

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] Circular RNA, along with related compositions and methods are described herein. In some ~~embodiments~~examples, the inventive circular RNA comprises group I intron fragments, spacers, an IRES, duplex forming regions, and an expression sequence. In some example~~embodiments~~, the expression sequence encodes one or more antigens. In certain example~~embodiments~~, the expression sequence is replaced with a non-coding sequence. In some example~~embodiments~~, circular RNA of the invention has improved expression, functional stability, ease of manufacturing, and/or half-life when compared to linear RNA. In some example~~embodiments~~, circular RNA of the invention has reduced immunogenicity. In some example~~embodiments~~, inventive methods and constructs result in improved circularization efficiency, splicing efficiency, and/or purity when compared to existing RNA circularization approaches.

[0006a] In one aspect, the present disclosure provides a circular RNA polynucleotide comprising, in the following order, a. a post-splicing 3' group I intron fragment, b. an Internal Ribosome Entry Site (IRES), c. (i) an expression sequence encoding one or more antigens, adjuvants, antigen-like or adjuvant-like polypeptides, or fragments thereof; or (ii) a non-coding expression sequence, and d. a post-splicing 5' group I intron fragment.

[0006b] In another aspect of the present disclosure provides a pharmaceutical composition comprising the circular RNA polynucleotide as described herein and,

a. a diluent, and optionally a salt buffer;

b. a polycationic or cationic compound, optionally selected from the group consisting of: cationic peptides or proteins, basic polypeptides, cell penetrating peptides (CPPs), Tat-derived peptides, Penetratin, VP22 derived or analog peptides, Pestivirus Erns, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs), PpT620, proline-rich peptides, arginine-rich peptides, lysine-rich peptides, MPG-peptide(s), Pep-1, L-oligomers, Calcitonin peptide(s), Antennapedia-derived peptides, pAntp, pIsl, FGF, Lactoferrin, Transportan, Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, histones, cationic polysaccharides, cationic polymers, cationic lipids, dendrimers, polyimine, polyallylamine, oligofectamine, or cationic or polycationic polymers, sugar backbone based polymers, silan backbone based polymers, modified polyaminoacids, modified acrylates, modified polybetaminoester (PBAE), modified amidoamines, dendrimers, blockpolymers consisting of a combination of one or more cationic blocks and of one or more hydrophilic or hydrophobic blocks, protamine, nucleoline, spermine or spermidine, poly-L-lysine (PLL), polyarginine, HIV-binding peptides, HIV-1 Tat (HIV), polyethyleneimine (PEI), DOTMA: [1-(2,3-sioleyloxy)propyl]-N,N,N-trimethylammonium chloride, DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, DOPE: Dioleoyl phosphatidylethanol-amine, DOSPA, DODAB, DOIC, DMEPC, DOGS: Dioctadecylamidoglycylspermin, DIMRI: Dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide, DOTAP: dioleoyloxy-3-(trimethylammonio)propane, DC-6-14: O,O-ditetradecanoyl-N-.alpha.-trimethylammonioacetyl)diethanolamine chloride, CLIP 1: rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride, CLIP6: rac-[2(2,3-dihexadecyloxypropyloxymethyloxy)ethyl]-trimethylammonium, CLIP9: rac-[2(2,3-dihexadecyloxypropyloxysuccinyloxy)ethyl]-trimethylammonium, beta-aminoacid-polymers or reversed polyamides, PVP (poly(N-ethyl-4-vinylpyridinium bromide)), pDMAEMA (poly(dimethylaminoethyl methylacrylate)), pAMAM (poly(amidoamine)), diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-pentanol polymers, polypropylamine dendrimers or pAMAM based dendrimers, polyimine(s), PEI: poly(ethyleneimine), poly(propyleneimine), polyallylamine, cyclodextrin based polymers, dextran based polymers, chitosan, and PMOXA-PDMS copolymers; and/or

c. a polymeric compound, optionally selected from the group consisting of: polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. For example, a polymer may include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-coglycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone (PVP), polysiloxanes, polystyrene, polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fiimarate, polyoxymethylene, poloxamers, poloxamines, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), trimethylene carbonate, poly(N-acryloylmorpholine) (PACM), poly(2-methyl-2-oxazoline) (PMOX), poly(2-ethyl-2-oxazoline) (PEOZ), and polyglycerol.

[0006c] In other aspect of the present disclosure provides a pharmaceutical composition comprising the circular RNA polynucleotide as described herein, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle, optionally wherein

- a. the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β -amino esters;
- b. wherein the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids;
- c. the nanoparticle comprises cholesterol;
- d. the nanoparticle comprises arachidonic acid or oleic acid;
- e. 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; and/or
- f. 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; or is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof; and/or
- g. 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.

[0006d] Yet other aspect of the present disclosure provides a vector for making a circular RNA polynucleotide comprising, in the following order,

- a. a 3' Group I intron fragment,
- b. optionally a 5' duplex forming region,
- c. an Internal Ribosome Entry Site (IRES),
- d. (i) an expression sequence encoding one or more adjuvants, antigens, adjuvant-like or antigen-like polypeptides, or fragments thereof; or (ii) a non-coding expression sequence,
- e. optionally a 3' duplex forming region, and
- f. a 5' Group I intron fragment.

[0006e] In some aspects, the present disclosure provides a cell comprising the vector as described herein.

[0006f] In some aspects, the present disclosure provides a vaccine, comprising: at least one circular RNA polynucleotide having an expression sequence encoding at least one viral antigenic polypeptide, adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, formulated in a lipid nanoparticle.

[0006g] In another aspect, the present disclosure provides a SARS-CoV2 vaccine, comprising: at least one circular RNA polynucleotide having an expression sequence encoding at least one SARS-CoV2 viral antigenic polypeptide or an immunogenic fragment thereof, formulated in a lipid nanoparticle.

[0006h] In another aspects, the present disclosure provides use of a circular RNA polypeptide, a pharmaceutical composition, or a vaccine as described herein, in the manufacture of a medicament for inducing an immune response or treatment in a subject in need thereof, optionally wherein the immune response is an antigen-specific immune response.

[0006i] In another aspects, the present disclosure provides use of a circular RNA polypeptide, a pharmaceutical composition, or the vaccine as described herein, in the manufacture of a medicament for inducing cross-reactivity against a variety of viruses or strains of a virus in a mammal in need thereof.

[0006j] In another aspects, the present disclosure provides a circular RNA polypeptide, a pharmaceutical composition, or a vaccine as described herein, for use in therapy.

[0007] In an ~~aspect~~example, provided herein is a circular RNA polynucleotide comprising, in the following order, a. a 3' group I intron fragment, b. an Internal Ribosome Entry Site (IRES), c. an expression sequence encoding one or more antigens, adjuvants, antigen-like or adjuvant-like polypeptides, or fragments thereof, and d. a 5' group I intron fragment. In some ~~embodiments~~examples, a 3' group I intron fragment includes a 3' group I intron splice site dinucleotide. In some ~~embodiments~~examples, a 5' group I intron fragment includes a 5' group I intron splice site dinucleotide.

[0008] In an ~~aspect~~example, provided herein is a circular RNA polynucleotide comprising, in the

following order, a. a 3' group I intron fragment, b. an Internal Ribosome Entry Site (IRES), c. a non-coding expression sequence, and d. a 5' group I intron fragment.

[0009] In an ~~aspect~~example, provided herein is a circular RNA polynucleotide produced from transcription of a vector comprising, in the following order, a. a 5' duplex forming region, b. a 3' group I intron fragment, c. an Internal Ribosome Entry Site (IRES), d. an expression sequence encoding for one or more antigens, adjuvants, antigen-like or adjuvant-like polypeptides, or fragments thereof, e. a 5' group I intron fragment, and f. a 3' duplex forming region.

[0010] In an ~~aspect~~example, provided herein is a circular RNA polynucleotide produced from transcription of a vector comprising, in the following order, a. a 5' duplex forming region, b. a 3' group I intron fragment, c. an Internal Ribosome Entry Site (IRES), d. a non-coding expression sequence, e. a 5' group I intron fragment, and f. a 3' duplex forming region.

[0011] In some ~~embodiment~~examples, the vector further comprises a triphosphorylated 5' terminus. In some ~~embodiment~~examples, the vector further comprises a monophosphorylated 5' terminus.

[0012] In some ~~embodiment~~examples, the circular RNA polynucleotide 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 ~~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 other ~~embodiment~~examples, the first and second duplex forming regions each have a length of about 30 nucleotides.

[0013] 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.

[0014] In some ~~embodiments~~examples, the circular RNA polynucleotide consists of natural nucleotides. In some ~~embodiments~~examples, the expression sequence is codon-optimized. In some ~~embodiments~~examples, the circular RNA polynucleotide is optimized to lack at least one microRNA binding site present in an equivalent pre-optimized polynucleotide. In some ~~embodiments~~examples, the circular RNA polynucleotide is optimized to lack at least one endonuclease susceptible site present in an equivalent pre-optimized polynucleotide. In some ~~embodiments~~examples, the circular RNA polynucleotide is optimized to lack at least one RNA-editing susceptible site present in an equivalent pre-optimized polynucleotide.

[0015] In some ~~embodiments~~examples, the circular RNA polynucleotide is from about 100 nucleotides to about 10 kilobases in length.

[0016] In some ~~embodiments~~examples, the circular RNA polynucleotide has an in vivo duration of therapeutic effect in humans of at least about 20 hours. In some ~~embodiments~~examples, the circular RNA polynucleotide has a functional half-life of at least about 20 hours. In some ~~embodiments~~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 sequence. In some ~~embodiments~~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 sequence. In some ~~embodiments~~examples, the circular

RNA polynucleotide has a *in vivo* duration of therapeutic effect in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence. In some [embodimentsexamples](#), 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 sequence.

[0017] In some [embodimentsexamples](#), the adjuvant or adjuvant-like polypeptide is selected from the group comprising toll-like receptor ligand, cytokine, FLT3-ligand, antibody, chemokines, chimeric protein, endogenous adjuvant released from a dying tumor, and checkpoint inhibition proteins. In some [embodimentsexamples](#), the adjuvant or adjuvant-like polypeptide is selected from the group comprising BCSP31, MOMP, FomA, MymA, ESAT6, PorB, PVL, Porin, OmpA, PepO, OmpU, Lumazine synthase, Omp16, Omp19, CobT, RpfE, Rv0652, HBHA, NhhA, DnaJ, Pneumolysin, Flagellin, IFN-alpha, IFN-gamma, IL-2, IL-12, IL-15, IL-18, IL-21, GM-CSF, IL-1b, IL-6, TNF-a, IL-7, IL-17, IL-1Beta, anti-CTLA4, anti-PD1, anti-41BB, PD-L1, Tim-3, Lag-3, TIGIT, GITR, and anti-CD3. In some [embodimentsexamples](#), the adjuvant or adjuvant-like polypeptide is selected from Table 10.

[0018] In one [aspectexample](#), provided herein is an RNA polynucleotide comprising, in the following order, a 3' intron fragment and a triphosphorylated 5' terminus. In some [embodimentsexamples](#), the RNA polynucleotide comprises a 5' spacer located upstream to the 3' intron fragment and downstream from the triphosphorylated 5' terminus.

[0019] In one [aspectexample](#), provided herein is an RNA polynucleotide comprising a 5' intron fragment and a triphosphorylated 5' terminus. In some [embodimentsexamples](#), the RNA polynucleotide comprises a 5' spacer located downstream to the 5' intron fragment.

[0020] In some [embodimentsexamples](#), the RNA polynucleotide further comprises a monophosphorylated 5' terminus.

[0021] In one [aspectexample](#), provided herein is an RNA polynucleotide comprising, in the following order, a 3' intron fragment and a monophosphorylated 5' terminus. In some [embodimentsexamples](#), the RNA polynucleotide comprises a 5' spacer located upstream to the 3' intron fragment and downstream from the monophosphorylated 5' terminus.

[0022] In one [aspectexample](#), provided herein is an RNA polynucleotide comprising a 5' intron fragment and a monophosphorylated 5' terminus. In some [embodimentsexamples](#), the RNA polynucleotide comprises a 5' spacer located downstream to the 5' intron fragment.

[0023] In some [embodimentsexamples](#), the RNA polynucleotide further comprises a triphosphorylated 5' terminus.

[0024] In some [embodimentsexamples](#), the RNA polynucleotide further comprises a polyA

purification tag. In some ~~embodiments~~examples, the RNA polynucleotide further comprises an initiation sequence.

[0025] In one ~~aspect~~example, provided herein is an RNA preparation comprising: a. the circular RNA polynucleotide of claim 1, claim 2, or both; and b. a linear RNA polynucleotide comprising, at least one of the following: i. a 3' intron polynucleotide comprising a monophosphorylated 5' terminus and a 3' intron fragment; ii. a 5' intron polynucleotide comprising a monophosphorylated 5' terminus and a 5' intron fragment; iii. a 3' intron polynucleotide comprising a triphosphorylated 5' terminus and a 3' intron fragment; and iv. a 5' intron polynucleotide comprising a triphosphorylated 5' terminus and a 3' intron fragment, wherein the circular RNA polynucleotide comprises at least 90% of the RNA preparation.

[0026] In some ~~embodiments~~examples, the 3' intron polynucleotide or 5' intron polynucleotide comprises a spacer. In some ~~embodiments~~examples, the 3' intron polynucleotide or 5' intron polynucleotide comprises a polyA sequence. In some ~~embodiments~~examples, the 3' intron polynucleotide or 5' intron polynucleotide comprises a UTR. In some ~~embodiments~~examples, wherein the 3' intron polynucleotide or 5' intron polynucleotide comprises an IRES.

[0027] In one ~~aspect~~example, provided herein is a pharmaceutical composition comprising a circular RNA polynucleotide disclosed herein, a diluent, and optionally a salt buffer.

[0028] In one ~~aspect~~example, provided herein is a pharmaceutical composition comprising an RNA preparation disclosed herein, a diluent, and optionally a salt buffer.

[0029] In one ~~aspect~~example, provided herein is a pharmaceutical composition comprising a circular RNA polynucleotide disclosed herein, and a polycationic, cationic, or polymeric compound.

[0030] In one ~~aspect~~example, provided herein is a pharmaceutical composition comprising an RNA preparation of disclosed herein, and a polycationic, cationic, or polymeric compound.

[0031] In some ~~embodiments~~examples, the polycationic or cationic compound is selected from the group consisting of: cationic peptides or proteins, basic polypeptides, cell penetrating peptides (CPPs), Tat-derived peptides, Penetratin, VP22 derived or analog peptides, Pestivirus Erns, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs), PpT620, proline-rich peptides, arginine-rich peptides, lysine-rich peptides, MPG-peptide(s), Pep-1, L-oligomers, Calcitonin peptide(s), Antennapedia-derived peptides, pAntp, pIsl, FGF, Lactoferrin, Transportan, Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, histones, cationic polysaccharides, cationic polymers, cationic lipids, dendrimers, polyimine, polyallylamine, oligofectamine, or cationic or polycationic polymers, sugar backbone based polymers, silan backbone based polymers, modified polyaminoacids,

modified acrylates, modified polybetaminoester (PBAE), modified amidoamines, dendrimers, block polymers consisting of a combination of one or more cationic blocks and of one or more hydrophilic or hydrophobic blocks. In some ~~embodiments~~examples, the polymeric compound is selected from the group consisting of: polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. For example, a polymer may include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-coglycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone (PVP), polysiloxanes, polystyrene, polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fiimarate, polyoxymethylene, poloxamers, poloxamines, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), trimethylene carbonate, poly(N-acryloylmorpholine) (PAcM), poly(2-methyl-2-oxazoline) (PMOX), poly(2-ethyl-2-oxazoline) (PEOZ), and polyglycerol. In some ~~embodiments~~examples, the polycationic or cationic compound is selected from the group comprising: protamine, nucleoline, spermine or spermidine, poly-L-lysine (PLL), polyarginine, HIV-

binding peptides, HIV-1 Tat (HIV), polyethyleneimine (PEI), DOTMA: [1-(2,3-sioleyloxy)propyl]-N,N,N-trimethylammonium chloride, DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, DOPE: Dioleoyl phosphatidylethanolamine, DOSPA, DODAB, DOIC, DMEPC, DOGS: Dioctadecylamidoglycylspermin, DIMRI: Dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide, DOTAP: dioleoyloxy-3-(trimethylammonio)propane, DC-6-14: O,O-ditetradecanoyl-N-.alpha.-trimethylammonioacetyl)diethanolamine chloride, CLIP 1: rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride, CLIP6: rac-[2(2,3-dihexadecyloxypropyloxymethyloxy)ethyl]-trimethylammonium, CLIP9: rac-[2(2,3-dihexadecyloxypropyloxysuccinyloxy)ethyl]-trimethylammonium, beta-aminoacid-polymers or reversed polyamides, PVP (poly(N-ethyl-4-vinylpyridinium bromide)), pDMAEMA (poly(dimethylaminoethyl methylacrylate)), pAMAM (poly(amidoamine)), diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-pentanol polymers, polypropylamine dendrimers or pAMAM based dendrimers, polyimine(s), PEI: poly(ethyleneimine), poly(propyleneimine), polyallylamine, cyclodextrin based polymers, dextran based polymers, chitosan, and PMOXA-PDMS copolymers.

[0032] In an aspectexample, provided herein is a pharmaceutical composition comprising a circular RNA polynucleotide disclosed herein, a nanoparticle, and optionally a targeting moiety operably connected to the nanoparticle.

[0033] In an aspectexample, provided herein is a pharmaceutical composition comprising a RNA preparation disclosed herein, a nanoparticle, and optionally a targeting moiety operably connected to the nanoparticle.

[0034] In some embodimentsexamples, 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 embodimentsexamples, 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.

[0035] In some embodimentsexamples, 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 embodimentsexamples, the targeting moiety is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof. In some embodimentsexamples, the circular RNA polynucleotide or RNA preparation is in an amount effective to treat an infection (e.g., a viral infection) in a human subject in need thereof. In some embodimentsexamples, the pharmaceutical composition has an enhanced safety profile when compared to a pharmaceutical composition comprising vectors comprising exogenous DNA encoding antigens. In some embodimentsexamples, less than 1%, by weight, of the polynucleotides in the composition are double stranded RNA, DNA splints, or triphosphorylated RNA. In some embodimentsexamples, 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.

[0036] 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 disclosed herein, a nanoparticle, and optionally a targeting moiety operably connected to the nanoparticle. 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 RNA preparation disclosed herein, a nanoparticle, and optionally a targeting moiety operably connected to the nanoparticle.

[0037] In some embodimentsexamples, the subject has an infection (e.g., a viral infection). In certain embodimentsexamples, the method of treating a subject in need further comprises co-administration of an anti-inflammatory agent.

[0038] In some embodimentsexamples, 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. In some embodimentsexamples, the targeting moiety is an scFv, nanobody, peptide, minibody, heavy chain variable region, light chain variable region or fragment thereof. In some embodimentsexamples, 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.

[0039] In some embodimentsexamples, the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, or a biodegradable nanoparticle. In some embodimentsexamples, the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β -amino esters. In some embodimentsexamples, the nanoparticle comprises one or more non-cationic lipids. In some embodimentsexamples, the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids. In some embodimentsexamples, the nanoparticle comprises cholesterol.

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

[0040] In an ~~aspect~~example, provided herein is 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), an expression sequence encoding for one or more adjuvants, antigens, or adjuvant-like or antigen-like polypeptides, or fragments thereof, a 5' Group I intron fragment, and a 3' duplex forming region.

[0041] In an ~~aspect~~example, provided herein is 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 noncoding sequence, a 5' Group I intron fragment, and a 3' duplex forming region.

[0042] In some ~~embodiments~~examples, the 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 some ~~embodiments~~examples, the first and second spacers each have a length of about 20 to about 60 nucleotides. In certain ~~embodiments~~examples, the first and second spacers each comprise an unstructured region at least 5 nucleotides long. In some ~~embodiments~~examples, the first and second spacers each comprise a structured region at least 7 nucleotides long. In some ~~embodiments~~examples, the first and second duplex forming regions each have a length of about 9 to 50 nucleotides. In some ~~embodiments~~examples, the vector is codon optimized. In certain ~~embodiments~~examples, the vector is lacking at least one microRNA binding site present in an equivalent pre-optimization polynucleotide.

[0043] In an ~~aspect~~example, provided herein is a prokaryotic cell comprising a vector disclosed herein. In an ~~aspect~~example, provided herein is a eukaryotic cell comprising a circular RNA polynucleotide disclosed herein. In some ~~embodiments~~examples, the eukaryotic cell is a human cell. In some ~~embodiments~~examples, the eukaryotic cell is an antigen presentic cell.

[0044] In an ~~aspect~~example, provided herein is a vaccine, comprising: at least one circular RNA polynucleotide having an expression sequence encoding at least one viral antigenic polypeptide, adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, formulated in a lipid nanoparticle. In some ~~embodiments~~examples, the adjuvant or adjuvant-like polypeptide is selected from Table 10. In some ~~embodiments~~examples, the antigenic polypeptide is a viral polypeptide from an adenovirus; Herpes simplex, type 1; Herpes simplex, type 2; encephalitis virus, papillomavirus, Varicella-zoster virus; Epstein-barr virus; Human cytomegalovirus; Human herpes virus, type 8; Human papillomavirus; BK virus; JC virus; Smallpox; polio virus; Hepatitis B virus; Human bocavirus; Parvovirus B19; Human

astrovirus; Norwalk virus; coxsackievirus; hepatitis A virus; poliovirus; rhinovirus; Severe acute respiratory syndrome virus; Hepatitis C virus; Yellow Fever virus; Dengue virus; West Nile virus; Rubella virus; Hepatitis E virus; Human Immunodeficiency virus (HIV); Influenza virus; Guanarito virus; Junin virus; Lassa virus; Machupo virus; Sabia virus; Crimean-Congo hemorrhagic fever virus; Ebola virus; Marburg virus; Measles virus; Mumps virus; Parainfluenza virus; Respiratory syncytial virus; Human metapneumo virus; Hendra virus; Nipah virus; Rabies virus; Hepatitis D; Rotavirus; Orbivirus; Coltivirus; Banna virus; Human Enterovirus; Hanta virus; West Nile virus; Middle East Respiratory Syndrome Corona Virus; Japanese encephalitis virus; Vesicular exanthernavirus; SARS-CoV-2; Eastern equine encephalitis, or a combination of any two or more of the foregoing. In some ~~embodiments~~examples, the viral antigenic polypeptide or an immunogenic fragment thereof is selected or derived from any one of SEQ ID NOs: 325-336. In some ~~embodiments~~examples, the viral antigenic polypeptide or an immunogenic fragment thereof has an amino acid sequence that has at least 90% identity to an amino acid sequence of any one of SEQ ID NOs: 325-336, and wherein the antigenic polypeptide or immunogenic fragment thereof has membrane fusion activity, attaches to cell receptors, causes fusion of viral and mammalian cellular membranes, and/or is responsible for binding of the virus to a cell being infected.

[0045] In an ~~aspect~~example, provided herein is a SARS-CoV2 vaccine, comprising: at least one circular RNA polynucleotide having an expression sequence encoding at least one SARS-CoV2 viral antigenic polypeptide or an immunogenic fragment thereof, formulated in a lipid nanoparticle. In some ~~embodiments~~examples, the SARS-CoV2 viral antigenic polypeptide is selected from: SARS-CoV2 spike protein, Nsp1 – Nsp16, ORF3a, ORF6, ORF7a, ORFb, ORF8, ORF10, SARS-CoV2 envelope protein, SARS-CoV2 Membrane protein, SARS-CoV2 nucleocapsid protein or any antigenic peptide of SARS-CoV2 or fragment of SARS-CoV2 peptide. In some ~~embodiments~~examples, the SARS-CoV2 viral antigenic polypeptide is derived from SARS-CoV2 virus strain G, strain GR, strain GH, strain L, strain V, or a combination thereof.

[0046] In some ~~embodiments~~examples, the expression sequence comprised in a vaccine disclosed herein (e.g., a SARS-CoV2 vaccine) is codon-optimized. In some ~~embodiments~~examples, the vaccine (e.g., SARS-CoV2 vaccine) is multivalent. In some ~~embodiments~~examples, the vaccine (e.g., SARS-CoV2 vaccine) is formulated in an effective amount to produce an antigen-specific immune response.

[0047] In some ~~embodiments~~examples, the circular RNA polynucleotide comprises a first expression sequence encoding a first viral antigenic polypeptide and a second expression sequence encoding a second viral antigenic polypeptide.

[0048] In one aspectexample, provided herein is a method of inducing an immune response in a subject, the method comprising administering to the subject a vaccine disclosed herein, in an amount effective to produce an antigen-specific immune response in the subject. In an aspectexample, provided herein is a method of inducing an immune response in a subject, the method comprising administering to the subject a SARS-CoV2 disclosed herein, in an amount effective to produce an antigen-specific immune response in the subject.

[0049] In some embodimentsexamples, the antigen-specific immune response comprises a T cell response or a B cell response. In some embodimentsexamples, the subject is administered a single dose of the vaccine. In some embodimentsexamples, the subject is administered a booster dose of the vaccine. In some embodimentsexamples, the vaccine is administered to the subject by intranasal administration, intradermal injection or intramuscular injection. In some embodimentsexamples, an anti-antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a pre-determined threshold level. In some embodimentsexamples, an anti-antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a pre-determined threshold level. In some embodimentsexamples, the anti-antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a pre-determined threshold level. In some embodimentsexamples, the anti-antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a pre-determined threshold level. In some embodimentsexamples, the pre-determined threshold level is an anti-antigenic polypeptide antibody titer produced in a subject who has not been administered a vaccine comprising the antigenic polypeptide. In some embodimentsexamples, the pre-determined threshold level is an anti-antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated vaccine or an inactivated vaccine comprising the antigenic polypeptide. In some embodimentsexamples, the pre-determined threshold level is an anti-antigenic polypeptide antibody titer produced in a subject who has been administered a recombinant protein vaccine or purified protein vaccine comprising the antigenic polypeptide.

[0050] In one aspectexample, provided herein is a circular RNA polynucleotide having an expression sequence encoding at least one viral antigenic polypeptide, adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof. In one aspectexample, provided herein is an expression vector comprising an engineered nucleic acid encoding at least one circular RNA polynucleotide disclosed herein.

[0051] In one aspectexample, provided herein is a circular RNA polynucleotide vaccine comprising the circular RNA polynucleotide disclosed herein, formulated in a lipid nanoparticle. In some embodimentsexamples, the nanoparticle has a mean diameter of 50-200 nm. In some embodimentsexamples, the

lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid. In some ~~embodiments~~examples, the lipid nanoparticle carrier comprises a molar ratio of about 20-60% cationic lipid, 0.5-15% PEG-modified lipid, 25-55% sterol, and 25% non-cationic lipid. In some ~~embodiments~~examples, the cationic lipid is an ionizable cationic lipid and the non-cationic lipid is a neutral lipid, and the sterol is a cholesterol. In some ~~embodiments~~examples, the cationic lipid is selected from 2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl-4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319). In some ~~embodiments~~examples, the nanoparticle has a polydispersity value of less than 0.4. In some ~~embodiments~~examples, the nanoparticle has a net neutral charge at a neutral pH value.

[0052] In some ~~embodiments~~examples of a disclosed vaccine, the circular RNA polynucleotide is co-formulated with an adjuvant in the same nanoparticle. In some ~~embodiments~~examples, the adjuvant is CpG, imiquimod, Aluminium, or Freund's adjuvant.

[0053] In one ~~aspect~~example, provided herein is a pharmaceutical composition for use in vaccination of a subject, comprising an effective dose of circular RNA polynucleotide encoding at least one viral antigen or adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, wherein the effective dose is sufficient to produce a 1,000-10,000 neutralization titer produced by neutralizing antibody against said antigen or adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, as measured in serum of the subject at 1-72 hours post administration. In one ~~aspect~~example, provided herein is a pharmaceutical composition for use in vaccination of a subject, comprising an effective dose of circular mRNA encoding at least one viral antigen or adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, wherein the effective dose is sufficient to produce detectable levels of antigen or adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, as measured in serum of the subject at 1-72 hours post administration. In some ~~embodiments~~examples, the pharmaceutical composition is for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the vaccine or the pharmaceutical composition in an amount effective to produce an antigen specific immune response in the subject.

[0054] In one ~~aspect~~example, provided herein is a method of inducing, producing, or enhancing an immune response in a subject, the method comprising administering to the subject the pharmaceutical composition disclosed herein, in an amount effective to induce, produce or enhance an antigen-specific immune response in the subject. In some

~~embodiment~~examples, the pharmaceutical composition immunizes the subject against the virus for up to 2 years. In some ~~embodiment~~examples, the pharmaceutical composition immunizes the subject against the virus for more than 2 years. In some ~~embodiment~~examples, the subject has been exposed to the virus, wherein the subject is infected with the virus, or wherein the subject is at risk of infection by the virus. In some ~~embodiment~~examples, the subject is immunocompromised.

[0055] In one ~~aspect~~example, provided herein is the use of a vaccine or pharmaceutical composition disclosed herein, in the manufacture of a medicament for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the vaccine in an amount effective to produce an antigen specific immune response in the subject.

[0056] In one ~~aspect~~example, provided herein is a method of inducing cross-reactivity against a variety of viruses or strains of a virus in a mammal, the method comprising administering to the mammal in need thereof the vaccine of any preceding claim or the pharmaceutical composition of any preceding claim. In some ~~embodiment~~examples, the method comprises administering at least two circular RNA polynucleotides having an expression sequence each encoding a consensus viral antigen to the mammal separately. In some ~~embodiment~~examples, the method comprises administering at least two circular RNA polynucleotides having an expression sequence each encoding a consensus viral antigen to the mammal simultaneously. In some ~~embodiment~~examples, the method comprises.

BRIEF DESCRIPTION OF THE DRAWINGS

[0057] **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.

[0058] **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.

[0059] **FIG. 3** depicts stability of select IRES constructs in HepG2 (**FIG. 3A**) or 1C1C7 (**FIG. 3B**) cells over 3 days as measured by luminescence.

[0060] **FIGs. 4A and 4B** depict protein expression from select IRES constructs in Jurkat cells, as measured by luminescence from secreted *Gaussia* luciferase in cell supernatants.

[0061] **FIGs. 5A and 5B** depict stability of select IRES constructs in Jurkat cells over 3 days as measured by luminescence.

WHAT IS CLAIMED IS:

1. A circular RNA polynucleotide comprising, in the following order,
 - a. a post-splicing 3' group I intron fragment,
 - b. an Internal Ribosome Entry Site (IRES),
 - c. (i) an expression sequence encoding one or more antigens, adjuvants, antigen-like or adjuvant-like polypeptides, or fragments thereof; or (ii) a non-coding expression sequence, and
 - d. a post-splicing 5' group I intron fragment.
- ~~2. A circular RNA polynucleotide comprising, in the following order,~~
 - ~~a. a 3' group I intron fragment,~~
 - ~~b. an Internal Ribosome Entry Site (IRES),~~
 - ~~c. a non coding expression sequence, and~~
 - ~~d. a 5' group I intron fragment.~~
- ~~3.2. The circular RNA polynucleotide of claim 1, further comprising a 5' duplex forming region between (a) the post-splicing 3' group I intron fragment and (b) the IRES. produced from transcription of a vector comprising, in the following order,~~
 - ~~a. a 5' duplex forming region,~~
 - ~~b. a 3' group I intron fragment,~~
 - ~~c. an Internal Ribosome Entry Site (IRES),~~
 - ~~d. an expression sequence encoding one or more antigens, adjuvants, antigen-like or adjuvant-like polypeptides, or fragments thereof,~~
 - ~~e. a 5' group I intron fragment, and~~
 - ~~f. a 3' duplex forming region.~~
- ~~4.3. The circular RNA polynucleotide of claim 1 or 2, further comprising a 3' duplex forming region between (c) the expression sequence and (d) the post-splicing 5' group I intron fragment. produced from the transcription of a vector comprising, in the following order,~~
 - ~~a. a 5' duplex forming region,~~
 - ~~b. a 3' group I intron fragment,~~
 - ~~c. an Internal Ribosome Entry Site (IRES),~~
 - ~~d. a non coding expression sequence,~~
 - ~~e. a 5' group I intron fragment, and~~

f. ~~_____ a 3' duplex forming region.~~

5.4. ~~_____~~ The circular RNA polynucleotide of claim ~~3-2~~ or ~~43~~, further comprising a first spacer between ~~the 5' duplex forming region and the 3' group I intron fragment~~ and the 5' duplex forming region, and/or a second spacer between ~~the 5' group I intron fragment and the 3' duplex forming region~~ and the 5' group I intron fragment.

6.5. ~~_____~~ The circular RNA polynucleotide of claim ~~45~~, wherein the first and/or second spacers each ~~have~~ has a length of about 10 to about 60 nucleotides, optionally wherein each has a length of about 20 to about 40 nucleotides.

7. ~~_____~~ ~~The circular RNA polynucleotide of any one of claims 3-6, wherein the first and second duplex forming regions each have a length of about 9 to about 19 nucleotides.~~

8. ~~_____~~ ~~The circular RNA polynucleotide of any one of claims 3-6, wherein the first and second duplex forming regions each have a length of about 30 nucleotides.~~

9.6. ~~_____~~ The circular RNA polynucleotide of any one of claims 1-85, 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.

~~10-7.~~ The circular RNA polynucleotide of any one of claims 1-96, consisting of natural nucleotides.

~~11-8.~~ The circular RNA polynucleotide of any one of claims 1-96, wherein the expression sequence is codon optimized, comprising modified RNA nucleotides and/or modified nucleosides,

a. optionally wherein the modified nucleoside is selected from m⁶A (N⁶-methyladenosine); s²U (2-thiouridine); Ψ (pseudouridine); Um (2'-O-methyluridine); m¹A (1-methyladenosine); m²A (2-methyladenosine); Am (2'-O-methyladenosine); ms²m⁶A (2-methylthio-N⁶-methyladenosine); i⁶A (N⁶-isopentenyladenosine); ms²i⁶A (2-methylthio-N⁶ isopentenyladenosine); io⁶A (N⁶-(cis-hydroxyisopentenyl)adenosine); ms²io⁶A (2-methylthio-N⁶-(cis-hydroxyisopentenyl)adenosine); g⁶A (N⁶-glycinylicarbamoyladenosine); t⁶A (N⁶-threonylicarbamoyladenosine); ms²t⁶A (2-methylthio-N⁶-threonylicarbamoyladenosine); m⁶t⁶A (N⁶-methyl-N⁶-threonylicarbamoyladenosine); hn⁶A (N⁶-hydroxynorvalylicarbamoyladenosine); ms²hn⁶A (2-methylthio-N⁶-hydroxynorvalylicarbamoyladenosine); Ar(p) (2'-O-ribosyladenosine (phosphate)); I (inosine); m¹I (1-methylinosine); m¹Im (1,2'-O-dimethylinosine); m³C (3-methylcytidine); Cm (2'-O-methylcytidine); s²C (2-thiocytidine); ac⁴C (N⁴-acetylcytidine); f⁵C (5-formylcytidine); m⁵Cm (5,2' -O-dimethylcytidine); ac⁴Cm (N⁴-acetyl-2'-O-methylcytidine); k²C (lysidine); m¹G (1-methylguanosine); m²G (N²-methylguanosine); m⁷G (7-methylguanosine); Gm (2'-O-methylguanosine); m²₂G (N²,N²-dimethylguanosine);

m²Gm (N²,2'-O-dimethylguanosine); m²₂Gm (N²,N²,2'-O-trimethylguanosine); Gr(p) (2'-O-ribosylguanosine(phosphate)); yW (wybutosine); o₂yW (peroxywybutosine); OHyW (hydroxywybutosine); OHyW* (undermodified hydroxywybutosine); imG (wyosine); mimG (methylwyosine); Q (queuosine); oQ (epoxyqueuosine); galQ (galactosyl-queuosine); manQ (mannosyl-queuosine); preQ₀ (7-cyano-7-deazaguanosine); preQ₁ (7-aminomethyl-7-deazaguanosine); G⁺ (archaeosine); D (dihydrouridine); m⁵Um (5,2'-O-dimethyluridine); s⁴U (4-thiouridine); m⁵s²U (5-methyl-2-thiouridine); s²Um (2-thio-2'-O-methyluridine); acp³U (3-(3-amino-3-carboxypropyl)uridine); ho⁵U (5-hydroxyuridine); mo⁵U (5-methoxyuridine); cmo⁵U (uridine 5-oxyacetic acid); mcmo⁵U (uridine 5-oxyacetic acid methyl ester); chm⁵U (5-(carboxyhydroxymethyl)uridine); mchm⁵U (5-(carboxyhydroxymethyl)uridine methyl ester); mcm⁵U (5-methoxycarbonylmethyluridine); mcm⁵Um (5-methoxycarbonylmethyl-2'-O-methyluridine); mcm⁵s²U (5-methoxycarbonylmethyl-2-thiouridine); nm⁵S²U (5-aminomethyl-2-thiouridine); mnm⁵U (5-methylaminomethyluridine); mnm⁵s²U (5-methylaminomethyl-2-thiouridine); mnm⁵se²U (5-methylaminomethyl-2-selenouridine); ncm⁵U (5-carbamoylmethyluridine); ncm⁵Um (5-carbamoylmethyl-2'-O-methyluridine); cmnm⁵U (5-carboxymethylaminomethyluridine); cmnm⁵Um (5-carboxymethylaminomethyl-2'-O-methyluridine); cmnm⁵s²U (5-carboxymethylaminomethyl-2-thiouridine); m⁶₂A (N⁶,N⁶-dimethyladenosine); Im (2'-O-methylinosine); m⁴C (N⁴-methylcytidine); m⁴Cm (N⁴,2'-O-dimethylcytidine); hm⁵C (5-hydroxymethylcytidine); m³U (3-methyluridine); cm⁵U (5-carboxymethyluridine); m⁶Am (N⁶,2'-O-dimethyladenosine); m⁶₂Am (N⁶,N⁶,O-2'-trimethyladenosine); m^{2,7}G (N²,7-dimethylguanosine); m^{2,2,7}G (N²,N²,7-trimethylguanosine); m³Um (3,2'-O-dimethyluridine); m⁵D (5-methyldihydrouridine); f⁵Cm (5-formyl-2'-O-methylcytidine); m¹Gm (1,2'-O-dimethylguanosine); m¹Am (1,2'-O-dimethyladenosine); tm⁵U (5-taurinomethyluridine); tm⁵s²U (5-taurinomethyl-2-thiouridine); imG-14 (4-demethylwyosine); imG2 (isowyosine); or ac⁶A (N⁶-acetyladenosine);

b. optionally wherein the modified nucleoside includes a compound selected from pyridin-4-one ribonucleoside, 5-aza-uridine, 2-thio-5-aza-uridine, 2-thiouridine, 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxyuridine, 3-methyluridine, 5-carboxymethyl-uridine, 1-carboxymethyl-pseudouridine, 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyluridine, 1-taurinomethyl-pseudouridine, 5-

taurinomethyl-2-thio-uridine, 1-taurinomethyl-4-thio-uridine, 5-methyl-uridine, 1-methyl-pseudouridine, 4-thio-1-methyl-pseudouridine, 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine, dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, 4-methoxy-2-thio-pseudouridine, 5-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine, N4-acetylcytidine, 5-formylcytidine, N4-methylcytidine, 5-hydroxymethylcytidine, 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine, 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, 4-methoxy-1-methyl-pseudoisocytidine, 2-aminopurine, 2,6-diaminopurine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-aminopurine, 7-deaza-8-aza-2-aminopurine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyladenosine, N6-methyladenosine, N6-isopentenyladenosine, N6-(cis-hydroxyisopentenyl)adenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl)adenosine, N6-glycylcarbamoyladenosine, N6-threonylcarbamoyladenosine, 2-methylthio-N6-threonyl carbamoyladenosine, N6,N6-dimethyladenosine, 7-methyladenine, 2-methylthio-adenine, 2-methoxy-adenine, inosine, 1-methyl-inosine, wyosine, wybutosine, 7-deaza-guanosine, 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine, 6-thio-7-methyl-guanosine, 7-methylinosine, 6-methoxy-guanosine, 1-methylguanosine, N2-methylguanosine, N2,N2-dimethylguanosine, 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, N2,N2-dimethyl-6-thio-guanosine, 5-methylcytosine, pseudouridine, 1-methylpseudouridine; and/or

c. optionally wherein the circular RNA polynucleotide contains at least about 80%, at least about 90%, at least about 95%, or at least about 99% naturally occurring nucleotides.

12. The circular RNA polynucleotide of any one of claims 1-11, wherein the circular RNA polynucleotide is from about 100 nucleotides to about 10 kilobases in length.

~~13. The circular RNA polynucleotide of any one of the claims 1-12, having an *in vivo* duration of therapeutic effect in humans of at least about 20 hours.~~

~~14. The circular RNA polynucleotide of any one of claims 1-13, having a functional half-life of at least about 20 hours.~~

~~15. The circular RNA polynucleotide of any one of claims 1-14, having 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.~~

~~16. The circular RNA polynucleotide of any one of claims 1-15, 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.~~

~~17. The circular RNA polynucleotide of any one of claims 1-16, having an *in vivo* duration of therapeutic effect in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.~~

~~18. The circular RNA polynucleotide of any one of claims 1-17, having an *in vivo* functional half life in humans greater than that of an equivalent linear RNA polynucleotide having the same expression sequence.~~

~~19.9.~~ The circular RNA polynucleotide of any one of claims 1-8, 3, and 5-18, wherein

a. the adjuvant or adjuvant-like polypeptide is selected from the group comprising toll-like receptor ligand, cytokine, FLt3-ligand, antibody, chemokines, chimeric protein, endogenous adjuvant released from a dying tumor, and checkpoint inhibition proteins;

b. the adjuvant or adjuvant-like polypeptide is selected from the group comprising BCSP31, MOMP, FomA, MymA, ESAT6, PorB, PVL, Porin, OmpA, PepO, OmpU, Lumazine synthase, Omp16, Omp19, CobT, RpfE, Rv0652, HBHA, NhhA, DnaJ, Pneumolysin, Flagellin, IFN-alpha, IFN-gamma, IL-2, IL-12, IL-15, IL-18, IL-21, GM-CSF, IL-1b, IL-6, TNF-a, IL-7, IL-17, IL-1Beta, anti-CTLA4, anti-PD1, anti-41BB, PD-L1, Tim-3, Lag-3, TIGIT, GITR, and anti-CD3; and/or

c. the adjuvant or adjuvant-like polypeptide is selected from Table 10.

~~20.10. The circular RNA polynucleotide of any one of claims 1-9, 3, and 5-19, comprising a cap, a triphosphorylated 5' terminus, or a monophosphorylated 5' terminus wherein the adjuvant or adjuvant-like polypeptide is selected from the group comprising BCSP31, MOMP, FomA, MymA, ESAT6, PorB, PVL, Porin, OmpA, PepO, OmpU, Lumazine synthase, Omp16, Omp19, CobT, RpfE, Rv0652, HBHA, NhhA, DnaJ, Pneumolysin, Flagellin, IFN alpha, IFN gamma, IL 2, IL 12, IL 15, IL 18, IL 21, GM-CSF, IL 1b, IL 6, TNF-a, IL 7, IL 17, IL 1Beta, anti-CTLA4, anti-PD1, anti-41BB, PD-L1, Tim-3, Lag-3, TIGIT, GITR, and anti-CD3.~~

~~21. The circular RNA polynucleotide of any one of claims 1, 3, and 5-20, wherein the adjuvant or adjuvant-like polypeptide is selected from Table 10.~~

~~22. An RNA polynucleotide comprising, in the following order, a 3' intron fragment and a triphosphorylated 5' terminus.~~

~~23. The RNA polynucleotide of claim 22, comprising a 5' spacer located upstream to the 3' intron fragment and downstream from the triphosphorylated 5' terminus.~~

~~24. An RNA polynucleotide comprising, in the following order, a 3' intron fragment and a monophosphorylated 5' terminus.~~

~~25. The RNA polynucleotide of claim 24, comprising a 5' spacer located upstream to the 3' intron fragment and downstream from the monophosphorylated 5' terminus.~~

~~26. An RNA polynucleotide, comprising a 5' intron fragment and a triphosphorylated 5' terminus.~~

~~27. The RNA polynucleotide of claim 26, comprising a 5' spacer located downstream to the 5' intron fragment.~~

~~28. An RNA polynucleotide, comprising a 5' intron fragment and a monophosphorylated 5' terminus.~~

~~29. The RNA polynucleotide of claim 28, comprising a 5' spacer located downstream to the 5' intron fragment.~~

~~30. The RNA polynucleotide of any one of claims 22-29, further comprising a polyA purification tag.~~

31. — ~~The RNA polynucleotide of any one of claims 22-30, further comprising an initiation sequence.~~

32. — ~~The circular RNA polynucleotide of claim 3 or 4, wherein the vector further comprises a triphosphorylated 5' terminus.~~

33. — ~~The circular RNA polynucleotide of claim 3 or 4, wherein the vector further comprises a monophosphorylated 5' terminus.~~

34. — ~~The RNA polynucleotide of any one of claims 24-25 and 28-29, further comprising a triphosphorylated 5' terminus.~~

35. — ~~The RNA polynucleotide of any one of claims 22-23 and 26-27, further comprising a monophosphorylated 5' terminus.~~

36. — ~~An RNA preparation comprising:~~

a. — ~~the circular RNA polynucleotide of claim 1, claim 2, or both; and~~

b. — ~~a linear RNA polynucleotide comprising, at least one of the following:~~

i. — ~~a 3' intron polynucleotide comprising a monophosphorylated 5' terminus and a 3' intron fragment;~~

ii. — ~~a 5' intron polynucleotide comprising a monophosphorylated 5' terminus and a 5' intron fragment;~~

iii. — ~~a 3' intron polynucleotide comprising a triphosphorylated 5' terminus and a 3' intron fragment; and~~

iv. — ~~a 5' intron polynucleotide comprising a triphosphorylated 5' terminus and a 3' intron fragment,~~

~~wherein the circular RNA polynucleotide comprises at least 90% of the RNA preparation.~~

37. — ~~The RNA preparation of claim 36, wherein the 3' intron polynucleotide or 5' intron polynucleotide comprises a spacer.~~

38. — ~~The RNA preparation of claim 36, wherein the 3' intron polynucleotide or 5' intron polynucleotide comprises a polyA sequence.~~

39. — ~~The RNA preparation of any one of claims 36-38, wherein the 3' intron polynucleotide or 5' intron polynucleotide comprises a UTR.~~

40. ~~The RNA preparation of any one of claims 39, wherein the 3' intron polynucleotide or 5' intron polynucleotide comprises an IRES.~~

41.11. A pharmaceutical composition comprising ~~a~~the circular RNA polynucleotide of any one of claims 1-1021 and,

a. a diluent, and optionally a salt buffer;

b. a polycationic or cationic compound, optionally selected from the group consisting of: cationic peptides or proteins, basic polypeptides, cell penetrating peptides (CPPs), Tat-derived peptides, Penetratin, VP22 derived or analog peptides, Pestivirus Erns, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs), PpT620, proline-rich peptides, arginine-rich peptides, lysine-rich peptides, MPG-peptide(s), Pep-1, L-oligomers, Calcitonin peptide(s), Antennapedia-derived peptides, pAntp, pIsl, FGF, Lactoferrin, Transportan, Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, histones, cationic polysaccharides, cationic polymers, cationic lipids, dendrimers, polyimine, polyallylamine, oligofectamine, or cationic or polycationic polymers, sugar backbone based polymers, silan backbone based polymers, modified polyaminoacids, modified acrylates, modified polybetaminoester (PBAE), modified amidoamines, dendrimers, blockpolymers consisting of a combination of one or more cationic blocks and of one or more hydrophilic or hydrophobic blocks, protamine, nucleoline, spermine or spermidine, poly-L-lysine (PLL), polyarginine, HIV-binding peptides, HIV-1 Tat (HIV), polyethyleneimine (PEI), DOTMA: [1-(2,3-sioleyloxy)propyl]-N,N,N-trimethylammonium chloride, DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, DOPE: Dioleoyl phosphatidylethanol-amine, DOSPA, DODAB, DOIC, DMEPC, DOGS: Dioctadecylamidoglycylspermin, DIMRI: Dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide, DOTAP: dioleoyloxy-3-(trimethylammonio)propane, DC-6-14: O,O-ditetradecanoyl-N-.alpha.-trimethylammonioacetyl)diethanolamine chloride, CLIP 1: rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride, CLIP6: rac-[2(2,3-dihexadecyloxypropyloxymethyloxy)ethyl]-trimethylammonium, CLIP9: rac-[2(2,3-dihexadecyloxypropyloxysuccinyloxy)ethyl]-trimethylammonium, beta-aminoacid-polymers or reversed polyamides, PVP (poly(N-ethyl-4-vinylpyridinium bromide)), pDMAEMA (poly(dimethylaminoethyl methylacrylate)), pAMAM (poly(amidoamine)), diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-

pentanol polymers, polypropylamine dendrimers or pAMAM based dendrimers, polyimine(s), PEI: poly(ethyleneimine), poly(propyleneimine), polyallylamine, cyclodextrin based polymers, dextran based polymers, chitosan, and PMOXA-PDMS copolymers; and/or

c. a polymeric compound, optionally selected from the group consisting of: polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. For example, a polymer may include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L-lactic acid-co-glycolic acid) (PLLGA), poly(D,L-lactide) (PDLA), poly(L-lactide) (PLLA), poly(D,L-lactide-co-caprolactone), poly(D,L-lactide-co-caprolactone-coglycolide), poly(D,L-lactide-co-PEO-co-D,L-lactide), poly(D,L-lactide-co-PPO-co-D,L-lactide), polyalkyl cyanoacrylate, polyurethane, poly-L-lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly-L-glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone (PVP), polysiloxanes, polystyrene, polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fiimarate, polyoxymethylene, poloxamers, poloxamines, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-

caprolactone), trimethylene carbonate, poly(N-acryloylmorpholine) (PACM), poly(2-methyl-2-oxazoline) (PMOX), poly(2-ethyl-2-oxazoline) (PEOZ), and polyglycerol.

42. — ~~A pharmaceutical composition comprising an RNA preparation of any one of claims 36-40, a diluent, and optionally a salt buffer.~~

43. — ~~A pharmaceutical composition comprising a circular RNA polynucleotide of any one of claims 1-21, and a polycationic, cationic, or polymeric compound.~~

44. — ~~A pharmaceutical composition comprising an RNA preparation of any one of claims 36-40, and a polycationic, cationic, or polymeric compound.~~

45. — ~~The pharmaceutical composition of claim 43 or 44, wherein the polycationic or cationic compound is selected from the group consisting of: cationic peptides or proteins, basic polypeptides, cell penetrating peptides (CPPs), Tat derived peptides, Penetratin, VP22 derived or analog peptides, Pestivirus Erns, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs), PpT620, proline rich peptides, arginine rich peptides, lysine-rich peptides, MPG peptide(s), Pep-1, L oligomers, Calcitonin peptide(s), Antennapedia-derived peptides, pAntp, pIsl, FGF, Lactoferrin, Transportan, Buforin 2, Bac715-24, SynB, SynB(1), pVEC, hCT derived peptides, SAP, histones, cationic polysaccharides, cationic polymers, cationic lipids, dendrimers, polyimine, polyallylamine, oligofectamine, or cationic or polycationic polymers, sugar backbone based polymers, silan backbone based polymers, modified polyaminoacids, modified acrylates, modified polybetaminoester (PBAE), modified amidoamines, dendrimers, block polymers consisting of a combination of one or more cationic blocks and of one or more hydrophilic or hydrophobic blocks.~~

46. — ~~The pharmaceutical composition of claim 43 or 44, wherein the polymeric compound is selected from the group consisting of: polyamines, polyethers, polyamides, polyesters, polycarbamates, polyureas, polycarbonates, polystyrenes, polyimides, polysulfones, polyurethanes, polyacetylenes, polyethylenes, polyethyleneimines, polyisocyanates, polyacrylates, polymethacrylates, polyacrylonitriles, and polyarylates. For example, a polymer may include poly(caprolactone) (PCL), ethylene vinyl acetate polymer (EVA), poly(lactic acid) (PLA), poly(L lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic acid-co-glycolic acid) (PLGA), poly(L lactic acid-co-glycolic acid) (PLLGA), poly(D,L lactide) (PDLA), poly(L lactide) (PLLA), poly(D,L lactide-co-caprolactone), poly(D,L lactide-co-caprolactone-co-glycolide), poly(D,L lactide-co-PEO-co-D,L lactide), poly(D,L lactide-co-~~

PPO-co D,L lactide), polyalkyl cyanoacrylate, polyurethane, poly L lysine (PLL), hydroxypropyl methacrylate (HPMA), polyethyleneglycol, poly L glutamic acid, poly(hydroxy acids), polyanhydrides, polyorthoesters, poly(ester amides), polyamides, poly(ester ethers), polycarbonates, polyalkylenes such as polyethylene and polypropylene, polyalkylene glycols such as poly(ethylene glycol) (PEG), polyalkylene oxides (PEO), polyalkylene terephthalates such as poly(ethylene terephthalate), polyvinyl alcohols (PVA), polyvinyl ethers, polyvinyl esters such as poly(vinyl acetate), polyvinyl halides such as poly(vinyl chloride) (PVC), polyvinylpyrrolidone (PVP), polysiloxanes, polystyrene, polyurethanes, derivatized celluloses such as alkyl celluloses, hydroxyalkyl celluloses, cellulose ethers, cellulose esters, nitro celluloses, hydroxypropylcellulose, carboxymethylcellulose, polymers of acrylic acids, such as poly(methyl(meth)acrylate) (PMMA), poly(ethyl(meth)acrylate), poly(butyl(meth)acrylate), 363-5-10-15-20-25-30-35 WO 2021/076805 PCT/US2020/055844 poly(isobutyl(meth)acrylate), poly(hexyl(meth)acrylate), poly(isodecyl(meth)acrylate), poly(lauryl(meth)acrylate), poly(phenyl(meth)acrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), poly(octadecyl acrylate) and copolymers and mixtures thereof, polydioxanone and its copolymers, polyhydroxyalkanoates, polypropylene fiimarate, polyoxymethylene, poloxamers, poloxamines, poly(ortho)esters, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), trimethylene carbonate, poly(N acryloylmorpholine) (PAcM), poly(2-methyl-2-oxazoline) (PMOX), poly(2-ethyl-2-oxazoline) (PEOZ), and polyglycerol.

47. The pharmaceutical composition of claim 43 or 44, wherein the polycationic or cationic compound is selected from the group comprising: protamine, nucleoline, spermine or spermidine, poly L lysine (PLL), polyarginine, HIV binding peptides, HIV-1 Tat (HIV), polyethyleneimine (PEI), DOTMA: [1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride, DMRIE, di-C14 amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, DOPE: Dioleoyl-phosphatidylethanol-amine, DOSPA, DODAB, DOIC, DMEPC, DOGS: Dioctadecylamidoglycylspermin, DIMRI: Dimyristooxypropyl dimethyl hydroxyethyl ammonium bromide, DOTAP: dioleoyloxy-3-(trimethylammonio)propane, DC-6-14: O,O-ditetradecanoyl-N- α -trimethylammonioacetate diethanolamine chloride, CLIP-1: rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)] dimethylammonium chloride, CLIP6: rac-[2(2,3-dihexadecyloxypropyloxymethyloxy)ethyl] trimethylammonium, CLIP9: rac-[2(2,3-dihexadecyloxypropyloxysuccinyloxy)ethyl] trimethylammonium, beta-aminoacid polymers or reversed polyamides, PVP (poly(N-ethyl-4-vinylpyridinium bromide)), pDMAEMA

~~(poly(dimethylaminoethyl methacrylate)), pAMAM (poly(amidoamine)), diamine end modified 1,4 butanediol diacrylate co 5 amino 1 pentanol polymers, polypropylamine dendrimers or pAMAM based dendrimers, polyimine(s), PEI: poly(ethyleneimine), poly(propyleneimine), polyallylamine, cyclodextrin based polymers, dextran based polymers, chitosan, and PMOXA PDMS copolymers.~~

48.12. A pharmaceutical composition comprising a the circular RNA polynucleotide of any one of the claims 1-~~1024~~, a nanoparticle, and optionally, a targeting moiety operably connected to the nanoparticle, optionally wherein

- a. the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β -amino esters;
- b. wherein the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids;
- c. the nanoparticle comprises cholesterol;
- d. the nanoparticle comprises arachidonic acid or oleic acid;
- e. 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; and/or
- f. 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; or is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof; and/or
- g. 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.

49. — A pharmaceutical composition comprising an RNA preparation of any one of claims ~~36-40~~, a nanoparticle, and optionally, a target moiety operably connected to the nanoparticle.

50. — The pharmaceutical composition of claim 48 or 49, 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.

~~51. The pharmaceutical composition of any one of claims 41-50, 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.~~

~~52. The pharmaceutical composition of any one of claims 48-51, wherein the targeting moiety is a scFv, nanobody, peptide, minibody, polynucleotide aptamer, heavy chain variable region, light chain variable region or fragment thereof.~~

~~53.~~13. The pharmaceutical composition of ~~any one of claims 41-52~~claim 11 or 12, wherein the circular RNA polynucleotide ~~or RNA preparation~~ is in an amount effective to treat or prevent an infection in a human subject in need thereof.

~~54.~~14. The pharmaceutical composition of any one of claims 41-~~53~~11-13, wherein

a. the pharmaceutical composition has an enhanced safety profile when compared to a pharmaceutical composition comprising vectors comprising exogenous DNA encoding the same antigen;

b. the pharmaceutical composition is less than 1%, by weight, of the polynucleotides in the composition are double stranded RNA, DNA splints, or triphosphorylated RNA; and/or

c. the pharmaceutical composition is 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.

~~55. The pharmaceutical composition of any one of claims 41-54, wherein less than 1%, by weight, of the polynucleotides in the composition are double stranded RNA, DNA splints, or triphosphorylated RNA.~~

~~56. The pharmaceutical composition of any one of claims 41-55, 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.~~

~~57. The pharmaceutical composition of any one of claims 48-56, wherein 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, DLinearbDAP, DLinCDAP, KLin K DMA, DLin K XTC2 DMA, HGT4003, and combinations thereof.~~

~~58. 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 1-21, a nanoparticle, and optionally a targeting moiety operably connected to the nanoparticle.~~

~~59. A method of treating a subject in need thereof comprising administering a therapeutically effective amount of a composition comprising the RNA preparation of any one of claims 36-40, a nanoparticle, and optionally, a targeting moiety operably connected to a nanoparticle.~~

~~60. The method of claim 58 or 59, wherein the targeting moiety is an scFv, nanobody, peptide, minibody, heavy chain variable region, light chain variable region or fragment thereof.~~

~~61. The method of any one of claims 58-60, wherein the nanoparticle is a lipid nanoparticle, a core-shell nanoparticle, or a biodegradable nanoparticle.~~

~~62. The method of any one of claims 58-61, wherein the nanoparticle comprises one or more cationic lipids, ionizable lipids, or poly β amino esters.~~

~~63. The method of any one of claims 58-62, wherein the nanoparticle comprises one or more non-cationic lipids.~~

~~64. The method of any one of claims 58-63, wherein the nanoparticle comprises one or more PEG-modified lipids, polyglutamic acid lipids, or hyaluronic acid lipids.~~

~~65. The method of any one of claims 58-64, wherein the nanoparticle comprises cholesterol.~~

~~66. The method of any one of claims 58-65, wherein the nanoparticle comprises arachidonic acid or oleic acid.~~

~~67. The method of any one of claims 58-66, wherein 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.~~

~~68. The method of any one of claims 58-67, wherein the nanoparticle encapsulates more than one circular RNA polynucleotide.~~

~~69-15. A vector for making a circular RNA polynucleotide comprising, in the following order,~~

- ~~a. a 5' duplex forming region, a 3' Group I intron fragment,~~
- ~~b. optionally a 5' duplex forming region,~~
- ~~c. an Internal Ribosome Entry Site (IRES),~~
- ~~d. (i) an expression sequence encoding one or more adjuvants, antigens, adjuvant-like or antigen-like polypeptides, or fragments thereof; or (ii) a non-coding expression sequence,~~
- ~~e. optionally a 3' duplex forming region, and~~
- ~~f. a 5' Group I intron fragment, and a 3' duplex forming region.~~

~~70. 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 noncoding sequence, a 5' Group I intron fragment, and a 3' duplex forming region.~~

~~71. The vector of claim 69 or 70, 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.~~

~~72. The vector of any one of claims 69-71, wherein the first and second spacers each have a length of about 20 to about 60 nucleotides.~~

~~73. The vector of any one of claims 69-72, wherein the first and second spacers each comprise an unstructured region at least 5 nucleotides long.~~

~~74. The vector of any one of claims 69-73, wherein the first and second spacers each comprise a structured region at least 7 nucleotides long.~~

~~75. The vector of any one of claims 69-74, wherein the first and second duplex forming regions each have a length of about 9 to 50 nucleotides.~~

- ~~76. The vector of any one of claims 69-75, wherein the vector is codon optimized.~~
- ~~77. The vector of any one of claims 69-76, lacking at least one microRNA binding site present in an equivalent pre-optimization polynucleotide.~~
- ~~78.16. A prokaryotic cell comprising a the vector of any one of claims 69-77~~claim 15.
- ~~79. A eukaryotic cell comprising a circular RNA polynucleotide of any one of claims 1-21.~~
- ~~80. The eukaryotic cell of claim 79, wherein the eukaryotic cell is a human cell.~~
- ~~81. The eukaryotic cell of claim 79 or 80, wherein the eukaryotic cell is an antigen presenting cell.~~
- ~~82.17. A vaccine, comprising: at least one circular RNA polynucleotide having an expression sequence encoding at least one viral antigenic polypeptide, adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, formulated in a lipid nanoparticle.~~
- ~~83. The vaccine of claim 82, wherein the adjuvant or adjuvant like polypeptide is selected from Table 10.~~
- ~~84. The vaccine of claim 82 or 83, wherein the antigenic polypeptide is a viral polypeptide from an adenovirus; Herpes simplex, type 1; Herpes simplex, type 2; encephalitis virus; papillomavirus; Varicella zoster virus; Epstein barr virus; Human cytomegalovirus; Human herpes virus, type 8; Human papillomavirus; BK virus; JC virus; Smallpox; polio virus; Hepatitis B virus; Human bocavirus; Parvovirus B19; Human astrovirus; Norwalk virus; coxsackievirus; hepatitis A virus; poliovirus; rhinovirus; Severe acute respiratory syndrome virus; Hepatitis C virus; Yellow Fever virus; Dengue virus; West Nile virus; Rubella virus; Hepatitis E virus; Human Immunodeficiency virus (HIV); Influenza virus; Guanarito virus; Junin virus; Lassa virus; Machupo virus; Sabia virus; Crimean Congo hemorrhagic fever virus; Ebola virus; Marburg virus; Measles virus; Mumps virus; Parainfluenza virus; Respiratory syncytial virus; Human metapneumo virus; Hendra virus; Nipah virus; Rabies virus; Hepatitis D; Rotavirus; Orbivirus; Coltivirus; Banna virus; Human Enterovirus; Hanta virus; West Nile virus; Middle East Respiratory Syndrome Corona Virus; Japanese encephalitis virus; Vesicular exanthernavirus; SARS-CoV-2; Eastern equine encephalitis, or a combination of any two or more of the foregoing.~~

~~85.18.~~ The vaccine of ~~any of claims 82-84~~claim 17, wherein the viral antigenic polypeptide or an immunogenic fragment thereof:

- a. is selected or derived from any one of SEQ ID NOs: 325-336; and/or
- b. has an amino acid sequence that has at least 90% identity to an amino acid sequence of any one of SEQ ID NOs: 325-336, and wherein the viral antigenic polypeptide or immunogenic fragment thereof has membrane fusion activity, attaches to cell receptors, causes fusion of viral and mammalian cellular membranes, and/or is responsible for binding of the virus to a cell being infected.

~~86.—The vaccine of any one of claims 82-85, wherein the viral antigenic polypeptide or an immunogenic fragment thereof has an amino acid sequence that has at least 90% identity to an amino acid sequence of any one of SEQ ID NOs: 325-336, and wherein the viral antigenic polypeptide or immunogenic fragment thereof has membrane fusion activity, attaches to cell receptors, causes fusion of viral and mammalian cellular membranes, and/or is responsible for binding of the virus to a cell being infected.~~

~~87.—The vaccine of any one of claims 82-86, wherein the expression sequence is codon-optimized.~~

~~88.—The vaccine of any one of claims 82-87, wherein the vaccine is multivalent.~~

~~89.—The vaccine of any one of claims 82-88, formulated in an effective amount to produce an antigen specific immune response.~~

~~90.—The vaccine of any one of claims 82-89, wherein the circular RNA polynucleotide comprises a first expression sequence encoding a first viral antigenic polypeptide and a second expression sequence encoding a second viral antigenic polypeptide.~~

~~91.—A method of inducing an immune response in a subject, the method comprising administering to the subject the vaccine of any one of claims 82-90, in an amount effective to produce an antigen specific immune response in the subject.~~

~~92.—The method of claim 91, wherein the antigen specific immune response comprises a T cell response or a B cell response.~~

~~93.—The method of claim 91 or 92, wherein the subject is administered a single dose of the vaccine.~~

~~94.—The method of any one of claims 91-93, wherein the subject is administered a booster dose of the vaccine.~~

~~95.—The method of any one of claims 91-94, wherein the vaccine is administered to the subject by intranasal administration, intradermal injection or intramuscular injection.~~

~~96.—The method of any one of claims 91-95, wherein an anti-antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a pre-determined threshold level.~~

~~97.—The method of any one of claims 91-96, wherein an anti-antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a pre-determined threshold level.~~

~~98.—The method of any one of claims 91-97, wherein the anti-antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a pre-determined threshold level.~~

~~99.—The method of any one of claims 91-98, wherein the anti-antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a pre-determined threshold level.~~

~~100.—The method of any one of claims 91-99, wherein the pre-determined threshold level is an anti-antigenic polypeptide antibody titer produced in a subject who has not been administered a vaccine comprising the antigenic polypeptide.~~

~~101.—The method of any one of claims 91-100, wherein the pre-determined threshold level is an anti-antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated vaccine or an inactivated vaccine comprising the antigenic polypeptide.~~

~~102.—The method of any one of claims 91-101, wherein the pre-determined threshold level is an anti-antigenic polypeptide antibody titer produced in a subject who has been administered a recombinant protein vaccine or purified protein vaccine comprising the antigenic polypeptide.~~

~~103.~~19. A SARS-CoV2 vaccine, comprising: at least one circular RNA polynucleotide having an expression sequence encoding at least one SARS-CoV2 viral antigenic polypeptide or an immunogenic fragment thereof, formulated in a lipid nanoparticle.

~~104.20.~~ The SARS-CoV2 vaccine of claim ~~102~~19, wherein the SARS-CoV2 viral antigenic polypeptide

a. is selected from: SARS-CoV2 spike protein, Nsp1 – Nsp16, ORF3a, ORF6, ORF7a, ORFb, ORF8, ORF10, SARS-CoV2 envelope protein, SARS-CoV2 Membrane protein, SARS-CoV2 nucleocapsid protein or any antigenic peptide of SARS-CoV2 or fragment of SARS-CoV2 peptide; and/or

b. the SARS-CoV2 viral antigenic polypeptide is derived from SARS-CoV2 virus strain G, strain GR, strain GH, strain L, strain V, or a combination thereof.

~~105. The SARS CoV2 vaccine of claim 103 or 104, wherein the SARS CoV2 viral antigenic polypeptide is derived from SARS CoV2 virus strain G, strain GR, strain GH, strain L, strain V, or a combination thereof.~~

~~106. The SARS CoV2 vaccine of any one of claims 103-105, wherein the expression sequence is codon optimized.~~

~~107. The SARS CoV2 vaccine of any one of claims 103-106, wherein the vaccine is multivalent.~~

~~108. The SARS CoV2 vaccine of any one of claims 103-107, formulated in an effective amount to produce an antigen specific immune response.~~

~~109. A method of inducing an immune response in a subject, the method comprising administering to the subject the SARS CoV2 vaccine of any one of claims 103-108, in an amount effective to produce an antigen specific immune response in the subject.~~

~~110. The method of claim 109, wherein the antigen specific immune response comprises a T-cell response or a B-cell response.~~

~~111. The method of claim 109 or 110, wherein the subject is administered a single dose of the vaccine.~~

~~112. The method of claim any one of claims 109-111, wherein the subject is administered a booster dose of the vaccine.~~

~~113. The method of any one of claims 109-112, wherein the vaccine is administered to the subject by intranasal administration, intradermal injection or intramuscular injection.~~

~~114. The method of anyone of claims 109-113, wherein an anti antigenic polypeptide antibody titer produced in the subject is increased by at least 1 log relative to a pre-determined threshold level.~~

~~115. The method of any one of claims 109-114, wherein an anti antigenic polypeptide antibody titer produced in the subject is increased by 1-3 log relative to a pre-determined threshold level.~~

~~116. The method of any one of claims 109-115, wherein the anti antigenic polypeptide antibody titer produced in the subject is increased at least 2 times relative to a pre-determined threshold level.~~

~~117. The method of anyone of claims 109-116, wherein the anti antigenic polypeptide antibody titer produced in the subject is increased 2-10 times relative to a pre-determined threshold level.~~

~~118. The method of any one of claims 109-117, wherein the pre-determined threshold level is an anti antigenic polypeptide antibody titer produced in a subject who has not been administered a vaccine comprising the antigenic polypeptide.~~

~~119. The method of any one of claims 109-118, wherein the pre-determined threshold level is an anti antigenic polypeptide antibody titer produced in a subject who has been administered a live attenuated vaccine or an inactivated vaccine comprising the antigenic polypeptide.~~

~~120. The method of any one of claims 109-119, wherein the pre-determined threshold level is an anti antigenic polypeptide antibody titer produced in a subject who has been administered a recombinant protein vaccine or purified protein vaccine comprising the antigenic polypeptide.~~

~~121. A circular RNA polynucleotide having an expression sequence encoding at least one viral antigenic polypeptide, adjuvant or adjuvant like polypeptide, or an immunogenic fragment thereof.~~

~~122. An expression vector comprising an engineered nucleic acid encoding at least one circular RNA polynucleotide of any one of claims 1-21.~~

~~123.—A circular RNA polynucleotide vaccine comprising the circular RNA polynucleotide of claim 121, formulated in a lipid nanoparticle.~~

~~124.—The circular RNA polynucleotide vaccine of claim 123, wherein the nanoparticle has a mean diameter of 50–200 nm.~~

~~125.—The circular RNA polynucleotide vaccine of claim 123 or 124, wherein the lipid nanoparticle comprises a cationic lipid, a PEG-modified lipid, a sterol and a non-cationic lipid.~~

~~126.—The circular RNA polynucleotide vaccine of any one of claims 123–125, wherein the lipid nanoparticle carrier comprises a molar ratio of about 20–60% cationic lipid, 0.5–15% PEG-modified lipid, 25–55% sterol, and 25% non-cationic lipid.~~

~~127.—The circular RNA polynucleotide vaccine of any one of claims 123–126, wherein the cationic lipid is an ionizable cationic lipid and the non-cationic lipid is a neutral lipid, and the sterol is a cholesterol.~~

~~128.—The circular RNA polynucleotide vaccine of any one of 123–127, wherein the cationic lipid is selected from 2,2-dilinoleyl 4-dimethylaminoethyl [1,3]-dioxolane (DLin-KC2-DMA), dilinoleyl-methyl 4-dimethylaminobutyrate (DLin-MC3-DMA), and di((Z)-non-2-en-1-yl) 9-((4-(dimethylamino)butanoyl)oxy)heptadecanedioate (L319).~~

~~129.—The circular RNA polynucleotide vaccine of any one of claims 123–128, wherein the nanoparticle has a polydispersity value of less than 0.4.~~

~~130.—The circular RNA polynucleotide vaccine of any one of claims 123–129, wherein the nanoparticle has a net neutral charge at a neutral pH value.~~

~~131.—A pharmaceutical composition for use in vaccination of a subject, comprising an effective dose of circular RNA polynucleotide encoding at least one viral antigen or adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, wherein the effective dose is sufficient to produce a 1,000–10,000 neutralization titer produced by neutralizing antibody against said antigen or adjuvant or adjuvant-like polypeptide, or an immunogenic fragment thereof, as measured in serum of the subject at 1–72 hours post administration.~~

~~132.—A pharmaceutical composition for use in vaccination of a subject, comprising an effective dose of circular mRNA encoding at least one viral antigen or adjuvant or adjuvant-~~

like polypeptide, or an immunogenic fragment thereof, wherein the effective dose is sufficient to produce detectable levels of antigen or adjuvant or adjuvant like polypeptide, or an immunogenic fragment thereof, as measured in serum of the subject at 1-72 hours post administration.

133. — A method of inducing, producing, or enhancing an immune response in a subject, the method comprising administering to the subject the pharmaceutical composition of claim 131 or 132, in an amount effective to induce, produce or enhance an antigen specific immune response in the subject.

134. — The method of claim 133, wherein the pharmaceutical composition immunizes the subject against the virus for up to 2 years.

135. — The method of claim 133 or 134, wherein the pharmaceutical composition immunizes the subject against the virus for more than 2 years.

136. — The method of any one of claims 133-135, wherein the subject has been exposed to the virus, wherein the subject is infected with the virus, or wherein the subject is at risk of infection by the virus.

137. — The method of any one of claims 133-136, wherein the subject is immunocompromised.

138. — The vaccine of any one of claims 82-90, 103-108, and 123-130, or the pharmaceutical composition of claim 131 or 132, for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the vaccine or the pharmaceutical composition in an amount effective to produce an antigen specific immune response in the subject.

139. — Use of the vaccine of any one of claims 82-90, 103-108, and 123-130, or the pharmaceutical composition of claim 131 or 132, in the manufacture of a medicament for use in a method of inducing an antigen specific immune response in a subject, the method comprising administering to the subject the vaccine in an amount effective to produce an antigen specific immune response in the subject.

140. — A method of inducing cross reactivity against a variety of viruses or strains of a virus in a mammal, the method comprising administering to the mammal in need thereof the vaccine of any preceding claim or the pharmaceutical composition of any preceding claim.

~~141. The method of claim 140, wherein at least two circular RNA polynucleotides having an expression sequence each encoding a consensus viral antigen are administered to the mammal separately.~~

~~142. The method of claim 140 or 141, wherein at least two circular RNA polynucleotides having an expression sequence each encoding a consensus viral antigen are administered to the mammal simultaneously.~~

~~143.21. The vaccine of any one of claims 17-2082 90, 103-108, and 123-130, wherein the circular RNA polynucleotide is co-formulated with an adjuvant in the same nanoparticle, optionally wherein the adjuvant is CpG, imiquimod, Aluminium, or Freund's adjuvant.~~

~~144. The vaccine of any one of claims 82-90, 103-108, 123-130, and 143, wherein the adjuvant is CpG, imiquimod, Aluminium, or Freund's adjuvant.~~

22. Use of a circular RNA polypeptide of any one of claims 1-10, a pharmaceutical composition of any one of claims 11-14, or a vaccine of any one of claims 17-21, in the manufacture of a medicament for inducing an immune response or treatment in a subject in need thereof, optionally wherein the immune response is an antigen-specific immune response.

23. Use of a circular RNA polypeptide of any one of claims 1-10, a pharmaceutical composition of any one of claims 11-14, or the vaccine of any one of claims 17-21, in the manufacture of a medicament for inducing cross-reactivity against a variety of viruses or strains of a virus in a mammal in need thereof.

24. The circular RNA polypeptide of any one of claims 1-10, the pharmaceutical composition of any one of claims 11-14, or the vaccine of any one of claims 17-21, for use in therapy.