

stimulatory signals during activation of exogenously expressed TCRs by antigens. However, existing recombinant DNA strategies often suffer from reduced cell surface expression of the chimeric proteins combining two co-stimulatory signaling domains, preventing realization of the functional potential of the chimeric proteins. The present application addresses such needs.

5 The present application discloses third-generation chimeric T cell co-stimulatory molecules that incorporate two signaling domains from CD28 and TNFR families and express at significantly improved levels than what have been conventionally reported for enhanced T cell functions, and methods of making the co-stimulatory molecules.

10 SUMMARY

Provided herein are novel chimeric co-stimulatory intracellular domains. The chimeric co-stimulatory intracellular domains provided herein comprise: (a) a first signaling domain that is based on the intracellular signaling domain of a CD28 family protein; and (b) at least a second signaling domain that comprises a mutant intracellular signaling domain of a tumor necrosis factor receptor (TNFR) family protein.

15 In some [examples](#)~~embodiments~~, the first signaling domain that is based on the intracellular signaling domain of a CD28 family proteins is selected from a CD28 protein, ICOS protein or a combination thereof. In some [examples](#)~~embodiments~~, the at least second signaling domain is based on a mutant of the intracellular signaling domain of a TNFR family protein selected from CD137 (4-1BB) and CD134 (OX-40).

The chimeric co-stimulatory intracellular domains provided herein comprise: (a) a first signaling domain that is based on the intracellular signaling domain of a CD28 protein, ICOS protein or a combination thereof; and (b) at least a second signaling domain that comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain.

25 In some [examples](#)~~embodiments~~, the mutant CD137 (4-1BB) intracellular domain or the mutant CD134 (OX-40) intracellular domain comprises a deletion, an insertion or a substitution of one or more amino acids in the membrane proximal portion of the CD137 or CD134 intracellular domain. Without being bound by theory, in some [examples](#)~~embodiments~~, the one or more amino acids in the membrane proximal portion can be ubiquitination sites involved in the ubiquitination and degradation of the CD137 or CD134 protein.

wherein the at least second signal transduction domain comprises a mutant intracellular signaling domain of a tumor necrosis factor receptor (TNFR) family protein.

The present disclosure provides a recombinant T cell co-stimulatory receptor (RTCR), comprising: (a) an extracellular domain; (b) a transmembrane domain; and (c) a chimeric intracellular domain comprising a first and at least a second signal transduction domains, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least second signal transduction domain comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain.

The present disclosure also provides a nucleic acid encoding the RTCR disclosed herein.

The present disclosure also provides a vector comprising the nucleic acid disclosed herein. The present disclosure also provides a cell comprising the nucleic acid or the vector disclosed herein.

In another aspect, the present disclosure refers to a cell comprising the nucleic acid or the vector as described herein, optionally wherein the cell is a modified T cell, or a modified natural killer T cell (NK-T cell), optionally wherein the modified T cell is an allogenic T cell or an autologous T cell, optionally wherein the modified T cell is a naïve T cell, an early memory T cell, a stem cell-like T cell, a stem memory T cell (TSCM), a central memory T cell (TCM), or a regulatory T cell (Treg), optionally wherein the cell further comprises a sequence encoding an artificial antigen receptor, a therapeutic polypeptide, an immune cell modulatory protein, or a combination thereof, optionally wherein the artificial antigen receptor comprises a chimeric antigen receptor (CAR), optionally wherein the cell expresses the RTCR either transiently or stably, and optionally wherein the cell co-expresses with the RTCR at least one or more of the endogenous co-stimulatory molecules selected from CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, CD40L, and a combination thereof.

The present disclosure also provides a modified T lymphocyte (T cell), comprising: (a) a modification of an endogenous sequence encoding a T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; and (b) a recombinant T cell co-stimulatory receptor (RTCR) disclosed herein. In another aspect, the present disclosure refers to a modified T cell, comprising: (a) a modification of an endogenous sequence encoding a TCR, wherein the modification reduces or eliminates a level of expression or activity of the TCR; and (b) a recombinant RTCR as described herein, optionally further comprising a modification of an endogenous sequence encoding a component of major

histocompatibility complex class I (MHC-I), wherein the modification reduces or eliminates a level of expression or activity of the MHC-I, and optionally wherein the modified T cell co-expresses with the RTCR at least one of the endogenous co-stimulatory molecules CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, CD40L, or a combination thereof.

5 The present disclosure also provides a composition comprising the RTCR disclosed herein. The present disclosure also provides a composition comprising the nucleic acid encoding the RTCR disclosed herein. The present disclosure also provides a composition comprising the vector comprising the nucleic acid disclosed herein. The present disclosure also provides a composition comprising the cell disclosed herein. The present disclosure also provides a
10 composition comprising the modified T cell disclosed herein.

 The present disclosure also provides a composition comprising a population of cells, wherein the population comprises a plurality of the cell comprising the nucleic acid encoding or a vector comprising the nucleic acid encoding the RTCR disclosed herein. The present disclosure also provides a composition comprising a population of cells, wherein the population comprises
15 a plurality of the modified T cell disclosed herein.

 The present disclosure provides a method of producing a plurality of modified T cells, wherein the method comprises: a) providing a plurality of primary T cells disclosed herein; b) providing a composition comprising the RTCR disclosed herein, the nucleic acid encoding the RTCR disclosed herein, or the vector comprising the nucleic acid encoding the RTCR disclosed
20 herein; and c) introducing into the plurality of primary T cells of (a) the composition of (b), to

produce a plurality of modified T cells under conditions that stably express the RTCR within the plurality of modified T cells. In some [examples](#)~~embodiments~~, the method of producing a plurality of modified T cells disclosed herein, further comprises a step of modifying an endogenous sequence encoding an endogenous T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the endogenous TCR. In some [examples](#)~~embodiments~~, the method of producing a plurality of modified T cells disclosed herein, further comprises a step of modifying an endogenous sequence, wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I). In some [examples](#)~~embodiments~~, the method of producing a plurality of modified T cells disclosed herein, further comprises: d) maintaining the plurality of modified T cells in a suitable cell culture media; and e) either: i) cryopreserving the plurality of modified T cells in a suitable cell freezing media; or ii) preparing the plurality of modified T cells for administering to a subject suffering from a disease or disorder.

The present disclosure also provides a method of treating a disease or disorder, comprising administering to a subject in need thereof a therapeutically effective number of the cell comprising the nucleic acid encoding or the vector comprising the nucleic acid encoding the RTCR disclosed herein, a therapeutically effective number of any one of the modified T cell disclosed herein, a therapeutically effective amount of any one of the compositions disclosed herein, or a therapeutically effective number of the plurality of modified T cells produced by the method disclosed herein. In another aspect, the present disclosure refers to a use of a therapeutically effective number of the cell as described herein, a therapeutically effective number of the modified T cell as described herein, a therapeutically effective amount of the composition as described herein, or a therapeutically effective number of the plurality of modified T cells produced by the method as described herein in the manufacture of a medicament for treating a disease or disorder, optionally wherein the subject is a mammal, and optionally wherein the mammal is a human, wherein the disease or disorder is a cancer, an autoimmune disease or disorder, an infectious disease, an inflammatory disease, a renal disease or disorder, a lung disease or disorder, a liver disease or disorder, a cardiovascular system disease or disorder, a neurodegenerative disease or disorder, or a metabolic disease or disorder, optionally wherein the cancer is a solid tumor or a hematologic cancer, optionally wherein the solid cancer is a sarcoma, a carcinoma or a melanoma or wherein the hematological cancer is a

leukemia, a lymphoma or a myeloma, and optionally wherein a) the cancer is selected from acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, B cell, T cell or FAB ALL, acute myeloid leukemia (AML), acute myelogenous leukemia, chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), Hodgkin's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, bladder cancer, breast cancer, colorectal cancer, endometrial cancer, head cancer, neck cancer, hereditary nonpolyposis cancer, liver cancer, lung cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, testicular cancer, adenocarcinomas, sarcomas, malignant melanoma, and hemangioma; b) the infectious disease is caused by a bacteria, a virus, a fungus, a protozoa, or a parasite; c) the neurodegenerative disorder is Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD) and PD-related disorders, prion disease, Motor neuron diseases (MND), Huntington's disease (HD), Spinocerebellar ataxia (SCA), or Spinal muscular atrophy (SMA); or d) the inflammatory disease is systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, or multiple sclerosis.

The present disclosure also provides a chimeric co-stimulatory intracellular protein (CIP) comprising a first and at least a second signal transduction domains, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least second signal transduction domain comprises a mutant intracellular signaling domain of a tumor necrosis factor receptor (TNFR) family protein.

The present disclosure also provides a chimeric co-stimulatory intracellular protein (CIP) comprising a first and at least a second signal transduction domains, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least second signal transduction domain comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain. In another aspect, the present disclosure refers to a chimeric co-stimulatory intracellular protein (CIP) comprising a first and at least a second signal transduction domain, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least second signal transduction domain comprises a mutant intracellular signaling domain of a tumor necrosis factor receptor (TNFR) family protein, wherein the mutant intracellular signaling domain of the TNFR family protein is a mutant

CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain, and optionally wherein the CIP further comprises a transmembrane domain.

CLAIMS

What is claimed is:

1. A recombinant T cell co-stimulatory receptor (RTCR), comprising:
 - (a) an extracellular domain;
 - (b) a transmembrane domain; and
 - (c) a chimeric intracellular domain comprising a first and at least a second signal transduction domains,

wherein the first and the at least second signal transduction domains are non-identical; and

wherein the at least second signal transduction domain comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain.

2. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises ~~(1)~~is a truncated CD137 intracellular domain comprising:-
 - 1a)~~3.~~ ~~The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises~~ an amino acid sequence according to amino acid position 13 to amino acid position 42 of the CD137 intracellular domain~~:-~~

 - 1b)~~4.~~ ~~The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises~~ a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from the N-terminus of the CD137 intracellular domain~~:-~~

 - 1c)~~5.~~ ~~The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises~~ a deletion of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain~~:-~~ and/or:-

~~1d)6. The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 3;~~

~~(2)7. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises a deletion of one, two, three, or four lysine residue(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain;~~

~~(3)8. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises one or more lysine mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, optionally;~~

~~9. The RTCR of claim 8, wherein the mutant CD137 intracellular domain comprises one or more lysine mutation(s) at amino acid positions selected from amino acid positions 1, 5, 6 and 12 of the N-terminus of the CD137 intracellular domain;~~

~~(4)10. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain; and/or;~~

~~(5)11. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, optionally;~~

~~12. The RTCR of claim 11, wherein the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, 3, 4, 5 and 6 of the N-terminus of the CD137 intracellular domain, and; optionally~~

~~13. The RTCR of claim 12, wherein the mutant CD137 intracellular domain further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain.~~

~~314.~~ The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises
(1)~~is~~ a truncated CD134 intracellular domain comprising:-

1a)~~15.~~ ~~The RTCR of claim 14, wherein the truncated CD134 intracellular domain~~
~~comprises~~ an amino acid sequence according to amino acid position 15 to amino acid
 position 37 of the CD134 intracellular domain;-

1b)~~16.~~ ~~The RTCR of claim 14, wherein the truncated CD134 intracellular domain~~
~~comprise~~ a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight,
 nine, ten, eleven, twelve, thirteen or more amino acids from the N-terminus of the CD134
 intracellular domain;-

1c)~~17.~~ ~~The RTCR of claim 14, wherein the truncated CD134 intracellular domain~~
~~comprises~~ a deletion of one, two, three, four, five, six, seven, eight, nine, ten, eleven,
 twelve, thirteen or more amino acids from amino acid position 1 to amino acid position
 14 of the N-terminus of the CD134 intracellular domain; and/or:-

1d)~~18.~~ ~~The RTCR of claim 14, wherein the truncated CD134 intracellular domain~~
~~comprises~~ an amino acid sequence according to SEQ ID NO: 6;-

(2)

~~19.~~ ~~The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises~~
 a deletion of a lysine residue from amino acid position 1 to amino acid position 14 of the N-
 terminus of the CD134 intracellular domain;-

(3)

~~20.~~ ~~The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises~~
 a lysine mutation at amino acid position 12 of the N-terminus of the CD134 intracellular
 domain;-

(4)

~~21.~~ ~~The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises~~
 a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid
 position 14 of the N-terminus of the CD134 intracellular domain; and/or:-

(5)

~~22. The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises~~ one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.

~~23. The, optionally RTCR of claim 22, wherein the mutant CD134 intracellular domain comprises~~ one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, and 5 of the N-terminus of the CD134 intracellular domain, and.

optionally

~~24. The RTCR of claim 23,~~ wherein the mutant CD134~~CD137~~ intracellular domain further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain.

~~425.~~ The RTCR of any one of claims 1-~~3~~24, wherein the chimeric intracellular domain comprises a first signal transduction domain derived from a protein of the CD28 family, optionally

~~26. The RTCR of claim 25,~~ wherein the first signal transduction domain is derived from CD28, CD28H, ICOS, or a combination thereof, and optionally wherein:

~~a) 27. The RTCR of claim 26, wherein~~ the first signal transduction domain is derived from ICOS, and optionally

~~28. The RTCR of claim 19, wherein the first signal transduction domain derived from ICOS~~ comprises an amino acid sequence according to SEQ ID NO: 9, optionally.

~~29. The RTCR of any one of claims 1-24,~~ wherein the chimeric intracellular domain comprises a first signal transduction domain comprising a portion of a CD28 intracellular domain combined with an ICOS domain according to SEQ ID NO: 9, and optionally.

~~30. The RTCR of claim 29,~~ wherein the first signal transduction domain comprises an amino acid sequence according to any one of SEQ ID NOs: 12 and 109; or;

b)

~~31. The RTCR of any one of claims 1-24, wherein the chimeric intracellular domain comprises a~~ first signal transduction domain is derived from CD28 and optionally;

~~32. The RTCR of claim 31, wherein the first signal transduction domain derived from CD28~~ comprises an amino acid sequence according to SEQ ID NO: 10, optionally;

~~33. The RTCR of any one of claims 31-32,~~ wherein the first signal transduction domain comprises an amino acid sequence according to any one of SEQ ID NOs: 121-122, and optionally

~~34. The RTCR of any one of claims 1-30,~~ wherein the chimeric intracellular domain comprises an amino acid sequence according to any one of SEQ ID NOs: 14-17.

35. The RTCR of any one of claims 1-~~4~~34, wherein the chimeric intracellular domain further comprises a third signal transduction domain, optionally;

~~36. The RTCR of claim 35,~~ wherein the third signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain, ~~or~~ an interleukin 2 receptor binding (IL-2RB) protein signaling domain, or a combination thereof, and optionally wherein;

a)

~~37. The RTCR of claim 36, wherein~~ the CD3 signaling domain is derived from ~~form~~ a CD3 ζ or a CD3 ϵ domain, or a combination thereof, optionally;

~~38. The RTCR of claim 37,~~ wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47, and 48; and;

b)

~~39. The RTCR of claim 36, wherein~~ the CD2 signaling domain is a mutant CD2 signaling domain, optionally;

~~40. The RTCR of claim 39,~~ wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain, and optionally;

~~41. The RTCR of any one of claims 39-40,~~ wherein the CD2 signaling domain comprises an amino acid sequence according to SEQ ID NO: 49; or;

c)

~~42. The RTCR of claim 36, wherein~~ the IL-2RB protein signaling domain comprises an amino acid sequence according to SEQ ID NO: 50.

643. The RTCR of any one of claims 1-~~5~~42, wherein the chimeric intracellular domain further comprises a fourth signal transduction domain, optionally;

~~44. The RTCR of claim 43,~~ wherein the fourth signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain, ~~or~~ an interleukin 2 receptor binding (IL-2RB) protein signaling domain, or a combination thereof, wherein the third and the fourth signal transduction domain are not identical, and optionally wherein;

a)

~~45. The RTCR of claim 44, wherein~~ the CD3 signaling domain is derived from ~~form~~ a CD3 ζ or a CD3 ϵ domain, or a combination thereof, optionally;

~~46. The RTCR of claim 45,~~ wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47, and 48;:-

b)

~~47. The RTCR of claim 44, wherein~~ the CD2 signaling domain is a mutant CD2 signaling domain, optionally;

~~48. The RTCR of claim 47,~~ wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain, and optionally;

~~49. The RTCR of any one of claims 47-48,~~ wherein the CD2 signaling domain comprises an amino acid sequence according to SEQ ID NO: 49; ~~or~~;

c)

~~50. The RTCR of claim 44, wherein~~ the IL-2RB protein signaling domain comprises an amino acid sequence according to SEQ ID NO: 50.

~~751.~~ The RTCR of any one of claims 1-~~650~~, wherein the extracellular domain comprises a protein or a portion thereof that induces activation and/or proliferation of an immune cell, optionally;

~~52. The RTCR of claims 51,~~ wherein the extracellular domain comprises any one of:

- a) a component of a T cell Receptor (TCR) complex;
- b) a component of a chimeric antigen receptor (CAR);
- c) a component of a T cell co-receptor, wherein the T cell co-receptor is a T cell co-stimulatory protein or a T cell inhibitory protein;
- d) a ligand that binds to a cell surface receptor or a component thereof;
- e) a component of a cytokine receptor;
- f) a component of a chemokine receptor;
- g) a component of an integrin receptor;
- h) a component of an endothelial cell surface protein receptor or a fragment thereof;
- i) a component of a neuronal guidance protein receptor; and
- j) a component of a complement receptor.

~~853.~~ The RTCR of claims ~~752~~, wherein the extracellular domain comprises any one of:

a) the component of the T cell co-receptor or the CAR is a component of PD1, CD28, CD2, OX-40, ICOS, CTLA-4, CD28, CD3, CD4, CD8, CD40L, Lag-3, Tim-3, ~~or~~ TIGIT, or a combination thereof;

b)

~~54. The RTCR of claims 52, wherein~~ the component of T cell co-receptor or the CAR binds to CD19, B cell maturation Ag (BCMA), PD-L1, PD-L2, IL-10, a proliferation-inducing

ligand (APRIL), BAFF, OX-40L, ICOS-L, B7-1, B7-2, CD40, CD58, CD59, nectin, CD155, or CD112, or a combination thereof;

c)

~~55. — The RTCR of claim 52, wherein~~ the cytokine receptor binds to IL-10, IL-27, TGF- β , IL-12, IL-1, IL-2, IL-4, IL-5, IFN- γ , IFN- α/β , or a combination thereof;

d)

~~56. — The RTCR of claim 52, wherein~~ the component of the complement receptor is a component of C3aR, C5aR, CD46/MCP, CD55, CD97, or DAF, or a combination thereof;
and/or;

e)

~~57. — The RTCR of claim 52, wherein~~ the extracellular domain comprises an amino acid sequence of a component of epithelial growth factor receptor (EGFR), vascular-endothelial growth factor receptor (VEGFR), chemokine receptor (CCR) 4, CCR5, CCR7, CCR10, netrin-1 receptor, semaphorin receptor, lymphocyte function-associated antigen-1 (LFA-1), leukocyte-specific $\beta 2$ integrin ($\alpha L\beta 2$, $\alpha M\beta 2$, $\alpha X\beta 2$, or $\alpha D\beta 2$), $\beta 7$ integrin ($\alpha 4\beta 7$ or $\alpha E\beta 7$), extracellular matrix (ECM)-binding $\beta 1$ integrin ($\alpha 1$ – $\alpha 6\beta 1$), L-selectin, or sialyl Lewis^x;

optionally

~~58. — The RTCR of any one of claims 51–54,~~ wherein the extracellular domain is a polypeptide, a glycoprotein, or an antibody or a fragment thereof, optionally;

~~59. — The RTCR of claim 58,~~ wherein the antibody or fragment thereof is a Fab fragment, a F(ab)2 fragment, a diabody, a nanobody, a sdAb, Fv, a V_HH fragment, or a single chain Fv fragment, and;

further optionally

~~60. — The RTCR of any one of claims 51–54 and 58–59,~~ wherein the extracellular domain binds to a target selected from a tumor antigen, a pathogen associated protein, and an antigen associated with an autoimmune, an inflammatory, a metabolic, or a neurodegenerative condition or disorder, optionally;

~~61. — The RTCR of claim 60,~~ wherein the tumor antigen is a tumor associated antigen (TAA), a tumor secreted antigen (TSA) or an unconventional antigen (UCA), optionally

~~62. The RTCR of any one of claims 51-61,~~ wherein the extracellular domain binds to a target with a binding affinity of 1 fM to 100 μ M; optionally

~~63. The RTCR of claim 62,~~ wherein the extracellular domain binds to a target with a binding affinity of 1 μ M to 10 μ M; and
further optionally

~~64. The RTCR of any one of claims 1-63,~~ wherein the extracellular domain comprises:

a) a signal peptide at the N-terminus, wherein the signal peptide is derived from a surface or a secretory protein; optionally wherein the signal peptide comprises:-

i)

~~65. The RTCR of claim 64, wherein the signal peptide is~~ PD-1 signal peptide (PD-1 SP); or:-

ii) an

~~66. The RTCR of claim 64, wherein the signal peptide is a~~ HLA class I histocompatibility antigen or a portion thereof; and/or:-

b)

~~67. The RTCR of any one of claims 1-66, wherein the extracellular domain comprises a~~ hinge region; optionally:-

~~68. The RTCR of claim 68,~~ wherein the hinge region is derived from CD8, PD-1, CD28, ICOS, or IgG.

969. The RTCR of any one of claims 1-~~868~~, wherein the transmembrane domain is derived from CD8, PD1, CD28, COS, or IgG.

1070. The RTCR of any one of claims 1-969, wherein the RTCR is for expression in a T cell, wherein the T cell co-expresses at least one of the endogenous co-stimulatory molecules selected

from CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, ~~and~~ CD40L, and a combination thereof.

~~1174.~~ A nucleic acid encoding the RTCR of any one of claims 1-~~10, or a~~70.

~~72.~~ A vector comprising saidthe nucleic acid ~~of claim 71~~.

~~1273.~~ A cell comprising the nucleic acid ~~of claim 71~~ or the vector of claim ~~11, optionally~~72.

~~74.~~ ~~The cell of claim 73,~~ wherein the cell is a modified T cell, or.

~~75.~~ ~~The cell of claim 73, wherein the cell is~~ a modified natural killer T cell (NK-T cell), optionally.

~~76.~~ ~~The cell of claim 74,~~ wherein the modified T cell is an allogenic T cell or.

~~77.~~ ~~The cell of claim 74, wherein the modified T cell is~~ an autologous T cell, optionally.

~~78.~~ ~~The cell of claim 74,~~ wherein the modified T cell is a naïve T cell, an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}), a central memory T cell (T_{CM}), or a regulatory T cell (T_{reg}).

optionally

~~79.~~ ~~The cell of any one of claims 73-78,~~ wherein the cell further comprises a sequence encoding an artificial antigen receptor, a therapeutic polypeptide, ~~or~~ an immune cell modulatory protein, or a combination thereof.

~~80.~~ ~~The cell of claim 79,~~ optionally wherein the artificial antigen receptor comprises a chimeric antigen receptor (CAR).

~~81.~~ ~~The cell of any one of claims 73-80,~~ optionally wherein the cell expresses the RTCR ~~of any one of claims 1-70~~ either transiently or stably, and.

optionally

~~82. — The cell of any one of claims 73-81,~~ wherein the cell co-expresses with the RTCR at least one or more of the endogenous co-stimulatory molecules selected from CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, ~~and~~ CD40L, and a combination thereof.

~~1383.~~ A modified T lymphocyte (T cell), comprising:

(a) a modification of an endogenous sequence encoding a T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; and

(b) a recombinant T cell co-stimulatory receptor (RTCR) according to any one of claims 1-~~1070.~~, optionally

~~84. — The modified T cell of claim 83,~~ further comprising a modification of an endogenous sequence encoding a component of major histocompatibility complex (MHC) class I (MHC-I), wherein the modification reduces or eliminates a level of expression or activity of the MHC-I, and optionally

~~85. — The modified T cell of any one of claims 83-84,~~ wherein the modified T cell co-expresses with the RTCR at least one of the endogenous co-stimulatory molecules CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, ~~and~~ CD40L, or a combination thereof.

~~1486.~~ A composition comprising:

a) the RTCR of any one of claims 1-~~10;70.~~

b)

~~87. — A composition comprising~~ the nucleic acid of claim ~~11;71.~~

c)

~~88. — A composition comprising~~ the vector of claim ~~11;72.~~

d)

~~89. — A composition comprising~~ the cell of claim 12;~~any one of claims 73-82.~~

e)

~~90. — A composition comprising~~ the modified T cell of claim 13; and/or~~any one of claims 83-85.~~

f)

~~91. — A composition comprising~~ a population of cells, wherein the population comprises a plurality of the cell of [claim 12](#)~~any one of claims 73–82~~ or wherein the population comprises a plurality of the modified T cell of [claim 13](#)~~any one of claims 83–85~~.

~~1592.~~ A method of producing a plurality of modified T cells, wherein the method comprises:

- a) providing a plurality of primary T cells;
- b) providing a composition comprising the RTCR of any one of claims 1–~~1070~~, the nucleic acid ~~of claim 71~~, or the vector of claim ~~1172~~; and
- c) introducing into the plurality of primary T cells of (a) the composition of (b), to produce a plurality of modified T cells under conditions that stably express the RTCR within the plurality of modified T cells;

optionally

~~93. — The method of claim 92,~~ wherein the method further comprises a step of modifying an endogenous sequence encoding an endogenous T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the endogenous TCR;

optionally

~~94. — The method of any one of claims 92–93,~~ wherein the method further comprises a step of modifying an endogenous sequence of the plurality of primary T cells, wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I);

optionally

~~95. — The method of any one of claims 92–94,~~ wherein the method further comprises:

- d) maintaining or expanding the plurality of modified T cells in a suitable cell culture media; and
- e) either:
 - i) cryopreserving the plurality of modified T cells in a suitable cell freezing media; or
 - ii) preparing the plurality of modified T cells for administration to a subject in need thereof.

~~1696. Use of~~~~A method of treating a disease or disorder, comprising administering to a subject in need thereof~~ a therapeutically effective number of the cell of [claim 12](#)~~any one of claims 73-82~~, a therapeutically effective number of the modified T cell of [claim 13](#)~~any one of claims 83-85~~, a therapeutically effective amount of the composition of [claim 14](#)~~any one of claims 86-91~~, or a therapeutically effective number of the plurality of modified T cells produced by the method of [claim 15 in the manufacture of a medicament for treating a disease or disorder](#),~~any one of claims 92-95.~~

[optionally](#)

~~97. The method of claim 96,~~ wherein the subject is a mammal, [and optionally](#).

~~98. The method of claim 97,~~ wherein the mammal is a human.

~~99. The method of any one of claims 96-98,~~ wherein the disease or disorder is a cancer, an autoimmune disease or disorder, an infectious disease, an inflammatory disease, a renal disease or disorder, a lung disease or disorder, a liver disease or disorder, a cardiovascular system disease or disorder, a neurodegenerative disease or disorder, or a metabolic disease or disorder, [optionally](#).

~~100. The method of claim 99,~~ wherein the cancer is a solid tumor or a hematologic cancer, [optionally](#)

~~101. The method of claim 100,~~ wherein the solid cancer is a sarcoma, a carcinoma or a melanoma [or](#).

~~102. The method of claim 100,~~ wherein the hematological cancer is a leukemia, a lymphoma or a myeloma,

[and optionally](#)

~~103. The method of claim 99,~~ wherein

[a\)](#) the cancer is selected from acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, B cell, T cell or FAB ALL, acute myeloid leukemia (AML), acute myelogenous leukemia, chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), Hodgkin's lymphoma, Hodgkin's

disease, non-Hodgkin's lymphoma, multiple myeloma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, bladder cancer, breast cancer, colorectal cancer, endometrial cancer, head cancer, neck cancer, hereditary nonpolyposis cancer, liver cancer, lung cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, testicular cancer, adenocarcinomas, sarcomas, malignant melanoma, and hemangioma₁;

~~b)104. The method of claim 99, wherein~~ the infectious disease is caused by a bacteria, a virus, a fungus, a protozoa, or a parasite₁;

~~c)105. The method of claim 99, wherein~~ the neurodegenerative disorder is Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD) and PD-related disorders, prion disease, Motor neuron diseases (MND), Huntington's disease (HD), Spinocerebellar ataxia (SCA), or Spinal muscular atrophy (SMA)₁; or

~~d)106. The method of claim 99, wherein~~ the inflammatory disease is systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, or multiple sclerosis.

~~17107.~~ A chimeric co-stimulatory intracellular protein (CIP) comprising a first and at least a second signal transduction domains,

wherein the first and the at least second signal transduction domains are non-identical; and

wherein the at least second signal transduction domain comprises a mutant intracellular signaling domain of a tumor necrosis factor receptor (TNFR) family protein₁.

~~108. The CIP of claim 107,~~ wherein the mutant intracellular signaling domain of ~~the~~ a TNFR family protein is a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain₁, and

~~109. The CIP of any one of claims 107-108, optionally~~ wherein the CIP further comprises a transmembrane domain.

~~18410.~~ The CIP of claim ~~17408~~, wherein:

a) the mutant CD137 intracellular domain comprises

~~(1)is~~ a truncated CD137 intracellular domain, optionally;

~~111. The CIP of claim 110,~~ wherein the truncated CD137 intracellular domain comprises:

li) an amino acid sequence according to amino acid position 13 to amino acid position 42 of the CD137 intracellular domain;

lii)

~~112. The CIP of claim 110, wherein the truncated CD137 intracellular domain comprises~~ a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from the N-terminus the CD137 intracellular domain;

liii)

~~113. The CIP of claim 110, wherein the truncated CD137 intracellular domain comprises~~ a deletion of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain; and/or;

(1)

~~114. The CIP of claim 110, wherein the truncated CD137 intracellular domain comprises~~ an amino acid sequence according to SEQ ID NO: 3;

(2)

~~115. The CIP of claim 108, wherein the mutant CD137 intracellular domain comprises~~ a deletion of one, two, three or four lysine residue(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain;

(3)

~~116.—The CIP of claim 108, wherein the mutant CD137 intracellular domain comprises~~
one or more lysine mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, optionally;

~~117.—The CIP of claim 116, wherein the mutant CD137 intracellular domain comprises~~
one or more lysine mutation(s) at amino acid positions selected from amino acid positions 1, 5, 6 and 12 of the N-terminus of the CD137 intracellular domain;

(4)

~~118.—The CIP of claim 108, wherein the mutant CD137 intracellular domain comprises~~
a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain; and/or;

(5)

~~119.—The CIP of claim 108, wherein the mutant CD137 intracellular domain comprises~~
one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain optionally;

~~120.—The CIP of claim 119, wherein the mutant CD137 intracellular domain comprises~~
one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, 3, 4, 5 and 6 of the N-terminus of the CD137 intracellular domain, and;

optionally

~~121.—The CIP of claim 120, wherein the mutant CD137 intracellular domain further~~
~~comprises~~ a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain; or;

b)

~~122.—The CIP of claim 108, wherein~~ the mutant CD134 intracellular domain comprises

(1) ~~is~~ a truncated CD134 intracellular domain, optionally;

~~123.—The CIP of claim 122,~~ wherein the truncated CD134 intracellular domain comprises;

li) an amino acid sequence according to amino acid position 15 to amino acid position 37 of the CD134 intracellular domain_{;-}

lii)

~~124. — The CIP of claim 122, wherein the truncated CD134 intracellular domain~~
~~comprise~~ a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from the N-terminus of the CD134 intracellular domain_{;-}

liii)

~~125. — The CIP of claim 122, wherein the truncated CD134 intracellular domain~~
~~comprises~~ a deletion of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain_{;-} and/or_{;-}

liv)

~~126. — The CIP of claim 122, wherein the truncated CD134 intracellular domain~~
~~comprises~~ an amino acid sequence according to SEQ ID NO: 6_{;-}

(2)

~~127. — The CIP of claim 108, wherein the mutant CD134 intracellular domain comprises~~
a deletion of a lysine residue from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain_{;-}

(3)

~~128. — The CIP of claim 127, wherein the mutant CD134 intracellular domain comprises~~
a lysine mutation at amino acid position 12 of the N-terminus of the CD134 intracellular domain_{;-}

(4)

~~129. — The CIP of claim 108, wherein the mutant CD134 intracellular domain comprises~~
a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain_{;-} and/or_{;-}

(5)

~~130. — The CIP of claim 108, wherein the mutant CD134 intracellular domain~~
~~comprises~~ one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain_{;-} optionally_{;-}

~~131. The CIP of claim 130, wherein the mutant CD134 intracellular domain comprises~~
one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino
acid positions 1, 2, and 5 of the N-terminus of the CD134 intracellular domain, ~~and~~

~~optionally~~

~~132. The CIP of claim 131,~~ wherein the mutant CD134 intracellular domain further
comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD134
intracellular domain.

~~133. The CIP of claim 17 or 18~~~~any one of claims 107-132~~, wherein the chimeric intracellular
domain comprises a first signal transduction domain derived from a protein of the CD28 family,
~~optionally~~

~~134. The CIP of claim 133,~~ wherein the first signal transduction domain is derived from
CD28, CD28H, ICOS, or a combination thereof, ~~and optionally wherein:~~

~~a)~~

~~135. The CIP of claim 134, wherein~~ the first signal transduction domain is derived from
ICOS, ~~optionally wherein~~

~~i)~~

~~136. The CIP of claim 135, wherein~~ the first signal transduction domain derived from ICOS
comprises an amino acid sequence according to SEQ ID NO: 9;
~~or~~

~~ii)~~

~~137. The CIP of any one of claims 107-134, wherein the chimeric intracellular domain~~
~~comprises a~~ first signal transduction domain ~~comprises~~~~comprising~~ a portion of a CD28
intracellular domain combined with an ICOS domain according to SEQ ID NO: 9;
~~or~~

~~iii)~~

~~138. The CIP of claim 137, wherein~~ the first signal transduction domain comprises an amino
acid sequence according to any one of SEQ ID NOs: 12 or 109;
~~or~~

~~b)~~

~~139. The CIP of any one of claims 107-134, wherein~~ the first signal transduction domain is derived from CD28, optionally wherein:-

i)

~~140. The CIP of claim 139, wherein~~ the first signal transduction domain derived from CD28 comprises an amino acid sequence according to SEQ ID NO: 10; or

ii)

~~141. The CIP of any one of claims 139-140, wherein~~ the first signal transduction domain comprises an amino acid sequence according to any one of SEQ ID NOs: 121-122;:-

and further optionally

~~142. The CIP of any one of claims 107-138,~~ wherein the CIP comprises an amino acid sequence according to any one of SEQ ID NOs: 14-17.

~~20143.~~ The CIP of any one of claims ~~17-19107-142,~~ wherein the CIP further comprises a third signal transduction domain, optionally:-

~~144. The CIP of claim 143,~~ wherein the third signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain, ~~or~~ an interleukin 2 receptor binding (IL-2RB) protein signaling domain, or a combination thereof, and optionally wherein:-

a)

~~145. The CIP of claim 144, wherein~~ the CD3 signaling domain is derived ~~from~~ from a CD3 ζ or a CD3 ϵ domain, or a combination thereof, optionally:-

~~146. The CIP of claim 145,~~ wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47, and 48;:-

b)

~~147. The CIP of claim 144, wherein~~ the CD2 signaling domain ~~comprises~~ is a mutant CD2 signaling domain, optionally:-

~~148. The CIP of claim 147,~~ wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain, and optionally:-

~~149.—The CIP of any one of claims 147-148,~~ wherein the CD2 signaling domain comprises an amino acid sequence according to SEQ ID NO: 49~~;~~; or

c)

~~150.—The CIP of claim 144, wherein~~ the IL-2RB protein signaling domain comprises an amino acid sequence according to SEQ ID NO: 50.

~~21151.~~ The CIP of any one of claims ~~17-20107-150,~~ wherein the chimeric intracellular domain further comprises a fourth signal transduction domain, optionally~~;~~

~~152.—The CIP of claim 135,~~ wherein the fourth signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-2RB) protein signaling domain or a combination thereof, wherein the third and the fourth signal transduction domain are not identical, optionally wherein~~;~~

a)

~~153.—The CIP of claim 152, wherein~~ the CD3 signaling domain is derived ~~from~~from a CD3 ζ or a CD3 ϵ domain, or a combination thereof, and optionally~~;~~

~~154.—The CIP of claim 153,~~ wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47, ; and 48~~;~~

b)

~~155.—The CIP of claim 152, wherein~~ the CD2 signaling domain is a mutant CD2 signaling domain, optionally~~;~~

~~156.—The CIP of claim 155,~~ wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain, optionally~~;~~

~~157.—The CIP of any one of claims 155-156,~~ wherein the CD2 signaling domain comprises an amino acid sequence according to SEQ ID NO: 49~~;~~; or

c)

~~158.—The CIP of claim 152, wherein~~ the IL-2RB protein signaling domain comprises an amino acid sequence according to SEQ ID NO: 50.

~~22~~¹⁵⁹. The CIP of any one of claims ~~17-21~~¹⁰⁷⁻¹⁵⁸, wherein the CIP is for expression in a T cell, wherein the T cell co-expresses at least one of the endogenous co-stimulatory molecules selected from CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, ~~and~~ CD40L, and a combination thereof.

23. The cell of claim 12, the modified T cell of claim 13, the composition of claim 14, or the plurality of modified T cells produced by the method of claim 15 for use in therapy.