

transfected cell, thus eliminating the concern that the introduced genetic material will disrupt the normal functioning of an essential gene, or cause a mutation that results in deleterious or oncogenic effects, and extraneous promoter sequences are not required for effective translation of the encoded protein, again avoiding possible deleterious side effects. In addition, it is not necessary for mRNA to enter the nucleus to perform its function, while DNA must overcome this major barrier.

[0004] Circular RNA is useful in the design and production of stable forms of RNA. The circularization of an RNA molecule provides an advantage to the study of RNA structure and function, especially in the case of molecules that are prone to folding in an inactive conformation (Wang and Ruffner, 1998). Circular RNA can also be particularly interesting and useful for *in vivo* applications, especially in the research area of RNA-based control of gene expression and therapeutics, including protein replacement therapy and vaccination.

[0005] Prior to this invention, there were three main techniques for making circularized RNA *in vitro*: the splint-mediated method, the permuted intron-exon method, and the RNA ligase-mediated method. However, the existing methodologies are limited by the size of RNA that can be circularized, thus limiting their therapeutic application.

SUMMARY

[0006] In one aspect, the present disclosure refers to a circular RNA polynucleotide comprising, in the following order, a post splicing 3' group I intron fragment, a first Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, and a post splicing 5' group I intron fragment, and optionally comprising a second IRES between the first expression sequence and the second expression sequence.

[0006a] In another aspect, the present disclosure refers to a use of the circular RNA polynucleotide as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for treating cancer and/or an autoimmune disorder.

[0006b] In another aspect, the present disclosure refers to a vector for making a circular RNA polynucleotide, comprising, in the following order, a 5' duplex forming region, a 3' Group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, a 5' Group I intron fragment, and a 3' duplex forming region, optionally comprising: a. a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence; and/or b. a first spacer between the 5' duplex forming region and the 3' group I intron fragment, and a second spacer between the 5' group I intron fragment and the 3' duplex forming region.

[0006c] In another aspect, the present disclosure refers to a eukaryotic cell comprising a circular RNA polynucleotide as described herein, optionally wherein the cell is a human cell, an immune cell, and/or a T cell.

[0006d] Circular RNA, along with related compositions and methods are described herein. In some embodimentexamples, the inventive circular RNA comprises post splicing group I intron fragments, spacers, an IRES, optional duplex forming regions, and more than one expression sequence. In some embodimentexamples, the inventive circular RNA comprises duplex forming regions. In some embodimentexamples, the expression sequences are separated by one or more polynucleotide sequences encoding a cleavage site. In some embodimentexamples, a cleavage site is a self-cleaving peptide. In some embodimentexamples, the self-cleaving peptide is a 2A self-cleaving peptide. In some embodimentexamples, the first and second expression sequences are separated by ribosomal skipping element. In some embodimentexamples, each expression sequence encodes a therapeutic protein. In some embodimentexamples, the first expression sequence encodes a cytokine or a functional fragment thereof. In some embodimentexamples, the first expression sequence encodes a transcription factor. In some embodimentexamples, the first expression sequence encodes an immune checkpoint inhibitor. In some embodimentexamples, the first expression sequence encodes for a chimeric antigen receptor. In some embodimentexamples, the first expression sequence encodes a first T-cell receptor (TCR) chain, and the second expression encodes a second TCR chain. In some embodimentexamples, circular RNA of the inventiondisclosure has improved expression, functional

stability, ease of manufacturing, and/or half-life when compared to linear RNA. In some [embodimentexamples](#), circular RNA of the [inventiondisclosure](#) has reduced immunogenicity. In some [embodimentexamples](#), inventive methods and constructs result in improved circularization efficiency, splicing efficiency, and/or purity when compared to existing RNA circularization approaches.

[0007] In some [embodimentexamples](#), the circular RNA polynucleotide comprises one or more microRNA binding sites. The microRNA binding site is recognized by a microRNA expressed in the liver. In some [embodimentexamples](#), the microRNA binding site is recognized by miR-122.

[0008] One [aspectexample](#) of the present application provides a circular RNA polynucleotide comprising, in the following order, a post splicing 3' group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, and a post splicing 5' group I intron fragment.

[0009] In some [embodimentexamples](#), the circular RNA polynucleotide comprises a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence. In some [embodimentexamples](#), the cleavage site is a self-cleaving spacer. In some [embodimentexamples](#), the self-cleaving spacer is a 2A self-cleaving peptide.

[0010] In some [embodimentexamples](#), the circular RNA polynucleotide comprises a second IRES between the first expression sequence and the second expression sequence. In some [embodimentexamples](#), the first IRES consists of or comprises a sequence according to any of SEQ ID NO: 1-72. In some [embodimentexamples](#), the second IRES consists of or comprises a sequence according to any of SEQ ID NO: 1-72.

[0011] In some [embodimentexamples](#), the first expression sequence encodes a first therapeutic protein, and the second expression sequence encodes a second therapeutic protein. In some [embodimentexamples](#), the first expression sequence or the second expression sequence encodes an antibody. In some [embodimentexamples](#), the first expression sequence or the second expression sequence encodes a chimeric antigen receptor. In some [embodimentexamples](#), the first expression sequence or the second expression sequence encodes a transcription factor. In some [embodimentexamples](#), wherein the first expression sequence or the second expression sequence encodes a cytokine. In some [embodimentexamples](#), wherein the first expression sequence or the second expression sequence encodes an immune inhibitory molecule. In some [embodimentexamples](#), the first expression sequence or the second expression sequence encodes an agonist of a costimulatory molecule. In some [embodimentexamples](#), wherein the first expression sequence or the second expression sequence encodes an inhibitor of an immune checkpoint molecule. In some [embodimentexamples](#), wherein the first expression sequence encodes the alpha chain of a T cell

receptor (TCR) and the second expression sequence encodes the beta chain of a T cell receptor (TCR).

[0012] In some [embodimentexamples](#), the first expression sequence encodes the beta chain of a T cell receptor (TCR) and the second expression sequence encodes the alpha chain of a T cell receptor (TCR). In some [embodimentexamples](#), the first expression sequence encodes the gamma chain of a T cell receptor (TCR) and the second expression sequence encodes the delta chain of a T cell receptor (TCR). In some [embodimentexamples](#), the first expression sequence encodes the delta chain of a T cell receptor (TCR) and the second expression sequence encodes the gamma chain of a T cell receptor (TCR). In some [embodimentexamples](#), the first expression sequence encodes a T cell receptor (TCR) and the second expression sequence encodes a chemokine. In some [embodimentexample](#), the first expression sequence encodes for a chemokine and the second expression sequence encodes for a T cell receptor (TCR). In some [embodimentexamples](#), the first expression sequence encodes a chimeric antigen receptor (CAR) and the second expression sequence encodes a PD1 or PDL1 antagonist. In some [embodimentexamples](#), the first expression sequence encodes a PD1 or PDL1 antagonist and the second expression sequence encodes a chimeric antigen receptor (CAR). In some [embodimentexamples](#), the first expression sequence encodes for a chimeric antigen receptor (CAR), and the second expression sequence encodes a chemokine. In some [embodimentexamples](#), the first expression sequence encodes for a chemokine, and the second expression sequence encodes for a chimeric antigen receptor (CAR). In some [embodimentexamples](#), the first expression sequence encodes a transcription factor and the second expression sequence encodes a cytokine.

[0013] In some [embodimentexamples](#), the first expression sequence encodes a T cell receptor (TCR) and the second expression sequence encodes a cytokine. In some [embodimentexamples](#), the first expression sequence encodes a cytokine and the second expression sequence encodes a T cell receptor (TCR). In some [embodimentexamples](#), the cytokine is selected from IL-2, IL-7, IL-12, and IL-15.

[0014] In some [embodimentexamples](#), the first expression sequence encodes for a T cell receptor (TCR) and the second expression sequence encodes for a transcription factor. In some [embodimentexamples](#), the first expression sequence encodes for a transcription factor and the second expression sequence encodes for a T cell receptor (TCR). In some [embodimentexamples](#), the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25.

[0015] In some [embodimentexamples](#), the first expression sequence encodes a chimeric antigen receptor (CAR) and the second expression sequence encodes a cytokine. In some

[embodimentexamples](#), the first expression sequence encodes a cytokine and the second expression sequence encodes a chimeric antigen receptor (CAR). In some [embodimentexamples](#), the cytokine is selected from IL-2, IL-7, IL-12, and IL-15.

[0016] In some [embodimentexamples](#), the first expression sequence encodes a cytokine and the second expression sequence encodes a transcription factor. In some [embodimentexamples](#), the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25. In some [embodimentexamples](#), the cytokine is selected from IL-10, IL-12, and TGF β .

[0017] In some [embodimentexamples](#), the first expression sequence encodes a transcription factor and the second expression sequence encodes a chemokine. In some [embodimentexamples](#), the first expression sequence encodes a chemokine and the second expression sequence encodes a transcription factor. In some [embodimentexamples](#), the transcription factor is selected from FOXP3, STAT5B, and HELIOS. In some [embodimentexamples](#), the chemokine is a CC chemokine, CXC chemokine, C chemokine, or a CX3C chemokine. In some [embodimentexamples](#), the chemokine is selected from CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL 1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, and CX3CL1.

[0018] In some [embodimentexamples](#), the first expression sequence encodes a tumor antigen and the second expression sequence encodes a cytokine. In some [embodimentexamples](#), the first expression sequence encodes a cytokine and the second expression sequence encodes a tumor antigen. In some [embodimentexamples](#), the antigen is a neoantigen. In some [embodimentexamples](#), the cytokine is IFN γ .

[0019] In some [embodimentexamples](#), the first expression sequence encodes a CAR and the second expression sequence encodes a CAR.

[0020] In some [embodimentexamples](#), the first expression sequence encodes a cytokine and the second expression sequence encodes a cytokine. In some [embodimentexamples](#), the first or second expression sequence encodes a cytokine selected from IL-10, TGF β , and IL-35. In some [embodimentexamples](#), the first or second expression sequence encodes a cytokine selected from IFN γ , IL-2, IL-7, IL-15, and IL-18.

[0021] In some [embodimentexamples](#), the first expression sequence encodes a T cell receptor (TCR) and the second expression sequence encodes a T cell receptor (TCR). In some [embodimentexamples](#), the first expression sequence encodes for a chemokine and the second expression sequence encodes for a chemokine. In some [embodimentexamples](#), the first or second

expression sequence encodes for an immunosuppressive enzyme. In some [embodimentexamples](#), the first expression sequence encodes a rate limiting enzyme and the second expression sequence encodes a flux-limiting enzyme. In some [embodimentexamples](#), the first expression sequence encodes a flux-limiting enzyme and the second expression sequence encodes a rate limiting enzyme.

[0022] In some [embodimentexamples](#), the first expression sequence encodes a transcription factor and the second expression sequence encodes a survival factor. In some [embodimentexamples](#), the first expression sequence encodes a survival factor and the second expression sequence encodes a transcription factor. In some [embodimentexamples](#), the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet,GATA3, RORgt, and cd25. In some [embodimentexamples](#), the survival factor is selected from BCL-XL.

[0023] In some [embodimentexamples](#), the first or second expression sequence encodes for a chaperone protein or complex. In some [embodimentexamples](#), the first expression sequence encodes a transcription factor and the second expression sequence encodes a chaperone protein or complex. In some [embodimentexamples](#), the first expression sequence encodes a chaperone protein or complex and the second expression sequence encodes for a transcription factor. In some [embodimentexamples](#), the chaperone protein or complex is selected from Skp, Spy, FkpA, SurA, Hsp60, Hsp70, GroEL, GroES, Hsp90, HtpG, Hsp100, ClpA, ClpX, ClpP, and Hsp104. In some [embodimentexamples](#), the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet,GATA3, RORgt, and cd25.

[0024] In some [embodimentexamples](#), one or both expression sequences encode a signaling protein.

[0025] In some [embodimentexamples](#), the first expression sequence encodes for an enzyme and the second expression encodes for the first expression sequence's negative regulatory inhibitor. In some [embodimentexamples](#), the first expression sequence encodes for a negative regulatory inhibitor protein of an enzyme encoded in the second expression sequence. In some [embodimentexamples](#), the negative regulatory inhibitor is selected from a p57kip2, BAX inhibitor, and TIPE2.

[0026] In some [embodimentexamples](#), the first expression sequence encodes a dominant negative protein and the second expression sequence encodes an immune protein. In some [embodimentexamples](#), the first expression sequence encodes an immune protein and the second expression sequence encodes a dominant negative protein. In some [embodimentexamples](#), the first or second expression sequence encodes for an anti-inflammatory protein.

[0027] In some [embodimentexamples](#), the first expression sequence encodes a transcription factor

and the second expression sequence is capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU). In some [embodiment examples](#), the first expression sequence is capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU) and the second expression sequence is a transcription factor. In some [embodiment examples](#), wherein the expression sequence capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU) is cytosine deaminase.

[0028] In some [embodiment examples](#), the circular RNA polynucleotide comprises a first spacer between the 5' duplex forming region and the post splicing 3' group I intron fragment, and a second spacer between the post splicing 5' group I intron fragment and the 3' duplex forming region. In some [embodiment examples](#), the first and second spacers each have a length of about 10 to about 60 nucleotides. In some [embodiment examples](#), the first and second duplex forming regions each have a length of about 9 to about 19 nucleotides. In some [embodiment examples](#), the first and second duplex forming regions each have a length of about 30 nucleotides. In some [embodiment examples](#), the IRES has a sequence of an IRES from Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, Simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, Human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus- 1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus- 1, Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus, Foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus, Drosophila C Virus, Human coxsackievirus B3, Crucifer tobamovirus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPA1, Human AML1/RUNX1, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAP1, Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, S. cerevisiae TFIID, S. cerevisiae YAP1, tobacco etch virus, turnip crinkle virus, EMCV-A, EMCV-B, EMCV-Bf, EMCV-Cf, EMCV pEC9, Picobirnavirus, HCV QC64, Human Cosavirus E/D, Human Cosavirus F, Human Cosavirus JMY, Rhinovirus NAT001, HRV14, HRV89, HRVC-02, HRV-A21, Salivirus A SH1, Salivirus FHB, Salivirus NG-J1, Human Parechovirus 1, Crohivirus B, Yc-3, Rosavirus M-7,

Shanbavirus A, Pasivirus A, Pasivirus A 2, Echovirus E14, Human Parechovirus 5, Aichi Virus, Hepatitis A Virus HA16, Phopivirus, CVA10, Enterovirus C, Enterovirus D, Enterovirus J, Human Pegivirus 2, GBV-C GT110, GBV-C K1737, GBV-C Iowa, Pegivirus A 1220, Pasivirus A 3, Sapelovirus, Rosavirus B, Bakunsa Virus, Tremovirus A, Swine Pasivirus 1, PLV-CHN, Pasivirus A, Sicinivirus, Hepacivirus K, Hepacivirus A, BVDV1, Border Disease Virus, BVDV2, CSFV-PK15C, SF573 Dicistrovirus, Hubei Picorna-like Virus, CRPV, Salivirus A BN5, Salivirus A BN2, Salivirus A 02394, Salivirus A GUT, Salivirus A CH, Salivirus A SZ1, Salivirus FHB, CVB3, CVB1, Echovirus 7, CVB5, EVA71, CVA3, CVA12, EV24 or an aptamer to eIF4G.

[0029] In some [embodimentexamples](#), the circular RNA polynucleotide comprises natural nucleotides. In some [embodimentexamples](#), the circular RNA polynucleotide consists of natural nucleotides. In some [embodimentexamples](#), the expression sequence is codon optimized.

[0030] In some [embodimentexamples](#), the circular RNA polynucleotide is optimized to lack at least one microRNA binding site present in an equivalent pre-optimized polynucleotide. In some [embodimentexamples](#), the circular RNA polynucleotide is optimized to lack at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide. In some [embodimentexamples](#), the circular RNA polynucleotide is optimized to lack at least one RNA-editing susceptible site present in an equivalent pre-optimized polynucleotide.

[0031] In some [embodimentexamples](#), the circular RNA polynucleotide of any one of the preceding claims, wherein the circular RNA polynucleotide is from about 100 nucleotides to about 15 kilobases in length.

[0032] In some [embodimentexamples](#), the circular RNA polynucleotide of has an *in vivo* duration of therapeutic effect in humans of at least about 20 hours. In some [embodimentexamples](#), the circular RNA polynucleotide of has a functional half-life of at least about 20 hours. In some [embodimentexamples](#), the circular RNA polynucleotide of has a duration of therapeutic effect in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequence. In some [embodimentexamples](#), the circular RNA polynucleotide of has a functional half-life in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequence. In some [embodimentexamples](#), the circular RNA polynucleotide of has an *in vivo* duration of therapeutic effect in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence. In some [embodimentexamples](#), the circular RNA polynucleotide of has an *in vivo* functional half-life in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.

[0033] In another [aspectexample](#), the present application provides a pharmaceutical composition comprising a circular RNA polynucleotide as described herein, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle. In some [embodimentexamples](#), the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, a biodegradable nanoparticle, a biodegradable lipid nanoparticle, a polymer nanoparticle, or a biodegradable polymer nanoparticle.

[0034] In some [embodimentexamples](#), the pharmaceutical composition comprises a targeting moiety, wherein the targeting moiety mediates receptor-mediated endocytosis or direct fusion into selected cells of a selected cell population or tissue in the absence of cell isolation or purification. In some [embodimentexamples](#), the pharmaceutical composition comprises a targeting moiety operably connected to the nanoparticle. In some [embodimentexamples](#), the targeting moiety is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof.

[0035] In some [embodimentexamples](#), the pharmaceutical composition comprises less than 1%, by weight, of the polynucleotides in the composition that are double stranded RNA, DNA splints, or triphosphorylated RNA. In some [embodimentexamples](#), the pharmaceutical composition comprises less than 1%, by weight, of the polynucleotides and proteins in the pharmaceutical composition that are double stranded RNA, DNA splints, triphosphorylated RNA, phosphatase proteins, protein ligases, and capping enzymes.

[0036] In another [aspectexample](#), the present application provides a method of treating a subject in need thereof comprising administering a therapeutically effective amount of a composition comprising the circular RNA polynucleotide described herein, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle.

[0037] In some [embodimentexamples](#), the composition comprises a targeting moiety, wherein the targeting moiety mediates receptor-mediated endocytosis selectively into cells of a selected cell population in the absence of cell selection or purification. In some [embodimentexamples](#), the targeting moiety is an scFv, nanobody, peptide, minibody, heavy chain variable region, light chain variable region or fragment thereof. In some [embodimentexamples](#), the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, or a biodegradable nanoparticle.

[0038] In some [embodimentexamples](#), the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β -amino esters. In some [embodimentexamples](#), the nanoparticle comprises one or more non-cationic lipids. In some [embodimentexamples](#), the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids. In some [embodimentexamples](#), the nanoparticle comprises cholesterol. In some [embodimentexamples](#), the

nanoparticle comprises arachidonic acid or oleic acid. In some [embodimentexamples](#), the nanoparticle comprises more than one circular RNA polynucleotide.

[0039] In some [embodimentexamples](#), the subject has a cancer selected from the group consisting of acute lymphocytic leukemia; acute myeloid leukemia (AML); alveolar rhabdomyosarcoma; B cell malignancies; bladder cancer (e.g., bladder carcinoma); bone cancer; brain cancer (e.g., medulloblastoma); breast cancer; cancer of the anus, anal canal, or anorectum; cancer of the eye; cancer of the intrahepatic bile duct; cancer of the joints; cancer of the neck; gallbladder cancer; cancer of the pleura; cancer of the nose, nasal cavity, or middle ear; cancer of the oral cavity; cancer of the vulva; chronic lymphocytic leukemia; chronic myeloid cancer; colon cancer; esophageal cancer, cervical cancer; fibrosarcoma; gastrointestinal carcinoid tumor; head and neck cancer (e.g., head and neck squamous cell carcinoma); Hodgkin lymphoma; hypopharynx cancer; kidney cancer; larynx cancer; leukemia; liquid tumors; liver cancer; lung cancer (e.g., non-small cell lung carcinoma and lung adenocarcinoma); lymphoma; mesothelioma; mastocytoma; melanoma; multiple myeloma; nasopharynx cancer; non-Hodgkin lymphoma; B-chronic lymphocytic leukemia; hairy cell leukemia; acute lymphocytic leukemia (ALL); Burkitt's lymphoma; ovarian cancer; pancreatic cancer; cancer of the peritoneum; cancer of the omentum; mesentery cancer; pharynx cancer; prostate cancer; rectal cancer; renal cancer; skin cancer; small intestine cancer; soft tissue cancer; solid tumors; synovial sarcoma; gastric cancer; testicular cancer; thyroid cancer; and ureter cancer. In some [embodimentexamples](#), the subject has an autoimmune disorder selected from scleroderma, Grave's disease, Crohn's disease, Sjorgen's disease, multiple sclerosis, Hashimoto's disease, psoriasis, myasthenia gravis, autoimmune polyendocrinopathy syndromes, Type I diabetes mellitus (T1DM), autoimmune gastritis, autoimmune uveoretinitis, polymyositis, colitis, thyroiditis, and the generalized autoimmune diseases typified by human Lupus.

[0040] In another [aspectexample](#), the present application provides a vector for making a circular RNA polynucleotide, comprising, in the following order, a 5' duplex forming region, a 3' Group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, a 5' Group I intron fragment, and a 3' duplex forming region.

[0041] In some [embodimentexamples](#), the vector of comprises a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence. In some [embodimentexamples](#), the cleavage site is a self-cleaving spacer. In some [embodimentexamples](#), the self-cleaving spacer is a 2A self-cleaving peptide.

[0042] In some [embodimentexamples](#), the vector of comprises a first spacer between the 5'

duplex forming region and the 3' group I intron fragment, and a second spacer between the 5' group I intron fragment and the 3' duplex forming region. In some [embodimentexamples](#), the first and second spacers each have a length of about 5 to about 60 nucleotides. In some [embodimentexamples](#), the first and second spacers each comprise an unstructured region at least 5 nucleotides long. In some [embodimentexamples](#), the first and second spacers each comprise a structured region at least 7 nucleotides long. In some [embodimentexamples](#), the first and second duplex forming regions each have a length of about 9 to 50 nucleotides.

[0043] In some [embodimentexamples](#), the vector is codon optimized. In some [embodimentexamples](#), the vector lacks at least one microRNA binding site present in an equivalent pre-optimization polynucleotide.

[0044] In another [aspectexample](#), the present application provides a eukaryotic cell comprising a circular RNA polynucleotide as described herein. In some [embodimentexamples](#), the eukaryotic cell is a human cell. In some [embodimentexamples](#), the eukaryotic cell is an immune cell. In some [embodimentexamples](#), the eukaryotic cell is a T cell.

[0045] In an [aspectexample](#), provided herein is a circular RNA polynucleotide comprising, in the following order, a post splicing 3' group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a polynucleotide sequence encoding a cleavage site, a second expression sequence, and a post splicing 5' group I intron fragment. In an [aspectexample](#), provided herein is a circular RNA polynucleotide comprising, in the following order, a post splicing 3' group I intron fragment, a first Internal Ribosome Entry Site (IRES), a first expression sequence, a second IRES, a second expression sequence, and a post splicing 5' group I intron fragment. In some [embodimentexamples](#), the first expression sequence and the second expression sequence encode different therapeutic proteins. In some [embodimentexamples](#), the first expression sequence and the second expression sequence encodes for the same therapeutic protein.

[0046] In an [aspectexample](#), provided herein is a circular RNA polynucleotide produced from transcription of a vector comprising, in the following order, an optional 5' duplex forming region, a post splicing 3' group I intron fragment, an Internal Ribosome Entry Site (IRES), an expression sequence, a polynucleotide sequence encoding a cleavage site, a second expression sequence, a 5' group I intron fragment, and an optional 3' duplex forming region. In an [aspectexample](#), provided herein is a circular RNA polynucleotide produced from transcription of a vector comprising, in the following order, an optional 5' duplex forming region, a 3' group I intron fragment, a first Internal Ribosome Entry Site (IRES), an expression sequence, a second IRES, a second expression sequence, a 5' group I intron fragment, and an optional 3'

duplex forming region. In some [embodimentexamples](#), a circular RNA polynucleotide or vector provided herein comprises 3' and 5' duplex forming regions.

[0047] In some [embodimentexamples](#), a circular RNA polynucleotide comprises a first spacer between the 5' duplex forming region and the post splicing 3' group I intron fragment, and a second spacer between the post splicing 5' group I intron fragment and the 3' duplex forming region. In some [embodimentexamples](#), the first and second spacers each have a length of about 10 to about 60 nucleotides. In certain [embodimentexamples](#), the first and second duplex forming regions each have a length of about 9 to about 19 nucleotides. In certain other [embodimentexamples](#), the first and second duplex forming regions each have a length of about 30 nucleotides.

[0048] In certain [embodimentexamples](#), the IRES is selected from Table 17 has a sequence of an IRES, or is a functional fragment or variant thereof. In some [embodimentexamples](#), the IRES has a sequence of an IRES from Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, Simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, Human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus- 1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus- 1, Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus, Foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus, Drosophila C Virus, Human coxsackievirus B3, Crucifer tobamovirus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPA1, Human AML1/RUNX1, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAP1, Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, S. cerevisiae TFIID, S. cerevisiae YAP1, tobacco etch virus, turnip crinkle virus, EMCV-A, EMCV-B, EMCV-Bf, EMCV-Cf, EMCV pEC9, Picobirnavirus, HCV QC64, Human Cosavirus E/D, Human Cosavirus F, Human Cosavirus JMY, Rhinovirus NAT001, HRV14, HRV89, HRVC-02, HRV-A21, Salivirus A SH1, Salivirus FHB, Salivirus NG-J1, Human Parechovirus 1, Crohivirus B, Yc-3, Rosavirus M-7, Shanbavirus A, Pasivirus A, Pasivirus A 2, Echovirus E14, Human Parechovirus 5, Aichi Virus, Hepatitis A Virus HA16, Phopivirus, CVA10, Enterovirus C, Enterovirus D, Enterovirus J, Human Pegivirus 2, GBV-C GT110,

GBV-C K1737, GBV-C Iowa, Pegivirus A 1220, Pasivirus A 3, Sapelovirus, Rosavirus B, Bakunsa Virus, Tremovirus A, Swine Pasivirus 1, PLV-CHN, Pasivirus A, Sicinivirus, Hepacivirus K, Hepacivirus A, BVDV1, Border Disease Virus, BVDV2, CSFV-PK15C, SF573 Dicistrovirus, Hubei Picorna-like Virus, CRPV, Salivirus A BN5, Salivirus A BN2, Salivirus A 02394, Salivirus A GUT, Salivirus A CH, Salivirus A SZ1, Salivirus FHB, CVB3, CVB1, Echovirus 7, CVB5, EVA71, CVA3, CVA12, EV24 or an aptamer to eIF4G.

[0049] In some [embodimentexamples](#), the first and second polyA sequences each have a length of 15-50nt. In some [embodimentexamples](#), the first and second polyA sequences each have a length of about 20-25nt.

[0050] In certain [embodimentexamples](#), the circular RNA polynucleotide consists of naturally occurring nucleotides. In some [embodimentexamples](#), the circular RNA contains at least about 80%, at least 90%, at least about 95%, or at least about 99% naturally occurring nucleotides. In certain [embodimentexamples](#), the expression sequence is codon-optimized. In certain [embodimentexamples](#), the circular RNA polynucleotide is optimized to lack at least one microRNA binding site present in an equivalent pre-optimized polynucleotide. In certain [embodimentexamples](#), the circular RNA polynucleotide is optimized to lack at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide. In certain [embodimentexamples](#), the circular RNA polynucleotide is optimized to lack at least one RNA-editing susceptible site present in an equivalent pre-optimized polynucleotide.

[0051] In some [embodimentexamples](#), the circular RNA polynucleotide is from about 100 nucleotides to about 15 kilobases in length. In certain [embodimentexamples](#), the circular RNA polynucleotide of the present disclosure, has an *in vivo* duration of therapeutic effect in humans of at least about 20 hours. In certain [embodimentexample](#), the circular RNA polynucleotide has a functional half-life of at least about 20 hours. In certain [embodimentexamples](#), the circular RNA polynucleotide has a duration of therapeutic effect in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequences. In certain [embodimentexamples](#), the circular RNA polynucleotide has a functional half-life in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequences. In certain [embodimentexamples](#), the circular RNA polynucleotide has an *in vivo* functional half-life in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequences. In some [embodimentexamples](#), the reference linear RNA polynucleotide is a linear, unmodified or nucleoside-modified, fully-processed mRNA comprising a cap1 structure and a polyA tail at least 80nt in length.

[0052] In some [embodimentexamples](#), the pharmaceutical composition has a functional half-life

in a human cell greater than or equal that of a pre-determined threshold value. In some [embodimentexamples](#), the pharmaceutical composition has a functional half-life *in vivo* in humans greater than that of a pre-determined threshold value. In some [embodimentexamples](#), the functional protein assay is an *in vitro* luciferase assay. In some [embodimentexamples](#), the functional protein assay comprises measuring levels of protein encoded by the expression sequence of the circular RNA polynucleotide in a patient serum or tissue sample. In some [embodimentexamples](#), wherein the pre-determined threshold value is the functional half-life of a reference linear RNA polynucleotide comprising the same expression sequence as the circular RNA polynucleotide. In some [embodimentexamples](#), the pharmaceutical composition has a functional half-life of at least about 20 hours.

[0053] In an [aspectexample](#), provided herein is a pharmaceutical composition comprising a circular RNA polynucleotide, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle. In some [embodimentexamples](#), the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, a biodegradable nanoparticle, a biodegradable lipid nanoparticle, a polymer nanoparticle, or a biodegradable polymer nanoparticle. In certain [embodimentexamples](#), the nanoparticle comprises one or more cationic lipids selected from the group C12-200, MC3, DLinDMA, DLinkC2DMA, cKK-E12, ICE (Imidazol- based), HGT5000, HGT5001, DODAC, DDAB, DMRIE, DOSPA, DOGS, DODAP, DODMA and DMDMA, DODAC, DLenDMA, DMRIE, CLinDMA, CpLinDMA, DMOBA, DOcarbDAP, DLinDAP, DLincarbDAP, DLinCDAP, KLin-K-DMA, DLin-K-XTC2-DMA, HGT4003, and combinations thereof.

[0054] In certain [embodimentexamples](#), the pharmaceutical composition comprises a targeting moiety, wherein the targeting moiety mediates receptor-mediated endocytosis or direct fusion into selected cells of a selected cell population or tissue in the absence of cell isolation or purification. In some [embodimentexamples](#), the targeting moiety is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof. In certain [embodimentexamples](#), the circular RNA polynucleotide is in an amount effective to treat an autoimmune disorder or cancer in a human subject in need thereof. In certain [embodimentexamples](#), the pharmaceutical composition has an enhanced safety profile when compared to a pharmaceutical composition comprising vectors comprising exogenous DNA encoding the same expression sequences.

[0055] In certain [embodimentexamples](#), less than 1%, by weight, of the polynucleotides in the composition are double stranded RNA, DNA splints, or triphosphorylated RNA. In certain [embodimentexamples](#), less than 1%, by weight, of the polynucleotides and proteins in the

pharmaceutical composition are double stranded RNA, DNA splints, triphosphorylated RNA, phosphatase proteins, protein ligases, and capping enzymes.

[0056] In an [aspectexample](#), provided herein is a method of treating a subject in need thereof comprising administering a therapeutically effective amount of a composition comprising the circular RNA polynucleotide, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle. In some [embodimentexamples](#), the subject has an autoimmune disorder or cancer.

[0057] In some [embodimentexamples](#), the targeting moiety is an scFv, nanobody, peptide, minibody, heavy chain variable region, light chain variable region or fragment thereof. In some [embodimentexamples](#), the composition comprises a targeting moiety, wherein the targeting moiety mediates receptor-mediated endocytosis into selected cells of a selected cell population in the absence of cell isolation or purification.

[0058] In some [embodimentexamples](#), the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, or a biodegradable nanoparticle. In some [embodimentexamples](#), the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β -amino esters. In some [embodimentexamples](#), the nanoparticle comprises one or more non-cationic lipids. In some [embodimentexamples](#), the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, structural lipid or hyaluronic acid lipids. In certain [embodimentexamples](#), the nanoparticle comprises cholesterol. In some [embodimentexamples](#), the structural lipid is a beta-sitosterol. In some [embodimentexamples](#), the structural lipid is not a beta sitosterol. In some [embodimentexamples](#), the nanoparticle comprises arachidonic acid or oleic acid. In some [embodimentexamples](#), the nanoparticle comprises more than one circular RNA polynucleotide.

[0059] In some [embodimentexamples](#), the structural lipid binds to C1q and/or promotes the binding of the transfer vehicle comprising said lipid to C1q compared to a control transfer vehicle lacking the structural lipid and/or increases uptake of C1q-bound transfer vehicle into an immune cell compared to a control transfer vehicle lacking the structural lipid.

[0060] In some [embodimentexamples](#), the PEG-modified lipid is DSPE-PEG, DMG-PEG, or PEG-1. In some [embodimentexamples](#), the PEG-modified lipid is DSPE-PEG(2000).

[0061] In some [embodimentexamples](#), the pharmaceutical composition further comprises a helper lipid. In some [embodimentexamples](#), the helper lipid is DSPC or DOPE.

[0062] In some [embodimentexamples](#), the pharmaceutical composition comprises DOPE, cholesterol, and DSPE-PEG.

[0063] In some [embodimentexamples](#), the transfer vehicle comprises about 0.5% to about 4% PEG-modified lipids by molar ratio. In some [embodimentexamples](#), the transfer vehicle comprises

about 1% to about 2% PEG-modified lipids by molar ratio.

[0064] In some [embodimentexamples](#), the molar ration of ionizable lipid:DSPC:cholesterol:DSPE-PEG(2000) is 62:4:33:1.

[0065] In some [embodimentexamples](#), the transfer vehicle comprises an ionizable lipid, a DOPE, cholesterol, and a DSPE-PEG(2000).

[0066] In some [embodimentexamples](#), the molar ration of ionizable lipid:DSPC:cholesterol:DSPE-PEG(2000) is 50:10:38.5:1.5.

[0067] In some [embodimentexamples](#), the transfer vehicle has a nitrogen:phosphate (N:P) ratio of about 3 to about 6.

[0068] In some [embodimentexamples](#), the transfer vehicle is formulated for endosomal release of the circular RNA polynucleotide.

[0069] In some [embodimentexamples](#), the transfer vehicle is capable of binding to APOE. In some [embodimentexamples](#), the transfer vehicle interacts with apolipoprotein E (APOE) less than an equivalent transfer vehicle loaded with a reference linear RNA having the same expression sequence as the circular RNA polynucleotide. In some [embodimentexamples](#), the exterior surface of the transfer vehicle is substantially free of APOE binding sites.

[0070] In some [embodimentexamples](#), the transfer vehicle has a diameter of less than about 120nm. In some [embodimentexamples](#), the transfer vehicle does not form aggregates with a diameter of more than 300nm.

[0071] In some [embodimentexamples](#), the transfer vehicle has a diameter of less than about 120nm. In some [embodimentexamples](#), the transfer vehicle does not form aggregates with a diameter of more than 300nm.

[0072] In some [embodimentexamples](#), the transfer vehicle has an in vivo half-life of less than about 30 hours.

[0073] In some [embodimentexamples](#), the transfer vehicle is capable of low density lipoprotein receptor (LDLR) dependent uptake into a cell. In some [embodimentexamples](#), the transfer vehicle is capable of LDLR independent uptake into a cell.

[0074] In some [embodimentexamples](#), the pharmaceutical composition is substantially free of linear RNA.

[0075] In some [embodimentexamples](#), the pharmaceutical composition further comprises a targeting moiety operably connected to the transfer vehicle. In some [embodimentexamples](#), the targeting moiety specifically binds an immune cell antigen or indirectly. In some [embodimentexamples](#), the immune cell antigen is a T cell antigen. In some [embodimentexamples](#), the T cell antigen is selected from the group consisting of CD2, CD3, CD5, CD7, CD8, CD4, beta7

integrin, beta2 integrin, and C1q.

[0076] In some [embodimentexamples](#), the pharmaceutical composition further comprises an adapter molecule comprising a transfer vehicle binding moiety and a cell binding moiety, wherein the targeting moiety specifically binds the transfer vehicle binding moiety and the cell binding moiety specifically binds a target cell antigen. In some [embodimentexamples](#), the target cell antigen is an immune cell antigen. In some [embodimentexamples](#), the immune cell antigen is a T cell antigen, an NK cell, an NKT cell, a macrophage, or a neutrophil. In some [embodimentexamples](#), the T cell antigen is selected from the group consisting of CD2, CD3, CD5, CD7, CD8, CD4, beta7 integrin, beta2 integrin, CD25, CD39, CD73, A2a Receptor, A2b Receptor, and C1q. In some [embodimentexamples](#), the immune cell antigen is a macrophage antigen. In some [embodimentexamples](#), the macrophage antigen is selected from the group consisting of mannose receptor, CD206, and C1q.

[0077] In some [embodimentexamples](#), the targeting moiety is a small molecule. In some [embodimentexamples](#), the small molecule binds to an ectoenzyme on an immune cell, wherein the ectoenzyme is selected from the group consisting of CD38, CD73, adenosine 2a receptor, and adenosine 2b receptor. In some [embodimentexamples](#), the small molecule is mannose, a lectin, acivicin, biotin, or digoxigenin.

[0078] In some [embodimentexamples](#), the targeting moiety is a single chain Fv (scFv) fragment, nanobody, peptide, peptide-based macrocycle, minibody, small molecule ligand such as folate, arginylglycylaspartic acid (RGD), or phenol-soluble modulin alpha 1 peptide (PSMA1), heavy chain variable region, light chain variable region or fragment thereof.

[0079] In some [embodimentexamples](#), the ionizable lipid has a half-life in a cell membrane less than about 2 weeks. In some [embodimentexamples](#), the ionizable lipid has a half-life in a cell membrane less than about 1 week. In some [embodimentexamples](#), the ionizable lipid has a half-life in a cell membrane less than about 30 hours. In some [embodimentexamples](#), the ionizable lipid has a half-life in a cell membrane less than the functional half-life of the circular RNA polynucleotide.

[0080] In another [aspectexample](#), the present application provides a method of treating or preventing a disease, disorder, or condition, comprising administering an effective amount of a pharmaceutical composition disclosed herein. In some [embodimentexamples](#), the disease, disorder, or condition is associated with aberrant expression, activity, or localization of a polypeptide selected from Tables 27 or 28. In some [embodimentexamples](#), the circular RNA polynucleotide encodes a therapeutic protein. In some [embodimentexamples](#), therapeutic protein expression in the spleen is higher than therapeutic protein expression in the liver. In some [embodimentexamples](#),

therapeutic protein expression in the spleen is at least about 2.9x therapeutic protein expression in the liver. In some [embodimentexamples](#), the therapeutic protein is not expressed at functional levels in the liver. In some [embodimentexamples](#), the therapeutic protein is not expressed at detectable levels in the liver. In some [embodimentexamples](#), therapeutic protein expression in the spleen is at least about 63% of total therapeutic protein expression.

[0081] In some [embodimentexamples](#), the linear RNA polynucleotide comprises a 3' anabaena group I intron fragment and a 5' anabaena group I intron fragment. In some [embodimentexamples](#), the reference RNA polynucleotide comprises a reference 3' anabaena group I intron fragment and a reference 5' anabaena group I intron fragment. In some [embodimentexamples](#), the reference 3' anabaena group I intron fragment and reference 5' anabaena group I intron fragment were generated using the L6-5 permutation site. In some [embodimentexamples](#), the 3' anabaena group I intron fragment and 5' anabaena group I intron fragment were not generated using the L6-5 permutation site. In some [embodimentexamples](#), the 3' anabaena group I intron fragment comprises or consists of a sequence selected from SEQ ID NO: 112-123 and 125-150. In some [embodimentexamples](#), the 5' anabaena group I intron fragment comprises a corresponding sequence selected from SEQ ID NO: 73-84 and 86-111. In some [embodimentexamples](#), the 5' anabaena group I intron fragment comprises or consists of a sequence selected from SEQ ID NO: 73-84 and 86-111. In some [embodimentexamples](#), the 3' anabaena group I intron fragment comprises or consists of a corresponding sequence selected from SEQ ID NO: 112-124 and 125-150.

[0082] In some [embodimentexamples](#), the IRES comprises a nucleotide sequence selected from SEQ ID NOs: 348-351. In some [embodimentexamples](#), the reference IRES is CVB3. In some [embodimentexamples](#), the IRES is not CVB3. In some [embodimentexamples](#), the IRES comprises a sequence selected from SEQ ID NOs: 1-64 and 66-72.

[0083] In another [aspectexample](#), the present application discloses a circular RNA polynucleotide produced from the linear RNA disclosed herein.

[0084] In another [aspectexample](#), the present application discloses a circular RNA comprising, from 5' to 3', a 3' group I intron fragment, an IRES, an expression sequence, and a 5' group I intron fragment, wherein the IRES comprises a nucleotide sequence selected from SEQ ID NOs: 348-351.

[0085] In some [embodimentexamples](#), the circular RNA polynucleotide further comprises a spacer between the 3' group I intron fragment and the IRES.

[0086] In some [embodimentexamples](#), the circular RNA polynucleotide further comprises a first and a second duplex forming regions capable of forming a duplex. In some [embodimentexamples](#), the first and second duplex forming regions each have a length of about 9 to 19 nucleotides. In

some [embodimentexamples](#), the first and second duplex forming regions each have a length of about 30 nucleotides.

[0087] In an [aspectexample](#), provided herein is a vector for making a circular RNA polynucleotide, comprising, in the following order, an optional 5' duplex forming region, a 3' Group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a polynucleotide sequence encoding a cleavage site, a second expression sequence, a 5' Group I intron fragment, and an optional 3' duplex forming region. In an [aspectexample](#), provided herein is a vector for making a circular RNA polynucleotide, comprising, in the following order, an optional 5' duplex forming region, a 3' Group I intron fragment, a first Internal Ribosome Entry Site (IRES), a first expression sequence, a second IRES, a second expression sequence, a 5' Group I intron fragment, and an optional 3' duplex forming region. In some [embodimentexamples](#), a polynucleotide contains a 3' duplex forming region and a 5' duplex forming region. In certain [embodimentexamples](#), vector comprises a first spacer between the 5' duplex forming region and the 3' group I intron fragment, and a second spacer between the 5' group I intron fragment and the 3' duplex forming region. In certain [embodimentexamples](#), the first and second spacers each have a length of about 5 to about 60 nucleotides. In certain [embodimentexamples](#), the first and second spacers each have a length of about 8 to about 60 nucleotides. In certain [embodimentexamples](#), the first and second spacers each comprise an unstructured region at least 5 nucleotides long. In certain [embodimentexamples](#), the first and second spacers each comprise a structured region at least 7 nucleotides long. In certain [embodimentexamples](#), the first and second duplex forming regions each have a length of about 9 to 50 nucleotides. In certain [embodimentexamples](#), the vector is codon optimized. In certain [embodimentexamples](#), the vector is lacking at least one microRNA binding site present in an equivalent pre-optimization polynucleotide.

[0088] In an [aspectexample](#), provided herein is a prokaryotic cell comprising a vector for making a circular RNA polynucleotide, comprising, in the following order, an optional 5' duplex forming region, a 3' Group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a polynucleotide sequence encoding a cleavage site, a second expression sequence, a 5' Group I intron fragment, and an optional 3' duplex forming region. In some [embodimentexamples](#), a polynucleotide contains a 3' duplex forming region and a 5' duplex forming region. In an [aspectexample](#), provided herein is a prokaryotic cell comprising a vector for making a circular RNA polynucleotide, comprising, in the following order, an optional 5' duplex forming region, a 3' Group I intron fragment, a first Internal Ribosome Entry Site (IRES), a first expression sequence, a second IRES, a second expression sequence, a 5' Group I intron fragment, and an

optional 3' duplex forming region. In some [embodimentexamples](#), a polynucleotide contains a 3' duplex forming region and a 5' duplex forming region.

[0089] In an [aspectexample](#), provided herein is a eukaryotic cell comprising a circular RNA polynucleotide of the present disclosure. In some [embodimentexamples](#), the eukaryotic cell is a human cell. In some [embodimentexamples](#), the eukaryotic cell is an immune cell. In some [embodimentexamples](#), the eukaryotic cell is a T cell, NK cell, an NKT cell, a macrophage, or a neutrophil.

BRIEF DESCRIPTION OF THE DRAWINGS

[0090] **FIG. 1** depicts luminescence in supernatants of HEK293 (FIGs. 1A, 1D, and 1E), HepG2 (FIG. 1B), or 1C1C7 (FIG. 1C) cells 24 hours after transfection with circular RNA comprising a *Gaussia* luciferase expression sequence and various IRES sequences.

[0091] **FIG. 2** depicts luminescence in supernatants of HEK293 (FIG. 2A), HepG2 (FIG. 2B), or 1C1C7 (FIG. 2C) cells 24 hours after transfection with circular RNA comprising a *Gaussia* luciferase expression sequence and various IRES sequences having different lengths.

[0092] **FIG. 3** depicts stability of select IRES constructs in HepG2 (FIG. 3A) or 1C1C7 (FIG. 3B) cells over 3 days as measured by luminescence.

[0093] **FIG. 4A** and **FIG. 4B** depict protein expression from select IRES constructs in Jurkat cells, as measured by luminescence from secreted *Gaussia* luciferase in cell supernatants.

[0094] **FIG. 5A** and **FIG. 5B** depict stability of select IRES constructs in Jurkat cells over 3 days as measured by luminescence.

[0095] **FIG. 6** depicts comparisons of 24 hour luminescence (FIG. 6A) or relative luminescence over 3 days (FIG. 6B) of modified linear, unpurified circular, or purified circular RNA encoding *Gaussia* luciferase.

[0096] **FIG. 7** depicts transcript induction of IFN γ (FIG. 7A), IL-6 (FIG. 7B), IL-2 (FIG. 7C), RIG-I (FIG. 7D), IFN- β 1 (FIG. 7E), and TNF α (FIG. 7F) after electroporation of Jurkat cells with modified linear, unpurified circular, or purified circular RNA.

[0097] **FIG. 8** depicts a comparison of luminescence of circular RNA and modified linear RNA encoding *Gaussia* luciferase in human primary monocytes (FIG. 8A) and macrophages (FIG. 8B and FIG. 8C).

[0098] **FIG. 9** depicts relative luminescence over 3 days (FIG. 9A) in supernatant of primary T cells after transduction with circular RNA comprising a *Gaussia* luciferase expression sequence and varying IRES sequences or 24 hour luminescence (FIG. 9B).

[0099] **FIG. 10** depicts 24 hour luminescence in supernatant of primary T cells (FIG.

WHAT IS CLAIMED IS:

1. A circular RNA polynucleotide comprising, in the following order, a post splicing 3' group I intron fragment, an ~~first~~ Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, and a post splicing 5' group I intron fragment, ~~and optionally comprising a second IRES between the first expression sequence and the second expression sequence.~~
- ~~1.2. The circular RNA polynucleotide of claim 1, further comprising a cleavage site between the first expression sequence and the second expression sequence, optionally wherein the cleavage site is a self-cleaving spacer or a 2A self-cleaving peptide.~~
- ~~2. The circular RNA polynucleotide of claim 1, comprising a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence.~~
- ~~3. The circular RNA polynucleotide of claim 2, wherein the cleavage site is a self-cleaving spacer.~~
- ~~4. The circular RNA polynucleotide of claim 3, wherein the self-cleaving spacer is a 2A self-cleaving peptide.~~
- ~~5. The circular RNA polynucleotide of claim 1, comprising a second IRES between the first expression sequence and the second expression sequence.~~
- ~~6.3. The circular RNA polynucleotide of claim ~~5~~ 1 or 2, wherein the first IRES ~~and/or the second IRES~~ consists of or comprises a sequence according to any of SEQ ID NO: 1-72.~~
- ~~7. The circular RNA of claim 5 or 6, wherein the second IRES consists of or comprises a sequence according to any of SEQ ID NO: 1-72.~~
- 8.4. The circular RNA polynucleotide of ~~any preceding claim~~ any one of claims 1-3, wherein the first expression sequence encodes a first therapeutic protein, and the second expression sequence encodes a second therapeutic protein, optionally wherein the first expression sequence or the second expression sequence encodes an antibody, a chimeric antigen receptor, a transcription factor, a cytokine, an immune inhibitory molecule, an agonist of a costimulatory molecule, or an inhibitor of an immune checkpoint molecule.
- ~~9. The circular RNA polynucleotide of any preceding claim, wherein the first expression sequence or the second expression sequence encodes an antibody.~~

10. ~~The circular RNA polynucleotide of any preceding claim, wherein the first expression sequence or the second expression sequence encodes a chimeric antigen receptor.~~
11. ~~The circular RNA polynucleotide of any preceding claim, wherein the first expression sequence or the second expression sequence encodes a transcription factor.~~
12. ~~The circular RNA polynucleotide of any preceding claim, wherein the first expression sequence or the second expression sequence encodes a cytokine.~~
13. ~~The circular RNA polynucleotide of any preceding claim, wherein the first expression sequence or the second expression sequence encodes an immune inhibitory molecule.~~
14. ~~The circular RNA polynucleotide of any preceding claim, wherein the first expression sequence or the second expression sequence encodes an agonist of a costimulatory molecule.~~
15. ~~The circular RNA polynucleotide of any preceding claim, wherein the first expression sequence or the second expression sequence encodes an inhibitor of an immune checkpoint molecule.~~
- 16.5. The circular RNA polynucleotide of any of claims 1-84, wherein
- a. ~~the first expression sequence encodes the alpha chain of a T cell receptor (TCR) and the second expression sequence encodes the beta chain of a T-cell receptor (TCR);~~
 - b. ~~the first expression sequence encodes the beta chain of a TCR and the second expression sequence encodes the alpha chain of a TCR;~~
 - c. ~~the first expression sequence encodes the gamma chain of a TCR and the second expression sequence encodes the delta chain of a TCR;~~
 - d. ~~the first expression sequence encodes the delta chain of a TCR and the second expression sequence encodes the gamma chain of a TCR;~~
 - e. ~~the first expression sequence encodes a TCR and the second expression sequence encodes a cytokine;~~
 - f. ~~the first expression sequence encodes a cytokine and the second expression sequence encodes a TCR;~~
 - g. ~~the first expression sequence encodes a TCR and the second expression sequence encodes a chemokine;~~
 - h. ~~the first expression sequence encodes for a chemokine and the second expression sequence encodes for a TCR;~~

- i. the first expression sequence encodes for a TCR and the second expression sequence encodes for a transcription factor;
- j. the first expression sequence encodes for a transcription factor and the second expression sequence encodes for a TCR;
- k. the first expression sequence encodes a chimeric antigen receptor (CAR) and the second expression sequence encodes a PD1 or PDL1 antagonist;
- l. the first expression sequence encodes a PD1 or PDL1 antagonist and the second expression sequence encodes a CAR;
- m. the first expression sequence encodes a CAR and the second expression sequence encodes a cytokine;
- n. the first expression sequence encodes a cytokine and the second expression sequence encodes a CAR;
- o. the first expression sequence encodes for a CAR, and the second expression sequence encodes a chemokine;
- p. the first expression sequence encodes for a chemokine, and the second expression sequence encodes for a CAR;
- q. the first expression sequence encodes a transcription factor and the second expression sequence encodes a cytokine;
- r. the first expression sequence encodes a cytokine and the second expression sequence encodes a transcription factor;
- s. the first expression sequence encodes a transcription factor and the second expression sequence encodes a chemokine; or
- ~~a-t.~~ the first expression sequence encodes a chemokine and the second expression sequence encodes a transcription factor.

6. The circular RNA polypeptide of claim 5, wherein:

- a. the cytokine is selected from IL-2, IL-7, IL-12, IL-15, IL-10, IL-12, and TGFβ;
- b. the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25; and/or
- ~~a-c.~~ the chemokine is selected from a CC chemokine, CXC chemokine, C chemokine, CX3C chemokine, CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18,

CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL 1, CXCL2, CXCL3, CXCL4, CXCL5, CXLC6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, and CX3CL1.

17. ~~The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes the beta chain of a T cell receptor (TCR) and the second expression sequence encodes the alpha chain of a T cell receptor (TCR).~~
18. ~~The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes the gamma chain of a T cell receptor (TCR) and the second expression sequence encodes the delta chain of a T cell receptor (TCR).~~
19. ~~The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes the delta chain of a T cell receptor (TCR) and the second expression sequence encodes the gamma chain of a T cell receptor (TCR).~~
20. ~~The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a T cell receptor (TCR) and the second expression sequence encodes a cytokine.~~
21. ~~The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a cytokine and the second expression sequence encodes a T cell receptor (TCR).~~
22. ~~The circular RNA polynucleotide of any of claims 20-21, wherein the cytokine is selected from IL-2, IL-7, IL-12, and IL-15.~~
23. ~~The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a T cell receptor (TCR) and the second expression sequence encodes a chemokine.~~
24. ~~The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for a chemokine and the second expression sequence encodes for a T cell receptor (TCR).~~
25. ~~The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for a T cell receptor (TCR) and the second expression sequence encodes for a transcription factor.~~

- ~~26. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for a transcription factor and the second expression sequence encodes for a T cell receptor (TCR).~~
- ~~27. The circular RNA polynucleotide of any of claims 25-26, wherein the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25.~~
- ~~28. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a chimeric antigen receptor (CAR) and the second expression sequence encodes a PD1 or PDL1 antagonist.~~
- ~~29. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a PD1 or PDL1 antagonist and the second expression sequence encodes a chimeric antigen receptor (CAR).~~
- ~~30. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a chimeric antigen receptor (CAR) and the second expression sequence encodes a cytokine.~~
- ~~31. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a cytokine and the second expression sequence encodes a chimeric antigen receptor (CAR).~~
- ~~32. The circular RNA polynucleotide of any of claims 30-31, wherein the cytokine is selected from IL-2, IL-7, IL-12, and IL-15.~~
- ~~33. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for a chimeric antigen receptor (CAR), and the second expression sequence encodes a chemokine.~~
- ~~34. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for a chemokine, and the second expression sequence encodes for a chimeric antigen receptor (CAR).~~
- ~~35. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a transcription factor and the second expression sequence encodes a cytokine.~~
- ~~36. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a cytokine and the second expression sequence encodes a transcription factor.~~

- ~~37. The circular RNA polynucleotide of any of claims 35-36, wherein the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25.~~
- ~~38. The circular RNA polynucleotide of any of claims 35-37, wherein the cytokine is selected from IL-10, IL-12, and TGFβ.~~
- ~~39. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a transcription factor and the second expression sequence encodes a chemokine.~~
- ~~40. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a chemokine and the second expression sequence encodes a transcription factor.~~
- ~~41. The circular RNA of any of claims 39-40, wherein the transcription factor is selected from FOXP3, STAT5B, and HELIOS.~~
- ~~42. The circular RNA polynucleotide of any of claims 39-41, wherein the chemokine is a CC chemokine, CXC chemokine, C chemokine, or a CX3C chemokine.~~
- ~~43. The circular RNA polynucleotide of any of claims 39-42, wherein the chemokine is selected from CCL1, CCL2, CCL3, CCL4, CCL5, CCL6, CCL7, CCL8, CCL9/CCL10, CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, and CX3CL1.~~
447. The circular RNA polynucleotide of any of claims 1-84, wherein
- a. the first expression sequence encodes a tumor antigen and the second expression sequence encodes a cytokine;
 - b. the first expression sequence encodes a cytokine and the second expression sequence encodes a tumor antigen;
 - c. the first expression sequence encodes a CAR and the second expression sequence encodes a CAR;
 - d. the first expression sequence encodes a cytokine and the second expression sequence encodes a cytokine;
 - e. the first expression sequence encodes a TCR and the second expression sequence encodes a TCR;

- f. the first expression sequence encodes for a chemokine and the second expression sequence encodes for a chemokine;
- g. the first or second expression sequence encodes for an immunosuppressive enzyme;
- h. the first expression sequence encodes a rate limiting enzyme and the second expression sequence encodes a flux-limiting enzyme;
- i. the first expression sequence encodes a flux-limiting enzyme and the second expression sequence encodes a rate limiting enzyme;
- j. the first expression sequence encodes a transcription factor and the second expression sequence encodes a survival factor;
- k. the first expression sequence encodes a survival factor and the second expression sequence encodes a transcription factor;
- l. the first or second expression sequence encodes for a chaperone protein or complex;
- m. the first expression sequence encodes a transcription factor and the second expression sequence encodes a chaperone protein or complex;
- n. the first expression sequence encodes a chaperone protein or complex and the second expression sequence encodes for a transcription factor;
- o. the first and/or second expression sequences encode a signaling protein;
- p. the first expression sequence encodes for an enzyme and the second expression encodes for the first expression sequence's negative regulatory inhibitor;
- q. the first expression sequence encodes for a negative regulatory inhibitor protein of an enzyme encoded in the second expression sequence;
- r. the first expression sequence encodes a dominant negative protein and the second expression sequence encodes an immune protein;
- s. the first expression sequence encodes an immune protein and the second expression sequence encodes a dominant negative protein;
- t. the first or second expression sequence encodes for an anti-inflammatory protein;
- u. the first expression sequence encodes a transcription factor and the second expression sequence is capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU); or
- a-v. the first expression sequence is capable of converting 5-FC into 5-FU and the second expression sequence is a transcription factor.

8. The circular RNA polypeptide of claim 7, wherein:
 - a. the antigen is a neoantigen;
 - b. the cytokine is IFN γ , IL-2, IL-7, IL-15, IL-18, IL-10, TGF β , or IL-35;
 - c. the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, ROR γ t, and cd25;
 - d. the survival factor is selected from BCL-XL;
 - e. the chaperone protein or complex is selected from Skp, Spy, FkpA, SurA, Hsp60, Hsp70, GroEL, GroES, Hsp90, HtpG, Hsp100, ClpA, ClpX, ClpP, and Hsp104;
 - f. the negative regulatory inhibitor is selected from a p57kip2, BAX inhibitor, and TIPE2; and/or
 - a.g. the expression sequence capable of converting 5-FC into 5-FU is cytosine deaminase.
- ~~45. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a cytokine and the second expression sequence encodes a tumor antigen.~~
- ~~46. The circular RNA polynucleotide of any of claims 44-45, wherein the antigen is a neoantigen.~~
- ~~47. The circular RNA polynucleotide of any of claims 44-46, wherein the cytokine is IFN γ .~~
- ~~48. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a CAR and the second expression sequence encodes a CAR.~~
- ~~49. The circular RNA polynucleotide of any of claims 1-8, wherein the first expression sequence encodes a cytokine and the second expression sequence encodes a cytokine.~~
- ~~50. The circular RNA polynucleotide of claim 49, wherein the first or second expression sequence encodes a cytokine selected from IL-10, TGF β , and IL-35.~~
- ~~51. The circular RNA polynucleotide of any of claims 49-50, wherein the first or second expression sequence encodes a cytokine selected from IFN γ , IL-2, IL-7, IL-15, and IL-18.~~
- ~~52. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a T cell receptor (TCR) and the second expression sequence encodes a T cell receptor (TCR).~~
- ~~53. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for a chemokine and the second expression sequence encodes for a chemokine.~~

- ~~54. The circular RNA polynucleotide of any one of claims 1-8, wherein the first or second expression sequence encodes for an immunosuppressive enzyme.~~
- ~~55. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a rate limiting enzyme and the second expression sequence encodes a flux limiting enzyme.~~
- ~~56. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a flux limiting enzyme and the second expression sequence encodes a rate limiting enzyme.~~
- ~~57. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a transcription factor and the second expression sequence encodes a survival factor.~~
- ~~58. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a survival factor and the second expression sequence encodes a transcription factor.~~
- ~~59. The circular RNA polynucleotide of any one of claims 57-58, wherein the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25.~~
- ~~60. The circular RNA polynucleotide of any one of claims 57-59, wherein the survival factor is selected from BCL-XL.~~
- ~~61. The circular RNA polynucleotide of any one of claims 1-8, wherein the first or second expression sequence encodes for a chaperone protein or complex.~~
- ~~62. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a transcription factor and the second expression sequence encodes a chaperone protein or complex.~~
- ~~63. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a chaperone protein or complex and the second expression sequence encodes for a transcription factor.~~
- ~~64. The circular RNA polynucleotide of any one of claims 61-63, wherein the chaperone protein or complex is selected from Skp, Spy, FkpA, SurA, Hsp60, Hsp70, GroEL, GroES, Hsp90, HtpG, Hsp100, ClpA, ClpX, ClpP, and Hsp104.~~
- ~~65. The circular RNA polynucleotide of any one of claims 61-64, wherein the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, and cd25.~~

- ~~66. The circular RNA polynucleotide of any one of claims 1-8, wherein one or both expression sequences encode a signaling protein.~~
- ~~67. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for an enzyme and the second expression encodes for the first expression sequence's negative regulatory inhibitor.~~
- ~~68. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes for a negative regulatory inhibitor protein of an enzyme encoded in the second expression sequence.~~
- ~~69. The circular RNA polynucleotide of any one of claims 67-68, wherein the negative regulatory inhibitor is selected from a p57kip2, BAX inhibitor, and TIPE2.~~
- ~~70. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a dominant negative protein and the second expression sequence encodes an immune protein.~~
- ~~71. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes an immune protein and the second expression sequence encodes a dominant negative protein.~~
- ~~72. The circular RNA polynucleotide of any one of claims 1-8, wherein the first or second expression sequence encodes for an anti-inflammatory protein.~~
- ~~73. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence encodes a transcription factor and the second expression sequence is capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU).~~
- ~~74. The circular RNA polynucleotide of any one of claims 1-8, wherein the first expression sequence is capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU) and the second expression sequence is a transcription factor.~~
- ~~75. The circular RNA polynucleotide of any one of claims 73-74, wherein the expression sequence capable of converting 5-fluorocytosine (5-FC) into 5-fluorouracil (5-FU) is cytosine deaminase.~~
769. The circular RNA polynucleotide of any one of the preceding claims, comprising a first spacer between the 5' duplex forming region and the post splicing 3' group I intron fragment, and a second spacer between the post splicing 5' group I intron fragment and the 3' duplex forming region.

- ~~77~~10. The circular RNA polynucleotide of claim ~~76~~9, wherein the first and second spacers each ~~have~~has a length of about 10 to about 60 nucleotides or about 9 to about 19 nucleotides.
- ~~78.~~ ~~The circular RNA polynucleotide of any one of the preceding claims, wherein the first and second duplex forming regions each have a length of about 9 to about 19 nucleotides.~~
- ~~79~~11. The circular RNA polynucleotide of ~~any one of claims 77-78~~claim 9 or 10, wherein the first and second duplex forming regions each have a length of about 30 nucleotides.
- ~~80~~12. The circular RNA polynucleotide of any one of the preceding claims, wherein the IRES has a sequence of an IRES from Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, Simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, Human poliovirus 1, Plautia stali intestine virus, Kashmir bee virus, Human rhinovirus 2, Homalodisca coagulata virus- 1, Human Immunodeficiency Virus type 1, Homalodisca coagulata virus- 1, Himetobi P virus, Hepatitis C virus, Hepatitis A virus, Hepatitis GB virus , Foot and mouth disease virus, Human enterovirus 71, Equine rhinitis virus, Ectropis obliqua picorna-like virus, Encephalomyocarditis virus, Drosophila C Virus, Human coxsackievirus B3, Crucifer tobamovirus, Cricket paralysis virus, Bovine viral diarrhea virus 1, Black Queen Cell Virus, Aphid lethal paralysis virus, Avian encephalomyelitis virus, Acute bee paralysis virus, Hibiscus chlorotic ringspot virus, Classical swine fever virus, Human FGF2, Human SFTPA1, Human AML1/RUNX1, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAP1, Human c-myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, S. cerevisiae TFIID, S. cerevisiae YAP1, tobacco etch virus, turnip crinkle virus, EMCV-A, EMCV-B, EMCV-Bf, EMCV-Cf, EMCV pEC9, Picobirnavirus, HCV QC64, Human Cosavirus E/D, Human Cosavirus F, Human Cosavirus JMY, Rhinovirus NAT001, HRV14, HRV89, HRVC-02, HRV-A21, Salivirus A SH1, Salivirus FHB, Salivirus NG-J1, Human Parechovirus 1, Crohivirus B, Yc-3, Rosavirus M-7, Shanbavirus A, Pasivirus A, Pasivirus A 2, Echovirus E14, Human Parechovirus 5, Aichi Virus, Hepatitis A Virus HA16, Phopivirus, CVA10, Enterovirus C, Enterovirus D, Enterovirus J, Human Pegivirus 2, GBV-C GT110, GBV-C K1737, GBV-C

Iowa, Pegivirus A 1220, Pasivirus A 3, Sapelovirus, Rosavirus B, Bakunsa Virus, Tremovirus A, Swine Pasivirus 1, PLV-CHN, Pasivirus A, Sicinivirus, Hepacivirus K, Hepacivirus A, BVDV1, Border Disease Virus, BVDV2, CSFV-PK15C, SF573 Dicistrovirus, Hubei Picorna-like Virus, CRPV, Salivirus A BN5, Salivirus A BN2, Salivirus A 02394, Salivirus A GUT, Salivirus A CH, Salivirus A SZ1, Salivirus FHB, CVB3, CVB1, Echovirus 7, CVB5, EVA71, CVA3, CVA12, EV24 or an aptamer to eIF4G.

~~8413.~~ The circular RNA polynucleotide of any one of the preceding claims, consisting of natural nucleotides.

~~1482.~~ The circular RNA polynucleotide of any one of the preceding claims, wherein the expression sequence is codon optimized.

~~8315.~~ The circular RNA polynucleotide of any one of the preceding claims, optimized to lack:

~~a.~~ _at least one microRNA binding site present in an equivalent pre-optimized polynucleotide;

~~b.~~ at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide; and/or

~~a-c.~~ at least one RNA-editing susceptible site present in an equivalent pre-optimized polynucleotide.

~~84.~~ ~~The circular RNA polynucleotide of any one of the preceding claims, optimized to lack at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide.~~

~~85.~~ ~~The circular RNA polynucleotide of any one of the preceding claims, optimized to lack at least one RNA editing susceptible site present in an equivalent pre-optimized polynucleotide.~~

~~8616.~~ The circular RNA polynucleotide of any one of the preceding claims, wherein the circular RNA polynucleotide is from about 100 nucleotides to about 15 kilobases in length.

~~1787.~~ The circular RNA polynucleotide of any one of the preceding claims, having an *in vivo* duration of therapeutic effect in humans of at least about 20 hours and/or a functional half-life of at least about 20 hours.

~~88.~~ ~~The circular RNA polynucleotide of any one of the preceding claims, having a functional half life of at least about 20 hours.~~

- ~~89~~18. The circular RNA polynucleotide of any one of the preceding claims, having:
- a. a duration of therapeutic effect in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequence;
 - b. a functional half-life in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequence;
 - c. an *in vivo* duration of therapeutic effect in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence; and/or
 - a.d. an *in vivo* functional half-life in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.
- ~~90.~~ The circular RNA polynucleotide of any one of the preceding claims, having a functional half life in a human cell greater than or equal to that of an equivalent linear RNA polynucleotide comprising the same expression sequence.
- ~~91.~~ The circular RNA polynucleotide of any one of the preceding claims, having an *in vivo* duration of therapeutic effect in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.
- ~~92.~~ The circular RNA polynucleotide of any one of the preceding claims, having an *in vivo* functional half life in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.
- 93~~19~~. A pharmaceutical composition comprising a circular RNA polynucleotide of any one of the preceding claims, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle.
- 94~~20~~. The pharmaceutical composition of claim 93~~19~~, wherein the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, a biodegradable nanoparticle, a biodegradable lipid nanoparticle, a polymer nanoparticle, or a biodegradable polymer nanoparticle.
- 95~~21~~. The pharmaceutical composition of claim 93~~or 94~~19 or 20, comprising a targeting moiety, wherein the targeting moiety mediates receptor-mediated endocytosis or direct fusion into selected cells of a selected cell population or tissue in the absence of cell isolation or purification, optionally wherein the targeting moiety is operably connected to the nanoparticle.
- ~~96.~~ The pharmaceutical composition of any one of claims ~~93-95~~, comprising a targeting moiety operably connected to the nanoparticle.

9722. The pharmaceutical composition of any one of claims ~~93-96~~19-21, wherein the targeting moiety is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof.

9823. The pharmaceutical composition of any one of claims ~~93-97~~19-22, wherein

- a. less than 1%, by weight, of the polynucleotides in the pharmaceutical composition are double stranded RNA, DNA splints, or triphosphorylated RNA; and/or
- a-b. less than 1%, by weight, of the polynucleotides and proteins in the pharmaceutical composition are double stranded RNA, DNA splints, triphosphorylated RNA, phosphatase proteins, protein ligases, and capping enzymes.

~~99.—The pharmaceutical composition of any one of claims 93-98, wherein less than 1%, by weight, of the polynucleotides and proteins in the pharmaceutical composition are double stranded RNA, DNA splints, triphosphorylated RNA, phosphatase proteins, protein ligases, and capping enzymes.~~

24. The pharmaceutical composition of any one of claims 19-23, wherein the nanoparticle comprises:

- a. one or more cationic lipids, ionizable lipids, or poly β -amino esters;
- b. one or more non-cationic lipids;
- c. one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids;
- d. cholesterol; and/or
- e. arachidonic acid or oleic acid.

25. The pharmaceutical composition of any one of claims 19-24, wherein the nanoparticle comprises more than one circular RNA polynucleotide.

~~100.—A method of treating a subject in need thereof comprising administering a therapeutically effective amount of a composition comprising the circular RNA polynucleotide of any one of claims 1-92, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle.~~

26. Use of the circular RNA polynucleotide of any one claims 1-18 or the pharmaceutical composition of any one of claims 19-25 in the manufacture of a medicament for treating cancer and/or an autoimmune disorder.

- ~~101. The method of claim 100, wherein the targeting moiety is an scFv, nanobody, peptide, minibody, heavy chain variable region, light chain variable region or fragment thereof.~~
- ~~102. The method of any one of claims 100-101, wherein the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, or a biodegradable nanoparticle.~~
- ~~103. The method of any one of claims 100-102, wherein the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β -amino esters.~~
- ~~104. The method of any one of claims 100-103, wherein the nanoparticle comprises one or more non-cationic lipids.~~
- ~~105. The method of any one of claims 100-104, wherein the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids.~~
- ~~106. The method of any one of claims 100-105, wherein the nanoparticle comprises cholesterol.~~
- ~~107. The method of any one of claims 100-106, wherein the nanoparticle comprises arachidonic acid or oleic acid.~~
- ~~108. The method of any one of claims 100-107, wherein the composition comprises a targeting moiety, wherein the targeting moiety mediates receptor-mediated endocytosis selectively into cells of a selected cell population in the absence of cell selection or purification.~~
- ~~109. The method of any one of claims 100-108, wherein the nanoparticle comprises more than one circular RNA polynucleotide.~~
- ~~110-27. The use of method of any one of claims 100-109~~claim 26, wherein the cancer and/or autoimmune disorder is:subject has

- a. a cancer selected from the group consisting of acute lymphocytic leukemia; acute myeloid leukemia (AML); alveolar rhabdomyosarcoma; B cell malignancies; bladder cancer (*e.g.*, bladder carcinoma); bone cancer; brain cancer (*e.g.*, medulloblastoma); breast cancer; cancer of the anus, anal canal, or anorectum; cancer of the eye; cancer of the intrahepatic bile duct; cancer of the joints; cancer of the neck; gallbladder cancer; cancer of the pleura; cancer of the nose, nasal cavity, or middle ear; cancer of the oral cavity; cancer of the vulva; chronic lymphocytic leukemia; chronic myeloid cancer; colon cancer; esophageal cancer, cervical cancer; fibrosarcoma; gastrointestinal carcinoid tumor; head and neck cancer (*e.g.*, head and neck squamous cell carcinoma); Hodgkin lymphoma; hypopharynx cancer; kidney cancer; larynx cancer; leukemia; liquid tumors; liver

cancer; lung cancer (*e.g.*, non-small cell lung carcinoma and lung adenocarcinoma); lymphoma; mesothelioma; mastocytoma; melanoma; multiple myeloma; nasopharynx cancer; non-Hodgkin lymphoma; B-chronic lymphocytic leukemia; hairy cell leukemia; acute lymphocytic leukemia (ALL); Burkitt's lymphoma; ovarian cancer; pancreatic cancer; cancer of the peritoneum; cancer of the omentum; mesentery cancer; pharynx cancer; prostate cancer; rectal cancer; renal cancer; skin cancer; small intestine cancer; soft tissue cancer; solid tumors; synovial sarcoma; gastric cancer; testicular cancer; thyroid cancer; and ureter cancer; and/or

a.b. an autoimmune disorder selected from scleroderma, Grave's disease, Crohn's disease, Sjorgen's disease, multiple sclerosis, Hashimoto's disease, psoriasis, myasthenia gravis, autoimmune polyendocrinopathy syndromes, Type I diabetes mellitus (T1DM), autoimmune gastritis, autoimmune uveoretinitis, polymyositis, colitis, thyroiditis, and the generalized autoimmune diseases typified by human Lupus.

~~111. The method of any one of claims 100-109, wherein the subject has an autoimmune disorder selected from scleroderma, Grave's disease, Crohn's disease, Sjorgen's disease, multiple sclerosis, Hashimoto's disease, psoriasis, myasthenia gravis, autoimmune polyendocrinopathy syndromes, Type I diabetes mellitus (T1DM), autoimmune gastritis, autoimmune uveoretinitis, polymyositis, colitis, thyroiditis, and the generalized autoimmune diseases typified by human Lupus.~~

~~112~~28. A vector for making a circular RNA polynucleotide, comprising, in the following order, a 5' duplex forming region, a 3' Group I intron fragment, an Internal Ribosome Entry Site (IRES), a first expression sequence, a second expression sequence, a 5' Group I intron fragment, and a 3' duplex forming region, optionally comprising:

a. a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence; and/or

a.b. a first spacer between the 5' duplex forming region and the 3' group I intron fragment, and a second spacer between the 5' group I intron fragment and the 3' duplex forming region.

- ~~113. The vector of claim 112, comprising a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence.~~
- ~~114. The vector of claim 112 or 113, wherein the cleavage site is a self-cleaving spacer.~~
- ~~115. The vector of any one of claims 112-114, wherein the self-cleaving spacer is a 2A self-cleaving peptide.~~
- ~~116. The vector of any one of claims 112-115, comprising a first spacer between the 5' duplex forming region and the 3' group I intron fragment, and a second spacer between the 5' group I intron fragment and the 3' duplex forming region.~~
- ~~117. The vector of any one of claims 112-116, wherein the first and second spacers each have a length of about 5 to about 60 nucleotides.~~
- ~~118. The vector of any one of claims 112-117, wherein the first and second spacers each comprise an unstructured region at least 5 nucleotides long.~~
- ~~119. The vector of any one of claims 112-118, wherein the first and second spacers each comprise a structured region at least 7 nucleotides long.~~
- ~~120. The vector of any one of claims 112-119, wherein the first and second duplex forming regions each have a length of about 9 to 50 nucleotides.~~
- ~~121. The vector of any one of claims 112-120 wherein the vector is codon optimized.~~
- ~~122. The vector of any one of claims 112-121, lacking at least one microRNA binding site present in an equivalent pre-optimization polynucleotide.~~
12329. A eukaryotic cell comprising a circular RNA polynucleotide according to any of claims ~~1-92~~18, optionally wherein the cell is a human cell, an immune cell, and/or a T cell.
- ~~124. The eukaryotic cell of claim 123, wherein the eukaryotic cell is a human cell.~~
- ~~125. The eukaryotic cell of claim 124, wherein the eukaryotic cell is an immune cell.~~
- ~~126. The eukaryotic cell of claim 125, wherein the eukaryotic cell is a T cell.~~