

COMPOSITIONS AND METHODS FOR MODULATING EXPRESSION OF GENES**CROSS-REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of U.S. Provisional Application No. 63/213,829,
5 filed June 23, 2021, which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing.

BACKGROUND

10 [0003] Numerous human diseases and disorders are caused by combinations of higher and/or lower expression levels of certain proteins compared to the expression levels of these proteins in humans without the disease or disorder. RNA therapeutics that can selectively increase the expression of a target protein and decrease the expression of another, different target protein,
15 with a single construct, may have a therapeutic effect. However, exogenous RNAs can trigger undesirable innate immune response. Accordingly, there is a need to develop RNA therapeutics with reduced immunogenicity to avoid unwanted innate immune activation.

BRIEF SUMMARY

20 [0004] Provided herein are compositions and methods for simultaneously modulating expression of two or three or more proteins using one recombinant polynucleic acid or RNA construct. For example, the compositions and methods provided herein can be used to increase expression of a first protein and decrease expression of a second protein. For example, the compositions and methods provided herein can be used to increase expression of
25 a first protein, decrease expression of a second protein and decrease expression of a third protein.

[0005] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or
30 more target RNAs, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the first RNA sequence of (i) and a corresponding second RNA sequence of (ii) with at most one of the at least two genetic elements. In some embodiments, the recombinant RNA construct comprises

one or more uridines. In some embodiments, the recombinant RNA construct does not comprise a modified uridine. In some embodiments, the nucleotide variant comprises a modified uridine. In some embodiments, the modified uridine comprises a N1-methylpseudouridine.

- 5 **[0006]** In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct does not comprise a nucleotide variant.
- 10 **[0007]** In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct does not comprise a modified uridine.
- 15 **[0008]** In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct does not comprise a N1-methylpseudouridine.
- 20 **[0009]** In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct comprises solely unmodified nucleotides or natural nucleotides.
- 25 **[0010]** In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct comprises uridines, wherein (a) all uridines comprised by the recombinant RNA constructs are unmodified or natural
- 30 nucleotide(s); or (b) at least one of the uridines comprised by the recombinant RNA constructs is an unmodified uridine.
- [0011]** In some aspects, provided herein, is a composition for use in modulating the expression of two or more genes in a cell. In some aspects, provided herein, is a pharmaceutical composition comprising a therapeutically effective amount of any one of the

compositions described herein and a pharmaceutically acceptable excipient. In some aspects, provided herein, is a vector comprising a recombinant polynucleic acid construct encoding any one of the compositions described herein. In some aspects, provided herein, is a cell comprising any one of the compositions described herein or any one of the vectors described
5 herein.

[0012] In some aspects, provided herein, is a method of simultaneously expressing an siRNA and an mRNA from a single RNA transcript in a cell, comprising introducing into the cell any one of the compositions described herein, or any one of the vectors described herein.

[0013] In some aspects, provided herein, is a method of treating a disease or condition
10 comprising administering to a subject in need thereof any one of the compositions described herein or any one of the pharmaceutical compositions described herein.

[0014] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to an
15 Interleukin-8 (IL-8) mRNA, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IGF-1 of (i) and at most one of the at least two siRNAs capable of binding to the IL-8 mRNA of (ii).

[0015] In some aspects, provided herein, is a composition comprising recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a
20 Interleukin-1 beta (IL-1 beta) mRNA, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the
25 mRNA encoding IGF-1 of (i) and at most one of the at least two siRNAs capable of binding to the IL-1beta mRNA of (ii).

[0016] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two
30 small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IL-4 of (i)

and at most one of the at least two siRNAs capable of binding to the TNF-alpha mRNA of (ii).

[0017] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA and Interleukin 17 (IL-17), wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IL-4 of (i) and at most one of the at least two siRNAs capable of binding to the TNF-alpha mRNA and IL-17 mRNA of (ii).

[0018] In some aspects, provided herein, is a composition comprising a recombinant polynucleic acid construct comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-24, 42, 125, 97-108, 121-122, and 127-128.

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INCORPORATION BY REFERENCE

[0019] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0020] The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0021] **Figure 1** depicts a schematic representation of construct design. A polynucleic acid (e.g., DNA) construct may comprise a T7 promoter sequence upstream of the gene of interest sequence for T7 RNA polymerase binding and successful *in vitro* transcription of both the gene of interest (e.g., IGF-1 or IL-4) and siRNA in a single RNA transcript. Signal peptide of the gene of interest is highlighted in a grey box. Linkers to connect mRNA to siRNA or siRNA to siRNA are indicated with boxes with horizontal stripes or boxes with checkered stripes, respectively. T7: T7 promoter, siRNA: small interfering RNA.

- [0022] **Figure 2A** is a plot for activation of the NF- κ B pathway in HEK-Blue™ hTLR7 cells by transfection with modified or unmodified compounds (Cpd.1 to Cpd.6; 0.3 μ g/well). The X-axis indicates different construct samples used to stimulate HEK-Blue™ hTLR7 cells. R848 was used as a positive control. The IL-4 mRNA without siRNA structure was used for comparison (modified and unmodified). Untransfected samples were used as background (BG) controls. The Y-axis indicates OD₆₂₀ BG corrected to show activation level of NF- κ B pathway in HEK-Blue™ hTLR7 cells. Data represent means $\pm$ standard error of the mean of 4 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar.
- 10 [0023] **Figure 2B** is a plot for activation of the JAK-STAT and ISG3 pathway in HEK-Blue™ IFN- α/β cells by supernatant of human embryonic kidney (HEK293) cells that had been transfected with modified or unmodified compounds (Cpd.1 to Cpd.6; 0.6 μ g/well). The X-axis indicates different samples used to stimulate HEK-Blue™ IFN- α/β cells. IFN-alpha was used as a positive control. Supernatant of untransfected HEK293 cells was used as background control. The Y-axis indicates OD₆₂₀ BG corrected to show activation level of the JAK-STAT and ISG3 pathway in HEK-Blue™ IFN- α/β cells. Data represent means $\pm$ standard error of the mean of 4 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar.
- 15 [0024] **Figure 3A** is a plot for activation of the NF- κ B pathway in HEK-Blue™ hTLR7 cells by transfection with modified or unmodified compounds (Cpd.4, Cpd.6 to Cpd.9; 0.3 μ g/well). Untransfected samples were used as background (BG) controls. R848 was used as a positive control. The X-axis indicates different construct samples used to stimulate HEK-Blue™ hTLR7 cells and the Y-axis indicates OD₆₂₀ (BG corrected) to show activation level of NF- κ B pathway. Data represent means $\pm$ standard error of the mean of 4 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar. Significance (***, <0.001) was assessed by Student's t-test of Cpd.7 unmod. (1x siRNA at 5' position) and Cpd. 8 unmod. (1x siRNA at 3' position) for NF- κ B pathway activation in relation to siRNA position.
- 20 [0025] **Figure 3B** is a plot for activation of the JAK-STAT and ISG3 pathway in HEK-Blue™ IFN- α/β cells by supernatant of human embryonic kidney (HEK293) cells that had been transfected with modified or unmodified compounds (Cpd.4, Cpd.6 to Cpd.9; 0.3 μ g/well). Supernatant of untransfected HEK293 cells was used as background (BG) control. IFN-alpha was used as a positive control. The X-axis indicates different samples used to stimulate HEK-Blue™ IFN- α/β cells and the Y-axis indicates OD₆₂₀ (BG corrected) to show
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activation level of the JAK-STAT and ISG3 pathway. Data represent means $\pm$ standard error of the mean of 4 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar. Significance (***, <0.001) was assessed by Student's t-test of Cpd.7 unmod. and Cpd.8 unmod. for JAK-STAT and ISG3 pathway activation in relation to siRNA position.

[0026] Figure 4A is a plot for activation of the NF- κ B pathway in HEK-BlueTM hTLR7 cells by transfection with modified or unmodified compounds (Cpd.10 to Cpd.12; 0.45 μ g/well). Untransfected samples were used as background controls. R848 was used as a positive control. The X-axis indicates different construct samples used to stimulate HEK-BlueTM hTLR7 cells and the Y-axis indicates OD₆₂₀ (BG corrected) to show activation level of NF- κ B pathway. Data represent means $\pm$ standard error of the mean of 4 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar.

[0027] Figure 4B is a plot for activation of the JAK-STAT and ISG3 pathway in HEK-BlueTM IFN- α/β cells by supernatant of human embryonic kidney (HEK293) cells that had been transfected with modified or unmodified compounds (Cpd.10 to Cpd.12; 0.6 μ g/well). Supernatant of untransfected HEK293 cells was used as background control. IFN-alpha was used as a positive control. The X-axis indicates different samples used to stimulate HEK-BlueTM IFN- α/β cells and the Y-axis indicates OD₆₂₀ (background corrected) to show activation level of the JAK-STAT and ISG3 pathway. Data represent means $\pm$ standard error of the mean of 4 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar.

[0028] Figure 5A is a plot for activation of the NF- κ B and AP-1 signaling pathway in HEK-BlueTM hTLR3 cells by transfection with modified or unmodified compounds (Cpd.3, Cpd.13 and Cpd.14; 0.6 μ g/well). Untransfected samples were used as background (BG) controls.

Poly(I:C) HMW was used as a positive control. The X-axis indicates different construct samples used to stimulate HEK-BlueTM hTLR3 cells and the Y-axis indicates OD₆₂₀ (BG corrected) to show activation level of NF- κ B/AP1 pathway. Data represent means $\pm$ standard error of the mean of 8 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar. Significance (***, <0.001) was assessed by Student's t-test of Cpd.3 unmod vs. Cpd.13 unmod. for TLR3 mediated NF- κ B/AP1 activation in relation to presence or absence of siRNA. Significance (*, <0.05) was assessed by Student's t-test of Cpd.3 unmod vs. Cpd.14 unmod. for TLR3 mediated NF- κ B/AP1 activation in relation to presence or absence of siRNA.

[0029] Figure 5B is a plot for activation of the NF- κ B/AP-1 and IRF mediated-signaling pathway in HEK-Blue™ hTLR8 cells by transfection with modified or unmodified compounds (Cpd.3, Cpd.13 and Cpd.14; 0.6 μ g/well). Untransfected samples were used as background (BG) controls. R848 was used as a positive control. The X-axis indicates different construct samples used to stimulate HEK-Blue™ hTLR8 cells and the Y-axis indicates OD₆₂₀ (BG corrected) to show activation level of NF- κ B/AP1 and IRF pathway. Data represent means $\pm$ standard error of the mean of 8 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar. Significance (***, <0.001) was assessed by Student's t-test of Cpd.3 unmod vs. Cpd.13 unmod. for TLR8 mediated NF- κ B/AP1/IRF activation in relation to presence or absence of siRNA. Significance (*, <0.05) was assessed by Student's t-test of Cpd.3 unmod vs. Cpd.14 unmod. for TLR3 mediated NF- κ B/AP1/IRF activation in relation to presence or absence of siRNA.

[0030] Figure 6A is a plot for IL-6 expression in Human Caucasian lung carcinoma (A549) cells as an immune response to the transfection of modified or unmodified compounds post 24 hours (Cpd.3, Cpd.4, Cpd.13 and Cpd.14; 0.3 μ g/well). The X-axis indicates different construct samples used to stimulate IL-6 expression in A549 cells and the Y-axis indicates human IL-6 levels (pg/mL). Data represent means $\pm$ standard error of the mean of 8 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar. Significance (***, <0.001) was assessed by Student's t-test of Cpd.3 unmod. and Cpd. 13 unmod. for IL-6 expression as an immune response in relation to presence or absence of siRNA. Significance (***, <0.001) was assessed by Student's t-test of Cpd.4 unmod. and Cpd. 14 unmod for IL-6 expression as an immune response in relation to presence or absence of siRNA.

[0031] Figure 6B is a plot for activation of the STAT-3 pathway in HEK-Blue™ Human IL-6 Reporter cells by equivalent volume (20 μ l) of supernatant derived from Human Caucasian lung carcinoma (A549) cells that had been transfected with modified or unmodified compounds (Cpd.3, Cpd.4, Cpd.13 and Cpd.14; 0.3 μ g/well). Supernatant (20 μ l) of untransfected A549 cells was used as background (BG) control. Recombinant IL-6 (100 ng/mL) was used as a positive control. The X-axis indicates different samples used to stimulate HEK-Blue™ Human IL-6 Reporter cells and the Y-axis indicates OD₆₂₀ (BG corrected) to show activation level of STAT-3 pathway. Data represent means $\pm$ standard error of the mean of 8 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar. Significance (**, <0.01) was assessed by

Student's t-test of Cpd.3 unmod. and Cpd. 13 unmod. for STAT3 pathway activation in relation to presence or absence of siRNA. Significance (***, <0.001) was assessed by Student's t-test of Cpd.4 unmod. and Cpd. 14 unmod. for STAT3 pathway activation in relation to presence or absence of siRNA.

- 5 **[0032]** Figure 7A is a plot for IL-6 expression in Human Blood Monocytes (THP-1) cells as an immune response to the transfection of modified or unmodified compounds post 24 hours (Cpd.3, Cpd.4, Cpd.13 and Cpd.14; 0.3 µg/well). The X-axis indicates different construct samples used to stimulate IL-6 expression in THP-1 cells and the Y-axis indicates human IL-6 levels (pg/mL). Data represent means ± standard error of the mean of 8 replicates per Cpd.
- 10 Cpd. with modification are presented as black bar and unmodified Cpd. are presented as dotted bar. Significance (***, <0.001) was assessed by Student's t-test of Cpd.3 unmod. and Cpd. 13 unmod. for IL-6 expression as an immune response in relation to presence or absence of siRNA. Significance (***, <0.001) was assessed by Student's t-test of Cpd.4 unmod. and Cpd. 14 unmod for IL-6 expression as an immune response in relation to presence or absence
- 15 of siRNA.

- [0033]** Figure 7B is a plot for activation of the STAT-3 pathway in HEK-Blue™ Human IL-6 Reporter cells by equivalent volume (20 µl) of supernatant derived from Human Blood Monocytes (THP-1) cells that had been reverse transfected with modified or unmodified compounds (Cpd.3, Cpd.4, Cpd.13 and Cpd.14; 0.3 µg/well). Supernatant of untransfected
- 20 THP1 cells was used as background (BG) control. Recombinant IL-6 (100 ng/mL) was used as a positive control. The X-axis indicates different samples used to stimulate HEK-Blue™ Human IL-6 Reporter cells and the Y-axis indicates OD₆₂₀ (BG corrected) to show activation level of STAT-3 pathway. Data represent means ± standard error of the mean of 8 replicates per Cpd. Cpd. with modification are presented as black bar and unmodified Cpd. are
- 25 presented as dotted bar. Significance (***, <0.001) was assessed by Student's t-test of Cpd.3 unmod. and Cpd. 13 unmod. for STAT3 pathway activation in relation to presence or absence of siRNA. Significance (***, <0.001) was assessed by Student's t-test of Cpd.4 unmod. and Cpd. 14 unmod. for STAT3 pathway activation in relation to presence or absence of siRNA.

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DETAILED DESCRIPTION

[0034] Provided herein are compositions and methods for modulating expression of two or more genes, comprising recombinant polynucleic acid or RNA constructs comprising at least one nucleic acid sequence encoding a gene of interest and at least two nucleic acid sequences each encoding or comprising a genetic element that modulates expression of a target RNA. A

cell or cells contacted with compositions comprising recombinant polynucleic acid or RNA constructs described herein can have reduced level of innate immune activity and lower immune response and can increase the efficiency of modulating expression of two or more genes in the cell or cells.

- 5 **[0035]** Recombinant polynucleic acid or RNA construct compositions provided herein may further comprise one or more linkers. In one instance, recombinant polynucleic acid or RNA constructs may comprise nucleic acid sequences encoding or comprising at least two genetic elements that modulate expression of one or more target RNAs and one or more linkers, wherein a linker may be present between each of at least two genetic elements that modulate
- 10 expression of one or more target RNAs. In another instance, recombinant polynucleic acid or RNA constructs may comprise nucleic acid sequences encoding one or more genes of interest and one or more linkers, wherein a linker may be present between each of one or more genes of interest. In some instances, recombinant polynucleic acid or RNA constructs may comprise nucleic acid sequences encoding one or more genes of interest, nucleic acid sequences
- 15 encoding or comprising at least two genetic elements that modulate expression of one or more target RNAs, and one or more linkers, wherein a linker may be present between nucleic acid sequences encoding one or more genes of interest and nucleic acid sequences encoding or comprising at least two genetic elements that modulate expression of one or more target RNAs, between each of at least two genetic elements that modulate expression of one or more
- 20 target RNAs, and/or between each of one or more genes of interest. In some embodiments, recombinant polynucleic acid or RNA constructs described herein may comprise at least three genetic elements that modulate expression of one or more target RNAs. . In some embodiments, recombinant polynucleic acid or RNA constructs described herein may comprise at least six genetic elements that modulate expression of one or more target RNAs.
- 25 **[0036]** Also provided herein are vectors comprising recombinant polynucleic acid constructs described herein or encoding recombinant RNA constructs described herein. Provided herein are cells comprising recombinant polynucleic acid or RNA construct composition or vectors described herein. Recombinant polynucleic acid or RNA construct compositions described herein can be formulated into pharmaceutical compositions. Further provided herein are
- 30 compositions and methods to modulate expression of two or more genes in parallel.
- [0037]** Provided herein are compositions and methods for treating a disease or a condition comprising administering to a subject in need thereof compositions or pharmaceutical compositions described herein. Recombinant polynucleic acid or RNA construct compositions provided herein may comprise a first RNA sequence and a second RNA sequence. In one

example, the first RNA sequence or the second RNA sequence may comprise one or more messenger RNAs (mRNAs) and can increase the level of proteins encoded by mRNAs. In another example, the first RNA sequence or the second RNA sequence may be a genetic element that modulates expression of a target RNA. For example, the first RNA sequence or the second RNA sequence may comprise small interfering RNAs (siRNAs) capable of binding to one or more target RNAs and can downregulate the levels of protein encoded by target RNAs. For example, mRNAs and target RNAs may be of genes associated diseases and conditions described herein.

[0038] Unless otherwise defined, 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. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods, and materials are described below.

15 Definitions

[0039] Certain specific details of this description are set forth in order to provide a thorough understanding of various embodiments. However, one skilled in the art will understand that the present disclosure may be practiced without these details. In other instances, well-known structures have not been shown or described in detail to avoid unnecessarily obscuring descriptions of the embodiments. Unless the context requires otherwise, throughout the specification and claims which follow, the word “comprise” and variations thereof, such as, “comprises” and “comprising” are to be construed in an open, inclusive sense, that is, as “including, but not limited to.” Further, headings provided herein are for convenience only and do not interpret the scope or meaning of the claimed disclosure.

25 [0040] As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the content clearly dictates otherwise. It should also be noted that the term “or” is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. The terms “and/or” and “any combination thereof” and their grammatical equivalents as used herein, can be used interchangeably. These terms can convey that any combination is specifically contemplated. Solely for illustrative purposes, the following phrases “A, B, and/or C” or “A, B, C, or any combination thereof” can mean “A individually; B individually; C individually; A and B; B and C; A and C; and A, B, and C.” The term “or” can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[0041] The term “about” or “approximately” can mean within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, “about” can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. Alternatively, particularly with respect to biological systems or processes, the term can mean within an order of magnitude, within 5-fold, or within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0042] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the present disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0043] Reference in the specification to “embodiments,” “certain embodiments,” “preferred embodiments,” “specific embodiments,” “some embodiments,” “an embodiment,” “one embodiment” or “other embodiments” mean that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the present disclosures. To facilitate an understanding of the present disclosure, a number of terms and phrases are defined below.

[0044] The term “RNA” as used herein includes RNA which encodes an amino acid sequence (e.g., mRNA, etc.) as well as RNA which does not encode an amino acid sequence (e.g., siRNA, shRNA, miRNA etc.). The RNA as used herein may be a coding RNA, i.e., an RNA which encodes an amino acid sequence. Such RNA molecules are also referred to as mRNA (messenger RNA) and are single-stranded RNA molecules. The RNA as used herein may be a non-coding RNA, i.e., an RNA which does not encode an amino acid sequence or is not translated into a protein. A non-coding RNA can include, but is not limited to, a small interfering RNA (siRNA), a short or small harpin RNA (shRNA), a microRNA (miRNA), a piwi-interacting RNA (piRNA), and a long non-coding RNA (lncRNA). siRNAs as used

herein may comprise a double-stranded RNA (dsRNA) region, a hairpin structure, a loop structure, or any combinations thereof. In some embodiments, siRNAs may comprise at least one shRNA, at least one dsRNA region, or at least one loop structure. In some embodiments, siRNAs may be processed from a dsRNA or an shRNA. In some embodiments, siRNAs may be processed or cleaved by an endogenous protein, such as DICER, from an shRNA. In some embodiments, a hairpin structure or a loop structure may be cleaved or removed from an siRNA. For example, a hairpin structure or a loop structure of an shRNA may be cleaved or removed. In some embodiments, RNAs described herein may be made by synthetic, chemical, or enzymatic methodology known to one of ordinary skill in the art, made by recombinant technology known to one of ordinary skill in the art, or isolated from natural sources, or made by any combinations thereof. The RNA may comprise modified or unmodified nucleotides or mixtures thereof, e.g., the RNA may optionally comprise chemical and naturally occurring nucleoside modifications known in the art (e.g., N¹-Methylpseudouridine also referred herein as methylpseudouridine).

15 **[0045]** The terms “nucleic acid sequence,” “polynucleic acid sequence,” and “nucleotide sequence” are used herein interchangeably and have the identical meaning herein and refer to DNA or RNA. In some embodiments, a nucleic acid sequence is a polymer comprising or consisting of nucleotide monomers, which are covalently linked to each other by phosphodiester-bonds of a sugar/phosphate-backbone. The terms “nucleic acid sequence,” “polynucleic acid sequence,” and “nucleotide sequence” may encompass unmodified nucleic acid sequences, i.e., comprise unmodified nucleotides or natural nucleotides. The terms “nucleic acid sequence,” “polynucleic acid sequence,” and “nucleotide sequence” may also encompass modified nucleic acid sequences, such as base-modified, sugar-modified or backbone-modified etc., DNA or RNA.

20 **[0046]** The terms “natural nucleotide” and “canonical nucleotide” are used herein interchangeably and have the identical meaning herein and refer to the naturally occurring nucleotide bases adenine (A), guanine (G), cytosine (C), uracil (U), thymine (T).

[0047] The term “unmodified nucleotide” is used herein to refer to natural nucleotides which are not naturally modified e.g., which are not epigenetically or post-transcriptionally modified *in vivo*. Preferably the term “unmodified nucleotides” is used herein to refer to natural nucleotides which are not naturally modified e.g., which are not epigenetically or post-transcriptionally modified *in vivo* and which are not chemically modified e.g., which are not chemically modified *in vitro*.

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[0048] The term “modified nucleotide” is used herein to refer to naturally modified nucleotides such as epigenetically or post-transcriptionally modified nucleotides and to chemically modified nucleotides e.g., nucleotides which are chemically modified *in vitro*.

5 **Recombinant RNA constructs**

[0049] Provided herein are compositions comprising recombinant RNA constructs comprising at least one nucleic acid sequence encoding a gene of interest and/or at least two nucleic acid sequences each comprising a genetic element that modulate expression of a target RNA. In some instances, a genetic element that modulates the expression of one or more target RNAs, e.g., a siRNA, may comprise a nucleic acid sequence comprising a sense siRNA strand and an anti-sense siRNA strand. For example, in some instances, recombinant RNA constructs may comprise at least 1 species of a genetic element that modulates the expression of one or more target RNAs, e.g., at least 1 species of siRNA, such as a nucleic acid sequence comprising a sense strand of siRNA and a nucleic acid sequence comprising an anti-sense strand of siRNA. 1 species of a genetic element that modulates the expression of one or more target RNAs, e.g., 1 species of siRNA, as described herein, can refer to 1 species of sense strand siRNA and 1 species of anti-sense strand siRNA. In some instances, a recombinant RNA construct may comprise at least two species of genetic elements that modulate the expression of one or more target RNAs, e.g., at least two species of siRNA. For example, a recombinant RNA construct may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, or more species of genetic elements that modulate the expression of one or more target RNAs, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more species of siRNA comprising a sense strand of siRNA and an anti-sense strand of siRNA. In some embodiments, recombinant RNA constructs may comprise 1 to 20 species of genetic elements that modulate the expression of one or more target RNAs, e.g., 1 to 20 species of siRNA. In some embodiments, recombinant RNA constructs may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or at least 10 species of genetic elements that modulate the expression of one or more target RNAs, e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or at least 10 species of siRNA. In some embodiments, recombinant RNA constructs may comprise at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at most 20 species of genetic elements that modulate the expression of one or more target RNAs, e.g., at most 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at most 20 species of siRNA. In some embodiments, recombinant RNA constructs may comprise at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at most 20 species of genetic elements that modulate the expression of one

or more target RNAs, *e.g.*, at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at most 20 species of siRNA.

[0050] In some embodiments, recombinant RNA constructs may comprise between 2 genetic elements that modulate the expression of one or more target RNAs and 10 genetic elements that modulate the expression of one or more target RNAs, between 3 genetic elements that modulate the expression of one or more target RNAs and 10 genetic elements that modulate the expression of one or more target RNAs, between 4 genetic elements that modulate the expression of one or more target RNAs and 10 genetic elements that modulate the expression of one or more target RNAs, between 5 genetic elements that modulate the expression of one or more target RNAs and 10 genetic elements that modulate the expression of one or more target RNAs, between 6 genetic elements that modulate the expression of one or more target RNAs and 10 genetic elements that modulate the expression of one or more target RNAs, between 7 genetic elements that modulate the expression of one or more target RNAs and 10 genetic elements that modulate the expression of one or more target RNAs, between 9 genetic elements that modulate the expression of one or more target RNAs and 10 genetic elements that modulate the expression of one or more target RNAs, preferably between 2 genetic elements that modulate the expression of one or more target RNAs and 6 genetic elements that modulate the expression of one or more target RNAs, between 3 genetic elements that modulate the expression of one or more target RNAs and 6 genetic elements that modulate the expression of one or more target RNAs, or between 4 genetic elements that modulate the expression of one or more target RNAs and 6 genetic elements that modulate the expression of one or more target RNAs. In a preferred embodiment, recombinant RNA constructs described herein comprise at least 2 species of genetic elements that modulate the expression of one or more target RNAs. In another preferred embodiment, recombinant RNA constructs described herein comprise at least 3 species of genetic elements that modulate the expression of one or more target RNAs. In yet another preferred embodiment, recombinant RNA constructs described herein comprise at least 6 species of genetic elements that modulate the expression of one or more target RNAs. For example, recombinant RNA constructs may comprise between 2 siRNAs and 10 siRNAs, between 3 siRNAs and 10 siRNAs, between 4 siRNAs and 10 siRNAs, between 5 siRNAs and 10 siRNAs, between 6 siRNAs and 10 siRNAs, between 7 siRNAs and 10 siRNAs, between 9 siRNAs and 10 siRNAs, preferably between 2 siRNAs and 6 siRNAs, between 3 siRNAs and 6 siRNAs, or between 4 siRNAs and 6 siRNAs. In a preferred embodiment, recombinant RNA constructs described herein comprise at least 2 species of siRNAs. In another preferred embodiment, recombinant RNA

constructs described herein comprise at least 3 species of siRNAs. In yet another preferred embodiment, recombinant RNA constructs described herein comprise at least 6 species of siRNAs.

[0051] Recombinant RNA construct compositions provided herein may further comprise one or more linkers. In one instance, recombinant RNA constructs may comprise nucleic acid sequences comprising at least two genetic elements that modulate expression of one or more target RNAs and one or more linkers, wherein a linker may be present between each of at least two genetic elements that modulate expression of one or more target RNAs. In another instance, recombinant RNA constructs may comprise nucleic acid sequences encoding one or more genes of interest and one or more linkers, wherein a linker may be present between each of one or more genes of interest. In some instances, recombinant RNA constructs may comprise nucleic acid sequences encoding one or more genes of interest, nucleic acid sequences comprising at least two genetic elements that modulate expression of one or more target RNAs, and one or more linkers, wherein a linker may be present between nucleic acid sequences encoding one or more genes of interest and nucleic acid sequences comprising at least two genetic elements that modulate expression of one or more target RNAs; between each of at least two genetic elements that modulate expression of one or more target RNAs; and/or between each of one or more genes of interest.

[0052] Provided herein are compositions for modulating expression of two or more genes comprising recombinant RNA constructs comprising at least one nucleic acid sequence encoding a gene of interest and/or at least two nucleic acid sequences each comprising a genetic element that modulates expression of a target RNA. Further provided herein are compositions for treating a disease or a condition comprising recombinant RNA constructs comprising at least one nucleic acid sequence encoding a gene of interest and/or at least two nucleic acid sequences each comprising a genetic element that modulates expression of a target RNA. Recombinant RNA construct compositions provided herein may comprise a first RNA sequence and a second RNA sequence. In one example, the first RNA sequence or the second RNA sequence may comprise one or more messenger RNAs (mRNAs) and can increase the level of proteins encoded by mRNAs. In another example, the first RNA sequence or the second RNA sequence may be a genetic element that modulates expression of a target RNA. In some embodiments, the genetic element that modulates expression of a target RNA may be a small interfering RNA (siRNA) capable of binding to one or more target RNAs. For example, the first RNA sequence or the second RNA sequence may comprise siRNAs capable of binding to one or more target RNAs and can downregulate the levels of

protein encoded by target RNAs. In some instances, the genetic element that modulates expression of a target RNA does not inhibit the expression of the gene of interest. In some instances, mRNAs and target RNAs may be of genes associated diseases and conditions described herein. Also provided herein are compositions and methods to modulate expression of two or more genes in parallel using a single RNA transcript.

[0053] Further provided herein are recombinant polynucleic acid or RNA constructs comprising a gene of interest and at least two genetic elements that reduces expression of another gene, such as siRNA, wherein the gene of interest and the genetic element that reduces expression of another gene such as siRNA may be present in a sequential manner from the 5' to 3' direction, as illustrated in **Figure 1**, or from 3' to 5' direction. In one example, the gene of interest (e.g., IGF-1 or IL-4) can be present 5' to or upstream of the genetic element that reduces expression of another gene such as siRNA, and the gene of interest can be linked to siRNA by a linker (mRNA to siRNA/shRNA linker, can be also referred as a "spacer"), as illustrated in **Figure 1**. In another example, the gene of interest may be present 3' to or downstream of the genetic element that reduces expression of another gene such as siRNA, and siRNA can be linked to the gene of interest by a linker (siRNA/shRNA to mRNA linker, can be also referred as a "spacer"). Recombinant polynucleic acid or RNA constructs provided herein may comprise more than one species of siRNAs and each of more than one species of siRNAs can be linked by a linker (siRNA to siRNA or shRNA to shRNA linker). In some embodiments, the sequence of mRNA to siRNA (or siRNA to mRNA) linker and the sequence of siRNA to siRNA (or shRNA to shRNA) linker may be different. In some embodiments, the sequence of mRNA to siRNA/shRNA (or siRNA/shRNA to mRNA) linker and the sequence of siRNA to siRNA (or shRNA to shRNA) linker may be the same. Recombinant polynucleic acid or RNA constructs provided herein may comprise more than one gene of interest and each of more than one gene of interest can be linked by a linker (mRNA to mRNA linker). In some instances, a gene of interest may comprise a signal peptide sequence at the N-terminus as shown in **Figure 1**. In some instances, a gene of interest may comprise unmodified (WT) signal peptide sequence or modified signal peptide sequence. Recombinant polynucleic acid constructs (e.g., DNA constructs) provided herein may also comprise a promoter sequence for RNA polymerase binding. For example, DNA constructs may comprise a promoter sequence for DNA-dependent RNA polymerase binding to express RNA constructs described herein. As an example, T7 promoter for T7 RNA polymerase binding is shown in **Figure 1**. In some embodiments, RNA constructs described herein may not comprise a promoter sequence.

[0054] A recombinant polynucleic acid or a recombinant RNA can refer to a polynucleic acid or RNA that is not naturally occurring and is synthesized or manipulated *in vitro*. A recombinant polynucleic acid or RNA can be synthesized in a laboratory and can be prepared by using recombinant DNA or RNA technology by using enzymatic modification of DNA or RNA, such as enzymatic restriction digestion, ligation, cloning, and/or *in vitro* transcription. A recombinant polynucleic acid can be transcribed *in vitro* to produce a messenger RNA (mRNA) and recombinant mRNAs can be isolated, purified, and used for transfection into a cell. A recombinant polynucleic acid or RNA used herein can encode a protein, polypeptide, a target motif, a signal peptide, and/or a non-coding RNA such as small interfering RNA (siRNA). In some embodiments, under suitable conditions, a recombinant polynucleic acid or RNA can be incorporated into a cell and expressed within the cell.

[0055] Exogenous nucleic acids such as recombinant polynucleic acids including recombinant RNAs can induce an innate immune response when introduced into cells. Exogenous RNAs can be recognized by different types of innate immune receptors including cell surface, endosomal, and cytosolic innate immune receptors. Endosomal innate immune receptors include, but are not limited to, toll-like receptor 3 (TLR3), TLR7, and TLR8. Examples of cytosolic innate immune receptors include, but are not limited to, retinoic acid inducible gene 1 (RIG1), melanoma differentiation-associated gene 5 (MDA5), NOD-like receptor, pyrin domain containing 3 (NLRP3), and nucleotide-binding and oligomerization domain containing 2 (NOD2). TLR3, RIG1 and MDA5 can recognize double-stranded RNAs (dsRNAs), and TLR7 and TLR8 can recognize single-stranded RNAs (ssRNAs) and RNA degradation products (e.g., uridine and guanosine-uridine-rich fragments). These RNA sensors (e.g., TLR3, TLR7, TLR8, RIG1, MDA5, NOD2, etc.) can activate interferon regulatory factor (IRF)-mediated signaling and nuclear factor κ B (NF- κ B)-mediated signaling to increase production of type I interferons (IFN- α/β) and pro-inflammatory cytokines or, in case of NLRP3, can initiate inflammasome formation and processes cytokines for maturation. As such, it is of great interest and importance to develop polynucleic acid and RNA therapeutics that induces less innate immune response. Recombinant polynucleic acid or recombinant RNA compositions, as described herein, can provide means to modulate two or more genes in parallel with reduced level of innate immune activity and lower immune response.

[0056] Provided herein are compositions and methods for modulating expression of two or more genes, comprising recombinant RNA constructs comprising a first RNA sequence encoding at least one gene of interest and a second RNA sequence comprising at least two

genetic elements that modulate expression of one or more target RNAs. Recombinant RNA constructs, as described herein, comprising an RNA sequence encoding at least one gene of interest and an RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs and can be advantageous in reducing or inducing lower levels of immune response over recombinant RNA constructs comprising an RNA sequence encoding at least one gene of interest and RNA sequence comprising less than two (e.g., 0 or 1) genetic elements that modulate expression of one or more target RNAs. For example, contacting a cell with recombinant RNA constructs described herein can result in an immune response that is lower than the immune response of the cell contacted with corresponding recombinant RNA constructs comprising the first RNA sequence encoding a gene of interest and a corresponding second RNA sequence comprising at most one genetic element that modulates expression of a target RNA. In some embodiments, the corresponding recombinant RNA constructs may comprise a second RNA sequence comprising one genetic element that modulates expression of a target RNA. In some embodiments, the corresponding recombinant RNA constructs may comprise a second RNA sequence that does not comprise any genetic elements that modulate expression of a target RNA. In some embodiments, the cell or the cells are of human origin.

[0057] Immune responses, as described herein, can be measured by any methods known in the art. In one example, immune responses can be assessed by measuring secretion of cytokines, expression of DC activation markers, or the ability to act as an adjuvant for an adaptive immune response. In another example, immune responses can be measured using any kits known in the art, e.g., commercially available kits. Details of methods for measuring immune responses are described in Examples. In some embodiments, the immune response described herein is a human immune response. In some embodiments, the immune response is a human TLR7 immune response, a human TLR3 immune response, or an IFN α / β immune response, or any combination thereof. In some embodiments, the immune response described herein is a human immune response. In some embodiments, the immune response is a human TLR7 immune response, or an IFN α / β immune response, or any combinations thereof.

[0058] A human TLR7 immune response can be measured by using a human TLR7 immunogenicity assay. In some embodiments, the human TLR7 immunogenicity assay can be performed in HEK293 cells that are engineered to express human TLR7 gene and a reporter gene. In some embodiments, the reporter gene can be a secreted reporter gene. For examples, a secreted reporter gene can include, but is not limited to, secreted embryonic alkaline phosphatase (SEAP). In some embodiments, the reporter gene can be under a control of a

promoter with one or more NF- κ B and/or activator protein 1(AP1) binding sites. In some embodiments, the promoter is an IFN- β minimal promoter. In some embodiments, the human TLR7 immunogenicity assay can measure activation of NF- κ B and/or AP1.

[0059] A human TLR3 immune response can be measured by using a human TLR3

5 immunogenicity assay. In some embodiments, the human TLR3 immunogenicity assay can be performed in HEK293 cells that are engineered to express human TLR3 gene and a reporter gene. In some embodiments, the reporter gene can be a secreted reporter gene. For examples, a secreted reporter gene can include, but is not limited to, secreted embryonic alkaline phosphatase (SEAP). In some embodiments, the reporter gene can be under a control of a
10 promoter with one or more NF- κ B and/or AP1 binding sites. In some embodiments, the promoter is an IFN- β minimal promoter. In some embodiments, the human TLR 3 immunogenicity assay can measure activation of NF- κ B and/or AP1.

[0060] An IFN α/β immune response can be measured by using an IFN α/β immunogenicity assay. In some embodiments, the IFN α/β immunogenicity assay can be performed in HEK293
15 cells that are engineered to express human signal transducer and activator of transcription 2 (STAT2) gene and/or IRF9 gene and a reporter gene. In some embodiments, the reporter gene can be a secreted reporter gene. For examples, a secreted reporter gene can include, but is not limited to, secreted embryonic alkaline phosphatase (SEAP). In some embodiments, the reporter gene can be under a control of a promoter with one or more STAT2 and/or IRF9
20 binding sites. In some embodiments, the promoter is an interferon stimulated gene factor 54 (ISG54) promoter. In some embodiments, the IFN α/β immunogenicity assay can measure activation of Janus kinase (JAK)-STAT and/or ISG3.

[0061] In some embodiments, contacting the human cell with recombinant RNA constructs described herein can result in an immune response that is lower than the immune response of
25 the human cell contacted with the corresponding recombinant RNA construct comprising at most one genetic element that modulates expression of one or more target RNAs according to a human TLR7 immunogenicity assay described herein. In some embodiments, contacting the human cell with recombinant RNA constructs described herein can result in an immune response that is lower than the immune response of the human cell contacted with the
30 corresponding recombinant RNA construct comprising at most one genetic element that modulates expression of one or more target RNAs according to a human TLR3 immunogenicity assay described herein. In some embodiments, contacting the human cell with recombinant RNA constructs described herein can result in an immune response that is lower than the immune response of the human cell contacted with the corresponding

recombinant RNA construct comprising at most one genetic element that modulates expression of one or more target RNAs according to an IFN α / β immunogenicity assay described herein. In some embodiments, the immune response in the human cell contacted with the recombinant RNA construct may be at least about 1.1 fold, about 1.2 fold, about 1.3 fold, about 1.4 fold, about 1.5 fold, about 1.6 fold, about 1.7 fold, about 1.8 fold, about 1.9 fold, about 2.0 fold, about 2.1 fold, about 2.2 fold, about 2.3 fold, about 2.4 fold, about 2.5 fold, about 2.6 fold, about 2.7 fold, about 2.8 fold, about 2.9 fold, about 3.0 fold, about 3.1 fold, about 3.2 fold, about 3.3 fold, about 3.4 fold, about 3.5 fold, about 3.6 fold, about 3.7 fold, about 3.8 fold, about 3.9 fold, about 4.0 fold, about 4.1 fold, about 4.2 fold, about 4.3 fold, about 4.4 fold, about 4.5 fold, about 4.6 fold, about 4.7 fold, about 4.8 fold, about 4.9 fold, about 5.0 fold, about 10 fold, about 15 fold, about 20 fold, about 25 fold, about 30 fold, about 35 fold, about 40 fold, about 45 fold, about 50 fold, about 55 fold, about 60 fold, about 65 fold, about 70 fold, about 75 fold, about 80 fold, about 85 fold, about 90 fold, about 95 fold, about 100 fold, about 105 fold, about 110 fold, about 115 fold, about 120 fold, about 125 fold, about 130 fold, about 135 fold, about 140 fold, about 145 fold, or at least about 150 fold less than the immune response in the human cell contacted with the corresponding recombinant RNA construct comprising at most one genetic element that modulates expression of one or more target RNAs. In some embodiments, the immune response in the human cell contacted with the recombinant RNA construct may be from about 1.1 fold to about 10 fold, from about 1.5 fold to about 15 fold, from about 2.0 fold to about 20 fold, from about 2.5 fold to about 25 fold, from about 3.0 fold to about 30 fold, from about 3.5 fold to about 35 fold, from about 4.0 fold to about 40 fold, from about 4.5 fold to about 45 fold, from about 5.0 fold to about 50 fold, from about 5.5 fold to about 55 fold, from about 6.0 fold to about 60 fold, from about 6.5 fold to about 65 fold, from about 7.0 fold to about 70 fold, from about 7.5 fold to about 75 fold, from about 8.0 fold to about 80 fold, from about 8.5 fold to about 85 fold, from about 9.0 fold to about 90 fold, from about 9.5 fold to about 95 fold, from about 10 fold to about 100 fold, from about 15 fold to about 150 fold, from about 20 fold to about 200 fold, from about 25 fold to about 250 fold, from about 30 fold to about 300 fold, from about 35 fold to about 350 fold, from about 40 fold to about 400 fold, from about 45 fold to about 450 fold, or from about 50 fold to about 500 fold less than the immune response in the human cell contacted with the corresponding recombinant RNA construct comprising at most one genetic element that modulates expression of one or more target RNAs. In some embodiments, the immune response in the human cell contacted with the recombinant RNA construct described herein is reduced by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%,

45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, or at least 99.9% as compared to the immune response in the human cell contacted with the corresponding recombinant RNA construct comprising at most one genetic element that modulates expression of one or more target RNAs.

- 5 [0062] In some instances, recombinant RNA constructs described herein may not trigger any detectable immune response as measured by any methods described herein. For example, contacting a human cell with recombinant RNA constructs described herein may not result in a substantial immune response according to a immunogenicity assay described herein (e.g., human TLR3 immunogenicity assay, human TLR7 immunogenicity assay, or IFN α / β immunogenicity assay, etc.).

- 10 [0063] Provided herein are compositions comprising recombinant RNA constructs comprising a first RNA sequence and a second RNA sequence, wherein the first RNA sequence and/or the second RNA sequence may encode a gene of interest or a genetic element that modulates expression of a target RNA. In one example, the first RNA sequence or the second RNA sequence may be an mRNA encoding a gene of interest. In another example, the first RNA sequence or the second RNA sequence may be a genetic element that reduces expression of a target RNA, such as a small interfering RNA (siRNA) capable of binding to a target RNA. In some embodiments, the siRNA capable of binding to a target RNA is not a part of an intron sequence encoded by the gene of interest. In some instances, the gene of interest is expressed without RNA splicing. In some instances, the siRNA capable of binding to a target RNA is not encoded by or comprised of an intron sequence of the gene of interest. In some instances, the siRNA capable of binding to a target RNA binds to an exon of a target RNA. In some instances, the siRNA capable of binding to a target RNA specifically binds to one target RNA.

- 25 [0064] Recombinant RNA constructs provided herein may comprise multiple copies of a gene of interest, wherein each of the multiple copies of a gene of interest encodes the same protein. Also provided herein are compositions comprising recombinant RNA constructs comprising multiple genes of interest, wherein each of the multiple genes of interest encodes a different protein. In some embodiments, recombinant RNA constructs provided herein may comprise a combination of multiple copies of a gene of interest encoding the same protein and multiple genes of interest each of which encodes a different protein.

30 [0065] Recombinant RNA constructs provided herein may comprise more than one nucleic acid sequences encoding a gene of interest. For example, recombinant RNA constructs may comprise 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more nucleic acid sequences encoding

a gene of interest. In some instances, each of the two or more nucleic acid sequences may encode the same gene of interest, wherein the mRNA encoded by the same gene of interest is different from the siRNA target mRNA. In some instances, each of the two or more nucleic acid sequences may encode a different gene of interest, wherein the mRNA encoded by the different gene of interest is not a target of siRNA comprised in the same RNA construct. In some instances, recombinant RNA constructs may comprise three or more nucleic acid sequences encoding a gene of interest, wherein each of the three or more nucleic acid sequences may encode the same gene of interest or a different gene of interest, and wherein mRNAs encoded by the same or the different gene of interest are not a target of siRNA comprised in the same RNA construct. For example, recombinant RNA constructs may comprise four nucleic acid sequences encoding a gene of interest, wherein three of the four nucleic acid sequences encode the same gene of interest and one of the four nucleic acid sequences encodes a different gene of interest, and wherein mRNAs encoded by the same or different gene of interest are not a target of siRNA comprised in the same RNA construct.

[0066] Recombinant RNA constructs provided herein may comprise multiple species of siRNAs, wherein each of the multiple species of siRNAs is capable of binding to the same target RNA. In some embodiments, each of the multiple species of siRNAs may bind to the same region of the same target RNA. In some embodiments, each of the multiple species of siRNAs may bind to a different region of the same target RNA. In some embodiments, some of the multiple species of siRNAs may bind to the same target RNA and some of the multiple species of siRNAs may bind to a different region of the same target RNA. Also provided herein are recombinant RNA constructs comprising multiple species of siRNAs, wherein each of the multiple species of siRNAs is capable of binding to a different target RNA. In some embodiments, the target RNA is a noncoding RNA. In some embodiments, the target RNA is a messenger (mRNA).

[0067] Recombinant RNA constructs provided herein may comprise at least two species of siRNA targeting an RNA of a gene associated with a disease or a condition described herein. For example, recombinant RNA constructs provided herein may comprise 2-10 species of siRNA targeting the same RNA or different RNAs. In some instances, each of the 2-10 species of siRNA targeting the same RNA may comprise the same sequence, i.e., each of the 2-10 species of siRNA binds to the same region of the target RNA. In some instances, each of the 2-10 species of siRNA targeting the same RNA may comprise different sequences, i.e., each of the 2-10 species of siRNA binds to different regions of the target RNA. For instance, recombinant RNA constructs provided herein, may comprise 3 species of siRNA targeting

one RNA and each of the 3 species of siRNA comprise the same nucleic acid sequence to target the same region of the RNA. In this example, each of the 3 species of siRNA may comprise the same nucleic acid sequence to target exon 1. In another example, each of the 3 species of siRNA may comprise different nucleic acid sequence to target different regions of the RNA. In this example, one of the 3 species of siRNA may comprise a nucleic acid sequence targeting exon 1 and another one of the 3 species of siRNA may comprise a nucleic acid sequence targeting exon 2, etc. In yet another example, each of the 3 species of siRNA may comprise different nucleic acid sequence to target different RNAs. In all aspects, siRNAs in recombinant RNA constructs provided herein may not affect the expression of the gene of interest, expressed by the mRNA in the same RNA construct compositions. In some embodiments, recombinant RNA constructs provided herein may comprise 6 species of siRNA capable of binding to one or more target RNAs. In some embodiments, the target RNA is an mRNA.

[0068] Provided herein are compositions comprising recombinant RNA constructs may further comprise a linker. In some instances, a linker described herein may have a structure of **Formula (I)** $X_mCAACAAX_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4. In some instances, a linker described herein may have a structure of **Formula (II)**: X_pTCCCX_r , wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13. In one embodiment, the first RNA sequence or the second RNA sequence may comprise at least two genetic elements that modulate the expression of one or more target RNAs and the linker RNA sequence may connect each of the at least two genetic elements that modulate the expression of one or more target RNAs (e.g., siRNA to siRNA linker or shRNA to shRNA linker). In another embodiment, the first RNA sequence may encode a gene of interest and the second RNA sequence may comprise at least two genetic elements that modulate the expression of one or more target RNA, and the linker RNA sequence may connect the gene of interest and the at least two genetic elements that modulate the expression of one or more target RNAs (e.g., mRNA to siRNA linker, siRNA to mRNA, mRNA to shRNA linker, or shRNA to mRNA linker). In some embodiments, the sequence of mRNA to siRNA/shRNA (or siRNA/shRNA to mRNA) linker and the sequence of siRNA to siRNA (or shRNA to shRNA) linker may be different. In some embodiments, the sequence of mRNA to siRNA/shRNA (or siRNA/shRNA to mRNA) linker and the sequence of siRNA to siRNA (or shRNA to shRNA) linker may be the same. In some embodiments, the first RNA sequence may encode a gene of interest and the second RNA sequence may comprise at least two genetic elements that modulate the expression of one or more target RNA, and the same

RNA linker sequence may connect the gene of interest and the at least two genetic elements that modulate the expression of one or more target RNAs (e.g., mRNA to siRNA/shRNA linker or siRNA/shRNA to mRNA linker) and between each of the at least two genetic elements that modulate the expression of one or more target RNAs (e.g., siRNA/shRNA to
5 siRNA/shRNA linker).

[0069] In some embodiments, the length of a linker is from about 4 to about 50, from about 4 to about 45, or from about 4 to about 40, from about 4 to about 35, or from about 4 to about 30 nucleotides. In some embodiments, the length of a linker is from about 4 to about 27 nucleotides. In some embodiments, the length of a linker is from about 4 to about 18
10 nucleotides. For example, the length of a linker is about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45,
15 about 46, about 47, about 48, about 49, or about 50 nucleotides. In some embodiments, the length of a linker can be at most about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37,
20 about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or at most about 50 nucleotides. In some embodiments, the length of a linker is 4 nucleotides. In some embodiments, the length of a linker is 7 nucleotides. In some embodiments, the length of a linker is 11 nucleotides. In some embodiments, the length of a linker is 12 nucleotides. In some embodiments, the length of a
25 linker is 18 nucleotides.

[0070] In some instances, a linker described herein may have a structure of **Formula (I)** $X_mCAACAAX_n$, wherein X is any nucleotide; m is an integer from 1 to 12, and n is an integer from 0 to 4; and m is 1 and n is 0. In some instances, a linker described herein may comprise a sequence comprising CAACAA (SEQ ID NO: 91), TCCC (SEQ ID NO: 89), or
30 ACAACAA (SEQ ID NO: 85). In some embodiments, a linker may comprise a sequence selected from the group consisting of ATCCCTACGTACCAACAA (SEQ ID NO: 87), ACGTACCAACAA (SEQ ID NO: 88), TCCC (SEQ ID NO: 89), ACAACAATCCC (SEQ ID NO: 90), and ACAACAA (SEQ ID NO: 85). In some embodiments, a linker may comprise a sequence comprising ACAACAA (SEQ ID NO: 85),

- ATAGTGAGTCGTATTATCCC (SEQ ID NO: 92),
ATAGTGAGTCGTATTAACAACAATCCC (SEQ ID NO: 93),
ATAGTGAGTCGTATTAACAACAA (SEQ ID NO: 94),
ATAGTGAGTCGTATTAATCCCTACGTACCAACAA (SEQ ID NO: 95), or
- 5 ATAGTGAGTCGTATTAACGTACCAACAA (SEQ ID NO: 27). In some embodiments, a linker may comprise a sequence comprising ACAACAA (SEQ ID NO: 85). In some embodiments, a linker described herein may comprise a sequence selected from the group consisting of SEQ ID NOs: 27, 28, 85-95. In some embodiments, a linker described herein may not comprise a sequence comprising
- 10 TTTATCTTAGAGGCATATCCCTACGTACCAACAA (SEQ ID NO: 28). In some embodiments, a linker described herein may not comprise a sequence comprising
ATAGTGAGTCGTATTAACGTACCAACAA (SEQ ID NO: 27). In some embodiments, a linker described herein does not comprise
TTTATCTTAGAGGCATATCCCTACGTACCAACAA (SEQ ID NO: 28) or
- 15 ATAGTGAGTCGTATTAACGTACCAACAA (SEQ ID NO: 27).
- [0071]** In some instances, a tRNA linker can be used. The tRNA system is evolutionarily conserved across living organism and utilizes endogenous RNases P and Z to process multicistronic constructs (Dong et al., 2016). In some instances, tRNA linkers described herein may comprise a nucleic acid sequence comprising
- 20 AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCC
GGGTTCGATTCCCGGCTGGTGCA (SEQ ID NO: 96). In some instances, a linker comprising a nucleic acid sequence comprising
ATAGTGAGTCGTATTAACGTACCAACAA (SEQ ID NO: 27) may be used to link the first RNA sequence and the second RNA sequence. In some embodiments, a linker
- 25 comprising a nucleic acid sequence comprising
TTTATCTTAGAGGCATATCCCTACGTACCAACAA (SEQ ID NO: 28) may be used to connect each of the 1-20 or more siRNA species.
- [0072]** In some instances, linkers described herein may not form a secondary structure. For example, linkers described herein may not bind to or basepairs with a nucleic acid sequence
- 30 of recombinant RNA constructs provided herein. In some embodiments, a linker RNA sequence described herein does not form a secondary structure according to RNAfold WebServer. In some embodiments, an siRNA sequence described herein may form a secondary structure according to RNAfold WebServer.

[0073] Further provided herein are recombinant RNA construct compositions comprising 1-20 or more siRNA species, wherein each of the 1-20 or more siRNA species are connected by a linker having a structure selected from the group consisting of **Formula (I)**:

$X_m\text{CAACAAX}_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4; and **Formula (II)**: $X_p\text{TCCCX}_r$, wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13.

[0074] In some instances, recombinant RNA constructs provided herein may be cleaved. For example, recombinant RNA constructs provided herein may be cleaved endogenously after cellular uptake. In some embodiments, recombinant RNA constructs may be cleaved by an intracellular protein or an endogenous protein. In some embodiments, recombinant RNA constructs may be cleaved by DICER, e.g., an endogenous DICER. In some embodiments, recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence, wherein the linker RNA sequence links the first RNA sequence and the second RNA sequence, may be cleaved between the first RNA sequence and the second RNA sequence. In some embodiments, recombinant RNA constructs provided herein comprise a first RNA sequence, a second RNA sequence, and a linker. In this embodiment, the first RNA sequence or the second RNA sequence may comprise at least two genetic elements that modulate the expression of one or more target RNAs and recombinant RNA constructs may be cleaved between each of at least two genetic elements that modulate the expression of one or more target RNAs. In another embodiment, the first RNA sequence may encode a gene of interest and the second RNA sequence may comprise at least two genetic elements that modulate the expression of one or more target RNA, and recombinant RNA constructs may be cleaved between the gene of interest and the at least two genetic elements that modulate the expression of one or more target RNAs. In some embodiments, the first RNA sequence may encode a gene of interest and the second RNA sequence may comprise at least two genetic elements that modulate the expression of one or more target RNA, and recombinant RNA constructs may be cleaved between the gene of interest and the at least two genetic elements that modulate the expression of one or more target RNAs and/or between each of the at least two genetic elements that modulate the expression of one or more target RNAs.

[0075] In some instances, the cleavage of recombinant RNA constructs is enhanced compared to the cleavage of a corresponding RNA construct that does not comprise a linker described herein. For example, the cleavage of recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and one or more of linkers described herein is enhanced

compared to the cleavage of an RNA construct that does not comprise a linker described herein. For example, the cleavage of recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence described herein is enhanced compared to the cleavage of an RNA construct that comprises a linker that does not have a structure selected from the group consisting of **Formula (I)**: $X_mCAACAAX_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4; and **Formula (II)**: X_pTCCCX_r , wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13. For example, the cleavage of recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence described herein is enhanced compared to the cleavage of an RNA construct that comprises a linker that does not comprise a sequence comprising ACAACAA (SEQ ID NO: 85). For example, the cleavage of recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence described herein is enhanced compared to the cleavage of an RNA construct comprising a linker that forms a secondary structure.

[0076] In some instances, the expression of a gene of interest from recombinant RNA constructs provided herein is enhanced compared to the expression of a gene of interest from a corresponding recombinant RNA construct that does not comprise a linker described herein. For example, the expression of a gene of interest from recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence described herein is enhanced compared to the expression of a gene of interest from an RNA construct that does not comprise a linker described herein. For example, the expression of a gene of interest from recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence described herein is enhanced compared to the expression of a gene of interest from an RNA construct that comprises a linker that does not have a structure selected from the group consisting of **Formula (I)**: $X_mCAACAAX_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4; and **Formula (II)**: X_pTCCCX_r , wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13. For example, the expression of a gene of interest from recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence described herein is enhanced compared to the expression of a gene of interest from an RNA construct that comprises a linker that does not comprise a sequence comprising ACAACAA (SEQ ID NO: 85). For example, the expression of a gene of interest from recombinant RNA constructs comprising a first RNA sequence, a second RNA sequence, and

a linker RNA sequence described herein is enhanced compared to the expression of a gene of interest from an RNA construct comprising a linker that forms a secondary structure.

[0077] In some embodiments, the relative increase or enhancement in the expression of a gene of interest or in the cleavage of recombinant RNA constructs is at least about 1.3 fold, about 1.4 fold, about 1.5 fold, about 1.6 fold, about 1.7 fold, about 1.8 fold, about 1.9 fold, about 2 fold, about 3 fold, about 4 fold, about 5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, about 15 fold, about 17 fold, about 18 fold, about 19 fold, about 20 fold, about 21 fold, about 22 fold, or at least about 25 fold. In some embodiments, the relative increase in the expression of the gene of interest or in the cleavage of recombinant RNA constructs is from about 1.3 fold to about 3 fold, from about 1.5 fold to about 4 fold, from about 2 fold to about 5 fold, from about 2 fold to about 10 fold, from about 2 fold to about 15 fold, from about 2 fold to about 17 fold, from about 2 fold to about 18 fold, from about 2 fold to about 19 fold, from about 2 fold to about 20 fold, from about 2 fold to about 21 fold, from about 2 fold to about 22 fold, from about 2 fold to about 25 fold, from about 2 fold to about 30 fold, from about 5 fold to about 10 fold, from about 5 fold to about 15 fold, from about 5 fold to about 17 fold, from about 5 fold to about 18 fold, from about 5 fold to about 19 fold, from about 5 fold to about 20 fold, from about 5 fold to about 21 fold, from about 5 fold to about 22 fold, from about 5 fold to about 25 fold, from about 5 fold to about 30 fold, from about 10 fold to about 15 fold, from about 10 fold to about 17 fold, from about 10 fold to about 18 fold, from about 10 fold to about 19 fold, from about 10 fold to about 20 fold, from about 10 fold to about 21 fold, from about 10 fold to about 22 fold, from about 10 fold to about 25 fold, from about 10 fold to about 30 fold, from about 15 fold to about 17 fold, from about 15 fold to about 18 fold, from about 15 fold to about 19 fold, from about 15 fold to about 20 fold, from about 15 fold to about 21 fold, from about 15 fold to about 22 fold, from about 15 fold to about 25 fold, from about 15 fold to about 30 fold, from about 17 fold to about 18 fold, from about 17 fold to about 19 fold, from about 17 fold to about 20 fold, from about 17 fold to about 21 fold, from about 17 fold to about 22 fold, from about 17 fold to about 25 fold, from about 17 fold to about 30 fold, from about 18 fold to about 19 fold, from about 18 fold to about 20 fold, from about 18 fold to about 21 fold, from about 18 fold to about 22 fold, from about 18 fold to about 25 fold, from about 18 fold to about 30 fold, from about 19 fold to about 20 fold, from about 19 fold to about 21 fold, from about 19 fold to about 22 fold, from about 19 fold to about 25 fold, from about 19 fold to about 30 fold, from about 20 fold to about 21 fold, from about 20 fold to about 22 fold, from about 20 fold to about 25 fold, from about 20 fold to about 30 fold, from about 21 fold to about 22 fold,

from about 21 fold to about 25 fold, from about 21 fold to about 30 fold, from about 22 fold to about 25 fold, from about 22 fold to about 30 fold, or from about 25 fold to about 30 fold. In some embodiments, the relative increase in the expression of the gene of interest or in the cleavage of recombinant RNA constructs is about 1.3 fold, about 1.4 fold, about 1.5 fold, about 1.6 fold, about 1.7 fold, about 1.8 fold, about 1.9 fold, about 2 fold, about 3 fold, about 4 fold, about 5 fold, about 6 fold, about 7 fold, about 8 fold, about 9 fold, about 10 fold, about 15 fold, about 17 fold, about 18 fold, about 19 fold, about 20 fold, about 21 fold, about 22 fold, about 25 fold, or about 30 fold. In some embodiments, the relative increase in the expression of the gene of interest or in the cleavage of recombinant RNA constructs is at most about 2 fold, about 3 fold, about 5 fold, about 10 fold, about 15 fold, about 17 fold, about 18 fold, about 19 fold, about 20 fold, about 21 fold, about 22 fold, about 25 fold, or about 30 fold.

[0078] In some embodiments, recombinant RNA constructs provided herein may be naked RNA. In some embodiments, recombinant RNA constructs provided herein may further comprise a 5' cap, a Kozak sequence, and/or internal ribosome entry site (IRES), and/or a poly(A) tail in a particular in order to improve translation. In some instances, recombinant RNA constructs may further comprise one or more regions promoting translation known to any skilled artisan. Non-limiting examples of the 5' cap can include an anti-reverse CAP analog, Clean Cap, Cap 0, Cap 1, Cap 2, or Locked Nucleic Acid cap (LNA-cap). In some instances, 5' cap may comprise $m_2^{7,3'-O}G(5')ppp(5')G$, $m7G$, $m7G(5')G$, $m7GpppG$, or $m7GpppGm$. In some instances, recombinant RNA constructs provided herein may comprise an IRES upstream or 5' of the RNA sequence encoding for a gene of interest. In some instances, recombinant RNA constructs provided herein may comprise an IRES immediately upstream or 5' of the RNA sequence encoding for a gene of interest. In some instances, recombinant RNA constructs provided herein may comprise an IRES downstream or 3' of the RNA sequence encoding at least one genetic element that modulates expression of a target RNA, wherein the RNA sequence encoding at least one genetic element that modulates expression of a target RNA is present upstream of the RNA sequence encoding for a gene of interest.

[0079] Recombinant RNA constructs provided herein may further comprise a poly(A) tail. In some instances, the poly(A) tail comprises 1 to 220 base pairs of poly(A). For example, the poly(A) tail comprises 1, 3, 5, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 205, 210, 215, or 220 base pairs of poly(A). In some embodiments, the poly(A) tail comprises 1 to 20, 1 to 40, 1 to 60, 1 to 80, 1 to 100, 1 to 120, 1 to 140, 1 to 160,

1 to 180, 1 to 200, 1 to 220, 20 to 40, 20 to 60, 20 to 80, 20 to 100, 20 to 120, 20 to 140, 20 to 160, 20 to 180, 20 to 200, 20 to 220, 40 to 60, 40 to 80, 40 to 100, 40 to 120, 40 to 140, 40 to 160, 40 to 180, 40 to 200, 40 to 220, 60 to 80, 60 to 100, 60 to 120, 60 to 140, 60 to 160, 60 to 180, 60 to 200, 60 to 220, 80 to 100, 80 to 120, 80 to 140, 80 to 160, 80 to 180, 80 to 200, 80 to 220, 100 to 120, 100 to 140, 100 to 160, 100 to 180, 100 to 200, 100 to 220, 120 to 140, 120 to 160, 120 to 180, 120 to 200, 120 to 220, 140 to 160, 140 to 180, 140 to 200, 140 to 220, 160 to 180, 160 to 200, 160 to 220, 180 to 200, 180 to 220, or 200 to 220 base pairs of poly(A). In some embodiments, the poly(A) tail comprises 1, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, or 220 base pairs of poly(A). In some embodiments, the poly(A) tail comprises at least 1, 20, 40, 60, 80, 100, 120, 140, 160, 180, or at least 200 base pairs of poly(A). In some embodiments, the poly(A) tail comprises at most 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, or at most 220 base pairs of poly(A). In some embodiments, the poly(A) tail comprises 120 base pairs of poly(A).

[0080] Recombinant RNA constructs provided herein may further comprise a Kozak sequence. A Kozak sequence may refer to a nucleic acid sequence motif that functions as a protein translation initiation site. Kozak sequences are described at length in the literature, *e.g.*, by Kozak, M., Gene 299(1-2):1-34, incorporated herein by reference herein in its entirety. In some embodiments, the Kozak sequence described herein may comprise a sequence comprising GCCACC (SEQ ID NO: 25). In some embodiments, recombinant RNA constructs provided herein may further comprise a nuclear localization signal (NLS).

[0081] In one aspect, recombinant RNA constructs described herein may not comprise a nucleotide variant. In some instances, recombinant RNA constructs described herein may comprise one or more uridines. In some instances, recombinant RNA constructs described herein may not comprise a modified uridine. In some instances, recombinant RNA constructs described herein may not comprise one or more N1-methylpseudouridines. In some embodiments, between 99% and 1%, between 98% and 2%, between 97% and 3%, between 96% and 4%, between 95% and 2%, between 94% and 6%, between 93% and 7%, between 92% and 8%, between 91% and 9%, between 90% and 10%, between 97% and 3%, of the one or more uridines comprised in the recombinant RNA constructs are unmodified. In some embodiments, at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or at least 99.9% of one or more uridines comprised in the recombinant RNA constructs are unmodified. In some embodiments, at most 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.6%, 99.7%, 99.8%, or at least 99.9% of one or more

uridines comprised in the recombinant RNA constructs are modified. In one embodiment, recombinant RNA constructs described herein comprise solely unmodified nucleotides. For example, recombinant RNA constructs described herein comprise only natural nucleotides. For example, recombinant RNA constructs described herein comprise only canonical

5 nucleotides. In a preferred embodiment, recombinant RNA constructs described herein comprise one or more uridines, wherein all of one or more uridines are unmodified. In another aspect, recombinant RNA constructs described herein may include one or more nucleotide variants, including nonstandard nucleotide(s), non-natural nucleotide(s), nucleotide analog(s), and/or modified nucleotides. Examples of modified nucleotides include, but are not limited to

10 diaminopurine, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-

15 methylcytosine, N6-methyladenosine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid

20 methylester, 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-diaminopurine, N1-methylpseudouridine, and the like. In some cases, nucleotides may include modifications in their phosphate moieties, including modifications to a triphosphate moiety. Non-limiting examples of such modifications include phosphate chains of greater length and modifications with thiol moieties. In some embodiments, phosphate chains can

25 comprise 4, 5, 6, 7, 8, 9, 10 or more phosphate moieties. In some embodiments, thiol moieties can include but are not limited to alpha-thiotriphosphate and beta-thiotriphosphates. In some embodiments, a recombinant RNA construct described herein does not comprise 5-methylcytosine and/or N6-methyladenosine.

[0082] In some instances, recombinant RNA constructs described herein may be modified at

30 the base moiety, sugar moiety, or phosphate backbone. For example, modifications can be at one or more atoms that typically are available to form a hydrogen bond with a complementary nucleotide and/or at one or more atoms that are not typically capable of forming a hydrogen bond with a complementary nucleotide. In some embodiments, backbone modifications include, but are not limited to, a phosphorothioate, a phosphorodithioate, a

phosphoroselenoate, a phosphorodiselenoate, a phosphoroanilothioate, a phosphoraniladate, a phosphoramidate, and a phosphorodiamidate linkage. A phosphorothioate linkage substitutes a sulfur atom for a non-bridging oxygen in the phosphate backbone and delay nuclease degradation of oligonucleotides. A phosphorodiamidate linkage (N3'→P5') allows prevents
5 nuclease recognition and degradation. In some embodiments, backbone modifications include having peptide bonds instead of phosphorous in the backbone structure, or linking groups including carbamate, amides, and linear and cyclic hydrocarbon groups. For example, N-(2-aminoethyl)-glycine units may be linked by peptide bonds in a peptide nucleic acid.

Oligonucleotides with modified backbones are reviewed in Micklefield, Backbone
10 modification of nucleic acids: synthesis, structure and therapeutic applications, Curr. Med. Chem., 8 (10): 1157-79, 2001 and Lyer et al., Modified oligonucleotides-synthesis, properties and applications, Curr. Opin. Mol. Ther., 1 (3): 344-358, 1999.

[0083] Recombinant RNA constructs provided herein may comprise a combination of modified and unmodified nucleotides. In some instances, the adenosine-, guanosine-, and
15 cytidine-containing nucleotides are unmodified or partially modified. In some instances, for modified RNA constructs, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of uridine nucleotides may be modified. In some embodiments, 5% to 25% of uridine nucleotides are modified in recombinant RNA constructs. Non-limiting examples of the modified uridine nucleotides may comprise pseudouridines, N¹-methylpseudouridines, or N1-
20 methylpseudo-UTP and any modified uridine nucleotides known in the art may be utilized. In some embodiments, recombinant RNA constructs may contain a combination of modified and unmodified nucleotides, wherein 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of uridine nucleotides may comprise pseudouridines, N¹-methylpseudouridines, N1-
25 methylpseudo-UTP, or any other modified uridine nucleotide known in the art. In some embodiments, recombinant RNA constructs may contain a combination of modified and unmodified nucleotides, wherein 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% of the uridine nucleotides may comprise N¹-methylpseudouridines.

[0084] Recombinant RNA constructs provided herein may be codon-optimized. In general, codon optimization refers to a process of modifying a nucleic acid sequence for expression in
30 a host cell of interest by replacing at least one codon (*e.g.*, more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more codons) of a native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Codon usage tables are readily available, for example, at the "Codon Usage Database," and these tables can be adapted in a number of ways. Computer algorithms for

codon optimizing a particular sequence for expression in a particular host cell are also available, such as Gene Forge[®] (Aptagen, PA) and GeneOptimizer[®] (ThermoFischer, MA) which is preferred. In some embodiments, recombinant RNA constructs may not be codon-optimized.

- 5 [0085] In some instances, recombinant RNA constructs may comprise a nucleic acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 1-12 and 97-108.

RNA interference and small interfering RNA (siRNA)

- 10 [0086] RNA interference (RNAi) or RNA silencing is a process in which RNA molecules inhibit gene expression or translation, by neutralizing target mRNA molecules. RNAi process is described in Mello & Conte (2004) Nature 431, 338-342, Meister & Tuschl (2004) Nature 431, 343-349, Hannon & Rossi (2004) Nature 431, 371-378, and Fire (2007) Angew. Chem. Int. Ed. 46, 6966-6984. Briefly, in a natural process, the reaction initiates with a cleavage of
15 long double-stranded RNA (dsRNA) into small dsRNA fragments or siRNAs with a hairpin structure (i.e., shRNAs) by a dsRNA-specific endonuclease Dicer. These small dsRNA fragments or siRNAs are then integrated into RNA-induced silencing complex (RISC) and guide the RISC to the target mRNA sequence. During interference, the siRNA duplex unwinds, and the anti-sense strand remains in complex with RISC to lead RISC to the target
20 mRNA sequence to induce degradation and subsequent suppression of protein translation. Unlike commercially available synthetic siRNAs, siRNAs in the present invention can utilize endogenous Dicer and RISC pathway in the cytoplasm of a cell to get cleaved from recombinant RNA constructs (e.g., recombinant RNA constructs comprising an mRNA and at least two siRNAs) and follow the natural process detailed above, as siRNAs in the
25 recombinant RNA constructs of the present invention may comprise a hairpin loop structure. In addition, as the rest of the recombinant RNA constructs (i.e., mRNA) is left intact after cleavage of siRNAs by Dicer, the desired protein expression from the gene of interest in the recombinant RNA constructs of the present invention is attained.

- [0087] Provided herein are compositions comprising recombinant RNA constructs
30 comprising at least two nucleic acid sequence each comprising a genetic element that modulates the expression of one or more target RNAs. In some instances, the genetic element that modulates the expression of one or more target RNAs may be a siRNA capable of binding to a target RNA. In some instances, provided here in are compositions comprising recombinant RNA constructs comprising at least one nucleic acid sequence comprising a

siRNA capable of binding to a target RNA. In some instances, the target RNA is a noncoding RNA. In some instances, the target RNA is an mRNA. In some embodiments, the siRNA is capable of binding to a target mRNA in the 5' untranslated region. In some embodiments, the siRNA is capable of binding to a target mRNA in the 3' untranslated region. In some
5 embodiments, the siRNA is capable of binding to a target mRNA in an exon.

[0088] In some embodiments, recombinant RNA constructs may comprise a nucleic acid sequence comprising a sense siRNA strand. In some embodiments, recombinant RNA constructs may comprise a nucleic acid sequence comprising an anti-sense siRNA strand. In some embodiments, recombinant RNA constructs may comprise a nucleic acid sequence
10 comprising a sense siRNA strand and a nucleic acid sequence comprising an anti-sense siRNA strand. Details of siRNA comprised in the present invention are described in Cheng, et al. (2018) J. Mater. Chem. B., 6, 4638-4644, which is incorporated by reference herein.

[0089] In some instances, a genetic element that modulates the expression of one or more target RNAs, e.g., a siRNA, may comprise a nucleic acid sequence comprising a sense siRNA
15 strand and an anti-sense siRNA strand. For example, in some instances, recombinant RNA constructs may comprise at least 1 species of siRNA, i.e., a nucleic acid sequence comprising a sense strand of siRNA and a nucleic acid sequence comprising an anti-sense strand of siRNA. 1 species of siRNA, as described herein, can refer to 1 species of sense strand siRNA and 1 species of anti-sense strand siRNA. In some instances, recombinant RNA constructs
20 may comprise more than 1 species of siRNA, e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, or more species of siRNA comprising a sense strand of siRNA and an anti-sense strand of siRNA. In some embodiments, recombinant RNA constructs may comprise 1 to 20 species of siRNA. In some embodiments, recombinant RNA constructs may comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or at least 10 species of siRNA. In some embodiments, recombinant RNA constructs may comprise
25 at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at most 20 species of siRNA. In some embodiments, recombinant RNA constructs may comprise between 2 siRNAs and 10 siRNAs, between 3 siRNAs and 10 siRNAs, between 4 siRNAs and 10 siRNAs, between 5 siRNAs and 10 siRNAs, between 6 siRNAs and 10 siRNAs, between 7 siRNAs and 10 siRNAs, between 9 siRNAs and 10 siRNAs, preferably between 2 siRNAs
30 and 6 siRNAs, between 3 siRNAs and 6 siRNAs, or between 4 siRNAs and 6 siRNAs. In a preferred embodiment, recombinant RNA constructs described herein comprise at least 2 species of siRNA. In another preferred embodiment, recombinant RNA constructs described herein comprise at least 3 species of siRNA. In yet another preferred embodiment, recombinant RNA constructs described herein comprise at least 6 species of siRNA.

[0090] Provided herein are compositions of recombinant RNA constructs comprising 1-20 or more siRNA species, wherein each of the 1-20 or more siRNA species is capable of binding to a target RNA. In some embodiments, a target RNA is an mRNA or a non-coding RNA. In some instances, each of the siRNA species binds to the same target RNA. In one instance, each of the siRNA species may comprise the same sequence and bind to the same region or sequence of the same target RNA. For example, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, or more siRNA species and each of the 1, 2, 3, 4, 5, 6, or more siRNA species may comprise the same sequence targeting the same region of a target RNA, i.e., recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, or more redundant species of siRNA. In another instance, each of the siRNA species may comprise a different sequence and bind to a different region or sequence of the same target RNA. For example, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, or more siRNA species and each of the 1, 2, 3, 4, 5, 6, or more siRNA species may comprise a different sequence targeting a different region of the same target RNA. In this example, one siRNA of the 1, 2, 3, 4, 5, 6, or more siRNA species may target exon 1 and another siRNA of the 1, 2, 3, 4, 5, 6, or more siRNA species may target exon 2 of the same mRNA, etc. In some instances, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, or more siRNA species capable of binding to the same and different regions of the same target RNA. For example, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, or more siRNA species and 2 of the 1, 2, 3, 4, 5, 6, or more siRNA species may comprise the same sequence and bind to the same regions of the target RNA and 3 or more of the 1, 2, 3, 4, 5, 6, or more siRNA species may comprise a different sequence and bind to different regions of the same target RNA. In some instances, each of the siRNA species binds to a different target RNA. In some instances, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, or more siRNA species capable of binding to the same and different target RNAs. For example, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, or more siRNA species and 2 of the 1, 2, 3, 4, 5, 6, or more siRNA species may comprise a sequence capable of binding to the same or different regions of the same target RNA and 3 or more of the 1, 2, 3, 4, 5, 6, or more siRNA species may comprise a sequence capable of binding to a different target RNA. In some embodiments, a target RNA may be an mRNA and/or a non-coding RNA.

[0091] Provided herein are compositions of recombinant RNA constructs comprising 1-20 or more siRNA species, wherein each of the 1-20 or more siRNA species are connected by a linker described herein. In some instances, the linker may be a non-cleavable linker. In some instances, the linker may be a cleavable linker such as a self-cleavable linker. In some

instances, the linker may be cleaved by a protein, e.g., an intracellular or an endogenous protein. In some instances, the linker has a structure selected from the group consisting of **Formula (I)**: $X_m\text{CAACAAX}_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4; and **Formula (II)**: $X_p\text{TCCCX}_r$, wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13. In some instances, the linker may comprise a sequence comprising ACAACAA (SEQ ID NO: 85), ATCCCTACGTACCAACAA (SEQ ID NO: 87), ACGTACCAACAA (SEQ ID NO: 88), TCCC (SEQ ID NO: 89), or ACAACAATCCC (SEQ ID NO: 90). In some embodiments, the linker may comprise a sequence comprising ACAACAA (SEQ ID NO: 85), ATAGTGAGTCGTATTATCCC (SEQ ID NO: 92), ATAGTGAGTCGTATTAACAACAATCCC (SEQ ID NO: 93), ATAGTGAGTCGTATTAACAACAA (SEQ ID NO: 94), ATAGTGAGTCGTATTAATCCCTACGTACCAACAA (SEQ ID NO: 95), or ATAGTGAGTCGTATTAACGTACCAACAA (SEQ ID NO: 27). In some embodiments, the linker may comprise a sequence comprising ACAACAA (SEQ ID NO: 85). In some embodiments, the linker does not comprise a sequence comprising TTTATCTTAGAGGCATATCCCTACGTACCAACAA (SEQ ID NO: 28) or ATAGTGAGTCGTATTAACGTACCAACAA (SEQ ID NO: 27).

[0092] In some instances, the length of a linker is from about 4 to about 50, from about 4 to about 45, or from about 4 to about 40, from about 4 to about 35, or from about 4 to about 30 nucleotides. In some embodiments, the length of a linker is from about 4 to about 27 nucleotides. In some embodiments, the length of a linker is from about 4 to about 18 nucleotides. For example, the length of a linker is about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or about 50 nucleotides. In some embodiments, the length of a linker can be at most about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, or at most about 50 nucleotides. In some embodiments, the

length of a linker is 4 nucleotides. In some embodiments, the length of a linker is 7 nucleotides. In some embodiments, the length of a linker is 11 nucleotides. In some embodiments, the length of a linker is 12 nucleotides. In some embodiments, the length of a linker is 18 nucleotides.

- 5 **[0093]** In some instances, the linker may have a structure of **Formula (I)** $X_mCAACAAX_n$, wherein X is any nucleotide; m is an integer from 1 to 12, and n is an integer from 0 to 4; and m is 1 and n is 0. In some instances, the linker may comprise a sequence comprising CAACAA (SEQ ID NO: 91), TCCC (SEQ ID NO: 89), or ACAACAA (SEQ ID NO: 85). In some embodiments, the linker may comprise a sequence selected from the group consisting of
- 10 ATCCCTACGTACCAACAA (SEQ ID NO: 87), ACGTACCAACAA (SEQ ID NO: 88), TCCC (SEQ ID NO: 89), ACAACAATCCC (SEQ ID NO: 90), and ACAACAA (SEQ ID NO: 85). In some embodiments, the linker may comprise a sequence comprising ACAACAA (SEQ ID NO: 85), ATAGTGAGTCGTATTATCCC (SEQ ID NO: 92), ATAGTGAGTCGTATTAACAACAATCCC (SEQ ID NO: 93),
- 15 ATAGTGAGTCGTATTAACAACAA (SEQ ID NO: 94), ATAGTGAGTCGTATTAATCCCTACGTACCAACAA (SEQ ID NO: 95), or ATAGTGAGTCGTATTAACGTACCAACAA (SEQ ID NO: 27). In some embodiments, the linker may comprise a sequence comprising ACAACAA (SEQ ID NO: 85). In some embodiments, the linker may comprise a sequence selected from the group consisting of SEQ
- 20 ID NOs: 27, 28, 85-95.

- [0094]** In some instances, the linker may be a tRNA linker. The tRNA system is evolutionarily conserved across living organism and utilizes endogenous RNases P and Z to process multicistronic constructs (Dong et al., 2016). In some embodiments, the tRNA linker may comprise a nucleic acid sequence comprising
- 25 AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCC GGGTTCGATTCCCGGCTGGTGCA (SEQ ID NO: 96). In some embodiments, a linker comprising a nucleic acid sequence comprising TTTATCTTAGAGGCATATCCCTACGTACCAACAA (SEQ ID NO: 28) may be used to connect each of the 1-20 or more siRNA species.

- 30 **[0095]** In some instances, specific binding of an siRNA to its target mRNA results in interference with the normal function of the target mRNA, leading to modulation, *e.g.*, downregulation, of expression level, function, and/or activity of a protein encoded by the target mRNA, and there is a sufficient degree of complementarity to avoid non-specific binding of the siRNA to non-target nucleic acid sequences under conditions in which specific

binding is desired, i.e., under physiological conditions in the case of *in vivo* assays or therapeutic treatment, and under conditions in which assays are performed in the case of *in vitro* assays.

[0096] A protein as used herein can refer to molecules typically comprising one or more peptides or polypeptides. A peptide or polypeptide is typically a chain of amino acid residues, linked by peptide bonds. A peptide usually comprises between 2 and 50 amino acid residues. A polypeptide usually comprises more than 50 amino acid residues. A protein is typically folded into 3-dimensional form, which may be required for the protein to exert its biological function. A protein as used herein can include a fragment of a protein, a functional variant of a protein, and a fusion protein. A functional variant as used herein may refer to a full-length molecule, a fragment thereof, or a variant thereof. For example, a variant molecule may comprise a sequence modified by insertion, deletion, and/or substitution of one or more amino acids, in the case of protein sequence, or one or more nucleotides, in the case of nucleic acid sequence. For example, a variant molecule may comprise or encode a mutant protein, including, but not limited to, a gain-of-function or a loss-of-function mutant. A fragment may be a shorter portion of a full-length sequence of a nucleic acid molecule like DNA or RNA, or a protein. Accordingly, a fragment, typically, comprises a sequence that is identical to the corresponding stretch within the full-length sequence. In some embodiments, a fragment of a sequence may comprise at least 5% to at least 80% of a full-length nucleotide or amino acid sequence from which the fragment is derived. In some embodiments, a protein can be a mammalian protein. In some embodiments, a protein can be a human protein. In some embodiments, a protein may be a protein secreted from a cell. In some embodiments, a protein may be a protein on cell membranes. In some embodiments, a protein as referred to herein can be a protein that is secreted and acts either locally or systemically as a modulator of target cell signaling via receptors on cell surfaces, often involved in immunologic reactions or other host proteins involved in viral infection. Nucleotide and amino acid sequences of proteins useful in the context of the present invention, including proteins that are encoded by a gene of interest, are known in the art and available in the literature. For example, nucleotide and amino acid sequences of proteins useful in the context of the present invention, including proteins that are encoded by a gene of interest are available in the UniProt database.

[0097] Provided herein are compositions of recombinant RNA constructs comprising an siRNA capable of binding to a target mRNA to modulate expression of the target mRNA. In some instances, expression of the target mRNA (e.g., the level of protein encoded by the target mRNA) is downregulated by the siRNA capable of binding to the target mRNA. In

some embodiments, expression of the target mRNA is inhibited by the siRNA capable of binding to the target mRNA. Inhibition or downregulation of expression of the target mRNA, as described herein, can refer to, but is not limited to, interference with the target mRNA to interfere with translation of the protein from the target mRNA; thus, inhibition or

5 downregulation of expression of the target mRNA can refer to, but is not limited to, a decreased level of proteins expressed from the target mRNA compared to a level of proteins expressed from the target mRNA in the absence of recombinant RNA constructs comprising siRNA capable of binding to the target mRNA. Levels of protein expression can be measured by using any methods well known in the art and these include, but are not limited to Western-

10 blotting, flow cytometry, ELISAs, radioimmunoassays (RIAs), and various proteomics techniques. An exemplary method to measure or detect a polypeptide is an immunoassay, such as an ELISA. This type of protein quantitation can be based on an antibody capable of capturing a specific antigen, and a second antibody capable of detecting the captured antigen. Exemplary assays for detection and/or measurement of polypeptides are described in Harlow,

15 E. and Lane, D. Antibodies: A Laboratory Manual, (1988), Cold Spring Harbor Laboratory Press.

[0098] Provided herein are compositions comprising recombinant RNA constructs comprising at least two nucleic acid sequences comprising siRNA capable of binding to one or more target mRNAs and at least one nucleic acid sequence encoding a gene of interest

20 wherein the target mRNA is different from an mRNA encoded by the gene of interest. Provided herein are compositions comprising recombinant RNA constructs comprising at least two nucleic acid sequences comprising siRNA capable of binding to one or more target mRNAs and at least one nucleic acid sequence encoding a gene of interest wherein the siRNA does not affect expression of the gene of interest. In some instances, the siRNA is not capable

25 of binding to an mRNA encoded by the gene of interest. In some instances, the siRNA does not inhibit the expression of the gene of interest. In some instances, the siRNA does not downregulate the expression of the gene of interest. Inhibiting or downregulating the expression of the gene of interest, as described herein, can refer to, but is not limited to, interfering with translation of proteins from recombinant RNA constructs; thus, inhibiting or

30 downregulating the expression of the gene of interest can refer to, but is not limited to, a decreased level of protein compared to a level of protein expressed in the absence of recombinant RNA constructs comprising siRNA capable of binding to the target mRNA. Levels of protein expression can be measured by using any methods well known in the art and these include, but are not limited to Western-blotting, flow cytometry, ELISAs, RIAs, and

various proteomics techniques. An exemplary method to measure or detect a polypeptide is an immunoassay, such as an ELISA. This type of protein quantitation can be based on an antibody capable of capturing a specific antigen, and a second antibody capable of detecting the captured antigen. Exemplary assays for detection and/or measurement of polypeptides are described in Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, (1988), Cold Spring Harbor Laboratory Press.

[0099] Provided herein are compositions comprising recombinant RNA constructs comprising at least two nucleic acid sequences comprising an siRNA capable of binding to one or more target mRNAs. A list of non-limiting examples of target mRNAs that the siRNA is capable of binding to includes an mRNA of a gene comprising Tumor Necrosis Factor alpha (TNF-alpha or TNF- α), Interleukin 8 (IL-8), Interleukin 1 beta (IL-1beta), Interleukin 17 (IL-17), Turbo Green Fluorescence Protein (Turbo GFP), or a functional variant thereof. In some embodiments, Turbo GFP sequence can be derived from marine copepod *Pontellina plumate*. A functional variant as used herein may refer to a full-length molecule, a fragment thereof, or a variant thereof. For example, a variant molecule may comprise a sequence modified by insertion, deletion, and/or substitution of one or more amino acids, in the case of protein sequence, or one or more nucleotides, in the case of nucleic acid sequence.

[0100] In some embodiments, TNF-alpha comprises a sequence listed in SEQ ID NO: 40. In some embodiments, IL-8 comprises a sequence listed in SEQ ID NO: 37. In some embodiments, IL-1beta comprises a sequence listed in SEQ ID NO: 38. In some embodiments, IL-17 comprises a sequence listed in SEQ ID NO: 39. In some embodiments, Turbo GFP comprises a sequence listed in SEQ ID NO: 41.

[0101] In some aspects, the siRNA comprises a sense strand encoded by a sequence selected from SEQ ID NOs: 57-70. In some aspects, the siRNA comprises an anti-sense strand encoded by a sequence selected from SEQ ID NOs: 71-84. In some aspects, the siRNA comprises a sense strand encoded by a sequence selected from SEQ ID NOs: 57-70, and the corresponding anti-sense strand encoded by a sequence selected from SEQ ID NOs: 71-84.

Gene of interest

[0102] Provided herein are recombinant RNA constructs comprising one or more copies of nucleic acid sequence encoding a gene of interest. For example, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of nucleic acid sequence encoding a gene of interest. In some instances, each of the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of nucleic acid sequence encoding a gene of interest encodes the same gene of interest. In some

instances, recombinant RNA constructs may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more copies of nucleic acid sequence encoding a cytokine.

[0103] Also provided herein are recombinant RNA constructs comprising two or more copies of nucleic acid sequence encoding a gene of interest, wherein each of the two or more nucleic acid sequence may encode a different gene of interest. In some cases, each of the two or more nucleic acid sequences encoding different gene of interest may comprise a nucleic acid sequence encoding a secretory protein. In some cases, each of the two or more nucleic acid sequences encoding different gene of interest may comprise a nucleic acid sequence encoding a cytokine, e.g., Interleukin 4 (IL-4). In some embodiments, each of the two or more nucleic acid sequences encoding different gene of interest may encode a different secretory protein. In some cases, each of the two or more nucleic acid encoding different gene of interest may comprise a nucleic acid sequence encoding Insulin-like Growth Factor 1 (IGF-1). Further provided herein are recombinant RNA constructs comprising a linker described herein. In some embodiments, the linker may connect each of the two or more nucleic acid sequences encoding a gene of interest. In some cases, the linker may be a non-cleavable linker. In some cases, the linker may be a cleavable linker. In some cases, the linker may be a self-cleavable linker. In some cases, the linker may be cleaved by a protein, e.g., an intracellular protein or an endogenous protein. In some instances, the linker is selected from the group consisting of **Formula (I)**: $X_mCAACAAX_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4; and **Formula (II)**: X_pTCCCX_r , wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13. In some instances, the linker comprises a sequence comprising ACAACAA (SEQ ID NO: 85). In some embodiments, the linker is selected from the group consisting of SEQ ID NOs: 27, 28, 85-95.

[0104] Other examples of the linker include, but are not limited to, a flexible linker, a 2A peptide linker (or 2A self-cleaving peptides) such as T2A, P2A, E2A, or F2A, and a tRNA linker, etc. The tRNA system is evolutionarily conserved across living organism and utilizes endogenous RNases P and Z to process multicistronic constructs (Dong et al., 2016). In some embodiments, the tRNA linker may comprise a nucleic acid sequence comprising AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCC GGGTTCGATTCCCGGCTGGTGCA (SEQ ID NO: 96).

[0105] Provided herein are recombinant RNA constructs comprising an RNA encoding for a gene of interest for modulating the expression of the gene of interest. For example, expression of a protein encoded by the mRNA of the gene of interest can be modulated. For example, the expression of the gene of interest is upregulated by expressing a protein encoded by mRNA of

the gene of interest in recombinant RNA constructs. For example, the expression of the gene of interest is upregulated by increasing the level of protein encoded by mRNA of the gene of interest in recombinant RNA constructs. The level of protein expression can be measured by using any methods well known in the art and these include, but are not limited to Western-blotting, flow cytometry, ELISAs, RIAs, and various proteomics techniques. An exemplary method to measure or detect a polypeptide is an immunoassay, such as an ELISA. This type of protein quantitation can be based on an antibody capable of capturing a specific antigen, and a second antibody capable of detecting the captured antigen. Exemplary assays for detection and/or measurement of polypeptides are described in Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, (1988), Cold Spring Harbor Laboratory Press.

[0106] Provided herein are recombinant RNA constructs comprising an RNA encoding for a gene of interest wherein the gene of the interest encodes a protein of interest. In some instances, the protein of interest is a therapeutic protein. In some instances, the protein of interest is of human origin i.e., is a human protein. In some instances, the gene of interest encodes a secretory protein. In some embodiments, the gene of interest encodes Insulin-like Growth Factor 1 (IGF-1). In some embodiments, the protein of interest is IGF-1. In some instances, the gene of interest encodes a cytokine. In some embodiments, the cytokine comprises an interleukin. In some embodiments, the protein of interest is Interleukin 4 (IL-4) or a functional variant thereof.

[0107] In some instances, recombinant RNA constructs comprising a nucleic acid sequence encoding a gene of interest may comprise a nucleic acid sequence encoding human insulin-like growth factor 1 (IGF-1). In some instances, IGF-1 as used herein may refer to the natural sequence of human IGF-1 (Uniprot database: P05019 and in the Genbank database: NM_001111285.3), a fragment, or a functional variant thereof. In one embodiment, recombinant RNA constructs can be naked RNA comprising a nucleic acid sequence encoding IGF-1. In this embodiment, recombinant RNA constructs may comprise a nucleic acid sequence encoding the mature human IGF-1. The natural DNA sequence encoding human IGF-1 may be codon-optimized. The natural sequence of human IGF-1 comprises a signal peptide having 21 amino acids (nucleotides 1-63), a pro-peptide having 27 amino acids (nucleotides 64-144), a mature human IGF-1 having 70 amino acids (nucleotides 145-354), and E-peptide having 77 amino acids (nucleotides 355-585). In some embodiments, recombinant RNA constructs may comprise a nucleic acid sequence encoding a pro-peptide (also called pro-domain) of IGF-1, a nucleic acid sequence encoding a mature protein of IGF-1, or an E-peptide (also called E-domain) of IGF-1 (i.e., IGF-1 with a carboxyl-terminal

extension). In some embodiments, recombinant RNA constructs do not comprise a nucleic acid sequence encoding an E-peptide of IGF-1. In some embodiments, recombinant RNA constructs may comprise a nucleic acid sequence encoding a pro-peptide of IGF-1, a nucleic acid sequence encoding a mature protein of IGF-1, and a nucleic acid sequence encoding the signal peptide of brain-derived neurotrophic factor (BDNF). In some embodiments, IGF-1 is a human IGF-1.

[0108] In some embodiments, recombinant RNA constructs may comprise a nucleic acid sequence encoding a pro-peptide of IGF-1, preferably of human IGF-1 having 27 amino acids, and a nucleic sequence encoding a mature IGF-1, preferably a mature human IGF-1 having 70 amino acids, and preferably do not comprise a nucleotide sequence encoding an E-peptide of IGF-1, and preferably do not comprise a nucleic acid sequence encoding a human E-peptide of IGF-1. In some embodiments, recombinant RNA constructs may comprise a nucleic acid sequence encoding a pro-peptide of IGF-1, preferably of human IGF-1 having 27 amino acids, a nucleic sequence encoding a mature IGF-1, preferably a mature human IGF-1 having 70 amino acids, and a nucleic acid sequence encoding the signal peptide of brain-derived neurotrophic factor (BDNF). In some embodiments, recombinant RNA constructs do not comprise a nucleic sequence encoding an E-peptide of IGF-1, more preferably does not comprise a nucleic acid sequence encoding a human E-peptide of IGF-1.

[0109] In some embodiments, recombinant RNA constructs provided herein may comprise a nucleic acid sequence encoding a pro-peptide of human IGF-1 having 27 amino acids and a nucleic acid sequence encoding a mature human IGF-1 having 70 amino acids, and preferably do not comprise a nucleic acid sequence encoding an E-peptide of human IGF-1, wherein the nucleic acid sequence encoding the pro-peptide of human IGF-1 having 27 amino acids and the nucleic acid sequence encoding the mature human IGF-1 having 70 amino acids, and the nucleic acid sequence encoding the E-peptide are as referred to in the Uniprot database as UniProtKB - P05019. In some embodiments, IGF-1 described herein may have an amino acid sequence comprising SEQ ID NO: 34 or SEQ ID NO: 36.

[0110] In some instances, recombinant RNA constructs provided herein may comprise an mRNA encoding IGF-1. In some embodiments, the mRNA encoding IGF-1 may refer to an mRNA comprising a nucleotide sequence encoding the pro-peptide of human IGF-1 having 27 amino acids and/or a nucleotide sequence encoding the mature human IGF-1 having 70 amino acids. The nucleotide sequence encoding the pro-peptide of human IGF-1 and the nucleotide sequence encoding the mature human IGF-1 may be codon-optimized. In some instances, recombinant RNA constructs provided herein may comprise 1 copy of IGF-1

mRNA. In some instances, recombinant RNA constructs provided herein may comprise 2 or more copies of IGF-1 mRNA.

[0111] In some instances, Interleukin 4 (IL-4) or IL-4 as used herein may refer to the natural sequence of human IL-4 (Uniprot database: P05112 and in the Genbank database:

5 NM_000589.4), a fragment, or a functional variant thereof. The natural DNA sequence encoding human IL-4 may be codon-optimized. The natural sequence of human IL-4 comprises a signal peptide having 24 amino acids (nucleotides 1-72) and a mature human IL-4 having 153 amino acids (nucleotides 73-459). In some embodiments, the signal peptide is unmodified IL-4 signal peptide. In some embodiments, the signal peptide is IL-4 signal peptide modified by insertion, deletion, and/or substitution of at least one amino acid. In some
10 embodiments, Interleukin 4 (IL-4) or IL-4 as used herein may refer to the mature human IL-4. In some embodiments, a mature protein can refer to a protein synthesized in the endoplasmic reticulum and secreted via the Golgi apparatus in a cell expressing and secreting the protein. In some embodiments, a mature IL-4 may refer to an IL-4 protein synthesized in the endoplasmic
15 reticulum and secreted via the Golgi apparatus in a cell expressing and secreting IL-4. In some embodiments, a mature human IL-4 may refer to an IL-4 protein synthesized in the endoplasmic reticulum and secreted via the Golgi apparatus in a human cell expressing and secreting human IL-4 and normally contains the amino acids encoded by nucleotide as shown in SEQ ID NO: 31. In some embodiments, IL-4 described herein may have an amino acid
20 sequence comprising SEQ ID NO: 32 or SEQ ID NO: 42.

[0112] The mRNA encoding IL-4 may refer to an mRNA comprising a nucleotide sequence encoding the pro-peptide of human IL-4 having 153 amino acids or a nucleotide sequence encoding the mature human IL-4 having 129 amino acids. The nucleotide sequence encoding the pro-peptide of human IL-4 and the nucleotide sequence encoding the mature human IL-4
25 may be codon-optimized. In some instances, recombinant RNA constructs provided herein may comprise 1 copy of IL-4 mRNA. In some instances, recombinant RNA constructs provided herein may comprise 2 or more copies of IL-4 mRNA.

Target Motif

30 **[0113]** Provided herein are compositions comprising recombinant RNA constructs comprising a target motif. A target motif or a targeting motif as used herein can refer to any short peptide present in the newly synthesized polypeptides or proteins that are destined to any parts of cell membranes, extracellular compartments, or intracellular compartments, except cytoplasm or cytosol. In some embodiments, a peptide may refer to a series of amino

acid residues connected one to the other, typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acid residues. Intracellular compartments include, but are not limited to, intracellular organelles such as nucleus, nucleolus, endosome, proteasome, ribosome, chromatin, nuclear envelope, nuclear pore, exosome, melanosome, Golgi apparatus, 5 peroxisome, endoplasmic reticulum (ER), lysosome, centrosome, microtubule, mitochondria, chloroplast, microfilament, intermediate filament, or plasma membrane. In some embodiments, a signal peptide can be referred to as a signal sequence, a targeting signal, a localization signal, a localization sequence, a transit peptide, a leader sequence, or a leader peptide. In some embodiments, a target motif is operably linked to a nucleic acid sequence 10 encoding a gene of interest. In some embodiments, the term “operably linked” can refer to a functional relationship between two or more nucleic acid sequences, *e.g.*, a functional relationship of a transcriptional regulatory or signal sequence to a transcribed sequence. For example, a target motif or a nucleic acid encoding a target motif is operably linked to a coding sequence if it is expressed as a preprotein that participates in targeting the polypeptide 15 encoded by the coding sequence to a cell membrane, intracellular, or an extracellular compartment. For example, a signal peptide or a nucleic acid encoding a signal peptide is operably linked to a coding sequence if it is expressed as a preprotein that participates in the secretion of the polypeptide encoded by the coding sequence. For example, a promoter is operably linked if it stimulates or modulates the transcription of the coding sequence. Non- 20 limiting examples of a target motif comprise a signal peptide, a nuclear localization signal (NLS), a nucleolar localization signal (NoLS), a lysosomal targeting signal, a mitochondrial targeting signal, a peroxisomal targeting signal, a microtubule tip localization signal (MtLS), an endosomal targeting signal, a chloroplast targeting signal, a Golgi targeting signal, an endoplasmic reticulum (ER) targeting signal, a proteasomal targeting signal, a membrane 25 targeting signal, a transmembrane targeting signal, a centrosomal localization signal (CLS) or any other signal that targets a protein to a certain part of cell membrane, extracellular compartments, or intracellular compartments.

[0114] A signal peptide is a short peptide present at the N-terminus of newly synthesized proteins that are destined towards the secretory pathway. The signal peptide of the present 30 invention can be 10-40 amino acids long. A signal peptide can be situated at the N-terminal end of the protein of interest or at the N-terminal end of a pro-protein form of the protein of interest. A signal peptide may be of eukaryotic origin. In some embodiments, a signal peptide may be a mammalian protein. In some embodiments, a signal peptide may be a human protein. In some instances, a signal peptide may be a homologous signal peptide (*i.e.*, from the same

protein) or a heterologous signal peptide (i.e., from a different protein or a synthetic signal peptide). In some instances, a signal peptide may be a naturally occurring signal peptide of a protein or a modified signal peptide.

[0115] Provided herein are compositions comprising recombinant RNA constructs

- 5 comprising a target motif, wherein the target motif may be selected from the group consisting of (a) a target motif heterologous to a protein encoded by the gene of interest; (b) a target motif heterologous to a protein encoded by the gene of interest, wherein the target motif heterologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid; (c) a target motif homologous to a protein
- 10 encoded by the gene of interest; (d) a target motif homologous to a protein encoded by the gene of interest, wherein the target motif homologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid; and (e) a naturally occurring amino acid sequence which does not have the function of a target motif in nature, wherein the naturally occurring amino acid sequence is optionally modified
- 15 by insertion, deletion, and/or substitution of at least one amino acid.

[0116] Provided herein are compositions comprising recombinant RNA constructs

- comprising a target motif, wherein the target motif is a signal peptide. In some embodiments, the signal peptide is selected from the group consisting of: (a) a signal peptide heterologous to a protein encoded by the gene of interest; (b) a signal peptide heterologous to a protein
- 20 encoded by the gene of interest, wherein the signal peptide heterologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid, with proviso that the protein is not an oxidoreductase; (c) a signal peptide homologous to a protein encoded by the gene of interest; (d) a signal peptide homologous to a protein encoded by the gene of interest, wherein the signal peptide
- 25 homologous to the protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid; and (e) a naturally occurring amino acid sequence which does not have the function of a signal peptide in nature, wherein the naturally occurring amino acid sequence is optionally modified by insertion, deletion, and/or substitution of at least one amino acid. In some instances, the amino acids 1-9 of the N-
- 30 terminal end of the signal peptide have an average hydrophobic score of above 2.

[0117] In some instances, a target motif heterologous to a protein encoded by the gene of interest or a signal peptide heterologous to a protein encoded by the gene of interest as used herein can refer to a naturally occurring target motif or signal peptide which is different from the naturally occurring target motif or signal peptide of a protein. For example, the target motif

or the signal peptide is not derived from the gene of interest. Usually a target motif or a signal peptide heterologous to a given protein is a target motif or a signal peptide from another protein, which is not related to the given protein. For example, a target motif or a signal peptide heterologous to a given protein has an amino acid sequence that is different from the amino acid sequence of the target motif or the signal peptide of the given protein by more than 50%, 60%, 70%, 80%, 90%, or by more than 95%. Although heterologous sequences may be derived from the same organism, they naturally (in nature) do not occur in the same nucleic acid molecule, such as in the same mRNA. The target motif or the signal peptide heterologous to a protein and the protein to which the target motif or the signal peptide is heterologous can be of the same or different origin. In some embodiments, they are of eukaryotic origin. In some embodiments, they are of the same eukaryotic organism. In some embodiments, they are of mammalian origin. In some embodiments, they are of the same mammalian organism. In some embodiments, they are human origin. For example, an RNA construct may comprise a nucleic acid sequence encoding the human IL-4 gene and a signal peptide of another human protein. In some embodiments, an RNA construct may comprise a signal peptide heterologous to a protein wherein the signal peptide and the protein are of the same origin, namely of human origin.

[0118] In some instance, a target motif homologous to a protein encoded by the gene of interest or a signal peptide homologous to a protein encoded by the gene of interest as used herein can refer to a naturally occurring target motif or signal peptide of a protein. A target motif or a signal peptide homologous to a protein is the target motif or the signal peptide encoded by the gene of the protein as it occurs in nature. A target motif or a signal peptide homologous to a protein is usually of eukaryotic origin. In some embodiments, a target motif or a signal peptide homologous to a protein is of mammalian origin. In some embodiments, a target motif or a signal peptide homologous to a protein is of human origin.

[0119] In some instances, a naturally occurring amino acid sequence which does not have the function of a target motif in nature or a naturally occurring amino acid sequence which does not have the function of a signal peptide in nature as used herein can refer to an amino acid sequence which occurs in nature and is not identical to the amino acid sequence of any target motif or signal peptide occurring in nature. A naturally occurring amino acid sequence which does not have the function of a target motif or a signal peptide in nature can be between 10-50 amino acids long. In some embodiments, a naturally occurring amino acid sequence which does not have the function of a target motif or a signal peptide in nature is of eukaryotic origin and not identical to any target motif or signal peptide of eukaryotic origin. In some

embodiments, a naturally occurring amino acid sequence which does not have the function of a target motif or a signal peptide in nature is of mammalian origin and not identical to any target motif or signal peptide of mammalian origin. In some embodiments, a naturally occurring amino acid sequence which does not have the function of a target motif or a signal peptide in nature is of human origin and not identical to any target motif or signal peptide of human origin occurring in nature. A naturally occurring amino acid sequence which does not have the function of a target motif or a signal peptide in nature is usually an amino acid sequence of the coding sequence of a protein. The terms “naturally occurring,” “natural,” and “in nature” as used herein have the equivalent meaning.

10 **[0120]** In some instances, amino acids 1-9 of the N-terminal end of the signal peptide as used herein can refer to the first nine amino acids of the N-terminal end of the amino acid sequence of a signal peptide. Analogously, amino acids 1-7 of the N-terminal end of the signal peptide as used herein can refer to the first seven amino acids of the N-terminal end of the amino acid sequence of a signal peptide and amino acids 1-5 of the N-terminal end of the signal peptide
15 can refer to the first five amino acids of the N-terminal end of the amino acid sequence of a signal peptide.

[0121] In some instances, amino acid sequence modified by insertion, deletion, and/or substitution of at least one amino acid can refer to an amino acid sequence which includes an amino acid substitution, insertion, and/or deletion of at least one amino acid within the amino acid sequence. For example, target motif heterologous to a protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid or signal peptide heterologous to a protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid as used herein can refer to an amino acid sequence of a naturally occurring target motif or signal peptide heterologous to a protein which includes an amino acid substitution, insertion, and/or deletion of at least one amino acid within its naturally occurring amino acid sequence. For example, target motif homologous to a protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid or signal peptide homologous to a protein encoded by the gene of interest is modified by insertion, deletion, and/or substitution of at least one amino acid as used herein can refer to a naturally occurring target motif or signal peptide homologous to a protein which includes an amino acid substitution, insertion, and/or deletion of at least one amino acid within its naturally occurring amino acid sequence. In some embodiments, naturally occurring amino acid sequence may be modified by insertion, deletion, and/or substitution of at least one amino acid and a naturally occurring amino acid sequence

can include an amino acid substitution, insertion, and/or deletion of at least one amino acid within its naturally occurring amino acid sequence. An amino acid substitution or a substitution may refer to replacement of an amino acid at a particular position in an amino acid or polypeptide sequence with another amino acid. For example, the substitution R34K refers to a polypeptide, in which the arginine (Arg or R) at position 34 is replaced with a lysine (Lys or K). For the preceding example, 34K indicates the substitution of an amino acid at position 34 with a lysine (Lys or K). In some embodiments, multiple substitutions are typically separated by a slash. For example, R34K/L38V refers to a variant comprising the substitutions R34K and L38V. An amino acid insertion or an insertion may refer to addition of an amino acid at a particular position in an amino acid or polypeptide sequence. For example, insert -34 designates an insertion at position 34. An amino acid deletion or a deletion may refer to removal of an amino acid at a particular position in an amino acid or polypeptide sequence. For example, R34- designates the deletion of arginine (Arg or R) at position 34.

[0122] In some instances, deleted amino acid is an amino acid with a hydrophobic score of below -0.8, -0.7, -0.6, -0.5, -0.4, -0.3, -0.2, -0.1, 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, or below 1.9. In some instances, the substitute amino acid is an amino acid with a hydrophobic score which is higher than the hydrophobic score of the substituted amino acid. For example, the substitute amino acid is an amino acid with a hydrophobic score of 2.8 and higher, or 3.8 and higher. In some instances, the inserted amino acid is an amino acid with a hydrophobic score of 2.8 and higher or 3.8 and higher.

[0123] In some instances, an amino acid sequence described herein may comprise 1 to 15 amino acid insertions, deletions, and/or substitutions. In some embodiments, an amino acid sequence described herein may comprise 1 to 7 amino acid insertions, deletions, and/or substitutions. In some instances, an amino acid sequence described herein may not comprise amino acid insertions, deletions, and/or substitutions. In some instances, an amino acid sequence described herein may comprise 1 to 15 amino acid insertions, deletions, and/or substitutions within the amino acids 1-30 of the N-terminal end of the amino acid sequence of the target motif or the signal peptide. In some embodiments, an amino acid sequence described herein may comprise 1 to 9 amino acid insertions, deletions, and/or substitutions within the amino acids 1-30 of the N-terminal end of the amino acid sequence of the target motif or the signal peptide. In some instances, an amino acid sequence described herein may comprise 1 to 15 amino acid insertions, deletions, and/or substitutions within the amino acids 1-20 of the N-terminal end of the amino acid sequence of the target motif or the signal peptide. In some

embodiments, an amino acid sequence described herein may comprise 1 to 9 amino acid insertions, deletions, and/or substitutions within the amino acids 1-20 of the N-terminal end of the amino acid sequence of the target motif or the signal peptide. In some instances, at least one amino acid of an amino acid sequence described herein may be optionally modified by deletion, and/or substitution.

[0124] In some instances, the average hydrophobic score of the first nine amino acids of the N-terminal end of the amino acid sequence of the modified signal peptide is increased 1.0 unit or above compared to the signal peptide without modification. In some instances, hydrophobic score or hydrophobicity score can be used synonymously to hydrophathy score herein and can refer to the degree of hydrophobicity of an amino acid as calculated according to the Kyte-Doolittle scale (Kyte J., Doolittle R.F.; J. Mol. Biol. 157:105-132(1982)). The amino acid hydrophobic scores according to the Kyte-Doolittle scale are as follows:

[0125] Table A. Amino Acid Hydrophobic Scores

Amino Acid	One Letter Code	Hydrophobic Score
Isoleucine	I	4.5
Valine	V	4.2
Leucine	L	3.8
Phenylalanine	F	2.8
Cysteine	C	2.5
Methionine	M	1.9
Alanine	A	1.8
Glycine	G	-0.4
Threonine	T	-0.7
Serine	S	-0.8
Tryptophan	W	-0.9
Tyrosine	Y	-1.3
Proline	P	-1.6
Histidine	H	-3.2
Glutamic acid	E	-3.5
Glutamine	Q	-3.5
Aspartic acid	D	-3.5
Asparagine	N	-3.5
Lysine	K	-3.9
Arginine	R	-4.5

[0126] In some instances, average hydrophobic score of an amino acid sequence can be calculated by adding the hydrophobic score according to the Kyte-Doolittle scale of each of the amino acid of the amino acid sequence divided by the number of the amino acids. For example, the average hydrophobic score of the amino acids 1-9 of the N-terminal end of the amino acid sequence of a signal peptide can be calculated by adding the hydrophobic score of each of the nine amino acids divided by nine.

[0127] The polarity is calculated according to Zimmerman Polarity index (Zimmerman J.M., Eliezer N., Simha R.; J. Theor. Biol. 21:170-201(1968)). In some embodiments, average polarity of an amino acid sequence can be calculated by adding the polarity value calculated according to Zimmerman Polarity index of each of the amino acid of the amino acid

- 5 sequence divided by the number of the amino acids. For example, the average polarity of the amino acids 1-9 of the N-terminal end of the amino acid sequence of a signal peptide can be calculated by adding the average polarity of each of the nine amino acids of the amino acids 1-9 of the N-terminal end, divided by nine. The polarity of amino acids according to Zimmerman Polarity index is as follows:

10 [0128] **Table B.** Amino Acid Polarity

Amino Acid	One Letter Code	Polarity
Isoleucine	I	0.13
Valine	V	0.13
Leucine	L	0.13
Phenylalanine	F	0.35
Cysteine	C	1.48
Methionine	M	1.43
Alanine	A	0
Glycine	G	0
Threonine	T	1.66
Serine	S	1.67
Tryptophan	W	2.1
Tyrosine	Y	1.61
Proline	P	1.58
Histidine	H	51.6
Glutamic acid	E	49.9
Glutamine	Q	3.53
Aspartic acid	D	49.7
Asparagine	N	3.38
Lysine	K	49.5
Arginine	R	52

- [0129] In some instances, a naturally occurring signal peptide of Insulin-like Growth Factor 1 (IGF-1) may be modified by one or more substitutions, deletions, and/or insertions, wherein the naturally occurring signal peptide of IGF-1 is referred to the amino acids 1-20 of the IGF-1
- 15 amino acid sequence in the Uniprot database as P05019 and in the Genbank database as NM_001111285.3. In some instances, the amino acid sequence of IGF-1 signal peptide may be modified by the one or more substitutions, deletions, and/or insertions selected from the group consisting of G2L, K3-, S5L, T9L, Q10L, and C15-. In some instances, a naturally occurring signal peptide of IGF-1 may be replaced with a signal peptide of another protein. In some
- 20 instances, a naturally occurring signal peptide of IGF-1 may be replaced with a naturally

- occurring signal peptide of another protein. For example, a naturally occurring signal peptide of IGF-1 may be replaced with a naturally occurring signal peptide of brain-derived neurotrophic factor (BDNF). In some embodiments, the wild type (WT) IGF-1 signal peptide amino acid sequence comprises a sequence comprising SEQ ID NO: 49. In some instances, a modified IGF-1 signal peptide has an amino acid sequence comprising a sequence comprising SEQ ID NO: 44 encoded by the DNA sequence as shown in SEQ ID NO: 45. In some embodiments, a modified IGF-1 signal peptide may comprise a signal peptide from another protein. In some instances, a modified IGF-1 signal peptide has an amino acid sequence comprising a sequence comprising SEQ ID NO: 51 encoded by the DNA sequence as shown in SEQ ID NO: 52.
- [0130]** SEQ ID NO: 44
Met-Leu-Ile-Leu-Leu-Leu-Pro-Leu-Leu-Leu-Phe-Lys-Cys-Phe-Cys-Asp-Phe-Leu-Lys
- [0131]** SEQ ID NO: 45
ATGCTGATTCTGCTGCTGCCCCCTGCTGCTGTTCAAGTGCTTCTGCGACTTCCTGAA
A
- [0132]** SEQ ID NO: 51
MTILFLTMVISYFGCMKA
- [0133]** SEQ ID NO: 52
ATGACCATCCTGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCC
- [0134]** In some instances, the pro-peptide of IGF-1 may be modified. In some embodiments, a naturally occurring amino acid sequence of the pro-peptide of IGF-1, which does not have the function of a signal peptide in nature (Uniprot database as P05019), is modified by deletion of ten amino acid residues (VKMHTMSSSH (SEQ ID NO: 48)) flanking 22-31 in the N-terminal end of the pro-peptide and has preferably the amino acid sequence as shown in SEQ ID NO: 46 encoded by the DNA sequence as shown in SEQ ID NO: 45.
- [0135]** SEQ ID NO: 46
Met-Leu-Phe-Tyr-Leu-Ala-Leu-Cys-Leu-Leu-Thr-Phe-Thr-Ser-Ser-Ala-Thr-Ala
- [0136]** SEQ ID NO: 47
ATGCTGTTCTATCTGGCCCTGTGCCTGCTGACCTTTACCAGCTCTGCTACCGCC
- [0137]** In some instances, an mRNA comprising a nucleic acid sequence encoding the pro-peptide of IGF-1 and a nucleic acid sequence encoding the mature IGF-1, but not comprising a nucleic acid sequence encoding an E-peptide of IGF-1 may refer to an mRNA which comprises a nucleotide sequence encoding the pro-peptide of human IGF-1 having 27 amino acids and a nucleotide sequence encoding the mature human IGF-1 having 70 amino acids,

but does not comprise a nucleotide sequence encoding an E-peptide of human IGF-1 *i.e.*, does not comprise a nucleotide sequence encoding an Ea-, Eb-, or Ec-domain. The nucleotide sequence encoding the pro-peptide of human IGF-1 having 27 amino acids and the nucleotide sequence encoding the mature human IGF-1 having 70 amino acids may be codon-optimized.

- 5 **[0138]** In some instances, a naturally occurring signal peptide of Interleukin 4 (IL-4) may be modified by one or more substitutions, deletions, and/or insertions, wherein the naturally occurring signal peptide of IL-4 is referred to the amino acids 1-24 of the IL-4 amino acid sequence in the Uniprot database as P05112 and in the Genbank database as NM_000589.4. In some instances, the wild type (WT) IL-4 signal peptide amino acid sequence comprises a
- 10 sequence as shown in SEQ ID NO: 53. In some instances, the WT IL-4 signal peptide is encoded by a DNA sequence as shown in SEQ ID NO: 54. In some instances, a modified IL-4 signal peptide has an amino acid sequence comprising a sequence as shown in SEQ ID NO: 55. In some instances, a modified IL-4 signal peptide is encoded by a DNA sequence as shown in SEQ ID NO: 56.

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Linkers

- [0139]** In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence, wherein: (i) the first RNA sequence is a first small interfering RNA (siRNA)
- 20 sequence; (ii) the second RNA sequence is a second siRNA sequence or a first messenger RNA (mRNA) sequence encoding a gene of interest (GOI); and (iii) the linker RNA sequence links the first RNA sequence and the second RNA sequence, wherein the linker RNA sequence has a structure selected from the group consisting of: Formula (I): $X_mCAACAAX_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4; and
- 25 Formula (II): X_pTCCCX_r , wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13.

- [0140]** In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence, wherein: (i) the first RNA sequence is a first small interfering RNA (siRNA)
- 30 sequence; (ii) the second RNA sequence is a second siRNA sequence or a first messenger RNA (mRNA) sequence encoding a gene of interest (GOI); and (iii) the linker RNA sequence links the first RNA sequence and the second RNA sequence, wherein the linker RNA sequence comprises a sequence comprising ACAACAA.

- [0141] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence, wherein: (i) the first RNA sequence is a first small interfering (siRNA) sequence; (ii) the second RNA sequence is a second siRNA sequence or a first messenger (mRNA) sequence encoding a gene of interest (GOI); and (iii) the linker RNA sequence links the first RNA sequence and the second RNA sequence, wherein (a) the linker RNA sequence is not TTTATCTTAGAGGCATATCCCTACGTACCAACAA or ATAGTGAGTCGTATTAAACGTACCAACAA; or (b) the linker RNA sequence does not form a secondary structure according to RNAfold WebServer.
- 10 [0142] In some embodiments, the second RNA sequence is a second siRNA sequence. In some embodiments, the linker RNA sequence comprises ACAACAA, ATCCCTACGTACCAACAA, ACGTACCAACAA, TCCC, or ACAACAATCCC. In some embodiments, the recombinant RNA construct further comprises a first mRNA sequence encoding a GOI. In some embodiments, the second RNA sequence is a first mRNA sequence
- 15 encoding a GOI.
- [0143] In some embodiments, the linker RNA sequence comprises ACAACAA, ATAGTGAGTCGTATTATCCC, ATAGTGAGTCGTATTAAACAACAATCCC, ATAGTGAGTCGTATTAAACAACAA, ATAGTGAGTCGTATTAAATCCCTACGTACCAACAA, or
- 20 ATAGTGAGTCGTATTAAACGTACCAACAA.
- [0144] In some embodiments, the recombinant RNA construct further comprises a second mRNA sequence encoding a GOI. In some embodiments, the recombinant RNA construct further comprises a second siRNA sequence. In some embodiments, the recombinant RNA construct comprises a third siRNA sequence. In some embodiments, the recombinant RNA
- 25 construct further comprises four, five, or more siRNA sequences. In some embodiments, each of the siRNA sequences binds to a target RNA and modulates the expression of the target RNA.
- [0145] In some embodiments, each of the siRNA sequences is capable of binding to: (a) different target RNAs; (b) different regions of the same target RNA; (c) the same region of
- 30 the same target RNA; or (d) any combinations thereof. In some embodiments, the siRNA sequences of (c) are the same. In some embodiments, the recombinant RNA construct comprises three, four, five, or more mRNA sequences, each encoding a GOI. In some embodiments, each of the mRNA sequences encodes the same GOI. In some embodiments, each of the mRNA sequences encodes a different GOI.

- [0146] In some embodiments, the length of the linker RNA sequence between siRNA sequences is from about 4 to about 27 nucleotides. In some embodiments, the length the linker RNA sequence between siRNA sequences is from about 4 to about 18 nucleotides. In some embodiments, m is 1 and n is 0. In some embodiments, the linker RNA sequence between siRNA sequences is ACAACAATCCC. In some embodiments, the linker RNA sequence is ACAACAA. In some embodiments, the linker RNA sequence comprises a sequence selected from the group consisting of SEQ ID NOs: 85, 87-95. In some embodiments, the linker RNA sequence comprises a sequence according to SEQ ID NO: 85.
- [0147] In some embodiments, the expression of the GOI is modulated. In some embodiments, the expression of the GOI is upregulated by expressing a protein encoded by the GOI. In some embodiments, the expression of the target RNA is modulated. In some embodiments, the expression of the target RNA is downregulated by the siRNA sequences capable of binding to the target RNA. In some embodiments, the siRNA sequences capable of binding to the target RNA does not inhibit the expression of the GOI.
- [0148] In some embodiments, the RNA linker sequence between siRNA sequences does not form a secondary structure according to RNAfold WebServer. In some embodiments, an siRNA sequence forms a secondary structure according to RNAfold WebServer.
- [0149] In some embodiments, the recombinant RNA construct is cleaved. In some embodiments, the recombinant RNA construct is cleaved by an intracellular protein. In some embodiments, the recombinant RNA construct is cleaved by an endogenous protein. In some embodiments, the recombinant RNA construct is cleaved by an endogenous DICER.
- [0150] In some embodiments, the cleavage of the recombinant RNA construct is enhanced compared to the cleavage of an RNA construct that does not comprise a linker having a structure selected from the group consisting of Formula (I) and Formula (II). In some embodiments, the cleavage of the recombinant RNA construct is enhanced compared to the cleavage of an RNA construct that does not comprise a linker comprising a sequence comprising ACAACAA. In some embodiments, the cleavage of the recombinant RNA construct is enhanced compared to the cleavage of an RNA construct comprising a linker that forms a secondary structure.
- [0151] In some embodiments, the expression of the gene of interest is enhanced compared to the expression of a gene of interest from an RNA construct that does not comprise a linker having a structure selected from the group consisting of Formula (I) and Formula (II). In some embodiments, the expression of the gene of interest is enhanced compared to the expression

of a gene of interest from an RNA construct that does not comprise a linker comprising a sequence comprising ACAACAA.

[0152] In some aspects, provided herein, is a composition comprising a recombinant polynucleic acid construct comprising a nucleic acid sequence selected from the group
5 consisting of SEQ ID NOs: 1-24 and 97-108.

Expression vector and production of RNA constructs

[0153] Provided herein are compositions comprising recombinant polynucleic acid constructs encoding recombinant RNA constructs described herein. Provided herein are compositions
10 comprising recombinant polynucleic acid constructs encoding recombinant RNA constructs comprising a first RNA sequence and a second RNA sequence. In some instances, the first RNA sequence or the second RNA sequence may encode a gene of interest. In some embodiments, the first RNA sequence or the second RNA sequence may be an mRNA encoding a gene of interest. In some instances, the first RNA sequence or the second RNA
15 sequence may comprise at least two genetic elements that modulate the expression of one or more target RNA. In some embodiments, the first RNA sequence or the second RNA sequence may comprise at least two siRNAs each capable of binding to a target RNA. For example, an mRNA encoding a gene of interest can be an mRNA of IL-4 or IGF-1. For example, a target RNA can be an mRNA of TNF-alpha, IL-17, IL-8, IL-1beta, or Turbo GFP.

20 [0154] In related aspects, recombinant polynucleic acid constructs encoding recombinant RNA constructs may encode 2, 3, 4, 5, or more siRNA species. In related aspects, recombinant polynucleic acid constructs encoding recombinant RNA constructs may encode 2 siRNA species directed to a target mRNA. In related aspects, recombinant polynucleic acid constructs encoding recombinant RNA constructs may encode 3 siRNAs, each directed to a
25 target mRNA. In related aspects, each of the siRNA species may comprise the same sequence, different sequence, or a combination thereof. For example, recombinant polynucleic acid constructs encoding recombinant RNA constructs may encode 3 siRNAs, each directed to the same region or sequence of the target mRNA. For example, recombinant polynucleic acid constructs encoding recombinant RNA constructs may encode 3 siRNAs, each directed to a
30 different region or sequence of the target mRNA. In some aspects, recombinant polynucleic acid constructs encoding recombinant RNA constructs may encode 3 siRNA species, wherein each of the 3 siRNA species is directed to a different target mRNA. In some embodiments, a target mRNA may be TNF-alpha mRNA, IL-8 mRNA, IL-17 mRNA, Turbo GFP mRNA, or

IL-1 β mRNA. In related aspects, recombinant polynucleic acid constructs may comprise a sequence selected from the group consisting of SEQ ID NOs: 13-24.

[0155] The polynucleic acid constructs, described herein, can be obtained by any method known in the art, such as by chemically synthesizing the DNA chain, by PCR, or by the Gibson Assembly method. The advantage of constructing polynucleic acid constructs by chemical synthesis or a combination of PCR method or Gibson Assembly method is that the codons may be optimized to ensure that the fusion protein is expressed at a high level in a host cell. Codon optimization can refer to a process of modifying a nucleic acid sequence for expression in a host cell of interest by replacing at least one codon (*e.g.*, more than 1, 2, 3, 4, 5, 10, 15, 20, 25, 50, or more codons) of a native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Codon usage tables are readily available, for example, at the "Codon Usage Database," and these tables can be adapted in a number of ways. Computer algorithms for codon optimizing a particular sequence for expression in a particular host cell are also available, such as Gene Forge[®] (Aptagen, PA) and GeneOptimizer[®] (ThermoFischer, MA). Once obtained polynucleotides can be incorporated into suitable vectors. Vectors as used herein can refer to naturally occurring or synthetically generated constructs for uptake, proliferation, expression or transmission of nucleic acids *in vivo* or *in vitro*, *e.g.*, plasmids, minicircles, phagemids, cosmids, artificial chromosomes/mini-chromosomes, bacteriophages, viruses such as baculovirus, retrovirus, adenovirus, adeno-associated virus, herpes simplex virus, bacteriophages. Methods used to construct vectors are well known to a person skilled in the art and described in various publications. In particular techniques for constructing suitable vectors, including a description of the functional and regulatory components such as promoters, enhancers, termination and polyadenylation signals, selection markers, origins of replication, and splicing signals, are known to the person skilled in the art. A variety of vectors are well known in the art and some are commercially available from companies such as Agilent Technologies, Santa Clara, Calif.; Invitrogen, Carlsbad, Calif.; Promega, Madison, Wis.; Thermo Fisher Scientific; or Invivogen, San Diego, Calif. A non-limiting examples of vectors for *in vitro* transcription includes pT7CFE1-CHis, pMX (such as pMA-T, pMA-RQ, pMC, pMK, pMS, pMZ), pEVL, pSP73, pSP72, pSP64, and pGEM (such as pGEM[®]-4Z, pGEM[®]-5Zf(+), pGEM[®]-11Zf(+), pGEM[®]-9Zf(-), pGEM[®]-3Zf(+/-), pGEM[®]-7Zf(+/-)). In some instances, recombinant polynucleic acid constructs may be DNA.

[0156] The polynucleic acid constructs, as described herein, can be circular or linear. For example, circular polynucleic acid constructs may include vector system such as pMX, pMA-

T, pMA-RQ, or pT7CFE1-CHis. For example, linear polynucleic acid constructs may include linear vector such as pEVL or linearized vectors. In some instances, recombinant polynucleic acid constructs may further comprise a promoter. In some instances, the promoter may be present upstream of or 5' to the sequence encoding for the first RNA sequence and the second RNA sequence. Non-limiting examples of a promoter can include T3, T7, SP6, P60, Syn5, and KP34. In some instances, recombinant polynucleic acid constructs provided herein may comprise a T7 promoter comprising a sequence comprising TAATACGACTCACTATA (SEQ ID NO: 26). In some instances, recombinant polynucleic acid constructs further comprises a sequence encoding a Kozak sequence. A Kozak sequence may refer to a nucleic acid sequence motif that functions as the protein translation initiation site. Kozak sequences are described at length in the literature, *e.g.*, by Kozak, M., Gene 299(1-2):1-34, incorporated herein by reference herein in its entirety. In some embodiments, recombinant polynucleic acid constructs comprises a sequence encoding a Kozak sequence comprising a sequence comprising GCCACC (SEQ ID NO: 25). In some instances, recombinant polynucleic acid constructs described herein may be codon-optimized.

[0157] Provided herein are compositions comprising recombinant polynucleic acid constructs encoding RNA constructs described herein comprising at least two nucleic acid sequences each encoding an siRNA capable of binding to one or more target RNAs and one or more nucleic acid sequence encoding a gene of interest, wherein the siRNA capable of binding to a target RNA is not a part of an intron sequence encoded by the gene of interest. In some instances, the gene of interest is expressed without RNA splicing. In some instances, the siRNA capable of binding to a target RNA is not encoded by or comprised of an intron sequence of the gene of interest. In some instances, the siRNA capable of binding to a target RNA binds to an exon of a target RNA. In some instances, the siRNA capable of binding to a target RNA specifically binds to one target RNA. In some instances, recombinant polynucleic acid constructs may comprise a nucleic acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 13-24.

[0158] Provided herein are methods of producing RNA construct compositions described herein. For example, recombinant RNA constructs may be produced by *in vitro* transcription from a polynucleic acid construct comprising a promoter for an RNA polymerase, at least one nucleic acid sequence encoding a gene of interest, at least two nucleic acid sequences encoding siRNAs capable of binding to one or more target mRNAs, and a nucleic acid sequence encoding poly(A) tail. *In vitro* transcription reaction may further comprise an RNA polymerase, a mixture of nucleotide triphosphates (NTPs), and/or a capping enzyme. Details

- of producing RNAs using *in vitro* transcription as well as isolating and purifying transcribed RNAs is well known in the art and can be found, for example, in Beckert & Masquida ((2011) Synthesis of RNA by In vitro Transcription. RNA. Methods in Molecular Biology (Methods and Protocols), vol 703. Humana Press). A non-limiting list of *in vitro* transcript kits includes
- 5 MEGAscript™ T3 Transcription Kit, MEGAscript T7 kit, MEGAscript™ SP6 Transcription Kit, MAXIsript™ T3 Transcription Kit, MAXIsript™ T7 Transcription Kit, MAXIsript™ SP6 Transcription Kit, MAXIsript™ T7/T3 Transcription Kit, MAXIsript™ SP6/T7 Transcription Kit, mMESSAGE mMACHINE™ T3 Transcription Kit, mMESSAGE mMACHINE™ T7 Transcription Kit, mMESSAGE mMACHINE™ SP6 Transcription Kit,
 - 10 MEGAscript™ T7 Transcription Kit, HiScribe™ T7 High Yield RNA Synthesis Kit, HiScribe™ T7 In Vitro Transcription Kit, AmpliScribe™ T7-Flash™ Transcription Kit, AmpliScribe™ T7 High Yield Transcription Kit, AmpliScribe™ T7-Flash™ Biotin-RNA Transcription Kit, T7 Transcription Kit, HighYield T7 RNA Synthesis Kit, DuraScribe® T7 Transcription Kit, etc.
 - 15 **[0159]** The *in vitro* transcription reaction can further comprise a transcription buffer system, nucleotide triphosphates (NTPs), and an RNase inhibitor. In some embodiments, the transcription buffer system may comprise dithiothreitol (DTT) and magnesium ions. The NTPs can be naturally occurring or non-naturally occurring (modified) NTPs. Non-limiting examples of non-naturally occurring (modified) NTPs include N¹-methylpseudouridine,
 - 20 pseudouridine, N¹-ethylpseudouridine, N¹-methoxymethylpseudouridine, N¹-propylpseudouridine, 2-thiouridine, 4-thiouridine, 5-methoxyuridine, 5-methyluridine, 5-carboxymethylesteruridine, 5-formyluridine, 5-carboxyuridine, 5-hydroxyuridine, 5-bromouridine, 5-Iodouridine, 5,6-dihydrouridine, 6-azauridine, thienouridine, 3-methyluridine, 1-carboxymethyl-pseudouridine, 4-thio-1-methyl-pseudouridine, 2-thio-1-
 - 25 methyl-pseudouridine, dihydrouridine, dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, 5-methylcytidine, 5-methoxycytidine, 5-hydroxymethylcytidine, 5-formylcytidine, 5-carboxycytidine, 5-hydroxycytidine, 5-iodocytidine, 5-bromocytidine, 2-thiocytidine, 5-azacytidine, pseudoisocytidine, 3-methyl-cytidine, N⁴-acetylcytidine, 5-formylcytidine, N⁴-
 - 30 methylcytidine, 5-hydroxymethylcytidine, 1-methyl-pseudoisocytidine, 4-methoxy-pseudoisocytidine, and 4-methoxy-1-methyl-pseudoisocytidine, N¹-methyladenosine, N⁶-methyladenosine, N⁶-methyl-2-aminoadenosine, N⁶-isopentenyladenosine, N⁶,N⁶-dimethyladenosine, 7-methyladenine, 2-methylthio-adenine, and 2-methoxy-adenine. Non-limiting examples of DNA-dependent RNA polymerase include T3, T7, SP6, P60, Syn5, and

KP34 RNA polymerases. In some embodiments, the RNA polymerase is selected from the group consisting of T3 RNA polymerase, T7 RNA polymerase, SP6 RNA polymerase, P60 RNA polymerase, Syn5 RNA polymerase, and KP34 RNA polymerase.

[0160] Transcribed RNAs, as described herein, may be isolated and purified from the *in vitro* transcription reaction mixture. For example, transcribed RNAs may be isolated and purified using column purification. Details of isolating and purifying transcribed RNAs from *in vitro* transcription reaction mixture is well known in the art and any commercially available kits may be used. A non-limiting list of RNA purification kits includes MEGAclear kit, Monarch® RNA Cleanup Kit, EasyPure® RNA Purification Kit, NucleoSpin® RNA Cleanup, etc.

Therapeutic applications

[0161] Provided herein are compositions useful in the treatment of a disease or condition. In some aspects, compositions are present or administered in an amount sufficient to treat or prevent a disease or condition. In some aspects, provided herein, is a method of treating a disease or condition comprising administering to a subject in need thereof the composition or the pharmaceutical composition described herein. In some aspects, provided herein, is the composition or the pharmaceutical composition described herein for use in a method of treating a disease or a condition in a subject in need thereof. In some aspects, provided herein, is the use of the composition or the pharmaceutical composition described herein for the manufacture of a medicament for treating a disease or a condition in a subject in need thereof. In some embodiments, the disease or condition comprises a skin disease or condition or a joint disease or condition. In some embodiments, the skin disease or condition comprises an inflammatory skin disorder. In some embodiments, an inflammatory skin disorder comprises psoriasis. In some embodiments, the joint degeneration comprises intervertebral disc disease (IVDD) or osteoarthritis (OA). Provided herein are recombinant polynucleic acid or RNA construct compositions comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the first RNA sequence encoding a gene of interest and a corresponding second RNA sequence comprising at most one of the at least two genetic elements that modulate expression of one or more target RNAs. In some instances, the first RNA sequence or the second RNA sequence may

encode a gene of interest. In some embodiments, the gene of interest may comprise IL-4 or IGF-1. In some instances, the first RNA sequence or the second RNA sequence may comprise at least two genetic elements that can reduce expression of a gene associated with a disease or condition described herein. In some embodiments, the genetic element that can reduce
5 expression of a gene associated with a disease or condition may comprise siRNA targeting TNF-alpha mRNA or a functional variant. In some embodiments, the genetic element that can reduce expression of a gene associated with a disease or condition may comprise siRNA targeting IL-8 mRNA or a functional variant. In some embodiments, the genetic element that can reduce expression of a gene associated with a disease or condition may comprise siRNA
10 targeting IL-1beta mRNA or a functional variant. In some embodiments, the genetic element that can reduce expression of a gene associated with a disease or condition may comprise siRNA targeting IL-17 mRNA or a functional variant.

[0162] Also provided herein are pharmaceutical compositions comprising any recombinant RNA construct composition described herein and a pharmaceutically acceptable excipient. A
15 pharmaceutical composition can denote a mixture or solution comprising a therapeutically effective amount of an active pharmaceutical ingredient together with one or more pharmaceutically acceptable excipients to be administered to a subject in need thereof. The term “pharmaceutically acceptable” denotes an attribute of a material which is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and neither
20 biologically nor otherwise undesirable and is acceptable for veterinary as well as human pharmaceutical use. The term “Pharmaceutically acceptable” can refer to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively nontoxic, i.e., the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any
25 of the components of the composition in which it is contained. A pharmaceutically acceptable excipient can denote any pharmaceutically acceptable ingredient in a pharmaceutical composition having no therapeutic activity and being non-toxic to the subject administered, such as disintegrators, binders, fillers, solvents, buffers, tonicity agents, stabilizers, antioxidants, surfactants, carriers, diluents, excipients, preservatives or lubricants used in
30 formulating pharmaceutical products. Pharmaceutical compositions can facilitate administration of the compound to an organism and can be formulated in a conventional manner using one or more pharmaceutically acceptable inactive ingredients that facilitate processing of the active compounds into preparations that can be used pharmaceutically. A proper formulation is dependent upon the route of administration chosen and a summary of

pharmaceutical compositions can be found, for example, in Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins 1999), herein incorporated by reference. In some embodiments, pharmaceutical compositions can be formulated by dissolving active substances (e.g., recombinant polynucleic acid or RNA constructs described herein) in aqueous solution for injection into disease tissues or disease cells. In some
10 embodiments, pharmaceutical compositions can be formulated by dissolving active substances (e.g., recombinant polynucleic acid or RNA constructs described herein) in aqueous solution for direct injection into disease tissues or disease cells.

[0163] Also provided herein are methods of treating a disease or condition in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of
15 polynucleic acid construct or recombinant RNA construct compositions or pharmaceutical compositions described herein. The terms "effective amount" or "therapeutically effective amount," as used herein, refer to a sufficient amount of an agent or a compound being administered which will relieve to some extent one or more of the symptoms of the disease or the condition being treated; for example a reduction and/or alleviation of one or more signs,
20 symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an "effective amount" for therapeutic uses can be an amount of an agent that provides a clinically significant decrease in one or more disease symptoms. An appropriate "effective" amount may be determined using techniques, such as a dose escalation study, in individual cases.

[0164] The terms "treat," "treating" or "treatment," as used herein, include alleviating, abating or ameliorating at least one symptom of a disease or a condition, preventing additional symptoms, inhibiting the disease or the condition, *e.g.*, arresting the development of the disease or the condition, relieving the disease or the condition, causing regression of the disease or the condition, relieving a condition caused by the disease or the condition, or
30 stopping the symptoms of the disease or the condition either prophylactically and/or therapeutically. In some embodiments, treating a disease or condition comprises reducing the size of disease tissues or disease cells. In some embodiments, treating a disease or a condition in a subject comprises increasing the survival of a subject. In some embodiments, treating a disease or condition comprises reducing or ameliorating the severity of a disease, delaying

onset of a disease, inhibiting the progression of a disease, reducing hospitalization of or hospitalization length for a subject, improving the quality of life of a subject, reducing the number of symptoms associated with a disease, reducing or ameliorating the severity of a symptom associated with a disease, reducing the duration of a symptom associated with a disease, preventing the recurrence of a symptom associated with a disease, inhibiting the development or onset of a symptom of a disease, or inhibiting of the progression of a symptom associated with a disease.

[0165] In some cases, a subject can encompass mammals. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. In some cases, the mammal is a human. In some cases, the subject may be an animal. In some cases, an animal may comprise human beings and non-human animals. In one embodiment, a non-human animal may be a mammal, for example a rodent such as rat or a mouse. In another embodiment, a non-human animal may be a mouse. In some instances, the subject is a mammal. In some instances, the subject is a human. In some instances, the subject is an adult, a child, or an infant. In some instances, the subject is a companion animal. In some instances, the subject is a feline, a canine, or a rodent. In some instances, the subject is a dog or a cat.

[0166] In some aspects, provided herein, is a method of treating a disease or condition in a subject, comprising administering to the subject recombinant RNA construct compositions or pharmaceutical compositions, described herein, comprising an mRNA encoding a gene of interest and at least two siRNAs capable of binding to a target mRNA. In some embodiments, the target mRNA comprises an mRNA of TNF-alpha, IL-1beta, IL-8, IL-17, Turbo GFP, or a functional variant thereof. In some embodiments, the mRNA encoding the gene of interest encodes IGF-1 or a functional variant thereof. In some embodiments, the mRNA encoding the gene of interest encodes a cytokine. In some embodiments, the cytokine is an IL-4 or a functional variant thereof.

[0167] In some aspects, provided herein, is a method of treating a disease or condition in a subject, the method comprising administering to the subject recombinant RNA construct compositions or pharmaceutical compositions, described herein, comprising an mRNA encoding IGF-1 and siRNA capable of binding to an mRNA of IL-8. In some aspects, provided herein, is a method of treating a disease or condition in a subject, the method comprising administering to the subject recombinant RNA construct compositions or

pharmaceutical compositions, described herein, comprising an mRNA encoding IGF-1 and siRNA capable of binding to an mRNA of IL-1beta. In some aspects, provided herein, is a method of treating a disease or condition in a subject, the method comprising administering to the subject recombinant RNA compositions or pharmaceutical compositions described herein

5 comprising an mRNA encoding IL-4 and siRNA capable of binding to an mRNA of TNF-alpha. In some aspects, provided herein, is a method of treating a disease or condition in a subject, the method comprising administering to the subject recombinant RNA construct compositions or pharmaceutical compositions, described herein, comprising an mRNA encoding IL-4 and siRNA capable of binding to an mRNA of IL-17. In some aspects,

10 provided herein, is a method of treating a disease or condition in a subject, the method comprising administering to the subject recombinant RNA construct compositions or pharmaceutical compositions, described herein, comprising an mRNA encoding IL-4, siRNA capable of binding to an mRNA of TNF-alpha, and siRNA capable of binding to an mRNA of IL-17. In some embodiments, the disease or condition comprises a skin disease or condition

15 or a joint disease or condition. In some embodiments, the skin disease or condition comprises an inflammatory skin disorder. In some embodiments, an inflammatory skin disorder comprises psoriasis. In some embodiments, the joint disease or condition comprises a joint degeneration. In some embodiments, the joint degeneration comprises intervertebral disc disease (IVDD) or osteoarthritis (OA).

20 **[0168]** In some aspects, compositions or pharmaceutical compositions administered to a subject in need thereof comprise recombinant polynucleic acid constructs or RNA constructs comprising: (i) an mRNA encoding IL-4; and (ii) at least two siRNAs capable of binding to a TNF-alpha mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6 or more siRNAs. In related aspects,

25 recombinant polynucleic acid constructs or RNA constructs may comprise 2 siRNAs directed to a TNF-alpha mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 3 siRNAs, each directed to a TNF-alpha mRNA. In related aspects, each of the at least 3 siRNAs may be the same, different, or a combination thereof. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise a sequence

30 as set forth in SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 104, or SEQ ID NO: 105 (Cpd.4-Cpd.9). In related aspects, recombinant polynucleic acid constructs may comprise a sequence as set forth in SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, or SEQ ID NO: 21 (Cpd.4-Cpd.9).

[0169] In some aspects, compositions or pharmaceutical compositions administered to a subject in need thereof comprise recombinant polynucleic acid constructs or RNA constructs comprising: (i) an mRNA encoding IGF-1; and (ii) at least two siRNA capable of binding to an IL-8 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 2 siRNAs directed to an IL-8 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 3 siRNAs, each directed to an IL-8 mRNA. In related aspects, each of the at least 3 siRNAs may be the same, different, or a combination thereof. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise a sequence as set forth in SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 97, or SEQ ID NO: 98 (Cpd.1 or Cpd.2). In related aspects, recombinant polynucleic acid constructs may comprise a sequence as set forth in SEQ ID NO: 13 or SEQ ID NO: 14 (Cpd.1 or Cpd.2).

[0170] In some aspects, compositions or pharmaceutical compositions administered to a subject in need thereof comprise recombinant polynucleic acid constructs or RNA constructs comprising: (i) an mRNA encoding IGF-1; and (ii) at least two siRNA capable of binding to an IL-1beta mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 2 siRNAs directed to an IL-1beta mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 3 siRNAs, each directed to an IL-1beta mRNA. In related aspects, each of the at least 3 siRNAs may be the same, different, or a combination thereof. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise a sequence as set forth in SEQ ID NO: 3 or SEQ ID NO: 99 (Cpd.3). In related aspects, recombinant polynucleic acid constructs may comprise a sequence as set forth in SEQ ID NO: 15 (Cpd.3).

[0171] In some aspects, compositions or pharmaceutical compositions administered to a subject in need thereof comprise recombinant polynucleic acid constructs or RNA constructs comprising: (i) an mRNA encoding IL-4; and (ii) at least two siRNA capable of binding to an IL-17 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 2 siRNAs directed to an IL-17 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 3 siRNAs, each directed to an IL-17 mRNA. In related aspects, each of the at least 3 siRNAs may be the same, different, or a combination thereof. In related aspects, recombinant

polynucleic acid constructs or RNA constructs may comprise a sequence as set forth in SEQ ID NO: 4 or SEQ ID NO: 100 (Cpd.4). In related aspects, recombinant polynucleic acid constructs may comprise a sequence as set forth in SEQ ID NO: 16 (Cpd.4).

[0172] In some aspects, compositions or pharmaceutical compositions administered to a subject in need thereof comprise recombinant polynucleic acid constructs or RNA constructs comprising: (i) an mRNA encoding IL-4; (ii) at least two siRNAs capable of binding to a TNF-alpha mRNA; and (iii) at least two siRNAs capable of binding to an IL-17 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6 or more siRNAs. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 2 siRNAs directed to a TNF-alpha mRNA and 2 siRNAs directed to an IL-17 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 3 siRNAs, each directed to a TNF-alpha mRNA or an IL-17 mRNA. In related aspects, each of the at least 3 siRNAs may be the same, different, or a combination thereof. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 6 siRNAs, each directed to a TNF-alpha mRNA or an IL-17 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 6 siRNAs, 3 directed to a TNF-alpha mRNA and 3 an IL-17 mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise a sequence as set forth in SEQ ID NO: 4 or SEQ ID NO: 100 (Cpd.4). In related aspects, recombinant polynucleic acid constructs may comprise a sequence as set forth in SEQ ID NO: 16 (Cpd.4).

[0173] In some aspects, compositions or pharmaceutical compositions administered to a subject in need thereof comprise recombinant polynucleic acid constructs or RNA constructs comprising: (i) an IGF-1 mRNA; and (ii) at least one siRNA capable of binding to a Turbo GFP mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may encode or comprise at least 1, 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 1 siRNA directed to a Turbo GFP mRNA. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise 2 or 3 siRNAs, each directed to a Turbo GFP mRNA. In related aspects, each of the at least 2 or at least 3 siRNAs may be the same, different, or a combination thereof. In related aspects, recombinant polynucleic acid constructs or RNA constructs may comprise a sequence as set forth in SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 106, SEQ ID NO: 107, or SEQ ID NO: 108 (Cpd.10-Cpd.12). In related

aspects, recombinant polynucleic acid constructs may comprise a sequence as set forth in SEQ ID NO: 22, SEQ ID NO: 23, or SEQ ID NO: 24 (Cpd.10-Cpd.12).

[0174] Recombinant RNA construct compositions described herein may be administered as a combination therapy. Combination therapies with two or more therapeutic agents or therapies may use agents and therapies that work by different mechanisms of action. Combination therapies using agents or therapies with different mechanisms of action can result in additive or synergetic effects. Combination therapies may allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the agent(s). Combination therapies can decrease the likelihood that resistant disease cells will develop. In some instances, combination therapies comprise a therapeutic agent or therapy that affects the immune response (*e.g.*, enhances or activates the response) and a therapeutic agent that affects (*e.g.*, inhibits or kills) the disease cells. In some instances, combination therapies may comprise (i) recombinant RNA compositions or pharmaceutical compositions described herein; and (ii) one or more additional therapies known in the art for the diseases described herein. In some embodiments, recombinant RNA compositions or pharmaceutical compositions described herein may be administered to a subject with a disease or condition prior to, concurrently with, and/or subsequently to, administration of one or more additional therapies for combination therapies. In some embodiments, the one or more additional therapies may comprise 1, 2, 3, or more additional therapeutic agents or therapies.

[0175] Compositions and pharmaceutical compositions described herein can be administered to a subject using any suitable methods known in the art. Suitable formulations for use in the present invention and methods of delivery are generally well known in the art. For example, compositions described herein can be administered to the subject in a variety of ways, including parenterally, intravenously, intradermally, intramuscularly, colonically, rectally, or intraperitoneally. In some embodiments, compositions described herein is administered by intraperitoneal injection, intramuscular injection, subcutaneous injection, or intravenous injection of the subject. In some embodiments, compositions described herein can be administered parenterally, intravenously, intramuscularly or orally. In some embodiments, compositions described herein can be administered via injection into disease tissues or cells.

In some embodiments, compositions described herein can be administered as an aqueous solution for injection into disease tissues or cells.

[0176] Any of compositions and pharmaceutical compositions described herein may be provided together with an instruction manual. The instruction manual may comprise guidance for the skilled person or attending physician how to treat (or prevent) a disease or a disorder

as described herein (*e.g.*, a cancer such as a head and neck cancer) in accordance with the present invention. In some embodiments, the instruction manual may comprise guidance as to the herein described mode of delivery/administration and delivery/administration regimen, respectively (*e.g.*, route of delivery/administration, dosage regimen, time of
5 delivery/administration, frequency of delivery/administration, etc.). In some embodiments, the instruction manual may comprise the instruction that how compositions of the present invention is to be administrated or injected and/or is prepared for administration or injection. In principle, what has been described herein elsewhere with respect to the mode of delivery/administration and delivery/administration regimen, respectively, may be comprised
10 as respective instructions in the instruction manual.

[0177] Compositions and pharmaceutical compositions described herein can be used in a gene therapy. In certain embodiments, compositions comprising recombinant polynucleic acids or RNA constructs described herein can be delivered to a cell in gene therapy vectors. Gene therapy vectors and methods of gene delivery are well known in the art. Non-limiting
15 examples of these methods include viral vector delivery systems including DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell, non-viral vector delivery systems including DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle, transposon system (for delivery and integration into the host genomes; Moriarity, et al. (2013) *Nucleic Acids Res* 41(8), e92, Aronovich, et al., (2011)
20 *Hum. Mol. Genet.* 20(R1), R14-R20), retrovirus-mediated DNA transfer (*e.g.*, Moloney Mouse Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus; see *e.g.*, Kay et al. (1993) *Science* 262, 117-119, Anderson (1992) *Science* 256,
25 808-813), and DNA virus-mediated DNA transfer including adenovirus, herpes virus, parvovirus and adeno-associated virus (*e.g.*, Ali et al. (1994) *Gene Therapy* 1, 367-384). Viral vectors also include but are not limited to adeno-associated virus, adenoviral virus, lentivirus, retroviral, and herpes simplex virus vectors. Vectors capable of integration in the host genome include but are not limited to retrovirus or lentivirus.

[0178] In some embodiments, compositions comprising recombinant polynucleic acid or RNA constructs described herein can be delivered to a cell via direct DNA transfer (Wolff et al. (1990) *Science* 247, 1465-1468). Recombinant polynucleic acid or RNA constructs can be delivered to cells following mild mechanical disruption of the cell membrane, temporarily permeabilizing the cells. Such a mild mechanical disruption of the membrane can be

accomplished by gently forcing cells through a small aperture (Sharei et al. PLOS ONE (2015) 10(4), e0118803). In another embodiment, compositions comprising recombinant polynucleic acid or RNA constructs described herein can be delivered to a cell via liposome-mediated DNA transfer (*e.g.*, Gao & Huang (1991) Biochem. Biophys. Res. Comm. 179, 280-285, Crystal (1995) Nature Med. 1, 15-17, Caplen et al. (1995) Nature Med. 3, 39-46). A liposome can encompass a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Recombinant polynucleic acid or RNA constructs can be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, or complexed with a liposome.

Modulation of gene expression

[0179] Provided herein are methods of expressing an mRNA and at least two siRNAs from a single RNA transcript in a cell, comprising introducing into the cell compositions comprising any recombinant polynucleic acid or RNA constructs described herein. Further provided herein are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs encoding or comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the at least two genetic elements that modulate expression of one or more target RNAs comprises a small interfering RNA (siRNA) capable of binding to a target messenger RNA (mRNA), and wherein the target mRNA is different from an mRNA encoded by the gene of interest, thereby modulating the expression of the target mRNA and the gene of interest from a single RNA transcript. In some instances, expression of a polynucleic acid, gene, DNA, or RNA, as used herein, can refer to transcription and/or translation of the polynucleic acid, gene, DNA, or RNA. In some instances, modulating, increasing, upregulating, decreasing, or downregulating expression of a polynucleic acid, gene such as a gene of interest, DNA, or RNA such as a target mRNA, as used herein, can refer to modulating, increasing, upregulating, decreasing, downregulating the level of protein encoded by a polynucleic acid, gene such as a gene of interest, DNA, or RNA such as a target mRNA by affecting transcription and/or translation of the polynucleic acid, gene such as a gene of interest, DNA, or RNA such as a target mRNA. In some instances, inhibiting expression of a polynucleic acid, gene such as a gene of interest, DNA, or RNA such as a

target mRNA can refer to affecting transcription and/or translation of the polynucleic acid, gene such as a gene of interest, DNA, or RNA such as a target mRNA such that the level of protein encoded by the polynucleic acid, gene such as a gene of interest, DNA, or RNA such as a target mRNA is reduced or abolished.

- 5 **[0180]** For example, provided herein, are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs encoding or comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA sequence encodes a
- 10 IL-4, and wherein the second RNA sequence encodes a small interfering RNA (siRNA) capable of binding to a TNF-alpha mRNA, thereby modulating the expression of the TNF-alpha mRNA and IL-4 from a single RNA transcript.

- [0181]** For example, provided herein, are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant
- 15 polynucleic acid or RNA constructs encoding or comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA sequence encodes a IGF-1, and wherein the second RNA sequence encodes a small interfering RNA (siRNA) capable of binding to an IL-8 mRNA, thereby modulating the expression of the IL-8 mRNA
- 20 and IGF-1 from a single RNA transcript.

- [0182]** For example, provided herein, are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs encoding or comprising a first RNA sequence encoding a
- 25 gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA sequence encodes a IGF-1, and wherein the second RNA sequence encodes a small interfering RNA (siRNA) capable of binding to an IL-1beta mRNA, thereby modulating the expression of the IL-1beta mRNA and IGF-1 from a single RNA transcript.

- [0183]** For example, provided herein, are methods of modulating expression of two or more
- 30 genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs encoding or comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA sequence encodes a IL-4, and wherein the second RNA sequence encodes a small interfering RNA (siRNA)

capable of binding to an IL-17 mRNA, thereby modulating the expression of the IL-17 mRNA and IL-4 from a single RNA transcript.

[0184] For example, provided herein, are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs encoding or comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA sequence encodes a IL-4, and wherein the second RNA sequence encodes a small interfering RNA (siRNA) capable of binding to a TNF-alpha mRNA, and siRNA capable of binding to an IL-17 mRNA, thereby modulating the expression of the TNF-alpha mRNA, IL-17 mRNA, and IL-4 from a single RNA transcript.

[0185] For example, provided herein, are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs encoding or comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence, wherein the linker RNA sequence links the first RNA sequence and the second RNA sequence, wherein the first RNA sequence encodes a IGF-1, and wherein the second RNA sequence encodes a small interfering RNA (siRNA) capable of binding to a Turbo GFP mRNA, thereby modulating the expression of the Turbo GFP mRNA and IGF-1 from a single RNA transcript.

[0186] Provided herein are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA encodes IL-4, and wherein the second RNA encodes a small interfering RNA (siRNA) capable of binding to a TNF-alpha mRNA; wherein the expression of IL-4 and TNF-alpha is modulated simultaneously, i.e., the expression of IL-4 is upregulated and the expression of TNF-alpha is downregulated simultaneously. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to the same region of a TNF-alpha mRNA. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to a different region of a TNF-alpha mRNA. In related aspects, each of the at least 3 siRNAs may be directed to the same, different, or a combination thereof. In related aspects, recombinant RNA constructs may

comprise a sequence shown in SEQ ID NOs: 4-9 or 100-105 (Cpd.4-Cpd.9). In related aspects, recombinant polynucleic acid constructs may comprise a sequence shown in SEQ ID NOs: 16-21 (Cpd.4-Cpd.9).

[0187] Also provided herein are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA encodes IGF-1, and wherein the second RNA encodes a small interfering RNA (siRNA) capable of binding to an IL-8 mRNA; wherein the expression of IGF-1 and IL-8 is modulated simultaneously, i.e., the expression of IGF-1 is upregulated and the expression of IL-8 is downregulated simultaneously. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to the same region of an IL-8 mRNA. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to a different region of an IL-8 mRNA. In related aspects, each of the at least 3 siRNAs may be directed to the same, different, or a combination thereof. In related aspects, recombinant RNA constructs may comprise a sequence comprising SEQ ID NO: 1 (Cpd.1), SEQ ID NO: 97 (Cpd.1), SEQ ID NO: 2 (Cpd.2), or SEQ ID NO: 98 (Cpd.2). In related aspects, recombinant polynucleic acid constructs may comprise a sequence comprising SEQ ID NO: 13 (Cpd.1) or SEQ ID NO: 14 (Cpd.2).

[0188] Also provided herein are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the first RNA encodes IGF-1, and wherein the second RNA encodes a small interfering RNA (siRNA) capable of binding to an IL-1beta mRNA; wherein the expression of IGF-1 and IL-1beta is modulated simultaneously, i.e., the expression of IGF-1 is upregulated and the expression of IL-1beta is downregulated simultaneously. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to the same region of an IL-1beta mRNA. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to a different region of an IL-

1 beta mRNA. In related aspects, each of the at least 3 siRNAs may be directed to the same, different, or a combination thereof. In related aspects, recombinant polynucleic acid constructs or recombinant RNA constructs may comprise a sequence comprising in SEQ ID NO: 3 or SEQ ID NO: 99 (Cpd.3). In related aspects, recombinant polynucleic acid constructs
5 may comprise a sequence comprising in SEQ ID NO: 15 (Cpd.3).

[0189] Also provided herein are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of
10 one or more target RNAs, wherein the first RNA encodes IL-4, and wherein the second RNA encodes a small interfering RNA (siRNA) capable of binding to an IL-17 mRNA; wherein the expression of IL-4 and IL-17 is modulated simultaneously, i.e., the expression of IL-4 is upregulated and the expression of IL-17 is downregulated simultaneously. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6,
15 or more siRNAs. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to the same region of an IL-17 mRNA. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to a different region of an IL-17 mRNA. In related aspects, each of the at least 3 siRNAs may be directed to the same, different, or a combination thereof. In related aspects,
20 recombinant RNA constructs may comprise a sequence comprising in SEQ ID NO: 4 or SEQ ID NO: 100 (Cpd.4). In related aspects, recombinant polynucleic acid constructs may comprise a sequence comprising in SEQ ID NO: 16 (Cpd.4).

[0190] Also provided herein are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs comprising a first RNA sequence encoding a gene of interest, and a second RNA sequence comprising at least two genetic elements that modulate expression of
25 one or more target RNAs, wherein the first RNA encodes IL-4, and wherein the second RNA encodes a small interfering RNA (siRNA) capable of binding to a TNF-alpha mRNA and siRNA capable of binding to an IL-17 mRNA; wherein the expression of IL-4, TNF-alpha, and IL-17 is modulated simultaneously, i.e., the expression of IL-4 is upregulated and the expression of TNF-alpha and IL-17 is downregulated simultaneously. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise at least 2, 3, 4, 5, 6,
30 or more siRNAs. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to the same region of a TNF-alpha mRNA. In

related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to the same region of an IL-17 mRNA. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to a different region of a TNF-alpha and/or an IL-17 mRNA. In related aspects, each of the at least 3 siRNAs may be directed to the same, different, or a combination thereof. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 6 siRNAs, wherein 3 of 6 siRNAs are directed to a different region of a TNF-alpha and the other 3 siRNAs are directed to a different region of an IL-17 mRNA. In related aspects, recombinant RNA constructs may comprise a sequence comprising in SEQ ID NO: 4 or SEQ ID NO: 100 (Cpd.4). In related aspects, recombinant polynucleic acid constructs may comprise a sequence comprising in SEQ ID NO: 16 (Cpd.4).

[0191] Also provided herein are methods of modulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs comprising a first RNA sequence, a second RNA sequence, and a linker RNA sequence, wherein the linker RNA sequence links the first RNA sequence and the second RNA sequence, wherein the first RNA encodes IGF-1, and wherein the second RNA encodes a small interfering RNA (siRNA) capable of binding to a Turbo GFP mRNA; wherein the expression of IGF-1 and Turbo GFP is modulated simultaneously, i.e., the expression of IGF-1 is upregulated and the expression of Turbo GFP is downregulated simultaneously. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise at least 1, 2, 3, 4, 5, 6, or more siRNAs. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to the same region of a Turbo GFP mRNA. In related aspects, recombinant polynucleic acid or RNA constructs may encode or comprise 3 siRNAs, each directed to a different region of a Turbo GFP mRNA. In related aspects, each of the at least 3 siRNAs may be directed to the same, different, or a combination thereof. In related aspects, recombinant RNA constructs may comprise a sequence selected from the group consisting of SEQ ID NOs: 10-12 and 106-108 (Cpd.10-Cpd.12). In related aspects, recombinant polynucleic acid constructs may comprise a sequence selected from the group consisting of SEQ ID NOs: 22-24 (Cpd.10-Cpd.12).

[0192] Provided herein are methods of upregulating and downregulating expression of two or more genes in a cell, comprising introducing into the cell compositions comprising recombinant polynucleic acid or RNA constructs encoding or comprising a first RNA sequence encoding a gene of interest (e.g., IL-4 or IGF-1), and a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target

RNAs, wherein the second RNA encodes a small interfering RNA (siRNA) capable of binding to a target mRNA (*e.g.*, TNF-alpha IL-8, IL-17, Turbo GFP, or IL-1beta); wherein the target mRNA is different from an mRNA encoded by the gene of interest, and wherein the expression of the target mRNA is downregulated and the expression of the gene of interest is upregulated simultaneously. In some embodiments, the expression of the target mRNA is downregulated by the siRNA capable of binding to the target mRNA. In some embodiments, the expression of the gene of interest is upregulated by expressing an mRNA or a protein encoded by the gene of interest.

10 **Illustrative embodiments**

[0193] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the first RNA sequence of (i) and a corresponding second RNA sequence of (ii) with at most one of the at least two genetic elements. In some embodiments, the recombinant RNA construct comprises one or more uridines. In some embodiments, the recombinant RNA construct does not comprise a modified uridine.

[0194] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct does not comprise a nucleotide variant. In some embodiments, the nucleotide variant comprises a modified uridine. In some embodiments, the modified uridine comprises a N1-methylpseudouridine.

[0195] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct does not comprise a modified uridine.

[0196] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or

more target RNAs, wherein the recombinant RNA construct does not comprise a N1-methylpseudouridine.

[0197] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct comprises solely unmodified nucleotides or natural nucleotides.

[0198] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising (i) a first RNA sequence encoding a gene of interest, and (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs, wherein the recombinant RNA construct comprises uridines, wherein: (a) all uridines comprised by the recombinant RNA constructs are unmodified or natural nucleotide(s); or (b) at least one of the uridines comprised by the recombinant RNA constructs is an unmodified uridine.

[0199] In some embodiments, the corresponding recombinant RNA construct does not comprise any of the genetic elements that modulate expression of one or more target RNAs. In some embodiments, the second RNA sequence comprises at least three genetic elements that modulate expression of one or more target RNAs. In some embodiments, the second RNA sequence comprises at least six genetic elements that modulate expression of one or more target RNAs. In some embodiments, the second RNA sequence comprises at least two or at least four genetic elements that modulate expression of one or more target RNAs. In some embodiments, the second RNA sequence comprises two or four or more genetic elements that modulate expression of one or more target RNAs.

[0200] In some embodiments, the first RNA sequence is a messenger RNA (mRNA) sequence. In some embodiments, each of the at least two genetic elements of the second RNA sequence comprises a secondary structure. In some embodiments, each of the at least two genetic elements of the second RNA sequence comprises a hairpin structure or a loop structure. In some embodiments, each of the at least two genetic elements of the second RNA sequence is a short or small hairpin RNA (shRNA). In some embodiments, each of the at least two genetic elements of the second RNA sequence is processed or cleaved by an intracellular protein. In some embodiments, each of the at least two genetic elements of the second RNA sequence is processed or cleaved by an endogenous protein of a cell. In some embodiments, each of the at least two genetic elements of the second RNA sequence is processed or cleaved by an endogenous Dicer. In some embodiments, each of the at least two genetic elements of

the second RNA sequence comprises a small interfering RNA (siRNA). In some embodiments, each of the at least two genetic elements of the second RNA sequence is capable of binding to the one or more target RNAs. In some embodiments, the second RNA sequence comprises at least two or at least four siRNAs that modulate expression of one or more target RNAs. In some embodiments, the second RNA sequence comprises two or four or more siRNAs that modulate expression of one or more target RNAs.

[0201] In some embodiments, the immune response is a human Toll-Like Receptor 7 (TLR7) immune response, an interferon alpha/beta (IFN α / β) immune response, a human Toll-Like Receptor 3 (TLR3) immune response, a human Toll-Like Receptor 8 (TLR8) immune response, or any combination thereof. In some embodiments, contacting the human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with the corresponding recombinant RNA construct according to a human Toll-Like Receptor 7 (TLR7) immunogenicity assay. In some embodiments, the human TLR7 immunogenicity assay measures activation of NF- κ B and/or AP1. In some embodiments, the human TLR7 immunogenicity assay is performed in HEK293 cells or a derivative thereof. In some embodiments, the HEK293 cells are engineered to express hTLR7 and a reporter gene. In some embodiments, the reporter gene is a secreted reporter gene. In some embodiments, the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP). In some embodiments, the reporter gene is under the control of a promoter with one or more NF- κ B and/or AP1 binding sites. In some embodiments, the promoter is an IFN- β minimal promoter. In some embodiments, the immune response in the human cell contacted with the recombinant RNA construct is at least 1.5 fold or at least 2 fold less than the immune response in the human cell contacted with the corresponding recombinant RNA construct.

[0202] In some embodiments, contacting the human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with the corresponding recombinant RNA construct according to an interferon alpha/beta (IFN α / β) immunogenicity assay. In some embodiments, the IFN α / β immunogenicity assay measures activation of JAK-STAT and/or ISG3. In some embodiments, the IFN α / β immunogenicity assay is performed in HEK293 cells or a derivative thereof. In some embodiments, the HEK293 cells are engineered to express human STAT2 and/or IRF9 genes and a reporter gene. In some embodiments, the reporter gene is a secreted reporter gene. In some embodiments, the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP). In some embodiments, the reporter gene is under the control of

a promoter with one or more STAT2 and/or IRF9 binding sites. In some embodiments, the promoter is an ISG54 promoter. In some embodiments, the immune response in the human cell contacted with the recombinant RNA construct is at least 1.5 fold, at least 2 fold, or at least 100 fold less than the immune response in the human cell contacted with the
5 corresponding recombinant RNA construct.

[0203] In some embodiments, contacting the human cell with the recombinant RNA construct does not result in a substantial immune response according to a human Toll-Like Receptor 3 (TLR3) immunogenicity assay. In some embodiments, the human TLR3 immunogenicity assay measures activation of NF- κ B and/or AP1. In some embodiments, the human TLR3
10 immunogenicity assay is performed in HEK293 cells or a derivative thereof. In some embodiments, the HEK293 cells are engineered to express hTLR3 and a reporter gene. In some embodiments, the reporter gene is a secreted reporter gene. In some embodiments, the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP). In some
15 embodiments, the reporter gene is under the control of a promoter with one or more NF- κ B and/or AP1 binding sites. In some embodiments, the promoter is an IFN- β minimal promoter.

[0204] In some embodiments, contacting the human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with the corresponding recombinant RNA construct according to a human Toll-Like Receptor 8 (TLR8) immunogenicity assay. In some embodiments, the human TLR8
20 immunogenicity assay measures activation of NF- κ B, AP1, and/or IRF. In some embodiments, the human TLR8 immunogenicity assay is performed in HEK293 cells or a derivative thereof. In some embodiments, the HEK293 cells are engineered to express hTLR8 and a reporter gene. In some embodiments, the reporter gene is a secreted reporter gene. In
25 some embodiments, the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP). In some embodiments, the reporter gene is under the control of a promoter with one or more NF- κ B and/or AP1 binding sites. In some embodiments, the promoter is an IFN- β minimal promoter.

[0205] In some embodiments, the immune response induces the expression of a proinflammatory cytokine in a cell. In some embodiments, the proinflammatory cytokine
30 comprises Interleukin 6 (IL-6). In some embodiments, the cell comprises a human lung epithelial carcinoma cell (A549) or a human monocyte leukemia cell (THP-1).

[0206] In some embodiments, the second RNA sequence comprises 2, 3, 4, 5, 6, or more species of siRNA, wherein the 2, 3, 4, 5, 6, or more species of siRNA include siRNAs that are capable of binding to: (i) different target RNAs; (ii) different regions of the same target RNA;

(iii) the same region of the same target RNA; or (iv) any combination thereof. In some embodiments, the second RNA sequence comprises at least 3 species of siRNA. In some embodiments, the second RNA sequence comprises at least 6 species of siRNA.

[0207] In some embodiments, the recombinant RNA construct further comprises one or more linkers. In some embodiments, each of the one or more linkers has a structure selected from the group consisting of: Formula (I): $X_mCAACAAX_n$, wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4; and Formula (II): X_pTCCCX_r , wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13. In some embodiments, each of the one or more linkers comprises a sequence comprising ACAACAA.

In some embodiments, each of the one or more linkers is not (a) TTTATCTTAGAGGCATATCCCTACGTACCAACAA or ATAGTGAGTCGTATTAACGTACCAACAA; or (b) does not form a secondary structure according to RNAfold WebServer. In some embodiments, each of the one or more linkers is present between (a) the first RNA sequence and the second RNA sequence, (b) each of the 2, 3, 4, 5, 6, or more species of siRNA of the second RNA sequence, or (c) both (a) and (b). In some embodiments, each of the one or more linkers comprises a sequence independently selected from the group consisting of SEQ ID NOs: 27, 28, 85-95.

[0208] In some embodiments, the expression of the gene of interest is modulated. In some embodiments, the expression of the gene of interest is upregulated in a cell comprising the recombinant RNA construct. In some embodiments, the expression of a protein encoded by the gene of interest is upregulated in a cell comprising the recombinant RNA construct.

[0209] In some embodiments, the expression of the one or more target RNAs is modulated. In some embodiments, the expression of the one or more target RNAs is downregulated by the genetic elements that modulate expression of the one or more target RNAs. In some embodiments, the genetic elements that modulate expression of the one or more target RNAs do not inhibit the expression of the gene of interest.

[0210] In some embodiments, the gene of interest is selected from the group consisting of Interleukin 4 (IL-4) and Insulin-like Growth Factor 1 (IGF-1). In some embodiments, the one or more target RNA comprises a noncoding RNA or a messenger RNA (mRNA). In some embodiments, each of the one or more target RNA is a noncoding RNA. In some embodiments, each of the one or more target RNA is an mRNA. In some embodiments, the target RNA is an mRNA encoding a protein comprising Interleukin 8 (IL-8), Interleukin 1 beta (IL-1 beta), Tumor Necrosis Factor alpha (TNF-alpha), Interleukin 17 (IL-17), or a functional variant thereof.

[0211] In some embodiments, the genetic elements that modulate expression of the one or more target RNAs binds to an exon of the one or more target RNAs. In some embodiments, the genetic elements that modulate expression of the one or more target RNAs specifically binds to one target RNA. In some embodiments, the genetic elements that modulate expression of the one or more target RNAs are not encoded by or comprised of an intron sequence of the gene of interest. In some embodiments, the gene of interest is expressed without RNA splicing.

[0212] In some embodiments, the first RNA sequence is present downstream or 3' of the second RNA sequence. In some embodiments, the first RNA sequence is present upstream or 5' of the second RNA sequence. In some embodiments, the RNA construct comprises an internal ribosome entry site (IRES) upstream or 5' of the first RNA sequence.

[0213] In some embodiments, the compositions described herein further comprises a poly(A) tail, a 5' cap, or a Kozak sequence. In some embodiments, the first RNA sequence and the second RNA sequence are both recombinant. In some embodiments, the siRNA comprises a sense strand sequence selected from SEQ ID NOs: 57-70.

[0214] In some aspects, provided herein, is a composition for use in modulating the expression of two or more genes in a cell. In some aspects, provided herein, is a pharmaceutical composition comprising a therapeutically effective amount of any one of the compositions described herein and a pharmaceutically acceptable excipient. In some aspects, provided herein, is a vector comprising a recombinant polynucleic acid construct encoding any one of the compositions described herein. In some aspects, provided herein, is a cell comprising any one of the compositions described herein or any one of the vectors described herein.

[0215] In some aspects, provided herein, is a method of simultaneously expressing an siRNA and an mRNA from a single RNA transcript in a cell, comprising introducing into the cell any one of the compositions described herein, or any one of the vectors described herein.

[0216] In some aspects, provided herein, is a method of treating a disease or condition comprising administering to a subject in need thereof any one of the compositions described herein or any one of the pharmaceutical compositions described herein.

[0217] In some embodiments, the disease or condition comprises a skin disease or condition or a joint disease or condition. In some embodiments, the skin disease or condition comprises an inflammatory skin disorder. In some embodiments, the inflammatory skin disorder comprises psoriasis. In some embodiments, the joint disease or condition comprises a joint

degeneration. In some embodiments, the joint degeneration comprises intervertebral disc disease (IVDD) or osteoarthritis (OA). In some embodiments, the subject is a human.

[0218] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to an Interleukin-8 (IL-8) mRNA, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IGF-1 of (i) and at most one of the at least two siRNAs capable of binding to the IL-8 mRNA of (ii).

[0219] In some aspects, provided herein, is a composition comprising recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Interleukin-1 beta (IL-1 beta) mRNA, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IGF-1 of (i) and at most one of the at least two siRNAs capable of binding to the IL-1beta mRNA of (ii).

[0220] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA, wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IL-4 of (i) and at most one of the at least two siRNAs capable of binding to the TNF-alpha mRNA of (ii).

[0221] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA and Interleukin 17 (IL-17), wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IL-4 of (i) and at most one of the at least two siRNAs capable of binding to the TNF-alpha mRNA and IL-17 mRNA of (ii).

[0222] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to an Interleukin-8 (IL-8) mRNA, wherein the recombinant RNA construct does not comprise a nucleotide variant.

[0223] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to an Interleukin-8 (IL-8) mRNA, wherein the recombinant RNA construct does not comprise a modified uridine.

[0224] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to an Interleukin-8 (IL-8) mRNA, wherein the recombinant RNA construct does not comprise a N1-methylpseudouridine.

[0225] In some aspects, provided herein, is a composition comprising a recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to an Interleukin-8 (IL-8) mRNA, wherein the recombinant RNA construct comprises uridines, wherein (a) all uridines comprised by the recombinant RNA constructs are unmodified or natural nucleotide(s); or (b) at least one of the uridines comprised by the recombinant RNA constructs is an unmodified uridine.

[0226] In some aspects, provided herein, is a composition comprising recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Interleukin-1 beta (IL-1 beta) mRNA, wherein the recombinant RNA construct does not comprise a nucleotide variant.

[0227] In some aspects, provided herein, is a composition comprising recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Interleukin-1 beta (IL-1 beta) mRNA, wherein the recombinant RNA construct does not comprise a modified uridine.

[0228] In some aspects, provided herein, is a composition comprising recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1

(IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Interleukin-1 beta (IL-1 beta) mRNA, wherein the recombinant RNA construct does not comprise a N1-methylpseudouridine.

[0229] In some aspects, provided herein, is a composition comprising recombinant RNA construct comprising: (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Interleukin-1 beta (IL-1 beta) mRNA, wherein the recombinant RNA construct comprises uridines, wherein (a) all uridines comprised by the recombinant RNA constructs are unmodified or natural nucleotide(s); or (b) at least one of the uridines comprised by the recombinant RNA constructs is an unmodified uridine.

[0230] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA, wherein the recombinant RNA construct does not comprise a nucleotide variant.

[0231] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA, wherein the recombinant RNA construct does not comprise a modified uridine.

[0232] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA, wherein the recombinant RNA construct does not comprise a N1-methylpseudouridine.

[0233] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA, wherein the recombinant RNA construct comprises uridines, wherein (a) all uridines comprised by the recombinant RNA constructs are unmodified or natural nucleotide(s); or (b) at least one of the uridines comprised by the recombinant RNA constructs is an unmodified uridine.

[0234] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-

alpha) mRNA and Interleukin 17 (IL-17), wherein the recombinant RNA construct does not comprise a nucleotide variant.

[0235] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA and Interleukin 17 (IL-17), wherein the recombinant RNA construct does not comprise a modified uridine.

[0236] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA and Interleukin 17 (IL-17), wherein the recombinant RNA construct does not comprise a N1-methylpseudouridine.

[0237] In some aspects, provided herein, is a composition comprising recombinant RNA construct: (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA and Interleukin 17 (IL-17), wherein the recombinant RNA construct comprises uridines, wherein (a) all uridines comprised by the recombinant RNA constructs are unmodified or natural nucleotide(s); or (b) at least one of the uridines comprised by the recombinant RNA constructs is an unmodified uridine.

[0238] In some aspects, provided herein, is a composition comprising a recombinant polynucleic acid construct comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-24, 42, 125, 97-108, 121-122, and 127-128.

[0239] In a preferred embodiment, the gene of interest encoded by the first RNA sequence is not an interleukin 2 (IL-2) and the one or more target RNAs modulated by the at least two genetic elements comprised by the second RNA sequence is not vascular endothelial growth factor A (VEGFA), an isoform of VEGFA, MHC class I chain-related sequence A (MICA), or MHC class I chain-related sequence B (MICB).

[0240] In a preferred embodiment, the gene of interest encoded by the first RNA sequence is not an interleukin 2 (IL-2), a fragment thereof, or a functional variant thereof and the one or more target RNAs modulated by the at least two genetic elements comprised by the second RNA sequence is not vascular endothelial growth factor A (VEGFA), an isoform of VEGFA, MHC class I chain-related sequence A (MICA), MHC class I chain-related sequence B (MICB), a fragment thereof, or a functional variant thereof.

EXAMPLES

[0241] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

[0242] Example 1: Construct design, sequence, and synthesis

5 [0243] Construct Design

[0244] Both siRNAs and genes of interest are simultaneously expressed from a single transcript generated by *in vitro* transcription (Table 1; SEQ ID NOs: 1-12 and 97-108). IL-4 and IGF-1 coding sequences originate from *Homo sapiens* and no changes in the resulting amino acid sequences were introduced for IL-4 (hIL-4: NP_000580.1; SEQ ID NO: 31 and 10 32; Cpd.4-Cpd.9) and some of IGF-1 constructs (Cpd.10-Cpd.13; IGF-1:NP_000609.1). To increase secretion of mRNA-induced IGF-1 (NP_000609.1) out of the transfected cell, the endogenous IGF-1 pre-domain (signal peptide; SEQ ID NO: 33 and 34) was exchanged by BDNF (NP_733931.1; SEQ ID NO: 35 and 36) signal peptide (BDNF-pro-IGF-1) in Cpd.1 to Cpd.4 mRNA constructs. Furthermore, the construct contained the sequence encoding the full 15 coding sequence of mature human IGF-1 with 70 amino acids (SEQ ID NO: 35 and 36). No C-terminal E-domain was added to the construct. The siRNA target sequence for IL-8 (NM_000584.3; SEQ ID NO: 37), IL-1beta (NM_000576.2; SEQ ID NO: 38), IL-17 (NM_002190.2; SEQ ID NO: 39) and TNF-alpha (NM_000594.3; SEQ ID NO: 40) originate from *Homo sapiens* and no changes to the sequences introduced. Turbo GFP sequence was 20 derived from marine copepod *Pontellina plumate* (SEQ ID NO: 41). To compare mRNA with siRNA structure and without siRNA structure, an IL-4 molecule with optimized signal peptide was used (SEQ ID NO: 42 and 43).

[0245] A polynucleic acid construct may comprise a Kozak sequence, (5' GCCACC 3'; SEQ ID NO:25). In addition, a polynucleic acid construct may comprise a T7 promoter sequence 25 (5' TAATACGACTCACTATA 3'; SEQ ID NO:26) upstream of the gene of interest sequence, for RNA polymerase binding and successful *in vitro* transcription of both the gene of interest and siRNA in a single transcript. An alternative promoter *e.g.*, SP6, T3, P60, Syn5, and KP34 may be used. A transcription template is generated by PCR to produce mRNA, using primers designed to flank the T7 promoter, gene of interest, and siRNA sequences. The 30 reverse primer includes a stretch of thymidine (T) base (120) to add the 120 bp length of poly(A) tail to the mRNA. Some of the polynucleotide or RNA constructs are engineered to include siRNA designs described in Cheng, et al. (2018) J. Mater. Chem. B., 6, 4638-4644, and further comprising one or more gene of interest upstream or downstream of the siRNA sequence with linkers to connect different RNA segments (gene of interest mRNA to siRNA

(SEQ ID NO:27) or siRNA to siRNA (SEQ ID NO:28)). Recombinant constructs may encode or comprise more than one siRNA sequence targeting the same or different target mRNA. Likewise, constructs may comprise nucleic acid sequences of two or more genes of interest.

[0246] Construct Synthesis

- 5 [0247] The constructs as shown in **Table 1** (Compound ID numbers Cpd.1-Cpd.14) were synthesized by GeneArt, Germany (Thermo Fisher Scientific) in pMA-RQ plasmid-backbone vector containing a T7 RNA polymerase promoter with codon optimization (GeneOptimizer algorithm). **Table 1** shows, for each compound (Cpd.), protein to be downregulated through siRNA binding to the corresponding mRNA (siRNA target), siRNA position in the
- 10 compound, the number of siRNAs in the compound, gene of interest and respective indication. The sequences of each construct are shown in **Table 2** and annotated as indicated below the table (SEQ ID NOs: 1-12, 42, 125, 97-108, and 121-122). The plasmid-backbone sequences of each construct are shown in **Table 3** and compound sequence are in bold and underlined (SEQ ID NO: 13-24 and 127-128).

- 15 [0248] **Table 1.** Summary of Compounds

Compound ID	siRNA Target	siRNA Position	# of siRNAs	Protein Target	Indication
1 unmodified*	IL-8	5'	1x	IGF-1	OA, IVDD
1 modified**					
2 unmodified*	IL-8	5'	3x	IGF-1	OA, IVDD
2 modified**					
3 unmodified*	IL-1beta	5'	3x	IGF-1	OA, IVDD
3 modified**					
4 unmodified*	TNF- α /IL-17	5'	6x	IL-4	Psoriasis
4 modified**					
5 unmodified*	TNF- α	5'	3x	IL-4	Psoriasis
5 modified**					
6 unmodified*	TNF- α	3'	3x	IL-4	Psoriasis
6 modified**					
7 unmodified*	TNF- α	5'	1x	IL-4	Psoriasis
7 modified**					
8 unmodified*	TNF- α	3'	1x	IL-4	Psoriasis
8 modified**					
9 unmodified*#	TNF- α	3'	3x	IL-4	Psoriasis
9 modified**#					
10 unmodified*	Turbo GFP	3'	1x	IGF-1	NA
10 modified**					
11 unmodified*	Turbo GFP	3'	2x	IGF-1	NA
11 modified**					
12 unmodified*	Turbo GFP	3'	3x	IGF-1	NA
12 modified**					
13 unmodified*	NA	NA	NA	IGF-1	NA
13 modified**					
14 unmodified*	NA	NA	NA	IL-4	NA
14 modified**					

- *unmodified: all uridines are unmodified uridines and all other nucleotides are unmodified or natural nucleotides, **modified: all uridines are modified (N¹-methylpseudouridine) and all other nucleotides are unmodified or natural nucleotides, #: A2 linker, IL-8: Interleukin-8, IGF-1: Insulin like growth factor-1, OA: Osteoarthritis; IVDD: Intervertebral disc disease; IL-1beta Interleukin 1 beta; TNF- α : Tumor necrosis factor-alpha, IL-17: Interleukin-17, IL-4: Interleukin-4, Turbo GFP: Turbo green fluorescent protein (derived from copepod *Pontellina plumate*).

[0249] Table 2. Sequences of Compounds

SEQ ID NO	Compound	Sequence (5' to 3')
1	Compound 1 unmodified	ATAGTGAGTCGTATTAACGTACCAACA CAAGGAAGTGCTAAAGAA ACTTG TTCTTTAGCACTTCCTTG TTTATCTTAGAGGCATATCCCTGCCACCATGAC CATCCTGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCCGT GAAGATGCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCCTGTGCCT GCTGACCTTTACCAGCTCTGCTACCGCCGGACCTGAGACACTTTGTGGCGC TGAACCTGGTGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTCTACTT CAACAAGCCACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGACCGG AATCGTGGACGAGTGCTGCTTCAGAAGCTGCGACCTGCGGCGGCTGGAAT GTATTGTGCCCTCTGAAGCCTGCCAAGAGCGCCTAATTTATCTTAGAGGC ATATCCCT
2	Compound 2 unmodified	ATAGTGAGTCGTATTAACGTACCAACA CAAGGAGTGCTAAAGAA ACTTG T TCTTTAGCACTCCTTG TTTATCTTAGAGGCATATCCCTACGTACCAACA AG AGAGTGATTGAGAGTGG ACTTG CCACTCTCAATCACTCTCT TTTATCTTAGA GGCATATCCCTACGTACCAACA AGAGAGCTCTGTCTGGACCACTTGGGTCC AGACAGAGCTCTCT TTTATCTTAGAGGCATATCCCTGCCACCATGACCATCC TGTCTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCCGTGAAGA TGCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCCTGTGCCTGCTGA CCTTTACCAGCTCTGCTACCGCCGGACCTGAGACACTTTGTGGCGCTGAAC TGGTGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTCTACTTCAACA AGCCACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGACCGGAATCG TGGACGAGTGCTGCTTCAGAAGCTGCGACCTGCGGCGGCTGGAATGTATT GTGCCCTCTGAAGCCTGCCAAGAGCGCCTAATTTATCTTAGAGGCATATC CCT
3	Compound 3 unmodified	ATAGTGAGTCGTATTAACGTACCAACA GAAAGATGATAAGCCC ACTCTAC TT GAGAGTGGGCTTATCATCTTTCT TTTATCTTAGAGGCATATCCCTACGTA CCAACA AGGTGATGTCTGGTCCATATGA ACTTG TCATATGGACCAGACATC ACCTTTATCTTAGAGGCATATCCCTACGTACCAACAAGATGATAAGCCAC TCTAACTTGTAGAGTGGGCTTATCATCT TTTATCTTAGAGGCATATCCCTGC CACCATGACCATCCTGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCAT GAAGGCCGTGAAGATGCACACCATGAGCAGCAGCCACCTGTTCTATCTGGC CCTGTGCCCTGCTGACCTTTACCAGCTCTGCTACCGCCGGACCTGAGACACT TTGTGGCGCTGAAGTGGTGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGG CTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCTAGAAGGGCTCC TCAGACCGGAATCGTGGACGAGTGCTGCTTCAGAAGCTGCGACCTGCGGCG GCTGGAATGTATTGTGCCCTCTGAAGCCTGCCAAGAGCGCCTAATTTAT CTTAGAGGCATATCCCT
4	Compound 4 unmodified	ATAGTGAGTCGTATTAACGTACCAACA GGCGTGGAGCTGAGAGATAA ACT TG TTATCTCTCAGCTCCACGCCT TTTATCTTAGAGGCATATCCCTACGTACC AACA AGGGCTGTACCTCATCTACTACTTGAGTAGATGAGGTACAGGCCCT TTATCTTAGAGGCATATCCCTACGTACCAACA AGGTATGAGCCCATCTATC TACTTGAGATAGATGGGCTCATACCT TTTATCTTAGAGGCATATCCCTACGT ACCAACA AGCAATGAGGACCTGAGAGATACTTGATCTCTCAGGGTCCTCA TTGCTTTATCTTAGAGGCATATCCCTACGTACCAACAAGCTGATGGGAACG TGGACTAACTTG TAGTCCAGTTCCTCATCAGCT TTTATCTTAGAGGCATATC CCTACGTACCAACA AGGTCTCAGATTACTACAA ACTTG TTGTAGTAATCT GAGGACCTTTATCTTAGAGGCATATCCCTGCCACCATGGGACTGACATCTC AACTGCTGCCTCCACTGTTCTTTCTGCTGGCCTGCGCCGGCAATTTGTGC ACGGCCACAAGTGCACATCACCTGCAAGAGATCATCAAGACCTGAACA GCCTGACCGAGCAGAAAACCTGTGCACCGAGCTGACCGTGACCGATATCT TTGCCGCCAGCAAGAACAACCGAGAAAGAGACATTCTGCAGAGCCGCCA CCGTGCTGAGACAGTTCTACAGCCACCACGAGAAGGACACCAGATGCCTGG

SEQ ID NO	Compound	Sequence (5' to 3')
		GAGCTACAGCCCAGCAGTTCCACAGACACAAGCAGCTGATCCGGTTCCTGA AGCGGCTGGACAGAAATCTGTGGGGACTCGCCGGCTGAATAGCTGCCCTG TGAAAGAGGCCAACCACTCTACCTGGAAAACCTCTGGAACGGCTGAAAA CCATCATGCGCGAGAAGTACAGCAAGTGCAGCAGCTGATTTATCTTAGAGG CATATCCCT
5	Compound 5 unmodified	ATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAGAGATAAACT TG TTATCTCTCAGCTCCACGCCT TTTATCTTAGAGGCATATCCCTACGTACC AACAAGGGCCTGTACCTCATCTACTACTT GAGTAGATGAGGTACAGGCCCT TTATCTTAGAGGCATATCCCTACGTACCAACAAG GATATGAGCCCATCTATC TACTTGAGATAGATGGGCTCATACCT TTTATCTTAGAGGCATATCCCTGCCA CCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCTGCTGGCCT GCGCCGGCAATTTTGTGCACGGCCACAAGTGCACATCACCTGCAAGAGA TCATCAAGACCCTGAACAGCCTGACCGAGCAGAAAAACCTGTGCACCGAGC TGACCGTGACCGATATCTTTGCCGCCAGCAAGAACAACCGAGAAAGAGA CATTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCACGAGA AGGACACCAGATGCCTGGGAGCTACAGCCAGCAGTTCCACAGACACAAGC AGCTGATCCGTTTCTGAAGCGGCTGGACAGAAATCTGTGGGGACTCGCCG GCCTGAATAGCTGCCCTGTGAAAGAGGCCAACCACTCTACCTGGAAAACCT TCCTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGCAGCA GCTGATTTATCTTAGAGGCATATCCCT
6	Compound 6 unmodified	GCCACCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCTGCTG GCCTGCGCCGGCAATTTTGTGCACGGCCACAAGTGCACATCACCTGCAA GAGATCATCAAGACCCTGAACAGCCTGACCGAGCAGAAAAACCTGTGCACC GAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACACAACCGAGAAA GAGACATTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCAC GAGAAGGACACCAGATGCCTGGGAGCTACAGCCAGCAGTTCCACAGACAC AAGCAGCTGATCCGGTTCCTGAAGCGGCTGGACAGAAATCTGTGGGGACTC GCCGGCCTGAATAGCTGCCCTGTGAAAGAGGCCAACCACTCTACCTGGAA AACTTCCTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGC AGCAGCTGAATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAG AGATAAACTTGTTATCTCTCAGCTCCACGCCT TTTATCTTAGAGGCATATCC CTACGTACCAACAAGGGCCTGTACCTCATCTACTACTT GAGTAGATGAGGT ACAGGCCCT TTTATCTTAGAGGCATATCCCTACGTACCAACAAG GATATGAGC CCATCTATCTACTTGAGATAGATGGGCTCATACCT TTTATCTTAGAGGCATA TCCCTTTTATCTTAGAGGCATATCCCT
7	Compound 7 unmodified	ATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAGAGATAAACT TG TTATCTCTCAGCTCCACGCCT TTTATCTTAGAGGCATATCCCTGCCACCA TGGGCTGACATCTCAGTTGCTGCCTCCACTGTTCTTTCTGCTGGCTGCG CCGGCAATTTTGTGCACGGCCACAAGTGCACATCACCTGCAAGAGATCA TCAAGACCCTGAACAGCCTGACCGAGCAGAAAAACCTGTGCACCGAGCTGA CCGTGACCGATATCTTTGCCGCCAGCAAGAACACAACCGAGAAAGAGACAT TCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCACGAGAAGG ACACCAGATGCCTGGGAGCTACAGCCAGCAGTTCCACAGACACAAGCAGC TGATCCGGTTCCTGAAGCGGCTGGACAGAAATCTGTGGGGACTCGCCGGCC TGAATAGCTGCCCTGTGAAAGAGGCCAACCACTCTACCTGGAAAACCTCC TGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGCAGCAGCT AGTTTATCTTAGAGGCATATCCCT
8	Compound 8 unmodified	GCCACCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCTGCTG GCCTGCGCCGGCAATTTTGTGCACGGCCACAAGTGCACATCACCTGCAA GAGATCATCAAGACCCTGAACAGCCTGACCGAGCAGAAAAACCTGTGCACC GAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACACAACCGAGAAA GAGACATTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCAC GAGAAGGACACCAGATGCCTGGGAGCTACAGCCAGCAGTTCCACAGACAC AAGCAGCTGATCCGGTTCCTGAAGCGGCTGGACAGAAATCTGTGGGGACTC GCCGGCCTGAATAGCTGCCCTGTGAAAGAGGCCAACCACTCTACCTGGAA AACTTCCTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGC AGCAGCTGAATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAG AGATAAACTTGTTATCTCTCAGCTCCACGCCT TTTATCTTAGAGGCATATCC CTTTTATCTTAGAGGCATATCCCT
9	Compound 9 unmodified	GCCACCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCTGCTG GCCTGCGCCGGCAATTTTGTGCACGGCCACAAGTGCACATCACCTGCAA

SEQ ID NO	Compound	Sequence (5' to 3')
		GAGATCATCAAGACCCTGAACAGCCTGACCGAGCAGAAAACCCTGTGCACC GAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACAACCGAGAAA GAGACATTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCAC GAGAAGGACACCAGATGCCTGGGAGCTACAGCCAGCAGTTCCACAGACAC AAGCAGCTGATCCGGTTCCTGAAGCGGCTGGACAGAAATCTGTGGGGACTC GCCGGCCTGAATAGCTGCCCTGTGAAAGAGGCCAACAGTCTACCCTGGAA AACTTCCTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGC AGCAGCTGAACAACAAGGCGTGGAGCTGAGAGATAAACTTGT TATCTCTCA GCTCCACGCCACAACAAGGGCCTGTACCTCATCTACTACTTGAGTAGATGA GGTACAGGCCACAACAAGGTATGAGCCCATCTATCTACTTGAGATAGATG GGCTCATACCAACAACATTTATCTTAGAGGCATATCCCT
10	Compound 10 unmodified	GCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAAGTGCTGC TTCTGCGACTTCCTGAAAGTGAAGATGCACACCATGAGCAGCAGCCACCTG TTCTATCTGGCCCTGTGCCTGCTGACCTTTACCAGCTCTGCTACCGCCGGA CCTGAGACACTTTGTGGCGCTGAACTGGTGGACGCCCTGCAGTTTGTGTGT GGCGACAGAGGCTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCT AGAAGGGCTCCTCAGACCGGAATCGTGGACGAGTGTGTTTCAGAAGCTGC GACCTGCGGCGGCTGGAAATGTATTGTGCCCCCTCTGAAGCCTGCCAAGAGC GCCTAAATAGTGAGTCGTATTAACGTACCAACAACA CAACAAGATGAAGAGCA CCAAACTTGTGGTGCTCTTCATCTTGTTGTTTATCTTAGAGGCATATCCC TTTTATCTTAGAGGCATATCCCT
11	Compound 11 unmodified	GCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAAGTGCTGC TTCTGCGACTTCCTGAAAGTGAAGATGCACACCATGAGCAGCAGCCACCTG TTCTATCTGGCCCTGTGCCTGCTGACCTTTACCAGCTCTGCTACCGCCGGA CCTGAGACACTTTGTGGCGCTGAACTGGTGGACGCCCTGCAGTTTGTGTGT GGCGACAGAGGCTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCT AGAAGGGCTCCTCAGACCGGAATCGTGGACGAGTGTGTTTCAGAAGCTGC GACCTGCGGCGGCTGGAAATGTATTGTGCCCCCTCTGAAGCCTGCCAAGAGC GCCTAAATAGTGAGTCGTATTAACGTACCAACAACA CAACAAGATGAAGAGCA CCAAACTTGTGGTGCTCTTCATCTTGTTGTTTATCTTAGAGGCATATCCC TACGTACCAACAACAACAAGATGAAGAGCACCAAACTTGTGGTGCTCTTC ATCTTGTTGTTTATCTTAGAGGCATATCCCTTTTATCTTAGAGGCATATCC CT
12	Compound 12 unmodified	GCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAAGTGCTGC TTCTGCGACTTCCTGAAAGTGAAGATGCACACCATGAGCAGCAGCCACCTG TTCTATCTGGCCCTGTGCCTGCTGACCTTTACCAGCTCTGCTACCGCCGGA CCTGAGACACTTTGTGGCGCTGAACTGGTGGACGCCCTGCAGTTTGTGTGT GGCGACAGAGGCTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCT AGAAGGGCTCCTCAGACCGGAATCGTGGACGAGTGTGTTTCAGAAGCTGC GACCTGCGGCGGCTGGAAATGTATTGTGCCCCCTCTGAAGCCTGCCAAGAGC GCCTAAATAGTGAGTCGTATTAACGTACCAACAACA CAACAAGATGAAGAGCA CCAAACTTGTGGTGCTCTTCATCTTGTTGTTTATCTTAGAGGCATATCCC TACGTACCAACAACAACAAGATGAAGAGCACCAAACTTGTGGTGCTCTTC ATCTTGTTGTTTATCTTAGAGGCATATCCCTACGTACCAACAACAACAAGA TGAAGAGCACCAAACTTGTGGTGCTCTTCATCTTGTTGTTTATCTTAGAG GCATATCCCTTTTATCTTAGAGGCATATCCCT
125	Compound 13 unmodified	GCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAAGTGCTGC TTCTGCGACTTCCTGAAAGTGAAGATGCACACCATGAGCAGCAGCCACCTG TTCTATCTGGCCCTGTGCCTGCTGACCTTTACCAGCTCTGCTACCGCCGGA CCTGAGACACTTTGTGGCGCTGAACTGGTGGACGCCCTGCAGTTTGTGTGT GGCGACAGAGGCTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCT AGAAGGGCTCCTCAGACCGGAATCGTGGACGAGTGTGTTTCAGAAGCTGC GACCTGCGGCGGCTGGAAATGTATTGTGCCCCCTCTGAAGCCTGCCAAGAGC GCCTAA
42	Compound 14 unmodified	GCCACCATGTTGCTGCTGCCTCTGTTCTTCTGCTGGCCTGCGCCGGCAAT TTTGTGCACGGCCACAAGTGCGACATACCCTGCAGAGATCATCAAGACC CTGAACAGCCTGACCGAGCAGAAAACCCTGTGCACCGAGCTGACCGTGACC GATATCTTTGCCGCCAGCAAGAACAACAACCGAGAAAGAGACATTCTGCAGA GCCGCCACCGTGCTGAGACAGTTCTACAGCCACCAGAGAGGACACAGA TGCCCTGGGAGCTACAGCCAGCAGTTCCACAGACACAAGCAGCTGATCCGG TTCCTGAAGCGGCTGGACAGAAATCTGTGGGGACTCGCCGGCCTGAATAGC

SEQ ID NO	Compound	Sequence (5' to 3')
		TGCCCTGTGAAAGAGGCCAACCACTACCTGGAAACTTCCTGGAACGG CTGAAACCATCATGCGGAGAAGTACAGCAAGTGCAGCAGCTGA

Bold = Sense siRNA strand

Bold and Italics = anti-Sense siRNA strand

Underline = Signal peptide

Italics = Kozak sequence

5

[0250] Table 3. Plasmid Sequences

SEQ ID NO	Compound #	Sequence (5' → 3' direction)
13	Compound 1 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCAT CGCCATTACGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCAGGCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCAT <i>ATAGTGAGTCGTATTAACGTACCAACAACAAGGAAGTGCTAAAGAA</i> <i>CTTGTTCTTTAGCACTTCCTTGTTTATCTTAGAGGCATATCCCTGCCACCAT</i> <i>GACCATCCTGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCC</i> <i>GTGAAGATGCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCCTGTGCC</i> <i>TGCTGACCTTTACCAGCTCTGCTACCGCCGGACCTGAGACACTTTGTGGCGC</i> <i>TGAACTGGTGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTCTACTTC</i> <i>AACAAGCCACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGACCGGAA</i> <i>TCGTGGACGAGTGCTGCTTCAGAAGCTGCGACCTGCGGCGGCTGGAATGTA</i> <i>TTGTGCCCCCTCTGAAGCCTGCCAAGAGCGCCTAATTTATCTTAGAGGCATAT</i> <i>CCCT</i> CTGGGCTCATGGGCTTCCGCTCACTGCCGCTTTCCAGTCGGGAA CCTGTCTGTCAGCTGCATTAAACATGGTCATAGCTGTTTCTTTCGCTATTGG GCGCTCTCCGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTTCGGGTAA AGCCTGGGCTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAA AAGGCCGCTTGTGGGCTTTTCCATAGGCTCCGCCCCCTGACGAGCATC ACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAG ATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACC CTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGC TTTCTCATAGCTCACGCTGTAGGTATCTCAGTTTCGGTGTAGGTGTTTCGCTC CAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCTTA TCCGGTAATATCGTCTTGAGTCCAACCCGGTAAGACACGACTATCGCCAC TGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGC TACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTA TTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAGAGAGTTGGTA GCTCTTGATCCGGCAACAAACACCGCTGGTAGCGGTGGTTTTTTTGTGTTG CAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATC TTTTCTACGGGCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGATTT TGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTA ATGAAGTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGT TACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTT CATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGG CTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACCG GCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAA GTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGA AGCTAGAGTAAGTAGTTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATT GCTACAGGCATCGTGGTGTACGCTCGTCTGTTTGGTATGGCTTCATTACGCT CCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAA AGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAAGTTGGCCGCA GTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTTTACTGTCACTGC CATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTG AGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGAT AATACCGGCCACATAGCAGAACTTAAAGTGCTCATCATTGGAACCGTT CTTCCGGGCGAAAACCTCAAGGATCTTACCGCTGTTGAGATCCAGTTTCAT GTAACCACTCGTGCACCAACTGATCTTCAGCATCTTTACTTTACACGAC

		GTTCTGGGTGAGCAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAA GGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTG AAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATT TAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG GGTAAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATATAGTGAGTCGTATTAAACGTACCAACAACAGGAGTGCTAAAGAAAC TTGTTCTTTAGCACTCCTTGTATTCTTTAGAGGCATATCCCTACGTACCAAC AAGAGAGTGATTGAGAGTGGACTTGCCACTCTCAATCACTCTCTTTATCTTA GAGGCATATCCCTACGTACCAACAAGAGAGCTCTGTCTGGACCACTTGGGTC CAGACAGAGCTCTCTTTATCTTTAGAGGCATATCCCTGCCACCATGACCATCC TGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCGTGAAGAT GCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCCTGTGCCTGTGACC TTTACCAGCTCTGCTACCGCCGGACCTGAGACACTTTGTGGCGCTGAAGTGG TGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTCTACTTCAACAAGCC CACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGACCGGAATCGTGGAC GAGTGCTGCTTCAGAAGCTGCGACCTGCGGGCGCTGGAAATGTATTGTGCC CTCTGAAGCCTGCCAAGAGCGCCTAATTTATCTTAGAGGCATATCCCTCTGG GCCTCATGGGCCTTCCGCTCACTGCCCCTTTCCAGTCGGGAAACCTGTCTGT GCCAGCTGCATTAACATGGTCATAGCTGTTTCTTTCGCTATTGGGCGCTCTC CGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCGGGTAAAGCCTGGG GTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGC GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAAT CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACAGG CGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCT TACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAT AGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTTCGCTCCAAGCTGG GCTGTGTGCACGAACCCCCGTTACGCCCCGACCGCTGCGCCTTATCCGGTAA CTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGT TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGA TCCGGCAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGC AGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC GGGGTCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATTTTGGTCATG AGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAGATT TTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATG CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATA GTTGCCTGACTCCCCGTGCTGTAGATAACTACGATACGGGAGGGCTTACCAT CTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACC GGCTCCAGA TTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCT GCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAG TAAGTAGTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGG CATCGTGGTGTACGCTCGTCTTGGTATGGCTTCATTACGCTCCGGTTCC CAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTA GCTCCTTCGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTTATC ACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTATGCCATCCGTA AGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGT GTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC GCCACATAGCAGAACTTTAAAAGTGCTCATCATTTGAAAACGTTCTTCGGGG CGAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCA CTCGTGCACCCAATGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGG GTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACA CGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTT ATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC
14	Compound 2 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG GGTAAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATATAGTGAGTCGTATTAAACGTACCAACAACAGGAGTGCTAAAGAAAC TTGTTCTTTAGCACTCCTTGTATTCTTTAGAGGCATATCCCTACGTACCAAC AAGAGAGTGATTGAGAGTGGACTTGCCACTCTCAATCACTCTCTTTATCTTA GAGGCATATCCCTACGTACCAACAAGAGAGCTCTGTCTGGACCACTTGGGTC CAGACAGAGCTCTCTTTATCTTTAGAGGCATATCCCTGCCACCATGACCATCC TGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCGTGAAGAT GCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCCTGTGCCTGTGACC TTTACCAGCTCTGCTACCGCCGGACCTGAGACACTTTGTGGCGCTGAAGTGG TGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTCTACTTCAACAAGCC CACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGACCGGAATCGTGGAC GAGTGCTGCTTCAGAAGCTGCGACCTGCGGGCGCTGGAAATGTATTGTGCC CTCTGAAGCCTGCCAAGAGCGCCTAATTTATCTTAGAGGCATATCCCTCTGG GCCTCATGGGCCTTCCGCTCACTGCCCCTTTCCAGTCGGGAAACCTGTCTGT GCCAGCTGCATTAACATGGTCATAGCTGTTTCTTTCGCTATTGGGCGCTCTC CGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCGGGTAAAGCCTGGG GTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGC GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAAT CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACAGG CGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCT TACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAT AGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTTCGCTCCAAGCTGG GCTGTGTGCACGAACCCCCGTTACGCCCCGACCGCTGCGCCTTATCCGGTAA CTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGT TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGA TCCGGCAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGC AGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC GGGGTCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATTTTGGTCATG AGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAGATT TTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATG CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATA GTTGCCTGACTCCCCGTGCTGTAGATAACTACGATACGGGAGGGCTTACCAT CTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACC GGCTCCAGA TTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCT GCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAG TAAGTAGTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGG CATCGTGGTGTACGCTCGTCTTGGTATGGCTTCATTACGCTCCGGTTCC CAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTA GCTCCTTCGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTTATC ACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTATGCCATCCGTA AGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGT GTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC GCCACATAGCAGAACTTTAAAAGTGCTCATCATTTGAAAACGTTCTTCGGGG CGAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCA CTCGTGCACCCAATGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGG GTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACA CGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTT ATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC
15	Compound 3	CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG GGTAAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATATAGTGAGTCGTATTAAACGTACCAACAACAGGAGTGCTAAAGAAAC TTGTTCTTTAGCACTCCTTGTATTCTTTAGAGGCATATCCCTACGTACCAAC AAGAGAGTGATTGAGAGTGGACTTGCCACTCTCAATCACTCTCTTTATCTTA GAGGCATATCCCTACGTACCAACAAGAGAGCTCTGTCTGGACCACTTGGGTC CAGACAGAGCTCTCTTTATCTTTAGAGGCATATCCCTGCCACCATGACCATCC TGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCGTGAAGAT GCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCCTGTGCCTGTGACC TTTACCAGCTCTGCTACCGCCGGACCTGAGACACTTTGTGGCGCTGAAGTGG TGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTCTACTTCAACAAGCC CACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGACCGGAATCGTGGAC GAGTGCTGCTTCAGAAGCTGCGACCTGCGGGCGCTGGAAATGTATTGTGCC CTCTGAAGCCTGCCAAGAGCGCCTAATTTATCTTAGAGGCATATCCCTCTGG GCCTCATGGGCCTTCCGCTCACTGCCCCTTTCCAGTCGGGAAACCTGTCTGT GCCAGCTGCATTAACATGGTCATAGCTGTTTCTTTCGCTATTGGGCGCTCTC CGCTTCCCTCGCTCACTGACTCGCTGCGCTCGGTGCTTCGGGTAAAGCCTGGG GTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGC GTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAAT CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACAGG CGTTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCCTGTTCCGACCTGCCGCT TACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAT AGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGTTTCGCTCCAAGCTGG GCTGTGTGCACGAACCCCCGTTACGCCCCGACCGCTGCGCCTTATCCGGTAA CTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGCGGTGCTACAGAGT TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGA TCCGGCAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGAAGCAGC AGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC GGGGTCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGATTTTGGTCATG AGATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAATAAGATT TTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATG CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTTCATCCATA GTTGCCTGACTCCCCGTGCTGTAGATAACTACGATACGGGAGGGCTTACCAT CTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACC GGCTCCAGA TTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCT GCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAG TAAGTAGTTCGCCAGTTAATAGTTTGGCGAACGTTGTTGCCATTGCTACAGG CATCGTGGTGTACGCTCGTCTTGGTATGGCTTCATTACGCTCCGGTTCC CAACGATCAAGGCGAGTTACATGATCCCCATGTTGTGCAAAAAGCGGTTA GCTCCTTCGGTCTCCGATCGTTGTGCAAGTAAGTTGGCCGAGTGTTATC ACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTATGCCATCCGTA AGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGT GTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGC GCCACATAGCAGAACTTTAAAAGTGCTCATCATTTGAAAACGTTCTTCGGGG CGAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCA CTCGTGCACCCAATGATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGG GTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACA CGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTT ATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC

	in pMA-RQ	ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAGGC CGCATATAGTGAGTCGTATTAACTACCAACAAGAAAGATGATAAGCCCACT CTACTTGAGAGTGGGCTTATCATCTTTCTTTATCTTAGAGGCATATCCCTAC GTACCAACAAGGTGATGTCTGGTCCATATGAACTTGTGCATATGGACCAGACA TCACCTTTATCTTAGAGGCATATCCCTACGTACCAACAAGATGATAAGCCCA CTCTAAGTTGTAGAGTGGGCTTATCATCTTTATCTTAGAGGCATATCCCTGC CACCATGACCATCCTGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATG AAGGCCGTGAAGATGCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCC TGTGCCTGTGACCTTTACCAGCTCTGCTACCGCGGACCTGAGACACTTTG TGGCGCTGAACCTGGTGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTC TACTTCAACAAGCCCACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGA CCGGAATCGTGGACGAGTGCTGCTTCAAGAGCTGCGAGCTGCGCGGCTGGA AATGTATTGTGCCCCCTCTGAAGCCTGCCAAGAGCGCTAGTTTATCTTAGAG GCATATCCCTCTGGGCCCTCATGGGCCCTCCGCTCACTGCCCGCTTTCCAGTC GGGAACCTGTGCTGCCAGCTGCATTAACATGGTCATAGCTGTTTCCTTGCG TATTGGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTGCTTC GGGTAAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAAC CGTAAAAAGGCCGCGTTGCTGGCGTTTTCATAGGCTCCGCCCCCTGACG AGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACT ATAAAGATAACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTT CCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCG TGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGTGTAGGTGCT TCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGC GCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACACGACTTAT CGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGG CGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGA ACAGTATTTGGTATCTCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAG TTGGTAGCTCTTGATCCGGCAAAACAAACACCGCTGGTAGCGGTGGTTTTTT TGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCT TTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAACCTCACGTTAAG GGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAA TTAAAAATGAAGTTTTTAAATCAATCTAAAGTATATATGAGTAACTTGGTCT GACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTAT TTCGTTTCATCCATAGTTGCTGACTCCCCGCTCGTGTAGATAACTACGATACG GGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCAGAACACCGC TCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGC GCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGTCTATTAATTGTTG CCGGGAAGCTAGAGTAAGTAGTTCCGCGATTAATAGTTTGCAGCAAGTTGTT GCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTATGGCTTCAT TCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCATGTTGTG CAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTG GCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTG TCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTC ATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATA CGGGATAATACCGCGCCACATAGCAGAAGTTTAAAGTGCTCATCATTTGGAA AACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAG TTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTTTTACTTTT ACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGG GAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCTTTTCAATA TTATTGAAGCATTTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAA TGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCCCGGAAAAG TGCCAC
16	Compound 4 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTTG

		GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAGAGATA AACTTGTATCTCTCAGCTCCACGCCCTTATCTTAGAGGCATATCCCTACGT ACCAACAAGGGCCTGTACCTCATCTACTACTTGAGTAGATGAGGTACAGGCC CTTTATCTTAGAGGCATATCCCTACGTACCAACAAGGTATGAGCCCATCTAT CTACTTGAGATAGATGGGCTCATAACCTTATCTTAGAGGCATATCCCTACGT ACCAACAAGCAATGAGGACCTGAGAGATACTTGATCTCTCAGGGTCCTCAT TGCTTTATCTTAGAGGCATATCCCTACGTACCAACAAGCTGATGGGAACGTG GACTAACTTGAGTCCACGTTCCCATCAGCTTTATCTTAGAGGCATATCCCT ACGTACCAACAAGGTCTCAGATTACTACAACTTGTGTAGTAATCTGAGG ACCTTTATCTTAGAGGCATATCCCTGCCACCATGGGACTGACATCTCAACTG CTGCCTCCACTGTTCTTTCTGCTGGCCTGCGCCGGCAATTTTGTGCACGGCC ACAAGTGCAGACATCACCTGCAAGAGATCATCAAGACCCTGAACAGCCTGAC CGAGCAGAAAACCTGTGCACCGAGCTGACCGTGACCGATATCTTTGCCGCC AGCAAGAACACAACCGAGAAAGAGACATTCTGCAGAGCCGCCACCGTGCTGA GACAGTTCTCAGCCACCACGAGAAGGACACCAGATGCCCTGGGAGCTCAGC CCAGCAGTTCCACAGACACAAGCAGCTGATCCGGTTCTGAAGCGGCTGGAC AGAAATCTGTGGGGACTCGCCGGCCTGAATAGCTGCCCTGTGAAAGAGGCCA ACCAGTCTACCCTGGAAACTTCTTGGAAACGGCTGAAAACCATATGCGCGA GAAGTACAGCAAGTGCAGCAGCTGATTATCTTAGAGGCATATCCCTCTGGG CCTCATGGGCCTTCCGCTCACTGCCCCGCTTCCAGTCGGGAACCTGTCTGT CCAGCTGCATTAACATGGTCATAGCTGTTTCTTGGCTATTGGGCGCTCTCC GCTTCTCGCTCACTGACTCGCTGCGCTCGGTCTGCTCGGTAAGCCTGGGG TGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAGGCCGCG TTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCAGAAAATC GACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGC GTTTCCCCCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCTGCGCCTT ACCGGATACCTGTCCGCTTCTCTCCCTCGGGAAGCGTGCGCCTTCTCATA GCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCTGCTCCAAGCTGGG CTGTGTGCACGAACCCCCGTTCCAGCCGACCGCTGCGCCTTATCCGGTAAC TATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAG CCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGTACAGAGTT CTTGAAGTGGTGGCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATC TGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGAT CCGGCAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTGTCAGCAGCA GATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACG GGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTCATGA GATTATCAAAAAGGATCTTACCTAGATCCTTTTAAATTAAAAATGAAGTTT TAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATGC TTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTATCATAG TTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATC TGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACCGGCTCCAGAT TTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCCTG CAACTTTATCCGCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGT AAGTAGTTCGCCAGTTAATAGTTTTCGCAACGTTGTTGCCATTGCTACAGGC ATCGTGGTGTACGCTCGTCTGTTTGGTATGGCTTATTGAGCTCCGCTTCCC AACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAGCGGTTAG CTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGAGTGTATCA CTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTGCTATCCGTAA GATGCTTTTCTGTGACTGGTGTGACTCAACCAAGTCATTCTGAGAATAGTG TATGCGGCGACCGAGTTGCTCTTGCCGCGCGTCAATACGGGATAATACCGCG CCACATAGCAGAACTTTAAAAGTGCTCATCATTTGAAAACGTTCTCGGGGC GAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCAC TCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGG TGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACAC GGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTATA TCAGGGTTATTGTCTCATGAGCGGATACATATTGAATGTATTAGAAAAAT AAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC
17	Compound 5 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAT CAAAAGAAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT

		CGCCATT CAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAGAGATA AACTTGTTATCTCTCAGCTCCACGCCTTTATCTTAGAGGCATATCCCTACGT ACCAACAAGGGCCTGTACCTCATCTACTACTTGAGTAGATGAGGTACAGGCC CTTTATCTTAGAGGCATATCCCTACGTACCAACAAGGTATGAGCCCATCTAT CTACTTGAGATAGATGGGCTCATACCTTTATCTTAGAGGCATATCCCTGCCA CCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCTGCTGGCCTG CGCCGGCAATTTTGTGCACGGCCACAAGTGCGACATCACCTGCAAGAGATG ATCAAGACCCTGAACAGCCTGACCGAGCAGAAAACCTGTGACCCGAGCTGA CCGTGACCGATATCTTTGCCGCCAGCAAGAACAACCGAGAAAGAGACATT CTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCACGAGAAGGAC ACCAGATGCTGGGAGCTACAGCCCAGCAGTTCCACAGACACAAGCAGCTGA TCCGGTTCCCTGAAGCGGCTGGACAGAAATCTGTGGGGACTCGCCGGCCTGAA TAGCTGCCCTGTGAAAGAGGCCAACCAGTCTACCTTGGAAAACCTCTGGAA CGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGACAGCAGCTGATTTA TCTTAGAGGCATATCCCTCTGGGCCTCATGGGCCTTCCGCTCACTGCCCGCT TTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAACATGGTCATAGCTGTT TCCTTGCGTATTGGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGCGCTC GGTCGTTCCGGGTAAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCAAAAGG CCAGGAACCGTA AAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC CCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCG ACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT CTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTC GGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCCGGT TAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCGCTTCAGCCCG ACCGCTGCCCTTATCCGGTAACCTATCGTCTTGAGTCCAACCCGTAAGACA CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGG TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACA CTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG AAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACACCGCTGGTAGCGGT GGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAG AAGATCCCTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAAACGAAAACCTC ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATC CTTTTAAATTA AAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAA CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT CTGTCTATTTTCGTTTATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACT ACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAG AACCACGCTCACCGGCTCCAGATTTATCAGCAATAAACACAGCCAGCCGGAAG GGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCTCCATCCAGTCTATT AATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCSCA ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTAT GGCTTCATT CAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCC ATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCCTCCGATCGTTGT CAGAA GTAAGTTGGCCGCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTCT TCTTACTGT CATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA ACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGG CGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCAT CATTGGAACCGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTG AGATCCAGTTCGATGTAACCCACTCGTGCACCAACTGATCTTCAGCATCTT TTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCCAAAATGCCGC AAAAAAGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTT TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA TATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCACATTTCC CCGAAAAGTGCCAC
18	Compound 6 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTGTTAAATTCGCGTTAAATTTTGTGTA ATCAGCTCATTTTTTAAACCAATAGGCGGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATT CAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG

		GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATGCCACCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCT GCTGGCCTGCGCCGGCAATTTTGTGCACGGCCACAAGTGCGACATCACCTTG CAAGAGATCATCAAGACCCTGAACAGCCTGACCAGCAGAAAAACCTGTGCA CCGAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACACAACCGAGAA AGAGACATTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCAC GAGAAGGACACCAGATGCCTGGGAGCTACAGCCCAGCAGTTCCACAGACACA AGCAGCTGATCCGGTTCTGAAAGCGGCTGGACAGAAATCTGTGGGGACTCGC CGGCCTGAATAGCTGCCCTGTGAAAGAGGCCAACCAGTCTACCCTGGAAAAAC TTCCTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGCGAGCA GCTGAATAGTGAGTCGTATTAACGTACCAACAAGCGTGAGCTGAGAGATA AACTTGTATCTCTCAGCTCCACGCCCTTATCTTAGAGGCATATCCCTACGT ACCAACAAGGGCCTGTACCTCATCTACTACTTGAGTAGATGAGGTACAGGCC CTTTATCTTAGAGGCATATCCCTACGTACCAACAAGGTATGAGCCCATCTAT CTACTTGAGATAGATGGGCTCATACCTTTATCTTAGAGGCATATCCCTTTTA TCTTAGAGGCATATCCCTCTGGGCCCTCATGGGCCCTCCGCTCACTGCCCGCT TTCCAGTCGGGAAACCTGTCTGCGCCAGCTGCATTAACATGGTCATAGCTGTT TCCTTGCGTATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTGCGCTC GGTCGTTCCGGTAAAGCCTGGGGTGCCATATGAGCAAAAGGCCAGCAAAAGG CCAGGAACCGTAAAAAGGCCGCTTGTGGCGTTTTTCCATAGGCTCCGCCC CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCG ACAGGACTATAAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT CTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTC GGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTG TAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTAGCCCCG ACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTAAGACA CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGG TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACCTACCGCTACA CTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG AAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAACACCGCTGGTAGCGGT GGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAG AAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGAACGAAAACTC ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATC CTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAA CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT CTGTCTATTTGTTTCATCCATAGTTGCCTGACTCCCCGTGCTGTAGATAACT ACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAG AACCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAG GGCCGAGCGCAGAAGTGGTCTGCACTTTATCCGCCTCCATCCAGTCTATT AATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCCGCAAGTTAATAGTTTGC ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTAT GGCTTCATTGAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCC ATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTGTGTCAGAA GTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTC TCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA ACCAAGTCATTCTGAGAATAGTGATGCGGCGACCGAGTTGCTCTTGCCCGG CGTCAATACGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGCTCAT CATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCCTGTTG AGATCCAGTTCGATGTAACCCACTCGTGACCCCACTGATCTTCAGCATCTT TTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGC AAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCCTT TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCC CCGAAAAGTGCCAC
19	Compound 7 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAT CAAAAGAATAGACCGAGATAGGGTTAGTGGCCGTACAGGGCGCTCCCAT CGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC

		CGCATATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAGAGATA <u>AAC TTGTTATCTCTCAGCTCCACGCCTTATCTTAGAGGCATATCCCTGCCA</u> <u>CCATGGGCGCTGACATCTCAGTTGCTGCCTCCACTGTTCTTTCTGCTGGCCTG</u> <u>CGCCGGCAATTTTGTGCACGGCCACAAGTGCGACATCACCTGCAAGAGATC</u> <u>ATCAAGACCCTGAACAGCCTGACCGAGCAGAAAACCTGTGCACCGAGCTGA</u> <u>CCGTGACCGATATCTTTGCCGCCAGCAAGAACACAACCGAGAAAGAGACATT</u> <u>CTGCAGAGCCGCCACCGTGTGAGACAGTTCTACAGCCACCACGAGAAGGAC</u> <u>ACCAGATGCCTGGGAGCTACAGCCCAGCAGTTCCACAGACACAAGCAGCTGA</u> <u>TCCGGTTCTGTGAAGCGGCTGGACAGAAATCTGTGGGGACTCGCCGGCCTGAA</u> <u>TAGCTGCCCTGTGAAAGAGGCCAACCCAGTCTACCCTGGAAAACCTCTGTGAA</u> <u>CGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGACGACGCTAGTTTA</u> <u>TCTTAGAGGCATATCCCT</u>CTGGGCTCATGGGCTTCCGCTCACTGCCCGCT TTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAACATGGTCATAGCTGTT TCCTTGCGTATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTGCGCTC GGTCGTTCCGGTAAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCAAAAGG CCAGGAACCGTAAAAAGGCCGCTTGTGCGCTTTTCCATAGGCTCCGCCC CCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGTGCGGAACCCG ACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT CTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTC GGGAAGCGTGCGCTTTCTCATAGCTCAGCTGTAGGTATCTCAGTTCCGGTG TAGGTCGTTCCGCTCCAAGCTGGGCTGTGTGCAGAACCCCCGTTCCAGCCCG ACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACA CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGG TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACA CTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG AAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGT GGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAG AAGATCCTTTGATCTTTTCTACGGGCTGTCAGCTCAGTGAACGAAAACCTC ACGTTAAGGATTTTGTGTCATGAGATTATCAAAAAGGATCTTCACTAGATC CTTTTAAATTAAAAATGAAGTTTAAATCAATCTAAAGTATATATGAGTAAA CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT CTGTCTATTTTCGTTCCATAGTTGCCTGACTCCCGCTCGTGTAGATAACT ACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCGCGAG AACCACGCTCACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCCGGAAG GGCCGAGCGCAGAAGTGGTCTGCACTTTATCCGCTCCATCCAGTCTATT AATTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCA ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGTTTGGTAT GGCTTCATTCAGCTCCGTTCCCAACGATCAAGGCGAGTTACATGATCCCC ATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAA GTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCATAATTC TCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA ACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGG CGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCAT CATTGGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTG AGATCCAGTTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTT TTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGC AAAAAAGGGAATAAGGGCGACACGGAATGTTGAATACTCATACTCTTCCTT TTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGATACA TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCC CGAAAAGTGCCAC
20	Compound 8 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCAT CGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATGCCACCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCT <u>GCTGGCCTGCGCCGGCAATTTTGTGCACGGCCACAGTGCGACATCACCTG</u> <u>CAAGAGATCATCAAGACCCTGAACAGCCTGACCGAGCAGAAAACCTGTGCA</u> <u>CCGAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACACAACCGAGAA</u> <u>AGAGACATTCGACAGAGCCGCCACCGTGTGAGACAGTTCTACAGCCACCAC</u>

		<u>GAGAAGGACACCAGATGCCTGGGAGCTACAGCCCAGCAGTTCCACAGACACA</u> <u>AGCAGCTGATCCGGTTCTCTGAAGCGGCTGGACAGAAATCTGTGGGGACTCGC</u> <u>CGGCCTGAATAGCTGCCCTGTGAAAGAGGCCAACCAGTCTACCCTGGAAAAC</u> <u>TTCTTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGCAGCA</u> <u>GCTGAATAGTGAGTCGTATTAACGTACCAACAAGGCGTGGAGCTGAGAGATA</u> <u>AACTTGTTATCTCTCAGCTCCACGCCTTTATCTTAGAGGCATATCCCTTTTA</u> <u>TCTTAGAGGCATATCCCT</u>CTGGGCCTCATGGGCCTTCCGCTCACTGCCCGCT TTCCAGTCGGGAAACCTGTCTGCCAGCTGCATTAACATGGTCATAGCTGTT TCCTTGCGTATTGGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGCGCTC GGTCGTTCCGGGTAAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCAAAAG CCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTCCATAGGCTCCGCC CCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGTGGCGAAACCCG ACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCT CTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCCTTCTCCCTTC GGGAAGCGTGGCGCTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTG TAGGTCGTTGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCC ACCGCTGCGCCTTATCCGTAACCTATCGTCTTGAGTCCAACCCGTAAGACA CGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCAGG TATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACA CTAGAAGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGG AAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACCACCGCTGGTAGCGGT GGTTTTTTTGTGTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAG AAGATCCTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAAACGAAACTC ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATC CTTTTAAATTAATAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAA CTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGAT CTGTCTATTTTCGTTTATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACT ACGATACGGGAGGGCTTACCATCTGGCCCCAGTGTGCAATGATACCCGAG AACCACGCTCACCAGCTCCAGATTTATCAGCAATAAACACCGCCAGCCGGAAG GGCCGAGCGCAGAAGTGGTCTGCACTTTATCCGCCTCCATCCAGTCTATT AATTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCACA ACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTGGTAT GGCTTCAATTCAGCTCCGTTCCCAACGATCAAGGCGAGTTACATGATCCCC ATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTGAGAA GTAAGTTGCCCGCAGTGTATCACTCATGGTTATGGCAGCACTGCATAATT TCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCA ACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCGG CGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAGTGCTCAT CATTTGAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCTGTTG AGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTT TTACTTTCAACAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCG AAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTT TTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACA TATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCC CCGAAAAGTGCCAC
21	Compound 9 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTACGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAACACGACGGCCAGTGAG CGGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAGGC CGCATGCCACCATGGGACTGACATCTCAACTGCTGCCTCCACTGTTCTTTCT <u>GCTGGCCTGCGCCGGCAATTTTGTGCACGGCCACAAGTGCGACATCACCTG</u> <u>CAAGAGATCATCAAGACCCTGAACAGCCTGACCGAGCAGAAAACCTGTGCA</u> <u>CCGAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACACAACCGAGAA</u> <u>AGAGACATTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACCAC</u> <u>GAGAAGGACACCAGATGCCTGGGAGCTACAGCCCAGCAGTTCCACAGACACA</u> <u>AGCAGCTGATCCGGTTCTCTGAAGCGGCTGGACAGAAATCTGTGGGGACTCGC</u> <u>CGGCCTGAATAGCTGCCCTGTGAAAGAGGCCAACCAGTCTACCCTGGAAAAC</u> <u>TTCTTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGCAGCA</u> <u>GCTGAACAACAAGGCGTGGAGCTGAGAGATAAATTTGTTATCTCTCAGCTCC</u>

		<u>ACGCCACAACAAGGGCCTGTACCTCATCTACTACTTGAGTAGATGAGGTACA</u> <u>GGCCCACAACAAGGTATGAGCCCATCTATCTACTTGAGATAGATGGGCTCAT</u> <u>ACCACAACAATTTATCTTAGAGGCATATCCCT</u>CTGGGCCTCATGGGCCTTCC GCTCACTGCCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAACA TGGTCATAGCTGTTTCCTTGCGTATTGGGCGCTCTCCGCTTCCTCGCTCACT GACTCGCTGCGCTCGGTCGTTTCGGGTAAAGCCTGGGCTGCCTAATGAGCAAA AGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTC CATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGA GGTGGCGAAACCCGACAGGACTATAAAGATACCAGCGCTTTCCCCCTGGAAG CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCC GCCTTTCTCCCTTCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGT ATCTCAGTTCGGGTGATAGTTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAAC CCCCGTTACGCCCAGCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCC AACCCGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGA TTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCC TAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAG CCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACCAACCA CCGCTGGTAGCGGTGGTTTTTTTTGTTGCAAGCAGCAGATTACGCGCAGAAA AAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGCTGACGCTCAG TGGAACGAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAGGA TCTTCACCTAGATCCTTTTAAATTAATAAATGAAGTTTAAATCAATCTAAAG TATATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCA CCTATCTCAGCGATCTGTATTTTCGTTTCATCCATAGTTGCCGACTCCCCG TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGC AATGATACCGCGAGAACCACGCTCACCGGCTCCAGATTTATCAGCAATAAAC CAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCT CCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGT TAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGC TCGTGCTTTGGTATGGCTTCATTACGCTCCGGTTCACACGATCAAGGCGAG TTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCTCC GATCGTTGTGAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCA GCACTGCATAATTCTCTTACTGTGATGCCATCCGTAAGATGCTTTTCTGTGA CTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAG TTGCTCTTGCCCGCGTCAATACGGGATAATACCGCGCCACATAGCAGAAT TTAAAGTGCTCATCATTTGGAACACGTTCTTCGGGGCGAAAACTCTCAAGGA TCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCACTG ATCTTCAGCATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGA AGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACACGGAATGTTGAATAC TCATACTCTTCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCT CATGAGCGGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTT CCGCGCACATTTCCCCGAAAAGTGCCAC
22	Compound 10 in pMA-RQ	CTTCGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAAG TTGGGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAACGACGCGCCAGT GAGCGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAA GGCCGCATGCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAA <u>GTGCTGCTTCTGCGACTTCCTGAAAGTGAAGATGCACACCATGAGCAGCAGC</u> <u>CACCTGTTCTATCTGGCCCTGTGCCTGCTGACCTTTACCAGCTCTGCTACCG</u> <u>CCGGACCTGAGACACTTTGTGGCGCTGAACTGGTGGACGCCCTGCAGTTTGT</u> <u>GTGTGGCGACAGAGGCTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGC</u> <u>TCTAGAAGGGCTCCTCAGACCGGAATCGTGGACGAGTGCTGTTTCAGAAGCT</u> <u>GCGACCTGCGGCGGCTGGAAATGTATTGTGCCCCCTCTGAAGCCTGCCAAGAG</u> <u>CGCCTAAATAGTGAGTCGTATTAACGTACCAACAACAAGATGAAGAGCA</u> <u>CCAAACTTGTTGGTGCTCTTCATCTTGTTGTTTATCTTAGAGGCATATCCCT</u> <u>TTTATCTTAGAGGCATATCCCT</u>CTGGGCCTCATGGGCCTTCCGCTCACTGCC CGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAACATGGTCATAGC TGTTTTCCTTGCGTATTGGGCGCTCTCCGCTTCCTCGCTCACTGACTCGCTGC GCTCGGTCGTTTCGGGTAAAGCCTGGGGTGCCATAATGAGCAAAAGGCCAGCAA AAGGCCAGGAACCGTAAAAAGGCCGCTTGCTGGCGTTTTTCCATAGGCTCC GCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGTGCGCGAAA CCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTG CGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTCC CTTCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTC

		GGTGTAGGTCGTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTTCAG CCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAA GACACGACTTATCGCCACTGGCAGCAGCCACTGGTAAACAGGATTAGCAGAGC GAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTGGTGGCCTAACTACGGC TACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCT TCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCCGCTGGTAG CGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCT CAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGAACGAAA ACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTA GATCCTTTTAAATTAAAAATGAAGTTTAAATCAATCTAAAGTATATATGAG TAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAG CGATCTGTCTATTTTCGTTTCATCCATAGTTGCCTGACTCCCCGTCTGTAGAT AACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCG CGAGAACCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCG GAAGGGCCGAGCGCAGAGTGGTCCTGCAACTTTATCCGCTCCATCCAGTC TATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTTCGCAGTTAATAGTTTG CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCAAGCCTGCTCGTTTG GTATGGCTTCATTCAGCTCCGGTTCCTCAACGATCAAGGCGAGTTACATGATC CCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGTC AGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCATA ATTCTCTTACTGTCTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTA CTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGC CCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGC TCATCATTTGAAAAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACCGCT GTTGAGATCCAGTTTCATGTAACCCACTCGTGACCCCACTGATCTTCAGCA TCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATG CCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTT CCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGGA TACATATTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACAT TTCCCCGAAAAGTGCCAC
23	Compound 11 in pMA-RQ	CTAAATTGAAGCGTTAATATTTTTGTTAAATTCGCGTTAAATTTTTGTTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGGCGGCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAACACGCGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATGCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAAGTG CTGCTTCTGCGACTTCTGAAAGTGAAGATGCACACCATGAGCAGCAGCCAC CTGTTCTATCTGGCCCTGTGCTGCTGACCTTTACCAGCTCTGCTACCGCCG GACCTGAGACACTTTTGTGGCGCTGAACTGGTGGACGCCCTGCACTTTGTGTG TGGCGACAGAGGCTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCT AGAAGGGCTCCTCAGACCGGAATCGTGGACGAGTGCTGTTTCAGAAGCTGCG ACCTGCGGCGGCTGGAAATGTATTGTGCCCTCTGAAGCCTGCCAAGAGCGC CTAAATAGTGAGTCGTATTAAACGTACCAACAACAAGATGAAGAGCACCA AACTTGTGGTGTCTTTCATCTTGTGTTTATCTTAGAGGCATATCCCTACG TACCAACAACAACAAGATGAAGAGCACCAAACTTGTGGTGTCTTTCATCTT GTTGTTTATCTTAGAGGCATATCCCTTTTATCTTAGAGGCATATCCCTCTGG GCCTCATGGGCTTCCGCTCACTGCGGCTTTCCAGTCGGGAACCTGTCTGT GCCAGCTGCATTAACATGGTCATAGCTGTTTCCTTGCCTATTGGGCGCTCTC CGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCTGTTCCGGTAAAGCCTGGG GTGCCTAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCG GTTGCTGGCGTTTTTTCATAGGCTCCGCCCCCTGACGAGCATCACAAAAAT CGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGG CGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCTGCCGCT TACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCAT AGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTGCTCGCTCAAGCTGG GCTGTGTGCACGAACCCCGTTACGCCGACCGCTGCGCTTATCCGGTAA CTATCGTCTTGAGTCCAAACCCGGTAAGACACGACTTATCGCCACTGGCAGCA GCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGT TCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTAT CTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGA

		TCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAG AGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTAC GGGGTCTGACGCTCAGTGAACGAAACTCACGTTAAGGGATTTTGGTCATG AGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAATAATGAAGTT TTAAATCAATCTAAAGTATATATGAGTAACTTGGTCTGACAGTTACCAATG CTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCCGTTATCCATA GTTGCCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCAT CTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACCGGCTCCAGA TTTATCAGCAATAAACAGCCAGCCGGAAGGGCCGAGCGCAGAAAGTGGTCCT GCAACTTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAG TAAGTAGTTCGCCAGTTAATAGTTTTCGCAACGTTGTTGCCATTGCTACAGG CATCGTGGTGTACGCTCGTCTGTTTGGTATGGCTTCACTCAGCTCCGGTTCC CAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTA GCTCCTTCGGTCTCCGATCGTTGTGAGAAGTAAGTTGGCCGCGAGTGTTATC ACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTGCCATCCGTA AGATGCTTTTCTGTGACTGGTGAAGTCAACCAAGTCACTCTGAGAATAGT GTATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCGC GCCACATAGCAGAACTTTAAAGTGCTCATCATTTGAAAACGTTCTTCGGGG CGAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCA CTCGTGACCCAACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTGG GTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGACA CGGAAATGTTGAATACTCATACTCTTCTTTTCAATATTATTGAAGCATTT ATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAA TAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCAC
24	Compound 12 in pMA-RQ	CTAAATTGTAAGCGTTAATATTTTGTAAAATTCGCGTTAAATTTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTCAAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGGGGCTCTTT CGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CGCATGCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAGTG <u>CTGCTTCTGCGACTTCTGAAAGTGAAGATGCACACCATGAGCAGCAGCCAC</u> <u>CTGTTCTATCTGGCCCTGTGCTGCTGACCTTTACCAGCTCTGCTACCGCCG</u> <u>GACCTGAGACACTTTGTGGCGCTGAACTGGTGGACGCCCTGCAGTTTGTGTG</u> <u>TGGCGACAGAGGCTTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCT</u> <u>AGAAGGGCTCCTCAGACCGGAATCGTGGACGAGTGTGTTTTCAGAAGCTGCG</u> <u>ACCTGCGGCGGCTGGAAATGTATTGTGCCCTCTGAAGCCTGCCAAGAGCGC</u> <u>CTAAATAGTGAGTCGTATTAACGTACCAACAACAAGATGAAGAGCACCA</u> <u>AACTTGTGGTGTCTTTCATCTTGTGTTTATCTTAGAGGCATATCCCTACG</u> <u>TACCAACAACAACAAGATGAAGAGCACCAAACTTGTGGTGTCTTTCATCTT</u> <u>GTTGTTTATCTTAGAGGCATATCCCTACGTACCAACAACAAGATGAAGA</u> <u>GCACCAAACTTGTGGTGTCTTTCATCTTGTGTTTATCTTAGAGGCATATC</u> <u>CCTTTTATCTTAGAGGCATATCCCT</u>CTGGGCCTCATGGGCCTTCCGCTCACT GCCCCGCTTTCCAGTCGGGAAACCTGTCTGTGCCAGCTGCATTAACATGGTCAT AGCTGTTTCCTTGCATATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGC TGCGCTCGGTCTGTTCCGGTAAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAG CAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGC TCCGCCCCCTGACGAGCATCACAAAATCGACGCTCAAGTCAGAGGTGGCG AAACCCGACAGGACTATAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTC GTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTC TCCCTTCGGGAAGCGTGGCGCTTTCATAGCTCACGCTGTAGGTATCTCAG TTCGGTGTAGGTCTTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTT CAGCCCCACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGG TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAG AGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAACTAC GGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTA CCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACACCCAGTTGG TAGCGGTGGTTTTTTTTGTTTGAAGCAGCAGATTACGCGCAGAAAAAAGGA TCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGAACG AAAACCTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCAC CTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATAT

		GAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCT CAGCGATCTGTCTATTTCTGTTTCATCCATAGTTGCCTGACTCCCCGTCTGTGA GATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATA CCGCGAGAACCACGCTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAG CCGGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCCTCCATCCA GTCTATTAAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGT TTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCGT TTGGTATGGCTTCATTACAGTCCGGTTCCTAACGATCAAGGCGAGTTACATG ATCCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTT GTCAGAAGTAAGTTGGCCGAGTGTATCACTCATGGTTATGGCAGCACTGC ATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTCTGTGACTGGTGA GTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCT TGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAG TGCTCATCATTGGAACGTTCTTCGGGGCGAAAACCTCTCAAGGATCTTACC GCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGACCCCAACTGATCTTCA GCATCTTTTACTTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAA ATGCCGCAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATCACTATCAT CTTCCTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGC GGATACATATTTGAATGTATTTAGAAAAATAACAAATAGGGGTTCCGCGCA CATTTCCCGAAAAGTGCCAC
127	Compound 13 in pMA-T	CTAAATTGTAAGCGTTAATATTTTGTAAATTCGCGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCAT CGCCATTACAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGGCCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGGCCAGTGAG CGCGACGTAATACGACTCACTATAGGGCGAATTGGCGGAAGGCCGTCAAGGC CACGTGTCTTGTCCAGAGCTCGGATCCGCCACCATGGGCAAGATTAGCAGCC <u>TGCTTACACAGCTGTTCAAGTGCTGCTTCTGCGACTTCTGAAAGTGAAGAT</u> <u>GCACACCATGAGCAGCAGCCACCTGTTCTATCTGGCCCTGTGCCTGCTGACC</u> <u>TTTACCAGCTCTGCTACCGCCGGACCTGAGACACTTGTGTGGCGCTGAAGTGG</u> <u>TGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGCTTCTACTTCAACAAGCC</u> <u>CACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAGACCGGAATCGTGGAC</u> <u>GAGTGTGTTTCAGAAGCTGCGACCTGCGGCGGCTGGAATGTATTGTGCC</u> <u>CTCTGAAGCCTGCCAAGAGCGCCTAA</u>GAATTTCGGTACCTGGAGCACAAACT GGCCTCATGGGCCTTCCGCTCACTGCCCGCTTTCAGTCGGGAAACCTGTCTG TGCCAGCTGCATTAACATGGTCATAGCTGTTTCCTTGCCTATTGGGCGCTCT CCGCTTCTCGCTCACTGACTCGCTGCGCTCGGTCTGTTTCGGGTAAAGCCTGG GGTGCCATAATGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCG CGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAA TCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAG GCGTTTTCCCTGGAAGCTCCCTCGTGCGCTCTCTGTTCCGACCCTGCCGC TTACCGGATACCTGTCCGCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCA TAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCAAGCTG GGCTGTGTGCACGAACCCCCGTTACGCCCCGACCGCTGCGCCTTATCCGGTA ACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGC AGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGAGGCGGTGCTACAGAG TTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTA TCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTG ATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTCGAAGCAG CAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTA CGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAGGGATTTTGGTCAT GAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTTAAATGAAGT TTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTACCAAT GCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCTGTTTCATCCAT AGTTGCCTGACTCCCCGTCTGTAGATAACTACGATACGGGAGGGCTTACCA TCTGGCCCCAGTGCTGCAATGATACCGCGAGAACCACGCTCACCGGCTCCAG ATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCGAGCGCAAGTGGTCC TGCAACTTTATCCGCCTCCATCCAGTCTATTAATTTGTTGCCGGGAAGCTAGA GTAAGTAGTTCCGCGATTAAAGTTTGGCGCAACGTTGTTGCCATTGCTACAG GCATCGTGGTGTACGCTCGTCTGTTTGGTATGGCTTCATTACGCTCCGGTTC CCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAGCGGTT

		AGCTCCTTCGGTCTCCGATCGTTGTCAGAAAGTAAGTTGGCCGAGTGTTAT CACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGT AAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAG TGTATGCGGCGACCGAGTTGCTCTTGGCCGGCGTCAATACGGGATAATACCG CGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGAAAAACGTTCTTCGGG GCGAAAACCTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCC ACTCGTGCACCCACTGATCTTCAGCATCTTTTACTTTTACCAGCGTTTCTG GGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAGGGAATAAGGGCGAC ACGGAATGTTGAATACTCATACTCTCTCTTTTCAATATTATGAAGCATT TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTAGAAAA ATAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAGTGCCAC
128	Compound 14 in pMA-T	CTAAATTGTAAGCGTTAATATTTTGTAAAATTTCGGTTAAATTTTGTAA ATCAGCTCATTTTTTAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAT CAAAAGAAATAGACCGAGATAGGGTTGAGTGGCCGCTACAGGGCGCTCCCATT CGCCATTAGGCTGCGCAACTGTTGGGAAGGGCGTTTCGGTGCGGCGCTCTT CGCTATTACGCCAGCTGGCGAAAGGGGATGTGCTGCAAGGCGATTAAAGTTG GGTAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAACGACGCCAGTCAG CGCGACGTAATACGACTCACTATAGGCGCAATTGGCGGAAGGCCGTCAAGGC CACGTGTCTTGTCCAGAGCTC GCCACCATGTTGCTGCTGCCTCTGTTCTTCC TGCTGGCTGCGCCGGCAATTTTGTGCACGGCCACAAGTGCGACATCACCTT GCAAGAGATCATCAAGACCTGAACAGCCTGACCAGCAGAAAAACCTGTGC ACCGAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACAACCGAGA AAGAGACATTCTGCAGAGCCGCCACCGTGCTGAGACAGTTCTACAGCCACA CGAGAAGGACACCAGATGCCTGGGAGCTACAGCCAGCAGTTCCACAGACAC AAGCAGCTGATCCGGTTCTTGAAGCGGCTGGACAGAAATCTGTGGGACTCG CCGGCTGAATAGCTGCCCTGTGAAAGAGGCCAACCAGTCTACCTGGAAAA CTTCTGGAACGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGACAGC AGCTGA GGTACCTGGAGCACAAGACTGGCCTCATGGGCTTCCGCTCACTGC CCGCTTTCCAGTCGGGAACCTGTCTGCGAGCTGCATTAACATGGTCATAG CTGTTTCTTGCATATTGGGCGCTCTCCGCTTCTCGCTCACTGACTCGCTG CGCTCGGTGCTTGGGTAAAGCCTGGGGTGCCTAATGAGCAAAAGGCCAGCA AAAGGCCAGGAACCGTAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTC CGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAA ACCCGACAGGACTATAAGATACAGGCGTTTCCCCCTGGAAGCTCCCTCGT GCGCTCTCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCTTTCTC CCTTCGGGAAGCGTGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTT CGGTGTAGGTGCTTCCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTCA GCCCCACCGCTGCGCCTTATCCGGTAACATATCGTCTTGAGTCCAACCCGGTA AGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCTAACTACGG CTACAGTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACC TTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAACAAACCACCGCTGGTA GCGGTGGTTTTTTTGTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATC TCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAA AACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCT AGATCCTTTTAAATTAATAATGAAGTTTAAATCAATCTAAAGTATATATGA GTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCA GCGATCTGTCTATTTCTGTTTATCCATAGTTGCCGACTCCCCGTCGTGTAGA TAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACC GCGAGAACCACGCTACCGGCTCCAGATTTATCAGCAATAAACAGCCAGCC GGAAGGGCCGAGCGCAGAAGTGGTCTGCAACTTTATCCGCTCCATCCAGT CTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTT GCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTCTGTTT GGTATGGCTTCATTAGCTCCGTTCCCAACGATCAAGGCGAGTTACATGAT CCCCCATGTTGTGCAAAAAGCGGTTAGCTCCTTCGGTCTCCGATCGTTGT CAGAAGTAAGTTGGCCGAGTGTTATCACTCATGGTTATGGCAGCACTGCAT AATTCTCTTACTGTATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGT ACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTG CCCGGCGTCAATACGGGATAATACCGGCCACATAGCAGAACTTTAAAAGTG CTCATCATTGAAAAACGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGC TGTTGAGATCCAGTTCGATGTAACCACTCGTGCACCAACTGATCTTCAGC ATCTTTTACTTTACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAT

		GCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCT TCCTTTTTCAATATTATTGAAGCATTATCAGGGTTATTGTCTCATGAGCGG ATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACA TTTCCCCGAAAAGTGCCAC
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Bold and underline = compound sequence

[0251] Example 2: *In vitro* transcription of RNA constructs and data analysis

[0252] PCR-based *in vitro* transcription is carried out using the pMA-RQ/pMA-T vectors

5 encoding Cpd.1-Cpd.14 to produce mRNA. A transcription template was generated by PCR using the forward and reverse primers in **Table 4** (SEQ ID NO: 29 and 30). The poly(A) tail was encoded in the template resulting in a 120 bp poly(A) tail. Optimizations were made as needed to achieve specific amplification given the repetitive sequence of siRNA flanking regions. Optimizations include: 1) decreasing the amount of plasmid DNA of vector, 2) 10 changing the DNA polymerase (Q5 hot start polymerase, New England Biolabs), 3) reducing denaturation time (30 seconds to 10 seconds) and extension time (45 seconds/kb to 10 seconds/kb) for each cycle of PCR, 4) increasing the annealing (10 seconds to 30 seconds) for each cycle of PCR, and 5) increasing the final extension time (up to 15 minutes) for each cycle of PCR. In addition, to avoid non-specific primer binding, the PCR reaction mixture 15 was prepared on ice including thawing reagents, and the number of PCR cycles was reduced to 25.

[0253] For *in vitro* transcription, T7 RNA polymerase (MEGAscript kit, Thermo Fisher Scientific) was used at 37°C for 2 hours. Synthesized RNAs were chemically modified with 100% N1-methylpseudo-UTP (modified RNA) to reduce immunogenicity or unmodified using canonical UTP (unmodified RNA). All synthesized RNAs were co-transcriptionally capped with an anti-reverse CAP analog (ARCA; [m^{2,3,4}-O-G(5')ppp(5')G]) at the 5' end (Jena Bioscience). After *in vitro* transcription, the RNAs were column-purified using MEGAclean kit (Thermo Fisher Scientific) and quantified using Nanophotometer-N60 (Implen).

[0254] Table 4. Primers for Template Generation

SEQ ID NO	Primer Direction	Sequence (5' to 3')
29	Forward	GCTGCAAGGCGATTAAGTTG
30	Reverse	U (2'OMe) U (2'OMe) U (2'OMe) TTTTTTTTTTTTTTTTTTTTTT TT TT TTTCAGCTATGACCATGTTAATGCAG

25

[0255] Using *in vitro* transcription, Cpd.1- Cpd.14 were generated as RNA constructs and tested in various *in vitro* models for immunogenicity modulation.

[0256] Determination of Molecular weight of constructs was performed as below. The molecular weight of each construct was determined from each sequence by determining the total number of each base (A, C, G, T or N1-UTP) present in each sequence and multiply the number by respective molecular weight (e.g., A: 347.2 g/mol; C 323.2 g/mol; G 363.2 g/mol; N1-UTP:338.2 g/mol). The molecular weight was determined by the sum of all weights obtained for each base and ARCA molecular weight of 817.4 g/mol. The molecular weight of each construct was used to calculate the amount of RNA used for transfection in each well to nanomolar (nM) concentration. The SEAP activity was assessed using QUANTI-Blue™ and reading the optical density (O.D.) at 620 nm. Supernatant of untransfected cells was used as background control and subtracted from obtained O.D. values in tested samples. Data were analyzed using GraphPad Prism 8 (San Diego, USA). Statistical analysis was carried out using Student's t-tests to compare significant difference between groups.

[0257] Example 3: Immunogenicity Assays

[0258] HEK-Blue™ hTLR7 immunogenicity assay for NF-κB activation

[0259] The immunogenicity of different compounds with modified RNA and unmodified RNA performed in two different cell lines represent major pathways of immunogenicity. The first assay utilizes HEK-Blue™ hTLR7 (Invivogen, Cat. Code: hkb-htlr7) cells which are designed for studying the activation of human TLR7 (hTLR7) by monitoring the activation of NF-κB/AP1. Endosomal TLR7 receptor detect uridine and guanosine bases in single stranded RNA and elicit an immune reaction as an anti-viral response. These cells are derived from the human embryonic kidney HEK293 cell line and engineered to express hTLR7 and a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IFN-β minimal promoter with five NF-κB and AP-1-binding sites. Upon TLR7 activation, SEAP is produced and can be determined in real-time with HEK-Blue™ Detection cell culture medium in cell culture supernatant. Stimulation of HEK-Blue™ hTLR7 cells was achieved by direct transfection of modified or unmodified compounds (Cpd.1 to Cpd.12; 0.3-0.45 µg/well).

[0260] HEK-Blue™ hTLR7 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% (v/v) Fetal Bovine Serum (FBS). The antibiotics Blasticidin (10 µg/mL) and Zeocin (100 µg/mL) were added to the media to select cells containing hTLR7 and SEAP transgene plasmids. Cells were seeded at 40,000 cell/well in a 96 well culture plate and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours prior to transfection. Cells were grown in DMEM growth medium containing 10% of FBS to reach confluency < 80% before transfection. Thereafter, HEK-293

cells were transfected with 0.3-0.45 µg/well of specific RNA constructs using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's instructions with the RNA to Lipofectamine ratio of 1:1 w/v. 100 µl of DMEM was removed and replaced with 50 µl of Opti-MEM and 50 µl RNA and Lipofectamine 2000 complex in Opti-MEM (Thermo Fisher Scientific). After 5 hours, the medium was replaced by fresh growth medium and the plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours. R848 (Resiquimod; 1 µg/ml; Invivogen, Cat. Code: tlrl-r848) hTLR7 agonist was used as positive control. After 24 hours of incubation, SEAP activity was assessed using QUANTI-Blue™ (20 µl cell culture supernatant + 180 µl QUANTI-Blue™ solution) and reading the optical density (O.D.) at 620 nm in SpectraMax i3 multi-mode plate reader (Molecular Device). Untransfected samples were used as background control.

[0261] HEK-Blue™ IFN-α/β cells immunogenicity assay to monitor the activation of the ISGF3 pathway

[0262] The HEK-Blue™ IFN-α/β cells (Invivogen, Cat. Code: hkb-ifnab) which are designed for studying the activation the JAK-STAT and ISG3 induced by type I interferons. The activation of type I IFN is a primary immunogenicity pathway of IVT mRNA as it is detected by major RNA sensors. HEK293 cells were engineered to stably express human STAT2 and IRF9 genes to activate type I IFN signaling pathway. The activation of the IFN pathway led to the secretion of SEAP which is under the control of the ISG54 promoter, and its subsequent detection in the cell culture supernatant. HEK-Blue™ IFN-α/β cells were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% (v/v) Fetal Bovine Serum (FBS). The antibiotics Blasticidin (10 µg/mL) and Zeocin (100 µg/mL) were added to the media to select cells containing STAT2, IRF9 and SEAP transgene plasmids. Cells were seeded at 40,000 cell/well in a 96 well culture plate and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours prior to transfection. Cells were grown in DMEM growth medium containing 10% of FBS to reach confluency < 80% before stimulation. Stimulation of HEK-Blue™ IFN-α/β cells was achieved by recombinant IFN-α (1 µg/ml) or IFN-α/β derived from 20 µl HEK293 cells supernatant which was previously transfected with modified and unmodified compounds (Cpd.1 to Cpd.12; 0.3-0.6 µg/well) with details below.

[0263] Human embryonic kidney cells 293 (HEK293T; ATCC, CRL-1573, Rockville, MD, USA) were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% (v/v) Fetal Bovine Serum (FBS). Cells were seeded at 20,000 or 40,000 cell/well in a 96 well culture plate and incubated at 37°C in a humidified atmosphere

containing 5% CO₂ for 24 hours prior to transfection. Cells were grown in DMEM growth medium containing 10% of FBS to reach confluency < 80% before transfection. Thereafter, HEK293 cells were transfected with modified and unmodified RNA compounds (Cpd.1 to Cpd.12; 0.3-0.6 µg/well) using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's instructions with the RNA to Lipofectamine ratio of 1:1 w/v. 100 µl of DMEM was removed and replaced with 50 µl of Opti-MEM and 50 µl RNA and Lipofectamine 2000 complex in Opti-MEM (Thermo Fisher Scientific). After 5 hours, the medium was replaced by fresh growth medium and the plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours. Cell culture supernatant (20 µl) were collected and added to culturing media of HEK-Blue™ IFN-α/β cells to measure secreted IFNs activity through the stimulation of JAK-STAT and ISG3 pathway. Recombinant human IFN-α10 (1 µg/ml) used as positive control (Invivogen, Cat. Code: rcyc-hifna10). After 2 hours of incubation, SEAP activity was assessed using QUANTI-Blue™ (20 µl cell culture supernatant + 180 µl QUANTI-Blue™ solution) and reading the optical density (O.D.) at 620 nm in SpectraMax i3 multi-mode plate reader (Molecular Device). Untransfected samples were used as background control and subtracted from obtained O.D. values in tested samples.

[0264] Results

[0265] Immunogenicity evaluation of Cpd.1 to Cpd.6

[0266] The activation of the NF-κB pathway in HEK-Blue™ hTLR7 cells by direct transfection of modified or unmodified compounds (Cpd.1 to Cpd.6; 0.3 µg/well) was confirmed experimentally. R848 (Resiquimod; 1 µg/ml), a hTLR7 agonist used as a positive control, induced high level (O.D. >2) of TLR7 activation as expected. The immunogenicity assay using HEK-Blue™ hTLR7 cells with modified and unmodified Cpd.1-Cpd.6 demonstrated that chemical modification with N1-methylpseudo-UTP significantly diminishes the immune activation compared to their unmodified counterparts (**, $P < 0.01$; **Figure 2A**). Unmodified compounds with 3x siRNAs (Cpd.2, Cpd.3, Cpd.5 and Cpd.6) or 6x siRNAs (Cpd.4) showed reduced hTLR7 activation compared to the unmodified compound without siRNA (IL-4 mRNA only) or Cpd. 1 with 1x siRNA (***, $P < 0.001$; **Figure 2A**). Likewise, stimulation of HEK-Blue™ IFN-α/β cells with recombinant IFN-α (1 µg/ml) or IFN-α/β derived from supernatant of HEK293 cells previously transfected with modified or unmodified Cpd.1-Cpd.6 (0.6 µg) was confirmed experimentally with IFNα as positive control. In line with hTLR7 activation, the modified compounds showed significantly reduced activation of IFNα/β pathway compared to their unmodified counterparts (***, $P < 0.001$; **Figure 2B**). The assay revealed that supernatants of cells transfected with unmodified

compounds with 3x or 6x siRNAs (Cpd.2-Cpd.6) displayed decreased activation of JAK-STAT and ISG3 pathway compared to unmodified compounds without siRNA or Cpd.1 with 1x siRNA (***, $P<0.001$; **Figure 2B**). In addition, Cpd.5, with 3x siRNA targeting TNF-alpha present downstream of or 3' to IL-4, lowered immunogenicity in a significant manner, compared to Cpd. 6, with 3x siRNA targeting TNF-alpha present upstream of or 5' to IL-4 (*, $P<0.05$; **Figure 2B**). In summary, both assays demonstrated strongly reduced *in vitro* immunogenicity providing a potentially better safety profile of unmodified compounds.

[0267] Immunogenicity evaluation of Cpd.6 to Cpd.9 and Cpd.4

[0268] Cpd.6-Cpd.9 and Cpd.4 represent constructs with IL-4 encoding mRNA with increasing number siRNAs targeting TNF-alpha. Cpd.7 and Cpd.8 contain 1x siRNA at 5' (or upstream) and 3' (or downstream) to IL-4 encoding sequence, respectively. Cpd.6 contains 3x siRNAs with A1 linkers whereas Cpd.9 comprises 3x siRNA with A2 linkers. Cpd.4 contains 6x siRNAs (3x targeting TNF-alpha and 3x targeting IL-17). Stimulation of HEK-Blue™ hTLR7 cells with direct transfection (0.3 µg/well) of modified or unmodified Cpd.6-Cpd.9 and Cpd.4 displayed varying activation level of NF-κB pathway, as measured by SEAP secretion in transfected cells 24 hours later. Modified Compounds displayed reduced immunogenicity for all tested constructs (***, $P<0.001$; **Figure 3A**). Although unmodified Cpd.7 with 1x siRNA present at the 5' to (or upstream of) IL-4 encoding sequence was found to be highly immunogenic, repositioning the same siRNA to 3' to (or downstream of) IL-4 resulted in 3-fold decline in the NF-κB pathway activation (***, $P<0.001$; **Figure 3A**). The addition of 3x or 6x siRNA in Cpd.6, Cpd.9, and Cpd.4 eliminated the need of using modified bases for RNA constructs to escape from hTLR7 binding (**Figure 3A**). The positive control R848 induced high level of TLR7 activation as expected. The immunogenicity assessment of Cpd.6-Cpd.8 and Cpd.4 in HEK-Blue™ IFN-α/β cells using supernatant of cell culture transfected with unmodified compounds with increased number of siRNA (3x in Cpd.6 or Cpd.9 or 6x in Cpd.4) showed that the level of IFN signaling was undetectable compared to using supernatant of cell culture transfected with unmodified compounds with 1x siRNA (Cpd.7 and Cpd.8; **Figure 3A**). As observed with hTRL7 activation, the alteration of the position of 1x siRNA in the compound (5' to 3' of gene of interest) caused 4-fold reduced immunogenicity (***, $P<0.001$; **Figure 3B**). In summary, both assays demonstrated siRNA copy number dependent (e.g., number of siRNA) decline in immune activation of unmodified compounds.

[0269] Immunogenicity evaluation of Cpd.10 to Cpd.12

[0270] Cpd.10 to Cpd.12 represent constructs with IGF-1 encoding mRNA and increasing number (1x, 2x and 3x, respectively) of siRNA targeting Turbo GFP. Immune stimulation of unmodified Cpd.10-Cpd.12 in HEK-Blue™ hTLR7 cells displayed dose dependent (number of siRNAs in the construct) reduction in activation of NF-κB pathway as measured by SEAP secretion as shown in **Figure 4A** (***, $P < 0.001$). Likewise, the immunogenicity assessment of Cpd.9-Cpd.11 in HEK-Blue™ IFN-α/β cells showed dose dependent (number of siRNAs in the construct) decrease in IFN signaling (**Figure 4B**). In summary, compounds with at least 2x siRNA reduces the level of the hTLR7 activation moderately and reduces IFN signaling significantly.

10 **[0271] HEK-Blue™ hTLR3 immunogenicity assay for NF-κB/AP-1 activation**

[0272] The combination constructs (mRNA + siRNA) possess a hairpin loop with shorter dsRNA structure (about 20 nucleotides) and could potentially induce unwanted immune response through endosomal TLR3 ligands that detect double stranded RNA (dsRNA) motifs. Endosomal TLR3 receptors detect dsRNA and elicit an immune reaction as an anti-microbial response. The HEK-Blue™ hTLR3 (Invivogen, Cat. Code: hkb-htlr3) cells which are designed for studying the activation of human TLR3 (hTLR3) by monitoring the activation of NF-κB/AP1 signalling cascade. These cells are derived from the human embryonic kidney HEK293 cell line and engineered to express hTLR3 and a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of an NF-κB and AP-1-inducible promoter. Upon TLR3 activation, SEAP is produced and can be determined in real-time with HEK-Blue™ Detection cell culture medium in cell culture supernatant. Stimulation of HEK-Blue™ hTLR3 cells was achieved by direct transfection of modified or unmodified compounds (Cpd.3, Cpd.13 and Cpd.14; 0.6 µg/well).

15 [0273] HEK-Blue™ hTLR3 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% (v/v) Fetal Bovine Serum (FBS). The antibiotics Blasticidin (10 µg/mL) and Zeocin (100 µg/mL) were added to the media to select cells containing hTLR3 and SEAP transgene plasmids. Cells were seeded at 40,000 cell/well in a 96 well culture plate and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours prior to transfection. Cells were grown in DMEM growth medium
25 containing 10% of FBS to reach confluency < 80% before transfection. Thereafter, HEK-293 cells were transfected with 0.6 µg/well of specific RNA constructs using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's instructions with the RNA to Lipofectamine ratio of 1:1 w/v. 100 µl of DMEM was removed and replaced with 90 µl of Opti-MEM and 10 µl RNA and Lipofectamine 2000 complex in Opti-MEM (Thermo Fisher

Scientific). The plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours. Poly(I:C) HMW (1 µg/ml; Invivogen, Cat. Code: tlrl-pic) was used as a positive control. After 24 hours of incubation, SEAP activity was assessed using QUANTI-Blue™ (20 µl cell culture supernatant + 180 µl QUANTI-Blue™ solution) and reading the optical density (O.D.) at 620 nm in SpectraMax i3 multi-mode plate reader (Molecular Device).

Untransfected samples were used as background control.

[0274] Results

[0275] Immunogenicity evaluation of Cpd.3, Cpd.13 and Cpd. 14

[0276] The activation of the NF-κB/AP1 pathway in HEK-Blue™ hTLR3 cells by direct transfection of modified or unmodified compounds (Cpd.3 and Cpd.13; 0.6 µg/well) was confirmed experimentally. Poly(I:C) HMW (1 µg/ml), a hTLR3 agonist was used as a positive control, induced high level (O.D. > 1.95) of TLR3 activation as expected. The immunogenicity assay using HEK-Blue™ hTLR3 cells with modified and unmodified Cpd.3, Cpd.13 and Cpd.14 demonstrated that chemical modification with N1-methylpseudo-UTP does not have any impact on TLR3 activation compared to their unmodified counterparts as the endosomal TLR3 ligand directed towards dsRNA instead of sensing uridine (U) or guanosine (G) motifs. Unmodified Cpd.3 with 3x siRNA targeting IL-1 beta with IGF-1 showed significantly reduced hTLR3 activation compared to the unmodified compound without siRNA Cpd.13 (***, $P < 0.001$; **Figure 5A**) or Cpd.14 (*, $P < 0.05$; **Figure 5A**). Similarly, the presence of 3x siRNA in unmodified Cpd.3 diminished the TLR8 mediated immune activation compared to modified Cpd.3 (***, $P < 0.001$; **Figure 5B**).

[0277] HEK-Blue™ hTLR8 immunogenicity assay for NF-κB/AP-1/IRF activation

[0278] The HEK-Blue™ hTLR8 (Invivogen, Cat. Code: hkb-htlr8) cells are designed for studying the activation of human TLR8 (hTLR8) by monitoring the activation of NF-κB/AP1/IRF signalling cascade. Endosomal TLR8 receptors detect uridine and guanosine motif in single-stranded RNA and elicit an immune reaction as an anti-viral response. HEK-Blue™ hTLR8 cells are derived from the human embryonic kidney HEK293 cell line and engineered to express hTLR8 and a secreted embryonic alkaline phosphatase (SEAP) reporter gene under the control of the IFN-β minimal promoter with five NF-κB and AP-1-binding sites. Upon TLR8 activation, SEAP is produced and can be determined in real-time with HEK-Blue™ Detection cell culture medium in cell culture supernatant. Stimulation of HEK-Blue™ hTLR7 cells was achieved by direct transfection of modified or unmodified compounds (Cpd.3, Cpd.13 and Cpd.14; 0.6 µg/well).

[0279] HEK-Blue™ hTLR8 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% (v/v) Fetal Bovine Serum (FBS). The antibiotics Blasticidin (10 µg/mL) and Zeocin (100 µg/mL) were added to the media to select cells containing hTLR8 and SEAP transgene plasmids. Cells were seeded at 40,000 cell/well in a 96 well culture plate and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours prior to transfection. Cells were grown in DMEM growth medium containing 10% of FBS to reach confluency < 80% before transfection. Thereafter, HEK-293 cells were transfected with 0.6 µg/well of specific RNA constructs using Lipofectamine 2000 (Thermo Fisher Scientific) following the manufacturer's instructions with the RNA to Lipofectamine ratio of 1:1 w/v. 100 µl of DMEM was removed and replaced with 90 µl of Opti-MEM and 10 µl RNA and Lipofectamine 2000 complex in Opti-MEM (Thermo Fisher Scientific). The plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours. R848 (Resiquimod; 1 µg/ml; Invivogen, Cat. Code: tlrl-r848) hTLR7/8 agonist was used as positive control. After 24 hours of incubation, SEAP activity was assessed using QUANTI-Blue™ (20 µl cell culture supernatant + 180 µl QUANTI-Blue™ solution) and reading the optical density (O.D.) at 620 nm in SpectraMax i3 multi-mode plate reader (Molecular Device). Untransfected samples were used as background control.

[0280] Results

[0281] Immunogenicity evaluation of Cpd.3, Cpd.13 and Cpd. 14

[0282] The activation of the NF-κB/AP1/IRF pathway in HEK-Blue™ hTLR8 cells by direct transfection of modified or unmodified compounds (Cpd.3 and Cpd.13; 0.6 µg/well) was confirmed experimentally. R848 (1 µg/ml), a hTLR7/8 agonist was used as a positive control, induced moderate level of TLR8 activation (O.D. > 0.45) unlike with HEK-Blue™ hTLR7 cells. The immunogenicity assay using HEK-Blue™ hTLR8 cells with modified and unmodified Cpd.3, Cpd.13 and Cpd.14 demonstrated that chemical modification with N1-methylpseudo-UTP does not have any impact on TLR8 activation compared to their unmodified counterparts. Although TLR8 ligands are known to detect the uridine (U) motifs, there was no difference in TLR8 activation observed for modified and unmodified constructs. This may be explained by the report that the binding affinity of TLR8 is observed to be guanosine motif specific in HEK-Blue™ hTLR8 cells (Hu et al., Bioorg Med Chem. 2018 January 01; 26(1): 77–83). Unmodified Cpd.3 with 3x siRNA targeting IL-1 beta with IGF-1 showed significantly reduced hTLR8 activation compared to the unmodified compounds without siRNA i.e., Cpd.13 (***, $P < 0.001$; **Figure 5B**) or Cpd.14 (*, $P < 0.05$; **Figure 5B**).

Similarly, the presence of 3x siRNA in unmodified Cpd.3 diminished the TLR8 mediated immune activation compared to modified Cpd.3 (***, $P < 0.001$; **Figure 5B**).

[0283] Immunogenicity assessment by stimulating endogenous IL-6 expression in A549 cells

5 **[0284]** Human lung epithelial carcinoma cells (A549; Sigma-Aldrich Cat. # 6012804) cells were utilized for studying the immune response against *in vitro* transcribed mRNA (IVT mRNA) by monitoring the endogenous expression of Interleukin 6 (IL-6) upon direct transfection of mRNA. Stimulation of immunogenicity against IVT mRNA are triggered by RNA sensors (e.g. TLRs, PKRs) and induces the expression of several proinflammatory
10 cytokines including IL-6, which plays a central role in the immune activation against IVT mRNA. A549 cells were maintained in Dulbecco's Modified Eagle's medium high glucose (DMEM, Sigma-Aldrich) supplemented with 10% (v/v) Fetal Bovine Serum (FBS, VWR, Cat #97068-091). A549 cells were seeded at 10,000 cell/well in a 96 well culture plate and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours prior to
15 transfection. Cells were then grown in DMEM growth medium containing 10% of FBS to reach confluency < 70% before transfection. Thereafter, A549 cells were transfected with specific mRNA constructs (0.3 µg) using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions with the mRNA to Lipofectamine ratio of 1:1 w/v. 100 µl of DMEM was removed and 50 µl of Opti-MEM (Thermo Fisher Scientific) was added to each
20 well followed by 50 µl mRNA and Lipofectamine 2000 complex in Opti-MEM. After 5 hours of incubation, the medium was replaced by fresh growth medium and the plates were incubated for 24 hours at 37°C in a humidified atmosphere containing 5% CO₂. Cell culture supernatant were collected to measure secreted human IL-6 using ELISA (ThermoFisher Cat. # 887066).

25 **[0285] Results**

[0286] Immunogenicity evaluation of Cpd.3, Cpd.4, Cpd.13 and Cpd. 14

[0287] The endogenous expression of IL-6 in A549 cells by direct transfection of modified or unmodified compounds (Cpd.3, Cpd.4, Cpd.13 and Cpd.14; 0.3 µg/well) was confirmed experimentally. Distinct differences in IL-6 levels between cells transfected with modified or
30 unmodified RNA compounds (**Figure 6A**) demonstrated that chemical modification with N1-methylpseudo-UTP diminishes the immune activation in A549 cells. For modified compounds, the presence of siRNA structures (3x in Cpd.3; 6x in Cpd.4) significantly further reduced IL-6 levels relative to the respective compounds without siRNA (see, e.g. Cpd.3 vs. Cpd.13 (**, <0.01); Cpd.4 vs. Cpd.14 (***, <0.001)). Remarkable reduction in IL-6 levels

was noted for unmodified compounds in relation to the presence or absence of siRNA. Unmodified Cpd.3 with 3x siRNA targeting IL-1 beta with IGF-1 showed significantly reduced IL-6 levels compared to the unmodified compound comprising IGF-1 without siRNA i.e., Cpd.13 (***, $P<0.001$; **Figure 6A**). Similarly, the presence of 6x siRNA (3x targeting TNF-alpha and 3x targeting IL-17) along with IL-4 mRNA in unmodified Cpd.4 demonstrated decreased IL-6 levels compared to unmodified Cpd.14 comprising IL-4-encoding mRNA without siRNA (***, $P<0.001$; **Figure 6A**). The comparison of unmodified Cpd.3 (3x) and Cpd.4 (6x) shows that increased siRNA decreases the IL-6 stimulation (*, $P<0.05$; **Figure 6A**).

[0288] Immunogenicity assessment by stimulating endogenous IL-6 expression in THP-1 cells

[0289] Human monocyte leukemia cell line THP-1 (Sigma-Aldrich, Cat. #88081201) were utilized for studying the immune response against *in vitro* transcribed mRNA (IVT mRNA) by monitoring the endogenous expression of IL-6 upon direct transfection of mRNA.

Stimulation of immunogenicity against IVT mRNA are triggered by RNA sensors (e.g. TLRs, PKRs) and induces the expression of several proinflammatory cytokines including IL-6, which plays a central role in the immune activation against IVT mRNA. THP-1 cells were maintained in growth medium (RPMI 1640 supplemented with 10% FBS and 2 mM glutamine). The cells were seeded at 40,000 THP-1 cells in a 96 well cell culture plate and were reverse-transfected with specific mRNA (0.3 µg/well) using Lipofectamine MessengerMax (Thermo Fisher Scientific). The plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours. Post transfection, the cell culture supernatant was collected and quantified for human IL-6 using ELISA (ThermoFisher Cat. # 887066).

[0290] Results

[0291] Immunogenicity evaluation of Cpd.3, Cpd.4, Cpd.13 and Cpd. 14

[0292] The endogenous expression of IL-6 in THP-1 cells by direct transfection of modified or unmodified compounds (Cpd.3, Cpd.4, Cpd.13, and Cpd.14; 0.3 µg/well) was confirmed experimentally, however the expression levels were low compared to the expression levels in A549 cells. Similar to A549 cells, differences in IL-6 levels were noted between modified and unmodified RNA compounds (**Figure 7A**). A significant reduction in IL-6 levels was observed for unmodified compounds in relation to the presence or absence of siRNA. Unmodified Cpd.3 with 3x siRNA targeting IL-1 beta with IGF-1 mRNA showed significantly reduced IL-6 levels compared to the unmodified compound comprising IGF-1 mRNA alone, i.e. Cpd.13 (***, $P<0.001$; **Figure 7A**). Similarly, the presence of 6x siRNA

(3x targeting TNF-alpha and 3x targeting IL-17) along with IL-4 mRNA in unmodified Cpd.4 demonstrated decreased IL-6 levels compared to unmodified Cpd.14 comprising IL-4-encoding mRNA without siRNA (***, $P < 0.001$; **Figure 7A**).

[0293] HEK-Blue™ IL-6 Reporter assay for STAT-3 activation

5 **[0294]** The functional activity of secreted IL-6 in A549 and THP-1 *in vitro* model were tested in HEK-Blue™ IL-6 reporter cells (Invivogen, Cat. Code: hkb-il6), which are designed for studying the IL-6 signaling by monitoring the activation of STAT-3 pathway. The HEK-Blue™ IL-6 cells are derived from the human embryonic kidney HEK293 cell line and engineered to express IL-6 receptor (IL-6R) and signal transducer activator of transcription 3
10 (STAT3). IL-6 signalling is detected by a reporter gene expressing a secreted embryonic alkaline phosphatase (SEAP) under the control of the IFN- β minimal promoter fused to four STAT3 binding sites. Upon IL-6 stimulation, HEK-Blue™ IL-6 cells trigger the activation of STAT3 and the subsequent secretion of SEAP can be determined in real-time with HEK-Blue™ Detection cell culture medium in cell culture supernatant. Stimulation of HEK-Blue™
15 IL-6 cells were achieved by equivalent volume (20 μ l) of IL-6 derived from cell culture supernatant of A549 cell or THP-1 cells which had been transfected with Cpd.3, Cpd.4, Cpd.13, or Cpd.14 (0.3 μ g/well) with below details.

[0295] HEK-Blue™ IL-6 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM, Sigma Aldrich) supplemented with 10% (v/v) Fetal Bovine Serum (FBS). The
20 antibiotics HEK-Blue™ selection (1:250 dilution with media) were added to the media to select cells containing IL6R, STAT3 and SEAP transgene plasmids. Cells were seeded at 40,000 cell/well in a 96 well culture plate and incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 24 hours prior to transfection. Cells were grown in DMEM growth medium containing 10% of FBS to reach confluency < 80% before transfection. Equivalent
25 volume of (20 μ l) of cell culture supernatant from A549 and THP-1 cells which had been transfected with Cpd.3, Cpd.4, Cpd.13, or Cpd.14 (0.3 μ g/well) were collected and added to culture media of HEK-Blue™ IL-6 cells to measure IL-6 receptor recruitment followed by STAT3 pathway activation. rhIL-6 (100 ng/mL) was used as a positive control. After 24 hours of incubation, SEAP activity was assessed using QUANTI-Blue™ (20 μ l cell culture
30 supernatant + 180 μ l QUANTI-Blue™ solution) and reading the optical density (O.D.) at 620 nm in SpectraMax i3 multi-mode plate reader (Molecular Device). Untransfected samples were used as background control and subtracted from obtained O.D. values in tested samples.

[0296] Results

[0297] Immunogenicity evaluation of Cpd.3, Cpd.4, Cpd.13 and Cpd. 14

- [0298]** Stimulation of HEK-Blue™ IL-6 cells with rhIL-6 or IL-6 derived from cell culture supernatant of A549 or THP-1 cells that had been transfected with modified or unmodified Cpd.3, Cpd.4, Cpd.13 or Cpd.14 was functional as SEAP production was observed in all samples (**Figures 6B and 7B**). As expected, the supernatant derived from A549 and THP-1 cells transfected with the RNA compounds modified with N1-methylpseudouridine induced less STAT-3 signaling compared to the supernatant derived from A549 and THP-1 cells transfected with unmodified compounds (**Figures 6B and 7B**). The supernatant derived from A549 and THP-1 cells transfected with unmodified Cpd.3 with 3x siRNA targeting IL-1 beta with IGF-1 mRNA showed significantly reduced IL-6 signaling compared to the supernatant derived from A549 and THP-1 cells transfected with unmodified compound comprising IGF-1 mRNA alone, i.e. Cpd.13 (**Figures 6B and 7B**). Likewise, the presence of 6x siRNA (3x targeting TNF-alpha and 3x targeting IL-17) along with IL-4 mRNA in unmodified Cpd.4 demonstrated a decreased IL-6 mediated STAT-3 activation compared to unmodified Cpd.14 comprising IL-4-encoding mRNA without siRNA (***, $P<0.001$; **Figure 6B and 7B**) for the supernatant derived from both A549 and THP-1 cells. The comparison of unmodified Cpd.3 (3x) and Cpd.4 (6x) shows that increased number of siRNA from 3x to 6x decreases the IL-6 mediated immune activation (A549 cells, *, $P<0.05$; **Figure 6B**; THP-1 cells, **, $P<0.01$; **Figure 7B**).
- [0299]** The examples and embodiments described herein are for illustrative purposes only and various modifications or changes suggested to persons skilled in the art are to be included within the spirit and purview of this application and scope of the appended claims.

Table 5. Table of Sequences Listed

Protein or Nucleic Acid	Sequence	SEQ ID NO:
Compound 1-14 nucleic acid sequences	See Table 2 and Table 3	1-24, 42, 125, and 127-128
Kozak sequence	GCCACC	25
T7 promoter	TAATACGACTCACTATA	26
A1-linker: mRNA to siRNA linker	ATAGTGAGTCGTATTAACTACCAACAA	27
A1-linker: siRNA to siRNA linker	TTTATCTTAGAGGCATATCCCTACGTACCAACAA	28
Forward Primer	GCTGCAAGGCGATTAAGTTG	29
Reverse Primer	U (2 'OMe) U (2 'OMe) U (2 'OMe) TTTTTTTTTTTTTTTTTTTTTTTTTT	30

Protein or Nucleic Acid	Sequence	SEQ ID NO:
	TTT TTTCAGCTA TGACCATGTTAATGCAG	
IL-4 Human IL-4 Nucleotide (Genbank NM_000589.4)	ATCGTTAGCTTCTCCTGATAAACTAATTGCCCTCACATTGTCACTGCAAA TCGACACCTATTAATGGGTCTCACCTCCCAACTGCTTCCCCCTCTGTTC TTCCTGCTAGCATGTGCCGGCAACTIONTGTCCACGGACACAAGTGCGATA TCACCTTACAGGAGATCATCAAACTTTGAACAGCCTCACAGAGCAGAA GACTCTGTGCACCGAGTTGACCGTAACAGACATCTTTGCTGCCTCCAAG AACACAACCTGAGAAGGAAAACCTTCTGCAGGGCTGCGACTGTGCTCCGGC AGTTCTACAGCCACCATGAGAAGGACACTCGCTGCCTGGGTGCGACTGC ACAGCAGTTCCACAGGCACAAGCAGCTGATCCGATTCTGAAACGGCTC GACAGGAACCTCTGGGGCCTGGCGGGCTTGAATTCTGTCTGTGAAGG AAGCCAAC CAGAGTACGTTGGAAAACTTCTTGGAAAGGCTAAAGACGAT CATGAGAGAGAAATATTCAAAGTGTTCGAGCTGAATATTTTAATTTATG AGTTTTTGATAGCTTTATTTTTTAAGTATTTATATATTTATAACTCATC ATAAAATAAAGTATATATAGAATCTAA	31
IL-4 Human IL-4 amino acid (Genbank NP_000580.1) Underlined: signal sequence	MGLTSQLLPFLFLLACAGNFVHGKCDITLQEI IKTLNSLTEQKTLCT ELTVTDIFAASKNTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQQFH RHKQLIRFLKRLDRNLWGLAGLNSCPVKANQSTLENFLERLKTIMREK YSKCSS	32
IGF-1 Human IGF-1 Nucleotide (Genbank NM_000618.4)	ATGGGAAAAATCAGCAGTCTTCCAACCCAATTATTTAAGTGCTGCTTTT GTGATTTCTTGAAGGTGAAGATGCACACCATGTCTCTCGCATCTCTT CTACCTGGCGCTGTGCCGTGCTCACCTTCACCAGCTCTGCCACGGCTGGA CCGGAGACGCTCTGCGGGGCTGAGCTGGTGGATGCTCTTCAGTTCTGTG GTGGAGACAGGGGCTTTTATTTCACAAGCCCACAGGGTATGGCTCCAG CAGTCGGAGGGCGCCTCAGACAGGCATCGTGGATGAGTGTGCTTCCGG AGCTGTGATCTAAGGAGGCTGGAGATGTATTGCGCACCCCTCAAGCCTG CCAAGTCAGCTCGCTCTGTCCGTGCCCAGCGCCACACCGACATGCCCAA GACCCAGAAGGAAGTACATTTGAAGAACGCAAGTAGAGGGAGTGCAGGA AACAAGA ACTACAGGATGTAG	33
IGF-1 Human IGF-1 amino acid (Genbank NP_000609.1) Underlined: signal sequence	<u>MGKISSLP</u> TQLFKCCFCDFLKVKMHTMSSSHLFYLA LCLLTFTSSATAG PETLCGAELVDALQFVCGDRGFYNKP TGYGSSSRRAPQTGI VDECCFR SCDLRRLEMYCAPLKPASARSVRAQRH TDMPKTQKEVHLKNASRG SAG NKNYRM	34
IGF-1 Human IGF-1 Nucleotide (Optimized IGF-1 with BDNF SP and without E-peptide)	ATGACCATCCTGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGA AGGCCGTGAAGATGCACACCATGAGCAGCAGCCACCTGTTCTATCTGGC CCTGTGCCGTGCTGACCTTTACCAGCTCTGCTACCGCCGGACCTGAGACA CTTTGTGGCGCTGAAC TTGGTGGACGCCCTGCAGTTTGTGTGTGGCGACA GAGGCTTCTACTTCAACAAGCCCACAGGCTACGGCAGCAGCTCTAGAAG GGCTCCTCAGACCGGAATCGTGGACGAGTGCTGCTTCAGAAGCTGCGAC CTGCGGCGGCTGGAAATGTATTGTGCCCTCTGAAGCCTGCCAAGAGCG CCTAA	35

Protein or Nucleic Acid	Sequence	SEQ ID NO:
IGF-1 Human IGF-1 amino acid (Optimized IGF-1 with BDNF SP and without E-peptide) <u>Underlined:</u> signal sequence	MTILFLTMVISYFGCMKAVKMHTMSSSHLFYLALCLLTFTSSATAGPET LCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCD LRRLEMYCAPLKPAKSA	36
Human IL-8 Nucleotide (Genbank NM_000584.3) <i>Bold and italicized:</i> siRNA binding regions	ATGACTTCCAAGCTGGCCGTGGCTCTCTTGGCAGCCTTCTGATTTCTG CAGCTCTGTGTGAAGGTGCAGTTTTCG CAAGGAGTGCTAAAGAA CTTAG ATGTCAGTGCATAAAGACATACTCCAAACCTTCCACCCCAAATTTATC AAAGAAGCT GAGAGTGATTGAGAGTGG ACCACACTGCGCCAACACAGAAA TTATTGTAAAGCTTTCTGATGGAA GAGAGCTCTGTCTGGAC CCCAAGGA AAACTGGGTGCAGAGGGTTGTGGAGAAGTTTTTGAAGAGGGCTGAGAAT TCATAA	37
Human IL-1beta Nucleotide (Genbank NM_000594.3) <i>Bold and italicized:</i> siRNA binding regions Second siRNA binding site underlined	ATGGCAGAAGTACCTGAGCTCGCCAGTGAAATGATGGCTTATTACAGTG GCAATGAGGATGACTTGTCTTTGAAGCTGATGGCCCTAAACAGATGAA GTGCTCCTTCCAGGACCTGGACCTCTGCCCTCTGGATGGCGGCATCCAG CTACGAATCTCCGACCACCACTACAGCAAGGGCTTCAGGCAGGCCGCGT CAGTTGTTGTGGCCATGGACAAGCTGAGGAAGATGCTGGTTCCCTGCCC ACAGACCTTCCAGGAGAATGACCTGAGCACCTTCTTTCCCTTCATCTTT GAAGAAGAACCTATCTTCTTCGACACATGGGATAACGAGGCTTATGTGC ACGATGCACCTGTACGATCACTGAACTGCACGCTCCGGGACTCACAGCA AAAAAGCTT GGTGATGTCTGGTCCATATGAA CTGAAAGCTCTCCACCTC CAGGGACAGGATATGGAGCAACAAGTGGTGTCTCCATGTCTTTGTAC AAGGAGAAGAAAGTAATGACAAAATACCTGTGGCCTTGGGCCTCAAGGA AAAGAATCTGTACCTGTCTGCGTGT GAAAGATGATAAGCCCACTCTA CAGCTGGAGAGTGTAGATCCCCAAAATACCCAAAGAAGAAGATGGAAA AGCGATTTGTCTTCAACAAGATAGAAATCAATAACAAGCTGGAATTTGA GTCTGCCCAGTTCCCCAACTGGTACATCAGCACCTCTCAAGCAGAAAAC ATGCCCCTCTTCTGGGAGGGACCAAAGCGGCCAGGATATAACTGACT TCACCATGCAATTTGTGTCTTCCTAA	38
Human IL-17 Nucleotide (Genbank NM_002190.2) <i>Bold and italicized:</i> siRNA binding regions	ATGACTCCTGGGAAGACCTCATTTGGTGTCACTGCTACTGCTGCTG AGCCTGGAGGCCATAGTGAAGGCAGGAATCACAATCCACGAAAT CCAGGATGCCCAAATTTCTGAGGACAAGAAGCTTCCCCCGGACTGTG ATGGTCAACCTGAACATCCATAACCGGAATACCAATACCAATCCC AAAA GGTCCTCAGATTACTACA CCGATCCACCTCACCTTGGAAT CTCCACC GCAATGAGGACCTGAGAGAT ATCCCTCTGTGATCTGG GAGGCAAAGTGCCGCCACTTGGGCTGCATCAAC GCTGATGGGAAC GTGGACTA CCACATGAACTCTGTCCCCATCCAGCAAGAGATCCTG GTCTGCGCAGGGAGCCTCCACACTGCCCCAACTCCTTCCGGCTG GAGAAGATACTGGTGTCCGTGGGCTGCACCTGTGTCACCCCGATT GTCCACCATGTGGCCTAA	39
Human TNF- α	ATGAGCACTGAAAGCATGATCCGGGACGTGGAGCTGGCCGAGGAG GCGCTCCCCAAGAAGACAGGGGGGGCCCCAGGGCTCCAGGCGGTGC	40

Protein or Nucleic Acid	Sequence	SEQ ID NO:
Nucleotide (Genbank NM_000594.3) <i>Bold and italicized:</i> siRNA binding regions	TTGTTCCCTCAGCCTCTTCTCCTTCCTGATCGTGGCAGGCGCCACC ACGCTCTTCTGCCTGCTGCACTTTGGAGTGATCGGCCCCCAGAGG GAAGAGTTCCCCAGGGACCTCTCTCTAATCAGCCCTCTGGCCCAG GCAGTCAGATCATCTTCTCGAACCCCGAGTGACAAGCCTGTAGCC CATGTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTCCAGTGGCTG AACCGCCGGGCCAATGCCCTCCTGGCCAAT <i>GGCGTGGAGCTGAGA GATAA</i> CCAGCTGGTGGTGCCATCAGA <i>GGGCCTGTACCTCATCTAC TCCCAGTCTCTTCAAGGGCCAAGGCTGCCCCCTCCACCCATGTG CTCCTCACCCACACCATCAGCCGCATCGCCGTCTCTACCAGACC AAGGTCAACCTCCTCTCTGCCATCAAGAGCCCCCTGCCAGAGGGAG ACCCAGAGGGGGCTGAGGCCAAGCCCT</i> <i>GGTATGAGCCCATCTAT CTGGGAGGGGTCTTCCAGCTGGAGAAGGGTGACCGACTCAGCGCT GAGATCAATCGGCCCGACTATCTCGACTTTGCCGAGTCTGGGCAG GTCTACTTTGGGATCATTGCCCTGTGA</i>	
Turbo GFP <i>Bold and italicized:</i> siRNA binding regions	ATGGAGAGCGACGAGAGCGGCCCTGCCCGCCATGGAGATCGAGTGC CGCATCACCGGCACCCTGAACGGCGTGGAGTTTCGAGCTGGTGGGC GGCGGAGAGGGCACCCCCGAGCAGGGCCGCATGAC <i>CAACAAGATG AAGAGCACCAA</i> AGGCGCCCTGACCTTCAGCCCCCTACCTGCTGAGC CACGTGATGGGCTACGGCTTCTACCACTTCGGCACCTACCCACAGC GGCTACGAGAACCCCTTCTGACGCCATCAACAACGGCGGCTAC ACCAACACCCGCATCGAGAAGTACGAGGACGGCGGCGTGCTGCAC GTGAGCTTCAGCTACCGCTACGAGGCCGGCCGCGTGATCGGCGAC TTCAAGGTGATGGGCACCGGCTTCCCCGAGGACAGCGTGATCTTC ACCGACAAGATCATCCGCAGCAACGCCACCGTGGAGCACCTGCAC CCCATGGGCGATAACGATCTGGATGGCAGCTTCACCCGCACCTTC AGCCTGCGCGACGGCGGCTACTACAGCTCCGTGGT <i>GGACAGCCAC ATGCACTTCA</i> AGAGCGCCATCCACCCAGCATCCTGCAGAACGGG GGCCCCATGTTTCGCCTTCCGCCGCGTGGAGGAGGATCACAGCAAC ACCGAGCTGGGCATCGTGGAGTACCAGCACGCCTTCAAGACCCCG GATGCAGATGCCGGTGAAGAATAA	41
IL-4 Human IL-4 Nucleotide (Optimized IL-4 with modified SP)	GCCACCATGTTGCTGCTGCCTCTGTTCTTCTGCTGGCCTGCGCC GGCAATTTTGTGCACGGCCACAAGTGCACATCACCTGCAAGAG ATCATCAAGACCCTGAACAGCCTGACCGAGCAGAAAACCCTGTGC ACCGAGCTGACCGTGACCGATATCTTTGCCGCCAGCAAGAACACA ACCGAGAAAGAGACATTCTGCAGAGCCGCCACCGTGCTGAGACAG TTCTACAGCCACCACGAGAAGGACACCAGATGCCTGGGAGCTACA GCCCAGCAGTTCCACAGACACAAGCAGCTGATCCGGTTCCTGAAG CGGCTGGACAGAAATCTGTGGGGACTCGCCGGCCTGAATAGCTGC CCTGTGAAAGAGGCCAACCAGTCTACCCTGGAAAACCTCCTGGAA CGGCTGAAAACCATCATGCGCGAGAAGTACAGCAAGTGCAGCAGC TGA	42
IL-4 Human IL-4 with modified SP Underlined: signal sequence	<u>MLLLPLFFLLACAGNFVHGKCDITLQEIIKTLNSLTEQKTLCTE LTVTDIFAASKNTTEKETFCRAATVLRQFYSHHEKDTRCLGATAQ QFHRHKQLIRFLKRLDRNLWGLAGLNSCPVKEANQSTLENFLERL KTIMREKYSKCSS</u>	43
A modified	MLILLPLLLFKCFCDFLK	44

Protein or Nucleic Acid	Sequence	SEQ ID NO:
signal peptide of IGF-1		
A modified signal peptide of IGF-1 - coding sequence	ATGCTGATTCTGCTGCTGCCCTGCTGCTGTTCAAGTGCTTCTGCGACTTCCTGAAA	45
A modified IGF-1 pro domain	MLFYALCLLTFTSSATA	46
A modified IGF-1 pro domain - coding sequence	ATGCTGTTCTATCTGGCCCTGTGCTGCTGACCTTTACCAGCTCTGCTACCGCC	47
IGF-1 pro domain sequence that is deleted	VKMHTMSSSH	48
WT IGF-1 signal peptide	MGKISSLPTQLFKCCFCDFLK	49
WT IGF-1 signal peptide coding sequence	ATGGGAAAAATCAGCAGTCTTCCAACCAATTATTTAAGTGCTGCTTTTGTGATTTCTTGAAG	50
A modified signal peptide of IGF-1	MTILFLTMVISYFGCMKA	51
A modified signal peptide of IGF-1 - coding sequence	ATGACCATCCTGTTTCTGACAATGGTCATCAGCTACTTCGGCTGCATGAAGGCC	52
WT IL-4 signal peptide	MGLTSQLLPPLFLLACAGNFVHG	53
WT IL-4 signal peptide coding sequence	ATGGGTCTCACCTCCCACTGCTTCCCCCTCTGTTCTTCTGCTAGCATGTGCCGGCAACTTTGTCCACGGA	54
A modified signal peptide of IL-4	MLLLPLFLLACAGNFVHG	55
A modified signal peptide of IL-4 - coding sequence	ATGTTGCTGCTGCCTCTGTTCTTCTGCTGGCCTGCGCCGGCAATTTTGTGCACGGC	56
IL-8 siRNA sense strand (Cpd.1)	CAAGGAAGTGCTAAAGAA	57
IL-8 siRNA sense strand (Cpd.2-1)	CAAGGAGTGCTAAAGAA	58
IL-8 siRNA sense strand (Cpd.2-2)	GAGAGTGATTGAGAGTGG	59
IL-8 siRNA sense strand (Cpd.2-3)	GAGAGCTCTGTCTGGACC	60

Protein or Nucleic Acid	Sequence	SEQ ID NO:
IL-1beta siRNA sense strand (Cpd.3-1)	GAAAGATGATAAGCCCACTCT	61
IL-1beta siRNA sense strand (Cpd.3-2)	GGTGATGTCTGGTCCATATGA	62
IL-1beta siRNA sense strand (Cpd.3-3)	GATGATAAGCCCACTCTA	63
TNF-alpha siRNA sense strand (Cpd.4-1, Cpd.5-1, Cpd.6-1, Cpd.7 and Cpd.8)	GGCGTGGAGCTGAGAGATAA	64
TNF-alpha siRNA sense strand (Cpd.4-2, Cpd.5-2 and Cpd.6-2)	GGGCCTGTACCTCATCTACT	65
TNF-alpha siRNA sense strand (Cpd.4-3, Cpd.5-3 and Cpd.6-3)	GGTATGAGCCCATCTATCT	66
IL-17 siRNA sense strand (Cpd.4-1)	GCAATGAGGACCCTGAGAGAT	67
IL-17 siRNA sense strand (Cpd.4-2)	GCTGATGGGAACGTGGACTA	68
IL-17 siRNA sense strand (Cpd.4-3)	GGTCCTCAGATTACTACAA	69
Turbo GFP siRNA sense strand (Cpd.9, Cpd.10-1, Cpd.10-2, Cpd.11-1, Cpd.11-2, Cpd.11-3)	AACAAGATGAAGAGCACCAA	70
IL-8 siRNA anti-sense strand (Cpd.1)	TTCTTTAGCACTTCCTTG	71
IL-8 siRNA anti-sense strand (Cpd.2-1)	TTCTTTAGCACTCCTTG	72
IL-8 siRNA anti-sense strand (Cpd.2-2)	CCACTCTCAATCACTCTC	73
IL-8 siRNA anti-sense strand	GGTCCAGACAGAGCTCTC	74

Protein or Nucleic Acid	Sequence	SEQ ID NO:
(Cpd.2-3)		
IL-1beta siRNA anti-sense strand (Cpd.3-1)	AGAGTGGGCTTATCATCTTTC	75
IL-1beta siRNA anti-sense strand (Cpd.3-2)	TCATATGGACCAGACATCACC	76
IL-1beta siRNA anti-sense strand (Cpd.3-3)	TAGAGTGGGCTTATCATC	77
TNF-alpha siRNA anti-sense strand (Cpd.4-1, Cpd.5-1, Cpd.6-1, Cpd.7 and Cpd.8)	TTATCTCTCAGCTCCACGCC	78
TNF-alpha siRNA anti-sense strand (Cpd.4-2, Cpd.5-2 and Cpd.6-2)	AGTAGATGAGGTACAGGCC	79
TNF-alpha siRNA anti-sense strand (Cpd.4-3, Cpd.5-3 and Cpd.6-3)	AGATAGATGGGCTCATACC	80
IL-17 siRNA anti-sense strand (Cpd.4-1)	ATCTCTCAGGGTCCTCATGTC	81
IL-17 siRNA anti-sense strand (Cpd.4-2)	TAGTCCACGTTCCCATCAGC	82
IL-17 siRNA anti-sense strand (Cpd.4-3)	TTGTAGTAATCTGAGGACC	83
Turbo GFP siRNA anti-sense strand (Cpd.9, Cpd.10-1, Cpd.10-2, Cpd.11-1, Cpd.11-2, Cpd.11-3)	TTGGTGCTCTTCATCTTGTTG	84
A2-linker	ACAACAA	85
Linker	ATAGTGAGTCGTATTA	86
Linker	ATCCCTACGTACCAACAA	87
Linker	ACGTACCAACAA	88
Linker	TCCC	89
Linker	ACAACAATCCC	90
Linker	CAACAA	91

Protein or Nucleic Acid	Sequence	SEQ ID NO:
Linker	ATAGTGAGTCGTATTATCCC	92
Linker	ATAGTGAGTCGTATTAAACAACATCCC	93
Linker	ATAGTGAGTCGTATTAAACAACAA	94
Linker	ATAGTGAGTCGTATTATCCCTACGTACCAACAA	95
tRNA linker	AACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACA GACCCGGGTTTCGATTCCCGGCTGGTGCA	96
Compound 1 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAAUUAACGUACCAACAA CAAGGAAGUGCUAAAGAA ACUUG <u>UUCUUUAGCACUCCUUG</u> UUUAUCUUAGAGGCAUAUCCCUGCCACCAUGACCAUCCU GUUUCUGACAAUGGUCAUCAGCUACUUCGGCUGCAUGAAGGCCGUGAAGAU CACCAGAGCAGCAGCCACCUGUUCUAUCUGGCCUGUGCCUGCUGACCUUAC CAGCUCUGCUACCGCCGGACCUGAGACACUUUGGGCGUGAACUGGGGACGC CCUGCAGUUUGUGUGGGCAGAGGCUUCUACUUAACAAGCCACAGGCUA CGGCAGCAGCUCUAGAAGGGCUCUACAGACCGGAAUCUGGGACGAGUGCUCU CAGAAGCUGCGACCUGCGGGCGGUGGAAUGUAUUGGCCCUUGAAGCCUGC CAAGAGCGCCUAAUUUAUCUUAGAGGCAUAUCCCU	97
Compound 2 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAAUUAACGUACCAACAA CAAGGAGUGCUAAAGAA ACUUG <u>UUCUUUAGCACUCCUUG</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAGAGAGUG AUUGAGAGUGGACUUGCCACUCUCAUACUCUCU UUUAUCUUAGAGGCAUAUCC CUACGUACCAACAAGAGAGCUCUGUCUGGACCACUUG <u>GGUCCAGACAGAGCUCU</u> <u>CUUUAUCUUAGAGGCAUAUCCCUGCCACCAUGACCAUCCU</u> GUUUCUGACAAUGG UCAUCAGCUACUUCGGCUGCAUGAAGGCCGUGAAGAUCAACCAUGAGCAGCA GCCACCUGUUCUAUCUGGCCUGUGCCUGCUGACCUUUAACAGCUCUGCUACCG CCGGACCUGAGACACUUUGGGCGCUGAACUGGGGACGCCUGCAGUUUGUGU GUGGCAGCAGAGGCUUCUACUUAACAAGCCACAGGCUACGGCAGCAGCUCUA GAAGGGCUCUACAGACCGGAAUCUGGGACGAGUGCUCUUCAGAAGCUGCGACC UGCGGGCGCUGGAAUGUAUUGGCCCUUGAAGCCUGCCAAGAGCGCCUAAU UUAUCUUAGAGGCAUAUCCCU	98
Compound 3 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAAUUAACGUACCAACAA GAAAGAUUAAGCCACUCU ACUUG AGAGUGGGCUUAUCAUCUUUC UUUAUCUUAGAGGCAUAUCCCUACGUACCAACA AGGUGAGUGUCUGGUCCAUAUGAACUUG <u>UCAUAUGGACCAGACAUCACC</u> UUUAUC UUAGAGGCAUAUCCCUACGUACCAACAAGAUUAAGCCACUCUACUUG <u>UAGAGUGGGCUUAUCAUC</u> UUUAUCUUAGAGGCAUAUCCCUGCCACCAUGACCAUCCU GUUUCUGACAAUGGUCAUCAGCUACUUCGGCUGCAUGAAGGCCGUGAAGAU CACCAGAGCAGCAGCCACCUGUUCUAUCUGGCCUGUGCCUGCUGACCUUAC CAGCUCUGCUACCGCCGGACCUGAGACACUUUGGGCGUGAACUGGGGACGC CCUGCAGUUUGUGUGGGCAGAGGCUUCUACUUAACAAGCCACAGGCUA CGGCAGCAGCUCUAGAAGGGCUCUACAGACCGGAAUCUGGGACGAGUGCUCU CAGAAGCUGCGACCUGCGGGCGGUGGAAUGUAUUGGCCCUUGAAGCCUGC CAAGAGCGCCUAAUUUAUCUUAGAGGCAUAUCCCU	99
Compound 4 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAAUUAACGUACCAACAA GGCGUGGAGCUGAGAGAUAA ACUUG <u>UUAUCUCACAGUCCACGCC</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAG GGCCUGUACCUCUACUACU ACUUG <u>AGUAGAUGAGGUACAGGCC</u> UUUAUCUUAG AGGCAUAUCCCUACGUACCAACAAGGUUAUGAGCCCAUCUAUCUACUUG <u>AGAUAAGUGGGCUAUAAC</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAGCAAGGA AGGCCUGAGAGAU ACUUG <u>AUCUCUCAGGGUCCUATUGC</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAGCUGAUGGCAUAUCCCUACGUACCAACAAGCUGAUGGGAACGUGGACUAACUUG <u>UAGUCCACG</u> <u>UUCCAUCAGC</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAGGUCCUCAG AUUACUACAA ACUUG <u>UUGUAGUAAUCUGAGGACC</u> UUUAUCUUAGAGGCAUAUCC CUGCCACCAUGGGACUGACAUCAACUGCUGCCUCCACUGUUCUUUCUGCUGG CCUGCGCCGGCAAUUUUGUGCAGGCCACAAGUGCGACAUACCCUGCCAAGAGA UCAUCAAGACCCUGAACCCUGACCGAGCAGAAAACCCUGUGCACCGGACUGA CCGUGACCGAUUAUCUUUGCCGCCAGCAAGAACACAACCGAGAAAGAGACAUUCU GCAGAGCCGCCACCGUGCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCA GAUGCCUGGGAGCUACAGCCACAGAUUCCACAGACACAAGCAGCUGAUCCGGU UCCUGAAGCGGUGGACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCC CUGUGAAAGAGGCCAACAGCUACCCUGGAAACUUCUGGAACGGCUGAAAA CCAUCAUGCGCGAGAAGUACAGCAAGUGCAGCAGCUGAUUUUAUCUUAGAGGCAU	100

Protein or Nucleic Acid	Sequence	SEQ ID NO:
	AUCCCU	
Compound 5 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAAUUAACGUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUGU <u>UAUCUCUCAGCUCACGCCC</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAG <u>GGCCUGUACCUCUACUACU</u> ACUUG <u>AGUAGAUGAGGUACAGGCC</u> UUUAUCUUAG AGGCAUAUCCCUACGUACCAACAAG <u>GGUAUGAGCCCAUCUACUACU</u> AG <u>AGAUAG</u> <u>AUGGGCUCAUACC</u> UUUAUCUUAGAGGCAUAUCCCU <u>GCCACCAUGGGACUGACAU</u> <u>CUCAACUGCUGCCUCCACUGUUCUUUCUGCUGGCCUGCGCCGGCAAUUUUGUGC</u> <u>ACGGCCACAAGUGCGACAUACCCUGCAAGAGAUCAUCAAGACCCUGAACAGCC</u> <u>UGACCGAGCAGAAAAACCCUGUGCACCAGCUGACCCUGACCGAUACUUUGCCG</u> <u>CCAGCAAGAACAACCCGAGAAAGAGACAUUCUGCAGAGCCGCCACCCUGUCUGA</u> <u>GACAGUUCUACAGCCACCACGAGAAGGACACCAGAUGCCUGGGAGCUACAGCCC</u> <u>AGCAGUCCACAGACACAAGCAGCUGAUCCGGUUCUGAAGCGGCUGGACAGAA</u> <u>AUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCUGUGAAAGAGGCCAACCAGU</u> <u>CUACCCUGGAAAACUUCUGGAACGGCUGAAAACCAUCAUGCGCGAGAAGUACA</u> <u>GCAAGUGCAGCAGCUGAUUUUAUCUUAGAGGCAUAUCCCU</u>	101
Compound 6 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGACUGACAUUCUACACUGCUGCCUCCACUGUUCUUUCUGCUGGCC</u> <u>UGCGCCGGCAAUUUUGUGCACGGCCACAAGUGCGACAUACCCUGCAAGAGAU</u> <u>AUCAAGACCCUGAACAGCCUGACCGAGCAGAAAAACCCUGUGCACCAGCUGACC</u> <u>GUGACCGAUACUUUGCCGCCAGCAAGAACAACCCGAGAAAGAGACAUUCUGC</u> <u>AGAGCCGCCACCCUGUCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGA</u> <u>UGCCUGGGAGCUACAGCCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUC</u> <u>CUGAAGCGGCUGGACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCU</u> <u>GUGAAAGAGGCCAACCCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACC</u> <u>AUCAUGCGCGAGAAGUACAGCAAGUGCAGCAGCUGAAUAGUGAGUCGUAAUAA</u> <u>GUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUG</u> <u>UAUCUCUCAGCUCACG</u> <u>CUUUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAGGGCCUGUACCUCUAC</u> <u>UACUUGAGUAGAUGAGGUACAGGCC</u> UUUAUCUUAGAGGCAUAUCCCUACGUAC CAACAA <u>GGUAUGAGCCCAUCUACUACU</u> AG <u>AGAUAGAUGGGCUCAUACC</u> UUUAU CUUAGAGGCAUAUCCCUUUUAUCUUAGAGGCAUAUCCCU	102
Compound 7 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAAUUAACGUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUGU <u>UAUCUCUCAGCUCACGCCC</u> UUUAUCUUAGAGGCAUAUCCCU <u>GCCACCAUGGGCC</u> <u>UGACAUUCUAGUUGCUGCCUCCACUGUUCUUUCUGCUGGCCUGCGCCGGCAAUU</u> <u>UUGUGCACGGCCACAAGUGCGACAUACCCUGCAAGAGAUCAUCAAGACCCUGA</u> <u>ACAGCCUGACCGAGCAGAAAAACCCUGUGCACCAGCUGACCCUGACCGAUACU</u> <u>UUGCCGCCAGCAAGAACAACAACCCGAGAAAGAGACAUUCUGCAGAGCCGCCACCG</u> <u>GUGCAGACAGUUCUACAGCCACCACGAGAAGGACACCAGAUGCCUGGGAGCUA</u> <u>CAGCCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUCUGAAGCGGCUGG</u> <u>ACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCUGUGAAAGAGGCCA</u> <u>ACCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACCAUCAUGCGCGAGA</u> <u>AGUACAGCAAGUGCAGCAGCUGAUUUUAUCUUAGAGGCAUAUCCCU</u>	103
Compound 8 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGACUGACAUUCUACACUGCUGCCUCCACUGUUCUUUCUGCUGGCC</u> <u>UGCGCCGGCAAUUUUGUGCACGGCCACAAGUGCGACAUACCCUGCAAGAGAU</u> <u>AUCAAGACCCUGAACAGCCUGACCGAGCAGAAAAACCCUGUGCACCAGCUGACC</u> <u>GUGACCGAUACUUUGCCGCCAGCAAGAACAACAACCCGAGAAAGAGACAUUCUGC</u> <u>AGAGCCGCCACCCUGUCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGA</u> <u>UGCCUGGGAGCUACAGCCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUC</u> <u>CUGAAGCGGCUGGACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCU</u> <u>GUGAAAGAGGCCAACCCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACC</u> <u>AUCAUGCGCGAGAAGUACAGCAAGUGCAGCAGCUGAAUAGUGAGUCGUAAUAA</u> <u>GUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUG</u> <u>UAUCUCUCAGCUCACG</u> <u>CUUUUAUCUUAGAGGCAUAUCCCUUUUAUCUUAGAGGCAUAUCCCU</u>	104
Compound 9 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand	<u>GCCACCAUGGGACUGACAUUCUACACUGCUGCCUCCACUGUUCUUUCUGCUGGCC</u> <u>UGCGCCGGCAAUUUUGUGCACGGCCACAAGUGCGACAUACCCUGCAAGAGAU</u> <u>AUCAAGACCCUGAACAGCCUGACCGAGCAGAAAAACCCUGUGCACCAGCUGACC</u> <u>GUGACCGAUACUUUGCCGCCAGCAAGAACAACAACCCGAGAAAGAGACAUUCUGC</u> <u>AGAGCCGCCACCCUGUCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGA</u> <u>UGCCUGGGAGCUACAGCCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUC</u> <u>CUGAAGCGGCUGGACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCU</u> <u>GUGAAAGAGGCCAACCCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACC</u> <u>GUGAAAGAGGCCAACCCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACC</u>	105

Protein or Nucleic Acid	Sequence	SEQ ID NO:
Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUCAUGCGCGAGAAGUACAGCAAGUGCAGCAGCUGAACACAAGGCGUGGAGCU GAGAGAUAA CUUG UUAUCUCUCAGCUC CACGCC ACAACAAGGGCCUGUACCUC AUCUACUACUUGAGUAGAUGAGGUACAGGCC ACAACAAGGUAGAGCCCAUCU AUCUACUUGAGAUAGAUGGGCUCAUACC ACAACAUAUUUAUCUAGAGGCAUAUCCU	
Compound 10 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUCAAGUGCUGCUUC</u> <u>UGCGACUUCUGAAAGUGAAGAUGCACACCAUGAGCAGCAGCCACCUGUUCUAU</u> <u>CUGGCCUGUGCCUGCUGACCUUUACCAGCUCUGCUACCGCCGGACCUGAGACA</u> <u>CUUUGUGGCGCUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGCGACAGAGGC</u> <u>UUCUACUUCAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCCUCAG</u> <u>ACCGGAAUCGUGGACGAGUGCUGUUUCAGAAGCUGCGACCUGCGGCGGCGUGGAA</u> <u>AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAAUAGUGAGUCGUAUU</u> <u>AACGUACCAACACAACAAGAUGAAGAGCACCAA</u> CUUG UUGGUGCUCUCAUC UUGUUG UUUAUCUAGAGGCAUAUCCCUUUUAUCUAGAGGCAUAUCCCU	106
Compound 11 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUCAAGUGCUGCUUC</u> <u>UGCGACUUCUGAAAGUGAAGAUGCACACCAUGAGCAGCAGCCACCUGUUCUAU</u> <u>CUGGCCUGUGCCUGCUGACCUUUACCAGCUCUGCUACCGCCGGACCUGAGACA</u> <u>CUUUGUGGCGCUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGCGACAGAGGC</u> <u>UUCUACUUCAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCCUCAG</u> <u>ACCGGAAUCGUGGACGAGUGCUGUUUCAGAAGCUGCGACCUGCGGCGGCGUGGAA</u> <u>AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAAUAGUGAGUCGUAUU</u> <u>AACGUACCAACACAACAAGAUGAAGAGCACCAA</u> CUUG UUGGUGCUCUCAUC UUGUUG UUUAUCUAGAGGCAUAUCCCUACGUACCAACA CAACAAGAUGAAGA GCACCAA CUUG UUGGUGCUCUCAUCUUGUUG UUUAUCUAGAGGCAUAUCCCU UUUAUCUAGAGGCAUAUCCCU	107
Compound 12 unmodified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUCAAGUGCUGCUUC</u> <u>UGCGACUUCUGAAAGUGAAGAUGCACACCAUGAGCAGCAGCCACCUGUUCUAU</u> <u>CUGGCCUGUGCCUGCUGACCUUUACCAGCUCUGCUACCGCCGGACCUGAGACA</u> <u>CUUUGUGGCGCUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGCGACAGAGGC</u> <u>UUCUACUUCAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCCUCAG</u> <u>ACCGGAAUCGUGGACGAGUGCUGUUUCAGAAGCUGCGACCUGCGGCGGCGUGGAA</u> <u>AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAAUAGUGAGUCGUAUU</u> <u>AACGUACCAACACAACAAGAUGAAGAGCACCAA</u> CUUG UUGGUGCUCUCAUC UUGUUG UUUAUCUAGAGGCAUAUCCCUACGUACCAACA CAACAAGAUGAAGA GCACCAA CUUG UUGGUGCUCUCAUCUUGUUG UUUAUCUAGAGGCAUAUCCCU UACGUACCAACA CAACAAGAUGAAGAGCACC AACUUG UUGGUGCUCUCAUC UUGUUG UUUAUCUAGAGGCAUAUCCCUUUUAUCUAGAGGCAUAUCCCU	108
Compound 1 modified Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAUUAAACGUACCAACA CAAGGAAGUGCUAAAGAA CUUG UUC UUUAGCACUUCUUG UUUAUCUAGAGGCAUAUCCCU <u>GCCACCAUGACCAUCCU</u> <u>GUUUCUGACAAUGGUCAUCAGCUACUUCGGCUGCAUGAAGGCCGUGAAGAU</u> <u>GCA</u> <u>CACCAUGAGCAGCAGCCACCUGUUCUAUCUGGCCUGUGCCUGCUGACCUUAC</u> <u>CAGCUCUGCUACCGCCGGACCUGAGACACUUUGUGGCGCUAACUGGUGGACGC</u> <u>CCUGCAGUUUGUGUGUGGCGACAGAGGCUUCUACUUCAACAAGCCACAGGCUA</u> <u>CGGCAGCAGCUCUAGAAGGGCUCCUCAGACCGGAAUCGUGGACGAGUGCUGCUU</u> <u>CAGAAGCUGCGACCUGCGGCGGCGUGGAAUGUAUUGUGCCCCUCUGAAGCCUGC</u> <u>CAAGAGCGCCUAAUUUAUCUAGAGGCAUAUCCCU</u> (all Us are modified; N ¹ -methylpseudouridine)	109

Protein or Nucleic Acid	Sequence	SEQ ID NO:
Compound 2 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAUUAACGUACCAACAACAAGGAGUGCUAAAGAAACUUG <u>UUUUUUUAGAGGCAUAUCCCUACGUACCAACAAGAGAGUGAUUAGAGUGGACUUGCCACUCUCAAUCACUCUCUUUAU</u> CUUAGAGGCAUAUCCCUACGUACCAACAAGAGAGCUCUGUCUGGACCACUUG <u>GGUCCAGACAGAGCUCU</u> CUUUAU <u>CUUAGAGGCAUAUCCCUGCCACCAUGACCAUCCUGUUUCUGACAAUGGUCAUCAGCUACUUCGGCUGCAUGAAGGCCGUGAAGAUGCACACCAUGAGCAGCA</u> GCCACCU <u>GUUCUAUCUGGCCUCUGGCCUGCUGACCUUUACCAGCUCUGCUACCG</u> CCGGACCUGAGACACUUUGUGGCGCUGAACUGGUGGACGCCUCGCAGUUUGUGUGUGGCGACAGAGGCUUCUACUUC <u>AAACAAGCCCCACAGGCUACGGCAGCAGCUCUA</u> GAAGGGCUCUCAGACCGGAUUCGUGGACGAGUGCUGCUUCAGAAAGCUGCGACCUGCGCGGCUGGAAUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAUUAUCUUAGAGGCAUAUCCCU (all Us are modified; N ¹ -methylpseudouridine)	110
Compound 3 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAUUAACGUACCAACAAGAAAGAUUAAGCCCACUCUACUUG <u>AGAGUGGGCUUAUCAUCUUUUUUUUAU</u> CUUAGAGGCAUAUCCCUACGUACCAACAAGGUGAUGUCUGGUCCAUAUGAACUUG <u>UCAUAUGGACCAGACAUCACC</u> UUUAU <u>CUUAGAGGCAUAUCCCUACGUACCAACAAGAUUAAGCCCACUCUAACUUGUAGAGUGGGCUUAUCAUC</u> UUUAU <u>CUUAGAGGCAUAUCCCUGCCACCAUGACCAUCCUGUUUCUGACAAUGGUCAUCAGCUACUUCGGCUGCAUGAAGGCCGUGAAGAUGC</u> CACCAUGAGCAGCAGCCACCUGUUUCUAUCUGGCCUCUGUGCCUGCUGACCUUUACAGCUCUGCUACCGCCGGACCUGAGACACUUUGUGGCGCUGAACUGGUGGACGC	111
Compound 4 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	CCUGCAGUUUGUGUGGGCAGAGGCUUCUACUUC <u>AAACAAGCCCCACAGGCUA</u> CGGCAGCAGCUCUAGAAGGGCUCUCAGACCGGAUUCGUGGACGAGUGCUGCUUCAGAAAGCUGCGACCUGCGCGGCUGGAAUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAUUUAUCUUAGAGGCAUAUCCCU (all Us are modified; N ¹ -methylpseudouridine)	112
Compound 5 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	AUAGUGAGUCGUAUUAACGUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUG <u>UAUCUCUCAGCUCACGCCUUUAU</u> CUUAGAGGCAUAUCCCUACGUACCAACAAGGGCCUGUACCUCUACUACUACUUG <u>AGUAGAUGAGGUACAGGCCUUUAU</u> CUUAGAGGCAUAUCCCUACGUACCAACAAGGUAUGAGGCCAUCAUCUACUUG <u>AGAUAGAUUGGGCUCAUACC</u> UUUAU <u>CUUAGAGGCAUAUCCCUACGUACCAACAAGCAAUGAGGACCCUGAGAGAUACUUGAUCUCUCAGGGUCCUAUUGCUUUUAU</u> CUUAGAGGCAUAUCCCUACGUACCAACAAGCGUAUGGGAAACGUGGACUAAUUG <u>UAGUCCACG</u> UUCCCAUCAGCUUUUAU <u>CUUAGAGGCAUAUCCCUACGUACCAACAAGGUCCUCAGAUUACUACAACUUGUUGUAGUAAUCUGAGGACCUUUUAU</u> CUUAGAGGCAUAUCCCUGCCACCAUGGGACUGACAUCCAACUGCUGCCUCCACUGUUUCUUGCUGGCCUGCGCCGGCAAUUUUGUGCAGCGCCACAAGUGCGACAUCAACCCUGCAAGAGAUCAUAGACCCUGAACAGCCUGACCGAGCAGAAAACCCUGUGCACCAGCUGACCGUGACCGGAUAUCUUUGCCGCCAGCAAGAACAACCGAGAAAGAGACAUUCUGCAGAGCCGCCACCUGUGCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGAUGCCUGGGAGCUCAGCCCAGCAGUCCACAGACACAAGCAGCUGAUCCGGUUCUGAAGCGGCGUGGACAGAAUUCUGUGGGGACUCGCGCGCCUGAAUAGCUGCCUGUGAAAGAGGCCAACAGUCUACCCUGGAAAACUCCUGGAACGGCUGAAAA	113

Protein or Nucleic Acid	Sequence	SEQ ID NO:
	(all Us are modified; N ¹ -methylpseudouridine)	
Compound 6 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGACUGACAUCUCAACUGCUGCCUCCACUGUUCUUUCUGCUGGCC</u> <u>UGCGCCGGCAAUUUUUGGCACGGCCACAAGUGCGACAUCACCCUGCAAGAGAUC</u> <u>AUCAAGACCCUGAACAGCCUGACCGAGCAGAAAACCCUGUGCACCAGCUGACC</u> <u>GUGACCGAUUAUCUUUGCCGCCAGCAAGAACAACACACCCGAGAAAGAGACAUUCUGC</u> <u>AGAGCCGCCACCGUGCUGAGACAGUUCUACAGCCACCACGAGAAGGACACACAGA</u> <u>UGCCUGGGAGCUACAGCCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUC</u> <u>CUGAAGCGGCUGGACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCU</u> <u>GUGAAAGAGGCCAACCCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACC</u> <u>AUCAUGCGCGAGAAGUACAGCAAGUGCAGCAGCUGAAUAGUGAGUCGUAAUAAAC</u> <u>GUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUG</u><i>UUAUCUCUCAGCUGCCACGC</i> <u><i>CUUUAUCUUAGAGGCAUAUCCCUACGUACCAACAAGGGCCUGUACCUCAUCUAC</i></u> <u><i>UACUUGAGUAGAUGAGGUACAGGCCCUUUUAUCUUAGAGGCAUAUCCCUACGUAC</i></u> <u><i>CAACAAGGUUAUGAGCCCAUCUAUCUACUUGAGAUAGAUGGGCUCAUACCUUUUAU</i></u> <u><i>CUUAGAGGCAUAUCCCUUUUAUCUUAGAGGCAUAUCCCU</i></u> (all Us are modified; N¹-methylpseudouridine)	114
Compound 7 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>AUAGUGAGUCGUAAUAAACGUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUG</u><i>U</i> <u><i>UUAUCUCUCAGCUGCCACGCCCUUUUAUCUUAGAGGCAUAUCCCUGCCACCAUGGGCC</i></u> <u><i>UGACAUCUCAGUUGCUGCCUCCACUGUUCUUUCUGCUGGCCUGCGCCGGCAAUU</i></u> <u><i>UUGUGCACGGCCACAAGUGCGACAUCACCCUGCAAGAGAUCAUCAAGACCCUGA</i></u> <u><i>ACAGCCUGACCGAGCAGAAAACCCUGUGCACCAGCUGACCUGACCGAUUAUCU</i></u> <u><i>UUGCCGCCAGCAAGAACAACACACCCGAGAAAGAGACAUUCUGCAGAGCCGCCACCG</i></u> <u><i>UGCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGAUGCCUGGGAGCUA</i></u> <u><i>CAGCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUCUGAAGCGGCUGG</i></u> <u><i>ACAGAAUUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCUUGUGAAAGAGGCCA</i></u> <u><i>ACCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACCAUCAUGCGCGAGA</i></u> <u><i>AGUACAGCAAGUGCAGCAGCUAGUUUAUCUUAGAGGCAUAUCCCU</i></u> (all Us are modified; N¹-methylpseudouridine)	115
Compound 8 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGACUGACAUCUCAACUGCUGCCUCCACUGUUCUUUCUGCUGGCC</u> <u>UGCGCCGGCAAUUUUUGGCACGGCCACAAGUGCGACAUCACCCUGCAAGAGAUC</u> <u>AUCAAGACCCUGAACAGCCUGACCGAGCAGAAAACCCUGUGCACCAGCUGACC</u> <u>GUGACCGAUUAUCUUUGCCGCCAGCAAGAACAACACACCCGAGAAAGAGACAUUCUGC</u> <u>AGAGCCGCCACCGUGCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGA</u> <u>UGCCUGGGAGCUACAGCCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUC</u> <u>CUGAAGCGGCUGGACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCU</u> <u>GUGAAAGAGGCCAACCCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACC</u> <u>AUCAUGCGCGAGAAGUACAGCAAGUGCAGCAGCUGAAUAGUGAGUCGUAAUAAAC</u> <u><i>GUACCAACAAGGCGUGGAGCUGAGAGAUAAACUUG</i></u><i>UUAUCUCUCAGCUGCCACGC</i> <u><i>CUUUAUCUUAGAGGCAUAUCCCUUUUAUCUUAGAGGCAUAUCCCU</i></u> (all Us are modified; N¹-methylpseudouridine)	116
Compound 9 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	<u>GCCACCAUGGGACUGACAUCUCAACUGCUGCCUCCACUGUUCUUUCUGCUGGCC</u> <u>UGCGCCGGCAAUUUUUGGCACGGCCACAAGUGCGACAUCACCCUGCAAGAGAUC</u> <u>AUCAAGACCCUGAACAGCCUGACCGAGCAGAAAACCCUGUGCACCAGCUGACC</u> <u>GUGACCGAUUAUCUUUGCCGCCAGCAAGAACAACACACCCGAGAAAGAGACAUUCUGC</u> <u>AGAGCCGCCACCGUGCUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGA</u> <u>UGCCUGGGAGCUACAGCCCAGCAGUUCACAGACACAAGCAGCUGAUCCGGUUC</u> <u>CUGAAGCGGCUGGACAGAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCU</u> <u>GUGAAAGAGGCCAACCCAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACC</u> <u>AUCAUGCGCGAGAAGUACAGCAAGUGCAGCAGCUGAACAACAAGGCGUGGAGCU</u> <u><i>GAGAGAUAAACUUG</i></u><i>UUAUCUCUCAGCUGCCACGCCACAACAAGGGCCUGUACCU</i> <u><i>AUCUACUACUUGAGUAGAUGAGGUACAGGCCCAACAAGGUUAUGAGCCCAUCU</i></u> <u><i>AUCUACUUGAGAUAGAUGGGCUCAUACCACAACAACUUUAUCUUAGAGGCAUAUC</i></u> <u><i>CCU</i></u> (all Us are modified; N¹-methylpseudouridine)	117
Compound 10 modified RNA sequence Bold = Sense siRNA strand	<u>GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUAAGUGCUGCUUC</u> <u>UGCGACUUCUGAAAUGAGAUGCACACCAUGAGCAGCAGCCACCGUUCUUA</u> <u>CUGGCCUUGUGCCUGCUGACCUUUUACAGCUCUGCUACCGCCGGACCUAGACA</u> <u>CUUUGUGGGCGUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGGCGACAGAGGC</u> <u>UUCUACUUAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCCUCAG</u>	118

Protein or Nucleic Acid	Sequence	SEQ ID NO:
Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	ACCGGAAUCGUGGACGAGUGCGUUUCAGAAGCUGCGACCUGCGGGCGGUGGAA AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAAUGAGUGGUAUU AACGUACCAACAA CAACAAGAUGAAGAGCACC AAACUUG <u>UUGGUGCUCUUAUC</u> <u>UUGUUG</u> UUUAUCUUAGAGGCAUAUCCCUUUUAUCUUAGAGGCAUAUCCCU (all Us are modified; N ¹ -methylpseudouridine)	
Compound 11 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUAAGUGCUGCUUC UGCGACUUCUGAAAGUGAAGAUGCACACCAUGAGCAGCAGCCACCUGUUCUAU CUGGCCCUGUGCCUGCUGACCUUUACCAGCUCUGCUACCGCCGGACCUGAGACA CUUUGUGGCGCUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGCGACAGAGGC UUCUACUUAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCUCAG ACCGGAAUCGUGGACGAGUGCGUUUCAGAAGCUGCGACCUGCGGGCGGUGGAA AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAAUGAGUGGUAUU AACGUACCAACAA CAACAAGAUGAAGAGCACC AAACUUG <u>UUGGUGCUCUUAUC</u> <u>UUGUUG</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAA CAACAAGAUGAAGA GCACCAA ACUUG <u>UUGGUGCUCUUAUCUUGUUG</u> UUUAUCUUAGAGGCAUAUCCCU (all Us are modified; N ¹ -methylpseudouridine)	119
Compound 12 modified RNA sequence Bold = Sense siRNA strand Bold and Italics = anti-Sense siRNA strand Underline = Signal peptide <i>Italics</i> = Kozak sequence	GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUAAGUGCUGCUUC UGCGACUUCUGAAAGUGAAGAUGCACACCAUGAGCAGCAGCCACCUGUUCUAU CUGGCCCUGUGCCUGCUGACCUUUACCAGCUCUGCUACCGCCGGACCUGAGACA CUUUGUGGCGCUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGCGACAGAGGC UUCUACUUAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCUCAG ACCGGAAUCGUGGACGAGUGCGUUUCAGAAGCUGCGACCUGCGGGCGGUGGAA AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAAAUGAGUGGUAUU AACGUACCAACAA CAACAAGAUGAAGAGCACC AAACUUG <u>UUGGUGCUCUUAUC</u> <u>UUGUUG</u> UUUAUCUUAGAGGCAUAUCCCUACGUACCAACAA CAACAAGAUGAAGA GCACCAA ACUUG <u>UUGGUGCUCUUAUCUUGUUG</u> UUUAUCUUAGAGGCAUAUCCCU UACGUACCAACAA CAACAAGAUGAAGAGCACC AAACUUG <u>UUGGUGCUCUUAUC</u> <u>UUGUUG</u> UUUAUCUUAGAGGCAUAUCCCUUUUAUCUUAGAGGCAUAUCCCU (all Us are modified; N ¹ -methylpseudouridine)	120
Compound 13 unmodified RNA sequence Underline = Signal peptide <i>Italics</i> = Kozak sequence	GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUAAGUGCUGCUUC UGCGACUUCUGAAAGUGAAGAUGCACACCAUGAGCAGCAGCCACCUGUUCUAU CUGGCCCUGUGCCUGCUGACCUUUACCAGCUCUGCUACCGCCGGACCUGAGACA CUUUGUGGCGCUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGCGACAGAGGC UUCUACUUAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCUCAG ACCGGAAUCGUGGACGAGUGCGUUUCAGAAGCUGCGACCUGCGGGCGGUGGAA AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAA	121
Compound 14 unmodified RNA sequence Underline = Signal peptide <i>Italics</i> = Kozak sequence	GCCACCAUGUUGCUGCUGCCUCUGUUCUUCUGCUGGCCUGCGCCGGCAAUUUU GUGCAGCGGCCACAAGUGCGACAUCACCCUGCAAGAGAUCAUCAAGACCCUGAAC AGCCUGACCGAGCAGAAAACCCUGUGCACCAGCUGACCGUGACCGAUUUCUUU GCCGCCAGCAAGAACACAACCCGAGAAAGAGACAUUCUGCAGAGCCGCCACCGUG CUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGAUGCCUGGGAGCUACA GCCCAGCAGUCCACAGACACAAGCAGCUGAUCCGGUUCUGAAGCGGCUGGAC AGAAAUUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCUGUGAAAGAGGCCAAC CAGUCUACCCUGGAAAACUUCUGGAACGGCUGAAAACCAUCAUGCGCGAGAGAG UACAGCAAGUGCAGCAGCUGA	122
Compound 13 modified RNA sequence Underline = Signal peptide <i>Italics</i> = Kozak sequence	GCCACCAUGGGCAAGAUUAGCAGCCUGCCUACACAGCUGUUAAGUGCUGCUUC UGCGACUUCUGAAAGUGAAGAUGCACACCAUGAGCAGCAGCCACCUGUUCUAU CUGGCCCUGUGCCUGCUGACCUUUACCAGCUCUGCUACCGCCGGACCUGAGACA CUUUGUGGCGCUGAACUGGUGGACGCCUGCAGUUUGUGUGUGGCGACAGAGGC UUCUACUUAACAAGCCACAGGCUACGGCAGCAGCUCUAGAAGGGCUCUCAG ACCGGAAUCGUGGACGAGUGCGUUUCAGAAGCUGCGACCUGCGGGCGGUGGAA AUGUAUUGUGCCCCUCUGAAGCCUGCCAAGAGCGCCUAA (all Us are modified; N ¹ -methylpseudouridine)	123
Compound 14 modified RNA sequence Underline = Signal	GCCACCAUGUUGCUGCUGCCUCUGUUCUUCUGCUGGCCUGCGCCGGCAAUUUU GUGCAGCGGCCACAAGUGCGACAUCACCCUGCAAGAGAUCAUCAAGACCCUGAAC AGCCUGACCGAGCAGAAAACCCUGUGCACCAGCUGACCGUGACCGAUUUCUUU GCCGCCAGCAAGAACACAACCCGAGAAAGAGACAUUCUGCAGAGCCGCCACCGUG GCGGCCAGCAAGAACACAACCCGAGAAAGAGACAUUCUGCAGAGCCGCCACCGUG CUGAGACAGUUCUACAGCCACCACGAGAAGGACACCAGAUGCCUGGGAGCUACA	124

Protein or Nucleic Acid	Sequence	SEQ ID NO:
peptide <i>Italics</i> = Kozak sequence	GCCCAGCAGUCCACAGACACAAGCAGCUGAUCCGGUCCUGAAGCGGCUGGAC AGAAAUCUGUGGGGACUCGCCGGCCUGAAUAGCUGCCCUGUGAAAGAGGCCAAC CAGUCUACCCUGGAAAACUCCUGGAACGGCUGAAAACCAUCAUGCGCGAGAAG UACAGCAAGUGCAGCAGCUGA (all Us are modified; N ¹ -methylpseudouridine)	
IGF-1 Human IGF-1 Nucleotide (Optimized IGF-1 with endogenous SP and without E- peptide)	<u>GCCACCATGGGCAAGATTAGCAGCCTGCCTACACAGCTGTTCAAGTGCTGCTTC</u> <u>TGCGACTTCCTGAAAGTGAAGATGCACACCATGAGCAGCAGCCACCTGTTCTAT</u> <u>CTGGCCCTGTGCCTGCTGACCTTTACCAGCTCTGCTACCGCCGGACCTGAGACA</u> <u>CTTTGTGGCGCTGAACTGGTGGACGCCCTGCAGTTTGTGTGTGGCGACAGAGGC</u> <u>TTCTACTTCAACAAGCCACAGGCTACGGCAGCAGCTCTAGAAGGGCTCCTCAG</u> <u>ACCGGAATCGTGGACGAGTGCTGTTTCAGAAGCTGCGACCTGCGGCGGCTGGAA</u> <u>ATGTATTGTGCCCTCTGAAGCCTGCCAAGAGCGCCTAA</u>	125
IGF-1 Human IGF-1 amino acid (Optimized IGF-1 with BDNF SP and without E- peptide) <u>Underlined</u> : signal sequence	MGKISSSLPTQLFKCCFCDFLKVKMHTMSSSHLFYLALCLLTFTSSATAG PETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFR SCDLRRLEMYCAPLKPAKSA	126

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising a recombinant RNA construct comprising:
 - (i) a first RNA sequence encoding a gene of interest, and
 - 5 (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs,wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the first RNA sequence of (i) and
10 a corresponding second RNA sequence of (ii) comprising at most one of the at least two genetic elements.
2. The composition of claim 1, wherein the recombinant RNA construct comprises one or more uridines.
3. The composition of claim 1 or 2, wherein the recombinant RNA construct does not
15 comprise a modified uridine.
4. A composition comprising a recombinant RNA construct comprising:
 - (i) a first RNA sequence encoding a gene of interest, and
 - (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs,20 wherein the recombinant RNA construct does not comprise a nucleotide variant.
5. A composition comprising a recombinant RNA construct comprising:
 - (i) a first RNA sequence encoding a gene of interest, and
 - (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs,25 wherein the recombinant RNA construct does not comprise a modified uridine.
6. A composition comprising a recombinant RNA construct comprising:
 - (i) a first RNA sequence encoding a gene of interest, and
 - (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs,30 wherein the recombinant RNA construct does not comprise a N1-methylpseudouridine.
7. A composition comprising a recombinant RNA construct comprising:
 - (i) a first RNA sequence encoding a gene of interest, and
 - (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs,

wherein the recombinant RNA construct comprises solely unmodified nucleotides or natural nucleotides.

8. A composition comprising a recombinant RNA construct comprising:
 - (i) a first RNA sequence encoding a gene of interest, and
 - 5 (ii) a second RNA sequence comprising at least two genetic elements that modulate expression of one or more target RNAs,wherein the recombinant RNA construct comprises uridines, wherein:
 - (a) all uridines comprised by the recombinant RNA constructs are unmodified or natural nucleotide(s); or
 - 10 (b) at least one of the uridines comprised by the recombinant RNA constructs is an unmodified uridine.
9. The composition of claim 4, wherein the nucleotide variant comprises a modified uridine.
10. The composition of any one of claims 3, 5, and 9, wherein the modified uridine comprises a N1-methylpseudouridine.
- 15 11. The composition of any one of claims 1-3, wherein the corresponding recombinant RNA construct does not comprise any of the genetic elements that modulate expression of one or more target RNAs.
12. The composition of any one of the preceding claims, wherein the second RNA sequence comprises at least three genetic elements that modulate expression of one or more target
20 RNAs.
13. The composition of any one of the preceding claims, wherein the second RNA sequence comprises at least six genetic elements that modulate expression of one or more target RNAs.
14. The composition of any one of the preceding claims, wherein the first RNA sequence is a
25 messenger RNA (mRNA) sequence.
15. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence comprises a secondary structure.
16. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence comprises a hairpin structure or a loop
30 structure.
17. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence is a short or small hairpin RNA (shRNA).

18. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence is processed or cleaved by an intracellular protein.
19. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence is processed or cleaved by an endogenous protein of a cell.
20. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence is processed or cleaved by an endogenous DICER.
21. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence comprises a small interfering RNA (siRNA).
22. The composition of any one of the preceding claims, wherein each of the at least two genetic elements of the second RNA sequence is capable of binding to the one or more target RNAs.
23. The composition of any one of claims 1-3 or 11-22, wherein the immune response is a human Toll-Like Receptor 7 (TLR7) immune response, an interferon alpha/beta (IFN α / β) immune response, a human Toll-Like Receptor 3 (TLR3) immune response, a human Toll-Like Receptor 8 (TLR8) immune response, or any combination thereof.
24. The composition of any one of claims 1-3 or 11-23, wherein contacting the human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with the corresponding recombinant RNA construct according to a human Toll-Like Receptor 7 (TLR7) immunogenicity assay.
25. The composition of claim 24, wherein the human TLR7 immunogenicity assay measures activation of NF- κ B and/or AP1.
26. The composition of claim 24 or 25, wherein the human TLR7 immunogenicity assay is performed in HEK293 cells or a derivative thereof.
27. The composition of claim 26, wherein the HEK293 cells are engineered to express hTLR7 and a reporter gene.
28. The composition of claim 27, wherein the reporter gene is a secreted reporter gene.
29. The composition of claim 28, wherein the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP).
30. The composition of any one of claims 27-29, wherein the reporter gene is under the control of a promoter with one or more NF- κ B and/or AP1 binding sites.

31. The composition of claim 30, wherein the promoter is an IFN- β minimal promoter.
32. The composition of any one of claims 24-31, wherein the immune response in the human cell contacted with the recombinant RNA construct is at least 1.5 fold or at least 2 fold less than the immune response in the human cell contacted with the corresponding recombinant RNA construct.
33. The composition of any one of claims 1-3 or 11-23, wherein contacting the human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with the corresponding recombinant RNA construct according to an interferon alpha/beta (IFN α/β) immunogenicity assay.
34. The composition of claim 33, wherein the IFN α/β immunogenicity assay measures activation of JAK-STAT and/or ISG3.
35. The composition of claim 33 or 34, wherein the IFN α/β immunogenicity assay is performed in HEK293 cells or a derivative thereof.
36. The composition of claim 35, wherein the HEK293 cells are engineered to express human STAT2 and/or IRF9 genes and a reporter gene.
37. The composition of claim 36, wherein the reporter gene is a secreted reporter gene.
38. The composition of claim 37, wherein the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP).
39. The composition of any one of claims 36-38, wherein the reporter gene is under the control of a promoter with one or more STAT2 and/or IRF9 binding sites.
40. The composition of claim 39, wherein the promoter is an ISG54 promoter.
41. The composition of any one of claims 33-40, wherein the immune response in the human cell contacted with the recombinant RNA construct is at least 1.5 fold, at least 2 fold, or at least 100 fold less than the immune response in the human cell contacted with the corresponding recombinant RNA construct.
42. The composition of any one of claims 1-3 or 11-23, wherein contacting the human cell with the recombinant RNA construct does not result in a substantial immune response according to a human Toll-Like Receptor 3 (TLR3) immunogenicity assay.
43. The composition of claim 42, wherein the human TLR3 immunogenicity assay measures activation of NF- κ B and/or AP1.
44. The composition of claim 42 or 43, wherein the human TLR3 immunogenicity assay is performed in HEK293 cells or a derivative thereof.
45. The composition of claim 44, wherein the HEK293 cells are engineered to express hTLR3 and a reporter gene.

46. The composition of claim 45, wherein the reporter gene is a secreted reporter gene.
47. The composition of claim 46, wherein the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP).
48. The composition of any one of claims 45-47, wherein the reporter gene is under the
5 control of a promoter with one or more NF- κ B and/or AP1 binding sites.
49. The composition of claim 48, wherein the promoter is an IFN- β minimal promoter.
50. The composition of any one of claims 1-3 or 11-23, wherein contacting the human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with the corresponding recombinant RNA
10 construct according to a human Toll-Like Receptor 8 (TLR8) immunogenicity assay.
51. The composition of claim 50, wherein the human TLR8 immunogenicity assay measures activation of NF- κ B, AP1, and/or IRF.
52. The composition of claim 50 or 51, wherein the human TLR8 immunogenicity assay is performed in HEK293 cells or a derivative thereof.
- 15 53. The composition of claim 52, wherein the HEK293 cells are engineered to express hTLR8 and a reporter gene.
54. The composition of claim 53, wherein the reporter gene is a secreted reporter gene.
55. The composition of claim 54, wherein the secreted reporter gene is secreted embryonic alkaline phosphatase (SEAP).
- 20 56. The composition of any one of claims 53-55, wherein the reporter gene is under the control of a promoter with one or more NF- κ B and/or AP1 binding sites.
57. The composition of claim 56, wherein the promoter is an IFN- β minimal promoter.
58. The composition of any one of claims 1-3 or 11-22, wherein the immune response induces the expression of a proinflammatory cytokine in a cell.
- 25 59. The composition of claim 58, wherein the proinflammatory cytokine comprises Interleukin 6 (IL-6).
60. The composition of claim 58 or 59, wherein the cell comprises a human lung epithelial carcinoma cell (A549) or a human monocyte leukemia cell (THP-1).
61. The composition of any one of claims 21-60, wherein the second RNA sequence
30 comprises 2, 3, 4, 5, 6, or more species of siRNA, wherein the 2, 3, 4, 5, 6, or more species of siRNA include siRNAs that are capable of binding to:
- (i) different target RNAs;
 - (ii) different regions of the same target RNA;
 - (iii) the same region of the same target RNA; or

- (iv) any combination thereof.
62. The composition of claim 61, wherein the second RNA sequence comprises at least 3 species of siRNA.
63. The composition of claim 61, wherein the second RNA sequence comprises at least 6 species of siRNA.
64. The composition of any one of the preceding claims, wherein the recombinant RNA construct further comprises one or more linkers.
65. The composition of claim 64, wherein each of the one or more linkers has a structure selected from the group consisting of:
- Formula (I): $X_mCAACAAX_n$,
wherein X is any nucleotide, m is an integer from 1 to 12, and n is an integer from 0 to 4;
and
Formula (II): X_pTCCCX_r ,
wherein X is any nucleotide, p is an integer from 0 to 17, and r is an integer from 0 to 13.
66. The composition of claim 64, wherein each of the one or more linkers comprises a sequence comprising ACAACAA.
67. The composition of claim 64, wherein each of the one or more linkers is not (a) TTTATCTTAGAGGCATATCCCTACGTACCAACAA or ATAGTGAGTCGTATTAACGTACCAACAA; or (b) does not form a secondary structure according to RNAfold WebServer.
68. The composition of any one of claims 64-67, wherein each of the one or more linkers is present between (a) the first RNA sequence and the second RNA sequence, (b) each of the 2, 3, 4, 5, 6, or more species of siRNA of the second RNA sequence, or (c) both (a) and (b).
69. The composition of any one of claims 64-68, wherein each of the one or more linkers comprises a sequence independently selected from the group consisting of SEQ ID NOs: 27, 28, 85-95.
70. The composition of any one of the preceding claims, wherein the expression of the gene of interest is modulated.
71. The composition of claim 70, wherein the expression of the gene of interest is upregulated in a cell comprising the recombinant RNA construct.
72. The composition of claim 70, wherein the expression of a protein encoded by the gene of interest is upregulated in a cell comprising the recombinant RNA construct.

73. The composition of any one of the preceding claims, wherein the expression of the one or more target RNAs is modulated.
74. The composition of claim 73, wherein the expression of the one or more target RNAs is downregulated by the genetic elements that modulate expression of the one or more target RNAs.
75. The composition of any one of the preceding claims, wherein the genetic elements that modulate expression of the one or more target RNAs do not inhibit the expression of the gene of interest.
76. The composition of any one of the preceding claims, wherein the gene of interest is selected from the group consisting of Interleukin 4 (IL-4) and Insulin-like Growth Factor 1 (IGF-1).
77. The composition of any one of the preceding claims, wherein the one or more target RNA comprises a noncoding RNA or a messenger RNA (mRNA).
78. The composition of any one of the preceding claims, wherein each of the one or more target RNA is a noncoding RNA.
79. The composition of any one of claims 1-77, wherein each of the one or more target RNA is an mRNA.
80. The composition of any one of claims 1-77, wherein the target RNA is an mRNA encoding a protein comprising Interleukin 8 (IL-8), Interleukin 1 beta (IL-1 beta), Tumor Necrosis Factor alpha (TNF-alpha), Interleukin 17 (IL-17), or a functional variant thereof.
81. The composition of any one of the preceding claims, wherein the genetic elements that modulate expression of the one or more target RNAs binds to an exon of the one or more target RNAs.
82. The composition of any one of the preceding claims, wherein the genetic elements that modulate expression of the one or more target RNAs specifically binds to one target RNA.
83. The composition of any one of the preceding claims, wherein the genetic elements that modulate expression of the one or more target RNAs are not encoded by or comprised of an intron sequence of the gene of interest.
84. The composition of any one of the preceding claims, wherein the gene of interest is expressed without RNA splicing.
85. The composition of any one of the preceding claims, wherein the first RNA sequence is present downstream or 3' of the second RNA sequence.

86. The composition of any one of the preceding claims, wherein the first RNA sequence is present upstream or 5' of the second RNA sequence.
87. The composition of any one of the preceding claims, wherein the RNA construct comprises an internal ribosome entry site (IRES) upstream or 5' of the first RNA sequence.
88. The composition of any one of the preceding claims, further comprising a poly(A) tail, a 5' cap, or a Kozak sequence.
89. The composition of any one of the preceding claims, wherein the first RNA sequence and the second RNA sequence are both recombinant.
90. The composition of any one of the preceding claims, wherein the siRNA comprises a sense strand sequence selected from SEQ ID NOs: 57-70.
91. The composition of any one of the preceding claims for use in modulating the expression of two or more genes in a cell.
92. A pharmaceutical composition comprising a therapeutically effective amount of the composition of any one of claims 1-90 and a pharmaceutically acceptable excipient.
93. A vector comprising a recombinant polynucleic acid construct encoding the composition of any one of claims 1-90.
94. A cell comprising the composition of any one of claims 1-90 or the vector of claim 93.
95. A method of simultaneously expressing an siRNA and an mRNA from a single RNA transcript in a cell, comprising introducing into the cell the composition of any one of claims 1-90, or the vector of claim 93.
96. A method of treating a disease or condition comprising administering to a subject in need thereof the composition of any one of claims 1-90 or the pharmaceutical composition of claim 92.
97. The method of claim 96, wherein the disease or condition comprises a skin disease or condition or a joint disease or condition.
98. The method of claim 97, wherein the skin disease or condition comprises an inflammatory skin disorder.
99. The method of claim 98, wherein the inflammatory skin disorder comprises psoriasis.
100. The method of claim 97, wherein the joint disease or condition comprises a joint degeneration.
101. The method of claim 100, wherein the joint degeneration comprises intervertebral disc disease (IVDD) or osteoarthritis (OA).
102. The method of any one of claims 96-101, wherein the subject is a human.

103. A composition comprising a recombinant RNA construct comprising:
- (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and
 - (ii) at least two small interfering RNAs (siRNAs) capable of binding to an Interleukin-8 (IL-8) mRNA,
- 5 wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IGF-1 of (i) and at most one of the at least two siRNAs capable of binding to the IL-8 mRNA of (ii).
104. A composition comprising recombinant RNA construct comprising:
- 10 (i) a messenger RNA (mRNA) encoding Insulin-like Growth Factor 1 (IGF-1), and
 - (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Interleukin-1 beta (IL-1 beta) mRNA,
- wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with
- 15 a corresponding recombinant RNA construct comprising the mRNA encoding IGF-1 of (i) and at most one of the at least two siRNAs capable of binding to the IL-1beta mRNA of (ii).
105. A composition comprising recombinant RNA construct:
- (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and
 - 20 (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis Factor alpha (TNF-alpha) mRNA,
- wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IL-4 of (i)
- 25 and at most one of the at least two siRNAs capable of binding to the TNF-alpha mRNA of (ii).
106. A composition comprising recombinant RNA construct:
- (i) a messenger RNA (mRNA) encoding Interleukin-4 (IL-4), and
 - (ii) at least two small interfering RNAs (siRNAs) capable of binding to a Tumor Necrosis
 - 30 Factor alpha (TNF-alpha) mRNA and Interleukin 17 (IL-17),
- wherein contacting a human cell with the recombinant RNA construct results in an immune response that is lower than the immune response of the human cell contacted with a corresponding recombinant RNA construct comprising the mRNA encoding IL-4 of (i)

and at most one of the at least two siRNAs capable of binding to the TNF-alpha mRNA and IL-17 mRNA of (ii).

107. A composition comprising a recombinant polynucleic acid construct comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 1-24, 42, 125, 97-108, 121-122, and 127-128.
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