CLAIMS

What is claimed is:

~~1. An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta.~~

~~2. The RNA of claim 1, wherein the extracellular domain comprises an antigen binding moiety.~~

~~3. The RNA of claim 2, wherein the antigen bind moiety binds to a tumor antigen.~~

~~4. The RNA of claim 3, wherein the tumor antigen is an antigen associated with a cancer selected from the group consisting of brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, liver cancer, kidney cancer, lymphoma, leukemia, lung cancer, melanoma, metastatic melanoma, mesothelioma, neuroblastoma, ovarian cancer, prostate cancer, pancreatic cancer, renal cancer, skin cancer, thymoma, sarcoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, uterine cancer, and any combination thereof.~~

~~5.~~1. ~~The RNA of claim 1~~ An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.ss1.OF.BBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 4.

~~6.~~2. ~~The RNA of claim 1~~ An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting SEQ ID NO: 6, and SEQ ID NO: 8.

~~7.~~3. ~~The RNA of claim 1~~ An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.19.OF.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 5.

~~8.~~4. ~~The RNA of claim 1~~ An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24.

~~9.~~5. ~~The RNA of claim 1~~ An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.GD2.OF.8TMBBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 28.

~~10.~~6. ~~The RNA of claim 1~~ An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13.

~~11.~~7. ~~The RNA of claim 1~~ An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.cMet.OF.8TMBBZ.2bgUTR.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 27.

~~12. The RNA of claim 1~~8. An *in vitro* transcribed RNA or synthetic RNA comprising a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA is transcribed comprises a

sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18.

~~13. The RNA of claim 1, w*herein t*he costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof~~.

~~14. The RNA of claim 1, wherein the nucleic acid sequence comprises a poly(A) tail comprising about 150 adenosine bases.~~

~~15. The RNA of claim 1, wherein the nucleic acid sequence comprises a 3'UTR comprising at least one repeat of a 3'UTR derived from human beta-globulin.~~

~~16. A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta.~~

~~17. The T cell of claim 16, wherein the extracellular domain comprises an antigen binding moiety.~~

~~18. The T cell of claim 17, wherein the antigen bind moiety binds to a tumor antigen.~~

~~19. The T cell of claim 18, wherein the tumor antigen is an antigen associated with a cancer selected from the group consisting of brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, liver cancer, kidney cancer, lymphoma, leukemia, lung cancer, melanoma, metastatic melanoma, mesothelioma, neuroblastoma, ovarian cancer, prostate cancer, pancreatic cancer, renal cancer, skin cancer, thymoma, sarcoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, uterine cancer, and any combination thereof.~~

~~20.~~ 9.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.ss1.OF.BBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 4.

~~21.~~ 10.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA

is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 6 and SEQ ID NO: 8.

~~22.~~ 11.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector wherein the vector is pD-A.19.OF.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 5.

~~23~~.12.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID

NO: 7, SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24.

~~24.~~ 13.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.GD2.OF.8TMBBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 28.

~~25.~~ 14.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13.

~~26.~~ 15.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.cMet.OF.8TMBBZ.2bgUTR.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 27.

~~27.~~ 16.~~The T cell of claim 16~~ A T cell comprising an *in vitro* transcribed RNA or synthetic RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta, wherein the DNA from which the RNA

is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18.

~~28. The T cell of claim 16, wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.~~

~~29. The T cell of claim 16, wherein the nucleic acid sequence comprises a poly(A) tail comprising about 150 adenosine bases.~~

~~30. The T cell of claim 16, wherein the nucleic acid sequence comprises a 3'UTR comprising at least one repeat of a 3'UTR derived from human beta-globulin.~~

~~31.A method of generating a population of RNA-engineered T cells transiently expressing exogenous RNA, the method comprising introducing an *in*~~

vitro transcribed RNA or synthetic RNA into a T cell, wherein the RNA comprises a nucleic acid sequence encoding an extracellular domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta.

32. *The m*ethod of claim 31, wherein the extracellular domain comprises an antigen binding moiety.

33. The method of claim 32, wherein the antigen bind moiety binds to a tumor antigen.

34. The method of claim 33, wherein the tumor antigen is *an* antigen associated with a cancer selected from the group consisting of brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, liver cancer, kidney cancer, lymphoma, leukemia, lung cancer, melanoma, metastatic melanoma, mesothelioma, neuroblastoma, ovarian cancer, prostate cancer, pancreatic cancer, renal cancer, skin cancer, thymoma, sarcoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, uterine cancer, and any combination thereof.

35. The method of claim 31, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.ss1.OF.BBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 4.

36. The method of claim 31, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 6 and SEQ ID NO: 8.

37. The method of claim 31, wherein the RNA is transcribed from an *in vitro* transcription vector wherein the vector is pD-A.19.OF.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 5.

38 . The method of claim 31, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ

~~ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24.~~

~~39. The method of claim 31, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.GD2.OF.8TMBBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 28.~~

~~40. The method of claim 31, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13.~~

~~41. The method of claim 31, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.cMet.OF.8TMBBZ.2bgUTR.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 27.~~

~~42. The method of claim 31, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18.~~

~~43. The method of claim 31, wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.~~

~~44. The method of claim 31, wherein the nucleic acid sequence comprises a poly(A) tail comprising about 150 adenosine bases.~~

~~45. The method of claim 31, wherein the nucleic acid sequence comprises a 3'UTR comprising at least one repeat of a 3'UTR derived from human beta-globulin.~~

~~46. A method of treating a cancer patient, the method comprising administering to the patient a T cell engineered to transiently express exogenous RNA, wherein the RNA comprises a nucleic acid sequence encoding an extracellular~~

~~domain, a transmembrane domain, a costimulatory signaling region, and a signaling domain of CD3-zeta.~~

~~47. The method of claim 46, wherein the extracellular domain comprises an antigen binding moiety.~~

~~48. The method of claim 47, wherein the antigen bind moiety binds to a tumor antigen.~~

~~49. The method of claim 48, wherein the tumor antigen is an antigen associated with a cancer selected from the group consisting of brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, liver cancer, kidney cancer, lymphoma, leukemia, lung cancer, melanoma, metastatic melanoma, mesothelioma, neuroblastoma, ovarian cancer, prostate cancer, pancreatic cancer, renal cancer, skin cancer, thymoma, sarcoma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, uterine cancer, and any combination thereof.~~

~~50. The method of claim 46 comprising repeating the administration of a T cell.~~

~~51. The method of claim 46 comprising administering a chemotherapeutic agent to the patient.~~

~~52. The method of claim 46, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.ss1.OF.BBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 4.~~

~~53. The method of claim 46, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 6 and SEQ ID NO: 8.~~

~~54. The method of claim 46, wherein the RNA is transcribed from an *in vitro* transcription vector wherein the vector is pD-A.19.OF.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 5.~~

~~55. The method of claim 46, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24.~~

~~56. The method of claim 46, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.GD2.OF.8TMBBZ.2bg.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 28.~~

~~57. The method of claim 46, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 and SEQ ID NO: 13.~~

~~58. The method of claim 46, wherein the RNA is transcribed from an *in vitro* transcription vector, wherein the vector is pD-A.cMet.OF.8TMBBZ.2bgUTR.150A, wherein the vector comprises the nucleic acid sequence of SEQ ID NO: 27.~~

~~59. The method of claim 46, wherein the DNA from which the RNA is transcribed comprises a sequence selected from the group consisting of SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18.~~

~~60. The method of claim 46, wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.~~

~~61. The method of claim 46, wherein the nucleic acid sequence comprises a poly(A) tail comprising 150 adenosine bases.~~

~~62. The method of claim 46, wherein the nucleic acid sequence comprises a 3'UTR comprising at least one repeat of a 3'UTR derived from human beta-globulin.~~