CLAIM

What is claimed is:

1. A vector for making circular RNA, said vector comprising the following elements operably connected to each other and arranged in the following sequence:
   a) a 5’ homology arm,
   b) a 3’ Group I intron fragment containing a 3’ splice site dinucleotide,
   c) a 5’ permissive spacer sequence,
   d) an internal ribosome entry site (IRES),
   e) a protein coding region or noncoding region,
   f) a 3’ permissive spacer sequence,
   g) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and
   h) a 3’ homology arm,
   said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells.

2. The vector of Claim 1, wherein the 3’ Group I intron fragment and the 5’ Group I intron fragment are from a *Cyanobacterium Anabaena sp.* pre-tRNA-Leu gene.

3. The vector of Claim 1, wherein the 3’ Group I intron fragment and the 5’ Group I intron fragment are from a T4 phage Td gene.

4. The vector of Claim 1, wherein the IRES is selected from an IRES sequence of Taura syndrome virus, Triatoma virus, Theiler's encephalomyelitis virus, simian Virus 40, Solenopsis invicta virus 1, Rhopalosiphum padi virus, Reticuloendotheliosis virus, ~~fuman~~ 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 (EMCV), Drosophila C Virus, Crucifer tobamo virus, 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 SFTPAl, Human AMLl/RUNXl, Drosophila antennapedia, Human AQP4, Human AT1R, Human BAG-1, Human BCL2, Human BiP, Human c-IAPl , Human c-

myc, Human eIF4G, Mouse NDST4L, Human LEF1, Mouse HIF1 alpha, Human n.myc, Mouse Gtx, Human p27kipl, Human PDGF2/c-sis, Human p53, Human Pim-1, Mouse Rbm3, Drosophila reaper, Canine Scamper, Drosophila Ubx, Salivirus, Cosavirus, Parechovirus, Human UNR, Mouse UtrA, Human VEGF-A, Human XIAP, Drosophila hairless, *S. cerevisiae* TFIID, *S. cerevisiae* YAP1, Human c-src, Human FGF-1, Simian picomavirus, Turnip crinkle virus, an aptamer to eIF4G, Coxsackievirus B3 (CVB3) or Coxsackievirus A (CVB1/2).

5. The vector of Claim 1, wherein the protein coding region ~~or noncoding region is a protein coding region that~~ encodes an eukaryotic protein or a prokaryotic protein.

6. The vector of Claim 1, wherein: ~~each homology arm is about 5-50 nucleotides in length.~~
   a) the 5' homology arm is 5-50 nucleotides in length,
   b) the 3' homology arm is 5-50 nucleotides in length,
   or both a) and b).

7. The vector of Claim 1, wherein: ~~at least one spacer sequence is a polyA sequence.~~
   a) the 5' permissive spacer sequence is a polyA sequence,
   b) the 3' permissive spacer sequence is a polyA sequence,
   or both a) and b).

8. The vector of Claim 1, wherein at least one spacer sequences is a polyA-C sequence.
   a) the 5' permissive spacer sequence is a polyA-C sequence,
   b) the 3' permissive spacer sequence is a polyA-C sequence,
   or both a) and b).

9. The vector of Claim 1, further comprising an RNA polymerase promoter ~~positioned upstream of the 5' homology arm~~.

10. The vector of Claim 1, wherein the vector is used to transcribe circular RNA with a size range of ~~about~~ 500 to ~~about~~ 10,000nt.

11. The vector of claim 1, wherein the 3' Group I intron fragment comprises a 3'-proximal Group I intron-derived sequence including the 3' splice site dinucleotide and, optionally, sequence corresponding to the adjacent natural exon, and the 5' Group I intron fragment comprises a 5'-proximal Group I intron-derived sequence including the 5' splice site dinucleotide and, optionally, sequence corresponding to the adjacent exon.

12. A circular RNA produced by the vector of Claim 1.

~~13. A method of purifying circular RNA, said method comprising: running circular RNA through a size-exclusion column in tris-EDTA or citrate buffer in a high performance liquid chromatography (HPLC) system, and treating the circular RNA with phosphatase after running the circular RNA through the size-exclusion column, thereby producing purified circular RNA.~~

~~14. The method of Claim 13, wherein the phosphatase treatment is followed by RNase R treatment.~~

~~15. The method of Claim 13, wherein the purified circular RNA is formulated into nanoparticles.~~

~~16. The method of Claim 13, wherein the circular RNA is run through the size-exclusion column at a pH in the range of about 4-8.~~

~~17. The method of Claim 16, wherein the circular RNA is run through the size-exclusion column at a flow rate of about 0.01-5.0 mL/minute.~~

13~~18~~. A pharmaceutical composition comprising an effective amount of the circular RNA of claim 12 and a pharmaceutically acceptable carrier.

14~~19~~. The pharmaceutical composition of claim 13~~18~~, further comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

15~~20~~. A vector for making circular RNA, said vector comprising the following elements operably connected to each other and arranged in the following sequence:

a) a 5’ homology arm,
b) a 3’ Group I intron fragment containing a 3’ splice site dinucleotide,
c) a 5’ permissive spacer sequence,
d) an internal ribosome entry site (IRES),
e) a protein coding region,
f) a 3’ permissive spacer sequence,
g) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and
h) a 3’ homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells.

16~~21~~. The vector of Claim 15~~20~~, wherein the protein coding region encodes a therapeutic protein, a chimeric antigen receptor, an antibody or a bispecific antibody.

17~~22~~. A composition comprising:

a) the vector for making circular RNA of Claim 15~~20~~, and

b) a delivery vehicle for delivering the vector to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

18~~23~~. A circular RNA produced by the vector of Claim 15~~20~~, wherein the vector is used to transcribe circular RNA with a size of at least 500 nucleotides.

19~~24~~. A eukaryotic cell comprising the circular RNA of Claim 18~~23~~.

20~~25~~. A cell population comprising the circular RNA of Claim 18~~23~~.

21~~26~~. The circular RNA of Claim 18~~23~~, wherein the circular RNA comprises in the following order, ~~a portion of a 3' group I intron fragment,~~ the 5' permissive spacer sequence, ~~an~~ the Internal Ribosome Entry Site (IRES), ~~a~~ the protein coding region, and the 3' permissive spacer sequence. ~~a portion of a 5' group I intron fragment.~~

22~~27~~. A composition comprising an effective amount of the circular RNA of Claim 18~~23~~ and a pharmaceutically acceptable carrier.

23~~28~~. A composition comprising:

a) an effective amount of the circular RNA of Claim 18~~23~~, and

b) a delivery vehicle for delivering the circular RNA to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

24~~29~~. A composition comprising a circular RNA-expressing cell in combination with one or more pharmaceutically or physiologically acceptable carriers, excipients or diluents, wherein the circular RNA is the circular RNA of Claim 18~~23~~.

25~~30~~. The composition of Claim 24~~29~~, comprising a plurality of circular RNA-expressing cells.

26~~31~~. The composition of Claim 22~~27~~, wherein the composition is a nanoparticle formulation.

27~~32~~. The circular RNA of Claim 21~~26~~, wherein the protein coding region encodes a chimeric antigen receptor.

28~~33~~. The composition of Claim 17~~22~~, which is formulated for intravenous delivery.

29~~34~~. A method of making circular RNA, said method comprising preparing the vector of Claim 15~~20~~, and transcribing the vector.

30~~35~~. A vector for making circular RNA, said vector comprising the following elements operably connected to each other and arranged in the following sequence:

a) a 5’ homology arm,

b) a 3’ Group I intron fragment containing a 3’ splice site dinucleotide,

c) a 5’ permissive spacer sequence,

d) an internal ribosome entry site (IRES),

e) a protein coding region encoding a chimeric antigen receptor,

f) a 3’ permissive spacer sequence,

g) a 5’ Group I intron fragment containing a 5’ splice site dinucleotide, and

h) a 3’ homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells.

31~~36~~. The circular RNA of Claim 18~~23~~ which is at least 1 kb.

32~~37~~. A composition comprising:

a) the vector for making circular RNA of Claim 30~~35~~, and

b) a delivery vehicle for delivering the vector to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

33~~38~~. A circular RNA produced by the vector of Claim 30~~35~~, wherein the vector is used to transcribe circular RNA with a size of at least 500 nucleotides.

34~~39~~. The circular RNA of Claim 33~~38~~, wherein the circular RNA comprises in the following order, ~~a portion of a 3’ group I intron fragment,~~ the 5’ permissive spacer sequence, ~~an~~ the Internal Ribosome Entry Site (IRES), a protein coding region encoding a chimeric antigen receptor, and the 3’ permissive spacer sequence. ~~a portion of a 5’ group I intron fragment.~~

35~~40~~. A composition comprising:

a) an effective amount of the circular RNA of Claim 33~~38~~, and

b) a delivery vehicle for delivering the circular RNA to a cell, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

3641. The composition of Claim 3540, wherein the composition is a nanoparticle formulation.

3742. A method for making a cell population comprising circular RNA, said method comprising contacting the cells in the population with a composition comprising:

a) circular RNA transcribed from a vector comprising the following elements operably connected to each other and arranged in the following sequence:

i) a 5' homology arm,

ii) a 3' Group I intron fragment containing a 3' splice site dinucleotide,

iii) a 5' permissive spacer sequence,

iv) an internal ribosome entry site (IRES),

v) a protein coding region,

vi) a 3' permissive spacer sequence,

vii) a 5' Group I intron fragment containing a 5' splice site dinucleotide, and

viii) a 3' homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells, and

b) a delivery vehicle for delivering the circular RNA to the cells, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

3843. A method for making a cell population comprising circular RNA, said method comprising contacting the cells in the population with a composition comprising:

a) a vector comprising the following elements operably connected to each other and arranged in the following sequence:

i) a 5' homology arm,

ii) a 3' Group I intron fragment containing a 3' splice site dinucleotide,

iii) a 5' permissive spacer sequence,

iv) an internal ribosome entry site (IRES),

v) a protein coding region,

vi) a 3' permissive spacer sequence,

vii) a 5' Group I intron fragment containing a 5' splice site dinucleotide, and

viii) a 3' homology arm,

said vector allowing production of a circular RNA that is translatable or biologically active inside eukaryotic cells, and

b) a delivery vehicle for delivering the vector to the cells, said delivery vehicle comprising a nanocarrier selected from the group consisting of a lipid, a polymer and a lipo-polymeric hybrid.

3944. The method of Claim 3742, wherein the circular RNA comprises in the following order, ~~a portion of a 3' group I intron fragment,~~ the 5' permissive spacer sequence, ~~an~~ the Internal Ribosome Entry Site (IRES), ~~a~~ the protein coding region, and the 3' permissive spacer sequence.~~a portion of a 5' group I intron fragment.~~

4045. The method of Claim 3944, wherein the protein coding region encodes a chimeric antigen receptor (CAR).

4146. The method of Claim 3742, wherein the protein coding region encodes a therapeutic protein, a chimeric antigen receptor, an antibody or a bispecific antibody.

4247. Use of ~~A method of expressing a protein in~~ a cell comprising ~~delivering to the cell~~ the circular RNA of Claim 1823 in the manufacture of a medicament for the treatment of a patient in need thereof, wherein the cell expresses a protein encoded by the protein coding region.

4348. Use of ~~A method of expressing a protein in~~ a cell comprising ~~delivering to the cell~~ the circular RNA of Claim 2126 in the manufacture of a medicament for the treatment of a patient in need thereof, wherein the cell expresses a protein encoded by the protein coding region.

4449. Use of ~~A method of expressing a protein in~~ a cell comprising ~~delivering to the cell~~ the circular RNA of Claim 3338 in the manufacture of a medicament for the treatment of a patient in need thereof, wherein the cell expresses a protein encoded by the protein coding region.