CLAIMS

1. A recombinant RNA replicon comprising:
   a picornavirus genome, wherein the picornavirus genome comprises a deletion or a truncation in one or more protein coding regions; and
   a heterologous polynucleotide.
2. The recombinant RNA replicon of claim 1, wherein the picornavirus genome comprises the deletion or the truncation in one or more VP coding regions.
3. The recombinant RNA replicon of claim 1 or 2, wherein the picornavirus genome comprises the deletion or the truncation in each of the VP1, VP3 and VP2 coding regions.
4. The recombinant RNA replicon of any one of claims 1-3, wherein the picornavirus genome comprises the deletion of the VP1 and VP3 coding regions and the truncation of the VP2 coding region.
5. The recombinant RNA replicon of any one of claims 1-4, wherein the picornavirus is selected from a senecavirus, a cardiovirus, and an enterovirus.
6. The recombinant RNA replicon of any one of claims 1-5, wherein the deletion or the truncation comprises at least 500 bp, at least 1000 bp, at least 1500 bp, at least 2000 bp, at least 2500 bp, or at least 3000 bp.
7. The recombinant RNA replicon of claim~~s~~ 6, wherein the deletion or the truncation comprises at least 2000 bp.
8. The recombinant RNA replicon of any one of claims 1-7, wherein a site of the deletion or a site of the truncation comprises the heterologous polynucleotide, or
9. ~~The recombinant RNA replicon of any one of claims 1-7,~~ wherein the heterologous polynucleotide is inserted between a 2A coding region and a 2B coding region, or
   ~~.~~
10. ~~The recombinant RNA replicon of any one of claims 1-7, wherein the heterologous polynucleotide is inserted~~ between a 3D coding region and a 3' untranslated region (UTR).
11. ~~The recombinant RNA replicon of any one of claims 1-10, wherein the heterologous polynucleotide comprises at least 1000bp, at least 2000 bp, or at least 3000 bp.~~
12. The recombinant RNA replicon of any one of claims 1-~~11~~8, wherein the picornavirus is a Seneca Valley Virus (SVV).

~~13. The recombinant RNA replicon of claim 12, wherein the deletion or the truncation comprises one or more nucleotides between nucleotide 1261 and 3477, inclusive of the endpoints, according to the numbering of SEQ ID NO: 1.~~

~~14. The recombinant RNA replicon of claim 12, wherein the deletion or the truncation comprises nucleotide 1261 to 3477, inclusive of the endpoints, according to the numbering of SEQ ID NO: 1.~~

~~15. The recombinant RNA replicon of claims 12 or 13, wherein the deletion or the truncation comprises at least 500 bp, at least 1000 bp, at least 1500 bp, or at least 2000 bp.~~

~~16. The recombinant RNA replicon of claim 15, wherein the deletion or the truncation comprises at least 2000 bp.~~

~~17. The recombinant RNA replicon of any one of claims 12 to 16, wherein the SVV genome comprises a 5' leader protein coding sequence.~~

~~18. The recombinant RNA replicon of any one of claims 12 to 17, wherein the SVV genome comprises a VP4 coding region.~~

~~19. The recombinant RNA replicon of any one of claims 12 to 18, wherein the SVV genome comprises a VP2 coding region or a truncation thereof.~~

~~20~~10. The recombinant RNA replicon of claim ~~1~~9, wherein the SVV genome comprises, from 5' to 3' direction, the 5' leader protein coding sequence, the VP4 coding region, and the VP2 coding region or a truncation thereof, and ~~.~~

~~21. The recombinant RNA replicon of claim 20,~~ wherein a portion of the SVV genome comprising the 5' leader protein coding sequence, the VP4 coding region, and the VP2 coding region or a truncation thereof has at least 90% sequence identity to nucleotide 1 to 1260 of SEQ ID NO: 1.

~~22. The recombinant RNA replicon of claim 20 or 21, wherein the SVV genome comprises, from 5' to 3' direction, the 5' leader protein coding sequence, the VP4 coding region, the VP2 coding region or a truncation thereof, and the heterologous polynucleotide.~~

~~23~~11. The recombinant RNA replicon of any one of claims 1-~~22~~10, wherein the SVV genome comprises a cis-acting replication element (CRE), and ~~.~~

~~24. The recombinant RNA replicon of claim 23, wherein the CRE comprises between 10-200 bp.~~

~~25. The recombinant RNA replicon of claim 23 or 24,~~ wherein the CRE comprises one or more nucleotides within the region corresponding to nucleotide 1000 to nucleotide 1260 according to SEQ ID NO: 1.

~~26. The recombinant RNA replicon of claim 23 or 24, wherein the CRE comprises one or more nucleotides within the region corresponding to nucleotide 1117 to nucleotide 1260 according to SEQ ID NO: 1.~~

~~27~~12. The recombinant RNA replicon of ~~any one of~~ claim 11~~s 23-26~~, wherein the CRE comprises a polynucleotide sequence having at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 149.

~~28. The recombinant RNA replicon of any one of claims 12-27, wherein the SVV genome further comprises a 2A coding region.~~

~~29. The recombinant RNA replicon of claim 28, wherein the 2A coding region is located between the VP2 coding region or a truncation thereof and the heterologous polynucleotide.~~

~~30. The recombinant RNA replicon of any one of claims 12-29, wherein the SVV genome comprises one or more of a 2B coding region, a 2C coding region, a 3A coding region, a 3B coding region, a 3Cpro coding region, and a 3D(RdRp) coding region.~~

~~31. The recombinant RNA replicon of any one of claims 12-29, wherein the SVV genome comprises a 2B coding region, a 2C coding region, a 3A coding region, a 3B coding region, a 3Cpro coding region, and a 3D(RdRp) coding region.~~

~~32. The recombinant RNA replicon of claim 31, wherein the SVV genome comprises, from 5' to 3', the 2B coding region, the 2C coding region, the 3A coding region, the 3B coding region, the 3Cpro coding region, and the 3D(RdRp) coding region.~~

~~33. The recombinant RNA replicon of claim 32, wherein a portion of the SVV genome comprising the 2B coding region, the 2C coding region, the 3A coding region, the 3B coding region, the 3Cpro coding region, and the 3D(RdRp) coding region has at least 90% sequence identity to nucleotide 3505 to 7310 according to SEQ ID NO: 1.~~

~~34. The recombinant RNA replicon of any one of claims 30-33, wherein the SVV genome comprises, from 5' to 3', the heterologous polynucleotide and the 2B coding region.~~

~~35~~13. The recombinant RNA replicon of any one of claims 1 to ~~11~~8, wherein the picornavirus is a coxsackievirus.

~~36~~14. The recombinant RNA replicon of claim 13~~35~~, wherein the deletion or the truncation comprises at least 500 bp, at least 1000bp, at least 1500 bp, at least 2000 bp, or at least 2600 bp~~one or more~~ nucleotides between nucleotide 717 to 3332, inclusive of the endpoints, according to the numbering of SEQ ID NO: 3.

~~37. The recombinant RNA replicon of claim 35, wherein the deletion or the truncation comprises nucleotide 717 to 3332 , inclusive of the endpoints, according to the numbering of SEQ ID NO: 3.~~

~~38. The recombinant RNA replicon of claim 35 or 36, wherein the deletion or the truncation comprises at least 500 bp, at least 1000bp, at least 1500 bp, at least 2000 bp, or at least 2600 bp.~~

~~39~~15. The recombinant RNA replicon of any one of claims 13 or 14~~35 to 38~~, wherein the coxsackievirus genome comprises a 5' UTR, and ~~.~~

~~40. The recombinant RNA replicon of any one of claims 35 to 39,~~ wherein a portion of the coxsackievirus genome comprising the 5' UTR has at least 90% sequence identity to SEQ ID NO: 4.

~~41. The recombinant RNA replicon of any one of claims 35 to 40, wherein the coxsackievirus genome comprises one or more of a 2A coding region, a 2B coding region, a 2C coding region, a 3A coding region, a 3B coding region, a VPg coding region, a 3C coding region, a 3D pol coding region, and a 3' UTR.~~

~~42. The recombinant RNA replicon of any one of claims 35 to 40, wherein the coxsackievirus genome comprises a 2A coding region, a 2B coding region, a 2C coding region, a 3A coding region, a 3B coding region, a VPg coding region, a 3C coding region, a 3D pol coding region, and a 3' UTR.~~

~~43. The recombinant RNA replicon of claim 42, wherein the coxsackievirus genome comprises, from 5' to 3' direction, the 2A coding region, the 2B coding region, the 2C coding region, the 3A coding region, the 3B coding region, the VPg coding region, the 3C coding region, the 3D pol coding region, and the 3' UTR.~~

~~44. The recombinant RNA replicon of claim 42, wherein a portion of the coxsackievirus genome comprising the 2A coding region, the 2B coding region, the 2C coding region, the 3A coding region, the 3B coding region, the VPg coding region, the 3C coding region, the 3D pol coding region, and the 3' UTR has at least 90% sequence identity to nucleotide 3492 to 7435 in SEQ ID NO: 3.~~

~~45. The recombinant RNA replicon of any one of claims 41 to 44, wherein the coxsackievirus genome comprises, from 5' to 3', the 5' UTR, the heterologous polynucleotide, and the 2A coding region.~~

~~46. The recombinant RNA replicon of any one of claims 1 to 11, wherein the picornavirus is an encephalomyocarditis virus (EMCV).~~

~~47~~16. The recombinant RNA replicon of any one of claims 8~~9 and 11~~-15~~46~~, wherein the recombinant RNA replicon comprises an internal ribosome entry site (IRES) inserted between the heterologous polynucleotide and the 2B coding region.

~~48~~17. The recombinant RNA replicon of any one of claims 1 to ~~47~~16, wherein the heterologous polynucleotide encodes one or more payload molecules, or ~~.~~

~~49. The recombinant RNA replicon of any one of claims 1 to 47, wherein the heterologous polynucleotide encodes~~ two or more payload molecules.

~~50~~18. The recombinant RNA replicon of claim ~~49~~17, wherein the two or more payload molecules are operably linked by one or more cleavage polypeptides, and wherein ~~.~~

~~51. The recombinant RNA replicon of claim 50, wherein~~ the cleavage polypeptide comprises a 2A family self-cleaving peptide, a 3C cleavage site, a furin site, an IGSF1 polypeptide, or a HIV protease site.

~~52. The recombinant RNA replicon of claim 51, wherein the cleavage polypeptide comprises an IGSF1 polypeptide, and wherein the IGSF1 polypeptide comprises an amino acid sequence having at least 90% identity to SEQ ID NO: 75.~~

~~53. The recombinant RNA replicon of claim 51, wherein the cleavage polypeptide comprises an HIV protease site.~~

~~54. The recombinant RNA replicon of claim 51, wherein the cleavage polypeptide comprises a 2A family self-cleaving peptide.~~

~~55. The recombinant RNA replicon of any one of claims 50 to 54, wherein the cleavage polypeptide comprises a furin site.~~

~~56. The recombinant RNA replicon of any one of claims 50 to 55, wherein the heterologous polynucleotide encodes a polypeptide comprising the two or more payload molecules and the cleavage polypeptide comprising, from N-terminus to C-terminus: N' payload molecule 1 cleavage polypeptide payload molecule 2 C'.~~

~~57. The recombinant RNA replicon of claim 53, wherein the heterologous polynucleotide further comprises a coding region that encodes an HIV protease,~~

~~and wherein the heterologous polynucleotide comprises a coding region that encodes a polypeptide comprising, from N-terminus to C-terminus: N' – Payload molecule 1 – HIV protease site – HIV protease – HIV protease site – Payload molecule 2 – C'.~~

~~58. The recombinant RNA replicon of claim 57, wherein the heterologous polynucleotide further comprises a coding region that encodes a third payload molecule, and wherein the heterologous polynucleotide comprises a coding region that encodes a polypeptide comprising, from N-terminus to C-terminus:~~

~~N' – Payload molecule 1 – HIV protease site – HIV protease – HIV protease site – Payload molecule 2 – HIV protease site – Payload molecule 3 – C'.~~

~~59. The recombinant RNA replicon of any one of claims 56 to 58, further comprising a cleavage polypeptide at the C-terminus of the encoded polypeptide.~~

~~60. The recombinant RNA replicon of any one of claim 48 to 59, wherein the payload molecules are selected from a fluorescent protein, an enzyme, a cytokine, a chemokine, an antigen, an antigen-binding molecule capable of binding to a cell surface receptor, and a ligand for a cell-surface receptor.~~

~~61~~19. The recombinant RNA replicon of any one of claims ~~48~~17 to ~~59~~18, wherein the payload molecules are selected from:

a) one or more cytokines comprising IFNγ, GM-CSF, IL-2, IL-12, IL-15, IL-18, IL-23, and IL-36γ;

b) one or more chemokines comprising CXCL10, CCL4, CCL5, and CCL21;

c) one or more antibodies comprising an anti-PD1-VHH-Fc antibody, an anti-CD47-VHH-Fc antibody, and an anti-TGFβ-VHH(or scFv)-Fc antibody;

d) one or more bipartite polypeptides comprising a bipartite polypeptide binding to DLL3 and an effector cell target antigen, a bipartite polypeptide binding to FAP and an effector cell target antigen, and a bipartite polypeptide binding to EpCAM and an effector cell target antigen;

e) one or more tumor-associated antigens comprising survivin, MAGE family proteins, and all antigens according to Table 6;

f) one or more tumor neoantigens;

g) one or more bipartite polypeptides binding to MHC-peptide antigen complex;

h) one or more fusogenic proteins comprising herpes simplex virus (HSV) UL27/glycoprotein B/gB, HSV UL53/glycoprotein K/gK, Respiratory syncytial virus (RSV) F protein, FASTp15, VSV-G, syncitin-1 (from human endogenous

retrovirus-W (HERV-W)) or syncitin-2 (from HERVFRDE1), paramyxovirus SV5-F, measles virus-H, measles virus-F, and the glycoprotein from a retrovirus or lentivirus, such as gibbon ape leukemia virus (GALV), murine leukemia virus (MLV), Mason-Pfizer monkey virus (MPMV) and equine infectious anemia virus (EIAV), optionally with the R transmembrane peptide removed (R- versions);

i) one or more other payload molecules comprising IL15R, PGDH, ADA, ADA2, HYAL1, HYAL2, CHIPS, MLKL (or its 4HB domain only), GSDMD (or its L192A mutant, or its amino acids 1-233 fragment, or its amino acids 1-233 fragment with L192A mutation), GSDME (or its amino acid 1-237 fragment), HMGB1 (or its Box B domain only), Melittin (e.g., alpha-Melittin), SMAC/Diablo (or its amino acid 56-239 fragment), Snake LAAO, Snake disintegrin, Leptin, FLT3L, TRAIL, Gasdermin D or a truncation thereof, and Gasdermin E or a truncation thereof;

j) one or more antigens from pathogens comprising Dengue virus, Chikungunya virus, Mycobacterium tuberculosis, Human immunodeficiency viruses, SARS-CoV-2, Coronavirus, Hepatitis B Virus, Togaviridae family virus, Flaviviridae family virus, Influenza A virus, Influenza B virus, and a veterinary virus; and~~or~~

k) any combination thereof.

~~62. The recombinant RNA replicon of any one of claims 49 to 59, wherein the two or more payload molecules are selected from the group consisting of a fluorescent protein, an enzyme, a cytokine, a chemokine, an antigen-binding molecule capable of binding to a cell surface receptor, and a ligand for a cell surface receptor.~~

~~63. The recombinant RNA replicon of any one of claims 49 to 59, wherein the heterologous polynucleotide encodes two or more payload molecules comprising:~~

~~a. IL-2 and IL-36γ;~~

~~b. CXCL10 and an antigen binding molecule binding to FAP and CD3;~~

~~c. IL-2 and an antigen binding molecule binding to DLL3 and CD3;~~

~~d. IL-36γ and an antigen binding molecule binding to DLL3 and CD3; or~~

~~e. IL-2, IL-36γ and an antigen binding molecule binding to DLL3 and CD3.~~

~~64~~20. The recombinant RNA replicon of any one of claims 1 to ~~63~~19, further comprising a microRNA (miRNA) target sequence (miR-TS) cassette comprising one or more miRNA target sequences~~.~~

~~65. The recombinant RNA replicon of claim 64, wherein the one or more miRNAs comprise miR-124, miR-1, miR-143, miR-128, miR-219, miR-219a, miR-122, miR-204, miR-217, miR-137, and miR-126~~.

~~66~~21. A recombinant DNA molecule comprising, from 5' to 3', a promoter sequence, a 5' junctional cleavage sequence, a polynucleotide sequence encoding the recombinant RNA replicon of any one of claims 1-~~65~~20, and a 3' junctional cleavage sequence.

~~67. The recombinant DNA molecule of claim 66, wherein the promoter sequence is a T7 promoter sequence.~~

~~68. The recombinant DNA molecule of claim 66 or 67, wherein the 5' junctional cleavage sequence is a ribozyme sequence and the 3' junctional cleavage sequence is a ribozyme sequence.~~

~~69. The recombinant DNA molecule of claim 68, wherein the 5' ribozyme sequence is a hammerhead ribozyme sequence and wherein the 3' ribozyme sequence is a hepatitis delta virus ribozyme sequence.~~

~~70. The recombinant DNA molecule of claim 66 or 67, wherein the 5' junctional cleavage sequence is a ribozyme sequence and the 3' junctional cleavage sequence is a restriction enzyme recognition sequence.~~

~~71. The recombinant DNA molecule of claim 70, wherein the 5' ribozyme sequence is a hammerhead ribozyme sequence, a Pistol ribozyme sequence, or a modified Pistol ribozyme sequence.~~

~~72. The recombinant DNA molecule of claim 70 or 71, wherein 3' restriction enzyme recognition sequence is a Type IIS restriction enzyme recognition sequence.~~

~~73. The recombinant DNA molecule of claim 72, wherein the Type IIS recognition sequence is a SapI recognition sequence.~~

~~74. The recombinant DNA molecule of claim 66 or 67, wherein the 5' junctional cleavage sequence is an RNAseH primer binding sequence and the 3' junctional cleavage sequence is a restriction enzyme recognition sequence.~~

~~75~~22. A method of producing ~~the~~ a recombinant RNA replicon ~~of any one of claims 1-65~~, comprising in vitro transcription of the DNA molecule of ~~any one of~~ claim 21~~s 66-74~~ and purification of the resulting recombinant RNA replicon.

~~76. A composition comprising an effective amount of the recombinant RNA replicon of any one of claims 1-65, and a carrier suitable for administration to a mammalian subject.~~

~~77~~23. A vector comprising the recombinant RNA replicon of any one of claims 1-~~65~~20.

~~78. The vector of claim 77, wherein the vector is a viral vector.~~

~~79. The vector of claim 77, wherein the vector is a non-viral vector.~~

~~80~~24. A particle comprising the recombinant RNA replicon of any one of claims 1-~~65~~20.

~~81~~25. The particle of claim ~~80~~24, wherein the particle is a lipid nanoparticle (LNP).~~selected from the group consisting of a nanoparticle, an exosome, a liposome, and a lipoplex.~~

~~82. The particle of claim 81, wherein the nanoparticle is a lipid nanoparticle (LNP) comprising a cationic lipid, one or more helper lipids, and a phospholipid-polymer conjugate.~~

~~83. The particle of claim 82, wherein the cationic lipid is selected from DLinDMA, DLin-KC2-DMA, DLin-MC3-DMA (MC3), COATSOME® SS-LC (former name: SS-18/4PE-13), COATSOME® SS-EC (former name: SS-33/4PE-15), COATSOME® SS-OC, COATSOME® SS-OP, Di((Z)-non-2-en-1-yl)9-((4-dimethylamino)butanoyl)oxy)heptadecanedioate (L-319), or N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP).~~

~~84. The particle of claim 82 or 83, wherein the helper lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); 1,2-dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE); 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC); 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE); and cholesterol.~~

~~85. The particle of claim 82, wherein the cationic lipid is 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and wherein the neutral lipid is 1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE) or 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE).~~

~~86. The particle of any one of claims 82-85, wherein the PEG-lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)] (DSPE-PEG); 1,2-dipalmitoyl-rac-glycerol methoxypolyethylene glycol (DPG-PEG); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene (DSG-PEG); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene (DSG-PEG); 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene (DMG-PEG); and 1,2-dimyristoyl-rac-glycero-3-~~

methylpolyoxyethylene (DMG-PEG), or 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG-amine).

87. The particle of any one of claims 82-86, wherein the PEG-lipid is selected from 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyleneglycol)-5000] (DSPE-PEG5K); 1,2-dipalmitoyl-rac-glycerol methoxypolyethylene-glycol-2000 (DPG-PEG2K); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene-5000 (DSG-PEG5K); 1,2-distearoyl-rac-glycero-3-methylpolyoxyethylene-2000 (DSG-PEG2K); 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene-5000 (DMG-PEG5K); and 1,2-dimyristoyl-rac-glycero-3-methylpolyoxyethylene-2000 (DMG-PEG2K).

88. The particle of claim 82, wherein the cationic lipid comprises COATSOME® SS-OC, wherein the one or more helper lipids comprise cholesterol (Chol) and DSPC, and wherein the phospholipid-polymer conjugate comprises DPG-PEG2000.

89. The particle of claim 88, wherein the ratio of SS-OC:DSPC:Chol:DPG-PEG2K (as a percentage of total lipid content) is A:B:C:D, wherein:

a. A = 40% - 60%, B = 10% - 25%, C = 20% - 30%, and D = 0% - 3% and wherein A+B+C+D = 100%;

b. A = 45% - 50%, B = 20% - 25%, C = 25% - 30%, and D = 0% - 1% and wherein A+B+C+D = 100%

c. A = 40% - 60%, B = 10% - 30%, C = 20% - 45%, and D = 0% - 3% and wherein A+B+C+D = 100%;

d. A = 40% - 60%, B = 10% - 30%, C = 25% - 45%, and D = 0% - 3% and wherein A+B+C+D = 100%;

e. A = 45% - 55%, B = 10% - 20%, C = 30% - 40%, and D = 1% - 2% and wherein A+B+C+D = 100%;

f. A = 45% - 50%, B = 10% - 15%, C = 35% - 40%, and D = 1% - 2% and wherein A+B+C+D = 100%;

g. A = 45% - 65%, B = 5% - 20%, C = 20% - 45%, and D = 0% - 3% and wherein A+B+C+D = 100%;

h. A = 50% - 60%, B = 5% - 15%, C = 30% - 45%, and D = 0% - 3% and wherein A+B+C+D = 100%;

i. A = 55% - 60%, B = 5% - 15%, C = 30% - 40%, and D = 1% - 2% and wherein A+B+C+D = 100%;

~~j. A = 55% - 60%, B = 5% - 10%, C = 30% - 35%, and D = 1% - 2% and wherein A+B+C+D = 100%.~~

~~90. The particle of claim 88, wherein the ratio of SS-OC:DSPC:Chol:DPG-PEG2K (as a percentage of total lipid content) is:~~

~~a. about 49:22:28.5:0.5;~~

~~b. about 49:11:38.5:1.5; or~~

~~c. about 58:7:33.5:1.5.~~

~~91. The particle of claim 88, wherein the ratio of SS-OC:DSPC:Chol:DPG-PEG2K (as a percentage of total lipid content) is about 49:22:28.5:0.5.~~

~~92. The particle of claim 82, wherein the cationic lipid is 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), and wherein the neutral lipid is 1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE) or 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE).~~

~~93. The particle of claim 82 or 92, further comprising a PEG-lipid, wherein the PEG-lipid is 1, 2-Distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol) (DSPE-PEG) or 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] (DSPE-PEG-amine).~~

~~94~~26. The particle of any one of claims ~~80~~24-~~93~~25, further comprising a second recombinant RNA molecule encoding ~~an oncolytic virus.~~

~~95. The particle of claim 94, wherein the oncolytic virus is a picornavirus.~~

~~96. The particle of claim 95, wherein the picornavirus is selected from a senecavirus, a cardiovirus, and an enterovirus.~~

~~97. The particle of claim 95, wherein the picornavirus is~~ a Seneca Valley Virus (SVV) or ~~.~~

~~98. The particle of claim 95, wherein the picornavirus is~~ a Coxsackievirus.

~~99. The particle of claim 95, wherein the picornavirus is an encephalomyocarditis virus (EMCV).~~

~~100~~27. A therapeutic composition comprising an effective amount of the recombinant RNA replicon of any one of claims 1-20 or a plurality of ~~lipid nano~~particles according to any one of claims ~~82~~24-~~99~~26.

~~101. The therapeutic composition of claim 100 wherein the plurality of LNPs have an average size of about 50 nm to about 120 nm.~~

~~102. The therapeutic composition of claim 100 wherein the plurality of LNPs have an average size of about 100 nm.~~

~~103. The therapeutic composition of any one of claims 100-102, wherein the plurality of LNPs have an average zeta potential of between about 20 mV to about -20 mV, about 10 mV to about -10 mV, about 5 mV to about -5 mV, or about 20 mV to about -40 mV, -50 mV to about -20 mV, about -40 mV to about -20 mV, or about -30 mV to about -20 mV.~~

~~104. The therapeutic composition of claim 103, wherein the plurality of LNPs have an average zeta potential of about -30 mV, about -31 mV, about -32 mV, about -33 mV, about -34 mV, about -35 mV, about -36 mV, about -37 mV, about -38 mV, about -39 mV, or about -40 mV.~~

~~105~~28. A method of killing a cancerous cell comprising exposing the cancerous cell to the particle of any one of claims ~~80~~24-~~97~~26, the vector of ~~any one of~~ claim~~s~~ 23~~77-79~~, the recombinant RNA replicon of any one of claims 1-~~65~~20, or compositions thereof.

~~106. The method of claim 105, wherein the method is performed in vivo, in vitro, or ex vivo.~~

~~107~~29. A method of treating a cancer in a subject comprising administering to the subject suffering from the cancer an effective amount of the particle of any one of claims ~~80~~24-~~97~~26, the vector of ~~any one of~~ claim~~s~~ 23~~77-79~~, the recombinant RNA replicon of any one of claims 1-~~65~~20, or compositions thereof.

~~108~~30. The method of claim ~~107~~29, wherein the particle, the recombinant RNA replicon, or composition thereof is administered intravenously, intranasally, as an inhalant, or is injected directly into a tumor.

~~109~~31. The method of claim ~~107~~29 or ~~108~~30, wherein the particle, the recombinant RNA replicon, or composition thereof is administered to the subject repeatedly.

~~110. The method of any of claims 107-109, wherein the subject is a mouse, a rat, a rabbit, a cat, a dog, a horse, a non-human primate, or a human.~~

~~111~~32. The method of ~~any of~~ claim~~s~~ ~~107~~31-~~110~~, wherein the cancer is selected from lung cancer, breast cancer, ovarian cancer, cervical cancer, prostate cancer, testicular cancer, colorectal cancer, colon cancer, pancreatic cancer (e.g., Castration resistant neuroendocrine prostate cancer), liver cancer, gastric cancer, head and neck cancer, thyroid cancer, malignant glioma, glioblastoma, melanoma, B-cell chronic lymphocytic leukemia, diffuse large B-cell lymphoma (DLBCL), sarcoma, a neuroblastoma, a neuroendocrine cancer, a rhabdomyosarcoma, a

medulloblastoma, a bladder cancer, marginal zone lymphoma (MZL), Merkel cell carcinoma, and renal cell carcinoma.

~~112~~33. The method of claim ~~111~~32, wherein:

a. the lung cancer is small cell lung cancer or non-small cell lung cancer;

b. the liver cancer is hepatocellular carcinoma (HCC); and/or

c. the prostate cancer is treatment-emergent neuroendocrine prostate cancer.

~~113~~34. The method of claims ~~111~~32, wherein the cancer is a neuroendocrine cancer.

~~114~~35. A method of immunizing a subject against a disease, comprising administering to the subject an effective amount of the particle of any one of claims ~~80~~24-~~97~~26, the vector of ~~any one of~~ claim~~s 77-79~~23, the recombinant RNA replicon of any one of claims 1-20~~65~~, or compositions thereof.

~~115~~36. The method of claim ~~114~~35, wherein the particle, the recombinant RNA replicon, or composition thereof is administered intravenously, intramuscularly, intradermally, intranasally, or as an inhalant.

~~116. The method of claim 114 or 115, wherein the particle, the recombinant RNA replicon, or composition thereof is administered to the subject repeatedly.~~

~~117. The method of any one of claims 114 to 116, wherein the disease is an infectious disease.~~

~~118. The method of claim 117, wherein the infectious disease is caused by one of the pathogens comprising Dengue virus, Chikungunya virus, Mycobacterium tuberculosis, Human immunodeficiency virus, SARS-CoV-2, Coronavirus, Hepatitis B virus, Togaviridae family virus, Flaviviridae family virus, Influenza A virus, Influenza B virus and a veterinary virus.~~

~~119. A recombinant RNA replicon comprising a picornavirus genome and a heterologous polynucleotide.~~

~~120. The recombinant RNA replicon of claim 119, wherein the heterologous polynucleotide is inserted between a 2A coding region and a 2B coding region.~~

~~121. The recombinant RNA replicon of claim 119, wherein the heterologous polynucleotide is inserted between a 5' UTR and a 2A coding region.~~

~~122. The recombinant RNA replicon of claim 119, wherein the heterologous polynucleotide is inserted between a 3D coding region and a 3' UTR.~~

~~123. The recombinant RNA replicon of any one of claims 119-122, wherein the picornavirus is selected from a senecavirus, a cardiovirus, and an enterovirus.~~