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 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 wherein the heterologous polynucleotide is inserted between a 2A coding region and a 2B coding region, or between a 3D coding region and a 3’ untranslated region (UTR).
9. The recombinant RNA replicon of any one of claims 1-8, wherein the picornavirus is a Seneca Valley Virus (SVV).
10. The recombinant RNA replicon of claim 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 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.

11. The recombinant RNA replicon of any one of claims 1-10, wherein the SVV genome comprises a cis-acting replication element (CRE), and wherein the CRE comprises one or more nucleotides within the region corresponding to nucleotide 1000 to nucleotide 1260 according to SEQ ID NO: 1.
12. The recombinant RNA replicon of claim 11, 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.
13. The recombinant RNA replicon of any one of claims 1 to 8, wherein the picornavirus is a coxsackievirus.
14. The recombinant RNA replicon of claim 13, 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 nucleotides between nucleotide 717 to 3332, inclusive of the endpoints, according to the numbering of SEQ ID NO: 3.
15. The recombinant RNA replicon of any one of claims 13 or 14, wherein the coxsackievirus genome comprises a 5' UTR, and wherein a portion of the coxsackievirus genome comprising the 5' UTR has at least 90% sequence identity to SEQ ID NO: 4.
16. The recombinant RNA replicon of any one of claims 8-15, wherein the recombinant RNA replicon comprises an internal ribosome entry site (IRES) inserted between the heterologous polynucleotide and the 2B coding region.
17. The recombinant RNA replicon of any one of claims 1 to 16, wherein the heterologous polynucleotide encodes one or more payload molecules, or two or more payload molecules.
18. The recombinant RNA replicon of claim 17, wherein the two or more payload molecules are operably linked by one or more cleavage polypeptides, and 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.
19. The recombinant RNA replicon of any one of claims 17 to 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

k) any combination thereof.

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

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 1-20, and a 3’ junctional cleavage sequence.
22. A method of producing a recombinant RNA replicon, comprising in vitro transcription of the DNA molecule of claim 21 and purification of the resulting recombinant RNA replicon.
23. A vector comprising the recombinant RNA replicon of any one of claims 1-20.
24. A particle comprising the recombinant RNA replicon of any one of claims 1-20.
25. The particle of claim 24, wherein the particle is a lipid nanoparticle (LNP).
26. The particle of any one of claims 24-25, further comprising a second recombinant RNA molecule encoding a Seneca Valley Virus (SVV) or a Coxsackievirus.
27. A therapeutic composition comprising an effective amount of the recombinant RNA replicon of any one of claims 1-20 or a plurality of particles according to any one of claims 24-26.
28. A method of killing a cancerous cell comprising exposing the cancerous cell to the particle of any one of claims 24-26, the vector of claim 23, the recombinant RNA replicon of any one of claims 1-20, or compositions thereof.
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 24-26, the vector of claim 23, the recombinant RNA replicon of any one of claims 1-20, or compositions thereof.
30. The method of claim 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.
31. The method of claim 29 or 30, wherein the particle, the recombinant RNA replicon, or composition thereof is administered to the subject repeatedly.
32. The method of claim 31, 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.

33. The method of claim 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.
34. The method of claims 32, wherein the cancer is a neuroendocrine cancer.
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 24-26, the vector of claim 23, the recombinant RNA replicon of any one of claims 1-20, or compositions thereof.
36. The method of claim 35, wherein the particle, the recombinant RNA replicon, or composition thereof is administered intravenously, intramuscularly, intradermally, intranasally, or as an inhalant.