3). In some aspects, the TS comprises five copies of 5' - UCUACGUC -3' (TS #3). In some aspects, the TS comprises six copies of 5' - UCUACGUC -3' (TS #3). In some aspects, the TS comprises seven copies of 5'

- UCUACGUC -3' (TS #3). In some aspects, the TS comprises eight copies of 5' - UCUACGUC -3' (TS #3).

[0159] In some aspects, the TS comprises two copies of 5' - UGUACGAC – 3' (TS #4). In some aspects, the TS comprises three copies of 5' - UGUACGAC – 3' (TS #4). In some aspects, the TS comprises four copies of 5' - UGUACGAC – 3' (TS #4). In some aspects, the TS comprises five copies of 5' - UGUACGAC – 3' (TS #4). In some aspects, the TS comprises six copies of 5' - UGUACGAC – 3' (TS #4). In some aspects, the TS comprises seven copies of 5' - UGUACGAC – 3' (TS #4). In some aspects, the TS comprises eight copies of 5' - UGUACGAC – 3' (TS #4).

[0160] In some aspects, the TS comprises two copies of 5' - UGUCCGUC -3' (TS #5). In some aspects, the TS comprises three copies of 5' - UGUCCGUC -3' (TS #5). In some aspects, the TS comprises four copies of 5' - UGUCCGUC -3' (TS #5). In some aspects, the TS comprises five copies of 5' - UGUCCGUC -3' (TS #5). In some aspects, the TS comprises six copies of 5' - UGUCCGUC -3' (TS #5). In some aspects, the TS comprises seven copies of 5' - UGUCCGUC -3' (TS #5). In some aspects, the TS comprises eight copies of 5' - UGUCCGUC -3' (TS #5).

[0161] In some aspects, the TS comprises two copies of 5' - UGUACGUG – 3' (TS #6). In some aspects, the TS comprises three copies of 5' - UGUACGUG – 3' (TS #6). In some aspects, the TS comprises four copies of 5' - UGUACGUG – 3' (TS #6). In some aspects, the TS comprises five copies of 5' - UGUACGUG – 3' (TS #6). In some aspects, the TS comprises six copies of 5' - UGUACGUG – 3' (TS #6). In some aspects, the TS comprises seven copies of 5' - UGUACGUG – 3' (TS #6). In some aspects, the TS comprises eight copies of 5' - UGUACGUG – 3' (TS #6).

[0162] In some aspects, the TS comprises two copies of 5' -UGGAAGUC -3' (TS #7). In some aspects, the TS comprises three copies of 5' -UGGAAGUC -3' (TS #7). In some aspects, the TS comprises four copies of 5' -UGGAAGUC -3' (TS #7). In some aspects, the TS comprises five copies of 5' -UGGAAGUC -3' (TS #7). In some aspects, the TS comprises six copies of 5' -UGGAAGUC -3' (TS #7). In some aspects, the TS comprises seven copies of 5' -UGGAAGUC -3' (TS #7). In some aspects, the TS comprises eight copies of 5' - UGGAAGUC -3' (TS #7).

[0163] In some aspects, the TS comprises two copies of 5' - UGUGCCUC -3' (TS #8). In some aspects, the TS comprises three copies of 5' - UGUGCCUC -3' (TS #8). In some aspects, the TS comprises four copies of 5' - UGUGCCUC -3' (TS #8). In some aspects, the TS comprises five copies of 5' - UGUGCCUC -3' (TS #8). In some aspects, the TS comprises six

copies of 5' - UGUGCCUC -3' (TS #8). In some aspects, the TS comprises seven copies of 5' - UGUGCCUC -3' (TS #8). In some aspects, the TS comprises eight copies of 5' - UGUGCCUC -3' (TS #8).

[0164] In some aspects, the TS comprises two copies of 5' -UGUAGCUA -3' (TS #9). In some aspects, the TS comprises three copies of 5' -UGUAGCUA -3' (TS #9). In some aspects, the TS comprises four copies of 5' -UGUAGCUA -3' (TS #9). In some aspects, the TS comprises five copies of 5' -UGUAGCUA -3' (TS #9). In some aspects, the TS comprises six copies of 5' -UGUAGCU A -3' (TS #9). In some aspects, the TS comprises seven copies of 5' -UGUAGCUA -3' (TS #9). In some aspects, the TS comprises eight copies of 5' - UGUAGCUA -3' (TS #9).

[0165] In some aspects, the TS comprises two copies of 5' -UGGUGGAGAA -3' (TS #10). In some aspects, the TS comprises three copies of 5' -UGGUGGAGAA -3' (TS #10). In some aspects, the TS comprises four copies of 5' -UGGUGGAGAA -3' (TS #10). In some aspects, the TS comprises five copies of 5' -UGGUGGAGAA -3' (TS #10). In some aspects, the TS comprises six copies of 5' -UGGUGGAGAA -3' (TS #10). In some aspects, the TS comprises seven copies of 5' -UGGUGGAGAA -3' (TS #10). In some aspects, the TS comprises eight copies of 5' -UGGUGGAGAA -3' (TS #10).

[0166] In some aspects, a synthetic circuit provided herein comprises a plurality of sensors. For instance, in some aspects, a payload sequence provided herein comprises a plurality of first type P sensors, e.g., a plurality of TS. In some aspects, each of the plurality of sensors on the payload sequence is the same. In some aspects, one or more of the plurality of sensors on the payload sequence are different. Where the payload sequence comprises a first type P sensor (*e.g.*, recognizing a regulator) and a second type P sensor (*e.g.*, recognizing a marker), in some aspects, the payload sequence comprises a plurality of first type P sensors. For example in some aspects, the payload sequence comprises about two first type P sensors, about three first type P sensors, about four first type P sensors, about five first type P sensors, about six first type P sensors, about seven first type P sensors, about eight first type P sensors, about nine first type sensors, about 10 first type P sensors, about 11 first type P sensors, about 12 first type P sensors, about 13 first type P sensors, about 14 first type P sensors, about 15 first type P sensors, about 16 first type P sensors, about 17 first type P sensors, about 18 first type P sensors, about 19 first type P sensors, or about 20 or more first type P sensors. In some aspects, the payload sequence comprises at least two first type P sensors. In some aspects, the payload sequence comprises at least three first type P sensors. In some aspects, the payload sequence comprises at least four first type P sensors. In some aspects, the payload sequence comprises at least five

first type P sensors. In some aspects, the payload sequence comprises at least six first type P sensors. In some aspects, the payload sequence comprises at least seven first type P sensors. In some aspects, the payload sequence comprises at least eight first type P sensors. In some aspects, the payload sequence comprises at least nine first type P sensors. In some aspects, the payload sequence comprises at least 10 first type P sensors. In some aspects, the payload sequence comprises at least 11 first type P sensors. In some aspects, the payload sequence comprises at least 12 first type P sensors. In some aspects, the payload sequence comprises at least 13 first type P sensors. In some aspects, the payload sequence comprises at least 14 first type P sensors. In some aspects, the payload sequence comprises at least 15 first type P sensors. In some aspects, the payload sequence comprises at least 16 first type P sensors. In some aspects, the payload sequence comprises at least 17 first type P sensors. In some aspects, the payload sequence comprises at least 18 first type P sensors. In some aspects, the payload sequence comprises at least 19 first type P sensors. In some aspects, the payload sequence comprises at least 20 first type P sensors.

[0167] In some aspects, each of the type P sensors is the same. For instance, in some aspects, a synthetic circuit described herein comprises a payload sequence and a regulator sequence, wherein the payload sequence comprises a plurality of the first type P sensor, and wherein each of the plurality of the first type P sensor specifically recognizes the same regulator (*e.g.*, each of the first type P sensor comprises the same binding site for the regulator). In some aspects, one or more of the first type P sensors are different. For instance, in some aspects, one or more of the first type P sensors recognize a different regulator. In some aspects, one or more of the first type P sensors recognize a different binding site on the same regulator.

[0168] In some aspects, where the payload sequence comprises a first type P sensor (*e.g.*, recognizing a regulator) and a second type P sensor (*e.g.*, recognizing a marker), the payload sequence comprises a plurality of second type P sensors. For example, in some aspects, the payload sequence comprises about two second type P sensors, about three second type P sensors, about four second type P sensors, about five second type P sensors, about six second type P sensors, about seven second type P sensors, about eight second type P sensors, about nine second type P sensors, about 10 second type P sensors, about 11 second type P sensors, about 12 second type P sensors, about 13 second type P sensors, about 14 second type P sensors, about 15 second type P sensors, about 16 second type P sensors, about 17 second type P sensors, about 18 second type P sensors, about 19 second type P sensors, or about 20 or more second type P sensors. In some aspects, the payload sequence comprises at least two second type P sensors. In some aspects, the payload sequence comprises at least three second type P sensors.

In some aspects, the payload sequence comprises at least four second type P sensors. In some aspects, the payload sequence comprises at least five second type P sensors. In some aspects, the payload sequence comprises at least six second type P sensors. In some aspects, the payload sequence comprises at least seven second type P sensors. In some aspects, the payload sequence comprises at least eight second type P sensors. In some aspects, the payload sequence comprises at least nine second type P sensors. In some aspects, the payload sequence comprises at least 10 second type P sensors. In some aspects, the payload sequence comprises at least 11 second type P sensors. In some aspects, the payload sequence comprises at least 12 second type P sensors. In some aspects, the payload sequence comprises at least 13 second type P sensors. In some aspects, the payload sequence comprises at least 14 second type P sensors. In some aspects, the payload sequence comprises at least 15 second type P sensors. In some aspects, the payload sequence comprises at least 16 second type P sensors. In some aspects, the payload sequence comprises at least 17 second type P sensors. In some aspects, the payload sequence comprises at least 18 second type P sensors. In some aspects, the payload sequence comprises at least 19 second type P sensors. In some aspects, the payload sequence comprises at least 20 second type P sensors.

[0169] In some aspects, each of the second type P sensors is the same. For instance, in some aspects, a synthetic circuit comprises a payload sequence and a regulator sequence, wherein the payload sequence comprises a plurality of the second type P sensors, and wherein each of the plurality of the second type P sensors specifically recognizes the same marker. In some aspects, one or more of the second type P sensors are different. In some aspects, one or more of the second type P sensors specifically recognizes a different marker. In some aspects, one or more of the second type P sensors recognize a different binding site on the same marker.

[0170] In some aspects, where a payload sequence comprises a first type P sensor (*e.g.*, recognizing a regulator) and a second type P sensor (*e.g.*, recognizing a marker), the payload sequence comprises a plurality of first type P sensors and a plurality of second type P sensors. In some aspects, the payload sequence comprises: (a) about two first type P sensors, about three first type P sensors, about four first type P sensors, about five first type P sensors, about six first type P sensors, about seven first type P sensors, about eight first type P sensors, about nine first type sensors, about 10 first type P sensors, about 11 first type P sensors, about 12 first type P sensors, about 13 first type P sensors, about 14 first type P sensors, about 15 first type P sensors, about 16 first type P sensors, about 17 first type P sensors, about 18 first type P sensors, about 19 first type P sensors, or about 20 or more first type P sensors; (b) about two second type P sensors, about three second type P sensors, about four second type P sensors, about five

second type P sensors, about six second type P sensors, about seven second type P sensors, about eight second type P sensors, about nine second type P sensors, about 10 second type P sensors, about 11 second type P sensors, about 12 second type P sensors, about 13 second type P sensors, about 14 second type P sensors, about 15 second type P sensors, about 16 second type P sensors, about 17 second type P sensors, about 18 second type P sensors, about 19 second type P sensors, or about 20 or more second type P sensors; or (c) both (a) and (b). As further described herein, in some aspects, each of the first type P sensors is the same. In some aspects, one or more of the first type P sensors are different. In some aspects, each of the second type P sensors is the same. In some aspects, one or more of the second type P sensors are different.

II.B.2. Type P Spacers

[0171] In some aspects, a payload sequence useful for the present disclosure further comprises a spacer sequence ("**type P spacer**"). In some aspects, the payload sequence comprises a plurality of type P spacers. For instance, in some aspects, the payload sequence comprises about two type P spacers, about three type P spacers, about four type P spacers, about five type P spacers, about six type P spacers, about seven type P spacers, about eight type P spacers, about nine type P spacers, or about 10 or more type P spacers. In some aspects, the payload sequence comprises at least two type P spacers. In some aspects, the payload sequence comprises at least three type P spacers. In some aspects, the payload sequence comprises at least four type P spacers. In some aspects, the payload sequence comprises at least five type P spacers. In some aspects, the payload sequence comprises at least six type P spacers. In some aspects, the payload sequence comprises at least seven type P spacers. In some aspects, the payload sequence comprises at least eight type P spacers. In some aspects, the payload sequence comprises at least nine type P spacers. In some aspects, the payload sequence comprises at least 10 type P spacers. In some aspects, each of the type P spacers are the same. In some aspects, one or more of the type P spacers are different.

[0172] In some aspects, the payload sequence therefore comprises first type P sensor (#1) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#1), first type P sensor (#2) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#2), first type P sensor (#3) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#3), and first type P sensor (#4) (e.g., 5'- UGGAUGAA - 3' (TS #1)). In some aspects, the payload sequence therefore comprises first type P sensor (#1) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#1), first type P sensor (#2) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#2), first type P sensor (#3) (e.g., 5'- UGGAUGAA

- 3' (TS #1)), type P spacer (#3), first type P sensor (#4) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#4), and first type P sensor (#5) (e.g., 5'- UGGAUGAA - 3' (TS #1)). In some aspects, the payload sequence therefore comprises first type P sensor (#1) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#1), first type P sensor (#2) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#2), first type P sensor (#3) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#3), first type P sensor (#4) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#4), first type P sensor (#5) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#5), and first type P sensor (#6) (e.g., 5'- UGGAUGAA - 3' (TS #1)). In some aspects, the payload sequence therefore comprises first type P sensor (#1) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#1), first type P sensor (#2) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#2), first type P sensor (#3) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#3), first type P sensor (#4) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#4), first type P sensor (#5) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#5), first type P sensor (#6) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#6), and first type P sensor (#7) (e.g., 5'- UGGAUGAA - 3' (TS #1)). In some aspects, the payload sequence therefore comprises first type P sensor (#1) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#1), first type P sensor (#2) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#2), first type P sensor (#3) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#3), first type P sensor (#4) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#4), first type P sensor (#5) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#5), first type P sensor (#6) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#6), first type P sensor (#7) (e.g., 5'- UGGAUGAA - 3' (TS #1)), type P spacer (#7), and first type P sensor (#8) (e.g., 5'- UGGAUGAA - 3' (TS #1)).

[0173] Accordingly, in some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence comprises a type P sensor (*e.g.*, specifically recognizing a regulator and/or marker) and a type P spacer. In some aspects, the type P spacer is upstream of the type P sensor (*e.g.*, the type P spacer is positioned closer to the 5'-end of the payload sequence as compared to the type P sensor). In some aspects, the type P spacer is downstream of the type P sensor (*e.g.*, the type P spacer is positioned closer to the 3'-end of the payload sequence as compared to the type P sensor). In some aspects, the type P sensor is downstream of the coding region of the payload sequence, and the type P spacer is positioned between the coding region of the payload sequence and the type P sensor (*e.g.*, after the stop codon of the coding region and before the beginning of the type P sensor). As used herein, the term "**coding region of the payload sequence**" refers to the portion of the payload sequence that specifically encodes for the payload.

[0174] Where a payload sequence comprises a plurality of type P sensors, in some aspects, the type P spacer is upstream of one or more of the plurality of type P sensors. In some aspects, the type P spacer is downstream of one or more of the plurality of type P sensor. In some aspects, the type P spacer is positioned in between at least two of the type P sensors. In some aspects, each of the plurality of type P sensors are separated by a type P spacer. For instance, in some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence comprises a first type P sensor (*e.g.*, specifically recognizes a regulator), a second type P sensor (*e.g.*, specifically recognizes a marker), and a type P spacer, wherein the type P spacer is is positioned in between the first type P sensor and the second type P sensor. As described herein, in some aspects, the payload sequence comprises a plurality of first type P sensors, wherein each of the plurality of first type P sensors are separated by a type P spacer. In some aspects, the payload sequence comprises a plurality of second type P sensors, wherein each of the plurality of second type P sensors are separated by a type P spacer. In some aspects, a payload sequence comprises a plurality of first type P sensors and a plurality of second type P sensors, wherein: (a) each of the plurality of first type P sensors are separated by a type P spacer, (b) each of the plurality of second type P sensors are separated by a type P spacer, and (c) both (a) and (b).

[0175] Where the payload sequence comprises a plurality of type P spacers, in some aspects, each of the type P spacers are the same. In some aspects, one or more of the type P spacers are different. Not to be bound by any one theory, in some aspects, type P spacers useful for the present disclosure are of suitable lengths such that the spacers aid in the binding of the type P sensors to their ligand (*e.g.*, regulator and/or markers). In some aspects, the type P spacer is between about 1 to about 100 nucleotides in length. In some aspects, the type P spacer is about 1 nucleotide in length, about 5 nucleotides in length, about 10 nucleotides in length, about 15 nucleotides in length, about 20 nucleotides in length, about 25 nucleotides in length, about 30 nucleotides in length, about 35 nucleotides in length, about 40 nucleotides in length, about 45 nucleotides in length, about 50 nucleotides in length, about 55 nucleotides in length, about 60 nucleotides in length, about 65 nucleotides in length, about 70 nucleotides in length, about 75 nucleotides in length, about 80 nucleotides in length, about 85 nucleotides in length, about 90 nucleotides in length, about 95 nucleotides in length, or about 100 nucleotides in length. In some aspects, the type P spacer is between about 1 to about 50 nucleotides length. In some aspects, the type P spacer is about 5 nucleotides in length. In some aspects, the type P spacer is about 10 nucleotides in length. In some aspects, the type P spacer is about 15 nucleotides in length. In some aspects, the type P spacer is about 20 nucleotides in length. In some aspects,

the type P spacer is about 25 nucleotides in length. In some aspects, the type P spacer is about 30 nucleotides in length. In some aspects, the type P spacer is about 35 nucleotides in length. In some aspects, the type P spacer is about 40 nucleotides in length. In some aspects, the type P spacer is about 45 nucleotides in length. In some aspects, the type P spacer is about 50 nucleotides in length.

[0176] Unless indicated otherwise, type P spacers useful for the present disclosure are not limited to any specific nucleotide sequences, as long as the type P spacers are of sufficient length to carry out their intended function (*e.g.*, aid in the binding of the type P sensors to their ligands). Accordingly, in some aspects, a type P spacer useful for the present disclosure comprises a randomly generated nucleotide sequence. In some aspects, where a plurality of type P spacers are used in separating a plurality of type P sensors, one or more of the plurality of type P spacers have a difference sequence, such that the plurality of type P spacers do not include randomly generated nucleotide sequences that repeat. In some aspects, the type P spacer is different than the type R spacer.

II.C. Modalities

[0177] As further described herein, synthetic circuits of the present disclosure comprise certain properties (*e.g.*, structural and/or functional) that make them particularly useful for selectively regulating the expression of a gene (or a protein encoded thereof) in a cell of interest (*e.g.*, target cell). For example, in some aspects, a synthetic circuit provided herein comprise a payload sequence and a regulator sequence, which have been programmed such that when both are present in a target cell, the payload (encoded by the payload sequence) is robustly expressed while expression of the regulator (encoded by the regulator sequence) is robustly reduced or inhibited. As described earlier in the present disclosure, in some aspects, a synthetic circuit described herein comprises a sensor (*e.g.*, type P sensor and/or type R sensor), which can be programmed to allow for the selective expression of the payload or regulator in specific cells of interest. In some aspects, in addition to such sensors, a synthetic circuit provided herein comprises a payload sequence and a regulator sequence, wherein the payload sequence and/or the regulator sequence are of a particular modality which is conducive in promoting the selective expression of the payload and/or regulator.

[0178] Unless indicated otherwise, a payload sequence comprises one or more of the following RNA modalities: linear RNA, circular RNA, self-replicating RNA, and non-replicating RNA. Unless indicated otherwise, a regulator sequence comprises one or more of

the following RNA modalities: linear RNA, circular RNA, and non-replicating RNA. For example, in some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence is a self-replicating RNA. In some aspects, a synthetic circuit provided herein comprises a regulator sequence, wherein the regulator sequence is a non-replicating RNA. Accordingly, in some aspects, a synthetic circuit provided herein comprises a payload sequence and a regulator sequence, wherein the payload sequence is a self-replicating RNA and the regulator sequence is a non-replicating RNA. In some aspects, a synthetic circuit provided herein comprises a payload sequence and a regulator sequence, wherein the payload sequence is a self-replicating RNA and the regulator sequence is a circular RNA (*i.e.*, not self-replicating).

[0179] Using self-replicating RNA to express payload sequence while using non-replicating RNA to express the regulator sequence improves performance of the RNA circuit.

[0180] Without wishing to be bound by a certain theory, expressing the payload sequence from repRNAs, under marker conditions that enable the payload sequence "ON state", improves performance of the circuit given repRNAs will replicate and robustly express high levels of payload protein for a long duration. This may be due to the self-replicating nature of self-replicating RNA.

[0181] Without wishing to be bound by a certain theory, expressing the regulator sequence from linear RNA instead of repRNAs improves performance of the circuit given:

[0182] (1) non-replicating linear RNA can rapidly express sufficient levels of regulator protein to effectively inhibit payload protein expression (from repRNAs), whereas the expression from repRNA is slower (e.g., requires replication) and may allow the payload repRNA to initiate replication, thereby causing "leaky expression" of payload in non-target cells; and

[0183] (2) once repRNA starts to replicate, it is more difficult to knock-down (*e.g.*, with miRNAs); therefore, compared to when expressing regulator protein from linear RNA, there may be "leaky expression" of regulator protein in target cells, and this may significantly reduce expression from payload repRNA.

[0184] Thus, the combination of repRNA payload sequence with linear RNA regulator sequence allows for very strong expression of payload in target cells, while minimizing expression of transgenes (payload) in non-target cells. Similar to the situation with using linear RNA to regulate a repRNA payload, utilizing a linear RNA regulator strand to regulate a circular RNA payload may also achieve an advantageous outcome, in that circular RNA is more durable than linear RNA.

[0185] As is apparent from the present disclosure, a synthetic circuit useful for the present disclosure can comprise various combinations of RNA modalities, so long as the payload can be selectively expressed in the cell of interest (*i.e.*, target cell).

[0186] For example, in some aspects, a synthetic circuit provided herein comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a self-replicating RNA and comprises a sensor that is capable of specifically recognizing the regulator (type P sensor); and wherein the regulator sequence is a non-replicating RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor). In some aspects, a synthetic circuit provided herein comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a self-replicating RNA and comprises (i) a first sensor that is capable of specifically recognizing the regulator (first type P sensor) and (ii) a second sensor that is capable of specifically recognizing a marker (second type P sensor); and wherein the regulator sequence is a non-replicating RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor).

[0187] In some aspects, a synthetic circuit provided herein comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a self-replicating RNA and comprises a sensor that is capable of specifically recognizing the regulator (type P sensor); and wherein the regulator sequence is a circular RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor). In some aspects, a synthetic circuit provided herein comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a self-replicating RNA and comprises (i) a first sensor that is capable of specifically recognizing the regulator (first type P sensor) and (ii) a second sensor that is capable of specifically recognizing a marker (second type P sensor); and wherein the regulator sequence is a circular RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor).

[0188] In some aspects, a synthetic circuit provided herein comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a circular RNA and comprises a sensor that is capable of specifically recognizing the regulator (type P sensor); and wherein the regulator sequence is a circular RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor). In some aspects, a synthetic circuit provided herein

comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a circular RNA and comprises (i) a first sensor that is capable of specifically recognizing the regulator (first type P sensor) and (ii) a second sensor that is capable of specifically recognizing a marker (second type P sensor); and wherein the regulator sequence is a circular RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor).

In some aspects, a synthetic circuit provided herein comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a circular RNA and comprises a sensor that is capable of specifically recognizing the regulator (type P sensor); and wherein the regulator sequence is a non-replicating RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor). In some aspects, a synthetic circuit provided herein comprises: (a) a first sequence encoding a payload (payload sequence) and (b) a second sequence encoding a regulator (regulator sequence); wherein the payload sequence is a non-replicating RNA and comprises (i) a first sensor that is capable of specifically recognizing the regulator (first type P sensor) and (ii) a second sensor that is capable of specifically recognizing a marker (second type P sensor); and wherein the regulator sequence is a non-replicating RNA and comprises a sensor that is capable of specifically recognizing a marker (type R sensor).

II.D. Additional Components

[0189] In some aspects, a synthetic circuit described herein comprises one or more additional components that aid in the function of the synthetic circuit. For instance, in some aspects, a synthetic circuit described herein comprises a payload sequence, wherein the payload sequence comprises a type P sensor and one or more additional components described herein. In some aspects, a synthetic circuit described herein comprises a regulator sequence, wherein the regulator sequence comprises a type R sensor and one or more additional components described herein. In some aspects, a synthetic circuit described herein comprise a payload sequence and a regulator sequence, wherein each of the payload sequence and the regulator sequence comprises one or more additional components described herein.

[0190] In some aspects, a payload sequence useful for the present disclosure comprises one or more additional components, wherein the one or more additional components enhance the expression of the encoded payload. In some aspects, the regulator sequence does not comprise one or more additional components that enhance the expression of the encoded regulator.

Therefore, in some aspects, when such a synthetic circuit is introduced into a target cell, the expression of the payload is increased as compared to the expression of the regulator. In some aspects, compared to the expression of the regulator, the expression of the payload is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%. In some aspects, the expression of the payload is increased by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 12.5-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold.

[0191] In some aspects, a payload sequence useful for the present disclosure comprises one or more additional components that increases the stability of the payload sequence. In some aspects, the regulator sequence does not comprise one or more additional components that increase the stability of the payload sequence. In some aspects, increased stability results in increased expression of the encoded protein. In some aspects, when such a synthetic circuit is introduced into a target cell, the expression of the payload is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%. In some aspects, the expression of the payload is increased by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 12.5-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold.

[0192] In some aspects, the one or more additional components that can be included in a synthetic circuit provided herein comprises an aptamer for a translational initiation factor. For instance, in some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence is a circular RNA and comprises an aptamer for a translational initiation factor. The inclusion of such additional component, particularly where the payload sequence is a circular RNA, can aid in the expression of the encoded payload when the synthetic circuit is introduced into a target cell. *See, e.g.*, Prats *et al.*, *Int J Mol Sci* 21(22): 8591 (Nov. 2020). Non-limiting examples of additional components that are useful for the present disclosure include: (1) an internal ribosome entry cite (IRES), (2) an untranslated region

(UTR), (3) a sequence encoding a signal peptide, (4) a translation initiation sequence, (5) a polyA sequence, (6) a sequence encoding a RNA binding protein, (7) a sequence encoding a 2A ribosome skip peptide, (8) a 5'-cap, (9) a translation enhancer element, or (10) any combination of (1) to (10). Additional disclosure related to such additional components are provided below.

Terminal Architecture Modifications: Untranslated Regions (UTRs)

[0193] In some aspects, a synthetic circuit described herein comprises a UTR. For example, in some aspects, a synthetic circuit described herein comprises a payload sequence, wherein the payload sequence comprises a UTR. In some aspects, the UTR is a 5'-UTR. In some aspects, the UTR is a 3'-UTR. In some aspects, the UTR comprises both a 5'-UTR and a 3'-UTR.

[0194] Untranslated regions (UTRs) of a gene are transcribed but not translated. The 5′-UTR starts at the transcription start site and continues to the start codon but does not include the start codon; whereas, the 3′-UTR starts immediately following the stop codon and continues until the transcriptional termination signal. There is growing body of evidence about the regulatory roles played by the UTRs in terms of stability of the nucleic acid molecule and translation. Accordingly, where a payload sequence described herein comprises a UTR, the stability of the payload sequence is increased, *e.g.*, as compared to a sequence without the UTR. As described herein, in some aspects, increased stability results in increased expression of the encoded protein.

5′-UTR and Translation Initiation

[0195] Natural 5′-UTRs bear features which play roles in translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5′-UTR also have been known to form secondary structures which are involved in elongation factor binding.

[0196] 5′-UTR secondary structures involved in elongation factor binding can interact with other RNA binding molecules in the 5′-UTR or 3′-UTR to regulate gene expression. For example, the elongation factor EIF4A2 binding to a secondarily structured element in the 5′-UTR is necessary for microRNA mediated repression (Meijer H A et al., Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The different secondary structures in the 5′-UTR can be incorporated into the flanking region to either stabilize or selectively destabilize mRNAs in specific tissues or cells.

[0197] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production of a nucleic acid sequence (*e.g.*, payload sequence of a synthetic circuit provided herein). For example, introduction of 5′-UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII, could be used to enhance expression of a nucleic acid molecule, such as a mRNA, in hepatic cell lines or liver. Likewise, use of 5′-UTR from other tissue-specific mRNA to improve expression in that tissue is possible—for muscle (MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (Tie-1, CD36), for myeloid cells (C/EBP, AML1, G-CSF, GM-CSF, CD11b, MSR, Fr-1, i-NOS), for leukocytes (CD45, CD18), for adipose tissue (CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (SP-A/B/C/D).

[0198] Other non-UTR sequences can also be incorporated into the UTRs (*e.g.*, 5'-UTR and/or 3'-UTR). For example, introns or portions of introns sequences can be incorporated into the flanking regions of a nucleic acid sequence (*e.g.*, payload sequence of a synthetic circuit provided herein).

[0199] In some aspects, one or more nucleotides within a UTR (*e.g.*, 5'-UTR and/or 3'-UTR) can be mutated, replaced and/or removed. For example, one or more nucleotides upstream of the start codon can be replaced with another nucleotide. The nucleotide or nucleotides to be replaced can be 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, 30, 35, 40, 45, 50, 55, 60 or more than 60 nucleotides upstream of the start codon. As another example, one or more nucleotides upstream of the start codon can be removed from the UTR.

3′ UTR and the AU Rich Elements

[0200] 3′-UTRs are known to have stretches of adenosines and uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III ARES are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been

documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3′-UTR of nucleic acid molecules can lead to HuR binding and thus, stabilization of the message *in vivo*.

[0201] In some aspects, introduction, removal, or modification of 3′-UTR AU rich elements (AREs) can be used to modulate the stability of a nucleic acid sequence. When engineering specific nucleic acid sequences (*e.g.*, payload sequence and/or regulator sequence described herein), one or more copies of an ARE can be introduced to make the nucleic acid sequence less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

Translation Enhancer Elements (TEEs)

[0202] In some aspects, a synthetic circuit provided herein comprises a translational enhancer element (TEE). As used herein, the term "**translational enhancer element**" refers to cis-acting sequences that increase the expression of a protein encoded by a nucleotide sequence. Non-limiting examples of TEEs that can be used with the present disclosure are known in the art, *see, e.g.*, US20130177581A, which is incorporated herein by reference in its entirety. In some aspects, a synthetic circuit provided herein comprises a payload sequence and a regulator sequence, wherein the payload sequence comprises a TEE. When such a synthetic circuit is introduced into a target cell, the expression of the payload is increased, *e.g.*, as compared to a corresponding synthetic circuit where the payload sequence does not comprise the TEE.

[0203] In some aspects, the TEE is positioned between the transcription promoter and the start codon of a sequence (*e.g.*, payload sequence). In some aspects, a TEE useful for the present disclosure has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% sequence identity with any of the TEEs provided in U.S. Publication Number US 20140147454, US20090226470, US20070048776, US20130177581, US20110124100, WO1999024595, WO2012009644, WO2009075886, WO2007025008, U.S. Pat. No. 6,310,197, U.S. Pat. No. 6,849,405, U.S. Pat. No. 7,456,273, U.S. Pat. No. 7,183,395, each of which is herein incorporated by reference in its entirety.

[0204] In some aspects, a synthetic circuit provided herein comprises multiple TEEs. For example, in some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence comprises at least 1, at least 2, at least 3, at least 4, at least 5, at

least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18 at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, or more than 60 TEE sequences. In some aspects, the TEE sequences in the 5′UTR of the RNA (*e.g.*, modified RNA) are the same or different TEE sequences. In some aspects, the TEE sequences are in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

5′ Capping

[0205] In some aspects, a synthetic circuit described herein comprises a 5'-cap structure. For example, in some aspects, a synthetic circuit described herein comprises a payload sequence, wherein the payload sequence comprises a 5'-cap structure. The 5′ cap structure of a mRNA is involved in nuclear export, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsible for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The cap further assists the removal of 5′ proximal introns removal during mRNA splicing.

[0206] Modifications to the RNA of the present disclosure can generate a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5′-ppp-5′ phosphorodiester linkages, modified nucleotides can be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, Mass.) can be used with α-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5′-ppp-5′ cap. Additional modified guanosine nucleotides can be used such as α-methyl-phosphonate and seleno-phosphate nucleotides.

[0207] Additional modifications include, but are not limited to, 2′-O-methylation of the ribose sugars of 5′-terminal and/or 5′-anteterminal nucleotides of the mRNA (as mentioned above) on the 2′-hydroxyl group of the sugar ring. Multiple distinct 5′-cap structures can be used to generate the 5′-cap of a nucleic acid molecule, such as an mRNA molecule.

[0208] Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (i.e. endogenous, wild-type or physiological) 5′-caps in their chemical structure, while retaining cap

function. Cap analogs can be chemically (*i.e.*, non-enzymatically) or enzymatically synthesized and/linked to a nucleic acid molecule.

[0209] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5′-5′-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3′-O-methyl group (*i.e.*, N7,3′-O-dimethyl-guanosine-5′-triphosphate-5′-guanosine (m7G-3′ mppp-G; which can equivalently be designated 3′ O-Me-m7G(5′)ppp(5′)G). The 3′-O atom of the other, unmodified, guanine becomes linked to the 5′-terminal nucleotide of the capped nucleic acid molecule (*e.g.,* an mRNA or mmRNA). The N7- and 3′-O-methylated guanine provides the terminal moiety of the capped nucleic acid molecule (*e.g.,* mRNA or mmRNA).

[0210] Another exemplary cap is mCAP, which is similar to ARCA but has a 2′-β-methyl group on guanosine (*i.e.*, N7,2′-O-dimethyl-guanosine-5′-triphosphate-5′-guanosine, m7Gm-ppp-G).

[0211] In some aspects, the cap is a dinucleotide cap analog. In some aspects, the dinucleotide cap analog is modified at different phosphate positions with a boranophosphate group or a phosphoroselenoate group such as the dinucleotide cap analogs described in U.S. Pat. No. 8,519,110, the contents of which are herein incorporated by reference in its entirety.

[0212] In some aspects, the cap is a cap analog is a N7-(4-chlorophenoxyethyl) substituted dicucleotide form of a cap analog known in the art and/or described herein. Non-limiting examples of a N7-(4-chlorophenoxyethyl) substituted dinucleotide form of a cap analog include a N7-(4-chlorophenoxyethyl)-G(5′)ppp(5′)G and a N7-(4-chlorophenoxyethyl)-m3′-OG(5′)ppp(5′)G cap analog (See *e.g.,* the various cap analogs and the methods of synthesizing cap analogs described in Kore et al. Bioorganic & Medicinal Chemistry 2013 21:4570-4574; the contents of which are herein incorporated by reference in its entirety). In some aspects, a cap analog of the present disclosure is a 4-chloro/bromophenoxyethyl analog.

[0213] While cap analogs allow for the concomitant capping of a nucleic acid molecule in an *in vitro* transcription reaction, up to about 20% of transcripts remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5′-cap structures of nucleic acids produced by the endogenous, cellular transcription machinery, can lead to reduced translational competency and reduced cellular stability.

[0214] In some aspects, providing an RNA with a 5′-cap or 5′-cap analog is achieved by *in vitro* transcription of a DNA template in the presence of said 5′-cap or 5′-cap analog, wherein said 5′-cap is co-transcriptionally incorporated into the generated RNA strand,

[0215] In some aspects, RNA can be generated, for example, by *in vitro* transcription, and the 5′-cap can be attached to the RNA post-transcriptionally using capping enzymes, for

example, capping enzymes of vaccinia virus. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects. Non-limiting examples of more authentic 5′ cap structures of the present disclosure are those which, among other things, have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5′ endonucleases and/or reduced 5′ decapping, as compared to synthetic 5′ cap structures known in the art (or to a wild-type, natural or physiological 5′ cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2′-O-methyltransferase enzyme can create a canonical 5′-5′-triphosphate linkage between the 5′-terminal nucleotide of an mRNA and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5′-terminal nucleotide of the mRNA contains a 2′-O-methyl. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, *e.g.,* to other 5′ cap analog structures known in the art. Cap structures include 7mG(5′)ppp(5′)N,pN2p, 7mG(5′)ppp(5′)NlmpNp, 7mG(5′)-ppp(5′)NlmpN2 mp and m(7)Gpppm(3)(6,6,2′)Apm(2′)Apm(2′)Cpm(2)(3,2′)Up.

[0216] In some aspects, 5′ terminal caps include endogenous caps or cap analogs. In some aspects, a 5′ terminal cap comprises a guanine analog. Useful guanine analogs include inosine, N1-methyl-guanosine, 2′ fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0217] In some aspects, the 5' cap comprises a 5' to 5' triphosphate linkage. In some aspects, the 5' cap comprises a 5' to 5' triphosphate linkage including thiophosphate modification. In some aspects, the 5' cap comprises a 2′-O or 3′-O-ribose-methylated nucleotide. In some aspects, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some aspects, the 5' cap comprises 7- methylguanylate. Exemplary cap structures include m7G(5')ppp(5')G, m7,2`O-mG(5')ppSp(5')G, m7G(5')ppp(5')2`O-mG, and m7,3`O-mG(5')ppp(5')2`O-mA.

[0218] In some aspects, a synthetic circuit described herein comprises a modified 5′ cap. For instance, in some aspects, the payload sequence of a synthetic circuit comprises a modified 5'-cap. A modification on the 5′ cap can increase the stability of mRNA, increase the half-life of the mRNA, and could increase the mRNA translational efficiency. In some aspects, the

modified 5′ cap comprises one or more of the following modifications: modification at the 2′ and/or 3′ position of a capped guanosine triphosphate (GTP), a replacement of the sugar ring oxygen (that produced the carbocyclic ring) with a methylene moiety (CH2), a modification at the triphosphate bridge moiety of the cap structure, or a modification at the nucleobase (G) moiety.

[0219] The 5′ cap structure that can be modified includes, but is not limited to, the caps described in U.S. Application No. 2014/0147454 and WO2018/160540 which is incorporated herein by reference in its entirety.

IRES Sequences

[0220] In some aspects, a synthetic circuit provided herein comprises an internal ribosome entry site (IRES). For example, in some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence comprises an IRES. First identified as a feature Picorna virus RNA, IRES plays an important role in initiating protein synthesis in absence of the 5′ cap structure. An IRES can act as the sole ribosome binding site, or can serve as one of multiple ribosome binding sites of an mRNA. Nucleic acids or mRNA containing more than one functional ribosome binding site can encode several peptides or polypeptides that are translated independently by the ribosomes ("multicistronic nucleic acid molecules"). When nucleic acids or mRNA are provided with an IRES, further optionally provided is a second translatable region. Examples of IRES sequences that can be used according to the disclosure include without limitation, those from picornaviruses (*e.g.,* FMDV), pest viruses (CFFV), polio viruses (PV), encephalomyocarditis viruses (ECMV), foot-and-mouth disease viruses (FMDV), hepatitis C viruses (HCV), classical swine fever viruses (CSFV), murine leukemia virus (MLV), simian immune deficiency viruses (SIV) or cricket paralysis viruses (CrPV).

Poly-A Tails

[0221] In some aspects, a synthetic circuit provided herein comprises a poly-A tail. In some aspects, a synthetic circuit provided herein comprises a payload sequence, wherein the payload sequence comprises a poly-A tail.

[0222] In some aspects, the length of the poly-A tail is greater than about 30 nucleotides in length. In some aspects, the poly-A tail is greater than about 35 nucleotides in length. In some aspects, the length is at least about 40 nucleotides. In some aspects, the length is at least about 45 nucleotides. In some aspects, the length is at least about 50 nucleotides. In some aspects,

the length is at least about 55 nucleotides. In some aspects, the length is at least about 60 nucleotides. In some aspects, the length is at least 70 nucleotides. In some aspects, the length is at least about 80 nucleotides. In some aspects, the length is at least about 90 nucleotides. In some aspects, the length is at least about 100 nucleotides. In some aspects, the length is at least about 120 nucleotides. In some aspects, the length is at least about 140 nucleotides. In some aspects, the length is at least about 160 nucleotides. In some aspects, the length is at least about 180 nucleotides. In some aspects, the length is at least about 200 nucleotides. In some aspects, the length is at least about 250 nucleotides. In some aspects, the length is at least about 300 nucleotides. In some aspects, the length is at least about 350 nucleotides. In some aspects, the length is at least about 400 nucleotides. In some aspects, the length is at least about 450 nucleotides. In some aspects, the length is at least about 500 nucleotides. In some aspects, the length is at least about 600 nucleotides. In some aspects, the length is at least about 700 nucleotides. In some aspects, the length is at least about 800 nucleotides. In some aspects, the length is at least about 900 nucleotides. In some aspects, the length is at least about 1000 nucleotides. In some aspects, the length is at least about 1100 nucleotides. In some aspects, the length is at least about 1200 nucleotides. In some aspects, the length is at least about 1300 nucleotides. In some aspects, the length is at least about 1400 nucleotides. In some aspects, the length is at least about 1500 nucleotides. In some aspects, the length is at least about 1600 nucleotides. In some aspects, the length is at least about 1700 nucleotides. In some aspects, the length is at least about 1800 nucleotides. In some aspects, the length is at least about 1900 nucleotides. In some aspects, the length is at least about 2000 nucleotides. In some aspects, the length is at least about 2500 nucleotides. In some aspects, the length is at least about 3000 nucleotides.

[0223] In some aspects, the poly-A tail comprises a polyA-G quartet. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In some aspects, the G-quartet is incorporated at the end of the poly-A tail. The resultant nucleic acid or mRNA can be assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G quartet results in protein production equivalent to at least 75% of that seen using a poly-A tail of 120 nucleotides alone.

Modified Nucleosides

[0224] In some aspects, a synthetic circuit provided herein comprises one or more modified nucleosides. In some aspects, a synthetic circuit provided herein comprises a payload sequence,

wherein the payload sequence comprises one or more modified nucleosides. In some aspects, the one or more modified nucleosides comprises 6-aza-cytidine, 2-thio-cytidine, α-thio-cytidine, pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, α-thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, pseudo-uridine, inosine, α-thio-guanosine, 8-oxo-guanosine, O6-methyl-guanosine, 7-deaza-guanosine, N1-methyl adenosine, 2-amino-6-chloro-purine, N6-methyl-2-amino-purine, 6-chloro-purine, N6-methyl-adenosine, α-thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine, pyrrolo-cytidine, 5-methyl-cytidine, N4-acetyl-cytidine, 5-methyl-uridine, 5-iodo-cytidine, and combinations thereof.

[0225] In some aspects, a synthetic circuit provided herein comprises one or more uridines which have been replaced by a modified nucleoside. In some aspects, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ) or 5-methyl-uridine (m5U).

Table 1. PUF TS RNA Sequences

	Sequence (RNA)	
WT	U G U A U A U A	WT TS
PUFF(UGG)	U G G A U G A A	TS#1
PUF1	U G U A C G U C	TS#2
PUF2	U C U A C G U C	TS#3
PUF3	U G U A C G A C	TS#4
PUF4	U G U C C G U C	TS#5
PUF5	U G U A C G U G	TS#6
PUF6	U G G A A G U C	TS#7
PUF7	U G U G C C U C	TS#8
PUF8	U G U A G C U A	TS#9

Table 2. PUF TS DNA Sequences

Name		**Sequence (DNA)**
PUF(NRE)	WT	TGTATATA
PUF(LacZ)	PUF(UGG)	TGGATGAA
PUF1	PUF1	TGTACGTC
PUF2	PUF2	TCTACGTC
PUF3	PUF3	TGTACGAC
PUF4	PUF4	TGTCCGTC
PUF5	PUF5	TGTACGTG
PUF6	PUF6	TGGAAGTC
PUF7	PUF7	TGTGCCTC
PUF8	PUF8	TGTACGTA

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PUF(LacZ) 2x-TTP		TGGTGGATGAAATGAA
PUF(LacZ)-Z10r		TGGTGGAGAA
PUF(LacZ)-Z9r_A		TGGAATGAA
PUF(LacZ)-Z9r_C		TGGTGCGAA
PUF(LacZ)-Z9r_G		TGGTGGGAA
PUF(LacZ)-Z9r_U		TGGTGTGAA

Table 3. PUF RBD sequences

Name		Description	SEQ ID NO:	Sequence
PUF	PUM-HD	WT PUF RBD	1	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGCRVIQKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF(LacZ)	PUF(UGG)		2	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGSYVIEHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

PUF(LacZ) 2x-TTP	16 repeats	3	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQLMVDVFGCRVIQ KFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVRELDGHVLKCVKD QNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLPDQTLPILEELHQ HTEQLVQDQYGSYVIEHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERA VLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHTEQLVQDQYGSY VIEHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGP HSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNG VDLG
PUF(LacZ) -Z10r	10 repeats	4	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGQVFALSTHPYGCRVIQRILEHCLPDQTLPILEELHQHTEQLVQDQYGSRVIEHVLEHGRP EDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQ YANYVVQKMIDVAEPGQRKIVMHKIRPHTEQLVQDQYGSYVIEHVLEHGRPEDKSKIVAEIRGN VLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVA EPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF(LacZ) -Z9r_A	9 repeats	5	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQHTEQLVQDQYGSYVIE HVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGPHSA LYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNGVDL G

PUF(LacZ)-Z9r_C		9 repeats	6	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHIMEFSQDQHGSYFIRLKLERATPAERQLVFNEILQHTEQLVQDQYGSYVIE HVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGPHSA LYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNGVDL G
PUF(LacZ)-Z9r_G		9 repeats	7	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHIMEFSQDQHGSRFIELKLERATPAERQLVFNEILQHTEQLVQDQYGSYVIE HVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGPHSA LYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNGVDL G
PUF(LacZ)-Z9r_U		9 repeats	8	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHIMEFSQDQHGNYFIQLKLERATPAERQLVFNEILQHTEQLVQDQYGSYVIE HVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGPHSA LYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNGVDL G
PUF1.1	PUF1.1	reprogrammed PUF	9	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

PUF1.2	reprogrammed PUF	10	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGGRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGGHVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF1.2-v4	reprogrammed PUF1.2 PUF	11	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGSHVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF2	reprogrammed PUF	12	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFAKWVVN KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF2-v2	reprogrammed PUF	13	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGSYVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASYVVR KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

PUF3	PUF3.1	reprogrammed PUF	14	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGVFVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF3-v16	PUF3.2	reprogrammed PUF	15	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGSHVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF3-v17	PUF3.3	reprogrammed PUF	16	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGCRVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGSYVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF4.1		reprogrammed PUF	17	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGTKVIRRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

PUF4.2	reprogrammed PUF	18	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGTTVIRRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF4.3	reprogrammed PUF	19	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCKVIRRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF4.4	reprogrammed PUF	20	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGGRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGGHVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGGRVIRRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF4.4-v2	PUF4	reprogrammed PUF	21	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGSHVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGSYVIRRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

PUF4.5	reprogrammed PUF	22	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGSYVIRRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF5	reprogrammed PUF	23	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSGFIDLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF5-v3	PUF5	24	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIELKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGSYVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF6	PUF6.1	25	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGSWVIDHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

PUF6-v6	PUF6.2	reprogrammed PUF	26	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGSYVIEHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF6-v11	PUF6.3	reprogrammed PUF	27	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSRVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGNHVVQKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGSYVIEHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF7		reprogrammed PUF	28	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGQFFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGQFVIRKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGTHVIERILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG
PUF7-v2	PUF7	reprogrammed PUF	29	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIRLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGSYVIRKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGSYVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGSRVIERILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

PUF8	PUF8	reprogrammed PUF	30	GRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIQLKLERATPAERQLVFNEILQAAYQ LMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQMYGTHVIEKALEFIPSDQQNEMVR ELDGHVLKCVKDQNGTFVVRKCIECVQPQSLQFIIDAFKGQVFALSTHPYGCRVIQRILEHCLP DQTLPILEELHQHTEQLVQDQYGNYVIQHVLEHGRPEDKSKIVAEIRGNVLVLSQHKFASNVVE KCVTHASRTERAVLIDEVCTMNDGPHSALYTMMKDQYANYVVQKMIDVAEPGQRKIVMHKIRPH IATLRKYTYGKHILAKLEKYYMKNGVDLG

Table 4a. Effector Sequences

Effector Name	SEQ ID No.	Protein Sequence
DDX6	31	MSTARTENPVIMGLSSQNGQLRGPVKPTGGPGGGGTQTQQQMNQLKNTNTINNGTQQQAQSMT TTIKPGDDWKKTLKLPPKDLRIKTSDVTSTKGNEFEDYCLKRELLMGIFEMGWEKPSPIQEESIPIAL SGRDILARAKNGTGKSGAYLIPLLERLDLKKDNIQAMVIVPTRELALQVSQICIQVSKHMGGAKVM ATTGGTNLRDDIMRLDDTVHVVIATPGRILDLIKKGVAKVDHVQMIVLDEADKLLSQDFVQIMEDII LTLPKNRQILLYSATFPLSVQKFMNSHLQKPYEINLMEELTLKGVTQYYAYVTERQKVHCLNTLFSR LQINQSIIFCNSSQRVELLAKKISQLGYSCFYIHAKMRQEHRNRVFHDFRNGLCRNLVCTDLFTRGIDI QAVNVVINFDFPKLAETYLHRIGRSGRFGHLGLAINLITYDDRFNLKSIEEQLGTEIKPIPSNIDKSLYV AEYHSEPVEDEKP
Dis3 iso2 (PIN)	32	MLKSKTFLKKTRAGGVMKIVREHYLRDDIGCGAPGCAACGGAHEGPALEPQPQDPASSVCPQPH YLLPDTNVLLHQIVSAWRPGTWASVASSLRLPGSLETYVEQEQGENANDRNDRAIRVAAKWYNE HLKKMSADNQLQVIFITNDRRNKEKAIEEGIPAFTCEEYVKSLTANPELIDRLACLSEEGNEIES
Smg6 (PIN)	33	MQMELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINELDGLAKGQETDHRAGGYA RVVQEKARKSIEFLEQRFESRDSCLRALTSRGNELESIAFRSEDITGQLGNNDDLILSCCLHYCK DKAKDFMPASKEEPIRLLREVVLLTDDRNLRVKALTRNVPVRDIPAFLTWAQVG
cNOT7	34	MPAATVDHSQRICEVWACNLDEEMKKIRQVIRKYNYVAMDTEFPGVVARPIGEFRSNADY QYQLLRCNVDLLKIIQLGLTFMNEQGEYPPGTSTWQFNFKFNLTEDMYAQDSIELLTTSG IQFKKHEEEGIETQYFAELLMTSGVVLCEGVKWLSFHSGYDFGYLIKILTNSNLPEEELD FFEILRLFFPVIYDVKYLMKSCKNLKGGLQEVAEQLELERIGPQHQAGSDSLLTGMAFFK MREMFFEDHIDDAKYCGHLYGLGSGSSYVQNGTGNAYEEEANKQSV
MCPIP1 PIN	35	MGGGTPKAPNLEPPLPEEEKEGSDLRPVVIDGSNVAMSHGNKEVFSCRGILLAVNWFLER GHTDITVFVPSWRKEQPRPDVPITDQHILRELEKKKILVFTPSRRVGGKRVVCYDDRFIV KLAYESDGIVVSNDTYRDLQGERQEWKRFIEERLLMYSFVNDKFMPPDDPLGRHGPSLDN FLRKKPLTLE

TTP (ZFP36)	36	MDLTAIYESLLSLSPDVPVPSDHGGTESSPGWGSSGPWSLSPSDSSPSGVTSRLPGRSTS LVEGRSCGWVPPPPGFAPLAPRLGPELSPSPTSPTATSTTPSRYKTELCRTFSESGRCRY GAKCQFAHGLGELRQANRHPKYKTELRHKFYLQGRCPYGSRCHFIHNPSEDLAAPGHPPV LRQSISFSGLPSGRRTSPPPPGLAGPSLSSSSFSPSSSPPPPGDLPLSPSAFSAAPGTPL ARRDPTPVCCPSCRRATPISVWGPLGGLVRTPSVQSLGSDPDEYASSGSSLGGSDSPVFE AGVFAPPQPVAAPRRLPIFNRISVSE
Ago2 (full protein)	37	MDIPKIDIYHYELDIKPEKCPRRVNREIVEHMVQHFKTQIFGDRKPVFDGRKNLYTAMPL PIGRDKVELEVTLPGEGKDRIFKVSIKWVSCVSLQALHDALSGRLPSVPFETIQALDVVM RHLPSMRYTPVGRSFFTASEGCSNPLGGGREVWFGFHQSVRPSLWKMMLNIDVSATAFYK AQPVIEFVCEVLDFKSIEEQQKPLTDSQRVKFTKEIKGLKVEITHCGQMKRKYRVCNVTR RPASHQTFPLQQESGQTVECTVAQYFKDRHKLVLRYPHLPCLQVGQEQKHTYLPLEVCNI VAGQRCIKKLTDNQTSTMIRATARSAPDDRQEEISKLMRSASFNTDPYVREFGIMVKDEMT DVTGRVLQPPSILYGGRNKAIATPVQGVWDMRNKQFHTGIEIKVWAIACFAPQRQCTEVH LKSFTEQLRKISRDAGMPIQGQPCFCKYAQGADSVEPMFRHLKNTYAGLQLVVVILPGKT PVYAEVKRVGDTVLGMATQCVQMKNVQRTTPQTLSNLCLKINVKLGGVNNILLPQGRPPV FQQPVIFLGADVTHPPAGDGKKPSIAAVVGSMDAHPNRYCATVRVQQHRQEIIQDLAAMV RELLIQFYKSTRFKPTRIIFYRDGVSEGQFQQVLHHELLAIREACIKLEKDYQPGITFIV VQKRHHTRLFCTDKNERVGKSGNIPAGTTVDTKITHPTEFDFYLCSHAGIQGTSRPSHYH VLWDDNRFSSDELQILTYQLCHTYVRCTRSVSIPAPAYYAHLVAFRARYHLVDKEHDSAE GSHTSGQSNGRDHQALAKAVQVHQDTLRTMYFA
angiogenin precursor (full length)	38	MVMGLGVLLLVFVLGLGLTPPTLAQDNSRYTHFLTQHYDAKPQGRDDRYCESIMRRRGLTSPCKDINTFI HGNKRSIKAICENKNGNPHRENLRISKSSFQVTTCKLHGGSPWPPCQYRATAGFRNVVVACENGLPVHLD QSIFRRP
APEX1 (full protein)	39	MPKRGKKGAVAEDGDELRTEPEAKKSKTAAKKNDKEAAGEGPALYEDPPDQKTSPSGKPA TLKICSWNVDGLRAWIKKKGLDWVKEEAPDILCLQETKCSENKLPAELQELPGLSHQYWS APSDKEGYSGVGLLSRQCPLKVSYGIGDEEHDQEGRVIVAEFDSFVLVTAYVPNAGRGLV RLEYRQRWDEAFRKFLKGLASRKPLVLCGDLNVAHEEIDLRNPKGNKKNAGFTPQERQGF GELLQAVPLADSFRHLYPNTPYAYTFWTYMMNARSKNVGWRLDYFLLSHSLLPALCDSKI RSKALGSDHCPITLYLAL
APEX2	40	MLRVVSWNINGIRRPLQGVANQEPSNCAAVAVGRIILDELDADIVCLQETKVTRDALTEPLAIVEGYNSYF SFSRNRSGYSGVATFCKDNATPVAAEEGLSGLFATQNGDVGCYGNMDEFTQEELRALDSEGRALLTQHKI RTWEGKEKTLTLINVYCPHADPGRPERLVFKMRFYRLLQIRAEALLAAGSHVIILGDLNTAHRPIDHWDA VNLECFEEDPGRKWMDSLLSNLGCQSASHVGPFIDSYRCFQPKQEGAFTCWSAVTGARHLNYGSRLDYVL GDRTLVIDTFQASFLLPEVMGSDHCPVGAVLSVSSVPAKQCPPLCTRFLPEFAGTQLKILRFLVPLEQSP VLEQSTLQHNNQTRVQTCQNKAQVRSTRPQPSQVGSSRGQKNLKSYFQPSPSCPQASPDIELPSLPLMSA LMTPKTPEEKAVAKVVKGQAKTSEAKDEKELRTSFWKSVLAGPLRTPLCGGHREPCVMRTVKKPGPNLGR RFYMCARPRGPPTDPSSRCNFFLWSRPS

BTG1	41	MHPFYTRAATMIGEIAAAVSFISKFLRTKGLTSERQLQTFSQSLQELLAEHYKHHWFPEKPCKGSGYRCI RINHKMDPLIGQAAQRIGLSSQELFRLLPSELTLWVDPYEVSYRIGEDGSICVLYEASPAGGSTQNSTNV QMVDSRISCKEELLLGRTSPSKNYNMMTVSG
CHTOP	42	MAAQSAPKVVLKSTTKMSLNERFTNMLKNKQPTPVNIRASMQQQQQLASARNRRLAQQMENRPSVQAALK LKQKSLKQRLGKSNIQARLGRPIGALARGAIGGRGLPIIQRGLPRGGLRGGRATRTLLRGGMSLRGQNLL RGGRAVAPRMGLRRGGVRGRGGPGRGGLGRGAMGRGGIGGGRGRGMIGRGRGGFGGRGRGRGRGRGALARP VLTKEQLDNQLDAYMSKTKGHLDAELDAYMAQTDPETND
CNOT2	43	MVRTDGHTLSEKRNYQVTNSMFGASRKKFVEGVDSDYHDENMYYSQSSMFPHRSEKDMLASPSTSGQLSQ FGASLYGQQSALGLPMRGMSNNTPQLNRSLSQGTQLPSHVTPTTGVPTMSLHTPPSPSRGILPMNPRNMM NHSQVGQGIGIPSRTNSMSSSGLGSPNRSSPSIICMPKQQPSRQPFTVNSMSGFGMNRNQAFGMNNSLSS NIFNGTDGSENVTGLDLSDFPALADRNRREGSGNPTPLINPLAGRAPYVGMVTKPANEQSQDFSIHNEDF PALPGSSYKDPTSSNDDSKSNLNTSGKTTSSTDGPKFPGDKSSTTQNNNQQKKGIQVLPDGRVTNIPQGM VTDQFGMIGLLTFIRAAETDPGMVHLALGSDLTTLGLNLNSPENLYPKFASPWASSPCRPQDIDFHVPSE YLTNIHIRDKLAAIKLGRYGEDLLFYLYYMNGGDVLQLLAAVELFNRDWRYHKEERVWITRAPGMEPTMK TNTYERGTYYFFDCLNWRKVAKEFHLEYDKLEERPHLPSTFNYNPAQQAF
CNOT4	44	MSRSPDAKEDPVECPLCMEPLEIDDINFFPCTCGYQICRFCWHRIRTDENGLCPACRKPYPEDPAVYKPL SQEELQRIKNEKKQKQNERKQKISENRKHLASVRVVQKNLVFVVGLSQRLADPEVLKRPEYFGKFGKIHK VVINNSTSYAGSQGPSASAYVTYIRSEDALRAIQCVNNVVVDGRTLKASLGTTKYCSYFLKNMQCPKPDC MYLHELGDEAASFTKEEMQAGKHQEYEQKLLQELYKLNPNFLQLSTGSVDKNKNKVTPLQSPIDKPSDSL SIGNGDNSQQISNSDTPSPPPGLSKSNPVIPISSSNHSARSPFEGAVTESQSLFSDIFRHPNPIPSGLPP FPSSPQTSSDWPTAPEPQSLFTSETIPVSSSTDWQAAFGFGSSKQPEDDLGFDPFDVTRKALADLIEKEL SVQDQPSLSPTSLQNSSSHTTTAKGPGSGFLHPAAATNANSLNSTFSVLPQRFPQFQQHRAVYNSFSFPG QAARYPWMAFPRNSIMHLNHTANPTSNSNFLDLNLPPQHNTGLGGIPVAGEEEVKVSTMPLSTSSHSLQQ GQQPTSLHTTVA
CPEB2	45	MPPPSPDSENGFYPGLPSSMNPAFFPSFSPVSPHGCTGLSVPPPAMNIPQQQPPPPAAPQQPQSRRSPVS PQLQQQHQAAAAAFLQQRNSYNHHQPLLKQSPWSNHQSSGWGTGSMSWGAMHGRDHRRTGNMGIPGTMNQ ISPLKKPFSGNVIAPPKFTRSTPSLTPKSWIEDNVFRTDNNSNTLLPLQVRMDRSRMYDSLNMHSLENSL IDIMRAEHDPLKGRLSYPHPGTDNLLMLNARSYGRRRGRSSLFPIDDGLLDDGHSDQVGVLNSPTCYSAH QNGERIERFSRKVFVGGLPPDIDEDEITASFRRFGPLVVDWPHKAESKSYFPPKGYAFLLFQEESSVQAL IDACIEEDGKLYLCVSSPTIKDKPVQIRPWNLSDSDFVMDGSQPLDPRKTIFVGGVPRPLRAVELAMIMD RLYGGVCYAGIDTDPELKYPKGAGRVAFSNQQSYIAAISARFVQLQHGDIDKRVEVKPYVLDDQMCDECQ GARCGGKFAPFFCANVTCLQYYCEFCWANIHSRAGREFHKPLVKEGADRPRQIHFRWN
CPEB4 (full protein)	46	MGDYGFGVLVQSNTGNKSAFPVRFHPHLQPPHHHQNATPSPAAFINNNTAANGSSAGSAWLFPAPATHNI QDEILGSEKAKSQQQEQQDPLEKQQLSPSPGQEAGILPETEKAKSEENQGDNSSENGNGKEKIRIESPVL TGFDYQEATGLGTSTQPLTSSASSLTGFSNWSAAIAPSSSTIINEDASFFHQGGVPAASANNGALLFQNF PHHVSPGFGGSFSPQIGPLSQHHPHHPHFQHHHSQHQQQRRSPASPHPPPFFTHRNAAFNQLPHLANNLNK PPSPWSSYQSPSPTPSSSWSPGGGGYGGWGGSQGRDHRRGLNGGITPLNSISPLKKNFASNHIQLQKYAR

		PSSAFAPKSWMEDSLNRADNIFPFPDRPRTFDMHSLESSLIDIMRAENDTIKGRLNYSYPGSDSSLLINA RTYGRRRGQSSLFPMEDGFLDDGRGDQPLHSGLGSPHCFSHQNGERVERYSRKVFVGGLPPDIDEDEITA SFRRFGPLIVDWPHKAESKSYFPPKGYAFLLFQDESSVQALIDACIEEDGKLYLCVSSPTIKDKPVQIRP WNLSDSDFVMDGSQPLDPRKTIFVGGVPRPLRAVELAMIMDRLYGGVCYAGIDTDPELKYPKGAGRVAFS NQQSYIAAISARFVQLQHGEIDKRVEVKPYVLDDQLCDECQGARCGGKFAPFFCANVTCLQYYCEYCWAA IHSRAGREFHKPLVKEGGDRPRHISFRWN
CPSF4L	47	MQEVIAGLERFTFAFEKDVEMQKGTGLLPFQGMDKSASAVCNFFTKGLCEKGKLCPFRHD RGEKMVVCKHWLRGLCKKGDHCKFLHQYDLTRMPECYFYSKFGDCSNKECSFLHVKPAFK SQDCPWYDQGFCKDGPLCKYRHVPRIMCLNYLVGFCPEGPKCQFAQKIREFKLLPGSKI
CPSF5	48	MSVVPPNRSQTGWPRGVTQFGNKYIQQTKPLTLERTINLYPLTNYTFGTKEPLYEKDSSVAARFQRMREE FDKIGMRRTVEGVLIVHEHRLPHVLLLQLGTTFFKLPGGELNPGEDEVEGLKRLMTEILGRQDGVLQDWV IDDCIGNWWRPNFEPPQYPYIPAHITKPKEHKKLFLVQLQEKALFAVPKNYKLVAAPLFELYDNAPGYGP IISSLPQLLSRFNFIYN
Dis3 iso1 (PIN)	49	MLKSKTFLKKTRAGGVMKIVREHYLRDDIGCGAPGCAACGGAHEGPALEPQPQDPASSVCPQPHYLLPD TNVLLHQIDVLEDPAIRNVIVLQTVLQEVRNRSAPVYKRIRDVTNNQEKHFYTFTNEHHRETYVEQEQGE NANDRNDRAIRVAAKWYNEHLKKMSADNQLQVIFITNDRRNKEKAIEEGIPAFTCEEYVVKSLTANPELIDR LACLSEEGNEIES
DNA2 (nuclease domain)	50	MXSAVDNILLKLAKFKIGFLRLGQQIQKVHPAIQQFTEQEICRSKSIKSLALLEELYNSQLIVATTCMGINH PIFSRKIFDFCIVDEASQISQPICLGPLFFSRRFVLVGDHQQLPPLVLNREARALGMSESLFKRLEQNKS AVVQLTVQYRMNSKIMSLSNKLTYEGKLECGSDKVANAVINLRHFKDVKLELEFYADYSDNPWLMGVFEP NNPVCFLNTDKVPAPEQVEKGGVSNVTEAKLIVFLTSIFVKAGCSPSDIGIIAPYRQQLKIINDLLARSI GMVEVNTVDKYQGRDKSIVLVSFVRSNKDGTVGELLKDWRRLNVAITRAKHKLILLGCVPSLNCYPPLEK LLNHLNSEKLISFFFCIWSHLIALL
ENDOD1 (full-length)	51	MGTARWLALGSLFALAGLLEGRLVGEEEAGFGECDKFFYAGTPPAGLAADSHVKICQRAEGAERFATLYS TRDRIPVYSAFRAPRPAPGGAEQRWLVEPQIDDPNSNLEEAINEAEAITSVNSLGSKQALNTDYLDSDYQ RGQLYPFSLSSDVQVATFTLTNSAPMTQSFQERWYVNLHSLMDRALTPQCGSGEDLYILTGTVPSDYRVVK DKVAVPEFVWLAACCAVPGGGWAMGFVKHTRDSDIIEDVMVKDLQKLLPFNPQLFQNNCGETEQDTEKMK KILEVVNQIQDEERMVQSQKSSSPLSSTRSKRSTLLPPEASEGSSSFLGKLMGFIATPFIKLFQLIYYLV VAILKNIVYFLWCVTKQVINGIESCLYRLGSATISYFMAIGEELVSIPWKVLKVVAKVIRALLRILCCLL KAICRVLSIPVRVLVDVATFPVYTMGAIPIVCKDIALGLGGTVSLLFDTAFGTLGGLFQVVFSVCKRIGY KVTFDNSGEL
ENDOG (full length)	52	MRALRAGLTLASGAGLGAVVEGWRRRREDARAAPGLLGRLPVLPVLPVAAAAELPPVPGGPRGPGELAKYGLP GLAQLKSRESYVLCYDPRTRGALWVVEQLRPERLRGDGDRRECDFREDDSVHAYHRATNADYRGSGFDRG HLAAAANHRWSQKAMDDTFYLSNVAPQVPHLNQNAWNNLEKYSRSLTRSYQNVVVCTGPLFLPRTEADGK SYVKYQVIGKNHVAVPTHFFKVLILEAAGGQIELRTYVMPNAPVDEAIPLERFLVPIESIERASGLLFVP NILARAGSLKAITAGSK

EndoV (full length)	53	MALEAAGGPPEETLSLWKREQARLKAHVVDRDTEAWQRDPAFSGLQRVGGVDVSFVKGDSVRACASLVVL SFPELEVVYEESRMVSLTAPYVSGFLAFREVPFLLELVQQLREKEPGLMPQVLLVDGNGVLHHRGFGVAC HLGVLTDLPCVGVAKKLLQVDGLENNALHKEKIRLLQTRGDSFPLLGDSGTVLGMALRSHDRSTRPLYIS VGHRMSLEAAVRLTCCCCRFRIPEPVRQADICSREHIRKSLGLPGPPTPRSPKAQRPVACPKGDSGEBSSA LC
ERCC1 (full protein)	54	MDPGKDKEGVPQPSGPPARKKFVIPLDEDEVPPGVAKPLFRSTQSLPTVDTSAQAAPQTY AEYAISQPLEGAGATCPTGSEPLAGETPNQALKPGAKSNSIIVSPRQRGNPVLKFVRNVP WEFGDVIPDYVLGQSTCALFLSLRYHNLHPDYIHGRLQSLGKNFALRVLLVQVDVKDPQQ ALKELAKMCILADCTLILAWSPEEAGRYLETYKAYEQKPADLLMEKLEQDFVSRVTECLT TVKSVNKTDSQTLLTTFGSLEQLIAASREDLALCPGLGPQKARRLFDVLHEPPFLKVP
ERN2 (full length)	55	MASAVRGSRPWPRLGLQLQFAALLLGTLSPQVHTLRPENLLLVSTLDGSLHALSKQTGDLKWTLRDDPVI EGPMYVTEMAFLSDPADGSLYILGTQKQQGLMKLPFTIPELVHASPCRSSDGVFYTGRKQDAWFVVDPES GETQMTLTTEGPSTPRLYIGRTQYTVTMHDPRAPALRWNTTYRRYSAPPMDGSPGKYMSHLASCGMGLLL TVDPGSGTVLWTQDLGVPVMGVYTWHQDGLRQLPHLTLARDTLHFLALRWGHIRLPASGPRDTATLFSTL DTQLLMTLYVGKDETGFYVSKALVHTGVALVPRGLTLAPADGPTTDEVTLQVSGEREGSPSTAVRYPSGS VALPSQWLLIGHHELPPVLHTTMLRVHPTLGSGTAETRPPENTQAPAFFLELLSLSREKLWDSELHPEEK TPDSYLGLGPQDLLAASLTAVLLGGWILFVMRQQQPQVVEKQQETPLAPADFAHISQDAQSLHSGASRRS QKRLQSPSKQAQPLDDPEAEQLTVVGKISFNPKDVLGRGAGGTFVFRGQFEGRAVAVKRLLRECFGLVRR EVQLLQESDRHPNVLRYFCTERGPQFHYIALELCRASLQEYVENPDLDRGGLEPEVVLQQLMSGLAHLHS LHIVHRDLKPGNILITGPDSQGLGRVVLSDFGLCKKLPAGRCSFSLHSGIPGTEGWMAPELLQLLPPDSP TSAVDIFSAGCVFYYVLSGGSHPFGDSLYRQANILTGAPCLAHLEEEVHDKVVARDLVGAMLSPLPQPRP SAPQVLAHPFFWSRAKQLQFFQDVSDWLEKSESEQEPLVRALEAGGCAVVRDNWHEHISMPLQTDLRKFRS YKGTSVRDLLRAVRNKKHHYRELPVEVRQALGQVPDGFVQYFTNRFPRLLLHTHRAMRSCASESLFLPYY PPDSEARRPCPGATGR
EXOG (Full Protein)	56	MAIKSIASRLRGSRRFLSGFVAGAVVGAAGAGLAALQFFRSQGAEGALTGKQPDGSAEKA VLEQFGFPLTGTEARCYTNHALSYDQAKRVPRWVLEHISKSKIMGDADRKHCKFKPDPNI PPTFSAFNEDYVGSGWSRGHMAPAGNNKFSSKAMAETFYLSNIVPQDFDNNSGYWNRIEM YCRELTERFEDVWVVSGPLTLPQTRGDGKKIVSYQVIGEDNVAVPSHLYKVILARRSSVS TEPLALGAFVVPNEAIGFQPQLTEFQVSLQDLEKLSGLVFFPHLDRTSDIRNICSVDTCK LLDFQEFTLYLSTRKIEGARSVLRLEKIMENLKNAEIEPDDYFMSRYEKKLEELKAKEQS GTQIRKPS
FEN1	57	MGIQGLAKLIADVAPSAIRENDIKSYFGRKVAIDASMSIYQFLIAVRQGGDVLQNEEGETTSHLMGMFYR TIRMMENGIKPVYVFDGKPPQLKSGELAKRSERRAEAEKQLQQAQAAGAEQEVEKFTKRLVKVTKQHNDE CKHLLSLMGIPYLDAPSEAEASCAALVKAGKVYAAATEDMDCLTFGSPVLMRHLTASEAKKLPIQEFHLS RILQELGLNQEQFVDLCILLGSDYCESIRGIGPKRAVDLIQKHKSIEEIVRRLDPNKYPVPENWLHKEAH QLFLEPEVLDPESVELKWSEPNEEELIKFMCGEKQFSEERIRSGVKRLSKSRQGSTQGRLDDFFKVTGSL SSAKRKEPEPKGSTKKKAKTGAAGKFKRGK

FIP1L1	58	MSAGEVERLVSELSGGTGGDEEEEWLYGDENEVERPEEENASANPPSGIEDETAENGVPKPKVTETEDDS DSDSDDDEDDVHVTIGDIKTGAPQYGSYGTAPVNLNIKTGGRVYGTTGTKVKGVDLDAPGSINGVPLLEV DLDSFEDKPWRKPGADLSDYFNYGFNEDTWKAYCEKQKRIRMGLEVIPVTSTTNKITVQQGRTGNSEKET ALPSTKAEFTSPPSLFKTGLPPSRRLPGAIDVIGQTITISRVEGRRRANENSNIQVLSERSATEVDNNFS KPPPFFPPGAPPTHLPPPPFLPPPPTVSTAPPLIPPPGFPPPPPGAPPPSLIPTIESGHSSGYDSRSARAF PYGNVAFPHLPGSAPSWPSLVDTSKQWDYYARREKDRDRERDRDRERDRDRDRERERTRERERERDHSPT PSVFNSDEERYRYREYAERGYERHRASREKEERHRERRHREKEETRHKSSRSNSRRRRHESEEGDSHRRHK HKKSKRSKEGKEAGSEPAPEQESTEATPAE
FUBP1	59	MADYSTVPPPSSGSAGGGGGGGGGVNDAFKDALQRARQIAAKIGGDAGTSLNSNDYGYGGQKRPLEDG DQPDAKKVAPQNDSFGTQLPPMHQQQRSVMTEEYKVPDGMVGFIIGRGGEQISRIQQESGCKIQIAPDSG GLPERSCMLTGTPESVQSAKRLLDQIVEKGRPAPGFHHGDGPGNAVQEIMIPASKAGLVIGKGGETIKQL QERAGVKMVMIQDGPQNTGADKPLRITGDPYKVQQAKEMVLELIRDQGGFREVRNEYGSRIGGNEGIDVP IPRFAVGIVIGRNGEMIKKIQNDAGVRIQFKPDDGTTPERIAQITGPPDRCQHAAEIITDLLRSVQAGNP GGPGPGGRGRGRGQGNWNMGPPGGLQEFNFIVPTGKTGLIIGKGGETIKSISQQSGARIELQRNPPPNAD PNMKLFTIRGTPQQIDYARQLIEEKIGGPVNPLGPPVPHGPHGVPGPHGPPGPPGPGTPMGPYNPAPYNP GPPGPAPHGPPAPYAPQGWGNAYPHWQQQAPPDPAKAGTDPNSAAWAAYYAHYYQQQAQPPPAAPAGAPT TTQTNGQGDQQNPAPAGQVDYTKAWEEYYKKMGQAVPAPTGAPPGGQPDYSAAWAEYYRQQAAYYAQTSP QGMPQHPPAPQGQ
HNRNPCL1	60	MASNVTNKMDPHSMNSRVFIGNLNTLVVKKSDVEAIFSKYGKIAGCSVHKGFAFVQYDKEKNARAAVAGE DGRMIASQVVDINLAAEPKVNRGNAGVKRSAAEMYGSSFDLDYGFQRDYYDGMYSFPARVPPPPPPIALAV VPSKRQRLSGNTSRRGKSGFNSKSGKRGSSKSGKLKGDDLQAIKQELTQIKQKVDSLLENLEKIEKEQSK QEVEVKNAKSEEEQSSSSMKKDETHVKMESEGGAEDSAEEGDPLDDDVNEDQGDDQLELIKDDEKEAEEG EDDRDSTNGQDDS
HRSP12	61	MSSLIRRVISTAKAPGAIGPYSQAVLVDRTIYISGQIGMDPSSGQLVSGGVAEEAKQALKNMGEILKAAG CDFTNVVKTTVLLADINDFNTVNEIYKQYFKSNFPARAAYQVAALPKGSRIEIEAVAIQGPLTTASL
KIAA0391 (full-length)	62	MTFYLFGIRSFPKLWKSPYLGLGPGHSYVSLFLADRCGIRNQQRLFSLKTMSPQNTKATNLIAKARYLRK DEGSNKQVYSVPHFFLAGAAKERSQMNSQTEDHALAPVRNTIQLPTQPLNSEEWDKLKEDLKENTGKTSF ESWIISQMAGCHSSIDVAKSLLAWVAAKNNGIVSYDLLVKYLYLCVFHMQTSEVIDVFEIMKARYKTLEP RGYSLLIRGLIHSDRWREALLLLEDIKKVITPSKKNYNDCIQGALLHQDVNTAWNLYQELLGHDIVPMLE TLKAFFDFGKDIKDDNYSNKLLDILSYLRNNQLYPGESFAHSIKTWFESVPGKQWKGQFTTVRKSGQCSG CGKTIESIQLSPEEYECLKGKIMRDVIDGGDQYRKTTPQELKRFENFIKSRPPFDVVIDGLNVAKMFPKV RESQLLLNVVSQLAKRNLRLLVLGRKHMLRRSSQWSRDEMEEVQKQASCFFADDISEDDPFLLYATLHSG NHCRFITRDLMRDHKACLPDAKTQRLFFKWQQGHQLAIVNRFPGSKLTFQRILSYDTVVQTTGDSWHIPY DEDLVERCSCEVPTKWLCLHQKT
LACTB2 (full-length)	63	MAAVLQRVERLSNRVVRVLGCNPGPMTLQGTNTYLVGTGPRRILIDTGEPAIPEYISCLKQALTEFNTAI QEIVVTHWHRDHSGGIGDICKSINNDTTYCIKKLPRNPQREEIIGNGEQQYVYLKDGDVIKTEGATLRVL YTPGHTDDHMALLLEEENAIFSGDCILGEGTTVFEDLYDYMNSLKELLKIKADIIYPGHGPVIHNAEAKI

		QQYISHRNIREQQILTLFRENFEKSFTVMELVKIIYKNTPENLHEMAKHNLLLHLKKLEKEGKIFSNTDP DKKWKAHL
LSM4	64	MLPLSLLKTAQNHPMLVELKNGETYNGHLVSCDNWMNINLREVICTSRDGDKFWRMPECYIRGSTIKYLR
MCPIP1 full length	65	IPDEIIDMVKEEVVAKGRGRGGLQQQKQQKGRGMGGAGRGVFGGRGRGGIPGTGRGQPEKKPGRQAGKQ MSGPCGEKPVLEASPTMSLWEFEDSHSRQGTPRPGQELAAEEASALELQMKVDFFRKLGY SSTEIHSVLQKLGVQADTNTVLGELVKHGTATERERQTSPDPCPQLPLVPRGGGTPKAPN LEPPLPEEEKEGSDLRPVVIDGSNVAMSHGNKEVFSCRGILLAVNWFLERGHTDITVFVP SWRKEQPRPDVPITDQHILRELEKKKILVFTPSRRVGGKRVVCYDDRFIVKLAYESDGIV VSNDTYRDLQGERQEWKRFIEERLLMYSFVNDKFMPPDDPLGRHGPSLDNFLRKKPLTLE HRKQPCPYGRKCTYGIKCRFFHPERPSCPQRSVADELRANALLSPPRAPSKDKNGRRPSP SSQSSLLTESEQCSLDGKKLGAQASPGSRQEGLTQTYAPSGRSLAPSGGSGSSFGPTDW LPQTLDSLPYVSQDCLDSGIGSLESQMSELWGVRGGGPGEPGPPRAPYTGYSPYGSELPA TAAFSAFGRAMGAGHFSVPADYPPAPPPREYWSEPYPLPPPTSVLQEPPVQSPGAGR SPWGRAGSLAKEQASVYTKLCGVFPPHLVEAVMGRFPQLLDPQQLAAEILSYKSQHPSE
MCPIP1 PIN with NTR	66	MSGPCGEKPVLEASPTMSLWEFEDSHSRQGTPRPGQELAAEEASALELQMKVDFFRKLGYSSTEIH SVLQKLGVQADTNTVLGELVKHGTATERERQTSPDPCPQLPLVPRGGGTPKAPNLEPPLPEEEKEGS DLRPVVIDGSNVAMSHGNKEVFSCRGILLAVNWFLERGHTDITVFVPSWRKEQPRPDVPITDQHILRE LEKKKILVFTPSRRVGGKRVVCYDDRFIVKLAYESDGIVVSNDTYRDLQGERQEWKRFIEERLLMYSF VNDKFMPPDDPLGRHGPSLDNFLRKKPL
MCPIP4	67	MEHPSKMEFFQKLGYDREDVLRVLGKLGEGALVNDVLQELIRTGSRPGALEHPAAPRLVP RGSCGVPDSAQRGPGTALEEDFRTLASSLRPIVIDGSNVAMSHGNKETFSCRGIKLAVDW FRDRGHTYIKVFVPSWRKDPPRADTPIREQHVLAELERQAVLVYTPSRKVHGKRLVCYDD RYIVKVAYEQDGVIVSNDNYRDLQSENPEWKWFIEQRLLMFSFVNDRFMPPDDPLGRHGP SLSNFLSRKPKPPEPSWQHCPYGKKCTYGIKCKFYHPERPHHAQLAVADELRAKTGARPG AGAEEQRPPRAPGGSAGARAAPREPFAHSLPPARGSPDLAALRGSFSRLAFSDDLGPLGP PLPVPACSLTPRLGGPDWVSAGGRVPGPLSLPSPESQFSPGDLPPPPGLQLQPRGEHRPR DLHGDLLSPRRPPDDPWARPPRSDRFPGRSVWAEPAWGDGATGGLSVYATEDDEGDARAR ARIALYSVFPRDQVDRVMAAFPELSDLARLILLVQRCQSAGAPLGKP
MEX3C	68	MPSGSSAALALAAAPAPLPQPPPPPPPPPPLPPPSGGPELEGDGLLLRERLAALGLDDPSPAEPGAPAL RAPAAAAQGQARRAAELSPEERAPPGRPGAPEAAELELEEDEEEGEEAELDGDLLEEEELEEAEEEDRSS LLLLSPPAATASQTQQIPGGSLGSVLLPAARFDAREAAAAAAAAAGVLYGGDDAQGMMAAMLSHAYGPGGC GAAAAALNGEQAALLRRKSVNTTECVPVPSSEHVAEIVGRQGCKIKALRAKTNTYIKTPVRGEEPIFVVT GRKEDVAMAKREILSAAEHFSMIRASRNKNGPALGGLSCSPNLPGQTTVQVRVPYRVVGLVVGPKGATIK RIQQQTHTYIVTPSRDKEPVFEVTGMPENVDRARAEEIEMHIAMRTGNYIELNEENDFHYNGTDVSFEGGT LGSAWLSSNPVPPSRARMISNYRNDSSSSLGSGSTDSYFGSNRLADFSPTSPFSTGNFWFGDTLPSVGSE DLAVDSPAFDSLPTSAQTIWTPFEPVNPLSGFGSDPSGNMKTQRRGSQPSTPRLSPTFPESIEHPLARRV

		RSDPPSTGNHVGLPIYIPAFSNGTNSYSSSNGGSTSSSPPESRRKHDCVICFENEVIAALVPCGHNLFCM ECANKICEKRTPSCPVCQTAVTQAIQIHS
MYEF2 (C-term isoformB)	69	MDGPGFGGMNRIGGGIGFGGLEAMNSMGGFGGVGRMGELYRGAMTSSMERDFGRGDIGINRGFGDSFGRL GGGMGSMNSVTGGMGMGLDRMSSSFDRMGPGIGAILERSIDMDRGFLSGPMGSGMREERIGSSKGNQIFVRN LPFDLTWQKLKEKFSQCGHVMFAEIKMENGKSKGCGTVRFDSPESAEKACRIMNGIKISGREIDVRLDRN A
MYEF2 (isoform a)	70	MADANKAEVPGATGGDSPHLQPAEPPGEPRREPHPAEAEKQQPQHSSSSNGVKMENDESAKEEKSDLKEEK STGSKKANRFHPYSKDKNSGAGEKKGPNRNRVFISNIPYDMKWQAIKDLMREKVGEVTYVELFKDAEGKS RGCGVVEFKDEEFVKKALETMNKYDLSGRPLNIKEDPDGENARRALQRTGGSFPGGHVPDMGSGLMNLPP SILNNPNIPPEVISNLQAGRLGSTIFVANLDFKVGWKKLKEVFSIAGTVKRADIKEDKDGKSRGMGTVTF EQAIEAVQAISMFNGQFLFDRPMHVKMDDKSVPHEEYRSHDGKTPQLPRGLGGIGMGLGPGGQPISASQL NIGGVMGNLGPGGMGMDGPGFGGMNRIGGGIGFGGLEAMNSMGGFGGVGRMGELYRGAMTSSMERDFGRG DIGINRGFGDSFGRLGSAMIGGFAGRIGSSNMGPVGSGISGGMGSMNSVTGGMGMGLDRMSSSFDRMGPG IGAILERSIDMDRGFLSGPMGSGMRERIGSKGNQIFVRNLPFDLTWQKLKEKFSQCGHVMPAEIKMENGK SKGCGTVRFDSPESAEKACRIMNGIKISGREIDVRLDRNA
NANOS2	71	MQLPPFDMWKDYFNLSQVVWALIASRGQRLETQEIEEPSPGPPLGQDQGLGAPGANGGLGTLCNFCKHNG ESRHVYSSHQLKTPDGVVVCPILRHYVCPVCGATGDQAHTLKYCPLNGGQQSLYRRSGRNSAGRRVKR
NANOS3	72	MGTFDLWTDYLGLAHLVRALSGKEGPETRLSPQPEPEPMLEPVSALEPMPAPESVPVPGPKDQKRSLESS PAPERLCSFCKHNGESRAIYQSHVLKDEAGRVLCPILRDYVCPQCGATRERAHTRRFCPLTGQGYTSVYS HTTRNSAGKKLVRPDKAKTQDTGHRRGGGGAGFRGAGKSEPSPSCSPSMST
NOB1	73	MAPVEHVVADAGAFLRHAALQDIGKNIYTIREVVTEIRDKATRRRLAVLPYELRFKEPLPEYVRLVTEFS KKTGDYPSLSATDIQVLALTYQLEAEFVGVSHLKQEPQKVKVSSSIQHPETPLHISGFHLPYKPKPPQET EKGHSACEPENLEFSSFMFWRNPLPNIDHELQELLIDRGEDVPSEEEEEENGFEDRKDDSDDDGGGWIT PSNIKQIQQELEQCDVPEDVRVGCLTTDFAMQNVLLQMGLHVLAVNGMLIREARSYILRCHGCFKTTSDM SRVFCSHCGNKTLKKVSVTVSDDGTLHMHFSRNPKVLNPRGLRYSLPTPKGGKYAINPHLTEDQRFPQLR LSQKARQKTNVFAPDYIAGVSPFVENDISSRSATLQVRDSTLGAGRRRLNPNASRKKFVKKR
NOP56 (full length)	74	MVLLHVLFEHAVGYALLALKEVEEISLLQPQVEESVTLNLGKFHSIVRLVAFCPFASSQVALENANAVSEG VVHEDLRLLLETHLPSKKKKVLLGVGDPKIGAAIQEELGYNCQTGGVIAEILRGVRLHPHNLVKGLTDLS ACKAQLGLGHSYSRAKVKFNVNRVDNMIIQSISLLDQLDKDINTFSMRVREWYGYHPPELVKIINDNATY CRLAQFIGNRRELNEDKLEKLEELTMDGAKAKAILDASRSSMGMDISAIDLINIESFSSRVVSLSEYRQS LHTYLRSKMSQVAPSLSALIGEAVGARLIAHAGSLTNLAKYPASTVQILGAEKALFRALKTRGNTPKYGL IFHSTFIGRAAAKNKGRISRYLANKCSIASRIDCFSEVPTSVFGEKLREQVEERLSFYETGEIPRKNLDV MKEAMVQAEEAAAEITRKLEKQEKKRLKKEKKRLAALALASSENSSSTPEECEEMSEKPKKKKKQKPQEV PQENGMEDPSISFSKPKKKKSFSKEELMSSDLEETAGSTSIPKRKKSTPKEETVNDPEEAGHRSGSKKKR KFSKEEPVSSGPEEAVGKSSSKKKKKFHKASQED
NTHL1	75	MCSPQESGMTALSARMLTRSRSLGPGAGPRGCREEPGPLRRREAAAEARKSHSPVKRPRKAQRLRVAYEGS DSEKGEGAEPLKVPVWEPQDWQQQLVNIRAMRNKKDAPVDHLGTEHCYDSSAPPKVRRYQVLLSLMLSSQ

		TKDQVTAGAMQRLRARGLTVDSILQTDDATLGKLIYPVGFWRSKVKYIKQTSAILQQHYGGDIPASVAEL VALPGVGPKMAHLAMAVAWGTVSGIAVDTHVHRIANRLRWTKKATKSPEETRAALEEWLPRELWHEINGL LVGFGQQTCLPVHPRCHACLNQALCPAAQGL
PARN	76	MEIIRSNFKSNLHKVYQAIEEADFFAIDGEFSGISDGPSVSALTNGFDTPEERYQKLKKHSMDFLLFQFG LCTFKYDYTDSKYITKSFNFYVFPKPFNRSSPDVKFVCQSSSIDFLASQGFDFNKVFRNGIPYLNQEEER QLREQYDEKRSQANGAGALSYVSPNTSKCPVTIPEDQKKFIDQVVEKIEDLLQSEENKNLDLEPCTGFQR KLIYQTLSWKYPKGIHVETLETEKKERYIVISKVDEEERKRREQQKHAKEQEELNDAVGFSRVIHAIANS GKLVIGHNMLLDVMHTVHQFYCPLPADLSEFKEMTTCVFPRLLDTKLMASTQPFKDIINNTSLAELEKRL KETPFNPPKVESAEGFPSYDTASEQLHEAGYDAYITGLCFISMANYLGSFLSPPKIHVSARSKLIEPFFN KLFLMRVMDIPYLNLEGPDLQPKRDHVLHVTFPKEWKTSDLYQLFSAFGNIQISWIDDTSAFVSLSQPEQ VKIAVNTSKYAESYRIQTYAEYMGRKQEEKQIKRKWTEDSWKEADSKRLNPQCIPYTLQNHYYRNNSFTA PSTVGKRNLSPSQEEAGLEDGVSGEISDTELEQTDSCAEPLSEGRKKAKKLKRMKKELSPAGSISKNSPA TLFEVPDTW
PDL6 (full protein)	77	MGRLSWQVAAAAAVGLALTLEALPWVLRWLRSRRRRPRREALFFPSQVTCTEALLRAPGA ELAELPEGCPCGLPHGESALSRLLRALLAARASLDLCLFAFSSPQLGRAVQLLHQRGVRV RVVTDCDYMALNGSQIGLLRKAGIQVRHDQDPGYMHHKFAIVDKRVLITGSLNWTTQAIQ NNRENVLITEDDEYVRLFLEEFERIWEQFNPTKYTFFPPKKSHGSCAPPVSRAGGRLLSW HRTCGTSSESQT
Pelota homolog (full length)	78	MKLVRKNIEKDNAGQVTLVPEEPEDMWHTYNLVQVGDSLRASTIRKVQTESSTGSVGSNRVRTTLTLCVEA IDFDSQACQLRVKGTNIQENEYVKMGAYHTIELEPNRQFTLAKKQWDSVVLERIEQACDPAWSADVAAVV MQEGLAHICLVTPSMTLTRAKVEVNIPRKRKGNCSQHDRALERFYEQVVQAIQRHIHFDVVKCILVASPG FVREQFCDYMFQQAVKTDNKLLLENRSKFLQVHASSGHKYSLKEALCDPTVASRLSDTKAAGEVKALDDF YKMLQHEPDDRAFYGLKQVEKANEAMAIDTLLISDELFRHQDVATRSRYVRLVDSVKENAGTVRIFSSLHV SGEQLSQLTGVAAILRFPVPELSDQEGDSSSEED
PRR3	79	MPKRKKQNHHQPPTQQQPPLPEREETGDEEDGSPIGPPSLLGPPPMANGKPGDPKSALHRGPPGSRGPLI PPLLSLPPPPWGRGPIRRGLGPRSSPYGRGWWGVNAEPPFPGPGHGGPTRGSFHKEQRNPRRLKSWSLIK NTCPPKDDPQVMEDKSDRPVCRHFAKKGHCRYEDLCAFYHPGVNGPPL
QKI	80	MVGEMETKEKPKPTPDYLMQLMNDKKLMSSLPNFCGIFNHLERLLDEEISRVRKDMYNDTLNGSTEKRSA ELPDAVGPIVQLQEKLYVPVKEYPDFNFVGRILGPRGLTAKQLEAETGCKIMVRGKGSMRDKKKEEQNRG KPNWEHLNEDLHVLITVEDAQNRAEIKLKRAVEEVKKLLVPAAEGEDSLKKMQLMELAILNGTYRDANIK SPALAFSLAATAQAAPRIITGPAPVLPPAALRTPTPAGPTIMPLIRQIQTAVMPNGTPHPTAAIVPPGPE AGLIYTPYEYPYTLAPATSILEYPIEPSGVLGAVATKVRRHDMRVHPYQRIVTADRAATGN
RAB1	81	MALQRTHSLLLLLLLTLGLGLVQPSYGQDGMYQRFLRQHVHPEETGGSDRYCNLMMQRRKMTLYHCKRF NTFIHEDIWNIRSICSTTNIQCKNGKMNCHEGVVKVTDCRDTGSSRAPNCRYRAIASTRRVVIACEGNPQ VPVHFDG

RAC1 (full length)	82	MALEKSLVRLLLLVLILLVLGWVQPSLGKESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGRCKP VNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACEG SPYVPVHFDASVEDST
RBM22	83	MATSLGSNTYNRQNWEDADFPILCQTCLGENPYIRMTKEKYGKECKICARPFTVFRWCPGVRMRFKKTEV CQTCSKLKNVCQTCLLDLEYGLPIQVRDAGLSFKDDMPKSDVNKEYYTQNMEREISNSDGTRPVGMLGKA TSTSDMLLKLARTTPYYKRNRPHICSFWVKGECKRGEECPYRHEKPTDPDDPLADQNIKDRYYGINDPVA DKLLKRASTMPRLDPPEDKTITTLYVGGLGDTITETDLRNHFYQFGEIRTITVVQRQQCAFIQFATRQAA EVAAEKSFNKLIVNGRRLNVKWGRSQAARGKEKEKDGTTDSGIKLEPVPGLPGALPPPPAAEEEASANYF NLPPSGPPAVVNIALPPPPPGIAPPPPPGFGPGPHMFHPMGPPPPFMRAPGPIHYPSQDPQRMGAHAGKHSSP
RBM7	84	MGAAAAEADRTLFVGNLETKVTEELLFELFHQAGPVIKVKIPKDKDGKPKQFAFVNFKHEVSVPYAMNLL NGIKLYGRPIKIQFRSGSSHAPQDVSLSYPQHHVGNSSPTSTSPSRYERTMDNMTSSAQIIQRSFSSPEN FQRQAVMNSALRQMSYGGKFGSSPLDQSGFSPSVQSHSHSFNQSSSSQWRQGTPSSQRKVRMNSYPYLAD RHYSREQRYTDHGSDHHYRGKRDDFFYEDRNHDDWSHDYDNRRDSSRDGKWRSSRH
RIDA	85	MSSLIRRVISTAKAPGAIGPYSQAVLVDRTIYISGQIGMDPSSGQLVSGGVAEEAKQALKNMGEILKAAG CDFTNVVKTTVLLADINDFNTVNEIYKQYFKSNFPARAAYQVAALPKGSRIEIEAVAIQGPLTTASL
RNAse L full protein	86	MESRDHNNPQEGPTSSSGRRAAVEDNHLLIKAVQNEDVDLVQQLLEGGANVNFQEEGGW TPLHNAVQMSREDIVELLLRHGADPVLRKKNGATPFILAAIAGSVKLLKLFLSKGADVNE CDFYGFTAFMEAAVYGKVKALKFLYKRGANVNLRRKTKEDQERLRKGGATALMDAAEKGH VEVLKILLDEMGADVNACDNMGRNALIHALLSSDDSDVEAITHLLLDHGADVNVRGERGK TPLILAVEKKHLGLVQRLLEQEHIEINDTDSDGKTALLLAVELKLKKIAELLCKRGASTD CGDLVMTARRNYDHSLVKVLLSHGAKEDFHPPAEDWKPQSSHWGAALKDLHRIYRPMIGK LKFFIDEKYKIADTSEGGIYLGFYEKQEVAVKTFCEGSPRAQREVSCLQSSRENSHLVTF YGSESHRGHLFVCVTLCEQTLEACLDVHRGEDVENEEDEFARNVLSSIFKAVQELHLSCG YTHQDLQPQNILIDSKKAAHLADFDKSIKWAGDPQEVKRDLEDLGRLVLYVVKKGSISFE DLKAQSNEEVVQLSPDEETKDLIHRLFHPGEHVRDCLSDLLGHPFFWTWESRYRTTLRNVG NESDIKTRKSESEILRLLQPGPSEHSKSFDKWTTKINECVMKKMNKPYEKRGNFYQNTVG DLLKFIRNLGEHIDEEKHKKMKLKIGDPSLYFQKTFPDLVIYVYTKLQNTEYRKHFPQTH SPNKPQCDGAGGASGLASPGC
RNAse l (full-length)	87	MALEKSLVRLLLLVLILLVLGWVQPSLGKESRAKKFQRQHMDSDSSPSSSSTYCNQMMRRRNMTQGLCKP VNTFVHEPLVDVQNVCFQEKVTCKNGQGNCYKSNSSMHITDCRLTNGSRYPNCAYRTSPKERHIIVACEG SPYVPVHFDASVEDST
RNAse l l (full protein)	88	METFPLLLLSLGLVLAEASESTMKIIKEEFTDEEMQYDMAKSGQEKQTIEILMNPILLVK NTSLSMSKDDMSSTLLTFRSLHYNDPKGNSSGNDKECCNDMTVWRKVSEANGSCKWSNNF IRSSTEVMRRVHRAPSCKFVQNPGISCCESLELENTVCQFTTGKQFPRCQYHSVTSLEKI LTVLTGHSLMSWLVCGSKL

RNAse2 (full protein)	89	MVPKLFTSQICLLLLLGLLAVEGSLHVKPPQFTWAQWFETQHINMTSQQCTNAMQVINNY QRRCKNQNTFLLTTFANVVNVCGNPNMTCPSNKTRKNCHHSGSQVPLIHCNLTTPSPQNI SNCRYAQTPANMFYIVACDNRDQRRDPPQYPVVPVHLDRII
RNAse4 (full protein)	90	MALQRTHSLLLLLLLTLLGLGLVQPSYGQDGMYQRFLRQHVHPEETGGSDRYCNLMMQRR KMTLYHCKRFNTFIHEDIWNIRSICSTTNIQCKNGKMNCHEGVVKVTDCRDTGSSRAPNC RYRAIASTRRVVIACEGNPQVPVHFDG
RNAse6 (full length)	91	MVLCFPLLLLLLLVLWGPVCPLHAWPKRLTKAHWFEIQHIQPSPLQCNRAMSGINNYTQHCKHQNTFLHDS FQNVAAVCDLLSIVCKNRRHNCHQSSKPVNMTDCRLTSGKYPQCRYSAAAQYKFFIVACDPPQKSDPPYK LVPVHLDSIL
RNAse6PL (full length)	92	MRPAALRGALLGCLCLALLCLGGADKRLRDNHEWKKLIMVQHWPETVCEKIQNDCRDPPDYWTIHGLWPD KSEGCNRSWPFNLEEIKKNWMEITDSSLPSPSMGPAPPRWMRSTPRRSTLAEAWNSTGSWTSTGGCALPP AALPSGDLCCRPSLTAGSRGVGVDLTALHQLLHVHYSATGIIPEECSEPTKPFQIILHHDHTEWVQSIGM PIWGTISSSESAIGKNEESQPACAVLSHDS
RNAse7	93	MAPARAGFCPLLLLLLLLGLWVAEIPVSAKPKGMTSSQWFKIQHMQPSPQACNSAMKNINKHTKRCKDLNT FLHEPFSSVAATCQTPKIACKNGDKNCHQSHGAVSLTMCKLTSGKHPNCRYKEKRQNKSYVVACKPPQKK DSQQFHLVPVHLDRVL
RNAse8 (full length)	94	MAPARAGCCPLLLLLLLGLWVAEVLVRAKPKDMTSSQWFKTQHVQPSPQACNSAMSIINKYTERCKDLNTF LHEPFSSVAITCQTPNIACKNSCKNCHQSHGPMSLTMGELTSGKYPNCRYKEKHLNTPYIVACDPPQQGD PGYPLVPVHLDKVV
RNAseK	95	MASLLCCGPKLAACGIVLSAWGVIMLIMLGIFFNVHSAVLIEDVPFTEKDFENGPQNIYN LYEQVSYNCFIAAGLYLLLGGFSFCQVRLNKRKEYMVR
RNAseT2 (full length)	96	MRPAALRGALLGCLCLALLCLGGADKRLRDNHEWKKLIMVQHWPETVCEKIQNDCRDPPDYWTIHGLWPD KSEGCNRSWPFNLEEIKDLLPEMRAYWPDVIHSFPNRSRFWKHEWEKHGTCAAQVDALNSQKKYFGRSLE LYRELDLNSVLLKLGIKPSINYYQVADFKDALARVYGVIPKIQCLPPSQDEEVQTIGQIELCLTKQDQQL QNCTEPGEQPSPKQEVWLANGAAESRGLRVCEDGPVFYPPPKKTKH
SLFN14	97	MESLKTDTEMPYPEVIVDVGRVIFGEENRKKMTNSCLKRSENSRIIRAICALLNSGGGVIKAEIDDKTYS YQCHGLGQDLETSFQKLLPSGSQKYLDYMQQGHNLLIFVKSWSPDVFSLPLRICSLRSNLYRRDVTSAIN LSASSALELLRKGFRAQRGRPRVKKLHPQQVLNRCIQEEEDMRIILASEFFKKDKLMYKEKLNFTESTHV EFKRFTTKKVIPRIKEMLPHYVSAFANTQGGYVLIGVDDKSKEVVGCKWEKVNPDLLKKEIENCIEKLPT FHFCCEKPKVNFTTKILNVYQKDVLDGYVCVIQVEPFCCVVFAEAPDSWIMKDNSVTRLTAEQWVVMMLD TQSAPPSLVTDYNSCLISSASSARKSPGYPIKVHKFKEALQRHLFPVTQEEVQFKPESLCKKLFSDHKEL EGLMKTLIHPCSQGIVIFSRSWAGDVGFRKEQNVLCDALLIAVNSPVVLYTILIDPNWPGGLEYARNTAH QLKQKLQTVGGYTGKVCIIPRLIHLSSTQSRPGEIPLRYPRSYRLADEEEMEDLLQALVVVSLSSRSLLS DQMGCEFFNLLIMEQSQLLSESLQKTRELFIYCFPGVRKTALAIKIMEKIKDLFHCKPKEILYVCESDSL KDFVTQQTTCQAVTRKTFMQGEFLKIKHIVMDETENFCSKYGNWYMKAKNITHPKAKGTGSENLHHGILW LFLDPFQIHHADVNGLPPPSAQFPRKTITSGIHCALEIAKVMKEEMKRIKENPPSNMSPDTILALFSETAY EEATCAQALPGVCETKTNLTTEQIANYVARKCHSLFQCGYLPKDIAILCRRGEDRGRYRLALLKAMELIE

		THRPSEVVFSPATGVWGSHIVLDSIQQFSGLERTVVFGLSPECDQSEEFHKLCFASRAIKHLYLLYEKRA AY
SNRPA	98	MAVPETRPNHTIYINNLNEKIKKDELKKSLYAIFSQFGQILDILVSRSLKMRGQAFVIFKEVSSATNALR SMQGFPFYDKPMRIQYAKTDSDIIAKMKGTFVERDRKREKRKPKSQETPATKKAVQGGGATPVVGAVQGP VPGMPPMTQAPRIMHHMPGQPPYMPPPGMIPPPGLAPGQIPPGAMPPQQLMPGQMPPAQPLSENPPNHIL FLTNLPEETNELMLSMLFNQFPGFKEVRLVPGRHDIAFVEFDNEVQAGAARDALQGFKITQNNAMKISFA KK
SRSF11	99	MSNTTVVPSTAGPGPSGGPGGGGGGGGGGTEVIQVTNVSPSASSEQMRTLFGFLGKIDELRLFPPDDS PLPVSSRVCFVKFHDPDSAVVAQHLTNTVFVDRALIVVPYAEGVIPDEAKALSLLAPANAVAGLLPGGGL LPTPNPLTQIGAVPLAALGAPTLDPALAALGLPGANLNSQSLAADQLLKLMSTVDPKLNHVAAGLVSPSL KSDTSSKEIEEAMKRVREAQSLISAAIEPDKKEEKRRHSRSRSRSRRRRTPSSSRHRRSRSRSRRRSHSK SRSRRRSKSPRRRRSRHSRERGRRSRSTSKTRDKKKEDKEKKRSKTPPKSYSTARRSRSASRERRRRRRSRS GTRSPKKPRSPKRKLSRSPSPRRHKKEKKKDKDKERSRDERERRSTSKKKKSKDKEKDRERKSESDKDVKQ VTRDYDEEEQGYDSEKEKKEKKPIETGSPKTKECSVEKGTGDSLRESKVNGDDHHEEDMDMSD
THRAP3	100	MSKTNKSKSGSRSSRSRSASRSRSRSFSKSRSRSRSLSRSRKRRLSSRSRSRSYSPAHNRERNHPRVYQN RDFRGHNRGYRRPYYFRGRNRGFYPWGQYNRGGYGNYRSNWQNYRQAYSPRRGRSRSRSPKRRSPSPRSR SHSRNSDKSSSDRSRRSSSSRSSSNHSRVESSKRKSAKEKKSSSKDSRPSQAAGDNQGDEAKEQTFSGGT SQDTKASESSKPWPDATYGTGSASRASAVSELSPRERSPALKSPLQSVVVRRRSPRPSPVPKPSPPLSST SQMGSTLPSGAGYQSGTHQGQFDHGSGSLSPSKKSPVGKSPPSTGSTYGSSQKEESAASGGAAYTKRYLE EQKTENGKDKEQKQTNTDKEKIKEKGSFSDTGLGDGKMKSDSFAPKTDSEKPFRGSQSPKRYKLRDDFEK KMADFHKEEMDDQDKDKAKGRKESEFDDEPKFMSKVIGANKNQEEEKSGKWEGLVVAPPGKEKQRKTEEL EEESFPERSKKEDRGKRSEGGHRGFVPEKNFRVTAYKAVQEKSSSPPPRKTSESRDKLGAKGDFPTGKSS FSITREAQVNVRMDSFDEDLARPSGLLAQERKLCRDLVHSNKKEQEFRSIFQHIQSAQSQRSPSELFAQH IVTIVHHVKEHHFGSSGMTLHERFTKYLKRGTEQEAAKNKKSPEIHRRIDISPSTFRKHGLAHDEMKSPR EPGYKAEGKYKDDPVDLRLDIERRKKHKERDLKRGKSRESVDSRDSSHSRERSAEKTEKTHKGSKKQKKH RRARDRSRSSSSSSQSSHSYKAEEYTEETEREESTTGFDKSRLGTKDFVGPSERGGGRARGTFQFRARG RGWGRGNYSGNNNNSNNDFQKRNREEEWDPEYTPKSKKYYLHDDREGEGSDKWVSRGRGRGAFPRGRGR FMFRKSSTSPKWAHDKFSGEEGEIEDDESGTENREEKDNIQPTTE
TOB1	101	MQLEIQVALNFIISYLYNKLPRRRVNIFGEELERLLKKKYEGHWYPEKPYKGSGFRCIHIGEKVDPVIEQ ASKESGLDIDDVRGNLPQDLSVWIDPFEVSYQIGEKGPVKVLYVDDNNENGCELDKEIKNSFNPEAQVFM PISDPASSVSSSPSPPFGHSAAVSPTFMPRSTQPLTFTTATFAATKFGSTKMKNSGRSNKVARTSPINLG LNVNDLLKQKAISSSMHSLYGLGLGSQQPQQQQPAQPPPPPPPPQQQQQQKTSALSPNAKEFIFPNMQ GQGSSTNGMFPGDSPLNLSPLQYSNAFDVFAAYGGLNEKSFVDGLNFSLNNMQYSNQQFQPVMAN
TOB2	102	MQLEIKVALNFIISYLYNKLPRRRADLFGEELERLLKKKYEGHWYPEKPLKGSGFRCVHIGEMVDPVVEL AAKRSGLAVEDVRANVPEELSVWIDPFEVSYQIGEKGAVKVLYLDDSEGCGAPELDKEIKSSFNPDAQVF VPIGSQDSSLSNSPSPSFGQSPSPTFIPRSAQPITFTTASFAATKFGSTKMKKGGGAASGGGVASSGAGG

		QQPPQQPRMARSPTNSLLKHKSLSLSMHSLNFITANPAPQSQLSPNAKEFVYNGGGSPSLFFDAADGQGS
		GTPGPFGGSGAGTCNSSSFDMAQVFGGGANSLFLEKTPFVEGLSYNLNTMQYPSQQFQPVVLAN
U2AF2	103	MSDFDEFERQLNENKQERDKENRHRKRSHSRSRSRDRKRRSRSRDRRNRDQRSASRDRRRRSKPLTRGAK
		EEHGGLIRSPRHEKKKKVRKYWDVPPPGFEHITPMQYKAMQAAGQIPATALLPTMTPDGLAVTPTPVPVV
		GSQMTRQARRLYVGNIPFGITEEAMMDFFNAQMRLGGLTQAPGNPVLAVQINQDKNFAFLEFRSVDETTQ
		AMAFDGIIFQGQSLKIRRPHDYQPLPGMSENPSVYVPGVVSTVVPDSAHKLFIGGLPNYLNDDQVKELLT
		SFGPLKAFNLVKDSATGLSKGYAFCEYVDINVTDQAIAGLNGMQLGDKKLLVQRASVGAKNATLSTINQT
		PVTLQVPGLMSSQVQMGGHPTEVLCLMNMVLPEELLDDEEYEEIVEDVRDECSKYGLVKSIEIPRPVDGV
		EVPGCGKIFVEFTSVFDCQKAMQGLTGRKFANRVVVTKYCDPDSYHRRDFW
UTP11L	104	MAAAFRKAAKSRQREHRERSQPGFRKHLGLLEKKKDYKLRADDYRKKQEYLKALRKKALEKNPDEFYYKM
		TRVKLQDGVHIIKETKEEVTPEQLKLMRTQDVKYIEMKRVAEAKKIERLKSELHLLDFQGKQQNKHVFFF
		DTKKEVEQFDVATHLQTAPELVDRVFNRPRIETLQKEKVKGVTNQTGLKRIAKERQKQYNCLTQRIERFK
		KLFVIAQKIQTRKDLMDKTQKVKVKKETVNSPAIYKFQSRRKR
YBEY	105	MSLVIRNLQRVIPIRRAPLRSKIEIVRRILGVQKFDLGIICVDNKNIQHINRIYRDRNVPTDVLSFPFHE
		HLKAGEFPQPDFPDDYNLGDIFLGVEYIFHQCKENEDYNDVLTVTATHGLCHLLGFTHGTEAEWQQMFQK
		EKAVLDELGRRTGTRLQPLTRGLFGGS
YTHDF2	106	MSASSLLEQRPKGQGNKVQNGSVHQKDGLNDDDFEPYLSPQARPNNAYTAMSDSYLPSYYSPSIGFSYSL
		GEAAWSTGGDTAMPYLTSYGQLSNGEPHFLPDAMFGQPGALGSTPFLGQHGFNFFPSGIDFSAWGNNSSQ
		GQSTQSSGYSSNYAYAPSSLGGAMIDGQSAFANETLNKAPGMNTIDQGMAALKLGSTEVASNVPKVVGSA
		VGSGSITSNIVASNSLPPATIAPPKPASWADIASKPAKQQPKLKTKNGIAGSSLPPPPIKHNMDIGTWDN
		KGPVAKAPSQALVQNIGQPTQGSPQPVGQQANNSPPVAQASVGQQTQPLPPPPQPAQLSVQQQAAQPTR
		WVAPRNRGSGFGHNGVDGNGVGQSQAGSGSTPSEPHPVLEKLRSINNYNPKDFDWNLKHGRVFIIKSYSE
		DDIHRSIKYNIWCSTEHGNKRLDAAYRSMNGKGPVYLLFSVNGSGHFCGVAEMKSAVDYNTCAGVWSQDK
		WKGRFDVRWIFVKDVPNSQLRHIRLENNENKPVTNSRDTQEVPLEKAKQVLKIIASYKHTTSIFDDFSHY
		EKRQEEEESVKKERQGRGK
ZFC3H1 (full length)	107	MATADTPAPASSGLSPKEEGELEDGEISDDDNNSQIRSRSSSSSSSGGGLLPYPRRRPPHSARGGGSGGGG
		GSSSSSSSSQQQLRNFSRSRHASERGHLRGPSSYRPKEPFRSHPPSVRMPSSSLSESSPRPSFWERSHLA
		LDRFRFRGRPYRGGSRWSRGRGVGERGGKPGCRPPLGGGAGSGFSSSQSWREPSPPRKSSKSFGRSPSRK
		QNYSSKNENCVEETFEDLLLKYKQIQLELECINKDEKLALSSKEENVQEDPKTLNFEDQTSTDNVSITKD
		SSKEVAPEEKTQVKTFQAFELKPLRQKLTLPGDKNRLKKVKDGAKPLSLKSDTTDSSQGLQDKEQNLTRR
		ISTSDILSEKKLGEDEEELSELQLRLLALQSASKKWQQKEQQVMKESKEKLTKTKTVQQKVKTSTKTHSA
		KKVSTTAKQALRKQQTKAWKKLQQQKEQERQKEEDQRKQAEEEERRKREEEIRKIRDLSNQEEQYNRFMK
		LVGGKRRSRSKSSDPDLRRSLDKQPTDSGGGIYQYDNYEEVAMDTDSETSSPAPSPVQPPFFSECSLGYF
		SPAPSLSLPPPPQVSSLPPLSQPYVEGLCVSLEPLPPLPPLPPEDPEQPPKPPFADEEEEEEMLLRE
		ELLKSLANKRAFKPEETSSNSDPPSPPVLNNSHPVPRSNLSIVSINTVSQPRIQNPKFHRGPRLLPRTVIS
		LPKHKSVVVTLNDSDDSESDGEASKSTNSVFGGLESMIKEARRTAEQASKPKVPPKSEKENDPLRTPEAL
		PEEKKIEYRLLKEEIANREKQRLIKSDQLKTSSSSPANSDVEIDGIGRIAMVTKQVTDAESKLKKHRILL

		MKDESVLKNLVQQEAKKKESVRNAEAKITKLTEQLQATEKILNVNRMFLKKLQEQIHRVQQRVTIKKALT
		LKYGEELARAKAVASKEIGKRKLEQDRFGPNKMMRLDSSPVSSPRKHSAELIAMEKRRLQKLEYEYALKI
		QKLKEARALKAKEQQNISPVVEEEPEFSLPQPSLHDLTQDKLTLDTEENDVDDEILSGSSREERRRSFLES
		NYFTKPNLKHTDTANKECINKLNKNTVEKPELFLGLKIGELQKLYSKADSLKQLILKTTTGITEKVLHGQ
		EISVDVDFVTAQSKTMEVKPCPFRPYHSPLLVFKSYRFSPYYRTKEKLPLSSVSYSNMIEPDQCFCRFDL
		TGTCNDDDCQWQHIQDYTLSRKQLFQDILSYNLSLIGCAETSTNEEITASAEKYVEKLFGVNKDRMSMDQ
		MAVLLVSNINESKGHTPPFTTYKDKRKWKPKFWRKPISDNSFSSDEEQSTGPIKYAFQPENQINVPALDT
		VVTPDDVRYFTNETDDIANLEASVLENPSHVQLWLKLAYKYLNQNEGECSESLDSALNVLARALENNKDN
		PEIWCHYLRLFSKRGTKDEVQEMCETAVEYAPDYQSFWTFLHLESTFEEKDYVCERMLEFLMGAAKQETS
		NILSFQLLEALLFRVQLHIFTGRCQSALAILQNALKSANDGIVAEYLKTSDRCLAWLAYIHLIEFNILPS
		KFYDPSNDNPSRIVNTESFVMPWQAVQDVKTNPDMLLAVFEDAVKACTDESLAVEERIEACLPLYTNMIA
		LHQLLERYEAAMELCKSLLESCPINCQLLEALVALYLQTNQHDKARAVWLTAFEKNPQNAEVFYHMCKFF
		ILQNRGDNLLPFLRKFIASFFKPGFEKYNNLDLFRYLLNIPGPIDIPSRLCKGNFDDDMFNHQVPYLWLI
		YCLCHPLQSSIKETVEAYEAALGVAMRCDIVQKIWMDYLVFANNRAAGSRNKVQEFKFFTDLVNRCLVTV
		PARYPIPFSSADYWSNYEFHNRVIFFYLSCVPKTQHSKTLERFCSVMPANSGLALRLLQHEWEESNVQIL
		KLQAKMFTYNIPTCLATWKIAIAAEIVLKGQREVHRLYQRALQKLPLCASLWKDQLLFEASEGGKTDNLR
		KLVSKCQEIGVSLNELLNLNSNKTESKNH
ZFP36L1	108	MTTTLVSATIFDLSEVLCKGNKMLNYSAPSAGGCLLDRKAVGTPAGGGFPRRHSVTLPSSKFHQNQLLSS
		LKGEPAPALSSRDSRFRDRSFSEGGERLLPTQKQPGGGQVNSSRYKTELCRPFEENGACKYGDKCQFAHG
		IHELRSLTRHPKYKTELCRTFHTIGFCPYGPRCHFIHNAEERRALAGARDLSADRPRLQHSFSFAGFPSA
		AATAAATGLLDSPTSITPPPILSADDLLGSPTLPDGTNNPFAFSSQELASLFAPSMGLPGGGSPTTFLFR
		PMSESPHMFDSPPSPQDSLSDQEGYLSSSSSSSHSGSDSPTLDNSRRLPIFSRLSISDD
ZFP36L2	109	MSTTLLSAFYDVDFLCKTEKSLANLNLNNMLDKKAVGTPVAAAPSSGFAPGFLRRHSASNLHALAHPAPS
		PGSCSPKFPGAANGSSCGSAAAGGPTSYGTLKEPSGGGGTALLNKENKFRDRSFSENGDRSQHLLHLQQQ
		QKGGGGSQINSTRYKTELCRPFEESGTCKYGEKCQFAHGFHELRSLTRHPKYKTELCRTFHTIGFCPYGP
		RCHFIHNADERRPAPSGGASGDLRAFGTRDALHLGFPREPRPKLHHSLSFSGFPSGHHQPPGGLESPLLL
		DSPTSRTPPPPSCSSASSCSSSASSCSSASAASTPSGAPTCCASAAAAAAAAALLYGTGGAEDLLAPGAPC
		AACSSASCANNAFAFGPELSSLITPLAIQTHNFAAVAAAAAYYRSQQQQQQQQQQQQGLAPPAQPPAPPSATL
		PAGAAAPPSPPFSFQLPRRLSDSPVFDAPPSPPDSLSDRDSYLSGSLSSGSLSGSESPSLDPGRRLPIFS
		RLSISDD

IV. Reprogrammable PUF Domain

[0226] The present disclosure also provides a novel PUF RNA binding domain that is capable of specifically being bound to a PUF target site, e.g., wild type TS or TS#1-#9. The novel PUF RNA binding domain is substituted or mutated from the wild type PUF RNA binding domain (i.e., SEQ ID NO: 1). In some aspects, the amino acid substitution in the PUF RBD comprises a substitution corresponding to an amino acid position in R1, R2, R3, R4, R5, R6, R7, or R8 of the wild type PUF RBD as set forth in SEQ ID NO: 1, or any combination thereof. In some aspects, the amino acid substitution in the PUF RBD as compared to the WT PUF RBD is in R1. In some aspects, the amino acid substitution in the PUF RBD as compared to the WT PUF RBD is in R2. In some aspects, the amino acid substitution in the PUF RBD as compared to the WT PUF RBD is in R3. In some aspects, the amino acid substitution in the PUF RBD as compared to the WT PUF RBD is in R4. In some aspects, the amino acid substitution in the PUF RBD as compared to the WT PUF RBD is in R5. In some aspects, the amino acid substitution in the PUF RBD is in R6. In some aspects, the amino acid substitution in the PUF RBD as compared to the WT PUF RBD is in R7. In some aspects, the amino acid substitution in the PUF RBD as compared to the WT PUF RBD is in R8. In some aspects, the amino acid substitution in the PUF RBD allows the PUF RBD to bind to the PUF TS site with greater affinity than the wild type PUF RBD as set forth in SEQ ID NO: 1. In some aspects, the amino acid substitution in the PUF RBD allows the PUF RBD to have less off-target binding than the wild type PUF RBD as set forth in SEQ ID NO: 1.

[0227] In some aspects, the PUF RBD comprises one or more sequences listed in Table 3. In some aspects, the PUF RBD comprises one or more sequences selected from SEQ ID Nos: 9-30.

[0228] In some aspects, the PUF RBD useful for the synthetic circuit comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the PUF RBD as set forth in any one of SEQ ID NOs: 9-30, wherein the PUF RBD is capable of binding to the PUF TS, wherein the PUF RBD is not SEQ ID NO: 1.

[0229] In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 2. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 3. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 4. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence

as set forth in SEQ ID NO: 5. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 6 In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 7. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 8. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 9. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 10. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 11. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 12. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 13. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 14. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 15. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 16. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 17. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 18. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 19. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 20. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 21. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 22. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 23. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 24. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 25. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 26. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 27. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 28. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence

as set forth in SEQ ID NO: 29. In some aspects, the PUF RBD useful for the synthetic circuit comprises the amino acid sequence as set forth in SEQ ID NO: 30.

VI. Therapeutic Applications and Delivery System

[0230] In some aspects, the present disclosure relates to the delivery of a synthetic circuit (*e.g.*, described herein) to cells. In some aspects, the delivery can occur *in vivo* (*e.g.*, by administering a synthetic circuit described herein to a subject) or *ex vivo* (*e.g.*, by culturing a synthetic circuit described herein with the cells *in vitro*). In some aspects, delivery of a synthetic circuit described herein can be performed using any suitable delivery system known in the art. In certain aspects, the delivery system is a vector. Accordingly, in some aspects, the present disclosure provides a vector comprising any of the synthetic circuits described herein. Suitable vectors that can be used are known in the art. *See, e.g.*, Sung *et al.*, *Biomater Res* 23(8) (2019) which is incorporated herein by reference in its entirety.

[0231] In some aspects, a synthetic circuit is delivered using a nanoparticle (*e.g.*, lipid nanoparticle or lipid like nanoparticle). Accordingly, in some aspects, the present disclosure relates to a synthetic circuit (*e.g.*, described herein) encapsulated within a nanoparticle, a composition comprising such a nanoparticle, and the use of such a nanoparticle to treat a disease or disorder in a subject in need thereof. More specifically, in some aspects, provided herein is a nanoparticle comprising (i) any of the synthetic circuits described herein and (ii) one or more types of nanoparticle components.

[0232] In some aspects of the disclosure, the synthetic circuits, and/or pharmaceutical compositions described herein (also collectively referred to herein as "**compositions**") are used to treat a disease or disorder. As is apparent from the present disclosure, any of the compositions provided herein can be used to treat a wide range of diseases or disorders. Any suitable disease or disorder whether in a therapeutic agent can be encoded by the payload sequence of a synthetic circuit provided herein. Accordingly, some aspects of the present disclosure relates to a method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject any of the compositions provided herein (*e.g.*, synthetic circuit).

[0233] In some aspects, any of the compositions described herein is administered to a subject in need thereof via a suitable route, such as intratumoral administration, intravenous administration (e.g., as a bolus or by continuous infusion over a period of time), by intramuscular, intraperitoneal, intracerebospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, inhalation, or

topical routes. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be nebulized and lyophilized powder can be nebulized after reconstitution. In some aspects, the pharmaceutical composition described herein is aerolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder. In some aspects, the pharmaceutical composition described herein is formulated for intratumoral injection. In some aspects, the pharmaceutical composition described herein is administered to a subject via a local route, for example, injected to a local site such as a tumor site or an infectious site. In some aspects, the subject is a human.

[0234] As will be apparent from the present disclosure, in some aspects, the compositions described herein are administered to a subject in an effective amount to confer a therapeutic effect, either alone or in combination with one or more other active agents. In some aspects, the compositions are administered to a subject suffering from a cancer, and the therapeutic effect comprises reduced tumor burden, reduction of cancer cells, increased immune activity, or combinations thereof. Whether the administered composition achieved the therapeutic effect can be determined using any suitable methods known in the art (*e.g.*, measuring tumor volume and/or T cell activity). Effective amounts vary, as recognized by those skilled in the art, depending on the particular condition being treated, the severity of the condition, the individual patient parameters including age, physical condition, size, gender and weight, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration and like factors within the knowledge of expertise of the health practitioner.

[0235] Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. Frequency of administration can be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of a target disease/disorder. Alternatively, sustained continuous release formulations of a composition described herein can be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[0236] In some aspects of the disclosure, the treatment is a single injection of the composition disclosed herein. In some aspects, the single injection is administered intratumorally to the subject in need thereof.

[0237] In some aspects of the disclosure, dosages for a composition described herein can be determined empirically in individuals who have been given one or more administration(s) of the composition. In some aspects, the individuals are given incremental dosages of the composition

described herein. To assess efficacy of the composition herein, an indicator of disease/disorder can be followed. For repeated administrations over several days or longer, depending on the condition, in some aspects, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved to alleviate a target disease or disorder, or symptom thereof.

[0238] In some aspects of the disclosure, the method comprises administering to a subject in need thereof one or multiple doses of a composition described herein.

[0239] As described herein, a synthetic circuit of the present disclosure is particularly useful in selectively expressing a payload in a target cell of interest. Accordingly, some aspects of the present disclosure relates to a method of inducing the selective expression of a payload in a cell, comprising contacting a population of cells with any of the compositions provided herein (*e.g.*, synthetic circuit). In some aspects, the payload is expressed in the cell when the cell meets the following condition: (i) comprises a sufficient level of a type R marker such that the type R marker is specifically recognized by the type R sensor and inducing the activation of the type R sensor, thereby, reducing or inhibiting the expression of the regulator; (ii) does not comprise a sufficient level of a type P marker such that the type P marker is not recognized by the type P sensor, allowing the type P sensor to remain in an inactive form; or (iii) both (i) and (ii). When a cell satisfies such conditions, the expression of the payload is increased in the cell as compared to a reference cell (*e.g.*, corresponding cell that does not meet any of the conditions described above). In some aspects, after the contacting, the expression of the payload in the cell is increased by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100%, as compared to the reference cell. In some aspects, after the contacting, the expression of the payload is increased in the cell by at least about 1.5-fold, at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 12.5-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, at least about 30-fold, at least about 35-fold, at least about 40-fold, at least about 45-fold, or at least about 50-fold, as compared to the reference cell.

[0240] In some aspects, the composition described herein is co-administered with at least one additional suitable therapeutic agent. In some aspects, the composition described herein and the at least one additional therapeutic agent are administered to the subject in a sequential manner, i.e., each therapeutic agent is administered at a different time. In some aspects, the composition

described herein and the at least one additional therapeutic agent are administered to the subject in a substantially simultaneous manner.

[0241] In some aspects, a therapeutic application of a synthetic circuit described herein comprises producing the encoded payload in a target cell. Accordingly, in some aspects, the present disclosure relates to a method of selectively producing a payload in a target cell. In some aspects, the method comprises contacting a target cell with any of the compositions described herein (*e.g.*, synthetic circuit) under conditions suitable for producing the encoded IL-12 protein. In some aspects, the method further comprises purifying the produced payload. In some aspects, the contacting occurs *in vivo* (*e.g.*, by administering the synthetic circuit, to a subject). In some aspects, the contacting occurs *ex vivo* (*e.g.*, by culturing cells with the synthetic circuit *in vitro*). Cells (*e.g.*, host cells) comprising the synthetic circuit are encompassed herein. Non-limiting examples of cells that can be used include immortal hybridoma cell, NS/0 myeloma cell, 293 cell, Chinese hamster ovary (CHO) cell, HeLa cell, human amniotic fluid-derived cell (CapT cell), COS cell, or combinations thereof.

VII. Kits for Use in Therapy

[0242] The present disclosure also provides kits for use in therapy. In some aspects, the kit includes one or more containers comprising a composition described herein.

[0243] In some aspects, the kit comprises instructions for use in accordance with any of the methods described herein. For example, the included instructions can comprise a description of administration of the pharmaceutical composition described herein to treat, delay the onset, or alleviate a target disease. In some aspects, the instructions comprise a description of administering the composition described herein to a subject at risk of a target disease.

[0244] In some aspects, the instructions comprise dosage information, dosing schedule, and route of administration. In some aspects, the containers are unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. In some aspects, the instructions are written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit). In some aspects, the instructions are machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk).

[0245] In some aspects, the kits described herein are in suitable packaging. In some aspects, suitable packing comprises vials, bottles, jars, flexible packaging (*e.g.*, seal Mylar or plastic bags), or combinations thereof. In some aspects, the packaging comprises packages for use in combination with a specific device such as an inhaler, nasal administration device (*e.g.*, an atomizer), or an infusion device such as a minipump. In some aspects, the kit comprises a sterile

access port (for example, the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, the container can also have a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, at least one active agent is a composition as described herein.

[0246] In some aspects, the kits further comprise additional components such as buffers and interpretive information. In some aspects, the kit comprises a container and a label or package insert(s) on or associated with the container. In some aspects, the disclosure provides articles of manufacture comprising the contents of the kits described herein.

VIII. General Techniques

[0247] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney, ed. 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Giffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; Method of Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel, et al., eds., 1987): PCR: The Polymerase Chain Reaction, (Mullis, et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty, ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanette and J.D. Capra, eds., Harwood Academic Publishers, 1995). Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. All publications cited herein (including those listed above and elsewhere in the present disclosure) are incorporated by reference in their entirety.

Examples

Example 1: Effector testing on a modRNA payload

[0248] The effectiveness of a linear non-replicating regulator sequence containing a human Pum1 (PUF) RNA binding domain (RBD) and various effector domains [i.e., cNOT7, TTP, DDX6, MCPIP1$_{PIN}$, Dis3$_{PIN}$ (isoform 2), and SMG6$_{PIN}$ (isoform 2)] to inhibit expression of a modified mRNA (modRNA) payload was assessed (*see* **FIG. 2**). The RBD of human PUF recognizes the following 8nt target RNA sequence, 5'-UGGAUGAA-3' (i.e., PUF$_{UGG}$ binding site; TS #1). The payload sequence contained either 1, 4, 8, or no PUF binding sites, which are termed target sites (TS). First, the mRNA circuitry was transfected into cells using electroporation then fluorescent cells were visualized every 2 hours for 30 hours to assess mVenus expression.

[0249] When assessed 6 hours post electroporation (EP) of cells, effectors cNOT7, TTP, and MCPIP1$_{PIN}$ downregulated payload (i.e., mVenus) expression, as shown in **FIG. 3**. Specifically, for TTP there is an uptick in payload expression after the initial downregulation. DDX6 only downregulates expression of the modRNA payload with 8X TS, but no less, and Dis3$_{PIN}$ (isoform 2), and SMG6$_{PIN}$ (isoform 2) did not downregulate payload expression, irrespective of the number of TS. Table 4b summarizes the effector results with PUF$_{UGG}$.

Table 4b.

Effector	Result	Function in cells
cNOT7	Downregulates Payload	Deadenylase
TTP	Downregulates payload, uptick in payload expression after initial downregulation	Recruits Deadenylation complex
MCPIP1$_{PIN}$	Downregulates Payload	Endonuclease domain
DDX6	Downregulates payload with 8X TS, but no less	Helicase involved in decapping and deadenylation
Dis3$_{PIN}$ (isoform 2)	No downregulation	Endonuclease domain
SMG6$_{PIN}$ (isoform 2)	No downregulation	Endonuclease domain

[0250] **FIGs. 4-5** show expression of the modRNA payload 0-30 hours post electroporation. **FIGs. 4-5** demonstrate that effectors cNOT7, TTP, and MCPIP1$_{PIN}$ downregulate payload

expression when the payload sequence contained 4x (**FIG. 4**) and 8x TS (**FIG. 5**). **FIG. 6** shows that cNOT7 downregulates payload expression slightly with 1x TS. Together, these results demonstrate that cNOT7 is the most efficient regulator of modRNA payload expression. These data support that increasing target sites in a modRNA payload sequence results in increased downregulation of payload expression by a regulator sequence containing a human PUF RBD with a cNOT7 effector domain.

[0251] All payload constructs (**FIGs. 4-6**) had unique, randomly generated 12 base pair spacer sequences before the first TS, between every TS, and after the final TS.

Example 2: Effector testing on a repRNA payload

[0252] The effectiveness of a linear non-replicating regulator sequence containing a human PUF RBD and the tested effector domains (i.e., cNOT7, TTP, DDX6, and MCPIP1$_{PIN}$) to inhibit expression of a self-amplifying replicon RNA (repRNA) payload was assessed (*see* **FIG. 7**). The RBD of human PUF recognizes the following 8nt target RNA sequence, 5'-UGGAUGAA-3' (i.e., PUF$_{UGG}$ binding site, TS #1). The payload sequence contained 1, 4, 8, or no PUF binding sites, which are termed target sites (TS).

[0253] First, the mRNA circuitry was transfected into cells using electroporation then fluorescent cells were visualized every 2 hours for 200 hours to assess mVenus reporter activity. When expression was assessed 20 hours post electroporation of cells, effectors TTP, cNOT7, MCPIP1$_{PIN}$, and DDX6 downregulated replicon payload expression when the payload sequence contained 8x TS, as shown in **FIG. 8**. **FIG. 9** shows the expression of the payload 0-200 hours post electroporation, further demonstrating that effectors cNOT7, TTP, and MCPIP1$_{PIN}$ downregulated payload expression when the payload sequence contained 8x TS. These data support that increasing target sites in a repRNA payload results in increased downregulation of payload expression by a regulator sequence containing a human PUF RBD with a cNOT7, TTP, or MCPIP1$_{PIN}$ effector domain.

[0254] Similarly, **FIG. 22** shows that TTP (C147R) and MCPIP1$_{PIN}$ were top-performing candidates in targeting replicon payload reporter constructs, with TTP outperforming MCPIPI (PIN) in targeting linear N1-methyl-pseudouridine-modified mRNA payloads in HEK293T cells that were transfected with replicon fluorescent reporter mRNA containing 8x PUF$_{UGG}$ TS. Furthermore, payload expression of the replicon fluorescent reporter was effectively reduced with an increasing dose (1000 fmols, 500 fmols, 250 fmols, 125 fmols, 75 fmols, 37.5 fmols, and 18.25 fmols) of the linear mRNA encoding PUF$_{UGG}$-TTP (C147R) regulator (**FIG. 23**).

[0255] All payload constructs (**FIGs. 8-9, 22, and 23**) had unique, randomly generated 12 base pair spacer sequences before the first TS, between every TS, and after the final TS. Further, for downregulation of repRNA payload expression, spacers are not necessary in between PUF TS but are necessary after the stop codon (*see* Example 8).

Example 3: Reprogramming PUF to degrade a payload with novel target sites

[0256] Novel human-derived, reprogrammed PUF RBDs (i.e., UGG, PUF1.1, PUF1.2, PUF1.3, PUF3.1, PUF3.2, PUF 3.3, PUF 6.1, PUF6.2, PUF6.3) that can bind to a predicted target site were generated as shown in Table 5. RNA target sequences were analyzed and the least identified sequences in the human transcriptome were selected. The mutations in the target site sequence, compared to the WT sequence, are shown in bold text. The corresponding amino acid mutations in the 8 structural repeats (R8, R7, R6, R5, R4, R3, R2, and R1) are also shown. As described previously, the C-terminal repeat R8 binds to the 5′-nucleotide residue N1.

[0257] **FIG. 10** shows the effectiveness of the following PUF RBDs: PUF_{UGG} cNOT7, PUF1.1-cNOT7, PUF1.2-cNOT7, PUF1.3-cNOT7, PUF3.1-cNOT7, PUF3.2-cNOT7, PUF3.3-cNOT7, PUF6.1-cNOT7, PUF6.2-cNOT7, and PUF6.3-cNOT7 for downregulating expression of modRNA payload sequences containing 8x TS, 6 hours after EP. All payload constructs had unique, randomly generated 12 base pair spacer sequences before the first TS, between every TS, and after the final TS.

Table 5.

PUF Name	**Target Site Sequence (3′-nucleotide residue) N 1 2 3 4 5 6 7 8**	**R8**	**R7**	**R6**	**R5**	**R4**	**R3**	**R2**	**R1**	**SEQ ID No:**
WT	U G U A U A U A	-	-	-	-	-	-	-	-	WT TS
PUF(UGG)	U G **G** A U **G** **A** A	-	-	SYE	-	-	SRE	CRQ	-	TS#1
PUF1.1	U G U A **C** **G** U **C**	-	-	-	-	GHR	SRE	-	GRR	TS#2
PUF1.2		-	-	-	-	SHR	SRE	-	SRR	
PUF1.3		-	-	-	-	SYR	SRE	-	SRR	
PUF3.1	U G U A **C** **G** **A** **C**	-	-	-	-	TFR	THE	VFQ	QFR	TS#4
PUF3.2		-	-	-	-	SHR	SRE	CRQ	SRR	
PUF3.3		-	-	-	-	SYR	SRE	CRQ	SRR	
PUF6.1	U G **G** A **A** **G** U **C**	-	-	SYE	-	-	SRE	-	QFR	TS#7
PUF6.2		-	-	SYE	-	-	SRE	-	QFR	
PUF6.3		-	-	SYE	-	-	SRE	-	SRR	

[0258] Table 6 shows the WT and additional reprogrammed PUF RBDs (i.e., PUF4.5, PUF5.2, PUF5.3, and PUF7.2) that can bind to a predicted target site. The mutations in the target site sequence, compared to the WT sequence, are shown in bold text. The corresponding amino acid mutation in the 8 structural repeats (R8, R7, R6, R5, R4, R3, R2, and R1) are also shown in bold text. As described previously, the C-terminal repeat R8 binds to the 5′-nucleotide residue N1.

Table 6.

PUF Name	Target Site Sequence (3′-nucleotide residue) N 1 2 3 4 5 6 7 8	R8	R7	R6	R5	R4	R3	R2	R1	SEQ ID No:
WT	U G U A U A U A	-	-	-	-	-	-	-	-	WT TS
PUF4.5	U G U **C C G** U **C**	-	-	-	**SY**R	**S**HR	SRE	-	**S**RR	TS#5
PUF5.2	U G U A **C G** U **G**	-	-	-	-	**SY**R	**SR**E	-	SGD	TS#6
PUF5.3		-	-	-	-	**SY**R	**SR**E	-	**SRE**	
PUF7.2	U G U **G C C** U **C**	-	-	-	**SR**E	**SY**R	**SY**R		**SR**R	TS#8

[0259] Successful reprogrammed PUFs are specific to their target sequence; however, not all predicted reprogrammed PUFs are able to bind their target sequence. The reprogrammed regulators of PUF1 and PUF3 effectively downregulate expression of their cognate 8x TS payload sequence. PUF_{UGG} has some off-target effects on PUF6 despite having two differences in nucleotides between sequences.

Example 4: Reprogrammed PUF Testing on a repRNA payload

[0260] The effectiveness of linear non-replicating regulator sequences containing reprogrammed human PUF RNA RBDs (1-8) and a cNOT7 effector domain to inhibit expression of a repRNA payload was assessed. The replicon payload sequence comprised sequences for nonstructural proteins (nsp) 1-4. Non-rep fLuciferase (P1021) was used as filler RNA, and a non-rep mCherry (P1087) was used as a transfection marker. The maximum RNA dosage was 1,926 ng.

[0261] Reprogrammed human PUF RNA RBDs (1-8) were shown to downregulate repRNA reporters with 8x TS. Payload expression was rescued in PUF4 and PUF5 constructs starting at 96 hour time point, with PUF2 and PUF7 constructs showing sustained payload downregulation (*see* **FIGs. 11A-11E**). Further, the kinetics of downregulation by reprogrammed regulators differ. Specifically, PUF2 and PUF7 constructs showed efficient downregulation of the reporter (**FIGs.**

12A-12B), whereas PUF1 and PUF4 constructs did not shown as efficient downregulation. Further, payload expression in the PUF4 construct increased after 3 days (**FIGs. 12C-12D**).

[0262] In another set of experiments, HEK293T cells were transfected with replicon fluorescent reporter mRNA containing either a positive control 8x PUF_{UGG} TS cassette, or a reprogrammed 8x PUFvarX TS cassette (i.e., PUFvar1, PUFvar2, PUFvar3, PUFvar4, or PUFvar5 as described in **Table 8**), with or without their respective cognate PUF-TTP regulator, and quantified for fluorescent expression by live-cell imaging. As shown in **FIG. 25**, PUFvarl demonstrated enhanced baseline fluorescent expression compared to PUF_{UGG}, and significant downregulation upon regulator addition. **FIG. 26** shows the effectiveness of reprogrammed PUF TS/regulator combinations at downregulating fluorescent expression.

[0263] All payload constructs had unique, randomly generated 12 base pair spacer sequences before the first TS, between every TS, and after the final TS. As previously described, for downregulation of repRNA payload expression, spacers are not necessary in between PUF TS but are necessary after the stop codon (*see* Example 8).

Table 7.

PUF Name	**Target Site Sequence (3′-nucleotide residue) N 1 2 3 4 5 6 7 8**	**Identity**
WT	U G U A U A U A	WT TS
PUF(UGG)	U G **G** A U **G A** A	TS#1
PUF1	U G U A **C G** U **C**	TS#2
PUF2	U **C** U A **C G** U **C**	TS#3
PUF3	U G U A **C G A C**	TS#4
PUF4	U G U **C C G** U**C**	TS#5
PUF5	U G U A **C G** U**G**	TS#6
PUF6	U G **G** A **A G** U**C**	TS#7
PUF7	U G U **G C C** U **C**	TS#8
PUF8	U G U A **G C** U A	TS#9

Table 8.

PUF Name	**Target Site Sequence (3′-nucleotide residue) N 1 2 3 4 5 6 7 8**	**R8**	**R7**	**R6**	**R5**	**R4**	**R3**	**R2**	**R1**	**SEQ ID No:**
WT	U G U A U A U A	-	-	-	-	-	-	-	-	WT TS
PUF(UGG)	U G **G** A U **G A** A	-	-	SYE	-	-	SRE	CRQ	-	TS#1
PUFvar1	U G U A **C G** U **C**	-	-	-	-	SHR	SRE	-	SRR	TS#2

PUFvar4	U **C** U A **C** **G** U **C**	-	SYR	-	-	SYR	SRE	-	SRR	TS#3
PUFvar3	U G U A **C** **G** **A** **C**	-	-	-	-	SHR	SRE	CRQ	SRR	TS#4
PUFvar5	U G U **G** **C** **C** U **C**	-	-	-	SRE	SYR	SYR		SRR	TS#8
PUFvar2	U G U A **G** **C** U A	-	-	-	-	TFR	THE	-	-	TS#9

Example 5: Assessment of target site placement, spacing, and number in a modRNA payload

[0264] The effects of target site placement, spacing, and number on downregulation of a modRNA payload by a sequence containing a human PUF RBD with cNOT7, TTP, or MCPIP1$_{PIN}$ effector domains was assessed (*see* **FIG. 13**). The payload sequence contained either 8x TS and spacers or 7x TS and no spacers. First, the mRNA circuitry was transfected into cells using electroporation then fluorescent cells were visualized every 2 hours for 30 hours to assess mVenus reporter activity. When assessed 6 hours post EP of cells, it was determined that full spacers between every target site (i.e., 8x TS with spacers) resulted in greater payload inhibition compared to a no-spacer construct (i.e., 7x TS no spacers), as shown in **FIG. 14.**

Example 6: Combining PUF TS and regulator with miR target sites

[0265] The following example demonstrates that regulator sequence and miR target sites within the payload sequence synergistically downregulate payload (i.e., reporter) RNA expression. Specifically, HEK293T and HUH7 cells were electroporated with a payload sequence containing 4x PUF target sites, 4x miRNA target sites, or a combination of both types of target sites (in either orientation, as shown in the upper and lower panels of **FIG. 15**). The payload sequences were repRNA with only a single spacer between the stop codon and the first target site of the array (in either orientation). The regulator sequence was a linear non-replicating RNA and contained a TTP effector domain and a PUF$_{UGG}$ RBD, which recognizes the PUF$_{UGG}$ binding site. HEK293T cells contains high levels of a miRNA, whereas, HUH7 cells only contain mid-levels of the miRNA. The schematic shown in **FIG. 15** demonstrates that there is a potentiated downregulation of the reporter (i.e., full knockdown of reporter RNA) when both regulator and miR target sites are present on the payload (reporter) sequence in HEK293T cells.

[0266] When assessed 24 hours post electroporation of cells, the addition of PUF target sites to a payload sequence containing miR target sites and regulator [low or high; for HEK293T cells, 250fmol (low) and 1000fmol (high); and for Huh7 cells, 500fmol (low) and 2000fmol (high)] enhanced downregulation when the level of the miR was high (i.e., in target cells), and the addition of a regulator (low or high) increased downregulation in off-target cell types (see **FIG. 16**). In off-

target cells (i.e., HUH7 cells), miR levels were insufficient for full knockdown of the payload (see **FIG. 16**). Further, the order of miR versus PUF target sites on a reporter RNA does not affect downregulation (see **FIG. 16**).

Example 7: Regulators with added synthetic repeats can downregulate repRNA payloads

[0267] The effects of added synthetic repeats (2 and 8) to a regulator sequence containing a human PUF RBD with a TTP effector domain on downregulation of repRNA payloads were assessed (*see* **FIGs. 17A-17B** and **18A-18B**). **FIG. 27A** is a schematic showing PUF_{UGG} with 8 repeats (8R) was modified to incorporate two additional synthetic PUF repeats, forming PUF_{UGG}-10R. **FIG. 17B** shows that regulator sequences with added synthetic (2 and 8) repeats can downregulate repRNA with 10 and 16 nt. **FIG. 18B** shows that regulator sequences with added synthetic repeats can downregulate repRNA with 8x 9nt TS, depending on nucleotide identity (cognate versus non-cognate). **FIG. 27B** similary shows that PUF_{UGG}-TTP 10R was able to significantly downregulate its cognate 10 nt target RNA, outperforming the canonical PUF_{UGG}-TTP 8R with its cognate target RNA.

Example 8: Assessment of spacers sequences in repRNA payloads

[0268] The effects of spacer sequences in repRNA payloads on downregulation of a payload sequence by a regulator sequence containing a human PUF RBD with a TTP effector domain were assessed (*see* **FIGs. 19-21**).

[0269] As shown in **FIGs. 19A-19B**, the replicon does not require spacers in between each target site (spacers throughout = ST); however, a minimum of one spacer after the stop codon (SASC) and before TS is required.

[0270] As shown in **FIG. 20**, the efficiency of downregulation of the payload sequence was shown to vary with the spacer sequence size length (12 or 20 bp) for payload sequences with 2x TS.

[0271] Additionally, the number of target sites (TS, 1-10) in repRNA payloads were tested. In these experiments, a spacer sequence was present after the stop codon (SASC). Downregulation of the payload was observed with 2-10x TS; however, 9 and 10x TS did not significantly improve beyond 8x TS (data not shown). **FIG. 21** shows the results for 2-8X TS.

Example 9: PUF-based regulator exerts precise control within a multi-input miRNA sensing circuit to ensure cell-type specific payload expression

[0272] The effectiveness of a PUF-based regulator on multi-input miRNA sensing circuit and resulting cell-type specific payload expression was assessed. Target sites on the 3' UTR of the mVenus payload facilitate the binding of either the PUF-based regulator (pink) or endogenous cell-specific miRNA (blue), enabling dual payload regulation (purple). *See* **FIG. 28A**.

[0273] Two cell models were used, in which HEK.293T cells exhibit high levels of miR-X activity, and Huh-7 cells exhibit moderate levels of miR-X activity. HEK.293T or Huh-7 cells were transfected with a replicon mVenus reporter containing miR TS specific for miR-X and 8x TS for PUF_{UGG}10nt, with or without the addition of the cognate regulator PUF_{UGG}-TTP 10R. Live-cell imaging showed that separately, the regulator and miR TS were able to reduce mVenus protein expression, with knockdown in HEK.293T but not in Huh-7, and when both components were incorporated together, mVenus expression levels approached near-zero in both HEK.293T and Huh-7 cells. (**FIG. 28B**).

[0274] Additionally, qPCR was performed with primers and probe sets targeting genomic RNA of replicon encoding the non-structural proteins (NSP1) or its subgenomic RNA encoding reporter mVenus. The results were consistent with observed protein regulation shown in **FIG. 28B**: in HEK.293T cells, inclusion of regulator results in increased degradation of replicon compared to miR TS alone (**FIG. 28C**). In Huh-7 cells, regulator induces similar levels of RNA degradation in presence and absence of miR TS (**FIG. 28D**). Payload constructs had one spacer after the stop codon.

WHAT IS CLAIMED IS:

1. A synthetic circuit comprising:

(a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain; and

(b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises a first sensor ("first type P sensor") comprising a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TS"); and

wherein upon binding of the PUF RBD to the PUF TS, the effector domain is capable of downregulating the payload sequence.

2. The synthetic circuit of claim 1, wherein the first type P sensor comprises a PUF TS comprising one or more nucleic acid sequences as set forth in 5'-UGUAUAUA -3' (WT TS), 5'-UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' - UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), or 5' -UGUAGCUA -3' (TS #9).

3. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5'-UGUAUAUA -3' (WT TS).

4. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5'- GGAUGAA - 3' (TS #1).

5. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5'-UGUACGUC -3' (TS #2).

6. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UCUACGUC -3' (TS #3).

7. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGUACGAC –3' (TS #4).

8. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGUCCGUC -3' (TS #5).

9. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGUACGUG –3' (TS #6).

10. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGGAAGUC -3' (TS #7).

11. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5' - UGUGCCUC -3' (TS #8).

12. The synthetic circuit of claim 1 or 2, wherein the first type P sensor comprises the nucleic acid sequence as set forth in 5' -UGUAGCUA -3' (TS #9).

13. The synthetic circuit of any one of claims 1 to 12, wherein the first type P sensor comprises at least 8 nucleotides in length.

14. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least one first type P sensor, at least two first type P sensors, at least three first type P sensors, at least four first type P sensors, at least five first type P sensors, at least six first type P sensors, at least seven first type P sensors, at least eight first type P sensors, at least nine first type P sensors, or at least ten first type P sensors.

15. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least two first type P sensors.

16. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least three first type P sensors.

17. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least four first type P sensors.

18. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least five first type P sensors.

19. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least six first type P sensors.

20. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least seven first type P sensors.

21. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least eight first type P sensors.

22. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least nine first type P sensors.

23. The synthetic circuit of any one of claims 1 to 13, wherein the payload sequence comprises at least ten first type P sensors.

24. The synthetic circuit of any one of claims 1 to 23, wherein the PUF RBD comprises one or more amino acid substitutions compared to the wild type PUF RBD as set forth in SEQ ID NO: 1.

25. The synthetic circuit of any one of claims 1 to 23, wherein the PUF RBD comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the wild type PUF RBD as set forth in SEQ ID NO: 1, wherein the PUF RBD is capable of binding to the PUF TS.

26. The synthetic circuit of claim 24 or 25, wherein the amino acid substitution in the PUF RBD comprises a substitution corresponding to an amino acid position in R1, R2, R3, R4, R5, R6, R7, or R8 of the wild type PUF RBD as set forth in SEQ ID NO: 1, or any combination thereof.

27. The synthetic circuit of any one of claims 1 to 26, wherein the PUF RBD comprises one or more sequences listed in Table 3.

28. The synthetic circuit of any one of claims 25 to 27, wherein the PUF RBD having the amino acid substitution is capable of binding to the PUF TS site with greater affinity than the wild type PUF RBD as set forth in SEQ ID NO: 1.

29. The synthetic circuit of any one of claims 25 to 27, wherein the PUF RBD having the amino acid substitution has less off-target binding than the wild type PUF RBD as set forth in SEQ ID NO: 1.

30. The synthetic circuit of any one of claims 1 to 29, wherein the effector domain comprises a degradation domain, a translation inhibition domain, a protein recruiting domain, or any combination thereof.

31. The synthetic circuit of claim 30, wherein the effector domain comprises a degradation domain.

32. The synthetic circuit of claim 30 or 31, wherein the degradation domain is derived from one or more sequences listed in Table 4a.

33. The synthetic circuit of claim 32, wherein the degradation domain comprises one or more sequences listed in Table 4a.

34. The synthetic circuit of any one of claims 1 to 33, wherein the effector domain comprises a degradation domain that is derived from cNOT7, TTP, MCPIP1PIN, DDX6, Dis3PIN, SMG6PIN, or any combination thereof.

35. The synthetic circuit of any one of claims 1 to 34, wherein the degradation domain is derived from cNOT7.

36. The synthetic circuit of claim 35, wherein the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at

least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 34.

37. The synthetic circuit of any one of claims 1 to 33, wherein the degradation domain is derived from TTP.

38. The synthetic circuit of any one of claims 37, wherein the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 36.

39. The synthetic circuit of any one of claims 1 to 33, wherein the degradation domain is derived from MCPIP1PIN.

40. The synthetic circuit of any one of claims 39, wherein the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 35.

41. The synthetic circuit of any one of claims 1 to 33, wherein the degradation domain is derived from DDX6.

42. The synthetic circuit of any one of claims 41, wherein the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 31.

43. The synthetic circuit of any one of claims 1 to 33, wherein the degradation domain is derived from Dis3PIN.

44. The synthetic circuit of any one of claims 43, wherein the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 32.

45. The synthetic circuit of any one of claims 1 to 33, wherein the degradation domain is derived from SMG6PIN.

46. The synthetic circuit of any one of claims 45, wherein the effector domain comprises an amino acid sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% or about 100% sequence identity to the sequence set forth in SEQ ID NO: 33.

47. The synthetic circuit of any one of claims 1 to 46, wherein the payload sequence comprises a spacer sequence (type P spacer).

48. The synthetic circuit of claim 47, wherein the type P spacer sequence is located within the first type P sensor.

49. The synthetic circuit of claim 47, wherein the type P spacer sequence is located at the 5' of the first type P sensor.

50. The synthetic circuit of claim 47, wherein the type P spacer sequence is located at the 3' of the first type P sensor.

51. The synthetic circuit of any one of claims 1 to 50, wherein the regulator sequence is human-derived.

52. The synthetic circuit of any one of claims 1 to 51, wherein the payload sequence comprises a therapeutic protein.

53. The synthetic circuit of any one of claims 1 to 52, wherein the payload sequence comprises a plurality of first type P sensor.

54. The synthetic circuit of any one of claims 1 to 52, wherein the payload sequence comprises two first type P sensors, three first type P sensors, four first type P sensors, five first type P sensors, six first type P sensors, seven first type P sensors, eight first type P sensors, nine first type P sensors, or ten or more first type P sensors.

55. The synthetic circuit of claim 54, wherein each of the first type P sensors is the same.

56. The synthetic circuit of claim 54, wherein one or more of the first type P sensors are different.

57. The synthetic circuit of any one of claims 47 to 56, wherein the payload sequence comprises a plurality of the type P spacer.

58. The synthetic circuit of any one of claims 47 to 57, wherein the type P spacer is located before the first type P sensor, between two first type P sensors, or after the final first type P sensor.

59. The synthetic circuit of any one of claims 47 to 57, wherein the type P spacer is located between every first type P sensor.

60. The synthetic circuit of any one of claims 1 to 59, wherein the payload sequence comprises a replicon RNA.

61. The synthetic circuit of any one of claims 1 to 59, wherein the payload sequence comprises a modified RNA.

62. The synthetic circuit of any one of claims 1 to 59, wherein the payload sequence comprises a circular RNA.

63. The synthetic circuit of claim 1 to 62, wherein the regulator sequence comprises a linear non-replicating RNA or a circular RNA.

64. The synthetic circuit of any one of claims 1 to 63, wherein the payload sequence comprises an additional sensor that is capable of specifically recognizing a marker (second type P sensor).

65. The synthetic circuit of any one of claims 1 to 64, wherein the regulator sequence comprises a sensor that is capable of specifically recognizing a marker (type R sensor).

66. The synthetic circuit of claim 65, wherein the regulator, the marker recognized by the second type P sensor (second type P marker), and/or the marker recognized by the type R sensor (type R marker) are not the same.

67. A synthetic circuit of any one of claims 1 to 66, wherein when the payload sequence and the regulator sequence are present in a target cell, the payload is expressed in the target cell for a first expression and the regulator is expressed in the target cell for a second expression, and wherein the first expression is greater than the second expression.

68. The synthetic circuit of claim 66 or 67, wherein the recognition of the type P marker by the second type P sensor inhibits the expression of the payload.

69. The synthetic circuit of any one of claims 66 or 67, wherein the recognition of the type R marker by the type R sensor inhibits the expression of the regulator.

70. The synthetic circuit of any one of claims 66 to 69, wherein: (a) a target cell does not express sufficient levels of the type P marker to turn on the second type P sensor, and (b) the target cell expresses sufficient levels of the type R marker to turn on the type R sensor.

71. The synthetic circuit of any one of claims 66 to 70, wherein: (a) a non-target cell expresses sufficient levels of the type P marker to turn on the second type P sensor, (b) the non-target cell does not express sufficient levels of the type R marker to turn on the type R sensor, or (c) both (a) and (b).

72. The synthetic circuit of any one of claims 1 to 71, wherein the type P marker, type R marker, or both comprise a microRNA, a protein, a metabolite, or combinations thereof.

73. The synthetic circuit of any one of claims 66 to 72, wherein the regulator sequence comprises a plurality of the type R sensor.

74. The synthetic circuit of claim 73, wherein the plurality of the type R sensor comprises two type R sensors, three type R sensors, four type R sensors, five type R sensors, six type R sensors, seven type R sensors, or eight or more type R sensors.

75. The synthetic circuit of claim 73 or 74, wherein each of the type R sensors is the same.

76. The synthetic circuit of claim 73 or 74, wherein one or more of the type R sensors are different.

77. The synthetic circuit of any one of claims 1 to 76, wherein the regulator sequence comprises a spacer sequence (type R spacer).

78. The synthetic circuit of claim 77, wherein the regulator sequence comprises a plurality of type R spacer.

79. The synthetic circuit of claim 77 or 78, wherein each of the type R spacer is the same.

80. The synthetic circuit of claim 77 or 78, wherein one or more of the type R spacers are different.

81. The synthetic circuit of any one of claims 73 to 80, which comprises the plurality of the type R sensor, wherein two or more of the type R sensors are separated by a type R spacer.

82. The synthetic circuit of claim 81, wherein each of the type R sensors are separated by a type R spacer.

83. The synthetic circuit of any one of claims 77 to 82, wherein at least one type R spacer is upstream of at least one type R sensor.

84. The synthetic circuit of any one of claims 77 to 82, wherein the type R spacer is between about 1 to about 50 nucleotides in length.

85. A synthetic circuit comprising:

(a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain which comprises a cNOT7 domain; and

(b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises four to eight first type P sensors, each of which comprises a target site (“TS”) that is capable of being specifically bound by the PUF domain ("PUF TSs"), wherein the TS comprises one or more nucleic acid sequences as set forth in 5’-UGUAUAUA -3’ (WT TS), 5’-UGGAUGAA - 3’ (TS #1), 5’- UGUACGUC -3’ (TS #2), 5’ - UCUACGUC -3’ (TS #3), 5’ -

UGUACGAC – 3’ (TS #4), 5’ - UGUCCGUC -3’ (TS #5), 5’ - UGUACGUG – 3’ (TS #6), 5’ - UGGAAGUC -3’ (TS #7), 5’ - UGUGCCUC -3’ (TS #8), or 5’ -UGUAGCUA -3’ (TS #9), wherein each of the TSs is linked to each other by a type P spacer; and

wherein upon binding of the PUF RBD to the PUF TSs, the effector domain is capable of downregulating the payload sequence.

86. A synthetic circuit comprising:

(a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain which comprises a TTP domain; and

(b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises four to eight first type P sensors, each of which comprises a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TSs"), wherein the TS comprises one or more nucleic acids selected from 5’-UGUAUAUA -3’ (WT TS), 5’-UGGAUGAA - 3’ (TS #1), 5’- UGUACGUC -3’ (TS #2), 5’ - UCUACGUC -3’ (TS #3), 5’ - UGUACGAC – 3’ (TS #4), 5’ - UGUCCGUC -3’ (TS #5), 5’ - UGUACGUG – 3’ (TS #6), 5’ - UGGAAGUC -3’ (TS #7), 5’ - UGUGCCUC -3’ (TS #8), 5’ -UGUAGCUA -3’ (TS #9), wherein each of the TSs is linked to each other by a type P spacer; and

wherein upon binding of the PUF RBD to the PUF TSs, the effector domain is capable of downregulating the payload sequence.

87. A synthetic circuit comprising:

(a) a first nucleotide sequence encoding a regulator ("regulator sequence"), wherein the regulator sequence comprises a nucleotide sequence encoding an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD") and a nucleotide sequence encoding an effector domain which comprises a $MCPIP1_{PIN}$ domain; and

(b) a second nucleotide sequence encoding a payload ("payload sequence") wherein the payload sequence comprises four to eight first type P sensors, each of which comprises a target site (TS) that is capable of being specifically bound by the PUF domain ("PUF TSs"), wherein the TS comprises one or more nucleic acids selected from 5’-UGUAUAUA -3’ (WT TS), 5’-UGGAUGAA - 3’ (TS #1), 5’- UGUACGUC -3’ (TS #2), 5’ - UCUACGUC -3’ (TS #3), 5’ - UGUACGAC – 3’ (TS #4), 5’ - UGUCCGUC -3’ (TS #5), 5’ - UGUACGUG – 3’ (TS #6), 5’ - UGGAAGUC -3’ (TS #7), 5’ - UGUGCCUC -3’ (TS #8), 5’ -UGUAGCUA -3’ (TS #9), wherein each of the TSs is linked to each other by a type P spacer; and

wherein upon binding of the PUF RBD to the PUF TSs, the effector domain is capable of downregulating the payload sequence.

88. A nucleotide sequence comprising a target site ("PUF TS"), wherein the TS is capable of being specifically bound by an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD"), wherein the nucleotide sequence comprises one or more nucleic acid sequences as set forth in 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3' (TS #1), 5'- UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), or 5' -UGUAGCUA -3' (TS #9), or any combination thereof.

89. A nucleotide sequence comprising a target site ("PUF TS") that is bound by an RNA binding domain (RBD) of a human Pum1 protein ("PUF RBD"), wherein the nucleotide sequence comprises at least two sites, at least three sites, at least four sites, at least five sites, at least six sites, at least seven sites, at least eight sites, at least nine sites, or at least ten sites, and wherein at least one of the sites comprises 5'-UGUAUAUA -3' (WT TS), 5'- UGGAUGAA - 3' (TS #1), 5'-UGUACGUC -3' (TS #2), 5' - UCUACGUC -3' (TS #3), 5' - UGUACGAC – 3' (TS #4), 5' - UGUCCGUC -3' (TS #5), 5' - UGUACGUG – 3' (TS #6), 5' -UGGAAGUC -3' (TS #7), 5' - UGUGCCUC -3' (TS #8), 5' -UGUAGCUA -3' (TS #9), or any combination thereof.

90. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5'-UGUACGUC -3' (TS #2).

91. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UCUACGUC -3' (TS #3).

92. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUACGAC – 3' (TS #4).

93. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUCCGUC -3' (TS #5).

94. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUACGUG – 3' (TS #6).

95. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGGAAGUC -3' (TS #7).

96. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUGCCUC -3' (TS #8).

97. A nucleotide sequence comprising two, three, four, five, six, seven, or eight PUF target sites, wherein each of PUF TSs comprises the nucleic acid sequence as set forth in 5' - UGUAGCUA -3' (TS #9).

98. The nucleotide sequence of any one of claims 77 to 97, wherein a spacer sequence is located before the first PUF TS, between every PUF TS, and after the final PUF TS.

99. The nucleotide sequence of claim 98, wherein a spacer sequence comprises at least about 5 bp, at least about 6 bp, at least about 7 bp, at least about 8 bp, at least about 9 bp, at least about 10 bp, at least about 11 bp, at least about 12 bp, at least about 13 bp, at least about 14 bp, or at least about 15 bp.

100. The nucleotide sequence of claim 99, wherein the spacer sequence comprises between 5 bp and 15 bp, 6 bp and 15 bp, 7 bp and 15 bp, 8 bp and 15 bp, 9 bp and 15 bp, 10 bp and 15 bp, 11 bp and 15 bp, 12 bp and 15 bp, 13 bp and 15 bp, 14 bp and 15 bp, 5bp and 14 bp, 6 bp and 14 bp, 7 bp and 14 bp, 8 bp and 14 bp, 9 bp and 14 bp, 10 bp and 14 bp, 11 bp and 14 bp, 12 bp and 14 bp, 13 bp and 14 bp, 5 bp and 13 bp, 6 bp and 13 bp, 7 bp and 13 bp, 8 bp and 13 bp, 9 bp and 13 bp, 10 bp and 13 bp, 11 bp and 13 bp, 12 bp and 13 bp, 5 bp and 12 bp, 6 bp and 12 bp, 7 bp and 12 bp, 8 bp and 12 bp, 9 bp and 12 bp, 10 bp and 12 bp, 11 bp and 12 bp, 5 bp and 11 bp, 6 bp and 11 bp, 7 bp and 11 bp, 8 bp and 11 bp, 9 bp and 11 bp, 10 bp and 11 bp, 5 bp and 10 bp, 6 bp and 10 bp, 7 bp and 10 bp, 8 bp and 10 bp, 9 bp and 10 bp, 5 bp and 9 bp, 6 bp and 9 bp, 7 bp and 9 bp, 8 bp and 9 bp, 5 bp and 8 bp, 6 bp and 8 bp, 7 bp and 8 bp, 5 bp and 7 bp, 6 bp and 7 bp, or 5bp and 6bp.

101. The nucleotide sequence of any one of claims 88 to 100, wherein the spacer sequence comprises the nucleic acid sequence set forth in SEQ ID NO: 110 (GAACGGGTTTGA).

102. A method of improving binding of a PUF RBD to a PUF TS in the synthetic circuit of any one of claims 1 to 87, comprising adding a spacer sequence in the nucleotide sequence of any one of claims 88 to 101, wherein the spacer sequence improves binding of the PUF RBD to the PUF TS.

103. A method of improving downregulation of a payload sequence in the synthetic circuit of any one of claims 1 to 87, comprising adding a spacer sequence in the nucleotide sequence of any one of claims 88 to 101, wherein the spacer sequence to improve downregulation of the payload sequence.

104. A reprogrammed human Pum1 (PUF) RNA binding domain (RBD) protein ("PUF RBD"), wherein there are one or more amino acid substitutions in the wild type PUF domain, and wherein the PUF RBD is capable of binding a target site ("PUF TS"), wherein the reprogrammed PUF RBD comprises the amino acid sequence as set forth in any one of SEQ ID NO: 2 - SEQ ID NO: 30.

105. The reprogrammed PUF domain protein of claim 104, which binds to the PUF TS with greater affinity than the wild type PUF domain.

106. The reprogrammed PUF domain protein of claim 104 or 105, which binds to the PUF TS at least about 1.5 fold, at least 2 fold, at least 2.5 fold, at least 3 fold, at least 3.5 fold, at least 4 fold, at least 4.5 fold, at least 5 fold, at least 5.5 fold, or at least 6 fold higher than the wild type PUF RBD domain as set forth in SEQ ID NO: 1.

107. The reprogrammed PUF domain of claim 104, which has reduced off-target binding than the wild type PUF RBD.

108. A pharmaceutical composition comprising the synthetic circuit of any one of claims 1 to 87 and a pharmaceutically acceptable carrier.

109. The pharmaceutical composition of claim 108, which is formulated for intratumoral, intrathecal, intramuscular, intravenous, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

110. A method of treating a disease or disorder in a subject in need thereof comprising administering the synthetic circuit of any one of claims 1-87 or the pharmaceutical composition of any one claims 108-109 to the subject.