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(54) Title: POLYNUCLEOTIDES COMPRISING DETARGETING SENSORS AND USES THEREOF

(57) Abstract: The present disclosure relates to polynucleotides comprising (a) a first region encoding a payload and (b) a second region comprising a sensor that is capable of recognizing a marker in a cell that expresses the marker ("detargeting cell"), wherein the marker is miR-22-3p or miR-185-5p ("detargeting sensor"). In some aspects, the recognition of the marker by the sensor in the cell results in reduced expression of the payload as compared to a reference cell (e.g., cell that does not express the marker or cell that expresses the marker at a low level). Also provided herein are replicons, nanoparticles, synthetic circuits, circular RNA, pharmaceutical compositions comprising such polynucleotides. Methods of using the polynucleotides, replicons, nanoparticles, synthetic circuits, circular RNA, and pharmaceutical compositions are also provided.

POLYNUCLEOTIDES COMPRISING DETARGETING SENSORS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit to U.S. Provisional Application No. 63/751,216, filed January 29, 2025 and U.S. Provisional Application No. 63/783,644, filed April 4, 2025, the entire disclosures of each of which are incorporated herein by reference for all purposes.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS-WEB

[0002] The content of the electronic sequence listing (Name: 4597_037PC02_SequenceListing_ST26.xml; Size: 77,509 bytes; and Date of Creation: January 27, 2026) is herein incorporated by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0003] Nucleic acid-based treatment (e.g., mRNA vaccines) is becoming an increasingly important approach for the treatment of a variety of diseases. Upon administration, the encoded protein can be produced to induce an immune response (e.g., tumor antigen to induce anti-tumor immune response) or to provide important function to maintain a healthy state. However, controlling expression of payload proteins from mRNA therapeutics in a tissue-specific and/or cell-specific manner remains a challenge. Additionally, current delivery platforms for delivering the nucleic acid molecules are ineffective or associated with undesirable side effects, limiting the full potential of nucleic acid-based therapy.

[0004] Therefore, there remains a need for improved delivery platforms for nucleic acids (e.g., mRNA) that are more effective and allow for tissue-specific and/or cell-specific delivery.

BRIEF SUMMARY OF THE DISCLOSURE

[0005] The present disclosure provides an isolated polynucleotide comprising (a) a first region encoding a payload and (b) a second region comprising a sensor that is capable of recognizing a marker in a cell that expresses the marker (“detargeting cell”), wherein the marker

is miR-185-5p or miR-22-3p ("detargeting sensor"). In some aspects, the recognition of the marker by the sensor in the cell results in reduced expression of the payload as compared to a reference cell (*e.g.*, cell that does not express the marker or cell that expresses the marker at a low level).

[0006] In some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof. In some aspects, the detargeting cell comprises an immune cell. In some aspects, the immune cell comprises hematopoietic stem cells and/or derivatives thereof. In some aspects, the immune cell comprises T cells, B cells, natural killer (NK) cells, macrophages, monocytes, dendritic cells, neutrophils, basophils, eosinophils, or combinations thereof.

[0007] In some aspects, the marker is present within the immune cell at a greater quantity as compared to non-immune cells or a cell type that does not express the marker or expresses the marker at a lower level than the immune cell. In some aspects, the expression level of the marker in the immune cell is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, greater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, greater than about 20-fold, or more, as compared to the corresponding value for the non-immune cells or a cell type that does not express the marker or expresses the marker at a lower level than the immune cell.

[0008] In some aspects, the immune cell comprises a spleen, lymph node, or both. In some aspects, the detargeting cell is a cardiomyocyte. In some aspects, the cardiomyocyte comprises atrial myocytes, ventricular myocytes, pacemaker cells (nodal cells), purkinje fibers, conducting cells, or any combination thereof.

[0009] In some aspects, the marker is present within a cardiomyocyte at a greater quantity as compared to non-cardiomyocytes, e.g., cancer cells, or a cell type that does not express the marker or expresses the marker at a lower level than the cardiomyocytes. In some aspects, the expression level of the marker present within the cardiomyocyte is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, greater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, greater

than about 20-fold, or more, as compared to the corresponding value for the non-cardiomyocyte or a cell type that does not express the marker or expresses the marker at a lower level than the cardiomyocytes.

[0010] In some aspects, the cardiomyocyte comprises a myocardium. In some aspects, the detargeting cell comprises an endothelial cell. In some aspects, the endothelial cell comprises vascular endothelial cells (e.g., arterial endotheliam cells, venous endothelial cells, liver endothelial cells, or capillary endothelial cells (e.g., continuous endothelium, fenestrated endothelium, or sinusoidal endothelium)), lymphatic endothelial cells, pulmonary endothelial cells, brain endothelial cells (blood-brain barrier), or any combination thereof.

[0011] In some aspects, the marker is present within an endothelial cell at a greater quantity as compared to non-endothelial cells or a cell type that does not express the marker or expresses the marker at a lower level than the endothelial cells. In some aspects, the expression level of the marker present within the endothelial cell is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, greater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, greater than about 20-fold, or more, as compared to the corresponding value for the non-endothelial cells or a cell type that does not express the marker or expresses the marker at a lower level than the endothelial cells. In some aspects, the endothelial cell comprises blood vessel, lymphatic vessel, liver, lung, kidney, brain, intestine, heart, and/or muscle.

[0012] In some aspects, the marker comprises miR-185-5p.

[0013] In some aspects, the marker comprises miR-22-3p.

[0014] In some aspects, the second region comprises multiple detargeting sensors. In some aspects, the multiple detargeting sensors are the same. In some aspects, two or more of the multiple detargeting sensors are different. In some aspects, one of the multiple detargeting sensors comprise a sensor that is capable of recognizing a second marker, which comprises miR-142-3p.

[0015] In some aspects, the multiple detargeting sensors comprise a first sensor that is capable of recognizing miR-22-3p and a second sensor that is capable of recognizing miR-142-3p.

[0016] In some aspects, the first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 6.

[0017] In some aspects, the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 3.

[0018] In some aspects, the multiple detargeting sensors comprise a first sensor that is capable of recognizing miR-185 and a second sensor that is capable of recognizing miR-142-3p.

[0019] In some aspects, the first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 9.

[0020] In some aspects, the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 3.

[0021] In some aspects, the multiple detargeting sensors comprise a first sensor that is capable of recognizing miR-22-3p and a second sensor that is capable of recognizing miR-185-5p.

[0022] In some aspects, the first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 6.

[0023] In some aspects, the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 9.

[0024] In some aspects, the multiple detargeting sensors comprises a first sensor that is capable of recognizing miR-22-3p, a second sensor that is capable of recognizing miR-142-3p, and a third sensor that is capable of recognizing miR-185-5p.

[0025] In some aspects, the first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 6.

[0026] In some aspects, the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 3.

[0027] In some aspects, the third sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 9.

[0028] In some aspects, an isolated polynucleotide of the present disclosure comprises at least two sensors comprising a miR-142 sensor and a miR-185 sensor (or a miR-22 sensor), at least

four sensors comprising two miR-142 sensors and two miR-185 sensors (or two miR-22 sensors), at least six sensors comprising three miR-142 sensors and three miR-185 sensors (or three miR-22 sensors), at least eight sensors comprising four miR-142 sensors and four miR-185 sensors (or four miR-22 sensors), at least ten sensors comprising five miR-142 sensors and five miR-185 sensors (or five miR-22 sensors), or at least 12 sensors comprising six miR-142 sensors and six miR-185 sensors (or six miR-22 sensors).

[0029] In some aspects, an isolated polynucleotide of the present disclosure comprises (i) at least one first sensor, at least two first sensors, at least three first sensors, at least four first sensors, at least five first sensors, at least six first sensors, at least seven first sensors, at least eight first sensors, at least nine first sensors, or at least ten first sensors and (ii) at least one second sensor, at least two second sensors, at least three second sensors, at least four second sensors, at least five second sensors, at least six second sensors, at least seven second sensors, at least eight second sensors, at least nine second sensors, or at least ten second sensors. In some aspects, the first sensor comprises a miR-185 sensor or a miR-22 sensor and the second sensor comprises a miR-142 sensor.

[0030] In some aspects, the payload comprises a cytokine, a ligand-binding protein, or both. In some aspects, the cytokine comprises an interleukin (IL)-12 protein.

[0031] In some aspects, any of the polynucleotides described herein further comprises: (1) an untranslated region (UTR), (2) a sequence encoding a signal peptide, (3) a translation initiation sequence, (4) a polyA sequence, (5) a sequence encoding a RNA binding protein, (6) a 5'-cap, (7) a sequence encoding a 2A ribosome skip peptide, (8) a translation enhancer element, or (9) any combination of (1) to (8).

[0032] In some aspects, the polynucleotide comprises the sequence set forth in SEQ ID NO: 12.

[0033] In some aspects, the polynucleotide consists essentially of the sequence set forth in SEQ ID NO: 12.

[0034] In some aspects, the polynucleotide consists of the sequence set forth in SEQ ID NO: 12.

[0035] Also provided herein is a synthetic circuit comprising any of the isolated polynucleotides described herein.

[0036] Also provided herein is a replicon comprising any of the isolated polynucleotides or the synthetic circuits described herein, wherein the replicon is self-replicating. In some aspects,

the replicon is derived from an alpha virus. In some aspects, the alpha virus comprises a Venezuelan equine encephalitis (VEE) virus.

[0037] Also provided herein is a circular RNA comprising any of the isolated polynucleotides described herein.

[0038] Also provided herein is a nanoparticle comprising (a) any of the isolated polynucleotides, synthetic circuits, replicons, or circular RNA described herein, and (b) a lipid and/or lipid-like material.

[0039] In some aspects, the lipid comprises an ionizable lipid, cationic lipid, lipidoid, non-cationic helper lipid, phospholipid, sterol or other structural lipids, or combinations thereof.

[0040] In some aspects, the ionizable lipid comprises ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315), heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102), heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Lipid 5), di((*Z*)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), 9Z,12Z-octadecadienoic acid, 3-[4,4-bis(octyloxy)-1-oxobutoxy]-2-[[[[3-(diethylamino)propoxy]carbonyl]oxy]methyl]propyl ester (LP-01), 5-(dimethylamino)-pentanoic acid, (6Z)-1,2-di-(4Z)-4-decen-1-yl-6-dodecen-1-yl ester (CL-1), 2-(((4-(dimethylamino)butanoyl)oxy)methyl)-2-((((Z)-tetradec-9-enoyl)oxy)methyl)propane-1,3-diyl (9Z,9'Z)-bis(tetradec-9-enoate) (TCL053), 3-(didodecylamino)-N1,N1,4 tridodecyl-1-piperazineethanamine (KL10), Nl-[2 (didodecylamino)ethyl]-N1,N4,N4-tridodecyl 1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2 dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), or combinations thereof.

[0041] In some aspects, the cationic lipid comprises l,2-dioleoyl-3 -trimethylammonium-propane (DOTAP), lipofectamine, N-[l-(2,3- dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2- (oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride

(DOTEVI), 2,3- dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l ,2-dimyristyloxyprop-3 -yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DMRIE), N-(l,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), l,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), l,2-distearoyl-3- trimethylammonium-propane (DSTAP), l,2-dipalmitoyl-3 -trimethylammonium-propane (DPTAP), l,2-dilinoleoyl-3 -trimethylammonium-propane (DLTAP), l,2-dimyristoyl-3- trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3- ethylphosphocholine (DSePC), l,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn- glycero-3-ethylphosphocholine (DOePC), l,2-di-(9Z-tetradecenoyl)-sn-glycero-3- ethylphosphocholine (14: 1 EPC), l-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18: 1 EPC), or any combination thereof.

[0042] In some aspects, the lipidoid comprises 1,1′-((2-(4-(2-((2-(bis(2-hydroxydodecyl) amino)ethyl) (2- hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200), 3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine2,5-dione (cKK-E12), 1,1′-[[2-[2-[4-[2-[[2-[2-[bis(2-hydroxytetradecyl)amino]ethoxy]ethyl](2-hydroxytetradecyl)amino]ethyl]-1-piperazinyl]ethoxy]ethyl]imino]bis-2-tetradecanol (C14-4), tetrakis(8-methylnonyl) 3,3′,3″,3‴- (((methylazanediyl) bis(propane-3,1 diyl))bis (azanetriyl))tetrapropionate (306Oi$_{10}$), G0-C14, 3,3',3",3'"-(ethane-1,2-diylbis(azanetriyl))tetrakis(N-(2-(bis(2-hydroxytetradecyl)amino)ethyl)propanamide) (G0-C14 analog), 5A2-SC8, 4A3-SC8, 3,6-bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02), (((3,6-dioxopiperazine-2,5-diyl)bis (butane-4,1-diyl))bis(azanetriyl))tetrakis(ethane2,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin), (((3,6-dioxopiperazine-2,5-diyl)bis(butane-4,1-diyl)) bis(azanetriyl))tetrakis (butane-4,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-C4-Deg-Lin), 1,3,5-tris[2-[(2-hydroxydodecyl)methylamino]ethyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TNT-b10), N1,N3,N5-tris(3-(didodecylamino)propyl)benzene1,3,5-tricarboxamide (TT3), Hexa(octan-3-yl) 9,9′,9″,9‴,9⁗,9⁗′- ((((benzene-1,3,5-tricarbonyl)ris(azanediyl)) tris (propane-3,1-diyl))tris(azanetriyl))hexanonanoate (FTT5), PL-1, 98N12-5, ethyl 5,5-di((Z)-heptadec-8-en-1-yl)-1-(3-(pyrrolidin-1-yl)propyl)-2,5-dihydro-1H-imidazole-2-carboxylate (A2-Iso5-2DC18 (A2)), A12-Iso5−2DC18 (A12), or any combination thereof.

[0043] In some aspects, the phospholipid is selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3 phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanola mine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (14:0-16:0 PC, MPPC), 1-myristoyl-2 stearoyl-sn-glycero-3-phosphocholine (14:0-18:0 PC, MSPC), 1-palmitoyl 2-acetyl-sn-glycero-3-phosphocholine (16:0-02:0 PC), 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine (16:0-14:0 PC, PMPC), 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (16:0-18:0 PC, PSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18: 1 PC, POPC), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (16:0-18:2 PC, PLPC), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (16:0-20:4 PC), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (14:0-22:6 PC), 1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholine (18:0-14:0 PC, SMPC), 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18:0-16:0 PC, SPPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (18:0-18: 1 PC, SOPC), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (18:0-18:2 PC), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (18:0-20:4 PC), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (18:0-22:6 PC), 1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholine (18: 1-14:0 PC, OMPC), 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18: 1-16:0 PC, OPPC), 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (18: 1-18:0 PC, OSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (16:0- 18: 1 PE, POPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (16:0-18:2 PE), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (16:0-20:4 PE), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (16:0-22:6 PE), 1-stearoyl-2-oleoyl-sn-

glycero-3-phosphoethanolamine (18:0-18: 1 PE), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (18:0-18:2 PE), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (18:0-20:4 PE), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (18:0-22:6 PE), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), and any combination thereof.

[0044] In some aspects, the sterol comprises a cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and combinations thereof.

[0045] In some aspects, the nanoparticle is pegylated.

[0046] In some aspects, any of the nanoparticles described herein further comprises a targeting ligand.

[0047] Also provided herein is a pharmaceutical composition comprising (a) any of the isolated polynucleotides, synthetic circuits, replicons, circular RNA, or nanoparticles described herein, and (b) a pharmaceutically acceptable carrier.

[0048] Some aspects of the present disclosure relate to methods of reducing an expression of a payload (payload expression) within an immune cell, a cardiomyocyte, and/or an endothelial cell of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) circular RNA, (e) nanoparticles, (f) pharmaceutical compositions described herein, or (g) any combination of (a) to (f).

[0049] In some aspects of the methods, reducing the payload expression comprises (a) reducing the amount of payload that is expressed by the immune cell, the cardiomyocyte, and/or the endothelial cell, (b) reducing the duration of payload expression by the immune cell, the cardiomyocyte, and/or the endothelial cell, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell, cardiomyocyte, or endothelial cell detargeting sensor).

[0050] In some aspects of the methods, after the administration, the amount of payload that is expressed by the immune cell, the cardiomyocyte, and/or the endothelial cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0051] In some aspects of the methods, after the administration, the duration of the payload expression by the immune cell, the cardiomyocyte, and/or the endothelial cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least

about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0052] In some aspects of the methods, the immune cell comprises a T cell, macrophage, or both. In some aspects, the immune cell comprises a hematopoietic stem cell and/or a derivative thereof. In some aspects, the immune cell comprises a T cell, a B cell, a natural killer (NK) cell, a macrophage, a monocyte, a dendritic cell, a neutrophil, a basophil, an eosinophil, or combinations thereof.

[0053] In some aspects of the methods, the cardiomyocyte comprises a heart muscle cell.

[0054] In some aspects of the methods, the endothelial cell comprises a vascular endothelial cell (e.g., arterial endothelial cell, venous endothelial cell, or capillary endothelial cell (e.g., continuous endothelium, fenestrated endothelium, or sinusoidal endothelium), a lymphatic endothelial cell, a corneal endothelial cell, a pulmonary endothelial cell, and/or a brain endothelial cell.

[0055] Some aspects of the present disclosure relate to methods of selectively expressing a payload in a non-immune cell, non-cardiomyocyte, or non-endothelial cell of a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) circular RNA, (e) nanoparticles, (f) pharmaceutical compositions described herein, or (g) any combination of (a) to (f).

[0056] In some aspects of the methods, after the administration, (a) an amount of the payload that is expressed in the non-immune cell, non-cardiomyocyte, or non-endothelial cell, (b) a duration of the expression of the payload in the non-immune cell, non-cardiomyocyte, or non-endothelial cell, or (c) both (a) and (b) are higher than the corresponding amount and/or duration in the immune cell, cardiomyocyte, or endothelial cell of the subject.

[0057] Some aspects of the present disclosure relate to methods of reducing an expression of a payload (payload expression) within a lymphoid tissue, a myocardium tissue, or an endothelial tissue of a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) circular RNA, (e) nanoparticles, (f) pharmaceutical compositions described herein, or (g) any combination of (a) to (f).

[0058] In some aspects of the methods, the lymphoid tissue comprises a spleen, lymph node, or both.

[0059] In some aspects of the methods, the myocardium tissue comprises a heart.

[0060] In some aspects of the methods, the endothelial tissue comprises blood vessel, lymphatic vessel, liver, lung, kidney, brain, intestine, heart, or muscle.

[0061] In some aspects of the methods, reducing the payload expression comprises (a) reducing the amount of payload that is expressed in the lymphoid tissue, myocardium tissue, or endothelial tissue, (b) reducing the duration of payload expression in the lymphoid tissue, myocardium tissue, or endothelial tissue, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor, the cardiomyocyte detargeting sensor, or the endothelial cell detargeting sensor, respectively).

[0062] In some aspects of the methods, after the administration, the amount of payload that is expressed in the lymphoid tissue, myocardium tissue, or endothelial tissue, is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0063] In some aspects of the methods, after the administration, the duration of the payload expression in the lymphoid tissue, myocardium tissue, or endothelial tissue, is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

[0064] Some aspects of the present disclosure relate to methods of treating a disease or disorder in a subject in need thereof, comprising administering to the subject any of the (a) isolated polynucleotides, (b) synthetic circuits, (c) replicons, (d) circular RNA, (e) nanoparticles, (f) the pharmaceutical compositions described herein, or (g) any combination of (a) to (f).

[0065] In some aspects of the methods, the disease or disorder comprises a cancer, inflammatory disorders, monogenic disorders, neurological disorders, psychiatric disorders, or combinations thereof.

[0066] In some aspects of the methods, the cancer comprises a melanoma, squamous cell cancer (e.g., esophageal squamous cell carcinoma), small-cell lung cancer, non-small cell lung cancer (NSCLC), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer (e.g., hepatocellular carcinoma), bladder (urothelial) cancer, hepatoma, breast cancer (e.g., triple-negative breast cancer, TNBC), colon cancer, colorectal cancer (e.g., colorectal cancer with high microsatellite instability, MSI-H CRC), endometrial or uterine cancer, salivary gland carcinoma, kidney cancer (e.g., renal cell carcinoma),, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, head and neck

cancer (e.g., head and neck squamous cell carcinoma, HNSCC), Merkel cell carcinoma (MCC), or combinations thereof. In some aspects of the methods, the cancer comprises melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, head and neck squamous cell carcinoma (HNSCC), bladder (urothelial) cancer, Merkel cell carcinoma (MCC), triple-negative breast cancer (TNBC), hepatocellular carcinoma, colorectal cancer with high microsatellite instability (MSI-H CRC), esophageal squamous cell carcinoma, or combinations thereof.

[0067] In some aspects of the methods, the isolated polynucleotide, the synthetic circuit, the replicon, the circular RNA, the nanoparticle, and/or the pharmaceutical composition is administered to the subject via intravenous, intratumoral, intrathecal, intramuscular, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

[0068] In some aspects of the methods, the isolated polynucleotide, the synthetic circuit, the replicon, the circular RNA, the nanoparticle, and/or the pharmaceutical composition is administered to the subject via intravenous administration. In some aspects, the isolated polynucleotides, the synthetic circuit, the replicon, the circular RNA, the nanoparticle, and/or the pharmaceutical composition is administered to the subject via systemic administration.

[0069] In some aspects of the methods, any of the methods described herein further comprises administering to the subject an additional therapeutic agent. In some aspects, the additional therapeutic agent comprises an immune checkpoint inhibitor. In some aspects, the immune checkpoint inhibitor comprises a PD-1 antagonist, a PD-L1 antagonist, a LAG3 antagonist, a CCR8 antagonist, or any combination thereof.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0070] **FIGs. 1A-1D** provide a comparison of IL-12 mRNA expression (mIL-12, pg/mg) in mouse spleen (**FIG. 1A**), bone marrow (**FIG. 1B**), heart (**FIG. 1C**) and adrenal gland (**FIG. 1D**), measured post intravenous (I.V.) administration of 0.1 mg/kg of either: VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12 I.V.); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor I.V.); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142+miR-22 sensor I.V.); or a VEE replicon

comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+ miR-142+miR-185 I.V.).

[0071] **FIGs. 1E-1H** show additional comparisons of IL-12 mRNA expression (mIL-12, pg/mg) in mouse spleen (**FIG. 1E**), bone marrow (**FIG. 1F**), adrenal gland (**FIG. 1G**), and liver **(FIG. 1H)** measured post intravenous (I.V.) administration of either: VEE replicon comprising repRNA-mIL-12 I.V. or repRNA-mIL-12+miR-142+miR-185 I.V. Data shown is at Tmax. Results are reported as Mean ± SEM.

[0072] **FIGs. 2A-2G** provide a comparison of serum mIL-12 (pg/mL, **FIG. 2A**), mIFN-α (pg/mL, **FIG. 2B**), and IFN-γ (pg/mL, **FIG. 2C**) measured 72, 6, and 72 hours, respectively, post I.V. administration of 0.1 mg/kg of a lipid nanoparticle loaded with either: VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12 I.V.); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor I.V.); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+mIr-142 sensor+miR-22 sensor I.V.); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor I.V.). Levels of mIL-12 (pg/mg, **FIG. 2D**), mIFN-α (pg/mg, **FIG. 2E**), and IFN-γ (pg/mg, **FIG. 2F**) in spleen measured following administration of VEE replicon comprising repRNA-mIL-12 I.V. ("uncircuited," left bar) or repRNA-mIL-12+miR-142+miR-185 I.V. (right bar) are shown. Body weight was monitored over time and is shown as relative to pre-dosing (day 0) body weight for mice following administration of vehicle, VEE replicon comprising repRNA-mIL-12 I.V. ("uncircuited") or repRNA-mIL-12+miR-142+miR-185 I.V. **(FIG. 2G)**. Results are reported as Mean ± SEM.

[0073] **FIGs. 3A-3D** provide a comparison of spleen histiocytosis (**FIG. 3A**), liver margination (**FIG. 3B**), kidney tubular dilation (**FIG. 3C**), and heart degeneration (**FIG. 3D**) post I.V. administration of a lipid nanoparticle loaded with either: VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor+miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor).

[0074] **FIGs. 4A** and **4B** show liver pathology for mice treated with 0.1 mg/kg repRNA-mIL-12 and 0.1 mg/kg repRNA-mIL-12+miR-142 sensor. Arrows indicate intravascular margination of leukocytes, perivascular histiocyte infiltration, or Kupffer cell hyperplasia.

[0075] **FIGs. 5A** and **5B** show liver pathology for mice treated with 0.1 mg/kg repRNA-mIL-12 and 0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-22 sensor. Arrows indicate intravascular margination of leukocytes, perivascular histiocyte infiltration, or Kupffer cell hyperplasia.

[0076] **FIG. 6A** shows liver pathology for mice treated with 0.1 mg/kg repRNA-mIL-12 and 0.1 mg/kg repRNA-mIL-12+miR-142 sensor +miR-185 sensor. Arrows indicate intravascular margination of leukocytes, perivascular histiocyte infiltration, or Kupffer cell hyperplasia. **FIG. 6B** shows a bar graph showing incidence of intravascular margination of leukocytes among vehicle (left), uncircuited mIL-12 (middle), and repRNA-mIL-12+miR-142 sensor+miR-185 sensor (right) treated mice. **FIG. 6C** shows a bar graph of Kuffler cell hyperplasia among vehicle (left), uncircuited mIL-12 (middle), and repRNA-mIL-12+miR-142 sensor+miR-185 sensor (right) treated mice.

[0077] **FIG. 7** shows liver pathology for mice treated with 0.1 mg/kg repRNA-mIL-12 and 0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-185 sensor. Arrows indicate intravascular margination of leukocytes, perivascular histiocyte infiltration, or Kupffer cell hyperplasia.

[0078] **FIG. 8** shows splenic pathology for mice treated with a lipid nanoparticle loaded with either: PBS, VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor). All doses were administered I.V. at 0.1 mg/kg.

[0079] **FIG. 9A** shows splenic pathology (i.e., marginal zone histiocytosis, decreased cellularity (lymphocyte) white pulp) for mice treated with a lipid nanoparticle loaded with either: PBS, VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185

sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor). All doses were administered I.V. at 0.1 mg/kg. RP = red pulp. WP = white pulp. **FIG. 9B** shows a bar graph showing incidence of histiocytosis marginal zone/red pulp among vehicle (left), uncircuited mIL-12 (middle), and repRNA-mIL-12+miR-142 sensor+miR-185 sensor (right) treated mice. **FIG. 9C** shows a bar graph of extramedullary hematopoiesis among vehicle (left), uncircuited mIL-12 (middle), and repRNA-mIL-12+miR-142 sensor+miR-185 sensor (right) treated mice.

[0080] **FIG. 10** provides a comparison of spleen weights for mice treated with a lipid nanoparticle loaded with either: PBS, VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor+miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor).

[0081] **FIG. 11** shows heart pathology for mice treated with a lipid nanoparticle loaded with either: PBS, VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor). All doses were administered I.V. at 0.1 mg/kg.

[0082] **FIG. 12** shows heart pathology for mice treated with PBS and 0.1 mg/kg repRNA-mIL-12.

[0083] **FIG. 13** shows heart pathology for mice treated with 0.1 mg/kg repRNA-mIL-12 and 0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-22 sensor.

[0084] **FIG. 14A** shows heart pathology for mice treated with 0.1 mg/kg repRNA-mIL-12 and 0.1 mg/kg repRNA-mIL-12+miR-142 sensor +miR-185 sensor. **FIG. 14B** shows a bar graph showing incidence of degeneration/inflammatory infiltrates among vehicle (left), uncircuited mIL-12 (middle), and repRNA-mIL-12+miR-142 sensor+miR-185 sensor (right) treated mice. **FIG. 14C** shows a bar graph of intravascular margination of inflammatory cells among vehicle (left), uncircuited mIL-12 (middle), and repRNA-mIL-12+miR-142 sensor+miR-185 sensor (right) treated mice.

[0085] **FIGs. 15A-15B** provide a comparison of serum mIL-12 (pg/mL, **FIG. 15A**) and serum IFN-γ (pg/mL, **FIG. 15B**) measured 6 and 72 hours, respectively, in a B16.F10 melanoma mouse model following I.V. administration of a lipid nanoparticle loaded with either: VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12 I.V.); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor I.V.); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor+miR-22 sensor I.V.); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor I.V.).

[0086] **FIGs. 16A-16E** provide a comparison of tumor IL-12 (**FIG. 16A**) and tumor IFN-γ (**FIG. 16B**) in a B16.F10 melanoma mouse model following I.V. administration of an optimized lipid nanoparticle loaded with either: VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor+miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor). Additional comparisons are also shown for IL-12 mRNA (**FIG. 16C**), IL-12 (**FIG. 16D**), and IFN-γ (**FIG. 16E**) levels from tumors in mice that received the repRNA-mIL-12+miR-142 sensor+miR-185 sensor compared to control mice that received mRNA encoding IL-12 alone ("uncircuited"). Area under the curve (pg/mg, AUC) was calculated based on 6, 24 and 72 hrs post dose timepoints. Results are reported as Mean ± SEM.

[0087] **FIG. 17** provides a comparison of tumor volume (mm^3) from a B16.F10 melanoma mouse model up to 40 days following I.V. administration of an optimized lipid nanoparticle loaded with either: VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor+miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor).

[0088] **FIG. 18** provides a comparison of body weight (%) in a B16.F10 melanoma mouse model up to 20 days following I.V. administration of an optimized lipid nanoparticle loaded with either: VEE replicon comprising mRNA encoding IL-12 alone (i.e., repRNA-mIL-12); VEE

replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor (i.e., repRNA-mIL-12+miR-142 sensor); VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., repRNA-mIL-12+miR-142 sensor+miR-22 sensor); or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-mIL-12+miR-142 sensor +miR-185 sensor).

[0089] **FIGs. 19A-19B** provide a comparison of sensor activity (Luminescence, RLU) following transfection of the following sensor constructs in primary liver endothelial cells (**FIG. 19A**) and primary small airway epithelial cells (**FIG. 19B**) for different donors: uncircuited, miR-22 sensor, miR-185 sensor, and mock transfected.

[0090] **FIG. 20A** shows payload expression (% Expression (normalized to no sensor control)) in five patient derived tumor organoids representing non-small cell lung cancer (NSCLC) and triple-negative breast cancer (TNBC) for the following sensor constructs: miR-142-3p, miR-142-3p + miR-22-3p, and miR-142-3p + miR-185-5p. **FIGs. 20B-20E** show hIL-12 expression in patient-derived NSCLC organoids transfected with a VEE replicon comprising mRNA encoding hIL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-hIL-12+miR-142+miR-185) or a VEE replicon comprising mRNA encoding hIL-12 alone (i.e., repRNA-mIL-12, "uncircuited hIL-12").

[0091] **FIGs. 21A-21B** show responsiveness of miR-22 (**FIG. 21A**) or miR-185 (**FIG. 21B**) sensors in responding to their cognate miRNAs as assessed in B16.F10 melanoma cells. Expression (Luminescence, RLU) in B16.F10 melanoma cells transfected with miR-22 (**FIG. 21A**) or miR-185 (**FIG. 21B**) sensors was assessed following co-transfection (0, 1, 10, 100, 1,000 nM) of the associated mimic. A negative control mimic (1,000 nM) and mRNAs transfected with no sensors were also tested.

[0092] **FIGs. 22A-22C** provide a comparison of tumor volume (mm^3) in a B16.F10 melanoma mouse model following I.V. administration of a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., miR-142-3p + miR-22-3p) or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., miR-142-3p + miR-185-5p) at a low dose (**FIG. 22A**), a medium dose (**FIG. 22B**), and a high dose (**FIG. 22C**). Vehicle, no sensor ("uncircuited mIL-12"), and IT control groups are also shown. **FIG. 22D** is a schematic showing tumor volume, dose, and maximum tolerated dose (MTD) in a B16.F10 melanoma mouse model following I.V. administration of uncircuited mIL-12 (low dose only) or miR-142-3p + miR-185-5p at a low, mid, and high dose.

Numbers on the graph indicate the average increase in days taken for the tumor to reach a size of 1000 mm^3 compared to the vehicle contol. Results are reported as Mean ± SEM.

[0093] **FIGs. 23A-23F** provide a comparison of serum cytokine levels. **FIGs. 23A-23C** provide a comparison of serum IL-12 levels (pg/mL) following I.V. administration of a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., miR-142-3p + miR-22-3p) or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., miR-142-3p + miR-185-5p) at a low dose (**FIG. 23A**), a medium dose (**FIG. 23B**), or a high dose (**FIG. 23C**). **FIGs. 23D-23F** provide a comparison of serum IFN-γ levels (pg/mL) following I.V. of a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., miR-142-3p + miR-22-3p) or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., miR-142-3p + miR-185-5p) at a low dose (**FIG. 23D**), a medium dose (**FIG. 23E**), or a high dose (**FIG. 23F**).

[0094] **FIG. 24** shows tumor cytokine IL-12 levels in a B16.F10 melanoma mouse model following I.V. administration of a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., miR-142-3p + miR-22-3p) or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., miR-142-3p + miR-185-5p) at low, medium, and high doses.

[0095] **FIG. 25A** shows plasma IL-12p70 (pg/mL) for up to 288 hrs from naïve Cynomolgus macaques following I.V. administration of a VEE replicon comprising mRNA encoding hIL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-hIL-12+miR-142+miR-185, circles) or a VEE replicon comprising mRNA encoding hIL-12 alone (i.e., repRNA-mIL-12, "uncircuited hIL-12," triangles). **FIGs. 25B-25C** show plasma IL-12 (**FIG. 25B**) and IFN-γ (**FIG. 25C**) area under the curve (AUC) following administration of repRNA-hIL-12+miR-142+miR-185 or uncircuited hIL-12. Results are reported as Mean ± SEM).

[0096] **FIG. 26** shows tumor control in a CT26 tumor model for colorectal cancer for days post dose of vehicle and doses 1-5 of the LNP loaded with the VEE replicon comprising mRNA encoding IL-12 with a miR-142 sensor and a miR-185 sensor (abbreviated as "dual miRNA-sensing circuit" within this figure and the subsequent figures).

[0097] **FIG. 27** shows the probability of survival for hours post dose of vehicle and doses 1-4 of the dual miRNA-sensing circuit.

[0098] **FIG. 28** shows serum IL-12 levels for hours post dose of vehicle and doses 1-5 of the dual miRNA-sensing circuit.

[0099] **FIG. 29** shows serum IFN-γ levels for hours post dose of vehicle and doses 1-45 of the dual miRNA-sensing circuit.

[0100] **FIG. 30** shows tumor IL-12 levels for hours post dose of vehicle and doses 1-5 of the dual miRNA-sensing circuit.

[0101] **FIG. 31** shows tumor IFN-γ levels for hours post dose of vehicle and doses 1-5 of the dual miRNA-sensing circuit.

[0102] **FIGs. 32A-32F** show cell populations of various T cell phenotypes post administration of the dual miRNA-sensing circuit. **FIG. 32A** and **FIG. 32D** show % of live CD45+ cells for CD4 and CD8 T cells on Day 3 (**FIG. 32A**) and Day 6 (**FIG. 32D**) post administration of vehicle and the dual miRNA-sensing circuit at low and high doses. **FIG. 32B** and **FIG. 32E** show % of live CD4 cells for T regs (CD25+ Foxp3+) on Day 3 (**FIG. 32B**) and Day 6 (**FIG. 32E**) post administration of vehicle and low and high doses of the dual miRNA-sensing circuit. **FIG. 32C** and **FIG. 32F** show the tumor CD8 to T reg ratio on Day 3 (**FIG. 32C**) and Day 6 (**FIG. 32F**) post administration of vehicle and low and high doses of the dual miRNA-sensing circuit.

[0103] **FIG. 33** shows tumor microenvironment infiltration by T cells (staining denoted by arrows) on Day 6 following administration of vehicle and low and high doses of the dual miRNA-sensing circuit. Hematoxylin and eosin (H&E) staining is shown. Immunohistochemical (IHC) staining is also shown for CD8 and CD4.

[0104] **FIG. 34** shows severity of CD4 staining in CT26 tumors on Day 3 and Day 6 following administration of vehicle and low (left) and high (right) doses of the dual miRNA-sensing circuit.

[0105] **FIG. 35** shows severity of CD8 staining in CT26 tumors on Day 3 and Day 6 following administration of vehicle and low (left) and high (right) doses of the dual miRNA-sensing circuit.

[0106] **FIGs. 36A-36C** show tumor volume (mm^3) days post dose of the dual miRNA-sensing circuit, anti-PD1, and the dual miRNA-sensing circuit + anti-PD1. Low (**FIG. 36A**), medium (**FIG. 36B**), and high doses (**FIG. 36C**) of the dual miRNA-sensing circuit are shown.

[0107] **FIGs. 37A-37C** show probability of survival days post dose of the dual miRNA-sensing circuit, anti-PD1, and the dual miRNA-sensing circuit + anti-PD1. Low (**FIG. 37A**), medium (**FIG. 37B**), and high doses (**FIG. 37C**) of the dual miRNA-sensing circuit are shown.

[0108] **FIG. 38A** shows tumor suppression (days to 1000 mm^3) following administration of vehicle, the dual miRNA-sensing circuit, anti-PD1, and the dual miRNA-sensing circuit + anti-PD1. **FIG. 38B** shows median survival (days) following administration of vehicle, the dual

miRNA-sensing circuit, anti-PD1, and the dual miRNA-sensing circuit + anti-PD1. **FIG. 38C** shows response rate (%) following administration of vehicle, the dual miRNA-sensing circuit, anti-PD1, and the dual miRNA-sensing circuit + anti-PD1. Low, medium, and high doses of the dual miRNA-sensing circuit are shown.

[0109] **FIGS. 39A-39D** show IL-12, IFN-gamma, IFN-alpha, and IP-10 levels in serum respectively, following administration of vehicle, the dual miRNA-sensing circuit, anti-PD1, and the dual miRNA-sensing circuit + anti-PD1. **FIG. 39A** shows serum IL-12 levels (pg/mL). **FIG. 39B** shows serum IFN-γ levels (pg/mL). **FIG. 39C** shows serum IFN-α levels (pg/mL). **FIG. 39D** shows serum IP-10 levels (pg/mL). Low, medium, and high doses of the dual miRNA-sensing circuit are shown.

[0110] **FIGs. 40A-40E** show populations of various T cell phenotypes post administration of vehicle, anti-PD1, the dual miRNA-sensing circuit, and the combination (shown left to right). **FIGs. 40A** and **40C** show number (#) of cells per gram tumor for CD8 T cells, CD4 T cells, and NK cells on Day 3 (**FIG. 40A**) and Day 6 (**FIG. 40C**). **FIGs. 40B** and **40D** show percentage (%) of live CD45+ cells for CD8 T cells, CD4 T cells, and NK cells on Day 3 (**FIG. 40B**) and Day 6 (**FIG. 40D**). **FIG. 40E** shows the CD8 T cell to T reg ratio on Day 3 and Day 6.

[0111] **FIGs. 41A-41F** show populations of CD4 T cell subsets following administration of vehicle, anti-PD1, the dual miRNA-sensing circuit, and the combination (shown left to right). **FIGs. 41A** and **41B** show percentage (%) of live CD4 T cells on Day 3 (**FIG. 41A**) and Day 6 (**FIG. 41B**) for T regs, IFNg+TNFa+, IFNg+TNFa-, Tbet+, and PD1+. **FIGs. 41C** and **41D** show percentage (%) of CD8 T cell subsets on Day 3 (**FIG. 41C**) and Day 6 (**FIG. 41D**) for Granzyme B+, IFNg+TNFa+, IFNg+TNFa-, Tbet+, and PD1+. **FIGs. 41E** and **41F** show percentage (%) of NK cell subsets on Day 3 (**FIG. 41E**) and Day 6 (**FIG. 41F**) for Granzyme B+, IFNg+TNFa+, IFNg+TNFa-, Tbet+, and PD1+.

[0112] **FIGs. 42A-42D** show serum IL-12 (**FIG. 42A**), IFN-γ (**FIG. 42B**), IFN-α levels (**FIG. 42C**), and IP-10 levels (**FIG. 42D**) 24h following administration of vehicle, anti-PD1, the dual miRNA-sensing circuit, and the combination.

[0113] **FIG. 43A** and **FIG. 43B** show tumor volume (mm^3) days post tumor inoculation/rechallenge for naïve mice (**FIG. 43A**) and rechallenged mice (**FIG. 43B**) following injection with $1x10^6$ CT26.WT cells. **FIG. 43D** and **FIG. 43E** show tumor volume (mm^3) days post tumor inoculation/rechallenge for naïve mice (**FIG. 43D**) and rechallenged mice (**FIG. 43E**) following injection with $5x10^6$ CT26.WT cells. **FIG. 43C** and **FIG. 43F** show probability of survival for days following injection with $1x10^6$ (**FIG. 43C**) and $5x10^6$ (**FIG. 43F**) CT26.WT cells.

[0114] **FIG. 44A** and **FIG. 44B** show serum (**FIG. 44A**) and tumor (**FIG. 44B**) levels of human IL-12 (hIL-12) following administration of the dual miRNA-sensing circuit (encoding human IL-12) for the patient-derived xenograft mouse models for the following indications: triple-negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), and melanoma.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0115] Immunotherapies have revolutionized the treatment of solid tumors, which represent 90% of adult human cancers. Therapeutic delivery of IL-12, a potent immunostimulatory cytokine, is robustly effective in preclinical models. However, systemically delivered IL-12 is poorly tolerated potentially due to its off-target activity. To overcome this challenge, an mRNA platform was developed to utilize programmable genetic circuits to regulate the expression of an encoded protein in response to microRNAs (miRNAs), enabling precise control of payload expression in the target tissues. Using this platform, a systemically delivered self-replicating mRNA encoding IL-12 was generated to bear programmable genetic circuitry that limits the expression of payload in off-target tissues while preserving expression in the tumor.

[0116] The present disclosure is generally directed to isolated polynucleotides that allow for selective regulation of the expression of a gene (*e.g.*, encoding a payload) in a tissue. More specifically, the polynucleotides described herein comprise (a) a first region encoding a payload and (b) a second region comprising a sensor that is capable of recognizing a marker in a cell that expresses the marker ("detargeting cell"). As further described herein, depending on the presence or absence of the marker, the sensor can regulate the expression of the encoded payload. Such polynucleotides can be particularly useful in reducing and/or preventing the expression of a payload within a specific cell, *e.g.*, an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof. Not to be bound by any one theory, in some aspects, the expression of a gene (*e.g.*, encoding a payload) within a specific cell (*e.g.*, an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof) is directly correlated with the expression of the gene by the immune cells present within the specific tissue. Additional aspects of the present disclosure are provided throughout the present application.

[0117] Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to the particular compositions or process steps described, as such can, of course, vary. As will be apparent to those of skill in the art upon reading this disclosure, each of the individual aspects described and illustrated herein has discrete components and features

which can be readily separated from or combined with the features of any of the other several aspects without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

[0118] The headings provided herein are not limitations of the various aspects of the disclosure, which can be defined by reference to the specification as a whole. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only, and is not intended to be limiting, since the scope of the present disclosure will be limited only by the appended claims.

I. Definitions

[0119] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. In case of conflict, the present application, including the definitions, will control. Unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular.

[0120] Throughout this disclosure, the term “a” or “an” entity refers to one or more of that entity; for example, “a polynucleotide,” is understood to represent one or more polynucleotides. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0121] Furthermore, “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0122] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0123] The term “about” is used herein to mean approximately, roughly, around, or in the regions of. When the term is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower), unless indicated otherwise.

[0124] The term "at least" prior to a number or series of numbers is understood to include the number adjacent to the term "at least," and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21-nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range. "At least" is also not limited to integers (*e.g.,* "at least 5%" includes 5.0%, 5.1%, 5.18% without consideration of the number of significant figures.

[0125] As used herein, the term "sensor" refers to any moiety that is capable of recognizing a marker described herein. As used herein, "recognizing" a marker can comprise the physical interaction between the marker and the sensor (*e.g.*, the marker binds to a specific marker recognition site within the sensor). As used herein, the term "detargeting cell" refers to a cell that expresses a marker. Upon recognition of the marker, the sensor is "turned on" and thereby, reduces or inhibits the expression of the encoded payload by the "detargeting cell," *e.g.*, when administered to a subject. Where the sensors are configured to bind to or configured to recognize miR-142-3p, such sensors are also referred to herein as a "miR-142 sensor." Where the sensors are configured to bind to or configured to recognize miR-22-3p, such sensors are also referred to herein as a "miR-22 sensor." Where the sensors are configured to bind to or configured to recognize miR-185-5p, such sensors are also referred to herein as a "miR-185 sensor." Where the sensors are configured to bind to or configured to recognize miR-22-3p, miR-185-5p, or miR-142-3p, such sensors are also referred to herein as a "detargeting sensor." A detargeting sensor disclosed herein, e.g., a sensor that is configured to bind to or configured to recognize a specific miRNA (e.g., miR-185, miR-22, or miR-142), can also bind to additional targets, e.g., additional miRNAs. Where the sensors result in the reduction or inhibition of the expression of the encoded payload within an immune cell (*e.g.*, by recognizing markers expressed by immune cells), such sensors are also referred to herein as an "immune cell detargeting sensor." Where the sensors result in the reduction or inhibition of the expression of the encoded payload within a cardiomyocyte (*e.g.*, by recognizing markers expressed by cardiomyocyte), such sensors are also referred to herein as a "cardiomyocyte detargeting sensor." Where the sensors result in the reduction or inhibition of the expression of the encoded payload within an endothelial cell (*e.g.*, by recognizing markers expressed by an endothelial cell), such sensors are also referred to herein as an "endothelial cell detargeting sensor." As further described elsewhere in the present disclosure, such immune cells are present in many tissues, *e.g.*, lymphoid tissues, such as the spleen. In some aspects, by reducing or inhibiting the

expression of the encoded payload by such immune cells, the sensors described herein can also be useful in reducing or inhibiting the expression of the encoded polypeptide within certain tissues, *e.g.*, lymphoid tissues. In some aspects, by reducing or inhibiting the expression of the encoded payload by such cardiomyocytes, the sensors described herein can also be useful in reducing or inhibiting the expression of the encoded polypeptide within certain tissues, *e.g.*, myocardium tissues. In some aspects, by reducing or inhibiting the expression of the encoded payload by such endothelial cells, the sensors described herein can also be useful in reducing or inhibiting the expression of the encoded polypeptide within certain tissues, *e.g.*, endothelial tissue.

[0126] As used herein, the term "marker" refers to any molecule that is expressed in a detargeting cell and can be recognized by a sensor described herein. Non-limiting examples of such markers are provided elsewhere in the present disclosure.

[0127] As used herein, the term "immune cell" refers to those cells that are part of the immune system and helps the body fight infections and other diseases. Non-limiting examples of immune cells useful for the present disclosure include cells that are derivatives of hematopoietic stem cells. In some aspects, cells that are derivatives of hematopoietic stem cells comprise lymphoid lineage cells, myeloid lineage cells, or both. In some aspects, lymphoid lineage cells comprise lymphocytes, plasma cells, or both. In some aspects, lymphocytes comprise T cells, B cells, natural killer (NK) cells, or combinations thereof. In some aspects, myeloid lineage cells comprise macrophages, monocytes, dendritic cells, neutrophils, basophils, eosinophils, or combinations thereof. As will be apparent to those skilled in the art, immune cells are found predominantly within a lymphoid tissue or in circulation (*e.g.*, within the blood). As used herein, "lymphoid tissue" refers to any tissue within a subject that is rich in immune cells. Non-limiting examples of lymphoid tissues include the bone marrow, thymus, lymph nodes, spleen, tonsils, appendix, Peyer's patches, and combinations thereof. As used herein, the term "non-immune cell" refers to any cells that are not derived from hematopoietic stem cells.

[0128] As used herein, the term "cardiomyocyte" refers to those cells that are responsible for heart contraction and relaxation. Non-limiting examples of cardiomyocytes useful for the present disclosure include atrial myocytes, ventricular myocytes, pacemaker cells (nodal cells), purkinje fibers, conducting cells, or any combination thereof. As used herein, "myocardium tissue" refers to any tissue within a subject that is rich in cardiomyocytes. In some aspects, myocardium tissue comprises the heart muscle. As used herein, the term "non-cardiomyocyte" refers to any cells that are not cardiomyocytes. Non-limiting examples of non-cardiomyocytes useful for the present disclosure include cancer cells.

[0129] As used herein, the term "endothelial cell" refers to those cells that line the inside surfaces of blood vessels, lymph vessels, and the heart. In some aspects, endothelial cells comprise vascular endothelial cells (e.g., arterial endotheliam cells, venous endothelial cells, liver endothelial cells, or capillary endothelial cells (e.g., continuous endothelium, fenestrated endothelium, or sinusoidal endothelium)), lymphatic endothelial cells, pulmonary endothelial cells, brain endothelial cells (blood-brain barrier), or any combination thereof. As used herein, "endothelial tissue" refers to any tissue within a subject that is rich in endothelial cells. In some aspects, endothelial tissue comprises a single layer of cells that line blood and/or lympathic vessels. As used herein, the term "non-endothelial cell" refers to any cells that are not endothelial cells.

[0130] As used herein, the term "payload" refers to any agent that can be encoded by a polynucleotide described. Non-limiting examples of useful payloads are provided elsewhere in the present disclosure.

[0131] The term "derived from," as used herein, refers to a component that is isolated from or made using a specified molecule or organism, or information (*e.g.*, amino acid or nucleic acid sequence) from the specified molecule or organism. For example, a nucleic acid sequence that is derived from a second nucleic acid sequence can include a nucleotide sequence that is identical or substantially similar to the nucleotide sequence of the second nucleic acid sequence. In the case of nucleotides or polypeptides, the derived species can be obtained by, for example, naturally occurring mutagenesis, artificial directed mutagenesis or artificial random mutagenesis. The mutagenesis used to derive nucleotides or polypeptides can be intentionally directed or intentionally random, or a mixture of each. The mutagenesis of a nucleotide or polypeptide to create a different nucleotide or polypeptide derived from the first can be a random event (*e.g.*, caused by polymerase infidelity) and the identification of the derived nucleotide or polypeptide can be made by appropriate screening methods, *e.g.*, as discussed herein. In some aspects, a nucleotide or amino acid sequence that is derived from a second nucleotide or amino acid sequence has a sequence identity of at least about 50%, at least about 51%, at least about 52%, at least about 53%, at least about 54%, at least about 55%, at least about 56%, at least about 57%, at least about 58%, at least about 59%, at least about 60%, at least about 61%, at least about 62%, at least about 63%, at least about 64%, at least about 65%, at least about 66%, at least about 67%, at least about 68%, at least about 69%, at least about 70%, at least about 71%, at least about 72%, at least about 73%, at least about 74%, at least about 75%, at least about 76%, at least about 77%, at least about 78%, at least about 79%, at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about

88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% to the second nucleotide or amino acid sequence, respectively, wherein the first nucleotide or amino acid sequence retains the biological activity of the second nucleotide or amino acid sequence.

[0132] "Nucleic acid," "nucleic acid molecule," "nucleotide sequence," "nucleic acid sequence," "polynucleotide," and grammatical variants thereof are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form, or a double-stranded helix. Single stranded nucleic acid sequences refer to single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA). Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA. A "nucleic acid composition" of the disclosure comprises one or more nucleic acids as described herein. As described herein, a polynucleotide of the present disclosure comprises DNA, RNA, or both. In some aspects, the term "polynucleotide" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, shRNA, siRNA, miRNA and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (*e.g.*, peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA. A polynucleotide of the present disclosure can be a deoxyribonucleic acid (DNA) molecule or

ribonucleic acid (RNA) molecule. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), inosine (I) and uracil (U). It is to be understood that in the disclosed sequences T and U are interchangeable depending on whether the sequence is a DNA or an RNA.

[0133] The term "replicon," as used herein, refers to a polynucleotide comprising an origin of replication which allows for replication of the polynucleotide in a cell of interest. As further described herein, in some aspects, the replicon is a self-replicating mRNA (*i.e.*, capable of directing its own amplification or replication within a target cell) (also referred to herein as "repRNA" or "RNA-VEE"). To direct its own amplification, the RNA molecule should encode the enzyme(s) necessary to catalyze RNA amplification (*e.g.*, alphavirus nonstructural proteins nsP1, nsP2, nsP3, nsP4) and also contain cis RNA sequences required for replication which are recognized and utilized by the encoded enzymes(s). An alphavirus RNA vector replicon should contain the following ordered elements: 5′ viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred to as 5′CSE, or 5′ cis replication sequence, or 5′ viral sequences required in cis for replication, or 5′ sequence which is capable of initiating transcription of an alphavirus), sequences which, when expressed, code for biologically active alphavirus nonstructural proteins (*e.g.*, nsP1, nsP2, nsP3, nsP4), and 3′ viral or cellular sequences required for nonstructural protein-mediated amplification (may also be referred as 3′CSE, or 3′ viral sequences required in cis for replication, or an alphavirus RNA polymerase recognition sequence). The alphavirus RNA vector replicon may contain a means to express one or more heterologous sequence(s), such as for example, an IRES or a viral (e.g., alphaviral) subgenomic promoter (*e.g.*, junction region promoter) which may, in certain aspects, be modified in order to increase or reduce viral transcription of the subgenomic fragment, or to decrease homology with defective helper or structural protein expression cassettes, and one or more heterologous sequence(s) to be expressed. A replicon can also contain additional sequences, for example, one or more heterologous sequence(s) encoding one or more polypeptides (*e.g.*, a protein-encoding gene or a 3′ proximal gene) and/or a polyadenylate tract. The replicon should not contain sequences encoding all of the alphavirus structural proteins (capsid, E1, E2). Non-limiting examples of heterologous sequences that can be expressed by replicon vectors are described, for example in U.S. Pat. No. 6,015,686, incorporated by reference in its entirety herein, and include, for example, antigens, lymphokines, cytokines, etc. As is apparent from the present disclosure, VEE replicons provided herein are repRNA. Accordingly, "VEE replicon," "RNA-VEE," and "repRNA" are used interchangeably in the present disclosure.

[0134] As used herein, the term "modRNA" refers to a synthetic modified RNA which can encode a payload (*e.g.*, described herein) and when delivered to a cell, can induce the expression of the encoded payload. Unless indicated otherwise, modRNAs described herein are not self-replicating. In some aspects, a modRNA described herein comprises a modification which can help improve one or more properties of the RNA (*e.g.*, increased stability and/or transcription). Non-limiting examples of such modifications are known in the art (*e.g.*, chemical modification of a nucleobase). *See, e.g.*, US 2020/0000881, which is incorporated herein by reference in its entirety. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising sensor, *e.g.*, an immune cell detargeting sensor) is not a modRNA.

[0135] The terms "pharmaceutically acceptable carrier," "pharmaceutically acceptable excipient," and grammatical variations thereof, encompass any of the agents approved by a regulatory agency of the U.S. Federal government or listed in the U.S. Pharmacopeia for use in animals, including humans, as well as any carrier or diluent that does not cause the production of undesirable physiological effects to a degree that prohibits administration of the composition to a subject and does not abrogate the biological activity and properties of the administered compound. Included are excipients and carriers that are useful in preparing a pharmaceutical composition and are generally safe, non-toxic, and desirable.

[0136] As used herein, the term "pharmaceutical composition" refers to one or more of the compounds described herein, such as, *e.g.*, a polynucleotide of the present disclosure, mixed or intermingled with, or suspended in one or more other chemical components, such as pharmaceutically acceptable carriers and excipients.

[0137] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length, *e.g.*, that are encoded by a polynucleotide described herein. The polymer can comprise modified amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids such as homocysteine, ornithine, p-acetylphenylalanine, D-amino acids, and creatine), as well as other modifications known in the art. The term "polypeptide," as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function.

[0138] Polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing.

[0139] A polypeptide can be a single polypeptide or can be a multi-molecular complex such as a dimer, trimer or tetramer. They can also comprise single chain or multi-chain polypeptides. Most commonly disulfide linkages are found in multi-chain polypeptides. The term polypeptide can also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid. In some aspects, a "peptide" can be less than or equal to about 50 amino acids long, *e.g.,* about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, or about 50 amino acids long.

[0140] The term "RNA" is used herein to mean a molecule which comprises at least one ribonucleotide residue. "Ribonucleotide" relates to a nucleotide with a hydroxyl group at the 2′-position of a β-D-ribofuranosyl group. The term comprises double-stranded RNA, single-stranded RNA, isolated RNA such as partially or completely purified RNA, essentially pure RNA, synthetic RNA, recombinantly generated RNA differs from naturally occurring RNA by addition, deletion, substitution and/or alteration of one or more nucleotides. In some aspects, the RNA comprises a linear RNA, circular RNA, self-replicating RNA, non-replicating RNA, or combinations thereof. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a detargeting sensor) is a linear RNA. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a detargeting sensor) is a circular RNA. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a detargeting sensor) is a self-replicating RNA. In some aspects, a polynucleotide described herein (*e.g.*, comprising a first region encoding a payload and a second region comprising a detargeting sensor) is a non-self-replicating RNA.

[0141] The term "mRNA" means "messenger-RNA" and relates to a "transcript" which is generated by using a DNA template and encodes a peptide or protein (*e.g.*, payload described herein). Typically, an mRNA comprises a 5′-UTR, a protein coding region and a 3′-UTR. mRNA only possesses limited half-life in cells and in vitro. In the context of the present disclosure, mRNA can be generated by in vitro transcription from a DNA template. The in vitro transcription methodology is known to the skilled person. For example, there are a variety of in vitro transcription kits commercially available. In some aspects of the disclosure, the RNA, preferably the mRNA, is modified with a 5′-cap structure.

[0142] As used herein, the term "identity" (*e.g.*, sequence identity) refers to the overall monomer conservation between polymeric molecules, *e.g.*, between polynucleotide molecules. The term "identical" without any additional qualifiers, *e.g.*, polynucleotide A is identical to polynucleotide B, implies the polynucleotide sequences are 100% identical (100% sequence identity). Describing two sequences as, *e.g.*, "70% identical," is equivalent to describing them as having, *e.g.*, "70% sequence identity."

[0143] Calculation of the percent identity of two polypeptide or polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second polypeptide or polynucleotide sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In some aspects, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The amino acids at corresponding amino acid positions, or bases in the case of polynucleotides, are then compared.

[0144] When a position in the first sequence is occupied by the same amino acid or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

[0145] Suitable software programs that can be used to align different sequences (*e.g.*, polynucleotide sequences) are available from various sources. One suitable program to determine percent sequence identity is bl2seq, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). Bl2seq performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, *e.g.*, Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at worldwideweb.ebi.ac.uk/Tools/psa.

[0146] Sequence alignments can be conducted using methods known in the art such as MAFFT, Clustal (ClustalW, Clustal X or Clustal Omega), MUSCLE, etc.

[0147] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0148] In some aspects, the percentage identity (%ID) or of a first amino acid sequence (or nucleic acid sequence) to a second amino acid sequence (or nucleic acid sequence) is calculated as %ID = 100 x (Y/Z), where Y is the number of amino acid residues (or nucleobases) scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.

[0149] One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data *(e.g.,* crystallographic protein structures), functional data *(e.g.,* location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.,* from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity can be curated either automatically or manually.

[0150] As used herein, the terms "isolated," "purified," "extracted," and grammatical variants thereof are used interchangeably and refer to the state of a preparation of desired composition of the present disclosure, *e.g.*, a polynucleotide of the present disclosure, that has undergone one or more processes of purification. In some aspects, isolating or purifying as used herein is the process of removing, partially removing (*e.g.*, a fraction) of a composition of the present disclosure from a sample containing contaminants.

[0151] The term "expression," as used herein, refers to a process by which a polynucleotide produces a gene product, *e.g.*, RNA or a polypeptide (*e.g.*, therapeutic protein, *e.g.*, coronavirus protein). It includes without limitation transcription of the polynucleotide into micro RNA binding site, small hairpin RNA (shRNA), small interfering RNA (siRNA), or any other RNA product. It

includes, without limitation, transcription of the polynucleotide into messenger RNA (mRNA), and the translation of mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be, *e.g.*, a nucleic acid, such as an RNA produced by transcription of a gene. As used herein, a gene product can be either a nucleic acid, RNA or miRNA produced by the transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, phosphorylation, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

II. Polynucleotides

[0152] Provided herein is a polynucleotide (*e.g.*, isolated polynucleotide) comprising (a) a first region encoding a payload and (b) a second region comprising a sensor, wherein the sensor is capable of recognizing a marker expressed in a detargeting cell. As is apparent from the present disclosure, in some aspects, a tissue comprises a population of such detargeting cells, such that the expression of the encoded payload within the tissue is directly correlated with the expression of the encoded payload by the detargeting cells. As described herein, when the sensor recognizes (*e.g.*, binds to) the marker, the sensor is "turned on" (*i.e.*, in an active form) and thereby, inhibits or reduces the expression of the encoded payload. In the absence of the marker, the sensor is "turned off" (*i.e.*, in an inactive form) and there is no inhibition or reduction in the expression of the encoded payload. Therefore, the recognition of the marker by the sensor in the cell results in reduced expression of the payload as compared to a reference cell (*e.g.*, cell that does not express the marker or cell that expresses the marker at a low level). In some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof.

II.A. Sensors and Markers

[0153] A sensor useful for the present disclosure can be programmed to recognize a wide range of markers expressed in a detargeting cell. In some aspects, the marker is expressed only by a detargeting cell. Unless indicated otherwise, increased expression of the marker can comprise (a) an increase in the total amount of the marker expressed, (b) an increase in how long the marker is expressed, or (c) both (a) and (b).

[0154] In some aspects, the marker comprises a microRNA. In some aspects, a polynucleotide described herein comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor, wherein the detargeting sensor is capable of recognizing a

microRNA expressed within a detargeting cell. In some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof.

[0155] In some aspects, the marker comprises microRNA-185 (miR-185-5p), microRNA-22 (miR-22-3p), or any combination thereof. In some aspects, the marker comprises miR-185-5p. In some aspects, the marker comprises miR-22-3p. Accordingly, in some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor, wherein the detargeting sensor is capable of recognizing miR-22-3p (i.e., a miR-22 sensor). In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor, wherein the detargeting sensor is capable of recognizing miR-185-5p (i.e., a miR-185 sensor).

[0156] In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising two or more detargeting sensors, wherein at least one of the detargeting sensors is capable of recognizing miR-22-3p (i.e., a miR-22 sensor) and at least one of the detargeting sensors is capable of recognizing miR-142-3p (i.e., a miR-142 sensor). In some aspects, the miR-142 sensor is located at the 5' of the miR-22 sensor.

[0157] In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising two or more detargeting sensors, wherein at least one of the detargeting sensors is capable of recognizing miR-185-5p (i.e., a miR-185 sensor) and at least one of the detargeting sensors is capable of recognizing miR-142-3p (i.e., a miR-142 sensor). In some aspects, the miR-142 sensor is located at the 5' of the miR-185 sensor.

[0158] In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising two or more detargeting sensors, wherein at least one of the detargeting sensors is capable of recognizing miR-22-3p (i.e., a miR-22 sensor), at least one of the detargeting sensors is capable of recognizing miR-185-5p (i.e., a miR-185 sensor), and at least one of the detargeting sensors is capable of recognizing miR-142-3p (i.e., a miR-142 sensor). In some aspects, the miR-142 sensor is located at the 5' of the miR-185 sensor, and the miR-185 sensor is located at the 5' of the miR-22 sensor.

[0159] In some aspects, a detargeting sensor comprises a sensor targeting miR-142-3p. Unless indicated otherwise, miR-142-3p useful for the present disclosure comprises any miR-142-3p from any vertebrate source (*e.g.*, humans). An exemplary sequence for human miR-142-3p is set forth in NCBI Gene ID: 406934. In some aspects, the miR-142 sensor comprises the nucleotide sequence TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 3). In some aspects, the miR-142 sensor consists essentially of the nucleotide sequence TCCATAAAGTAGGAAACACTACA (SEQ ID NO:

3). In some aspects, the miR-142 sensor consists of the nucleotide sequence TCCATAAAGTAGGAAACACTACA (SEQ ID NO: 3). In some aspects, the miR-142 sensor is RNA, thus all Ts in SEQ ID NO: 3 is replaced with U.

[0160] In some aspects, a detargeting sensor comprises a sensor targeting miR-22-3p. Unless indicated otherwise, miR-22-3p useful for the present disclosure comprises any miR-22-3p from any vertebrate source (*e.g.*, humans). An exemplary sequence for human miR-22 is set forth in NCBI Gene ID: 407004. In some aspects, the miR-22 sensor comprises the nucleotide sequence ACAGTTCTTCAACTGGCAGCTT (SEQ ID NO: 6). In some aspects, the miR-22 sensor consists essentially of the nucleotide sequence ACAGTTCTTCAACTGGCAGCTT (SEQ ID NO: 6). In some aspects the miR-22 sensor consists of the nucleotide sequence ACAGTTCTTCAACTGGCAGCTT (SEQ ID NO: 6). In some aspects, the miR-22 sensor is RNA, thus all Ts in SEQ ID NO: 6 is replaced with U.

[0161] In some aspects, the miR-185 sensor comprises a sensor targeting miR-185-5p. Unless indicated otherwise, miR-185-5p useful for the present disclosure comprises any miR-185-5p from any vertebrate source (*e.g.*, humans). An exemplary sequence for human miR-185 is set forth in NCBI Gene ID: 406961. In some aspects, the miR-185 sensor comprises the nucleotide sequence TCAGGAACTGCCTTTCTCTCCA (SEQ ID NO: 9). In some aspects, the miR-185 sensor consists essentially of the nucleotide sequence TCAGGAACTGCCTTTCTCTCCA (SEQ ID NO: 9). In some aspects the miR-185 sensor consists of the nucleotide sequence TCAGGAACTGCCTTTCTCTCCA (SEQ ID NO: 9). In some aspects, the miR-185 sensor is RNA, thus all Ts in SEQ ID NO: 9 is replaced with U.

[0162] In some aspects, a polynucleotide provided herein comprises multiple detargeting sensors. For instance, in some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising multiple detargeting sensors (*e.g.*, a miR-142 sensor, a miR-185 sensor, a miR-22 sensor, or any combination thereof), wherein the recognition of the marker by detargeting sensors reduces the expression of the payload. In some aspects, the multiple detargeting sensors comprise about two detargeting sensors, about three detargeting sensors, about four detargeting sensors, about five detargeting sensors, about six detargeting sensors, about seven detargeting sensors, about eight detargeting sensors, about nine detargeting sensors, about 10 detargeting sensors, about 11 detargeting sensors, about 12 detargeting sensors, about 13 detargeting sensors, about 14 detargeting sensors, about 15 detargeting sensors, about 16 detargeting sensors, about 17 detargeting sensors, about 18 detargeting sensors, about 19 detargeting sensors, or about 20 or more detargeting sensors.

[0163] In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising a first detargeting sensor comprising a miR-142 sensor and a second detargeting sensor comprising a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising three detargeting sensors, four detargeting sensors, five deterageting sensors, six detargeting sensors, seven detargeting sensors, eight detargeting sensors, nine detargeting sensors, 10 detargeting sensors, 11 detargeting sensors, 12 detargeting sensors, wherein each of the first two detargeting sensors is a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising three detargeting sensors, four detargeting sensors, five deterageting sensors, six detargeting sensors, seven detargeting sensors, eight detargeting sensors, nine detargeting sensors, 10 detargeting sensors, 11 detargeting sensors, 12 detargeting sensors, wherein each of the first three detargeting sensors is a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising three detargeting sensors, four detargeting sensors, five deterageting sensors, six detargeting sensors, seven detargeting sensors, eight detargeting sensors, nine detargeting sensors, 10 detargeting sensors, 11 detargeting sensors, 12 detargeting sensors, wherein each of the first four detargeting is are a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising three detargeting sensors, four detargeting sensors, five deterageting sensors, six detargeting sensors, seven detargeting sensors, eight detargeting sensors, nine detargeting sensors, 10 detargeting sensors, 11 detargeting sensors, 12 detargeting sensors, wherein each of the first five detargeting sensors is a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising three detargeting sensors, four detargeting sensors, five deterageting sensors, six detargeting sensors, seven detargeting sensors, eight detargeting sensors, nine detargeting sensors, 10 detargeting sensors, 11 detargeting sensors, 12 detargeting sensors, wherein each of the first six detargeting sensors is a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor.

[0164] In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising four detargeting sensors, wherein each of the first two detargeting sensors are a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising six detargeting sensors, wherein each of the first three detargeting sensors are a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising eight detargeting sensors, wherein each of the first four detargeting sensors are a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising ten detargeting sensors, wherein each of the first five detargeting sensors are a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor. In some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload and (b) a second region comprising 12 detargeting sensors, wherein each of the first six detargeting sensors are a miR-142 sensor and each of the rest of the detargeting sensors is a miR-185 sensor or a miR-22 sensor.

[0165] In some aspects, each of the multiple detargeting sensors is the same (*e.g.*, all have the same sequence and recognize the same marker. In some aspects, the multiple detargeting sensors comprises two identical sensors (e.g., miR-142 sensors) in combination of another two identical sensors (e.g., miR-185 sensors). In some aspects, the multiple detargeting sensors comprise three identical sensors (e.g., miR-142 sensors) in combination of another three identical sensors (e.g., miR-185 sensors). In some aspects, the multiple detargeting sensors comprise four identical sensors (e.g., miR-142 sensors) in combination of another four identical sensors (e.g., miR-185 sensors). In some aspects, the multiple detargeting sensors comprises five identical sensors (e.g., miR-142 sensors) in combination of another five identical sensors (e.g., miR-185 sensors). In some aspects, one or more of the detargeting sensors are different. For example, in some aspects, one or more of the multiple detargeting sensors can recognize the same marker expressed by an immune cell but differ in their sequence. In some aspects, one or more of the multiple detargeting sensors differ in their sequence and recognize a different marker.

[0166] In some aspects, a polynucleotide described herein can comprise one or more additional sensors that recognize a marker expressed by a same cell type and/or different cell type. In some aspects, a polynucleotide useful for the present disclosure comprises (a) a first region

encoding a payload and (b) a second region comprising (i) a first detargeting sensor (*e.g.*, a miR-22 sensor or a miR-185 sensor) and (ii) a second sensor (e.g. *e.g.*, a miR-142 sensor), that recognizes a marker expressed by a same cell type and/or different cell type. As is apparent from the present disclosure, in some aspects, such a polynucleotide can be useful in regulating (*e.g.*, reducing or inhibiting) the expression of the payload within the same or multiple cell types.

[0167] In some aspects, the detargeting cell comprises a cardiomyocyte, e.g., atrial myocytes, ventricular myocytes, pacemaker cells (nodal cells), purkinje fibers, conducting cells, or any combination thereof. In some aspects, the marker (e.g., miR-185 or miR-22) is present within a cardiomyocyte at a greater quantity as compared to non-cardiomyocytes or the cell type that does not express the marker or expresses the marker at a lower level than the cardiomyocytes. In some aspects, the expression level of the marker present within the cardiomyocyte is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, grater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, or greater than about 20-fold or more, as compared to the corresponding value for a non-cardiomyocyte or a cell type that does not express the marker. Therefore, the expression level of the payload within cardiomyocytes is reduced by greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, grater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, or greater than about 20-fold or more, as compared to the corresponding value for a non-cardiomyocyte or a cell type that does not express the marker or expresses the marker at a lower level than the cardiomyocytes.

[0168] In some aspects, the detargeting cell comprises an endothelial cell. In some aspects, the endothelial cell comprises vascular endothelial cells (e.g., arterial endotheliam cells, venous endothelial cells, liver endothelial cells, or capillary endothelial cells (e.g., continuous endothelium, fenestrated endothelium, or sinusoidal endothelium)), lymphatic endothelial cells, pulmonary endothelial cells, brain endothelial cells (blood-brain barrier), or any combination thereof. In some aspects, the endothelial cell comprises blood vessel, lymphatic vessel, liver, lung, kidney, brain, intestine, heart, and/or muscle. In some aspects, the marker (e.g., miR-185 or miR-

22) is present within an endothelial cell at a greater quantity as compared to a non-endothelial cell or a cell type that does not express the marker. In some aspects, the expression level of the marker present within the endothelial cell is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, grater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, or greater than about 20-fold or more, as compared to the corresponding value for a non-endothelial cell or a cell type that does not express the marker. Therefore, the expression level of the payload present within endothelial cells is reduced by greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, grater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, or greater than about 20-fold or more, as compared to the corresponding value for a non-endothelial cell or a cell type that does not express the marker.

[0169] In some aspects, the detargeting cell comprises an immune cell. In some aspects, the expression level of the marker (e.g., miR-142) present within the immune cell is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, greater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, greater than about 20-fold, or more, as compared to the corresponding value for a non-immune cell or a cell type that does not express the marker. Therefore, the expression level of the payload by the polynucleotide comprising a detargeting sensor is reduced in the immune cells by about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, greater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, greater than about 20-fold, or more, as compared to the corresponding value for a non-immune cell

or a cell type that does not express the marker or expresses the marker at a lower level than the immune cell or expresses the marker at a lower level than the endothelial cells.

[0170] In some examples, the non-immune cell is present within a non-lymphoid tissue. Examples of non-lymphoid tissues where microRNA are known to regulate mRNA, and thereby protein expression, include, but are not limited to, liver (miR-122), muscle (miR-133, miR-206, miR-208), endothelial cells (miR-17-92, miR-126), myeloid cells (miR-16, miR-21, miR-223, miR-24, miR-27), adipose tissue (let-7, miR-30c), heart (miR-1d, miR-149), kidney (miR-192, miR-194, miR-204), and lung epithelial cells (let-7, miR-133, miR-126).

[0171] In some aspects, the detargeting cells comprise multiple cell types, e.g., immune cells, cardiomyocytes, and/or endothelial cells when multiple sensors are used to target multiple markers. For example, as described herein, a polynucleotide of the present disclosure comprises at least two different sensors, e.g., miR-142 sensor and miR-185 sensor or miR-142 sensor and miR-22 sensor, to detarget multiple cell types such as immune cells, endothelial cells, and/or cardiomyocytes.

II.B. Payloads

[0172] As described herein, polynucleotides provided herein (*e.g.*, comprising a detargeting sensor) comprise a first region that encodes a payload. As will be apparent to those skilled in the arts, any suitable payloads known in the art can be used with the present disclosure. Non-limiting examples of such payloads include a therapeutic protein, a detectable protein (*e.g.*, reporter or fluorescent protein), immunomodulatory protein, a ligand-binding protein, or combinations thereof.

[0173] In some aspects, the payload comprises a therapeutic protein. A non-limiting example of such a therapeutic protein comprises a cytokine. In some aspects, a polynucleotide described herein comprises (a) a first region encoding a cytokine and (b) a second region comprising a detargeting sensor, wherein recognition of the marker by the detargeting sensor results in reduced expression of the cytokine in the detargeting cell. In some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof.

[0174] In some aspects, the therapeutic protein is a cytokine and the marker comprises a microRNA. In some aspects, a polynucleotide described herein comprises (a) a first region encoding a cytokine and (b) a second region comprising a detargeting sensor that is capable of recognizing a microRNA expressed in a detargeting cell, wherein the recognition of the microRNA

by the sensor results in reduced expression of the cytokine in the detargeting cell. In some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof.

[0175] In some aspects, the cytokine comprises an interleukin (IL)-12. In some aspects, a polynucleotide described herein comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising a detargeting sensor, wherein the recognition of the marker by the sensor results in reduced expression of the IL-12 protein in the detargeting cell. In some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof. Exemplary amino acid and/or nucleic acid sequences of IL-12 are provided in WO2022150712A1, which is incorporated herein by reference in its entirety.

[0176] In some aspects, the cytokine is not IL-12. In some aspects, the cytokine is selected from (i) common gamma chain family of cytokines; (ii) IL-1 family of cytokines; (iii) hematopoietic cytokines; (iv) interferons (*e.g.*, type I, type II, or type III); (v) TNF family of cytokines; (vi) IL-17 family of cytokines; (vii) damage-associated molecular patterns (DAMPs); (viii) tolerogenic cytokines; or (ix) combinations thereof. In some aspects, the cytokine comprises IL-2, IL-4, IL-7, IL-10, IL-15, IL-21, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36ra, IL-37, IL-38, IL-3, IL-5, IL-6, IL-11, IL-13, IL-23, granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), leukemia inhibitory factor (LIF), stem cell factor (SCF), thrombopoietin (TPO), macrophage-colony stimulating factor (M-CSF), erythropoieticn (EPO), Flt-3, IFN-α, IFN-β, IFN-γ, IL-19, IL-20, IL-22, IL-24, TNF-α, TNF-β, BAFF, APRIL, lymphotoxin beta (TNF-γ), IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F, IL-25, TSLP, IL-35, IL-27, TGF-β, or combinations thereof.

[0177] In some aspects, the payload comprises a ligand-binding protein. Non-limiting examples of ligand binding proteins include a chimeric antigen receptor (CAR), T cell receptor (TCR), chimeric antibody-T cell receptor (caTCR), chimeric signaling receptor (CSR), T cell receptor mimic (TCR mimic), or combinations thereof.

[0178] In some aspects, the payload comprises an antibody or an antigen-binding fragment thereof (collectively referred to herein as "antibody"). The antibody can be derived from natural sources, or partly or wholly synthetically produced. In some aspects, the antibody is a monoclonal antibody. In some aspects, the monoclonal antibody is an IgG antibody. In some aspects, the monoclonal antibody is an IgG1, IgG2, IgG3, or IgG4. In some aspects, the antibody is a polyclonal antibody. In some aspects, the antibody is selected from Fab, Fab', and $F(ab')_2$, $F(ab1)_2$, Fv, dAb, and Fd fragments. In some aspects, the antibody is an scFv or $(scFv)_2$ fragment. In some aspects,

the antibody is a NANOBODY® (single-domain antibody). In some aspects, the antibody is a bispecific or multi-specific antibody.

[0179] In some aspects, the payload comprises a detectable protein (*e.g.*, reporter or fluorescent protein). Non-limiting examples of such detectable proteins include: luciferase, wt-GFP, green fluorescent protein (*e.g.*, EGFP, Emerald, Superfolder GFP, Azami Green, mWasabi, TagGFP, TurboGFP, AcGFP, ZsGreen, T-Sapphire, etc.), blue fluorescent protein, (*e.g.*, EBFP, EBFP2, Azurite, mTagBFP, etc.), cyan fluorescent protein (*e.g.*, ECFP, mECFP, Cerulean, mTurquoise, CyPet, AmCyan1, Midori-Ishi Cyan, TagCFP, mTFP1 (Teal), etc.), yellow fluorescent protein (*e.g.*, EYFP, Topaz, Venus, mCitrine, YPet, TagYFP, PhiYFP, ZsYellow1, mBanana, etc.), orange fluorescent protein (*e.g.*, Kusabira Orange, Kusabira Orange2, mOrange, mOrange2, dTomato, dTomato-Tandem, TagRFP, TagRFP-T, DsRed, DsRed2, DsRed-Express (T1), DsRed-Monomer, mTangerine, etc.), or red fluorescent protein (*e.g.*, mRuby, mApple, mStrawberry, AsRed2, mRFP1, JRed, mCherry, HcRedl, mRaspberry, dKeima-Tandem, HcRed-Tandem, mPlum, AQ143, etc.).

II.C. Additional Components

[0180] In some aspects, a polynucleotide provided herein (*e.g.*, encoding a payload and comprising a detargeting sensor) further comprises one or more additional components. In some aspects, such additional components can help in the function and/or expression of the polynucleotide. Accordingly, in some aspects, a polynucleotide of the present disclosure comprises (a) a first region encoding a payload, (b) a second region comprising a detargeting sensor, and (c) one or more additional component described herein, wherein the recognition of the marker by the sensor reduces the expression of the payload. Non-limiting examples of such additional components include: (1) an untranslated region (UTR), (2) a sequence encoding a signal peptide, (3) a translation initiation sequence, (4) a polyA sequence, (5) a sequence encoding a RNA binding protein, (6) a 5'-cap, (7) a sequence encoding a 2A ribosome skip peptide, (8) a translation enhancer element, (9) a spacer sequence, or (10) any combination of (1) to (9). Additional disclosure related to such additional components are provided below.

Untranslated Regions (UTRs)

[0181] In some aspects, a polynucleotide described herein further comprises a UTR. In some aspects, the UTR is a 5'-UTR. In some aspects, the UTR is a 3'-UTR. In some aspects, the UTR comprises both a 5'-UTR and a 3'-UTR.

[0182] Natural 5′-UTRs bear features which play roles in translation initiation. They harbor signatures like Kozak sequences which are commonly known to be involved in the process by which the ribosome initiates translation of many genes. Kozak sequences have the consensus CCR(A/G)CCAUGG, where R is a purine (adenine or guanine) three bases upstream of the start codon (AUG), which is followed by another 'G'. 5′-UTR also have been known to form secondary structures which are involved in elongation factor binding.

[0183] 5′-UTR secondary structures involved in elongation factor binding can interact with other RNA binding molecules in the 5′-UTR or 3′-UTR to regulate gene expression. For example, the elongation factor EIF4A2 binding to a secondarily structured element in the 5′-UTR is necessary for microRNA mediated repression (Meijer H A et al., Science, 2013, 340, 82-85, herein incorporated by reference in its entirety). The different secondary structures in the 5′-UTR can be incorporated into the flanking region to either stabilize or selectively destabilize mRNAs in specific tissues or cells.

[0184] By engineering the features typically found in abundantly expressed genes of specific target organs, one can enhance the stability and protein production. For example, introduction of 5′-UTR of liver-expressed mRNA, such as albumin, serum amyloid A, Apolipoprotein A/B/E, transferrin, alpha fetoprotein, erythropoietin, or Factor VIII, could be used to enhance expression of a nucleic acid molecule, such as a mRNA, in hepatic cell lines or liver. Likewise, use of 5′-UTR from other tissue-specific mRNA to improve expression in that tissue is possible—for muscle (MyoD, Myosin, Myoglobin, Myogenin, Herculin), for endothelial cells (Tie-1, CD36), for myeloid cells (C/EBP, AML1, G-CSF, GM-CSF, CD11b, MSR, Fr-1, i-NOS), for leukocytes (CD45, CD18), for adipose tissue (CD36, GLUT4, ACRP30, adiponectin) and for lung epithelial cells (SP-A/B/C/D).

[0185] Other non-UTR sequences can also be incorporated into the UTRs (*e.g.*, 5'-UTR and/or 3'-UTR). For example, introns or portions of introns sequences can be incorporated into the flanking regions of a polynucleotide described herein.

[0186] In some aspects, one or more nucleotides within a UTR (*e.g.*, 5'-UTR and/or 3'-UTR) can be mutated, replaced and/or removed. For example, one or more nucleotides upstream of the start codon can be replaced with another nucleotide. The nucleotide or nucleotides to be replaced can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60 or more than 60 nucleotides upstream of the start codon. As another example, one or more nucleotides upstream of the start codon can be removed from the UTR.

[0187] 3′-UTRs are known to have stretches of adenosines and uridines embedded in them. These AU rich signatures are particularly prevalent in genes with high rates of turnover. Based on their sequence features and functional properties, the AU rich elements (AREs) can be separated into three classes (Chen et al, 1995): Class I AREs contain several dispersed copies of an AUUUA motif within U-rich regions. C-Myc and MyoD contain class I AREs. Class II AREs possess two or more overlapping UUAUUUA(U/A)(U/A) nonamers. Molecules containing this type of AREs include GM-CSF and TNF-a. Class III AREs are less well defined. These U rich regions do not contain an AUUUA motif. c-Jun and Myogenin are two well-studied examples of this class. Most proteins binding to the AREs are known to destabilize the messenger, whereas members of the ELAV family, most notably HuR, have been documented to increase the stability of mRNA. HuR binds to AREs of all the three classes. Engineering the HuR specific binding sites into the 3′-UTR of nucleic acid molecules can lead to HuR binding and thus, stabilization of the message *in vivo*.

[0188] In some aspects, introduction, removal, or modification of 3′-UTR AU rich elements (AREs) can be used to modulate the stability of a nucleic acid sequence. When engineering specific polynucleotides, one or more copies of an ARE can be introduced to make the nucleic acid sequence less stable and thereby curtail translation and decrease production of the resultant protein. Likewise, AREs can be identified and removed or mutated to increase the intracellular stability and thus increase translation and production of the resultant protein.

Translation Enhancer Elements (TEEs)

[0189] In some aspects, a polynucleotide provided herein further comprises a translational enhancer element (TEE). As used herein, the term “translational enhancer element” refers to cis-acting sequences that increase the expression of a protein encoded by a nucleotide sequence. Non-limiting examples of TEEs that can be used with the present disclosure are known in the art, *see, e.g.*, US20130177581A, which is incorporated herein by reference in its entirety.

[0190] In some aspects, the TEE is positioned between the transcription promoter and the start codon of a sequence. In some aspects, a TEE useful for the present disclosure has at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% sequence identity with any of the TEEs provided in U.S. Publication Number US 20140147454, US20090226470, US20070048776, US20130177581, US20110124100, WO1999024595, WO2012009644, WO2009075886, WO2007025008, U.S. Pat. No. 6,310,197, U.S. Pat. No. 6,849,405, U.S. Pat. No. 7,456,273, U.S. Pat. No. 7,183,395, each of which is herein incorporated by reference in its entirety.

[0191] In some aspects, a polynucleotide provided herein comprises multiple TEEs. For example, in some aspects, a polynucleotide of the present disclosure (*e.g.*, encoding a payload and comprising a detargeting sensor) comprises at least about 1, at least about 2, at least about 3, at least about 4, at least about 5, at least about 6, at least about 7, at least about 8, at least about 9, at least about 10, at least about 11, at least about 12, at least about 13, at least about 14, at least about 15, at least about 16, at least about 17, at least about 18 at least about 19, at least about 20, at least about 21, at least about 22, at least about 23, at least about 24, at least about 25, at least about 30, at least about 35, at least about 40, at least about 45, at least about 50, at least about 55, or more than about 60 TEE sequences. In some aspects, the TEE sequences in the 5′UTR of the RNA (*e.g.*, modified RNA) are the same or different TEE sequences. In some aspects, the TEE sequences are in a pattern such as ABABAB or AABBAABBAABB or ABCABCABC or variants thereof repeated once, twice, or more than three times. In these patterns, each letter, A, B, or C represent a different TEE sequence at the nucleotide level.

RNA Binding Proteins (RBPs)

[0192] In some aspects, a polynucleotide provided herein further comprises a sequence encoding an RNA binding protein. RNA binding proteins (RBPs) can regulate numerous aspects of co- and post-transcription gene expression such as, but not limited to, RNA splicing, localization, translation, turnover, polyadenylation, capping, modification, export and localization. RNA-binding domains (RBDs), such as, but not limited to, RNA recognition motif (RR) and hnRNP K-homology (KH) domains, typically regulate the sequence association between RBPs and their RNA targets (Ray *et al., Nature* 2013. 499:172-177; herein incorporated by reference in its entirety). In some aspects, the canonical RBDs bind short RNA sequences. In some aspects, the canonical RBDs recognize RNA structure.

[0193] Non limiting examples of RNA binding proteins and related nucleic acid and protein sequences are described in US 2014/0147454, which is herein incorporated by reference in its entirety.

5′-Capping

[0194] In some aspects, a polynucleotide described herein further comprises a 5'-cap structure. The 5′ cap structure of a mRNA is involved in nuclear export, increasing mRNA stability and binds the mRNA Cap Binding Protein (CBP), which is responsible for mRNA stability in the cell and translation competency through the association of CBP with poly(A) binding protein to form the mature cyclic mRNA species. The cap further assists the removal of 5′ proximal introns removal during mRNA splicing.

[0195] Modifications to the RNA of the present disclosure can generate a non-hydrolyzable cap structure preventing decapping and thus increasing mRNA half-life. Because cap structure hydrolysis requires cleavage of 5′-ppp-5′ phosphorodiester linkages, modified nucleotides can be used during the capping reaction. For example, a Vaccinia Capping Enzyme from New England Biolabs (Ipswich, Mass.) can be used with α-thio-guanosine nucleotides according to the manufacturer's instructions to create a phosphorothioate linkage in the 5′-ppp-5′ cap. Additional modified guanosine nucleotides can be used such as α-methyl-phosphonate and seleno-phosphate nucleotides.

[0196] Additional modifications include, but are not limited to, 2′-O-methylation of the ribose sugars of 5′-terminal and/or 5′-anteterminal nucleotides of the mRNA (as mentioned above) on the 2′-hydroxyl group of the sugar ring. Multiple distinct 5′-cap structures can be used to generate the 5′-cap of a nucleic acid molecule, such as an mRNA molecule.

[0197] Cap analogs, which herein are also referred to as synthetic cap analogs, chemical caps, chemical cap analogs, or structural or functional cap analogs, differ from natural (*i.e.*, endogenous, wild-type or physiological) 5′-caps in their chemical structure, while retaining cap function. Cap analogs can be chemically (*i.e.*, non-enzymatically) or enzymatically synthesized and/linked to a nucleic acid molecule.

[0198] For example, the Anti-Reverse Cap Analog (ARCA) cap contains two guanines linked by a 5′-5′-triphosphate group, wherein one guanine contains an N7 methyl group as well as a 3′-O-methyl group (*i.e.*, N7,3′-O-dimethyl-guanosine-5′-triphosphate-5′-guanosine (m7G-3′ mppp-G; which can equivalently be designated 3′ O-Me-m7G(5′)ppp(5′)G). The 3′-O atom of the other, unmodified, guanine becomes linked to the 5′-terminal nucleotide of the capped nucleic acid molecule (*e.g.*, an mRNA or mmRNA). The N7- and 3′-O-methylated guanine provides the terminal moiety of the capped nucleic acid molecule (*e.g.*, mRNA or mmRNA).

[0199] Another exemplary cap is mCAP, which is similar to ARCA but has a 2′-β-methyl group on guanosine (*i.e.*, N7,2′-O-dimethyl-guanosine-5′-triphosphate-5′-guanosine, m7Gm-ppp-G).

[0200] In some aspects, the cap is a dinucleotide cap analog. In some aspects, the dinucleotide cap analog is modified at different phosphate positions with a boranophosphate group or a phosphoroselenoate group such as the dinucleotide cap analogs described in U.S. Pat. No. 8,519,110, the contents of which are herein incorporated by reference in its entirety.

[0201] In some aspects, the cap is a cap analog is a N7-(4-chlorophenoxyethyl) substituted dicucleotide form of a cap analog known in the art and/or described herein. Non-limiting examples

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

[0202] While cap analogs allow for the concomitant capping of a nucleic acid molecule in an *in vitro* transcription reaction, up to about 20% of transcripts remain uncapped. This, as well as the structural differences of a cap analog from an endogenous 5′-cap structures of nucleic acids produced by the endogenous, cellular transcription machinery, can lead to reduced translational competency and reduced cellular stability.

[0203] In some aspects, providing an RNA with a 5′-cap or 5′-cap analog is achieved by in vitro transcription of a DNA template in the presence of said 5′-cap or 5′-cap analog, wherein said 5′-cap is co-transcriptionally incorporated into the generated RNA strand,

[0204] In some aspects, RNA can be generated, for example, by in vitro transcription, and the 5′-cap can be attached to the RNA post-transcriptionally using capping enzymes, for example, capping enzymes of vaccinia virus. In some aspects, the nucleotide sequence encoding IL-12 is capped post-transcriptionally, using enzymes, in order to generate more authentic 5′-cap structures. As used herein, the phrase "more authentic" refers to a feature that closely mirrors or mimics, either structurally or functionally, an endogenous or wild type feature. That is, a "more authentic" feature is better representative of an endogenous, wild-type, natural or physiological cellular function and/or structure as compared to synthetic features or analogs, etc., of the prior art, or which outperforms the corresponding endogenous, wild-type, natural or physiological feature in one or more respects. Non-limiting examples of more authentic 5′ cap structures of the present disclosure are those which, among other things, have enhanced binding of cap binding proteins, increased half-life, reduced susceptibility to 5′ endonucleases and/or reduced 5′ decapping, as compared to synthetic 5′ cap structures known in the art (or to a wild-type, natural or physiological 5′ cap structure). For example, recombinant Vaccinia Virus Capping Enzyme and recombinant 2′-O-methyltransferase enzyme can create a canonical 5′-5′-triphosphate linkage between the 5′-terminal nucleotide of an mRNA and a guanine cap nucleotide wherein the cap guanine contains an N7 methylation and the 5′-terminal nucleotide of the mRNA contains a 2′-O-methyl. This cap results in a higher translational-competency and cellular stability and a reduced activation of cellular pro-inflammatory cytokines, as compared, *e.g.,* to other 5′ cap analog structures known in the art. Cap

structures include 7mG(5′)ppp(5′)N,pN2p, 7mG(5′)ppp(5′)NlmpNp, 7mG(5′)-ppp(5′)NlmpN2 mp and m(7)Gpppm(3)(6,6,2′)Apm(2′)Apm(2′)Cpm(2)(3,2′)Up.

[0205] In some aspects, 5′ terminal caps include endogenous caps or cap analogs. In some aspects, a 5′ terminal cap comprises a guanine analog. Useful guanine analogs include inosine, N1-methyl-guanosine, 2′ fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0206] In some aspects, the 5' cap comprises a 5' to 5' triphosphate linkage. In some aspects, the 5' cap comprises a 5' to 5' triphosphate linkage including thiophosphate modification. In some aspects, the 5' cap comprises a 2´-O or 3´-O-ribose-methylated nucleotide. In some aspects, the 5' cap comprises a modified guanosine nucleotide or modified adenosine nucleotide. In some aspects, the 5' cap comprises 7- methylguanylate. Exemplary cap structures include m7G(5')ppp(5')G, m7,2`O-mG(5')ppSp(5')G, m7G(5')ppp(5')2`O-mG, and m7,3`O-mG(5')ppp(5')2`O-mA.

[0207] In some aspects, a polynucleotide described herein comprises a modified 5′ cap. A modification on the 5′ cap can increase the stability of mRNA, increase the half-life of the mRNA, and could increase the mRNA translational efficiency. In some aspects, the modified 5′ cap comprises one or more of the following modifications: modification at the 2′ and/or 3′ position of a capped guanosine triphosphate (GTP), a replacement of the sugar ring oxygen (that produced the carbocyclic ring) with a methylene moiety (CH2), a modification at the triphosphate bridge moiety of the cap structure, or a modification at the nucleobase (G) moiety.

[0208] The 5′ cap structure that can be modified includes, but is not limited to, the caps described in U.S. Application No. 2014/0147454 and WO2018/160540 which is incorporated herein by reference in its entirety.

Linker

[0209] In some aspects, a polynucleotide provided herein comprises a nucleotide sequence encoding a linker. In some aspects, the linker is located within the region encoding a payload. In some aspects, the linker is located between two IL-12 polypeptides . In some aspects, the linker is from about 5 to about 50 amino acids in length. In some aspects, the linker is from about 8 to about 40 amino acids in length. In some aspects, the linker is from about 5 to about 15 amino acids in length. In some aspects, the linker is a flexible linker. In some aspects, the linker comprises 10 to 25 amino acids. In some aspects, the linker is composed of glycine and serine. In some aspects, the linker is composed of glycine, serine, and other amino acids. In some aspects, the linker is (GS)n, (G2S)n, (G3S)n, or (G4S)n, wherein n is 1-100, e.g., 1-20, 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10.

Poly-A Tails

[0210] In some aspects, a polynucleotide provided herein comprises a poly-A tail.

[0211] In some aspects, the length of the poly-A tail is greater than about 30 nucleotides in length. In some aspects, the poly-A tail is greater than about 35 nucleotides in length. In some aspects, the length is at least about 40 nucleotides. In some aspects, the length is at least about 45 nucleotides. In some aspects, the length is at least about 55 nucleotides. In some aspects, the length is at least about 60 nucleotides. In some aspects, the length is at least 70 nucleotides. In some aspects, the length is at least about 80 nucleotides. In some aspects, the length is at least about 90 nucleotides. In some aspects, the length is at least about 100 nucleotides. In some aspects, the length is at least about 120 nucleotides. In some aspects, the length is at least about 140 nucleotides. In some aspects, the length is at least about 160 nucleotides. In some aspects, the length is at least about 180 nucleotides. In some aspects, the length is at least about 200 nucleotides. In some aspects, the length is at least about 250 nucleotides. In some aspects, the length is at least about 300 nucleotides. In some aspects, the length is at least about 350 nucleotides. In some aspects, the length is at least about 400 nucleotides. In some aspects, the length is at least about 450 nucleotides. In some aspects, the length is at least about 500 nucleotides. In some aspects, the length is at least about 600 nucleotides. In some aspects, the length is at least about 700 nucleotides. In some aspects, the length is at least about 800 nucleotides. In some aspects, the length is at least about 900 nucleotides. In some aspects, the length is at least about 1000 nucleotides. In some aspects, the length is at least about 1100 nucleotides. In some aspects, the length is at least about 1200 nucleotides. In some aspects, the length is at least about 1300 nucleotides. In some aspects, the length is at least about 1400 nucleotides. In some aspects, the length is at least about 1500 nucleotides. In some aspects, the length is at least about 1600 nucleotides. In some aspects, the length is at least about 1700 nucleotides. In some aspects, the length is at least about 1800 nucleotides. In some aspects, the length is at least about 1900 nucleotides. In some aspects, the length is at least about 2000 nucleotides. In some aspects, the length is at least about 2500 nucleotides. In some aspects, the length is at least about 3000 nucleotides.

[0212] In some aspects, the poly-A tail comprises a polyA-G quartet. The G-quartet is a cyclic hydrogen bonded array of four guanine nucleotides that can be formed by G-rich sequences in both DNA and RNA. In some aspects, the G-quartet is incorporated at the end of the poly-A tail. The resultant nucleic acid or mRNA can be assayed for stability, protein production and other parameters including half-life at various time points. It has been discovered that the polyA-G

quartet results in protein production equivalent to at least 75% of that seen using a poly-A tail of 120 nucleotides alone.

Modified Nucleosides

[0213] In some aspects, a polynucleotide provided herein comprises one or more modified nucleosides. In some aspects, the one or more modified nucleosides comprises 6-aza-cytidine, 2-thio-cytidine, α-thio-cytidine, pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, α-thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, pseudo-uridine, inosine, α-thio-guanosine, 8-oxo-guanosine, O6-methyl-guanosine, 7-deaza-guanosine, N1-methyl adenosine, 2-amino-6-chloro-purine, N6-methyl-2-amino-purine, 6-chloro-purine, N6-methyl-adenosine, α-thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine, pyrrolo-cytidine, 5-methyl-cytidine, N4-acetyl-cytidine, 5-methyl-uridine, 5-iodo-cytidine, and combinations thereof.

[0214] In some aspects, a polynucleotide provided herein comprises one or more uridines which have been replaced by a modified nucleoside. In some aspects, the modified nucleoside replacing uridine is pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ) or 5-methyl-uridine (m5U).

[0215] In some aspects, a polynucleotide provided herein further comprises a spacer sequence. Accordingly, in some aspects, a polynucleotide provided herein comprises (a) a first region encoding a payload, (b) a second region comprising a detargeting sensor, *e.g.*, a miR-22 sensor or a miR-185 sensor), and (c) a spacer sequence. In some aspects, the spacer sequence comprises the nucleotide sequence GCGGCCGCTAAA (SEQ ID NO: 10). In some aspects, the spacer sequence consists essentially of the nucleotide sequence GCGGCCGCTAAA (SEQ ID NO: 10). In some aspects, the spacer sequence consists of the nucleotide sequence GCGGCCGCTAAA (SEQ ID NO: 10). In some aspects, the spacer sequence is positioned upstream of the second region comprising a detargeting sensor. In some aspects, the spacer sequence is positioned in between the first region encoding a payload and the second region comprising a detargeting sensor.

III. Synthetic Circuits

[0216] Some aspects of the present disclosure relate to genetic circuits that comprise any of the polynucleotides described herein. As used herein, the term "genetic circuit" refers to a controllable gene expression system. As described herein, a genetic circuit useful for the present disclosure comprises a synthetic genetic circuit ("synthetic circuit"). As used herein, the term "synthetic circuit" refers to an engineered, non-natural genetic circuit. As is apparent from the

present disclosure, synthetic circuits described herein have been specifically programmed to reduce or inhibit the expression of a payload within the detargeting cell of a subject.

[0217] In some aspects, provided herein is a synthetic circuit comprising an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor (*e.g.*, immune cell, cardiomyocyte, or endothelial cell detargeting sensor), wherein the recognition of the marker by the sensor reduces or inhibits the expression of the encoded payload. In some aspects, the synthetic circuit consists essentially of an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor (*e.g.*, immune cell, cardiomyocyte, or endothelial cell detargeting sensor), wherein the recognition of the marker by the sensor reduces or inhibits the expression of the encoded payload. In some aspects, the synthetic circuit consists of an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor (*e.g.*, immune cell, cardiomyocyte, or endothelial cell detargeting sensor), wherein the recognition of the marker by the sensor reduces or inhibits the expression of the encoded payload. Accordingly, in some aspects, a synthetic circuit useful for the present disclosure is single stranded.

[0218] As provided elsewhere in the present disclosure, in some aspects, a marker that is expressed in a detargeting cell and thereby, is bound by or recognized by a detargeting sensor, comprises a microRNA. In some aspects, a synthetic circuit described herein comprises an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor, which is capable of recognizing a microRNA expressed in a detargeting cell. In some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof. In some aspects, the payload is IL-12. In some aspects, the microRNA comprises miR-142-3p. Accordingly, in some aspects, a synthetic circuit described herein comprises an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising a detargeting sensor, which is capable of recognizing a miR-142-3p. As is apparent from the present disclosure, the recognition of the miR-142-3p by the detargeting sensor can result in reduced expression of the IL-12 protein by the detargeting cell.

[0219] In some aspects, the microRNA comprises miR-22-3p and optionally miR-142-3p. Accordingly, in some aspects, a synthetic circuit described herein comprises an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising a detargeting sensor, which is capable of recognizing a miR-22-3p and

optionally miR-142-3p. As is apparent from the present disclosure, the recognition of the miR-22-3p and optionally miR-142-3p by the detargeting sensor can result in reduced expression of the IL-12 protein by the detargeting cell.

[0220] In some aspects, the microRNA comprises miR-185-5p and optionally miR-142-3p. Accordingly, in some aspects, a synthetic circuit described herein comprises an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising a detargeting sensor, which is capable of recognizing a miR-185-5p and optionally miR-142-3p. As is apparent from the present disclosure, the recognition of the miR-185-5p and optionally miR-142-3p by the detargeting sensor can result in reduced expression of the IL-12 protein by the detargeting cell.

[0221] In some aspects, the microRNA comprises miR-142-3p, miR-22-3p, miR-185-5p, or any combination thereof. Accordingly, in some aspects, a synthetic circuit described herein comprises an isolated polynucleotide, which comprises (a) a first region encoding an IL-12 protein and (b) a second region comprising a detargeting sensor, which is capable of recognizing miR-142-3p, miR-22-3p, miR-185-5p, or any combination thereof. As is apparent from the present disclosure, the recognition of the miR-142-3p, miR-22-3p, miR-185-5p, or any combination thereof by the detargeting sensor can result in reduced expression of the IL-12 protein by the detargeting cell.

III. Replicons

[0222] Some aspects of the present disclosure relate to a replicon comprising any of the polynucleotides described herein. As used herein, the term "**replicon**" refers to a viral nucleic acid that is capable of directing the generation of copies of itself. Unless indicated otherwise, a replicon comprises a RNA or a DNA. Accordingly, in some aspects, a replicon useful for the present disclosure comprises an isolated polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor, wherein the recognition of the marker by the sensor reduces the expression of the payload.

[0223] In some aspects, a replicon useful for the present disclosure is derived from an alpha virus. Alphaviruses comprise a set of genetically, structurally, and serologically related arthropod-borne viruses of the Togaviridae family. Twenty-six known viruses and virus subtypes have been classified within the alphavirus genus, including, Sindbis virus (SIN), Semliki Forest virus (SFV), Ross River virus (RRV), and Venezuelan equine encephalitis virus (VEE). In some aspects, the replicon is derived from VEE. Accordingly, some aspects of the present disclosure relate to a VEE

replicon comprising any of the polynucleotides described herein. For instance, in some aspects, a VEE replicon provided herein comprises a polynucleotide, which comprises (a) a first region encoding a payload and (b) a second region comprising a detargeting sensor, wherein the recognition of the marker by the sensor reduces the expression of the payload.

[0224] The VEE virus is a viral pathogen typically carried by mosquitos that causes VEE or encephalomyelitis predominately in equine species. Humans, however, can also contract VEE, and people with weakened immune systems are especially at risk of having severe complications if infected with VEE. The virion of VEE is spherical and possesses a lipid membrane with glycoprotein surface proteins spread around the outer surface. VEE has a genome of approximately 11.45 kb, excluding the 5′-terminal cap and 3′-terminal poly(A) tract, and comprises four nonstructural proteins (nsPs) and five structural proteins. The non-structural proteins include nsP1, nsP2, nsP3, and nsP4, while the structural region encodes proteins C, E3, E2, 6K, and E1. In some aspects, the self-amplifying replicon RNA is a WT replicon RNA derived from VEE. Exemplary VEE replicon sequences are provided in Tables 2 and 3.

[0225] In some aspects, the VEE replicon useful for the present disclosure includes any VEE replicons known in the art. In some aspects, the VEE replicon comprises one or more mutations. For instance, in some aspects, the VEE replicon comprises mutations with the non-structural proteins nsP2 and nsP3, wherein the mutations promote subgenomic expression in human cells. In some aspects, the VEE replicon comprises mutations that allow for greater expression of the encoded payload compared to a corresponding VEE replicon without the mutations. In some aspects, the VEE replicon comprises at least one point mutation in a nucleic acid position 3936 and/or 4758 of any one of the sequences provided in Table 2. In some aspects, the VEE replicon comprises at least one of the following point mutations: guanine to cytosine at position 3936 (G3936C) and adenine to guanine at position 4758 (A4758G) of any one of the sequences provided in Table 2. The G3936C mutation would result in a glycine to arginine change at amino acid residue 1309 (G1309R). The A4758G mutation would result in a serine to glycine change at amino acid residue 1583 (S1583G). Additional disclosure regarding such VEE replicons is provided, *e.g.*, in US20200281994A1, which is incorporated herein by reference in its entirety.

Exemplary Replicons

[0226] Also provided herein is a replicon comprising any of the isolated polynucleotides or the synthetic circuits described herein, wherein the replicon is self-replicating. In some aspects,

the replicon is derived from an alpha virus. In some aspects, the alpha virus comprises a Venezuelan equine encephalitis (VEE) virus.

[0227] In some aspects, the replicon disclosed herein comprises a VEE genomic 5'UTR (e.g., SEQ ID NO: 22), VEE non-structural proteins (nsPs, e.g., nucleotides 45-7,523 of the sequence set forth in SEQ ID NO: 12), a subgenomic promoter (e.g., SEQ ID NO: 23), a VEE subgenomic 5'UTR (e.g., SEQ ID NO: 19), a Kozak sequence upstream of a start codon (e.g.,GCCACCATG), a sequence that encodes a payload (e.g., human IL-12 (e.g., SEQ ID NO: 17), a stop codon, a spacer (e.g., SEQ ID NO: 10), a detargeting sensor comprising at least one miR-142 sensor (e.g., SEQ ID NO: 3) and at least one miR-185 sensor (e.g., SEQ ID NO: 9), a 3'UTR (e.g., SEQ ID NO: 20), a PolyA tail (e.g., SEQ ID NO: 21), or any combinations thereof.

[0228] In some aspects, the polynucleotidereplicon disclosed herein comprises, from 5' to 3', a VEE genomic 5'UTR (e.g., SEQ ID NO: 22), VEE non-structural proteins (nsPs, e.g., nucleotides 45-7,523 of the sequence set forth in SEQ ID NO: 12), a subgenomic promoter (e.g., SEQ ID NO: 23), a VEE subgenomic 5'UTR (e.g., SEQ ID NO: 19), a Kozak sequence upstream of a start codon (e.g., GCCACCATG), a sequence that encodes a payload (e.g., cytokine such as human IL-12) (e.g., SEQ ID NO: 17), a stop codon, a spacer (e.g., SEQ ID NO: 10), four tandem miR-142 sensors (e.g., comprising SEQ ID NO: 3), four tandem miR-185 sensors (e.g., comprising SEQ ID NO: 9), a 3'UTR (e.g., SEQ ID NO: 20), and a PolyA tail (e.g., SEQ ID NO: 21).

[0229] In some aspects, the replicon disclosed herein comprises, from 5' to 3',

(1) a genomic promoter comprising a nucleotide sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 22;

(2) non-structural proteins nsP1, nsP2, nsP3, and/or nsP4 (nucleotides 45-7,523 of the sequence set forth in SEQ ID NO: 12);

(3) a subgenomic promoter comprising a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence set forth in SEQ ID NO: 23;

(4) a subgenomic 5'UTR comprising a nucleotide sequence having at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about

93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence set forth in SEQ ID NO: 19;

(5) a Kozak sequence optionally comprising a sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the following sequence: GCCACCATG;

(6) a coding sequence for a cytokine, optionally a sequence encoding human IL-12 (including a N-terminal signal peptide) comprising a nucleotide sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to SEQ ID NO: 17;

(7) a spacer, optionally comprising SEQ ID NO: 10;

(8) a detargeting sensor comprising 1, 2, 3, or 4 tandem first sensors and 1, 2, 3, or 4 tandem second sensors, optionally wherein each of the first sensors comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 6; and optionally wherein each of the second sensors comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 9, further optionally wherein the detargeting sensor comprises four tandem first sensors and four tandem second sensors, each first sensor comprising the sequence of SEQ ID NO: 6 and each second sensor comprising the sequence of SEQ ID NO: 9;

(9) 3' UTR comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence set forth in SEQ ID NO: 20; and

(10) a poly-A tail comprising at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about

94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% sequence identity to the sequence set forth in SEQ ID NO: 21.

[0230] In some aspects, the replicon disclosed herein comprises the sequence set forth in SEQ ID NO: 12. In some aspects, the replicon disclosed herein consists essentially of the sequence set forth in SEQ ID NO: 12. In some aspects, the replicon disclosed herein consists of the sequence set forth in SEQ ID NO: 12.

[0231] Additional Exemplary VEE replicon sequences are provided in Tables 2 and 3.

IV. Nanoparticles

[0232] In some aspects, the present disclosure relates to the delivery of a polynucleotide described herein (*e.g.*, encoding a payload and comprising a detargeting sensor), such that the expression of the payload is reduced or inhibited in a detargeting cell. In some aspects, the delivery can occur *in vivo* (*e.g.*, by administering a polynucleotide described herein to a subject) or *ex vivo* (*e.g.*, by culturing a polynucleotide described herein with the cells *in vitro*). In some aspects, delivery of a polynucleotide described herein can be performed using any suitable delivery system known in the art. In certain aspects, the delivery system is a vector. Accordingly, in some aspects, the present disclosure provides a vector comprising any of the polynucleotides described herein. Suitable vectors that can be used are known in the art. *See, e.g.*, Sung *et al.*, *Biomater Res* 23(8) (2019) which is incorporated herein by reference in its entirety.

[0233] In some aspects, a polynucleotide described herein is delivered using a nanoparticle (*e.g.*, lipid nanoparticle or lipid like nanoparticle). Accordingly, in some aspects, the present disclosure relates to a polynucleotide (*e.g.*, described herein) encapsulated within a nanoparticle, a composition comprising such a nanoparticle, and the use of such a nanoparticle. More specifically, in some aspects, provided herein is a nanoparticle comprising (i) any of the polynucleotides described herein and (ii) one or more types of nanoparticle components.

IV.A. Nanoparticle (NP)

[0234] A "nanoparticle" (NP), as used herein, refers to a particle, such as a vesicle, having characteristic dimensions measured in nanometers (nm). Nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations. Non-limiting examples of NPs include lipid nanoparticles (LNPs), lipid-like nanoparticles (LLNs), polymeric nanoparticles (PNPs), and inorganic nanoparticles.

IV.B. Lipid Nanoparticle (LNP)

[0235] A "lipid nanoparticle" (LNP), as used herein, refers to a nanoparticle composed of lipids. Lipid nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations. Non-limiting examples of LNPs include cationic lipid nanoparticles, ionizable lipid nanoparticles, liposomes, bolaamphihiles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and monolayer membrane structures (*e.g.,* archaeosomes and micelles).

[0236] As used herein a "cationic lipid nanoparticle" refers to a nanoparticle comprising a cationic lipid. As used herein an "ionizable lipid nanoparticle" refers to a nanoparticle comprising an ionizable lipid. In aspects of the disclosure, LNPs comprise one or more of the following lipids: a "non-cationic helper lipid," a "phospholipid," a "sterol other structural lipid," and a "PEG/pegylated lipid."

[0237] Exemplary LNPs comprise one or more of the following components: (i) an ionizable/cationic lipid; (ii) phospholipid or a non-cationic helper lipid; (iii) a sterol or other structural lipid;(iv) a PEG/PEGylated lipid and (v) a targeted delivery molecule (lipid composition/targeting ligand).

IV.C. Lipid Like Nanoparticle (LLN)

[0238] A "lipid like nanoparticle" (LLN), as used herein, refers to a nanoparticle comprising a lipid, and a lipid-like material or a lipidoid, as described herein. In aspects of the disclosure, LLNs comprise one or more of the following lipids: a "non-cationic helper lipid," a "phospholipid," a "sterol other structural lipid", and a "PEG/PEGylated lipid." Lipid like nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations.

[0239] Exemplary LLNs comprise one or more of the following components: (i) an ionizable/cationic lipid-like material or lipidoid; (ii) phospholipid or a non-cationic helper lipid; (iii) a sterol or other structural lipid; (iv) a PEG/PEGylated lipid; and (v) a targeted delivery molecule (lipid composition/targeting ligand).

IV.D. Lipids or Lipid-Like Material

Ionizable lipid

[0240] Non-limiting examples of ionizable lipids include: Non-limiting examples of ionizable lipids include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315), heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102), heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Lipid

5), di((*Z*)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), 9Z,12Z-octadecadienoic acid, 3-[4,4-bis(octyloxy)-1-oxobutoxy]-2-[[[[3-(diethylamino)propoxy]carbonyl]oxy]methyl]propyl ester (LP-01), 5-(dimethylamino)-pentanoic acid, (6Z)-1,2-di-(4Z)-4-decen-1-yl-6-dodecen-1-yl ester (CL-1), 2-(((4-(dimethylamino)butanoyl)oxy)methyl)-2-((((Z)-tetradec-9-enoyl)oxy)methyl)propane-1,3-diyl (9Z,9'Z)-bis(tetradec-9-enoate) (TCL053), 3-(didodecylamino)-N1,N1,4 tridodecyl-1-piperazineethanamine (KL10), Nl-[2 (didodecylamino)ethyl]-N1,N4,N4-tridodecyl 1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2 dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), or any combination thereof.

Cationic lipid

[0241] Non-limiting examples of a cationic lipid include: l,2-dioleoyl-3 -trimethylammonium-propane (DOTAP), lipofectamine, N-[l-(2,3- dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2- (oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTEVI), 2,3- dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l ,2-dimyristyloxyprop-3 -yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DMRIE), N-(l,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), l,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), l,2-distearoyl-3-trimethylammonium-propane (DSTAP), l,2-dipalmitoyl-3 -trimethylammonium-propane (DPTAP), l,2-dilinoleoyl-3 -trimethylammonium-propane (DLTAP), l,2-dimyristoyl-3-trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3- ethylphosphocholine (DSePC), l,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn- glycero-3-ethylphosphocholine

(DOePC), 1,2-di-(9Z-tetradecenoyl)-sn-glycero-3- ethylphosphocholine (14: 1 EPC), l-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18: 1 EPC), or any combination thereof.

[0242] In some aspects of the disclosure, LNPs primarily comprise cationic lipids along with other lipid ingredients. These typically include other lipid molecules belonging but not limited to the phophatidylcholine (PC) class (*e.g.,* 1,s-Distearoyl-sn-glycero-3-phophocholine (DSPC), and 1,2-Dioleoyl-sn-glycero-3-phophoethanolamine (DOPE), sterols (*e.g.,* cholesterol) and Polyethylene glycol (PEG)-lipid conjugates (*e.g.,* 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000 (DSPE-PEG2000), and C14-PEG2000.

DOTAP

[0243] In aspects of the disclosure, the catonic lipid is DOTAP. DOTAP can be used for the highly efficient transfection of DNA including yeast artificial chromosomes (YACs) into eukaryotic cells for transient or stable gene expression, and is also suitable for the efficient transfer of other negatively charged molecules, such as RNA, oligonucleotides, nucleotides, ribonucleoprotein (RNP) complexes, and proteins into research samples of mammalian cells.

Lipofectamine

[0244] In aspects of the disclosure, the catonic lipid is lipofectamine. Lipofectamine, as used herein, is a common transfection reagent, produced and sold by Invitrogen, used in molecular and cellular biology. It is used to increase the transfection efficiency of RNA (including mRNA and siRNA) or plasmid DNA into in vitro cell cultures by lipofection. Lipofectamine contains lipid subunits that can form liposomes or lipid nanoparticles in an aqueous environment, which entrap the transfection payload, *e.g.,* modRNA. The RNA-containing liposomes (positively charged on their surface) can fuse with the negatively charged plasma membrane of living cells, due to the neutral co-lipid mediating fusion of the liposome with the cell membrane, allowing nucleic acid cargo molecules to cross into the cytoplasm for replication or expression.

Lipid-like material or lipidoid

[0245] Unless indicated otherwise, "lipid-like material" and "lipidoid" can be used interchangeably. Non-limiting examples of lipid-like materials and/or lipidoids include: 1,1′-((2-(4-(2-((2-(bis(2-hydroxydodecyl) amino)ethyl) (2- hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200), 3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine2,5-dione (cKK-E12), 1,1′-[[2-[2-[4-[2-[[2-[2-[bis(2-hydroxytetradecyl)amino]ethoxy]ethyl](2-hydroxytetradecyl)amino]ethyl]-1-piperazinyl]ethoxy]ethyl]imino]bis-2-tetradecanol (C14-4), tetrakis(8-methylnonyl) 3,3′,3″,3‴-

(((methylazanediyl) bis(propane-3,1 diyl))bis (azanetriyl))tetrapropionate (306Oi$_{10}$), G0-C14, 3,3',3",3'"-(ethane-1,2-diylbis(azanetriyl))tetrakis(N-(2-(bis(2-hydroxytetradecyl)amino)ethyl)propanamide) (G0-C14 analog), 5A2-SC8, 4A3-SC8, 3,6-bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02), (((3,6-dioxopiperazine-2,5-diyl)bis (butane-4,1-diyl))bis(azanetriyl))tetrakis(ethane2,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin), (((3,6-dioxopiperazine-2,5-diyl)bis(butane-4,1-diyl)) bis(azanetriyl))tetrakis (butane-4,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-C4-Deg-Lin), 1,3,5-tris[2-[(2-hydroxydodecyl)methylamino]ethyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TNT-b10), N1,N3,N5-tris(3-(didodecylamino)propyl)benzene1,3,5-tricarboxamide (TT3), Hexa(octan-3-yl) 9,9′,9″,9‴,9⁗,9⁗′- ((((benzene-1,3,5-tricarbonyl)ris(azanediyl)) tris (propane-3,1-diyl))tris(azanetriyl))hexanonanoate (FTT5), PL-1 [disclosed in Nature Communications, 12-7264 (2021), which is is hereby incorporated by reference], 98N12-5 [disclosed in Molecular Therapy vol. 17 no. 5 May 2009, which is hereby incorporated by reference], ethyl 5,5-di((Z)-heptadec-8-en-1-yl)-1-(3-(pyrrolidin-1-yl)propyl)-2,5-dihydro-1H-imidazole-2-carboxylate (A2-Iso5-2DC18 (A2)) and A12-Iso5−2DC18 (A12), or any combination thereof.

[0246] TT3, as used herein, is capable of forming nanoparticles for delivery of various biologic active agents into the cells. In addition, the present disclosure also demonstrates that an unloaded TT3-LLN can induce immunogenic cell death (ICD) in cancer cells *in vivo* and *in vitro*. Immunogenic cell death, as described herein, refers to a form of cell death that can induce an effective immune response through activation of dendritic cells (DCs) and consequent activation of specific T cell response. In some aspects of the disclosure, the cells that undergo immunogenic cell death are tumor cells. Immunogenic tumor cell death can trigger an effective anti-tumor immune response.

[0247] In some aspects, the lipidoid comprises TT3. In some aspects, the lipidoid comprises FTT5 (Formal Name: 9,9′,9″,9‴,9⁗,9⁗′-[1,3,5-benzenetriyltris(carbonylimino-3,1-propanediylnitrilo)]hexakis-nonanoic acid, 1,1′,1″,1‴,1⁗,1⁗′-hexakis(1-ethylhexyl) ester), which can also be referred to as Functionalized N1,N3,N5-tris(2-aminoethyl)benzene-1,3,5-Tricarboxamide 5.

Phospholipid, or other non-cationic helper lipid

[0248] Unless indicated otherwise, "phospholipid" and "other non-cationic helper lipid" can be used interchangeably and non-limiting examples include: 1,2-dilinoleoyl-sn-glycero-3 phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn

glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, and any combinations thereof.

[0249] In some aspects, the phospholipid is selected from the group consisting of 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (14:0-16:0 PC, MPPC), 1-myristoyl-2 stearoyl-sn-glycero-3-phosphocholine (14:0-18:0 PC, MSPC), 1-palmitoyl 2-acetyl-sn-glycero-3-phosphocholine (16:0-02:0 PC), 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine (16:0-14:0 PC, PMPC), 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (16:0-18:0 PC, PSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18: 1 PC, POPC), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (16:0-18:2 PC, PLPC), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (16:0-20:4 PC), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (14:0-22:6 PC), 1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholine (18:0-14:0 PC, SMPC), 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18:0-16:0 PC, SPPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (18:0-18: 1 PC, SOPC), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (18:0-18:2 PC), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (18:0-20:4 PC), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (18:0-22:6 PC), 1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholine (18: 1-14:0 PC, OMPC), 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18: 1-16:0 PC, OPPC), 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (18: 1-18:0 PC, OSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (16:0- 18: 1 PE, POPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (16:0-18:2 PE), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (16:0-20:4 PE), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (16:0-22:6 PE), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (18:0-18: 1 PE), 1-stearoyl-2-linoleoyl-sn-glycero-3-

phosphoethanolamine (18:0-18:2 PE), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (18:0-20:4 PE), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (18:0-22:6 PE), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), and any combination thereof.

[0250] In some aspects, the phospholipid is DSPC. In some aspects, the phospholipid is DOPE.

Sterol or other structural lipid

[0251] As used herein, a "**sterol or other structural lipid**," refers to cholesterol or cholesterol analogs that could be used to fill lipid membrane packing defects and provide structural integrity. Non-limiting examples of sterols include: a cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha- tocopherol, and combinations thereof. In some aspects, the sterol is cholesterol.

PEG/Pegylated lipid

[0252] As used herein, a "**PEG lipid**" and a "**pegylated lipid**" are used interchangeably.

[0253] Non-limiting examples of PEG lipids include: 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159), 1,2-dimyristoyl-sn-glycerol methoxypolyethylene glycol (PEG-DMG), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (PEG-DSPE), PEG-disteryl glycerol (PEG-DSG), PEG-dipalmetoleyl, PEG-dioleyl, PEG-distearyl, PEG-diacylglycamide (PEG-DAG), PEG-dipalmitoyl phosphatidylethanolamine (PEG-DPPE), or PEG-1,2-dimyristyloxlpropyl-3-amine (PEG-c-DMA). In some aspects, the lipid moiety of the PEG-lipids includes those having lengths of from about C14 to about C22.

[0254] In some aspects, the PEG moiety has a size of about 1000, 2000, 5000, 10,000, 15,000 or 20,000 daltons. In certain aspects, C14-PEG2000 comprises 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DMPE-PEG2000), or both.

[0255] In some aspects, the PEG-lipids can be embedded in the LNP prior to the encapsulation of the polynucleotide. In some aspects, the PEG lipids (or other lipid ingredients disclosed herein) can be added to the LNP after the encapsulation of the polynucleotide. For example, in some aspects, a polynucleotide described herein is encapsulated in the LNP, and then the PEG lipid (other lipid ingredients disclosed herein) is attached to the LNP using, *e.g.*, micelles.

[0256] In some aspects, the nanoparticle does not comprise any pegylated lipid. In some aspects, the lipid nanoparticle does not comprise any pegylated lipid.

Targeted delivery molecule (lipid composition/targeting ligand)

[0257] In aspects of the disclosure, nanoparticles, (*e.g.*, LNPs and LLNs), as described herein, comprise a targeted delivery molecule (lipid composition/targeting ligand). As used herein, a "targeted delivery molecule (lipid composition/targeting ligand)," and a "targeted delivery molecule" are used interchangeably and in some aspects, the targeted delivery molecule could be an additional lipid or lipid like component, as described herein. In some aspects, the targeted delivery molecule could change the overall charge of the nanoparticles. In some aspects, the targeted delivery molecule could be a non-covalently or covalently bound ligand to the nanoparticle. In some aspects, the targeted delivery ligand could be a small molecule or a large molecule.

[0258] Non-limiting examples of targeted delivery molecules are disclosed in Pharmaceuticals (Basel). Jul 20;15(7):897. (2022), Nat Rev Drug Discov 20, 101–124 (2021), and Advanced Drug Delivery Reviews, Volume 188, (2022), which are hereby incorporated by reference. Non-limiting examples of targeted delivery molecules include: 1,2-dioleoyl-3 - trimethylammonium-propane (DOTAP), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), 1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt) (18:1 PA), 1,2-dimyristoyl-sn-glycero-3-phosphate (sodium salt) (14:0 PA), bis(monooleoylglycero)phosphate (S,R Isomer) (ammonium salt) (18BMP), 2-((2,3-bis(oleoyloxy)propyl)dimethylammonio)ethyl ethyl phosphate (DOCPe), folic acid, N-acetylgalactosamine (GalNAc), anti-CD3 antibodies.

Molar Ratios

[0259] In some aspects, a nanoparticle described herein comprises a lipid (*e.g.*, an ionizable lipid, a cationic lipid, a non-cationic helper lipid, a phospholipid, a sterol or other structural lipid, or PEG lipid) and/or lipidoid, as described herein at a molar ratio of about 10% to about 50% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 10%, about 20%, about 30%, about 40%, or about 50% in the lipid composition.

[0260] In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 10% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 20% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 30% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a lipid and/or lipidoid, as described herein, at a molar ratio of about 40% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a

lipid and/or lipidoid, as described herein, at a molar ratio of about 40% in the lipid and/or lipid like composition.

[0261] In some aspects, a nanoparticle described herein comprises a pegylated lipid at a molar ratio of about 0% to about 10% in the lipid and/or lipid like composition. In some aspects, a lipid nanoparticle comprises a pegylated lipid at a molar ratio of about 0%, about 0.25%, about 0.5%, about 0.75%, about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, or about 10% in the lipid and/or lipid like composition.

[0262] In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 0.25% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 0.5% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 0.75% in lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 1.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 2.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 3.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 4.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 5.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 6.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 7.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 8.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 9.0% in the lipid and/or lipid like composition. In some aspects, a nanoparticle provided herein comprises a pegylated lipid at a molar ratio of about 10.0% in the lipid and/or lipid like composition.

[0263] For example, in some aspects, the pegylated lipid comprises C14-PEG2000. In some aspects, the C14-PEG2000 is present in the lipid nanoparticle at a molar ratio of about 0.25% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 0.5% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 0.75% in the lipid and/or lipid like composition. In some aspects,

the C14-PEG2000 is present at a molar ratio of about 1% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 2% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 3% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 4% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 5% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 6% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 7% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 8% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 9% in the lipid and/or lipid like composition. In some aspects, the C14-PEG2000 is present at a molar ratio of about 10% in the lipid and/or lipid like composition.

Particle Size

[0264] Particle size of nanoparticles can affect drug release rate, bio-distribution, mucoadhesion, cellular uptake of water and buffer exchange to the interior of the nanoparticles, and protein diffusion. In some aspects of the disclosure, the diameter of the NPs ranges from about 30 to about 500 nm. In some aspects of the disclosure, the diameter of the NPs ranges from about 30 to about 500 nm, about 50 to about 400 nm, about 70 to about 300 nm, about 100 to about 200 nm, about 100 to about 175 nm, or about 100 to about 160 nm. In some aspects of the disclosure, the diameter of the NPs ranges from 100-160 nm. In some aspects of the disclosure, the diameter of the NPs can be about 30 nm, about 40 nm, about 50 nm, about 60 nm, about 70 nm, about 80 nm, about 90 nm, about 100 nm, about 101 nm, about 102 nm, about 103 nm, about 104 nm, about 105 nm, about 106 nm, about 107 nm, about 108 nm, about 109 nm, about 110 nm, about 111 nm, about 112 nm, about 113 nm, about 114 nm, about 115 nm, about 116 nm, about 117 nm, about 118 nm, about 119 nm, about 120 nm., about 130 nm, about 140 nm, about 150 nm, or about 160 nm. In certain aspects, the lipid nanoparticle has a diameter of about 140 nm.

Zeta Potential

[0265] As used herein, "**zeta potential**" refers to the measure of the effective electric charge on the nanoparticle surface. The magnitude of the zeta potential provides information about particle stability. In some aspects of the disclosure, the zeta potential of the nanoparticles ranges from about -20 to about 20 mv. In some aspects of the disclosure, the zeta potential of the NPs can be about -6.0 mv, about -5.9 mv, about -5.8 mv, about -5.7 mv, about -5.6 mv, about -5.5 mv, about -5.4 mv, about -5.3 mv, about -5.2 mv, about -5.1 mv, about -5.0 mv, about -4.9 mv, about -4.8

mv, about -4.7 mv, about -4.6 mv, about -4.5 mv, about -4.4 mv, about -4.3 mv, about -4.2 mv, about -4.1 mv, about -4.0 mv, about -3.9 mv, about -3.8 mv, about -3.7 mv, about -3.6 mv, about -3.5 mv, about -3.4 mv, about -3.3 mv, about -3.2 mv, about -3.1 mv, about -3.0 mv, about -2.9 mv, about -2.8 mv, about -2.7 mv, about -2.6 mv, about -2.5 mv, about -2.4 mv, about -2.3 mv, about -2.2 mv, about -2.1 mv, about -2.0 mv, about -1.9 mv, about -1.8 mv, about -1.7 mv, about -1.6 mv, about -1.5 mv, about -1.4 mv, about -1.3 mv, about -1.2 mv, about -1.1 mv, about -1.0 mv, about -0.9 mv, about -0.8 mv, about -0.7 mv, about -0.6 mv, about -0.5 mv, about -0.4 mv, about -0.3 mv, about -0.2 mv, about -0.1 mv, about 0.0 mv, 0.1 about mv, about 0.2 mv, about 0.3 mv, about 0.4 mv, about 0.5 mv, about 0.6 mv, about 0.7 mv, about 0.8 mv, about 0.9 mv, about 1.0 mv, about 1.1 mv, about 1.2 mv, about 1.3 mv, about 1.4 mv, about 1.5 mv, about 1.6 mv, about 1.7 mv, about 1.8 mv, about 1.9 mv, about 2.0 mv, about 2.1 mv, about 2.2 mv, about 2.3 mv, about 2.4 mv, about 2.5 mv, about 2.6 mv, about 2.7 mv, about 2.8 mv, about 2.9 mv, about 3.0 mv, about 3.1 mv, about 3.2 mv, about 3.3 mv, about 3.4 mv, about 3.5 mv, about 3.6 mv, about 3.7 mv, about 3.8 mv, about 3.9 mv, about 4.0 mv, about 4.1 mv, about 4.2 mv, about 4.3 mv, about 4.4 mv, about 4.5 mv, about 4.6 mv, about 4.7 mv, about 4.8 mv, about 4.9 mv, about 5.0 mv, about 5.1 mv, about 5.2 mv, about 5.3 mv, about 5.4 mv, about 5.5 mv, about 5.6 mv, about 5.7 mv, about 5.8 mv, about 5.9 mv, or about 6.0 mv.

Mass Ratio

[0266] In some aspects, the mass ratio between the lipid of the LNPs or the LLN and the polynucleotide ranges from about 1:2 to about 15:1. In some aspects, the mass ratio between the lipid and the polynucleotide can be about 1:2, about 1:1.9, about 1:1.8, about 1:1.7, about 1:1.6, about 1:1.5, about 1:1.4, about 1:1.3, about 1:1.2, about 1:1.1, about 1:1, about 1.1:1, about 1.2:1, about 1.3:1, about 1.4:1, about 1.5:1, about 1.6:1, about 1.7:1, about 1.8:1, about 1.9:1, about 2:1, about 2.5:1, about 3:1, about 3.5:1, about 4:1, about 4.5:1, about 5:1, about 5.5:1, about 6:1, about 6.5:1, about 7:1, about 7.5:1, about 8:1, about 8.5:1, about 9:1, about 9.5:1, about 10:1, about 10.5:1, about 11:1, about 11.5:1, about 12:1, about 12.5:1, about 13:1, about 13.5:1, about 14:1, about 14.5:1, or about 15:1. In some aspects of the disclosure, the mass ratio between the lipid and the polynucleotide is about 10:1.

V. Pharmaceutical Composition

[0267] In some aspects, the disclosure relates to a pharmaceutical composition comprising any of the polynucleotides, replicons, and/or nanoparticles described herein. In some aspects, the pharmaceutical composition further comprises a pharmaceutically acceptable carrier (excipient).

"Acceptable", as used herein, means that the carrier must be compatible with the active ingredient of the composition and not deleterious to the subject to be treated. In some aspects, the carrier is capable of stabilizing the active ingredient. Pharmaceutically acceptable excipients (carriers) include buffers, which are well known in the art. *See, e.g.*, Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkoins, Ed. K. E. Hoover.

[0268] The pharmaceutical compositions to be used for *in vivo* administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. The nanoparticles can be placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0269] In some aspects, the pharmaceutical composition can be formulated for intratumoral, intrathecal, intramuscular, intravenous, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration. In some aspects of the disclosure, the pharmaceutical composition can be formulated for intratumoral injection. Intratumoral injection, as used herein, refers to direct injections into the tumor. A high concentration of composition can be achieved in situ, while using small amounts of drugs. Local delivery of immunotherapies allows multiple combination therapies, while preventing significant system exposure and off-target toxicities.

[0270] In some aspects, the pharmaceutical composition can be formulated for intramuscular injection, intravenous injection, or subcutaneous injection.

[0271] In some aspects, the pharmaceutical composition comprises pharmaceutically acceptable carriers, buffer agents, excipients, salts, or stabilizers in the form of lyophilized formulations or aqueous solutions. *See, e.g.,* Remington: The Science and Practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover. Acceptable carriers and excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations used, and comprises buffers such as phosphate, citrate, and other organic acds; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl, or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine,

arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.,* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

[0272] In some aspects, the pharmaceutical composition described herein can be prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl. Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos 4,485,045 and 4,544,545, which are hereby incorporated by reference in their entirety. Liposomes with enhanced circulation time are disclosed in U.S. Pat. No. 5,013,556, which is hereby incorporated by reference in its entirety. In some aspects, liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[0273] In some aspects, the pharmaceutical composition is formulated in sustained-release format. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the nanoparticles which matrices are in the form of shaped articles, *e.g.*, films or microcapsules. Examples of sustained-release matrices include, but are not limited to, polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPROM DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[0274] In some aspects, suitable surface-active agents include, but are not limited to, non-ionic agents, such as polyoxyethylenesorbitans (*e.g.,* TWEEN™ 20, 40, 60, 80 or 85) and other sorbitans (*e.g.,* SPAN™ 20, 30, 60, 80, or 85). In some aspects, compositions with a surface-active agent comprise between 0.05 and 5% surface-active agent. In some aspects the composition comprises 0.1 and 2.5%. It will be appreciated that other ingredients can be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

[0275] In some aspects, the pharmaceutical composition is in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral, or rectal administration, or administration by inhalation or insufflation.

[0276] For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, *e.g.*, conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and other pharmaceutical diluents, *e.g.*, water, to form a solid preformulation composition containing a homogenous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition can be readily subdivided into equally effective unit dosage forms such as tablets, pills, and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from about 0.1 to about 500 mg of the active ingredient of the present disclosure. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[0277] Suitable emulsions can be prepared using commercially available fat emulsions, such as INTRALIPID™, LIPOSYN™, INFONUTROL™, LIPOFUNDIN™, and LIPIPHYSAN™. The active ingredient can be either dissolved in a pre-mixed emulsion composition or alternatively it can be dissolved in an oil (*e.g.*, soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil, or almond oil) and an emulsion formed upon mixing with a phospholipid (*e.g.*, egg phospholipids, soybean phospholipids, or soybean lecithin) and water. It will be appreciated that other ingredients can be added, for example glycerol or glucose, to adjust tonicity of the emulsion. Suitable emulsions will typically contain up to about 20% oil, for example, between about 5 and about 20%. The fat emulsion can comprise fat droplets having a suitable size and can have a pH in the range of about 5.5 to about 8.0.

[0278] Pharmaceutical compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions can contain suitable pharmaceutically acceptable excipients as set out above. In some aspects, the composition is administered by the oral or nasal respiratory route for local or systemic effect.

[0279] Compositions in pharmaceutically acceptable solvents can be nebulized by use of gases. Nebulized solutions can be breathed directly from the nebulizing device or the nebulizing device can be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions can be administered from devices which deliver the formulation in an appropriate manner.

VI. Therapeutic Applications

VI.A. Selective Expression

[0280] Some aspects of the present disclosure relate to methods of selectively reducing expression of a payload in a detargeting cell. As described herein, the polynucleotides described herein comprise a detargeting sensor, such that when the polynucleotides are administered to a subject, the expression of the encoded payload by the detargeting cell is reduced or inhibited.

[0281] Accordingly, some aspects of the present disclosure are related to a method of reducing or avoiding the expression of a payload within a detargeting cell of a subject in need thereof, comprising administering to the subject any of the isolated polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein. As described herein, in some aspects, the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof. In some aspects, provided herein is a method of reducing or avoiding the expression of a payload (payload expression) within an immune cell, a cardiomyocyte, and/or an endothelial cell of a subject in need thereof, comprising administering to the subject any of the isolated polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein.

[0282] In some aspects, the expression of the payload within detargeting cell is avoided entirely (*i.e.*, the payload is not expressed in the immune cell after the administration). In some aspects, the expression of the payload in the detargeting cell is reduced. For instance, in some aspects, after administration, the expression of the payload in the immune cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%.

[0283] As demonstrated herein, in some aspects, reduced expression of the payload within an immune cell can be associated with an overall decreased expression of the payload within the subject. Not to be bound by any one theory, in some aspects, by reducing the expression of the payload within a detargeting cell (e.g., an immune cell, a cardiomyocyte, and/or an endothelial

cell), the expression of the payload can be reduced. For example, as further described elsewhere in the present disclosure, in some aspects, a payload comprises an IL-12 protein. IL-12 is a secretory protein that when produced (*e.g.*, by immune cells within the spleen) is secreted into the periphery (*e.g.*, in the blood). Accordingly, it will be apparent to those skilled in the art that a reduced IL-12 expression within a detargeting cell (e.g., an immune cell, a cardiomyocyte, and/or an endothelial cell) can be associated with a reduced IL-12 expression in other tissues (*e.g.*, blood).

[0284] In some aspects, the reduced expression of the payload (*e.g.*, IL-12) can be associated with decreased toxicity. Accordingly, in some aspects, the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions allow for greater tolerability with reduced toxicity when administered to a subject. Toxicity and/or tolerability can be assessed by any suitable methods known in the art. In some aspects, toxicity and/or tolerability can be assessed by monitoring a subject's body weight after administration, wherein reduced toxicity and/or increased tolerability is associated with reduced body weight loss. In some aspects, reduced toxicity and/or increased tolerability can be assessed by tissue histology, and is associated with reduced spleen histiocytosis, liver margination, kidney tubular dilation, and/or heart degeneration. In some aspects, reduced toxicity and/or increased tolerability is associated with reduced spleen volume.

[0285] As used herein, "reducing the expression" refers to (i) a decrease in the total amount of the payload that is expressed in a detargeting cell (*e.g.*, an immune cell (*e.g.*, present in a tissue (*e.g.*, spleen and/or bone marrow)), a cardiomyocyte (*e.g.*, present in a tissue (*e.g.*, heart)), a liver cell, and/or an endothelial cell); (ii) a decrease in how long (*i.e.*, duration) the payload is expressed by the immune cells (*e.g.*, present in a tissue (*e.g.*, spleen)); or (iii) both (i) and (ii).

[0286] Accordingly, in some aspects, after administration, the total amount of payload that is expressed in a detargeting cell (*e.g.*, an immune cell (*e.g.*, present in a tissue (*e.g.*, spleen and/or bone marrow)), a cardiomyocyte (*e.g.*, present in a tissue (*e.g.*, heart or liver)), and/or an endothelial cell); is reduced. In some aspects, the total amount of payload that is expressed by the detargeting cells is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%. In some aspects, the total amount of payload that is expressed by the detargeting cells is reduced compared to the total amount of the payload that is expressed by the corresponding detargeting cells of a reference subject. In some aspects, after the administration, the total amount of payload that is expressed by the detargeting cells is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%,

at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding amount expressed by the detargeting cells of the reference subject. In some aspects, the reference subject is a subject who received a corresponding polynucleotide that lacks a detargeting sensor described herein.

[0287] As described herein, in some aspects, after administration, the payload is only transiently expressed by the detargeting cells. Accordingly, in some aspects, administering any of the polynucleotides, replicons, nanoparticles, and/or pharmaceutical compositions, to a subject, results in reduced duration of payload expression by the detargeting cells. In some aspects, the duration of payload expression by the immune cells is decreased by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%. In some aspects, after administration, the duration of payload expression by the detargeting cells of the subject is reduced, as compared to the duration of the payload expression by the the detargeting cells of the reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the detargeting sensor). In some aspects, the duration of payload expression in the the detargeting cells of the subject is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding duration in the detargeting cells of the reference subject.

[0288] In some aspects, after administration, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced in the detargeting cells. In some aspects, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%. In some aspects, after administration, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced in the detargeting cells as compared to the corresponding value for the detargeting cells of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the detargeting sensor). In some aspects, both (i) the total amount of the payload that is expressed and (ii) the duration of payload expression is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to the corresponding value for the detargeting cells of the reference subject.

[0289] Some aspects of the present disclosure are directed to methods of selectively expressing a payload in a subject in need thereof (*e.g.*, in tumors). In some aspects, such methods comprise administering to the subject any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein, wherein after the administration, the payload expression in the detargeting cells is reduced, as compared to the corresponding expression in e.g., tumors. As used herein, "selective expression" or "preferential expression," or grammatical variants thereof, refers to an increased expression (*e.g.*, of a payload) in a first tissue (*e.g.*, tumor) as compared to a second tissue (*e.g.*, lymphoid tissue, such as the spleen). In some aspects, the payload is selectively or preferentially expressed in a tumor where the expression (*e.g.*, total amount of the payload and/or duration of payload expression) in the tumor is increased as compared to the corresponding expression in a detargeting cell (*e.g.*, immune cells within the spleen).

VI.B. Methods of Treating

[0290] As is apparent from the present disclosure, the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein can be useful in treating a wide range of diseases or disorders. Accordingly, in some aspects, provided herein is a method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions provided herein. As described herein, after the administration, the payload is preferentially expressed in a subject in need thereof (e.g., in tumors).

[0291] The polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions provided herein can be used to treat any suitable diseases or disorders known in the art. Non-limiting examples of diseases and disorders that can be treated include cancer, inflammatory disorders, monogenic disorders, neurological disorders, psychiatric disorders, or combinations thereof.

[0292] In some aspects, the disease or disorder comprises a cancer. Non-limiting examples of cancers include: a melanoma, squamous cell cancer (e.g., esophageal squamous cell carcinoma), small-cell lung cancer, non-small cell lung cancer (NSCLC), adenocarcinoma of the lung, large cell carcinoma, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer (e.g., hepatocellular carcinoma), bladder (urothelial) cancer, hepatoma, breast cancer (e.g., triple-negative breast cancer, TNBC), colon cancer, colorectal cancer (e.g., colorectal cancer with

high microsatellite instability, MSI-H CRC), endometrial or uterine cancer, salivary gland carcinoma, kidney cancer (e.g., renal cell carcinoma), prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, head and neck cancer (e.g., head and neck squamous cell carcinoma, HNSCC), Merkel cell carcinoma (MCC), or combinations thereof. In some aspects, the breast cancer is triple-negative breast cancer.

[0293] In some aspects, the disease or disorder comprises a cancer. Non-limiting examples of cancers include: melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, head and neck squamous cell carcinoma (HNSCC), bladder (urothelial) cancer, Merkel cell carcinoma (MCC), triple-negative breast cancer (TNBC), hepatocellular carcinoma, colorectal cancer with high microsatellite instability (MSI-H CRC), esophageal squamous cell carcinoma, or combinations thereof.

[0294] In some aspects, the subject has a metastatic solid tumor. In some aspects, the subject has an advanced-stage solid tumor. In some aspects, the subject has an advanced stage and unresectable solid tumor. In some aspects, the subject has an advanced stage and metastatic solid tumor. In some embodiments, the subject has an unresectable and metastatic solid tumor. In some aspects, the subject has an advanced-stage, unresectable, and metastatic solid tumor. In some aspects, the subject has a metastatic solid tumor cancer. In some aspects, the subject has an advanced-stage, unresectable, or metastatic solid tumor cancer. In some aspects, the subject has anti-programmed cell death 1 (PD-1) and/or anti-programmed cell death 1 ligand 1 (PD-L1) resistant solid tumor cancer. In some aspects, the subject has a solid tumor cancer with acquired resistance or innate resistance to anti-PD-1 and/or anti-PD-L1 therapy. In some aspects, the subject has a solid tumor cancer with innate resistance to anti-PD-1 and/or anti-PD-L1 therapy. In some aspects, the subject has the refractory or resistant cancer is one that does not respond to a specified treatment. In some aspects, the subject has a cancer that does not respond to the anti-PD-1 and/or anti- PD-L1 therapy. In some aspects, the subject has become less responsive to the anti-PD-1 and/or anti- PD-L1 therapy since first receiving it. In some aspects, the subject has not received the therapy, but has a type of cancer that does not typically respond to the therapy.

[0295] In some aspects, any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions can be administered to the subject via intravenous, intratumoral, intrathecal, intramuscular, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

[0296] In some aspects, any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions can be administered to the subject in combination with one or more additional therapeutic agents. In some aspects, the additional therapeutic agent comprises an anti-cancer agent. Non-limiting examples of anti-cancer agents include: a chemotherapy, a radiation therapy, a surgical therapy, an immunotherapy, or combinations thereof.

[0297] In some aspects, an anti-cancer agent comprises an immune checkpoint inhibitor (*i.e.*, blocks signaling through the particular immune checkpoint pathway). Non-limiting examples of immune checkpoint inhibitors that can be used in the present methods comprise a CTLA-4 antagonist (*e.g.*, anti-CTLA-4 antibody), PD-1 antagonist (*e.g.*, anti-PD-1 antibody, anti-PD-L1 antibody), LAG-3 antagonist (*e.g.*, anti-LAG-3 antibody), CCR-8 antagonist (e.g., anti-CCR-8 antibody) or combinations thereof. Non-limiting examples of such immune checkpoint inhibitors include the following: anti-PD1 antibody (*e.g.*, nivolumab (OPDIVO®), pembrolizumab (KEYTRUDA®; MK-3475), pidilizumab (CT-011), PDR001, MEDI0680 (AMP-514), TSR-042, REGN2810, JS001, AMP-224 (GSK-2661380), PF-06801591, BGB-A317, BI 754091, SHR-1210, and combinations thereof); anti-PD-L1 antibody (*e.g.*, atezolizumab (TECENTRIQ®; RG7446; MPDL3280A; RO5541267), durvalumab (MEDI4736, IMFINZI®), BMS-936559, avelumab (BAVENCIO®), LY3300054, CX-072 (Proclaim-CX-072), FAZ053, KN035, MDX-1105, and combinations thereof); and anti-CTLA-4 antibody (*e.g.*, ipilimumab (YERVOY®), tremelimumab (ticilimumab; CP-675,206), AGEN-1884, ATOR-1015, and combinations thereof).

[0298] In some aspects, the chemotherapeutic agent is carboplatin, cisplatin, docetaxel, gemcitabine, nab-paclitaxel, pemetrexed, vinorelbine, or combinations thereof. In some aspects, the radiation therapy is ionizing radiation, gamma-radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, systemic radioactive isotopes, radiosensitizers, or combinations thereof. In some aspects, the surgical therapy is a curative surgery (*e.g.*, tumor removal surgery), a preventative surgery, a laparoscopic surgery, a laser surgery, or combinations thereof. In some aspects, the immunotherapy is adoptive cell transfer, therapeutic cancer vaccines, or combinations thereof.

[0299] In some aspects, the chemotherapeutic agent is platinating agents, such as carboplatin, oxaliplatin, cisplatin, nedaplatin, satraplatin, lobaplatin, triplatin, tetranitrate, picoplatin, prolindac, aroplatin and other derivatives; topoisomerase I inhibitors, such as camptothecin, topotecan, irinotecan/SN38, rubitecan, belotecan, and other derivatives; topoisomerase II inhibitors, such as etoposide (VP-16), daunorubicin, a doxorubicin agent (*e.g.*,

doxorubicin, doxorubicin HCl, doxorubicin analogs, or doxorubicin and salts or analogs thereof in liposomes), mitoxantrone, aclarubicin, epirubicin, idarubicin, amrubicin, amsacrine, pirarubicin, valrubicin, zorubicin, teniposide and other derivatives; antimetabolites, such as folic family (methotrexate, pemetrexed, raltitrexed, aminopterin, and relatives); purine antagonists (thioguanine, fludarabine, cladribine, 6-mercaptopurine, pentostatin, clofarabine and relatives) and pyrimidine antagonists (cytarabine, floxuridine, azacitidine, tegafur, carmofur, capacitabine, gemcitabine, hydroxyurea, 5-fluorouracil (5fu), and relatives); alkylating agents, such as nitrogen mustards (*e.g.,* cyclophosphamide, melphalan, chlorambucil, mechlorethamine, ifosfamide, trofosfamide, prednimustine, bendamustine, uramustine, estramustine, and relatives); nitrosoureas (*e.g.,* carmustine, lomustine, semustine, fotemustine, nimustine, ranimustine, streptozocin, and relatives); triazenes (*e.g.,* dacarbazine, altretamine, temozolomide, and relatives); alkyl sulphonates (*e.g.,* busulfan, mannosulfan, treosulfan, and relatives); procarbazine; mitobronitol, and aziridines (*e.g.,* carboquone, triaziquone, thiotepa, triethylenemalamine, and relatives); antibiotics, such as hydroxyurea, anthracyclines (*e.g.,* doxorubicin agent, daunorubicin, epirubicin and other derivatives); anthracenediones (*e.g.,* mitoxantrone and relatives); streptomyces family (*e.g.,* bleomycin, mitomycin c, actinomycin, plicamycin); ultraviolet light; and combinations thereof.

[0300] In some aspects, the additional therapeutic agent is an antibody. Antibodies (preferably monoclonal antibodies) achieve their therapeutic effect against cancer cells through various mechanisms. They can have direct effects in producing apoptosis or programmed cell death. They can block components of signal transduction pathways such as *e.g.,* growth factor receptors, effectively arresting proliferation of tumor cells. In cells that express monoclonal antibodies, they can bring about anti-idiotype antibody formation. Indirect effects include recruiting cells that have cytotoxicity, such as monocytes and macrophages. This type of antibody-mediated cell kill is called antibody-dependent cell mediated cytotoxicity (ADCC). Antibodies also bind complement, leading to direct cell toxicity, known as complement dependent cytotoxicity (CDC). Combining surgical methods with immunotherapeutic drugs or methods is an successful approach, as *e.g.,* demonstrated in Gadri et al. 2009: Synergistic effect of dendritic cell vaccination and anti-CD20 antibody treatment in the therapy of murine lymphoma. J Immunother. 32(4): 333-40. The following list provides some non-limiting examples of anti-cancer antibodies and potential antibody targets (in brackets) which can be used in combination with the present disclosure: Abagovomab (CA-125), Abciximab (CD41), Adecatumumab (EpCAM), Afutuzumab (CD20), Alacizumab pegol (VEGFR2), Altumomab pentetate (CEA), Amatuximab (MORAb-009),

Anatumomab mafenatox (TAG-72), Apolizumab (HLA-DR), Arcitumomab (CEA), Bavituximab (phosphatidylserine), Bectumomab (CD22), Belimumab (BAFF), Bevacizumab (VEGF-A), Bivatuzumab mertansine (CD44 v6), Blinatumomab (CD19), Brentuximab vedotin (CD30 TNFRSF8), Cantuzumab mertansin (mucin CanAg), Cantuzumab ravtansine (MUC1), Capromab pendetide (prostatic carcinoma cells), Carlumab (CNT0888), Catumaxomab (EpCAM, CD3), Cetuximab (EGFR), Citatuzumab bogatox (EpCAM), Cixutumumab (IGF-1 receptor), Claudiximab (Claudin), Clivatuzumab tetraxetan (MUC1), Conatumumab (TRAIL-R2), Dacetuzumab (CD40), Dalotuzumab (insulin-like growth factor I receptor), Denosumab (RANKL), Detumomab (B-lymphoma cell), Drozitumab (DR5), Ecromeximab (GD3 ganglioside), Edrecolomab (EpCAM), Elotuzumab (SLAMF7), Enavatuzumab (PDL192), Ensituximab (NPC-1C), Epratuzumab (CD22), Ertumaxomab (HER2/neu, CD3), Etaracizumab (integrin αvβ3), Farletuzumab (folate receptor 1), FBTA05 (CD20), Ficlatuzumab (SCH 900105), Figitumumab (IGF-1 receptor), Flanvotumab (glycoprotein 75), Fresolimumab (TGF-β), Galiximab (CD80), Ganitumab (IGF-I), Gemtuzumab ozogamicin (CD33), Gevokizumab (IL-1β), Girentuximab (carbonic anhydrase 9 (CA-IX)), Glembatumumab vedotin (GPNMB), Ibritumomab tiuxetan (CD20), Icrucumab (VEGFR-1), Igovoma (CA-125), Indatuximab ravtansine (SDC1), Intetumumab (CD51), Inotuzumab ozogamicin (CD22), Ipilimumab (CD152), Iratumumab (CD30), Labetuzumab (CEA), Lexatumumab (TRAIL-R2), Libivirumab (hepatitis B surface antigen), Lintuzumab (CD33), Lorvotuzumab mertansine (CD56), Lucatumumab (CD40), Lumiliximab (CD23), Mapatumumab (TRAIL-R1), Matuzumab (EGFR), Mepolizumab (IL-5), Milatuzumab (CD74), Mitumomab (GD3 ganglioside), Mogamulizumab (CCR4), Moxetumomab pasudotox (CD22), Nacolomab tafenatox (C242 antigen), Naptumomab estafenatox (5T4), Narnatumab (RON), Necitumumab (EGFR), Nimotuzumab (EGFR), Nivolumab (IgG4), Ofatumumab (CD20), Olaratumab (PDGF-R α), Onartuzumab (human scatter factor receptor kinase), Oportuzumab monatox (EpCAM), Oregovomab (CA-125), Oxelumab (OX-40), Panitumumab (EGFR), Patritumab (HER3), Pemtumoma (MUC1), Pertuzuma (HER2/neu), Pintumomab (adenocarcinoma antigen), Pritumumab (vimentin), Racotumomab (N-glycolylneuraminic acid), Radretumab (fibronectin extra domain-B), Rafivirumab (rabies virus glycoprotein), Ramucirumab (VEGFR2), Rilotumumab (HGF), Rituximab (CD20), Robatumumab (IGF-1 receptor), Samalizumab (CD200), Sibrotuzumab (FAP), Siltuximab (IL-6), Tabalumab (BAFF), Tacatuzumab tetraxetan (alpha-fetoprotein), Taplitumomab paptox (CD19), Tenatumomab (tenascin C), Teprotumumab (CD221), Ticilimumab (CTLA-4), Tigatuzumab (TRAIL-R2), TNX-650 (IL-13), Tositumomab (CD20), Trastuzumab (HER2/neu), TRBS07

(GD2), Tremelimumab (CTLA-4), Tucotuzumab celmoleukin (EpCAM), Ublituximab (MS4A1), Urelumab (4-1BB), Volociximab (integrin α5β1), Votumumab (tumor antigen CTAA16.88), Zalutumumab (EGFR), Zanolimumab (CD4).

[0301] In some aspects, the additional therapeutic agent is a cytokine, chemokine, costimulatory molecule, fusion protein, or combinations thereof. Examples of chemokines include, but are not limited to, CCR7 and its ligands CCL19 and CCL21, furthermore CCL2, CCL3, CCL5, and CCL16. Other examples are CXCR4, CXCR7 and CXCL12. Furthermore, costimulatory or regulatory molecules such as *e.g.,* B7 ligands (B7.1 and B7.2) are useful. Also useful are other cytokines such as *e.g.,* interleukins especially (*e.g.,* IL-1 to IL17), interferons (*e.g.,* IFNalpha1 to IFNalpha8, IFNalpha10, IFNalpha13, IFNalpha14, IFNalpha16, IFNalpha17, IFNalpha21, IFNbeta1, IFNW, IFNE1 and IFNK), hematopoietic factors, TGFs (*e.g.,* TGF-α, TGF-β, and other members of the TGF family), finally members of the tumor necrosis factor family of receptors and their ligands as well as other stimulatory molecules, comprising but not limited to 41BB, 41BB-L, CD137, CD137L, CTLA-4GITR, GITRL, Fas, Fas-L, TNFR1, TRAIL-R1, TRAIL-R2, p75NGF-R, DR6, LT.beta.R, RANK, EDAR1, XEDAR, Fn114, Troy/Trade, TAJ, TNFRII, HVEM, CD27, CD30, CD40, 4-1BB, OX40, GITR, GITRL, TACI, BAFF-R, BCMA, RELT, and CD95 (Fas/APO-1), glucocorticoid-induced TNFR-related protein, TNF receptor-related apoptosis-mediating protein (TRAMP) and death receptor-6 (DR6). Especially CD40/CD40L and OX40/OX40L are important targets for combined immunotherapy because of their direct impact on T cell survival and proliferation.

[0302] In some aspects, the additional therapeutic agent is a kinase inhibitor. The growth and survival of cancer cells is closely interlocked with the deregulation of kinase activity. To restore normal kinase activity and therefor reduce tumor growth a broad range of inhibitors is in used. The group of targeted kinases comprises receptor tyrosine kinases *e.g.,* BCR-ABL, B-Raf, EGFR, HER-2/ErbB2, IGF-IR, PDGFR-α, PDGFR-β, c-Kit, Flt-4, Flt3, FGFR1, FGFR3, FGFR4, CSF1R, c-Met, RON, c-Ret, ALK, cytoplasmic tyrosine kinases *e.g.,* c-SRC, c-YES, Abl, JAK-2, serine/threonine kinases *e.g.,* ATM, Aurora A & B, CDKs, mTOR, PKCi, PLKs, b-Raf, S6K, STK11/LKB1 and lipid kinases *e.g.,* PI3K, SK1. Small molecule kinase inhibitors are *e.g.,* PHA-739358, Nilotinib, Dasatinib, and PD166326, NSC 743411, Lapatinib (GW-572016), Canertinib (CI-1033), Semaxinib (SU5416), Vatalanib (PTK787/ZK222584), Sutent (SU11248), Sorafenib (BAY 43-9006) and Leflunomide (SU101). For more information see *e.g.,* Zhang et al. 2009: Targeting cancer with small molecule kinase inhibitors. Nature Reviews Cancer 9, 28-39.

[0303] In some aspects, the additional therapeutic agent is a toll-like receptor. The members of the Toll-like receptor (TLRs) family are an important link between innate and adaptive immunity and the effect of many adjuvants rely on the activation of TLRs. A large number of established vaccines against cancer incorporate ligands for TLRs for boosting vaccine responses. Besides TLR2, TLR3, TLR4 especially TLR7 and TLR8 have been examined for cancer therapy in passive immunotherapy approaches. The closely related TLR7 and TLR8 contribute to antitumor responses by affecting immune cells, tumor cells, and the tumor microenvironment and can be activated by nucleoside analogue structures. All TLRs have been used as stand-alone immunotherapeutics or cancer vaccine adjuvants and can be synergistically combined with the formulations and methods of the present disclosure.

[0304] In some aspects, the additional therapeutic agent is an angiogenesis inhibitor. Angiogenesis inhibitors prevent the extensive growth of blood vessels (angiogenesis) that tumors require to survive. The angiogenesis promoted by tumor cells to meet their increasing nutrient and oxygen demands for example can be blocked by targeting different molecules. Non-limiting examples of angiogenesis-mediating molecules or angiogenesis inhibitors which can be combined with the present disclosure are soluble VEGF (VEGF isoforms VEGF121 and VEGF165, receptors VEGFR1, VEGFR2 and co-receptors Neuropilin-1 and Neuropilin-2) 1 and NRP-1, angiopoietin 2, TSP-1 and TSP-2, angiostatin and related molecules, endostatin, vasostatin, calreticulin, platelet factor-4, TIMP and CDAI, Meth-1 and Meth-2, IFN-α, -β and -γ, CXCL10, IL-4, -12 and -18, prothrombin (kringle domain-2), antithrombin III fragment, prolactin, VEGI, SPARC, osteopontin, maspin, canstatin, proliferin-related protein, restin and drugs like *e.g.,* bevacizumab, itraconazole, carboxyamidotriazole, TNP-470, CM101, IFN-α, platelet factor-4, suramin, SU5416, thrombospondin, VEGFR antagonists, angiostatic steroids+heparin, cartilage-derived angiogenesis Inhibitory factor, matrix metalloproteinase inhibitors, 2-methoxyestradiol, tecogalan, tetrathiomolybdate, thalidomide, thrombospondin, prolactina Vβ3 inhibitors, linomide, tasquinimod.

[0305] In some aspects, the additional therapeutic agent is a virus-based vaccine. There are a number of virus-based cancer vaccines available or under development which can be used in a combined therapeutic approach together with the formulations of the present disclosure. One advantage of the use of such viral vectors is their intrinsic ability to initiate immune responses, with inflammatory reactions occurring as a result of the viral infection creating the danger signal necessary for immune activation. An ideal viral vector should be safe and should not introduce an anti-vector immune response to allow for boosting anti-tumor specific responses. Recombinant

viruses such as vaccinia viruses, herpes simplex viruses, adenoviruses, adeno-associated viruses, retroviruses and avipoxviruses have been used in animal tumor models and based on their encouraging results, human clinical trials have been initiated. Especially important virus-based vaccines are virus-like particles (VLPs), small particles that contain certain proteins from the outer coat of a virus. Virus-like particles do not contain any genetic material from the virus and cannot cause an infection but they can be constructed to present tumor antigens on their coat. VLPs can be derived from various viruses such as *e.g.,* the hepatitis B virus or other virus families including Parvoviridae (*e.g.,* adeno-associated virus), Retroviridae (*e.g.,* HIV), and Flaviviridae (*e.g.,* Hepatitis C virus).

[0306] In some aspects, the additional therapeutic agent is a peptide-based target therapy. Peptides can bind to cell surface receptors or affected extracellular matrix surrounding the tumor. Radionuclides which are attached to these peptides (*e.g.,* RGDs) eventually kill the cancer cell if the nuclide decays in the vicinity of the cell. Especially oligo- or multimers of these binding motifs are of great interest, since this can lead to enhanced tumor specificity and avidity.

VII. Kits for Use in Therapy

[0307] The present disclosure also provides kits for use in immunotherapy against a disease or disorder, such as a cancer (*e.g.,* melanoma, lung cancer, colorectal cancer, or renal-cell cancer), and/or treating or reducing the risk for the disease or disorder (*e.g.*, cancer). In some aspects, the kit includes one or more containers comprising any of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein.

[0308] In some aspects, the kit comprises instructions for use in accordance with any of the methods described herein. For example, the included instructions can comprise a description of administration of the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein to treat, delay the onset, or alleviate a target disease. In some aspects, the instructions comprise a description of administering the polynucleotides, replicons (*e.g.*, VEE replicon), nanoparticles, and/or pharmaceutical compositions described herein to a subject at risk of the target disease/disorder (*e.g.*, cancer).

[0309] In some aspects, the instructions comprise dosage information, dosing schedule, and route of administration. In some aspects, the containers are unit doses, bulk packages (*e.g.,* multi-dose packages) or sub-unit doses. In some aspects, the instructions are written instructions on a label or package insert (*e.g.,* a paper sheet included in the kit). In some aspects, the instructions are machine-readable instructions (*e.g.,* instructions carried on a magnetic or optical storage disk).

[0310] In some aspects, the label or package insert indicates that the composition disclosed herein is used for treating, delaying the onset, and/or alleviating a disease or disorder associated with cancer, such as those described herein. Instructions can be provided for practicing any of the methods described herein.

[0311] In some aspects, the kits described herein are in suitable packaging. In some aspects, suitable packing comprises vials, bottles, jars, flexible packaging (*e.g.,* seal Mylar or plastic bags), or combinations thereof. In some aspects, the packaging comprises packages for use in combination with a specific device such as an inhaler, nasal administration device (*e.g.,* an atomizer), or an infusion device such as a minipump. In some aspects, the kit comprises a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, the container can also have a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). In some aspects, at least one active agent is a composition as described herein.

[0312] In some aspects, the kits further comprise additional components such as buffers and interpretive information. In some aspects, the kit comprises a container and a label or package insert(s) on or associated with the container. In some aspects, the disclosure provides articles of manufacture comprising the contents of the kits described herein.

General Techniques

[0313] The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Molecular Cloning: A Laboratory Manual, second edition (Sambrook, et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J. E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R. I. Freshney, ed. 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Giffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; Method of Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel, et al., eds., 1987): PCR: The Polymerase Chain Reaction, (Mullis, et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short

Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty, ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane, Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanette and J.D. Capra, eds., Harwood Academic Publishers, 1995). Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. All publications cited herein (including those listed above and elsewhere in the present disclosure) are incorporated by reference in their entirety.

Examples

EXAMPLE 1: EFFECTS OF DUAL miRNA-SENSING CIRCUITS ON mRNA IN OFF-TARGET ORGANS

[0314] To assess the ability of miRNA sensors to detarget healthy tissues (e.g., spleen, bone marrow, heart, liver, and adrenal gland) LNPs loaded with one of the following mRNA constructs ("mRNA-LNPs") described in Table 1, were administered intravenously to naïve C57Bl/6J mice. Serum and tissues were collected at different time points post-dosing and subjected to bioanalysis. Tissue RT-qPCR was used to detect nsP4 mRNA and was performed 6 hrs post injection for spleen and bone marrow and 72 hrs post injection for heart to detect mRNA encoding exogenous mIL-12.

Table 1: mRNA Constructs Loaded into Lipid Nanoparticle

Construct	Description	
repRNA-mIL-12	VEE replicon comprising mRNA encoding IL-12 alone	Uncircuited mRNA
repRNA-mIL-12+miR-142 sensor	VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor	Circuited mRNA
repRNA-mIL-12+miR-142 sensor+miR-22 sensor	VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor	
repRNA-mIL-12+miR-142 sensor+miR-185 sensor	VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor	

[0315] As shown in **FIGs. 1A-1D**, mice that received the mRNA-LNP with a miR-142 sensor alone had reduced IL-12 mRNA expression within the spleen (**FIG. 1A**) bone marrow (**FIG.**

1B), and to a lesser extent adrenal gland (**FIG. 1D**), but not heart (**FIG. 1C**) compared to control mice that received mRNA encoding IL-12 alone (uncircuited).

[0316] Mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor had reduced IL-12 mRNA expression within the spleen (**FIG. 1A**), heart (**FIG. 1C**), and adrenal gland (**FIG. 1D**) compared to control mice that received the mRNA-LNP alone and mice that received the mRNA-LNP with a miR-142 sensor only. Mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor had similar IL-12 mRNA expression within bone marrow (**FIG. 1B**) compared to mice that received the mRNA-LNP with a miR-142 sensor only.

[0317] Mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor had reduced IL-12 mRNA expression within the spleen (**FIG. 1A**) compared to control mice that received the mRNA-LNP alone and mice that received the mRNA-LNP with a miR-142 sensor only. Mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor had similar IL-12 mRNA expression within bone marrow (**FIG. 1B**) compared to mice that received the mRNA-LNP with a miR-142 sensor only.

[0318] These results confirm that the addition of a miR-22 or a miR-185 sensor detarget a broader set of off-target organs than a miR-142 sensor alone. Notably, mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor showed robust reduced expression of mRNA levels in multiple off-target tissues in these naïve mice; specifically, a 73% reduction in spleen (**FIG. 1E**), a 75% reduction in bone marrow (**FIG. 1F**), and a 58% reduction in liver (**FIG. 1H**) compared to control mice that received the mRNA-LNP alone.

[0319] Because human interleukin 12 (hIL-12) has limited biological activity in mice, a murine surrogate drug product encoding murine IL-12 was developed for use in syngeneic mouse tumor models. This surrogate, is otherwise analogous to the replicon expressing human IL-12 and bearing dual sensor construct, differing only in its murine IL-12 payload sequence. This surrogate (SEQ ID NO: 13) is used throughout the mouse experiments described herein, unless otherwise noted.

Table 2. Exemplary VEE Replicon Sequences

VEE Replicon Sequence #1 (SEQ ID NO: 14)	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAA AGTTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGG AGCTTCCCGCAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATG CTAATGCCAGAGCGTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGT GGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATG TATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATC CGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAAT AACTGATAAGGAATTGGACAAGAAAATGAAGGAGCTGGCCGCCGTCATGAGC GACCCTGACCTGGAAACTGAGACTATGTGCCTCCACGACGACGAGTCGTGTC

	GCTACGAAGGGCAAGTCGCTGTTTACCAGGATGTATACGCGGTTGACGGACC GACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAGTCGCCTACTGGATA GGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATATCCAT CATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGG CCTATGCAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTT AGAAAGAAGTATTTGAAACCATCCAACAATGTTCTATTCTCTGTTGGCTCGA CCATCTACCACGAGAAGAGGGACTTACTGAGGAGCTGGCACCTGCCGTCTGT ATTTCACTTACGTGGCAAGCAAAATTACACATGTCGGTGTGAGACTATAGTT AGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCCTGTATG GGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTG CAAAGTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACG TATGTGCCAGCTACATTGTGTGACCAAATGACTGGCATACTGGCAACAGATG TCAGTGCGGACGACGCGCAAAAACTGCTGGTTGGGCTCAACCAGCGTATAGT CGTCAACGGTCGCACCCAGAGAAACACCAATACCATGAAAAATTACCTTTTG CCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAAGGAAGATC AAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTG TTGTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGAT ACCCAAACCATCATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCA GGATAGGCAGTAACACATTGGAGATCGGGCTGAGAACAAGAATCAGGAAAAT GTTAGAGGAGCACAAGGAGCCGTCACCTCTCATTACCGCCGAGGACGTACAA GAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAAGCCGAGGAGT TGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA GGCAGACGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACA CCTCGTGGCTTGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCT CTTACGCTGTGCTTTCTCCGCAGGCTGTACTCAAGAGTGAAAAATTATCTTG CATCCACCCTCTCGCTGAACAAGTCATAGTGATAACACACTCTGGCCGAAAA GGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGGTGCCAGAGGGAC ATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATTGT GTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACAT GGAGGAGCGCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCG AGCACGACGGCGAATACCTGTACGACATCGACAGGAAACAGTGCGTCAAGAA AGAACTAGTCACTGGGCTAGGGCTCACAGGCGAGCTGGTGGATCCTCCCTTC CATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGCCGCTCCTTACCAAG TACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCATCAT TAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAAC TGTGCAGAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATG CCAGAACTGTGGACTCAGTGCTCTTGAATGGATGCAAACACCCCGTAGAGAC CCTGTATATTGACGAAGCTTTTGCTTGTCATGCAGGTACTCTCAGAGCGCTC ATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGCGGGGATCCCAAACAGT GCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGAGATTTG CACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACT TCGGTCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGA AAGAGACTAAGATTGTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGA CGATCTCATTCTCACTTGTTTCAGAGGGTGGGTGAAGCAGTTGCAAATAGAT TACAAAGGCAACGAAATAATGACGGCAGCTGCCTCTCAAGGGCTGACCCGTA AAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTGTACGCACC CACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTG TGGAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACC CTGGGAATTTCACTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCAT CATGAGGCACATCTTGGAGAGACCGGACCCTACCGACGTCTTCCAGAATAAG GCAAACGTGTGTTGGGCCAAGGCTTTAGTGCCGGTGCTGAAGACCGCTGGCA TAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTGAAACGGACAA AGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTA

	GGAATAATCACTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAA AGAAGTGGTCCGTCAGCTCTCTCGCAGGTACCCACAACTGCCTCGGGCAGTT GCCACTGGAAGAGTCTATGACATGAACACTGGTACACTGCGCAATTATGATC CGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCATGCTTTAGTCCT CCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAATTG AAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAA TGGTTGACTGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGA TTTAGGCATCCCAGGTGATGTGCCCAAATATGACATAATATTTGTTAATGTG AGGACCCCATATAAATACCATCACTATCAGCAGTGTGAAGACCATGCCATTA AGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTGAATCCCGGCGGAAC CTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCATCATT GGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCT CACTTGAAGAGACGGAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGC CCGTACGCACAATTCTTACAAGCTTTCATCAACCTTGACCAACATTTATACA GGTTCCAGACTCCACGAAGCCGGATGTGCACCCTCATATCATGTGGTGCGAG GGGATATTGCCACGGCCACCGAAGGAGTGATTATAAATGCTGCTAACAGCAA AGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTCCCGGAA AGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTG CAGCTAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGA GGTTGAAGGTGACAAACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATT GTCAACGATAACAATTACAAGTCAGTAGCGATTCCACTGTTGTCCACCGGCA TCTTTTCCGGGAACAAAGATCGACTAACCCAATCATTGAACCATTTGCTGAC AGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGGACAAGAAA TGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGA TATGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAG GGTGCATCCGAAGAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGAT GGCAAAACTTTCTCATATTTGGAAGGGACCAAGTTTCACCAGGCGGCCAAGG ATATAGCAGAAATTAATGCCATGTGGCCCGTTGCAACGGAGGCCAATGAGCA GGTATGCATGTATATCCTCGGAGAAAGCATGAGCAGTATTAGGTCGAAATGC CCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCC AGAACAAATTACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACT GGTGTGCAGAAGATCCAATGCTCCCAGCCTATATTGTTCTCACCGAAAGTGC CTGCGTATATTCATCCAAGGAAGTATCTCGTGGAAACACCACCGGTAGACGA GACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGGACACCTGAACAA CCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGATCA TCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGAC CCACCAGGTGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCT AGCTCATCCTGGTCCATTCCTCATGCATCCGACTTTGATGTGGACAGTTTAT CCATACTTGACACCCTGGAGGGAGCTAGCGTGACCAGCGGGGCAACGTCAGC CGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTCTGGCGCGACCGGTG CCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGCACAA GAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTC CACCCCGCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTT ACCCCGTCACGCACTCCTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCA ACCCGCCAGGCGTAAATAGGGTGATTACAAGAGAGGAGTTTGAGGCGTTCGT AGCACAACAACAATGACGGTTTGATGCGGGTGCATACATCTTTTCCTCCGAC ACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGCTATCCG AAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGA CCAAGAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCT GCTAACAGAAGCAGATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAA CAGCTAGACGTATTCTGCAAGGCCTAGGGCATTATTTGAAGGCAGAAGGAAA AGTGGAGTGCTACCGAACCCTGCATCCTGTTCCTTTGTATTCATCTAGTGTG AACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAACGCCATGT

	TGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGA TGCCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGT TTTTGCCCTGCAAAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAAC CCACAATACGATCGGCAGTGCCTTCAGCGATCCAGAACACGCTCCAGAACGT CCTGGCAGCTGCCACAAAAAGAAATTGCAATGTCACGCAAATGAGAGAATTG CCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAGAAATATGCGT GTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCT CTTTTTGCGAAGACACATAATTTGAATATGTTGCAGGACATACCAATGGACA GGTTTGTAATGGACTTAAAGAGAGACGTGAAAGTGACTCCAGGAACAAAACA TACTGAAGAACGGCCCAAGGTACAGGTGATCCAGGCTGCCGATCCGCTAGCA ACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGAGATTAAATGCGG TCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTTGA CGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGAC ATCGCGTCGTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAA TGATTCTGGAAGACTTAGGTGTGGACGCAGAGCTGTTGACGCTGATTGAGGC GGCTTTCGGCGAAATTTCATCAATACATTTGCCCACTAAAACTAAATTTAAA TTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACTGTTTGTGAACACAG TCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCGGATC ACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCG GACAAATTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGA TTATAGATGCTGTGGTGGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTAT TTTGTGTGACTCCGTGACCGGCACAGCGTGCCGTGTGGCAGACCCCCTAAAA AGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGACGATGAACATGATGATG ACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGTGGGTAT TCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACT TCCATCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCA GCTACCTGAGAGGGGCCCCTATAACTCTCTACGGCTAACCTGAATGGACTAC GACATAGTCTAGTCCGCCAAGTCTAGCATATGGGCGCGCCCTCAGCATCGAT TGAATTGGCCACCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATC AAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACG AGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGAC CGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATC CTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCG ACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCG CGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCC CTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCC CCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTC CGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGG CTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACA AGGCCAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTT GGACATCACCTCCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGC GCCGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGTAGGAAT TGGCAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATT TTTATTTTATTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTC AAAAAAAAAAAAAAAAAAAAAAAAACGCGTCGAGGGGAATTAATTCTTGAA GACGAAAGGGCCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATT TGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAAC CCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACA TTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTT GCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTG CACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAG TTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTA TGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCC

	GCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAA GCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACC ATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGA AGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGA TCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACC ACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAAC TACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAA AGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCT GATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGG GGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCA GGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTG ATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTG ATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGA TAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCA GACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCG TAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTT GCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGA GCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACT TCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACC AGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGA CGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCA CACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCG TGAGCATTGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTAT CCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGG GAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGA GCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCC AGCAACGCGAGCTCTAATACGACTCACTATAG
VEE- Replicon Sequence #2 (SEQ ID NO: 15)	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAA AGTTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGG AGCTTCCCGCAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATG CTAATGCCAGAGCGTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGT GGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATG TATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATC CGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAAT AACTGATAAGGAATTGGACAAGAAAATGAAGGAGCTCGCCGCCGTCATGAGC GACCCTGACCTGGAAACTGAGACTATGTGCCTCCACGACGACGAGTCGTGTC GCTACGAAGGGCAAGTCGCTGTTTACCAGGATGTATACGCGGTTGACGGACC GACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAGTCGCCTACTGGATA GGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATATCCAT CATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGG CCTATGCAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTT AGAAAGAAGTATTTGAAACCATCCAACAATGTTCTATTCTCTGTTGGCTCGA CCATCTACCACGAGAAGAGGGACTTACTGAGGAGCTGGCACCTGCCGTCTGT ATTTCACTTACGTGGCAAGCAAAATTACACATGTCGGTGTGAGACTATAGTT AGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCCTGTATG GGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTG CAAAGTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACG TATGTGCCAGCTACATTGTGTGACCAAATGACTGGCATACTGGCAACAGATG TCAGTGCGGACGACGCGCAAAAACTGCTGGTTGGGCTCAACCAGCGTATAGT CGTCAACGGTCGCACCCAGAGAAACACCAATACCATGAAAAATTACCTTTTG CCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAAGGAAGATC AAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTG TTGTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGAT ACCCAAACCATCATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCA

	GGATAGGCAGTAACACATTGGAGATCGGGCTGAGAACAAGAATCAGGAAAAT GTTAGAGGAGCACAAGGAGCCGTCACCTCTCATTACCGCCGAGGACGTACAA GAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAAGCCGAGGAGT TGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA AGCCGATGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACA CCTCGTGGCTTGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCT CTTACGCTGTGCTTTCTCCGCAGGCTGTACTCAAGAGTGAAAAATTATCTTG CATCCACCCTCTCGCTGAACAAGTCATAGTGATAACACACTCTGGCCGAAAA GGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGGTGCCAGAGGGAC ATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATTGT GTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACAT GGAGGAGCGCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCG AGCACGACGGCGAATACCTGTACGACATCGACAGGAAACAGTGCGTCAAGAA AGAACTAGTCACTGGGCTAGGGCTCACAGGCGAGCTGGTGGATCCTCCCTTC CATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGCCGCTCCTTACCAAG TACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCATCAT TAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAAC TGTGCAGAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATG CCAGAACTGTGGACTCAGTGCTCTTGAATGGATGCAAACACCCCGTAGAGAC CCTGTATATTGACGAAGCTTTTGCTTGTCATGCAGGTACTCTCAGAGCGCTC ATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGCGGGGATCCCAAACAGT GCGGTTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGAGATTTG CACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACT TCGGTCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGA AAGAGACTAAGATTGTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGA CGATCTCATTCTCACTTGTTTCAGAGGGTGGGTGAAGCAGTTGCAAATAGAT TACAAAGGCAACGAAATAATGACGGCAGCTGCCTCTCAAGGGCTGACCCGTA AAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTGTACGCACC CACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTG TGGAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACC CTGGGAATTTCACTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCAT CATGAGGCACATCTTGGAGAGACCGGACCCTACCGACGTCTTCCAGAATAAG GCAAACGTGTGTTGGGCCAAGGCTTTAGTGCCGGTGCTGAAGACCGCTGGCA TAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTGAAACGGACAA AGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTA GGAATAATCACTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAA AGAAGTGGTCCGTCAGCTCTCTCGCAGGTACCCACAACTGCCTCGGGCAGTT GCCACTGGAAGAGTCTATGACATGAACACTGGTACACTGCGCAATTATGATC CGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCATGCTTTAGTCCT CCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAATTG AAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAA TGGTTGACTGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGA TTTAGGCATCCCAGGTGATGTGCCCAAATATGACATAATATTTGTTAATGTG AGGACCCCATATAAATACCATCACTATCAGCAGTGTGAAGACCATGCCATTA AGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTGAATCCCGGCGGAAC CTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCATCATT GGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCT CACTTGAAGAGACGGAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGC CCGTACGCACAATCCTTACAAGCTTTCATCAACCTTGACCAACATTTATACA GGTTCCAGACTCCACGAAGCCGGATGTGCACCCTCATATCATGTGGTGCGAG GGGATATTGCCACGGCCACCGAAGGAGTGATTATAAATGCTGCTAACAGCAA AGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTCCCGGAA AGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTG

	CAGCTAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGA GGTTGAAGGTGACAAACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATT GTCAACGATAACAATTACAAGTCAGTAGCGATTCCACTGTTGTCCACCGGCA TCTTTTCCGGGAACAAAGATCGACTAACCCAATCATTGAACCATTTGCTGAC AGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGGACAAGAAA TGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGA TATGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAG GGTGCATCCGAAGAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGAT GGCAAAACTTTCTCATATTTGGAAGGGACCAAGTTTCACCAGGCGGCCAAGG ATATAGCAGAAATTAATGCCATGTGGCCCGTTGCAACGGAGGCCAATGAGCA GGTATGCATGTATATCCTCGGAGAAAGCATGAGCAGTATTAGGTCGAAATGC CCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCC AGAACAAATTACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACT GGTGTGCAGAAGATCCAATGCTCCCAGCCTATATTGTTCTCACCGAAAGTGC CTGCGTATATTCATCCAAGGAAGTATCTCGTGGAAACACCACCGGTAGACGA GACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGGACACCTGAACAA CCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGATCA TCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGAC CCACCAGGTGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCT AGCTCATCCTGGTCCATTCCTCATGCATCCGACTTTGATGTGGACAGTTTAT CCATACTTGACACCCTGGAGGGAGCTAGCGTGACCAGCGGGGCAACGTCAGC CGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTCTGGCGCGACCGGTG CCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGCACAA GAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTC CACCCCGCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTT ACCCCGTCACGCACTCCTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCA ACCCGCCAGGCGTAAATAGGGTGATTACAAGAGAGGAGTTTGAGGCGTTCGT AGCACAACAACAATGACGGTTTGATGCGGGTGCATACATCTTTTCCTCCGAC ACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGCTATCCG AAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGA CCAAGAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCT GCTAACAGAAGCAGATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAA CAGCTAGACGTATTCTGCAAGGCCTAGGGCATTATTTGAAGGCAGAAGGAAA AGTGGAGTGCTACCGAACCCTGCATCCTGTTCCTTTGTATTCATCTAGTGTG AACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAACGCCATGT TGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGA TGCCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGT TTTTGCCCTGCAAAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAAC CCACAATACGATCGGCAGTGCCTTCAGCGATCCAGAACACGCTCCAGAACGT CCTGGCAGCTGCCACAAAAAGAAATTGCAATGTCACGCAAATGAGAGAATTG CCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAGAAATATGCGT GTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCT CTTTTTGCGAAGACACATAATTTGAATATGTTGCAGGACATACCAATGGACA GGTTTGTAATGGACTTAAAGAGAGACGTGAAAGTGACTCCAGGAACAAAACA TACTGAAGAACGGCCCAAGGTACAGGTGATCCAGGCTGCCGATCCGCTAGCA ACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGAGATTAAATGCGG TCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTTGA CGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGAC ATCGCGTCGTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAA TGATTCTGGAAGACTTAGGTGTGGACGCAGAGCTGTTGACGCTGATTGAGGC GGCTTTCGGCGAAATTTCATCAATACATTTGCCCACTAAAACTAAATTTAAA TTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACTGTTTGTGAACACAG

	TCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCGGATC ACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCG GACAAATTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGA TTATAGATGCTGTGGTGGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTAT TTTGTGTGACTCCGTGACCGGCACAGCGTGCCGTGTGGCAGACCCCCTAAAA AGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGACGATGAACATGATGATG ACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGTGGGTAT TCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACT TCCATCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCA GCTACCTGAGAGGGGCCCCTATAACTCTCTACGGCTAACCTGAATGGACTAC GACATAGTCTAGTCCGCCAAGGCCACCGATATCTATGTTACGTGCAAAGGTG ATTGTCACCCCCCGAAAGACCATATTGTGACACACCCTCAGTATCACGCCCA AACATTTACAGCCGCGGTGTCAAAAACCGCGTGGACGTGGTTAACATCCCTG CTGGGAGGATCAGCCGTAATTATTATAATTGGCTTGGTGCTGGCTACTATTG TGGCCATGTACGTGCTGACCAACCAGAAACATAATTGAATACAGCAGCAATT GGCAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATTT TTATTTTATTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGAAGAGCGC AGCTCTGGCCCGTGTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAA TATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGG GGTGTTATGAGCCATATTCAACGGGAAACGTCGAGGCCGCGATTAAATTCCA ACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGCA ATCAGGTGCGACAATCTATCGCTTGTATGGGAAGCCCGATGCGCCAGAGTTG TTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGAGATGG TCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTT TATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATCCCCGGAAAA ACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTG ATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTCCTGTTTGTAATTG TCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATG AATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGC CTGTTGAACAAGTCTGGAAAGAAATGCATAAACTTTTGCCATTCTCACCGGA TTCAGTCGTCACTCATGGTGATTTCTCACTTGATAACCTTATTTTTGACGAG GGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGAT ACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTGAGTTTTCTCCTTCATT ACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAA TTGCAGTTTCATTTGATGCTCGATGAGTTTTTCTAATCAGAATTGGTTAATT GGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGCAAGCTC ATGACCAAAATCCCTTAACGTGAGTTACGCGTCGTTCCACTGAGCGTCAGAC CCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAA TCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCC GGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCG CAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCA AGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGT GGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA TAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACAC AGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGA GCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCG GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAA ACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCG TCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGC AACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGC TCTTCTAAGTAATACGACTCACTATA
VEE Replicon Sequence #3	gctcttctaagTAATACGACTCACTATAATGGGCGGCGCATGAGAGAAGCCC AGACCAATTACCTACCCAAAATGGAGAAAGTTCACGTTGACATCGAGGAAGA

(SEQ ID NO: 16)	CAGCCCATTCCTCAGAGCTTTGCAGCGGAGCTTCCCGCAGTTTGAGGTAGAA GCCAAGCAGGTCACTGATAATGACCATGCTAATGCCAGAGCGTTTTCGCATC TGGCTTCAAAACTGATCGAAACGGAGGTGGACCCATCCGACACGATCCTTGA CATTGGAAGTGCGCCCGCCCGCAGAATGTATTCTAAGCACAAGTATCATTGT ATCTGTCCGATGAGATGTGCGGAAGATCCGGACAGATTGTATAAGTATGCAA CTAAGCTGAAGAAAAACTGTAAGGAAATAACTGATAAGGAATTGGACAAGAA AATGAAGGAGCTCGCCGCCGTCATGAGCGACCCTGACCTGGAAACTGAGACT ATGTGCCTCCACGACGACGAGTCGTGTCGCTACGAAGGGCAAGTCGCTGTTT ACCAGGATGTATACGCGGTTGACGGACCGACAAGTCTCTATCACCAAGCCAA TAAGGGAGTTAGAGTCGCCTACTGGATAGGCTTTGACACCACCCCTTTTATG TTTAAGAACTTGGCTGGAGCATATCCATCATACTCTACCAACTGGGCCGACG AAACCGTGTTAACGGCTCGTAACATAGGCCTATGCAGCTCTGACGTTATGGA GCGGTCACGTAGAGGGATGTCCATTCTTAGAAAGAAGTATTTGAAACCATCC AACAATGTTCTATTCTCTGTTGGCTCGACCATCTACCACGAGAAGAGGGACT TACTGAGGAGCTGGCACCTGCCGTCTGTATTTCACTTACGTGGCAAGCAAAA TTACACATGTCGGTGTGAGACTATAGTTAGTTGCGACGGGTACGTCGTTAAA AGAATAGCTATCAGTCCAGGCCTGTATGGGAAGCCTTCAGGCTATGCTGCTA CGATGCACCGCGAGGGATTCTTGTGCTGCAAAGTGACAGACACATTGAACGG GGAGAGGGTCTCTTTTCCCGTGTGCACGTATGTGCCAGCTACATTGTGTGAC CAAATGACTGGCATACTGGCAACAGATGTCAGTGCGGACGACGCGCAAAAAC TGCTGGTTGGGCTCAACCAGCGTATAGTCGTCAACGGTCGCACCCAGAGAAA CACCAATACCATGAAAAATTACCTTTTGCCCGTAGTGGCCCAGGCATTTGCT AGGTGGGCAAAGGAATATAAGGAAGATCAAGAAGATGAAAGGCCACTAGGAC TACGAGATAGACAGTTAGTCATGGGGGTGTTGTTGGGCTTTTAGAAGGCACAA GATAACATCTATTTATAAGCGCCCGGATACCCAAACCATCATCAAAGTGAAC AGCGATTTCCACTCATTCGTGCTGCCCAGGATAGGCAGTAACACATTGGAGA TCGGGCTGAGAACAAGAATCAGGAAAATGTTAGAGGAGCACAAGGAGCCGTC ACCTCTCATTACCGCCGAGGACGTACAAGAAGCTAAGTGCGCAGCCGATGAG GCTAAGGAGGTGCGTGAAGCCGAGGAGTTGCGCGCAGCTCTACCACCTTTGG CAGCTGATGTTGAGGAGCCCACTCTGGAAGCCGATGTCGACTTGATGTTACA AGAGGCTGGGGCCGGCTCAGTGGAGACACCTCGTGGCTTGATAAAGGTTACC AGCTACGATGGCGAGGACAAGATCGGCTCTTACGCTGTGCTTTCTCCGCAGG CTGTACTCAAGAGTGAAAAATTATCTTGCATCCACCCTCTCGCTGAACAAGT CATAGTGATAACACACTCTGGCCGAAAAGGGCGTTATGCCGTGGAACCATAC CATGGTAAAGTAGTGGTGCCAGAGGGACATGCAATACCCGTCCAGGACTTTC AAGCTCTGAGTGAAAGTGCCACCATTGTGTACAACGAACGTGAGTTCGTAAA CAGGTACCTGCACCATATTGCCACACATGGAGGAGCGCTGAACACTGATGAA GAATATTACAAAACTGTCAAGCCCAGCGAGCACGACGGCGAATACCTGTACG ACATCGACAGGAAACAGTGCGTCAAGAAAGAACTAGTCACTGGGCTAGGGCT CACAGGCGAGCTGGTGGATCCTCCCTTCCATGAATTCGCCTACGAGAGTCTG AGAACACGACCAGCCGCTCCTTACCAAGTACCAACCATAGGGGTGTATGGCG TGCCAGGATCAGGCAAGTCTGGCATCATTAAAAGCGCAGTCACCAAAAAAGA TCTAGTGGTGAGCGCCAAGAAAGAAAACTGTGCAGAAATTATAAGGGACGTC AAGAAAATGAAAGGGCTGGACGTCAATGCCAGAACTGTGGACTCAGTGCTCT TGAATGGATGCAAACACCCCGTAGAGACCCTGTATATTGACGAAGCTTTTGC TTGTCATGCAGGTACTCTCAGAGCGCTCATAGCCATTATAAGACCTAAAAAG GCAGTGCTCTGCGGGGATCCCAAACAGTGCGGTTTTTTTAACATGATGTGCC TGAAAGTGCATTTTAACCACGAGATTTGCACACAAGTCTTCCACAAAAGCAT CTCTCGCCGTTGCACTAAATCTGTGACTTCGGTCGTCTCAACCTTGTTTTAC GACAAAAAAATGAGAACGACGAATCCGAAAGAGACTAAGATTGTGATTGACA CTACCGGCAGTACCAAACCTAAGCAGGACGATCTCATTCTCACTTGTTTCAG AGGGTGGGTGAAGCAGTTGCAAATAGATTACAAAGGCAACGAAATAATGACG GCAGCTGCCTCTCAAGGGCTGACCCGTAAAGGTGTGTATGCCGTTCGGTACA AGGTGAATGAAAATCCTCTGTACGCACCCACCTCAGAACATGTGAACGTCCT

	ACTGACCCGCACGGAGGACCGCATCGTGTGGAAAACACTAGCCGGCGACCCA
	TGGATAAAAACACTGACTGCCAAGTACCCTGGGAATTTCACTGCCACGATAG
	AGGAGTGGCAAGCAGAGCATGATGCCATCATGAGGCACATCTTGGAGAGACC
	GGACCCTACCGACGTCTTCCAGAATAAGGCAAACGTGTGTTGGGCCAAGGCT
	TTAGTGCCGGTGCTGAAGACCGCTGGCATAGACATGACCACTGAACAATGGA
	ACACTGTGGATTATTTTGAAACGGACAAAGCTCACTCAGCAGAGATAGTATT
	GAACCAACTATGCGTGAGGTTCTTTGGACTCGATCTGGACTCCGGTCTATTT
	TCTGCACCCACTGTTCCGTTATCCATTAGGAATAATCACTGGGATAACTCCC
	CGTCGCCTAACATGTACGGGCTGAATAAAGAAGTGGTCCGTCAGCTCTCTCG
	CAGGTACCCACAACTGCCTCGGGCAGTTGCCACTGGAAGAGTCTATGACATG
	AACACTGGTACACTGCGCAATTATGATCCGCGCATAAACCTAGTACCTGTAA
	ACAGAAGACTGCCTCATGCTTTAGTCCTCCACCATAATGAACACCCACAGAG
	TGACTTTTCTTCATTCGTCAGCAAATTGAAGGGCAGAACTGTCCTGGTGGTC
	GGGGAAAAGTTGTCCGTCCCAGGCAAAATGGTTGACTGGTTGTCAGACCGGC
	CTGAGGCTACCTTCAGAGCTCGGCTGGATTTAGGCATCCCAGGTGATGTGCC
	CAAATATGACATAATATTTGTTAATGTGAGGACCCCATATAAATACCATCAC
	TATCAGCAGTGTGAAGACCATGCCATTAAGCTTAGCATGTTGACCAAGAAAG
	CTTGTCTGCATCTGAATCCCGGCGGAACCTGTGTCAGCATAGGTTATGGTTA
	CGCTGACAGGGCCAGCGAAAGCATCATTGGTGCTATAGCGCGGCAGTTCAAG
	TTTTCCCGGGTATGCAAACCGAAATCCTCACTTGAAGAGACGGAAGTTCTGT
	TTGTATTCATTGGGTACGATCGCAAGGCCCGTACGCACAATCCTTACAAGCT
	TTCATCAACCTTGACCAACATTTATACAGGTTCCAGACTCCACGAAGCCGGA
	TGTGCACCCTCATATCATGTGGTGCGAGGGGATATTGCCACGGCCACCGAAG
	GAGTGATTATAAATGCTGCTAACAGCAAAGGACAACCTGGCGGAGGGGTGTG
	CGGAGCGCTGTATAAGAAATTCCCGGAAAGCTTCGATTTACAGCCGATCGAA
	GTAGGAAAAGCGCGACTGGTCAAAGGTGCAGCTAAACATATCATTCATGCCG
	TAGGACCAAACTTCAACAAAGTTTCGGAGGTTGAAGGTGACAAACAGTTGGC
	AGAGGCTTATGAGTCCATCGCTAAGATTGTCAACGATAACAATTACAAGTCA
	GTAGCGATTCCACTGTTGTCCACCGGCATCTTTTCCGGGAACAAAGATCGAC
	TAACCCAATCATTGAACCATTTGCTGACAGCTTTAGACACCACTGATGCAGA
	TGTAGCCATATACTGCAGGGACAAGAAATGGGAAATGACTCTCAAGGAAGCA
	GTGGCTAGGAGAGAAGCAGTGGAGGAGATATGCATATCCGACGACTCTTCAG
	TGACAGAACCTGATGCAGAGCTGGTGAGGGTGCATCCGAAGAGTTCTTTGGC
	TGGAAGGAAGGGCTACAGCACAAGCGATGGCAAAACTTTCTCATATTTGGAA
	GGGACCAAGTTTCACCAGGCGGCCAAGGATATAGCAGAAATTAATGCCATGT
	GGCCCGTTGCAACGGAGGCCAATGAGCAGGTATGCATGTATATCCTCGGAGA
	AAGCATGAGCAGTATTAGGTCGAAATGCCCCGTCGAAGAGTCGGAAGCCTCC
	ACACCACCTAGCACGCTGCCTTGCTTGTGCATCCATGCCATGACTCCAGAAA
	GAGTACAGCGCCTAAAAGCCTCACGTCCAGAACAAATTACTGTGTGCTCATC
	CTTTCCATTGCCGAAGTATAGAATCACTGGTGTGCAGAAGATCCAATGCTCC
	CAGCCTATATTGTTCTCACCGAAAGTGCCTGCGTATATTCATCCAAGGAAGT
	ATCTCGTGGAAACACCACCGGTAGACGAGACTCCGGAGCCATCGGCAGAGAA
	CCAATCCACAGAGGGGACACCTGAACAACCACCACTTATAACCGAGGATGAG
	ACCAGGACTAGAACGCCTGAGCCGATCATCATCGAAGAGGAAGAAGAGGATA
	GCATAAGTTTGCTGTCAGATGGCCCGACCCACCAGGTGCTGCAAGTCGAGGC
	AGACATTCACGGGCCGCCCTCTGTATCTAGCTCATCCTGGTCCATTCCTCAT
	GCATCCGACTTTGATGTGGACAGTTTATCCATACTTGACACCCTGGAGGGAG
	CTAGCGTGACCAGCGGGGCAACGTCAGCCGAGACTAACTCTTACTTCGCAAA
	GAGTATGGAGTTTCTGGCGCGACCGGTGCCTGCGCCTCGAACAGTATTCAGG
	AACCCTCCACATCCCGCTCCGCGCACAAGAACACCGTCACTTGCACCCAGCA
	GGGCCTGCTCGAGAACCAGCCTAGTTTCCACCCCGCCAGGCGTGAATAGGGT
	GATCACTAGAGAGGAGCTCGAGGCGCTTACCCCGTCACGCACTCCTAGCAGG
	TCGGTCTCGAGAACCAGCCTGGTCTCCAACCCGCCAGGCGTAAATAGGGTGA
	TTACAAGAGAGGAGTTTGAGGCGTTCGTAGCACAACAACAATGACGGTTTGA

	TGCGGGTGCATACATCTTTTCCTCCGACACCGGTCAAGGGCATTTACAACAA AAATCAGTAAGGCAAACGGTGCTATCCGAAGTGGTGTTGGAGAGGACCGAAT TGGAGATTTCGTATGCCCCGCGCCTCGACCAAGAAAAAGAAGAATTACTACG CAAGAAATTACAGTTAAATCCCACACCTGCTAACAGAAGCAGATACCAGTCC AGGAAGGTGGAGAACATGAAAGCCATAACAGCTAGACGTATTCTGCAAGGCC TAGGGCATTATTTGAAGGCAGAAGGAAAAGTGGAGTGCTACCGAACCCTGCA TCCTGTTCCTTTGTATTCATCTAGTGTGAACCGTGCCTTTTCAAGCCCCAAG GTCGCAGTGGAAGCCTGTAACGCCATGTTGAAAGAGAACTTTCCGACTGTGG CTTCTTACTGTATTATTCCAGAGTACGATGCCTATTTGGACATGGTTGACGG AGCTTCATGCTGCTTAGACACTGCCAGTTTTTGCCCTGCAAAGCTGCGCAGC TTTCCAAAGAAACACTCCTATTTGGAACCCACAATACGATCGGCAGTGCCTT CAGCGATCCAGAACACGCTCCAGAACGTCCTGGCAGCTGCCACAAAAAGAAA TTGCAATGTCACGCAAATGAGAGAATTGCCCGTATTGGATTCGGCGGCCTTT AATGTGGAATGCTTCAAGAAATATGCGTGTAATAATGAATATTGGGAAACGT TTAAAGAAAACCCCATCAGGCTTACTGAAGAAAACGTGGTAAATTACATTAC CAAATTAAAAGGACCAAAAGCTGCTGCTCTTTTTGCGAAGACACATAATTTG AATATGTTGCAGGACATACCAATGGACAGGTTTGTAATGGACTTAAAGAGAG ACGTGAAAGTGACTCCAGGAACAAAACATACTGAAGAACGGCCCAAGGTACA GGTGATCCAGGCTGCCGATCCGCTAGCAACAGCGTATCTGTGCGGAATCCAC CGAGAGCTGGTTAGGAGATTAAATGCGGTCCTGCTTCCGAACATTCATACAC TGTTTGATATGTCGGCTGAAGACTTTGACGCTATTATAGCCGAGCACTTCCA GCCTGGGGATTGTGTTCTGGAAACTGACATCGCGTCGTTTGATAAAAGTGAG GACGACGCCATGGCTCTGACCGCGTTAATGATTCTGGAAGACTTAGGTGTGG ACGCAGAGCTGTTGACGCTGATTGAGGCGGCTTTCGGCGAAATTTCATCAAT ACATTTGCCCACTAAAACTAAATTTAAATTCGGAGCCATGATGAAATCTGGA ATGTTCCTCACACTGTTTGTGAACACAGTCATTAACATTGTAATCGCAAGCA GAGTGTTGAGAGAACGGCTAACCGGATCACCATGTGCAGCATTCATTGGAGA TGACAATATCGTGAAAGGAGTCAAATCGGACAAATTAATGGCAGACAGGTGC GCCACCTGGTTGAATATGGAAGTCAAGATTATAGATGCTGTGGTGGGCGAGA AAGCGCCTTATTTCTGTGGAGGGTTTATTTTGTGTGACTCCGTGACCGGCAC AGCGTGCCGTGTGGCAGACCCCCTAAAAAGGCTGTTTAAGCTTGGCAAACCT CTGGCAGCAGACGATGAACATGATGATGACAGGAGAAGGGCATTGCATGAAG AGTCAACACGCTGGAACCGAGTGGGTATTCTTTCAGAGCTGTGCAAGGCAGT AGAATCAAGGTATGAAACCGTAGGAACTTCCATCATAGTTATGGCCATGACT ACTCTAGCTAGCAGTGTTAAATCATTCAGCTACCTGAGAGGGGCCCCTATAA CTCTCTACGGCTAACCTGAATGGACTACGACATAGTCTAGTCCGCCAAGGCC ACCGATATCTATGTTACGTGCAAAGGTGATTGTCACCCCCCGAAAGACCATA TTGTGACACACCCTCAGTATCACGCCCAAACATTTACAGCCGCGGTGTCAAA AACCGCGTGGACGTGGTTAACATCCCTGCTGGGAGGATCAGCCGTAATTATT ATAATTGGCTTGGTGCTGGCTACTATTGTGGCCATGTACGTGCTGACCAACC AGAAACATAATTGAATACAGCAGCAATTGGCAAGCTGCTTACATAGAACTCG CGGCGATTGGCATGCCGCCTTAAAATTTTTATTTTATTTTTCTTTTCTTTTC CGAATCGGATTTTGTTTTTAATATTTCAAAAAAAAAAAAAAAAAAAAAAAA AAAAAAAAAAAAAAAAaaagaagagcGCAGCTCTGGCCCGTGTCTCAAAATC TCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACT GTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTCAACGG GAAACGTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGT ATAAATGGGCTCGCGATAATGTCGGGCAATCAGGTGCGACAATCTATCGCTT GTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGC GTTGCCAATGATGTTACAGATGAGATGGTCAGACTAAACTGGCTGACGGAAT TTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATG GTTACTCACCACTGCGATCCCCGGAAAAACAGCATTCCAGGTATTAGAAGAA TATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCC GGTTGCATTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATT

	TCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGT GATTTTGATGACGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAA TGCATAAACTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTT CTCACTTGATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGAT GTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGA ACTGCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATA TGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGAT GAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTA CGCTGACTTGACGGGACGGCGCAAGCTCATGACCAAAATCCCTTAACGTGAG TTACGCGTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCT TCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAAC CACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTT TCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTA GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACAT ACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTC GTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGG TCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCT ACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCC CGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGA GAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTG TCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGG GGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTG GCCTTTTGCTGGCCTTTTGCTCACAT

Table 3. Exemplary VEE Replicon Sequences and Additional Exemplary Sequences

miR-142 sensor sequence (SEQ ID NO: 3)	TCCATAAAGTAGGAAACACTACA
miR-22 sensor sequence (SEQ ID NO: 6)	ACAGTTCTTCAACTGGCAGCTT
miR-185 sensor sequence (SEQ ID NO: 9)	TCAGGAACTGCCTTTCTCTCCA
Spacer sequence (SEQ ID NO: 10)	GCGGCCGCTAAA
Replicon expressing human IL-12 and bearing miR-142 sensors and miR-22 sensors (SEQ ID NO: 11)	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAA AGTTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGG AGCTTCCCGCAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATG CTAATGCCAGAGCGTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGT GGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATG TATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATC CGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAAT AACTGATAAGGAATTGGACAAGAAAATGAAGGAGCTCGCCGCCGTCATGAGC GACCCTGACCTGGAAACTGAGACTATGTGCCTCCACGACGACGAGTCGTGTC GCTACGAAGGGCAAGTCGCTGTTTACCAGGATGTATACGCGGTTGACGGACC GACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAGTCGCCTACTGGATA GGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATATCCAT CATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGG CCTATGCAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTT AGAAAGAAGTATTTGAAACCATCCAACAATGTTCTATTCTCTGTTGGCTCGA CCATCTACCACGAGAAGAGGGACTTACTGAGGAGCTGGCACCTGCCGTCTGT

	ATTTCACTTACGTGGCAAGCAAAATTACACATGTCGGTGTGAGACTATAGTT AGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCCTGTATG GGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTG CAAAGTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACG TATGTGCCAGCTACATTGTGTGACCAAATGACTGGCATACTGGCAACAGATG TCAGTGCGGACGACGCGCAAAAACTGCTGGTTGGGCTCAACCAGCGTATAGT CGTCAACGGTCGCACCCAGAGAAACACCAATACCATGAAAAATTACCTTTTG CCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAAGGAAGATC AAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTG TTGTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGAT ACCCAAACCATCATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCA GGATAGGCAGTAACACATTGGAGATCGGGCTGAGAACAAGAATCAGGAAAAT GTTAGAGGAGCACAAGGAGCCGTCACCTCTCATTACCGCCGAGGACGTACAA GAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAAGCCGAGGAGT TGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA AGCCGATGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACA CCTCGTGGCTTGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCT CTTACGCTGTGCTTTCTCCGCAGGCTGTACTCAAGAGTGAAAAATTATCTTG CATCCACCCTCTCGCTGAACAAGTCATAGTGATAACACACTCTGGCCGAAAA GGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGGTGCCAGAGGGAC ATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATTGT GTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACAT GGAGGAGCGCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCG AGCACGACGGCGAATACCTGTACGACATCGACAGGAAACAGTGCGTCAAGAA AGAACTAGTCACTGGGCTAGGGCTCACAGGCGAGCTGGTGGATCCTCCCTTC CATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGCCGCTCCTTACCAAG TACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCATCAT TAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAAC TGTGCAGAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATG CCAGAACTGTGGACTCAGTGCTCTTGAATGGATGCAAACACCCCGTAGAGAC CCTGTATATTGACGAAGCTTTTGCTTGTCATGCAGGTACTCTCAGAGCGCTC ATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGCGGGGATCCCAAACAGT GCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGAGATTTG CACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACT TCGGTCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGA AAGAGACTAAGATTGTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGA CGATCTCATTCTCACTTGTTTCAGAGGGTGGGTGAAGCAGTTGCAAATAGAT TACAAAGGCAACGAAATAATGACGGCAGCTGCCTCTCAAGGGCTGACCCGTA AAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTGTACGCACC CACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTG TGGAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACC CTGGGAATTTCACTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCAT CATGAGGCACATCTTGGAGAGACCGGACCCTACCGACGTCTTCCAGAATAAG GCAAACGTGTGTTGGGCCAAGGCTTTAGTGCCGGTGCTGAAGACCGCTGGCA TAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTGAAACGGACAA AGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTA GGAATAATCACTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAA AGAAGTGGTCCGTCAGCTCTCTCGCAGGTACCCACAACTGCCTCGGGCAGTT GCCACTGGAAGAGTCTATGACATGAACACTGGTACACTGCGCAATTATGATC CGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCATGCTTTAGTCCT CCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAATTG AAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAA TGGTTGACTGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGA

	TTTAGGCATCCCAGGTGATGTGCCCAAATATGACATAATATTTGTTAATGTG AGGACCCCATATAAATACCATCACTATCAGCAGTGTGAAGACCATGCCATTA AGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTGAATCCCGGCGGAAC CTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCATCATT GGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCT CACTTGAAGAGACGGAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGC CCGTACGCACAATCCTTACAAGCTTTCATCAACCTTGACCAACATTTATACA GGTTCCAGACTCCACGAAGCCGGATGTGCACCCTCATATCATGTGGTGCGAG GGGATATTGCCACGGCCACCGAAGGAGTGATTATAAATGCTGCTAACAGCAA AGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTCCCGGAA AGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTG CAGCTAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGA GGTTGAAGGTGACAAACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATT GTCAACGATAACAATTACAAGTCAGTAGCGATTCCACTGTTGTCCACCGGCA TCTTTTCCGGGAACAAAGATCGACTAACCCAATCATTGAACCATTTGCTGAC AGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGGACAAGAAA TGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGA TATGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAG GGTGCATCCGAAGAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGAT GGCAAAACTTTCTCATATTTGGAAGGGACCAAGTTTCACCAGGCGGCCAAGG ATATAGCAGAAATTAATGCCATGTGGCCCGTTGCAACGGAGGCCAATGAGCA GGTATGCATGTATATCCTCGGAGAAAGCATGAGCAGTATTAGGTCGAAATGC CCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCC AGAACAAATTACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACT GGTGTGCAGAAGATCCAATGCTCCCAGCCTATATTGTTCTCACCGAAAGTGC CTGCGTATATTCATCCAAGGAAGTATCTCGTGGAAACACCACCGGTAGACGA GACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGGACACCTGAACAA CCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGATCA TCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGAC CCACCAGGTGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCT AGCTCATCCTGGTCCATTCCTCATGCATCCGACTTTGATGTGGACAGTTTAT CCATACTTGACACCCTGGAGGGAGCTAGCGTGACCAGCGGGGCAACGTCAGC CGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTCTGGCGCGACCGGTG CCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGCACAA GAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTC CACCCCGCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTT ACCCCGTCACGCACTCCTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCA ACCCGCCAGGCGTAAATAGGGTGATTACAAGAGAGGAGTTTGAGGCGTTCGT AGCACAACAACAATGACGGTTTGATGCGGGTGCATACATCTTTTCCTCCGAC ACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGCTATCCG AAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGA CCAAGAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCT GCTAACAGAAGCAGATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAA CAGCTAGACGTATTCTGCAAGGCCTAGGGCATTATTTGAAGGCAGAAGGAAA AGTGGAGTGCTACCGAACCCTGCATCCTGTTCCTTTGTATTCATCTAGTGTG AACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAACGCCATGT TGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGA TGCCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGT TTTTGCCCTGCAAAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAAC CCACAATACGATCGGCAGTGCCTTCAGCGATCCAGAACACGCTCCAGAACGT CCTGGCAGCTGCCACAAAAAGAAATTGCAATGTCACGCAAATGAGAGAATTG CCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAGAAATATGCGT GTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA

	AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCT CTTTTTGCGAAGACACATAATTTGAATATGTTGCAGGACATACCAATGGACA GGTTTGTAATGGACTTAAAGAGAGACGTGAAAGTGACTCCAGGAACAAAACA TACTGAAGAACGGCCCAAGGTACAGGTGATCCAGGCTGCCGATCCGCTAGCA ACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGAGATTAAATGCGG TCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTTGA CGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGAC ATCGCGTCGTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAA TGATTCTGGAAGACTTAGGTGTGGACGCAGAGCTGTTGACGCTGATTGAGGC GGCTTTCGGCGAAATTTCATCAATACATTTGCCCACTAAAACTAAATTTAAA TTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACTGTTTGTGAACACAG TCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCGGATC ACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCG GACAAATTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGA TTATAGATGCTGTGGTGGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTAT TTTGTGTGACTCCGTGACCGGCACAGCGTGCCGTGTGGCAGACCCCCTAAAA AGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGACGATGAACATGATGATG ACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGTGGGTAT TCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACT TCCATCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCA GCTACCTGAGAGGGGCCCCTATAACTCTCTACGGCTAACCTGAATGGACTAC GACATAGTCTAGTCCGCCAAGGCCACCATGAGGGTCCCCGCTCAGCTCCTGG GGCTCCTGCTGCTCTGGCTCCCAGGTGCACGATGTGCCATCTGGGAGCTGAA GAAAGACGTGTACGTGGTGGAGCTTGACTGGTACCCCGACGCCCCCGGTGAA ATGGTGGTTCTGACCTGCGACACACCAGAGGAGGACGGCATCACCTGGACCC TGGACCAGTCCAGCGAGGTCCTGGGATCTGGCAAGACCCTGACCATCCAGGT TAAGGAATTTGGCGACGCCGGCCAGTACACCTGCCACAAAGGCGGCGAGGTC CTTTCGCACAGTCTGCTGCTGCTGCATAAAAAGGAGGACGGCATTTGGAGCA CCGACATTCTGAAGGATCAGAAAGAGCCCAAGAACAAGACCTTTCTGAGATG TGAGGCCAAAAACTACTCTGGACGCTTCACCTGTTGGTGGCTGACCACCATC AGCACAGACCTGACCTTCTCGGTGAAGTCTAGTAGGGGCAGCAGTGACCCCC AGGGCGTAACATGCGGCGCCGCTACCCTGAGCGCCGAGAGAGTGAGAGGCGA TAACAAGGAGTACGAGTACTCCGTGGAGTGCCAAGAGGACTCAGCCTGCCCC GCCGCCGAGGAGTCGCTGCCCATCGAGGTGATGGTGGATGCAGTGCACAAGC TGAAGTACGAGAACTACACCAGTAGCTTTTTCATCAGAGATATCATTAAACC CGACCCTCCCAAGAACCTGCAGCTGAAGCCCTTAAAGAACAGCCGGCAGGTG GAAGTGTCATGGGAGTACCCAGACACCTGGAGCACTCCGCACAGCTACTTCA GCCTGACCTTCTGCGTTCAGGTGCAGGGAAAAAGCAAGAGAGAGAAGAAAGA CAGAGTGTTCACCGACAAGACCAGCGCAACCGTGATCTGTAGAAAGAACGCC TCGATCAGCGTGAGAGCCCAGGACAGATACTACAGCAGCAGCTGGAGCGAGT GGGCCAGTGTACCTTGCAGCGGTGGAAGCGGCGGAGGCTCTGGCGGAGGGAG TGGCGGAGGCAGCAGAAATCTTCCAGTAGCTACCCCCGACCCCGGCATGTTT CCCTGTCTGCACCATTCTCAAAACCTGTTACGGGCCGTGAGCAACATGCTGC AGAAGGCCAGACAGACACTGGAGTTTTACCCCTGTACCTCAGAGGAGATCGA TCATGAGGACATTACTAAGGACAAGACCAGCACCGTGGAAGCCTGCCTGCCC CTAGAGCTAACCAAGAACGAGAGCTGCCTGAACTCTAGAGAGACAAGCTTCA TCACGAACGGCTCATGCCTGGCCAGTAGGAAAACCAGCTTCATGATGGCTCT GTGCCTGAGCTCCATATATGAGGACCTTAAGATGTACCAGGTGGAGTTCAAA ACCATGAACGCCAAGCTGCTGATGGACCCAAAGAGACAGATCTTCCTTGACC AGAACATGCTGGCCGTTATCGATGAGCTGATGCAGGCCCTGAACTTCAACAG CGAGACCGTGCCCCAGAAAAGCAGCCTGGAGGAACCCGACTTCTACAAAACC AAGATCAAGCTGTGCATTCTGCTGCATGCCTTCCGCATTAGAGCCGTGACCA TCGATAGGGTGATGAGCTACCTGAACGCCAGCTGATAAGCGGCCGCTAAATC CATAAAGTAGGAAACACTACATCCATAAAGTAGGAAACACTACATCCATAAA

	GTAGGAAACACTACATCCATAAAGTAGGAAACACTACAGCAACAGTTCTTCA ACTGGCAGCTTACAGTTCTTCAACTGGCAGCTTACAGTTCTTCAACTGGCAG CTTACAGTTCTTCAACTGGCAGCTTTATGTTACGTGCAAAGGTGATTGTCAC CCCCCGAAAGACCATATTGTGACACACCCTCAGTATCACGCCCAAACATTTA CAGCCGCGGTGTCAAAAACCGCGTGGACGTGGTTAACATCCCTGCTGGGAGG ATCAGCCGTAATTATTATAATTGGCTTGGTGCTGGCTACTATTGTGGCCATG TACGTGCTGACCAACCAGAAACATAATTGAATACAGCAGCAATTGGCAAGCT GCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATTTTTATTTTA TTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Replicon expressing human IL-12 and bearing dual sensor construct (VEE Genomic 5'UTR – VEE non-structural proteins (nsPs) – VEE Subgenomic 5'UTR – Kozak sequence – Coding sequence for human IL-12 and signal peptide – Double stop codons – Spacer – 4 tandem miR-142-3p sensors – 4 tandem miR-185-5p sensors – 3'UTR – PolyA tail) (SEQ ID NO: 12)	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAA AGTTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGG AGCTTCCCGCAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATG CTAATGCCAGAGCGTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGT GGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATG TATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATC CGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAAT AACTGATAAGGAATTGGACAAGAAAATGAAGGAGCTCGCCGCCGTCATGAGC GACCCTGACCTGGAAACTGAGACTATGTGCCTCCACGACGACGAGTCGTGTC GCTACGAAGGGCAAGTCGCTGTTTACCAGGATGTATACGCGGTTGACGGACC GACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAGTCGCCTACTGGATA GGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATATCCAT CATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGG CCTATGCAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTT AGAAAGAAGTATTTGAAACCATCCAACAATGTTCTATTCTCTGTTGGCTCGA CCATCTACCACGAGAAGAGGGACTTACTGAGGAGCTGGCACCTGCCGTCTGT ATTTCACTTACGTGGCAAGCAAAATTACACATGTCGGTGTGAGACTATAGTT AGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCCTGTATG GGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTG CAAAGTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACG TATGTGCCAGCTACATTGTGTGACCAAATGACTGGCATACTGGCAACAGATG TCAGTGCGGACGACGCGCAAAAACTGCTGGTTGGGCTCAACCAGCGTATAGT CGTCAACGGTCGCACCCAGAGAAACACCAATACCATGAAAAATTACCTTTTG CCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAAGGAAGATC AAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTG TTGTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGAT ACCCAAACCATCATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCA GGATAGGCAGTAACACATTGGAGATCGGGCTGAGAACAAGAATCAGGAAAAT GTTAGAGGAGCACAAGGAGCCGTCACCTCTCATTACCGCCGAGGACGTACAA GAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAAGCCGAGGAGT TGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA AGCCGATGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACA CCTCGTGGCTTGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCT CTTACGCTGTGCTTTCTCCGCAGGCTGTACTCAAGAGTGAAAAATTATCTTG CATCCACCCTCTCGCTGAACAAGTCATAGTGATAACACACTCTGGCCGAAAA GGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGGTGCCAGAGGGAC ATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATTGT GTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACAT GGAGGAGCGCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCG AGCACGACGGCGAATACCTGTACGACATCGACAGGAAACAGTGCGTCAAGAA AGAACTAGTCACTGGGCTAGGGCTCACAGGCGAGCTGGTGGATCCTCCCTTC CATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGCCGCTCCTTACCAAG TACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCATCAT TAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAAC

	TGTGCAGAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATG CCAGAACTGTGGACTCAGTGCTCTTGAATGGATGCAAACACCCCGTAGAGAC CCTGTATATTGACGAAGCTTTTGCTTGTCATGCAGGTACTCTCAGAGCGCTC ATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGCGGGGATCCCAAACAGT GCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGAGATTTG CACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACT TCGGTCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGA AAGAGACTAAGATTGTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGA CGATCTCATTCTCACTTGTTTCAGAGGGTGGGTGAAGCAGTTGCAAATAGAT TACAAAGGCAACGAAATAATGACGGCAGCTGCCTCTCAAGGGCTGACCCGTA AAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTGTACGCACC CACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTG TGGAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACC CTGGGAATTTCACTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCAT CATGAGGCACATCTTGGAGAGACCGGACCCTACCGACGTCTTCCAGAATAAG GCAAACGTGTGTTGGGCCAAGGCTTTAGTGCCGGTGCTGAAGACCGCTGGCA TAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTGAAACGGACAA AGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTA GGAATAATCACTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAA AGAAGTGGTCCGTCAGCTCTCTCGCAGGTACCCACAACTGCCTCGGGCAGTT GCCACTGGAAGAGTCTATGACATGAACACTGGTACACTGCGCAATTATGATC CGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCATGCTTTAGTCCT CCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAATTG AAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAA TGGTTGACTGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGA TTTAGGCATCCCAGGTGATGTGCCCAAATATGACATAATATTTGTTAATGTG AGGACCCCATATAAATACCATCACTATCAGCAGTGTGAAGACCATGCCATTA AGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTGAATCCCGGCGGAAC CTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCATCATT GGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCT CACTTGAAGAGACGGAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGC CCGTACGCACAATCCTTACAAGCTTTCATCAACCTTGACCAACATTTATACA GGTTCCAGACTCCACGAAGCCGGATGTGCACCCTCATATCATGTGGTGCGAG GGGATATTGCCACGGCCACCGAAGGAGTGATTATAAATGCTGCTAACAGCAA AGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTCCCGGAA AGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTG CAGCTAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGA GGTTGAAGGTGACAAACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATT GTCAACGATAACAATTACAAGTCAGTAGCGATTCCACTGTTGTCCACCGGCA TCTTTTCCGGGAACAAAGATCGACTAACCCAATCATTGAACCATTTGCTGAC AGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGGACAAGAAA TGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGA TATGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAG GGTGCATCCGAAGAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGAT GGCAAAACTTTCTCATATTTGGAAGGGACCAAGTTTCACCAGGCGGCCAAGG ATATAGCAGAAATTAATGCCATGTGGCCCGTTGCAACGGAGGCCAATGAGCA GGTATGCATGTATATCCTCGGAGAAAGCATGAGCAGTATTAGGTCGAAATGC CCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCC AGAACAAATTACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACT GGTGTGCAGAAGATCCAATGCTCCCAGCCTATATTGTTCTCACCGAAAGTGC CTGCGTATATTCATCCAAGGAAGTATCTCGTGGAAACACCACCGGTAGACGA GACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGGACACCTGAACAA

	CCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGATCA
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	GAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTC
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	AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCT
	CTTTTTGCGAAGACACATAATTTGAATATGTTGCAGGACATACCAATGGACA
	GGTTTGTAATGGACTTAAAGAGAGACGTGAAAGTGACTCCAGGAACAAAACA
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	TCCATCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCA
	GCTACCTGAGAGGGGCCCCTATAACTCTCTACGGCTAACCTGAATGGACTAC
	GACATAGTCTAGTCCGCCAAGGCCACCATGAGGGTCCCCGCTCAGCTCCTGG
	GGCTCCTGCTGCTCTGGCTCCCAGGTGCACGATGTGCCATCTGGGAGCTGAA
	GAAAGACGTGTACGTGGTGGAGCTTGACTGGTACCCCGACGCCCCCGGTGAA
	ATGGTGGTTCTGACCTGCGACACACCAGAGGAGGACGGCATCACCTGGACCC
	TGGACCAGTCCAGCGAGGTCCTGGGATCTGGCAAGACCCTGACCATCCAGGT
	TAAGGAATTTGGCGACGCCGGCCAGTACACCTGCCACAAAGGCGGCGAGGTC
	CTTTCGCACAGTCTGCTGCTGCTGCATAAAAAGGAGGACGGCATTTGGAGCA

	CCGACATTCTGAAGGATCAGAAAGAGCCCAAGAACAAGACCTTTCTGAGATG TGAGGCCAAAAACTACTCTGGACGCTTCACCTGTTGGTGGCTGACCACCATC AGCACAGACCTGACCTTCTCGGTGAAGTCTAGTAGGGGCAGCAGTGACCCCC AGGGCGTAACATGCGGCGCCGCTACCCTGAGCGCCGAGAGAGTGAGAGGCGA TAACAAGGAGTACGAGTACTCCGTGGAGTGCCAAGAGGACTCAGCCTGCCCC GCCGCCGAGGAGTCGCTGCCCATCGAGGTGATGGTGGATGCAGTGCACAAGC TGAAGTACGAGAACTACACCAGTAGCTTTTTCATCAGAGATATCATTAAACC CGACCCTCCCAAGAACCTGCAGCTGAAGCCCTTAAAGAACAGCCGGCAGGTG GAAGTGTCATGGGAGTACCCAGACACCTGGAGCACTCCGCACAGCTACTTCA GCCTGACCTTCTGCGTTCAGGTGCAGGGAAAAAGCAAGAGAGAGAAGAAAGA CAGAGTGTTCACCGACAAGACCAGCGCAACCGTGATCTGTAGAAAGAACGCC TCGATCAGCGTGAGAGCCCAGGACAGATACTACAGCAGCAGCTGGAGCGAGT GGGCCAGTGTACCTTGCAGCGGTGGAAGCGGCGGAGGCTCTGGCGGAGGGAG TGGCGGAGGCAGCAGAAATCTTCCAGTAGCTACCCCCGACCCCGGCATGTTT CCCTGTCTGCACCATTCTCAAAACCTGTTACGGGCCGTGAGCAACATGCTGC AGAAGGCCAGACAGACACTGGAGTTTTACCCCTGTACCTCAGAGGAGATCGA TCATGAGGACATTACTAAGGACAAGACCAGCACCGTGGAAGCCTGCCTGCCC CTAGAGCTAACCAAGAACGAGAGCTGCCTGAACTCTAGAGAGACAAGCTTCA TCACGAACGGCTCATGCCTGGCCAGTAGGAAAACCAGCTTCATGATGGCTCT GTGCCTGAGCTCCATATATGAGGACCTTAAGATGTACCAGGTGGAGTTCAAA ACCATGAACGCCAAGCTGCTGATGGACCCAAAGAGACAGATCTTCCTTGACC AGAACATGCTGGCCGTTATCGATGAGCTGATGCAGGCCCTGAACTTCAACAG CGAGACCGTGCCCCAGAAAAGCAGCCTGGAGGAACCCGACTTCTACAAAACC AAGATCAAGCTGTGCATTCTGCTGCATGCCTTCCGCATTAGAGCCGTGACCA TCGATAGGGTGATGAGCTACCTGAACGCCAGCTGATAAGCGGCCGCTAAATC CATAAAGTAGGAAACACTACATCCATAAAGTAGGAAACACTACATCCATAAA GTAGGAAACACTACATCCATAAAGTAGGAAACACTACAGCATCAGGAACTGC CTTTCTCTCCATCAGGAACTGCCTTTCTCTCCATCAGGAACTGCCTTTCTCT CCATCAGGAACTGCCTTTCTCTCCATATGTTACGTGCAAAGGTGATTGTCAC CCCCCGAAAGACCATATTGTGACACACCCTCAGTATCACGCCCAAACATTTA CAGCCGCGGTGTCAAAAACCGCGTGGACGTGGTTAACATCCCTGCTGGGAGG ATCAGCCGTAATTATTATAATTGGCTTGGTGCTGGCTACTATTGTGGCCATG TACGTGCTGACCAACCAGAAACATAATTGAATACAGCAGCAATTGGCAAGCT GCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATTTTTATTTTA TTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTCAAAAAAAAA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Murine Surrogate Replicon expressing murine IL-12 and bearing dual sensor construct (VEE Genomic 5'UTR – VEE non-structural proteins (nsPs) – VEE Subgenomic 5'UTR – Kozak sequence – Coding sequence for murine IL-12 and signal peptide – Double stop codons – Spacer – 4 tandem miR-142-3p sensors	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAAATGGAGAA AGTTCACGTTGACATCGAGGAAGACAGCCCATTCCTCAGAGCTTTGCAGCGG AGCTTCCCGCAGTTTGAGGTAGAAGCCAAGCAGGTCACTGATAATGACCATG CTAATGCCAGAGCGTTTTCGCATCTGGCTTCAAAACTGATCGAAACGGAGGT GGACCCATCCGACACGATCCTTGACATTGGAAGTGCGCCCGCCCGCAGAATG TATTCTAAGCACAAGTATCATTGTATCTGTCCGATGAGATGTGCGGAAGATC CGGACAGATTGTATAAGTATGCAACTAAGCTGAAGAAAAACTGTAAGGAAAT AACTGATAAGGAATTGGACAAGAAAATGAAGGAGCTCGCCGCCGTCATGAGC GACCCTGACCTGGAAACTGAGACTATGTGCCTCCACGACGACGAGTCGTGTC GCTACGAAGGGCAAGTCGCTGTTTACCAGGATGTATACGCGGTTGACGGACC GACAAGTCTCTATCACCAAGCCAATAAGGGAGTTAGAGTCGCCTACTGGATA GGCTTTGACACCACCCCTTTTATGTTTAAGAACTTGGCTGGAGCATATCCAT CATACTCTACCAACTGGGCCGACGAAACCGTGTTAACGGCTCGTAACATAGG CCTATGCAGCTCTGACGTTATGGAGCGGTCACGTAGAGGGATGTCCATTCTT AGAAAGAAGTATTTGAAACCATCCAACAATGTTCTATTCTCTGTTGGCTCGA CCATCTACCACGAGAAGAGGGACTTACTGAGGAGCTGGCACCTGCCGTCTGT ATTTCACTTACGTGGCAAGCAAAATTACACATGTCGGTGTGAGACTATAGTT AGTTGCGACGGGTACGTCGTTAAAAGAATAGCTATCAGTCCAGGCCTGTATG

– 4 tandem miR-185-5p sensors – 3’UTR – PolyA tail) (SEQ ID NO: 13)	GGAAGCCTTCAGGCTATGCTGCTACGATGCACCGCGAGGGATTCTTGTGCTG CAAAGTGACAGACACATTGAACGGGGAGAGGGTCTCTTTTCCCGTGTGCACG TATGTGCCAGCTACATTGTGTGACCAAATGACTGGCATACTGGCAACAGATG TCAGTGCGGACGACGCGCAAAAACTGCTGGTTGGGCTCAACCAGCGTATAGT CGTCAACGGTCGCACCCAGAGAAACACCAATACCATGAAAAATTACCTTTTG CCCGTAGTGGCCCAGGCATTTGCTAGGTGGGCAAAGGAATATAAGGAAGATC AAGAAGATGAAAGGCCACTAGGACTACGAGATAGACAGTTAGTCATGGGGTG TTGTTGGGCTTTTAGAAGGCACAAGATAACATCTATTTATAAGCGCCCGGAT ACCCAAACCATCATCAAAGTGAACAGCGATTTCCACTCATTCGTGCTGCCCA GGATAGGCAGTAACACATTGGAGATCGGGCTGAGAACAAGAATCAGGAAAAT GTTAGAGGAGCACAAGGAGCCGTCACCTCTCATTACCGCCGAGGACGTACAA GAAGCTAAGTGCGCAGCCGATGAGGCTAAGGAGGTGCGTGAAGCCGAGGAGT TGCGCGCAGCTCTACCACCTTTGGCAGCTGATGTTGAGGAGCCCACTCTGGA AGCCGATGTCGACTTGATGTTACAAGAGGCTGGGGCCGGCTCAGTGGAGACA CCTCGTGGCTTGATAAAGGTTACCAGCTACGATGGCGAGGACAAGATCGGCT CTTACGCTGTGCTTTCTCCGCAGGCTGTACTCAAGAGTGAAAAATTATCTTG CATCCACCCTCTCGCTGAACAAGTCATAGTGATAACACACTCTGGCCGAAAA GGGCGTTATGCCGTGGAACCATACCATGGTAAAGTAGTGGTGCCAGAGGGAC ATGCAATACCCGTCCAGGACTTTCAAGCTCTGAGTGAAAGTGCCACCATTGT GTACAACGAACGTGAGTTCGTAAACAGGTACCTGCACCATATTGCCACACAT GGAGGAGCGCTGAACACTGATGAAGAATATTACAAAACTGTCAAGCCCAGCG AGCACGACGGCGAATACCTGTACGACATCGACAGGAAACAGTGCGTCAAGAA AGAACTAGTCACTGGGCTAGGGCTCACAGGCGAGCTGGTGGATCCTCCCTTC CATGAATTCGCCTACGAGAGTCTGAGAACACGACCAGCCGCTCCTTACCAAG TACCAACCATAGGGGTGTATGGCGTGCCAGGATCAGGCAAGTCTGGCATCAT TAAAAGCGCAGTCACCAAAAAAGATCTAGTGGTGAGCGCCAAGAAAGAAAAC TGTGCAGAAATTATAAGGGACGTCAAGAAAATGAAAGGGCTGGACGTCAATG CCAGAACTGTGGACTCAGTGCTCTTGAATGGATGCAAACACCCCGTAGAGAC CCTGTATATTGACGAAGCTTTTGCTTGTCATGCAGGTACTCTCAGAGCGCTC ATAGCCATTATAAGACCTAAAAAGGCAGTGCTCTGCGGGGATCCCAAACAGT GCGGTTTTTTTAACATGATGTGCCTGAAAGTGCATTTTAACCACGAGATTTG CACACAAGTCTTCCACAAAAGCATCTCTCGCCGTTGCACTAAATCTGTGACT TCGGTCGTCTCAACCTTGTTTTACGACAAAAAAATGAGAACGACGAATCCGA AAGAGACTAAGATTGTGATTGACACTACCGGCAGTACCAAACCTAAGCAGGA CGATCTCATTCTCACTTGTTTCAGAGGGTGGGTGAAGCAGTTGCAAATAGAT TACAAAGGCAACGAAATAATGACGGCAGCTGCCTCTCAAGGGCTGACCCGTA AAGGTGTGTATGCCGTTCGGTACAAGGTGAATGAAAATCCTCTGTACGCACC CACCTCAGAACATGTGAACGTCCTACTGACCCGCACGGAGGACCGCATCGTG TGGAAAACACTAGCCGGCGACCCATGGATAAAAACACTGACTGCCAAGTACC CTGGGAATTTCACTGCCACGATAGAGGAGTGGCAAGCAGAGCATGATGCCAT CATGAGGCACATCTTGGAGAGACCGGACCCTACCGACGTCTTCCAGAATAAG GCAAACGTGTGTTGGGCCAAGGCTTTAGTGCCGGTGCTGAAGACCGCTGGCA TAGACATGACCACTGAACAATGGAACACTGTGGATTATTTTGAAACGGACAA AGCTCACTCAGCAGAGATAGTATTGAACCAACTATGCGTGAGGTTCTTTGGA CTCGATCTGGACTCCGGTCTATTTTCTGCACCCACTGTTCCGTTATCCATTA GGAATAATCACTGGGATAACTCCCCGTCGCCTAACATGTACGGGCTGAATAA AGAAGTGGTCCGTCAGCTCTCTCGCAGGTACCCACAACTGCCTCGGGCAGTT GCCACTGGAAGAGTCTATGACATGAACACTGGTACACTGCGCAATTATGATC CGCGCATAAACCTAGTACCTGTAAACAGAAGACTGCCTCATGCTTTAGTCCT CCACCATAATGAACACCCACAGAGTGACTTTTCTTCATTCGTCAGCAAATTG AAGGGCAGAACTGTCCTGGTGGTCGGGGAAAAGTTGTCCGTCCCAGGCAAAA TGGTTGACTGGTTGTCAGACCGGCCTGAGGCTACCTTCAGAGCTCGGCTGGA TTTAGGCATCCCAGGTGATGTGCCCAAATATGACATAATATTTGTTAATGTG AGGACCCCATATAAATACCATCACTATCAGCAGTGTGAAGACCATGCCATTA

	AGCTTAGCATGTTGACCAAGAAAGCTTGTCTGCATCTGAATCCCGGCGGAAC CTGTGTCAGCATAGGTTATGGTTACGCTGACAGGGCCAGCGAAAGCATCATT GGTGCTATAGCGCGGCAGTTCAAGTTTTCCCGGGTATGCAAACCGAAATCCT CACTTGAAGAGACGGAAGTTCTGTTTGTATTCATTGGGTACGATCGCAAGGC CCGTACGCACAATCCTTACAAGCTTTCATCAACCTTGACCAACATTTATACA GGTTCCAGACTCCACGAAGCCGGATGTGCACCCTCATATCATGTGGTGCGAG GGGATATTGCCACGGCCACCGAAGGAGTGATTATAAATGCTGCTAACAGCAA AGGACAACCTGGCGGAGGGGTGTGCGGAGCGCTGTATAAGAAATTCCCGGAA AGCTTCGATTTACAGCCGATCGAAGTAGGAAAAGCGCGACTGGTCAAAGGTG CAGCTAAACATATCATTCATGCCGTAGGACCAAACTTCAACAAAGTTTCGGA GGTTGAAGGTGACAAACAGTTGGCAGAGGCTTATGAGTCCATCGCTAAGATT GTCAACGATAACAATTACAAGTCAGTAGCGATTCCACTGTTGTCCACCGGCA TCTTTTCCGGGAACAAAGATCGACTAACCCAATCATTGAACCATTTGCTGAC AGCTTTAGACACCACTGATGCAGATGTAGCCATATACTGCAGGGACAAGAAA TGGGAAATGACTCTCAAGGAAGCAGTGGCTAGGAGAGAAGCAGTGGAGGAGA TATGCATATCCGACGACTCTTCAGTGACAGAACCTGATGCAGAGCTGGTGAG GGTGCATCCGAAGAGTTCTTTGGCTGGAAGGAAGGGCTACAGCACAAGCGAT GGCAAAACTTTCTCATATTTGGAAGGGACCAAGTTTCACCAGGCGGCCAAGG ATATAGCAGAAATTAATGCCATGTGGCCCGTTGCAACGGAGGCCAATGAGCA GGTATGCATGTATATCCTCGGAGAAAGCATGAGCAGTATTAGGTCGAAATGC CCCGTCGAAGAGTCGGAAGCCTCCACACCACCTAGCACGCTGCCTTGCTTGT GCATCCATGCCATGACTCCAGAAAGAGTACAGCGCCTAAAAGCCTCACGTCC AGAACAAATTACTGTGTGCTCATCCTTTCCATTGCCGAAGTATAGAATCACT GGTGTGCAGAAGATCCAATGCTCCCAGCCTATATTGTTCTCACCGAAAGTGC CTGCGTATATTCATCCAAGGAAGTATCTCGTGGAAACACCACCGGTAGACGA GACTCCGGAGCCATCGGCAGAGAACCAATCCACAGAGGGGACACCTGAACAA CCACCACTTATAACCGAGGATGAGACCAGGACTAGAACGCCTGAGCCGATCA TCATCGAAGAGGAAGAAGAGGATAGCATAAGTTTGCTGTCAGATGGCCCGAC CCACCAGGTGCTGCAAGTCGAGGCAGACATTCACGGGCCGCCCTCTGTATCT AGCTCATCCTGGTCCATTCCTCATGCATCCGACTTTGATGTGGACAGTTTAT CCATACTTGACACCCTGGAGGGAGCTAGCGTGACCAGCGGGGCAACGTCAGC CGAGACTAACTCTTACTTCGCAAAGAGTATGGAGTTTCTGGCGCGACCGGTG CCTGCGCCTCGAACAGTATTCAGGAACCCTCCACATCCCGCTCCGCGCACAA GAACACCGTCACTTGCACCCAGCAGGGCCTGCTCGAGAACCAGCCTAGTTTC CACCCCGCCAGGCGTGAATAGGGTGATCACTAGAGAGGAGCTCGAGGCGCTT ACCCCGTCACGCACTCCTAGCAGGTCGGTCTCGAGAACCAGCCTGGTCTCCA ACCCGCCAGGCGTAAATAGGGTGATTACAAGAGAGGAGTTTGAGGCGTTCGT AGCACAACAACAATGACGGTTTGATGCGGGTGCATACATCTTTTCCTCCGAC ACCGGTCAAGGGCATTTACAACAAAAATCAGTAAGGCAAACGGTGCTATCCG AAGTGGTGTTGGAGAGGACCGAATTGGAGATTTCGTATGCCCCGCGCCTCGA CCAAGAAAAAGAAGAATTACTACGCAAGAAATTACAGTTAAATCCCACACCT GCTAACAGAAGCAGATACCAGTCCAGGAAGGTGGAGAACATGAAAGCCATAA CAGCTAGACGTATTCTGCAAGGCCTAGGGCATTATTTGAAGGCAGAAGGAAA AGTGGAGTGCTACCGAACCCTGCATCCTGTTCCTTTGTATTCATCTAGTGTG AACCGTGCCTTTTCAAGCCCCAAGGTCGCAGTGGAAGCCTGTAACGCCATGT TGAAAGAGAACTTTCCGACTGTGGCTTCTTACTGTATTATTCCAGAGTACGA TGCCTATTTGGACATGGTTGACGGAGCTTCATGCTGCTTAGACACTGCCAGT TTTTGCCCTGCAAAGCTGCGCAGCTTTCCAAAGAAACACTCCTATTTGGAAC CCACAATACGATCGGCAGTGCCTTCAGCGATCCAGAACACGCTCCAGAACGT CCTGGCAGCTGCCACAAAAAGAAATTGCAATGTCACGCAAATGAGAGAATTG CCCGTATTGGATTCGGCGGCCTTTAATGTGGAATGCTTCAAGAAATATGCGT GTAATAATGAATATTGGGAAACGTTTAAAGAAAACCCCATCAGGCTTACTGA AGAAAACGTGGTAAATTACATTACCAAATTAAAAGGACCAAAAGCTGCTGCT CTTTTTGCGAAGACACATAATTTGAATATGTTGCAGGACATACCAATGGACA

	GGTTTGTAATGGACTTAAAGAGAGACGTGAAAGTGACTCCAGGAACAAAACA TACTGAAGAACGGCCCAAGGTACAGGTGATCCAGGCTGCCGATCCGCTAGCA ACAGCGTATCTGTGCGGAATCCACCGAGAGCTGGTTAGGAGATTAAATGCGG TCCTGCTTCCGAACATTCATACACTGTTTGATATGTCGGCTGAAGACTTTGA CGCTATTATAGCCGAGCACTTCCAGCCTGGGGATTGTGTTCTGGAAACTGAC ATCGCGTCGTTTGATAAAAGTGAGGACGACGCCATGGCTCTGACCGCGTTAA TGATTCTGGAAGACTTAGGTGTGGACGCAGAGCTGTTGACGCTGATTGAGGC GGCTTTCGGCGAAATTTCATCAATACATTTGCCCACTAAAACTAAATTTAAA TTCGGAGCCATGATGAAATCTGGAATGTTCCTCACACTGTTTGTGAACACAG TCATTAACATTGTAATCGCAAGCAGAGTGTTGAGAGAACGGCTAACCGGATC ACCATGTGCAGCATTCATTGGAGATGACAATATCGTGAAAGGAGTCAAATCG GACAAATTAATGGCAGACAGGTGCGCCACCTGGTTGAATATGGAAGTCAAGA TTATAGATGCTGTGGTGGGCGAGAAAGCGCCTTATTTCTGTGGAGGGTTTAT TTTGTGTGACTCCGTGACCGGCACAGCGTGCCGTGTGGCAGACCCCCTAAAA AGGCTGTTTAAGCTTGGCAAACCTCTGGCAGCAGACGATGAACATGATGATG ACAGGAGAAGGGCATTGCATGAAGAGTCAACACGCTGGAACCGAGTGGGTAT TCTTTCAGAGCTGTGCAAGGCAGTAGAATCAAGGTATGAAACCGTAGGAACT TCCATCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCA GCTACCTGAGAGGGGCCCCTATAACTCTCTACGGCTAACCTGAATGGACTAC GACATAGTCTAGTCCGCCAAGgccaccATGAGGGTCCCCGCTCAGCTCCTGG GGCTCCTGCTGCTCTGGCTCCCAGGTGCACGATGTGCCATGTGGGAGCTGGA GAAAGACGTTTATGTTGTAGAGGTGGACTGGACTCCCGATGCCCCTGGAGAA ACAGTGAACCTCACCTGTGACACGCCTGAAGAAGATGACATCACCTGGACCT CAGACCAGAGACATGGAGTCATAGGCTCTGGAAAGACCCTGACCATCACTGT CAAAGAGTTTCTAGATGCTGGCCAGTACACCTGCCACAAAGGAGGCGAGACT CTGAGCCACTCACATCTGCTGCTCCACAAGAAGGAAAATGGAATTTGGTCCA CTGAAATTTTAAAAAATTTCAAAAACAAGACTTTCCTGAAGTGTGAAGCACC AAATTACTCCGGACGGTTCACGTGCTCATGGCTGGTGCAAAGAAACATGGAC TTGAAGTTCAACATCAAGAGCAGTAGCAGTTCCCCTGACTCTCGGGCAGTGA CATGTGGAATGGCGTCTCTGTCTGCAGAGAAGGTCACACTGGACCAAAGGGA CTATGAGAAGTATTCAGTGTCCTGCCAGGAGGATGTCACCTGCCCAACTGCC GAGGAGACACTGCCCATTGAACTGGCGTTGGAAGCACGGCAGCAGAATAAAT ATGAGAACTACAGCACCAGCTTCTTCATCAGGGACATCATCAAACCAGACCC GCCCAAGAACTTGCAGATGAAGCCTTTGAAGAACTCACAGGTGGAGGTCAGC TGGGAGTACCCTGACTCCTGGAGCACTCCCCATTCCTACTTCTCCCTCAAGT TCTTTGTTCGAATCCAGCGCAAGAAAGAAAAGATGAAGGAGACAGAGGAGGG GTGTAACCAGAAAGGTGCGTTCCTCGTAGAGAAGACATCTACCGAAGTCCAA TGCAAAGGCGGGAATGTCTGCGTGCAAGCTCAGGATCGCTATTACAATTCCT CATGCAGCAAGTGGGCATGTGTTCCCTGCAGGGTCCGATCCGGAGGTTCCGG TGGTGGATCCGGAGGTGGCTCCGGCGGCGGATCCAGGGTCATTCCAGTCTCT GGACCTGCCAGGTGTCTTAGCCAGTCCCGAAACCTGCTGAAGACCACAGATG ACATGGTGAAGACGGCCAGAGAAAAACTGAAACATTATTCCTGCACTGCTGA AGACATCGATCATGAAGACATCACACGGGACCAAACCAGCACATTGAAGACC TGTTTACCACTGGAACTACACAAGAACGAGAGTTGCCTGGCTACTAGAGAGA CTTCTTCCACAACAAGAGGGAGCTGCCTGCCCCCACAGAAGACGTCTTTGAT GATGACCCTGTGCCTTGGTAGCATCTATGAGGACTTGAAGATGTACCAGACA GAGTTCCAGGCCATCAACGCAGCACTTCAGAATCACAACCATCAGCAGATCA TTCTAGACAAGGGCATGCTGGTGGCCATCGATGAGCTGATGCAGTCTCTGAA TCATAATGGCGAGACTCTGCGCCAGAAACCTCCTGTGGGAGAAGCAGACCCT TACAGAGTGAAAATGAAGCTCTGCATCCTGCTTCACGCCTTCAGCACCCGCG TCGTGACCATCAACAGGGTGATGGGCTATCTGAGCTCCGCCtgataaGCGGC CGCTAAATCCATAAAGTAGGAAACACTACATCCATAAAGTAGGAAACACTAC ATCCATAAAGTAGGAAACACTACATCCATAAAGTAGGAAACACTACAGCATC AGGAACTGCCTTTCTCTCCATCAGGAACTGCCTTTCTCTCCATCAGGAACTG

	CCTTTCTCTCCATCAGGAACTGCCTTTCTCTCCATATGTTACGTGCAAAGGT GATTGTCACCCCCCGAAAGACCATATTGTGACACACCCTCAGTATCACGCCC AAACATTTACAGCCGCGGTGTCAAAAACCGCGTGGACGTGGTTAACATCCCT GCTGGGAGGATCAGCCGTAATTATTATAATTGGCTTGGTGCTGGCTACTATT GTGGCCATGTACGTGCTGACCAACCAGAAACATAATTGAATACAGCAGCAAT TGGCAAGCTGCTTACATAGAACTCGCGGCGATTGGCATGCCGCCTTAAAATT TTTATTTTATTTTTCTTTTCTTTTCCGAATCGGATTTTGTTTTTAATATTTC AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAaa
A1 Construct – includes (1) leader sequence; (2) IL-12β; (3) GS linker; and (4) IL-12α (SEQ ID NO: 17)	ATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCCCAGGTG CACGATGTGCCATCTGGGAGCTGAAGAAAGACGTGTACGTGGTGGAGCTTGA CTGGTACCCCGACGCCCCCGGTGAAATGGTGGTTCTGACCTGCGACACACCA GAGGAGGACGGCATCACCTGGACCCTGGACCAGTCCAGCGAGGTCCTGGGaT CTGGCAAGACCCTGACCATCCAGGTTAAGGAATTTGGCGACGCCGGCCAGTA CACCTGCCACAAAGGCGGCGAGGTCCTTTCGCACAGTCTGCTGCTGCTGCAT AAAAAGGAGGACGGCATTTGGAGCACCGACATTCTGAAGGATCAGAAAGAGC CCAAGAACAAGACCTTTCTGAGATGTGAGGCCAAAAACTACTCTGGACGCTT CACCTGTTGGTGGCTGACCACCATCAGCACAGACCTGACCTTCTCGGTGAAG TCTAGTAGGGGCAGCAGTGACCCCCAGGGCGTAACATGCGGCGCCGCTACCC TGAGCGCCGAGAGAGTGAGAGGCGATAACAAGGAGTACGAGTACTCCGTGGA GTGCCAAGAGGACTCAGCCTGCCCCGCCGCCGAGGAGTCGCTGCCCATCGAG GTGATGGTGGATGCAGTGCACAAGCTGAAGTACGAGAACTACACCAGTAGCT TTTTCATCAGAGATATCATTAAACCCGACCCTCCCAAGAACCTGCAGCTGAA GCCCTTAAAGAACAGCCGGCAGGTGGAAGTGTCATGGGAGTACCCAGACACC TGGAGCACTCCGCACAGCTACTTCAGCCTGACCTTCTGCGTTCAGGTGCAGG GAAAAAGCAAGAGAGAGAAGAAAGACAGAGTGTTCACCGACAAGACCAGCGC AACCGTGATCTGTAGAAAGAACGCCTCGATCAGCGTGAGAGCCCAGGACAGA TACTACAGCAGCAGCTGGAGCGAGTGGGCCAGTGTACCTTGCAGCGGTGGaA GCGGCGGaGGCTCTGGCGGaGGGAGTGGCGGaGGCAGCAGAAATCTTCCaGT AGCTACCCCCGACCCCGGCATGTTTCCCTGTCTGCACCATTCTCAAAACCTG TTACGGGCCGTGAGCAACATGCTGCAGAAGGCCAGACAGACACTGGAGTTTT ACCCCTGTACCTCAGAGGAGATCGATCATGAGGACATTACTAAGGACAAGAC CAGCACCGTGGAAGCCTGCCTGCCCCTAGAGCTAACCAAGAACGAGAGCTGC CTGAACTCTAGAGAGACAAGCTTCATCACGAACGGCTCATGCCTGGCCAGTA GGAAAACCAGCTTCATGATGGCTCTGTGCCTGAGCTCCATATATGAGGACCT TAAGATGTACCAGGTGGAGTTCAAAACCATGAACGCCAAGCTGCTGATGGAC CCAAAGAGACAGATCTTCCTTGACCAGAACATGCTGGCCGTTATCGATGAGC TGATGCAGGCCCTGAACTTCAACAGCGAGACCGTGCCCCAGAAAAGCAGCCT GGAGGAACCCGACTTCTACAAAACCAAGATCAAGCTGTGCATTCTGCTGCAT GCCTTCCGCATTAGAGCCGTGACCATCGATAGGGTGATGAGCTACCTGAACG CCAGC
Exemplary Kozak sequence GCCACC upstream of the start codon (AUG)	GCCACCATG
Exemplary subgenomic 5' UTR sequence (SEQ ID NO: 19)	ATGGACTACGACATAGTCTAGTCCGCCAAG
Exemplary 3' UTR sequence (SEQ ID NO: 20)	TATGTTACGTGCAAAGGTGATTGTCACCCCCCGAAAGACCATATTGTGACAC ACCCTCAGTATCACGCCCAAACATTTACAGCCGCGGTGTCAAAAACCGCGTG GACGTGGTTAACATCCCTGCTGGGAGGATCAGCCGTAATTATTATAATTGGC TTGGTGCTGGCTACTATTGTGGCCATGTACGTGCTGACCAACCAGAAACATA ATTGAATACAGCAGCAATTGGCAAGCTGCTTACATAGAACTCGCGGCGATTG

	GCATGCCGCCTTAAAATTTTTATTTTATTTTTCTTTTCTTTTCCGAATCGGA TTTTGTTTTTAATATTTC
Exemplary poly-A tail sequence (SEQ ID NO: 21)	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
Exemplary genomic 5' UTR sequence (SEQ ID NO: 22)	ATGGGCGGCGCATGAGAGAAGCCCAGACCAATTACCTACCCAAA
Exemplary subgenomic promoter sequence (SEQ ID NO: 23)	TCCATCATAGTTATGGCCATGACTACTCTAGCTAGCAGTGTTAAATCATTCA GCTACCTGAGAGGGGCCCCTATAACTCTCTACGGCTAACCTGAATGGA

EXAMPLE 2: EFFECTS OF DUAL miRNA-SENSING CIRCUITS ON SERUM LEVELS OF IL-12, IFN-α, AND IFN-γ

[0320] Next, the effects of dual miRNA-sensing circuits on systemic IL-12, IFN-α, and IFN-γ were assessed. Electrochemiluminescence was used to detect the payload mIL-12, as well as the downstream biomarkers IFN-γ and IFN-α. The above constructs described in Table 1 were loaded into separate nanoparticles and administered intravenously to naïve mice. Serum levels of mIL-12 (pg/mL, **FIG. 2A**), mIFN-α (pg/mL, **FIG. 2B**), and IFN-γ (pg/mL, **FIG. 2C**) were then measured 72, 6, and 72 hours, respectively, post I.V. administration. Levels of IL-12 (pg/mg, **FIG. 2D**), IFN-α (pg/mg, **FIG. 2E**), and IFN-γ (pg/mg, **FIG. 2F**) in spleen following administration of repRNA-mIL-12 I.V. or the dual miRNA-sensing circuit (i.e., repRNA-mIL-12+miR-142+miR-185) I.V. were measured. Body weight was also monitored over time and was expressed as relative to pre-dosing (day 0) body weight (**FIG. 2G**).

[0321] As shown in **FIGs. 2A-2C**, mice that received the mRNA-LNP with a miR-142 sensor alone had reduced serum levels of IFN-α (**FIG. 2B**) compared to all other groups; whereas serum IL-12 (**FIG. 2A**) and IFN-γ (**FIG. 2C)** levels were comparable to control mice that received mRNA encoding IL-12 alone (uncircuited).

[0322] Notably, mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor had reduced serum levels of IL-12 (**FIG. 2A**) and IFN-γ (**FIG. 2C**) compared to all other groups (i.e., control mice that received mRNA encoding IL-12 alone (uncircuited), mice that received the mRNA-LNP with a miR-142 sensor only, and mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor); whereas serum IFN-α (**FIG. 2B)** were reduced compared to control mice.

[0323] Mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor had reduced serum levels of IFN-α (**FIG. 2B**) IL-12 (by ~4-fold, **FIG. 2A**), and IFN-γ (by ~8-fold **FIG. 2C**) compared to control mice.

[0324] Notably, the presence of the mRNA-LNP with a miR-142 sensor and a miR-185 sensor lowered test article-related body weight loss as well as recovery time in naïve C57BL6/J mice (see **FIG. 2G**); when the mRNA-LNP with a miR-142 sensor and a miR-185 sensor were adminsitered to naïve mice, the mice show less body weight loss (see **FIG. 2G**).

[0325] This correlated with the lower levels of IL-12, IFN-γ, and IFN-α in circulation (e.g., a reduction of 75%, 87%, and 90%, respectively, in serum) as well as in the spleen (e.g., a reduction of 69%, 39%, and 96%, respectively) and other off-target tissues (as described in Example 1, compared to the uncircuted mRNA). Data shown is at the respective Tmax.

EXAMPLE 3: EFFECTS OF DUAL miRNA-SENSING CIRCUITS ON TISSUE PATHOLOGY ASSOCIATED WITH SYSTEMIC IL-12

[0326] Tissue (i.e, liver, spleen, and heart) histology profiles were further assessed in mice. Tissues were collected at different time points post-dosing, formalin-fixed, paraffin-embedded and subjected to microscopic histopathological examination by a board-certified pathologist. Specifically, spleen histiocytosis (e.g., hisiocytosis marginal zone/red pulp) (**FIG. 3A**), liver margination (e.g., intravascular margination of inflammatory cells) (**FIG. 3B**), kidney tubular dilation (**FIG. 3C**), and heart degeneration (e.g., degeneration/inflammatory infiltrates) (**FIG. 3D**) were assessed.

[0327] As shown in **FIGs. 3A-3D**, mice that received the mRNA-LNP with a miR-142 sensor alone had reduced spleen histiocytosis (**FIG. 3A**), liver margination (**FIG. 3B**), and kidney tubular dilation (**FIG. 3C**), compared to control mice that received mRNA encoding IL-12 alone (uncircuited), whereas heart degeneration (**FIG. 3D**) was comparable.

[0328] Notably, mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor showed a reduction (to grade 0) for spleen histiocytosis (**FIG. 3A**), liver margination (**FIG. 3B**), kidney tubular dilation (**FIG. 3C**), and heart degeneration (**FIG. 3D**) compared to control mice and mice that received the mRNA-LNP with a miR-142 sensor only. Further, of all groups, mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor showed the greatest reduction (to grade 0) in spleen histiocytosis (**FIG. 3A)** and kidney tubular dilation (**FIG. 3C**).

[0329] Mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor had reduced spleen histiocytosis (**FIG. 3A**), liver margination (**FIG. 3B**), and heart degeneration (**FIG. 3D**) compared to control mice and mice that received the mRNA-LNP with a miR-142 sensor only. Mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor had reduced kidney tubular dilation (**FIG. 3C**) compared to control mice.

[0330] Mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor and mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor both showed a reduction to grade 0 for liver margination and heart degeneration.

[0331] Liver pathology was assessed as shown in Table 4. Findings included intravascular margination of leukocytes, perivascular histiocyte infiltration, or Kupffer cell hyperplasia (see representative images in **FIGs. 4A-4B**, **FIGs. 5A-5B**, **FIG. 6**, and **FIG. 7**). Notably, liver obtained from mice treated with 0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-22 sensor appeared histologically normal (see **FIGs. 5A-5B**).

Table 4. Liver Pathology Assessment Summary

Condition	Representative Figure(s)
0.1 mg/kg repRNA-mIL-12	FIGs. 4A-4B, FIGs. 5A-5B, FIG. 6, and FIG. 7
0.1 mg/kg repRNA-mIL-12+miR-142 sensor	FIGs. 4A-4B
0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-22 sensor	FIGs. 5A-5B
0.1 mg/kg repRNA-mIL-12+miR-142 sensor +miR-185 sensor	FIG. 6
0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-185 sensor	FIG. 7

[0332] Additionally, administration of the dual circuits (i.e., repRNA-mIL-12+miR-142 sensor+miR-22 sensor and repRNA-mIL-12+miR-142 sensor+miR-185 sensor) rescued splenomegaly (see **FIG. 8**, **FIG. 9**, and **FIG. 10**) associated with systemic IL-12. The greatest amelioration in splenomegaly was observed in spleens obtained from mice treated with repRNA-mIL-12+miR-142 sensor+miR-22 sensor followed by mice treated with repRNA-mIL-12+miR-142 sensor+miR-185 sensor (see **FIGs. 8-10**).

[0333] Heart pathology was assessed as shown in Table 5. Findings included intravascular mononuclear cell infiltrates with perivascular extension (see **FIG. 12**, **FIG. 13**, and **FIG. 14**).

Table 5. Heart Pathology Assessment Summary

Condition	Representative Figure(s)
PBS	FIG. 12
0.1 mg/kg repRNA-mIL-12	FIG. 12, FIG. 13, and FIG. 14
0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-22 sensor	FIG. 13
0.1 mg/kg repRNA-mIL-12+miR-142 sensor+miR-185 sensor	FIG. 14

[0334] These results, together with the serum biomarker results (described in Example 2), show that the dual miRNA-sensing circuits are especially able to suppress IL-12 pharmacology effects in off-target tissues.

[0335] Specifically, the presence of the miR-142 sensor and a miR-185 sensor alleviated histopathological findings associated with systemic IL-12 exposure in liver, spleen, and heart of naïve mice seen at 17 days post-dose. Regarding histopathological findings associated with systemic IL-12 exposure in liver, mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor showed no incidence of intravascular margination of inflammatory cells (**FIG. 6B**) and Kupffer cell hyperplasia (**FIG. 6C**) compared to mice that received mRNA encoding IL-12 alone (uncircuited), thus being equivalent to vehicle control (**FIGs. 6B-6C**). Regarding histopathological findings associated with systemic IL-12 exposure in spleen, mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor showed reduced severity of histocytosis marginal zone/red pulp (**FIG. 9B**) and no incidence of extramedullary hematopoiesis (**FIG. 9C**) compared to mice that received mRNA encoding IL-12 alone (uncircuited). The absence of extramedullary hematopoiesis (**FIG. 9C**) was equivalent to vehicle control. Regarding histopathological findings associated with systemic IL-12 exposure in heart, mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor showed reduced severity of degeneration/inflammatory infiltrates (**FIG. 14B**) and no incidence of intravascular margination of inflammatory cells (**FIG. 14C**) compared to mice that received mRNA encoding IL-12 alone (uncircuited). The absence of degeneration/inflammatory infiltrates (**FIG. 14B**) was equivalent to vehicle control.

[0336] Furthermore, mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor showed robust expression of IL-12 in tumors and downregulation in healthy tissues and organs (94% lower in spleen, 96% lower in bone barrow, and 97% lower in heart), yielding lower circulating levels of IL-12 (88% lower in serum), when compared to mice that received mRNA encoding IL-12 alone (uncircuited). Notably, studies have shown IL-12 mRNA is present in the

serum at the same levels whether a circuit is included or not, but adding the circuit effectively limited how much IL-12 is present in the serum, due to the circuit leading to post-transcriptional silencing. Data not shown.

EXAMPLE 4: EFFECTS OF DUAL miRNA-SENSING CIRCUITS EXPRESS ON IL-12 IN TUMORS, TOLERABILITY, AND TUMOR GROWTH

[0337] The effects of dual miRNA-sensing circuits on payload expression within tumors in a B16.F10 melanoma model were assessed. B16.F10 melanoma cells were subcutaneously implanted into the right flank of C57Bl/6J mice to establish tumors. The constructs described in Table 1 were loaded into separate lipid nanoparticles and administered intravenously to mice implanted subcutaneously with B16-F10 melanoma cells. Once the tumors developed within the animals (i.e., reached a size of 100-150 mm^3), the above-described constructs (see Table 1) were administered intravenously. Serum IL-12 (**FIG. 15A**) and serum IFN-γ (**FIG. 15B**) levels from a B16.F10 melanoma model were assessed 6 and 72 hours, respectively, post-administration. IL-12 (**FIG. 16A**) and IFN-γ (**FIG. 16B**) level in tumor were assessed 6 and 72 hours, respectively, post-administration. RT-qPCR was used to detect nsP4 mRNA. Electrochemiluminescence was used to detect the payload mIL-12, as well as the downstream biomarker IFN-γ. The tumor volume was then measured periodically for 40 days (**FIG. 17**).

[0338] As shown in **FIG. 15A**, mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor had a ~39x reduction in serum levels of IL-12 compared to control mice that received mRNA encoding IL-12 alone (uncircuited), and mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor had a ~10x reduction in serum levels of IL-12 compared to control mice. As shown in **FIG. 15B**, mice that received the mRNA-LNP with a miR-142 sensor and a miR-22 sensor had a ~23x reduction in serum levels of IFN-γ compared to control mice, and mice that received the mRNA-LNP with a miR-142 sensor and a miR-185 sensor had a ~2x reduction in serum levels of IFN-γ compared to control mice.

[0339] While miRNA-sensing circuits reduced circulating levels of IL-12 and IFN-γ, as shown in **FIGs. 16A** and **16B** (and in **FIGs. 16D** and **16E**), miRNA-sensing circuits do not impair expression of IL-12 payload (or downstream IFN-γ) levels in tumors. IL-12 mRNA levels in tumors from mice that received the repRNA-mIL-12+miR-142 sensor+miR-185 sensor was also shown to be comparable (**FIG. 16C**) to control mice that received mRNA encoding IL-12 alone (uncircuited). As shown in **FIG. 17**, tumor growth was inhibited relative to treatment with PBS in mice administered with circuited mRNAs. Notably, mice administered with the repRNA-mIL-

12+miR-142 sensor+miR-22 sensor showed significant improvement in tolerability relative to uncircuited mRNA as demonstrated by no loss in body weight post-dosing relative to initial weight (see **FIG. 18**).

[0340] Together these results demonstrate tissue-specific control of therapeutic mRNA expression, and specifically that the repRNA-mIL-12+miR-142 sensor+miR-22 sensor is effective at detargeting off-target organs, while maintaining IL-12 expression in tumors. Further, the repRNA-mIL-12+miR-142 sensor+miR-22 sensor abrogates weight loss and pathological findings induced by systemic treatment, while inhibiting tumor growth.

EXAMPLE 5: EX-VIVO ASSESSMENT OF DETARGETING SENSORS

[0341] miRNA sensor activity was further tested in *ex-vivo* models. Specifically, miRNA sensor activity was assessed in two off-target cells: primary liver endothelial cells (**FIG. 19A**) and lung epithelial cells (**FIG. 19B**). Both miRNA-sensing circuits (i.e., miR-22 sensor and miR-185 sensor) displayed high miRNA sensor activity (high knockdown) compared to the control (i.e., uncircuited) condition in primary liver endothelial cells. When tested in primary small airway epethelial cells, both miRNA-sensing circuits (i.e., miR-22 sensor and miR-185 sensor) displayed high miRNA sensor activity (high knockdown) in at least two of the tested donors compared to the control (i.e., uncircuited) condition.

[0342] miR-22-3p and miR-185-5p were evaluated by in situ hybridization (ISH). Specifically, tissue/tumor microarrays (TMA) from two tumor types were evaluated. Tumor types included lung cancer; mostly non-small cell lung cancer (NSCLC) and other tumor types (as described in Table 6), and triple negative breast cancers (as described in Table 7). These tumors were mostly early-stage tumors. All tumor cores were positive with probe SR-RNU6-S1 (positive control) indicating that the quality of miRNA in the Formalin-fixed paraffin-embedded (FFPE) tumor cores were intact (at least for high abundant miRNA). All tumor cores were negative with probe SR-Scramble-S1 (negative control) indicating the specificity of the test probes used in the experiment. In the lung tumor TMA, miR-22-3p had no expression via ISH, and in the breast tumor TMA, miR-22-3p was absent. miR-185-5p was absent in the evaluated triple-negative breast cancer (TNBC) and lung tumor TMA. miR-142-3p was absent in the TNC tumors, but was detected within the immune cells of the tumor microenvironment (grades 1-3, 64%)

Table 6. Incidence for miRNA expression in patient tumor samples as evaluated by in situ Hybridization: NSCLC (Lung)

miR 185-5p		**miR-22-3p**	
Cancer	**Grade 0**	**Cancer**	**Grade 0**
AdenoCarcinoma (N=32)	32/32 (100%)	AdenoCarcinoma (N=32)	32/32 (100%)
AdenoSquamous Carcinoma (N=3)	3/3 (100%)	AdenoSquamous Carcinoma (N=3)	3/3 (100%)
SCC (N=22)	22/22 (100%)	SCC (N=22)	22/22 (100%)
Small Cell Carcinoma (N=1)	1/1 (100%)	Small Cell Carcinoma (N=1)	1/1 (100%)
Large Cell Carcinoma (N=1)	1/1 (100%)	Large Cell Carcinoma (N=1)	1/1 (100%)

Table 7. Incidence for miRNA expression in patient tumor samples as evaluated by in situ Hybridization: TNBC (Breast)

miR 185-5p					**miR-22-3p**	
Cancer	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Cancer**	**Grade 0**
Invasive Ductal Carcinomas (N=57)	56/57 (98%)	-	-	1/57 (2%)*	Invasive Ductal Carcinomas (N=57)	57/57 (100%)
Adjacent Normal Breast Tissue (N=2)	2/2 (100%)	-	-	-	Adjacent Normal Breast Tissue (N=2)	2/2 (100%)

[0343] The mouse IL-12 payload expression (% Expression (normalized to no sensor control)) in five patient derived tumor organoids representing non-small cell lung cancer (NSCLC) and triple-negative breast cancer (TNBC) are shown in **FIG. 20A** for the following sensor constructs: miR-142-3p, miR-142-3p + miR-22-3p, and miR-142-3p + miR-185-5p. In most cases, the payload expression from circuited mRNA constructs was preserved to at least 50% relative to a no sensor control.

[0344] Further, tumor cells obtained from patient tumor surgical resections were cultured in a basement membrane extract to form 3D organoids. Organoids were transfected with either a VEE replicon comprising mRNA encoding hIL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-hIL-12+miR-142+miR-185) or a VEE replicon comprising mRNA encoding hIL-12 alone (i.e., repRNA-mIL-12, "uncircuited hIL-12") for 24 hours. Post

transfection, supernatants were collected, and hIL-12 payload expression was analyzed using ELISA. Transfection of circuited and uncircuited mRNA into patient-derived NSCLC organoids yielded comparably robust expression of hIL-12 payload (**FIGs. 20B-20E**).

The responsiveness of miR-22 (**FIG. 21A**) or miR-185 (**FIG. 21B**) sensors in responding to their cognate miRNAs was assessed in B16.F10 melanoma cells. Expression (Luminescence, RLU) in B16.F10 melanoma cells transfected with miR-22 (**FIG. 21A**) or miR-185 (**FIG. 21B**) sensors was assessed following co-transfection (0, 1, 10, 100, 1,000 nM) of the associated mimic. A negative control mimic (1,000 nM) and mRNAs transfected with no sensors were also tested and does showcase the specificity of these sensors. A dose-dependent reduction in payload expression with cognate miRNA mimics was observed.

EXAMPLE 6: IN VIVO EFFECTS OF DETARGETING SENSORS

[0345] As previously described, the dual miRNA-sensing circuits improve tolerability in a B16.F10 mouse model. Also, the dual miRNA-sensing circuits show anti-tumor efficacy in a B16.F10 mouse model. B16.F10 melanoma cells were subcutaneously implanted into the right flank of C57Bl/6J mice to establish tumors. When the tumors reached a size of 100-150 mm^3, the mice were intravenously administered with vehicle control or different doses of uncircuited or circuited constructs. Body weights and clinical signs were monitored over time. Tumor volumes were measured thrice a week with calipers and calculated using the formula V = (L*W*H) * 0.5 where L, W, and H represent length, width and height measurements of the tumor. **FIGs. 22A-22C** provide a comparison of tumor volume (mm^3) in a B16.F10 melanoma mouse model following I.V. administration of a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-22 sensor (i.e., miR-142-3p + miR-22-3p) or a VEE replicon comprising mRNA encoding IL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., miR-142-3p + miR-185-5p) at a low dose (**FIG. 22A**), a medium dose (**FIG. 22B**), or a high dose (**FIG. 22C**). Dose-dependence was seen with the miR-142-3p + miR-22-3p combination sensor. With the miR-142-3p + miR-185-5p combination sensor, escalating the dose to a medium dose (**FIG. 22B**), resulted in improved efficacy and a further escalation to a high dose (**FIG. 22C**), did not appear distinguishable from the medium dose group for tumor growth inhibition.

[0346] While the low dose for mRNA encoding IL-12 extends the average days taken for the tumor to reach a size of 1000 mm^3 by 25 days compared to the vehicle control, the medium and high doses for mRNA encoding IL-12 alone (uncircuited) exceeded the maximum tolerated dose (MTD), and were therefore not tested (**FIG. 22D**). Notably, the miR-142-3p + miR-185-5p

combination sensor extended the maximum tolerated dose, and a dose-dependent increase in tumor control and survival was seen (**FIG. 22D**). Specifically, at the low, medium, and high dose, the miR-142-3p + miR-185-5p combination sensor extended the average days taken for the tumor to reach a size of 1000 mm^3 by 16 days, > 26 days, and > 26 days, respectively (**FIG. 22D**).

[0347] As previously described, the effects of the dual miRNA-sensing circuits on serum (**FIGs. 23A-23F**) and tumor (**FIG. 24**) cytokine levels were assessed. Specifically, **FIGs. 23A-23C** provide a comparison of serum IL-12 levels following I.V. administration of the dual miRNA sensors (miR-142-3p + miR-22-3p or miR-142-3p + miR-185-5p) at a low dose (**FIG. 23A**), a medium dose (**FIG. 23B**), or a high dose (**FIG. 23C**). **FIGs. 23D-23F** provide a comparison of serum IFN-γ levels following I.V. administration of the dual miRNA sensors at a low dose (**FIG. 23D**), a medium dose (**FIG. 23E**), or a high dose (**FIG. 23F**). The highest tumor IL-12 level seen was for the control when comparing similar doses (a low dose, **FIG. 24**). Only miR-22-3p dual sensor showed a dose dependent increase in tumor IL-12. The levels for miR-185-5p were comparable across dose groups. The tumor IL-12 levels had no correlation with efficacy. The uncircuited group had the lowest tumor IL-12 but had the best efficacy at a low dose. The miR-22-3p dual sensor group at the highest dose (high dose) had the highest tumor IL-12.

EXAMPLE 7: IN VIVO EFFECTS OF DETARGETING SENSORS

[0348] In addition, systemically delivered dual miRNA-sensing circuits showed robust activity in nonhuman primates (NHPs). Naïve Cynomolgus macaques were intravenously (I.V.) administered with either VEE replicon comprising repRNA-hIL-12 or repRNA-mIL-12+miR-142+miR-185. Plasma was collected at different time points post-dosing and subjected to bioanalysis. RT-qPCR was used to detect nsP4 mRNA. Electrochemiluminescence was used to detect the payload hIL-12, as well as the downstream biomarker IFN-g.

[0349] Plasma IL-12 kinetics were assessed up to 288 hrs from naïve Cynomolgus macaques following I.V. administration of a VEE replicon comprising mRNA encoding hIL-12 and comprising a miR-142 sensor and a miR-185 sensor (i.e., repRNA-hIL-12+miR-142+miR-185) or a VEE replicon comprising mRNA encoding hIL-12 alone (i.e., repRNA-mIL-12, "uncircuited hIL-12" (**FIG. 25A**). The presence of the dual sensors resulted in an 89% reduction in circulating hIL-12 and an 86% reduction in circulating IFN-γ. Overall, intravenous administration of repRNA-hIL-12+miR-142+miR-185 robustly represses payload expression in Cynomolgus monkeys, and is well tolerated at the tested doses.

EXAMPLE 8: TUMOR CONTROL IN A COLORECTAL CARCINOMA MOUSE MODEL

[0350] A CT26 tumor model for colorectal cancer (*i.e.*, CT26 colorectal carcinoma implanted in BALB/cJ mice) was used to assess tumor control. Mice were given a single intravenous (IV, tail vein) injection of vehicle (PBS) or the mRNA-LNP with the mRNA encoding IL-12 with a miR-142 sensor and a miR-185 sensor (or interchangeably referred as the "mRNA-LNP with the dual miRNA-sensing circuit" hereinafter in Examples 8-13 and is abbreviated as "the dual miRNA-sensing circuit" in the figures associated with Examples 8-13) at various escalating doses (1-5, with dose 1 being the lowest and dose 5 being the highest). The following in life measurements were assessed: moribundity, tumor volume, body weight, and clinical observations. For each group N = 20. Five mice were terminated on Day 1 (24h) and five mice were terminated on Day 3 (72h); serum and tumors were subsequently collected. Ten mice were terminated on Day 35 or when animals reach tumor size/body condition endpoint, whichever was earlier, and no samples were collected. As described above, IL-12 and IFN-γ protein levels in serum and tumor were assessed by MSD.

[0351] Dose-dependent efficacy, specifically tumor control (**FIG. 26**) and survival (**FIG. 27**), was observed in the colorectal carcinoma tumor model. The overall response rates (ORR), defined as Complete response (CR; reduction in tumor volume to undetectable for study duration) + Partial response (PR; reduction in tumor volume from last measurement for at least two consecutive tumor measurements), for each dose are summarized in Table 8, below.

Table 8. Tumor Response

Findings	Vehicle	Dose 1 (lowest)	Dose 2	Dose 3	Dose 4	Dose 5 (highest)
Overall Response Rate (ORR)	0%	20%	40%	40%	90%	100%

[0352] Serum IL-12 Tmax was 24h, and Cmax was dose-dependent and comparable for the top two doses (doses 4 and 5, **FIG. 28**). Serum IFN-γ Tmax was 24h, and the bottom 3 doses (doses 1-3) had comparable Cmax, as did the top two doses (doses 4 and 5, **FIG. 29**).

[0353] Tumor IL-12 Tmax was 24h, and Cmax was dose-dependent and comparable for the top two doses (doses 4 and 5, **FIG. 30**). The tumor IL-12 levels were below the limit of quantification (BLOQ) for the lowest dose (dose 1, **FIG. 30**). Overall tumor IFN-γ levels were relatively low; however, the Cmax levels were comparable (**FIG. 31**).

[0354] These results show that the mRNA-LNP with the dual miRNA-sensing circuit resulted in desired anti-tumor properties after IV dosing.

EXAMPLE 9: IMMUNE ACTIVATION IN A COLORECTAL CARCINOMA MOUSE MODEL

[0355] A CT26 tumor model for colorectal cancer (*i.e.*, CT26 colorectal carcinoma implanted in BALB/cJ mice) was used to assess immune activation. Mice were given a single intravenous (IV, tail vein) injection of vehicle (PBS) or the mRNA-LNP with the dual miRNA-sensing circuit at a low and high dose. The following in life measurements were assessed: moribundity, tumor volume, body weight, and clinical observations. For each group N = 18. Nine mice from each group were terminated on Days 3 and 6, and tumors were collected. From these mice, tumors from six mice each were used for flow cytometry. Specifically, tumors from 2 mice were pooled to generate one sample for flow cytometry for a total of three samples for each group. Tumors from the other three mice were fixed in formalin, and hematoxylin and eosin (H&E) staining was conducted. Immunohistochemical (IHC) staining was also conducted for CD4, CD8, Foxp3, PD-L1, and F4/80.

[0356] Infiltration of CD8+ T cells and a reduction in Tregs in CT26 tumors was observed. At both doses and timepoints tested, the frequency of (CD4+ CD25+ Foxp3+) T regs decreased See **FIGs. 32A-32F**. This data shows that by promoting tumor-infiltrating lymphocytes (TILs) in this murine tumor model, the tumor microenvironment was reprogrammed.

[0357] **FIG. 33** shows tumor microenvironment (TME) infiltration by T cells (staining denoted by arrows) within CT26 murine tumors from mice at both doses and timepoints tested. **FIG. 34** and **FIG. 35** show quantification of TME infiltration by T cells. Higher CD4 numbers (**FIG. 34**) were observed on day 6 for the high dose. Higher CD8 numbers (**FIG. 35**) were observed on day 6 for both the low and high doses. Additionally, the treated groups were observed to have higher T cell infiltration towards the center of the tumors, compared to the vehicle group where most cells were restricted around tumor margins.

[0358] Together, these results show that administration of the mRNA-LNP with the dual miRNA-sensing circuit promoted CD8+ T cell infiltration, enhanced Th1 and cytotoxic responses, and reduced T regulatory cells, indicating strong immunomodulatory effects in CT26 CRC model.

EXAMPLE 10: EVALUATION OF ANTI-TUMOR ACTIVITY OF THE DUAL miRNA-SENSING CIRCUIT IN COMBINATION WITH PD-1 BLOCKADE IN THE B16.F10 MELANOMA MODEL

[0359] Previous in vitro studies have demonstrated that the mRNA-LNP with the dual miRNA-sensing circuit is able to generate mIL-12 in several mouse origin tumor cell lines and subsequent mIL-12 generated can activate the IL-12 signaling pathway. In vivo, intravenous delivery is dependent on the host immune system to elicit full antitumor activity, therefore use of syngeneic tumor models in mice with an intact immune system are warranted to demonstrate anti-tumor activity. The IL-12/IFN-γ axis is not usually present in advanced melanoma, intratumoral delivered IL-12 has shown to restore this axis thus promoting anti-PD-1 immunotherapy in patients who are predicted to be unresponsive to treatment. Thus, this study bridges the gap and demonstrates that IL-12 from intravenous delivery (IV, tail vein) of the mRNA-LNP with the dual miRNA-sensing circuit also restores the IL-12/IFN-γ axis in a tumor model that is refractory to PD-1 blockade.

[0360] C57BL/6J mice with established syngeneic B16.F10 melanoma tumors (100-150 mm^3) were dosed intravenously with control (PBS) or a single dose of subtherapeutic doses (low medium, and high) of the mRNA-LNP with the dual miRNA-sensing circuit based on a previous study alone or in combination with 10 mg/kg anti-PD-1 administered intraperitoneally two times per week (2QW) and evaluated for general tolerability and tumor volume up to Day 34 post administration (N = 10 per group). Efficacy measures included survival, tolerability (i.e., body weights), tumor measurements, IL-12 in serum, and downstream serum inflammatory cytokine (IFN-γ) measurements. Animals were humanely euthanized if tumor volumes exceeded 2000 mm^3 or ulcerated, or if were observed to be moribund.

[0361] Enhanced efficacy was observed for the combination therapy compared to monotherapy, as shown by tumor suppression (**FIGs. 36A-36C** and **FIG. 38A**), survival (**FIGs. 37A-37C** and **FIG. 38B**), and overall response rates (ORR, **FIG. 38C**) at multiple doses tested in the B16.F10 syngeneic melanoma model. The overall response rates (ORR), as described previously, for the highest dose tested of the mRNA-LNP with dual miRNA-sensing circuit combined with anti PD-1 was 60% compared to 10% with the monotherapy. The combination groups showed an improvement in tumor responses at the highest dose level tested, where the overall response rate (ORR) increased from 10% in the monotherapy group to 60% with the addition of anti-PD-1, suggesting synergy between the two treatments (**FIG. 38C**). There were no statistically significant increases in circulating IL-12, IFN-γ, or IFN-α levels in serum in the combination groups when compared with monotherapy (**FIGs. 39A-39C**). At the highest dose, IP-10 levels in the monotherapy group were slightly (~1.5X) higher than the corresponding combination therapy group (*p = 0.016, **FIG. 39D**).

This study demonstrated enhanced anti-tumor activity of subtherapeutic doses of the mRNA-LNP with dual miRNA-sensing circuit against established melanoma tumors with the addition of anti PD-1 blocking agent without an additive increase in systemic IL-12, suggesting that these two drugs act through non-overlapping complementary mechanisms to enhance anti-tumor responses.

EXAMPLE 11: EVALUATION OF CD8 T CELL INFILTRATION OF THE DUAL miRNA-SENSING CIRCUIT IN COMBINATION WITH PD-1 BLOCKADE IN THE B16.F10 MELANOMA MODEL

[0362] A CT26 tumor model for colorectal cancer (*i.e.*, CT26 colorectal carcinoma implanted in BALB/cJ mice) was used to further assess the mRNA-LNP with the dual miRNA-sensing circuit alone or in combination with an anti-PD-1.

[0363] Mice were given a single intravenous (IV, tail vein) injection of vehicle (PBS) or the mRNA-LNP with the dual miRNA-sensing circuit at the medium dose described in Example 10, alone or in combination with 10 mg/kg anti-PD-1 administered intraperitoneally two times per week. The following in life measurements were assessed: moribundity, tumor volume, body weight, and clinical observations. For each group N = 18. Nine mice from each group were terminated on Days 3 and 6, and tumors were collected. From these mice, tumors from six mice each were used for flow cytometry. Specifically, tumors from 2 mice were pooled to generate one sample for flow cytometry for a total of three samples for each group. Tumors from the other three mice were fixed in formalin, and hematoxylin and eosin (H&E) staining was conducted. Immunohistochemical (IHC) staining was also conducted for CD4, CD8, Foxp3, PD-L1, and F4/80.

[0364] These studies showed monotherapy as well as combination with anti-PD1 promotes the infiltration of CD8 T cells in B16.F10 tumors. Specifically, the density of tumor infiltrating CD4 T cells, CD8 T cells, and NK cells were comparable among all groups at both timepoints. Differences in the relative proportion of these subsets are apparent on day 6 in different treatment groups. An increase in CD8 T cell frequencies, associated with a decrease in NK cells is seen with monotherapy as well as combination groups. CD4 frequencies were comparable at both time points. See **FIGs. 40A-40E**.

[0365] Monotherapy as well as combination with anti-PD1 was also shown to promote Th1 type and cytotoxic responses in B16.F10 tumors. While the frequencies of most CD8, CD4, and NK cell subsets were largely comparable at day 3, differences between groups were most apparent at day 6.

[0366] In the CD4 T cell subsets, monotherapy as well as combination with anti-PD1 showed increases in Th1-type responses (IFNg+, TNFa+ and T-bet+ subsets) as well as PD1+ cells, which is a marker of activated cells (**FIGs. 41A-41B**). While T regs were comparable between all groups, the CD8 to T reg ratio was higher in these two groups at day 6 due to the CD8 T cell numbers being higher.

[0367] In the CD8 T cell subsets, monotherapy and combination with anti-PD1 led to an increase in the proportion of IFN-γ and TNF-α secreting cells as well as T-bet+ cells. (**FIGs. 41C-41D**). These populations reflect Th1 type responses.There was also an increase in Granzyme B+ cells, which reflect cytotoxic cells as well as cells that express PD1, a marker of activation.

[0368] In the NK cell subsets, monotherapy and combination with anti-PD1 led to an increase in the proportion of IFN-γ and TNF-α secreting cells as well as T-bet+ cells (reflect Th1 type responses; **FIGs. 41E-41F**). There was also an increase in Granzyme B+ cells (reflecting cytotoxic cells).

[0369] Compared to monotherapy, IL-12 levels were higher (statistically significant) in the combination group, while IFN-γ, IP-10, and IFN-α levels were comparable (**FIGs. 42A-42D**).

[0370] Without wishing to be bound by a particular theory, several sources suggest that combining interleukin-12 (IL-12) with anti-PD-1 (programmed cell death protein 1) therapy can lead to an increase in IL-12 levels. This increase is thought to be an indirect effect where anti-PD-1 therapy, by blocking the PD-1/PD-L1 pathway, helps reactivate T cells, leading to increased production of IFN-γ. IFN-γ in turn acts on other immune cells like DCs, promoting them to produce IL-12. This creates a positive feedback loop within the tumor microenvironment, further stimulating the immune system and potentially enhancing the anti-tumor effects. The IFN-γ levels were comparable possibly due to saturation of responder cells (primarily T cells and NK cells) at the IL-12 levels achieved. This also explains why the levels of IP-10, which are primarily induced by IFN-γ, are also comparable. IFN-α results from an innate immune response to the replicon and is therefore expected to be similar between these two groups.

EXAMPLE 12: DURABLE ANTI-TUMOR IMMUNITY INDUCED BY THE DUAL miRNA-SENSING CIRCUIT IN TUMOR RECHALLENGE STUDIES

[0371] Surviving mice from Example 8 were used to investigate the mRNA-LNP with the dual miRNA-sensing circuit associated with immunological memory. Specifically, this study evaluated the immunological memory associated with the mRNA-LNP with the dual miRNA-sensing circuit by rechallenging 13 mice that achieved a complete response in the CT26 tumor

model from Example 8. After >100 days of tumor clearance, animals were subcutaneously injected with varying cell numbers of CT26.WT colorectal cancer cells on the opposite flank. Tumor growth was measured and compared to the control groups, represented by naïve BALB/cJ mice.

[0372] After inoculation with varying numbers of CT26.WT cells, no measurable tumors were detected in any of the rechallenged mice (**FIG. 43B** and **FIG. 43E**). In contrast, naïve mice had measurable tumors by study Day 5 (**FIG. 43A** and **FIG. 43D**), and tumors continued to grow until animals reached humane endpoints (tumor size >1,500 mm^3). Specifically, following inoculation with varying numbers of CT26.WT cells, tumor volumes were recorded three times weekly through Day 35. Tumor volumes were compared over time and across cell concentrations. The complete responders from Example 8 did not show any established tumors irrespective of the cell number used to rechallenge. While small palpable bumps (<20mm^3) were observed shortly after inoculation, these were undetectable by Day 16 and remained undetectable for the rest of the study duration. In comparison, steadily growing tumors were observed in naïve animals as expected. Naïve mice were observed to have an average tumor volume of ~100mm^3 on study Day 5, and tumor volumes continued to increase until animals reached humane endpoints.

[0373] These results were also reflected in the survival data, as all mice from the rechallenge group, but none from the naive cohort survived till the end of the study. Specifically, survival analyses in the form of Kaplan-Meier curves were generated to show survival of mice rechallenged with syngeneic CT26.WT colorectal cancer cells after remaining tumor free for >100 days. As shown in **FIG. 43C** and **FIG. 43F**, naïve mice injected with either $1x10^6$ or $5x10^6$ CT26.WT cells were all euthanized by day 35 due to reaching tumor endpoints (tumor size >1,500mm^3 or tumor developing ulcerations). In contrast, all the rechallenged mice survived till the end of the study.

[0374] This study demonstrates that the mRNA-LNP with the dual miRNA-sensing circuit induces a durable immune response and is associated with strong immunological memory in the CT26.WT model. None of the rechallenged mice developed established tumors irrespective of the original treatment dose or number of tumor cells used to rechallenge.

EXAMPLE 13: EVALUATION OF TRANSFECTABILITY AND PAYLOAD EXPRESSION

[0375] Patient-derived xenografts (PDX) from multiple indications were used to assess transfectability and payload expression of the mRNA-LNP with the dual miRNA-sensing circuit (encoding human IL-12).

[0376] Two PDX mice models for each of the following indications were used: triple-negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), and melanoma. For each model N = 4. PDX mice were given a single intravenous (IV, tail vein) injection of the mRNA-LNP with the dual miRNA-sensing circuit. Serum and tumor from untreated PDX mice were used as "Vehicle" controls. PDX mice were terminated 24h following administration and human IL-12 (hIL-12) levels in serum and tumor were subsequently collected. As shown in **FIG. 44A** and **FIG. 44B**, hIL-12 expression was induced in patient-derived xenografts from multiple indications demonstrated the versatility of the mRNA-LNP with the dual miRNA-sensing circuit.

[0377] It is to be appreciated that the Detailed Description section, and not the Summary and Abstract sections, is intended to be used to interpret the claims. The Summary and Abstract sections can set forth one or more, but not all exemplary aspects of the present disclosure as contemplated by the inventor(s), and thus, are not intended to limit the present disclosure and the appended claims in any way.

[0378] The present disclosure has been described above with the aid of functional building blocks illustrating the implementation of specified functions and relationships thereof. The boundaries of these functional building blocks have been arbitrarily defined herein for the convenience of the description. Alternate boundaries can be defined so long as the specified functions and relationships thereof are appropriately performed.

[0379] The foregoing description of the specific aspects will so fully reveal the general nature of the disclosure that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications such specific aspects, without undue experimentation, without departing from the general concept of the present disclosure. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed aspects, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0380] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary aspects, but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising (a) a first region encoding a payload and (b) a second region comprising a sensor that is configured to recognize a marker in a cell that expresses the marker ("detargeting cell"), wherein the marker is miR-185-5p or miR-22-3p ("detargeting sensor").

2. The polynucleotide of claim 1, wherein the recognition of the marker by the sensor in the cell results in reduced expression of the payload as compared to a reference cell (*e.g.*, cell that does not express the marker or cell that expresses the marker at a low level).

3. The isolated polynucleotide of claim 2, wherein the detargeting cell comprises an immune cell, a cardiomyocyte, an endothelial cell, or any combination thereof.

4. The isolated polynucleotide of claim 3, wherein the detargeting cell comprises an immune cell.

5. The isolated polynucleotide of claim 4, wherein the immune cell comprises hematopoietic stem cells and/or derivatives thereof.

6. The isolated polynucleotide of claim 4 or 5, wherein the immune cell comprises T cells, B cells, natural killer (NK) cells, macrophages, monocytes, dendritic cells, neutrophils, basophils, eosinophils, or combinations thereof.

7. The isolated polynucleotide of any one of claims 3 to 6, wherein the marker is present within the immune cell at a greater quantity as compared to non-immune cells or a cell type that does not express the marker or expresses the marker at a lower level than the immune cell.

8. The isolated polynucleotide of claim 7, wherein the expression level of the marker in the immune cell is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, grater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, or greater than about 20-fold or more, as compared to the corresponding value for the non-immune cells or a cell type that does not express the marker or expresses the marker at a lower level than the immune cell.

9. The isolated polynucleotide of claim 7 or 8, wherein the immune cell comprises a spleen, lymph node, or both.

10. The isolated polynucleotide of any one of claims 1 to 3, wherein the detargeting cell is a cardiomyocyte.

11. The isolated polynucleotide of claim 10, wherein the cardiomyocyte comprises atrial myocytes, ventricular myocytes, pacemaker cells (nodal cells), purkinje fibers, conducting cells, or any combination thereof.

12. The isolated polynucleotide of claim 10 or 11, wherein the marker is present within a cardiomyocyte at a greater quantity as compared to non-cardiomyocytes (e.g., cancer cells) or a cell type that does not express the marker or expresses the marker at a lower level than the cardiomyocytes.

13. The isolated polynucleotide of claim 12, wherein the expression level of the marker present within the cardiomyocyte is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, grater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, or greater than about 20-fold or more, as compared to the corresponding value for the non-cardiomyocyte or a cell type that does not express the marker or expresses the marker at a lower level than the cardiomyocytes.

14. The isolated polynucleotide of any one of claims 10 to 13, wherein the cardiomyocyte comprises a myocardium.

15. The isolated polynucleotide of any one of claims 1 to 3, wherein the detargeting cell comprises an endothelial cell.

16. The isolated polynucleotide of claim 15, wherein the endothelial cell comprises vascular endothelial cells (e.g., arterial endothelial cells, venous endothelial cells, liver endothelial cells, or capillary endothelial cells (e.g., continuous endothelium, fenestrated endothelium, or sinusoidal endothelium)), lymphatic endothelial cells, pulmonary endothelial cells, brain endothelial cells (blood-brain barrier), or any combination thereof.

17. The isolated polynucleotide of claim 15 or 16, wherein the marker is present within an endothelial cell at a greater quantity as compared to non-endothelial cells or a cell type that does not express the marker or expresses the marker at a lower level than the endothelial cells.

18. The isolated polynucleotide of claim 17, wherein the expression level of the marker present within the endothelial cell is greater than about 1-fold, greater than about 2-fold, greater than about 3-fold, greater than about 4-fold, greater than about 5-fold, greater than about 6-fold, greater than about 7-fold, greater than about 8-fold, greater than about 9-fold, greater than about 10-fold, greater than about 11-fold, greater than about 12-fold, greater than about 13-fold, greater than about 14-fold, greater than about 15-fold, grater than about 16-fold, greater than about 17-fold, greater than about 18-fold, greater than about 19-fold, or greater than about 20-fold or more, as compared to the corresponding value for the non-endothelial cells or the cell type that does not express the marker or expresses the marker at a lower level than the endothelial cells.

19. The isolated polynucleotide of any one of 15 to 18, wherein the endothelial cell comprises blood vessel, lymphatic vessel, liver, lung, kidney, brain, intestine, heart, and/or muscle.

20. The isolated polynucleotide of any one of claims 1 to 19, wherein the marker comprises miR-22-3p.

21. The isolated polynucleotide of any one of claims 1 to 19, wherein the marker comprises miR-185-5p.

22. The isolated polynucleotide of any one of claims 1 to 21, wherein the second region comprises multiple detargeting sensors, wherein at least one of the detargeting sensors comprises a miR-185 sensor or a miR-22 sensor.

23. The isolated polynucleotide of claim 22, wherein each of the multiple detargeting sensors is the same.

24. The isolated polynucleotide of claim 22, wherein two or more of the multiple detargeting sensors are different.

25. The isolated polynucleotide of claim 22 or 24, wherein one of the multiple detargeting sensors comprises a sensor that is capable of recognizing a second marker, which comprises miR-142-3p.

26. The isolated polynucleotide of claim 25, wherein the multiple detargeting sensors comprises a first sensor that is capable of recognizing miR-142-3p and a second sensor that is capable of recognizing miR-22-3p .

27. The isolated polynucleotide of any one of claims 1 to 26, wherein the sensor or the first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 6.

28. The isolated polynucleotide of any one of claims 25 to 27, wherein the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 3.

29. The isolated polynucleotide of claim 25, wherein the multiple detargeting sensors comprises a first sensor that is capable of recognizing miR-142-3p and a second sensor that is capable of recognizing miR-185-5p .

30. The isolated polynucleotide of claim 1 to 26, wherein the sensor or first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 9.

31. The isolated polynucleotide of claim 29 or 30, wherein the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 3.

32. The isolated polynucleotide of claim 22 or 24, wherein the multiple detargeting sensors comprises a first sensor that is capable of recognizing miR-22-3p and a second sensor that is capable of recognizing miR-185-5p.

33. The isolated polynucleotide of claim 32, wherein the first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 6.

34. The isolated polynucleotide of claim 32 or 33, wherein the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least

about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 9.

35. The isolated polynucleotide of claim 22 or 24, wherein the multiple detargeting sensors comprises a first sensor that is capable of recognizing miR-22-3p, a second sensor that is capable of recognizing miR-142-3p, and a third sensor that is capable of recognizing miR-185-5p.

36. The isolated polynucleotide of claim 35, wherein the first sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 6.

37. The isolated polynucleotide of claim 35 or 36, wherein the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 3.

38. The isolated polynucleotide of any one of claims 35 to 37, wherein the second sensor comprises a nucleotide sequence having at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% sequence identity to the nucleic acid sequence as set forth in SEQ ID NO: 9.

39. The isolated polynucleotide of any one of claims 1 to 38, which comprises at least two sensors comprising a miR-142 sensor and a miR-185 sensor (or a miR-22 sensor), at least four sensors comprising two miR-142 sensors and two miR-185 sensors (or two miR-22 sensors), at least six sensors comprising three miR-142 sensors and three miR-185 sensors (or three miR-22 sensors), at least eight sensors comprising four miR-142 sensors and four miR-185 sensors (or four miR-22 sensors), at least ten sensors comprising five miR-142 sensors and five miR-185 sensors (or five miR-22 sensors), or at least 12 sensors comprising six miR-142 sensors and six miR-185 sensors (or six miR-22 sensors).

40. The isolated polynucleotide of any one of claims 24 to 38, which comprises (i) at least one first sensor, at least two first sensors, at least three first sensors, at least four first sensors, at least five first sensors, at least six first sensors, at least seven first sensors, at least eight first sensors, at least nine first sensors, or at least ten first sensors and (ii) at least one second sensor, at least two second sensors, at least three second sensors, at least four second sensors, at least five second

sensors, at least six second sensors, at least seven second sensors, at least eight second sensors, at least nine second sensors, or at least ten second sensors.

41. The isolated polynucleotide of claim 40, wherein the first sensor comprises a miR-185 sensor or a miR-22 sensor and the second sensor comprises a miR-142 sensor

42. The isolated polynucleotide of any one of claims 1 to 41, wherein the payload comprises a cytokine, a ligand-binding protein, or both.

43. The isolated polynucleotide of claim 42, wherein the cytokine comprises an interleukin (IL)-12 protein.

44. The isolated polynucleotide of any one of claims 1 to 43, which further comprises: (1) an untranslated region (UTR), (2) a sequence encoding a signal peptide, (3) a translation initiation sequence, (4) a polyA sequence, (5) a sequence encoding a RNA binding protein, (6) a 5'-cap, (7) a sequence encoding a 2A ribosome skip peptide, (8) a translation enhancer element, or (9) any combination of (1) to (8).

45. The isolated polynucleotide of any one of claims 1 to 44, which comprises the sequence set forth in SEQ ID NO: 12.

46. The isolated polynucleotide of any one of claims 1 to 45, which consists essentially of the sequence set forth in SEQ ID NO: 12.

47. The isolated polynucleotide of any one of claims 1 to 46, which consists of the sequence set forth in SEQ ID NO: 12.

48. A synthetic circuit comprising the isolated polynucleotide of any one of claims 1 to 47.

49. A replicon comprising the isolated polynucleotide of any one of claims 1 to 48 or the synthetic circuit of any one of claims 45, wherein the replicon is self-replicating.

50. The replicon of claim 49, which is derived from an alpha virus.

51. The replicon of claim 50, wherein the alpha virus comprises a Venezuelan equine encephalitis (VEE) virus.

52. A circular RNA comprising the isolated polynucleotide of any one of claims 1 to 47.

53. A nanoparticle comprising (a) the isolated polynucleotide of any one of claims 1 to 47, the synthetic circuit of claim 48, the replicon of any one of claims 49 to 51, the circular RNA of claim 52, and (b) a lipid and/or lipid-like material.

54. The nanoparticle of claim 53, wherein the lipid comprises an ionizable lipid, cationic lipid, lipidoid, non-cationic helper lipid, phospholipid, sterol or other structural lipids, or combinations thereof.

55. The nanoparticle of claim 54, wherein the ionizable lipid comprises ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315), heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102), heptadecan-9-yl 8-((2-hydroxyethyl)(8-(nonyloxy)-8-oxooctyl)amino)octanoate (Lipid 5), di((*Z*)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319), 9Z,12Z-octadecadienoic acid, 3-[4,4-bis(octyloxy)-1-oxobutoxy]-2-[[[[3-(diethylamino)propoxy]carbonyl]oxy]methyl]propyl ester (LP-01), 5-(dimethylamino)-pentanoic acid, (6Z)-1,2-di-(4Z)-4-decen-1-yl-6-dodecen-1-yl ester (CL-1), 2-(((4-(dimethylamino)butanoyl)oxy)methyl)-2-((((Z)-tetradec-9-enoyl)oxy)methyl)propane-1,3-diyl (9Z,9'Z)-bis(tetradec-9-enoate) (TCL053), 3-(didodecylamino)-N1,N1,4 tridodecyl-1-piperazineethanamine (KL10), Nl-[2 (didodecylamino)ethyl]-N1,N4,N4-tridodecyl 1,4-piperazinediethanamine (KL22), 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25), 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA), 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA), heptatriaconta-6,9,28,31-tetraen-19-yl-4-(dimethylamino)butanoate (DLin-MC3-DMA), 2,2-dilinoleyl-4-(2 dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA), 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA), 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA), (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)), and (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)), or combinations thereof.

56. The nanoparticle of claim 54 or 55, wherein the cationic lipid comprises l,2-dioleoyl-3 -trimethylammonium-propane (DOTAP), lipofectamine, N-[l-(2,3- dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), l-[2- (oleoyloxy)ethyl]-2-oleyl-3-(2-hydroxyethyl)imidazolinium chloride (DOTEVI), 2,3- dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-l-propanaminium trifluoroacetate (DOSPA), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N-(l ,2-dimyristyloxyprop-3 -yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DMRIE), N-(l,2-dioleoyloxyprop-3-yl)-N,N-dimethyl-N-hydroxy ethyl ammonium bromide (DORIE), N,N-dioleyl-N,N-dimethylammonium

chloride (DODAC), 1,2-dilauroyl-sn-glycero-3-ethylphosphocholine (DLePC), 1,2-distearoyl-3-trimethylammonium-propane (DSTAP), 1,2-dipalmitoyl-3 -trimethylammonium-propane (DPTAP), 1,2-dilinoleoyl-3 -trimethylammonium-propane (DLTAP), 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP), 1,2-distearoyl -sn-glycero-3- ethylphosphocholine (DSePC), 1,2-dipalmitoyl-sn-glycero-3-ethylphosphocholine (DPePC), 1,2-dimyristoyl -sn-glycero-3-ethylphosphocholine (DMePC), 1,2-dioleoyl-sn- glycero-3-ethylphosphocholine (DOePC), 1,2-di-(9Z-tetradecenoyl)-sn-glycero-3- ethylphosphocholine (14: 1 EPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine (16:0-18: 1 EPC), or any combination thereof.

57. The nanoparticle of any one of claims 54 to 56, wherein the lipidoid comprises 1,1′-((2-(4-(2-((2-(bis(2-hydroxydodecyl) amino)ethyl) (2- hydroxydodecyl)amino)ethyl) piperazin-1-yl)ethyl)azanediyl) bis(dodecan-2-ol) (C12-200), 3,6-bis(4-(bis(2-hydroxydodecyl)amino)butyl)piperazine2,5-dione (cKK-E12), 1,1′-[[2-[2-[4-[2-[[2-[2-[bis(2-hydroxytetradecyl)amino]ethoxy]ethyl](2-hydroxytetradecyl)amino]ethyl]-1-piperazinyl]ethoxy]ethyl]imino]bis-2-tetradecanol (C14-4), tetrakis(8-methylnonyl) 3,3′,3″,3‴-(((methylazanediyl) bis(propane-3,1 diyl))bis (azanetriyl))tetrapropionate (306Oi$_{10}$), G0-C14, 3,3',3",3"'-(ethane-1,2-diylbis(azanetriyl))tetrakis(N-(2-(bis(2-hydroxytetradecyl)amino)ethyl)propanamide) (G0-C14 analog), 5A2-SC8, 4A3-SC8, 3,6-bis(4-(bis((9Z,12Z)-2-hydroxyoctadeca9,12-dien-1-yl)amino)butyl)piperazine-2,5-dione (OF-02), (((3,6-dioxopiperazine-2,5-diyl)bis (butane-4,1-diyl))bis(azanetriyl))tetrakis(ethane2,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-Deg-Lin), (((3,6-dioxopiperazine-2,5-diyl)bis(butane-4,1-diyl)) bis(azanetriyl))tetrakis (butane-4,1-diyl) (9Z,9′Z,9″Z,9‴Z,12Z,12′Z,12″Z,12‴Z)-tetrakis (octadeca-9,12-dienoate) (OF-C4-Deg-Lin), 1,3,5-tris[2-[(2-hydroxydodecyl)methylamino]ethyl]-1,3,5-triazine-2,4,6(1H,3H,5H)-trione (TNT-b10), N1,N3,N5-tris(3-(didodecylamino)propyl)benzene1,3,5-tricarboxamide (TT3), Hexa(octan-3-yl) 9,9′,9″,9‴,9⁗,9⁗′- ((((benzene-1,3,5-tricarbonyl)ris(azanediyl)) tris (propane-3,1-diyl))tris(azanetriyl))hexanonanoate (FTT5), PL-1, 98N12-5, ethyl 5,5-di((Z)-heptadec-8-en-1-yl)-1-(3-(pyrrolidin-1-yl)propyl)-2,5-dihydro-1H-imidazole-2-carboxylate (A2-Iso5-2DC18 (A2)), A12-Iso5−2DC18 (A12), or any combination thereof.

58. The nanoparticle of any one of claims 54 to 57, wherein the phospholipid is selected from the group consisting of 1,2-dilinoleoyl-sn-glycero-3 phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycerol-phosphocholine (DMPC), 1,2-dioleoyl-sn glycerol-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphocholine

(DSPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-dioleoyl-sn-glycero-3-phosphoethanola mine (DOPE), 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), sphingomyelin, 1-myristoyl-2-palmitoyl-sn-glycero-3-phosphocholine (14:0-16:0 PC, MPPC), 1-myristoyl-2 stearoyl-sn-glycero-3-phosphocholine (14:0-18:0 PC, MSPC), 1-palmitoyl 2-acetyl-sn-glycero-3-phosphocholine (16:0-02:0 PC), 1-palmitoyl-2-myristoyl-sn-glycero-3-phosphocholine (16:0-14:0 PC, PMPC), 1-palmitoyl-2-stearoyl-sn-glycero-3-phosphocholine (16:0-18:0 PC, PSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (16:0-18: 1 PC, POPC), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphocholine (16:0-18:2 PC, PLPC), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (16:0-20:4 PC), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (14:0-22:6 PC), 1-stearoyl-2-myristoyl-sn-glycero-3-phosphocholine (18:0-14:0 PC, SMPC), 1-stearoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18:0-16:0 PC, SPPC), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (18:0-18: 1 PC, SOPC), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (18:0-18:2 PC), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphocholine (18:0-20:4 PC), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-phosphocholine (18:0-22:6 PC), 1-oleoyl-2-myristoyl-sn-glycero-3-phosphocholine (18: 1-14:0 PC, OMPC), 1-oleoyl-2-palmitoyl-sn-glycero-3-phosphocholine (18: 1-16:0 PC, OPPC), 1-oleoyl-2-stearoyl-sn-glycero-3-phosphocholine (18: 1-18:0 PC, OSPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (16:0- 18: 1 PE, POPE), 1-palmitoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (16:0-18:2 PE), 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (16:0-20:4 PE), 1-palmitoyl-2-docosahexaenoyl-sn-glycero-3-phosphoethanolamine (16:0-22:6 PE), 1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (18:0-18: 1 PE), 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphoethanolamine (18:0-18:2 PE), 1-stearoyl-2-arachidonoyl-sn-glycero-3-phosphoethanolamine (18:0-20:4 PE), 1-stearoyl-2-docosahexaenoyl-sn-glycero-3-

phosphoethanolamine (18:0-22:6 PE), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemsPC), and any combination thereof.

59. The nanoparticle of any one of claims 54 to 58, wherein the sterol comprises a cholesterol, fecosterol, sitosterol, ergosterol, campesterol, stigmasterol, brassicasterol, tomatidine, tomatine, ursolic acid, alpha-tocopherol, and combinations thereof.

60. The nanoparticle of any one of claims 53 to 59, wherein the nanoparticle is pegylated.

61. The nanoparticle of any one of claims 53 to 60, which further comprises a targeting ligand.

62. A pharmaceutical composition comprising (a) the isolated polynucleotide of any one of claims 1 to 47, the synthetic circuit of claim 48, the replicon of any one of claims 49 to 51, the circular RNA of claim 52, or the nanoparticle of any one of claims 53 to 61, and (b) a pharmaceutically acceptable carrier.

63. A method of reducing an expression of a payload (payload expression) within an immune cell, a cardiomyocyte, and/or an endothelial cell of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 47, (b) the synthetic circuit of claim 48, (c) the replicon of any one of claims 49 to 51, (d) the circular RNA of claim 52, (e) the nanoparticle of any one of claims 53 to 61, (f) the pharmaceutical composition of claim 62, or (g) any combination of (a) to (f).

64. The method of claim 63, wherein reducing the payload expression comprises (a) reducing the amount of payload that is expressed by the immune cell, the cardiomyocyte, and/or the endothelial cell, (b) reducing the duration of payload expression by the immune cell, the cardiomyocyte, and/or the endothelial cell, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell, cardiomyocyte, or endothelial cell detargeting sensor).

65. The method of claim 63 or 64, wherein after the administration, the amount of payload that is expressed by the immune cell, the cardiomyocyte, and/or the endothelial cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

66. The method of any one of claims 63 to 65, wherein after the administration, the duration of the payload expression by the immune cell, the cardiomyocyte, and/or the endothelial cell is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least

about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

67. The method of any one of claims 63 to 66, wherein the immune cell comprises a T cell, macrophage, or both.

68. The method of any one of claims 63 to 67, wherein the cardiomyocyte comprises a heart muscle cell.

69. The method of any one of claims 63 to 68, wherein the endothelial cell comprises a vascular endothelial cell (e.g., arterial endothelial cell, venous endothelial cell, or capillary endothelial cell (e.g., continuous endothelium, fenestrated endothelium, or sinusoidal endothelium), a lymphatic endothelial cell, a corneal endothelial cell, a pulmonary endothelial cell, and/or a brain endothelial cell.

70. A method of selectively expressing a payload in a non-immune cell, non-cardiomyocyte, or non-endothelial cell of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 47, (b) the synthetic circuit of claim 48, (c) the replicon of any one of claims 49 to 51, (d) the circular RNA of claim 52, (e) the nanoparticle of any one of claims 53 to 61, (f) the pharmaceutical composition of claim 62, or (g) any combination of (a) to (f).

71. The method of claim 70, wherein after the administration, (a) an amount of the payload that is expressed in the non-immune cell, non-cardiomyocyte, or non-endothelial cell, (b) a duration of the expression of the payload in the non-immune cell, non-cardiomyocyte, or non-endothelial cell, or (c) both (a) and (b) are higher than the corresponding amount and/or duration in the immune cell, cardiomyocyte, or endothelial cell of the subject.

72. A method of reducing an expression of a payload (payload expression) within a lymphoid tissue, a myocardium tissue, or an endothelial tissue of a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 47, (b) the synthetic circuit of claim 48, (c) the replicon of any one of claims 49 to 51, (d) the circular RNA of claim 52, (e) the nanoparticle of any one of claims 53 to 61, (f) the pharmaceutical composition of claim 62, or (g) any combination of (a) to (f).

73. The method of claim 72, wherein the lymphoid tissue comprises a spleen, lymph node, or both.

74. The method of claim 72, wherein the myocardium tissue comprises a heart.

75. The method of claim 72, wherein the endothelial tissue comprises blood vessel, lymphatic vessel, liver, lung, kidney, brain, intestine, heart, or muscle.

76. The method of any one of claims 72 to 75, wherein reducing the payload expression comprises (a) reducing the amount of payload that is expressed in the lymphoid tissue, myocardium tissue, or endothelial tissue, (b) reducing the duration of payload expression in the lymphoid tissue, myocardium tissue, or endothelial tissue, or (c) both (a) and (b), as compared to that of a reference subject (*e.g.*, a subject who received a corresponding polynucleotide that lacks the immune cell detargeting sensor, the cardiomyocyte detargeting sensor, or the endothelial cell detargeting sensor, respectively).

77. The method of claim 76, wherein after the administration, the amount of payload that is expressed in the lymphoid tissue, myocardium tissue, or endothelial tissue, is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

78. The method of claim 76 or 77, wherein after the administration, the duration of the payload expression in the lymphoid tissue, myocardium tissue, or endothelial tissue, is reduced by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100%, as compared to that of the reference subject.

79. A method of treating a disease or disorder in a subject in need thereof, comprising administering to the subject (a) the isolated polynucleotide of any one of claims 1 to 47, (b) the synthetic circuit of claim 48, (c) the replicon of any one of claims 49 to 51, (d) the circular RNA of claim 52, (e) the nanoparticle of any one of claims 53 to 61, (f) the pharmaceutical composition of claim 62, or (g) any combination of (a) to (f).

80. The method of claim 79, wherein the disease or disorder comprises a cancer, inflammatory disorders, monogenic disorders, neurological disorders, psychiatric disorders, or combinations thereof.

81. The method of claim 79 or 80, wherein the cancer comprises a melanoma, squamous cell cancer (e.g., esophageal squamous cell carcinoma), small-cell lung cancer, non-small cell lung cancer (NSCLC), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma,

cervical cancer, ovarian cancer, liver cancer (e.g., hepatocellular carcinoma), bladder (urothelial) cancer, hepatoma, breast cancer (e.g., triple-negative breast cancer, TNBC), colon cancer, colorectal cancer (e.g., colorectal cancer with high microsatellite instability, MSI-H CRC), endometrial or uterine cancer, salivary gland carcinoma, kidney cancer (e.g., renal cell carcinoma), prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, head and neck cancer (e.g., head and neck squamous cell carcinoma, HNSCC), Merkel cell carcinoma (MCC), or combinations thereof.

82. The method of claim 81, wherein the cancer comprises melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, head and neck squamous cell carcinoma (HNSCC), bladder (urothelial) cancer, Merkel cell carcinoma (MCC), triple-negative breast cancer (TNBC), hepatocellular carcinoma, colorectal cancer with high microsatellite instability (MSI-H CRC), esophageal squamous cell carcinoma, or combinations thereof.

83. The method of any one of claims 79 to 82, wherein the isolated polynucleotide, the synthetic circuit, the replicon, the circular RNA, the nanoparticle, and/or the pharmaceutical composition is administered to the subject via intravenous, intratumoral, intrathecal, intramuscular, subcutaneous, inhalation, intradermal, intralymphatic, intraocular, intraperitoneal, intrapleural, intraspinal, intravascular, nasal, percutaneous, sublingual, submucosal, transdermal, or transmucosal administration.

84. The method of any one of claims 79 to 82, wherein the isolated polynucleotide, the synthetic circuit, the replicon, the circular RNA, the nanoparticle, and/or the pharmaceutical composition is administered to the subject via intravenous administration.

85. The method of claim 83 or 84, further comprising administering to the subject an additional therapeutic agent.

86. The method of claim 85, wherein the additional therapeutic agent comprises an immune checkpoint inhibitor.

87. The method of claim 86, wherein the immune checkpoint inhibitor comprises a PD-1 antagonist, a PD-L1 antagonist, a LAG3 antagonist, a CCR8 antagonist, or any combination thereof.

FIG. 1A

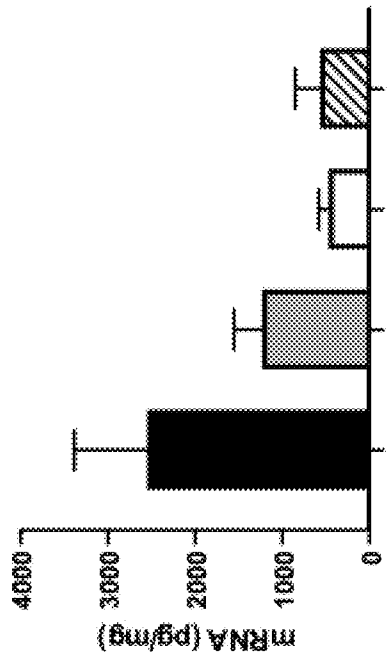


FIG. 1B

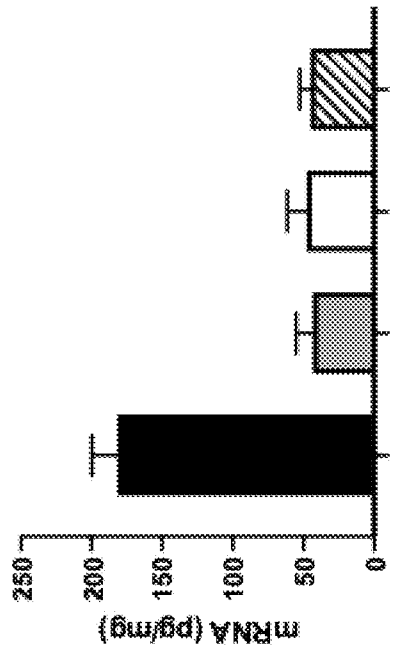


FIG. 1C

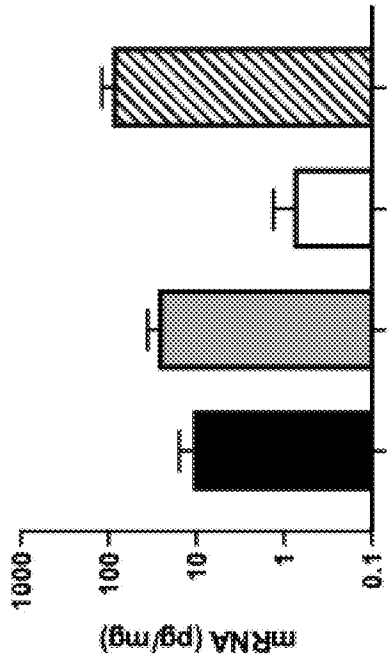
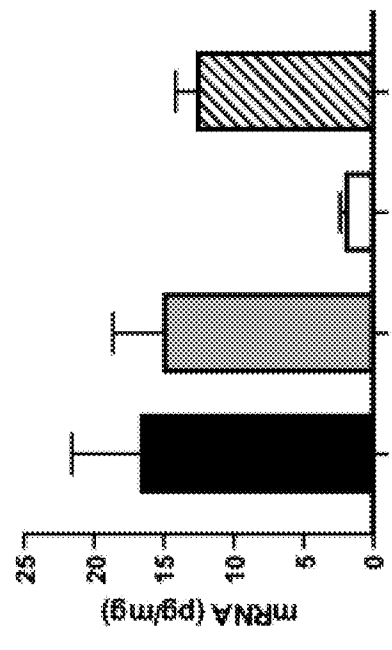


FIG. 1D



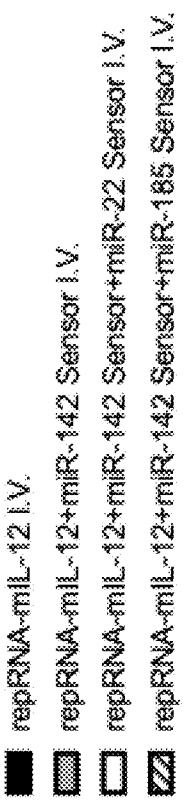


FIG. 1E

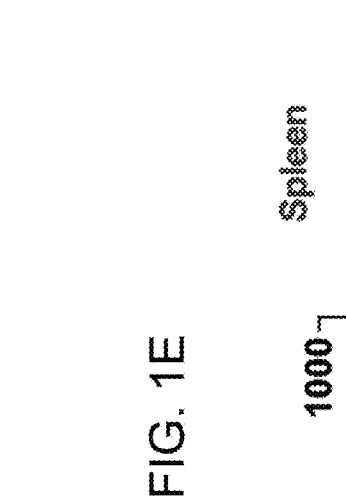


FIG. 1F

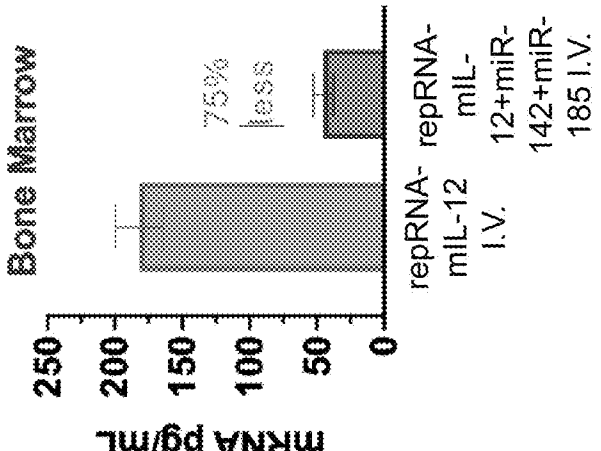


FIG. 1G

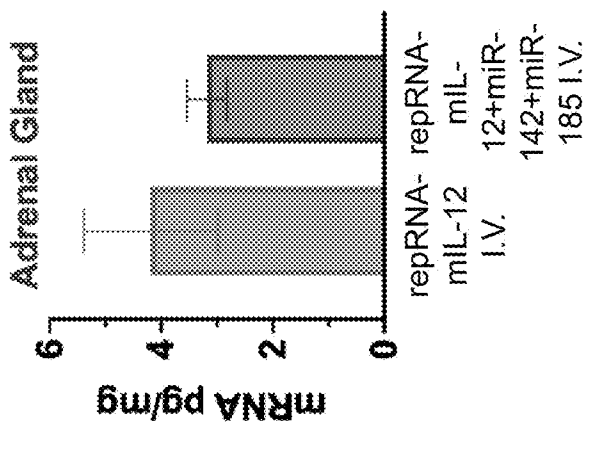


FIG. 1H

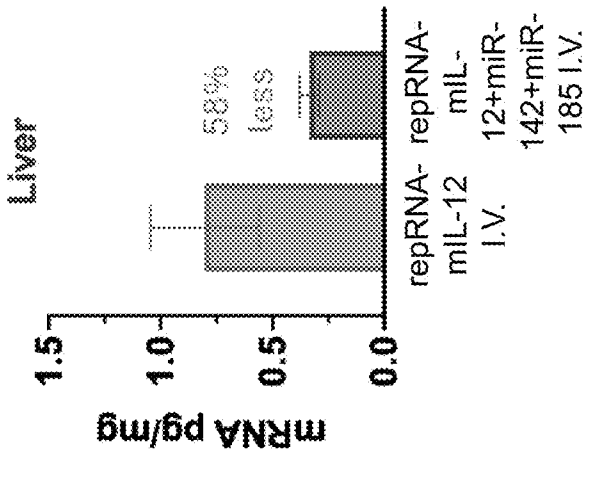


FIG. 2A

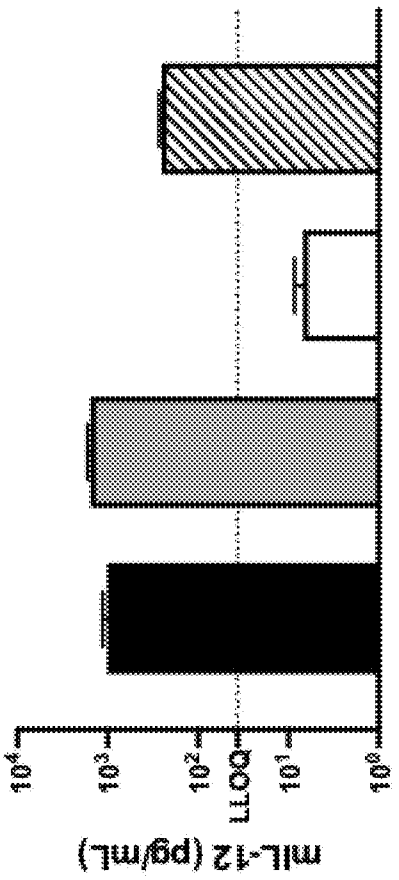
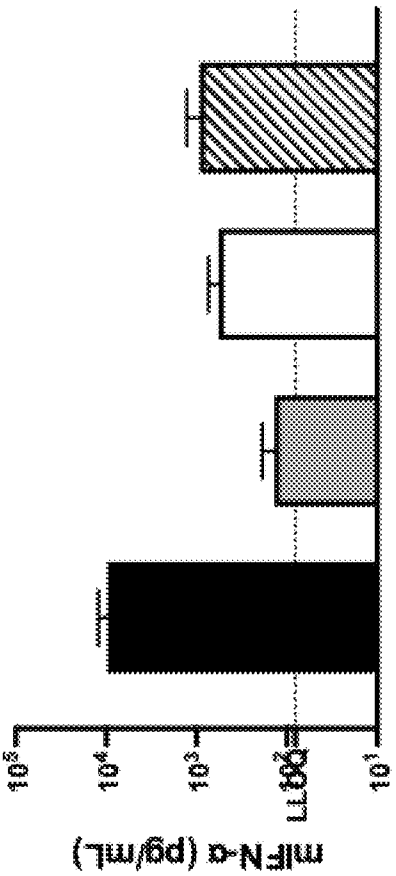


FIG. 2B



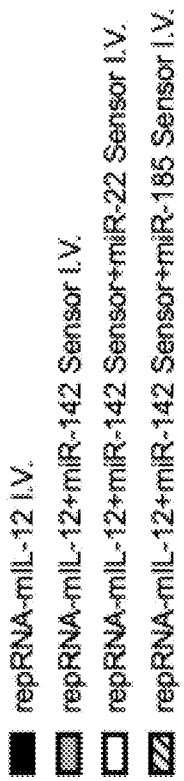


FIG. 2C

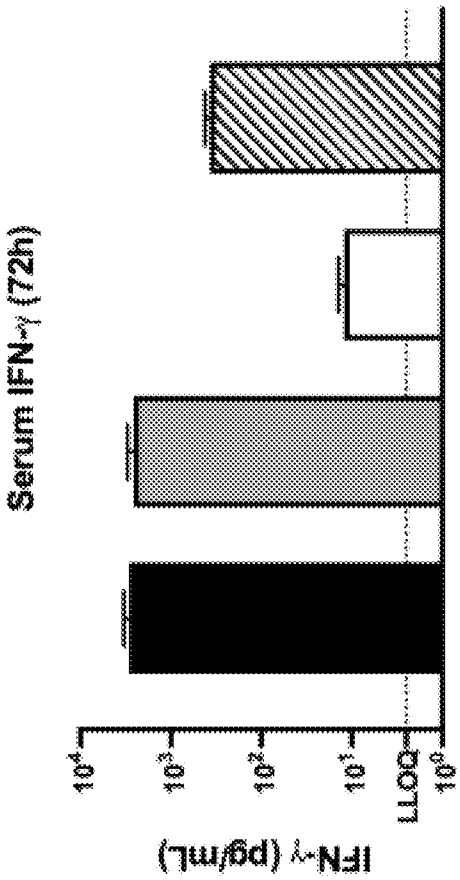


FIG. 2D

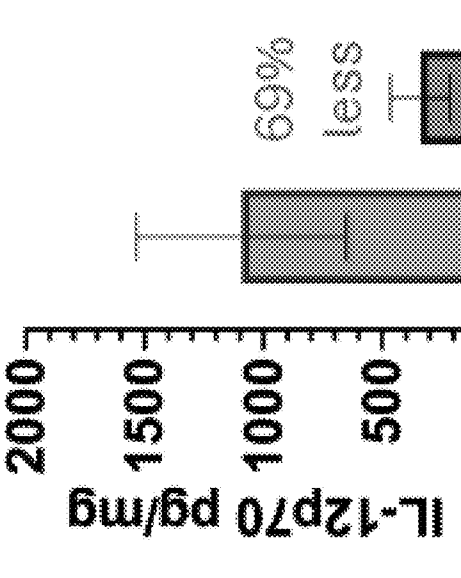


FIG. 2E

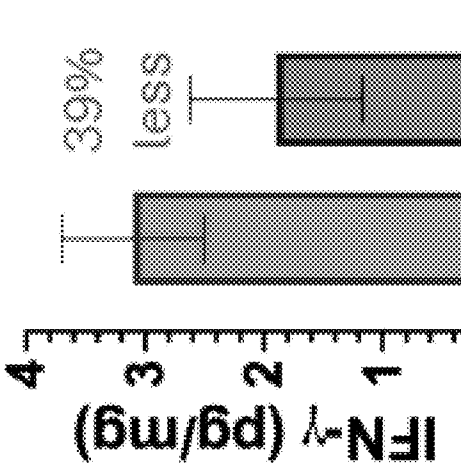
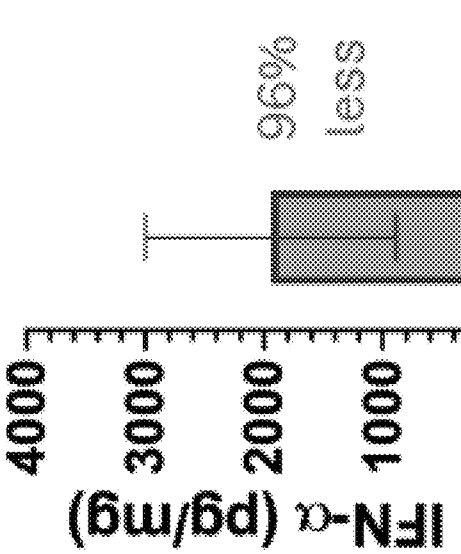


FIG. 2F



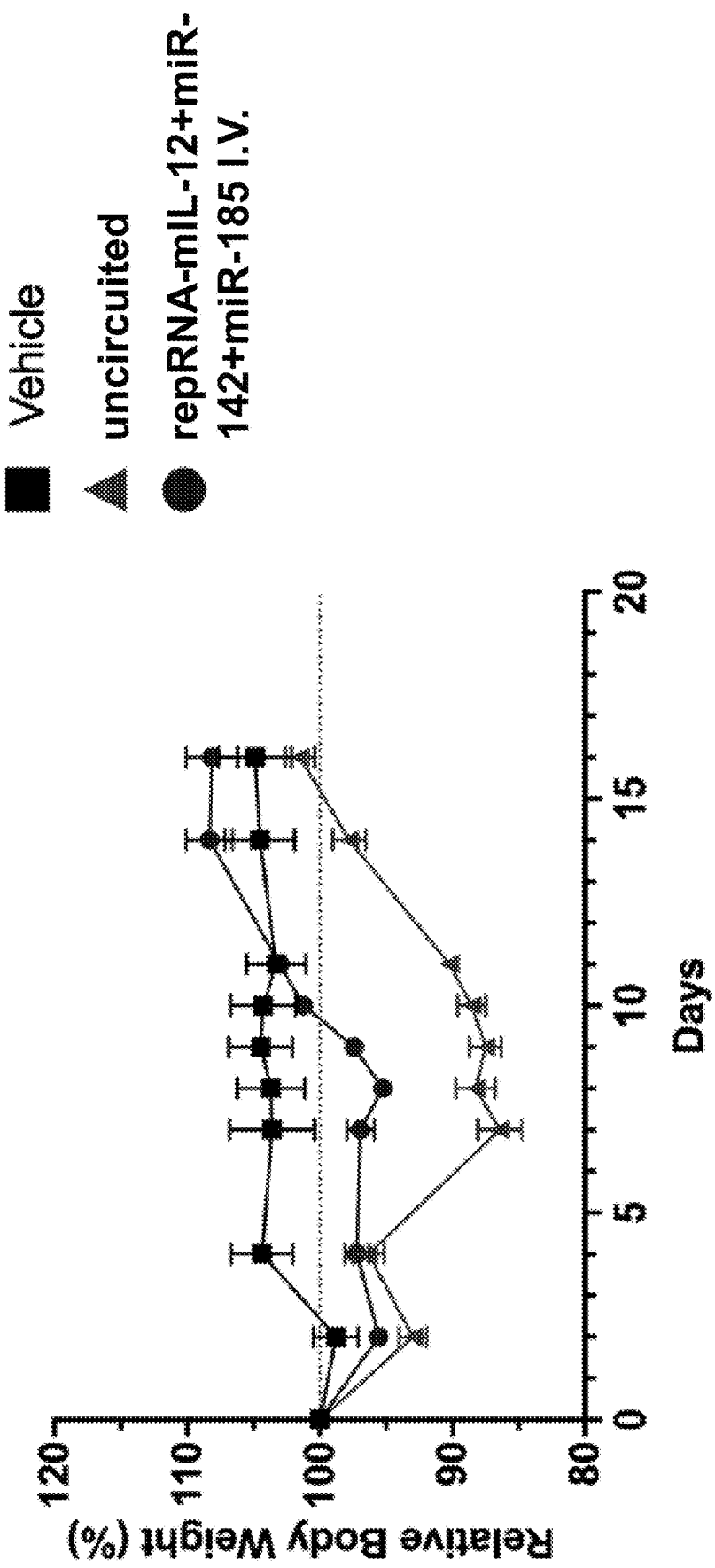

Vehicle
uncircuited
repRNA-mIL-12+miR-142+miR-185 I.V.
Relative Body Weight (%)
120
110
100
90
80
0
5
10
15
20
Days

FIG. 2G

FIG. 3A

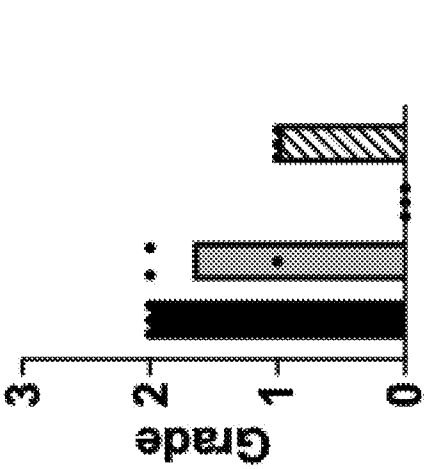


FIG. 3B

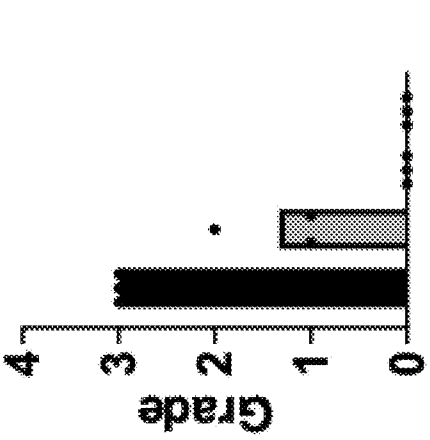
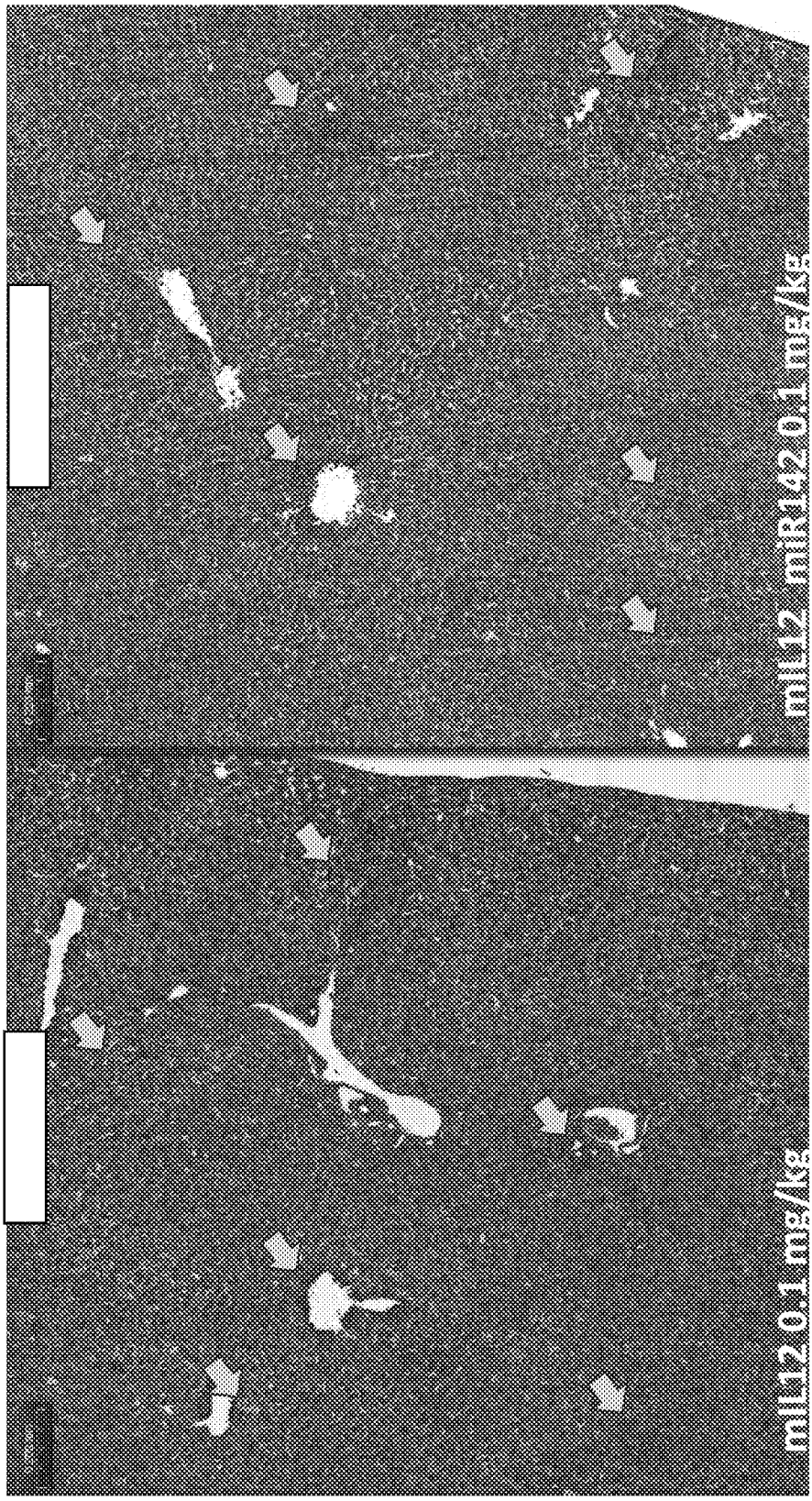


FIG. 3C

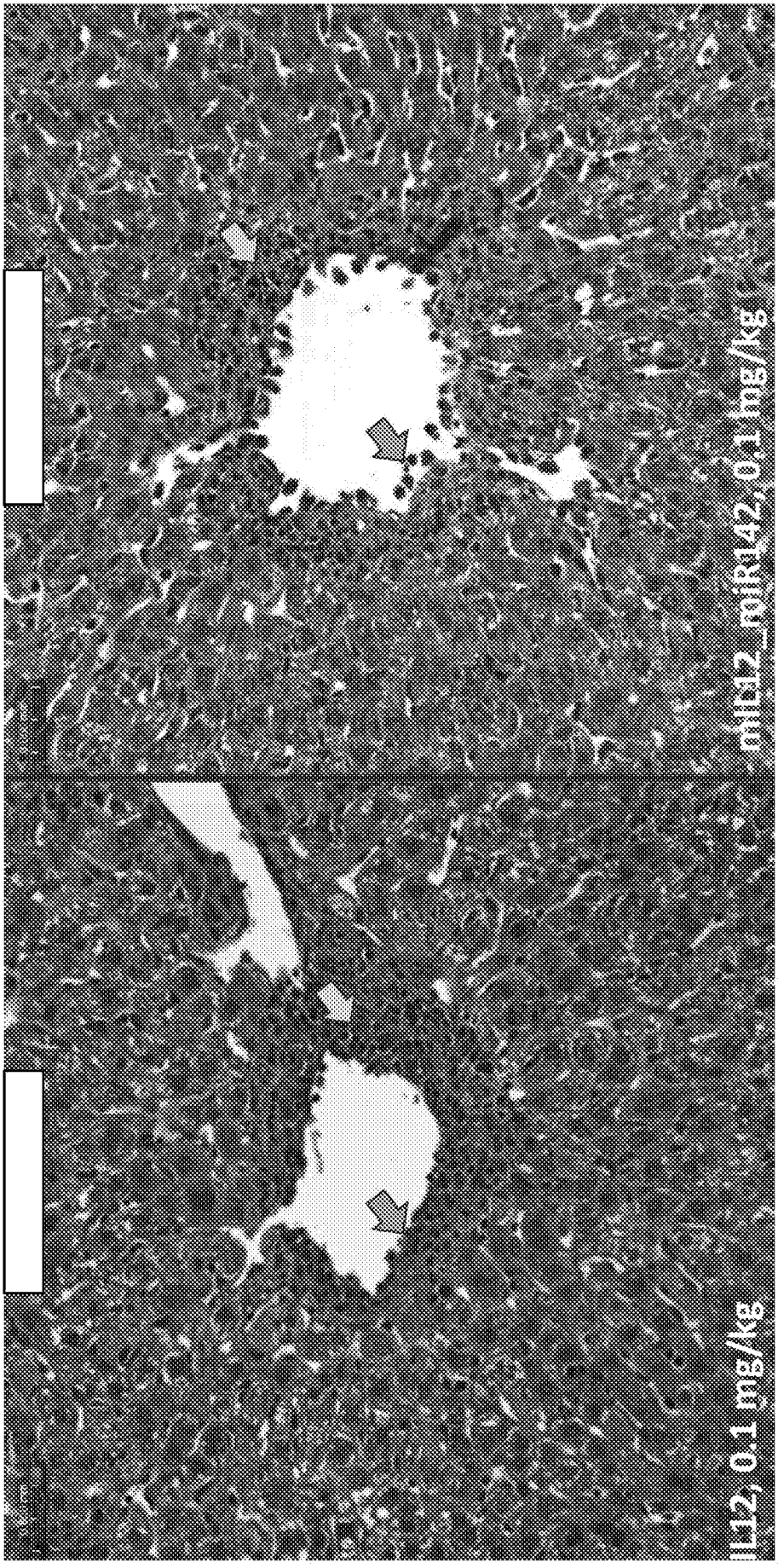
FIG. 3D

FIG. 4A



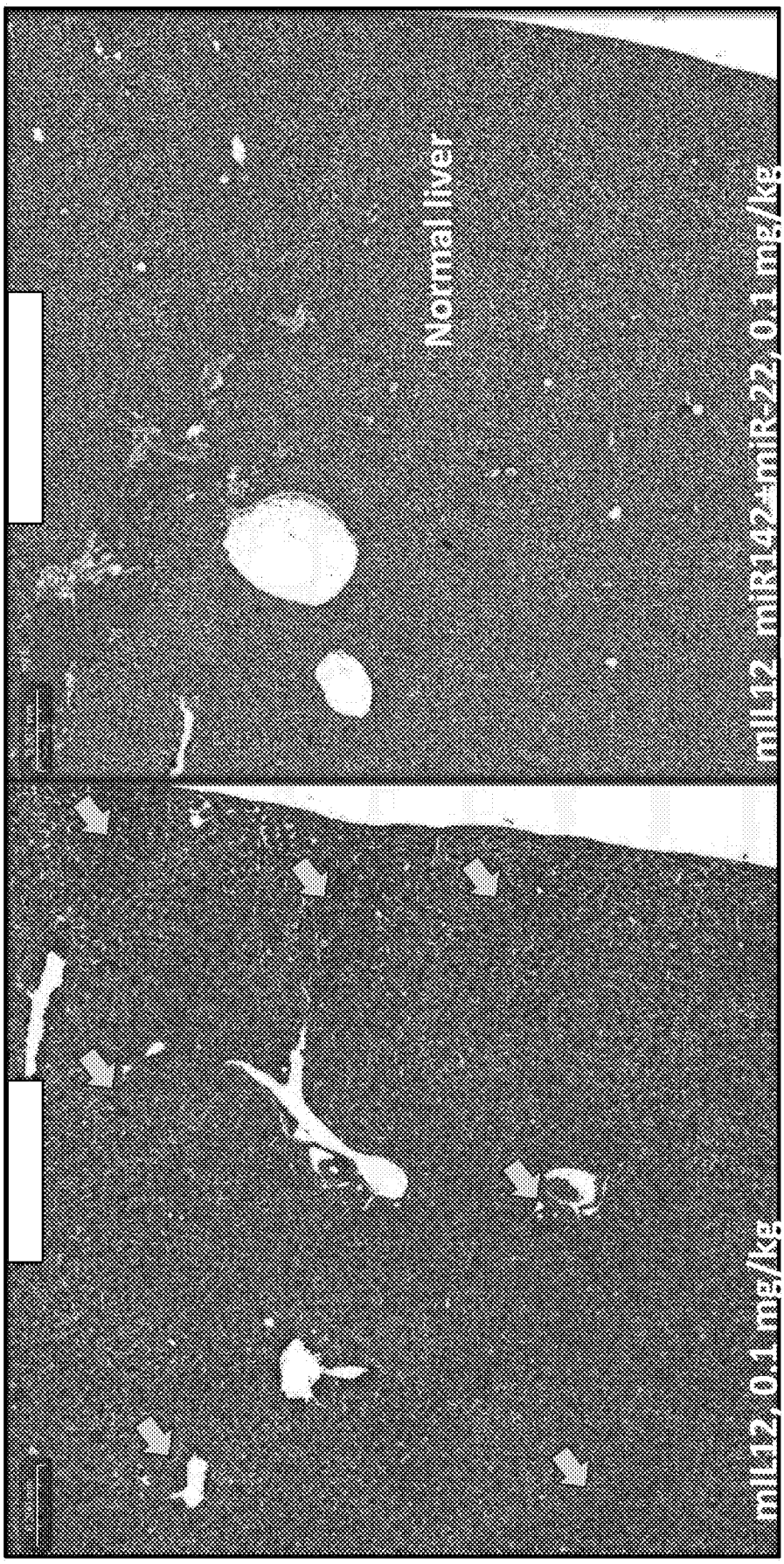
*, Findings include intravascular margination of leukocytes, perivascular histiocyte infiltration, Kupffer cell hyperplasia

FIG. 4B



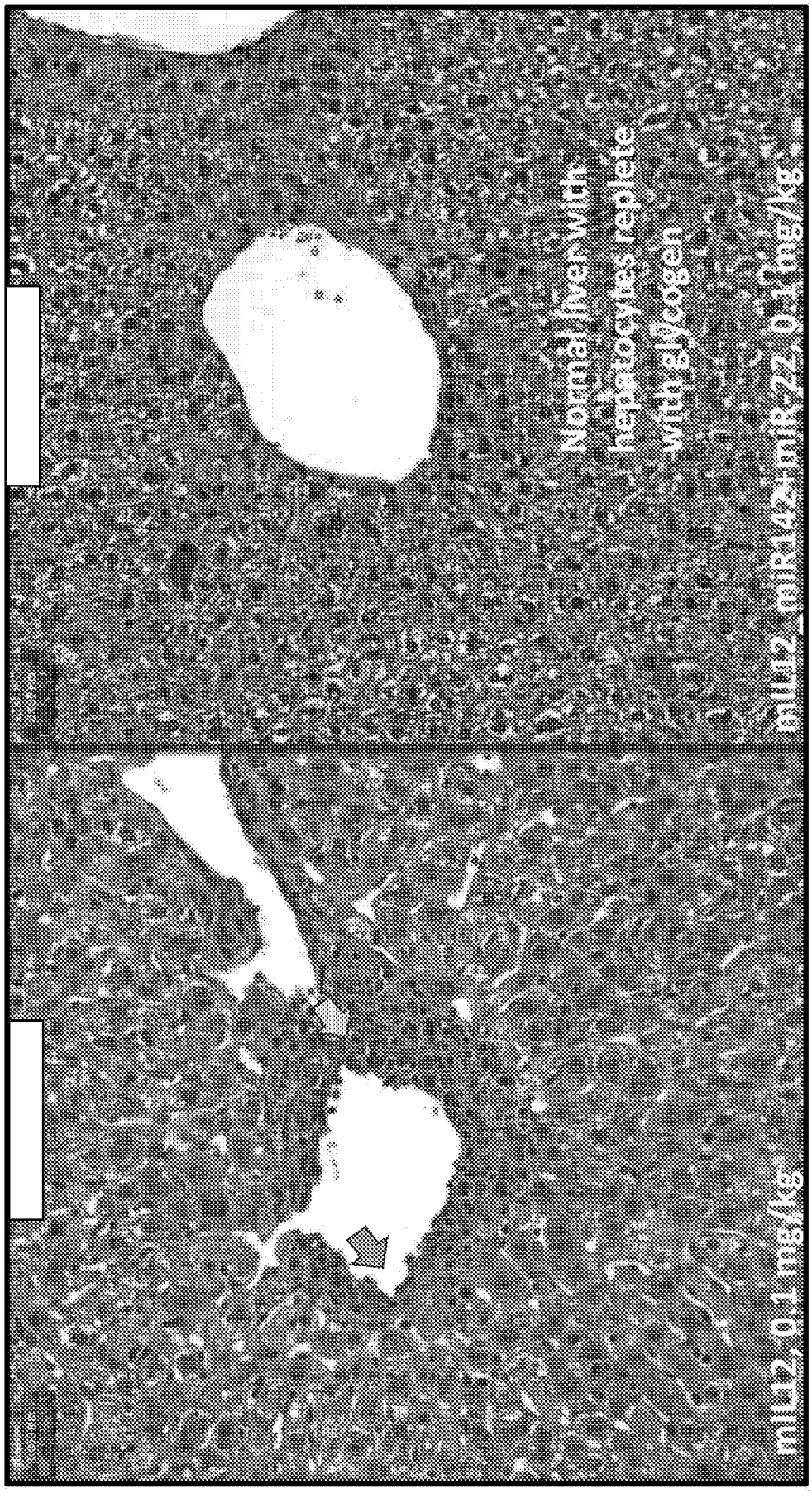
*, green arrow = intravascular margination of leukocytes, yellow arrow = perivascular histiocyte infiltration

FIG. 5A



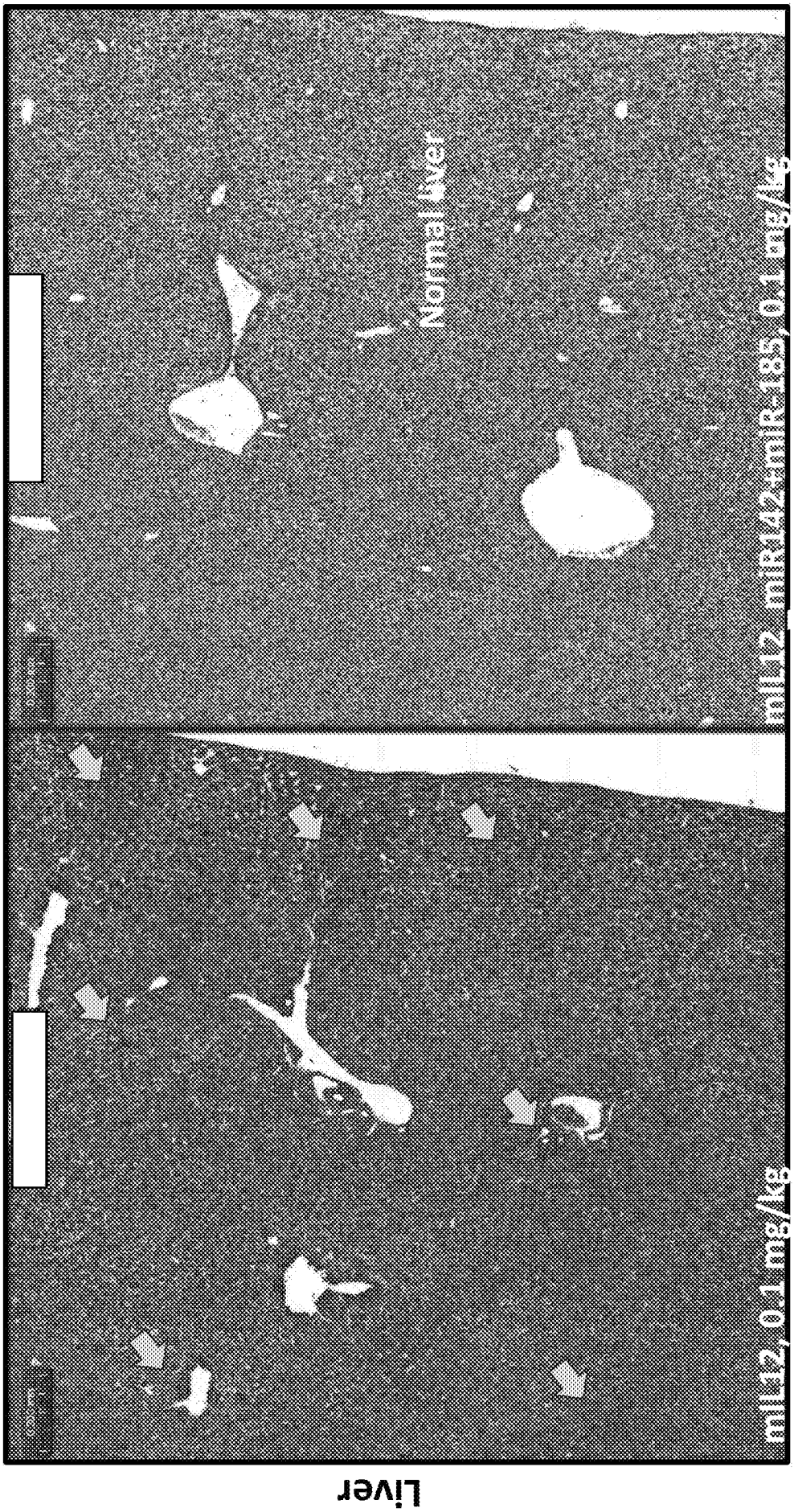
Yellow arrows, findings include intravascular margination of leukocytes, perivascular histiocyte infiltration, Kupffer cell hyperplasia

FIG. 5B



*, green arrow = intravascular margination of leukocytes, yellow arrow = perivascular histiocyte infiltration

FIG. 6A



Yellow arrows, findings include intravascular margination of leukocytes, perivascular histiocyte infiltration, Kupffer cell hyperplasia

FIG. 6B

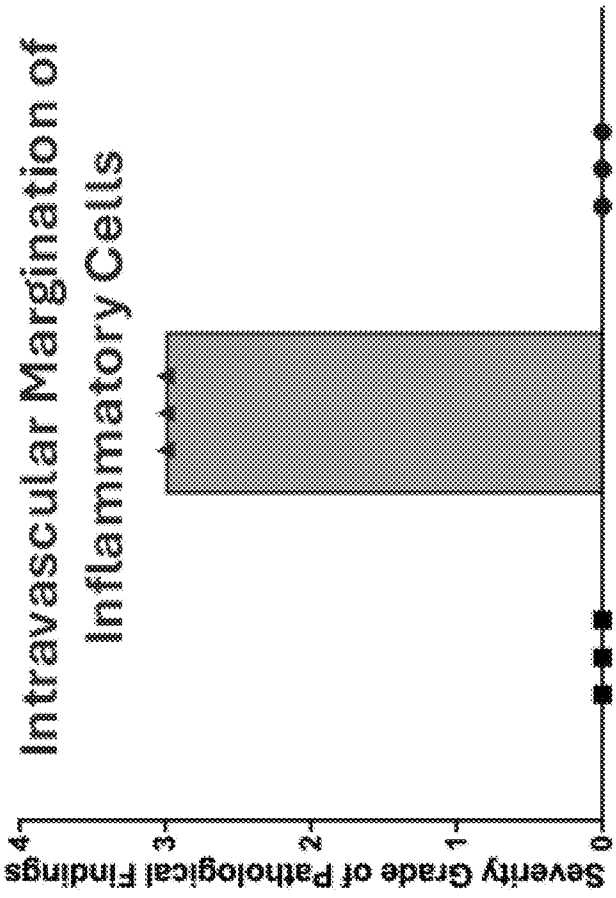

Intravascular Margination of Inflammatory Cells
Severity Grade of Pathological Findings
0
1
2
3
4
vehicle
uncircuited
repRNA-mIL-12+miR-142+miR-185 I.V.

FIG. 6C

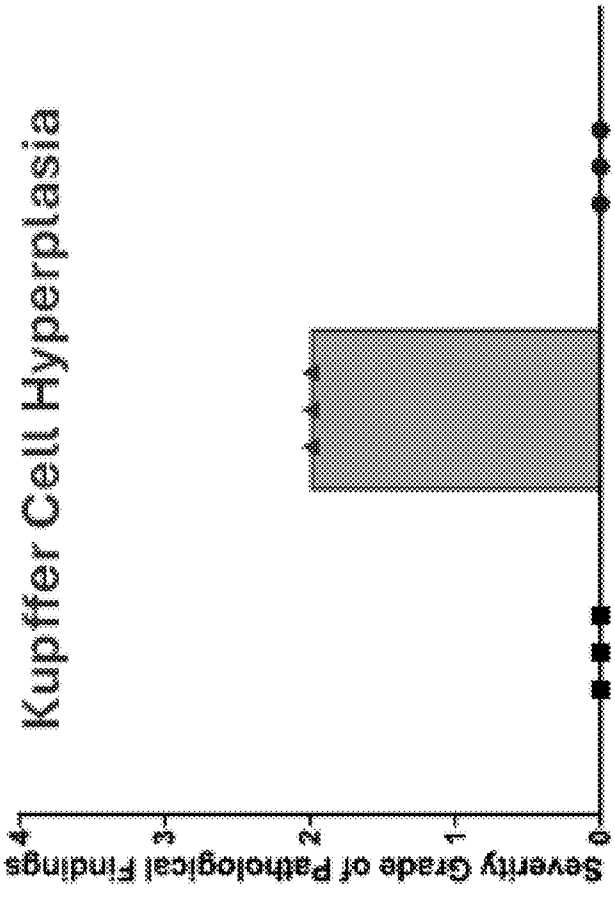
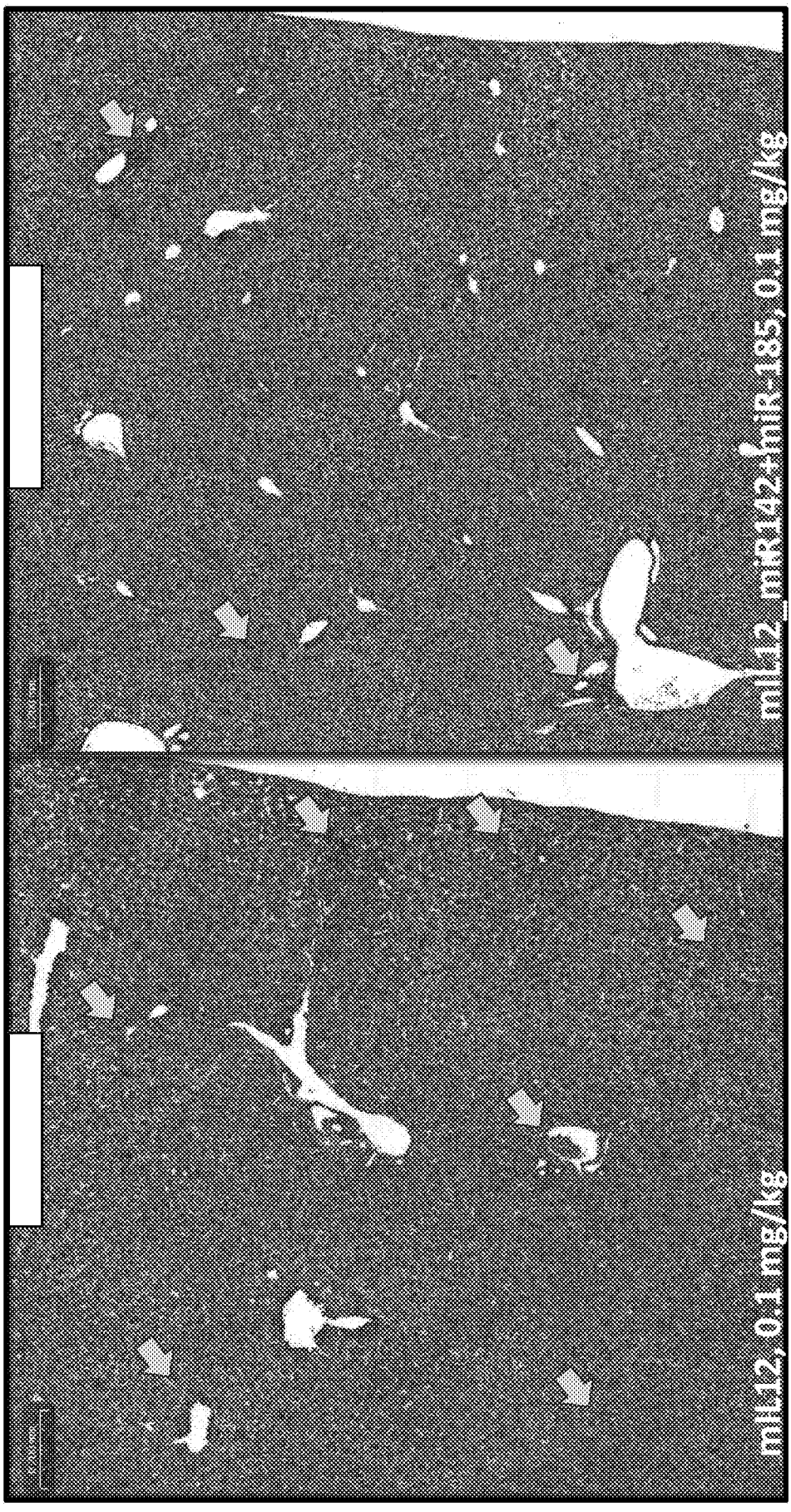

Kupffer Cell Hyperplasia
Severity Grade of Pathological Findings
0
1
2
3
4
vehicle
uncircuited
repRNA-mIL-12+miR-142+miR-185 I.V.

FIG. 7



Yellow arrows, findings include intravascular margination of leukocytes, perivascular histiocyte infiltration, Kupffer cell hyperplasia

FIG. 8

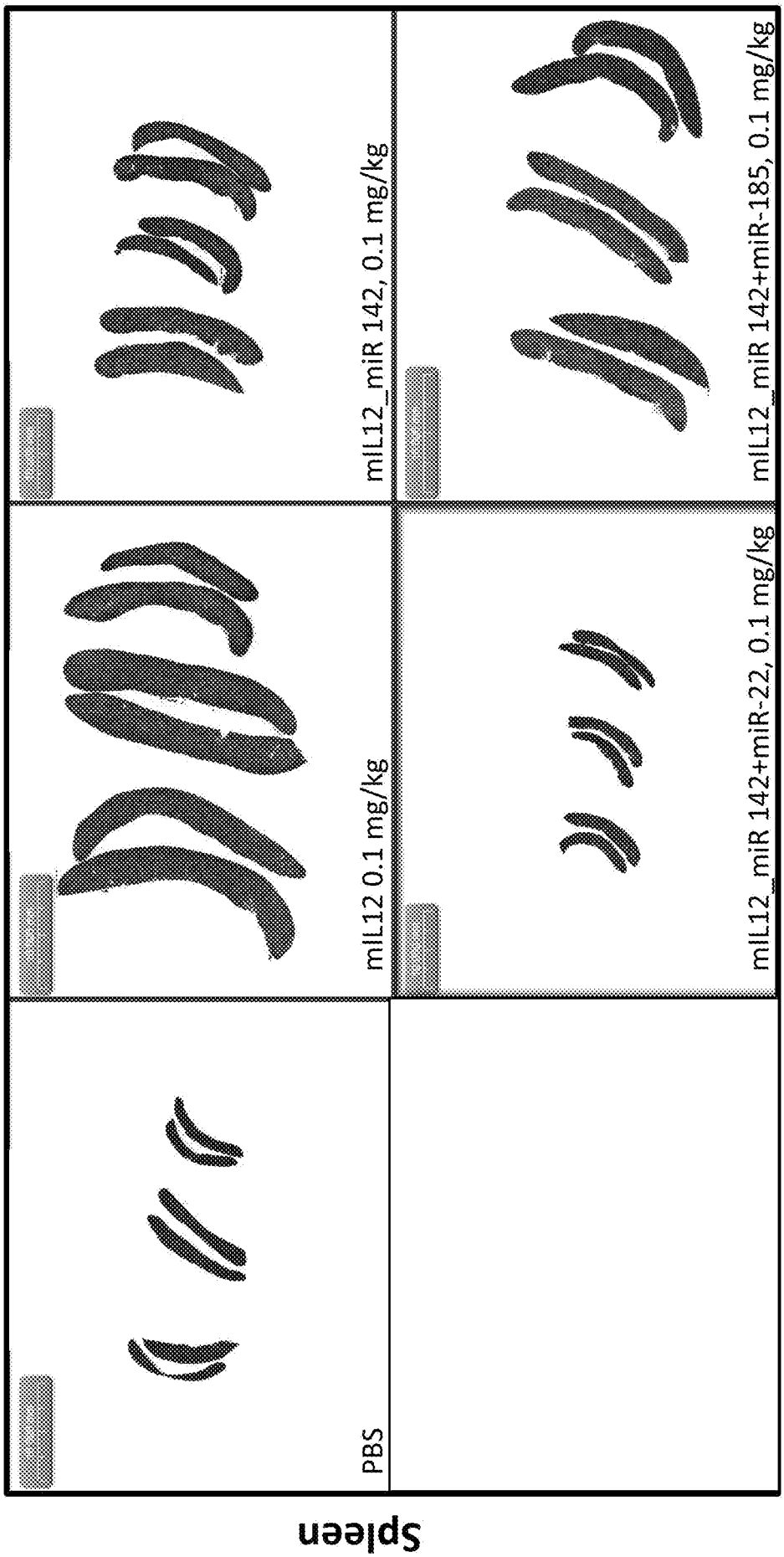
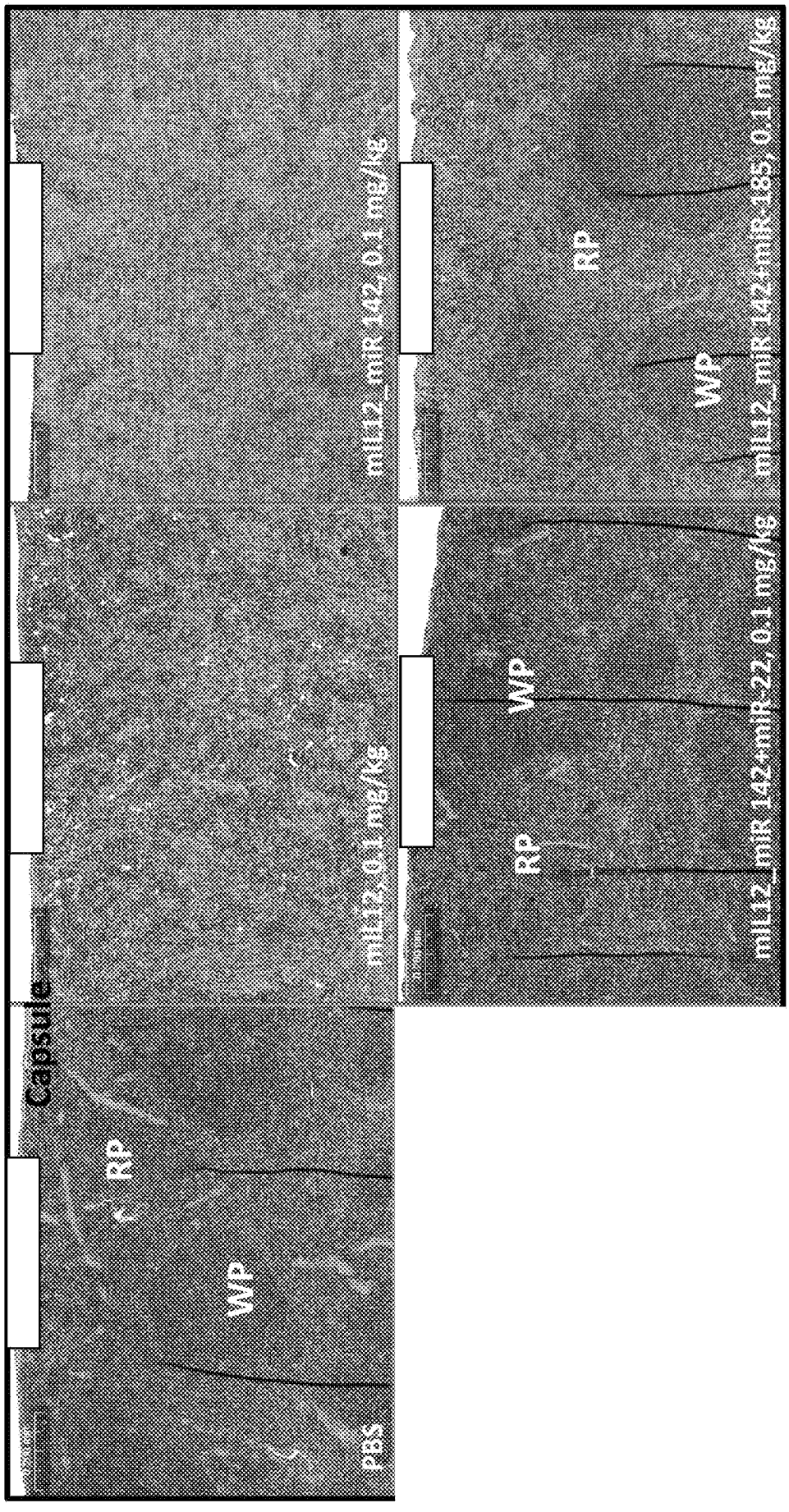

Spleen
PBS
mIL12 0.1 mg/kg
mIL12_miR 142, 0.1 mg/kg
mIL12_miR 142+miR-22, 0.1 mg/kg
mIL12_miR 142+miR-185, 0.1 mg/kg

FIG. 9A



*, Marginal zone histiocytosis, decreased cellularity (lymphocyte) white pulp; RP = red pulp; WP = white pulp

FIG. 9B

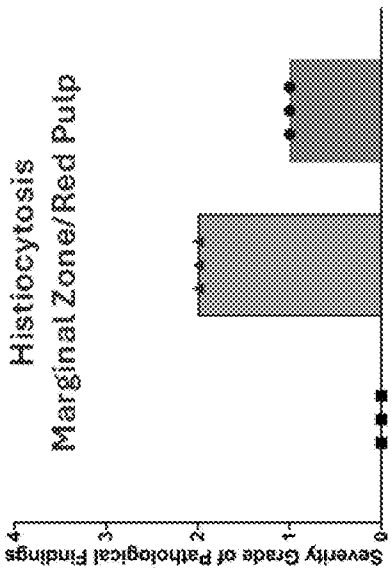
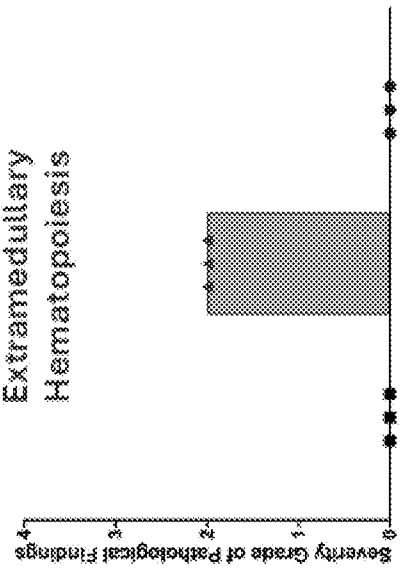

Histiocytosis
Marginal Zone/Red Pulp
Severity Grade of Pathological Findings
0
1
2
3
4
vehicle
uncircuited
repRNA-mIL-12+miR-142+miR-185 I.V.

FIG. 9C


Extramedullary Hematopoiesis
Severity Grade of Pathological Findings
0
1
2
3
4
vehicle
uncircuited
repRNA-mIL-12+miR-142+miR-185 I.V.

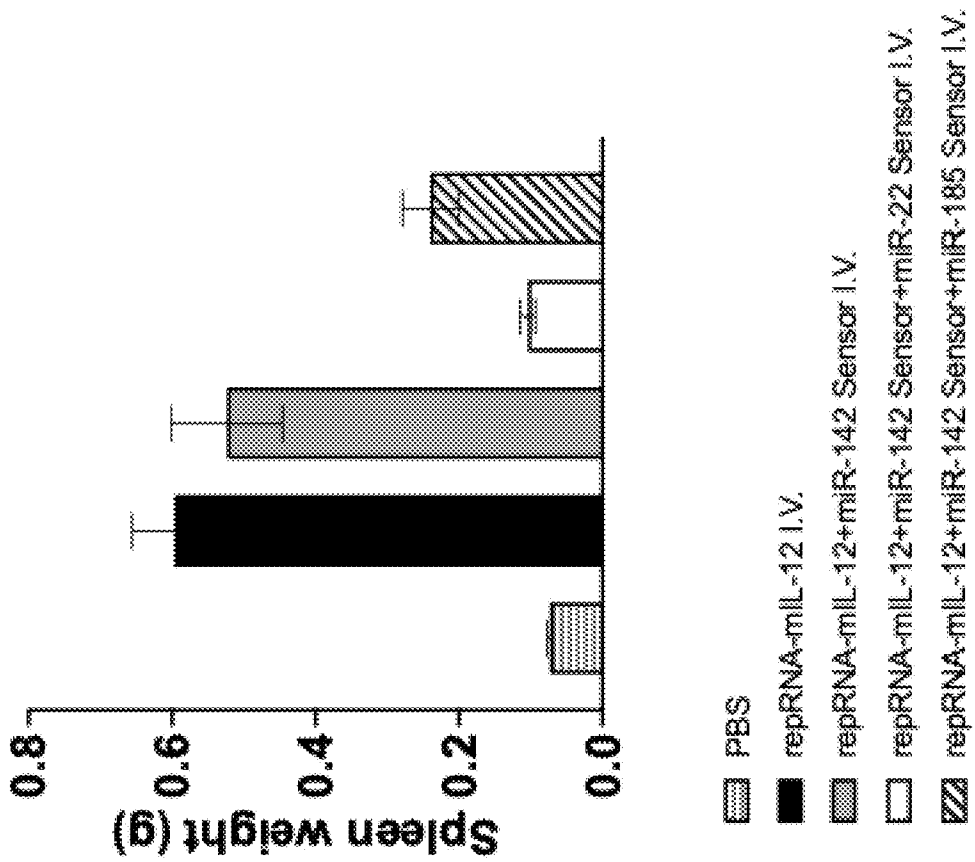
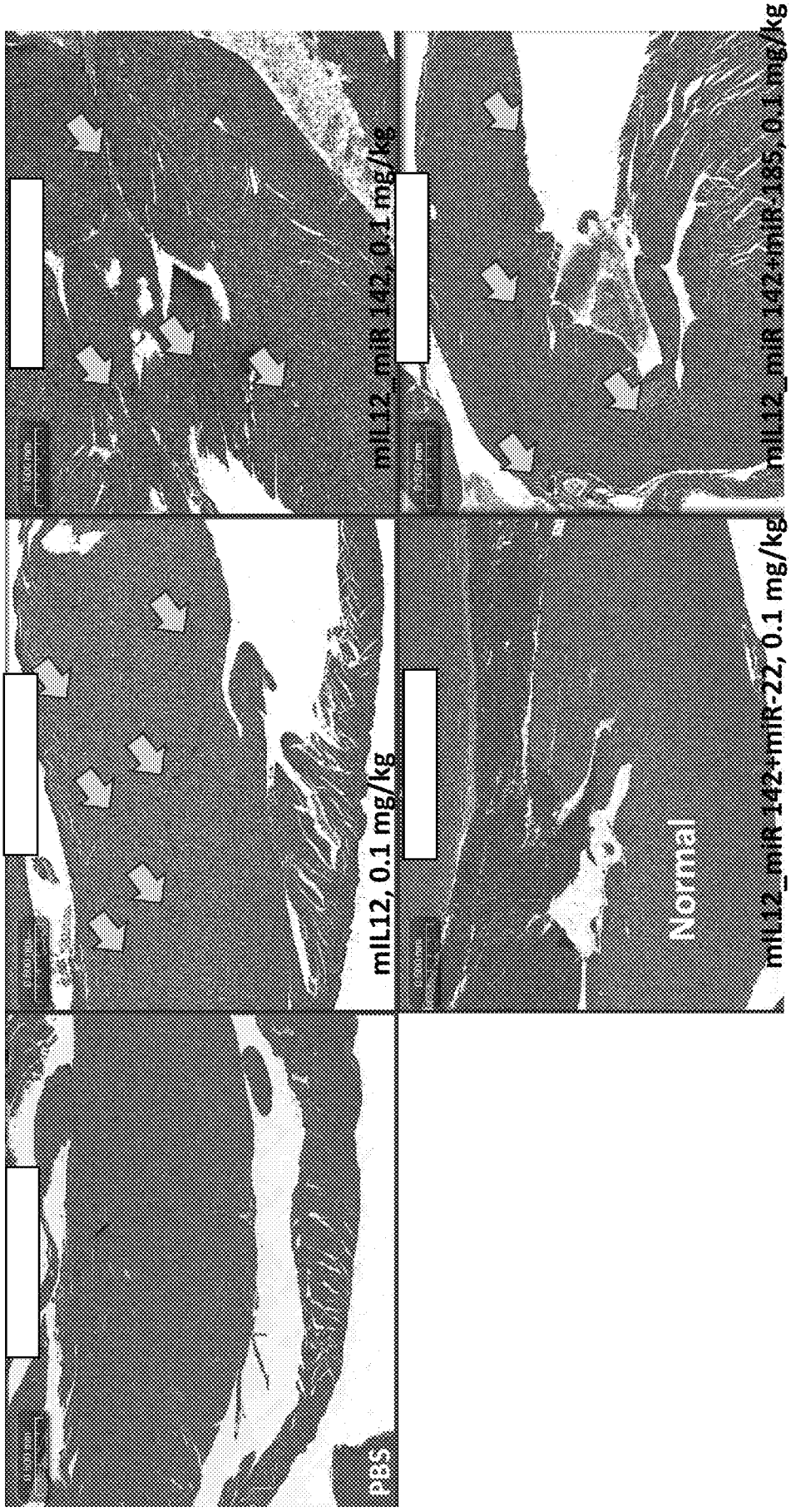

Spleen Weights
Spleen weight (g)
0.0
0.2
0.4
0.6
0.8
PBS
repRNA-mIL-12 I.V.
repRNA-mIL-12+miR-142 Sensor I.V.
repRNA-mIL-12+miR-142 Sensor+miR-22 Sensor I.V.
repRNA-mIL-12+miR-142 Sensor+miR-185 Sensor I.V.

FIG. 10

FIG. 11



*, intravascular mononuclear cell infiltrates with perivascular extension

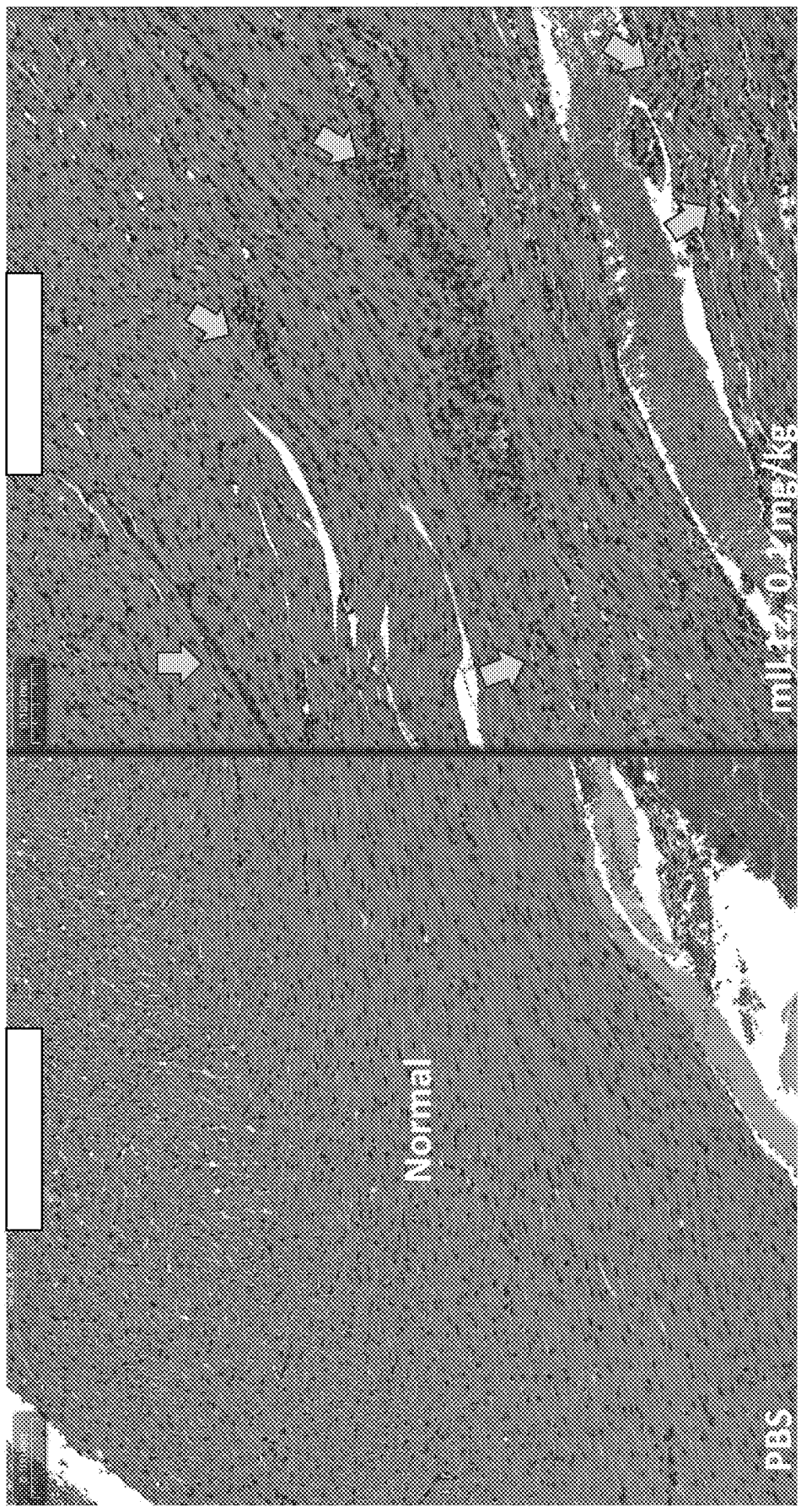


FIG. 12

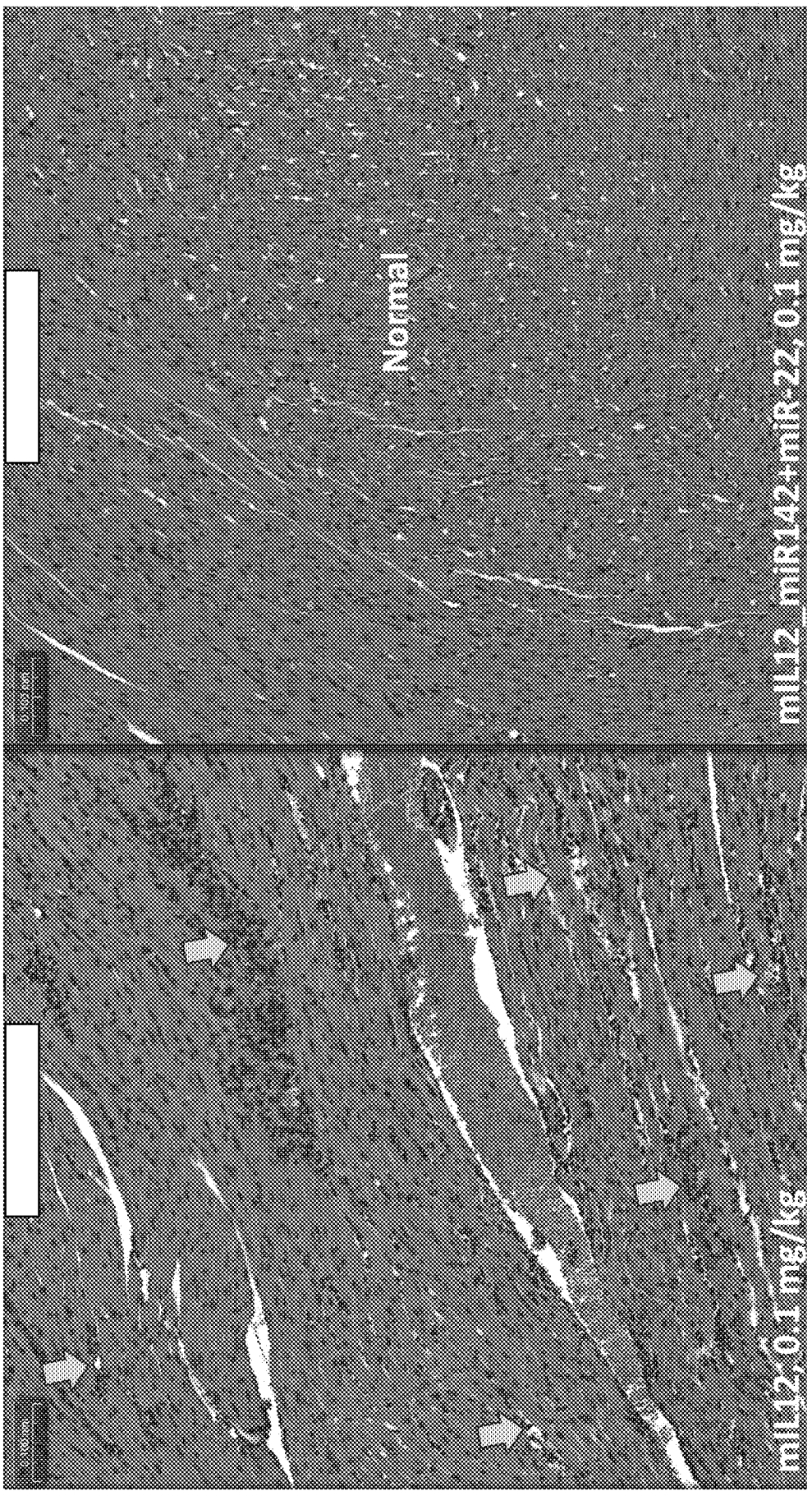


FIG. 13

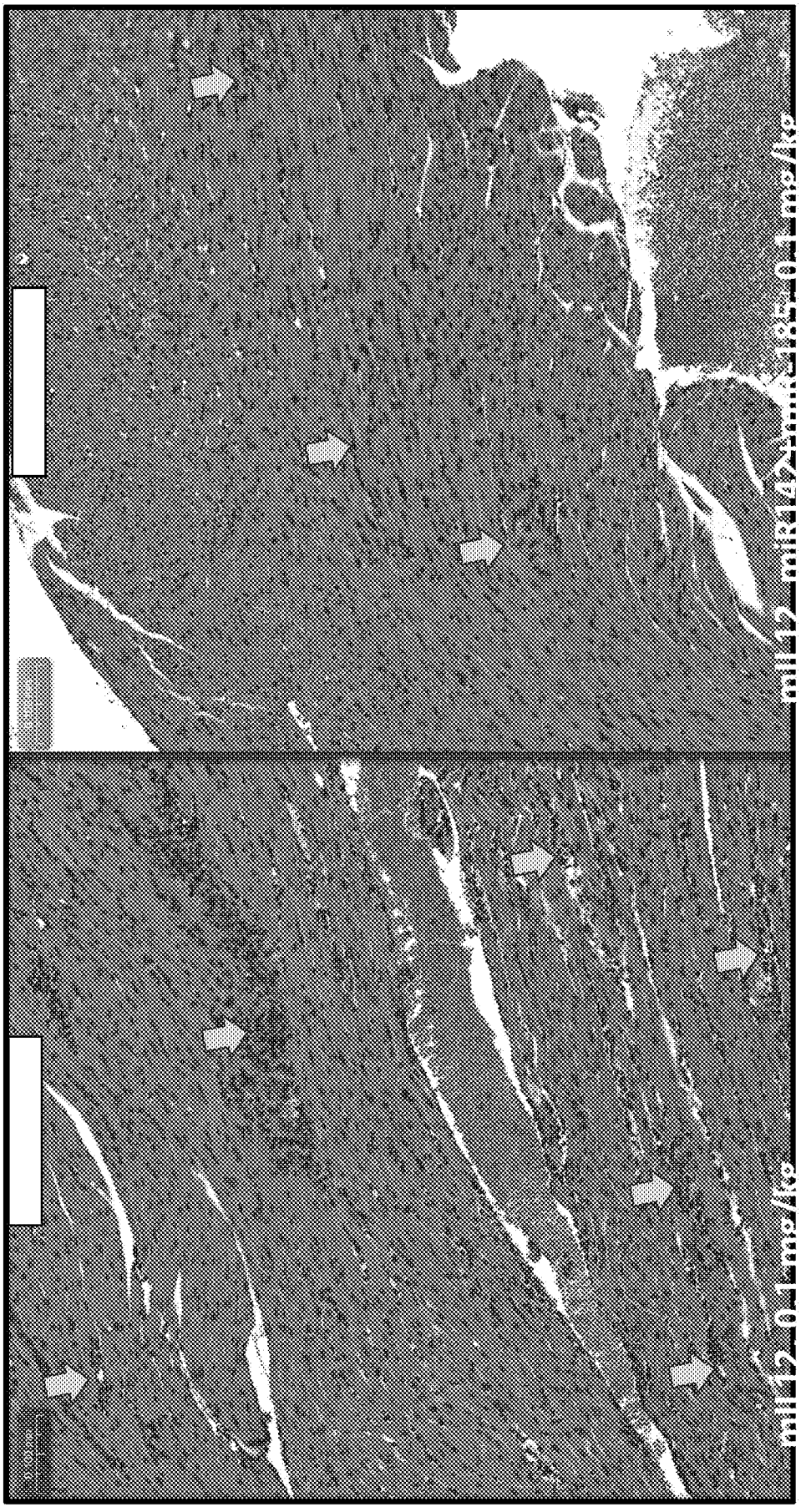


FIG. 14A

FIG. 14B

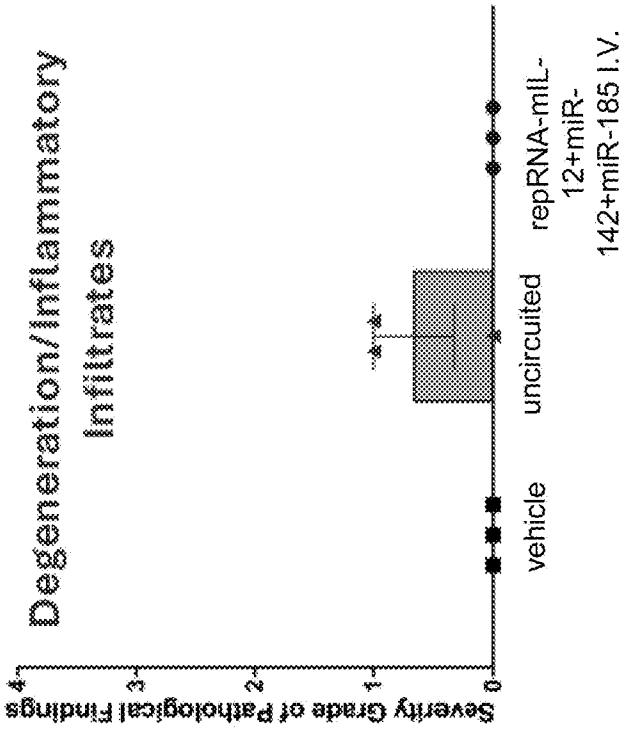
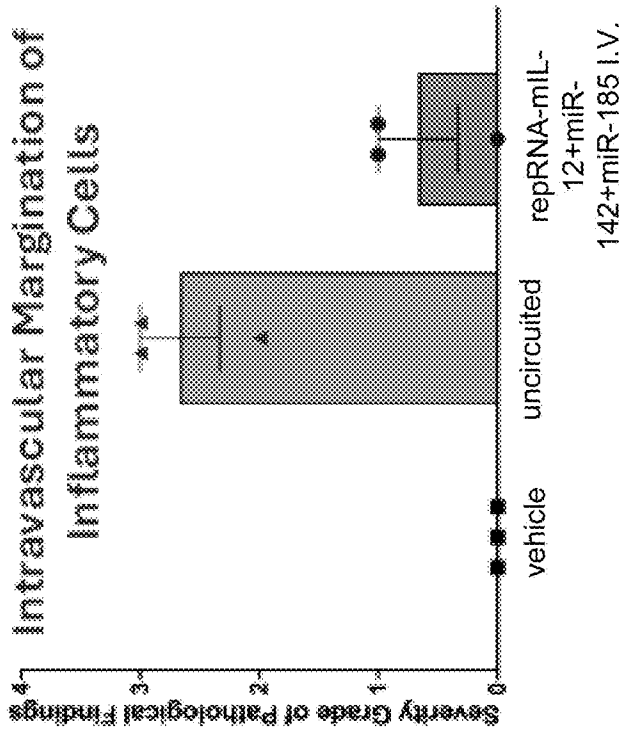


FIG. 14C



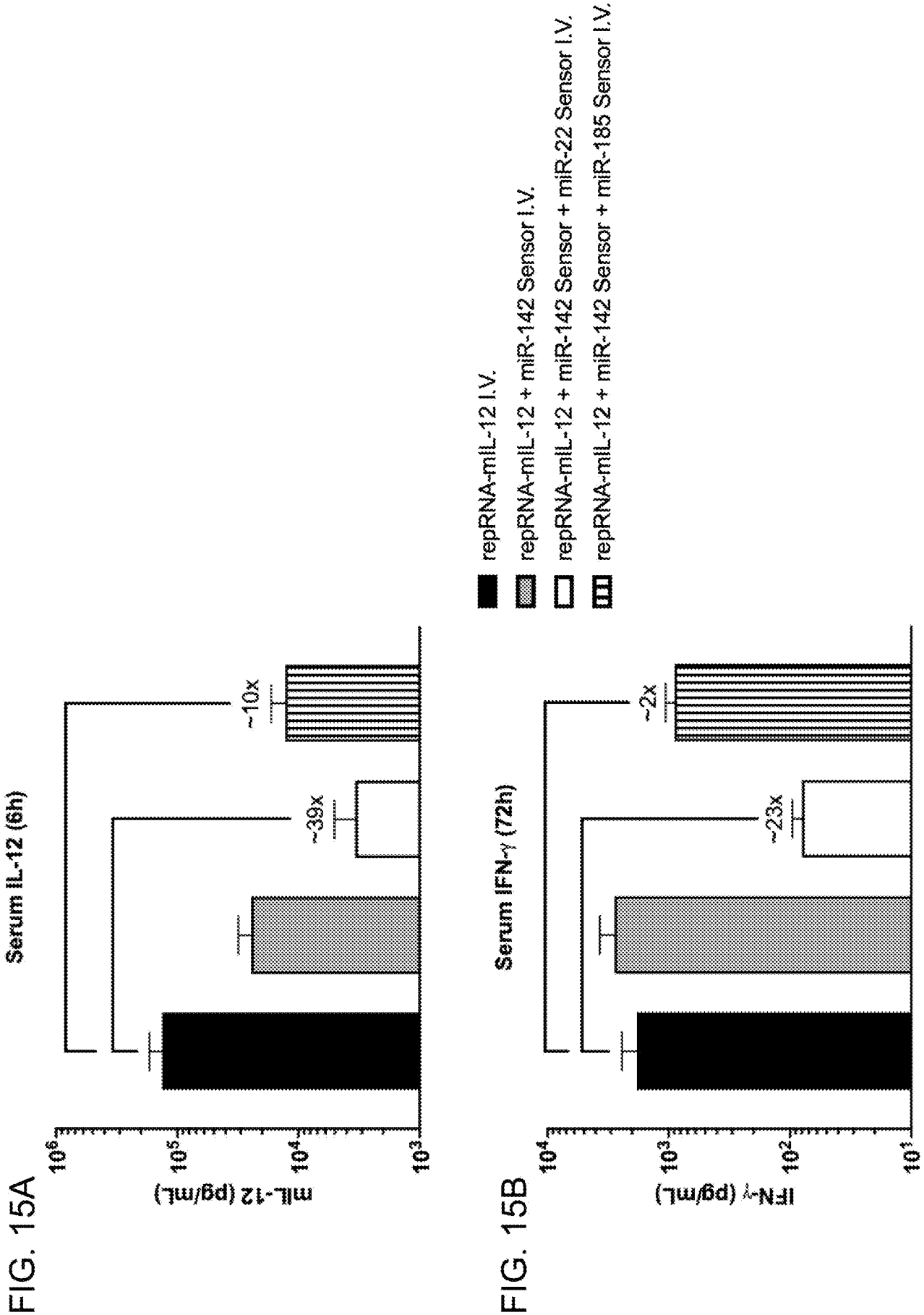


FIG. 15A

FIG. 15B

FIG. 16A

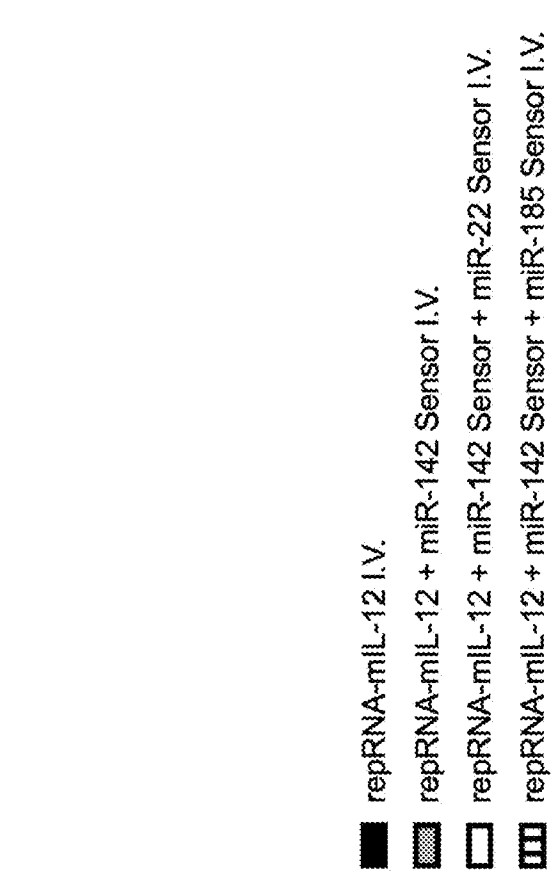


FIG. 16B

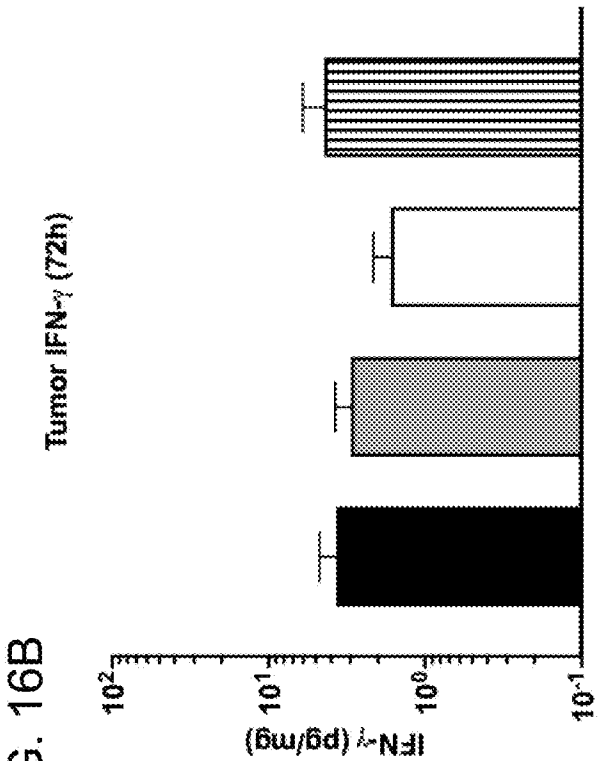


FIG. 16C

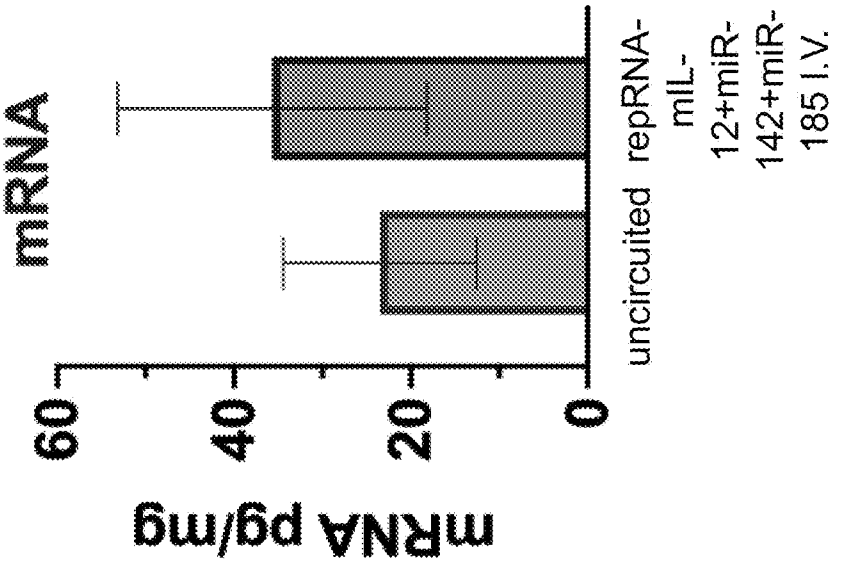


FIG. 16D

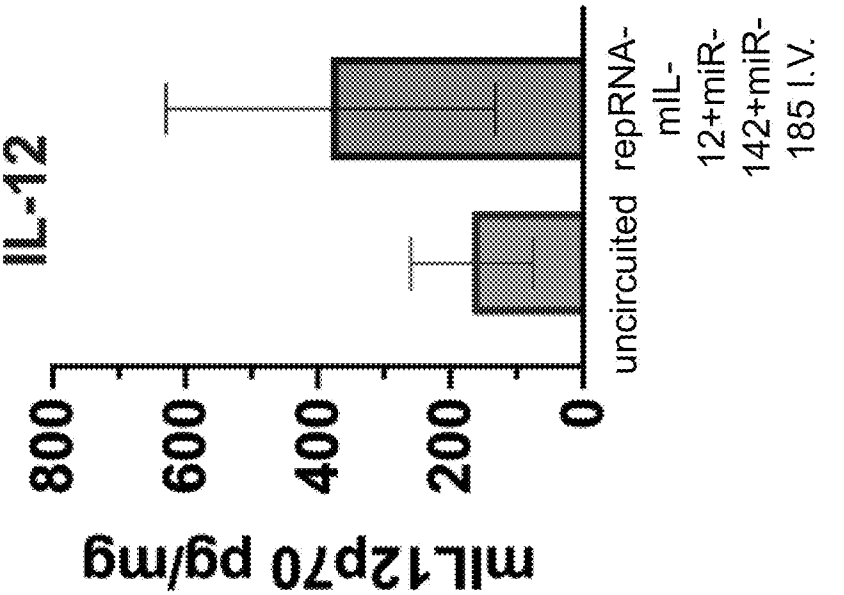


FIG. 16E

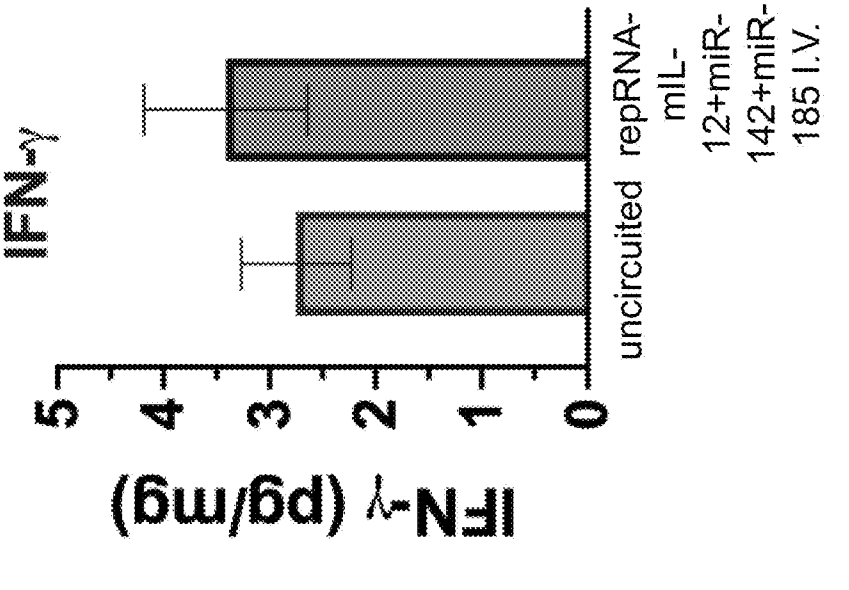
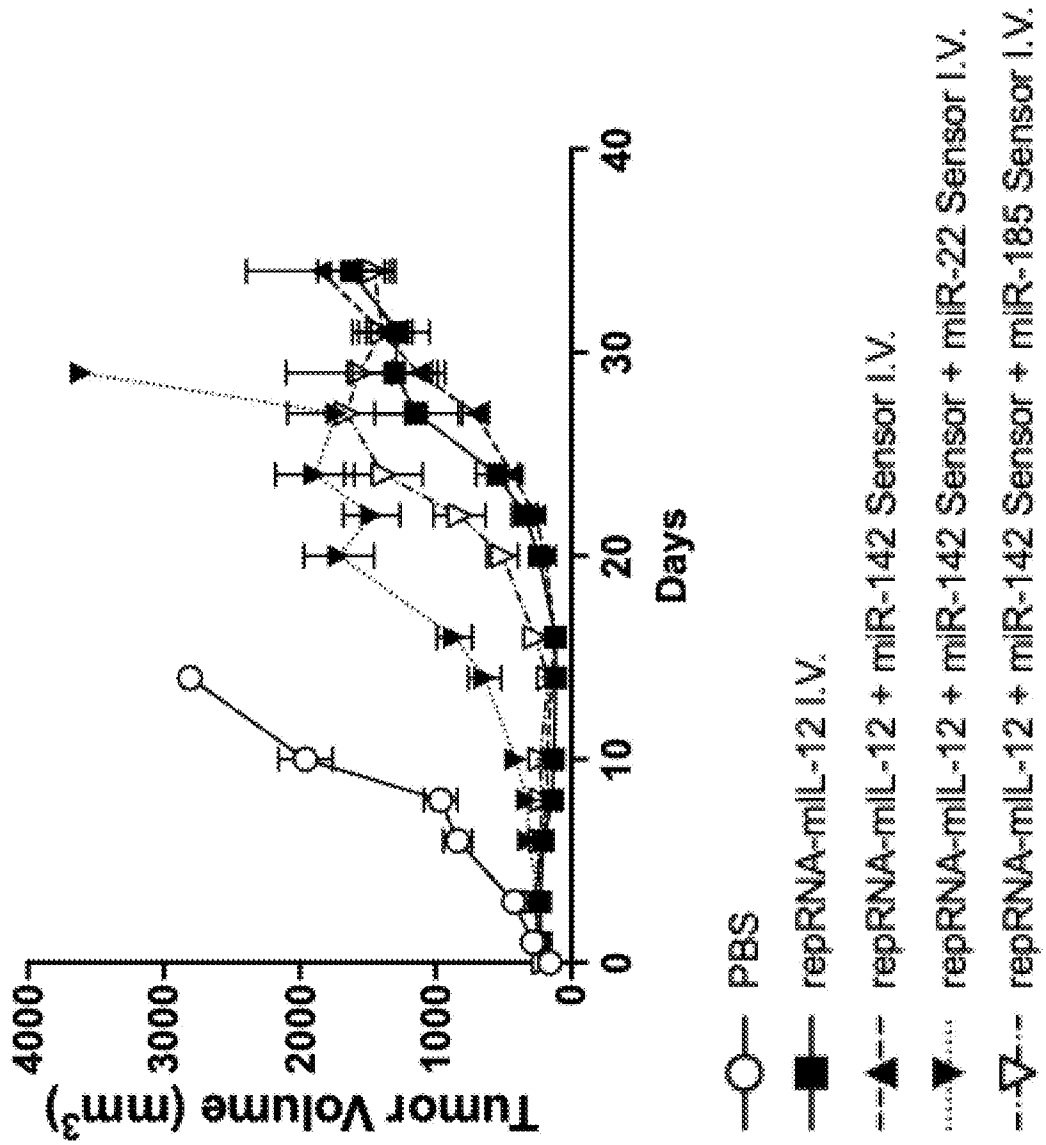


FIG. 17


Tumor Volume (mm³)
4000
3000
2000
1000
0
0
10
20
30
40
Days
PBS
repRNA-mIL-12 I.V.
repRNA-mIL-12 + miR-142 Sensor I.V.
repRNA-mIL-12 + miR-142 Sensor + miR-22 Sensor I.V.
repRNA-mIL-12 + miR-142 Sensor + miR-185 Sensor I.V.

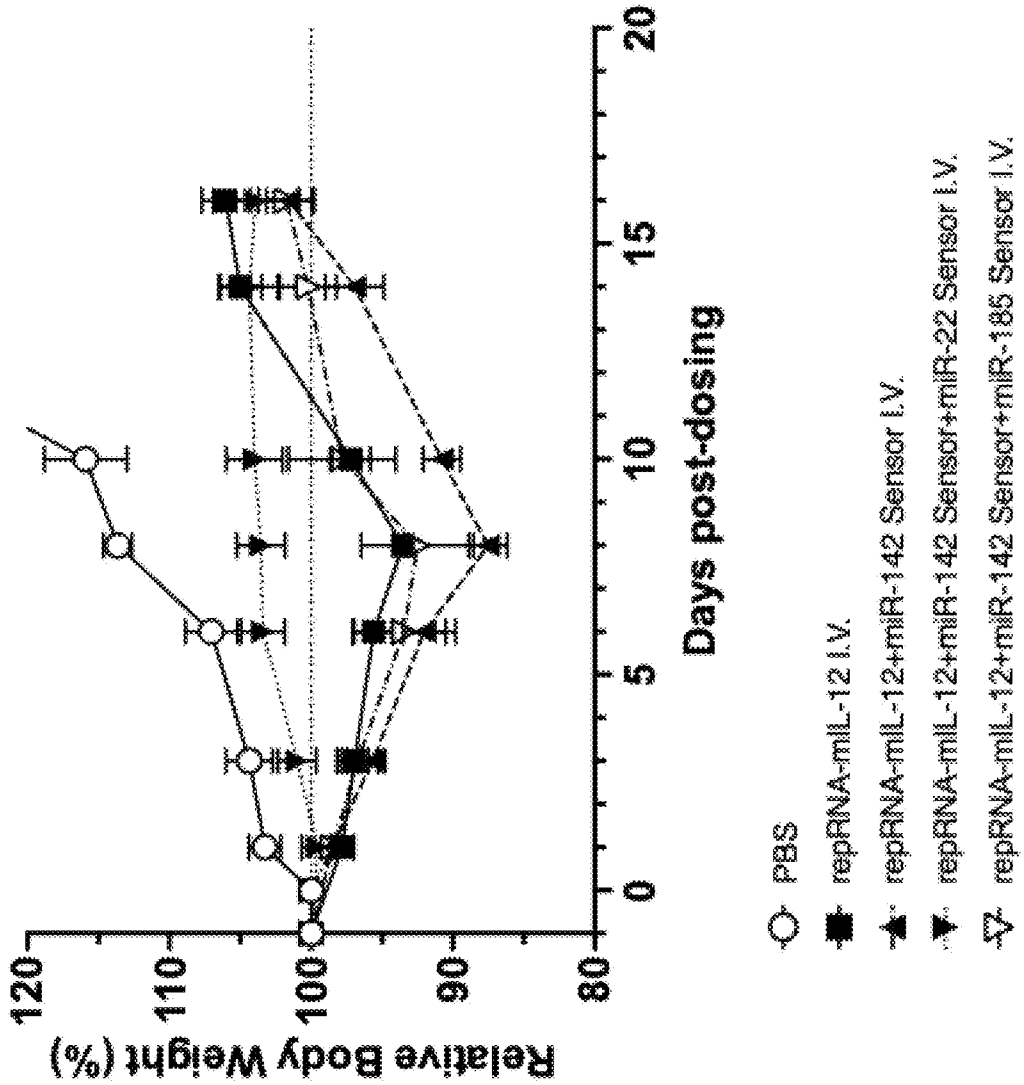

Body Weight Change
Relative Body Weight (%)
120
110
100
90
80
0
5
10
15
20
Days post-dosing
PBS
repRNA-mIL-12 I.V.
repRNA-mIL-12+miR-142 Sensor I.V.
repRNA-mIL-12+miR-142 Sensor+miR-22 Sensor I.V.
repRNA-mIL-12+miR-142 Sensor+miR-185 Sensor I.V.

FIG. 18

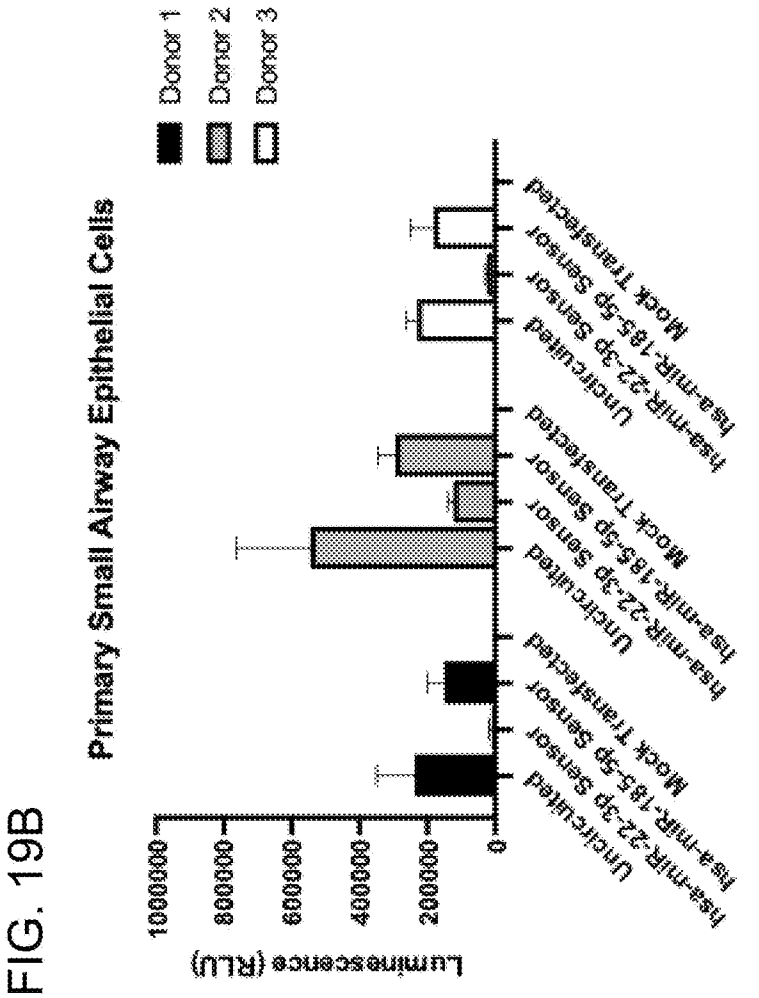


FIG. 19B

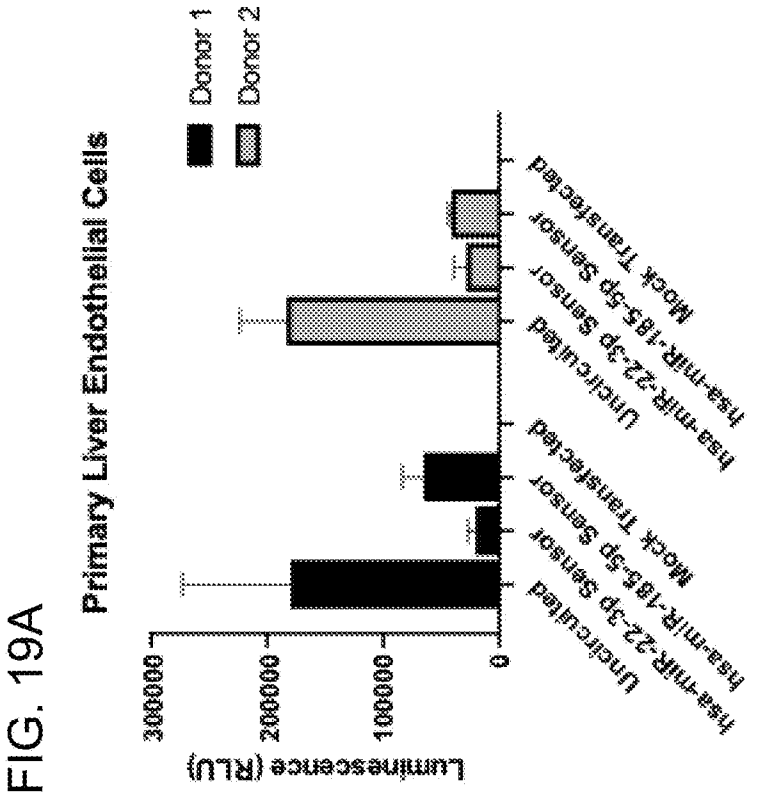


FIG. 19A

FIG. 20A

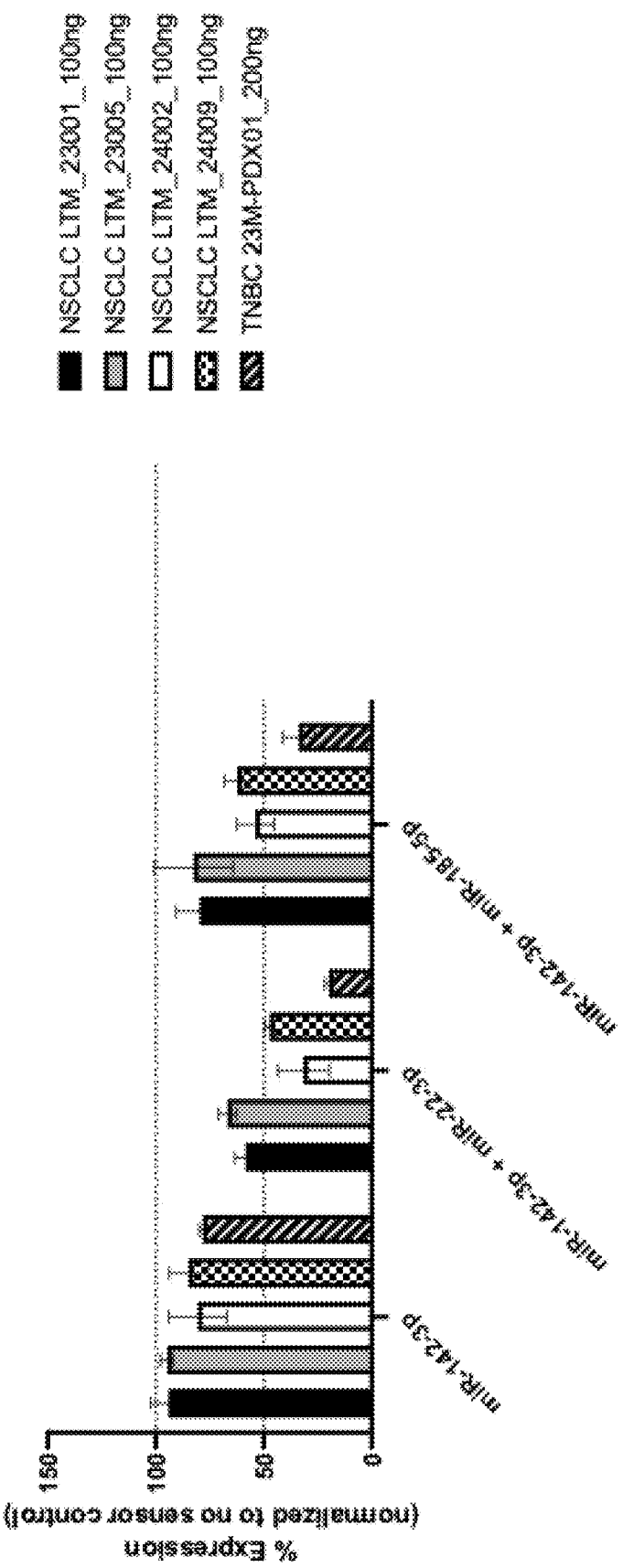

NSCLC LTM_23001_100ng
NSCLC LTM_23005_100ng
NSCLC LTM_24002_100ng
NSCLC LTM_24009_100ng
TNBC 23M-PDX01_200ng
% Expression
(normalized to no sensor control)
150
100
50
0
miR-142-3p
miR-142-3p + miR-22-3p
miR-142-3p + miR-185-5p

FIG. 20B

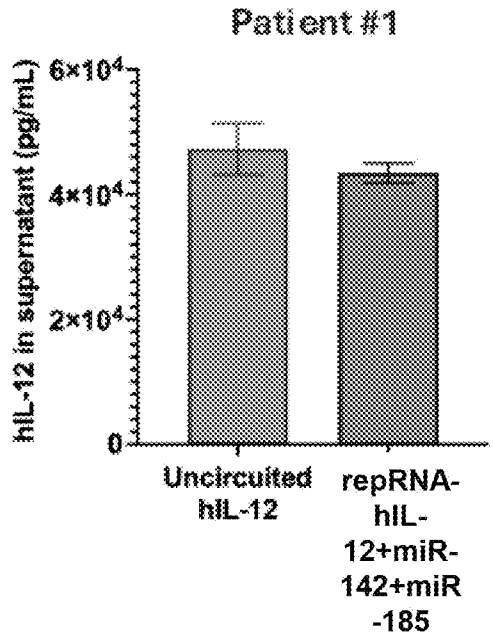


FIG. 20C

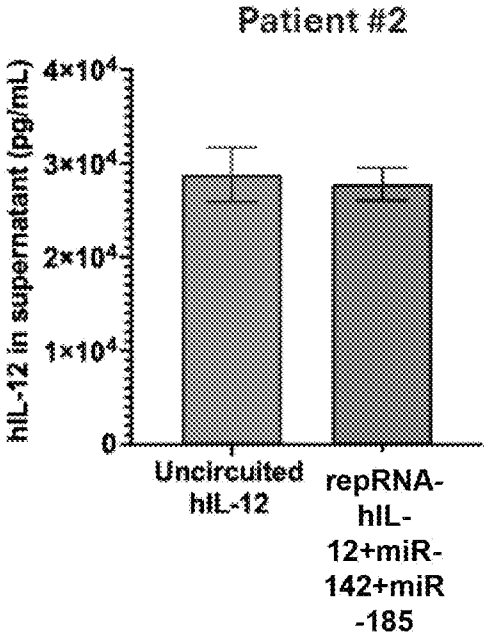


FIG. 20D

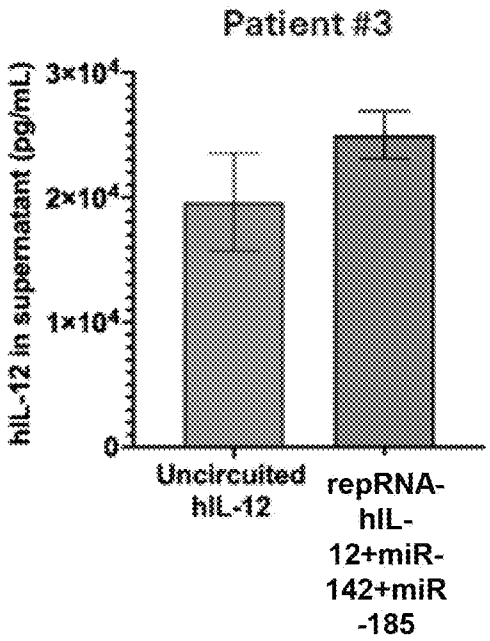


FIG. 20E

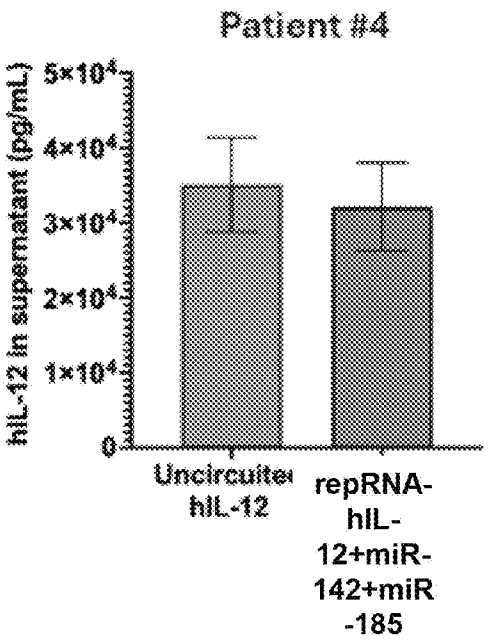


FIG. 21A

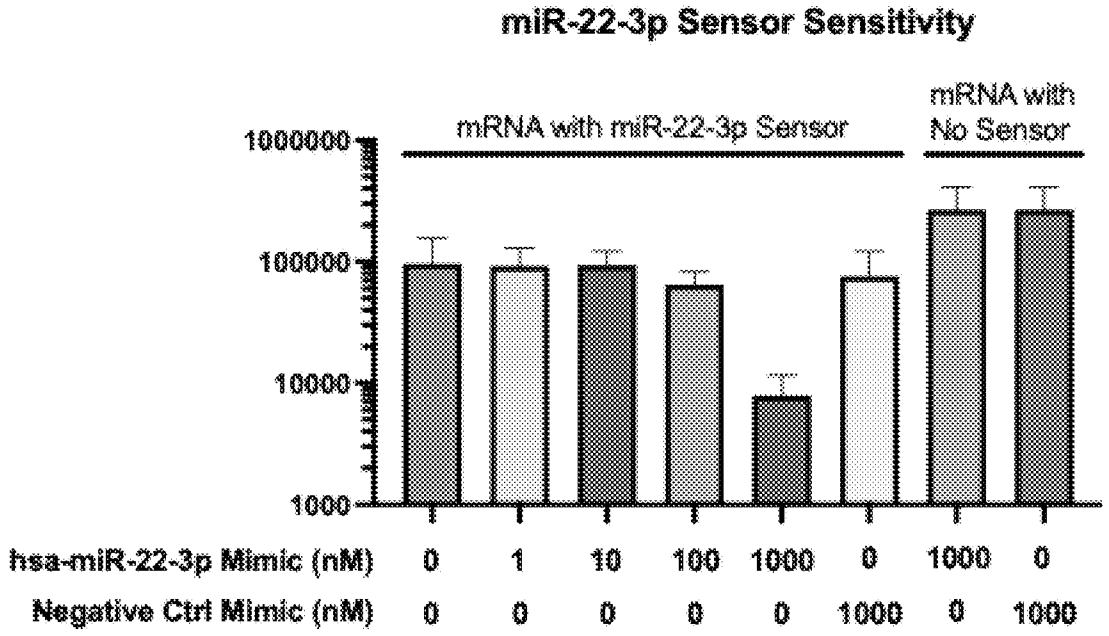


FIG. 21B

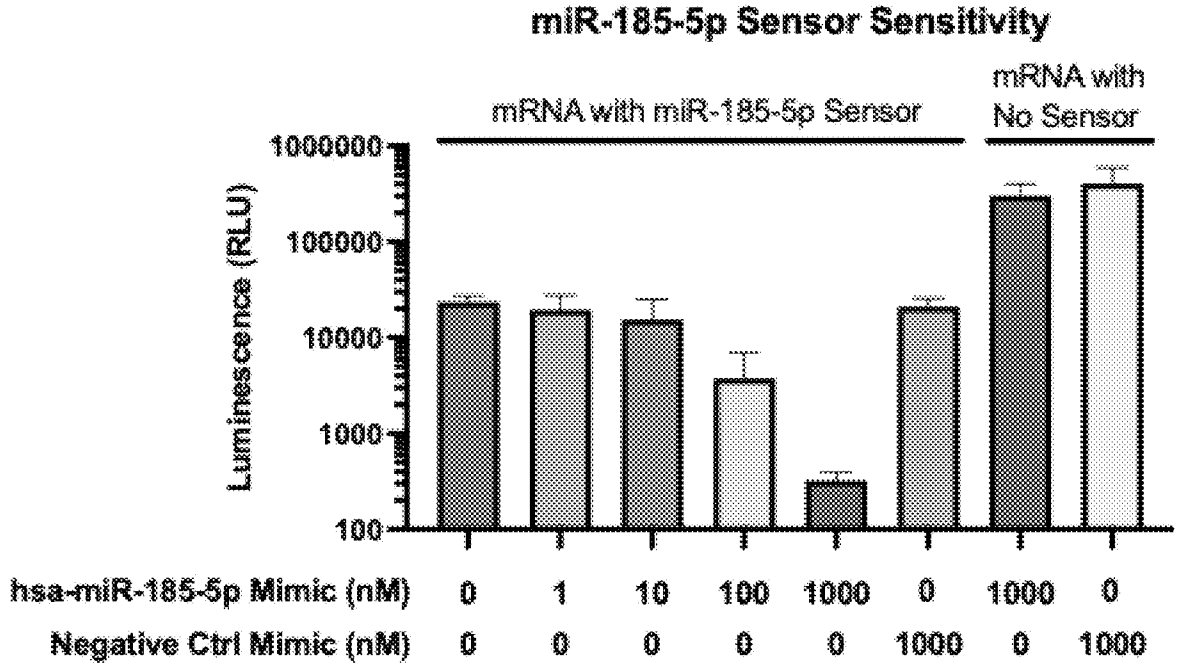


FIG. 22A

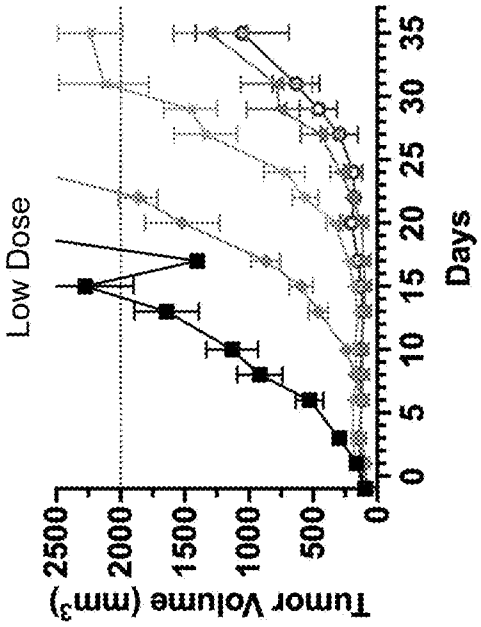


FIG. 22B

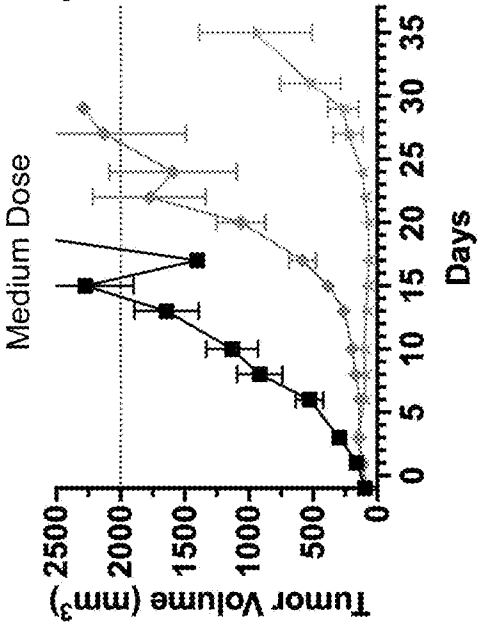
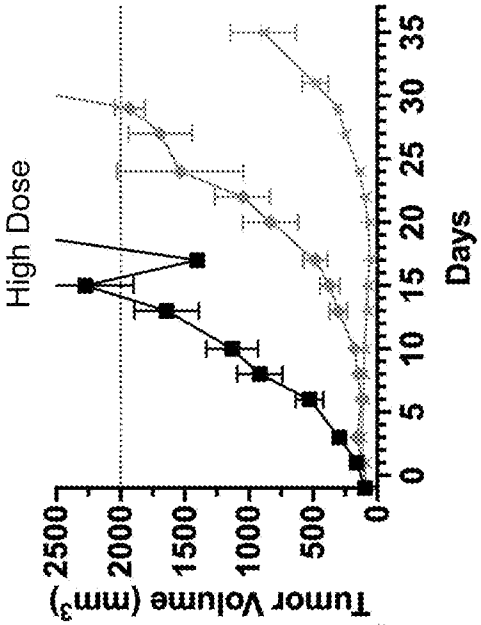


FIG. 22C



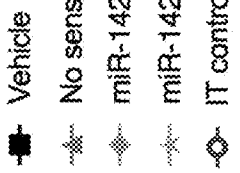


FIG. 22D

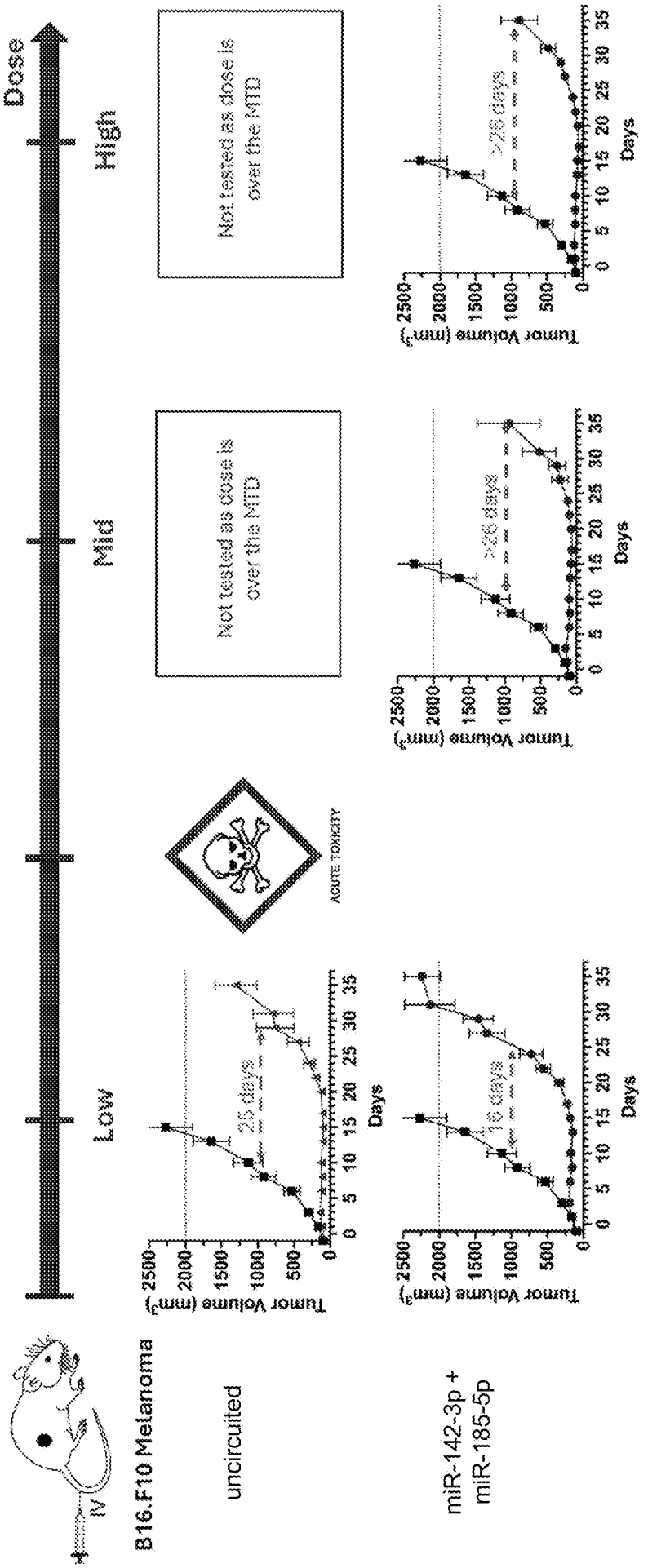

Dose
Low
Mid
High
IV
B16.F10 Melanoma
uncircuited
miR-142-3p +
miR-185-5p
Not tested as dose is over the MTD
Not tested as dose is over the MTD
ACUTE TOXICITY
25 days
>26 days
16 days
>26 days
Tumor Volume (mm³)
Days

FIG. 23A

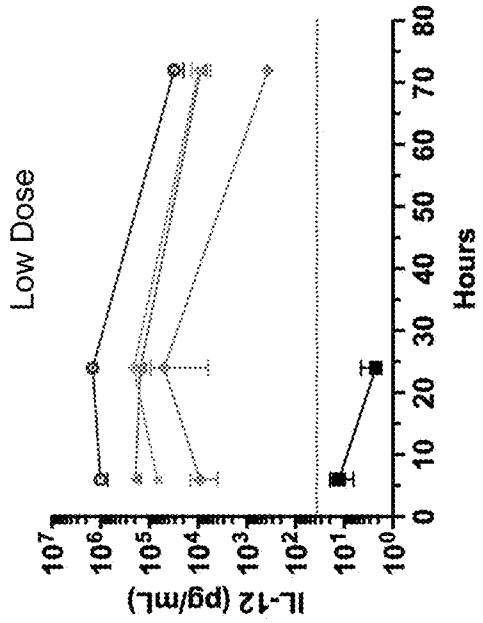


FIG. 23B

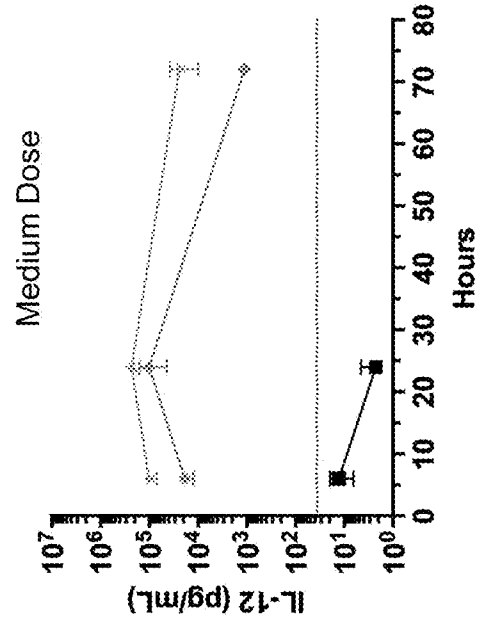
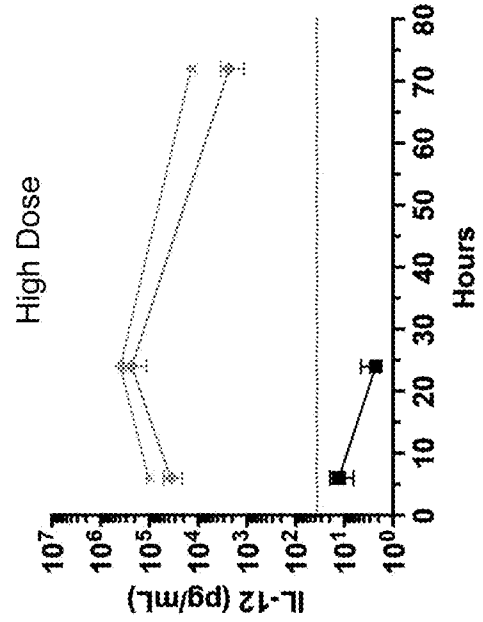


FIG. 23C



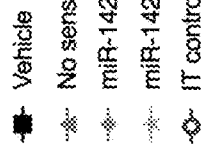


FIG. 23D

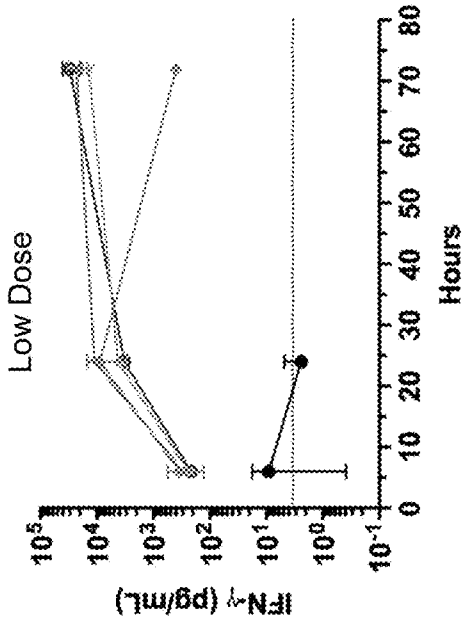


FIG. 23E

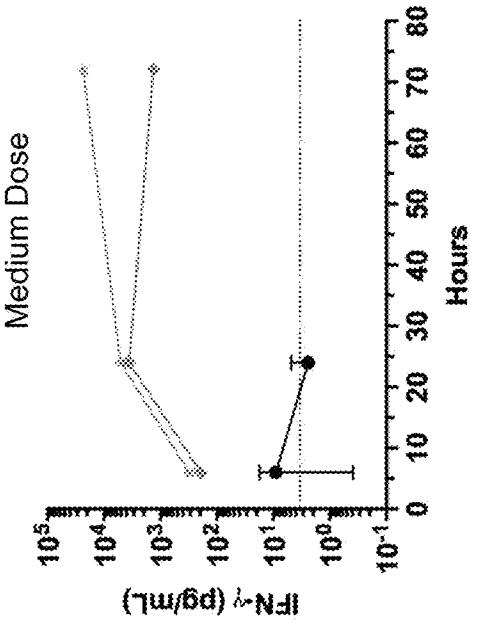
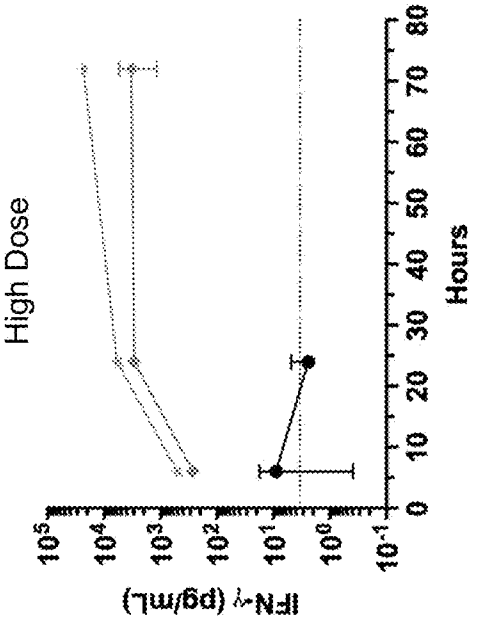


FIG. 23F



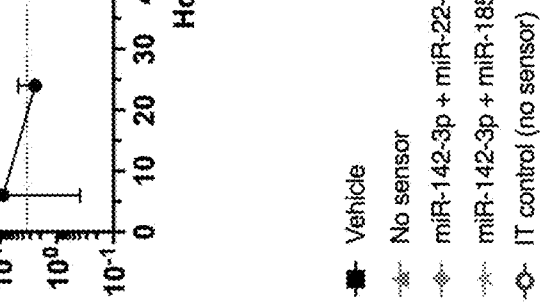


FIG. 24

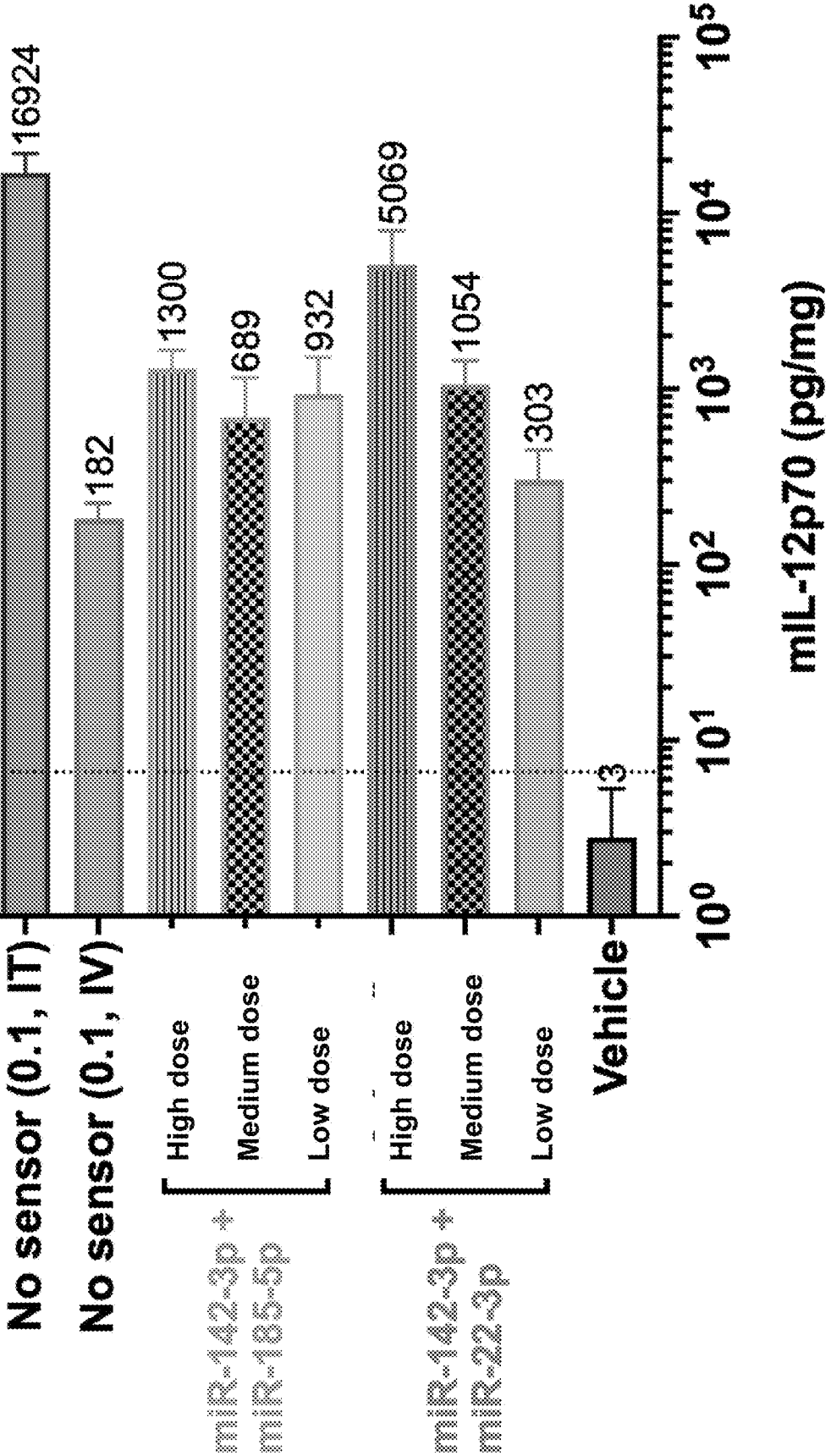

Tumor IL-12
LLOQ
No sensor (0.1, IT)
No sensor (0.1, IV)
miR-142-3p + miR-185-5p
High dose
Medium dose
Low dose
miR-142-3p + miR-22-3p
High dose
Medium dose
Low dose
Vehicle
16924
182
1300
689
932
5069
1054
303
3
10^0
10^1
10^2
10^3
10^4
10^5
mIL-12p70 (pg/mg)

FIG. 25A

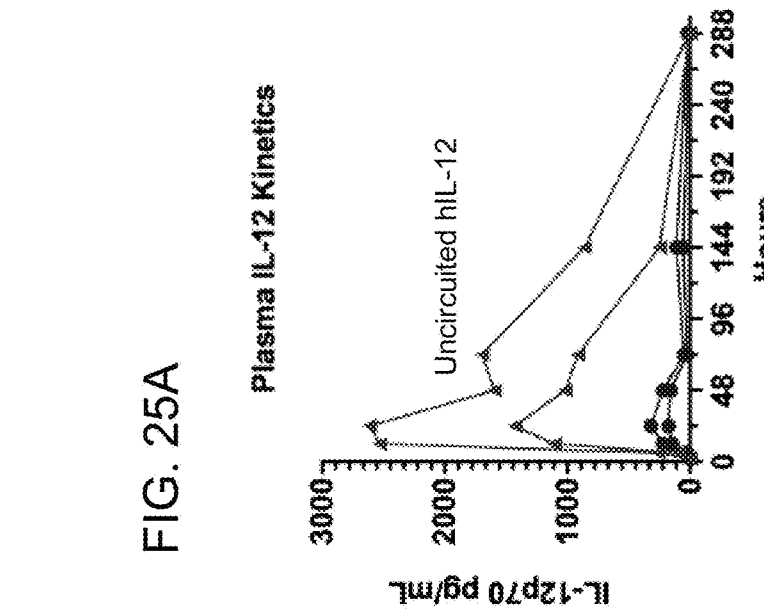


FIG. 25B

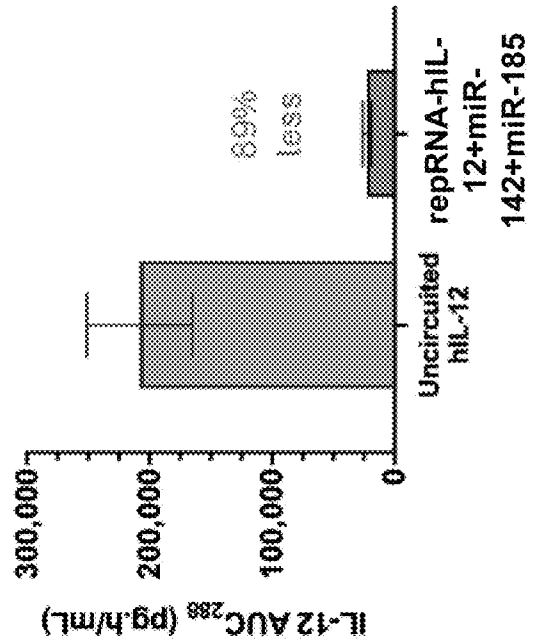


FIG. 25C

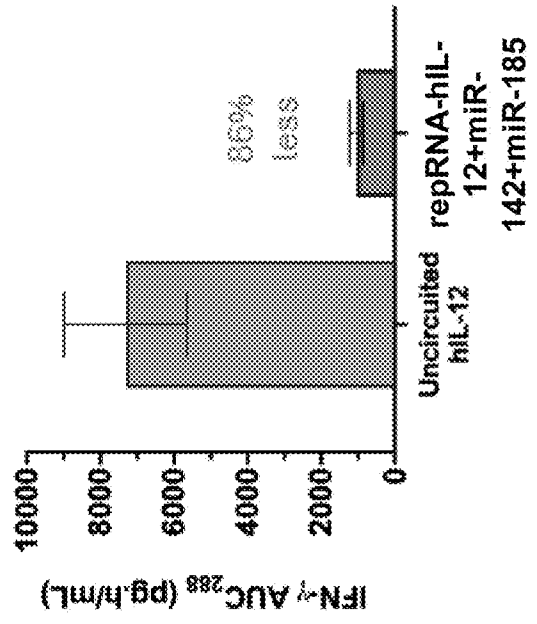


FIG. 26

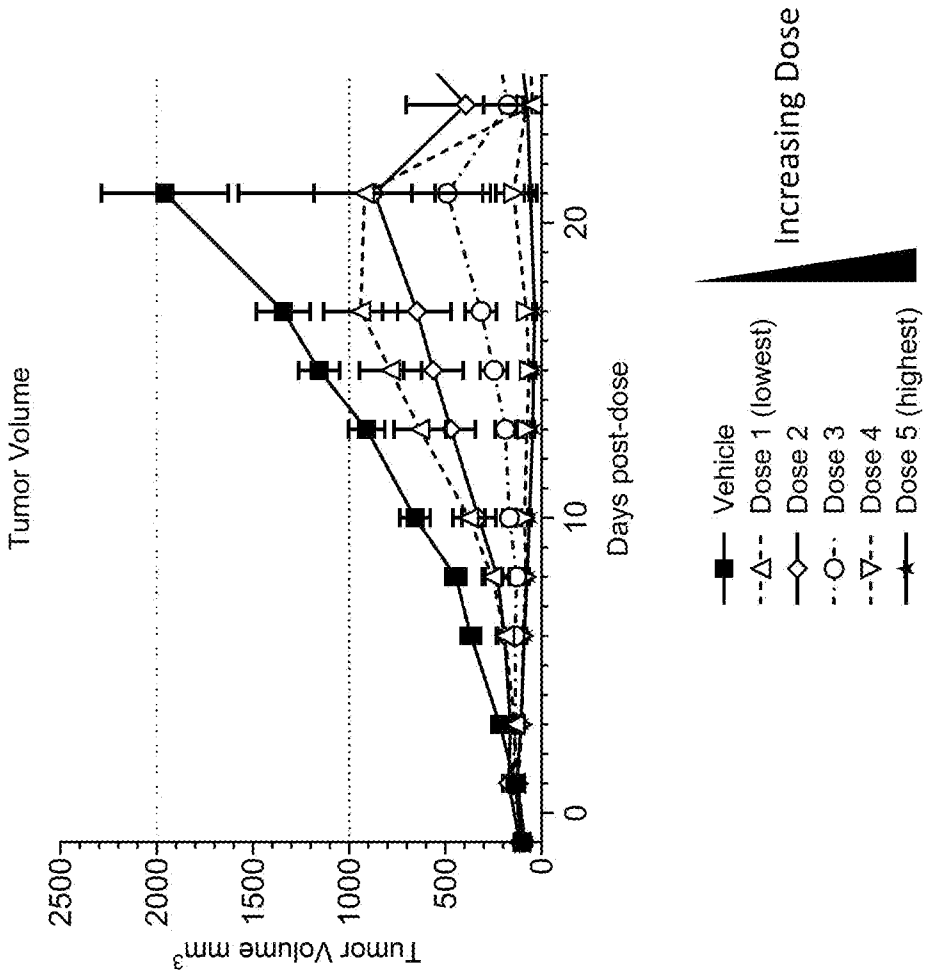

Tumor Volume
Tumor Volume mm3
2500
2000
1500
1000
500
0
0
10
20
Days post-dose
Increasing Dose
Vehicle
Dose 1 (lowest)
Dose 2
Dose 3
Dose 4
Dose 5 (highest)

FIG. 27

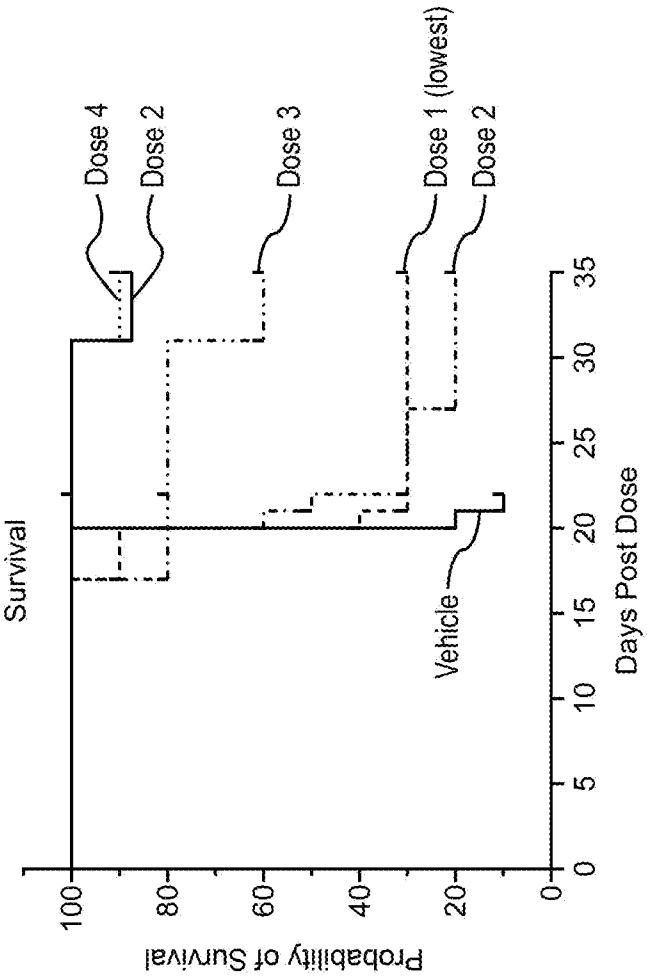

Survival
Dose 4
Dose 2
Dose 3
Dose 1 (lowest)
Dose 2
Vehicle
Probability of Survival
100
80
60
40
20
0
0
5
10
15
20
25
30
35
Days Post Dose

FIG. 28

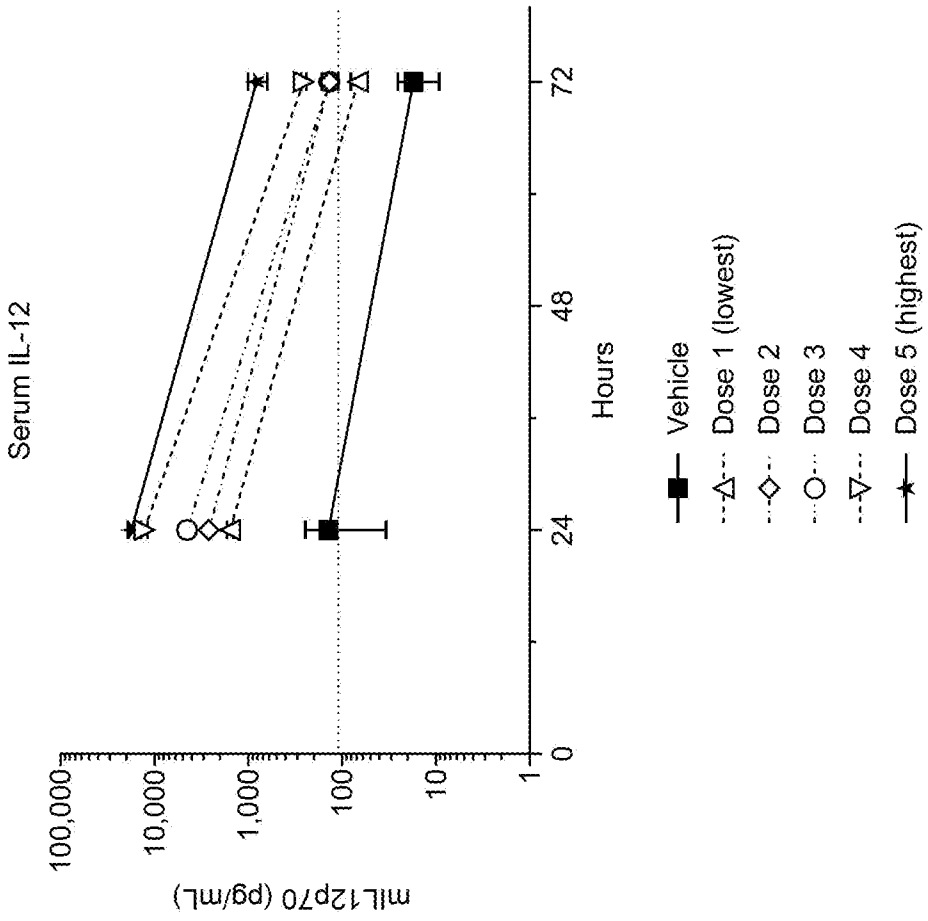

Serum IL-12
mIL12p70 (pg/mL)
100,000
10,000
1,000
100
10
1
0
24
48
72
Hours
Vehicle
Dose 1 (lowest)
Dose 2
Dose 3
Dose 4
Dose 5 (highest)

FIG. 29

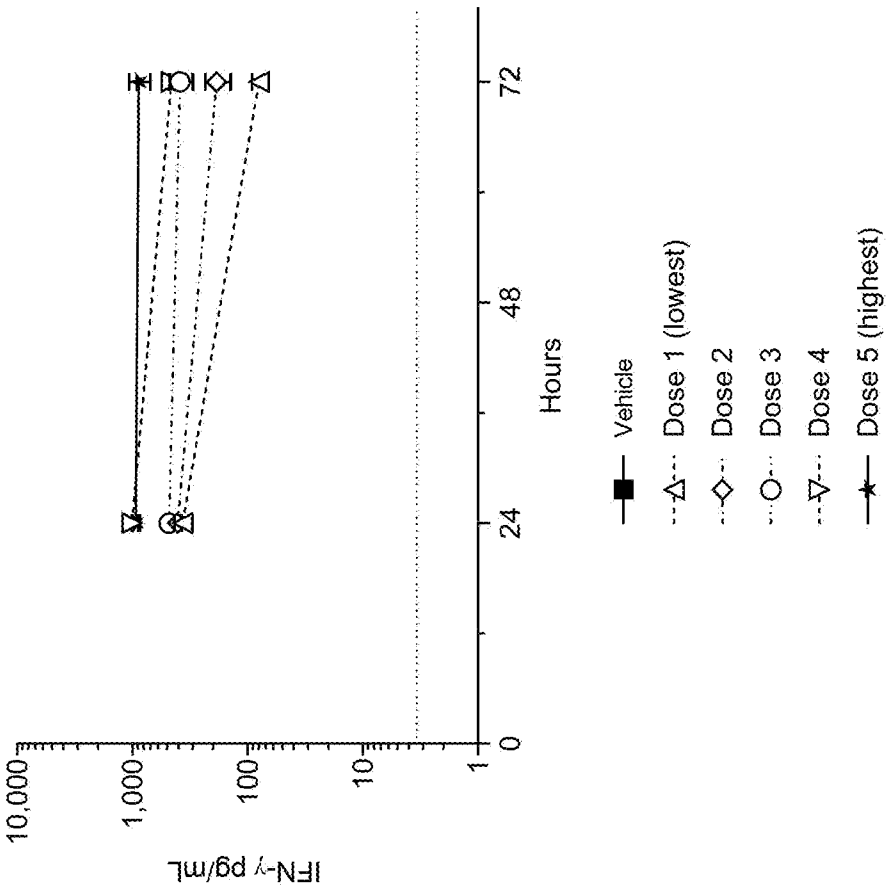

Serum IFN-γ
IFN-γ pg/mL
10,000
1,000
100
10
1
0
24
48
72
Hours
Vehicle
Dose 1 (lowest)
Dose 2
Dose 3
Dose 4
Dose 5 (highest)

FIG. 30

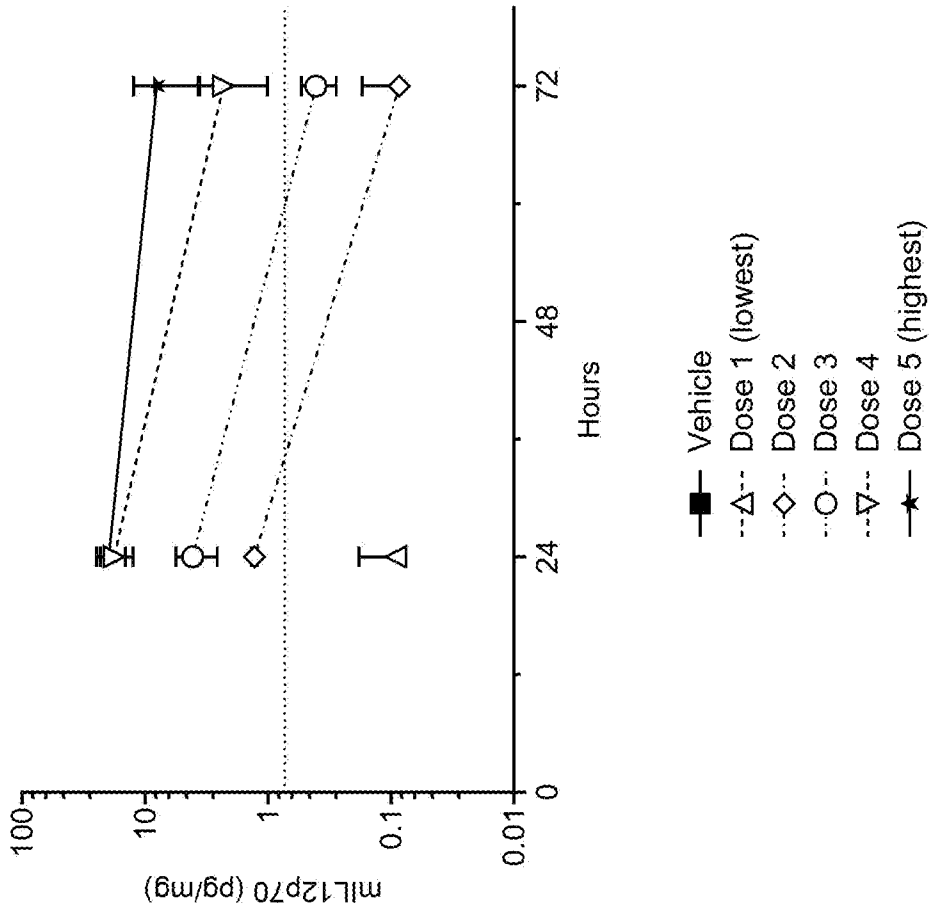

Tumor IL-12
mIL12p70 (pg/mg)
100
10
1
0.1
0.01
0
24
48
72
Hours
Vehicle
Dose 1 (lowest)
Dose 2
Dose 3
Dose 4
Dose 5 (highest)

FIG. 31

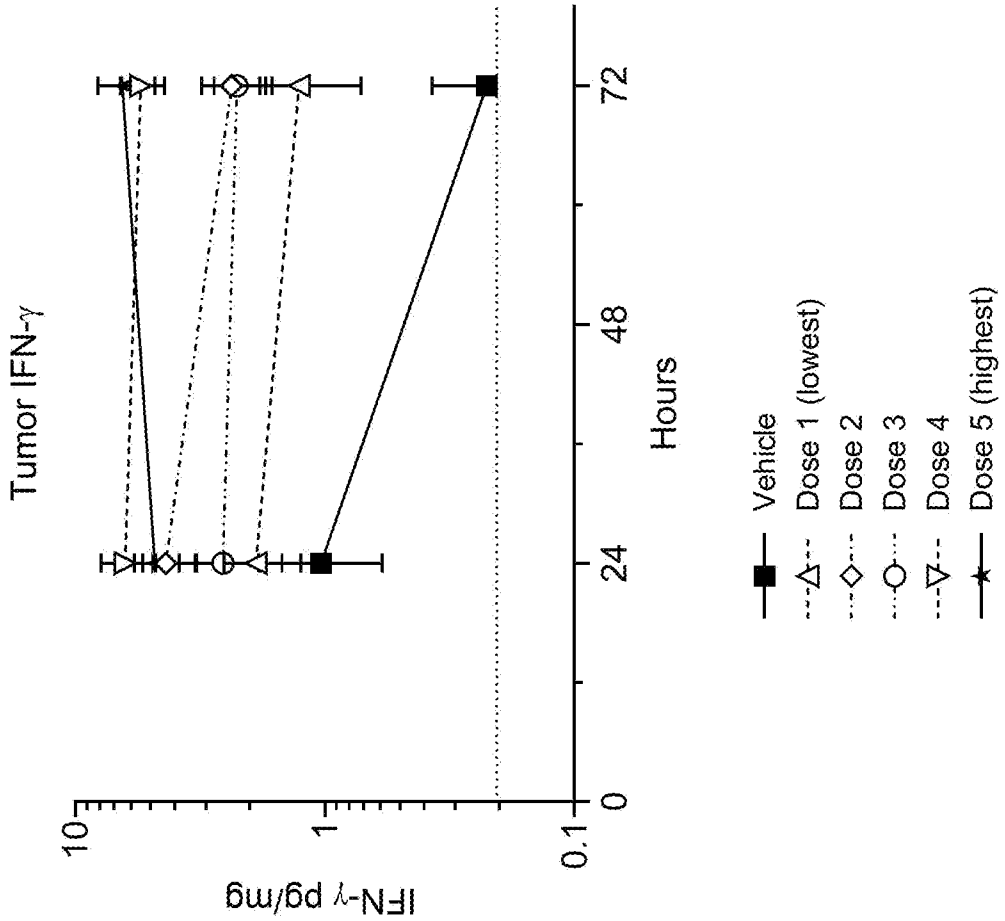

Tumor IFN-γ
IFN-γ pg/mg
10
1
0.1
0
24
48
72
Hours
Vehicle
Dose 1 (lowest)
Dose 2
Dose 3
Dose 4
Dose 5 (highest)

FIG. 32A

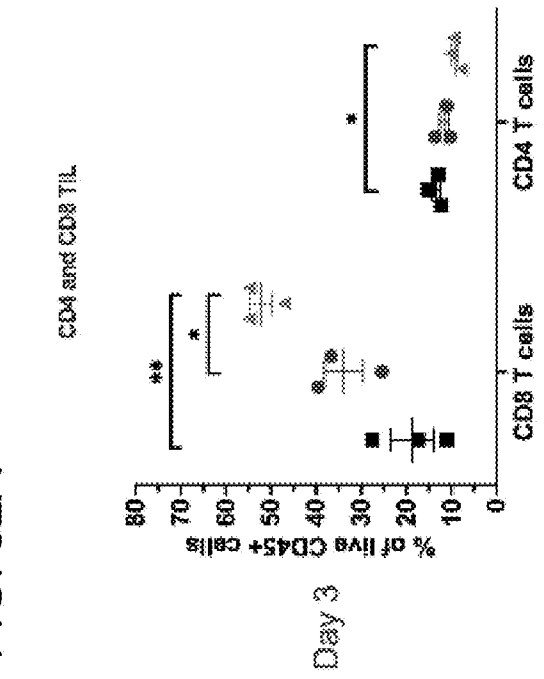


FIG. 32B

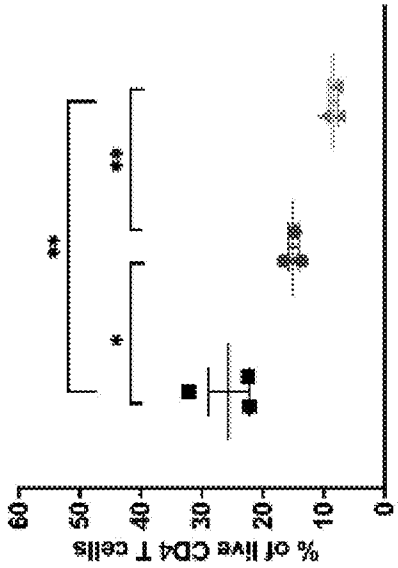


FIG. 32C

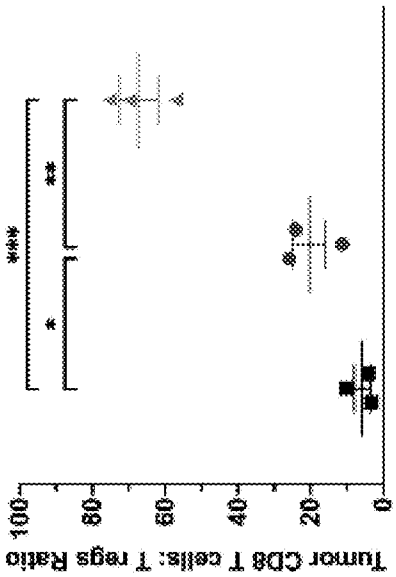


FIG. 32D

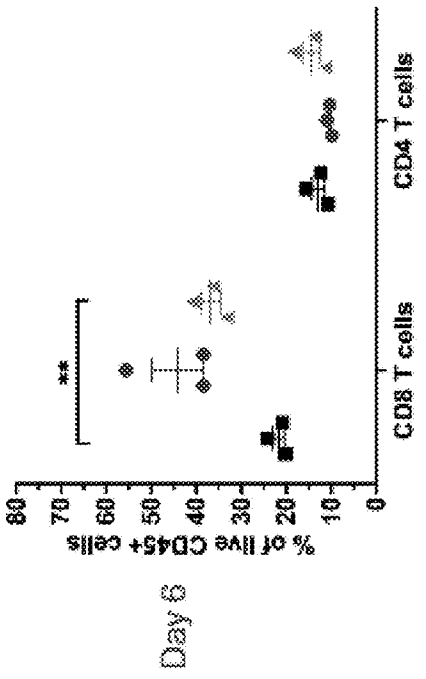


FIG. 32E

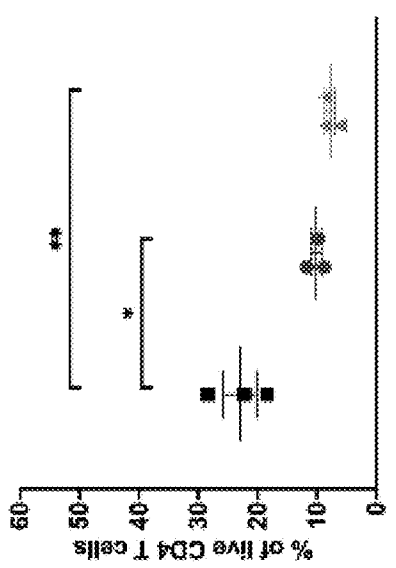


FIG. 32F

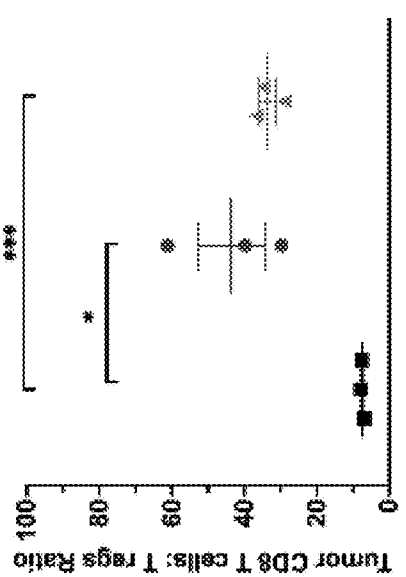


FIG. 33

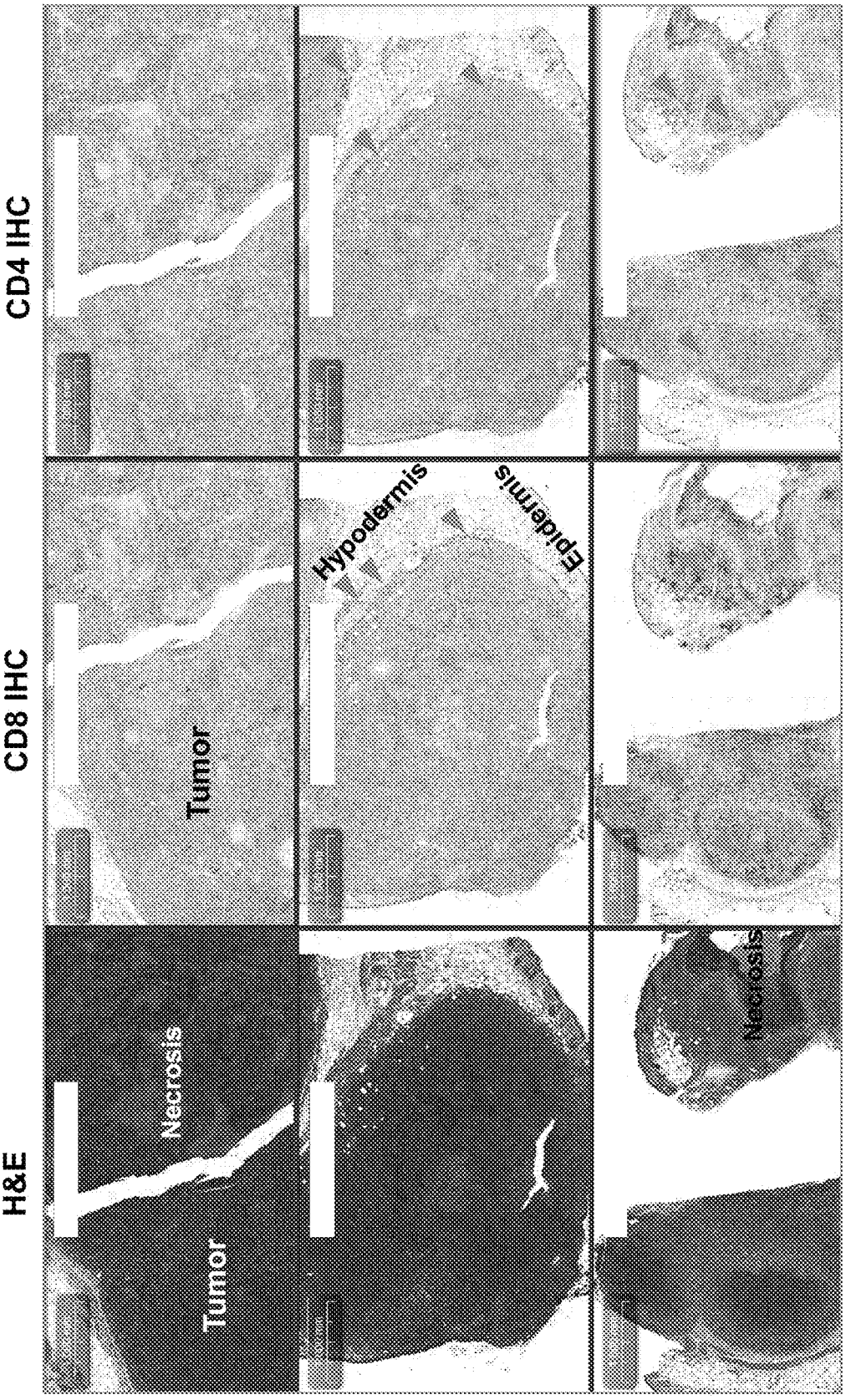

H&E
CD8 IHC
CD4 IHC
Tumor
Necrosis
Tumor
Hypodermis
Epidermis
Necrosis
Vehicle Day 6
Low Dose Day 6
High Dose Day 6

FIG. 34

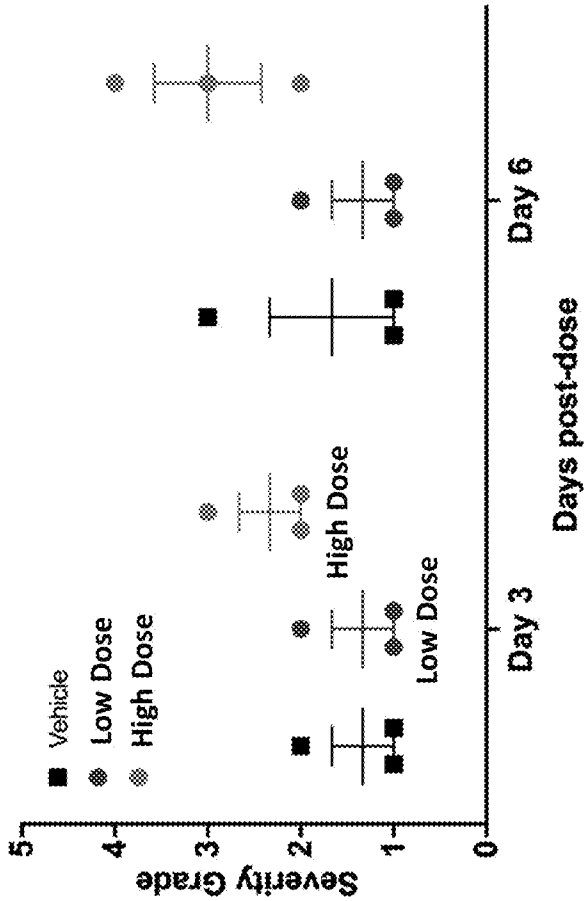

Severity of CD4 Staining in CT26 Tumors
Vehicle
Low Dose
High Dose
Severity Grade
5
4
3
2
1
0
Low Dose
High Dose
Day 3
Day 6
Days post-dose

FIG. 35

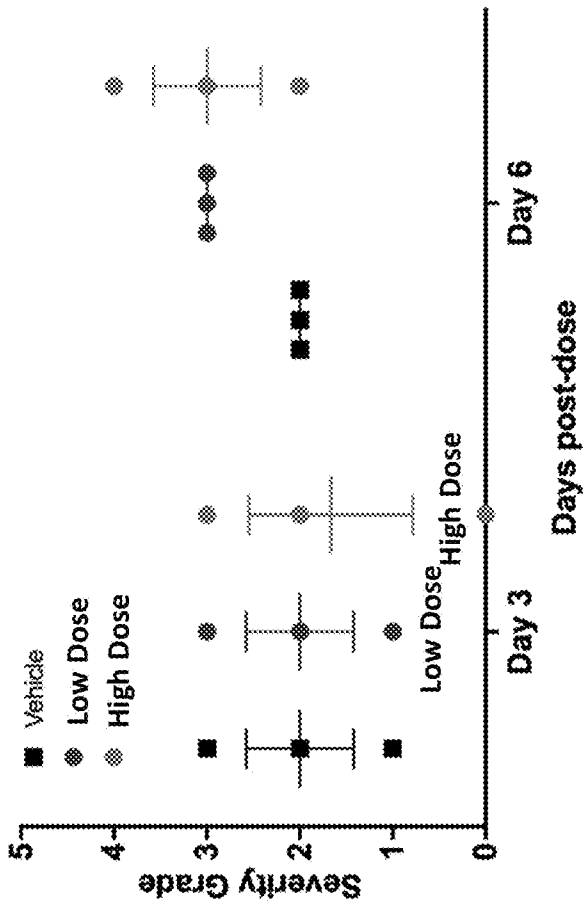

Severity of CD8 Staining in CT26 Tumors
Vehicle
Low Dose
High Dose
Severity Grade
5
4
3
2
1
0
Low Dose
High Dose
Day 3
Day 6
Days post-dose

FIG. 36A

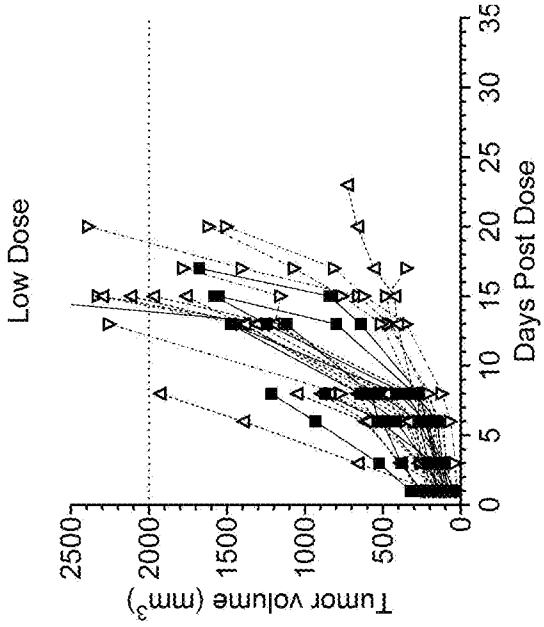


FIG. 36B



FIG. 36C

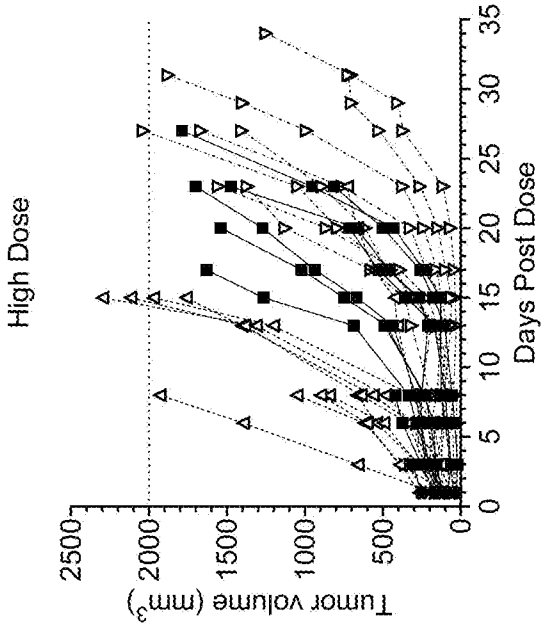


FIG. 37A



FIG. 37B

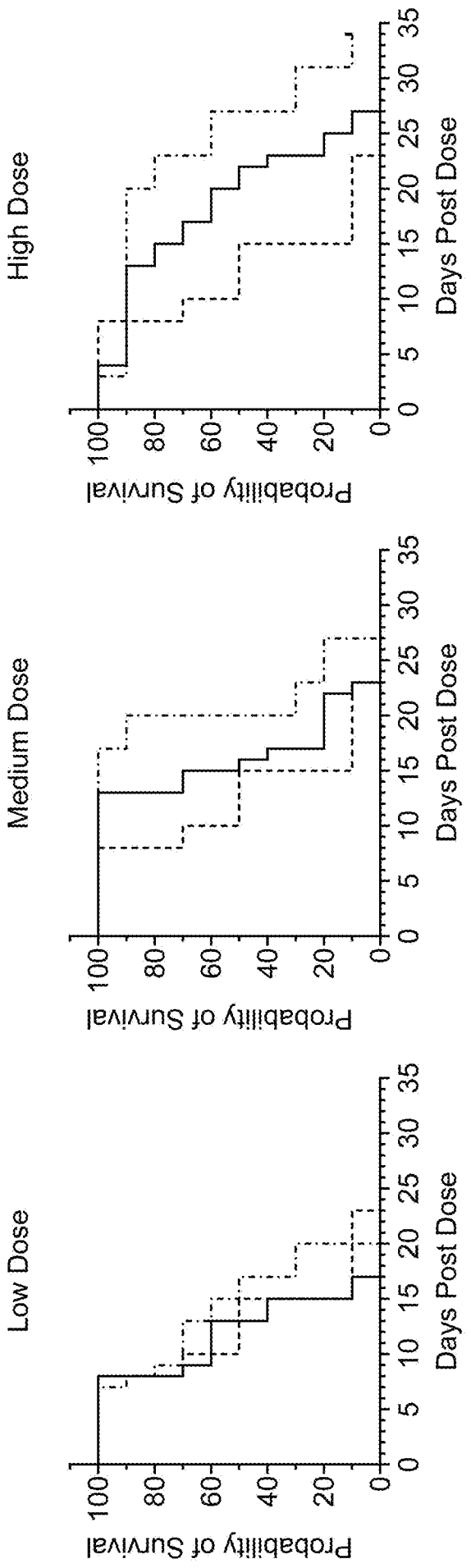


FIG. 37C

FIG. 38A

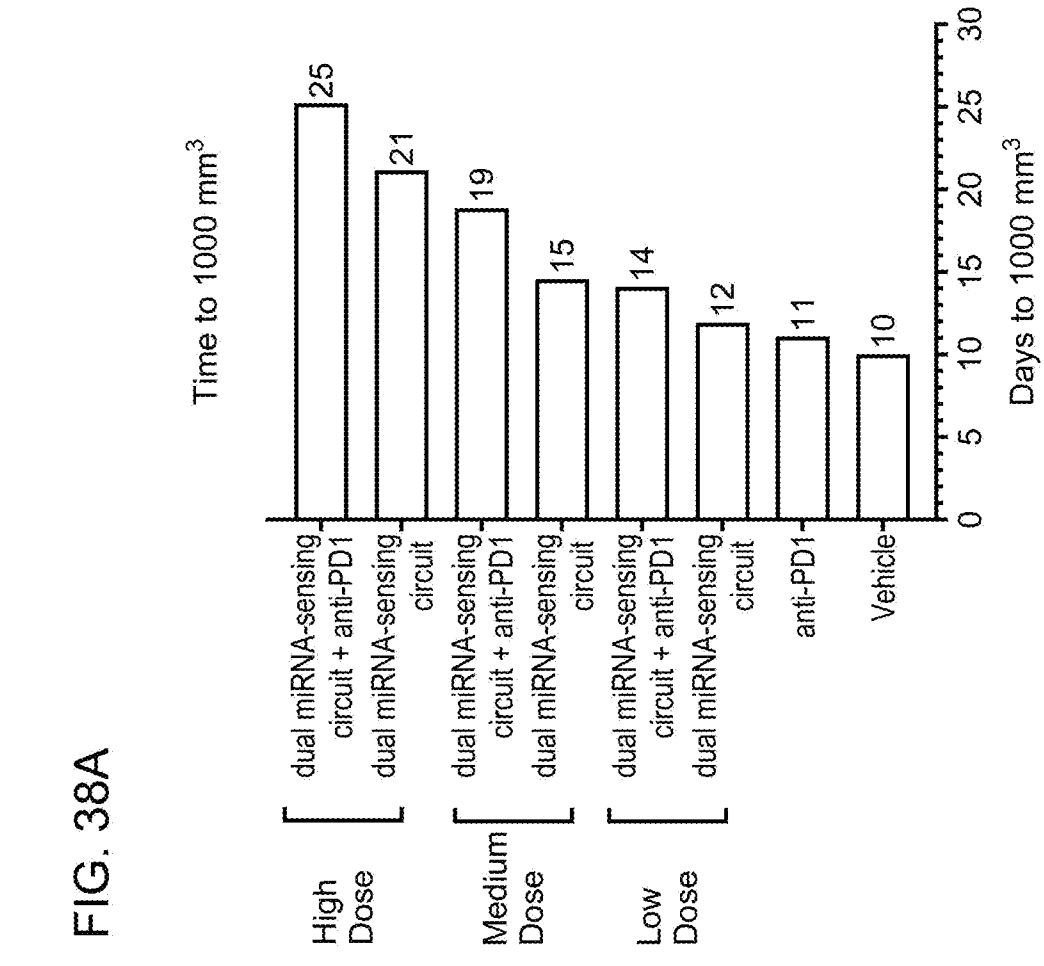


FIG. 38B

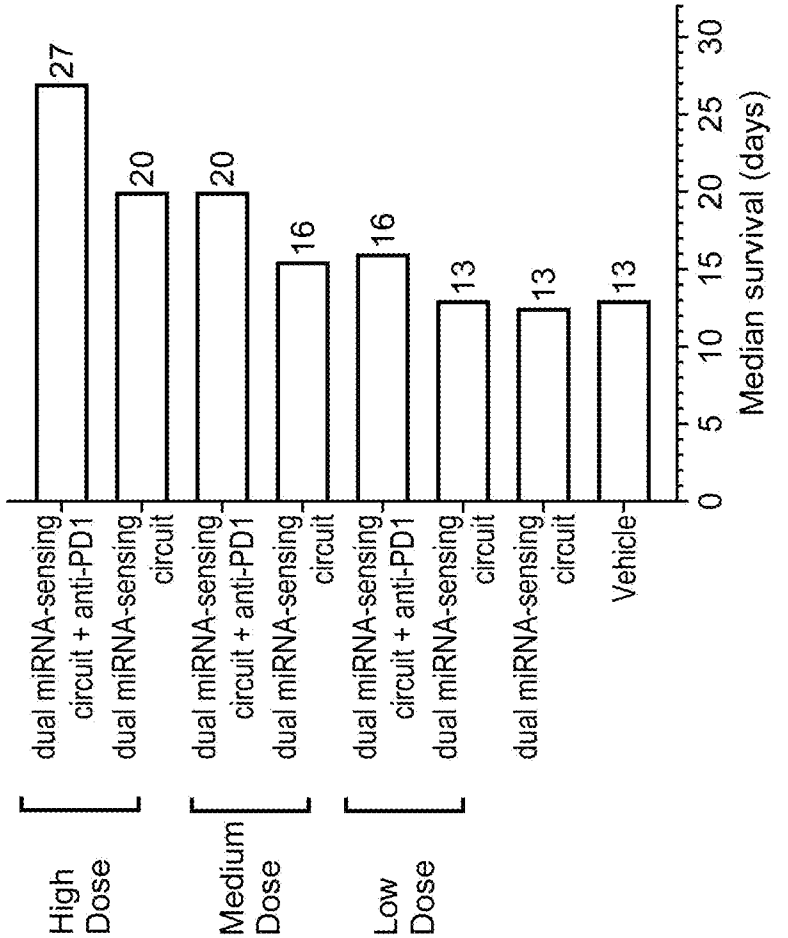


FIG. 38C

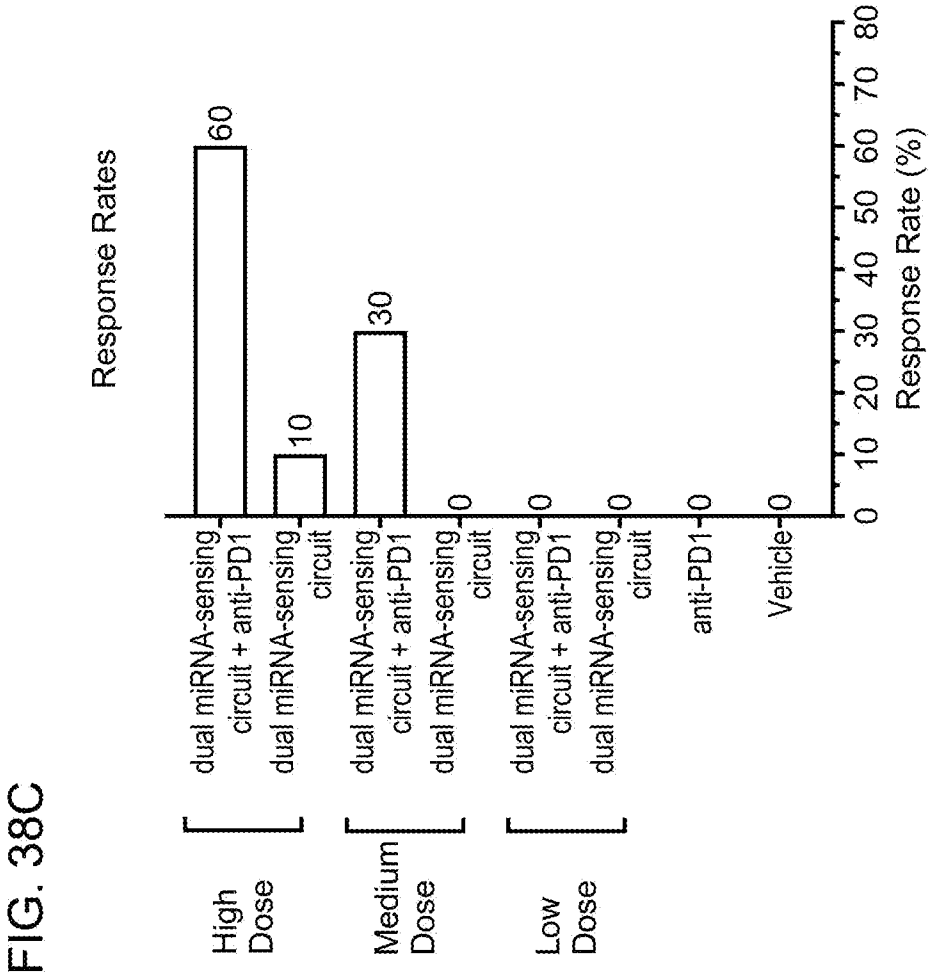

Response Rates
High Dose
Medium Dose
Low Dose
dual miRNA-sensing circuit + anti-PD1
dual miRNA-sensing circuit
dual miRNA-sensing circuit + anti-PD1
dual miRNA-sensing circuit
dual miRNA-sensing circuit + anti-PD1
dual miRNA-sensing circuit
anti-PD1
Vehicle
60
10
30
0
0
0
0
0
0 10 20 30 40 50 60 70 80
Response Rate (%)

FIG. 39A

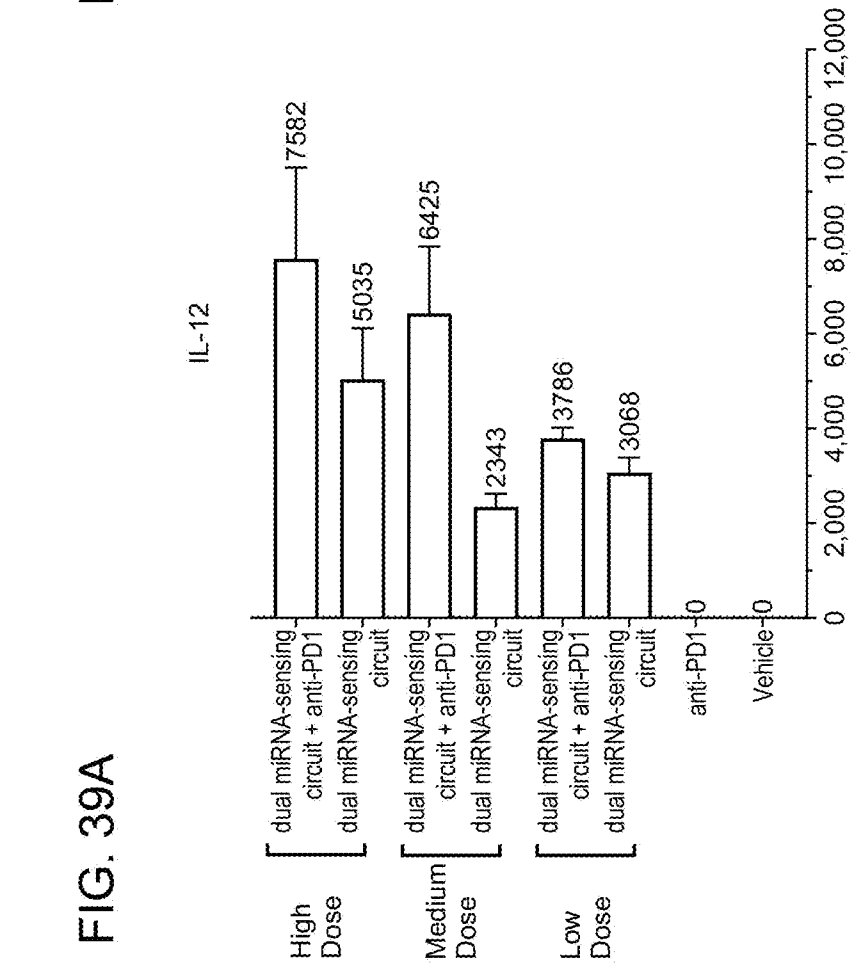


FIG. 39B

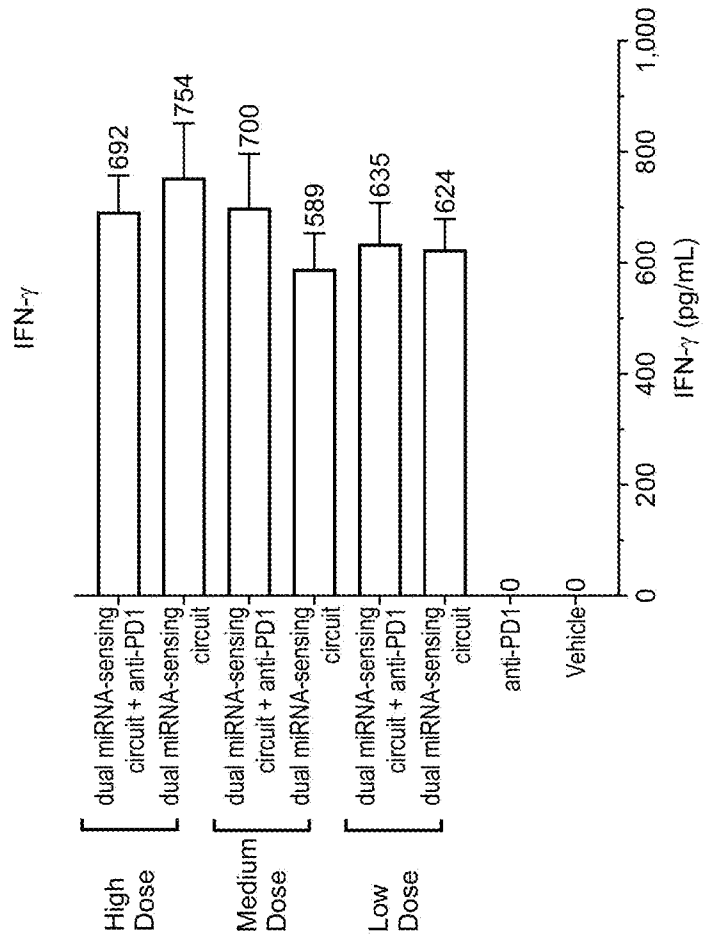


FIG. 39C

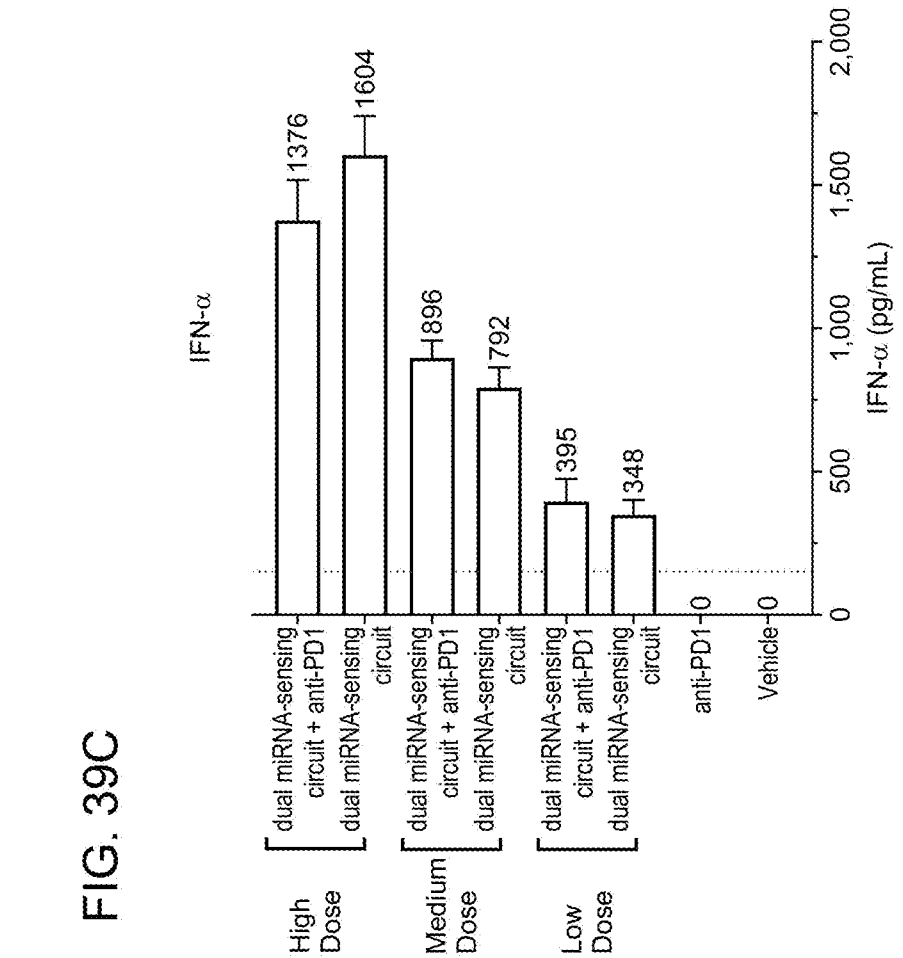


FIG. 39D

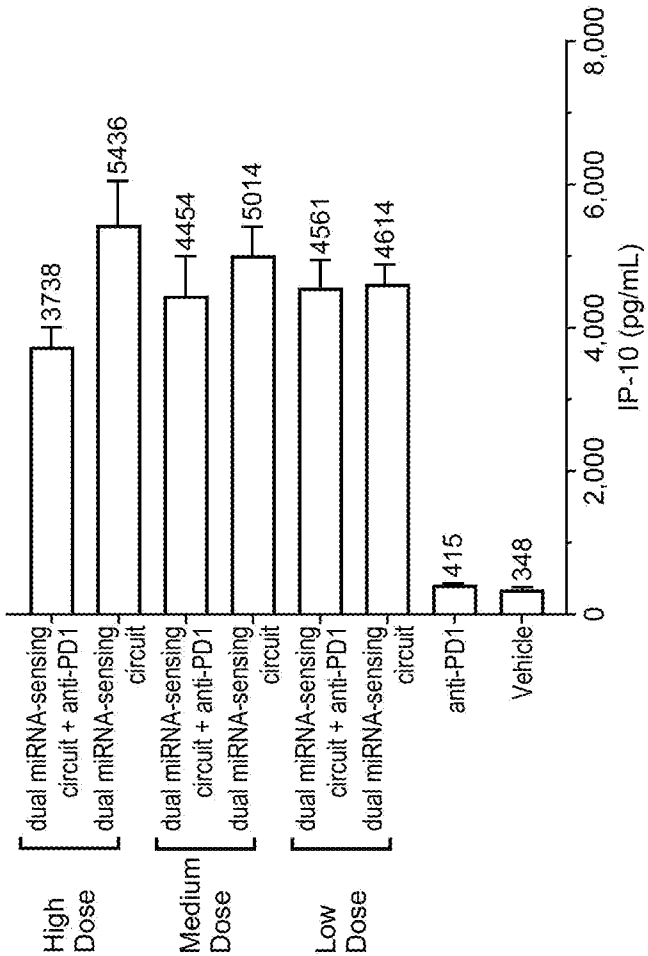


FIG. 40A

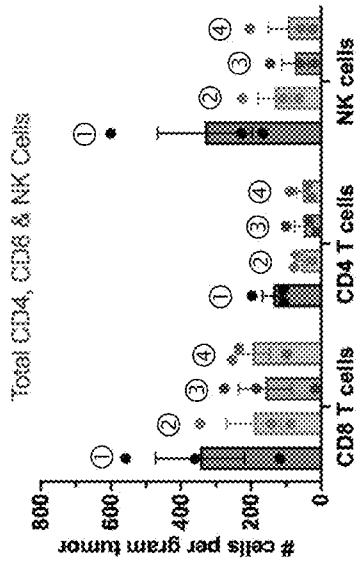


FIG. 40B

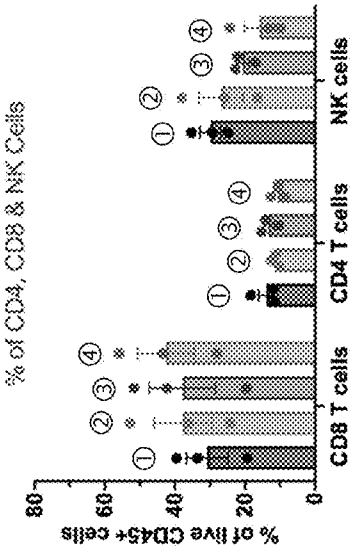


FIG. 40C

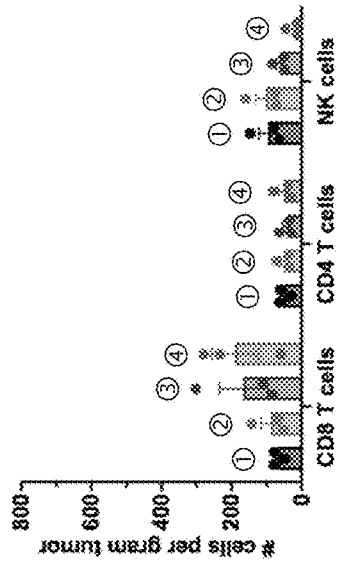


FIG. 40D

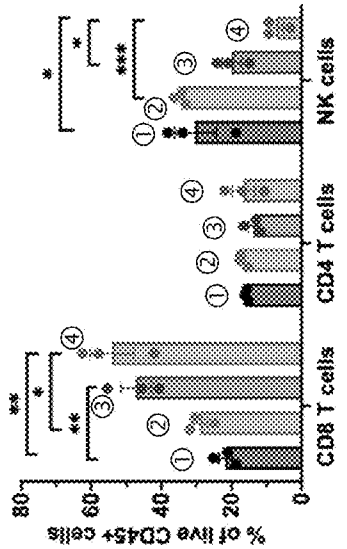
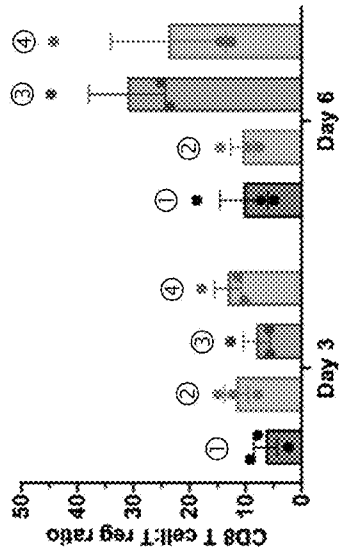


FIG. 40E



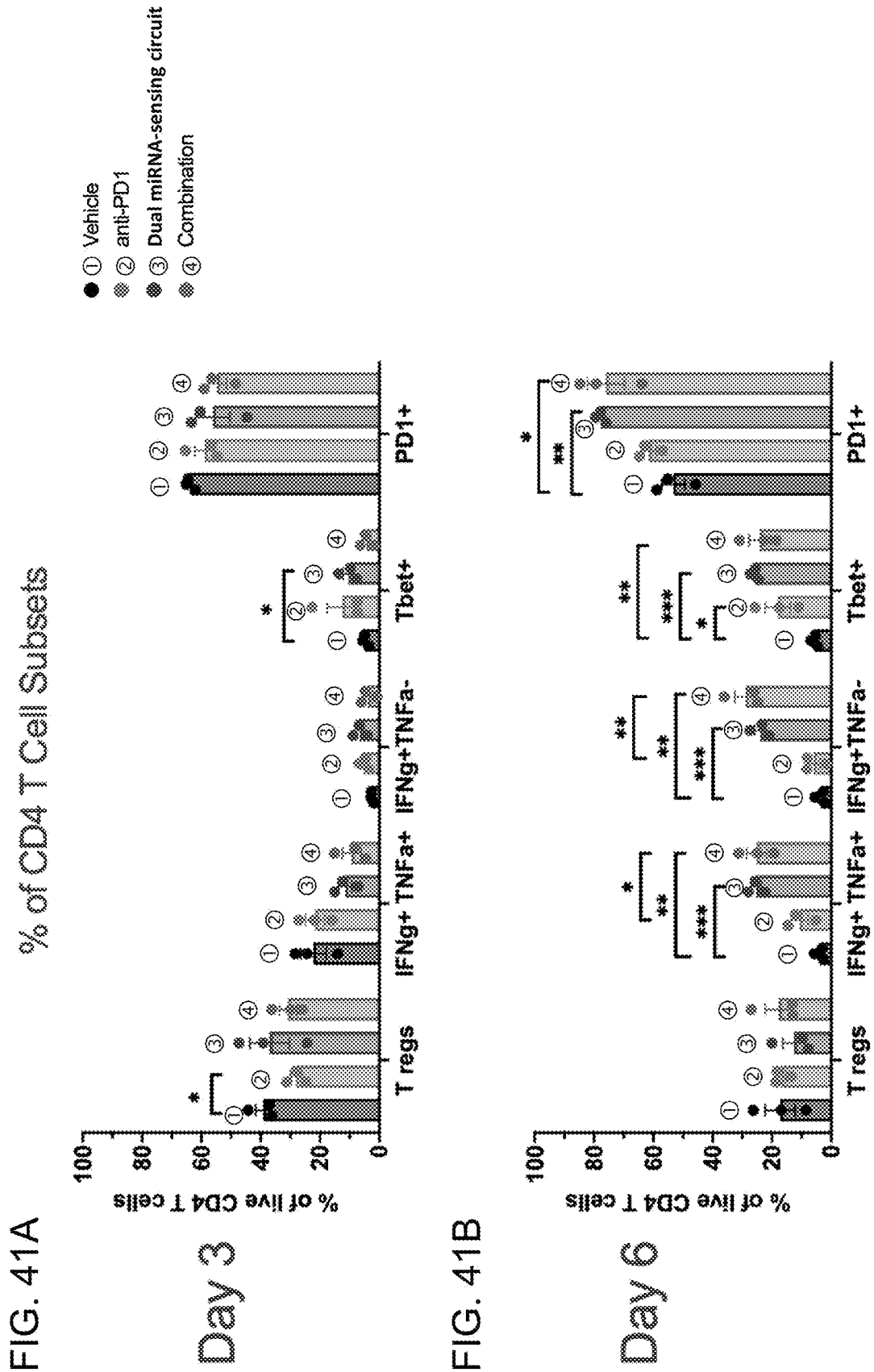


FIG. 41A

FIG. 41B

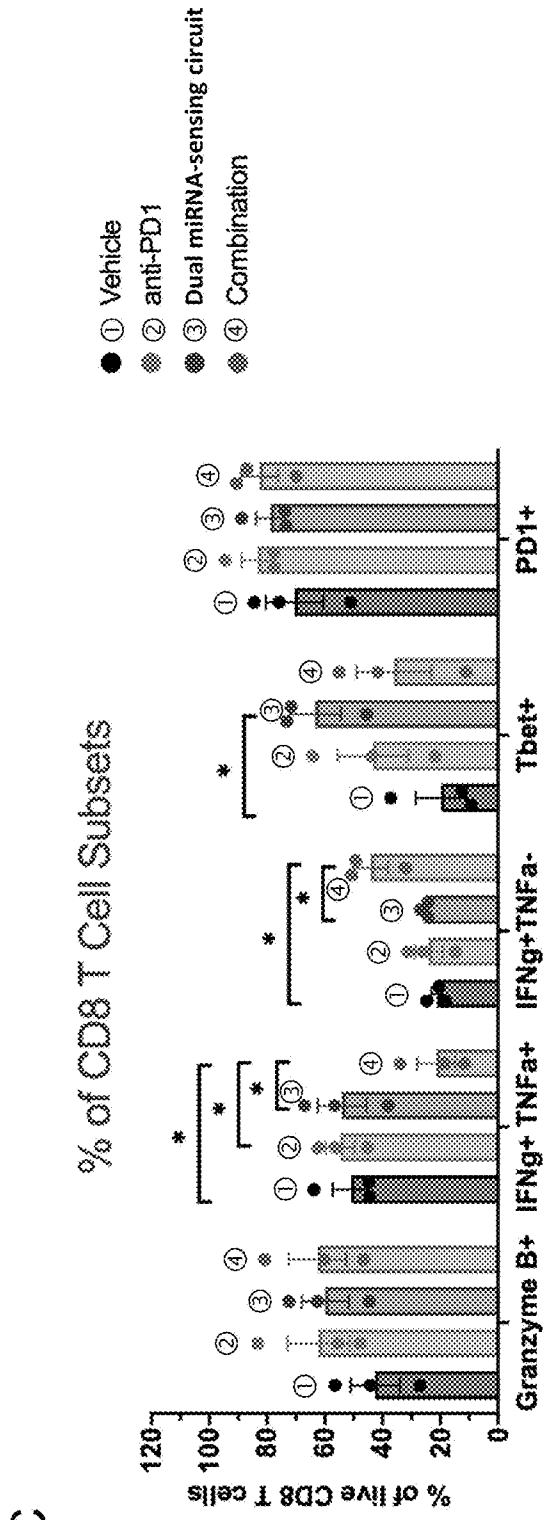


FIG. 41C

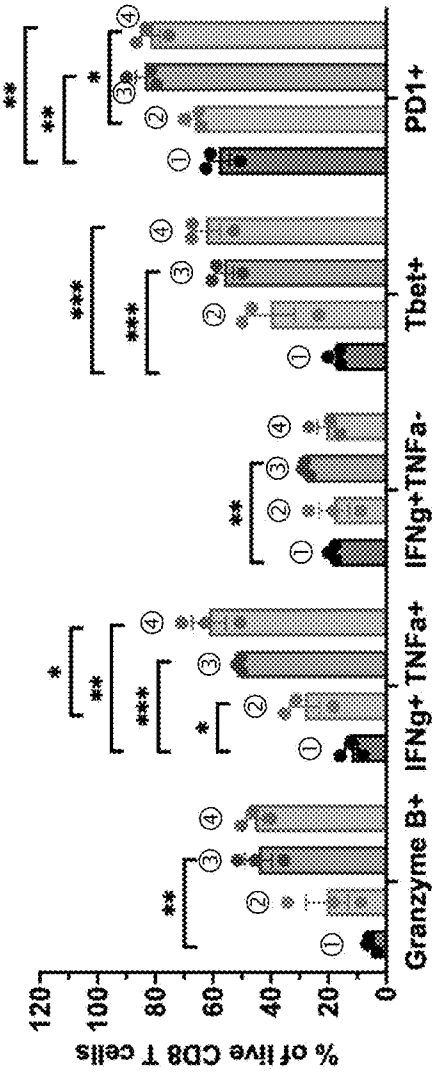


FIG. 41D

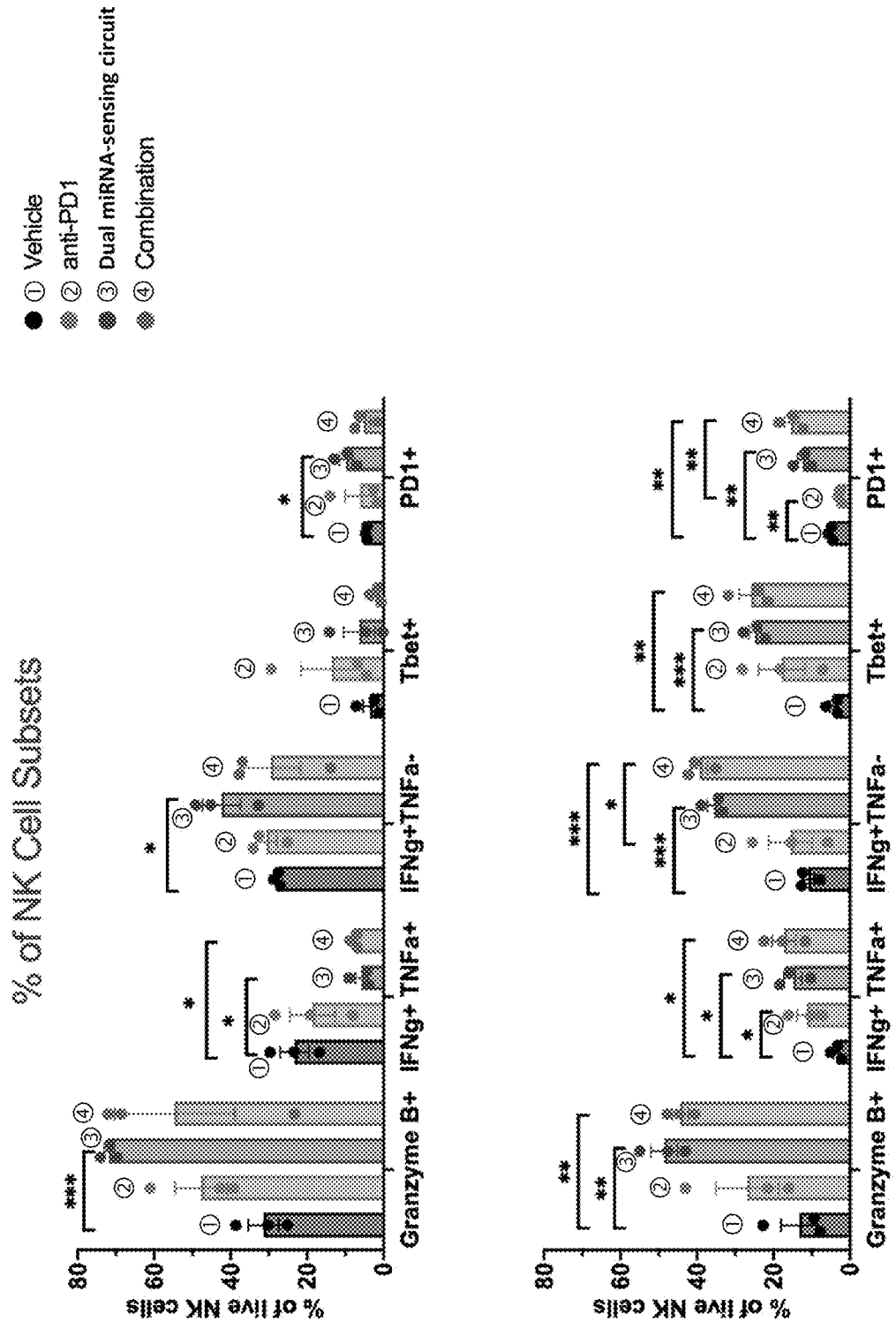


FIG. 41E

FIG. 41F

FIG. 42A

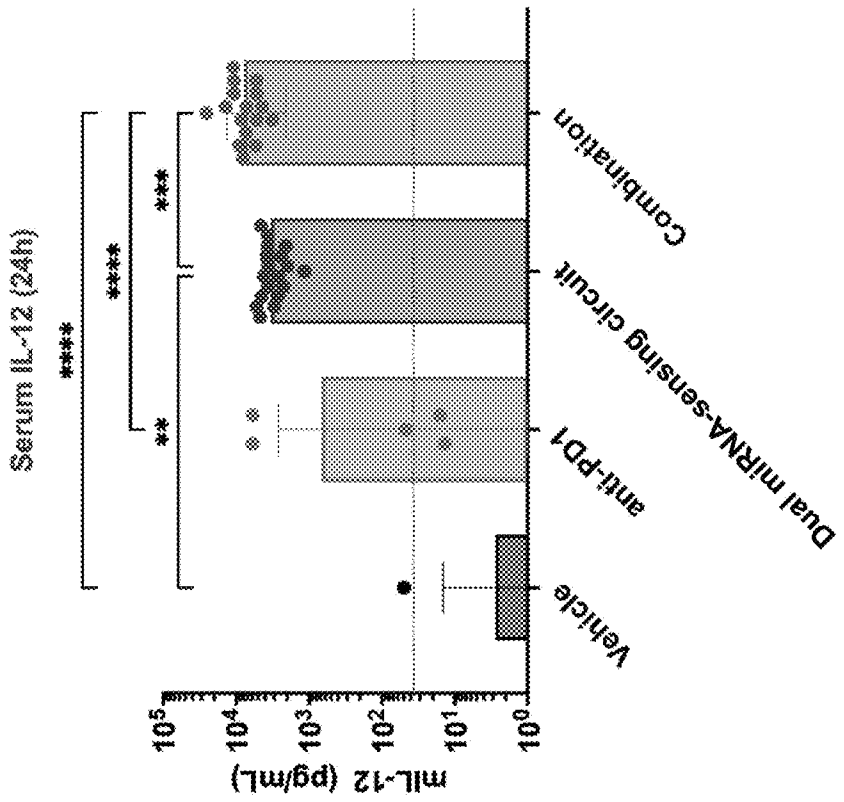


FIG. 42B

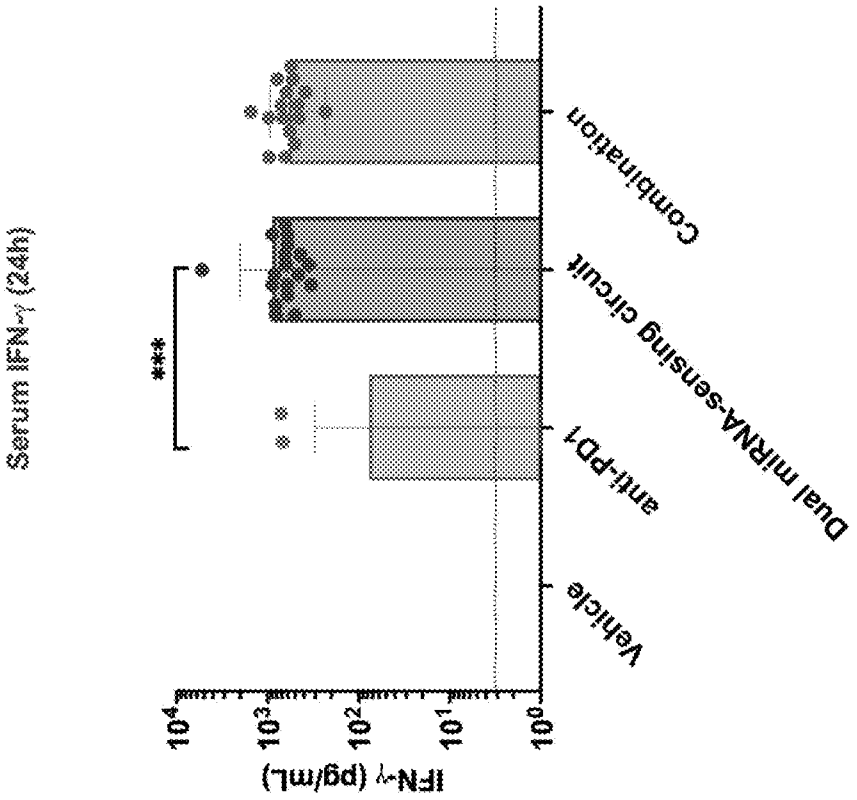


FIG. 42C

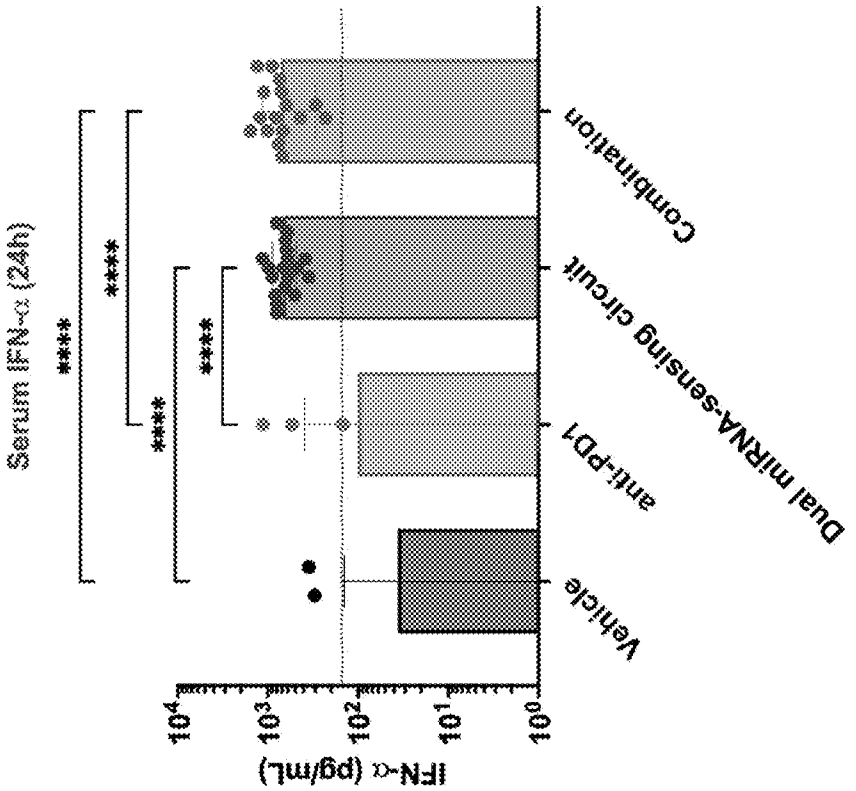


FIG. 42D

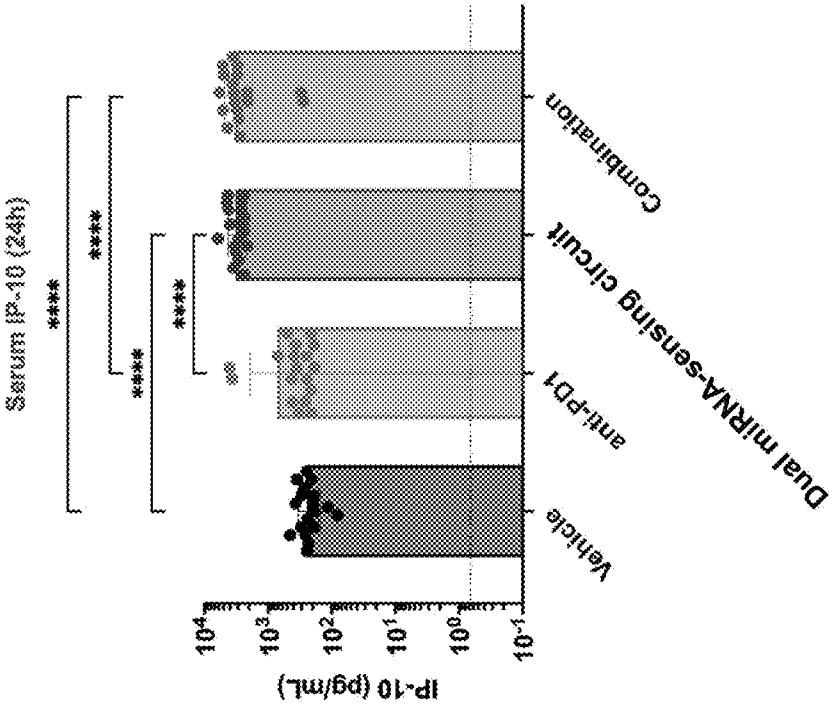


FIG. 43A

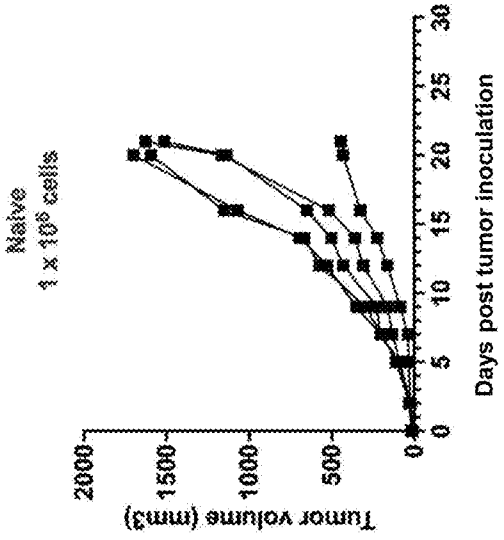


FIG. 43B

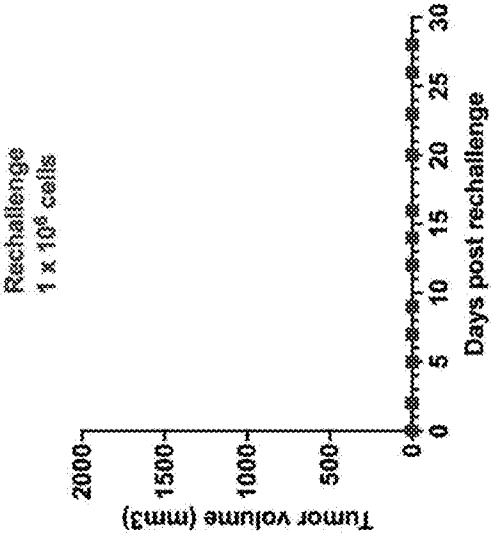


FIG. 43C

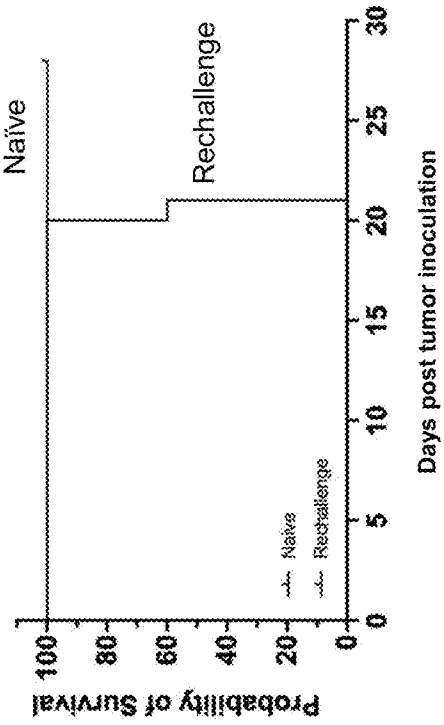


FIG. 43D

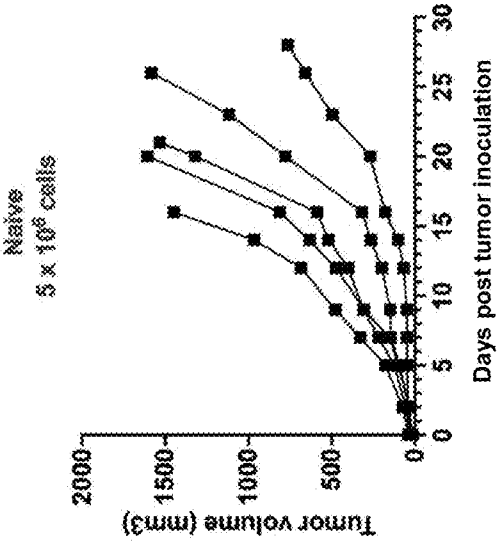


FIG. 43E

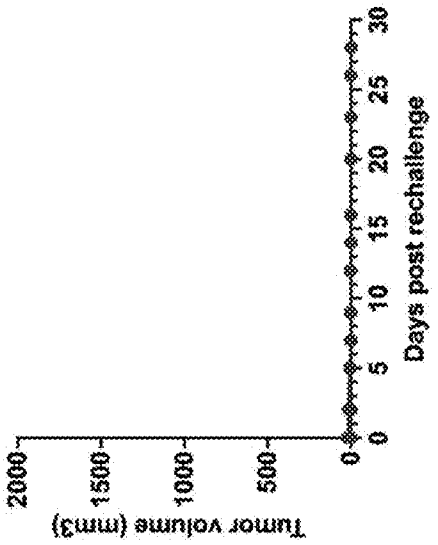


FIG. 43F

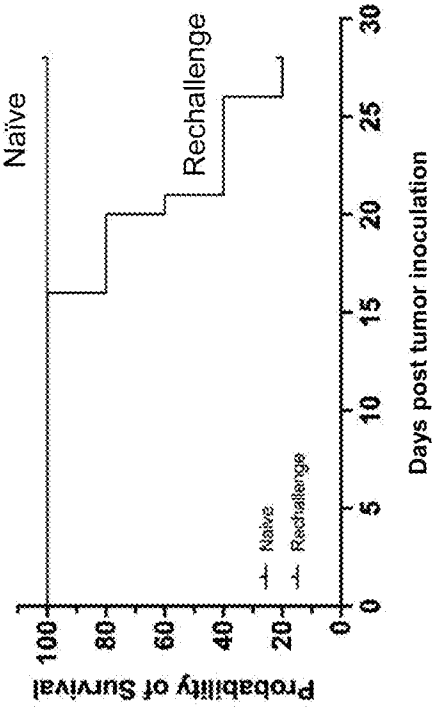


FIG. 44A

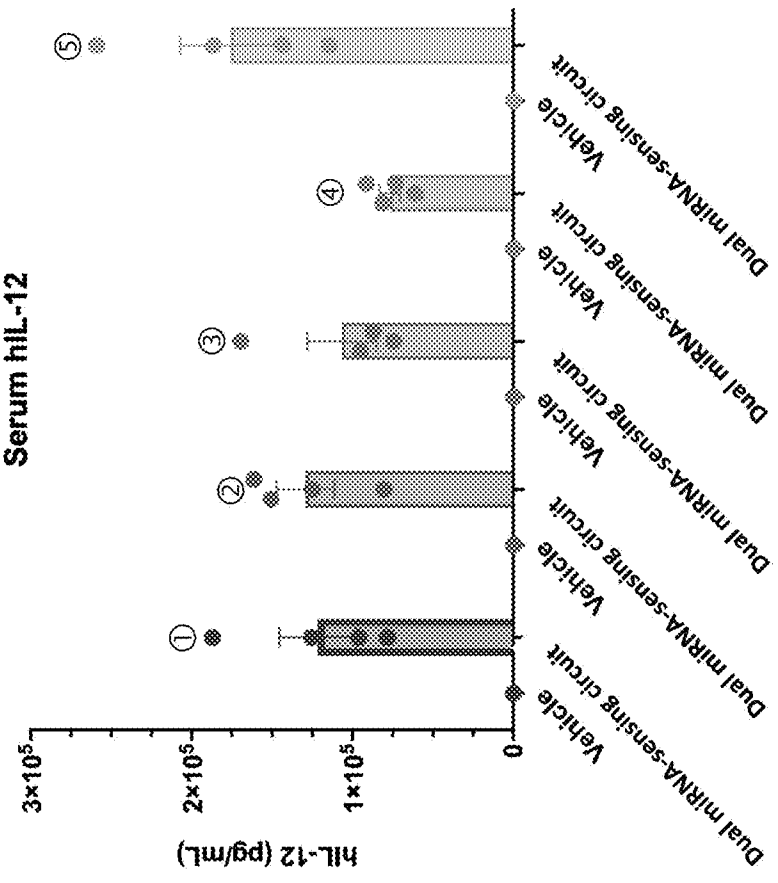
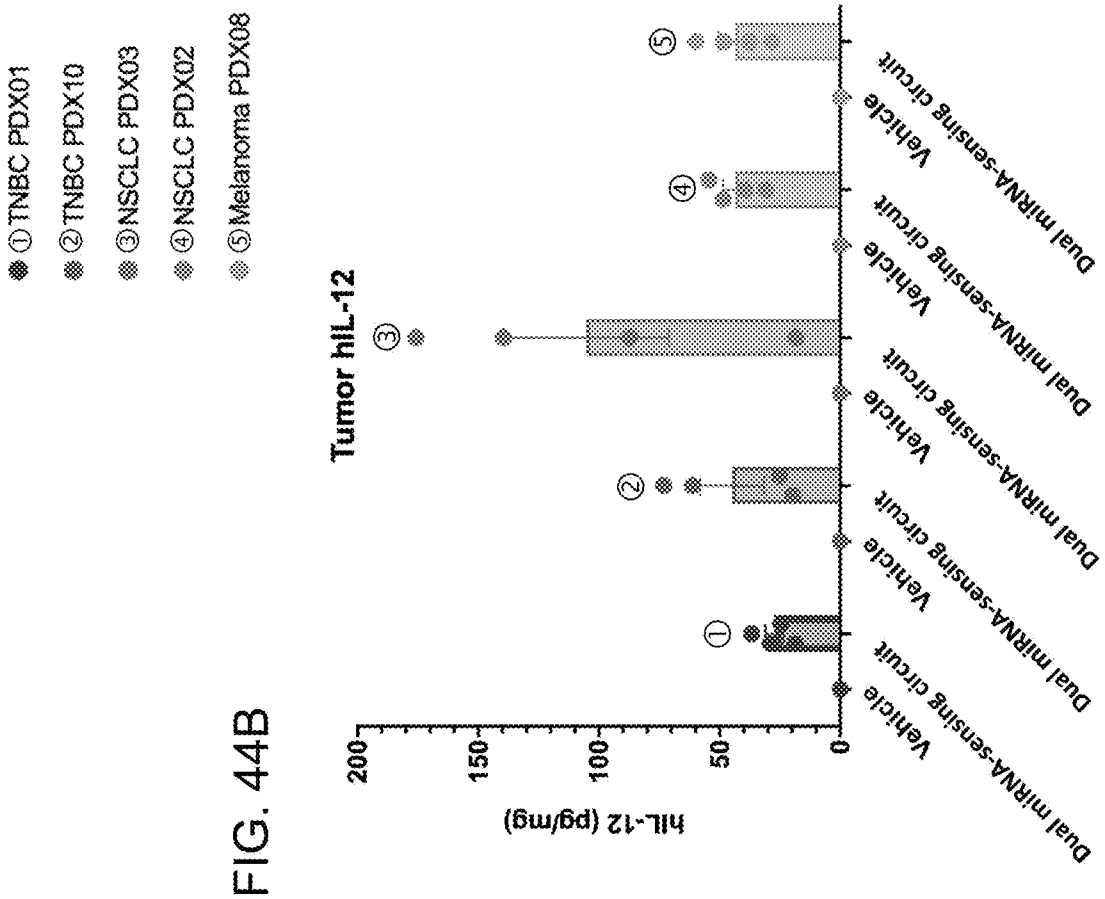


FIG. 44B



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2026/012893

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/69 A61K48/00 C12N5/0783 C12N5/077 C12N5/071
C12N15/11 C12N15/113 C12N15/88 C12Q1/68 C12Q1/6825
C12Q1/6886

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A01K C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2024/216162 A1 (STRAND THERAPEUTICS INC [US]) 17 October 2024 (2024-10-17) see claims 1-163, Fig. 18-21 and examples 1-8 -----	1-87
Y	TORRES-PAZ YAZMÍN ESTELA ET AL: "Overexpression of microRNA-21-5p and microRNA-221-5p in Monocytes Increases the Risk of Developing Coronary Artery Disease", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 24, no. 10, 12 May 2023 (2023-05-12), page 8641, XP093383890, Basel, CH ISSN: 1422-0067, DOI: 10.3390/ijms24108641 see abstract and discussion section pages 5-8 -----	1-87
	-/--	

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 April 2026	16/04/2026

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Vix, Olivier

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2026/012893

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2023/201354 A2 (ENCODED THERAPEUTICS INC [US]) 19 October 2023 (2023-10-19) The query sequence SEQ ID NO:6 has 100.00 % identity (100.00 % similarity) over 22 positions in a common overlap (range (q:s): 1-22:1-22) with subject GSN:BNW49901 (Liver de-targeting element test RNA 1, SEQ ID 1. Claim 1, length: 22) from WO2023201354-A2 published on 2023-10-19.	1-87
Y	US 2023/013773 A1 (HOGE STEPHEN G [US] ET AL) 19 January 2023 (2023-01-19) The query sequence SEQ ID NO:6 has 100.00 % identity over 22 positions in a common overlap (range (q:s): 1-22:1-22) with subject GSN:BMD90675 (Human microRNA hsa-miR-22-3p, SEQ 3921., length: 22) from US2022395562-A1 published on 2022-12-15. The query sequence SEQ ID NO:9 has 100.00 % identity over 22 positions in a common overlap (range (q:s): 1-22:1-22) with subject GSN:BMI38088 (Human microRNA hsa-miR-185-5p, SEQ 3805, length: 22) from US2023013773-A1 published on 2023-01-19.	1-87
Y	ANDO YUGO ET AL: "Overexpression of microRNA-21 is associated with elevated pro-inflammatory cytokines in dominant-negative TGF-[beta] receptor type II mouse", JOURNAL OF AUTOIMMUNITY, vol. 41, 1 March 2013 (2013-03-01), pages 111-119, XP093383892, GB ISSN: 0896-8411, DOI: 10.1016/j.jaut.2012.12.013 see abstract	1-87
Y	US 2020/281994 A1 (IRVINE DARRELL J [US] ET AL) 10 September 2020 (2020-09-10) see claims 1-21	1-87
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INTERNATIONAL SEARCH REPORT

International application No
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INTERNATIONAL SEARCH REPORT

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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

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