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 examples, 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 examples, the inventive circular RNA comprises duplex forming regions. In some examples, the expression sequences are separated by one or more polynucleotide sequences encoding a cleavage site. In some examples, a cleavage site is a self-cleaving peptide. In some examples, the self-cleaving peptide is a 2A self-cleaving peptide. In some examples, the first and second expression sequences are separated by ribosomal skipping element. In some examples, each expression sequence encodes a therapeutic protein. In some examples, the first expression sequence encodes a cytokine or a functional fragment thereof. In some examples, the first expression sequence encodes a transcription factor. In some examples, the first expression encodes an immune checkpoint inhibitor. In some examples, the first expression sequence encodes for a chimeric antigen receptor. In some examples, the first expression sequence encodes a first T-cell receptor (TCR) chain, and the second expression encodes a second TCR chain. In some examples, circular RNA of the disclosure has improved expression, functional

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

[0007] In some examples, 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 examples, the microRNA binding site is recognized by miR-122.

[0008] One example 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 examples, the circular RNA polynucleotide comprises a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence. In some examples, the cleavage site is a self-cleaving spacer. In some examples, the self-cleaving spacer is a 2A self-cleaving peptide.

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

[0011] In some examples, the first expression sequence encodes a first therapeutic protein, and the second expression sequence encodes a second therapeutic protein. In some examples, the first expression sequence or the second expression sequence encodes an antibody. In some examples, the first expression sequence or the second expression sequence encodes a chimeric antigen receptor. In some examples, the first expression sequence or the second expression sequence encodes a transcription factor. In some examples, wherein the first expression sequence or the second expression sequence encodes a cytokine. In some examples, wherein the first expression sequence or the second expression sequence encodes an immune inhibitory molecule. In some examples, the first expression sequence or the second expression sequence encodes an agonist of a costimulatory molecule. In some examples, wherein the first expression sequence or the second expression sequence encodes an inhibitor of an immune checkpoint molecule. In some examples, 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 examples, 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 examples, 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 examples, 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 examples, the first expression sequence encodes a T cell receptor (TCR) and the second expression sequence encodes a chemokine. In some examples, the first expression sequence encodes for a chemokine and the second expression sequence encodes for a T cell receptor (TCR). In some examples, the first expression sequence encodes a chimeric antigen receptor (CAR) and the second expression sequence encodes a PD1 or PDL1 antagonist. In some examples, the first expression sequence encodes a PD1 or PDL1 antagonist and the second expression sequence encodes a chimeric antigen receptor (CAR). In some examples, the first expression sequence encodes for a chimeric antigen receptor (CAR), and the second expression sequence encodes a chemokine. In some examples, the first expression sequence encodes for a chemokine, and the second expression sequence encodes for a chimeric antigen receptor (CAR). In some examples, the first expression sequence encodes a transcription factor and the second expression sequence encodes a cytokine.

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

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

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

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

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

[0017] In some examples, the first expression sequence encodes a transcription factor and the second expression sequence encodes a chemokine. In some examples, the first expression sequence encodes a chemokine and the second expression sequence encodes a transcription factor. In some examples, the transcription factor is selected from FOXP3, STAT5B, and HELIOS. In some examples, the chemokine is a CC chemokine, CXC chemokine, C chemokine, or a CX3C chemokine. In some examples, 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, CXLC6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, and CX3CL1.

[0018] In some examples, the first expression sequence encodes a tumor antigen and the second expression sequence encodes a cytokine. In some examples, the first expression sequence encodes a cytokine and the second expression sequence encodes a tumor antigen. In some examples, the antigen is a neoantigen. In some examples, the cytokine is IFNγ.

[0019] In some examples, the first expression sequence encodes a CAR and the second expression sequence encodes a CAR.

[0020] In some examples, the first expression sequence encodes a cytokine and the second expression sequence encodes a cytokine. In some examples, the first or second expression sequence encodes a cytokine selected from IL-10, TGFβ, and IL-35. In some examples, the first or second expression sequence encodes a cytokine selected from IFNγ, IL-2, IL-7, IL-15, and IL-18.

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

expression sequence encodes for an immunosuppressive enzyme. In some examples, the first expression sequence encodes a rate limiting enzyme and the second expression sequence encodes a flux-limiting enzyme. In some examples, the first expression sequence encodes a flux-limiting enzyme and the second expression sequence encodes a rate limiting enzyme.

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

[0023] In some examples, the first or second expression sequence encodes for a chaperone protein or complex. In some examples, the first expression sequence encodes a transcription factor and the second expression sequence encodes a chaperone protein or complex. In some examples, the first expression sequence encodes a chaperone protein or complex and the second expression sequence encodes for a transcription factor. In some examples, 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 examples, the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet,GATA3, RORgt, and cd25.

[0024] In some examples, one or both expression sequences encode a signaling protein.

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

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

[0027] In some examples, the first expression sequence encodes a transcription factor

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

[0028] In some 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 examples, the first and second spacers each have a length of about 10 to about 60 nucleotides. In some examples, the first and second duplex forming regions each have a length of about 9 to about 19 nucleotides. In some examples, the first and second duplex forming regions each have a length of about 30 nucleotides. In some 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-IAPl, 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 examples, the circular RNA polynucleotide comprises natural nucleotides. In some examples, the circular RNA polynucleotide consists of natural nucleotides. In some examples, the expression sequence is codon optimized.

[0030] In some examples, the circular RNA polynucleotide is optimized to lack at least one microRNA binding site present in an equivalent pre-optimized polynucleotide. In some examples, the circular RNA polynucleotide is optimized to lack at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide. In some examples, 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 examples, 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 examples, the circular RNA polynucleotide of has an *in vivo* duration of therapeutic effect in humans of at least about 20 hours. In some examples, the circular RNA polynucleotide of has a functional half-life of at least about 20 hours. In some examples, 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 examples, 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 examples, 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 examples, 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 example, 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 examples, 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 examples, 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 examples, the pharmaceutical composition comprises a targeting moiety operably connected to the nanoparticle. In some examples, 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 examples, 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 examples, 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 example, 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 examples, 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 examples, the targeting moiety is an scFv, nanobody, peptide, minibody, heavy chain variable region, light chain variable region or fragment thereof. In some examples, the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, or a biodegradable nanoparticle.

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

nanoparticle comprises arachidonic acid or oleic acid. In some examples, the nanoparticle comprises more than one circular RNA polynucleotide.

[0039] In some examples, 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 examples, 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 (TIDM), autoimmune gastritis, autoimmune uveoretinitis, polymyositis, colitis, thyroiditis, and the generalized autoimmune diseases typified by human Lupus.

[0040] In another example, 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 examples, the vector of comprises a polynucleotide sequence encoding a cleavage site between the first expression sequence and the second expression sequence. In some examples, the cleavage site is a self-cleaving spacer. In some examples, the self-cleaving spacer is a 2A self-cleaving peptide.

[0042] In some examples, 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 examples, the first and second spacers each have a length of about 5 to about 60 nucleotides. In some examples, the first and second spacers each comprise an unstructured region at least 5 nucleotides long. In some examples, the first and second spacers each comprise a structured region at least 7 nucleotides long. In some examples, the first and second duplex forming regions each have a length of about 9 to 50 nucleotides.

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

[0044] In another example, the present application provides a eukaryotic cell comprising a circular RNA polynucleotide as described herein. In some examples, the eukaryotic cell is a human cell. In some examples, the eukaryotic cell is an immune cell. In some examples, the eukaryotic cell is a T cell.

[0045] In an example, 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 example, 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 examples, the first expression sequence and the second expression sequence encode different therapeutic proteins. In some examples, the first expression sequence and the second expression sequence encodes for the same therapeutic protein.

[0046] In an example, 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 example, 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 examples, a circular RNA polynucleotide or vector provided herein comprises 3' and 5' duplex forming regions.

[0047] In some examples, 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 examples, the first and second spacers each have a length of about 10 to about 60 nucleotides. In certain examples, the first and second duplex forming regions each have a length of about 9 to about 19 nucleotides. In certain other examples, the first and second duplex forming regions each have a length of about 30 nucleotides.

[0048] In certain examples, the IRES is selected from Table 17 has a sequence of an IRES, or is a functional fragment or variant thereof. In some 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-IAPl, 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 examples, the first and second polyA sequences each have a length of 15-50nt. In some examples, the first and second polyA sequences each have a length of about 20-25nt.

[0050] In certain examples, the circular RNA polynucleotide consists of naturally occurring nucleotides. In some examples, 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 examples, the expression sequence is codon-optimized. In certain examples, the circular RNA polynucleotide is optimized to lack at least one microRNA binding site present in an equivalent pre-optimized polynucleotide. In certain examples, the circular RNA polynucleotide is optimized to lack at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide. In certain examples, 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 examples, the circular RNA polynucleotide is from about 100 nucleotides to about 15 kilobases in length. In certain examples, 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 examples, the circular RNA polynucleotide has a functional half-life of at least about 20 hours. In certain examples, 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 examples, 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 examples, 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 examples, 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 examples, 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 examples, the pharmaceutical composition has a functional half-life *in vivo* in humans greater than that of a pre-determined threshold value. In some examples, the functional protein assay is an *in vitro* luciferase assay. In some examples, 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 examples, 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 examples, the pharmaceutical composition has a functional half-life of at least about 20 hours.

[0053] In an example, 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 examples, 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 examples, 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 examples, 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 examples, the targeting moiety is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof. In certain examples, 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 examples, 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 examples, less than 1%, by weight, of the polynucleotides in the composition are double stranded RNA, DNA splints, or triphosphorylated RNA. In certain examples, 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 example, 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 examples, the subject has an autoimmune disorder or cancer.

[0057] In some examples, the targeting moiety is an scFv, nanobody, peptide, minibody, heavy chain variable region, light chain variable region or fragment thereof. In some examples, 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 examples, the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, or a biodegradable nanoparticle. In some examples, the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β-amino esters. In some examples, the nanoparticle comprises one or more non-cationic lipids. In some examples, the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, structural lipid or hyaluronic acid lipids. In certain examples, the nanoparticle comprises cholesterol. In some examples, the structural lipid is a beta-sitosterol. In some examples, the structural lipid is not a beta sitosterol. In some examples, the nanoparticle comprises arachidonic acid or oleic acid. In some examples, the nanoparticle comprises more than one circular RNA polynucleotide.

[0059] In some examples, 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 examples, the PEG-modified lipid is DSPE-PEG, DMG-PEG, or PEG-1. In some examples, the PEG-modified lipid is DSPE-PEG(2000).

[0061] In some examples, the pharmaceutic composition further comprises a helper lipid. In some examples, the helper lipid is DSPC or DOPE.

[0062] In some examples, the pharmaceutic composition comprises DOPE, cholesterol, and DSPE-PEG.

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

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

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

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

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

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

[0068] In some examples, the transfer vehicle is formulated for endosomal release of the circular RNA polynucleotide.

[0069] In some examples, the transfer vehicle is capable of binding to APOE. In some examples, 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 examples, the exterior surface of the transfer vehicle is substantially free of APOE binding sites.

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

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

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

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

[0074] In some examples, the pharmaceutical composition is substantially free of linear RNA.

[0075] In some examples, the pharmaceutical composition further comprises a targeting moiety operably connected to the transfer vehicle. In some examples, the targeting moiety specifically binds an immune cell antigen or indirectly. In some examples, the immune cell antigen is a T cell antigen. In some examples, 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 examples, 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 examples, the target cell antigen is an immune cell antigen. In some examples, the immune cell antigen is a T cell antigen, an NK cell, an NKT cell, a macrophage, or a neutrophil. In some examples, 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 examples, the immune cell antigen is a macrophage antigen. In some examples, the macrophage antigen is selected from the group consisting of mannose receptor, CD206, and C1q.

[0077] In some examples, the targeting moiety is a small molecule. In some examples, 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 examples, the small molecule is mannose, a lectin, acivicin, biotin, or digoxigenin.

[0078] In some examples, 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 examples, the ionizable lipid has a half-life in a cell membrane less than about 2 weeks. In some examples, the ionizable lipid has a half-life in a cell membrane less than about 1 week. In some examples, the ionizable lipid has a half-life in a cell membrane less than about 30 hours. In some examples, 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 example, 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 examples, the disease, disorder, or condition is associated with aberrant expression, activity, or localization of a polypeptide selected from Tables 27 or 28. In some examples, the circular RNA polynucleotide encodes a therapeutic protein. In some examples, therapeutic protein expression in the spleen is higher than therapeutic protein expression in the liver. In some examples,

therapeutic protein expression in the spleen is at least about 2.9x therapeutic protein expression in the liver. In some examples, the therapeutic protein is not expressed at functional levels in the liver. In some examples, the therapeutic protein is not expressed at detectable levels in the liver. In some examples, therapeutic protein expression in the spleen is at least about 63% of total therapeutic protein expression.

[0081] In some examples, the linear RNA polynucleotide comprises a 3' anabaena group I intron fragment and a 5' anabaena group I intron fragment. In some examples, the reference RNA polynucleotide comprises a reference 3' anabaena group I intron fragment and a reference 5' anabaena group I intron fragment. In some examples, 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 examples, 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 examples, 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 examples, the 5' anabaena group I intron fragment comprises a corresponding sequence selected from SEQ ID NO: 73-84 and 86-111. In some examples, 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 examples, 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 examples, the IRES comprises a nucleotide sequence selected from SEQ ID NOs: 348-351. In some examples, the reference IRES is CVB3. In some examples, the IRES is not CVB3. In some examples, the IRES comprises a sequence selected from SEQ ID NOs: 1-64 and 66-72.

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

[0084] In another example, 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 examples, the circular RNA polynucleotide further comprises a spacer between the 3' group I intron fragment and the IRES.

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

some examples, the first and second duplex forming regions each have a length of about 30 nucleotides.

[0087] In an example, 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 example, 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 examples, a polynucleotide contains a 3' duplex forming region and a 5' duplex forming region. In certain examples, 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 examples, the first and second spacers each have a length of about 5 to about 60 nucleotides. In certain examples, the first and second spacers each have a length of about 8 to about 60 nucleotides. In certain examples, the first and second spacers each comprise an unstructured region at least 5 nucleotides long. In certain examples, the first and second spacers each comprise a structured region at least 7 nucleotides long. In certain examples, the first and second duplex forming regions each have a length of about 9 to 50 nucleotides. In certain examples, the vector is codon optimized. In certain examples, the vector is lacking at least one microRNA binding site present in an equivalent pre-optimization polynucleotide.

[0088] In an example, 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 examples, a polynucleotide contains a 3' duplex forming region and a 5' duplex forming region. In an example, 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 examples, a polynucleotide contains a 3' duplex forming region and a 5' duplex forming region.

[0089] In an example, provided herein is a eukaryotic cell comprising a circular RNA polynucleotide of the present disclosure. In some examples, the eukaryotic cell is a human cell. In some examples, the eukaryotic cell is an immune cell. In some examples, 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 IFNγ (FIG. 7A), IL-6 (FIG. 7B), IL-2 (FIG. 7C), RIG-I (FIG. 7D), IFN-β1 (FIG. 7E), and TNFα (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, 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.
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.
3. The circular RNA polynucleotide of claim 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.
4. The circular RNA polynucleotide of 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.
5. The circular RNA polynucleotide of any of claims 1-4, 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 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
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

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.

7. The circular RNA polynucleotide of any of claims 1-4, 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. 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-fluorocytosinde (5-FC) into 5-fluorouracil (5-FU); or
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 IFNγ, IL-2, IL-7, IL-15, IL-18, IL-10, TGFβ, or IL-35;
c. the transcription factor is selected from FOXP3, STAT5B, HELIOS, Tbet, GATA3, RORgt, 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

g. the expression sequence capable of converting 5-FC into 5-FU is cytosine deaminase.

9. 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.

10. The circular RNA polynucleotide of claim 9, wherein the first and second spacers each has a length of about 10 to about 60 nucleotides or about 9 to about 19 nucleotides.

11. The circular RNA polynucleotide of claim 9 or 10, wherein the first and second duplex forming regions each have a length of about 30 nucleotides.

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-IAPl, 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.

13. The circular RNA polynucleotide of any one of the preceding claims, consisting of natural nucleotides.
14. The circular RNA polynucleotide of any one of the preceding claims, wherein the expression sequence is codon optimized.
15. 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
    c. at least one RNA-editing susceptible site present in an equivalent pre-optimized polynucleotide.
16. 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.
17. 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.
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
d. an *in vivo* functional half-life in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.

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.
20. The pharmaceutical composition of claim 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.
21. The pharmaceutical composition of claim 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.
22. The pharmaceutical composition of any one of claims 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.
23. The pharmaceutical composition of any one of claims 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
    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.
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.

26. Use of the circular RNA polynucleotide of any one of 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.

27. The use of claim 26, wherein the cancer and/or autoimmune disorder is:

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

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 (TIDM), autoimmune gastritis, autoimmune uveoretinitis, polymyositis, colitis, thyroiditis, and the generalized autoimmune diseases typified by human Lupus.

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
    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.
29. A eukaryotic cell comprising a circular RNA polynucleotide according to any of claims 1-18, optionally wherein the cell is a human cell, an immune cell, and/or a T cell.