ROR-1 SPECIFIC CHIMERIC ANTIGEN RECEPTORS AND USES THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/696,075, filed on July 10, 2018, which application is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 8, 2019, is named 50471_716_601_SL.txt and is 360,082 bytes in size.

BACKGROUND OF THE DISCLOSURE

[0003] Recombinant polypeptides such as chimeric polypeptides have been a valuable for research, diagnostic, manufacturing and therapeutic applications. Modified effector cells expressing antigen binding polypeptides such as CARs are useful in the treatment of diseases and disorders such as infectious disease, autoimmune disorders and cancers.

INCORPORATION BY REFERENCE

[0004] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY OF THE DISCLOSURE

[0005] Disclosed herein, in certain embodiments, are isolated nucleic acids, vectors, immune effector cells, methods and systems comprising chimeric antigen receptors (CAR).

[0006] Provided herein is an isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain.

[0007] In some embodiments, the CAR comprises at least one of: (a) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 3-14; or (b) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of

amino acid sequences as shown in SEQ ID NOs: 75-82. In some cases, the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15-74. In other cases, the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73. In some examples, the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74. In some embodiments, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 83. In other embodiments, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 84. In some cases, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 85. In some examples, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 86. In other embodiments, the transmembrane domain is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of the amino acid sequences as shown in SEQ ID NOs: 87-88.

[0008] In some cases, the costimulatory signaling domain comprises 4-1BB. In other embodiments, the costimulatory signaling domain of 4-1BB comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 89. In other cases, the costimulatory signaling domain comprises CD28. In some examples, the costimulatory signaling domain of CD28 comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 90. In some embodiments, the CD3 zeta signaling domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 93. In other embodiments, the isolated nuclei acid further comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NOs: 94-108.

[0009] In some examples, the isolated nucleic acid further comprises a cell tag. In some embodiments, the cell tag is a truncated epidermal growth factor receptor. In some cases, the

truncated epidermal growth factor receptor is HER1t and comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 109. In other embodiments, the truncated epidermal growth factor receptor is HER1t-1 and comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 110. In some embodiments, the cell tag comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 111. In other cases, the cell tag comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 112. In some examples, the isolated nucleic acid further comprises an additional polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

[0010] Provided herein is a vector comprising a backbone and a nucleic acid sequence encoding: (1) a cell tag; (2) a cytokine; and (3) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain.

[0011] In some embodiments, the vector is a lentivirus vector, a retroviral vector, or a non-viral vector. In some cases, the cell tag is a truncated epidermal growth factor receptor. In some embodiments, the truncated epidermal growth factor receptor is a HER1t, HER1t-1 or a functional variant thereof. In some examples, the truncated epidermal growth factor receptor comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 109 or SEQ ID NO: 110. In other examples, the cell tag comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 111. In some examples, the cell tag comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 112.

[0012] In some cases, the cytokine is IL-15. In some embodiments, the IL-15 is a membrane bound IL-15. In some embodiments, the membrane bound IL-15 comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the

amino acid sequence of SEQ ID NO: 113. In other cases, the vector further comprises a nucleotide sequence encoding a self-cleaving Thosea asigna virus (T2A) peptide. In some embodiments, the backbone is Sleeping Beauty transposon DNA plasmid. In some examples, the vector further comprises a promoter. In some embodiments, the promoter is hEF1a1. In other embodiments, the CAR comprises at least one of: (a) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 3-14; or (b) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 75-82.

[0013] In some embodiments, ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15-74. In some examples, the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73. In other examples, the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74. In some cases, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 83. In other cases, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 84.

[0014] In some cases, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 85. In other cases, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 86. In some embodiments, the transmembrane domain is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of the amino acid sequences as shown in SEQ ID NOs: 87-88.

[0015] In some examples, the costimulatory signaling domain comprises 4-1BB. In some embodiments, the costimulatory signaling domain of 4-1BB comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 89. In other embodiments, the CD3 zeta signaling domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,

99% or 100% identity with the amino acid sequence of SEQ ID NO: 93. In some cases, the vector further comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NOs: 94-108.

[0016] In some cases, the vector comprises a plasmid. In some embodiments, each vector comprises an expression plasmid. In some embodiments, the non-viral vector is a Sleeping Beauty transposon. In some examples, the vector further comprises an additional vector comprising a polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

[0017] Provided herein is an immune effector cell comprising the isolated nucleic acid. In some embodiments, an immune effector cell comprises the vector.

[0018] Further provided herein is an immune effector cell comprising (1) a cell tag; (2) a cytokine; and (3) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain.

[0019] In some embodiments, the cytokine is IL-15. In some embodiments, the IL-15 is a membrane bound IL-15. In some cases, the membrane bound IL-15 comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 113. In other cases, the cell tag is a truncated epidermal growth factor receptor. In some embodiments, the truncated epidermal growth factor receptor is a HER1t, HER1t-1 or a functional variant thereof. In some cases, the truncated epidermal growth factor receptor comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 109 or SEQ ID NO: 110. In some examples, the cell tag comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 111. In other embodiments, the cell tag comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 112.

[0020] In some embodiments, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 83. In other embodiments, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ

ID NO: 84. In some cases, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 85. In other cases, the spacer is a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 86.

[0021] In some examples, the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), or a regulatory T cell. In other examples, the CAR comprises at least one of a polypeptide having amino acid sequences as shown in SEQ ID NOs: 3-14 or 75-82. In some cases, the CAR comprises at least one of a polypeptide having amino acid sequences as shown in SEQ ID NOs: 15-74. In other cases, the immune effector cell further comprises gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

[0022] Provided herein is a method for stimulating a T cell-mediated immune response to a target cell population or tissue in a human subject in need thereof, comprising administering to the human subject an effective amount of a cell genetically modified to express a CAR, wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; (e) a CD3 zeta signaling domain; and (f) a truncated epidermal growth factor receptor (HER1t).

[0023] In some embodiments, the human has been diagnosed with at least one of lung cancer, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, adrenal cancer, melanoma, uterine cancer, testicular cancer, or bladder cancer. In some cases, the human has been diagnosed with non-Hodgkin's lymphoma, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), multiple myeloma (MM), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML).

[0024] Provided herein is an isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises: (a) a ROR-1 antigen binding domain with at least one of amino acid sequences of as shown in SEQ ID NO: 3-14 , 75-82, or 15-74; (b) a spacer with at least one of amino acid sequences of SEQ ID NOs: 83-86; (c) a costimulatory signaling domain comprising CD28 with the amino acid sequence of SEQ ID NO: 90; (d) a HER1 tag which comprises at least one of HER1t with the amino acid sequence of SEQ ID NO: 109 and HER1t-1 with the amino acid sequence of SEQ ID NO: 110; and (e) a CD3 zeta signaling domain with the amino acid sequence of SEQ ID NO: 93.

[0025] Provided herein is an isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises: (a) a ROR-1 antigen binding domain with at least one of amino acid sequences as shown in SEQ ID NO: 3-14, 85-82, or 15-74; (b) a spacer with at least one of amino acid sequences of SEQ ID NO: 83-86; (c) a costimulatory signaling domain comprising 4-1BB with the amino acid sequence of SEQ ID NO: 89; (d) a HER1 tag which comprises at least one of HER1t with the amino acid sequence of SEQ ID NO: 109 and HER1t-1 with the amino acid sequence of SEQ ID NO: 110; and (e) a CD3 zeta signaling domain with the amino acid sequence of SEQ ID NO: 93.

[0026] In some examples, a vector comprises any one or more of the isolated polynucleotides. In some embodiments, the vector is a lentivirus vector, a retroviral vector, or a non-viral vector. In some embodiments, the non-viral vector is a Sleeping Beauty transposon. In other examples, the vector is a plurality of vectors. In some embodiments, the vector further comprises an additional vector comprising a polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

[0027] Provided herein is a system for expressing a CAR in an immune effector cell, the system comprising one or more vectors encoding an isolated nucleic acid. In some examples, the immune effector cell is a T cell or NK cell. In some embodiments, the system further comprises a nucleic acid encoding at least one additional gene. In some embodiments, the additional gene comprises a cytokine. In some cases, the cytokine comprises at least one of IL-2, IL-15, IL-12, IL-21, and a fusion of IL-15 and IL-15Rα. In other embodiments, the cytokine is in secreted form. In some embodiments, the cytokine is in membrane bound form. In some embodiments, the system comprises at least one vector. In other cases, the at least one vector is a lentivirus vector, a retroviral vector, or a non-viral vector. In some embodiments, the non-viral vector is a Sleeping Beauty transposon. In some embodiments, the system further comprises a Sleeping Beauty transposase. In some examples, the Sleeping Beauty transposase is SB11, SB100X or SB110. In other examples, the system further comprises an additional vector comprising a polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation

domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker. In some embodiments, the immune effector cell is a mammalian cell.

[0028] Provided herein is a system for expressing a CAR in an immune effector cell, the system comprising (1) a first vector comprising a backbone and a nucleic acid sequence encoding: (a) a cell tag; (b) a cytokine; and (c) a chimeric antigen receptor (CAR), wherein the CAR comprises (i) a ROR-1 antigen binding domain; (ii) a spacer; (iii) a transmembrane domain; (iv) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (v) a CD3 zeta signaling domain; and (2) a second vector comprising a polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

[0029] Provided herein is a method of expressing a CAR in an immune effector cell comprising contacting the immune effector cell with a system.

[0030] Provided herein is a method of stimulating the proliferation and/or survival of engineered T-cells comprising: (a) obtaining a sample of cells from a subject, the sample comprising T-cells or T-cell progenitors; (b) transfecting the cells with one or more vectors encoding an isolated nucleic acid as provided in any one of claims 1-22 and 79-80 and a vector encoding a transposase , to provide a population of engineered ROR-1 CAR-expressing T-cells; and (c) optionally culturing the population of ROR-1 CAR T-cells ex vivo for 2 days or less.

[0031] In some embodiments, the vector further encodes a cytokine and a cell tag. In some cases, the cytokine is a fusion protein comprising IL- 15 and IL- 15Rα. In other embodiments, the method further comprises an additional vector comprising a polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

[0032] In some examples, the DNA binding domain comprises at least one of GAL4 (GAL4 DBD), a LexA DBD, a transcription factor DBD, a steroid/thyroid hormone nuclear receptor superfamily member DBD, a bacterial LacZ DBD, and a yeast DBD. In other embodiments, the

DNA binding domain comprises an amino acid sequence as shown in SEQ ID NO: 134. In some embodiments, the transactivation domain comprises at least one of a VP16 transactivation domain and a B42 acidic activator transactivation domain. In other cases, the transactivation domain comprises an amino acid sequence as shown in SEQ ID NO: 131. In some embodiments, at least one of the first nuclear receptor ligand binding domain and the second nuclear receptor ligand binding domain comprises at least one of an ecdysone receptor (EcR), a ubiquitous receptor, an orphan receptor 1, a NER-1, a steroid hormone nuclear receptor 1, a retinoid X receptor interacting protein-15, a liver X receptor β, a steroid hormone receptor like protein, a liver X receptor, a liver X receptor α, a farnesoid X receptor, a receptor interacting protein 14, and a famesol receptor.

[0033] In some cases, at least one of the first nuclear receptor ligand binding domain and the second nuclear receptor ligand binding domain comprise any one of amino acid sequences as shown in SEQ ID NOs: 135-136. In some examples, the first gene switch polypeptide comprises a GAL4 DBD fused to an EcR nuclear receptor ligand binding domain, and the second gene switch polypeptide comprises a VP16 transactivation domain fused to a retinoid receptor X (RXR) nuclear receptor ligand binding domain. In some embodiments, the Gal4 DBD fused to the EcR nuclear receptor ligand binding domain comprise an amino acid sequence as shown in any one of SEQ ID NOs: 137-138, and the VP16 transactivation domain fused to the retinoid receptor X (RXR) nuclear receptor ligand binding domain comprises an amino acid sequence as shown in SEQ ID NO: 133.

[0034] In some embodiments, the linker is a cleavable linker, a ribosome skipping linker sequence or an IRES linker. In some examples, the linker is an IRES linker and said IRES linker has a sequence as shown in any one of SEQ ID NOs: 146-147. In other embodiments, the linker is a cleavable linker or a ribosome skipping linker sequence. In other cases, the cleavable linker or the ribosome skipping linker sequence comprises one or more of a 2A linker, p2A linker, T2A linker, F2A linker, E2A linker, GSG-2A linker, GSG linker (SEQ ID NO: 129), SGSG linker (SEQ ID NO: 130), furinlink linker variants and derivatives thereof. In some embodiments, the cleavable linker or the ribosome skipping linker sequence has a sequence as shown in any one of SEQ ID NOs: 116-126. In some embodiments, at least one of the one or more vectors and the additional vector is a lentivirus vector, a retroviral vector, or a non-viral vector. In some cases, the non-viral vector is a Sleeping Beauty transposon. In some embodiments, the method further comprises a Sleeping Beauty transposase. In some embodiments, the Sleeping Beauty transposase is SB11, SB100X or SB110.

[0035] Provided herein is a method of culturing engineered T-cells comprising: (a) obtaining a sample of cells from a subject, the sample comprising T-cells or T-cell progenitors; (b)

transfecting the cells with one or more vectors encoding an isolated nucleic acid as provided in any one of claims 1-22 and 79-80 and a vector encoding a transposase, to provide a population of engineered ROR1 CAR-expressing T-cells; and (c) culturing the population of engineered ROR1 CAR-expressing T-cells ex vivo in a medium that selectively enhances proliferation of the engineered ROR1 CAR-expressing T-cells, wherein the engineered ROR1 CAR-expressing T-cells are cultured for no more than 21 days.

[0036] In some examples, the engineered ROR1 CAR-expressing T-cells are cultured for no more than 14 days. In some cases, the one or more vectors further encodes a cytokine and a cell tag. In some embodiments, the method further comprises an additional vector comprising a polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker. In other embodiments, the DNA binding domain comprises at least one of GAL4 (GAL4 DBD), a LexA DBD, a transcription factor DBD, a steroid/thyroid hormone nuclear receptor superfamily member DBD, a bacterial LacZ DBD, and a yeast DBD. In some embodiments, the DNA binding domain comprises an amino acid sequence as shown in SEQ ID NO: 134.

[0037] In other embodiments, the transactivation domain comprises at least one of a VP16 transactivation domain and a B42 acidic activator transactivation domain. In some embodiments, the transactivation domain comprises an amino acid sequence as shown in SEQ ID NO: 131. In some cases, at least one of the first nuclear receptor ligand binding domain and the second nuclear receptor ligand binding domain comprises at least one of an ecdysone receptor (EcR), a ubiquitous receptor, an orphan receptor 1, a NER-1, a steroid hormone nuclear receptor 1, a retinoid X receptor interacting protein-15, a liver X receptor β, a steroid hormone receptor like protein, a liver X receptor, a liver X receptor α, a farnesoid X receptor, a receptor interacting protein 14, and a famesol receptor. In other cases, at least one of the first nuclear receptor ligand binding domain and the second nuclear receptor ligand binding domain comprise any one of amino acid sequences as shown in SEQ ID NOs: 135-136. In some embodiments, the first gene switch polypeptide comprises a GAL4 DBD fused to an EcR nuclear receptor ligand binding domain, and the second gene switch polypeptide comprises a VP16 transactivation domain fused to a retinoid receptor X (RXR) nuclear receptor ligand binding domain. In some examples, the Gal4 DBD fused to the EcR nuclear receptor ligand binding domain comprise an amino acid sequence as shown in any one of SEQ ID NOs: 137-138, and the VP16 transactivation domain

fused to the retinoid receptor X (RXR) nuclear receptor ligand binding domain comprises an amino acid sequence as shown in SEQ ID NO: 133.

[0038] In some cases, the linker is a cleavable linker, a ribosome skipping linker sequence or an IRES linker. In other embodiments, the linker is an IRES linker and said IRES linker has a sequence as shown in any one of SEQ ID NOs: 146-147. In some embodiments, the linker is a cleavable linker or a ribosome skipping linker sequence. In some examples, the cleavable linker or the ribosome skipping linker sequence comprises one or more of a 2A linker, p2A linker, T2A linker, F2A linker, E2A linker, GSG-2A linker, GSG linker (SEQ ID NO: 129), SGSG linker (SEQ ID NO: 130), furinlink linker variants and derivatives thereof. In other examples, the cleavable linker or the ribosome skipping linker sequence has a sequence as shown in any one of SEQ ID NOs: 116-126. In some embodiments, at least one of the one or more vectors, the vector, and the additional vector is a lentivirus vector, a retroviral vector, or a non-viral vector. In some embodiments, the non-viral vector is a Sleeping Beauty transposon. In other embodiments, the method further comprises a Sleeping Beauty transposase. In some cases, the Sleeping Beauty transposase is SB11, SB100X or SB110. In some embodiments, culturing the engineered ROR1 CAR-expressing T-cells in (c) comprises culturing the engineered ROR1 CAR-expressing T-cells in the presence of artificial antigen presenting cells (aAPCs) that stimulate expansion of the engineered ROR1 CAR-T expressing T-cells. In other cases, the aAPCs are engineered K562 cells. In some embodiments, the aAPCs comprises (i) a ROR1 antigen expressed on the engineered CAR cells; (ii) CD64; (ii) CD86; (iii) CD 137L; and/or (v) membrane-bound IL- 15, expressed on the surface of the aAPCs.

[0039] Provided herein is a method of treating cancer in a subject in need thereof comprising administering to the subject one or more doses of an effective amount of engineered T-cells, wherein the engineered T-cells comprise ROR-1 CAR and membrane bound IL-15.

[0040] In some embodiments, a first dose of an effective amount of engineered T-cells is administered intraperitoneally. In other embodiments, a second dose of an effective amount of engineered T-cells is administered intravenously. In some embodiments, the cancer is non-Hodgkin's lymphoma, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), multiple myeloma (MM), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML). In some cases, the cancer is lung cancer, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, adrenal cancer, melanoma, uterine cancer, testicular cancer, or bladder cancer. In some examples, the ROR-1 CAR is encoded by any one of sequences as shown in SEQ ID NOs: 3-14, 75-82, or 15-74. In some embodiments, the membrane bound IL-15 is encoded by SEQ ID NO: 260. In some embodiments, the effective amount of engineered T-cells is at least 10^2 cells/kg. In other embodiments, the effective amount

of engineered T-cells is at least 10^4 cells/kg. In some cases, the effective amount of engineered T-cells is at least 10^5 cells/kg.

BRIEF DESCRIPTION OF THE FIGURES

[0041] The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0042] **FIGs. 1A-1E** depict exemplary gene expression cassettes for ROR1 CARs and cytokines in different configurations. In certain cases, the ROR1 CAR and/or cytokine can be co-expressed with a cell or kill tag for conditional *in vivo* ablation. Exemplary gene expression cassettes or combinations thereof for constitutive expression of ROR1 CAR and mbIL-15 can include c + e or d + e.

[0043] **FIGs. 2A-2E** depict exemplary gene expression cassettes for ROR1 CARs and cytokines in different configurations with gene switch components. Exemplary gene expression cassettes or combinations thereof for inducible expression can include a + e, b + e, c + e, or d + e.

[0044] **FIG. 3** depicts an exemplary ROR1 CAR design. ROR1 CAR based on the standard CAR design did not show any expression of ROR1 CAR after gene transfer. Various optimization strategies as described in Example 1 were employed to develop a surface expressed, functional ROR1 CAR.

[0045] **FIG. 4** depicts an illustration of ROR1 CARs with spacers of different lengths.

[0046] **FIG. 5** depicts the combination of signal peptides tested in a ROR1 CAR (murine scFv) construct that utilized conventional CD8α stalk and shows expression of ROR1 CAR (murine scFv) with different signal peptides. Expression of transgenes was measured at Day 1 post nucleofection. Cells were gated as live CD3+ cells. Short CD8α (conventional) stalk does not permit surface expression regardless of signal peptide used (human or mouse).

[0047] **FIG. 6** shows expression of ROR1 CAR (murine scFv) with different combinations of signal peptides and different spacer and spacer lengths. ROR1 CARs utilizing three different longer spacer lengths with strong surface expressions were identified.

[0048] **FIG. 7** shows expression of ROR1 CAR (murine scFv) with CD8α 3X stalk with different signal peptides. Expression of murine scFv based ROR1 CAR with 3X CD8α stalk was improved for certain (mIgGVH, β2M and Azurocidin) signal peptides.

[0049] FIG. 8 shows different ROR 1 CAR (murine scFv) with various combinations of stalk, intracellular domain and orientations of the VH/VL chains evaluated and expression of ROR 1 CAR (murine scFv) with various combinations of stalk, intracellular domain and orientations of the VH/VL chains. As observed, reversing orientation from VL-VH to VH-VL enables surface expression form hGMCSFR and hIGHV3-23 signal peptides.

[0050] FIG. 9A and FIG. 9B show specificity of ROR1(murine scFv)-CD8-3x stalk.CD28z CAR -T cells in various tumor cell lines as measured in CD107a degranulation assay and IFN-α expression assay.

[0051] FIG. 10A shows quantitative analysis of JeKo-1 tumor burden as measured by *in vivo* bioluminescence (IVIS) imaging at day 7 post CAR-T treatment. NSG mice (N = 5-7 mice per group) were administered with JeKo tumor cell line via i.p. injection on Day 0. Tumor bearing mice were treated with different hROR1 CAR-T cells treatment via single i.p. injection seven days after tumor cell administration and tumor burden was quantified via IVIS through the course of treatment. Data shown are mean ± SEM.

[0052] FIG. 10B shows tumor burden pre-treatment.

[0053] FIG. 11 shows quantitative analysis of JeKo-1 tumor burden as measured by *in vivo* bioluminescence (IVIS) imaging (as described above) at day 36.

[0054] FIG. 12 shows quantification of human CD3+ T cells in peripheral blood of tumor bearing NSG mice treated with various hROR1 CAR-T cells. The CD3+ T cell expansion in blood was measured at days 15, 22, 29, 36, 43 and 50.

[0055] FIG. 13A and FIG. 13B demonstrates that ROR1+ tumor cell lines were equally killed by hROR1 CAR T cells co-expressing constitutive or RTS-mbIL-15 *in vitro*.

[0056] FIG. 14 demonstrates that administration of hROR1 CAR RTS-mbIL-15-T cells + veledimex promotes anti-tumor effect in a JeKo-1 xenograft mouse model in a dose-dependent manner.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0057] The following description and examples illustrate embodiments of the invention in detail. It is to be understood that this invention is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this invention, which are encompassed within its scope.

[0058] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have

the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0059] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0060] Although various features of the invention can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the invention can be described herein in the context of separate embodiments for clarity, the invention can also be implemented in a single embodiment.

[0061] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, *e.g.*, to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0062] In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[0063] Reference in the specification to "some embodiments," "an embodiment," "one embodiment" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the inventions.

[0064] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[0065] The term "about" in relation to a reference numerical value and its grammatical equivalents as used herein can include the numerical value itself and a range of values plus or minus 10% from that numerical value. For example, the amount "about 10" includes 10 and any amounts from 9 to 11. For example, the term "about" in relation to a reference numerical value

can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

[0066] By "isolated" is meant the removal of a nucleic acid from its natural environment. By "purified" is meant that a given nucleic acid, whether one that has been removed from nature (including genomic DNA and mRNA) or synthesized (including cDNA) and/or amplified under laboratory conditions, has been increased in purity, wherein "purity" is a relative term, not "absolute purity." It is to be understood, however, that nucleic acids and proteins can be formulated with diluents or adjuvants and still for practical purposes be isolated. For example, nucleic acids typically are mixed with an acceptable carrier or diluent when used for introduction into cells.

[0067] "Polynucleotide" or "oligonucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double and single stranded DNA, triplex DNA, as well as double and single stranded RNA. It also includes modified, for example, by methylation and/or by capping, and unmodified forms of the polynucleotide. The term is also meant to include molecules that include non-naturally occurring or synthetic nucleotides as well as nucleotide analogs.

[0068] "Polypeptide" is used interchangeably with the terms "polypeptides" and "protein(s)," and refers to a polymer of amino acid residues. A "mature protein" is a protein which is full-length and which, optionally, includes glycosylation or other modifications typical for the protein in a given cellular environment.

[0069] Nucleic acids and/or nucleic acid sequences are "homologous" when they are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. Proteins and/or protein sequences are homologous when their encoding DNAs are derived, naturally or artificially, from a common ancestral nucleic acid or nucleic acid sequence. The homologous molecules can be termed homologs. For example, any naturally occurring proteins, as described herein, can be modified by any available mutagenesis method. When expressed, this mutagenized nucleic acid encodes a polypeptide that is homologous to the protein encoded by the original nucleic acid. Homology is generally inferred from sequence identity between two or more nucleic acids or proteins (or sequences thereof). The precise percentage of identity between sequences that is useful in establishing homology varies with the nucleic acid and protein at issue, but as little as 25% sequence identity is routinely used to establish homology. Higher levels of sequence identity, *e.g.*, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or 99% or more can also be used to establish homology.

[0070] The terms “identical” or “sequence identity” in the context of two nucleic acid sequences or amino acid sequences of polypeptides refers to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. In one class of embodiments, the polypeptides herein are at least 80%, 85%, 90%, 98% 99% or 100% identical to a reference polypeptide, or a fragment thereof, *e.g.*, as measured by BLASTP (or CLUSTAL, or any other available alignment software) using default parameters. Similarly, nucleic acids can also be described with reference to a starting nucleic acid, *e.g.*, they can be 50%, 60%, 70%, 75%, 80%, 85%, 90%, 98%, 99% or 100% identical to a reference nucleic acid or a fragment thereof, *e.g.*, as measured by BLASTN (or CLUSTAL, or any other available alignment software) using default parameters. When one molecule is the to have certain percentage of sequence identity with a larger molecule, it means that when the two molecules are optimally aligned, the percentage of residues in the smaller molecule finds a match residue in the larger molecule in accordance with the order by which the two molecules are optimally aligned.

[0071] “Transposon” or “transposable element” (TE) is a vector DNA sequence that can change its position within the genome, sometimes creating or reversing mutations and altering the cell’s genome size. Transposition often results in duplication of the TE. Class I TEs are copied in two stages: first, they are transcribed from DNA to RNA, and the RNA produced is then reverse transcribed to DNA. This copied DNA is then inserted at a new position into the genome. The reverse transcription step is catalyzed by a reverse transcriptase, which can be encoded by the TE itself. The characteristics of retrotransposons are similar to retroviruses, such as HIV. The cut-and-paste transposition mechanism of class II TEs does not involve an RNA intermediate. The transpositions are catalyzed by several transposase enzymes. Some transposases non-specifically bind to any target site in DNA, whereas others bind to specific DNA sequence targets. The transposase makes a staggered cut at the target site resulting in single-strand 5’ or 3’ DNA overhangs (sticky ends). This step cuts out the DNA transposon, which is then ligated into a new target site; this process involves activity of a DNA polymerase that fills in gaps and of a DNA ligase that closes the sugar-phosphate backbone. This results in duplication of the target site. The insertion sites of DNA transposons can be identified by short direct repeats which can be created by the staggered cut in the target DNA and filling in by DNA polymerase, followed by a series of inverted repeats important for the TE excision by transposase. Cut-and-paste TEs can be duplicated if their transposition takes place during S phase of the cell cycle when a donor site has already been replicated, but a target site has not yet been replicated. Transposition can be classified as either “autonomous” or “non-autonomous” in both Class I and Class II TEs. Autonomous TEs can move by themselves while non-

autonomous TEs require the presence of another TE to move. This is often because non-autonomous TEs lack transposase (for class II) or reverse transcriptase (for class I).

[0072] "Transposase" refers an enzyme that binds to the end of a transposon and catalyzes the movement of the transposon to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. In some embodiments, the transposase's catalytic activity can be utilized to move gene(s) from a vector to the genome.

[0073] The nucleic acid sequences and vectors disclosed or contemplated herein can be introduced into a cell by "transfection," "transformation," "nucleofection" or "transduction." "Transfection," "transformation," or "transduction," as used herein, refer to the introduction of one or more exogenous polynucleotides into a host cell by using physical or chemical methods. Many transfection techniques are known in the art and include, for example, calcium phosphate DNA co-precipitation (see, *e.g.*, Murray E. J. (ed.), Methods in Molecular Biology, Vol. 7, Gene Transfer and Expression Protocols, Humana Press (1991)); DEAE-dextran; electroporation; cationic liposome-mediated transfection; tungsten particle-facilitated microparticle bombardment (Johnston, Nature, 346: 776-777 (1990)); and strontium phosphate DNA co-precipitation (Brash et al., Mol. Cell Biol., 7: 2031-2034 (1987)); and nucleofection (Trompeter et al., J. Immunol. Methods 274:245-256 (2003). Phage or viral vectors can be introduced into host cells, after growth of infectious particles in suitable packaging cells, many of which are commercially available.

[0074] "Promoter" refers to a region of a polynucleotide that initiates transcription of a coding sequence. Promoters are located near the transcription start sites of genes, on the same strand and upstream on the DNA (towards the 5' region of the sense strand). Some promoters are constitutive as they are active in all circumstances in the cell, while others are regulated becoming active in response to specific stimuli, *e.g.*, an inducible promoter.

[0075] The term "promoter activity" refers to the extent of expression of nucleotide sequence that is operably linked to the promoter whose activity is being measured. Promoter activity can be measured directly by determining the amount of RNA transcript produced, for example by Northern blot analysis or indirectly by determining the amount of product coded for by the linked nucleic acid sequence, such as a reporter nucleic acid sequence linked to the promoter.

[0076] "Inducible promoter" as used herein refers to a promoter which is induced into activity by the presence or absence of transcriptional regulators, *e.g.*, biotic or abiotic factors. Inducible promoters are useful because the expression of genes operably linked to them can be turned on or off at certain stages of development of an organism or in a particular tissue. Examples of inducible promoters are alcohol-regulated promoters, tetracycline-regulated promoters, steroid-regulated promoters, metal-regulated promoters, pathogenesis-regulated promoters, temperature-

regulated promoters and light-regulated promoters. In one embodiment, the inducible promoter is part of a genetic switch.

[0077] The term "enhancer," as used herein, refers to a DNA sequence that increases transcription of, for example, a nucleic acid sequence to which it is operably linked. Enhancers can be located many kilobases away from the coding region of the nucleic acid sequence and can mediate the binding of regulatory factors, patterns of DNA methylation, or changes in DNA structure. A large number of enhancers from a variety of different sources are well known in the art and are available as or within cloned polynucleotides (from, *e.g.*, depositories such as the ATCC as well as other commercial or individual sources). A number of polynucleotides comprising promoters (such as the commonly-used CMV promoter) also comprise enhancer sequences. Enhancers can be located upstream, within, or downstream of coding sequences. The term "Ig enhancers" refers to enhancer elements derived from enhancer regions mapped within the immunoglobulin (Ig) locus (such enhancers include for example, the heavy chain (mu) 5' enhancers, light chain (kappa) 5' enhancers, kappa and mu intronic enhancers, and 3' enhancers (see generally Paul W. E. (ed), Fundamental Immunology, 3rd Edition, Raven Press, New York (1993), pages 353-363; and U.S. Pat. No. 5,885,827).

[0078] "Coding sequence" as used herein refers to a segment of a polynucleotide that codes for a polypeptide. The region or sequence is bounded nearer the 5' end by a start codon and nearer the 3' end with a stop codon. Coding sequences can also be referred to as open reading frames.

[0079] "Operably linked" as used herein refers to refers to the physical and/or functional linkage of a DNA segment to another DNA segment in such a way as to allow the segments to function in their intended manners. A DNA sequence encoding a gene product is operably linked to a regulatory sequence when it is linked to the regulatory sequence, such as, for example, promoters, enhancers and/or silencers, in a manner which allows modulation of transcription of the DNA sequence, directly or indirectly. For example, a DNA sequence is operably linked to a promoter when it is ligated to the promoter downstream with respect to the transcription initiation site of the promoter, in the correct reading frame with respect to the transcription initiation site and allows transcription elongation to proceed through the DNA sequence. An enhancer or silencer is operably linked to a DNA sequence coding for a gene product when it is ligated to the DNA sequence in such a manner as to increase or decrease, respectively, the transcription of the DNA sequence. Enhancers and silencers can be located upstream, downstream or embedded within the coding regions of the DNA sequence. A DNA for a signal sequence is operably linked to DNA coding for a polypeptide if the signal sequence is expressed as a preprotein that participates in the secretion of the polypeptide. Linkage of DNA

sequences to regulatory sequences is typically accomplished by ligation at suitable restriction sites or via adapters or linkers inserted in the sequence using restriction endonucleases known to one of skill in the art.

[0080] The term “transcriptional regulator” refers to a biochemical element that acts to prevent or inhibit the transcription of a promoter-driven DNA sequence under certain environmental conditions (*e.g.*, a repressor or nuclear inhibitory protein), or to permit or stimulate the transcription of the promoter-driven DNA sequence under certain environmental conditions (*e.g.*, an inducer or an enhancer).

[0081] The term “induction” refers to an increase in nucleic acid sequence transcription, promoter activity and/or expression brought about by a transcriptional regulator, relative to some basal level of transcription.

[0082] A “target” gene or “heterologous” gene, or “gene of interest (GOI)” refers to a gene introduced into the host cell by gene transfer.

[0083] "Recombinase" as used herein refers to a group of enzymes that can facilitate site-specific recombination between defined sites, where the sites are physically separated on a single DNA molecule or where the sites reside on separate DNA molecules. The DNA sequences of the defined recombination sites are not necessarily identical. Initiation of recombination depends on protein-DNA interaction, within the group there are large number of proteins that catalyze phage integration and excision (*e.g.*, λ integrase, .ϕC31), resolution of circular plasmids (*e.g.*, Tn3, gamma delta, Cre, Flp), DNA inversion for expression of alternate genes (*e.g.*, Hin, Gin, Pin), assembly of genes during development (*e.g.*, Anabaena nitrogen fixation genes), and transposition (*e.g.*, IS607 transposon). Most site-specific recombinases fall into one of the two families, based on evolutionary and mechanistic relatedness. These are. λ integrase family or tyrosine recombinases (*e.g.*, Cre, Flp, Xer D) and resolvase/integrase family or serine recombinase family (*e.g.*, ϕC31, TP901-1, Tn3, gamma delta).

[0084] “Recombination attachment sites” are specific polynucleotide sequences that are recognized by the recombinase enzymes described herein. Typically, two different sites are involved (termed "complementary sites"), one present in the target nucleic acid (*e.g.*, a chromosome or episome of a eukaryote or prokaryote) and another on the nucleic acid that is to be integrated at the target recombination site. The terms “attB” and “attP,” which refer to attachment (or recombination) sites originally from a bacterial target and a phage donor, respectively, are used herein although recombination sites for particular enzymes can have different names. The recombination sites typically include left and right arms separated by a core or spacer region. Thus, an attB recombination site consists of BOB', where B and B' are the left and right arms, respectively, and O is the core region. Similarly, attP is POP', where P and P'

are the arms and O is again the core region. Upon recombination between the attB and attP sites, and concomitant integration of a nucleic acid at the target, the recombination sites that flank the integrated DNA are referred to as “attL” and “attR.” The attL and attR sites, using the terminology above, thus consist of BOP' and POB', respectively. In some representations herein, the "O" is omitted and attB and attP, for example, are designated as BB' and PP', respectively.

[0085] An “expression vector” or “vector” is any genetic element, *e.g.*, a plasmid, chromosome, virus, transposon, behaving either as an autonomous unit of polynucleotide replication within a cell. (*i.e.* capable of replication under its own control) or being rendered capable of replication by insertion into a host cell chromosome, having attached to it another polynucleotide segment, so as to bring about the replication and/or expression of the attached segment. Suitable vectors include, but are not limited to, plasmids, transposons, bacteriophages and cosmids. Vectors can contain polynucleotide sequences which are necessary to effect ligation or insertion of the vector into a desired host cell and to effect the expression of the attached segment. Such sequences differ depending on the host organism; they include promoter sequences to effect transcription, enhancer sequences to increase transcription, ribosomal binding site sequences and transcription and translation termination sequences. Alternatively, expression vectors can be capable of directly expressing nucleic acid sequence products encoded therein without ligation or integration of the vector into host cell DNA sequences.

[0086] Vector also can comprise a “selectable marker gene.” The term “selectable marker gene,” as used herein, refers to a nucleic acid sequence that allows cells expressing the nucleic acid sequence to be specifically selected for or against, in the presence of a corresponding selective agent. Suitable selectable marker genes are known in the art and described in, *e.g.*, International Patent Application Publications WO 1992/08796 and WO 1994/28143; Wigler et al., Proc. Natl. Acad. Sci. USA, 77: 3567 (1980); O’Hare et al., Proc. Natl. Acad. Sci. USA, 78: 1527 (1981); Mulligan & Berg, Proc. Natl. Acad. Sci. USA, 78: 2072 (1981); Colberre-Garapin et al., J. Mol. Biol., 150:1 (1981); Santerre et al., Gene, 30: 147 (1984); Kent et al., Science, 237: 901-903 (1987); Wigler et al., Cell, 11: 223 (1977); Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA, 48: 2026 (1962); Lowy et al., Cell, 22: 817 (1980); and U.S. Pat. Nos. 5,122,464 and 5,770,359.

[0087] In some embodiments, the vector is an “episomal expression vector” or “episome,” which is able to replicate in a host cell, and persists as an extrachromosomal segment of DNA within the host cell in the presence of appropriate selective pressure (see, *e.g.*, Conese et al., Gene Therapy, 11:1735-1742 (2004)). Representative commercially available episomal expression vectors include, but are not limited to, episomal plasmids that utilize Epstein Barr

Nuclear Antigen 1 (EBNA1) and the Epstein Barr Virus (EBV) origin of replication (oriP). The vectors pREP4, pCEP4, pREP7, and pcDNA3.1 from Invitrogen (Carlsbad, Calif.) and pBK-CMV from Stratagene (La Jolla, Calif.) represent non-limiting examples of an episomal vector that uses T-antigen and the SV40 origin of replication in lieu of EBNA1 and oriP.

[0088] "Cancer cell" refers to a cell undergoing early, intermediate or advanced stages of multi-step neoplastic progression as previously described (Pitot et al., Fundamentals of Oncology, 15-28 (1978)). This includes cells in early, intermediate and advanced stages of neoplastic progression including "pre-neoplastic" cells (*i.e.*, "hyperplastic" cells and dysplastic cells), and neoplastic cells in advanced stages of neoplastic progression of a dysplastic cell.

[0089] "Metastatic" cancer cell refers to a cancer cell that is translocated from a primary cancer site (*i.e.*, a location where the cancer cell initially formed from a normal, hyperplastic or dysplastic cell) to a site other than the primary site, where the translocated cancer cell lodges and proliferates.

[0090] "Cancer" refers to a plurality of cancer cells that may or may not be metastatic, such as ovarian cancer, breast cancer, lung cancer, prostate cancer, cervical cancer, pancreatic cancer, colon cancer, stomach cancer, esophagus cancer, mouth cancer, tongue cancer, gum cancer, skin cancer (*e.g.*, melanoma, basal cell carcinoma, Kaposi's sarcoma, etc.), muscle cancer, heart cancer, liver cancer, bronchial cancer, cartilage cancer, bone cancer, testis cancer, kidney cancer, endometrium cancer, uterus cancer, bladder cancer, bone marrow cancer, lymphoma cancer, spleen cancer, thymus cancer, thyroid cancer, brain cancer, neuron cancer, mesothelioma, gall bladder cancer, ocular cancer (*e.g.*, cancer of the cornea, cancer of uvea, cancer of the choroids, cancer of the macula, vitreous humor cancer, etc.), joint cancer (such as synovium cancer), glioblastoma, lymphoma, leukemia, non-Hodgkin's lymphoma, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), multiple myeloma (MM), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML).

ROR-1

[0091] ROR1 is a transmembrane protein within the receptor tyrosine kinase (RTK) family. The ROR1 gene encodes two well-defined isoforms: a short 393 amino acid (aa) intracellular protein (isoform 2) and a long 937 aa type-1 transmembrane protein (isoform 1). The long cell surface isoform is expressed on primary human B-chronic lymphocytic leukemia (B-CLL) and mantle cell lymphomas, a subset of B-acute lymphocytic leukemia, and many tumors, including those associated with a metastatic phenotype.

[0092] ROR1 is principally expressed during embryonic development but its expression attenuates during fetal development. However, ROR1 is aberrantly expressed by some B-cell malignancies such as but not limited to, lymphomas, CLL, and B-ALL, and by many solid tumor malignancies, such as but not limited to, adrenal, bladder, breast, colon, lung, pancreas, prostate, ovary, skin, testes, uterus, and neuroblastoma.

Chimeric Antigen Receptors

[0093] In embodiments described herein, a CAR can comprise an extracellular antibody-derived single-chain variable domain (scFv) for target recognition, wherein the scFv can be connected by a flexible linker to a transmembrane domain and/or an intracellular signaling domain(s) that includes, for instance, CD3ζ for T-cell activation. Normally when T cells are activated *in vivo* they receive a primary antigen induced TCR signal with secondary costimulatory signaling from CD28 that induces the production of cytokines (*i.e.*, IL-2 and IL-21), which then feed back into the signaling loop in an autocrine/paracrine fashion. As such, CARs can include a signaling domain, for instance, a CD28 cytoplasmic signaling domain or other costimulatory molecule signaling domains such as 4-1BB signaling domain. Chimeric CD28 co-stimulation improves T-cell persistence by up-regulation of anti-apoptotic molecules and production of IL-2, as well as expanding T cells derived from peripheral blood mononuclear cells (PBMC).

[0094] In one embodiment, CARs are fusions of single-chain variable fragments (scFv) derived from monoclonal antibodies specific for various epitopes of ROR-1 for example, fused to transmembrane domain and CD3-zeta endodomain. Such molecules result in the transmission of a zeta signal in response to recognition by the scFv of its target.

[0095] In an embodiment, a CAR can have an ectodomain (extracellular), a transmembrane domain and an endodomain (intracellular). In one embodiment of the CAR ectodomain, a signal peptide directs the nascent protein into the endoplasmic reticulum. This is if the receptor is to be glycosylated and anchored in the cell membrane for example. Any eukaryotic signal peptide sequence is envisaged to be functional. Generally, the signal peptide natively attached to the amino-terminal most component is used (*e.g.*, in a scFv with orientation light chain - linker - heavy chain, the native signal of the light-chain is used). In embodiments, the signal peptide is GM-CSFRa (SEQ ID NO: 94) or IgK (SEQ ID NO: 95). Other signal peptides that can be used include signal peptides from CD8alpha (SEQ ID NO: 97) and CD28. In one embodiment, the signal peptide is Mouse Ig VH region 3 (SEQ ID NO: 101), Azurocidin (SEQ ID NO: 103), IGHV3-23 (SEQ ID NO: 106), IGKV1-D33 (HuL1) (SEQ ID NO: 107) or IGKV1-D33 (L14F) (HuH7) (SEQ ID NO: 108).

[0096] The antigen recognition domain can be a scFv. There can, however, be alternatives. An antigen recognition domain from native T-cell receptor (TCR) alpha and beta single chains are envisaged, as they have simple ectodomains (*e.g.* CD4 ectodomain) and as well as other recognition components such as a linked *e.g.*, cytokine (which leads to recognition of cells bearing the cytokine receptor). Almost anything that binds a given target, such as *e.g.*, viral associated antigen, with high affinity can be used as an antigen recognition region.

[0097] In general, CARs exist in a dimerized form and are expressed as a fusion protein that links the extracellular scFv (VH linked to VL) region, a spacer, a transmembrane domain, and intracellular signaling motifs. The endodomain of the first generation CAR induces T cell activation solely through CD3-ζ signaling. The second generation CAR provides activation signaling through CD3-ζ and CD28, or other endodomains such as 4- 1BB or OX40. The 3rd generation CAR activates T cells via a CD3-ζ-containing combination of three signaling motifs such as CD28, 4-1BB, or OX40.

[0098] In embodiments, the present invention provides chimeric antigen receptor (CAR) comprising an extracellular domain, a transmembrane domain and an intracellular signaling domain. In embodiments, the extracellular domain comprises a target-specific binding element otherwise referred to as an antigen binding moiety or scFv and a spacer. In embodiments, the intracellular signaling domain or otherwise the cytoplasmic signaling domain comprises, a costimulatory signaling region and a zeta chain portion.

[0099] The costimulatory signaling region refers to a portion of the CAR comprising the intracellular signaling domain of a costimulatory molecule. Costimulatory molecules are cell surface molecules other than antigens receptors or their ligands that are required for an efficient response of lymphocytes to antigen.

[00100] In embodiments, between the extracellular domain and the transmembrane domain of the CAR, there is incorporated a stalk domain or stalk region. As used herein, the term "stalk domain" or “stalk region” generally means any oligonucleotide- or polypeptide that functions to link the transmembrane domain to, either the scFv or, the cytoplasmic domain in the polypeptide chain. A stalk domain can include a flexible hinge such as a Fc hinge and optionally one or two constant domains of Fc. In some instances, the stalk region comprises the hinge region from IgG1. In alternative instances, the stalk region comprises the CH2CH3 region of immunoglobulin and optionally portions of CD3. In some cases, the stalk region comprises a CD8α hinge region (SEQ ID NO: 83), an IgG4-Fc 12 amino acid hinge region (ESKYGPPCPPCP (SEQ ID NO: 285)) or IgG4 hinge regions as described in WO/2016/073755.

[00101] In other embodiments, between the extracellular domain and the transmembrane domain of the CAR, there is an incorporated spacer. A spacer can comprise a stalk region and a stalk extension region as depicted in **FIG 3 and 4**. In some embodiments, the spacer extends the distance between different domains of a chimeric polypeptide resulting in improved expression or functional activity of the polypeptide compared to an otherwise identical polypeptide lacking the spacer. In some instances, a spacer comprises any polypeptide that functions to link the transmembrane region to, either the extracellular region or, the cytoplasmic region in the chimeric polypeptide. In some embodiments, the spacer is flexible enough to allow the antigen or ligand-binding region to align in different orientations to facilitate antigen or ligand receptor recognition.

[00102] In some embodiments, the stalk region can be from about 20 to about 300 amino acids in length and comprises at least one dimerization site, and a stalk extension region can comprise from about 1 to about 10 times the length of the stalk region as measured by number of amino acids.

[00103] In some cases, a stalk region can be about 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or greater amino acids in length. In other cases, the stalk region can be about: 100, 125, 150, 175, 200, 225, 250, 275 or 300 amino acids in length.

[00104] In one embodiment, a spacer can include a single stalk region. In another embodiment, a spacer can comprise a stalk region (designated as "S") and stalk extension region(s), which is herein designated as "S'n." For example, a spacer can comprise one (1) stalk region and S'n, wherein n can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In further embodiments, the stalk region can be linked to stalk extension region S'n via a linker. In one embodiment, when the CAR is a ROR1 CAR, n is not 0.

[00105] In some cases, a stalk extension region can comprise from about 1 to about 10 times the length of the stalk region as measured by number of amino acids. For example, a stalk extension region can comprise about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times the length of the stalk region as measured by number of amino acids. In some cases, a stalk extension region can comprise greater than 10 times the length of the stalk region as measured by number of amino acids. In some examples, a stalk extension region can comprise up to 2 times the length of the stalk region as measured by number of amino acids but comprise fewer dimerization sites than the stalk region.

[00106] A stalk extension region of a subject antigen-binding polypeptide can contain at least one fewer dimerization site as compared to a stalk region. For example, if a stalk region comprises two dimerization sites, a stalk extension region can comprise one or zero dimerization sites. As another example, if a stalk region comprises one dimerization site, a stalk extension

region can comprise zero dimerization sites. In some examples, a stalk extension region lacks a dimerization site. In some examples, a stalk extension region can comprise up to 2 times the length of the stalk region as measured by number of amino acids but comprise no dimerization sites. In some examples, a stalk extension region can comprise up to 3 times the length of the stalk region as measured by number of amino acids but comprise no dimerization sites. In some examples, a stalk extension region can comprise up to 4 times the length of the stalk region as measured by number of amino acids but comprise zero dimerization sites. In some cases, one or more dimerization site(s) can be membrane proximal. In other cases, one or more dimerization site(s) can be membrane distal.

[00107] Each of the stalk extension regions can, in some examples, be from about 20 to about 60 amino acids in length. In other examples, stalk extension regions can be about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, greater amino acids in length, or any integer within or outside of that range. In some cases, each stalk extension region has a sequence which has at least about 60% identity to the stalk region. In some examples, each stalk extension region has a sequence which has at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity to the stalk region.

[00108] The transmembrane domain can be derived from either a natural or a synthetic source. Where the source is natural, the domain can be derived from any membrane-bound or transmembrane protein. Suitable transmembrane domains can include the transmembrane region(s) of alpha, beta or zeta chain of the T-cell receptor; or a transmembrane region from CD28, CD3 epsilon, CD3ζ, CD45, CD4, CD5, CD8alpha, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 or CD154. Alternatively the transmembrane domain can be synthetic, and can comprise hydrophobic residues such as leucine and valine. In some embodiments, a triplet of phenylalanine, tryptophan and valine is found at one or both termini of a synthetic transmembrane domain. Optionally, a short oligonucleotide or polypeptide linker, in some embodiments, between 2 and 10 amino acids in length can form the linkage between the transmembrane domain and the cytoplasmic signaling domain of a CAR. In some embodiments, the linker is a glycine-serine linker. In some embodiments, the transmembrane domain comprises a CD8α transmembrane domain or a CD3ζ transmembrane domain. In some embodiments, the transmembrane domain comprises a CD8α transmembrane domain. In other embodiments, the transmembrane domain comprises a CD3ζ transmembrane domain.

[00109] The intracellular domain can comprise one or more costimulatory domains. Exemplary costimulatory domains include, but are not limited to, CD8, CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134), CD3-zeta or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory

domains selected from CD8, CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134) or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, OX40 (CD134) or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory domains selected from CD8, CD28, 4-1BB (CD137), DAP10, DAP12 or fragment or combination thereof. In some instances, a CAR described herein comprises one or more, or two or more of costimulatory domains selected from CD28, 4-1BB (CD137), or fragment or combination thereof. In some instances, a CAR described herein comprises costimulatory domains CD28 and 4-1BB (CD137) or their respective fragments thereof. In some instances, a CAR described herein comprises costimulatory domains CD28 and OX40 (CD134) or their respective fragments thereof. In some instances, a CAR described herein comprises costimulatory domains CD8 and CD28 or their respective fragments thereof. In some instances, a CAR described herein comprises costimulatory domains CD28 or a fragment thereof. In some instances, a CAR described herein comprises costimulatory domains 4-1BB (CD137) or a fragment thereof. In some instances, a CAR described herein comprises costimulatory domains OX40 (CD134) or a fragment thereof. In some instances, a CAR described herein comprises costimulatory domains CD8 or a fragment thereof. In some instances, a CAR described herein comprises at least one costimulatory domain DAP10 or a fragment thereof. In some instances, a CAR described herein comprises at least one costimulatory domain DAP12 or a fragment thereof.

[00110] The intracellular signaling domain, also known as cytoplasmic domain, of the CAR of the present disclosure, is responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been placed. The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, can be cytolytic activity or helper activity including the secretion of cytokines. Thus, the term “intracellular signaling domain” refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion can be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal. In some embodiments, the intracellular domain further comprises a signaling domain for T-cell activation. In some instances, the signaling domain for T-cell activation comprises a domain derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta,

CD3 epsilon, CD5, CD22, CD79a, CD79b or CD66d. In some cases, the signaling domain for T-cell activation comprises a domain derived from CD3ζ.

[00111] In embodiments, provided herein is an isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a stalk domain; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; (e) a CD3 zeta signaling domain; and optionally (f) a truncated epidermal growth factor receptor (HER1t or HER1t1).

[00112] Included in the scope of the invention are nucleic acid sequences that encode functional portions of the CAR described herein. Functional portions encompass, for example, those parts of a CAR that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to a nucleic acid sequence encoding the parent CAR, a nucleic acid sequence encoding a functional portion of the CAR can encode a protein comprising, for example, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent CAR.

[00113] In embodiments, the CAR contains additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent CAR. Desirably, the additional amino acids do not interfere with the biological function of the functional portion, *e.g.*, recognize target cells, detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity of the CAR, as compared to the biological activity of the parent CAR.

[00114] The term "functional variant," as used herein, refers to a polypeptide, or a protein having substantial or significant sequence identity or similarity to the reference polypeptide, and retains the biological activity of the reference polypeptide of which it is a variant. Functional variants encompass, for example, those variants of the CAR described herein (the parent CAR) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to a nucleic acid sequence encoding the parent CAR, a nucleic acid sequence encoding a functional variant of the CAR can be for example, about 10% identical, about 25% identical, about 30% identical, about 50% identical, about 65% identical, about 80% identical, about 90% identical, about 95% identical, or about 99% identical to the nucleic acid sequence encoding the parent CAR.

[00115] A CAR described herein include (including functional portions and functional variants thereof) glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, *e.g.*, a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

Antigen Binding Moiety

[00116] In embodiments, a CAR described herein comprises a target-specific binding element otherwise referred to as an antigen-binding moiety. In embodiments, a CAR described herein engineered to target an antigen of interest by way of engineering a desired antigen-binding moiety that specifically binds to an antigen on a cell.

[00117] In embodiments, the antigen binding moiety of a CAR described herein is specific to ROR-1 (ROR-1 CAR). The ROR-1-specific CAR, when expressed on the cell surface, redirects the specificity of T cells to human ROR-1. In embodiments, the antigen binding domain comprises a single chain antibody fragment (scFv) comprising a variable domain light chain (VL) and variable domain heavy chain (VH) of a target antigen specific monoclonal anti- ROR-1 antibody joined by a flexible linker, such as a glycine-serine linker or a Whitlow linker. In embodiments, the scFv is mROR-1 that has been humanized. In some embodiments, the scFv is hROR1(VH-VL)_14, hROR1(VL-VH)_05, hROR1(VH-VL)_14-3, hROR1(VH-VL)_14-4, hROR1(VH_5-VL_14), hROR1(VH_5-VL_16), hROR1(VH_18-VL_04), or hROR1(VH_18-VL_14). In some embodiments, the antigen binding moiety can comprise VH and VL that are directionally linked, for example, from N to C terminus, VH-linker-VL or VL-linker-VH.

[00118] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of amino acid sequences as shown in SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74 (hROR1 VL).

[00119] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of amino acid sequences as shown in SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73 (hROR1 VH).

[00120] In embodiments, a CAR described herein comprises antigen binding moieties VL (SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74) and VH (SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73) with a Gly-Ser linker (SEQ ID NOs: 127, 129, or 130) or functional variants of the linker.

[00121] In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of amino acid sequences as shown in SEQ ID NOs: 3-14 and 75-82 (VH, VL and linker).

[00122] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 15 (hROR1 VH_04).

[00123] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 16 (hROR1 VL_04).

[00124] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 17 (hROR1 VH_05).

[00125] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 18 (hROR1 VL_05).

[00126] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 19 (hROR1 VH_06).

[00127] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 20 (hROR1 VL_06).

[00128] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 21 (hROR1 VH_07).

[00129] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 22 (hROR1 VL_07).

[00130] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 23 (hROR1 VH_08).

[00131] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 24 (hROR1 VL_08)

[00132] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 25 (hROR1 VH_09).

[00133] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 26 (hROR1 VL_09).

[00134] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 27 (hROR1 VH_10).

[00135] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 28 (hROR1 VL_10).

[00136] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 29 (hROR1 VH_11).

[00137] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 30 (hROR1 VL_11).

[00138] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 31 (hROR1 VH_12).

[00139] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 32 (hROR1 VL_12).

[00140] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 33 (hROR1 VH_13).

[00141] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 34 (hROR1 VL_13).

[00142] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 35 (hROR1 VH_14).

[00143] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 36 (hROR1 VL_14).

[00144] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%,

98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 37 (hROR1 VH_14-1).

[00145] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 38 (hROR1 VL_14-1).

[00146] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 39 (hROR1 VH_14-2).

[00147] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 40 (hROR1 VL_14-2).

[00148] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 41 (hROR1 VH_14-3).

[00149] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 42 (hROR1 VL_14-3).

[00150] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 43 (hROR1 VH_14-4).

[00151] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 44 (hROR1 VL_14-4).

[00152] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 45 (hROR1 VH_14-5).

[00153] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 46 (hROR1 VL_14-5).

[00154] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 47 (hROR1 VH_15).

[00155] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 48 (hROR1 VL_15).

[00156] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 49 (hROR1 VH_16).

[00157] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 50 (hROR1 VL_16).

[00158] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 51 (hROR1 VH_17).

[00159] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 52 (hROR1 VL_17).

[00160] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 53 (hROR1 VH_18).

[00161] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 54 (hROR1 VL_18).

[00162] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 55 (hROR1 VH_19).

[00163] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 56 (hROR1 VL_19).

[00164] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 57 (hROR1 VH_20).

[00165] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 58 (hROR1 VL_20).

[00166] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 59 (hROR1 VH_21).

[00167] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 60 (hROR1 VL_21).

[00168] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 61 (hROR1 VH_22).

[00169] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 62 (hROR1 VL_22).

[00170] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 63 (hROR1 VH_23).

[00171] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 64 (hROR1 VL_23).

[00172] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 65 (hROR1 VH_24).

[00173] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 66 (hROR1 VL_24).

[00174] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 67 (hROR1 VH_25).

[00175] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 68 (hROR1 VL_25).

[00176] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 69 (hROR1 VH_26).

[00177] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 70 (hROR1 VL_26).

[00178] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 71 (hROR1 VH_27).

[00179] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 72 (hROR1 VL_27).

[00180] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 73 (hROR1 VH_28).

[00181] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 74 (hROR1 VL_28).

[00182] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having the amino acid sequence of SEQ ID NO: 17 (hROR1 VH_5) and a VL polypeptide having the amino acid sequence of SEQ ID NO: 18 (hROR1 VL_5).

[00183] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having the amino acid sequence of SEQ ID NO: 35 (hROR1 VH_14) and a VL polypeptide having the amino acid sequence of SEQ ID NO: 36 (hROR1 VL_14).

[00184] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having the amino acid sequence of SEQ ID NO: 41 (hROR1 VH_14-3) and a VL polypeptide having the amino acid sequence of SEQ ID NO: 42 (hROR1 VL_14-3).

[00185] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having the amino acid sequence of SEQ ID NO: 43 (hROR1 VH_14-4) and a VL polypeptide having the amino acid sequence of SEQ ID NO: 44 (hROR1 VL_14-4).

[00186] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having the amino acid sequence of SEQ ID NO: 17 (hROR1 VH_5) and a VL polypeptide having the amino acid sequence of SEQ ID NO: 36 (hROR1 VL_14).

[00187] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having the amino acid sequence of SEQ ID NO: 53 (hROR1 VH_18) and a VL polypeptide having the amino acid sequence of SEQ ID NO: 16 (hROR1 VL_04).

[00188] In embodiments, a CAR described herein comprises an antigen-binding moiety comprising a VH polypeptide having the amino acid sequence of SEQ ID NO: 53 (hROR1 VH_18) and a VL polypeptide having the amino acid sequence of SEQ ID NO: 36 (hROR1 VL_14).

[00189] In embodiments, the antigen binding moiety has GM-CSFRa signal peptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 94.

[00190] In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 3. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 4. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 5. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 6. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 7. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 8. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 9. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 10. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 11. In embodiments,

a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 12. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 13. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 14.

[00191] In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 75. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 76. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 77. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 78. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 79. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 80. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 81. In embodiments, a CAR described herein comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 82.

Spacer

[00192] In embodiments, the ROR-1 CAR of the present disclosure comprises a spacer comprising one or more stalk regions and optionally one or more stalk extension regions, that provides a separation between the antigen binding moiety and the T cell membrane. In embodiments, the spacer establishes an optimal effector-target inter-membrane distance. In embodiments, the stalk domain provides flexibility for antigen binding domain to reach its target. In one embodiment, the stalk domain is a CD8alpha hinge domain. In some

embodiments, the term “spacer,” “stalk regions” and “stalk domain” can be used interchangeably herein.

[00193] In embodiments, the CD8alpha hinge domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 88.

[00194] In other embodiments, between the extracellular domain and the transmembrane domain of the CAR, there is incorporated a spacer. As described herein, a spacer can comprise a stalk region and a stalk extension region as depicted in **FIG. 3 and FIG. 4**. In one embodiment, a spacer can include a single stalk region. In another embodiment, a spacer can comprise a stalk region (designated as “S”) and stalk extension region(s), which is herein designated as “S’n.” For example, a spacer can comprise one (1) stalk region and S’n, wherein n can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20. In one embodiment, when the scFv of the ROR1 CAR is mROR1 (SEQ ID NOs: 3-14) or hROR1 (SEQ ID NOs: 75-82), wherein n is not 0.

[00195] In further embodiments, the stalk region can be linked to stalk extension region S’n via a linker. A linker as described herein can include for instance, a GSG linker (SEQ ID NO: 129), SGSG linker (SEQ ID NO: 130), (G4S)3 linker (SEQ ID NO: 127), (G4S)4 linker (SEQ ID NO: 294) and/or a Whitlow linker (SEQ ID NO: 128).

[00196] In one embodiment, stalk region and stalk extension region(s) can be derived or designed from a polypeptide of natural or of synthetic origin. The stalk region and/or stalk extension region(s) can comprise hinge domain(s) derived from a cell surface protein or derivatives or variants thereof. In some embodiments, the stalk region and/or stalk extension region(s) can comprise a hinge domain derived from CD28 or CD8alpha (CD8α). In some embodiments, each of the stalk region and stalk extension region(s) can be derived from at least one of a CD8alpha hinge domain, a CD28 hinge domain, a CTLA-4 hinge domain, a LNGFR extracellular domain, IgG1 hinge, IgG4 hinge and CH2-CH3 domain. The stalk and stalk extension region(s) can be separately derived from any combination of CD8alpha hinge domain, CD28 hinge domain, CTLA-4 hinge domain, LNGFR extracellular domain, IgG1 hinge, IgG4 hinge or CH2-CH3 domain. As an example, the stalk region can be derived from CD8alpha hinge domain and at least one stalk extension region can be derived from CD28 hinge domain thus creating a hybrid spacer. As another example, the stalk region can be derived from an IgG1 hinge or IgG4 hinge and at least one stalk extension region can be derived from a CH2-CH3 domain of IgG.

[00197] In certain embodiments, the stalk region can comprise one or more dimerization sites to form homo or hetero dimerized chimeric polypeptides. In other embodiments, the stalk

region or one or more stalk extension regions can contain mutations that eliminate dimerization sites altogether. In some embodiments, a stalk extension region(s) can contain at least one fewer dimerization site as compared to a stalk region. For example, if a stalk region comprises two dimerization sites, a stalk extension region can comprise one or zero dimerization sites. As another example, if a stalk region comprises one dimerization site, a stalk extension region can comprise zero dimerization sites. In some examples, the stalk extension region(s) lacks a dimerization site.

[00198] In some aspects of the embodiments disclosed herein, a stalk region of a subject antigen binding polypeptide comprises a sequence with at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or greater identity to a CD8alpha hinge domain. A CD8alpha hinge domain can comprise a polypeptide sequence with at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or greater identity to the sequence shown in SEQ ID NO: 83. In some cases, a stalk extension region comprises a polypeptide sequence with at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or greater identity to the sequence shown in SEQ ID NO: 83. In some cases, a stalk extension region comprises a nucleotide sequence with at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or greater identity to the sequence shown in SEQ ID NO: 230. In some examples, a stalk region and stalk extension region can together comprise a polynucleotide sequence with at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, or greater identity to any one of the sequences shown in SEQ ID NOs: 230-233.

Transmembrane Domain

[00199] In embodiments, the CAR comprises a transmembrane domain that is fused to the extracellular domain of the CAR stalk domain. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the CAR is used. In embodiments, the transmembrane domain is a hydrophobic alpha helix that spans the membrane.

[00200] The transmembrane domain can be derived from either a natural or a synthetic source. Where the source is natural, the domain can be derived from any membrane-bound or transmembrane protein. Transmembrane regions of particular use in this invention can be derived from (*i.e.* comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8alpha, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. Alternatively the transmembrane domain can be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In embodiments, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short

oligonucleotide or polypeptide linker, in embodiments, between 2 and 10 amino acids in length can form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR. In embodiments, the linker is a glycine-serine linker.

[00201] In embodiments, the transmembrane domain in a CAR described herein is the CD8alpha transmembrane domain. In embodiments, the CD8alpha transmembrane domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 87.

[00202] In embodiments, the transmembrane domain in a CAR described herein is the CD28 transmembrane domain. In embodiments, the CD28 transmembrane domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 88.

Cytoplasmic Domain (Co-Stimulatory Domain and Signaling Domain)

[00203] The cytoplasmic domain, also known as the intracellular signaling domain of a CAR described herein, is responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been placed. The term “effector function” refers to a specialized function of a cell. Effector function of a T cell, for example, can be cytolytic activity or helper activity including the secretion of cytokines. Thus the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion can be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[00204] Examples of intracellular signaling domains for use in a CAR described herein can include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability.

[00205] Signals generated through the TCR alone are generally insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic signaling

sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

[00206] Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner can contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs.

[00207] Examples of ITAM-containing primary cytoplasmic signaling sequences that are of particular use in the invention include those derived from TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. In embodiments, the cytoplasmic signaling molecule in a CAR described herein comprises a cytoplasmic signaling sequence derived from CD3 zeta.

[00208] In embodiments, the cytoplasmic domain of the CAR can be designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of a CAR described herein. For example, the cytoplasmic domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling region. The costimulatory signaling region refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, DAP10, DAP12 and a ligand that specifically binds with CD83, and the like. In embodiments, costimulatory molecules can be used together, *e.g.*, CD28 and 4-1BB or CD28 and OX40. Thus, while the invention in exemplified primarily with 4-1BB and CD28 as the co-stimulatory signaling element, other costimulatory elements are within the scope of the invention.

[00209] The cytoplasmic signaling sequences within the cytoplasmic signaling portion of a CAR described herein can be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, between 2 and 10 amino acids in length can form the linkage. A glycine-serine doublet provides a particularly suitable linker.

[00210] In one embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta and the signaling domain of CD28. In another embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In yet another embodiment, the cytoplasmic domain comprises the signaling domain of CD3-zeta and the signaling domains of CD28 and 4-1BB.

[00211] In one embodiment, the cytoplasmic domain in a CAR described herein comprises the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide sequence of SEQ ID NO:89, and the signaling domain of CD3-zeta comprises a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the nucleic acid sequence of SEQ ID NO: 93.

[00212] In one embodiment, the cytoplasmic domain in a CAR described herein is designed to comprise the signaling domain of CD28 and the signaling domain of CD3-zeta, wherein the signaling domain of CD28 comprises a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide sequence of SEQ ID NO: 90, and the signaling domain of CD3-zeta comprises a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide sequence of SEQ ID NO: 93.

[00213] In one embodiment, the cytoplasmic domain in a CAR described herein is designed to comprise the signaling domain of DNAX-activation protein 10 (DAP10), wherein the signaling domain of DAP10 comprises a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide sequence of SEQ ID NO: 91.

[00214] In one embodiment, the cytoplasmic domain in a CAR described herein is designed to comprise the signaling domain of DNAX-activation protein 12 (DAP12), wherein the signaling domain of DAP10 comprises a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the polypeptide sequence of SEQ ID NO: 92.

[00215] In one embodiment, the cytoplasmic domain in a CAR described herein comprises the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises the amino acid sequence set forth in SEQ ID NO: 89 and the signaling domain of CD3-zeta comprises the amino acid sequence set forth in SEQ ID NO: 93.

Additional Genetic Elements

[00216] Although cellular therapies hold great promise for the treatment of human disease, significant toxicities from the cells themselves or from their transgene products have hampered clinical investigation. In embodiments described herein, immune effector cells comprising a CAR described herein that have been infused into a mammalian subject, *e.g.*, a human, can be

ablated in order to regulate the effect of such immune effector cells should toxicity arise from their use. Therefore, certain in embodiments, in addition to the therapeutic ROR-1-specific chimeric antigen receptor described herein, a second gene is also introduced into an engineered immune effector cell described herein. In some embodiments, the second gene is a "cell tag." In some embodiments, the cell tag is a "kill switch." In some embodiments, the second gene is effectively a "kill switch" that allows for the depletion of ROR-1 CAR or ROR-1 CAR/mbIL-15 containing cells. In certain embodiments, the "kill switch" is a HER1 tag or a CD20 tag which comprise a HER1 polypeptide or a CD20 polypeptide which comprises at least an antibody binding epitope of HER1 or CD20 or functional fragment thereof, and optionally a signal polypeptide sequence or fragment thereof.

[00217] In certain embodiments, the second gene is a HER1 tag which is Epidermal Growth Factor Receptor (HER1) or a fragment or variant thereof. In embodiments, the second gene is a HER1 tag which is truncated human Epidermal Growth Factor Receptor 1 (for instance HER1t or HER1t1). In some cases, the second gene is a variant of a truncated human Epidermal Growth Factor Receptor 1. In embodiments, at least one of HER1, HER1t and HER1t1 provides a safety mechanism by allowing for depletion of infused CAR-T cells through administering FDA approved cetuximab or any antibody that recognizes HER1, HER1t and/or HER1t1. In embodiments, the HER1t gene comprises a nucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the nucleic acid sequence of SEQ ID NO: 256. In embodiments, the HER1t1 gene comprises a nucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the nucleic acid sequence of SEQ ID NO: 257. The truncated HER1 sequence, for instance HER1t and HER1t1 eliminate the potential for EGF ligand binding, homo- and hetero- dimerization of EGFR, and EGFR mediated signaling while keeping cetuximab binding to the receptor intact (Ferguson, K., 2008. A structure-based view of Epidermal Growth Factor Receptor regulation. *Annu Rev Biophys*, Volume 37, pp. 353-373).

[00218] In some embodiments, the HER1t tag has a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the sequence of SEQ ID NO: 109. In some embodiments, the HER1t1 tag has a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the sequence of SEQ ID NO: 110.

[00219] In further embodiments, in addition to the therapeutic ROR-1-specific chimeric antigen receptor of the invention the second gene introduced is a CD20 tag. In some cases, the CD20 tag is a full-length CD20 polypeptide, or a truncated CD20 polypeptide (CD20t-1). In some cases, the CD20 tag, for instance CD20 or CD20t-1 also provides a safety mechanism by

allowing for depletion of infused CAR-T cells through administering FDA-approved rituximab therapy. In certain embodiments, the CD20 tag has a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the sequence of SEQ ID NO: 111. In certain embodiments, the CD20 tag is a CD20t-1 tag and has a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the sequence of SEQ ID NO: 112. In some embodiments, the CD20 tag is encoded by a CD20 gene which comprises a nucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the nucleic acid sequence of SEQ ID NO: 258. In some embodiments, the CD20 tag is encoded by a CD20t-1 gene which comprises a nucleotide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the nucleic acid sequence of SEQ ID NO: 259.

[00220] In embodiments, a CAR vector comprising a CAR described herein further comprises a full length CD20 tag comprising a nucleic acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the nucleic acid sequence of SEQ ID NO: 258.

[00221] In embodiments, the gene encoding the kill tag, for instance the HER1t, HER1t-1, CD20 or CD20t-1 tag, is genetically fused to the ROR-1 CAR at 3' end via in-frame with a self-cleaving peptide, for example but not restricted to *Thosea asigna* virus (T2A) peptide. In embodiments, the T2A peptide has an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 116.

[00222] In embodiments, the kill tag gene is cloned into a lentiviral plasmid backbone in frame with the ROR-1 CAR gene. In other embodiments, the kill tag is cloned into a separate lentiviral vector. In other embodiments, both genes are cloned into a Sleeping Beauty transposon vector. In yet other embodiments, the kill tag such as HER1t, HER1t-1, CD20 or CD20t-1 is cloned into a separate Sleeping Beauty transposon vector. In certain embodiments, the kill tags have a signal peptide, for instance, GM-CSFRa signal peptide wherein the GM-CSFRa signal peptide has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 94. In certain embodiments, the signal peptide is IgK having a sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the nucleic acid sequence of SEQ ID NO: 95.

[00223] In one embodiment, the signal peptide is Mouse Ig VH region 3 (SEQ ID NO: 101), Azurocidin (SEQ ID NO: 103), IGHV3-23 (SEQ ID NO: 106), IGKV1-D33 (HuL1) (SEQ ID NO: 107) or IGKV1-D33 (L14F) (HuH7) (SEQ ID NO: 108).

[00224] Exemplary gene expression cassettes encoding a CAR and a kill tag as described herein are shown in **FIG. 1** and **FIG. 2**.

Exemplary CAR Open Reading Frames

[00225] Exemplary CAR and human ROR-1 receptor open reading frames encompassed by methods and compositions described herein are in **Table 1**:

Table 1.

SEQ ID NO	CAR ORF
75	hROR1(VH-VL)_14.CD8a(3x).CD28z
76	hROR1(VL-VH)_05.CD8a(3x).CD28z
77	hROR1(VH-VL)_14-3.CD8a(3x).CD28z
78	hROR1(VH-VL)_14-4.CD8a(3x).CD28z
79	hROR1(VH_5-VL_14).CD8a(3x).CD28z
80	hROR1(VH_5-VL_16).CD8a(3x).CD28z
82	hROR1(VH_18-VL_04).CD8a(3x).CD28z
82	hROR1(VH_18-VL_14).CD8a(3x).CD28z

[00226] In embodiments, provided herein is an isolated nucleic acid encoding a CAR, wherein the CAR comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with an amino acid of SEQ ID NOs: 3-14 or SEQ ID NOs: 75-82.

[00227] In embodiments, the CAR hROR1(VH-VL)_14.CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 17 (hROR1 VH_5) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 18 (hROR1 VL_5).

[00228] In embodiments, the CAR hROR1(VL-VH)_05.CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 35 (hROR1 VH_14) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 36 (hROR1 VL_14).

[00229] In embodiments, the CAR hROR1(VH-VL)_14-3.CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 41 (hROR1 VH_14-3) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 42 (hROR1 VL_14-3).

[00230] In embodiments, the CAR hROR1(VH-VL)_14-4.CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 43 (hROR1 VH_14-4) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 44 (hROR1 VL_14-4).

[00231] In embodiments, the CAR hROR1(VH_5-VL_14).CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 17 (hROR1 VH_5) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 36 (hROR1 VL_14).

[00232] In embodiments, the CAR hROR1(VH_5-VL_16).CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 17 (hROR1 VH_05) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 50 (hROR1 VL_16).

[00233] In embodiments, the CAR hROR1(VH_18-VL_04).CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 53 (hROR1 VH_18) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 16 (hROR1 VL_04).

[00234] In embodiments, the CAR hROR1(VH_18-VL_14).CD8a(3x).CD28z comprises an antigen-binding moiety comprising a VH polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 53 (hROR1 VH_18) and a VL polypeptide having at least 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 36 (hROR1 VL_14).

[00235] In each of the embodiments in Table 1, the signal peptide of the CAR can comprise a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of Mouse Ig VH region 3 (SEQ ID NO: 101), Azurocidin (SEQ ID NO: 103), IGHV3-23 (SEQ ID NO: 106), IGKV1-D33 (HuL1) (SEQ ID NO: 107) or IGKV1-D33 (L14F) (HuH7) (SEQ ID NO: 108).

[00236] In each of the embodiments in **Table 1** with "CD8a," the transmembrane region of the CAR comprises CD8alpha transmembrane domain comprising a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 87, and the spacer is CD8a comprising a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with any one of the amino acid sequences as shown in SEQ ID NOs: 83-86.

[00237] In each of the embodiments in **Table 1** with "CD28m," the intracellular domain of the CAR comprises CD28 with an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 90.

[00238] In each of the embodiments in **Table 1** with "T2A", the CAR ORF comprises a self-cleaving *Thosea asigna* virus (T2A) peptide, which enables the production of multiple gene products from a single vector. In embodiments, the T2A peptide has an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 116.

[00239] In the embodiments in **Table 1** with "HER1t," the CAR ORF comprises truncated human Epidermal Growth Factor Receptor 1 (HER1t), which provides a safety mechanism by allowing for depletion of infused CAR-T cells through administering FDA approved cetuximab therapy. The HER1t gene as described herein can comprise a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 109. Unless otherwise noted in **Table 1**, HER1t tags have GM-CSFRa signal peptide ("GM-CSFRsp") (SEQ ID NO: 94). In one embodiment, the signal peptide is Mouse Ig VH region 3 (SEQ ID NO: 101), Azurocidin (SEQ ID NO: 103), IGHV3-23 (SEQ ID NO: 106), IGKV1-D33 (HuL1) (SEQ ID NO: 107) or IGKV1-D33 (L14F) (HuH7) (SEQ ID NO: 108). In certain embodiments, the HER1t can be substituted with another tag, for instance, HER1t-1. The HER1t-1 gene as described herein can comprise a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 110. In the embodiments in **Table 1** with "IgKsp," the

signal peptide is IgK having an amino acid sequence at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 95.

[00240] In embodiments in **Table 1** with "4-1BB," the CAR ORF comprises costimulatory molecule having a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 89.

[00241] In embodiments in **Table 1** with "FL CD20," the CAR ORF comprises a full length CD20 tag comprising a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 111. CD20 provides a safety mechanism by allowing for depletion of infused CAR-T cells through administering FDA-approved rituximab therapy. In other embodiments, FL CD20 can be substituted with CD20t-1 comprising a polypeptide sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 112.

[00242] In certain embodiments in **Table 1**, the CAR ORF can be under the control of an inducible promoter for gene transcription. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch. In some embodiments, the CAR ORF and gene switch components can be configured as depicted in **FIGs. 1A-1E and FIGs. 2A-2E**.

Cytokines

[00243] In some embodiments, a CAR described herein is administered to a subject with one or more additional therapeutic agents that include but are not limited to cytokines. In some cases, the cytokine comprises at least one chemokine, interferon, interleukin, lymphokine, tumor necrosis factor, or variant or combination thereof. In some cases, the cytokine is an interleukin. In some cases the interleukin is at least one of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33 and functional variants and fragments thereof. In some embodiments, the cytokines can be membrane bound or secreted. In embodiments, the cytokine is soluble IL-15, soluble IL-15/IL-15Rα complex (*e.g.*, ALT-803). In certain cases, the interleukin can comprise membrane bound IL-15 (mbIL-15) or a fusion of IL-15 and IL-15Rα. In some embodiments, a mbIL-15 is a membrane-bound chimeric IL-15 which can be co-expressed with a modified immune effector cell described herein. In some embodiments, the mbIL-15 comprises a full-length IL-15 (*e.g.*, a native IL-15 polypeptide)

or fragment or variant thereof, fused in frame with a full length IL-15Rα, functional fragment or variant thereof. In some cases, the IL-15 is indirectly linked to the IL-15Rα through a linker. In some instances, the mbIL-15 is as described in Hurton et al., "Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells," PNAS 2016. In some cases, the cytokine is expressed in the same immune effector cell as the CAR.

[00244] In further embodiments, an immune effector cell expressing a CAR described herein expresses membrane-bound IL-15 ("mIL-15 or mbIL-15"). In aspects of the invention, the mbIL-15 comprises a fusion protein between IL-15 and IL-15Rα. In further embodiments, the mbIL-15 comprises an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 113. In certain cases, the CAR and the cytokine is expressed in separate vectors. In specific cases, the vectors can be lentiviral vectors, retroviral vectors or Sleeping Beauty transposons.

[00245] In some embodiments, the mbIL-15 is expressed with a cell tag such as HER1t, HER-1t-1, CD20t-1 or CD20 as described herein. The mbIL-15 can be expressed in-frame with HER1t, HER-1t-1, CD20t-1 or CD20.

[00246] In some embodiments, the mbIL-15 can be under the control of an inducible promoter for gene transcription. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch.

[00247] In another aspect, the interleukin can comprise IL-12. In some embodiments, the IL-12 is a single chain IL-12 (scIL-12), protease sensitive IL-12, destabilized IL-12, membrane bound IL-12, intercalated IL-12. In some instances, the IL-12 variants are as described in WO2015/095249, WO2016/048903, WO2017/062953, all of which is incorporated by reference in their entireties.

[00248] In some embodiments, the cytokines described above can be under the control of an inducible promoter for gene transcription. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch.

Gene Switch

[00249] Provided herein are gene switch polypeptides, polynucleotides encoding ligand-inducible gene switch polypeptides, and methods and systems incorporating these polypeptides and/or polynucleotides. The term "gene switch" refers to the combination of a response element associated with a promoter, and for instance, an ecdysone receptor (EcR) based system which, in the presence of one or more ligands, modulates the expression of a gene into which the response element and promoter are incorporated. Tightly regulated inducible gene expression systems or gene switches are useful for various applications such as gene therapy, large scale production of proteins in cells, cell based high throughput screening assays, functional genomics and regulation of traits in transgenic plants and animals. Such inducible gene expression systems can include ligand inducible heterologous gene expression systems.

[00250] An early version of EcR-based gene switch used Drosophila melanogaster EcR (DmEcR) and Mus musculus RXR (MmRXR) polypeptides and showed that these receptors in the presence of steroid, ponasteroneA, transactivate reporter genes in mammalian cell lines and transgenic mice (Christopherson et al., 1992; No et al., 1996). Later, Suhr et al., 1998 showed that non-steroidal ecdysone agonist, tebufenozide, induced high level of transactivation of reporter genes in mammalian cells through Bombyx mori EcR (BmEcR) in the absence of exogenous heterodimer partner.

[00251] International Patent Applications No. PCT/US97/05330 (WO 97/38117) and PCT/US99/08381 (WO99/58155) disclose methods for modulating the expression of an exogenous gene in which a DNA construct comprising the exogenous gene and an ecdysone response element is activated by a second DNA construct comprising an ecdysone receptor that, in the presence of a ligand therefor, and optionally in the presence of a receptor capable of acting as a silent partner, binds to the ecdysone response element to induce gene expression. In this example, the ecdysone receptor was isolated from Drosophila melanogaster. Typically, such systems require the presence of the silent partner, preferably retinoid X receptor (RXR), in order to provide optimum activation. In mammalian cells, insect ecdysone receptor (EcR) is capable of heterodimerizing with mammalian retinoid X receptor (RXR) and, thereby, be used to regulate expression of target genes or heterologous genes in a ligand dependent manner. International Patent Application No. PCT/US98/14215 (WO 99/02683) discloses that the ecdysone receptor isolated from the silk moth Bombyx mori is functional in mammalian systems without the need for an exogenous dimer partner.

[00252] U.S. Pat. No. 6,265,173 discloses that various members of the steroid/thyroid superfamily of receptors can combine with Drosophila melanogaster ultraspiracle receptor (USP) or fragments thereof comprising at least the dimerization domain of USP for use in a gene expression system. U.S. Pat. No. 5,880,333 discloses a Drosophila melanogaster EcR and

ultraspiracle (USP) heterodimer system used in plants in which the transactivation domain and the DNA binding domain are positioned on two different hybrid proteins. In each of these cases, the transactivation domain and the DNA binding domain (either as native EcR as in International Patent Application No. PCT/US98/14215 or as modified EcR as in International Patent Application No. PCT/US97/05330) were incorporated into a single molecule and the other heterodimeric partners, either USP or RXR, were used in their native state.

[00253] International Patent Application No. PCT/US01/0905 discloses an ecdysone receptor-based inducible gene expression system in which the transactivation and DNA binding domains are separated from each other by placing them on two different proteins results in greatly reduced background activity in the absence of a ligand and significantly increased activity over background in the presence of a ligand. This two-hybrid system is a significantly improved inducible gene expression modulation system compared to the two systems disclosed in applications PCT/US97/05330 and PCT/US98/14215. The two-hybrid system is believed to exploit the ability of a pair of interacting proteins to bring the transcription activation domain into a more favorable position relative to the DNA binding domain such that when the DNA binding domain binds to the DNA binding site on the gene, the transactivation domain more effectively activates the promoter (see, for example, U.S. Pat. No. 5,283,173). The two-hybrid gene expression system comprises two gene expression cassettes; the first encoding a DNA binding domain fused to a nuclear receptor polypeptide, and the second encoding a transactivation domain fused to a different nuclear receptor polypeptide. In the presence of ligand, it is believed that a conformational change is induced which promotes interaction of the first polypeptide with the second polypeptide thereby resulting in dimerization of the DNA binding domain and the transactivation domain. Since the DNA binding and transactivation domains reside on two different molecules, the background activity in the absence of ligand is greatly reduced.

[00254] Certain modifications of the two-hybrid system could also provide improved sensitivity to non-steroidal ligands for example, diacylhydrazines, when compared to steroidal ligands for example, ponasterone A (“PonA”) or muristerone A (“MurA”). That is, when compared to steroids, the non-steroidal ligands provided higher gene transcription activity at a lower ligand concentration. Furthermore, the two-hybrid system avoids some side effects due to overexpression of RXR that can occur when unmodified RXR is used as a switching partner. In a preferred two-hybrid system, native DNA binding and transactivation domains of EcR or RXR are eliminated and as a result, these hybrid molecules have less chance of interacting with other steroid hormone receptors present in the cell, thereby resulting in reduced side effects.

[00255] The ecdysone receptor (EcR) is a member of the nuclear receptor superfamily and is classified into subfamily 1, group H (referred to herein as "Group H nuclear receptors"). The members of each group share 40-60% amino acid identity in the E (ligand binding) domain (Laudet et al., A Unified Nomenclature System for the Nuclear Receptor Subfamily, 1999; Cell 97: 161-163). In addition to the ecdysone receptor, other members of this nuclear receptor subfamily 1, group H include: ubiquitous receptor (UR), Orphan receptor 1 (OR-1), steroid hormone nuclear receptor 1 (NER-1), RXR interacting protein-15 (RIP-15), liver x receptor β (LXRβ), steroid hormone receptor like protein (RLD-1), liver x receptor (LXR), liver x receptor α (LXRα), farnesoid x receptor (FXR), receptor interacting protein 14 (RIP-14), and farnesol receptor (HRR-1).

[00256] In some cases, an inducible promoter ("IP") can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as Intrexon Corporation's RHEOSWITCH® gene switch. In some cases, a gene switch can be selected from ecdysone-based receptor components as described in, but without limitation to, any of the systems described in: PCT/US2001/009050 (WO 2001/070816); U.S. Pat. Nos. 7,091,038; 7,776,587; 7,807,417; 8,202,718; PCT/US2001/030608 (WO 2002/029075); U.S. Pat. Nos. 8,105,825; 8,168,426; PCT/1J52002/005235 (WO 2002/066613); U.S. App. No. 10/468,200 (U.S. Pub. No. 20120167239); PCT/US2002/005706 (WO 2002/066614); U.S. Pat. Nos. 7,531,326; 8,236,556; 8,598,409; PCT/U52002/005090 (WO 2002/066612); U.S. Pat. No. 8,715,959 (U.S. Pub. No. 20060100416); PCT/US2002/005234 (WO 2003/027266); U.S. Pat. Nos. 7,601,508; 7,829,676; 7,919,269; 8,030,067; PCT/U52002/005708 (WO 2002/066615); U.S. App. No. 10/468,192 (U.S. Pub. No. 20110212528); PCT/US2002/005026 (WO 2003/027289); U.S. Pat. Nos. 7,563,879; 8,021,878; 8,497,093; PCT/US2005/015089 (WO 2005/108617); U.S. Pat. No. 7,935,510; 8,076,454; PCT/U52008/011270 (WO 2009/045370); U.S. App. No. 12/241,018 (U.S. Pub. No. 20090136465); PCT/US2008/011563 (WO 2009/048560); U.S. App. No. 12/247,738 (U.S. Pub. No. 20090123441); PCT/US2009/005510 (WO 2010/042189); U.S. App. No. 13/123,129 (U.S. Pub. No. 20110268766); PCT/US2011/029682 (WO 2011/119773); U.S. App. No. 13/636,473 (U.S. Pub. No. 20130195800); PCT/US2012/027515 (WO 2012/122025); and, U.S. Pat. No. 9,402,919; each of which is incorporated by reference in its entirety.

[00257] Provided are systems for modulating the expression of a CAR and/or a cytokine in a host cell, comprising polynucelotides encoding for gene-switch polypeptides disclosed herein. Further provided herein are polynucleotides encoding gene switch polypeptides for ligand-inducible control of gene expression, wherein the gene switch polypeptides comprise (a) a first gene switch polypeptide comprising a DNA-binding domain (DBD) fused to a nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide comprising a transactivation

domain fused to a nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker. In some embodiments, the linker is a cleavable or ribosome skipping linker sequence selected from the group consisting of 2A, GSG-2A, GSG linker (SEQ ID NO: 129), SGSG linker (SEQ ID NO: 130), furinlink variants and derivatives thereof. In certain embodiments, the 2A linker is a p2A linker, a T2A linker, F2A linker, or E2A linker.

[00258] In some embodiments, the DNA binding domain (DBD) comprises at least one of GAL4 (GAL4 DBD), a LexA DBD, a transcription factor DBD, a steroid/thyroid hormone nuclear receptor superfamily member DBD, a bacterial LacZ DBD, and a yeast DBD. In some cases, the transactivation domain comprises at least one of a VP16 transactivation domain, and a B42 acidic activator transactivation domain. In other cases, the nuclear receptor ligand binding domain comprises at least one of a ecdysone receptor (EcR), a ubiquitous receptor, an orphan receptor 1, a NER-1, a steroid hormone nuclear receptor 1, a retinoid X receptor interacting protein-15, a liver X receptor β, a steroid hormone receptor like protein, a liver X receptor, a liver X receptor α, a farnesoid X receptor, a receptor interacting protein 14, and a famesol receptor. In some embodiments, the nuclear receptor ligand binding domain is derived from the Ecdysone Receptor polypeptide sequence of SEQ ID NOs: 135 and 136.

[00259] In yet another embodiment, the first gene switch polypeptide comprises a GAL4 DBD fused to an EcR nuclear receptor ligand binding domain, and the second gene switch polypeptide comprises a VP16 transactivation domain fused to a retinoid receptor X (RXR) nuclear receptor ligand binding domain. In some cases, the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker, which is selected from the group consisting of 2A, GSG-2A, GSG linker (SEQ ID NO: 129), SGSG linker (SEQ ID NO: 130), furinlink variants and derivatives thereof.

[00260] In certain embodiments, two or more polypeptides encoded by a polynucleotide described herein can be separated by an intervening sequence encoding a linker polypeptide. In certain cases, the linker is a cleavage-susceptible linker. In some embodiments, polypeptides of interest are expressed as fusion proteins linked by a cleavage-susceptible linker polypeptide. In certain embodiments, cleavage-susceptible linker polypeptide(s) can be any one or more of: F/T2A, T2A, p2A, 2A, GSG-p2A, GSG linker (SEQ ID NO: 129), and furinlink variants. In certain embodiments, the linker polypeptide comprises any one of the amino acid sequences as shown in SEQ ID NOs: 116-130.

[0126] In some cases, a viral 2A sequence can be used. 2A elements can be shorter than IRES, having from 5 to 100 base pairs. In some cases, a 2A sequence can have 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 100 nucleotides in length. 2A linked genes

can be expressed in one single open reading frame and "self-cleavage" can occur co-translationally between the last two amino acids, GP, at the C-terminus of the 2A polypeptide, giving rise to equal amounts of co-expressed proteins.

[0127] A viral 2A sequence can be about 20 amino acids. In some cases, a viral 2A sequence can contain a consensus motif Asp-Val/Ile-Glu-X-Asn-Pro-Gly-Pro (SEQ ID NO: 286). A consensus motif sequence can act co-translationally. For example, formation of a normal peptide bond between a glycine and proline residue can be prevented, which can result in ribosomal skipping and cleavage of a nascent polypeptide. This effect can produce multiple genes at equimolar levels.

[0128] A 2A peptide can allow translation of multiple proteins in a single open reading frame into a polypeptide that can be subsequently cleaved into individual polypeptide through a ribosome-skipping mechanism (Funston, Kallioinen et al. 2008). In some embodiments, a 2A sequence can include: F/T2A, T2A, p2A, 2A, T2A, E2A, F2A, and BmCPV2A, BmIFV2A, and any combination thereof.

[0129] In some cases, a vector can comprise an IRES sequence and a 2A linker sequence. In other cases, expression of multiple genes linked with 2A peptides can be facilitated by a spacer sequence (GSG (SEQ ID NO: 129)) ahead of the 2A peptides. In some cases, constructs can combine a spacers, linkers, adaptors, promotors, or combinations thereof. For example, a construct can have a spacer (SGSG (SEQ ID NO: 130) or GSG (SEQ ID NO: 129)) and furin linker (R-A-K-R (SEQ ID NO: 125)) cleavage site with different 2A peptides. A spacer can be an I-Ceui. In some cases, a linker can be engineered. For example, a linker can be designed to comprise chemical characteristics such as hydrophobicity. In some cases, at least two linker sequences can produce the same protein. In other cases, multiple linkers can be used in a vector. For example, genes of interest can be separated by at least two linkers.

[0130] In certain embodiments, two or more polypeptides encoded by a polynucleotide described herein can be separated by an intervening sequence encoding a linker polypeptide. In certain cases, the linker is a cleavage-susceptible linker. In some embodiments, polypeptides of interest are expressed as fusion proteins linked by a cleavage-susceptible linker polypeptide. In certain embodiments, cleavage-susceptible linker polypeptide(s) can be any one or two of: Furinlink, fmdv, p2a, GSG-p2a, and/or fp2a described in SEQ ID NOs: 119, 120, 121, 125, and 128.

[0131] In some embodiments, a linker can be utilized in a polynucleotide described herein. A linker can be a flexible linker, a rigid linker, an *in vivo* cleavable linker, or any combination thereof. In some cases, a linker can link functional domains together (as in flexible and rigid linkers) or releasing free functional domain *in vivo* as in *in vivo* cleavable linkers.

[0132] Linkers can improve biological activity, increase expression yield, and achieving desirable pharmacokinetic profiles. A linker can also comprise hydrazone, peptide, disulfide, or thioesther.

[0133] In some cases, a linker sequence described herein can include a flexible linker. Flexible linkers can be applied when a joined domain requires a certain degree of movement or interaction. Flexible linkers can be composed of small, non-polar (*e.g.*, Gly) or polar (*e.g.*, Ser or Thr) amino acids. A flexible linker can have sequences consisting primarily of stretches of Gly and Ser residues ("GS" linker). An example of a flexible linker can have the sequence of (Gly-Gly-Gly-Gly-Ser)n (SEQ ID NO: 287). By adjusting the copy number "n", the length of this exemplary GS linker can be optimized to achieve appropriate separation of functional domains, or to maintain necessary inter-domain interactions. For example, (Gly-Gly-Gly-Gly-Ser)n , wherein n is 3 is (G4S)3 linker as shown in SEQ ID NO: 127. Besides GS linkers, other flexible linkers can be utilized for recombinant fusion proteins. In some cases, flexible linkers can also be rich in small or polar amino acids such as Gly and Ser, but can contain additional amino acids such as Thr and Ala to maintain flexibility. In other cases, polar amino acids such as Lys and Glu can be used to improve solubility.

[0134] Flexible linkers included in linker sequences described herein, can be rich in small or polar amino acids such as Gly and Ser to provide good flexibility and solubility. Flexible linkers can be suitable choices when certain movements or interactions are desired for fusion protein domains. In addition, although flexible linkers do not have rigid structures in some cases, they can serve as a passive linker to keep a distance between functional domains. The length of a flexible linkers can be adjusted to allow for proper folding or to achieve optimal biological activity of the fusion proteins.

[0135] A linker described herein can further include a rigid linker in some cases. A rigid linker can be utilized to maintain a fixed distance between domains of a polypeptide. Examples of rigid linkers can be: Alpha helix-forming linkers, Pro-rich sequence, (XP)n, X-Pro backbone, A(EAAAK)nA (n = 2-5) (SEQ ID NO: 288), to name a few. Rigid linkers can exhibit relatively stiff structures by adopting α-helical structures or by containing multiple Pro residues in some cases.

[0136] A linker described herein can be cleavable in some cases. In other cases a linker is not cleavable. Linkers that are not cleavable can covalently join functional domains together to act as one molecule throughout an *in vivo* processes or an *ex vivo* process. A linker can also be cleavable *in vivo*. A cleavable linker can be introduced to release free functional domains *in vivo*. A cleavable linker can be cleaved by the presence of reducing reagents, proteases, to name a few. For example, a reduction of a disulfide bond can be utilized to produce a cleavable

linker. In the case of a disulfide linker, a cleavage event through disulfide exchange with a thiol, such as glutathione, could produce a cleavage. In other cases, an *in vivo* cleavage of a linker in a recombinant fusion protein can also be carried out by proteases that can be expressed *in vivo* under pathological conditions (*e.g.*, cancer or inflammation), in specific cells or tissues, or constrained within certain cellular compartments. In some cases, a cleavable linker can allow for targeted cleavage. For example, the specificity of many proteases can offer slower cleavage of a linker in constrained compartments. A cleavable linker can also comprise hydrazone, peptides, disulfide, or thioesther. For example, a hydrazone can confer serum stability. In other cases, a hydrazone can allow for cleavage in an acidic compartment. An acidic compartment can have a pH up to 7. A linker can also include a thioether. A thioether can be nonreducible A thioether can be designed for intracellular proteolytic degradation.

[0137] In certain embodiments, an fmdv linker polypeptide comprises a sequence that can be at least about 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 126. In certain embodiments, an fmdv linker polypeptide is one or more of the linkers encoded in a single vector linking two or more fusion proteins. In certain cases, an fmdv linker polypeptide can be encoded by a polynucleotide open reading frame (ORF) nucleic acid sequence. In some cases, an ORF encoding fmdv comprises or consists of a sequence of SEQ ID NO: 272. In certain embodiments, a polynucleotide encoding fmdv is at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 272.

[0138] In certain cases, a linker polypeptide can be a “p2a” linker. In certain embodiments, a p2a polypeptide can comprise a sequence that can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 119. In certain embodiments, the p2a linker polypeptide can be one or more of the linkers encoded in a single vector linking two or more fusion proteins. In some cases, a p2a linker polypeptide can be encoded by a polynucleotide open reading frame (ORF) nucleic acid sequence. In certain embodiments, an ORF encoding p2a comprises or consists of the sequence of SEQ ID NO: 266. In certain cases, a polynucleotide encoding p2a can be or can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 266.

[0139] In some cases, a linker polypeptide can be a “GSG-p2a” linker. In certain embodiments, a GSG-p2a linker polypeptide can comprise a sequence that can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 120. In certain embodiments, a GSG-p2a linker polypeptide can be one or more of the linkers encoded in a single vector linking two or more fusion proteins. In

some cases, a GSG-p2a linker polypeptide can be encoded by a polynucleotide open-reading frame (ORF) nucleic acid sequence. An ORF encoding GSG p2a can comprises the sequence of SEQ ID NO: 267. In some cases, a polynucleotide encoding GSG-p2a can be or can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 267.

[0140] A linker polypeptide can be an "fp2a" linker as provided herein. In certain embodiments, a fp2a linker polypeptide can comprise a sequence that can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 121. In certain cases, an fp2a linker polypeptide can be one or more of the linkers encoded in a single vector linking two or more fusion proteins. In some cases, a fp2a linker polypeptide can be encoded by a polynucleotide open reading frame (ORF) nucleic acid sequence. In certain embodiments, a polynucleotide encoding an fp2a linker can be or can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 268.

[0141] In some cases, a linker can be engineered. For example, a linker can be designed to comprise chemical characteristics such as hydrophobicity. In some cases, at least two linker sequences can produce the same protein. A sequence can be or can be about 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% , or 100% identical to a sequence of SEQ ID NO: 116 to SEQ ID NO: 130. In other cases, multiple linkers can be used in a vector. For example, genes of interest, and one or more gene switch polypeptide sequences described herein can be separated by at least two linkers. In some cases, genes can be separated by 2, 3, 4, 5, 6, 7, 8, 9, or up to 10 linkers.

[0142] A linker can be an engineered linker. Methods of designing linkers can be computational. In some cases, computational methods can include graphic techniques. Computation methods can be used to search for suitable peptides from libraries of three-dimensional peptide structures derived from databases. For example, a Brookhaven Protein Data Bank (PDB) can be used to span the distance in space between selected amino acids of a linker.

[0143] In some embodiments are polynucleotides encoding a polypeptide construct comprising a furin polypeptide and a 2A polypeptide, wherein the furin polypeptide and the 2A polypeptide are connected by a polypeptide linker comprising at least three hydrophobic amino acids. In some cases, at least three hydrophobic amino acids are selected from the list consisting of glycine (Gly)(G), alanine (Ala)(A), valine (Val)(V), leucine (Leu)(L), isoleucine (Ile)(I), proline (Pro)(P), phenylalanine (Phe)(F), methionine (Met)(M), tryptophan (Trp)(W). In some cases, a polypeptide linker can also include one or more GS linker sequences, for instance (GS)n

(SEQ ID NO: 289), (SG)n (SEQ ID NO: 290), (GSG)n (SEQ ID NO: 291) and (SGSG)n (SEQ ID NO: 292) wherein n can be any number from zero to fifteen.

[0144] Provided are methods of obtaining an improved expression of a polypeptide construct comprising: providing a polynucleotide encoding said polypeptide construct comprising a first functional polypeptide and a second functional polypeptide, wherein said first functional polypeptide and second functional polypeptide are connected by a linker polypeptide comprising a sequence with at least 60% identity to the sequence APVKQ (SEQ ID NO: 293); and expressing said polynucleotide in a host cell, wherein said expressing results in an improved expression of the polypeptide construct as compared to a corresponding polypeptide construct that does not have a linker polypeptide comprising a sequence with at least 60% identity to the sequence APVKQ (SEQ ID NO: 293).

[0145] In other instances, the linker can be an IRES. The term “internal ribosome entry site (IRES)” as used herein can be intended to mean internal ribosomal entry site. In a vector comprising an IRES sequence, a first gene can be translated by a cap-dependent, ribosome scanning, mechanism with its own 5’-UTR, whereas translation of a subsequent gene can be accomplished by direct recruitment of a ribosome to an IRES in a cap-independent manner. An IRES sequence can allow eukaryotic ribosomes to bind and begin translation without binding to a 5’ capped end. An IRES sequence can allow expression of multiple genes from one transcript (Mountford and Smith 1995).

[00261] Exemplary IRES sequences can be found in SEQ ID NO: 146 and 147. In certain cases, a polynucleotide encoding 2xRbm3 IRES a can be or can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 146. In certain cases, a polynucleotide encoding EMCV IRES a can be or can be about at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 147.

[00262] In some embodiments are systems for modulating the expression of a CAR and a cytokine in a host cell, comprising a first gene expression cassette comprising a first polynucleotide encoding a first polypeptide; a second gene expression cassette comprising a second polynucleotide encoding a second polypeptide; and a ligand; wherein the first and second polypeptides comprise one or more of: (i) a transactivation domain; (ii) a DNA-binding domain; and (iii) a ligand binding domain; (iv) CAR; (vi) cytokine, and/or (vii) cell tag such that upon contacting the host cell with the first gene expression cassette and the second gene expression cassette in the presence of the ligand, the CAR and the cytokine are expressed in the host cell. In some cases, the CAR is a ROR-1 CAR and the cytokine is mbIL-15. In some cases, ROR-1CAR, mbIL-15 are co-expressed with one cell tag. In some cases, ROR-1 CAR and mbIL-15

are each co-expressed with a cell tag. In other cases, ROR-1 CAR is expressed with a cell tag and mbIL-15 is expressed with a second cell tag. Exemplary configurations of gene expression cassettes are depicted in **FIGs. 1 and 2**. In other cases, the CAR is a ROR-1 CAR and the cytokine is IL-12. In some cases, ROR-1 CAR, IL-12 are co-expressed with one cell tag. In some cases, ROR-1 CAR and IL-12 are each co-expressed with a cell tag. In other cases, ROR-1 CAR is expressed with a cell tag and IL-12 is expressed with a second cell tag.

[00263] In some embodiments are systems for modulating the expression of a CAR and a cytokine in a host cell, comprising a first gene expression cassette comprising a first polynucleotide encoding a first polypeptide; a second gene expression cassette comprising a second polynucleotide encoding a second polypeptide; and a ligand; wherein the first polypeptide comprise one or more of: (i) a transactivation domain; (ii) a DNA-binding domain; and (iii) a ligand binding domain and the second polypeptide comprise one or more of (i) CAR; (ii) cytokine, and/or (iii) cell tag such that upon contacting the host cell with the first gene expression cassette and the second gene expression cassette in the presence of the ligand, the CAR and/or the cytokine are expressed in the host cell. In some cases, the CAR is a ROR-1 CAR and the cytokine is mbIL-15. In some cases, ROR-1 CAR and mbIL-15 are each co-expressed with a cell tag. In other cases, ROR-1 CAR is expressed with a first cell tag and mbIL-15 is expressed with a second cell tag.

[00264] Exemplary configurations of systems for modulating the expression of a ROR-1 CAR and a cytokine in a host cell are depicted in **FIGs. 1A-1E and FIGs 2A-2E**. In some embodiments, the gene expression cassettes are introduced into an immune effector cell using viral or viral based systems. Examples of non-viral based delivery systems as described herein include SB11 transposon system, the SB100X transposon system, the SB110 transposon system, the piggyBac transposon system. In one embodiment, the gene expression cassettes are introduced into an immune effector cell in one or more Sleeping Beauty transposons.

[00265] Exemplary embodiments of gene expression cassettes that encode for constitutive expression of ROR-1 CAR, cytokine (such as mbIL-15 or IL-12) and cell tag are depicted in **FIGs. 1A-1E**. **FIGs. 1A-1B** depict exemplary gene expression cassette designs for ROR-1 CAR, mbIL-15 and cell tag in various configurations. In this embodiment, the gene expression cassette is introduced into an immune effector cell in one Sleeping Beauty transposon. **FIGs. 1C-1D** depict gene expression cassette configurations where ROR-1 CAR can be in one gene expression cassette and mbIL-15 and cell tag are in a second gene expression cassette. In this embodiment, the gene expression cassette is introduced into an immune effector cell in one or more Sleeping Beauty transposons.

[00266] Exemplary embodiments of gene expression cassettes that encode for expression of ROR-1 CAR, inducible cytokine (such as mbIL-15) and/or cell tag are depicted in **FIGs. 2A-2E**. **FIGs 2A-2D** depict exemplary gene expression cassette designs for ROR-1 CAR, mbIL-15 and cell tag in various configurations under the control of an inducible promoter. **FIG. 2E** is an exemplary embodiment of a gene expression cassette encoding gene-switch polypeptides as described herein. In this embodiment, the gene expression cassette(s) is introduced into an immune effector cell in one or more Sleeping Beauty transposons.

Ligands

[00267] In some embodiments, a ligand used for inducible gene switch regulation can be selected from any of, but without limitation to, following: N-[(1R)-1-(1,1-dimethylethyl)butyl]-N'-(2-ethyl-3-methoxybenzoyl)-3,5-dimethylbenzohydrazide (also referred to as veledimex), (2S,3R,5R,9R,10R,13R,14S,17R)-17- [(2S,3R)-3,6-dihydroxy-6-methylheptan- 2-yl]-2,3,14-trihydroxy-10,13-dimethyl- 2,3,4,5,9,11,12,15,16,17-decahydro- 1H-cyclopenta[a]phenanthren-6-one; N'-(3,5-Dimethylbenzoyl)-N'-[(3R)-2,2-dimethyl-3-hexanyl]-2-ethyl-3-methoxybenzohydrazide; 5-Methyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(3,5-dimethyl-benzoyl)-N′-(1-ethyl-2,2-dimethyl-propyl)-hydrazide; 5-Methyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(3,5-dimethoxy-4-methyl-benzoyl)-N′-(1-ethyl-2,2-dimethyl-propyl)-hydrazide; 5-Methyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(1-tert-butyl-butyl)-N′-(3,5-dimethyl-benzoyl)-hydrazide; 5-Methyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(1-tert-butyl-butyl)-N′-(3,5-dimethoxy-4-methyl-benzoyl)-hydrazide; 5-Ethyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(3,5-dimethyl-benzoyl)-N′-(1-ethyl-2,2-dimethyl-propyl)-hydrazide; 5-Ethyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(3,5-dimethoxy-4-methyl-benzoyl)-N′-(1-ethyl-2,2-dimethyl-propyl)-hydrazide; 5-Ethyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(1-tert-butyl-butyl)-N′-(3,5-dimethyl-benzoyl)-hydrazide; 5-Ethyl-2,3-dihydro-benzo[1,4]dioxine-6-carboxylic acid N′-(1-tert-butyl-butyl)-N′-(3,5-dimethoxy-4-methyl-benzoyl)-hydrazide; 3,5-Dimethyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N′-(3-methoxy-2-methyl-benzoyl)-hydrazide; 3,5-Dimethoxy-4-methyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N′-(3-methoxy-2-methyl-benzoyl)-hydrazide; 3,5-Dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N′-(3-methoxy-2-methyl-benzoyl)-hydrazide; 3,5-Dimethoxy-4-methyl-benzoic acid N-(1-tert-butyl-butyl)-N′-(3-methoxy-2-methyl-benzoyl)-hydrazide; 3,5-Dimethyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N′-(2-ethyl-3-methoxy-benzoyl)-hydrazide; 3,5-Dimethoxy-4-methyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N′-(2-ethyl-3-methoxy-benzoyl)-hydrazide; 3,5-Dimethyl-benzoic acid N-(1-

tert-butyl-butyl)-N′-(2-ethyl-3-methoxy-benzoyl)-hydrazide; 3,5-Dimethoxy-4-methyl-benzoic acid N-(1-tert-butyl-butyl)-N′-(2-ethyl-3-methoxy-benzoyl)-hydrazide; 2-Methoxy-nicotinic acid N-(1-tert-butyl-pentyl)-N′-(4-ethyl-benzoyl)-hydrazide; 3,5-Dimethyl-benzoic acid N-(2,2-dimethyl-1-phenyl-propyl)-N′-(4-ethyl-benzoyl)-hydrazide; 3,5-Dimethyl-benzoic acid N-(1-tert-butyl-pentyl)-N′-(3-methoxy-2-methyl-benzoyl)-hydrazide; and 3,5-Dimethoxy-4-methyl-benzoic acid N-(1-tert-butyl-pentyl)-N′-(3-methoxy-2-methyl-benzoyl)-hydrazide.

[00268] In some cases, a ligand used for dose-regulated control of ecdysone receptor-based inducible gene switch can be selected from any of, but without limitation to, an ecdysteroid, such as ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A, and the like, 9-cis-retinoic acid, synthetic analogs of retinoic acid, N,N'-diacylhydrazines such as those disclosed in U.S. Pat. Nos. 6,013,836; 5,117,057; 5,530,028; and 5,378,726 and U.S. Published Application Nos. 2005/0209283 and 2006/0020146; oxadiazolines as described in U.S. Published Application No. 2004/0171651; dibenzoylalkyl cyanohydrazines such as those disclosed in European Application No. 461,809; N-alkyl-N,N'-diaroylhydrazines such as those disclosed in U.S. Pat. No. 5,225,443; N-acyl-N-alkylcarbonylhydrazines such as those disclosed in European Application No. 234,994; N-aroyl-N-alkyl-N'-aroylhydrazines such as those described in U.S. Pat. No. 4,985,461; arnidoketones such as those described in U.S. Published Application No. 2004/0049037; each of which is incorporated herein by reference and other similar materials including 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, oxysterols, 22(R) hydroxycholesterol, 24(S) hydroxycholesterol, 25-epoxycholesterol, T0901317, 5-alpha-6-alpha-epoxycholesterol-3-sulfate (ECHS), 7-ketocholesterol-3-sulfate, framesol, bile acids, 1,1-biphosphonate esters, juvenile hormone III, and the like. Examples of diacylhydrazine ligands useful in the present disclosure include RG-115819 (3,5 -Dimethyl-benzoic acid N-(1-ethy1-2,2-dimethyl-propy1)-N'-(2-methyl-3-methoxy-benzoy1)-hydrazide-), RG-115932 ((R)-3,5-Dimethyl-benzoic acid N-(1-tert-butyl-buty1)-N'-(2-ethy1-3-methoxy-benzoy1)-hydrazide), and RG-115830 (3,5 -Dimethyl-b enzoic acid N-(1-tert-butyl-buty1)-N'-(2-ethy1-3-methoxy-benzoy1)-hydrazide). See, *e.g.*, U.S. patent application Ser. No. 12/155,111, and PCT Appl. No. PCT/US2008/006757, both of which are incorporated herein by reference in their entireties.

Non-Viral Based Delivery Systems

[00269] A nucleic acid encoding a CAR described invention can also be introduced into immune effector cells using non-viral based delivery systems, such as the "Sleeping Beauty

(SB) Transposon System," which refers a synthetic DNA transposon system for introducing DNA sequences into the chromosomes of vertebrates. An exemplary SB transposon system is described for example, in U.S. Pat. Nos. 6,489,458 and 8,227,432. The Sleeping Beauty transposon system comprises a Sleeping Beauty (SB) transposase and SB transposon(s). As used herein, the Sleeping Beauty transposon system can comprise Sleeping Beauty transposase polypeptides as well as derivatives, variants and/or fragments that retain activity, and Sleeping Beauty transposon polynucleotides, derivatives, variants, and/or fragments that retain activity. In certain embodiments, the Sleeping Beauty transposase is provided as an mRNA. In some aspects, the mRNA encodes for a SB10, SB11, SB100x or SB110 transposase. In some aspects, the mRNA comprises a cap and a poly-A tail.

[00270] DNA transposons translocate from one DNA site to another in a simple, cut-and-paste manner. Transposition is a precise process in which a defined DNA segment is excised from one DNA molecule and moved to another site in the same or different DNA molecule or genome. As with other Tc1/mariner-type transposases, SB transposase inserts a transposon into a TA dinucleotide base pair in a recipient DNA sequence. The insertion site can be elsewhere in the same DNA molecule, or in another DNA molecule (or chromosome). In mammalian genomes, including humans, there are approximately 200 million TA sites. The TA insertion site is duplicated in the process of transposon integration. This duplication of the TA sequence is a hallmark of transposition and used to ascertain the mechanism in some experiments. The transposase can be encoded either within the transposon or the transposase can be supplied by another source, in which case the transposon becomes a non-autonomous element. Non-autonomous transposons are most useful as genetic tools because after insertion they cannot independently continue to excise and re-insert. SB transposons envisaged to be used as non-viral vectors for introduction of genes into genomes of vertebrate animals and for gene therapy. Briefly, the Sleeping Beauty (SB) system (Hackett et al., Mol Ther 18:674-83, (2010)) was adapted to genetically modify the immune effector cells (Cooper et al., Blood 105:1622-31, (2005)). In one embodiment, this involved two steps: (i) the electro-transfer of DNA plasmids expressing a SB transposon [*i.e.*, chimeric antigen receptor (CAR) to redirect T-cell specificity (Jin et al., Gene Ther 18:849-56, (2011); Kebriaei et al., Hum Gene Ther 23:444-50, (2012)) and SB transposase and (ii) the propagation and expansion of T cells stably expressing integrants on designer artificial antigen-presenting cells (AaPC) derived from the K562 cell line (also known as AaPCs (Activating and Propagating Cells). In another, embodiment, the second step (ii) is eliminated and the genetically modified T cells are cryopreserved or immediately infused into a patient.

[00271] In one embodiment, the SB transposon systems are described for example in Hudecek et al., Critical Reviews in Biochemistry and Molecular Biology, 52:4, 355-380 (2017), Singh et al., Cancer Res (8):68 (2008). April 15, 2008 and Maiti et al., J Immunother. 36(2): 112–123 (2013), incorporated herein by reference in their entireties.

[00272] In certain embodiments, a ROR-1 CAR and mbIL-15 are encoded in a transposon DNA plasmid vector, and the SB transposase is encoded in a separate vector. In certain embodiments, a ROR-1 CAR described herein is encoded in a transposon DNA plasmid vector, mb-IL15 is encoded in a second transposon DNA plasmid vector, and the SB transposase is encoded in a third DNA plasmid vector. In some embodiment, the CAR is encoded with a kill tag, for instance, HER1t, HER1t1, CD20 or CD20t-1. In some embodiments, the mbIL-15 is encoded with a kill tag, for instance, HER1t, HER1t1, CD20 or CD20t-1. Exemplary configurations of the ROR-1 CAR, kill tag and/or mbIL15 are depicted in **FIG. 1**.

[00273] In embodiments, the ROR-1 CAR can be co-expressed with mbIL-15 and the cell tag from a transposon DNA plasmid vector. In further embodiments, the ROR-1 CAR can be under the control of an inducible promoter. In another embodiment, the mbIL-15 can be under the control of an inducible promoter. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch. In certain embodiments, the ROR-1 CAR, mbIL-15 and kill tag can be configured in one, two or more transposons. Exemplary configurations of the ROR-1 CAR or mbIL15 under the control of an inducible promoter are depicted in **FIG 2**.

[00274] In embodiments, the ROR-1 CARs and other genetic elements are delivered to a cell using the SB11 transposon system, the SB100X transposon system, the SB110 transposon system, the piggyBac transposon system (see, *e.g.*, U.S. Patent No. 9,228,180, Wilson et al, "PiggyBac Transposon-mediated Gene Transfer in Human Cells," Molecular Therapy 15:139-145 (2007), incorporated herein by reference in its entirety) and/or the piggyBat transposon system (see, *e.g.*, Mitra et al., "Functional characterization of piggyBat from the bat Myotis lucifugus unveils an active mammalian DNA transposon," Proc. Natl. Acad. Sci USA 110:234-239 (2013). Additional transposases and transposon systems are provided in U.S. Patent Nos.; 7,148,203; 8,227,432; U.S. Patent Publn. No. 2011/0117072; Mates et al., *Nat Genet*, 41(6):753-61 (2009). doi: 10.1038/ng.343. Epub 2009 May 3, *Gene Ther*., 18(9):849-56 (2011). doi: 10.1038/gt.2011.40. Epub 2011 Mar 31 and in Ivics et al., *Cell*. 91(4):501-10, (1997), each of which is incorporated herein by reference in their entirety.

[00275] In other embodiments, the ROR-1 CAR and other genetic elements such as cytokines, mbIL-15 and/or HER1t/HER1t1/CD20/CD20t-1 tag, can be integrated into the immune effector

cell's DNA through a recombinase and integrating expression vectors. Such vectors can randomly integrate into the host cell's DNA, or can include a recombination site to enable the specific recombination between the expression vector and the host cell's chromosome. Such integrating expression vectors can utilize the endogenous expression control sequences of the host cell's chromosomes to effect expression of the desired protein. In some embodiments, targeted integration is promoted by the presence of sequences on the donor polynucleotide that are homologous to sequences flanking the integration site. For example, targeted integration using the donor polynucleotides described herein can be achieved following conventional transfection techniques, *e.g.* techniques used to create gene knockouts or knockins by homologous recombination. In other embodiments, targeted integration is promoted both by the presence of sequences on the donor polynucleotide that are homologous to sequences flanking the integration site, and by contacting the cells with donor polynucleotide in the presence of a site-specific recombinase. By a site-specific recombinase, or simply a recombinase, it is meant a polypeptide that catalyzes conservative site-specific recombination between its compatible recombination sites. As used herein, a site-specific recombinase includes native polypeptides as well as derivatives, variants and/or fragments that retain activity, and native polynucleotides, derivatives, variants, and/or fragments that encode a recombinase that retains activity.

[00276] The recombinases can be introduced into a target cell before, concurrently with, or after the introduction of a targeting vector. The recombinase can be directly introduced into a cell as a protein, for example, using liposomes, coated particles, or microinjection. Alternately, a polynucleotide, either DNA or messenger RNA, encoding the recombinase can be introduced into the cell using a suitable expression vector. The targeting vector components described above are useful in the construction of expression cassettes containing sequences encoding a recombinase of interest. However, expression of the recombinase can be regulated in other ways, for example, by placing the expression of the recombinase under the control of a regulatable promoter (*i.e.*, a promoter whose expression can be selectively induced or repressed).

[00277] A recombinase can be from the Integrase or Resolvase families. The Integrase family of recombinases has over one hundred members and includes, for example, FLP, Cre, and lambda integrase. The Integrase family, also referred to as the tyrosine family or the lambda integrase family, uses the catalytic tyrosine's hydroxyl group for a nucleophilic attack on the phosphodiester bond of the DNA. Typically, members of the tyrosine family initially nick the DNA, which later forms a double strand break. Examples of tyrosine family integrases include Cre, FLP, SSV1, and lambda (λ) integrase. In the resolvase family, also known as the serine

recombinase family, a conserved serine residue forms a covalent link to the DNA target site (Grindley, et al., (2006) Ann Rev Biochem 16:16).

[00278] In one embodiment, the recombinase is an isolated polynucleotide sequence comprising a nucleic acid sequence that encodes a recombinase selecting from the group consisting of a SPβc2 recombinase, a SF370.1 recombinase, a Bxb1 recombinase, an A118 recombinase and a ϕRv1 recombinase. Examples of serine recombinases are described in detail in U.S. Patent No. 9,034,652, hereby incorporated by reference in its entirety.

[00279] Recombinases for use in the practice of the present invention can be produced recombinantly or purified as previously described. Polypeptides having the desired recombinase activity can be purified to a desired degree of purity by methods known in the art of protein ammonium sulfate precipitation, purification, including, but not limited to, size fractionation, affinity chromatography, HPLC, ion exchange chromatography, heparin agarose affinity chromatography (*e.g.*, Thorpe & Smith, Proc. Nat. Acad. Sci. 95:5505-5510, 1998.)

[00280] In one embodiment, the recombinases can be introduced into the eukaryotic cells that contain the recombination attachment sites at which recombination is desired by any suitable method. Methods of introducing functional proteins, *e.g.*, by microinjection or other methods, into cells are well known in the art. Introduction of purified recombinase protein ensures a transient presence of the protein and its function, which is often a preferred embodiment. Alternatively, a gene encoding the recombinase can be included in an expression vector used to transform the cell, in which the recombinase-encoding polynucleotide is operably linked to a promoter which mediates expression of the polynucleotide in the eukaryotic cell. The recombinase polypeptide can also be introduced into the eukaryotic cell by messenger RNA that encodes the recombinase polypeptide. It is generally preferred that the recombinase be present for only such time as is necessary for insertion of the nucleic acid fragments into the genome being modified. Thus, the lack of permanence associated with most expression vectors is not expected to be detrimental. One can introduce the recombinase gene into the cell before, after, or simultaneously with, the introduction of the exogenous polynucleotide of interest. In one embodiment, the recombinase gene is present within the vector that carries the polynucleotide that is to be inserted; the recombinase gene can even be included within the polynucleotide.

[00281] In one embodiment, a method for site-specific recombination comprises providing a first recombination site and a second recombination site; contacting the first and second recombination sites with a prokaryotic recombinase polypeptide, resulting in recombination between the recombination sites, wherein the recombinase polypeptide can mediate recombination between the first and second recombination sites, the first recombination site is attP or attB, the second recombination site is attB or attP, and the recombinase is selected from

the group consisting of a Listeria monocytogenes phage recombinase, a Streptococcus pyogenes phage recombinase, a Bacillus subtilis phage recombinase, a Mycobacterium tuberculosis phage recombinase and a Mycobacterium smegmatis phage recombinase, provided that when the first recombination attachment site is attB, the second recombination attachment site is attP, and when the first recombination attachment site is attP, the second recombination attachment site is attB

[00282] Further embodiments provide for the introduction of a site-specific recombinase into a cell whose genome is to be modified. One embodiment relates to a method for obtaining site-specific recombination in an eukaryotic cell comprises providing a eukaryotic cell that comprises a first recombination attachment site and a second recombination attachment site; contacting the first and second recombination attachment sites with a prokaryotic recombinase polypeptide, resulting in recombination between the recombination attachment sites, wherein the recombinase polypeptide can mediate recombination between the first and second recombination attachment sites, the first recombination attachment site is a phage genomic recombination attachment site (attP) or a bacterial genomic recombination attachment site (attB), the second recombination attachment site is attB or attP, and the recombinase is selected from the group consisting of a Listeria monocytogenes phage recombinase, a Streptococcus pyogenes phage recombinase, a Bacillus subtilis phage recombinase, a Mycobacterium tuberculosis phage recombinase and a Mycobacterium smegmatis phage recombinase, provided that when the first recombination attachment site is attB, the second recombination attachment site is attP, and when the first recombination attachment site is attP, the second recombination attachment site is attB. In an embodiment the recombinase is selected from the group consisting of an A118 recombinase, a SF370.1 recombinase, a SPβc2 recombinase, a ϕRv1 recombinase, and a Bxb1 recombinase. In one embodiment the recombination results in integration.

[00283] Regardless of the method used to introduce exogenous nucleic acids into a host cell, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays can be performed. Such assays include, for example, “molecular biological” assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; “biochemical” assays, such as detecting the presence or absence of a particular peptide, *e.g.*, by immunological means (ELISAs and Western blots) or by assays described herein to identify peptides or proteins or nucleic acids falling within the scope of the invention.

Viral Based Delivery Systems

[00284] Also provided herein are viral-based delivery systems, in which a nucleic acid of the present invention is inserted. Representative viral expression vectors include, but are not limited to, the adenovirus-based vectors (*e.g.*, the adenovirus-based Per.C6 system available from Crucell, Inc. (Leiden, The Netherlands)), lentivirus-based vectors (*e.g.*, the lentiviral-based pLPI from Life Technologies (Carlsbad, Calif.)) and retroviral vectors (*e.g.*, the pFB-ERV plus pCFB-EGSH), herpes viruses. In an embodiment, the viral vector is a lentivirus vector. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. In general, and in embodiments, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (*e.g.*, WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[00285] In embodiments, provided is a lentiviral vector comprising a backbone and a nucleic acid sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a stalk domain; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; (e) a CD3 zeta signaling domain. Optionally, the vector further comprises a nucleic acid encoding a truncated epidermal growth factor receptor (HER1t or HER1t1), CD20t-1 or a full length CD20.

[00286] In some cases is provided a vector comprising a backbone and a nucleic acid sequence encoding (1) a truncated epidermal growth factor receptor for instance HER1t or HERt-1 or a functional variant thereof; and (2) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain.

[00287] In some cases is provided a vector comprising a backbone and a nucleic acid sequence encoding (1) full length CD20, truncated CD20 or functional variants thereof, and (2) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain.

[00288] In embodiments, the nucleic acid encoding the ROR-1 specific CAR is cloned into a vector comprising lentiviral backbone components. Exemplary backbone components include, but are not limited to, pFUGW, and pSMPUW. The pFUGW lentiviral vector backbone is a self inactivating (SIN) lentiviral vector backbone and has unnecessary HIV-1 viral sequences

removed resulting in reduced potential for the development of neoplasia, harmful mutations, and regeneration of infectious particles. In embodiments, the vector encoding the ROR-1 CAR also encodes mbIL-15 in a single construct. In embodiments, the ROR-1 CAR and mbIL-15 are encoded on two separate lentiviral vectors. In some embodiments, the mbIL-15 is expressed with a truncated epidermal growth factor receptor tag. In embodiments, the ROR-1 CAR can be co-expressed with mbIL-15 and the cell tag from a single lentiviral vector. In further embodiments, the ROR-1 CAR can be under the control of an inducible promoter. In another embodiment, the mbIL-15 can be under the control of an inducible promoter. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch.

[00289] In one embodiment, a ROR-1 CAR described herein comprises anti- ROR-1 scFv, human CD8 hinge and transmembrane domain, and human 4-1BB and CD3zeta signaling domains. In another embodiment, the ROR-1 CAR of the invention comprises anti- ROR-1 scFv, human CD8 hinge and transmembrane domain, human 4-1BB and CD3zeta signaling domains and optionally, a truncated epidermal growth factor receptor (HER1t or HER1t-1) tag. Other suitable vectors include integrating expression vectors, which can randomly integrate into the host cell's DNA, or can include a recombination site to enable the specific recombination between the expression vector and the host cell's chromosome. Such integrating expression vectors can utilize the endogenous expression control sequences of the host cell's chromosomes to effect expression of the desired protein. Examples of vectors that integrate in a site specific manner include, for example, components of the flp-in system from Invitrogen (Carlsbad, Calif.) (*e.g.*, pcDNA™5/FRT), or the cre-lox system, such as can be found in the pExchange-6 Core Vectors from Stratagene (La Jolla, Calif.). Examples of vectors that randomly integrate into host cell chromosomes include, for example, pcDNA3.1 (when introduced in the absence of T-antigen) from Invitrogen (Carlsbad, Calif.), and pCI or pFN10A (ACT) FLEXI™ from Promega (Madison, Wis.). Additional promoter elements, *e.g.*, enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

[00290] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto.

[00291] Another example of a suitable promoter is human elongation growth factor 1 alpha 1 (hEF1a1). In embodiments, the vector construct comprising a CAR described herein comprises hEF1a1 functional variants.

[00292] However, other constitutive promoter sequences can also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention as previously described. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter. In one aspect, the inducible promoter can be a gene switch ligand inducible promoter. In some cases, an inducible promoter can be a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch, such as RHEOSWITCH® gene switch.

[00293] In order to assess the expression of a CAR described herein or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors or non-viral vectors. In other aspects, the selectable marker can be carried on a separate piece of DNA and used in a co-transfection procedure. Both selectable markers and reporter genes can be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neomycin resistance gene (neo) and ampicillin resistance gene and the like. In some embodiments, a truncated epidermal growth factor receptor (HER1t or HER1t-1) tag can be used as a selectable marker gene.

[00294] Reporter genes can be used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide

whose expression is manifested by some easily detectable property, *e.g.*, enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (*e.g.*, Ui-Tei et al., FEBS Letters 479: 79-82 (2000)). Suitable expression systems are well known and can be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions can be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

[00295] In embodiments, a viral vector described herein can comprise a hEF1a1 promoter to drive expression of transgenes, a bovine growth hormone polyA sequence to enhance transcription, a woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), as well as LTR sequences derived from the pFUGW plasmid.

[00296] Methods of introducing and expressing genes into a cell are well known. In the context of an expression vector, the vector can be readily introduced into a host cell, *e.g.*, mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means.

[00297] Physical methods for introducing a polynucleotide into a host cell, for instance an immune effector cell, include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (2001)). In embodiments, a method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection or polyethylenimine (PEI) Transfection. In some embodiments, a method for introduction of a polynucleotide into a host cell is electroporation.

[00298] Biological methods for introducing a polynucleotide of interest into a host cell, for instance an immune effector cell, include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, *e.g.*, human cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex virus I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362.

[00299] Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (*e.g.*, an artificial membrane vesicle).

[00300] In the case where a viral delivery system is utilized, an exemplary delivery vehicle is a liposome. Lipid formulations can be used for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo*, or *in vivo*). In another aspect, the nucleic acid can be associated with a lipid. The nucleic acid associated with a lipid can be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they can be present in a bilayer structure, as micelles, or with a "collapsed" structure. They can also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which can be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[00301] Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, Mo.; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, N.Y.); cholesterol ("Chol") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids can be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et al., Glycobiology 5: 505-10 (1991)). However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids can

assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Cells Comprising ROR-1 CARs and Vectors

[00302] Provided herein are engineered cells expressing a CAR described herein. In certain embodiments, an engineered cell described herein is an immune effector cell. In embodiments, provided herein is an immune effector cell comprising a vector comprising a backbone and a nucleic acid sequence encoding (1) a truncated epidermal growth factor receptor (HER1t or HER1t1) and (2) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and e) a CD3 zeta signaling domain.

[00303] In certain embodiments is an immune effector cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; e) a CD3 zeta signaling domain; and (f) a truncated epidermal growth factor receptor (HER1t or HER1t1).

[00304] In embodiments, provided herein is an immune effector cell comprising (1) a cell tag for use as a kill switch, selection marker, a biomarker, or a combination thereof, and (2) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain. In embodiments, the cell tag is HER1t, HER1t1, CD20t-1 or CD20.

[00305] In embodiments, an immune effector cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), and a regulatory T cell. In embodiments, the cell exhibits an anti-tumor activity when the ROR-1 antigen binding domain binds to ROR-1.

Modified Immune Effector Cells

[00306] Provided are immune effector cells modified to express one or more heterologous genes or polypeptides described herein. Provided are immune effector cells modified to express a ROR-1 CAR described herein and at least one of a HER1t, HER1t1, CD20 and CD20t-1 tag. In some cases is provided an immune effector cell modified to express ROR-1 CAR, mbIL-15 and at least one of a HER1t, HER1t1, CD20 and CD20t-1 tag disclosed herein.

[00307] “T cell” or “T lymphocyte” as used herein is a type of lymphocyte that plays a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B

cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface.

[00308] In some embodiments, modified immune effector cells are modified immune cells that comprise T cells and/or natural killer cells. T cells or T lymphocytes are a subtype of white blood cells that are involved in cell-mediated immunity. Exemplary T cells include T helper cells, cytotoxic T cells, TH17 cells, stem memory T cells (TSCM), naïve T cells, memory T cells, effector T cells, regulatory T cells, or natural killer T cells.

[00309] T helper cells (TH cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. In some instances, TH cells are known as CD4+ T cells due to expression of the CD4 glycoprotein on the cell surfaces. Helper T cells become activated when they are presented with peptide antigens by MHC class II molecules, which are expressed on the surface of antigen-presenting cells (APCs). Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response. These cells can differentiate into one of several subtypes, including TH1, TH2, TH3, TH17, Th9, or TFH, which secrete different cytokines to facilitate different types of immune responses. Signaling from the APC directs T cells into particular subtypes.

[00310] Cytotoxic T cells (TC cells or CTLs) destroy virus-infected cells and tumor cells, and are also implicated in transplant rejection. These cells are also known as CD8+ T cells since they express the CD8 glycoprotein on their surfaces. These cells recognize their targets by binding to antigen associated with MHC class I molecules, which are present on the surface of all nucleated cells. Through IL-10, adenosine, and other molecules secreted by regulatory T cells, the CD8+ cells can be inactivated to an anergic state, which prevents autoimmune diseases.

[00311] Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with “memory” against past infections. Memory T cells comprise subtypes: stem memory T cells (TSCM), central memory T cells (TCM cells) and two types of effector memory T cells (TEM cells and TEMRA cells). Memory cells can be either CD4+ or CD8+. Memory T cells can express the cell surface proteins CD45RO, CD45RA and/or CCR7.

[00312] Regulatory T cells (Treg cells), formerly known as suppressor T cells, play a role in the maintenance of immunological tolerance. Their major role is to shut down T cell-mediated immunity toward the end of an immune reaction and to suppress autoreactive T cells that escaped the process of negative selection in the thymus.

[00313] Natural killer T cells (NKT cells) bridge the adaptive immune system with the innate immune system. Unlike conventional T cells that recognize peptide antigens presented by major histocompatibility complex (MHC) molecules, NKT cells recognize glycolipid antigen presented by a molecule called CD1d. Once activated, these cells can perform functions ascribed to both Th (T helper) and Tc (cytotoxic T) cells (*i.e.*, cytokine production and release of cytolytic/cell killing molecules). They are also able to recognize and eliminate some tumor cells and cells infected with herpes viruses.

[00314] Natural killer (NK) cells are a type of cytotoxic lymphocyte of the innate immune system. In some instances, NK cells provide a first line defense against viral infections and/or tumor formation. NK cells can detect MHC presented on infected or cancerous cells, triggering cytokine release, and subsequently induce lysis and apoptosis. NK cells can further detect stressed cells in the absence of antibodies and/or MHC, thereby allowing a rapid immune response.

Modified Immune Effector Cell Doses

[00315] In some embodiments, an amount of modified immune effector cells is administered to a subject in need thereof and the amount is determined based on the efficacy and the potential of inducing a cytokine-associated toxicity. In some cases, an amount of modified immune effector cells comprises about 10^2 to about 10^9 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^3 to about 10^9 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^4 to about 10^9 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^5 to about 10^9 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^5 to about 10^8 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^5 to about 10^7 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^6 to about 10^9 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^6 to about 10^8 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^7 to about 10^9 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^5 to about 10^6 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^6 to about 10^7 modified immune effector cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^7 to about 10^8 modified immune effector

cells/kg. In some cases, an amount of modified immune effector cells comprises about 10^8 to about 10^9 modified immune effector cells/kg. In some instances, an amount of modified immune effector cells comprises about 10^9 modified immune effector cells/kg. In some instances, an amount of modified immune effector cells comprises about 10^8 modified immune effector cells/kg. In some instances, an amount of modified immune effector cells comprises about 10^7 modified immune effector cells/kg. In some instances, an amount of modified immune effector cells comprises about 10^6 modified immune effector cells/kg. In some instances, an amount of modified immune effector cells comprises about 10^5 modified immune effector cells/kg.

[00316] In some embodiments, are CAR-T cells which are ROR-1-specific CAR-T cells. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^2 to about 10^9 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^3 to about 10^9 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^4 to about 10^9 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^6 to about 10^9 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg. In some cases, an amount of ROR-1-specific CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^9 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^8 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^7 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^6 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^5 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^4 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^3 CAR-T cells/kg. In some instances, an amount of ROR-1-specific CAR-T cells comprises about 10^2 CAR-T cells/kg.

Immune Effector Cell Sources

[00317] In certain aspects, the embodiments described herein include methods of making and/or expanding the antigen-specific redirected immune effector cells (*e.g.*, T-cells, Tregs, NK-cell or NK T-cells) that comprises transfecting the cells with an expression vector containing a DNA (or RNA) construct encoding the CAR, then, optionally, stimulating the cells with feeder cells, recombinant antigen, or an antibody to the receptor to cause the cells to proliferate. In certain aspects, the cell (or cell population) engineered to express a CAR is a stem cell, iPS cell, T cell differentiated from iPS cell, immune effector cell or a precursor of these cells.

[00318] Sources of immune effector cells can include both allogeneic and autologous sources. In some cases immune effector cells can be differentiated from stem cells or induced pluripotent stem cells (iPSCs). Thus, cell for engineering according to the embodiments can be isolated from umbilical cord blood, peripheral blood, human embryonic stem cells, or iPSCs. For example, allogeneic T cells can be modified to include a chimeric antigen receptor (and optionally, to lack functional TCR). In some aspects, the immune effector cells are primary human T cells such as T cells derived from human peripheral blood mononuclear cells (PBMC). PBMCs can be collected from the peripheral blood or after stimulation with G-CSF (Granulocyte colony stimulating factor) from the bone marrow, or umbilical cord blood. In one aspect, the immune effector cells are Pan T cells. Following transfection or transduction (*e.g.*, with a CAR expression construct), the cells can be immediately infused or can be cryo-preserved. In certain aspects, following transfection or transduction, the cells can be preserved in a cytokine bath that can include IL-2 and/or IL-21 until ready for infusion. In certain aspects, following transfection, the cells can be propagated for days, weeks, or months *ex vivo* as a bulk population within about 1, 2, 3, 4, 5 days or more following gene transfer into cells. In a further aspect, following transfection, the transfectants are cloned and a clone demonstrating presence of a single integrated or episomally maintained expression cassette or plasmid, and expression of the chimeric antigen receptor is expanded *ex vivo*. The clone selected for expansion demonstrates the capacity to specifically recognize and lyse antigen-expressing target cells. The recombinant T cells can be expanded by stimulation with IL-2, or other cytokines that bind the common gamma-chain (*e.g.*, IL-7, IL-12, IL-15, IL-21, and others). The recombinant T cells can be expanded by stimulation with artificial antigen presenting cells. The recombinant T cells can be expanded on artificial antigen presenting cell or with an antibody, such as OKT3, which cross links CD3 on the T cell surface. Subsets of the recombinant T cells can be further selected with the use of magnetic bead based isolation methods and/or fluorescence activated cell sorting

technology and further cultured with the AaPCs. In a further aspect, the genetically modified cells can be cryopreserved.

[00319] T cells can also be obtained from a number of sources, including bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available in the art, can be used. In certain embodiments of the present invention, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll® separation. In embodiments, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one embodiment, the cells collected by apheresis can be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one embodiment of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative embodiment, the wash solution lacks calcium and can lack magnesium or can lack many if not all divalent cations. Initial activation steps in the absence of calcium lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step can be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells can be resuspended in a variety of biocompatible buffers, such as, for example, Ca^{2+}-free, Mg^{2+}-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample can be removed and the cells directly resuspended in culture media.

[00320] In another embodiment, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL® gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as $CD3^+$, $CD28^+$, $CD4^+$, $CD8^+$, $CD45RA^+$, and $CD45RO^+$ T cells, can be further isolated by positive or negative selection techniques. In another embodiment, CD14+ cells are depleted from the T-cell population. For example, in one embodiment, T cells are isolated by incubation with anti-CD3/anti-CD28 (*i.e.*, 3x28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one embodiment, the time period is about 30 minutes. In a further embodiment, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further embodiment, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another embodiment, the time period is 10 to 24 hours. In one embodiment, the incubation time period is 24 hours. For

isolation of T cells from patients with leukemia, use of longer incubation times, such as 24 hours, can increase cell yield. Longer incubation times can be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immune-compromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads or other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain embodiments, it can be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

[00321] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for $CD4^+$ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CD11b, CD16, HLA-DR, and CD8. In certain embodiments, it can be desirable to enrich for or positively select for regulatory T cells which typically express $CD4^+$, $CD25^+$, $CD62L^{hi}$, $GITR^+$, and $FoxP3^+$. Alternatively, in certain embodiments, T regulatory cells are depleted by anti-CD25 conjugated beads or other similar method of selection.

[00322] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (*e.g.*, particles such as beads) can be varied. In certain embodiments, it can be desirable to significantly decrease the volume in which beads and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one embodiment, a concentration of 2 billion cells/ml is used. In one embodiment, a concentration of 1 billion cells/ml is used. In a further embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high

concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that can weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (*i.e.*, leukemic blood, tumor tissue, etc.). Such populations of cells can have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of $CD8^+$ T cells that normally have weaker CD28 expression.

[00323] In a related embodiment, it can be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (*e.g.*, particles such as beads), interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, $CD4^+$ T cells express higher levels of CD28 and are more efficiently captured than $CD8^+$ T cells in dilute concentrations. In one embodiment, the concentration of cells used is $5x10^6$/ml. In other embodiments, the concentration used can be from about $1x10^5$/ml to $1x10^6$/ml, and any integer value in between.

[00324] In other embodiments, the cells can be incubated on a rotator for varying lengths of time at varying speeds at either 2-10° C or at room temperature.

[00325] T cells for stimulation can also be frozen after a washing step. After the washing step that removes plasma and platelets, the cells can be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80° C at a rate of 1°C per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other methods of controlled freezing can be used as well as uncontrolled freezing immediately at -20°C or in liquid nitrogen. In certain embodiments, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present invention.

[00326] Also provided in certain embodiments is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as

those described herein. In one embodiment a blood sample or an apheresis is taken from a generally healthy subject. In certain embodiments, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain embodiments, the T cells can be expanded, frozen, and used at a later time. In certain embodiments, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments. In a further embodiment, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation. These drugs inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin) (Liu et al., *Cell* 66:807-815, (1991); Henderson et al., *Immun* 73:316-321, (1991); Bierer et al., *Curr*. *Opin*. *Immun* 5:763-773, (1993)). In a further embodiment, the cells are isolated for a patient and frozen for later use in conjunction with (*e.g.*, before, simultaneously or following) bone marrow or stem cell transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAMPATH. In another embodiment, the cells are isolated prior to and can be frozen for later use for treatment following B-cell ablative therapy such as agents that react with CD20, *e.g.*, Rituxan.

[00327] In a further embodiment of the present invention, T cells are obtained from a patient directly following treatment. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients would normally be recovering from the treatment, the quality of T cells obtained can be optimal or improved for their ability to expand *ex vivo*. Likewise, following *ex vivo* manipulation using the methods described herein, these cells can be in a preferred state for enhanced engraftment and *in vivo* expansion. Thus, it is contemplated within the context of the present invention to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain embodiments, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined

window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

Activation and Expansion of T cells

[00328] Whether prior to or after engineering of the T cells to express a CAR described herein, the T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 5,883,223; 6,905,874; 6,797,514; 6,867,041; and U.S. Patent Application Publication No. 20060121005.

[00329] Generally, the T cells described herein are expanded by contact with a surface having attached thereto an agent that stimulates a CD3/TCR complex associated signal and a ligand that stimulates a co-stimulatory molecule on the surface of the T cells. In particular, T cell populations can be stimulated as described herein, such as by contact with an anti-CD3 antibody, or antigen-binding fragment thereof, or an anti-CD2 antibody immobilized on a surface, or by contact with a protein kinase C activator (*e.g.*, bryostatin) in conjunction with a calcium ionophore. For co-stimulation of an accessory molecule on the surface of the T cells, a ligand that binds the accessory molecule is used. For example, a population of T cells can be contacted with an anti-CD3 antibody and an anti-CD28 antibody, under conditions appropriate for stimulating proliferation of the T cells. To stimulate proliferation of either $CD4^+$ T cells or $CD8^+$ T cells, an anti-CD3 antibody and an anti-CD28 antibody. Examples of an anti-CD28 antibody include 9.3, B-T3, XR-CD28 (Diaclone, Besancon, France) can be used as can other methods commonly known in the art (Berg et al., *Transplant Proc*. 30(8):3975-3977, (1998); Haanen et al., *J. Exp. Med.* 190(9):13191328, (1999); Garland et al., *J. Immunol Meth*. 227(1-2):53-63, (1999)).

[00330] In certain embodiments, the primary stimulatory signal and the co-stimulatory signal for the T cell can be provided by different protocols. For example, the agents providing each signal can be in solution or coupled to a surface. When coupled to a surface, the agents can be coupled to the same surface (*i.e.*, in "*cis*" formation) or to separate surfaces (*i.e.*, in "*trans*" formation). Alternatively, one agent can be coupled to a surface and the other agent in solution. In one embodiment, the agent providing the co-stimulatory signal is bound to a cell surface and the agent providing the primary activation signal is in solution or coupled to a surface. In certain embodiments, both agents can be in solution. In another embodiment, the agents can be in soluble form, and then cross-linked to a surface, such as a cell expressing Fc receptors or an antibody or other binding agent which will bind to the agents. In this regard, see for example,

U.S. Patent Application Publication Nos. 20040101519 and 20060034810 for artificial antigen presenting cells (aAPCs) that are contemplated for use in activating and expanding T cells in the present invention.

[00331] In one embodiment, the two agents are immobilized on beads, either on the same bead, *i.e.*, "*cis*," or to separate beads, *i.e.*, "*trans*." By way of example, the agent providing the primary activation signal is an anti-CD3 antibody or an antigen-binding fragment thereof and the agent providing the co-stimulatory signal is an anti-CD28 antibody or antigen-binding fragment thereof; and both agents are co-immobilized to the same bead in equivalent molecular amounts. In one embodiment, a 1:1 ratio of each antibody bound to the beads for $CD4^+$ T cell expansion and T cell growth is used. In certain aspects of the present invention, a ratio of anti CD3:CD28 antibodies bound to the beads is used such that an increase in T cell expansion is observed as compared to the expansion observed using a ratio of 1:1. In one particular embodiment an increase of from about 1 to about 3 fold is observed as compared to the expansion observed using a ratio of 1:1. In one embodiment, the ratio of CD3:CD28 antibody bound to the beads ranges from 100:1 to 1:100 and all integer values there between. In one aspect of the present invention, more anti-CD28 antibody is bound to the particles than anti-CD3 antibody, *i.e.*, the ratio of CD3:CD28 is less than one. In certain embodiments of the invention, the ratio of anti CD28 antibody to anti CD3 antibody bound to the beads is greater than 2:1. In one particular embodiment, a 1:100 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:75 CD3:CD28 ratio of antibody bound to beads is used. In a further embodiment, a 1:50 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:30 CD3:CD28 ratio of antibody bound to beads is used. In embodiments, a 1:10 CD3:CD28 ratio of antibody bound to beads is used. In another embodiment, a 1:3 CD3:CD28 ratio of antibody bound to the beads is used. In yet another embodiment, a 3:1 CD3:CD28 ratio of antibody bound to the beads is used.

[00332] Ratios of particles to cells from 1:500 to 500:1 and any integer values in between can be used to stimulate T cells or other target cells. As those of ordinary skill in the art can readily appreciate, the ratio of particles to cells can depend on particle size relative to the target cell. For example, small sized beads could only bind a few cells, while larger beads could bind many. In certain embodiments the ratio of cells to particles ranges from 1:100 to 100:1 and any integer values in-between and in further embodiments the ratio comprises 1:9 to 9:1 and any integer values in between, can also be used to stimulate T cells. The ratio of anti-CD3- and anti-CD28-coupled particles to T cells that result in T cell stimulation can vary as noted above, however certain values include 1:100, 1:50, 1:40, 1:30, 1:20, 1:10, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, and 15:1 with one ratio being at least 1:1 particles

per T cell. In one embodiment, a ratio of particles to cells of 1:1 or less is used. In one particular embodiment, the particle:cell ratio is 1:5. In further embodiments, the ratio of particles to cells can be varied depending on the day of stimulation. For example, in one embodiment, the ratio of particles to cells is from 1:1 to 10:1 on the first day and additional particles are added to the cells every day or every other day thereafter for up to 10 days, at final ratios of from 1:1 to 1:10 (based on cell counts on the day of addition). In one particular embodiment, the ratio of particles to cells is 1:1 on the first day of stimulation and adjusted to 1:5 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:5 on the third and fifth days of stimulation. In another embodiment, the ratio of particles to cells is 2:1 on the first day of stimulation and adjusted to 1:10 on the third and fifth days of stimulation. In another embodiment, particles are added on a daily or every other day basis to a final ratio of 1:1 on the first day, and 1:10 on the third and fifth days of stimulation. One of skill in the art will appreciate that a variety of other ratios can be suitable for use in the present invention. In particular, ratios will vary depending on particle size and on cell size and type.

[00333] In further embodiments described herein, the immune effector cells, such as T cells, are combined with agent-coated beads, the beads and the cells are subsequently separated, and then the cells are cultured. In an alternative embodiment, prior to culture, the agent-coated beads and cells are not separated but are cultured together. In a further embodiment, the beads and cells are first concentrated by application of a force, such as a magnetic force, resulting in increased ligation of cell surface markers, thereby inducing cell stimulation.

[00334] By way of example, cell surface proteins can be ligated by allowing paramagnetic beads to which anti-CD3 and anti-CD28 are attached (3x28 beads) to contact the T cells. In one embodiment the cells (for example, 10^4 to 10^9 T cells) and beads (for example, DYNABEADS® M-450 CD3/CD28 T paramagnetic beads at a ratio of 1:1, or MACS® MicroBeads from Miltenyi Biotec) are combined in a buffer, for example, PBS (without divalent cations such as, calcium and magnesium). Again, those of ordinary skill in the art can readily appreciate any cell concentration can be used. For example, the target cell can be very rare in the sample and comprise only 0.01% of the sample or the entire sample (*i.e.*, 100%) can comprise the target cell of interest. Accordingly, any cell number is within the context of the present invention. In certain embodiments, it can be desirable to significantly decrease the volume in which particles and cells are mixed together (*i.e.*, increase the concentration of cells), to ensure maximum contact of cells and particles. For example, in one embodiment, a concentration of about 2 billion cells/ml is used. In another embodiment, greater than 100 million cells/ml is used. In a further embodiment, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million

cells/ml is used. In yet another embodiment, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further embodiments, concentrations of 125 or 150 million cells/ml can be used. Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that can weakly express target antigens of interest, such as CD28-negative T cells. Such populations of cells can have therapeutic value and would be desirable to obtain in certain embodiments. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

[00335] In one embodiment described herein, the mixture can be cultured for several hours (about 3 hours) to about 14 days or any hourly integer value in between. In another embodiment, the mixture can be cultured for 21 days. In one embodiment of the invention the beads and the T cells are cultured together for about eight days. In another embodiment, the beads and T cells are cultured together for 2-3 days. Several cycles of stimulation can also be desired such that culture time of T cells can be 60 days or more. Conditions appropriate for T cell culture include an appropriate media (*e.g.*, Minimal Essential Media or RPMI Media 1640 or, X-vivo 15, (Lonza)) that can contain factors necessary for proliferation and viability, including serum (*e.g.*, fetal bovine or human serum), interleukin-2 (IL-2), insulin, IFN-.gamma., IL-4, IL-7, GM-CSF, IL-10, IL-12, IL-15, TGFbeta, and TNF-alpha or any other additives for the growth of cells known to the skilled artisan. Other additives for the growth of cells include, but are not limited to, surfactant, plasmanate, and reducing agents such as N-acetyl-cysteine and 2-mercaptoethanol. Media can include RPMI 1640, AIM-V, DMEM, MEM, alpha-MEM, F-12, X-Vivo 15, and X-Vivo 20, Optimizer, with added amino acids, sodium pyruvate, and vitamins, either serum-free or supplemented with an appropriate amount of serum (or plasma) or a defined set of hormones, and/or an amount of cytokine(s) sufficient for the growth and expansion of T cells. Antibiotics, *e.g.*, penicillin and streptomycin, are included only in experimental cultures, not in cultures of cells that are to be infused into a subject. The target cells are maintained under conditions necessary to support growth, for example, an appropriate temperature (*e.g.*, 37° C.) and atmosphere (*e.g.*, air plus 5% CO_2).

[00336] T cells that have been exposed to varied stimulation times can exhibit different characteristics. For example, typical blood or apheresed peripheral blood mononuclear cell products have a helper T cell population (T_H, $CD4^+$) that is greater than the cytotoxic or suppressor T cell population (T_C, $CD8^+$). *Ex vivo* expansion of T cells by stimulating CD3 and CD28 receptors produces a population of T cells that prior to about days 8-9 consists predominately of T_H cells, while after about days 8-9, the population of T cells comprises an increasingly greater population of T_C cells. Accordingly, depending on the purpose of

treatment, infusing a subject with a T cell population comprising predominately of T_H cells can be advantageous. Similarly, if an antigen-specific subset of T_C cells has been isolated it can be beneficial to expand this subset to a greater degree.

[00337] Further, in addition to CD4 and CD8 markers, other phenotypic markers vary significantly, but in large part, reproducibly during the course of the cell expansion process. Thus, such reproducibility enables the ability to tailor an activated T cell product for specific purposes.

[00338] In some cases, immune effector cells of the embodiments (*e.g.*, T-cells) are co-cultured with activating and propagating cells (AaPCs), to aid in cell expansion. AaPCs can also be referred to as artificial Antigen Presenting cells (aAPCs). For example, antigen presenting cells (APCs) are useful in preparing therapeutic compositions and cell therapy products of the embodiments. In one aspect, the AaPCs can be genetically modified K562 cells. For general guidance regarding the preparation and use of antigen-presenting systems, see, *e.g.*, U.S. Pat. Nos. 6,225,042, 6,355,479, 6,362,001 and 6,790,662; U.S. Patent Application Publication Nos. 2009/0017000 and 2009/0004142; and International Publication No. WO2007/103009, each of which is incorporated by reference. In yet a further aspect of the embodiments, culturing the genetically modified CAR cells comprises culturing the genetically modified CAR cells in the presence of dendritic cells or activating and propagating cells (AaPCs) that stimulate expansion of the CAR-expressing immune effector cells. In still further aspects, the AaPCs comprise a CAR-binding antibody or fragment thereof expressed on the surface of the AaPCs. The AaPCs can comprise additional molecules that activate or co-stimulate T-cells in some cases. The additional molecules can, in some cases, comprise membrane-bound Cγ cytokines. In yet still further aspects, the AaPCs are inactivated or irradiated, or have been tested for and confirmed to be free of infectious material. In still further aspects, culturing the genetically modified CAR cells in the presence of AaPCs comprises culturing the genetically modified CAR cells in a medium comprising soluble cytokines, such as IL-15, IL-21 and/or IL-2. The cells can be cultured at a ratio of about 10:1 to about 1:10; about 3:1 to about 1:5; about 1:1 to about 1:3 (immune effector cells to AaPCs); or any range derivable therein. For example, the co-culture of T cells and AaPCs can be at a ratio of about 1:1, about 1:2 or about 1:3.

[00339] In one aspect, the AaPCs can express CD137L. In some aspects, the AaPCs can further express the antigen that is targeted by the CAR cell, for example ROR-1 (full length, truncate or any variant thereof. In other aspects, the AaPCs can further express CD64, CD86, or mIL15. In certain aspects, the AaPCs can express at least one anti-CD3 antibody clone, such as, for example, OKT3 and/or UCHT1. In one aspect, the AaPCs can be inactivated (*e.g.*, irradiated). In one aspect, the AaPCs can have been tested for and confirmed to be free of

infectious material. Methods for producing such AaPCs are known in the art. In one aspect, culturing the CAR-modified T cell population with AaPCs can comprise culturing the cells at a ratio of about 10:1 to about 1:10; about 3:1 to about 1:5; about 1:1 to about 1:3 (T cells to AaPCs); or any range derivable therein. For example, the co-culture of T cells and AaPCs can be at a ratio of about 1:1, about 1:2 or about 1:3. In one aspect, the culturing step can further comprise culturing with an aminobisphosphonate (*e.g.*, zoledronic acid).

[00340] In one aspect, the population of genetically modified CAR cells is immediately infused into a subject or cryopreserved. In another aspect, the population of genetically modified CAR cells is placed in a cytokine bath prior to infusion into a subject. In a further aspect, the population of genetically modified CAR cells is cultured and/or stimulated for no more than 1, 2, 3, 4, 5, 6, 7, 14, 21, 28, 35 42 days, 49, 56, 63 or 70 days. In an embodiment, a stimulation includes the co-culture of the genetically modified CAR T cells with AaPCs to promote the growth of CAR positive T cells. In another aspect, the population of genetically modified CAR cells is stimulated for not more than: 1X stimulation, 2X stimulation, 3X stimulation, 4X stimulation, 5X stimulation, 5X stimulation, 6X stimulation, 7X stimulation, 8X stimulation, 9X stimulation or 10X stimulation. In some instances, the genetically modified cells are not cultured *ex vivo* in the presence of AaPCs. In some specific instances, the method of the embodiment further comprises enriching the cell population for CAR-expressing immune effector cells (*e.g.*, T-cells) after the transfection and/or culturing step. The enriching can comprise fluorescence-activated cell sorting (FACS) to sort for CAR-expressing cells. In a further aspect, the sorting for CAR-expressing cells comprises use of a CAR-binding antibody. The enriching can also comprise depletion of CD56+ cells. In yet still a further aspect of the embodiment, the method further comprises cryopreserving a sample of the population of genetically modified CAR cells.

[00341] In some cases, AaPCs are incubated with a peptide of an optimal length that allows for direct binding of the peptide to the MHC molecule without additional processing. Alternatively, the cells can express an antigen of interest (*i.e.*, in the case of MHC-independent antigen recognition). Furthermore, in some cases, APCs can express an antibody that binds to either a specific CAR polypeptide or to CAR polypeptides in general (*e.g.*, a universal activating and propagating cell (uAPC). Such methods are disclosed in WO/2014/190273, which is incorporated herein by reference. In addition to peptide-MHC molecules or antigens of interest, the AaPC systems can also comprise at least one exogenous assisting molecule. Any suitable number and combination of assisting molecules can be employed. The assisting molecule can be selected from assisting molecules such as co-stimulatory molecules and adhesion molecules. Exemplary co-stimulatory molecules include CD70 and B7.1 (B7.1 was previously known as B7

and also known as CD80), which among other things, bind to CD28 and/or CTLA-4 molecules on the surface of T cells, thereby affecting, for example, T-cell expansion, Th1 differentiation, short-term T-cell survival, and cytokine secretion such as interleukin (IL)-2. Adhesion molecules can include carbohydrate-binding glycoproteins such as selectins, transmembrane binding glycoproteins such as integrins, calcium-dependent proteins such as cadherins, and single-pass transmembrane immunoglobulin (Ig) superfamily proteins, such as intercellular adhesion molecules (ICAMs)that promote, for example, cell-to-cell or cell-to-matrix contact. Exemplary adhesion molecules include LFA-3 and ICAMs, such as ICAM-1. Techniques, methods, and reagents useful for selection, cloning, preparation, and expression of exemplary assisting molecules, including co-stimulatory molecules and adhesion molecules, are exemplified in, *e.g.*, U.S. Pat. Nos. 6,225,042, 6,355,479, and 6,362,001, incorporated herein by reference.

[00342] Cells selected to become AaPCs, preferably have deficiencies in intracellular antigen-processing, intracellular peptide trafficking, and/or intracellular MHC Class I or Class II molecule-peptide loading, or are poikilothermic (*i.e.*, less sensitive to temperature challenge than mammalian cell lines), or possess both deficiencies and poikilothermic properties. Preferably, cells selected to become AaPCs also lack the ability to express at least one endogenous counterpart (*e.g.*, endogenous MHC Class I or Class II molecule and/or endogenous assisting molecules as described above) to the exogenous MHC Class I or Class II molecule and assisting molecule components that are introduced into the cells. Furthermore, AaPCs preferably retain the deficiencies and poikilothermic properties that were possessed by the cells prior to their modification to generate the AaPCs. Exemplary AaPCs either constitute or are derived from a transporter associated with antigen processing (TAP)-deficient cell line, such as an insect cell line. An exemplary poikilothermic insect cells line is a Drosophila cell line, such as a Schneider 2 cell line (see, *e.g.*, Schneider 1972 Illustrative methods for the preparation, growth, and culture of Schneider 2 cells, are provided in U.S. Pat. Nos. 6,225,042, 6,355,479, and 6,362,001.

[00343] In one embodiment, AaPCs are also subjected to a freeze-thaw cycle. In an exemplary freeze-thaw cycle, the AaPCs can be frozen by contacting a suitable receptacle containing the AaPCs with an appropriate amount of liquid nitrogen, solid carbon dioxide (*i.e.*, dry ice), or similar low-temperature material, such that freezing occurs rapidly. The frozen APCs are then thawed, either by removal of the AaPCs from the low-temperature material and exposure to ambient room temperature conditions, or by a facilitated thawing process in which a lukewarm water bath or warm hand is employed to facilitate a shorter thawing time. Additionally, AaPCs can be frozen and stored for an extended period of time prior to thawing. Frozen AaPCs can also be thawed and then lyophilized before further use. Preferably, preservatives that might

detrimentally impact the freeze-thaw procedures, such as dimethyl sulfoxide (DMSO), polyethylene glycols (PEGs), and other preservatives, are absent from media containing AaPCs that undergo the freeze-thaw cycle, or are essentially removed, such as by transfer of AaPCs to media that is essentially devoid of such preservatives.

[00344] In further embodiments, xenogenic nucleic acid and nucleic acid endogenous to the AaPCs, can be inactivated by crosslinking, so that essentially no cell growth, replication or expression of nucleic acid occurs after the inactivation. In one embodiment, AaPCs are inactivated at a point subsequent to the expression of exogenous MHC and assisting molecules, presentation of such molecules on the surface of the AaPCs, and loading of presented MHC molecules with selected peptide or peptides. Accordingly, such inactivated and selected peptide loaded AaPCs, while rendered essentially incapable of proliferating or replicating, retain selected peptide presentation function. Preferably, the crosslinking also yields AaPCs that are essentially free of contaminating microorganisms, such as bacteria and viruses, without substantially decreasing the antigen-presenting cell function of the AaPCs. Thus crosslinking maintains the important AaPC functions of while helping to alleviate concerns about safety of a cell therapy product developed using the AaPCs. For methods related to crosslinking and AaPCs, see for example, U.S. Patent Application Publication No. 20090017000, which is incorporated herein by reference.

[00345] In certain embodiments there are further provided an engineered antigen presenting cell (APC). Such cells can be used, for example, as described above, to propagate immune effector cells *ex vivo*. In further aspects, engineered APCs can, themselves be administered to a patient and thereby stimulate expansion of immune effector cells *in vivo*. Engineered APCs of the embodiments can, themselves, be used as a therapeutic agent. In other embodiments, the engineered APCs can used as a therapeutic agent that can stimulate activation of endogenous immune effector cells specific for a target antigen and/or to increase the activity or persistence of adoptively transferred immune effector cells specific to a target antigen.

[00346] As used herein the term “engineered APC” refers to cell(s) that comprises at least a first transgene, wherein the first transgene encodes a HLA. Such engineered APCs can further comprise a second transgene for expression of an antigen, such that the antigen is presented at the surface on the APC in complex with the HLA. In some aspects, the engineered APC can be a cell type that presented antigens (*e.g.*, a dendritic cell). In further aspects, engineered APC can be produced from a cell type that does not normally present antigens, such a T-cell or T-cell progenitor (referred to as “T-APC"). Thus, in some aspects, an engineered APC of the embodiments comprises a first transgene encoding a target antigen and a second transgene encoding a human leukocyte antigen (HLA), such that the HLA is expressed on the surface of

the engineered APC in complex with an epitope of the target antigen. In certain specific aspects, the HLA expressed in the engineered APC is HLA-A2.

[00347] In some aspects, an engineered APC of the embodiments can further comprise at least a third transgene encoding co-stimulatory molecule. The co-stimulatory molecule can be a co-stimulatory cytokine that can be a membrane-bound Cγ cytokine. In certain aspects, the co-stimulatory cytokine is IL-15, such as membrane-bound IL-15. In some further aspects, an engineered APC can comprise an edited (or deleted) gene. For example, an inhibitory gene, such as PD-1, LIM-3, CTLA-4 or a TCR, can be edited to reduce or eliminate expression of the gene. An engineered APC of the embodiments can further comprise a transgene encoding any target antigen of interest.

Point-of-Care

[00348] In one embodiment of the present disclosure, the immune effector cells described herein are modified at a point-of-care site. In some cases, modified immune effector cells are also referred to as engineered T cells. In some cases, the point-of-care site is at a hospital or at a facility (*e.g.*, a medical facility) near a subject in need of treatment. The subject undergoes apheresis and peripheral blood mononuclear cells (PBMCs) or a sub population of PBMC can be enriched for example, by elutriation or Ficoll separation. Enriched PBMC or a subpopulation of PBMC can be cryopreserved in any appropriate cryopreservation solution prior to further processing. In one instance, the elutriation process is performed using a buffer solution containing human serum albumin. Immune effector cells, such as T cells can be isolated by selection methods described herein. In one instance, the selection method for T cells includes beads specific for CD3 or beads specific for CD4 and CD8 on T cells. In one case, the beads can be paramagnetic beads. The harvested immune effector cells can be cryopreserved in any appropriate cryopreservation solution prior to modification. The immune effector cells can be thawed up to 24 hours, 36 hours, 48 hours. 72 hours or 96 hours ahead of infusion. The thawed cells can be placed in cell culture buffer, for example in cell culture buffer (*e.g.* RPMI) supplemented with fetal bovine serum (FBS) or human serum AB or placed in a buffer that includes cytokines such as IL-2 and IL-21, prior to modification. In another aspect, the harvested immune effector cells can be modified immediately without the need for cryopreservation.

[00349] In some cases, the immune effector cells are modified by engineering/introducing a chimeric receptor, one or more cell tag(s), and/or cytokine(s) into the immune effector cells and then rapidly infused into a subject. In some cases, the sources of immune effector cells can

include both allogeneic and autologous sources. In one case, the immune effector cells can be T cells or NK cells. In one case, the chimeric receptor can be a ROR-1 CAR. In another case, the cytokine can be mbIL-15. In one case, the mbIL-15 is of SEQ ID NO: 113, or variant or fragment thereof. In yet another case, expression of mbIL-15 is modulated by ligand inducible gene-switch expression systems described herein. For example, a ligand such as veledimex can be delivered to the subject to modulate the expression of mbIL-15. In another case, the cytokine can be IL-12. In yet another case, expression of IL-12 is modulated by ligand inducible gene-switch expression systems described herein. For example, a ligand such as veledimex can be delivered to the subject to modulate the expression of IL-12.

[00350] In another aspect, veledimex is provided at 5 mg, 10 mg, 15 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 70 mg, 80 mg, 90 mg or 100 mg. In a further aspect, lower doses of veledimex are provided, for example, 0.5 mg, 1 mg, 5 mg, 10 mg, 15 mg or 20 mg. In one embodiment, veledimex is administered to the subject 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 days prior to infusion of the modified immune effector cells. In a further embodiment, veledimex is administered about once every 12 hours, about once every 24 hours, about once every 36 hours or about once every48 hours, for an effective period of time to a subject post infusion of the modified immune effector cells. In one embodiment, an effective period of time for veledimex administration is about: 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 days. In other embodiments, veledimex can be re-administered after a rest period, after a drug holiday or when the subject experiences a relapse.

[00351] In certain cases, where an adverse effect on a subject is observed or when treatment is not needed, the cell tag can be activated, for example via cetuximab, for conditional *in vivo* ablation of modified immune effector cells comprising cell tags such as truncated epidermal growth factor receptor tags as described herein.

[00352] In some embodiments, such immune effectors cells are modified by the constructs as described in **FIGs. 1-2** through electroporation. In one instance, electroporation is performed with electroporators such as Lonza's Nucleofector™ electroporators. In other embodiments, the vector comprising the above-mentioned constructs is a non-viral or viral vector. In one case, the non-viral vector includes a Sleeping Beauty transposon-transposase system. In one instance, the immune effector cells are electroporated using a specific sequence. For example, the immune effector cells can be electroporated with one transposon followed by the DNA encoding the transposase followed by a second transposon. In another instance, the immune effector cells can be electroporated with all transposons and transposase at the same time. In another instance, the immune effector cells can be electroporated with a transposase followed by both transposons or

one transposon at a time. While undergoing sequential electroporation, the immune effector cells can be rested for a period of time prior to the next electroporation step.

[00353] In some cases, the modified immune effector cells do not undergo a propagation and activation step. In some cases, the modified immune effector cells do not undergo an incubation or culturing step (*e.g. ex vivo* propagation). In certain cases, the modified immune effector cells are placed in a buffer that includes IL-2 and IL21 prior to infusion. In other instances, the modified immune effector cells are placed or rested in cell culture buffer, for example in cell culture buffer (*e.g.* RPMI) supplemented with fetal bovine serum (FBS) prior to infusion. Prior to infusion, the modified immune effector cells can be harvested, washed and formulated in saline buffer in preparation for infusion into the subject.

[00354] In one instance, the subject has been lymphodepleted prior to infusion. In other instances, lymphodepletion is not required and the modified immune effector cells are rapidly infused into the subject. Exemplary lymphodepletion regimens are listed in **Tables 2** and **3** below:

Table 2. Regimen 1

D-6	Admit / IV Hydration
D-5	Fludarabine 25 mg/m2, Cyclophosphamide 250 mg/m2
D-4	Fludarabine 25 mg/m2, Cyclophosphamide 250 mg/m2
D-3	Fludarabine 25 mg/m2 IV, Cyclophosphamide 250 mg/m2
D-2	REST
D-1	REST
D0	T-cell infusion

Table 3. Regimen 2

D-6	Admit / IV Hydration
D-5	Fludarabine 30 mg/m2, Cyclophosphamide 500 mg/m2
D-4	Fludarabine 30 mg/m2, Cyclophosphamide 500 mg/m2
D-3	Fludarabine 30 mg/m2 IV, Cyclophosphamide 500 mg/m2
D-2	REST
D-1	REST
D0	T-cell infusion

[00355] In a further instance, the subject undergoes minimal lymphodepletion. Minimal lymphodepletion herein refers to a reduced lymphodepletion protocol such that the subject can be infused within 1 day, 2 days or 3 days following the lymphodepletion regimen. In one instance, a reduced lymphodepletion protocol can include lower doses of fludarabine and/or cyclophosphamide. In another instance, a reduced lymphodepletion protocol can include a shortened period of lymphodepletion, for example 1 day or 2 days.

[00356] In one embodiment, the immune effector cells are modified by engineering/introducing a chimeric receptor and a cytokine into said immune effector cells and then rapidly infused into a subject. In other cases, the immune effector cells are modified by engineering/introducing a chimeric receptor and a cytokine into said cells and then infused within at least: 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 hours into a subject. In other cases, immune effector cells are modified by engineering/introducing a chimeric receptor and a cytokine into the immune effector cells and then infused in 0 days, <1 day, <2 days, <3 days, <4 days, <5 days, <6 days or <7 days into a subject.

[00357] In some embodiments, an amount of modified effector cells is administered to a subject in need thereof and the amount is determined based on the efficacy and the potential of inducing a cytokine-associated toxicity. In another embodiment, the modified effector cells are CAR^+ and $CD3^+$ cells. In some cases, an amount of modified effector cells comprises about 10^4 to about 10^9 modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10^4 to about 10^5 modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10^5 to about 10^6 modified effector cells/kg. In some cases, an amount of modified effector cells comprises about 10^6 to about 10^7 modified effector cells/kg. In some cases, an amount of modified effector cells comprises $>10^4$ but $\leq 10^5$ modified effector cells/kg. In some cases, an amount of modified effector cells comprises $>10^5$ but $\leq 10^6$ modified effector cells/kg. In some cases, an amount of modified effector cells comprises $>10^6$ but $\leq 10^7$ modified effector cells/kg.

[00358] In one embodiment, the modified immune effector cells are targeted to the cancer via regional delivery directly to the tumor tissue. For example, in ovarian cancer, the modified immune effector cells can be delivered intraperitoneally (IP) to the abdomen or peritoneal cavity. Such IP delivery can be performed via a port or pre-existing port placed for delivery of chemotherapy drugs. Other methods of regional delivery of modified immune effector cells can include catheter infusion into resection cavity, ultrasound guided intratumoral injection, hepatic artery infusion or intrapleural delivery.

[00359] In one embodiment, a subject in need thereof, can begin therapy with a first dose of modified immune effector cells delivered via IP followed by a second dose of modified immune effector cells delivered via IV. In a further embodiment, the second dose of modified immune effector cells can be followed by subsequent doses which can be delivered via IV or IP. In one embodiment, the duration between the first and second or further subsequent dose can be about: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 days. In one embodiment, the duration between the first and second or further subsequent

dose can be about: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 months. In another embodiment, the duration between the first and second or further subsequent dose can be about: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 years.

[00360] In another embodiment, a catheter can be placed at the tumor or metastasis site for further administration of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 doses of modified immune effector cells. In some cases, doses of modified effector cells can comprise about 10^2 to about 10^9 modified effector cells/kg. In cases where toxicity is observed, doses of modified effector cells can comprise about 10^2 to about 10^5 modified effector cells/kg. In some cases, doses of modified effector cells can start at about 10^2 modified effector cells/kg and subsequent doses can be increased to about: 10^4, 10^5, 10^6, 10^7, 10^8 or 10^9 modified effector cells/kg.

[00361] In other embodiments, a method of stimulating the proliferation and/or survival of engineered cells comprises obtaining a sample of cells from a subject, and transfecting cells of the sample of cells with one or more polynucleotides that comprise one or more transposons. In one embodiment, the transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, and a transposase effective to integrate said one or more polynucleotides into the genome of said cells, to provide a population of engineered cells. In an embodiment, the transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, gene switch polypeptides for ligand-inducible control of the cytokine and a transposase effective to integrate said one or more polynucleotides into the genome of said cells, to provide a population of engineered cells. In an embodiment, the gene switch polypeptides comprise i) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain, and ii) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain. In some embodiments, the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker. In one instance, lymphodepletion is not required prior to administration of the engineered cells to a subject.

[00362] In one instance, a method of *in vivo* propagation of engineered cells comprises obtaining a sample of cells from a subject, and transfecting cells of the sample of cells with one or more polynucleotides that comprise one or more transposons. In one embodiment, the transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, and a transposase effective to integrate said one or more polynucleotides into the genome of said cells, to provide a population of engineered cells. In an embodiment, the transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, gene switch polypeptides for ligand-inducible control of the cytokine and a transposase effective to integrate said one or more

polynucleotides into the genome of said cells, to provide a population of engineered cells. In an embodiment, the gene switch polypeptides comprise i) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain, and ii) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain. In some embodiments, the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker. In one instance, lymphodepletion is not required prior to administration of the engineered cells to a subject.

[00363] In another embodiment, a method of enhancing *in vivo* persistence of engineered cells in a subject in need thereof comprises obtaining a sample of cells from a subject, and transfecting cells of the sample of cells with one or more polynucleotides that comprise one or more transposons. In some cases, one or more transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, and a transposase effective to integrate the DNA into the genome of said cells, to provide a population of engineered cells. In some cases, one or more transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, gene switch polypeptides for ligand-inducible control of the cytokine and a transposase effective to integrate the DNA into the genome of said cells, to provide a population of engineered cells. In some cases, the gene switch polypeptides comprise i) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain, and ii) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain, wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker. In one instance, lymphodepletion is not required prior to administration of the engineered cells to a subject.

[00364] In another embodiment, a method of treating a subject with a solid tumor comprises obtaining a sample of cells from a subject, transfecting cells of the sample with one or more polynucleotides that comprise one or more transposons, and administering the population of engineered cells to the subject. In one instance, lymphodepletion is not required prior to administration of the engineered cells to a subject. In some cases, the one or more transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, and a transposase effective to integrate the DNA into the genome of the cells. In some cases, the one or more transposons encode a chimeric antigen receptor (CAR), a cytokine, one or more cell tags, gene switch polypeptides for ligand-inducible control of the cytokine and a transposase effective to integrate the DNA into the genome of the cells. In some cases, the gene switch polypeptides comprise: i) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain, and ii) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain,

wherein the first gene switch polypeptide and second gene switch polypeptide are connected by a linker. In some cases, the cells are transfected via electroporation. In some cases, the polynucleotides encoding the gene switch polypeptides are modulated by a promoter. In some cases, the promoter is a tissue-specific promoter or an EF1A promoter or functional variant thereof. In some cases, the tissue-specific promoter comprises a T cell specific response element or an NFAT response element. In some cases, the cytokine comprises at least one of IL-1, IL-2, IL-15, IL-12, IL-21, a fusion of IL-15, IL-15R or an IL-15 variant. In some cases, the cytokine is in secreted form. In some cases, the cytokine is in membrane-bound form. In some cases, the cells are NK cells, NKT cells, T-cells or T-cell progenitor cells. In some cases, the cells are administered to a subject (*e.g.* by infusing the subject with the engineered cells). In some cases, the method further comprises administering an effective amount of a ligand (*e.g.* veledimex) to induce expression of the cytokine. In some cases, the CAR is capable of binding at least ROR1. In some cases, the transposase is salmonid-type Tc1-like transposase. In some cases, the transposase is SB11 or SB100x transposase. In other cases, the transposase is PiggyBac. In some cases, the cell tag comprises at least one of a HER1 truncated variant or a CD20 truncated variant.

Therapeutic Applications

[00365] In embodiments described herein, is an immune effector cell (*e.g.*, T cell) transduced with Sleeping Beauty transposon(s) and Sleeping Beauty transposase. For example, the Sleeping Beauty transposon or transposons can include a CAR that combines an antigen recognition domain of ROR-1 with a spacer of CD8 alpha hinge and variants thereof, an intracellular domain of CD3-zeta, CD28, 4-1BB, or any combinations thereof and the intracellular domain CD3zeta, one or more cell tags, one or more cytokines and optionally, components of the gene switch system as described herein. Therefore, in some instances, the transduced T cell can elicit a CAR-mediated T-cell response.

[00366] In embodiments described herein, is provided the use of a CAR to redirect the specificity of a primary T cell to a ROR-1 surface antigen. Thus, the present invention also provides a method for stimulating a T cell-mediated immune response to a target cell population or tissue in a mammal comprising the step of administering to the mammal a T cell that expresses a CAR, wherein the CAR comprises a binding moiety that specifically interacts with ROR-1, a spacer, a zeta chain portion comprising for example the intracellular domain of human CD3zeta, and a costimulatory signaling region.

[00367] In one embodiment, the present disclosure includes a cellular therapy where T cells are genetically modified to express the ROR-1-specific CARs of the invention and the CAR T cell is infused to a recipient in need thereof. The infused cell is able to kill cells overexpressing ROR-1 in the recipient. Unlike antibody therapies, CAR T cells as described herein are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained effect on tumor cells.

[00368] The invention additionally provides a method for detecting a disease that comprises overexpression of ROR-1 in a subject, comprising a) providing i) a sample from a subject, and ii) any one or more of the antibodies, or antigen-binding fragments thereof, that are described herein, b) contacting the sample with the antibody under conditions for specific binding of the antibody with its antigen, and c) detecting an increased level of binding of the antibody to the sample compared to a control sample lacking the disease, thereby detecting the disease in the subject. In one embodiment, the disease is cancer. In a preferred embodiment, the cancer is selected from the group of ovarian cancer and breast cancer. While not intending to limit the method of detection, in one embodiment, detecting binding of the antibody to the sample is immunohistochemical, enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western blot, immunoprecipitation, and/or radiographic imaging.

[00369] Also provided herein is a method for treating a disease that comprises overexpression of ROR-1, comprising administering to a subject having the disease a therapeutically effective amount of any one or more of the antibodies, or antigen-binding fragments thereof, that are described herein. In one embodiment, the disease is cancer, as exemplified by ovarian cancer and breast cancer.

[00370] In one embodiment, the ROR-1 CAR T cells described herein can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time. In another embodiment, the CAR T cells described herein can evolve into specific memory T cells that can be reactivated.

[00371] The CAR-modified T cells described herein can also serve as a type of vaccine for *ex vivo* immunization and/or *in vivo* therapy in a mammal. In embodiments, the mammal is a human. With respect to *ex vivo* immunization, at least one of the following occurs *in vitro* prior to administering the immune effector cell into a mammal: i) expansion of the cells, ii) introducing a nucleic acid encoding a CAR to the cells, and/or iii) cryopreservation of the cells.

[00372] *Ex vivo* procedures are well known and are discussed more fully below. Briefly, cells are isolated from a mammal (for example, a human) and genetically modified (*i.e.*, transduced or transfected *in vitro*) with a vector expressing a CAR disclosed herein. The CAR-modified cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient can be a human and the CAR-modified cell can be autologous with respect to the

recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

[00373] The procedure for *ex vivo* expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, incorporated herein by reference, can be applied to the cells of the present invention. Other suitable methods are known in the art, therefore the present invention is not limited to any particular method of *ex vivo* expansion of the cells. Briefly, *ex vivo* culture and expansion of T cells comprises: (1) collecting CD34+ hematopoietic stem and progenitor cells from a mammal from peripheral blood harvest or bone marrow explants; and (2) expanding such cells *ex vivo*. In addition to the cellular growth factors described in U.S. Pat. No. 5,199,942, other factors such as flt3-L, IL-1, IL-3 and c-kit ligand, can be used for culturing and expansion of the cells.

[00374] In addition to using a cell-based vaccine in terms of *ex vivo* immunization, the present invention also provides compositions and methods for *in vivo* immunization to elicit an immune response directed against an antigen in a patient.

[00375] Generally, the cells activated and expanded as described herein can be utilized in the treatment and prevention of diseases that arise in individuals who are immunocompromised. In particular, the CAR-modified T cells of the invention are used in the treatment of ROR-1 malignancies, such as for example, ROR-1. In certain embodiments, the cells of the invention are used in the treatment of patients at risk for developing ROR-1. Thus, the methods for the treatment or prevention of ROR-1 comprising administering to a subject in need thereof, a therapeutically effective amount of the CAR-modified T cells of the invention. In embodiments, the cells activated and expanded as described herein can be utilized in the treatment of ROR-1.

[00376] Briefly, pharmaceutical compositions described herein can comprise a target cell population as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions can comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. In embodiments, compositions of the present invention are formulated for intravenous administration.

[00377] Pharmaceutical compositions described herein can be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages can be determined by clinical trials.

[00378] When "an immunologically effective amount", or "therapeutic amount" is indicated, the precise amount of the compositions described herein to be administered can be determined by a physician with consideration of individual differences in age, weight, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the T cells described herein can be administered at a dosage of 10^4 to 10^9 cells/kg body weight, 10^5 to 10^6 cells/kg body weight, including all integer values within those ranges. T cell compositions can also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, *e.g.*, Rosenberg et al., New Eng. J. of Med. 319:1676, (1988)). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[00379] In certain embodiments, it can be desired to administer activated T cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate T cells therefrom, and reinfuse the patient with these activated and expanded T cells. This process can be carried out multiple times every few weeks. In certain embodiments, T cells can be activated from blood draws of from 10 cc to 400 cc. In certain embodiments, T cells are activated from blood draws of 20 cc, 30 cc, 40 cc, 50 cc, 60 cc, 70 cc, 80 cc, 90 cc, or 100 cc. Not to be bound by theory, using this multiple blood draw/multiple reinfusion protocol can serve to select out certain populations of T cells. In another embodiment, it can be desired to administer activated T cells of the subject composition following lymphodepletion of the patient, either via radiation or chemotherapy.

[00380] The administration of compositions described herein can be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein can be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one embodiment, the T cell compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In another embodiment, the ROR-1 CAR-T cell compositions of the present invention are administered by i.v. injection. The compositions of T cells can be injected directly into a lymph node, or site of primary tumor or metastasis.

[00381] The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for human administration can be performed according to art-accepted practices. For example, the dose of the above treatment can be in the range of 1×10^4 CAR+ cells/kg to 5×10^6 CAR+ cells/kg. Exemplary doses can be 1×10^2 CAR+ cells/kg, 1×10^3 CAR+ cells/kg, 1×10^4

CAR+ cells/kg, 1×10^5 CAR+ cells/kg, 3×10^5 CAR+ cells/kg, 1×10^6 CAR+ cells/kg,5×10^6 CAR+ cells/kg, 1×10^7 CAR+ cells/kg, 1×10^8 CAR+ cells/kg or 1×10^9 CAR+ cells/kg. The appropriate dose can be adjusted accordingly for an adult or a pediatric patient.

[00382] Alternatively, a typical amount of immune effector cells administered to a mammal (*e.g.*, a human) can be, for example, in the range of one hundred, one thousand, ten thousand, one million to 100 billion cells; however, amounts below or above this exemplary range are within the scope of the invention. For example, the dose of inventive host cells can be about 1 million to about 50 billion cells (*e.g.*, about 5 million cells, about 25 million cells, about 500 million cells, about 1 billion cells, about 5 billion cells, about 20 billion cells, about 30 billion cells, about 40 billion cells, or a range defined by any two of the foregoing values), about 10 million to about 100 billion cells (*e.g.*, about 20 million cells, about 30 million cells, about 40 million cells, about 60 million cells, about 70 million cells, about 80 million cells, about 90 million cells, about 10 billion cells, about 25 billion cells, about 50 billion cells, about 75 billion cells, about 90 billion cells, or a range defined by any two of the foregoing values), about 100 million cells to about 50 billion cells (*e.g.*, about 120 million cells, about 250 million cells, about 350 million cells, about 450 million cells, about 650 million cells, about 800 million cells, about 900 million cells, about 3 billion cells, about 30 billion cells, about 45 billion cells, or a range defined by any two of the foregoing values).

[00383] Therapeutic or prophylactic efficacy can be monitored by periodic assessment of treated patients. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression of disease symptoms occurs. However, other dosage regimens can be useful and are within the scope of the invention. The desired dosage can be delivered by a single bolus administration of the composition, by multiple bolus administrations of the composition, or by continuous infusion administration of the composition.

[00384] The composition comprising the immune effector cells expressing the disclosed nucleic acid sequences, or a vector comprising the those nucleic acid sequences, can be administered with one or more additional therapeutic agents, which can be co-administered to the mammal. By "co-administering" is meant administering one or more additional therapeutic agents and the composition comprising the inventive host cells or the inventive vector sufficiently close in time to enhance the effect of one or more additional therapeutic agents, or vice versa. In this regard, the composition comprising the immune effector cells described herein or a vector described herein can be administered simultaneously with one or more additional therapeutic agents, or first, and the one or more additional therapeutic agents can be administered second, or vice versa. Alternatively, the composition comprising the disclosed immune effector cells or the

vectors described herein and the one or more additional therapeutic agents can be administered simultaneously.

[00385] An example of a therapeutic agents that can be included in or co-administered with the composition (or included in kits) comprising the inventive host cells and/or the inventive vectors are interleukins, cytokines, interferons, adjuvants and chemotherapeutic agents. In embodiments, the additional therapeutic agents are IFN-alpha, IFN-beta, IFN-gamma, GM-CSF, G-CSF, M-CSF, LT-beta, TNF-alpha, growth factors, and hGH, a ligand of human Toll-like receptor TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, and TLR10.

[00386] "Antifoaming agents" reduce foaming during processing which can result in coagulation of aqueous dispersions, bubbles in the finished film, or generally impair processing. Exemplary anti-foaming agents include silicon emulsions or sorbitan sesquoleate.

[00387] "Antioxidants" include, for example, butylated hydroxytoluene (BHT), sodium ascorbate, ascorbic acid, sodium metabisulfite and tocopherol. In certain embodiments, antioxidants enhance chemical stability where required.

[00388] Formulations described herein can benefit from antioxidants, metal chelating agents, thiol containing compounds and other general stabilizing agents. Examples of such stabilizing agents, include, but are not limited to: (a) about 0.5% to about 2% w/v glycerol, (b) about 0.1% to about 1% w/v methionine, (c) about 0.1% to about 2% w/v monothioglycerol, (d) about 1 mM to about 10 mM EDTA, (e) about 0.01% to about 2% w/v ascorbic acid, (f) 0.003% to about 0.02% w/v polysorbate 80, (g) 0.001% to about 0.05% w/v. polysorbate 20, (h) arginine, (i) heparin, (j) dextran sulfate, (k) cyclodextrins, (l) pentosan polysulfate and other heparinoids, (m) divalent cations such as magnesium and zinc; or (n) combinations thereof.

[00389] "Binders" impart cohesive qualities and include, *e.g.*, alginic acid and salts thereof; cellulose derivatives such as carboxymethylcellulose, methylcellulose (*e.g.*, Methocel®), hydroxypropylmethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose (*e.g.*, Klucel®), ethylcellulose (*e.g.*, Ethocel®), and microcrystalline cellulose (*e.g.*, Avicel®); microcrystalline dextrose; amylose; magnesium aluminum silicate; polysaccharide acids; bentonites; gelatin; polyvinylpyrrolidone/vinyl acetate copolymer; crospovidone; povidone; starch; pregelatinized starch; tragacanth, dextrin, a sugar, such as sucrose (*e.g.*, Dipac®), glucose, dextrose, molasses, mannitol, sorbitol, xylitol (*e.g.*, Xylitab®), and lactose; a natural or synthetic gum such as acacia, tragacanth, ghatti gum, mucilage of isapol husks, polyvinylpyrrolidone (*e.g.*, Polyvidone® CL, Kollidon® CL, Polyplasdone® XL-10), larch arabogalactan, Veegum®, polyethylene glycol, waxes, sodium alginate, and the like.

[00390] A "carrier" or "carrier materials" include any commonly used excipients in pharmaceutics and should be selected on the basis of compatibility with compounds disclosed

herein, such as, compounds of ibrutinib and an anticancer agent, and the release profile properties of the desired dosage form. Exemplary carrier materials include, *e.g.*, binders, suspending agents, disintegration agents, filling agents, surfactants, solubilizers, stabilizers, lubricants, wetting agents, diluents, and the like. "Pharmaceutically compatible carrier materials" can include, but are not limited to, acacia, gelatin, colloidal silicon dioxide, calcium glycerophosphate, calcium lactate, maltodextrin, glycerine, magnesium silicate, polyvinylpyrrollidone (PVP), cholesterol, cholesterol esters, sodium caseinate, soy lecithin, taurocholic acid, phosphotidylcholine, sodium chloride, tricalcium phosphate, dipotassium phosphate, cellulose and cellulose conjugates, sugars sodium stearoyl lactylate, carrageenan, monoglyceride, diglyceride, pregelatinized starch, and the like. See, *e.g.*, Remington: The Science and Practice of Pharmacy, Nineteenth Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania 1975; Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y., 1980; and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed. (Lippincott Williams & Wilkins1999).

[00391] "Dispersing agents," and/or "viscosity modulating agents" include materials that control the diffusion and homogeneity of a drug through liquid media or a granulation method or blend method. In some embodiments, these agents also facilitate the effectiveness of a coating or eroding matrix. Exemplary diffusion facilitators/dispersing agents include, *e.g.*, hydrophilic polymers, electrolytes, Tween ® 60 or 80, PEG, polyvinylpyrrolidone (PVP; commercially known as Plasdone®), and the carbohydrate-based dispersing agents such as, for example, hydroxypropyl celluloses (*e.g.*, HPC, HPC-SL, and HPC-L), hydroxypropyl methylcelluloses (*e.g.*, HPMC K100, HPMC K4M, HPMC K15M, and HPMC K100M), carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose acetate stearate (HPMCAS), noncrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol (PVA), vinyl pyrrolidone/vinyl acetate copolymer (S630), 4-(1,1,3,3-tetramethylbutyl)-phenol polymer with ethylene oxide and formaldehyde (also known as tyloxapol), poloxamers (*e.g.*, Pluronics F68®, F88®, and F108®, which are block copolymers of ethylene oxide and propylene oxide); and poloxamines (*e.g.*, Tetronic 908®, also known as Poloxamine 908®, which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine (BASF Corporation, Parsippany, N.J.)), polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, polyvinylpyrrolidone/vinyl acetate copolymer (S-630), polyethylene glycol, *e.g.*, the polyethylene glycol can have a molecular weight of about 300 to about 6000, or

about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, polysorbate-80, sodium alginate, gums, such as, *e.g.*, gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, *e.g.*, sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone, carbomers, polyvinyl alcohol (PVA), alginates, chitosans and combinations thereof. Plasticizers such as cellulose or triethyl cellulose can also be used as dispersing agents. Dispersing agents particularly useful in liposomal dispersions and self-emulsifying dispersions are dimyristoyl phosphatidyl choline, natural phosphatidyl choline from eggs, natural phosphatidyl glycerol from eggs, cholesterol and isopropyl myristate.

[00392] Combinations of one or more erosion facilitator with one or more diffusion facilitator can also be used in the present compositions.

[00393] The term “diluent” refers to chemical compounds that are used to dilute the compound of interest prior to delivery. Diluents can also be used to stabilize compounds because they can provide a more stable environment. Salts dissolved in buffered solutions (which also can provide pH control or maintenance) are utilized as diluents in the art, including, but not limited to a phosphate buffered saline solution. In certain embodiments, diluents increase bulk of the composition to facilitate compression or create sufficient bulk for homogenous blend for capsule filling. Such compounds include *e.g.*, lactose, starch, mannitol, sorbitol, dextrose, microcrystalline cellulose such as Avicel®; dibasic calcium phosphate, dicalcium phosphate dihydrate; tricalcium phosphate, calcium phosphate; anhydrous lactose, spray-dried lactose; pregelatinized starch, compressible sugar, such as Di-Pac® (Amstar); mannitol, hydroxypropylmethylcellulose, hydroxypropylmethylcellulose acetate stearate, sucrose-based diluents, confectioner’s sugar; monobasic calcium sulfate monohydrate, calcium sulfate dihydrate; calcium lactate trihydrate, dextrates; hydrolyzed cereal solids, amylose; powdered cellulose, calcium carbonate; glycine, kaolin; mannitol, sodium chloride; inositol, bentonite, and the like.

[00394] “Filling agents” include compounds such as lactose, calcium carbonate, calcium phosphate, dibasic calcium phosphate, calcium sulfate, microcrystalline cellulose, cellulose powder, dextrose, dextrates, dextran, starches, pregelatinized starch, sucrose, xylitol, lactitol, mannitol, sorbitol, sodium chloride, polyethylene glycol, and the like.

[00395] “Lubricants” and “glidants” are compounds that prevent, reduce or inhibit adhesion or friction of materials. Exemplary lubricants include, *e.g.*, stearic acid, calcium hydroxide, talc, sodium stearyl fumerate, a hydrocarbon such as mineral oil, or hydrogenated vegetable oil such as hydrogenated soybean oil (Sterotex®), higher fatty acids and their alkali-metal and alkaline

earth metal salts, such as aluminum, calcium, magnesium, zinc, stearic acid, sodium stearates, glycerol, talc, waxes, Stearowet®, boric acid, sodium benzoate, sodium acetate, sodium chloride, leucine, a polyethylene glycol (*e.g.*, PEG-4000) or a methoxypolyethylene glycol such as Carbowax™, sodium oleate, sodium benzoate, glyceryl behenate, polyethylene glycol, magnesium or sodium lauryl sulfate, colloidal silica such as Syloid™, Cab-O-Sil®, a starch such as corn starch, silicone oil, a surfactant, and the like.

[00396] "Plasticizers" are compounds used to soften the microencapsulation material or film coatings to make them less brittle. Suitable plasticizers include, *e.g.*, polyethylene glycols such as PEG 300, PEG 400, PEG 600, PEG 1450, PEG 3350, and PEG 800, stearic acid, propylene glycol, oleic acid, triethyl cellulose and triacetin. In some embodiments, plasticizers can also function as dispersing agents or wetting agents.

[00397] "Solubilizers" include compounds such as triacetin, triethylcitrate, ethyl oleate, ethyl caprylate, sodium lauryl sulfate, sodium doccusate, vitamin E TPGS, dimethylacetamide, N-methylpyrrolidone, N-hydroxyethylpyrrolidone, polyvinylpyrrolidone, hydroxypropylmethyl cellulose, hydroxypropyl cyclodextrins, ethanol, n-butanol, isopropyl alcohol, cholesterol, bile salts, polyethylene glycol 200-600, glycofurol, transcutol, propylene glycol, and dimethyl isosorbide and the like.

[00398] "Stabilizers" include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[00399] "Suspending agents" include compounds such as polyvinylpyrrolidone, *e.g.*, polyvinylpyrrolidone K12, polyvinylpyrrolidone K17, polyvinylpyrrolidone K25, or polyvinylpyrrolidone K30, vinyl pyrrolidone/vinyl acetate copolymer (S630), polyethylene glycol, *e.g.*, the polyethylene glycol can have a molecular weight of about 300 to about 6000, or about 3350 to about 4000, or about 7000 to about 5400, sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, hydroxymethylcellulose acetate stearate, polysorbate-80, hydroxyethylcellulose, sodium alginate, gums, such as, *e.g.*, gum tragacanth and gum acacia, guar gum, xanthans, including xanthan gum, sugars, cellulosics, such as, *e.g.*, sodium carboxymethylcellulose, methylcellulose, sodium carboxymethylcellulose, hydroxypropylmethylcellulose, hydroxyethylcellulose, polysorbate-80, sodium alginate, polyethoxylated sorbitan monolaurate, polyethoxylated sorbitan monolaurate, povidone and the like.

[00400] "Surfactants" include compounds such as sodium lauryl sulfate, sodium docusate, Tween 60 or 80, triacetin, vitamin E TPGS, sorbitan monooleate, polyoxyethylene sorbitan monooleate, polysorbates, polaxomers, bile salts, glyceryl monostearate, copolymers of ethylene oxide and propylene oxide, *e.g.*, Pluronic® (BASF), and the like. Some other surfactants

include polyoxyethylene fatty acid glycerides and vegetable oils, *e.g.*, polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, *e.g.*, octoxynol 10, octoxynol 40. In some embodiments, surfactants can be included to enhance physical stability or for other purposes.

[00401] "Viscosity enhancing agents" include, *e.g.*, methyl cellulose, xanthan gum, carboxymethyl cellulose, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, hydroxypropylmethyl cellulose acetate stearate, hydroxypropylmethyl cellulose phthalate, carbomer, polyvinyl alcohol, alginates, acacia, chitosans and combinations thereof.

[00402] "Wetting agents" include compounds such as oleic acid, glyceryl monostearate, sorbitan monooleate, sorbitan monolaurate, triethanolamine oleate, polyoxyethylene sorbitan monooleate, polyoxyethylene sorbitan monolaurate, sodium docusate, sodium oleate, sodium lauryl sulfate, sodium doccusate, triacetin, Tween 80, vitamin E TPGS, ammonium salts and the like.

KITS AND COMPOSITIONS

[00403] One aspect of the disclosure relates to kits and compositions including a first vector including coding regions that encode the ROR-1-specific CARs of the invention and optionally genes included for safety reasons, *e.g.*, HER1t or HER1t-1 and functional variants thereof, or CD20 or CD20t-1, and functional variants thereof. The kits and compositions can further include cytokines. In another aspect, the kits and compositions can include RHEOSWITCH® gene switch components. These kits and compositions can include multiple vectors each encoding different proteins or subsets of proteins. These vectors can be viral, non-viral, episomal, or integrating. In some embodiments, the vectors are transposons, *e.g.*, Sleeping Beauty transposons.

[00404] In some embodiments, the kits and compositions include not only vectors but also cells and agents such as interleukins, cytokines, interleukins and chemotherapeutics, adjuvants, wetting agents, or emulsifying agents. In one embodiment the cells are T cells. In one embodiment the kits and composition includes IL-2. In one embodiment, the kits and compositions include IL-21. In one embodiment, the kits and compositions include Bcl-2, STAT3 or STAT5 inhibitors. In embodiments, the kit includes IL-15, or mbIL-15.

[00405] Disclosed herein, in certain embodiments, are kits and articles of manufacture for use with one or more methods described herein. Such kits include a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method

described herein. Suitable containers include, for example, bottles, vials, syringes, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass or plastic.

[00406] The articles of manufacture provided herein contain packaging materials. Examples of pharmaceutical packaging materials include, but are not limited to, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for a selected formulation and intended mode of administration and treatment.

[00407] For example, the container(s) include CAR-T cells (*e.g.*, ROR-1-specific CAR-T cells described herein), and optionally in addition with cytokines and/or chemotherapeutic agents disclosed herein. Such kits optionally include an identifying description or label or instructions relating to its use in the methods described herein.

[00408] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included.

[00409] In some embodiments, a label is on or associated with the container. In one embodiment, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, *e.g.*, as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

EXAMPLES

[00410] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

Example 1 Nucleofection of T cells with Sleeping Beauty System

[00411] To generate the genetically modified T cells, cryopreserved pan T cells were thawed, washed and resuspended with pre-warmed Phenol Red free RPMI 1640 media supplemented with FBS and Glutamax (R20 media) and placed in in a humidified incubator at 37°C with 5% CO2. Cells were counted and centrifuged and resuspended in nucleofection buffer. To generate CAR- T cells, a total of 15 μg of the transposon plasmid(s) comprising CAR constructs were combined with 5 μg of plasmid encoding SB transposase for each nucleofection cuvette reaction. Electroporation of the T cells was achieved using the Amaxa 2b Nucleofection device or 4D Nucleofector (Lonza, Walkersville, MD). Following electroporation, the contents from each cuvette were transferred to pre-warmed R20 media and placed in incubator at 37°C. A

sample of each T cell culture was taken for flow cytometric analysis to characterize CAR expression where applicable at a specified time point. CAR^+ T cells were numerically expanded ex vivo for further characterization. For ex vivo numerical expansion of the generated CAR+ T cells, the T cells were further co-cultured with activating and propagating cells (AaPCs). Briefly, irradiated AaPCs derived from K562 cell line engineered to express CD86, 41BBL, mbIL15 along with ROR1 antigen were co-cultured with CAR^+T cells in complete media with IL-21and IL-2 for subsequent weekly AaPC additions.

[00412] Flow cytometric analysis for CAR expression was performed at Day 1 after electroporation and prior to each AaPC stimulation using fluorescently labeled recombinant ROR1-Fc fusion protein, biotin labelled soluble extracellular domain of ROR1 or Protein-L labelled with AF647. Cells were gated on live CD3+ cells.

Table 4. Murine ROR1 scFv CAR constructs with various signal peptides

Construct No.	CAR ORF
1	mIgVH (SP)-Murine ROR1 scFv-CD8a-CD28z
2	IgKappa (SP)-Murine ROR1 scFv-CD8a-CD28z
3	CD28(SP)- Murine ROR1 scFv-CD8a-CD28z
4	CD8a(SP)- Murine ROR1 scFv-CD8a-CD28z

[00413] The above mentioned CARs were constructed. FIG. 5 demonstrates that short CD8a stalk does not permit surface expression of ROR1 CAR regardless of signal peptide utilized. Other signal peptides that were tested included GM-CSFRα, β2M, azurocidin, Human Serum Albumin (HSA), A2M receptor associated protein, IgHV3-23, IgHV3-33, and IgKV1-D33.

Example 2: Optimization of ROR1 CAR

[00414] In all approximately 120 CAR vectors were designed and built based on various permutations of signal peptides, orientation of VL and VH chain of anti-ROR1 antibody, spacers of varying lengths, transmembrane and signaling domains. For example, 15 signal peptides, 2 orientations, and 7 different spacers of varying lengths, 2 different transmembrane and signaling domains were tested in various combinations. The expression of ROR1 CAR was found to be dependent on signal peptide, orientation and stalk type length. From these, 3 CAR constructs were identified with strong surface expression.

[00415] CAR-T cells expressing ROR1 CARs of different spacer lengths, signal peptides, and VH-VL orientation were generated by electroporation of SB system plasmids in healthy donor

PBMCs as described in Example 1. CAR-T cells were numerically expanded ex vivo by co-culture with ROR1 expressing AaPC by once weekly stimulation as described in Example 1. Expression of CAR was measured by multi parameter flow cytometry at Day 1 and Day 8 post nucleofection. CAR-T test articles evaluated are listed in **Table 5**.

[00416] **FIG. 6** shows flow cytometry data on ROR1 CAR expression of different ROR1 CAR (murine scFv)-T cells with. Cells were gated on live CD3+ cells. As shown in **FIG. 6**, varying degrees of murine ROR1 CAR expression was observed one day post gene transfer depending on the spacer utilized for construction of CAR molecule. Enrichment of CAR+T cells was observed upon co-culture of CAR+T cells with ROR1+ AaPC line *in vitro*. For murine ROR1 CAR-T, better surface expression of CAR on T cells was observed using longer spacers.

Table 5. ROR1 CAR constructs as utilized in FIGs. 6-7.

Construct No.	CAR ORF
5	mIgVH (SP)-Murine ROR1 scFv-CD8a-2X-CD28z
6	mIgVH (SP)-Murine ROR1 scFv-CD8a-3X-CD28z
7	mIgVH (SP)-Murine ROR1 scFv-CD8a-4X-CD28z
8	mIgVH (SP)-Murine ROR1 scFv-LNGFR-CD28z
9	hIgK(SP)- Murine ROR1 scFv-CD8a-3X-CD28z
10	hCD8a(SP)-Murine ROR1 scFv-CD8a-3X-CD28z
11	hCD28(SP) -Murine ROR1 scFv-CD8a-3X-CD28z
12	hβ2M (SP)-Murine ROR1 scFv-CD8a-3X-CD28z
13	HAS(SP) -Murine ROR1 scFv-CD8a-3X-CD28z
14	hAzurocidin-Murine ROR1 scFv-CD8a-3X-CD28z

[00417] FIG. 7 demonstrates varying degrees of ROR1 CAR expression using CD8a-3X spacer and different combinations of signal peptides. As shown in FIG. 7, signal peptides such as mIgVH, CD8a, β2M and Azurocidin allowed for better surface expression of CAR on T cells. The different signal peptides had no effect on surface expression of ROR1 CAR when CD8a-1X spacer was used.

Table 6. C ROR1 CAR constructs as utilized in FIG. 8.

Construct No.	CAR ORF	Orientation
15	mgIVH(SP)-murine ROR1scFv –FcM-CD28z	VL-VH
16	hGMCSFR- murine ROR1scFv –FcM-CD28z	VL-VH
17	GM-CSFR(SP)-Murine ROR1 scFv-CD8a-3X-CD28z	VH-whitlow linker-VL

18	IgHV3-23- Murine ROR1 scFv-CD8a-3X-CD28z	VL-VH
19	IgHV3-23 (SP)- Murine ROR1 scFv-CD8a-3X-CD28z	VH-whitlow linker-VL

[00418] FIG. 8 demonstrates that reversing orientation from VL-VH to VH-VL enables surface expression of murine ROR1CAR from GMCSFR and IGHV3-23 signal peptides. Based on the above described data, CD8a-3X stalk was selected as a spacer, IgHV3-23 was selected as a signal peptide, with VL-VH as a preferred orientation, and CD8TM –CD28z signaling domain in the murine ROR1 CAR.

Example 3 Murine ROR1 CAR with CD8a-3x stalk

[00419] The functional capability of murine ROR1 CAR-T cells was tested in an *in vitro* model. Mouse EL4 cell line was transduced to express human ROR1 on cell surface (EL4-ROR1), and secretion of IFN-γ and was measured upon co-culture of murine ROR1 scFv-CD8-3x-CD28z (CAR)+ T cells with $ROR1^+$ target cells. FIG. 9 shows murine ROR1 CAR with CD8-3x stalk demonstrates antigen specific IFN-γ expression upon coculture with ROR+ target cells.

[00420] Furthermore, the capability of the ROR1 CAR-T to recognize target cells with or without ROR1 expression was assessed in CD107a degranulation assay. CD107a , also known as lysosomal-associate membrane protein-1 (LAMP-1), is constitutively expressed in the late endosomes-lysosomes of cells but transiently expressed on the cell surface on degranulating cells. As shown in FIGs. 9A-9B, significant expression of CD107a degranulation was observed upon coculture with EL4-ROR1 cell line while effector cells only and coculture with EL4 cell line had minimal degranulation observed.

Example 4 Humanization of ROR1 scFv

[00421] To reduce immunogenicity of murine ROR1 scFv and to prevent immune-mediated CAR T cell deletion *in vivo*, efforts were undertaken to humanize the murine ROR1 scFv.

[00422] A total of 168 humanized ROR1 scFvs were designed based on the sequence of murine ROR1 scFv and tested. 77 of the humanized antibody clones were successfully expressed. Out of the 77 humanized clones, 42 retained the ability to bind ROR1 antigen. 21 clones were further selected for affinity maturation. 10 humanized antibodies showed binding affinities ≤ 2.0nM in IgG format. Additional scFv variants based on combination of different humanized VH and VL chains were also designed and tested.

[00423] Binding affinity of various humanized ROR1 scFv clones were assessed by surface plasmon resonance (SPR) assay using Biacore 3000. Variable regions of humanized ROR1 antibodies were fused to mouse constant chain regions to generate chimeric mouse IgG1 mAbs. Extracellular domain (ECD) of ROR1 fused to human Fc region was immobilized on sensor chip CM5. Different concentrations of humanized antibodies were injected in solution phase and data was analyzed using BIAevaluation to calculate Kd of the antibodies.

Table 7. Binding affinity of humanized clones of murine ROR1 scFv

Humanized clones	**Affinity (Kd)**
Clone 04	~55 nM
Clone 05	~35 nM
Clone 07	~90 nM
Clone 14	1.45 nM
Clone 16	3.03 nM
Clone 18	12.7 nM
Hum ROR1_14VH_(G4S)3x_VL_16	131nM
Hum ROR1_05VH_(G4S)3x_VL_14	3.55E-10 M
Hum ROR1_05VH_(G4S)3x_VL_16	3.37E-10 M
Hum ROR1_07VH_(G4S)3x_VL_05	7.16E-09 M
Hum ROR1_07VH_(G4S)3x_VL_16	4.08E-09 M
Hum ROR1_07VH_(G4S)3x_VL_18	1.17E-08 M
Hum ROR1_18VH_(G4S)3x_VL_04	3.72E-10 M
Hum ROR1_18VH_(G4S)3x_VL_07	3.87E-09 M
Hum ROR1_18VH_(G4S)3x_VL_14	2.09E-10 M
Hum ROR1_18VH_(G4S)3x_VL_16	1.60E-09 M
Hum_design14_variant3	1.3E-09 M
Hum_design14_variant4	4.08E-10 M
Hum_design14_variant5	4.52E-09 M

[00424] Based on top candidates from the humanization campaign, the following CAR constructs were designed and tested.

Table 8. Humanized ROR1 CAR constructs as utilized in FIGs. 10A-10B, FIG.11, FIG. 12 and Table 9.

Construct No.	**CAR ORF**

20	MurineROR1_v2 (VL-VH). CD8a(3x).CD28z
21	IGVH3-33sp. hROR1(VH-VL)_14.CD8a(3x).CD28z
22	mIgVH3 sp. hROR1 (VH-VL)_14. CD8a(3x).CD28z
23	hROR1(VL-VH)_05.CD8a(3x).CD28z
24	hROR1(VH-VL)_14-3.CD8a(3x).CD28z
25	hROR1(VH-VL)_14-4.CD8a(3x).CD28z
26	hROR1(VH_5-VL_14).CD8a(3x).CD28z
27	hROR1(VH_5-VL_16).CD8a(3x).CD28z
28	hROR1(VH_18-VL_04).CD8a(3x).CD28z
29	hROR1(VH_18-VL_14).CD8a(3x).CD28z
30	Mock transfected T cells

[00425] JeKo-1 tumor cells expressing fFLUC ($0.5x10^6$ cells) were administered IP into NSG mice on Day 0. On Day 8, mice with established tumor burden (as confirmed by IVIS imaging) were randomized to receive a single IV injection with either: Saline (HBSS), or T cells transfected with CAR constructs as described above. CAR-T cells were numerically expanded by 2x weekly stimulations ex vivo by coculture with ROR1+ AaPC. Effectiveness against tumor growth was evaluated by *in vivo* bioluminescence (IVIS) imaging performed every 7 days and 36 days post CAR-T cell dosing to assess tumor burden (FIGs. 10A, 10B and 11). Whole blood samples in EDTA were collected once per week and subjected to multi-parameter flow cytometry for evaluation of humanized ROR1 CAR T cell persistence (FIG. 12), expansion and determination of the different T cell subsets.

[00426] A lead humanized ROR1 CAR (hROR1(VH_5-VL_14).CD8a(3x).CD28z) was selected based on similarity to the murine ROR1 CAR in scFv affinity, ROR1-Fc binding, CAR expression, CAR T-cell expression, *in vitro* ROR-1 specific cytokine production, *in vitro* ROR-1 specific cytotoxicity and *in vivo* ROR-1 anti-tumor activity.

[00427] A summary of the data is provided in **Table 9** below.

Table 9. Summary of Constructs tested.

Construct #	ROR1-His	ROR1-Fc	*In vivo*			*In vitro*			
	Affinity (KD,M)	Affinity (KD,M)	Tumor growth	CD8 in circulation	ROR1-CAR in	ROR1-FC	Cytotoxicity	Cytokines	Expansion

			inhibition		circulation	binding			
20	7.07E-08	2.31E-08	2	1	2	3	3	3	3
21	1.45E-09	ND	2	1	1	2	3	3	3
22	1.45E-09	ND	2	2	2	2	3	3	3
23	3.74E-08	3.50E-08	1	1	1	2	3	3	3
24	1.30E-09	9.59E-10	2	2	1	2	3	3	3
25	4.08E-10	2.84E-10	1	3	2	3	2	3	3
26	3.55E-10	1.93E-10	3	3	3	3	3	3	3
27	3.37E-10	2.73E-10	0	2	0	0	0	3	3
28	3.72E-10	2.71E-10	1	1	1	2	3	3	3
29	2.09E-10	2.01E-10	3	1	1	3	3	3	3

ND – not determined

Relative performance score from 0 to 3 with 0 being worst and 3 being the best performance

[00428] Example 5 *In vitro* and *in vivo* experiments

[00429] Various DNA plasmids expressing a SB transposon system, i.e. SB11, membrane bound IL-15 (mbIL-15), cell tag and lead chimeric antigen receptor (CAR), were transfected into peripheral blood mononuclear cells (PBMC) via nucleofection to redirect T cell specificity. Constitutive expression of mbIL-15 or ligand inducible expression of mbIL-15 in combination with constitutive expression of lead ROR1 CAR was examined as described in **Table 10**.

Table 10 Combination of Transposons as utilized in FIGs. 13A-13B.

Combination No.	**Transposon #1**	**Transposon #2**
1	Constitutive CAR (FIG. 1C)	Constitutive mbIL-15.HER1t (FIG. 1E)
2	Constitutive CAR.HER1t (FIG. 1D)	Constitutive mbIL-15.HER1t (FIG. 1E)
3	Constitutive CAR.HER1t. Inducible mbIL-15.HER1t	Gene Switch components (FIG. 2E)

Table 11. Summary of combinations tested.

Combination	**1**	**2**	**3**
CAR	**hROR1**	**hROR1**	**hROR1**
mbIL-15	**const**	**const.**	**RTS®**
Expansion	**++**	**+**	**+**
CAR+ %	+++	+	++
mbIL-15 (%)	+	+	++
CD107	++	+/-	+
IFN-γ	++	+/-	+
TNF-α	+	-	+/-

[00430] Relative performance score from – to +++, '-' being undetectable performance and +++ being the best performance.

[00431] RTS-mbIL-15 in combination with hROR1 CAR was further tested *in vitro* using SKOV3_fLUC (**FIG. 13A**) and JeKo1_fLUC (**FIG. 13B**) tumor cell lines. As before, the 3 combinations of SB transposons encoding for hROR1 CAR and mbIL-15 were electroporated in healthy donor T cells. CAR+T cells were numerically expanded ex vivo by co-culture with ROR1 expressing AaPC by once weekly stimulation as previously described for 2X stimulation. For ligand inducible mbIL-15 hROR1 CAR, the T cells were incubated in complete media containing IL-21 and IL-2 at following 2X stimulation of the cells. Two days after, the hROR1 CAR T cells were incubated in a cytokine free complete media and treated with veledimex or DMSO overnight. The next day, cells were counted and the frequency of ROR1 expressing T cells was estimated. T cells were then normalized for CAR expression.

[00432] For T cells constitutively expressing hROR1 CAR and mbIL-15, following ex vivo expansion using two weekly stimulations by coculture with AaPC, cells were cultured for an additional 3 days and counted. As before, T cells were normalized for CAR expression.

[00433] hROR1 CAR T cells were then mixed with each tumor cell line at different ratios: 4:1, 2:1, 0.5:1 and 0.25:1. 72 hours later, cells were removed from the incubator and pelleted. Supernatant was collected to measure cytokine production, and cell pellet was treated with OneGlo reagent for Luciferase assay. **FIGs. 13A-13B** demonstrates that ROR1+ tumor cell lines were equally killed by hROR1 CAR T cells with constitutive or RTS-mbIL-15 *in vitro*.

[00434] An *in vivo* study was conducted in mice treated with hROR-1 CAR T cells with RTS-mbIL-15. As before, JeKo-1 tumor cells ($10x10^6$ cells) were administered IP into NSG mice on Day 0. On Day 8, mice with established tumor burden (as confirmed by IVIS imaging) were randomized to receive a single IP injection with either: Saline (HBSS), or hROR-1 CAR T cells with RTS-mbIL-15 (+/- veledimex). Effectiveness against tumor growth was evaluated by *in vivo* bioluminescence (IVIS) imaging performed at specific time points post CAR-T cell dosing to assess tumor burden (**FIG. 14**). Whole blood samples in EDTA were collected once per week and subjected to multi-parameter flow cytometry for evaluation of humanized ROR1 CAR T cell persistence, expansion and determination of the different T cell subsets.

[00435] FIG. 14 demonstrates that huROR1 RTS CART promotes an anti-tumor effect in a JEKO-1 xenograft mouse model in a dose-dependent manner.

[00436] Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of this invention.

[00437] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the present disclosure. It should be understood that various alternatives to the embodiments described herein, or combinations of one or more of these embodiments or aspects described therein can be employed in practicing the present disclosure. It is intended that the following claims define the scope of the present disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

SEQUENCES

[00438] Provided in **Table 12** is a representative list of certain sequences included in embodiments provided herein.

Table 12. Exemplary Sequences

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
CAR Sequences				

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Human ROR1	1	PLLALLAALLLAARG AAAQETELSVSAELV PTSSWNISSELNKDSY LTLDEPMNNITTSLGQ TAELHCKVSGNPPPTI RWFKNDAPVVQEPR RLSFRSTIYGSRLRIRN LDTTDTGYFQCVATN GKEVVSSTGVLFVKF GPPPTASPGYSDEYEE DGFCQPYRGIACARFI GNRTVYMESLHMQG EIENQITAAFTMIGTSS HLSDKCSQFAIPSLCH YAFPYCDETSSVPKPR DLCRDECEILENVLC QTEYIFARSNPMILMR LKLPNCEDLPQPESPE AANCIRIGIPMADPIN KNHKCYNSTGVDYR GTVSVTKSGRQCQPW NSQYPHTHTFTALRFP ELNGGHSYCRNPGNQ KEAPWCFTLDENFKS DLCDIPACDSKDSKE KNKMEILYILVPSVAI PLAIALLFFFICVCRN NQKSSSAPVQRQPKH VRGQNVEMSMLNAY KPKSKAKELPLSAVR FMEELGECAFGKIYK GHLYLPGMDHAQLV AIKTLKDYNNPQQWT EFQQEASLMAELHHP NIVCLLGAVTQEQPV CMLFEYINQGDLHEF LIMRSPHSDVGCSSDE DGTVKSSLDHGDFLH IAIQIAAGMEYLSSHF FVHKDLAARNILIGEQ LHVKISDLGLSREIYS ADYYRVQSKSLLPIR WMPPEAIMYGKFSSD SDIWSFGVVLWEIFSF GLQPYYGFSNQEVIE MVRKRQLLPCSEDCP PRMYSLMTECWNEIP SRRPRFKDIHVRLRS WEGLSSHTSSTTPSGG NATTQTTSLSASPVSN LSNPRYPNYMFPSQGI TPQGQIAGFIGPPIPQN	148	ATGCACCGGCCGCGCCGCCGCGGGACG CGCCCGCCGCTCCTGGCGCTGCTGGCC GCGCTGCTGCTGGCCGCACGCGGGGCT GCTGCCCAAGAAACAGAGCTGTCAGTC AGTGCTGAATTAGTGCCTACCTCATCA TGGAACATCTCAAGTGAACTCAACAAA GATTCTTACCTGACCCTCGATGAACCA ATGAATAACATCACCACGTCTCTGGGC CAGACAGCAGAACTGCACTGCAAAGTC TCTGGGAATCCACCTCCCACCATCCGC TGGTTCAAAAATGATGCTCCTGTGGTC CAGGAGCCCCGGAGGCTCTCCTTTCGG TCCACCATCTATGGCTCTCGGCTGCGG ATTAGAAACCTCGACACCACAGACACA GGCTACTTCCAGTGCGTGGCAACAAAC GGCAAGGAGGTGGTTTCTTCCACTGGA GTCTTGTTTGTCAAGTTTGGCCCCCCTC CCACTGCAAGTCCAGGATACTCAGATG AGTATGAAGAAGATGGATTCTGTCAGC CATACAGAGGGATTGCATGTGCAAGAT TTATTGGCAACCGCACCGTCTATATGG AGTCTTTGCACATGCAAGGGGAAATAG AAAATCAGATCACAGCTGCCTTCACTA TGATTGGCACTTCCAGTCACTTATCTGA TAAGTGTTCTCAGTTCGCCATTCCTTCC CTGTGCCACTATGCCTTCCCGTACTGCG ATGAAACTTCATCCGTCCCAAAGCCCC GTGACTTGTGTCGCGATGAATGTGAAA TCCTGGAGAATGTCCTGTGTCAAACAG AGTACATTTTTGCAAGATCAAATCCCA TGATTCTGATGAGGCTGAAACTGCCAA ACTGTGAAGATCTCCCCCAGCCAGAGA GCCCAGAAGCTGCGAACTGTATCCGGA TTGGAATTCCCATGGCAGATCCTATAA ATAAAAATCACAAGTGTTATAACAGCA CAGGTGTGGACTACCGGGGGACCGTCA GTGTGACCAAATCAGGGCGCCAGTGCC AGCCATGGAATTCCCAGTATCCCCACA CACACACTTTCACCGCCCTTCGTTTCCC AGAGCTGAATGGAGGCCATTCCTACTG CCGCAACCCAGGGAATCAAAAGGAAG CTCCCTGGTGCTTCACCTTGGATGAAA ACTTTAAGTCTGATCTGTGTGACATCCC AGCGTGCGATTCAAAGGATTCCAAGGA GAAGAATAAAATGGAAATCCTGTACAT ACTAGTGCCAAGTGTGGCCATTCCCCT GGCCATTGCTTTACTCTTCTTCTTCATT TGCGTCTGTCGGAATAACCAGAAGTCA TCGTCGGCACCAGTCCAGAGGCAACCA AAACACGTCAGAGGTCAAAATGTAGA GATGTCAATGCTGAATGCATATAAACC CAAGAGCAAGGCTAAAGAGCTACCTCT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		QRFIPINGYPIPPGYAA FPAAHYQPTGPPRVIQ HCPPPKSRSPSSASGS TSTGHVTSLPSSGSNQ EANIPLLPHMSIPNHP GGMGITVFGNKSQKP YKIDSKQASLLGDANI HGHTESMISAEL		TTCTGCTGTACGCTTTATGGAAGAATT GGGTGAGTGTGCCTTTGGAAAAATCTA TAAAGGCCATCTCTATCTCCCAGGCAT GGACCATGCTCAGCTGGTTGCTATCAA GACCTTGAAAGACTATAACAACCCCCA GCAATGGACGGAATTTCAACAAGAAG CCTCCCTAATGGCAGAACTGCACCACC CCAATATTGTCTGCCTTCTAGGTGCCGT CACTCAGGAACAACCTGTGTGCATGCT TTTTGAGTATATTAATCAGGGGGATCT CCATGAGTTCCTCATCATGAGATCCCC ACACTCTGATGTTGGCTGCAGCAGTGA TGAAGATGGGACTGTGAAATCCAGCCT GGACCACGGAGATTTTCTGCACATTGC AATTCAGATTGCAGCTGGCATGGAATA CCTGTCTAGTCACTTCTTTGTCCACAAG GACCTTGCAGCTCGCAATATTTTAATC GGAGAGCAACTTCATGTAAAGATTTCA GACTTGGGGCTTTCCAGAGAAATTTAC TCCGCTGATTACTACAGGGTCCAGAGT AAGTCCTTGCTGCCCATTCGCTGGATG CCCCCTGAAGCCATCATGTATGGCAAA TTCTCTTCTGATTCAGATATCTGGTCCT TTGGGGTTGTCTTGTGGGAGATTTTCA GTTTTGGACTCCAGCCATATTATGGATT CAGTAACCAGGAAGTGATTGAGATGGT GAGAAAACGGCAGCTCTTACCATGCTC TGAAGACTGCCCACCCAGAATGTACAG CCTCATGACAGAGTGCTGGAATGAGAT TCCTTCTAGGAGACCAAGATTTAAAGA TATTCACGTCCGGCTTCGGTCCTGGGA GGGACTCTCAAGTCACACAAGCTCTAC TACTCCTTCAGGGGGAAATGCCACCAC ACAGACAACCTCCCTCAGTGCCAGCCC AGTGAGTAATCTCAGTAACCCCAGATA TCCTAATTACATGTTCCCGAGCCAGGG TATTACACCACAGGGCCAGATTGCTGG TTTCATTGGCCCGCCAATACCTCAGAA CCAGCGATTCATTCCCATCAATGGATA CCCAATACCTCCTGGATATGCAGCGTT TCCAGCTGCCCACTACCAGCCAACAGG TCCTCCCAGAGTGATTCAGCACTGCCC ACCTCCCAAGAGTCGGTCCCCAAGCAG TGCCAGTGGGTCGACTAGCACTGGCCA TGTGACTAGCTTGCCCTCATCAGGATC CAATCAGGAAGCAAATATTCCTTTACT ACCACACATGTCAATTCCAAATCATCC TGGTGGAATGGGTATCACCGTTTTTGG CAACAAATCTCAAAAACCCTACAAAAT TGACTCAAAGCAAGCATCTTTACTAGG AGACGCCAATATTCATGGACACACCGA ATCTATGATTTCTGCAGAACTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Human ROR1 (1-437)	2	MHRPRRRGTRPPLLALLAALLLAARGAAAQETELSVSAELVPTSSWNISSELNKDSYLTLDEPMNNITTSLGQTAELHCKVSGNPPPTIRWFKNDAPVVQEPRRLSFRSTIYGSRLRIRNLDTTDTGYFQCVATNGKEVVSSTGVLFVKFGPPPTASPGYSDEYEEDGFCQPYRGIACARFIGNRTVYMESLHMQGEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCHYAFPYCDETSSVPKPRDLCRDECEILENVLCQTEYIFARSNPMILMRLKLPNCEDLPQPESPEAANCIRIGIPMADPINKNHKCYNSTGVDYRGTVSVTKSGRQCQPWNSQYPHTHTFTALRFPELNGGHSYCRNPGNQKEAPWCFTLDENFKSDLCDIPACDSKDSKEKNKMEILYILVPSVAIPLAIALLFFFICVCRNNQKSSSA	149	ATGCACCGGCCGCGCCGCCGCGGGACGCGCCCGCCGCTCCTGGCGCTGCTGGCCGCGCTGCTGCTGGCCGCACGCGGGGCTGCTGCCCAAGAAACAGAGCTGTCAGTCAGTGCTGAATTAGTGCCTACCTCATCATGGAACATCTCAAGTGAACTCAACAAAGATTCTTACCTGACCCTCGATGAACCAATGAATAACATCACCACGTCTCTGGGCCAGACAGCAGAACTGCACTGCAAAGTCTCTGGGAATCCACCTCCCACCATCCGCTGGTTCAAAAATGATGCTCCTGTGGTCCAGGAGCCCCGGAGGCTCTCCTTTCGGTCCACCATCTATGGCTCTCGGCTGCGGATTAGAAACCTCGACACCACAGACACAGGCTACTTCCAGTGCGTGGCAACAAACGGCAAGGAGGTGGTTTCTTCCACTGGAGTCTTGTTTGTCAAGTTTGGCCCCCCTCCCACTGCAAGTCCAGGATACTCAGATGAGTATGAAGAAGATGGATTCTGTCAGCCATACAGAGGGATTGCATGTGCAAGATTTATTGGCAACCGCACCGTCTATATGGAGTCTTTGCACATGCAAGGGGAAATAGAAAATCAGATCACAGCTGCCTTCACTATGATTGGCACTTCCAGTCACTTATCTGATAAGTGTTCTCAGTTCGCCATTCCTTCCCTGTGCCACTATGCCTTCCCGTACTGCGATGAAACTTCATCCGTCCCAAAGCCCCGTGACTTGTGTCGCGATGAATGTGAAATCCTGGAGAATGTCCTGTGTCAAACAGAGTACATTTTTGCAAGATCAAATCCCATGATTCTGATGAGGCTGAAACTGCCAAACTGTGAAGATCTCCCCCAGCCAGAGAGCCCAGAAGCTGCGAACTGTATCCGGATTGGAATTCCCATGGCAGATCCTATAAATAAAAATCACAAGTGTTATAACAGCACAGGTGTGGACTACCGGGGGACCGTCAGTGTGACCAAATCAGGGCGCCAGTGCCAGCCATGGAATTCCCAGTATCCCCACACACACACTTTCACCGCCCTTCGTTTCCCAGAGCTGAATGGAGGCCATTCCTACTGCCGCAACCCAGGGAATCAAAAGGAAGCTCCCTGGTGCTTCACCTTGGATGAAAACTTTAAGTCTGATCTGTGTGACATCCCAGCGTGCGATTCAAAGGATTCCAAGGAGAAGAATAAAATGGAAATCCTGTACATACTAGTGCCAAGTGTGGCCATTCCCCTGGCCATTGCTTTACTCTTCTTCTTCATTTGCGTCTGTCGGAATAACCAGAAGTCATCGTCGGCA
Murine ROR-1	3	DIKMTQSPSSMYASLGERVTITCKASPDINSYLSWFQQKPGKSPKT	150	GACATCAAGATGACCCAGAGCCCCAGCTCTATGTACGCCAGCCTGGGCGAGCGCGTGACCATCACATGCAAGGCCAGCCCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
(VL-VH). IgG4 Fc-CD28m-Z		LIYRANRLVDGVPSR FSGGGSGQDYSLTINS LEYEDMGIYYCLQYD EFPYTFGGGTKLEMK GSTSGSGKPGSGEGST KGEVKLVESGGGLVK PGGSLKLSCAASGFTF SSYAMSWVRQIPEKR LEWVASISRGGTTYY PDSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS ESKYGPPCPPCPAPEF EGGPSVFLFPPKPKDT LMISRTPEVTCVVVD VSQEDPEVQFNWYV DGVEVHNAKTKPREE QFQSTYRVVSVLTVL HQDWLNGKEYKCKV SNKGLPSSIEKTISKA KGQPREPQVYTLPPS QEEMTKNQVSLTCLV KGFYPSDIAVEWESN GQPENNYKTTPPVLD SDGSFFLYSRLTVDKS RWQEGNVFSCSVMH EALHNHYTQKSLSLS LGKMFWVLVVVGGV LACYSLLVTVAFIIFW VRSKRSRGGHSDYM NMTPRRPGPTRKHYQ PYAPPRDFAAYRSRV KFSRSADAPAYQQGQ NQLYNELNLGRREEY DVLDKRRGRDPEMG GKPRRKNPQEGLYNE LQKDKMAEAYSEIG MKGERRRGKGHDGL YQGLSTATKDTYDAL HMQALPPR		GACATCAACAGCTACCTGTCCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACCGGCT GGTGGACGGCGTGCCAAGCAGATTTTC CGGCGGAGGCAGCGGCCAGGACTACA GCCTGACCATCAACAGCCTGGAATACG AGGACATGGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGAAATGAAG GGCAGCACCTCCGGCAGCGGCAAGCCT GGCAGCGGCGAGGGCAGCACCAAGGG CGAAGTGAAGCTGGTGGAAAGCGGCG GAGGCCTGGTGAAACCTGGCGGCAGCC TGAAGCTGAGCTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCT GGGTCCGACAGATCCCCGAGAAGCGG CTGGAATGGGTGGCCAGCATCAGCAGG GGCGGCACCACCTACTACCCCGACAGC GTGAAGGGCCGGTTCACCATCAGCCGG GACAACGTGCGGAACATCCTGTACCTG CAGATGAGCAGCCTGCGGAGCGAGGA CACCGCCATGTACTACTGCGGCAGATA CGACTACGACGGCTACTACGCCATGGA TTACTGGGGCCAGGGCACCAGCGTGAC CGTGTCTAGCGAGAGCAAGTACGGCCC TCCCTGCCCCCCTTGCCCTGCCCCCGAG TTCGAGGGCGGACCCAGCGTGTTCCTG TTCCCCCCCAAGCCCAAGGACACCCTG ATGATCAGCCGGACCCCCGAGGTGACC TGTGTGGTGGTGGACGTGTCCCAGGAG GACCCCGAGGTCCAGTTCAACTGGTAC GTGGACGGCGTGGAGGTGCACAACGC CAAGACCAAGCCCCGGGAGGAGCAGT TCCAGAGCACCTACCGGGTGGTGTCCG TGCTGACCGTGCTGCACCAGGACTGGC TGAACGGCAAGGAATACAAGTGTAAG GTGTCCAACAAGGGCCTGCCCAGCAGC ATCGAGAAAACCATCAGCAAGGCCAA GGGCCAGCCTCGGGAGCCCCAGGTGTA CACCCTGCCCCCTAGCCAAGAGGAGAT GACCAAGAATCAGGTGTCCCTGACCTG CCTGGTGAAGGGCTTCTACCCCAGCGA CATCGCCGTGGAGTGGGAGAGCAACG GCCAGCCCGAGAACAACTACAAGACC ACCCCCCCTGTGCTGGACAGCGACGGC AGCTTCTTCCTGTACAGCAGGCTGACC GTGGACAAGAGCCGGTGGCAGGAGGG CAACGTCTTTAGCTGCTCCGTGATGCA CGAGGCCCTGCACAACCACTACACCCA GAAGAGCCTGTCCCTGAGCCTGGGCAA GATGTTCTGGGTGCTGGTCGTGGTGGG TGGCGTGCTGGCCTGCTACAGCCTGCT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GGTGACAGTGGCCTTCATCATCTTTTG GGTGAGGAGCAAGCGGAGCAGAGGCG GCCACAGCGACTACATGAACATGACCC CCCGGAGGCCTGGCCCCACCCGGAAGC ACTACCAGCCCTACGCCCCTCCCAGGG ACTTCGCCGCCTACCGGAGCCGGGTGA AGTTCAGCCGGAGCGCCGACGCCCCTG CCTACCAGCAGGGCCAGAACCAGCTGT ACAACGAGCTGAACCTGGGCCGGAGG GAGGAGTACGACGTGCTGGACAAGCG GAGAGGCCGGGACCCTGAGATGGGCG GCAAGCCCCGGAGAAAGAACCCTCAG GAGGGCCTGTATAACGAACTGCAGAA AGACAAGATGGCCGAGGCCTACAGCG AGATCGGCATGAAGGGCGAGCGGCGG AGGGGCAAGGGCCACGACGGCCTGTA CCAGGGCCTGAGCACCGCCACCAAGG ATACCTACGACGCCCTGCACATGCAGG CCCTGCCCCCCAGA
Murine ROR1 (VL-VH). IgG4 Fcm-CD28m-Z	4	DIKMTQSPSSMYASL GERVTITCKASPDINS YLSWFQQKPGKSPKT LIYRANRLVDGVPSR FSGGGSGQDYSLTINS LEYEDMGIYYCLQYD EFPYTFGGGTKLEMK GSTSGSGKPGSGEGST KGEVKLVESGGGLVK PGGSLKLSCAASGFTF SSYAMSWVRQIPEKR LEWVASISRGGTTYY PDSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS QGTSVTVSSESKYGPP CPPCPAPEFLGGPSVF LFPPKPKDTLMISRTP EVTCVVVDVSQEDPE VQFNWYVDGVEVHN AKTKPREEQFNSTYR VVSVLTVLHQDWLN GKEYKCKVSNKGLPS SIEKTISKAKGQPREP QVYTLPPSQEEMTKN QVSLTCLVKGFYPSDI AVEWESNGQPENNY KTTPPVLDSDGSFFLY SRLTVDKSRWQEGN VFSCSVMHEALHNHY TQKSLSLSLGKMFWV LVVVGGVLACYSLLV	151	GACATCAAGATGACCCAGAGCCCCAGC TCTATGTACGCCAGCCTGGGCGAGCGC GTGACCATCACATGCAAGGCCAGCCCC GACATCAACAGCTACCTGTCCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACCGGCT GGTGGACGGCGTGCCAAGCAGATTTTC CGGCGGAGGCAGCGGCCAGGACTACA GCCTGACCATCAACAGCCTGGAATACG AGGACATGGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGAAATGAAG GGCAGCACCAGCGGCAGCGGCAAGCC TGGAAGCGGCGAGGGCTCCACCAAGG GCGAAGTGAAGCTGGTGGAAAGCGGC GGAGGCCTGGTGAAACCTGGCGGCAG CCTGAAGCTGAGCTGCGCCGCCAGCGG CTTCACCTTCAGCAGCTACGCCATGAG CTGGGTCCGACAGATCCCCGAGAAGCG GCTGGAATGGGTGGCCAGCATCAGCAG GGGCGGCACCACCTACTACCCCGACAG CGTGAAGGGCCGGTTCACCATCAGCCG GGACAACGTGCGGAACATCCTGTACCT GCAGATGAGCAGCCTGCGGAGCGAGG ACACCGCCATGTACTACTGCGGCAGAT ACGACTACGACGGCTACTACGCCATGG ATTACTGGGGCCAGGGCACCAGCGTGA CCGTGTCTAGCCAGGGAACCTCCGTGA CAGTGTCCAGCGAGTCCAAATATGGTC CCCCATGCCCACCATGCCCAGCACCTG AGTTCCTGGGGGGACCATCAGTCTTCC TGTTCCCCCCAAAACCCAAGGACACTC TCATGATCTCCCGGACCCCTGAGGTCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		TVAFIIFWVRSKRSRG GHSDYMNMTPRRPG PTRKHYQPYAPPRDF AAYRSRVKFSRSADA PAYQQGQNQLYNEL NLGRREEYDVLDKRR GRDPEMGGKPRRKNP QEGLYNELQKDKMA EAYSEIGMKGERRRG KGHDGLYQGLSTATK DTYDALHMQALPPR		CGTGCGTGGTGGTGGACGTGAGCCAGG AAGACCCCGAGGTCCAGTTCAACTGGT ACGTGGATGGCGTGGAGGTGCATAATG CCAAGACAAAGCCCCGGGAGGAGCAG TTCAATAGCACCTACCGGGTGGTGTCC GTGCTGACCGTGCTGCACCAGGACTGG CTGAACGGCAAGGAATACAAGTGTAA GGTGTCCAACAAGGGCCTGCCCAGCAG CATCGAGAAAACCATCAGCAAGGCCA AGGGCCAGCCTCGGGAGCCCCAGGTGT ACACCCTGCCCCCTAGCCAAGAGGAGA TGACCAAGAATCAGGTGTCCCTGACCT GCCTGGTGAAGGGCTTCTACCCCAGCG ACATCGCCGTGGAGTGGGAGAGCAAC GGCCAGCCCGAGAACAACTACAAGAC CACCCCCCCTGTGCTGGACAGCGACGG CAGCTTCTTCCTGTACAGCAGGCTGAC CGTGGACAAGAGCCGGTGGCAGGAGG GCAACGTCTTTAGCTGCTCCGTGATGC ACGAGGCCCTGCACAACCACTACACCC AGAAGAGCCTGTCCCTGAGCCTGGGCA AGATGTTCTGGGTGCTGGTCGTGGTGG GTGGCGTGCTGGCCTGCTACAGCCTGC TGGTGACAGTGGCCTTCATCATCTTTTG GGTGAGGAGCAAGCGGAGCAGAGGCG GCCACAGCGACTACATGAACATGACCC CCCGGAGGCCTGGCCCCACCCGGAAGC ACTACCAGCCCTACGCCCCTCCCAGGG ACTTCGCCGCCTACCGGAGCCGGGTGA AGTTCAGCCGGAGCGCCGACGCCCCTG CCTACCAGCAGGGCCAGAACCAGCTGT ACAACGAGCTGAACCTGGGCCGGAGG GAGGAGTACGACGTGCTGGACAAGCG GAGAGGCCGGGACCCTGAGATGGGCG GCAAGCCCCGGAGAAAGAACCCTCAG GAGGGCCTGTATAACGAACTGCAGAA AGACAAGATGGCCGAGGCCTACAGCG AGATCGGCATGAAGGGCGAGCGGCGG AGGGGCAAGGGCCACGACGGCCTGTA CCAGGGCCTGAGCACCGCCACCAAGG ATACCTACGACGCCCTGCACATGCAGG CCCTGCCCCCCAGA
Murine ROR1 (VL-VH). CD8α. CD28z	5	DIKMTQSPSSMYASL GERVTITCKASPDINS YLSWFQQKPGKSPKT LIYRANRLVDGVPSR FSGGGSGQDYSLTINS LEYEDMGIYYCLQYD EFPYTFGGGTKLEMK GSTSGSGKPGSGEGST KGEVKLVESGGGLVK PGGSLKLSCAASGFTF	152	GACATCAAGATGACCCAGAGCCCCAGC TCTATGTACGCCAGCCTGGGCGAGCGC GTGACCATCACATGCAAGGCCAGCCCC GACATCAACAGCTACCTGTCCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACCGGCT GGTGGACGGCGTGCCAAGCAGATTTTC CGGCGGAGGCAGCGGCCAGGACTACA GCCTGACCATCAACAGCCTGGAATACG AGGACATGGGCATCTACTACTGCCTGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		SSYAMSWVRQIPEKR LEWVASISRGGTTYY PDSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS KPTTTPAPRPPTPAPTI ASQPLSLRPEACRPAA GGAVHTRGLDFACDI YIWAPLAGTCGVLLL SLVITLYCNHRNRSK RSRGGHSDYMNMTP RRPGPTRKHYQPYAP PRDFAAYRSRVKFSR SADAPAYQQGQNQL YNELNLGRREEYDVL DKRRGRDPEMGGKP RRKNPQEGLYNELQK DKMAEAYSEIGMKG ERRRGKGHDGLYQG LSTATKDTYDALHM QALPPR		AGTACGACGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGAAATGAAG GGCAGCACCTCCGGCAGCGGCAAGCCT GGCAGCGGCGAGGGCAGCACCAAGGG CGAAGTGAAGCTGGTGGAAAGCGGCG GAGGCCTGGTGAAACCTGGCGGCAGCC TGAAGCTGAGCTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCT GGGTCCGACAGATCCCCGAGAAGCGG CTGGAATGGGTGGCCAGCATCAGCAGG GGCGGCACCACCTACTACCCCGACAGC GTGAAGGGCCGGTTCACCATCAGCCGG GACAACGTGCGGAACATCCTGTACCTG CAGATGAGCAGCCTGCGGAGCGAGGA CACCGCCATGTACTACTGCGGCAGATA CGACTACGACGGCTACTACGCCATGGA TTACTGGGGCCAGGGCACCAGCGTGAC CGTGTCTAGCAAGCCCACCACCACCCC TGCCCCTAGACCTCCAACCCCAGCCCC TACAATCGCCAGCCAGCCCCTGAGCCT GAGGCCCGAAGCCTGTAGACCTGCCGC TGGCGGAGCCGTGCACACCAGAGGCCT GGATTTCGCCTGCGACATCTACATCTG GGCCCCTCTGGCCGGCACCTGTGGCGT GCTGCTGCTGAGCCTGGTCATCACCCT GTACTGCAACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCCACAGCGAC TACATGAACATGACCCCCCGGAGGCCT GGCCCCACCCGGAAGCACTACCAGCCC TACGCCCCTCCCAGGGACTTCGCCGCC TACCGGAGCCGGGTGAAGTTCAGCCGG AGCGCCGACGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAGGAGTACGA CGTGCTGGACAAGCGGAGAGGCCGGG ACCCTGAGATGGGCGGCAAGCCCCGG AGAAAGAACCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGC ACCGCCACCAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGCCCCCCAGA
Murine ROR1 (VL-VH). CD8α(2x).CD28z	6	DIKMTQSPSSMYASL GERVTITCKASPDINS YLSWFQQKPGKSPKT LIYRANRLVDGVPSR FSGGGSGQDYSLTINS LEYEDMGIYYCLQYD EFPYTFGGGTKLEMK GSTSGSGKPGSGEGST KGEVKLVESGGGLVK	153	GACATCAAGATGACCCAGAGCCCCAGC TCTATGTACGCCAGCCTGGGCGAGCGC GTGACCATCACATGCAAGGCCAGCCCC GACATCAACAGCTACCTGTCCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACCGGCT GGTGGACGGCGTGCCAAGCAGATTTTC CGGCGGAGGCAGCGGCCAGGACTACA GCCTGACCATCAACAGCCTGGAATACG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		PGGSLKLSCAASGFTF SSYAMSWVRQIPEKR LEWVASISRGGTTYY PDSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAG GAVHTRGLDFACDIY IWAPLAGTCGVLLLS LVITLYCNHRNRSKR SRGGHSDYMNMTPR RPGPTRKHYQPYAPP RDFAAYRSRVKFSRS ADAPAYQQGQNQLY NELNLGRREEYDVLD KRRGRDPEMGGKPR RKNPQEGLYNELQKD KMAEAYSEIGMKGER RRGKGHDGLYQGLST ATKDTYDALHMQAL PPR		AGGACATGGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGAAATGAAG GGCAGCACCTCCGGCAGCGGCAAGCCT GGCAGCGGCGAGGGCAGCACCAAGGG CGAAGTGAAGCTGGTGGAAAGCGGCG GAGGCCTGGTGAAACCTGGCGGCAGCC TGAAGCTGAGCTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCT GGGTCCGACAGATCCCCGAGAAGCGG CTGGAATGGGTGGCCAGCATCAGCAGG GGCGGCACCACCTACTACCCCGACAGC GTGAAGGGCCGGTTCACCATCAGCCGG GACAACGTGCGGAACATCCTGTACCTG CAGATGAGCAGCCTGCGGAGCGAGGA CACCGCCATGTACTACTGCGGCAGATA CGACTACGACGGCTACTACGCCATGGA TTACTGGGGCCAGGGCACCAGCGTGAC CGTGTCTAGCAAACCTACTACAACTCC TGCCCCCCGGCCTCCTACACCAGCTCC TACTATCGCCTCCCAGCCACTCAGTCTC AGACCCGAGGCTTCTAGGCCAGCGGCC GGAGGCGCGGTCCACACCCGCGGGCTG GACTTTGCATCCGATAAGCCCACCACC ACCCCTGCCCCTAGACCTCCAACCCCA GCCCCTACAATCGCCAGCCAGCCCCTG AGCCTGAGGCCCGAAGCCTGTAGACCT GCCGCTGGCGGAGCCGTGCACACCAGA GGCCTGGATTTCGCCTGCGACATCTAC ATCTGGGCCCCTCTGGCCGGCACCTGT GGCGTGCTGCTGCTGAGCCTGGTCATC ACCCTGTACTGCAACCACCGGAATAGG AGCAAGCGGAGCAGAGGCGGCCACAG CGACTACATGAACATGACCCCCCGGAG GCCTGGCCCCACCCGGAAGCACTACCA GCCCTACGCCCCTCCCAGGGACTTCGC CGCCTACCGGAGCCGGGTGAAGTTCAG CCGGAGCGCCGACGCCCCTGCCTACCA GCAGGGCCAGAACCAGCTGTACAACG AGCTGAACCTGGGCCGGAGGGAGGAG TACGACGTGCTGGACAAGCGGAGAGG CCGGGACCCTGAGATGGGCGGCAAGC CCCGGAGAAAGAACCCTCAGGAGGGC CTGTATAACGAACTGCAGAAAGACAA GATGGCCGAGGCCTACAGCGAGATCG GCATGAAGGGCGAGCGGCGGAGGGGC AAGGGCCACGACGGCCTGTACCAGGG CCTGAGCACCGCCACCAAGGATACCTA CGACGCCCTGCACATGCAGGCCCTGCC CCCCAGA
Murine ROR1	7	DIKMTQSPSSMYASL GERVTITCKASPDINS	154	GACATCAAGATGACCCAGAGCCCCAGC TCTATGTACGCCAGCCTGGGCGAGCGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
(VL-VH). CD8a(3x) .CD28z		YLSWFQQKPGKSPKT LIYRANRLVDGVPSR FSGGGSGQDYSLTINS LEYEDMGIYYCLQYD EFPYTFGGGTKLEMK GSTSGSGKPGSGEGST KGEVKLVESGGGLVK PGGSLKLSCAASGFTF SSYAMSWVRQIPEKR LEWVASISRGGTTYY PDSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEACRPAAG GAVHTRGLDFACDIY IWAPLAGTCGVLLLS LVITLYCNHRNRSKR SRGGHSDYMNMTPR RPGPTRKHYQPYAPP RDFAAYRSRVKFSRS ADAPAYQQGQNQLY NELNLGRREEYDVLD KRRGRDPEMGGKPR RKNPQEGLYNELQKD KMAEAYSEIGMKGER RRGKGHDGLYQGLST ATKDTYDALHMQAL PPR		GTGACCATCACATGCAAGGCCAGCCCC GACATCAACAGCTACCTGTCCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACCGGCT GGTGGACGGCGTGCCAAGCAGATTTTC CGGCGGAGGCAGCGGCCAGGACTACA GCCTGACCATCAACAGCCTGGAATACG AGGACATGGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGAAATGAAG GGCAGCACCTCCGGCAGCGGCAAGCCT GGCAGCGGCGAGGGCAGCACCAAGGG CGAAGTGAAGCTGGTGGAAAGCGGCG GAGGCCTGGTGAAACCTGGCGGCAGCC TGAAGCTGAGCTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCT GGGTCCGACAGATCCCCGAGAAGCGG CTGGAATGGGTGGCCAGCATCAGCAGG GGCGGCACCACCTACTACCCCGACAGC GTGAAGGGCCGGTTCACCATCAGCCGG GACAACGTGCGGAACATCCTGTACCTG CAGATGAGCAGCCTGCGGAGCGAGGA CACCGCCATGTACTACTGCGGCAGATA CGACTACGACGGCTACTACGCCATGGA TTACTGGGGCCAGGGCACCAGCGTGAC CGTGTCTAGCAAGCCTACCACCACCCC CGCACCTCGTCCTCCAACCCCTGCACC TACGATTGCCAGTCAGCCTCTTTCACTG CGGCCTGAGGCCAGCAGACCAGCTGCC GGCGGTGCCGTCCATACAAGAGGACTG GACTTCGCGTCCGATAAACCTACTACC ACTCCAGCCCCAAGGCCCCCAACCCCA GCACCGACTATCGCATCACAGCCTTTG TCACTGCGTCCTGAAGCCAGCCGGCCA GCTGCAGGGGGGGCCGTCCACACAAG GGGACTCGACTTTGCGAGTGATAAGCC CACCACCACCCCTGCCCCTAGACCTCC AACCCCAGCCCCTACAATCGCCAGCCA GCCCCTGAGCCTGAGGCCCGAAGCCTG TAGACCTGCCGCTGGCGGAGCCGTGCA CACCAGAGGCCTGGATTTCGCCTGCGA CATCTACATCTGGGCCCCTCTGGCCGG CACCTGTGGCGTGCTGCTGCTGAGCCT GGTCATCACCCTGTACTGCAACCACCG GAATAGGAGCAAGCGGAGCAGAGGCG GCCACAGCGACTACATGAACATGACCC CCCGGAGGCCTGGCCCCACCCGGAAGC ACTACCAGCCCTACGCCCCTCCCAGGG ACTTCGCCGCCTACCGGAGCCGGGTGA AGTTCAGCCGGAGCGCCGACGCCCCTG CCTACCAGCAGGGCCAGAACCAGCTGT ACAACGAGCTGAACCTGGGCCGGAGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GAGGAGTACGACGTGCTGGACAAGCG GAGAGGCCGGGACCCTGAGATGGGCG GCAAGCCCCGGAGAAAGAACCCTCAG GAGGGCCTGTATAACGAACTGCAGAA AGACAAGATGGCCGAGGCCTACAGCG AGATCGGCATGAAGGGCGAGCGGCGG AGGGGCAAGGGCCACGACGGCCTGTA CCAGGGCCTGAGCACCGCCACCAAGG ATACCTACGACGCCCTGCACATGCAGG CCCTGCCCCCCAGA
Murine ROR1 (VL-VH). CD8a(4x) .CD28z	8	DIKMTQSPSSMYASL GERVTITCKASPDINS YLSWFQQKPGKSPKT LIYRANRLVDGVPSR FSGGGSGQDYSLTINS LEYEDMGIYYCLQYD EFPYTFGGGTKLEMK GSTSGSGKPGSGEGST KGEVKLVESGGGLVK PGGSLKLSCAASGFTF SSYAMSWVRQIPEKR LEWVASISRGGTTYY PDSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEACRPAAG GAVHTRGLDFACDIY IWAPLAGTCGVLLLS LVITLYCNHRNRSKR SRGGHSDYMNMTPR RPGPTRKHYQPYAPP RDFAAYRSRVKFSRS ADAPAYQQGQNQLY NELNLGRREEYDVLD KRRGRDPEMGGKPR RKNPQEGLYNELQKD KMAEAYSEIGMKGER RRGKGHDGLYQGLST ATKDTYDALHMQAL PPR	155	GACATCAAGATGACCCAGAGCCCCAGC TCTATGTACGCCAGCCTGGGCGAGCGC GTGACCATCACATGCAAGGCCAGCCCC GACATCAACAGCTACCTGTCCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACCGGCT GGTGGACGGCGTGCCAAGCAGATTTTC CGGCGGAGGCAGCGGCCAGGACTACA GCCTGACCATCAACAGCCTGGAATACG AGGACATGGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGAAATGAAG GGCAGCACCTCCGGCAGCGGCAAGCCT GGCAGCGGCGAGGGCAGCACCAAGGG CGAAGTGAAGCTGGTGGAAAGCGGCG GAGGCCTGGTGAAACCTGGCGGCAGCC TGAAGCTGAGCTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCT GGGTCCGACAGATCCCCGAGAAGCGG CTGGAATGGGTGGCCAGCATCAGCAGG GGCGGCACCACCTACTACCCCGACAGC GTGAAGGGCCGGTTCACCATCAGCCGG GACAACGTGCGGAACATCCTGTACCTG CAGATGAGCAGCCTGCGGAGCGAGGA CACCGCCATGTACTACTGCGGCAGATA CGACTACGACGGCTACTACGCCATGGA TTACTGGGGCCAGGGCACCAGCGTGAC CGTGTCTAGCAAGCCTACCACCACCCC CGCACCTCGTCCTCCAACCCCTGCACC TACGATTGCCAGTCAGCCTCTTTCACTG CGGCCTGAGGCCAGCAGACCAGCTGCC GGCGGTGCCGTCCATACAAGAGGACTG GACTTCGCGTCCGATAAACCTACTACC ACTCCAGCCCCAAGGCCCCCAACCCCA GCACCGACTATCGCATCACAGCCTTTG TCACTGCGTCCTGAAGCCAGCCGGCCA GCTGCAGGGGGGGCCGTCCACACAAG GGGACTCGACTTTGCGAGTGATAAACC TACTACAACTCCTGCCCCCCGGCCTCCT ACACCAGCTCCTACTATCGCCTCCCAG CCACTCAGTCTCAGACCCGAGGCTTCT AGGCCAGCGGCCGGAGGCGCGGTCCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CACCCGCGGGCTGGACTTTGCATCCGA TAAGCCCACCACCACCCCTGCCCCTAG ACCTCCAACCCCAGCCCCTACAATCGC CAGCCAGCCCCTGAGCCTGAGGCCCGA AGCCTGTAGACCTGCCGCTGGCGGAGC CGTGCACACCAGAGGCCTGGATTTCGC CTGCGACATCTACATCTGGGCCCCTCT GGCCGGCACCTGTGGCGTGCTGCTGCT GAGCCTGGTCATCACCCTGTACTGCAA CCACCGGAATAGGAGCAAGCGGAGCA GAGGCGGCCACAGCGACTACATGAAC ATGACCCCCCGGAGGCCTGGCCCCACC CGGAAGCACTACCAGCCCTACGCCCCT CCCAGGGACTTCGCCGCCTACCGGAGC CGGGTGAAGTTCAGCCGGAGCGCCGAC GCCCCTGCCTACCAGCAGGGCCAGAAC CAGCTGTACAACGAGCTGAACCTGGGC CGGAGGGAGGAGTACGACGTGCTGGA CAAGCGGAGAGGCCGGGACCCTGAGA TGGGCGGCAAGCCCCGGAGAAAGAAC CCTCAGGAGGGCCTGTATAACGAACTG CAGAAAGACAAGATGGCCGAGGCCTA CAGCGAGATCGGCATGAAGGGCGAGC GGCGGAGGGGCAAGGGCCACGACGGC CTGTACCAGGGCCTGAGCACCGCCACC AAGGATACCTACGACGCCCTGCACATG CAGGCCCTGCCCCCCAGA
Murine ROR1 (VL-VH). LNGFR ECD. CD8TM. CD28z	9	DIKMTQSPSSMYASL GERVTITCKASPDINS YLSWFQQKPGKSPKT LIYRANRLVDGVPSR FSGGGSGQDYSLTINS LEYEDMGIYYCLQYD EFPYTFGGGTKLEMK GSTSGSGKPGSGEGST KGEVKLVESGGGLVK PGGSLKLSCAASGFTF SSYAMSWVRQIPEKR LEWVASISRGGTTYY PDSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS KEACPTGLYTHSGEC CKACNLGEGVAQPC GANQTVCEPCLDSVT FSDVVSATEPCKPCTE CVGLQSMSAPCVEAD DAVCRCAYGYYQDE TTGRCEACRVCEAGS GLVFSCQDKQNTVCE ECPDGTYSDEANHVD	156	GACATCAAGATGACCCAGAGCCCCAGC TCTATGTACGCCAGCCTGGGCGAGCGC GTGACCATCACATGCAAGGCCAGCCCC GACATCAACAGCTACCTGTCCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACCGGCT GGTGGACGGCGTGCCAAGCAGATTTTC CGGCGGAGGCAGCGGCCAGGACTACA GCCTGACCATCAACAGCCTGGAATACG AGGACATGGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGAAATGAAG GGCAGCACCTCCGGCAGCGGCAAGCCT GGCAGCGGCGAGGGCAGCACCAAGGG CGAAGTGAAGCTGGTGGAAAGCGGCG GAGGCCTGGTGAAACCTGGCGGCAGCC TGAAGCTGAGCTGCGCCGCCAGCGGCT TCACCTTCAGCAGCTACGCCATGAGCT GGGTCCGACAGATCCCCGAGAAGCGG CTGGAATGGGTGGCCAGCATCAGCAGG GGCGGCACCACCTACTACCCCGACAGC GTGAAGGGCCGGTTCACCATCAGCCGG GACAACGTGCGGAACATCCTGTACCTG CAGATGAGCAGCCTGCGGAGCGAGGA CACCGCCATGTACTACTGCGGCAGATA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		PCLPCTVCEDTERQL RECTRWADAECEEIP GRWITRSTPPEGSDST APSTQEPEAPPEQDLI ASTVAGVVTTVMGSS QPVVTRGTTDNIYIW APLAGTCGVLLLSLVI TLYCNHRNRSKRSRG GHSDYMNMTPRRPG PTRKHYQPYAPPRDF AAYRSRVKFSRSADA PAYQQGQNQLYNEL NLGRREEYDVLDKRR GRDPEMGGKPRRKNP QEGLYNELQKDKMA EAYSEIGMKGERRRG KGHDGLYQGLSTATK DTYDALHMQALPPR		CGACTACGACGGCTACTACGCCATGGA TTACTGGGGCCAGGGCACCAGCGTGAC CGTGTCTAGCAAGGAGGCATGCCCCAC AGGCCTGTACACACACAGCGGTGAGTG CTGCAAAGCCTGCAACCTGGGCGAGGG TGTGGCCCAGCCTTGTGGAGCCAACCA GACCGTGTGTGAGCCCTGCCTGGACAG CGTGACGTTCTCCGACGTGGTGAGCGC GACCGAGCCGTGCAAGCCGTGCACCGA GTGCGTGGGGCTCCAGAGCATGTCGGC GCCGTGCGTGGAGGCCGACGACGCCGT GTGCCGCTGCGCCTACGGCTACTACCA GGATGAGACGACTGGGCGCTGCGAGG CGTGCCGCGTGTGCGAGGCGGGCTCGG GCCTCGTGTTCTCCTGCCAGGACAAGC AGAACACCGTGTGCGAGGAGTGCCCCG ACGGCACGTATTCCGACGAGGCCAACC ACGTGGACCCGTGCCTGCCCTGCACCG TGTGCGAGGACACCGAGCGCCAGCTCC GCGAGTGCACACGCTGGGCCGACGCCG AGTGCGAGGAGATCCCTGGCCGTTGGA TTACACGGTCCACACCCCCAGAGGGCT CGGACAGCACAGCCCCCAGCACCCAG GAGCCTGAGGCACCTCCAGAACAAGA CCTCATAGCCAGCACGGTGGCAGGTGT GGTGACCACAGTGATGGGCAGCTCCCA GCCCGTGGTGACCCGAGGCACCACCGA CAACATCTACATCTGGGCCCCTCTGGC CGGCACCTGTGGCGTGCTGCTGCTGAG CCTGGTCATCACCCTGTACTGCAACCA CCGGAATAGGAGCAAGCGGAGCAGAG GCGGCCACAGCGACTACATGAACATGA CCCCCCGGAGGCCTGGCCCCACCCGGA AGCACTACCAGCCCTACGCCCCTCCCA GGGACTTCGCCGCCTACCGGAGCCGGG TGAAGTTCAGCCGGAGCGCCGACGCCC CTGCCTACCAGCAGGGCCAGAACCAGC TGTACAACGAGCTGAACCTGGGCCGGA GGGAGGAGTACGACGTGCTGGACAAG CGGAGAGGCCGGGACCCTGAGATGGG CGGCAAGCCCCGGAGAAAGAACCCTC AGGAGGGCCTGTATAACGAACTGCAG AAAGACAAGATGGCCGAGGCCTACAG CGAGATCGGCATGAAGGGCGAGCGGC GGAGGGGCAAGGGCCACGACGGCCTG TACCAGGGCCTGAGCACCGCCACCAAG GATACCTACGACGCCCTGCACATGCAG GCCCTGCCCCCCAGA
Murine ROR1 (VH-VL).	10	EVKLVESGGGLVKPG GSLKLSCAASGFTFSS YAMSWVRQIPEKRLE WVASISRGGTTYYPD	157	GAAGTGAAGCTGGTGGAAAGCGGCGG AGGCCTGGTGAAACCTGGCGGCAGCCT GAAGCTGAGCTGCGCCGCCAGCGGCTT CACCTTCAGCAGCTACGCCATGAGCTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
CD8a(3x).41BBz		SVKGRFTISRDNVRNI LYLQMSSLRSEDTAM YYCGRYDYDGYYAM DYWGQGTSVTVSSGS TSGSGKPGSGEGSTK GDIKMTQSPSSMYAS LGERVTITCKASPDIN SYLSWFQQKPGKSPK TLIYRANRLVDGVPS RFSGGGSGQDYSLTIN SLEYEDMGIYYCLQY DEFPYTFGGGTKLEM KKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEACRP AAGGAVHTRGLDFA CDIYIWAPLAGTCGV LLLSLVITLYCNHRNK RGRKKLLYIFKQPFM RPVQTTQEEDGCSCR FPEEEEGGCELRVKFS RSADAPAYQQGQNQ LYNELNLGRREEYDV LDKRRGRDPEMGGK PRRKNPQEGLYNELQ KDKMAEAYSEIGMK GERRRGKGHDGLYQ GLSTATKDTYDALH MQALPPR		GGTCCGACAGATCCCCGAGAAGCGGCT GGAATGGGTGGCCAGCATCAGCAGGG GCGGCACCACCTACTACCCCGACAGCG TGAAGGGCCGGTTCACCATCAGCCGGG ACAACGTGCGGAACATCCTGTACCTGC AGATGAGCAGCCTGCGGAGCGAGGAC ACCGCCATGTACTACTGCGGCAGATAC GACTACGACGGCTACTACGCCATGGAT TACTGGGGCCAGGGCACCAGCGTGACC GTGTCTAGCGGCAGCACCTCCGGCAGC GGCAAGCCTGGCAGCGGCGAGGGCAG CACCAAGGGCGACATCAAGATGACCC AGAGCCCCAGCTCTATGTACGCCAGCC TGGGCGAGCGCGTGACCATCACATGCA AGGCCAGCCCCGACATCAACAGCTACC TGTCCTGGTTCCAGCAGAAGCCCGGCA AGAGCCCCAAGACCCTGATCTACCGGG CCAACCGGCTGGTGGACGGCGTGCCAA GCAGATTTTCCGGCGGAGGCAGCGGCC AGGACTACAGCCTGACCATCAACAGCC TGGAATACGAGGACATGGGCATCTACT ACTGCCTGCAGTACGACGAGTTCCCCT ACACCTTCGGAGGCGGCACCAAGCTGG AAATGAAGAAGCCTACCACCACCCCCG CACCTCGTCCTCCAACCCCTGCACCTA CGATTGCCAGTCAGCCTCTTTCACTGC GGCCTGAGGCCAGCAGACCAGCTGCCG GCGGTGCCGTCCATACAAGAGGACTGG ACTTCGCGTCCGATAAACCTACTACCA CTCCAGCCCCAAGGCCCCCAACCCCAG CACCGACTATCGCATCACAGCCTTTGT CACTGCGTCCTGAAGCCAGCCGGCCAG CTGCAGGGGGGGCCGTCCACACAAGG GGACTCGACTTTGCGAGTGATAAGCCC ACCACCACCCCTGCCCCTAGACCTCCA ACCCCAGCCCCTACAATCGCCAGCCAG CCCCTGAGCCTGAGGCCCGAAGCCTGT AGACCTGCCGCTGGCGGAGCCGTGCAC ACCAGAGGCCTGGATTTCGCCTGCGAC ATCTACATCTGGGCCCCTCTGGCCGGC ACCTGTGGCGTGCTGCTGCTGAGCCTG GTCATCACCCTGTACTGCAACCACCGG AATAAGAGAGGCCGGAAGAAACTGCT GTACATCTTCAAGCAGCCCTTCATGCG GCCCGTGCAGACCACCCAGGAAGAGG ACGGCTGCAGCTGCCGGTTCCCCGAGG AAGAGGAAGGCGGCTGCGAACTGCGG GTGAAGTTCAGCCGGAGCGCCGACGCC CCTGCCTACCAGCAGGGCCAGAACCAG CTGTACAACGAGCTGAACCTGGGCCGG AGGGAGGAGTACGACGTGCTGGACAA GCGGAGAGGCCGGGACCCTGAGATGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCGGCAAGCCCCGGAGAAAGAACCCT CAGGAGGGCCTGTATAACGAACTGCAG AAAGACAAGATGGCCGAGGCCTACAG CGAGATCGGCATGAAGGGCGAGCGGC GGAGGGGCAAGGGCCACGACGGCCTG TACCAGGGCCTGAGCACCGCCACCAAG GATACCTACGACGCCCTGCACATGCAG GCCCTGCCCCCCAGA
Murine ROR1 (VH-VL). IgG4 Fcm. CD8aTM. 41BBz	11	EVKLVESGGGLVKPG GSLKLSCAASGFTFSS YAMSWVRQIPEKRLE WVASISRGGTTYYPD SVKGRFTISRDNVRNI LYLQMSSLRSEDTAM YYCGRYDYDGYYAM DYWGQGTSVTVSSGS TSGSGKPGSGEGSTK GDIKMTQSPSSMYAS LGERVTITCKASPDIN SYLSWFQQKPGKSPK TLIYRANRLVDGVPS RFSGGGSGQDYSLTIN SLEYEDMGIYYCLQY DEFPYTFGGGTKLEM KESKYGPPCPPCPAPE FEGGPSVFLFPPKPKD TLMISRTPEVTCVVV DVSQEDPEVQFNWY VDGVEVHNAKTKPR EEQFQSTYRVVSVLT VLHQDWLNGKEYKC KVSNKGLPSSIEKTIS KAKGQPREPQVYTLP PSQEEMTKNQVSLTC LVKGFYPSDIAVEWE SNGQPENNYKTTPPV LDSDGSFFLYSRLTVD KSRWQEGNVFSCSV MHEALHNHYTQKSLS LSLGKMIYIWAPLAG TCGVLLLSLVITLYCN HRNKRGRKKLLYIFK QPFMRPVQTTQEEDG CSCRFPEEEEGGCELR VKFSRSADAPAYQQG QNQLYNELNLGRREE YDVLDKRRGRDPEM GGKPRRKNPQEGLYN ELQKDKMAEAYSEIG MKGERRRGKGHDGL YQGLSTATKDTYDAL HMQALPPR	158	GAAGTGAAGCTGGTGGAAAGCGGCGG AGGCCTGGTGAAACCTGGCGGCAGCCT GAAGCTGAGCTGCGCCGCCAGCGGCTT CACCTTCAGCAGCTACGCCATGAGCTG GGTCCGACAGATCCCCGAGAAGCGGCT GGAATGGGTGGCCAGCATCAGCAGGG GCGGCACCACCTACTACCCCGACAGCG TGAAGGGCCGGTTCACCATCAGCCGGG ACAACGTGCGGAACATCCTGTACCTGC AGATGAGCAGCCTGCGGAGCGAGGAC ACCGCCATGTACTACTGCGGCAGATAC GACTACGACGGCTACTACGCCATGGAT TACTGGGGCCAGGGCACCAGCGTGACC GTGTCTAGCGGCAGCACCTCCGGCAGC GGCAAGCCTGGCAGCGGCGAGGGCAG CACCAAGGGCGACATCAAGATGACCC AGAGCCCCAGCTCTATGTACGCCAGCC TGGGCGAGCGCGTGACCATCACATGCA AGGCCAGCCCCGACATCAACAGCTACC TGTCCTGGTTCCAGCAGAAGCCCGGCA AGAGCCCCAAGACCCTGATCTACCGGG CCAACCGGCTGGTGGACGGCGTGCCAA GCAGATTTTCCGGCGGAGGCAGCGGCC AGGACTACAGCCTGACCATCAACAGCC TGGAATACGAGGACATGGGCATCTACT ACTGCCTGCAGTACGACGAGTTCCCCT ACACCTTCGGAGGCGGCACCAAGCTGG AAATGAAGGAGAGCAAGTACGGCCCT CCCTGCCCCCCTTGCCCTGCCCCCGAGT TCGAGGGCGGACCCAGCGTGTTCCTGT TCCCCCCCAAGCCCAAGGACACCCTGA TGATCAGCCGGACCCCCGAGGTGACCT GTGTGGTGGTGGACGTGTCCCAGGAGG ACCCCGAGGTCCAGTTCAACTGGTACG TGGACGGCGTGGAGGTGCACAACGCC AAGACCAAGCCCCGGGAGGAGCAGTT CCAGAGCACCTACCGGGTGGTGTCCGT GCTGACCGTGCTGCACCAGGACTGGCT GAACGGCAAGGAATACAAGTGTAAGG TGTCCAACAAGGGCCTGCCCAGCAGCA TCGAGAAAACCATCAGCAAGGCCAAG GGCCAGCCTCGGGAGCCCCAGGTGTAC ACCCTGCCCCCTAGCCAAGAGGAGATG ACCAAGAATCAGGTGTCCCTGACCTGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CTGGTGAAGGGCTTCTACCCCAGCGAC ATCGCCGTGGAGTGGGAGAGCAACGG CCAGCCCGAGAACAACTACAAGACCA CCCCCCCTGTGCTGGACAGCGACGGCA GCTTCTTCCTGTACAGCAGGCTGACCG TGGACAAGAGCCGGTGGCAGGAGGGC AACGTCTTTAGCTGCTCCGTGATGCAC GAGGCCCTGCACAACCACTACACCCAG AAGAGCCTGTCCCTGAGCCTGGGCAAG ATGATCTACATCTGGGCCCCTCTGGCC GGCACCTGTGGCGTGCTGCTGCTGAGC CTGGTCATCACCCTGTACTGCAACCAC CGGAATAAGAGAGGCCGGAAGAAACT GCTGTACATCTTCAAGCAGCCCTTCAT GCGGCCCGTGCAGACCACCCAGGAAG AGGACGGCTGCAGCTGCCGGTTCCCCG AGGAAGAGGAAGGCGGCTGCGAACTG CGGGTGAAGTTCAGCCGGAGCGCCGAC GCCCCTGCCTACCAGCAGGGCCAGAAC CAGCTGTACAACGAGCTGAACCTGGGC CGGAGGGAGGAGTACGACGTGCTGGA CAAGCGGAGAGGCCGGGACCCTGAGA TGGGCGGCAAGCCCCGGAGAAAGAAC CCTCAGGAGGGCCTGTATAACGAACTG CAGAAAGACAAGATGGCCGAGGCCTA CAGCGAGATCGGCATGAAGGGCGAGC GGCGGAGGGGCAAGGGCCACGACGGC CTGTACCAGGGCCTGAGCACCGCCACC AAGGATACCTACGACGCCCTGCACATG CAGGCCCTGCCCCCCAGA
Murine ROR1_v2 VL	12	DVQITQSPSSLYASLG ERVTITCKASPDINSY LSWFQQKPGKSPKTLI YRANRLVDGVPSRFS GGGSGQDYSLTINSLE YEDMGIYYCLQYDEF PYTFGGGTKLEMK	159	GACGTGCAGATCACCCAGAGCCCCAGC AGCCTGTATGCCAGCCTGGGCGAGAGA GTGACCATTACCTGCAAGGCCAGCCCC GACATCAACAGCTACCTGAGCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACAGACT GGTGGATGGCGTGCCCAGCAGATTCAG CGGCGGAGGCTCTGGCCAGGACTACAG CCTGACCATCAACTCCCTGGAATACGA GGACATGGGCATCTACTACTGCCTGCA GTACGACGAGTTCCCCTACACCTTCGG AGGCGGCACCAAGCTGGAAATGAAG
Murine ROR1_v2 VH	13	EVKLVESGGGLVKPG GSLKLSCAASGFTFSS YAMSWVRQIPEKRLE WVASISRGGTTYYPD SVKGRFTISRDNVRNI LYLQMSSLRSEDTAM YYCGRYDYDGYYAM DYWGQGTSVTVSS	160	GAAGTGAAGCTGGTGGAATCTGGCGGC GGACTCGTGAAGCCTGGCGGCTCTCTG AAGCTGTCTTGTGCCGCCAGCGGCTTC ACCTTCAGCAGCTACGCCATGAGCTGG GTGCGGCAGATCCCCGAGAAGCGGCTG GAATGGGTGGCCAGCATCAGCAGAGG CGGAACCACCTACTACCCCGACTCTGT GAAGGGCCGGTTCACCATCAGCCGGGA CAACGTGCGGAACATCCTGTACCTGCA GATGAGCAGCCTGCGGAGCGAGGACA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCGCCATGTACTACTGTGGCAGATACG ACTACGACGGCTACTATGCCATGGATT ACTGGGGCCAGGGCACCAGCGTGACC GTGTCATCT
Murine ROR1_v2 (VL-VH). CD8a(3x).CD28z	14	DVQITQSPSSLYASLG ERVTITCKASPDINSY LSWFQQKPGKSPKTLI YRANRLVDGVPSRFS GGGSGQDYSLTINSLE YEDMGIYYCLQYDEF PYTFGGGTKLEMKGS TSGSGKPGSGEGSTK GEVKLVESGGGLVKP GGSLKLSCAASGFTFS SYAMSWVRQIPEKRL EWVASISRGGTTYYP DSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTSVTVSS KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEACRPAAG GAVHTRGLDFACDIY IWAPLAGTCGVLLLS LVITLYCNHRNRSKR SRGGHSDYMNMTPR RPGPTRKHYQPYAPP RDFAAYRSRVKFSRS ADAPAYQQGQNQLY NELNLGRREEYDVLD KRRGRDPEMGGKPR RKNPQEGLYNELQKD KMAEAYSEIGMKGER RRGKGHDGLYQGLST ATKDTYDALHMQAL PPR	161	GACGTGCAGATCACCCAGAGCCCCAGC AGCCTGTATGCCAGCCTGGGCGAGAGA GTGACCATTACCTGCAAGGCCAGCCCC GACATCAACAGCTACCTGAGCTGGTTC CAGCAGAAGCCCGGCAAGAGCCCCAA GACCCTGATCTACCGGGCCAACAGACT GGTGGATGGCGTGCCCAGCAGATTCAG CGGCGGAGGCTCTGGCCAGGACTACAG CCTGACCATCAACTCCCTGGAATACGA GGACATGGGCATCTACTACTGCCTGCA GTACGACGAGTTCCCCTACACCTTCGG AGGCGGCACCAAGCTGGAAATGAAGG GCAGCACAAGCGGCAGCGGCAAGCCT GGATCTGGCGAGGGAAGCACCAAGGG CGAAGTGAAGCTGGTGGAATCTGGCGG CGGACTCGTGAAGCCTGGCGGCTCTCT GAAGCTGTCTTGTGCCGCCAGCGGCTT CACCTTCAGCAGCTACGCCATGAGCTG GGTGCGGCAGATCCCCGAGAAGCGGCT GGAATGGGTGGCCAGCATCAGCAGAG GCGGAACCACCTACTACCCCGACTCTG TGAAGGGCCGGTTCACCATCAGCCGGG ACAACGTGCGGAACATCCTGTACCTGC AGATGAGCAGCCTGCGGAGCGAGGAC ACCGCCATGTACTACTGTGGCAGATAC GACTACGACGGCTACTATGCCATGGAT TACTGGGGCCAGGGCACCAGCGTGACC GTGTCATCTAAGCCTACCACCACCCCC GCACCTCGTCCTCCAACCCCTGCACCT ACGATTGCCAGTCAGCCTCTTTCACTG CGGCCTGAGGCCAGCAGACCAGCTGCC GGCGGTGCCGTCCATACAAGAGGACTG GACTTCGCGTCCGATAAACCTACTACC ACTCCAGCCCCAAGGCCCCCAACCCCA GCACCGACTATCGCATCACAGCCTTTG TCACTGCGTCCTGAAGCCAGCCGGCCA GCTGCAGGGGGGGCCGTCCACACAAG GGGACTCGACTTTGCGAGTGATAAGCC CACCACCACCCCTGCCCCTAGACCTCC AACCCCAGCCCCTACAATCGCCAGCCA GCCCCTGAGCCTGAGGCCCGAAGCCTG TAGACCTGCCGCTGGCGGAGCCGTGCA CACCAGAGGCCTGGATTTCGCCTGCGA CATCTACATCTGGGCCCCTCTGGCCGG CACCTGTGGCGTGCTGCTGCTGAGCCT GGTCATCACCCTGTACTGCAACCACCG GAATAGGAGCAAGCGGAGCAGAGGCG GCCACAGCGACTACATGAACATGACCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCCGGAGGCCTGGCCCCACCCGGAAGC ACTACCAGCCCTACGCCCCTCCCAGGG ACTTCGCCGCCTACCGGAGCCGGGTGA AGTTCAGCCGGAGCGCCGACGCCCCTG CCTACCAGCAGGGCCAGAACCAGCTGT ACAACGAGCTGAACCTGGGCCGGAGG GAGGAGTACGACGTGCTGGACAAGCG GAGAGGCCGGGACCCTGAGATGGGCG GCAAGCCCCGGAGAAAGAACCCTCAG GAGGGCCTGTATAACGAACTGCAGAA AGACAAGATGGCCGAGGCCTACAGCG AGATCGGCATGAAGGGCGAGCGGCGG AGGGGCAAGGGCCACGACGGCCTGTA CCAGGGCCTGAGCACCGCCACCAAGG ATACCTACGACGCCCTGCACATGCAGG CCCTGCCCCCCAGA
hROR1 VH_04	15	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSAISRGGTTYYA DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	162	GAGGTGCAGCTCGTGGAATCCGGCGGT GGCCTGGTGCAGCCGGGCGGCAGTCTT CGACTCTCCTGTGCGGCGTCAGGCTTT ACGTTCAGTTCTTATGCCATGAGCTGG GTGAGGCAAGCTCCCGGTAAGGGACTG GAGTGGGTCTCTGCTATCAGCCGGGGA GGTACGACCTACTACGCTGACTCCGTA AAAGGAAGATTTACCATAAGTCGTGAC AATTCCAAAAACACTCTATACTTACAG ATGAACTCGCTCAGGGCCGAAGATACC GCAGTCTACTATTGTGGGAGATACGAT TACGACGGCTACTATGCTATGGATTAT TGGGGTCAGGGTACGCTCGTGACGGTG TCCTCC
hROR1 VL_04	16	DIQMTQSPSSLSASVG DRVTITCQASPDINSY LNWYQQKPGKAPKL LIYRANNLETGVPSRF SGSGSGTDFTLTISSL QPEDIATYYCLQYDE FPYTFGQGTKLEIK	163	GATATTCAAATGACGCAAAGTCCCAGC AGCCTCTCCGCCTCCGTTGGAGACAGG GTGACTATTACATGCCAAGCCAGCCCC GATATTAATAGCTACTTAAATTGGTAT CAGCAGAAACCTGGGAAGGCACCTAA ACTTCTCATCTACCGCGCTAACAATCT GGAGACCGGCGTGCCGTCTAGATTTTC CGGCTCTGGATCAGGGACCGATTTTAC TCTGACAATTAGTTCCCTGCAACCCGA AGACATCGCCACTTATTATTGCCTGCA ATATGATGAGTTTCCTTACACATTTGGT CAGGGAACTAAACTAGAGATTAAG
hROR1 VH_05	17	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSSISRGGTTYYP DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	164	GAAGTGCAACTGGTCGAGTCTGGGGGC GGCCTTGTGCAACCTGGAGGCAGCCTT CGACTCAGTTGCGCCGCGTCTGGTTTT ACCTTCTCCTCTTACGCGATGAGCTGG GTTCGCCAGGCCCCCGGCAAGGGACTT GAGTGGGTTAGTTCGATCTCCCGCGGA GGCACCACATATTATCCTGACTCGGTT AAGGGACGCTTCACTATCTCTAGGGAC AATTCAAAGAACACACTGTATCTCCAA ATGAACTCCTTGCGGGCCGAGGACACT

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				GCTGTGTATTATTGCGGACGATACGAC TACGATGGGTATTACGCCATGGATTAC TGGGGGCAAGGTACACTGGTCACTGTG AGTTCG
hROR1 VL_05	18	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKLL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDIATYYCLQYDEFP YTFGQGTKLEIK	165	GATATTCAGATGACCCAGTCACCTTCG AGTCTGAGCGCATCCGTGGGCGACAGA GTGACCATTACCTGTAAGGCCAGCCCG GACATTAACAGCTACCTATCGTGGTAT CAGCAAAAGCCTGGTAAGGCCCCTAAA CTCCTTATCTACAGGGCTAATAGGTTG GTAGACGGGGTGCCTAGCCGGTTCTCT GGTTCCGGCAGCGGTACGGACTTTACT CTGACCATAAGCTCTCTGCAACCAGAA GACATCGCAACATACTACTGTTTACAA TACGACGAATTTCCTTATACCTTTGGCC AGGGGACCAAGTTAGAGATCAAG
hROR1 VH_06	19	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAIIWVRQAPGKGLE WVARISRGGTTRYAD SVKGRFTISADTSKET AYLQMNSLRAEDTA VYYCGRYDYDGYYA MDYWGQGTLVTVSS	166	GAGGTTCAGCTGGTCGAGTCCGGGGGA GGCTTAGTGCAGCCAGGAGGCAGTCTG CGGCTCTCTTGCGCTGCAAGTGGCTTC ACATTCAGTTCATACGCAATCATCTGG GTTCGACAGGCTCCTGGTAAGGGCCTC GAATGGGTCGCAAGGATATCACGAGGT GGAACCACTAGATACGCAGACTCTGTT AAGGGCAGGTTCACAATTAGCGCGGAT ACCTCCAAGGAGACTGCTTATTTACAG ATGAACTCTCTGAGAGCCGAGGACACT GCTGTTTACTACTGCGGCCGATACGAT TACGACGGATATTACGCAATGGATTAC TGGGGCCAGGGCACGCTGGTGACAGTT TCATCG
hROR1 VL_06	20	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKLL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDIATYYCLQYDEFP YTFGQGTKLEIK	167	GATATCCAGATGACTCAGAGTCCCAGT AGCCTGTCGGCAAGCGTCGGAGATCGG GTCACAATTACCTGCAAAGCTAGTCCT GATATTAATTCTTACTTGTCCTGGTATC AGCAGAAGCCTGGTAAGGCCCCTAAGT TGCTCATCTATCGGGCTAACCGGCTGG TGGACGGTGTTCCCTCTAGATTCTCAG GGAGTGGAAGCGGCACTGACTTCACCC TGACTATATCGAGCCTTCAGCCAGAGG ACATTGCCACATACTACTGTCTGCAAT ATGATGAATTTCCATATACATTCGGAC AAGGTACAAAGTTAGAAATTAAG
hROR1 VH_07	21	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAIIWVRQAPGKGLE WVARISRGGTTRYAD SVKGRFTISADTSKET AYLQMNSLRAEDTA VYYCGRYDYDGYYA MDYWGQGTLVTVSS	168	GAAGTCCAACTGGTGGAGTCTGGCGGG GGCTTGGTGCAGCCCGGTGGCTCCCTT AGGCTGTCTTGCGCTGCCAGCGGGTTC ACATTCAGCTCCTATGCGATTATATGG GTCCGACAGGCACCCGGCAAGGGATTG GAGTGGGTGGCTCGCATCAGCAGAGGC GGCACTACTCGTTACGCCGACTCCGTG AAAGGCAGATTCACCATCAGTGCAGAC ACATCCAAGGAAACCGCATATCTTCAG ATGAATAGCCTGCGAGCGGAGGATACC

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				GCCGTCTATTATTGCGGACGCTATGAT TACGACGGTTATTATGCTATGGACTAC TGGGGCCAGGGCACACTTGTGACCGTC AGTAGC
hROR1 VL_07	22	DIQMTQSPSSLSASVG DRVTITCRASPDINSY VAWYQQKPGKAPKL LIYRANFLESGVPSRF SGSRSGTDFTLTISSL QPEDFATYYCLQYDE FPYTFGQGTKVEIK	169	GACATTCAAATGACGCAAAGCCCTAGT AGCTTGTCAGCTTCTGTGGGGGACCGT GTCACAATCACTTGTCGGGCCTCTCCA GATATAAACTCCTACGTTGCTTGGTAT CAGCAGAAGCCCGGAAAGGCTCCGAA ATTGTTGATTTATCGCGCTAATTTCTTA GAGTCAGGAGTGCCCAGCCGGTTCTCA GGGTCTCGCTCTGGAACCGACTTCACA CTCACTATTTCTAGCCTACAGCCTGAG GATTTTGCAACTTACTACTGTCTACAGT ACGACGAGTTTCCGTACACTTTCGGAC AGGGGACCAAGGTGGAGATCAAG
hROR1 VH_08	23	QVQLVQSGGGLVKP GGSLRLSCAASGFTFS SYAMSWVRQIPGKGL EWVSSISRGGTTYYP DSVKGRFTISRDNVK NTLYLQMSSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTMVTV SS	170	CAAGTACAGCTCGTGCAGAGCGGCGGT GGCCTGGTGAAGCCAGGAGGTAGTCTT AGACTGAGCTGTGCGGCTTCTGGTTTC ACGTTCAGCAGTTATGCTATGTCCTGG GTTAGGCAAATCCCCGGCAAAGGATTG GAGTGGGTTAGCAGTATCTCAAGGGGG GGAACCACATATTATCCTGACTCTGTC AAAGGACGGTTTACAATCAGCCGCGAT AACGTTAAAAATACCCTCTACCTCCAG ATGTCTTCGCTCCGCGCTGAAGATACA GCGGTTTACTACTGTGGCAGATACGAC TACGACGGTTATTACGCCATGGACTAC TGGGGACAGGGAACTATGGTCACAGTT AGCTCT
hROR1 VL_08	24	DIKMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKTL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ YEDMAIYYCLQYDEF PYTFGDGTKVEIK	171	GACATCAAAATGACGCAGTCACCTAGT AGCCTCTCCGCCTCGGTTGGCGATCGG GTAACCATTACCTGCAAAGCATCTCCA GACATAAATAGTTATCTTAGTTGGTAT CAACAGAAACCTGGCAAAGCTCCTAAG ACCCTCATCTACCGCGCTAACCGCCTC GTGGATGGTGTTCCAAGTCGGTTCTCA GGAAGCGGCAGTGGCACAGACTTTACA CTGACAATTAGTTCCCTCCAGTATGAG GATATGGCCATATATTACTGCCTTCAG TATGATGAGTTTCCATACACATTCGGA GACGGTACAAAGGTGGAGATCAAG
hROR1 VH_09	25	QVSLRESGGGLVQPG RSLRLSCTASGFTFSS YAMTWVRQAPGKGL EWVASISRGGTTHFA DSVKGRFTISRDNSN NTLYLQMDNVRDED TAIYYCGRYDYDGYY AMDYWGRGTLVTVS S	172	CAAGTGAGCCTCCGGGAGAGTGGGGG CGGTCTGGTCCAACCAGGACGGTCACT GCGGCTGTCATGCACTGCCAGCGGCTT CACATTTAGCTCTTACGCCATGACTTG GGTCCGCCAAGCTCCCGGTAAGGGACT GGAGTGGGTGGCCAGCATTAGCAGGG GTGGTACAACCCACTTCGCGGATTCAG TTAAGGGGAGATTCACTATCTCCAGGG ATAATTCCAACAACACGCTGTACCTTC AGATGGATAACGTGAGAGACGAGGAT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCGCGATATACTACTGTGGCCGCTAT GACTACGATGGTTATTATGCTATGGAT TACTGGGGGCGGGGCACCCTGGTGACT GTGTCCTCG
hROR1 VL_09	26	DIVMTQSPSSLSASVG DRVTITCRASPDINSY LAWYQQKPGKAPKL LIYRANSLQSGVPSRF SGSGSGTEFTLTISSLQ PEDFATYYCLQYDEF PYTFGQGTKLEMK	173	GATATCGTGATGACACAGTCACCTAGC TCCCTGAGCGCAAGCGTGGGGGATAGG GTTACCATAACTTGCAGGGCCAGTCCC GACATCAATAGTTATTTGGCCTGGTAT CAACAGAAGCCTGGGAAGGCACCTAA GTTGCTTATTTATAGGGCTAACTCGTTA CAGAGCGGTGTGCCAAGTCGGTTCTCA GGCTCAGGGTCCGGGACCGAGTTCACC CTGACCATCAGTAGCTTGCAGCCAGAA GATTTTGCCACCTACTACTGTCTTCAAT ACGATGAGTTTCCTTACACTTTTGGAC AGGGCACCAAACTAGAGATGAAG
hROR1 VH_10	27	QVQLVESGGGVVQP GRSLRLSCAASGFTFS SYAMNWVRQAPAKG LEWVAIISRGGTQYY ADSVKGRFTISRDNS KNTLYLQMNGLRAE DTAVYYCGRYDYDG YYAMDYWGQGTLVT VSS	174	CAGGTTCAACTGGTAGAATCCGGCGGA GGTGTAGTGCAGCCTGGAAGGTCATTA CGGTTAAGTTGCGCCGCCTCCGGGTTC ACATTTAGCAGCTATGCTATGAACTGG GTGCGCCAGGCCCCTGCGAAAGGACTC GAATGGGTTGCCATCATCAGCCGAGGA GGCACACAGTATTATGCCGATTCTGTG AAGGGTCGTTTTACTATTTCCAGAGAC AACAGTAAAAATACGCTGTACCTGCAA ATGAACGGATTGAGGGCTGAGGATACC GCCGTGTACTACTGTGGACGCTACGAC TATGATGGGTACTACGCGATGGACTAT TGGGGGCAAGGAACCCTTGTAACCGTT AGTTCA
hROR1 VL_10	28	EIVLTQSPDFQSVTPK EKVTITCRASPDINSY LSWYQQKPDQSPKLL IKRANQSFSGVPSRFS GSGSGTDFTLTINSLE AEDAAAYYCLQYDE FPYTFGPGTKVDIK	175	GAGATCGTTTTGACACAGAGCCCCGAT TTCCAGAGCGTCACGCCCAAGGAGAAG GTCACCATCACCTGCCGAGCCAGCCCC GACATCAACAGTTATCTTTCATGGTAT CAACAGAAACCTGATCAGAGCCCTAAG CTGCTGATTAAGCGCGCCAACCAGAGC TTCTCAGGGGTTCCTTCACGGTTTTCCG GGTCAGGCAGCGGGACTGACTTCACGT TGACCATTAACTCTTTGGAGGCTGAGG ATGCTGCTGCCTATTACTGCCTTCAGTA CGACGAGTTCCCCTATACATTTGGTCCT GGAACAAAAGTGGATATAAAG
hROR1 VH_11	29	QVQLVQSGAEVKKP GASVKVSCKASGFTF SSYAMHWVRQAPGQ GLEWMGNISRGGTTN YAEKFKNRVTMTRD TSISTAYMELSRLRSD DTAVYYCGRYDYDG YYAMDYWGQGTLVT VSS	176	CAGGTGCAGCTCGTCCAGAGCGGAGCC GAAGTGAAGAAGCCGGGAGCATCAGT GAAAGTTTCCTGCAAAGCAAGTGGCTT CACTTTCAGCAGTTACGCGATGCACTG GGTGCGGCAGGCACCAGGTCAGGGAC TGGAATGGATGGGGAACATCTCTCGCG GCGGAACAACCAATTACGCAGAGAAG TTTAAGAATCGCGTTACGATGACCAGA GACACTTCTATTAGTACAGCCTATATG GAGTTGTCGCGTCTGAGAAGCGACGAT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCGCTGTCTACTATTGCGGCCGGTAC GATTATGACGGCTACTATGCAATGGAT TACTGGGGACAGGGCACACTTGTGACA GTGTCTAGT
hROR1 VL_11	30	DIVMTQSPLSLPVTPG EPASISCRSSPDINSYL EWYLQKPGQSPQLLI YRANDRFSGVPDRFS GSGSGTDFTLKISRVE AEDVGVYYCLQYDE FPYTFGQGTKVEIK	177	GACATTGTGATGACTCAGTCTCCACTC AGCCTGCCTGTCACGCCCGGCGAACCC GCTTCTATCTCTTGTAGGAGTAGCCCTG ATATCAACAGCTACCTCGAATGGTATC TCCAGAAACCTGGTCAGAGCCCCCAGC TCTTGATCTATAGAGCAAACGACAGGT TCTCTGGCGTGCCTGATAGGTTTTCCGG TAGTGGCAGCGGAACCGACTTCACACT TAAGATTTCAAGGGTCGAGGCCGAGGA CGTGGGGGTGTATTACTGCTTACAGTA CGATGAGTTTCCGTATACATTCGGGCA AGGCACAAAGGTGGAAATTAAG
hROR1 VH_12	31	EVQLVESGGGLVQPG GSLRLSCTGSGFTFSS YAMHWLRQVPGEGL EWVSGISRGGTIDYA DSVKGRFTISRDDAK KTLSLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTMVTV SS	178	GAAGTGCAACTGGTCGAAAGTGGAGG GGGACTAGTGCAGCCCGGAGGGTCACT GAGGCTATCATGCACCGGCTCTGGTTT TACTTTTTCCAGCTATGCCATGCACTGG CTCAGACAGGTTCCGGGGGAAGGACTG GAGTGGGTTAGCGGAATCTCCAGAGGC GGAACTATTGACTACGCAGACAGCGTG AAAGGTAGGTTTACCATCAGCAGGGAC GATGCTAAAAAGACCCTGTCACTTCAA ATGAATAGCCTGAGAGCTGAGGATACG GCCGTGTATTACTGTGGACGCTATGAC TACGATGGATATTACGCAATGGACTAC TGGGGCCAGGGAACAATGGTGACCGTC TCAAGC
hROR1 VL_12	32	EIVLTQSPATLSVSPG ERATLSCRASPDINSY LAWYQQKPGQAPRL LFSRANNRATGIPARF TGSGSGTDFTLTISSL EPEDFAIYYCLQYDEF PYTFGQGTKVEIK	179	GAGATCGTCCTGACCCAGAGCCCAGCT ACTTTGTCAGTTTCGCCAGGCGAGCGG GCCACACTGAGCTGTAGGGCTTCTCCT GATATCAATTCTTACCTGGCCTGGTATC AACAGAAACCGGGACAGGCCCCTCGC CTGCTGTTCTCCCGCGCCAACAATAGG GCGACTGGCATACCAGCTCGGTTTACT GGGAGTGGGTCAGGCACTGATTTCACG CTTACAATCAGTAGCCTGGAGCCCGAA GACTTCGCCATCTACTACTGTTTACAAT ACGATGAGTTCCCCTATACCTTCGGCC AAGGGACCAAGGTGGAGATCAAG
hROR1 VH_13	33	EVQLVESGGGVVQPG RSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVASISRGGTQYYA DSVKGRFTISRDNSK NTLYLQMNGLRAED TAVYYCGRYDYDGY YAMDYWGQGTLVTV SS	180	GAAGTGCAGCTAGTAGAAAGTGGTGGT GGGGTCGTGCAGCCAGGCCGCTCGCTC AGGCTGTCTTGCGCTGCGAGTGGTTTC ACATTCTCTTCATACGCCATGAGCTGG GTGAGACAGGCTCCCGGCAAGGGCCTC GAATGGGTCGCATCTATAAGCAGAGGC GGAACCCAGTACTACGCTGACAGTGTG AAGGGTCGCTTTACAATCTCACGGGAC AACAGTAAAAACACCCTCTATCTACAG ATGAATGGCTTGCGAGCTGAAGACACG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCTGTGTATTATTGCGGGCGCTATGAC TATGATGGTTACTACGCTATGGATTAC TGGGGCCAGGGCACCCTGGTTACTGTT TCATCA
hROR1 VL_13	34	EIVLTQSPDFQSVTPK EKVTITCRASPDINSY LPWYQQKPDQSPKLL IKRANQSFSGVPSRFS GSGSGTDFTLTINSLE AEDAAAYYCLQYDE FPYTFGPGTKVDIK	181	GAAATAGTCCTGACCCAGAGCCCAGAC TTCCAGTCCGTGACCCCTAAGGAGAAG GTTACTATCACTTGCAGGGCAAGCCCT GACATAAATTCATACCTGCCATGGTAT CAGCAGAAGCCAGACCAGTCGCCGAA GCTATTAATCAAACGCGCCAACCAGTC TTTTAGCGGCGTACCATCCCGATTCTCA GGTTCGGGGTCCGGGACCGATTTCACA CTCACGATAAACTCCCTTGAGGCAGAG GATGCAGCGGCTTACTACTGTTTACAG TACGACGAGTTTCCATATACGTTCGGC CCCGGCACGAAGGTAGATATCAAG
hROR1 VH_14	35	EVQLVESGGGLVQPG GSLRLSCATSGFTFSS YAMSWMRQAPGKGL EWVASISRGGTTYYA DSVKGRFTISVDKSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	182	GAAGTGCAGCTGGTGGAGTCTGGCGGC GGTCTGGTGCAGCCCGGCGGCTCTCTG CGCCTCTCCTGTGCCACCTCTGGTTTTA CATTCTCCTCCTACGCTATGTCCTGGAT GCGGCAAGCCCCCGGCAAGGGCCTAG AGTGGGTCGCCTCAATCAGCAGGGGCG GGACGACTTATTATGCCGATTCAGTTA AGGGGAGATTCACAATTTCCGTGGATA AATCCAAGAATACCTTATACCTCCAGA TGAACTCTCTGCGGGCCGAAGATACGG CCGTATATTATTGTGGGAGGTATGACT ACGACGGATATTACGCCATGGATTATT GGGGGCAGGGGACACTTGTTACAGTGA GTTCC
hROR1 VL_14	36	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LNWYQQKPGKAPKL LIYRANRLVDGVPSR FSGSGSGTDYTLTISS LQPEDFATYYCLQYD EFPYTFGAGTKVEIK	183	GATATACAGATGACACAGAGCCCTTCA AGTTTATCTGCAAGCGTCGGCGATCGT GTTACAATAACTTGCAAGGCATCTCCC GACATCAATTCCTACCTCAACTGGTAT CAGCAGAAGCCTGGGAAGGCTCCTAA GCTGCTTATTTACAGAGCAAATCGCCT GGTGGACGGCGTGCCCAGTCGGTTTTC CGGGTCTGGGAGCGGAACGGATTACAC ACTGACCATCTCAAGCCTGCAACCCGA AGACTTCGCTACATATTACTGCCTTCA GTATGATGAGTTCCCATATACCTTCGG CGCTGGGACCAAGGTGGAGATAAAG
hROR1 VH_14-1	37	EVQLVESGGGLVQPG GSLRLSCASSGFTFSS YAMSWRRQAPGKGL EWVAGISRGGTTSYA DSVKGRFTISSDDSKN TLYLQMNSLRAEDTA VYYCGRYDYDGYYA MDYWGQGTLVTVSS	184	GAGGTCCAGCTCGTCGAATCTGGCGGA GGTTTAGTGCAACCAGGCGGGTCGCTC CGATTAAGTTGTGCGTCCAGTGGCTTC ACCTTCTCCAGCTACGCCATGTCGTGG AGGCGACAGGCTCCTGGCAAAGGCTTG GAGTGGGTTGCTGGTATCTCCCGAGGA GGCACCACTAGTTACGCTGACAGTGTA AAAGGACGTTTCACTATTTCCTCTGAC GACAGCAAGAACACACTCTATCTGCAA ATGAATAGTCTCCGTGCTGAGGACACA

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				GCCGTGTATTATTGCGGGCGGTATGAT TACGACGGCTACTACGCTATGGACTAC TGGGGCCAGGGAACTCTGGTCACTGTG AGCTCT
hROR1 VL_14-1	38	DIQMTQSPSSLSASVG DRVTITCRASPDINSY LSWYQQKPGKAPKLL IYRANTLESGVPSRFS GSGSGTDFTLTISSLQ PEDFATYYCLQYDEF PYTFGQGTKIEIK	185	GATATACAGATGACTCAAAGTCCTAGC TCCTTGAGCGCCTCAGTGGGAGATCGG GTCACTATAACTTGTAGAGCCTCACCA GATATAAACTCCTATCTCTCTTGGTATC AGCAGAAGCCCGGCAAAGCACCAAAG CTCTTGATCTATAGAGCTAATACGCTA GAGAGCGGAGTGCCTTCACGGTTTTCT GGTTCCGGGAGCGGAACCGACTTTACC CTTACAATTTCTAGCCTCCAGCCAGAG GACTTCGCAACTTACTATTGTCTCCAGT ATGATGAATTTCCTTACACCTTCGGCC AAGGGACCAAGATCGAGATAAAG
hROR1 VH_14-2	39	EVQLVESGGGLVQPG GSLRLSCASSGFTFSS YAMSWVRQAPGKGL EWVAGISRGGTTSYA DSVKGRFTISADTSKN TLYLQMNSLRAEDTA VYYCGRYDYDGYYA MDYWGQGTLVTVSS	186	GAGGTGCAGCTCGTTGAGTCCGGTGGG GGGCTGGTGCAGCCTGGCGGGTCTCTC CGCCTCTCTTGTGCCTCCTCCGGCTTTA CCTTCAGCAGCTATGCTATGTCATGGG TGCGGCAGGCACCAGGCAAAGGTCTG GAATGGGTCGCTGGGATCAGTAGAGGC GGCACAACCTCCTATGCCGACAGCGTT AAGGGGAGGTTCACAATCTCGGCTGAT ACAAGCAAGAACACTCTGTATCTCCAA ATGAACAGTCTCCGGGCAGAGGACACC GCGGTCTATTACTGCGGCCGGTACGAC TACGACGGGTACTACGCAATGGACTAT TGGGGACAGGGAACTCTGGTTACTGTC AGCTCT
hROR1 VL_14-2	40	DIQMTQSPSSLSASVG DRVTITCRASPDINSY LSWYQQKPGKAPKLL IYRANTLESGVPSRFS GSGSGTDFTLTISSLQ PEDFATYYCLQYDEF PYTFGTGTKLEIK	187	GATATCCAGATGACTCAAAGCCCATCT TCTCTCAGCGCAAGCGTGGGTGACCGA GTGACCATCACCTGCCGGGCGTCTCCT GATATCAACTCATACCTGTCCTGGTAT CAGCAGAAGCCCGGAAAGGCCCCTAA GCTGCTGATCTACCGCGCAAATACACT GGAGAGCGGGGTCCCAAGCAGATTCA GTGGGTCCGGCAGTGGTACGGACTTTA CTCTGACCATCAGCTCCCTGCAACCGG AGGACTTTGCTACTTATTACTGTCTCCA GTACGACGAGTTCCCATACACTTTCGG AACAGGCACTAAGCTGGAGATCAAA
hROR1 VH_14-3	41	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVASISRGGTTYYA DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	188	GAGGTTCAACTTGTGGAATCCGGCGGC GGGTTAGTCCAGCCCGGCGGGAGCTTG CGGCTGTCCTGCGCCGCCTCTGGATTC ACTTTTAGCTCCTATGCTATGTCTTGGG TAAGGCAGGCCCCTGGTAAAGGACTAG AGTGGGTGGCCTCGATCTCCCGTGGTG GCACTACATACTACGCCGACTCCGTTA AAGGCCGGTTTACCATCTCCCGTGACA ACTCTAAAAATACTTTGTACCTGCAAA TGAACTCCCTGCGGGCAGAAGACACAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCGTGTACTATTGCGGGCGTTACGATT ACGACGGATATTACGCAATGGACTACT GGGGCCAGGGCACACTGGTCACCGTGA GCAGC
hROR1 VL_14-3	42	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LNWYQQKPGKAPKL LIYRANRLVDGVPSR FSGSGSGTDFTLTISSL QPEDIATYYCLQYDE FPYTFGGGTKVEIK	189	GATATACAAATGACTCAGTCCCCTAGT AGCCTTAGTGCTAGTGTGGGAGACAGA GTGACCATCACCTGCAAAGCATCTCCT GATATCAATTCCTACCTTAACTGGTATC AACAGAAGCCTGGCAAAGCTCCAAAG CTCCTGATTTATCGCGCGAACAGATTG GTCGATGGGGTCCCTTCCAGATTCAGC GGCTCAGGGTCAGGGACCGATTTCACC CTCACAATTAGTTCACTTCAGCCCGAG GACATCGCCACGTATTATTGCCTTCAG TACGATGAGTTCCCTTACACCTTTGGC GGGGGAACTAAAGTCGAAATTAAG
hROR1 VH_14-4	43	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVASISRGGTTYYP DSVKGRFTISRDNVR NILYLQMSSLRSEDTA MYYCGRYDYDGYYA MDYWGQGTLVTVSS	190	GAAGTGCAGCTTGTGGAGTCAGGAGG AGGGCTAGTTCAGCCAGGCGGCTCTCT GAGACTATCTTGTGCTGCCTCCGGCTTC ACATTTAGCTCTTATGCAATGTCCTGG GTCCGCCAGGCCCCTGGTAAAGGCCTG GAATGGGTTGCTTCTATCTCTAGAGGC GGAACCACTTACTACCCTGATTCAGTG AAGGGGAGATTCACAATTAGTAGGGA CAACGTGCGGAACATCCTCTACCTACA GATGTCAAGTTTACGCAGTGAGGACAC TGCGATGTATTACTGCGGTCGATACGA TTATGATGGATATTATGCAATGGATTA TTGGGGCCAGGGCACTCTGGTCACAGT ATCTTCC
hROR1 VL_14-4	44	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LNWYQQKPGKAPKL LIYRANRLVDGVPSR FSGSGSGTDYTLTISS LQPEDFATYYCLQYD EFPYTFGAGTKVEIK	191	GACATCCAGATGACCCAATCACCATCG AGTCTTAGTGCATCCGTTGGGGATAGA GTGACAATCACTTGTAAGGCATCCCCG GACATCAACTCATATCTTAATTGGTAT CAGCAAAAGCCGGGCAAGGCCCCTAA GCTCCTGATTTATAGGGCCAACCGCCT TGTGGATGGAGTCCCCTCCCGCTTTAG TGGAAGCGGCTCTGGCACAGACTACAC CCTGACTATCAGCTCCTTGCAGCCTGA GGATTTTGCTACCTACTACTGTCTTCAG TACGATGAATTTCCATACACTTTCGGT GCTGGGACAAAAGTGGAGATCAAA
hROR1 VH_14-5	45	EVQLVESGGGLVQPG GSLRLSCATSGFTFSS YAMSWMRKAPGKGL EYVASISRGGTTYYA DSVKGRFTISVDKSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	192	GAAGTCCAGCTGGTTGAGTCTGGCGGA GGCCTCGTGCAGCCCGGTGGTTCCTTG CGACTGTCATGCGCTACCAGCGGGTTC ACATTCAGCTCTTATGCAATGTCCTGG ATGCGGAAGGCACCGGGTAAGGGCCT GGAGTATGTGGCCTCAATCTCCCGAGG AGGCACCACATACTATGCCGATTCTGT GAAAGGCCGATTCACCATTTCTGTGGA TAAGTCTAAAAACACTCTCTACCTCCA GATGAACTCCCTACGTGCCGAAGACAC

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				AGCCGTGTATTATTGCGGGCGATACGA TTATGACGGTTATTATGCGATGGATTA CTGGGGTCAAGGCACACTGGTAACAGT GTCTTCC
hROR1 VL_14-5	46	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LNWYQQKPGKAPKL LIYRANRLVDGVPSR FSGSGSGTDYTLTISS LQPEDFATYYCLQYD EFPYTFGAGTKVEIK	193	GATATTCAGATGACACAATCACCTAGC TCACTGTCAGCGAGCGTCGGTGACCGG GTTACTATCACATGCAAAGCCTCACCC GATATCAATTCATACCTTAACTGGTAT CAACAAAAACCAGGAAAGGCTCCAAA GCTGCTAATTTATCGGGCCAATCGGTT GGTGGATGGCGTCCCGTCGAGGTTTAG TGGCTCCGGGAGCGGGACAGACTACAC TCTTACAATTTCTTCTCTCCAGCCAGAG GACTTCGCAACCTACTACTGCTTGCAG TACGATGAATTTCCATATACCTTCGGC GCAGGGACAAAAGTGGAAATCAAA
hROR1 VH_15	47	EVQLVESGGGLVQPG GSLRLSCVTSGFTFSS YAMSWVRQAPGKGL EWVASISRGGTTYYS DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	194	GAGGTGCAGCTTGTAGAAAGCGGGGG GGGCCTGGTGCAACCTGGCGGGTCCCT GCGGCTTAGTTGCGTTACGAGCGGATT TACATTTTCCAGTTATGCCATGTCTTGG GTGAGACAAGCCCCCGGTAAGGGTCTG GAGTGGGTGGCAAGCATTAGCCGAGG CGGCACTACATACTACAGTGATAGTGT GAAAGGCCGTTTCACAATCAGTAGAGA TAATTCTAAAAACACCCTGTACTTGCA GATGAACAGCCTGCGCGCCGAGGATAC AGCCGTGTACTACTGTGGAAGATACGA CTACGATGGATATTATGCGATGGATTA CTGGGGACAGGGAACCCTTGTCACCGT TTCCTCT
hROR1 VL_15	48	DIVLTQSPATLSLSPG ERATLSCKASPDINSY MNWYQQKPGQAPRL LISRANRLVDGVPAR FSGSGSGTDFTLTISSL EPEDFAVYYCLQYDE FPYTFGQGTKVEIK	195	GACATAGTGTTGACGCAGTCCCCTGCC ACCCTGAGCCTGAGCCCCGGAGAGCGA GCAACGTTAAGTTGCAAGGCCAGTCCA GATATTAACTCATACATGAATTGGTAT CAACAGAAACCAGGCCAGGCTCCTAG ACTTCTCATATCTCGGGCAAATCGACT GGTGGATGGAGTACCCGCAAGATTCAG CGGCAGCGGCAGCGGAACGGATTTCAC GCTCACCATCTCTTCCCTTGAGCCTGAG GACTTTGCAGTCTATTATTGCTTGCAGT ATGATGAGTTCCCCTACACATTCGGGC AAGGCACAAAAGTGGAAATTAAG
hROR1 VH_16	49	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVASISRGGTTYYD PKFQDRATISADNSK NTAYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	196	GAGGTGCAGCTGGTGGAGAGCGGAGG GGGCCTTGTCCAACCAGGAGGTAGCCT CAGGCTGTCTTGCGCTGCCTCAGGATT TACTTTTTCATCCTACGCAATGAGCTGG GTGCGGCAAGCCCCAGGGAAGGGATT AGAATGGGTTGCCAGCATTTCTAGGGG GGGGACGACCTACTACGATCCGAAGTT TCAGGATCGCGCCACTATCTCAGCCGA TAACTCCAAGAATACTGCCTACTTACA GATGAACAGCCTGCGGGCCGAAGACA

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				CGGCCGTCTACTATTGCGGCCGATATG ATTACGACGGCTATTACGCCATGGATT ACTGGGGGCAAGGGACTCTGGTCACAG TGAGCTCT
hROR1 VL_16	50	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LNWYQQKPGKAPKV LIYRANRLVDGVPSR FSGSGSGTDYTLTISS LQPEDFATYYCLQYD EFPYTFGQGTKVEIK	197	GATATTCAGATGACCCAGTCGCCCAGC AGTCTCTCGGCCTCAGTGGGCGACCGG GTCACTATCACTTGCAAAGCAAGTCCT GATATAAACTCCTATCTTAATTGGTATC AGCAGAAGCCCGGCAAGGCACCTAAG GTTCTGATATATCGCGCAAATCGGCTC GTGGATGGAGTACCCAGCCGATTTTCC GGCAGCGGCTCAGGCACTGACTACACA CTGACAATCAGCAGCTTGCAGCCTGAA GATTTCGCCACATACTATTGTCTACAGT ACGACGAGTTCCCTTATACATTCGGCC AGGGGACCAAGGTCGAGATCAAG
hROR1 VH_17	51	EVQLVESGGGLVQPG GSLRLSCTGSGFTFSS YAMSWLRQVPGEGL EWVSSISRGGTTDYA DSVKGRFTISRDDAK KTLSLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTMVTV SS	198	GAGGTCCAACTCGTGGAGAGCGGAGG GGGGCTAGTGCAACCAGGTGGCTCCCT CCGCTTGTCCTGTACGGGCTCGGGGTT CACATTTTCATCCTATGCCATGAGCTG GCTGAGACAGGTGCCTGGCGAGGGCCT GGAATGGGTGTCTAGTATCAGCAGAGG GGGTACAACTGATTACGCAGATTCCGT CAAGGGACGTTTTACCATCTCAAGAGA CGATGCCAAGAAGACATTATCACTCCA AATGAACTCACTGAGGGCCGAGGACA CCGCTGTGTACTATTGTGGGAGATACG ACTACGACGGATACTATGCCATGGACT ATTGGGGACAAGGCACGATGGTGACG GTATCTAGC
hROR1 VL_17	52	EIVLTQSPATLSVSPG ERATLSCKASPDINSY LAWYQQKPGQAPRL LFSRANRLVDGIPARF TGSGSGTDFTLTISSL EPEDFAIYYCLQYDEF PYTFGQGTKVEIK	199	GAGATAGTGCTAACCCAGTCTCCCGCA ACCCTGTCTGTGTCCCCCGGAGAGCGC GCTACTCTGAGCTGCAAAGCCAGCCCG GACATTAATTCCTACCTTGCCTGGTATC AGCAGAAGCCTGGACAGGCCCCAAGA TTGCTCTTTTCACGCGCCAACCGCCTGG TAGATGGTATTCCAGCTAGGTTTACGG GCTCAGGCAGCGGAACAGACTTCACTC TCACTATTAGCTCATTGGAGCCTGAGG ACTTTGCAATTTACTATTGTCTTCAGTA CGACGAGTTCCCATATACTTTCGGCCA GGGCACAAAAGTAGAGATCAAG
hROR1 VH_18	53	EVQLVESGGGLVQPG GSLRLSCSASGFTFSS YAMSWVRQVPGKGL VWISSISRGGTTYYAD SVRGRFIISRDNAKNT LYLEMNNLRGEDTA VYYCARYDYDGYYA MDYWGQGTLVTVSS	200	GAGGTTCAACTCGTGGAGTCTGGAGGC GGGCTAGTGCAGCCTGGCGGCTCCCTG CGACTGTCTTGCAGCGCATCAGGCTTT ACATTCAGTTCTTATGCCATGAGCTGG GTGAGGCAGGTGCCCGGCAAGGGTCTG GTGTGGATCAGCTCAATCTCCAGGGGC GGGACTACATATTACGCCGATTCGGTC AGGGGTCGTTTTATCATTAGCAGGGAT AATGCCAAGAACACCTTGTATTTGGAG ATGAACAACCTAAGAGGCGAAGACAC

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				CGCTGTGTACTATTGTGCCCGTTACGA CTACGATGGGTACTACGCCATGGACTA TTGGGGCCAGGGAACCTTGGTGACTGT GTCAAGT
hROR1 VL_18	54	DIQLTQSPDSLAVSLG ERATINCKASPDINSY LSWYQQRPGQPPRLL IHRANRLVDGVPDRF SGSGFGTDFTLTITSL QAEDVAIYYCLQYDE FPYTFGQGTKLEIK	201	GACATACAGTTGACTCAGTCACCGGAT TCGCTGGCAGTTTCGCTGGGTGAGAGA GCAACCATCAACTGCAAAGCATCTCCC GATATCAACTCTTATCTGTCTTGGTATC AGCAGCGTCCGGGACAACCCCCTAGGC TGCTTATTCACCGAGCCAACAGGCTGG TGGACGGGGTGCCAGACCGCTTCTCGG GATCAGGATTTGGAACCGATTTTACCC TAACAATTACTAGTCTCCAAGCGGAAG ACGTGGCGATCTATTATTGTCTACAAT ATGACGAGTTCCCCTACACCTTCGGCC AGGGCACGAAGTTGGAGATCAAG
hROR1 VH_19	55	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVASISRGGTTYYA DSVKGRFTISADTSKN TAYLQMNSLRAEDTA VYYCARYDYDGYYA MDYWGQGTLVTVSS	202	GAGGTCCAGCTCGTCGAATCCGGTGGA GGGCTAGTTCAGCCAGGCGGCTCATTG CGTTTGTCTTGTGCCGCCTCCGGTTTCA CATTCTCTTCTTACGCTATGTCCTGGGT CCGACAAGCCCCAGGAAAAGGCTTGG AATGGGTGGCCAGTATCAGTAGAGGTG GGACTACATATTATGCCGACTCCGTGA AGGGCAGATTCACCATCTCAGCTGACA CCAGTAAGAACACTGCCTACCTACAGA TGAACAGCCTTCGGGCCGAGGACACCG CTGTGTATTACTGTGCCCGGTACGATT ATGATGGATATTATGCTATGGACTATT GGGGTCAGGGGACCTTGGTGACCGTCT CTAGC
hROR1 VL_19	56	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKLL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDFATYYCLQYDEF PYTFGQGTKVEIK	203	GACATTCAGATGACTCAATCGCCGAGT TCTCTTAGCGCTTCTGTTGGGGACCGG GTGACAATCACATGCAAGGCCTCTCCC GATATAAACTCCTATCTAAGCTGGTAT CAGCAGAAGCCAGGGAAGGCCCCCAA GTTGTTAATCTATCGCGCCAACAGACT GGTGGATGGGGTGCCCTCTCGATTCTC CGGGAGTGGCAGTGGGACTGATTTTAC ACTGACCATTTCCTCATTGCAGCCCGA AGACTTCGCTACCTATTACTGCTTGCA GTACGATGAGTTCCCATATACATTCGG TCAGGGGACTAAAGTGGAGATAAAA
hROR1 VH_20	57	EVQLLESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSSISRGGTTYYA DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCARYDYDGYY AMDYWGQGTLVTVS S	204	GAGGTACAGCTGCTGGAATCTGGTGGG GGGCTGGTCCAGCCAGGGGGGTCACTA CGACTGAGCTGCGCTGCCTCCGGTTTT ACATTCAGCAGCTATGCAATGTCATGG GTCAGACAGGCACCAGGTAAAGGCCTC GAATGGGTATCCTCCATCTCACGTGGT GGGACCACTTACTATGCCGATAGTGTG AAGGGCAGGTTCACGATCTCAAGAGAT AATTCAAAGAATACACTCTATCTACAA ATGAACAGTTTAAGGGCCGAGGACACC

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				GCTGTTTACTATTGTGCCAGATATGACT ACGACGGTTATTATGCTATGGATTACT GGGGACAAGGAACGCTGGTAACTGTTA GCTCT
hROR1 VL_20	58	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGEAPKLL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDFATYYCLQYDEF PYTFGQGTKVEIK	205	GACATCCAAATGACCCAGTCGCCTTCC TCCTTGTCTGCATCTGTCGGAGATCGG GTGACGATCACTTGCAAAGCGAGTCCA GACATCAACTCATATCTGTCCTGGTAT CAGCAGAAGCCGGGAGAGGCACCTAA GCTCCTGATCTACAGAGCAAACAGATT AGTGGATGGTGTGCCCTCACGGTTTTC TGGCTCCGGGTCCGGCACCGATTTCAC CTTGACCATCTCATCCCTACAGCCCGA GGATTTCGCTACTTACTATTGCTTACAG TATGATGAGTTTCCATACACCTTCGGTC AAGGCACCAAGGTTGAGATTAAG
hROR1 VH_21	59	EVQLLETGGGLVKPG GSLRLSCAASGFTFSS YAMSWIRQAPGKGLE WVASISRGGTTYYGD SVKGRFTISRDHAKN SLYLQMNSLRVEDTA VYYCVRYDYDGYYA MDYWGLGTLVTVSS	206	GAAGTTCAACTGCTTGAGACCGGAGGC GGCCTGGTAAAACCTGGGGGCTCACTG AGGCTGAGTTGTGCCGCTTCTGGGTTC ACCTTTTCATCCTATGCGATGTCATGGA TACGGCAGGCTCCTGGGAAGGGGCTTG AGTGGGTTGCATCAATTTCACGAGGTG GGACAACTTATTATGGGGATTCCGTTA AAGGTAGATTTACGATCTCTAGAGACC ATGCCAAAAATTCTCTCTATCTCCAGA TGAATAGTCTTAGGGTGGAGGACACCG CTGTGTACTACTGTGTCCGGTACGACT ATGATGGGTACTATGCTATGGACTATT GGGGGCTCGGCACTCTGGTCACTGTTA GCTCT
hROR1 VL_21	60	AIRMTQSPSFLSASVG DRVTITCKASPDINSY LSWYQQRPGKAPKLL IYRANRLVDGVPSRFS GGGSGTDFTLTISSLQ PEDIATYYCLQYDEFP YTFGQGTKLEIK	207	GCCATCCGCATGACACAATCTCCCTCC TTCCTTTCTGCCAGTGTCGGGGACAGA GTGACTATCACATGCAAAGCCAGCCCA GATATTAATTCGTACCTGTCTTGGTATC AGCAGAGGCCCGGCAAGGCACCAAAG CTGTTGATATATCGGGCCAACCGCTTA GTGGACGGTGTCCCCTCTCGATTCAGC GGAGGCGGTAGCGGGACGGACTTTAC ACTGACCATCTCCAGTCTCCAACCCGA GGATATTGCCACTTACTATTGTCTTCAG TATGACGAGTTCCCCTACACATTTGGA CAGGGCACCAAGCTAGAAATTAAG
hROR1 VH_22	61	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVASISRGGTTYYA ESLEGRFTISRDDSKN SLYLQMNSLKTEDTA VYYCARYDYDGYYA MDYWGQGTLVTVSS	208	GAGGTTCAGCTGGTGGAGTCTGGTGGG GGGCTCGTACAGCCGGGTGGCTCCCTA AGGCTGAGTTGCGCTGCCTCAGGCTTT ACCTTCTCAAGCTACGCGATGTCCTGG GTGAGACAGGCCCCTGGCAAAGGACT GGAGTGGGTGGCAAGCATTAGCCGGG GCGGAACTACCTATTACGCTGAGTCGT TAGAGGGGCGGTTTACTATCTCCAGAG ACGATTCAAAGAACTCGTTATACTTGC AGATGAACAGCCTCAAGACCGAGGAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCGCCGTGTACTACTGCGCCCGGTAC GACTATGACGGGTACTATGCTATGGAT TATTGGGGACAAGGCACCCTCGTGACC GTCTCTAGC
hROR1 VL_22	62	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKTL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDFATYYCLQYDEF PYTFGQGTKLEIK	209	GACATCCAGATGACACAGTCCCCTTCT TCACTTTCCGCTTCTGTGGGCGACAGG GTGACGATCACGTGTAAGGCCTCGCCA GACATTAATTCGTACTTATCGTGGTATC AGCAGAAACCGGGTAAAGCTCCGAAG ACTCTGATCTATAGAGCAAATAGGCTC GTAGACGGTGTCCCATCTAGATTTAGT GGGAGCGGCAGCGGAACCGACTTCACT CTCACCATCTCATCCCTGCAACCGGAG GATTTCGCTACTTACTATTGCTTGCAGT ATGACGAGTTTCCATATACGTTTGGTC AGGGAACCAAATTAGAGATCAAA
hROR1 VH_23	63	QVTLRESGPALVKPT QTLTLTCAASGFTFSS YAMSWIRQPPGKALE WLASISRGGTTYYNP SLKDRLTISKDTSANQ VVLKVTNMDPADTA TYYCARYDYDGYYA MDYWGQGTTVTVSS	210	CAGGTAACACTCCGAGAGAGTGGGCC AGCTCTCGTGAAGCCCACGCAGACTTT AACACTAACGTGTGCGGCAAGCGGCTT TACATTTTCGAGCTACGCGATGAGCTG GATAAGGCAACCTCCTGGGAAGGCGTT GGAGTGGTTGGCCTCAATTAGCCGGGG TGGCACCACTTACTACAATCCTAGTCTT AAGGACAGACTTACTATTTCAAAAGAT ACGTCCGCCAACCAGGTGGTACTGAAG GTCACAAATATGGACCCAGCTGACACT GCTACTTACTACTGCGCCCGGTACGAT TACGATGGTTACTACGCTATGGATTAC TGGGGTCAAGGAACCACAGTGACCGTC AGTTCA
hROR1 VL_23	64	DIQMTQSPSTLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKLL IYRANRLVDGVPSRFS GSGSGTAFTLTISSLQ PDDFATYYCLQYDEF PYTFGGGTKVEIK	211	GATATCCAGATGACGCAGTCCCCTTCA ACCCTCAGTGCCAGCGTTGGTGACCGG GTTACTATCACCTGTAAGGCTAGTCCC GATATAAATTCCTATTTGTCTTGGTATC AGCAGAAGCCAGGCAAGGCTCCTAAG CTGCTCATCTACCGGGCTAACAGGTTA GTTGACGGTGTGCCCTCCCGATTTTCCG GCAGTGGCAGCGGGACCGCTTTCACTC TTACAATCTCATCTCTTCAACCGGACG ACTTCGCTACGTACTACTGCCTCCAAT ATGATGAGTTTCCATACACATTCGGAG GAGGCACAAAAGTCGAAATCAAG
hROR1 VH_24	65	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSAISRGGTTYYA DSVKGRFTISADTSKE TAYLQMNSLRAEDTA VYYCGRYDYDGYYA MDYWGQGTLVTVSS	212	GAAGTCCAGCTGGTGGAGTCCGGCGGA GGCTTGGTTCAGCCCGGAGGATCTTTG CGACTGTCTTGCGCCGCCAGCGGTTTC ACTTTCAGCAGCTATGCCATGAGTTGG GTTAGACAAGCTCCCGGCAAGGGGCTG GAATGGGTTAGTGCTATTAGCCGGGGA GGGACAACATATTACGCTGACTCTGTC AAAGGCCGATTCACCATCTCTGCTGAC ACGAGCAAAGAAACCGCCTACCTCCAA ATGAACAGCCTGCGAGCTGAGGACACT

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				GCCGTCTACTATTGTGGTCGATATGATT ATGATGGGTACTATGCAATGGACTATT GGGGGCAGGGCACACTGGTGACCGTG AGCTCT
hROR1 VL_24	66	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKLL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDIATYYCLQYDEFP YTFGQGTKLEIK	213	GATATTCAGATGACGCAGAGTCCCTCC TCCCTATCTGCCTCTGTTGGAGATCGA GTCACCATTACGTGTAAAGCGTCTCCC GATATCAACAGCTACCTCTCTTGGTAT CAGCAGAAACCAGGGAAGGCCCCAA GCTGCTGATCTATAGAGCTAATCGCTT AGTGGATGGAGTGCCAAGCAGGTTCTC CGGGTCCGGCAGTGGAACCGATTTCAC CTTGACAATAAGTAGCTTGCAACCTGA GGATATTGCAACATACTACTGTCTACA GTACGACGAGTTCCCCTACACCTTCGG CCAAGGGACAAAGCTGGAGATTAAG
hROR1 VH_25	67	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSAISRGGTTYYA DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS S	214	GAAGTGCAGCTCGTGGAGAGCGGCGG CGGTCTGGTACAGCCAGGGGGGTCACT GCGTCTCTCATGTGCTGCGAGTGGCTTT ACGTTCTCTTCCTACGCTATGTCCTGGG TCAGGCAGGCACCGGGGAAGGGCTTA GAGTGGGTTAGTGCAATCTCTAGGGGC GGTACAACCTACTATGCCGACTCTGTC AAGGGCAGGTTTACAATTTCAAGAGAT AATTCTAAGAATACTCTTTACCTACAG ATGAATAGCTTGCGGGCGGAAGACAC AGCAGTCTATTATTGTGGCCGCTATGA CTACGACGGATACTATGCCATGGACTA CTGGGGCCAAGGCACTTTGGTCACGGT GAGCTCT
hROR1 VL_25	68	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKLL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDIATYYCLQYDEFP YTFGQGTKLEIK	215	GACATCCAGATGACCCAGAGCCCTAGT TCATTGTCTGCCAGTGTGGGGGATAGG GTCACTATCACGTGTAAGGCTTCCCCT GACATCAATTCATACCTGTCATGGTAT CAGCAGAAGCCTGGAAAAGCCCCTAA ACTGCTGATCTACCGCGCGAATAGGCT TGTGGACGGCGTTCCAAGCCGCTTCTC TGGCTCTGGATCAGGGACCGACTTCAC CCTCACGATCTCCAGCCTCCAACCCGA GGATATCGCCACCTATTATTGCCTTCA GTACGATGAGTTCCCCTATACATTCGG CCAGGGGACAAAGCTGGAAATCAAA
hROR1 VH_26	69	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSAISRGGTTYYA DSVKGRFTISADTSKE TAYLQMNSLRAEDTA VYYCGRYDYDGYYA MDYWGQGTLVTVSS	216	GAGGTCCAGCTCGTCGAGTCGGGTGGG GGCTTGGTGCAACCCGGTGGCAGTTTG CGCCTGAGCTGCGCCGCGAGCGGGTTC ACTTTCAGTTCGTATGCCATGAGTTGG GTGCGACAAGCGCCCGGCAAAGGACT GGAGTGGGTGTCAGCCATTAGCCGGGG CGGTACTACCTACTATGCGGACTCGGT CAAGGGAAGATTCACCATCAGCGCTGA TACCAGTAAGGAAACCGCTTATCTTCA GATGAACTCCCTGCGTGCCGAGGATAC

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				AGCAGTCTACTATTGCGGGCGCTACGA TTATGACGGATATTATGCCATGGATTA CTGGGGGCAGGGCACTCTGGTCACAGT CAGCTCT
hROR1 VL_26	70	DIQMTQSPSSLSASVG DRVTITCQASPDINSY LNWYQQKPGKAPKL LIYRANNLETGVPSRF SGSGSGTDFTLTISSL QPEDIATYYCLQYDE FPYTFGQGTKLEIK	217	GATATTCAGATGACGCAGTCTCCCTCT TCCCTGAGCGCCTCCGTCGGCGATAGA GTTACGATCACCTGTCAGGCCAGCCCA GATATCAACTCCTATCTGAATTGGTAT CAGCAAAAGCCTGGGAAGGCTCCCAA GTTGCTGATCTACAGAGCCAATAACTT AGAGACTGGCGTGCCGTCTCGGTTCAG CGGGTCCGGCAGTGGAACCGACTTTAC ACTGACCATTTCCAGCCTCCAACCTGA GGATATCGCCACATATTATTGTCTCCA GTATGACGAGTTCCCTTACACATTTGG TCAAGGAACTAAACTGGAAATCAAA
hROR1 VH_27	71	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSAISRGGTTYYA DSVKGRFTISADTSKE TAYLQMNSLRAEDTA VYYCGRYDYDGYYA MDYWGQGTLVTVSS	218	GAGGTGCAGCTGGTCGAAAGTGGAGG CGGACTCGTGCAGCCCGGCGGTAGTCT GCGATTGAGCTGTGCCGCGTCCGGCTT TACTTTCTCATCTTACGCTATGAGTTGG GTCCGCCAGGCCCCAGGCAAAGGACTG GAGTGGGTATCAGCCATCAGTAGGGGG GGAACTACCTATTACGCAGATTCTGTG AAGGGACGCTTCACCATCAGCGCGGAC ACTAGCAAGGAGACTGCCTACCTGCAA ATGAATAGTCTGAGAGCCGAGGATACC GCCGTGTACTATTGTGGCAGGTATGAC TACGATGGCTATTATGCTATGGATTAC TGGGGCCAGGGGACGTTAGTGACAGTA AGCTCT
hROR1 VL_27	72	DIQMTQSPSSLSASVG DRVTITCRASPDINSY VAWYQQKPGKAPKL LIYRANFLESGVPSRF SGSRSGTDFTLTISSL QPEDFATYYCLQYDE FPYTFGQGTKVEIK	219	GATATTCAGATGACCCAATCCCCTTCTT CTCTGAGCGCTTCTGTGGGCGATAGAG TTACAATAACCTGTCGGGCGTCCCCAG ACATTAACTCTTATGTAGCATGGTATC AGCAAAAGCCTGGAAAGGCACCAAAG TTACTGATCTACCGGGCCAATTTTCTGG AGTCGGGCGTGCCCTCACGATTTAGCG GTAGCAGATCAGGCACAGACTTTACTC TGACCATTAGCTCTCTGCAACCCGAGG ACTTCGCCACCTACTACTGTTTGCAGTA TGACGAGTTTCCATACACTTTTGGTCA AGGAACCAAAGTCGAAATCAAA
hROR1 VH_28	73	QIQLVQSGAEVKKPG ASVKVSCAASGFTFSS YAMSWVRQAPGKSF KWMGSISRGGTTYYS ADFKGRFAITKDTSAS TAYMELSSLRSEDTA VYYCARYDYDGYYA MDYWGQGTLVTVSS	220	CAGATACAGCTGGTGCAGTCTGGTGCC GAGGTTAAAAAGCCCGGAGCCTCGGTT AAAGTGAGTTGTGCGGCAAGCGGATTC ACGTTCAGTTCCTACGCTATGTCCTGG GTGCGGCAGGCTCCTGGCAAGTCATTT AAGTGGATGGGGTCGATCTCACGGGGT GGAACCACCTATTACTCTGCCGACTTC AAGGGGAGATTTGCGATTACAAAAGAT ACAAGCGCCTCTACGGCCTACATGGAG TTAAGTAGCCTTAGAAGCGAAGACACG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCGGTGTACTACTGCGCCAGATATGAC TATGACGGCTACTACGCCATGGACTAC TGGGGCCAGGGCACACTGGTTACAGTC AGCTCT
hROR1 VL_28	74	DIVMTQSPDSLAVSL GERATISCKASPDINS YLSWYQQKPGQPPKL LIYRANRLVDGVPDR FSGSGSRTDFTLTISSL QAEDVAVYYCLQYD EFPYTFGQGTKVEIK	221	GATATCGTGATGACACAAAGCCCAGAC AGTCTGGCAGTGTCCCTCGGCGAGCGC GCTACCATCTCATGCAAAGCTAGTCCC GACATCAATTCCTATCTGTCCTGGTATC AGCAAAAACCAGGCCAACCCCCCAAG CTGCTTATCTATCGGGCTAACCGATTA GTCGATGGGGTGCCAGATAGATTTTCA GGCTCTGGTTCCCGGACAGATTTTACT CTCACGATCTCCTCACTACAGGCAGAA GATGTTGCAGTGTATTACTGCCTGCAA TACGACGAGTTCCCCTACACCTTCGGC CAAGGCACGAAAGTGGAGATCAAG
hROR1 (VH-VL)_14. CD8a(3x).CD28z	75	EVQLVESGGGLVQPG GSLRLSCATSGFTFSS YAMSWMRQAPGKGL EWVASISRGGTTYYA DSVKGRFTISVDKSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS SGGGGSGGGGSGGG GSDIQMTQSPSSLSAS VGDRVTITCKASPDIN SYLNWYQQKPGKAP KLLIYRANRLVDGVP SRFSGSGSGTDYTLTI SSLQPEDFATYYCLQ YDEFPYTFGAGTKVEI KKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEACRP AAGGAVHTRGLDFA CDIYIWAPLAGTCGV LLLSLVITLYCNHRNR SKRSRGGHSDYMNM TPRRPGPTRKHYQPY APPRDFAAYRSRVKF SRSADAPAYQQGQN QLYNELNLGRREEYD VLDKRRGRDPEMGG KPRRKNPQEGLYNEL QKDKMAEAYSEIGM KGERRRGKGHDGLY	222	GAAGTGCAGCTGGTGGAGTCTGGCGGC GGTCTGGTGCAGCCCGGCGGCTCTCTG CGCCTCTCCTGTGCCACCTCTGGTTTTA CATTCTCCTCCTACGCTATGTCCTGGAT GCGGCAAGCCCCCGGCAAGGGCCTAG AGTGGGTCGCCTCAATCAGCAGGGGCG GGACGACTTATTATGCCGATTCAGTTA AGGGGAGATTCACAATTTCCGTGGATA AATCCAAGAATACCTTATACCTCCAGA TGAACTCTCTGCGGGCCGAAGATACGG CCGTATATTATTGTGGGAGGTATGACT ACGACGGATATTACGCCATGGATTATT GGGGGCAGGGGACACTTGTTACAGTGA GTTCCGGTGGTGGGGGGTCTGGAGGCG GGGGCAGTGGAGGCGGAGGGTCTGAT ATACAGATGACACAGAGCCCTTCAAGT TTATCTGCAAGCGTCGGCGATCGTGTT ACAATAACTTGCAAGGCATCTCCCGAC ATCAATTCCTACCTCAACTGGTATCAG CAGAAGCCTGGGAAGGCTCCTAAGCTG CTTATTTACAGAGCAAATCGCCTGGTG GACGGCGTGCCCAGTCGGTTTTCCGGG TCTGGGAGCGGAACGGATTACACACTG ACCATCTCAAGCCTGCAACCCGAAGAC TTCGCTACATATTACTGCCTTCAGTATG ATGAGTTCCCATATACCTTCGGCGCTG GGACCAAGGTGGAGATAAAGAAGCCT ACCACCACCCCCGCACCTCGTCCTCCA ACCCCTGCACCTACGATTGCCAGTCAG CCTCTTTCACTGCGGCCTGAGGCCAGC AGACCAGCTGCCGGCGGTGCCGTCCAT ACAAGAGGACTGGACTTCGCGTCCGAT AAACCTACTACCACTCCAGCCCCAAGG CCCCCAACCCCAGCACCGACTATCGCA TCACAGCCTTTGTCACTGCGTCCTGAA GCCAGCCGGCCAGCTGCAGGGGGGGC

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		QGLSTATKDTYDALH MQALPPR		CGTCCACACAAGGGGACTCGACTTTGC GAGTGATAAGCCCACCACCACCCCTGC CCCTAGACCTCCAACCCCAGCCCCTAC AATCGCCAGCCAGCCCCTGAGCCTGAG GCCCGAAGCCTGTAGACCTGCCGCTGG CGGAGCCGTGCACACCAGAGGCCTGG ATTTCGCCTGCGACATCTACATCTGGG CCCCTCTGGCCGGCACCTGTGGCGTGC TGCTGCTGAGCCTGGTCATCACCCTGT ACTGCAACCACCGGAATAGGAGCAAG CGGAGCAGAGGCGGCCACAGCGACTA CATGAACATGACCCCCCGGAGGCCTGG CCCCACCCGGAAGCACTACCAGCCCTA CGCCCCTCCCAGGGACTTCGCCGCCTA CCGGAGCCGGGTGAAGTTCAGCCGGA GCGCCGACGCCCCTGCCTACCAGCAGG GCCAGAACCAGCTGTACAACGAGCTGA ACCTGGGCCGGAGGGAGGAGTACGAC GTGCTGGACAAGCGGAGAGGCCGGGA CCCTGAGATGGGCGGCAAGCCCCGGA GAAAGAACCCTCAGGAGGGCCTGTATA ACGAACTGCAGAAAGACAAGATGGCC GAGGCCTACAGCGAGATCGGCATGAA GGGCGAGCGGCGGAGGGGCAAGGGCC ACGACGGCCTGTACCAGGGCCTGAGCA CCGCCACCAAGGATACCTACGACGCCC TGCACATGCAGGCCCTGCCCCCCAGA
hROR1 (VL-VH)_05. CD8a(3x).CD28z	76	DIQMTQSPSSLSASVG DRVTITCKASPDINSY LSWYQQKPGKAPKLL IYRANRLVDGVPSRFS GSGSGTDFTLTISSLQ PEDIATYYCLQYDEFP YTFGQGTKLEIKGGG GSGGGGSGGGGSEVQ LVESGGGLVQPGGSL RLSCAASGFTFSSYA MSWVRQAPGKGLEW VSSISRGGTTYYPDSV KGRFTISRDNSKNTLY LQMNSLRAEDTAVY YCGRYDYDGYYAMD YWGQGTLVTVSSKPT TTPAPRPPTPAPTIASQ PLSLRPEASRPAAGG AVHTRGLDFASDKPT TTPAPRPPTPAPTIASQ PLSLRPEASRPAAGG AVHTRGLDFASDKPT TTPAPRPPTPAPTIASQ PLSLRPEACRPAAGG AVHTRGLDFACDIYI	223	GATATTCAGATGACCCAGTCACCTTCG AGTCTGAGCGCATCCGTGGGCGACAGA GTGACCATTACCTGTAAGGCCAGCCCG GACATTAACAGCTACCTATCGTGGTAT CAGCAAAAGCCTGGTAAGGCCCCTAAA CTCCTTATCTACAGGGCTAATAGGTTG GTAGACGGGGTGCCTAGCCGGTTCTCT GGTTCCGGCAGCGGTACGGACTTTACT CTGACCATAAGCTCTCTGCAACCAGAA GACATCGCAACATACTACTGTTTACAA TACGACGAATTTCCTTATACCTTTGGCC AGGGGACCAAGTTAGAGATCAAGGGG GGCGGCGGAAGTGGTGGAGGGGGAAG TGGTGGAGGAGGAAGCGAAGTGCAAC TGGTCGAGTCTGGGGGCGGCCTTGTGC AACCTGGAGGCAGCCTTCGACTCAGTT GCGCCGCGTCTGGTTTTACCTTCTCCTC TTACGCGATGAGCTGGGTTCGCCAGGC CCCCGGCAAGGGACTTGAGTGGGTTAG TTCGATCTCCCGCGGAGGCACCACATA TTATCCTGACTCGGTTAAGGGACGCTT CACTATCTCTAGGGACAATTCAAAGAA CACACTGTATCTCCAAATGAACTCCTT GCGGGCCGAGGACACTGCTGTGTATTA TTGCGGACGATACGACTACGATGGGTA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		WAPLAGTCGVLLLSL VITLYCNHRNRSKRS RGGHSDYMNMTPRR PGPTRKHYQPYAPPR DFAAYRSRVKFSRSA DAPAYQQGQNQLYN ELNLGRREEYDVLDK RRGRDPEMGGKPRR KNPQEGLYNELQKDK MAEAYSEIGMKGERR RGKGHDGLYQGLST ATKDTYDALHMQAL PPR		TTACGCCATGGATTACTGGGGGCAAGG TACACTGGTCACTGTGAGTTCGAAGCC TACCACCACCCCCGCACCTCGTCCTCC AACCCCTGCACCTACGATTGCCAGTCA GCCTCTTTCACTGCGGCCTGAGGCCAG CAGACCAGCTGCCGGCGGTGCCGTCCA TACAAGAGGACTGGACTTCGCGTCCGA TAAACCTACTACCACTCCAGCCCCAAG GCCCCCAACCCCAGCACCGACTATCGC ATCACAGCCTTTGTCACTGCGTCCTGA AGCCAGCCGGCCAGCTGCAGGGGGGG CCGTCCACACAAGGGGACTCGACTTTG CGAGTGATAAGCCCACCACCACCCCTG CCCCTAGACCTCCAACCCCAGCCCCTA CAATCGCCAGCCAGCCCCTGAGCCTGA GGCCCGAAGCCTGTAGACCTGCCGCTG GCGGAGCCGTGCACACCAGAGGCCTG GATTTCGCCTGCGACATCTACATCTGG GCCCCTCTGGCCGGCACCTGTGGCGTG CTGCTGCTGAGCCTGGTCATCACCCTG TACTGCAACCACCGGAATAGGAGCAA GCGGAGCAGAGGCGGCCACAGCGACT ACATGAACATGACCCCCCGGAGGCCTG GCCCCACCCGGAAGCACTACCAGCCCT ACGCCCCTCCCAGGGACTTCGCCGCCT ACCGGAGCCGGGTGAAGTTCAGCCGG AGCGCCGACGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAGGAGTACGA CGTGCTGGACAAGCGGAGAGGCCGGG ACCCTGAGATGGGCGGCAAGCCCCGG AGAAAGAACCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGC ACCGCCACCAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGCCCCCCAGA TGA
hROR1 (VH-VL)_14-3.CD8a(3x).CD28z	77	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVASISRGGTTYYA DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS SGGGGSGGGGSGGG GSDIQMTQSPSSLSAS VGDRVTITCKASPDIN SYLNWYQQKPGKAP KLLIYRANRLVDGVP	224	GAGGTTCAACTTGTGGAATCCGGCGGC GGGTTAGTCCAGCCCGGCGGGAGCTTG CGGCTGTCCTGCGCCGCCTCTGGATTC ACTTTTAGCTCCTATGCTATGTCTTGGG TAAGGCAGGCCCCTGGTAAAGGACTAG AGTGGGTGGCCTCGATCTCCCGTGGTG GCACTACATACTACGCCGACTCCGTTA AAGGCCGGTTTACCATCTCCCGTGACA ACTCTAAAAATACTTTGTACCTGCAAA TGAACTCCCTGCGGGCAGAAGACACAG CCGTGTACTATTGCGGGCGTTACGATT ACGACGGATATTACGCAATGGACTACT GGGGCCAGGGCACACTGGTCACCGTGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		SRFSGSGSGTDFTLTIS SLQPEDIATYYCLQY DEFPYTFGGGTKVEIK KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEACRPAAG GAVHTRGLDFACDIY IWAPLAGTCGVLLLS LVITLYCNHRNRSKR SRGGHSDYMNMTPR RPGPTRKHYQPYAPP RDFAAYRSRVKFSRS ADAPAYQQGQNQLY NELNLGRREEYDVLD KRRGRDPEMGGKPR RKNPQEGLYNELQKD KMAEAYSEIGMKGER RRGKGHDGLYQGLST ATKDTYDALHMQAL PPR		GCAGCGGGGGCGGAGGAAGTGGAGGA GGCGGTAGTGGTGGGGGAGGAAGCGA TATACAAATGACTCAGTCCCCTAGTAG CCTTAGTGCTAGTGTGGGAGACAGAGT GACCATCACCTGCAAAGCATCTCCTGA TATCAATTCCTACCTTAACTGGTATCAA CAGAAGCCTGGCAAAGCTCCAAAGCTC CTGATTTATCGCGCGAACAGATTGGTC GATGGGGTCCCTTCCAGATTCAGCGGC TCAGGGTCAGGGACCGATTTCACCCTC ACAATTAGTTCACTTCAGCCCGAGGAC ATCGCCACGTATTATTGCCTTCAGTAC GATGAGTTCCCTTACACCTTTGGCGGG GGAACTAAAGTCGAAATTAAGAAGCCT ACCACCACCCCCGCACCTCGTCCTCCA ACCCCTGCACCTACGATTGCCAGTCAG CCTCTTTCACTGCGGCCTGAGGCCAGC AGACCAGCTGCCGGCGGTGCCGTCCAT ACAAGAGGACTGGACTTCGCGTCCGAT AAACCTACTACCACTCCAGCCCCAAGG CCCCCAACCCCAGCACCGACTATCGCA TCACAGCCTTTGTCACTGCGTCCTGAA GCCAGCCGGCCAGCTGCAGGGGGGGC CGTCCACACAAGGGGACTCGACTTTGC GAGTGATAAGCCCACCACCACCCCTGC CCCTAGACCTCCAACCCCAGCCCCTAC AATCGCCAGCCAGCCCCTGAGCCTGAG GCCCGAAGCCTGTAGACCTGCCGCTGG CGGAGCCGTGCACACCAGAGGCCTGG ATTTCGCCTGCGACATCTACATCTGGG CCCCTCTGGCCGGCACCTGTGGCGTGC TGCTGCTGAGCCTGGTCATCACCCTGT ACTGCAACCACCGGAATAGGAGCAAG CGGAGCAGAGGCGGCCACAGCGACTA CATGAACATGACCCCCCGGAGGCCTGG CCCCACCCGGAAGCACTACCAGCCCTA CGCCCCTCCCAGGGACTTCGCCGCCTA CCGGAGCCGGGTGAAGTTCAGCCGGA GCGCCGACGCCCCTGCCTACCAGCAGG GCCAGAACCAGCTGTACAACGAGCTGA ACCTGGGCCGGAGGGAGGAGTACGAC GTGCTGGACAAGCGGAGAGGCCGGGA CCCTGAGATGGGCGGCAAGCCCCGGA GAAAGAACCCTCAGGAGGGCCTGTATA ACGAACTGCAGAAAGACAAGATGGCC GAGGCCTACAGCGAGATCGGCATGAA GGGCGAGCGGCGGAGGGGCAAGGGCC ACGACGGCCTGTACCAGGGCCTGAGCA CCGCCACCAAGGATACCTACGACGCCC TGCACATGCAGGCCCTGCCCCCCAGA
hROR1 (VH-VL)	78	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS	225	GAAGTGCAGCTTGTGGAGTCAGGAGG AGGGCTAGTTCAGCCAGGCGGCTCTCT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
_14-4.CD8a(3x).CD28z		YAMSWVRQAPGKGLEWVASISRGGTTYYPDSVKGRFTISRDNVRNILYLQMSSLRSEDTAMYYCGRYDYDGYYAMDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCKASPDINSYLNWYQQKPGKAPKLLIYRANRLVDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCLQYDEFPYTFGAGTKVEIKKPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDKPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR		GAGACTATCTTGTGCTGCCTCCGGCTTCACATTTAGCTCTTATGCAATGTCCTGGGTCCGCCAGGCCCCTGGTAAAGGCCTGGAATGGGTTGCTTCTATCTCTAGAGGCGGAACCACTTACTACCCTGATTCAGTGAAGGGGAGATTCACAATTAGTAGGGACAACGTGCGGAACATCCTCTACCTACAGATGTCAAGTTTACGCAGTGAGGACACTGCGATGTATTACTGCGGTCGATACGATTATGATGGATATTATGCAATGGATTATTGGGGCCAGGGCACTCTGGTCACAGTATCTTCCGGCGGCGGTGGTTCTGGCGGTGGTGGAAGCGGAGGGGGGGGTCCGACATCCAGATGACCCAATCACCATCGAGTCTTAGTGCATCCGTTGGGGATAGAGTGACAATCACTTGTAAGGCATCCCCGGACATCAACTCATATCTTAATTGGTATCAGCAAAAGCCGGGCAAGGCCCCTAAGCTCCTGATTTATAGGGCCAACCGCCTTGTGGATGGAGTCCCCTCCCGCTTTAGTGGAAGCGGCTCTGGCACAGACTACACCCTGACTATCAGCTCCTTGCAGCCTGAGGATTTTGCTACCTACTACTGTCTTCAGTACGATGAATTTCCATACACTTTCGGTGCTGGGACAAAAGTGGAGATCAAAAAGCCTACCACCACCCCCGCACCTCGTCCTCCAACCCCTGCACCTACGATTGCCAGTCAGCCTCTTTCACTGCGGCCTGAGGCCAGCAGACCAGCTGCCGGCGGTGCCGTCCATACAAGAGGACTGGACTTCGCGTCCGATAAACCTACTACCACTCCAGCCCCAAGGCCCCCAACCCCAGCACCGACTATCGCATCACAGCCTTTGTCACTGCGTCCTGAAGCCAGCCGGCCAGCTGCAGGGGGGGCCGTCCACACAAGGGGACTCGACTTTGCGAGTGATAAGCCCACCACCACCCCTGCCCCTAGACCTCCAACCCCAGCCCCTACAATCGCCAGCCAGCCCCTGAGCCTGAGGCCCGAAGCCTGTAGACCTGCCGCTGGCGGAGCCGTGCACACCAGAGGCCTGGATTTCGCCTGCGACATCTACATCTGGGCCCCTCTGGCCGGCACCTGTGGCGTGCTGCTGCTGAGCCTGGTCATCACCCTGTACTGCAACCACCGGAATAGGAGCAAGCGGAGCAGAGGCGGCCACAGCGACTACATGAACATGACCCCCCGGAGGCCTGGCCCCACCCGGAAGCACTACCAGCCCTACGCCCCTCCCAGGGACTTCGCCGCCTACCGGAGCCGGGTGAAGTTCAGCCGGAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCCGGAGGGAGGAGTACGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CGTGCTGGACAAGCGGAGAGGCCGGG ACCCTGAGATGGGCGGCAAGCCCCGG AGAAAGAACCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGC ACCGCCACCAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGCCCCCCAGA
hROR1 (VH_5-VL_14). CD8a(3x) .CD28z	79	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSSISRGGTTYYP DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS SGGGGSGGGGSGGG GSDIQMTQSPSSLSAS VGDRVTITCKASPDIN SYLNWYQQKPGKAP KLLIYRANRLVDGVP SRFSGSGSGTDYTLTI SSLQPEDFATYYCLQ YDEFPYTFGAGTKVEI KKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEACRP AAGGAVHTRGLDFA CDIYIWAPLAGTCGV LLLSLVITLYCNHRNR SKRSRGGHSDYMNM TPRRPGPTRKHYQPY APPRDFAAYRSRVKF SRSADAPAYQQGQN QLYNELNLGRREEYD VLDKRRGRDPEMGG KPRRKNPQEGLYNEL QKDKMAEAYSEIGM KGERRRGKGHDGLY QGLSTATKDTYDALH MQALPPR	226	GAAGTGCAACTGGTCGAGTCTGGGGGC GGCCTTGTGCAACCTGGAGGCAGCCTT CGACTCAGTTGCGCCGCGTCTGGTTTT ACCTTCTCCTCTTACGCGATGAGCTGG GTTCGCCAGGCCCCCGGCAAGGGACTT GAGTGGGTTAGTTCGATCTCCCGCGGA GGCACCACATATTATCCTGACTCGGTT AAGGGACGCTTCACTATCTCTAGGGAC AATTCAAAGAACACACTGTATCTCCAA ATGAACTCCTTGCGGGCCGAGGACACT GCTGTGTATTATTGCGGACGATACGAC TACGATGGGTATTACGCCATGGATTAC TGGGGGCAAGGTACACTGGTCACTGTG AGTTCGGGGGGCGGCGGAAGTGGTGG AGGGGGAAGTGGTGGAGGAGGAAGCG ATATACAGATGACACAGAGCCCTTCAA GTTTATCTGCAAGCGTCGGCGATCGTG TTACAATAACTTGCAAGGCATCTCCCG ACATCAATTCCTACCTCAACTGGTATC AGCAGAAGCCTGGGAAGGCTCCTAAG CTGCTTATTTACAGAGCAAATCGCCTG GTGGACGGCGTGCCCAGTCGGTTTTCC GGGTCTGGGAGCGGAACGGATTACAC ACTGACCATCTCAAGCCTGCAACCCGA AGACTTCGCTACATATTACTGCCTTCA GTATGATGAGTTCCCATATACCTTCGG CGCTGGGACCAAGGTGGAGATAAAGA AGCCTACCACCACCCCCGCACCTCGTC CTCCAACCCCTGCACCTACGATTGCCA GTCAGCCTCTTTCACTGCGGCCTGAGG CCAGCAGACCAGCTGCCGGCGGTGCCG TCCATACAAGAGGACTGGACTTCGCGT CCGATAAACCTACTACCACTCCAGCCC CAAGGCCCCCAACCCCAGCACCGACTA TCGCATCACAGCCTTTGTCACTGCGTCC TGAAGCCAGCCGGCCAGCTGCAGGGG GGGCCGTCCACACAAGGGGACTCGACT TTGCGAGTGATAAGCCCACCACCACCC CTGCCCCTAGACCTCCAACCCCAGCCC CTACAATCGCCAGCCAGCCCCTGAGCC TGAGGCCCGAAGCCTGTAGACCTGCCG CTGGCGGAGCCGTGCACACCAGAGGCC TGGATTTCGCCTGCGACATCTACATCT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GGGCCCCTCTGGCCGGCACCTGTGGCG TGCTGCTGCTGAGCCTGGTCATCACCC TGTACTGCAACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCCACAGCGAC TACATGAACATGACCCCCCGGAGGCCT GGCCCCACCCGGAAGCACTACCAGCCC TACGCCCCTCCCAGGGACTTCGCCGCC TACCGGAGCCGGGTGAAGTTCAGCCGG AGCGCCGACGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAGGAGTACGA CGTGCTGGACAAGCGGAGAGGCCGGG ACCCTGAGATGGGCGGCAAGCCCCGG AGAAAGAACCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGC ACCGCCACCAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGCCCCCCAGA
hROR1 (VH_5-VL_16). CD8a(3x) .CD28z	80	EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YAMSWVRQAPGKGL EWVSSISRGGTTYYP DSVKGRFTISRDNSK NTLYLQMNSLRAEDT AVYYCGRYDYDGYY AMDYWGQGTLVTVS SGGGGSGGGGSGGG GSDIQMTQSPSSLSAS VGDRVTITCKASPDIN SYLNWYQQKPGKAP KVLIYRANRLVDGVP SRFSGSGSGTDYTLTI SSLQPEDFATYYCLQ YDEFPYTFGQGTKVEI KKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEASRP AAGGAVHTRGLDFAS DKPTTTPAPRPPTPAP TIASQPLSLRPEACRP AAGGAVHTRGLDFA CDIYIWAPLAGTCGV LLLSLVITLYCNHRNR SKRSRGGHSDYMNM TPRRPGPTRKHYQPY APPRDFAAYRSRVKF SRSADAPAYQQGQN QLYNELNLGRREEYD	227	GAAGTGCAACTGGTCGAGTCTGGGGGC GGCCTTGTGCAACCTGGAGGCAGCCTT CGACTCAGTTGCGCCGCGTCTGGTTTT ACCTTCTCCTCTTACGCGATGAGCTGG GTTCGCCAGGCCCCCGGCAAGGGACTT GAGTGGGTTAGTTCGATCTCCCGCGGA GGCACCACATATTATCCTGACTCGGTT AAGGGACGCTTCACTATCTCTAGGGAC AATTCAAAGAACACACTGTATCTCCAA ATGAACTCCTTGCGGGCCGAGGACACT GCTGTGTATTATTGCGGACGATACGAC TACGATGGGTATTACGCCATGGATTAC TGGGGGCAAGGTACACTGGTCACTGTG AGTTCGGGGGGCGGCGGAAGTGGTGG AGGGGGAAGTGGTGGAGGAGGAAGCG ATATTCAGATGACCCAGTCGCCCAGCA GTCTCTCGGCCTCAGTGGGCGACCGGG TCACTATCACTTGCAAAGCAAGTCCTG ATATAAACTCCTATCTTAATTGGTATCA GCAGAAGCCCGGCAAGGCACCTAAGG TTCTGATATATCGCGCAAATCGGCTCG TGGATGGAGTACCCAGCCGATTTTCCG GCAGCGGCTCAGGCACTGACTACACAC TGACAATCAGCAGCTTGCAGCCTGAAG ATTTCGCCACATACTATTGTCTACAGTA CGACGAGTTCCCTTATACATTCGGCCA GGGGACCAAGGTCGAGATCAAGAAGC CTACCACCACCCCCGCACCTCGTCCTC CAACCCCTGCACCTACGATTGCCAGTC AGCCTCTTTCACTGCGGCCTGAGGCCA GCAGACCAGCTGCCGGCGGTGCCGTCC ATACAAGAGGACTGGACTTCGCGTCCG

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		VLDKRRGRDPEMGG KPRRKNPQEGLYNEL QKDKMAEAYSEIGM KGERRRGKGHDGLY QGLSTATKDTYDALH MQALPPR		ATAAACCTACTACCACTCCAGCCCCAA GGCCCCCAACCCCAGCACCGACTATCG CATCACAGCCTTTGTCACTGCGTCCTG AAGCCAGCCGGCCAGCTGCAGGGGGG GCCGTCCACACAAGGGGACTCGACTTT GCGAGTGATAAGCCCACCACCACCCCT GCCCCTAGACCTCCAACCCCAGCCCCT ACAATCGCCAGCCAGCCCCTGAGCCTG AGGCCCGAAGCCTGTAGACCTGCCGCT GGCGGAGCCGTGCACACCAGAGGCCT GGATTTCGCCTGCGACATCTACATCTG GGCCCCTCTGGCCGGCACCTGTGGCGT GCTGCTGCTGAGCCTGGTCATCACCCT GTACTGCAACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCCACAGCGAC TACATGAACATGACCCCCCGGAGGCCT GGCCCCACCCGGAAGCACTACCAGCCC TACGCCCCTCCCAGGGACTTCGCCGCC TACCGGAGCCGGGTGAAGTTCAGCCGG AGCGCCGACGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAGGAGTACGA CGTGCTGGACAAGCGGAGAGGCCGGG ACCCTGAGATGGGCGGCAAGCCCCGG AGAAAGAACCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGC ACCGCCACCAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGCCCCCCAGA
hROR1 (VH_18-VL_04).CD8a(3x).CD28z	81	EVQLVESGGGLVQPG GSLRLSCSASGFTFSS YAMSWVRQVPGKGL VWISSISRGGTTYYAD SVRGRFIISRDNAKNT LYLEMNNLRGEDTA VYYCARYDYDGYYA MDYWGQGTLVTVSS GGGGSGGGGSGGGG SDIQMTQSPSSLSASV GDRVTITCQASPDINS YLNWYQQKPGKAPK LLIYRANNLETGVPSR FSGSGSGTDFTLTISSL QPEDIATYYCLQYDE FPYTFGQGTKLEIKKP TTTPAPRPPTPAPTIAS QPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEASRPAAG	228	GAGGTTCAACTCGTGGAGTCTGGAGGC GGGCTAGTGCAGCCTGGCGGCTCCCTG CGACTGTCTTGCAGCGCATCAGGCTTT ACATTCAGTTCTTATGCCATGAGCTGG GTGAGGCAGGTGCCCGGCAAGGGTCTG GTGTGGATCAGCTCAATCTCCAGGGGC GGGACTACATATTACGCCGATTCGGTC AGGGGTCGTTTTATCATTAGCAGGGAT AATGCCAAGAACACCTTGTATTTGGAG ATGAACAACCTAAGAGGCGAAGACAC CGCTGTGTACTATTGTGCCCGTTACGA CTACGATGGGTACTACGCCATGGACTA TTGGGGCCAGGGAACCTTGGTGACTGT GTCAAGTGGCGGGGGCGGCAGCGGAG GCGGTGGCAGCGGAGGCGGCGGTTCTG ATATTCAAATGACGCAAAGTCCCAGCA GCCTCTCCGCCTCCGTTGGAGACAGGG TGACTATTACATGCCAAGCCAGCCCCG ATATTAATAGCTACTTAAATTGGTATC AGCAGAAACCTGGGAAGGCACCTAAA CTTCTCATCTACCGCGCTAACAATCTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEACRPAAG GAVHTRGLDFACDIY IWAPLAGTCGVLLLS LVITLYCNHRNRSKR SRGGHSDYMNMTPR RPGPTRKHYQPYAPP RDFAAYRSRVKFSRS ADAPAYQQGQNQLY NELNLGRREEYDVLD KRRGRDPEMGGKPR RKNPQEGLYNELQKD KMAEAYSEIGMKGER RRGKGHDGLYQGLST ATKDTYDALHMQAL PPR		GAGACCGGCGTGCCGTCTAGATTTTCC GGCTCTGGATCAGGGACCGATTTTACT CTGACAATTAGTTCCCTGCAACCCGAA GACATCGCCACTTATTATTGCCTGCAA TATGATGAGTTTCCTTACACATTTGGTC AGGGAACTAAACTAGAGATTAAGAAG CCTACCACCACCCCCGCACCTCGTCCT CCAACCCCTGCACCTACGATTGCCAGT CAGCCTCTTTCACTGCGGCCTGAGGCC AGCAGACCAGCTGCCGGCGGTGCCGTC CATACAAGAGGACTGGACTTCGCGTCC GATAAACCTACTACCACTCCAGCCCCA AGGCCCCCAACCCCAGCACCGACTATC GCATCACAGCCTTTGTCACTGCGTCCT GAAGCCAGCCGGCCAGCTGCAGGGGG GGCCGTCCACACAAGGGGACTCGACTT TGCGAGTGATAAGCCCACCACCACCCC TGCCCCTAGACCTCCAACCCCAGCCCC TACAATCGCCAGCCAGCCCCTGAGCCT GAGGCCCGAAGCCTGTAGACCTGCCGC TGGCGGAGCCGTGCACACCAGAGGCCT GGATTTCGCCTGCGACATCTACATCTG GGCCCCTCTGGCCGGCACCTGTGGCGT GCTGCTGCTGAGCCTGGTCATCACCCT GTACTGCAACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCCACAGCGAC TACATGAACATGACCCCCCGGAGGCCT GGCCCCACCCGGAAGCACTACCAGCCC TACGCCCCTCCCAGGGACTTCGCCGCC TACCGGAGCCGGGTGAAGTTCAGCCGG AGCGCCGACGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAGGAGTACGA CGTGCTGGACAAGCGGAGAGGCCGGG ACCCTGAGATGGGCGGCAAGCCCCGG AGAAAGAACCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGC ACCGCCACCAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGCCCCCCAGA
hROR1 (VH_18-VL_14).CD8a(3x).CD28z	82	EVQLVESGGGLVQPG GSLRLSCSASGFTFSS YAMSWVRQVPGKGL VWISSISRGGTTYYAD SVRGRFIISRDNAKNT LYLEMNNLRGEDTA VYYCARYDYDGYYA MDYWGQGTLVTVSS GGGGSGGGGSGGGG SDIQMTQSPSSLSASV	229	GAGGTTCAACTCGTGGAGTCTGGAGGC GGGCTAGTGCAGCCTGGCGGCTCCCTG CGACTGTCTTGCAGCGCATCAGGCTTT ACATTCAGTTCTTATGCCATGAGCTGG GTGAGGCAGGTGCCCGGCAAGGGTCTG GTGTGGATCAGCTCAATCTCCAGGGGC GGGACTACATATTACGCCGATTCGGTC AGGGGTCGTTTTATCATTAGCAGGGAT AATGCCAAGAACACCTTGTATTTGGAG ATGAACAACCTAAGAGGCGAAGACAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		GDRVTITCKASPDINS YLNWYQQKPGKAPK LLIYRANRLVDGVPS RFSGSGSGTDYTLTIS SLQPEDFATYYCLQY DEFPYTFGAGTKVEIK KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEACRPAAG GAVHTRGLDFACDIY IWAPLAGTCGVLLLS LVITLYCNHRNRSKR SRGGHSDYMNMTPR RPGPTRKHYQPYAPP RDFAAYRSRVKFSRS ADAPAYQQGQNQLY NELNLGRREEYDVLD KRRGRDPEMGGKPR RKNPQEGLYNELQKD KMAEAYSEIGMKGER RRGKGHDGLYQGLST ATKDTYDALHMQAL PPR		CGCTGTGTACTATTGTGCCCGTTACGA CTACGATGGGTACTACGCCATGGACTA TTGGGGCCAGGGAACCTTGGTGACTGT GTCAAGTGGCGGGGGCGGCAGCGGAG GCGGTGGCAGCGGAGGCGGCGGTTCTG ATATACAGATGACACAGAGCCCTTCAA GTTTATCTGCAAGCGTCGGCGATCGTG TTACAATAACTTGCAAGGCATCTCCCG ACATCAATTCCTACCTCAACTGGTATC AGCAGAAGCCTGGGAAGGCTCCTAAG CTGCTTATTTACAGAGCAAATCGCCTG GTGGACGGCGTGCCCAGTCGGTTTTCC GGGTCTGGGAGCGGAACGGATTACAC ACTGACCATCTCAAGCCTGCAACCCGA AGACTTCGCTACATATTACTGCCTTCA GTATGATGAGTTCCCATATACCTTCGG CGCTGGGACCAAGGTGGAGATAAAGA AGCCTACCACCACCCCCGCACCTCGTC CTCCAACCCCTGCACCTACGATTGCCA GTCAGCCTCTTTCACTGCGGCCTGAGG CCAGCAGACCAGCTGCCGGCGGTGCCG TCCATACAAGAGGACTGGACTTCGCGT CCGATAAACCTACTACCACTCCAGCCC CAAGGCCCCCAACCCCAGCACCGACTA TCGCATCACAGCCTTTGTCACTGCGTCC TGAAGCCAGCCGGCCAGCTGCAGGGG GGGCCGTCCACACAAGGGGACTCGACT TTGCGAGTGATAAGCCCACCACCACCC CTGCCCCTAGACCTCCAACCCCAGCCC CTACAATCGCCAGCCAGCCCCTGAGCC TGAGGCCCGAAGCCTGTAGACCTGCCG CTGGCGGAGCCGTGCACACCAGAGGCC TGGATTTCGCCTGCGACATCTACATCT GGGCCCCTCTGGCCGGCACCTGTGGCG TGCTGCTGCTGAGCCTGGTCATCACCC TGTACTGCAACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCCACAGCGAC TACATGAACATGACCCCCCGGAGGCCT GGCCCCACCCGGAAGCACTACCAGCCC TACGCCCCTCCCAGGGACTTCGCCGCC TACCGGAGCCGGGTGAAGTTCAGCCGG AGCGCCGACGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAGGAGTACGA CGTGCTGGACAAGCGGAGAGGCCGGG ACCCTGAGATGGGCGGCAAGCCCCGG AGAAAGAACCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGGGCAAGGGC CACGACGGCCTGTACCAGGGCCTGAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCGCCACCAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGCCCCCCAGA
CD8α hinge	83	KPTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGG AVHTRGLDFACD	230	AAGCCCACCACCACCCCTGCCCCTAGACCT CCAACCCCAGCCCCTACAATCGCCAGCCAG CCCCTGAGCCTGAGGCCCGAAGCCTGTAG ACCTGCCGCTGGCGGAGCCGTGCACACCA GAGGCCTGGATTTCGCCTGCGAC
CD8α 2x	84	KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAG GAVHTRGLDFACD	231	AAACCTACTACAACTCCTGCCCCCCGG CCTCCTACACCAGCTCCTACTATCGCCT CCCAGCCACTCAGTCTCAGACCCGAGG CTTCTAGGCCAGCGGCCGGAGGCGCGG TCCACACCCGCGGGCTGGACTTTGCAT CCGATAAGCCCACCACCACCCCTGCCC CTAGACCTCCAACCCCAGCCCCTACAA TCGCCAGCCAGCCCCTGAGCCTGAGGC CCGAAGCCTGTAGACCTGCCGCTGGCG GAGCCGTGCACACCAGAGGCCTGGATT TCGCCTGCGAC
CD8α 3x	85	KPTTTPAPRPPTPAPTI ASQPLSLRPEASRPAA GGAVHTRGLDFASDK PTTTPAPRPPTPAPTIA SQPLSLRPEASRPAAG GAVHTRGLDFASDKP TTTPAPRPPTPAPTIAS QPLSLRPEACRPAAG GAVHTRGLDFACD	232	AAGCCTACCACCACCCCCGCACCTCGT CCTCCAACCCCTGCACCTACGATTGCC AGTCAGCCTCTTTCACTGCGGCCTGAG GCCAGCAGACCAGCTGCCGGCGGTGCC GTCCATACAAGAGGACTGGACTTCGCG TCCGATAAACCTACTACCACTCCAGCC CCAAGGCCCCCAACCCCAGCACCGACT ATCGCATCACAGCCTTTGTCACTGCGT CCTGAAGCCAGCCGGCCAGCTGCAGGG GGGGCCGTCCACACAAGGGGACTCGA CTTTGCGAGTGATAAGCCCACCACCAC CCCTGCCCCTAGACCTCCAACCCCAGC CCCTACAATCGCCAGCCAGCCCCTGAG CCTGAGGCCCGAAGCCTGTAGACCTGC CGCTGGCGGAGCCGTGCACACCAGAG GCCTGGATTTCGCCTGCGAC
CD8α 4x	86	TTPAPRPPTPAPTIASQ PLSLRPEASRPAAGG AVHTRGLDFASDKPT TTPAPRPPTPAPTIASQ PLSLRPEASRPAAGG AVHTRGLDFASDKPT TTPAPRPPTPAPTIASQ PLSLRPEASRPAAGG AVHTRGLDFASDKPT TTPAPRPPTPAPTIASQ PLSLRPEACRPAAGG AVHTRGLDFACD	233	AAGCCTACCACCACCCCCGCACCTCGT CCTCCAACCCCTGCACCTACGATTGCC AGTCAGCCTCTTTCACTGCGGCCTGAG GCCAGCAGACCAGCTGCCGGCGGTGCC GTCCATACAAGAGGACTGGACTTCGCG TCCGATAAACCTACTACCACTCCAGCC CCAAGGCCCCCAACCCCAGCACCGACT ATCGCATCACAGCCTTTGTCACTGCGT CCTGAAGCCAGCCGGCCAGCTGCAGGG GGGGCCGTCCACACAAGGGGACTCGA CTTTGCGAGTGATAAACCTACTACAAC TCCTGCCCCCCGGCCTCCTACACCAGC TCCTACTATCGCCTCCCAGCCACTCAGT CTCAGACCCGAGGCTTCTAGGCCAGCG GCCGGAGGCGCGGTCCACACCCGCGG GCTGGACTTTGCATCCGATAAGCCCAC CACCACCCCTGCCCCTAGACCTCCAAC CCCAGCCCCTACAATCGCCAGCCAGCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCTGAGCCTGAGGCCCGAAGCCTGTAG ACCTGCCGCTGGCGGAGCCGTGCACAC CAGAGGCCTGGATTTCGCCTGCGAC
CD8α TM	87	IYIWAPLAGTCGVLLLS LVITLYCNHRN	234	ATCTACATCTGGGCCCCTCTGGCCGGCACC TGTGGCGTGCTGCTGCTGAGCCTGGTCATC ACCCTGTACTGCAACCACCGGAAT
CD28 TM	88	FWVLVVVGGVLACYSL LVTVAFIIFWV	235	TTTTGGGTGCTGGTGGTGGTTGGTGGAGTC CTGGCTTGCTATAGCTTGCTAGTAACAGTG GCCTTTATTATTTTCTGGGTG
4-1BB signaling domain	89	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL	236	AAGAGAGGCCGGAAGAAACTGCTGTACAT CTTCAAGCAGCCCTTCATGCGGCCCGTGCA GACCACCCAGGAAGAGGACGGCTGCAGCT GCCGGTTCCCCGAGGAAGAGGAAGGCGGC TGCGAACTG
CD28 signaling domain	90	RSKRSRGGHSDYMNMT PRRPGPTRKHYQPYAPP RDFAAYRS	237	AGGAGCAAGCGGAGCAGAGGCGGCCACAG CGACTACATGAACATGACCCCCCGGAGGC CTGGCCCCACCCGGAAGCACTACCAGCCCT ACGCCCCTCCCAGGGACTTCGCCGCCTACC GGAGC
DNAX-activation protein 10 (DAP10) Signaling Domain	91	LCARPRRSPAQEDGK VYINMPGRG	238	CTGTGCGCACGCCCACGCCGCAGCCCC GCCCAAGAAGATGGCAAAGTCTACATC AACATGCCAGGCAGGGGC
DNAX-activation protein 12 (DAP12) Signaling Domain	92	YFLGRLVPRGRGAAE AATRKQRITETESPYQ ELQGQRSDVYSDLNT QRPYYK	239	TACTTCCTGGGCCGGCTGGTCCCTCGG GGGCGAGGGGCTGCGGAGGCAGCGAC CCGGAAACAGCGTATCACTGAGACCGA GTCGCCTTATCAGGAGCTCCAGGGTCA GAGGTCGGATGTCTACAGCGACCTCAA CACACAGAGGCCGTATTACAAA
CD3ς signaling domain	93	RVKFSRSADAPAYQQG QNQLYNELNLGRREEY DVLDKRRGRDPEMGGK PRRKNPQEGLYNELQK DKMAEAYSEIGMKGER RRGKGHDGLYQGLSTA TKDTYDALHMQALPPR	240	CGGGTGAAGTTCAGCCGGAGCGCCGACGC CCCTGCCTACCAGCAGGGCCAGAACCAGC TGTACAACGAGCTGAACCTGGGCCGGAGG GAGGAGTACGACGTGCTGGACAAGCGGAG AGGCCGGGACCCTGAGATGGGCGGCAAGC CCCGGAGAAAGAACCCTCAGGAGGGCCTG TATAACGAACTGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATCGGCATGAAGG GCGAGCGGCGGAGGGGCAAGGGCCACGAC GGCCTGTACCAGGGCCTGAGCACCGCCAC CAAGGATACCTACGACGCCCTGCACATGC AGGCCCTGCCCCCCAGA
SIGNAL PEPTIDES				
GMCSFR alpha	94	MLLLVTSLLLCELPHP AFLLIP	241	ATGCTTCTCCTGGTGACAAGCCTTCTGC TCTGTGAGTTACCACACCCAGCATTCC TCCTGATCCCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Ig Kappa	95	MRLPAQLLGLLMLWVPGSSG	242	ATGAGGCTCCCTGCTCAGCTCCTGGGGCTGCTAATGCTCTGGGTCCCAGGATCCAGTGGG
Immuno-globulin E	96	MDWTWILFLVAAATRVHS	243	ATGGATTGGACCTGGATTCTGTTTCTGGTGGCCGCTGCCACAAGAGTGCACAGC
CD8α	97	MALPVTALLLPLALLLHAARP	244	ATGGCGCTGCCCGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCG
TVB2 (T21A)	98	MGTSLLCWMALCLLGADHADA	245	ATGGGCACCAGCCTCCTCTGCTGGATGGCCCTGTGTCTCCTGGGGGCAGATCACGCAGATGCT
CD52	99	MKRFLFLLLTISLLVMVQIQTGLS	246	ATGAAGCGCTTCCTCTTCCTCCTACTCACCATCAGCCTCCTGGTTATGGTACAGATACAAACTGGACTCTCA
Low-affinity nerve growth factor receptor (LNGFR, TNFRSF16)	100	MGAGATGRAMDGPRLLLLLLLGVSLGGA	247	ATGGGGGCAGGTGCCACCGGCCGCGCCATGGACGGGCCGCGCCTGCTGCTGTTGCTGCTTCTGGGGGTGTCCCTTGGAGGTGCC
Mouse Ig VH region 3 signal peptide	101	MGWSCIILFLVATATGVHS	248	ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCTACCGCCACCGGCGTGCACAGC
β2M signal peptide	102	MSRSVALAVLALLSLSGLEA	249	ATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCT
Azurocidin signal peptide	103	MTRLTVLALLAGLLASSRA	250	ATGACCCGGCTGACAGTCCTGGCCCTGCTGGCTGGTCTGCTGGCGTCCTCGAGGGCC
Human Serum Albumin signal peptide	104	MKWVTFISLLFLFSSAYS	251	ATGAAGTGGGTAACCTTTATTTCCCTTCTTTTTCTCTTTAGCTCGGCTTATTCC
A2M receptor associated protein signal peptide	105	MGKNKLLHPSLVLLLVLLPTDA	252	ATGGGGAAGAACAAACTCCTTCATCCAAGTCTGGTTCTTCTCCTCTTGGTCCTCCTGCCCACAGACGCC
IGHV3-23signal peptide	106	MEFGLSWLFLVAILKGVQC	253	ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGT
IGKV1-D33 (HuL1)si	107	MDMRVPAQLLGLLLWLSGARC	254	ATGGACATGAGGGTCCCTGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCTCAGGTGCCAGATGT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
gnal peptide				
IGHV3-33(L14F) (HuH7)signal peptide	108	MEFGLSWVFLVALFRGVQC	255	ATGGAGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTCTTTTTAGAGGTGTCCAGTGT
KILL SWITCH				
HER1t	109	RKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPREFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNTLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLFM	256	CGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTGGCATTTAGGGGTGACTCCTTCACACATACTCCTCCTCTGGATCCACAGGAACTGGATATTCTGAAAACCGTAAAGGAAATCACAGGGTTTTTGCTGATTCAGGCTTGGCCTGAAAACAGGACGGACCTCCATGCCTTTGAGAACCTAGAAATCATACGCGGCAGGACCAAGCAACATGGTCAGTTTTCTCTTGCAGTCGTCAGCCTGAACATAACATCCTTGGGATTACGCTCCCTCAAGGAGATAAGTGATGGAGATGTGATAATTTCAGGAAACAAAAATTTGTGCTATGCAAATACAATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAACCAAAATTATAAGCAACAGAGGTGAAAACAGCTGCAAGGCCACAGGCCAGGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGCCCAGGGACTGCGTCTCTTGCCGGAATGTCAGCCGAGGCAGGGAATGCGTGGACAAGTGCAACCTTCTGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACTCTGAGTGCATACAGTGCCACCCAGAGTGCCTGCCTCAGGCCATGAACATCACCTGCACAGGACGGGGACCAGACAACTGTATCCAGTGTGCCCACTACATTGACGGCCCCCACTGCGTCAAGACCTGCCCGGCAGGAGTCATGGGAGAAAACAACACCCTGGTCTGGAAGTACGCAGACGCCGGCCATGTGTGCCACCTGTGCCATCCAAACTGCACCTACGGATGCACTGGGCCAGGTCTTGAAGGCTGTCCAACGAATGGGCCTAAGATCCCGTCCATCGCCACTGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGTGGTGGCCCTGGGGATCGGCCTCTTCATG
HER1t- 1	110	RKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENR	257	CGCAAAGTGTGTAACGGAATAGGTATTGGTGAATTTAAAGACTCACTCTCCATAAATGCTACGAATATTAAACACTTCAAAAACTGCACCTCCATCAGTGGCGATCTCCACATCCTGCCGGTGGCATTTAGGGGT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		TDLHAFENLEIIRGRT KQHGQFSLAVVSLNI TSLGLRSLKEISDGDV IISGNKNLCYANTINW KKLFGTSGQKTKIISN RGENSCKATGQVCH ALCSPEGCWGPEPRD CVSGGGGSGGGSGG GGSGGGGSFWVLVV VGGVLACYSLLVTVA FIIFWVRSKRS		GACTCCTTCACACATACTCCTCCTCTGG ATCCACAGGAACTGGATATTCTGAAAA CCGTAAAGGAAATCACAGGGTTTTTGC TGATTCAGGCTTGGCCTGAAAACAGGA CGGACCTCCATGCCTTTGAGAACCTAG AAATCATACGCGGCAGGACCAAGCAA CATGGTCAGTTTTCTCTTGCAGTCGTCA GCCTGAACATAACATCCTTGGGATTAC GCTCCCTCAAGGAGATAAGTGATGGAG ATGTGATAATTTCAGGAAACAAAAATT TGTGCTATGCAAATACAATAAACTGGA AAAAACTGTTTGGGACCTCCGGTCAGA AAACCAAAATTATAAGCAACAGAGGT GAAAACAGCTGCAAGGCCACAGGCCA GGTCTGCCATGCCTTGTGCTCCCCCGA GGGCTGCTGGGGCCCGGAGCCCAGGG ACTGCGTCTCTGGTGGCGGTGGCTCGG GCGGTGGTGGGTCGGGTGGCGGCGGAT CTGGTGGCGGTGGCTCGTTTTGGGTGC TGGTGGTGGTTGGTGGAGTCCTGGCTT GCTATAGCTTGCTAGTAACAGTGGCCT TTATTATTTTCTGGGTGAGGAGTAAGA GGAGC
FL CD20	111	MTTPRNSVNGTFPAE PMKGPIAMQSGPKPL FRRMSSLVGPTQSFF MRESKTLGAVQIMNG LFHIALGGLLMIPAGI YAPICVTVWYPLWG GIMYIISGSLLAATEK NSRKCLVKGKMIMNS LSLFAAISGMILSIMDI LNIKISHFLKMESLNFI RAHTPYINIYNCEPAN PSEKNSPSTQYCYSIQ SLFLGILSVMLIFAFFQ ELVIAGIVENEWKRT CSRPKSNIVLLSAEEK KEQTIEIKEEVVGLTE TSSQPKNEEDIEIIPIQE EEEEETETNFPEPPQD QESSPIENDSSP	258	ATGACAACACCCAGAAATTCAGTAAAT GGGACTTTCCCGGCAGAGCCAATGAAA GGCCCTATTGCTATGCAATCTGGTCCA AAACCACTCTTCAGGAGGATGTCTTCA CTGGTGGGCCCCACGCAAAGCTTCTTC ATGAGGGAATCTAAGACTTTGGGGGCT GTCCAGATTATGAATGGGCTCTTCCAC ATTGCCCTGGGGGGTCTTCTGATGATC CCAGCAGGGATCTATGCACCCATCTGT GTGACTGTGTGGTACCCTCTCTGGGGA GGCATTATGTATATTATTTCCGGATCAC TCCTGGCAGCAACGGAGAAAAACTCCA GGAAGTGTTTGGTCAAAGGAAAAATG ATAATGAATTCATTGAGCCTCTTTGCTG CCATTTCTGGAATGATTCTTTCAATCAT GGACATACTTAATATTAAAATTTCCCA TTTTTTAAAAATGGAGAGTCTGAATTTT ATTAGAGCTCACACACCATATATTAAC ATATACAACTGTGAACCAGCTAATCCC TCTGAGAAAAACTCCCCATCTACCCAA TACTGTTACAGCATACAATCTCTGTTCT TGGGCATTTTGTCAGTGATGCTGATCTT TGCCTTCTTCCAGGAACTTGTAATAGCT GGCATCGTTGAGAATGAATGGAAAAG AACGTGCTCCAGACCCAAATCTAACAT AGTTCTCCTGTCAGCAGAAGAAAAAAA AGAACAGACTATTGAAATAAAAGAAG AAGTGGTTGGGCTAACTGAAACATCTT CCCAACCAAAGAATGAAGAAGACATT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GAAATTATTCCAATCCAAGAAGAGGAA GAAGAAGAAACAGAGACGAACTTTCC AGAACCTCCCCAAGATCAGGAATCCTC ACCAATAGAAAATGACAGCTCTCCT
CD20t-1	112	MTTPRNSVNGTFPAE PMKGPIAMQSGPKPL FRRMSSLVGPTQSFF MRESKTLGAVQIMNG LFHIALGGLLMIPAGI YAPICVTVWYPLWG GIMYIISGSLLAATEK NSRKCLVKGKMIMNS LSLFAAISGMILSIMDI LNIKISHFLKMESLNFI RAHTPYINIYNCEPAN PSEKNSPSTQYCYSIQ SLFLGILSVMLIFAFFQ ELVIAGIVENEWKRT CSRPKSNIVLLSAEEK KEQTIEIKEEVVGLTE TSSQPKNEEDIE	259	ATGACCACACCACGGAACTCTGTGAAT GGCACCTTCCCAGCAGAGCCAATGAAG GGACCAATCGCAATGCAGAGCGGACC CAAGCCTCTGTTTCGGAGAATGAGCTC CCTGGTGGGCCCAACCCAGTCCTTCTTT ATGAGAGAGTCTAAGACACTGGGCGCC GTGCAGATCATGAACGGACTGTTCCAC ATCGCCCTGGGAGGACTGCTGATGATC CCAGCCGGCATCTACGCCCCTATCTGC GTGACCGTGTGGTACCCTCTGTGGGGC GGCATCATGTATATCATCTCCGGCTCTC TGCTGGCCGCCACAGAGAAGAACAGC AGGAAGTGTCTGGTGAAGGGCAAGAT GATCATGAATAGCCTGTCCCTGTTTGC CGCCATCTCTGGCATGATCCTGAGCAT CATGGACATCCTGAACATCAAGATCAG CCACTTCCTGAAGATGGAGAGCCTGAA CTTCATCAGAGCCCACACCCCTTACAT CAACATCTATAATTGCGAGCCTGCCAA CCCATCCGAGAAGAATTCTCCAAGCAC ACAGTACTGTTATTCCATCCAGTCTCTG TTCCTGGGCATCCTGTCTGTGATGCTGA TCTTTGCCTTCTTTCAGGAGCTGGTCAT CGCCGGCATCGTGGAGAACGAGTGGA AGAGGACCTGCAGCCGCCCCAAGTCCA ATATCGTGCTGCTGTCCGCCGAGGAGA AGAAGGAGCAGACAATCGAGATCAAG GAGGAGGTGGTGGGCCTGACCGAGAC ATCTAGCCAGCCTAAGAATGAGGAGG ATATCGAG
mbIL-15				
mbIL15	113	MDWTWILFLVAAAT RVHSNWVNVISDLKK IEDLIQSMHIDATLYT ESDVHPSCKVTAMKC FLLELQVISLESGDASI HDTVENLIILANNSLS SNGNVTESGCKECEE LEEKNIKEFLQSFVHI VQMFINTSSGGGSGG GGSGGGGSGGGGSG GGSLQITCPPPMSVEH ADIWVKSYSLYSRER YICNSGFKRKAGTSSL TECVLNKATNVAHW TTPSLKCIRDPALVHQ RPAPPSTVTTAGVTPQ	260	ATGGATTGGACCTGGATTCTGTTTCTG GTGGCCGCTGCCACAAGAGTGCACAGC AACTGGGTGAATGTGATCAGCGACCTG AAGAAGATCGAGGATCTGATCCAGAG CATGCACATTGATGCCACCCTGTACAC AGAATCTGATGTGCACCCTAGCTGTAA AGTGACCGCCATGAAGTGTTTTCTGCT GGAGCTGCAGGTGATTTCTCTGGAAAG CGGAGATGCCTCTATCCACGACACAGT GGAGAATCTGATCATCCTGGCCAACAA TAGCCTGAGCAGCAATGGCAATGTGAC AGAGTCTGGCTGTAAGGAGTGTGAGGA GCTGGAGGAGAAGAACATCAAGGAGT TTCTGCAGAGCTTTGTGCACATCGTGC AGATGTTCATCAATACAAGCTCTGGCG GAGGATCTGGAGGAGGCGGATCTGGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		PESLSPSGKEPAASSP SSNNTAATTAAIVPGS QLMPSKSPSTGTTEIS SHESSHGTPSQTTAK NWELTASASHQPPGV YPQGHSDTTVAISTST VLLCGLSAVSLLACY LKSRQTPPLASVEME AMEALPVTWGTSSRD EDLENCSHHL		GGAGGAGGCAGTGGAGGCGGAGGATC TGGCGGAGGATCTCTGCAGATTACATG CCCTCCTCCAATGTCTGTGGAGCACGC CGATATTTGGGTGAAGTCCTACAGCCT GTACAGCAGAGAGAGATACATCTGCA ACAGCGGCTTTAAGAGAAAGGCCGGC ACCTCTTCTCTGACAGAGTGCGTGCTG AATAAGGCCACAAATGTGGCCCACTGG ACAACACCTAGCCTGAAGTGCATTAGA GATCCTGCCCTGGTCCACCAGAGGCCT GCCCCTCCATCTACAGTGACAACAGCC GGAGTGACACCTCAGCCTGAATCTCTG AGCCCTTCTGGAAAAGAACCTGCCGCC AGCTCTCCTAGCTCTAATAATACCGCC GCCACAACAGCCGCCATTGTGCCTGGA TCTCAGCTGATGCCTAGCAAGTCTCCT AGCACAGGCACAACAGAGATCAGCAG CCACGAATCTTCTCACGGAACACCTTC TCAGACCACCGCCAAGAATTGGGAGCT GACAGCCTCTGCCTCTCACCAGCCTCC AGGAGTGTATCCTCAGGGCCACTCTGA TACAACAGTGGCCATCAGCACATCTAC AGTGCTGCTGTGTGGACTGTCTGCCGT GTCTCTGCTGGCCTGTTACCTGAAGTCT AGACAGACACCTCCTCTGGCCTCTGTG GAGATGGAGGCCATGGAAGCCCTGCCT GTGACATGGGGAACAAGCAGCAGAGA TGAGGACCTGGAGAATTGTTCTCACCA CCTG
IL-15	114	NWVNVISDLKKIEDLI QSMHIDATLYTESDV HPSCKVTAMKCFLLE LQVISLESGDASIHDT VENLIILANNSLSSNG NVTESGCKECEELEE KNIKEFLQSFVHIVQM FINTS	261	AACTGGGTGAATGTGATCAGCGACCTG AAGAAGATCGAGGATCTGATCCAGAG CATGCACATTGATGCCACCCTGTACAC AGAATCTGATGTGCACCCTAGCTGTAA AGTGACCGCCATGAAGTGTTTTCTGCT GGAGCTGCAGGTGATTTCTCTGGAAAG CGGAGATGCCTCTATCCACGACACAGT GGAGAATCTGATCATCCTGGCCAACAA TAGCCTGAGCAGCAATGGCAATGTGAC AGAGTCTGGCTGTAAGGAGTGTGAGGA GCTGGAGGAGAAGAACATCAAGGAGT TTCTGCAGAGCTTTGTGCACATCGTGC AGATGTTCATCAATACAAGC
IL-15Rα	115	ITCPPPMSVEHADIWV KSYSLYSRERYICNSG FKRKAGTSSLTECVL NKATNVAHWTTPSL KCIRDPALVHQRPAPP STVTTAGVTPQPESLS PSGKEPAASSPSSNNT AATTAAIVPGSQLMP SKSPSTGTTEISSHESS HGTPSQTTAKNWELT	262	ATTACATGCCCTCCTCCAATGTCTGTGG AGCACGCCGATATTTGGGTGAAGTCCT ACAGCCTGTACAGCAGAGAGAGATAC ATCTGCAACAGCGGCTTTAAGAGAAAG GCCGGCACCTCTTCTCTGACAGAGTGC GTGCTGAATAAGGCCACAAATGTGGCC CACTGGACAACACCTAGCCTGAAGTGC ATTAGAGATCCTGCCCTGGTCCACCAG AGGCCTGCCCCTCCATCTACAGTGACA ACAGCCGGAGTGACACCTCAGCCTGAA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		ASASHQPPGVYPQGHSDTTVAISTSTVLLCGLSAVSLLACYLKSRQTPPLASVEMEAMEALPVTWGTSSRDEDLENCSHHL		TCTCTGAGCCCTTCTGGAAAAGAACCTGCCGCCAGCTCTCCTAGCTCTAATAATACCGCCGCCACAACAGCCGCCATTGTGCCTGGATCTCAGCTGATGCCTAGCAAGTCTCCTAGCACAGGCACAACAGAGATCAGCAGCCACGAATCTTCTCACGGAACACCTTCTCAGACCACCGCCAAGAATTGGGAGCTGACAGCCTCTGCCTCTCACCAGCCTCCAGGAGTGTATCCTCAGGGCCACTCTGATACAACAGTGGCCATCAGCACATCTACAGTGCTGCTGTGTGGACTGTCTGCCGTGTCTCTGCTGGCCTGTTACCTGAAGTCTAGACAGACACCTCCTCTGGCCTCTGTGGAGATGGAGGCCATGGAAGCCCTGCCTGTGACATGGGGAACAAGCAGCAGAGATGAGGACCTGGAGAATTGTTCTCACCACCTG
LINKERS				
T2A	116	EGRGSLLTCGDVEENPGP	263	GAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCT
Furin-GSG-T2A	117	RAKRGSGEGRGSLLTCGDVEENPGP	264	AGAGCTAAGAGGGGAAGCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGAGAATCCTGGACCT
Furin-SGSG-T2A	118	RAKRSGSGEGRGSLLTCGDVEENPGP	265	AGGGCCAAGAGGAGTGGCAGCGGCGAGGGCAGAGGAAGTCTTCTAACATGCGGTGACGTGGAGGAGAATCCCGGCCCT
Porcine tescho-virus-1 2A region (P2A)	119	ATNFSLLKQAGDVEENPGP	266	GCAACGAACTTCTCTCTCCTAAAACAGGCTGGTGATGTGGAGGAGAATCCTGGTCCA
GSG-p2a	120	GSGATNFSLLKQAGDVEENPGP	267	GGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT
fp2a	121	RAKRAPVKQGSGATNFSLLKQAGDVEENPGP	268	CGTGCAAAGCGTGCACCGGTGAAACAGGGAAGCGGAGCTACTAACTTCAGCCTGCTGAAGCAGGCTGGAGACGTGGAGGAGAACCCTGGACCT
Equine rhinitis A virus 2A region (E2A)	122	QCTNYALLKLAGDVESNPGP	269	CAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGAGCAACCCTGGACCT
Foot-and-mouth disease virus 2A region (F2A)	123	VKQTLNFDLLKLAGDVESNPGP	270	GTCAAACAGACCCTAAACTTTGATCTGCTAAAACTGGCCGGGGATGTGGAAAGTAATCCCGGCCCC
Linker	124	APVKQGSG		
Furinlink1	125	RAKR	271	CGTGCAAAGCGT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Fmdv	126	RAKRAPVKQTLNFDLLKLAGDVESNPGP	272	AGAGCCAAGAGGGCACCGGTGAAACAGACTTTGAATTTTGACCTTCTGAAGTTGGCAGGAGACGTTGAGTCCAACCCTGGGCCC
(G4S)3 Linker	127	GGGGSGGGGSGGGGS	273	GGTGGCGGTGGCTCGGGCGGTGGTGGGTCGGGTGGCGGCGGATCT
Whitlow Linker	128	GSTSGSGKPGSGEGSTKG	274	GGCAGCACCTCCGGCAGCGGCAAGCCTGGCAGCGGCGAGGGCAGCACCAAGGGC
GSG linker	129	GSG	275	GGAAGCGGA
SGSG linker	130	SGSG	276	AGTGGCAGCGGC
RTS-COMPONENTS				
VP16 activation domain	131	GPKKKRKVAPPTDVSLGDELHLDGEDVAMAHADALDDFDLDMLGDGDSPGPGFTPHDSAPYGALDMADFEFEQMFTDALGIDEYGG	277	GGCCCCAAGAAGAAAAGGAAGGTGGCCCCCCCCACCGACGTGAGCCTGGGCGACGAGCTGCACCTGGACGGCGAGGACGTGGCCATGGCCCACGCCGACGCCCTGGACGACTTCGACCTGGACATGCTGGGCGACGGCGACAGCCCCGGCCCCGGCTTCACCCCCCACGACAGCGCCCCCTACGGCGCCCTGGACATGGCCGACTTCGAGTTCGAGCAGATGTTCACCGACGCCCTGGGCATCGACGAGTACGGCGGC
Retinoid x receptor (RxR)	132	EMPVDRILEAELAVEQKSDQGVEGPGGTGGSGSSPNDPVTNICQAADKQLFTLVEWAKRIPHFSSLPLDDQVILLRAGWNELLIASFSHRSIDVRDGILLATGLHVHRNSAHSAGVGAIFDRVLTELVSKMRDMRMDKTELGCLRAIILFNPEVRGLKSAQEVELLREKVYAALEEYTRTTHPDEPGRFAKLLLRLPSLRSIGLKCLEHLFFFRLIGDVPIDTFLMEMLESPSDS	278	GAGATGCCCGTGGACAGGATTCTGGAGGCCGAACTCGCCGTGGAGCAGAAAAGCGACCAGGGCGTGGAGGGCCCCGGCGGAACCGGCGGCAGCGGCAGCAGCCCCAACGACCCCGTGACCAACATCTGCCAGGCCGCCGACAAGCAGCTGTTCACCCTGGTGGAGTGGGCCAAGAGGATTCCCCACTTCAGCAGCCTGCCCCTGGACGACCAGGTGATCCTGCTGAGGGCCGGATGGAACGAGCTGCTGATCGCCAGCTTCAGCCACAGGAGCATCGACGTGAGGGACGGCATCCTGCTGGCCACCGGCCTGCACGTCCATAGGAACAGCGCCCACAGCGCCGGAGTGGGCGCCATCTTCGACAGGGTGCTGACCGAGCTGGTGAGCAAGATGAGGGACATGAGGATGGACAAGACCGAGCTGGGCTGCCTGAGGGCCATCATCCTGTTCAACCCCGAGGTGAGGGGCCTGAAAAGCGCCCAGGAGGTGGAGCTGCTGAGGGAGAAGGTGTACGCCGCCCTGGAGGAGTACACCAGGACCACCCACCCCGACGAGCCCGGCAGATTCGCCAAGCTGCTGCTGAGGCTGCCCAGCCTGAGGAGCATCGGCCTGAAGTGCCTGGAGCACCTGTTCTTCTTCAGGCTGATCGGCGACGTGCCCATCGACACCTTCCTGATGGAGATGCTGGAGAGCCCCAGCGACAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
VP16-linker-RxR	133	GPKKKRKVAPPTDVSLGDELHLDGEDVAMAHADALDDFDLDMLGDGDSPGPGFTPHDSAPYGALDMADFEFEQMFTDALGIDEYGGEFEMPVDRILEAELAVEQKSDQGVEGPGGTGGSGSSPNDPVTNICQAADKQLFTLVEWAKRIPHFSSLPLDDQVILLRAGWNELLIASFSHRSIDVRDGILLATGLHVHRNSAHSAGVGAIFDRVLTELVSKMRDMRMDKTELGCLRAIILFNPEVRGLKSAQEVELLREKVYAALEEYTRTTHPDEPGRFAKLLLRLPSLRSIGLKCLEHLFFFRLIGDVPIDTFLMEMLESPSDS	279	GGCCCCAAGAAGAAAAGGAAGGTGGCCCCCCCCACCGACGTGAGCCTGGGCGACGAGCTGCACCTGGACGGCGAGGACGTGGCCATGGCCCACGCCGACGCCCTGGACGACTTCGACCTGGACATGCTGGGCGACGGCGACAGCCCCGGCCCCGGCTTCACCCCCCACGACAGCGCCCCCTACGGCGCCCTGGACATGGCCGACTTCGAGTTCGAGCAGATGTTCACCGACGCCCTGGGCATCGACGAGTACGGCGGCGAATTCGAGATGCCCGTGGACAGGATTCTGGAGGCCGAACTCGCCGTGGAGCAGAAAAGCGACCAGGGCGTGGAGGGCCCCGGCGGAACCGGCGGCAGCGGCAGCAGCCCCAACGACCCCGTGACCAACATCTGCCAGGCCGCCGACAAGCAGCTGTTCACCCTGGTGGAGTGGGCCAAGAGGATTCCCCACTTCAGCAGCCTGCCCCTGGACGACCAGGTGATCCTGCTGAGGGCCGGATGGAACGAGCTGCTGATCGCCAGCTTCAGCCACAGGAGCATCGACGTGAGGGACGGCATCCTGCTGGCCACCGGCCTGCACGTCCATAGGAACAGCGCCCACAGCGCCGGAGTGGGCGCCATCTTCGACAGGGTGCTGACCGAGCTGGTGAGCAAGATGAGGGACATGAGGATGGACAAGACCGAGCTGGGCTGCCTGAGGGCCATCATCCTGTTCAACCCCGAGGTGAGGGCCTGAAAAGCGCCCAGGAGGTGGAGCTGCTGAGGGAGAAGGTGTACGCCGCCCTGGAGGAGTACACCAGGACCACCCACCCCGACGAGCCCGGCAGATTCGCCAAGCTGCTGCTGAGGCTGCCCAGCCTGAGGAGCATCGGCCTGAAGTGCCTGGAGCACCTGTTCTTCTTCAGGCTGATCGGCGACGTGCCCATCGACACCTTCCTGATGGAGATGCTGGAGAGCCCCAGCGACAGC
GAL4 DNA Binding Domain	134	MKLLSSIEQACDICRLKKLKCSKEKPKCAKCLKNNWECRYSPKTKRSPLTRAHLTEVESRLERLEQLFLLIFPREDLDMILKMDSLQDIKALLTGLFVQDNVNKDAVTDRLASVETDMPLTLRQHRISATSSSEESSNKGQRQLTVSPEF	280	ATGAAGCTGCTGAGCAGCATCGAGCAGGCTTGCGACATCTGCAGGCTGAAGAAGCTGAAGTGCAGCAAGGAGAAGCCCAAGTGCGCCAAGTGCCTGAAGAACAACTGGGAGTGCAGATACAGCCCCAAGACCAAGAGGAGCCCCCTGACCAGGGCCCACCTGACCGAGGTGGAGAGCAGGCTGGAGAGGCTGGAGCAGCTGTTCCTGCTGATCTTCCCCAGGGAGGACCTGGACATGATCCTGAAGATGGACAGCCTGCAAGACATCAAGGCCCTGCTGACCGGCCTGTTCGTGCAGGACAACGTGAACAAGGACGCCGTGACCGACAGGCTGGCCAGCGTGGAGACCGACATGCCCCTGACCCTGAGGCAGCACAGGATCAGCGCCACCAGCAGCAGCGAGGAGAGCAGCAACAAGGGCCAGAGGCAGCTGACCGTGAGCCCCGAGTTT
Ecdysone Receptor Ligand Binding Domain – VY	135	IRPECVVPETQCAMKRKEKKAQKEKDKLPVSTTTVDDHMPPIMQCEPPPPEAARIHEVVPRFLSDKLLVTNRQKNIPQLTANQQFLIARLI	281	ATCAGGCCCGAGTGCGTGGTGCCCGAGACCCAGTGCGCCATGAAAAGGAAGGAGAAGAAGGCCCAGAAGGAGAAGGACAAGCTGCCCGTGAGCACCACCACCGTCGATGACCACATGCCCCCCATCATGCAGTGCGAGCCCCCCCCCCCCGAGGCCGCCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
variant (EcR)		WYQDGYEQPSDEDL KRITQTWQQADDENE ESDTPFRQITEMTILT VQLIVEFAKGLPGFA KISQPDQITLLKACSS EVMMLRVARRYDAA SDSILFANNQAYTRD NYRKAGMAEVIEDLL HFCRCMYSMALDNIH YALLTAVVIFSDRPGL EQPQLVEEIQRYYLN TLRIYILNQLSGSARS SVIYGKILSILSELRTL GMQNSNMCISLKLKN RKLPPFLEEIWDVAD MSHTQPPPILESPTNL		GGATTCACGAGGTCGTGCCCAGGTTCC TGAGCGACAAGCTGCTGGTGACCAACA GGCAGAAGAACATCCCCCAGCTGACCG CCAACCAGCAGTTCCTGATCGCCAGGC TGATCTGGTATCAGGACGGCTACGAGC AGCCCAGCGACGAGGACCTGAAAAGG ATCACCCAGACCTGGCAGCAGGCCGAC GACGAGAACGAGGAGAGCGACACCCC CTTCAGGCAGATCACCGAGATGACCAT CCTGACCGTGCAGCTGATCGTGGAGTT CGCCAAGGGCCTGCCCGGATTCGCCAA GATCAGCCAGCCCGACCAGATCACCCT GCTGAAGGCTTGCAGCAGCGAGGTGAT GATGCTGAGGGTGGCCAGGAGGTACG ACGCCGCCAGCGACAGCATCCTGTTCG CCAACAACCAGGCTTACACCAGGGACA ACTACAGGAAGGCTGGCATGGCCGAG GTGATCGAGGACCTCCTGCACTTCTGC AGATGTATGTACAGCATGGCCCTGGAC AACATCCACTACGCCCTGCTGACCGCC GTGGTGATCTTCAGCGACAGGCCCGGC CTGGAGCAGCCCCAGCTGGTGGAGGA GATCCAGAGGTACTACCTGAACACCCT GAGGATCTACATCCTGAACCAGCTGAG CGGCAGCGCCAGGAGCAGCGTGATCTA CGGCAAGATCCTGAGCATCCTGAGCGA GCTGAGGACCCTGGGAATGCAGAACA GCAATATGTGTATCAGCCTGAAGCTGA AGAACAGGAAGCTGCCCCCCTTCCTGG AGGAGATTTGGGACGTGGCCGACATGA GCCACACCCAGCCCCCCCCCATCCTGG AGAGCCCCACCAACCTG
Ecdysone Receptor Ligand Binding Domain – VY variant (EcR)	136	RPECVVPETQCAMKR KEKKAQKEKDKLPVS TTTVDDHMPPIMQCE PPPPEAARIHEVVPRF LSDKLLVTNRQKNIP QLTANQQFLIARLIW YQDGYEQPSDEDLKR ITQTWQQADDENEES DTPFRQITEMTILTVQ LIVEFAKGLPGFAKIS QPDQITLLKACSSEV MMLRVARRYDAASD SILFANNQAYTRDNY RKAGMAEVIEDLLHF CRCMYSMALDNIHY ALLTAVVIFSDRPGLE QPQLVEEIQRYYLNT LRIYILNQLSGSARSS VIYGKILSILSELRTLG MQNSNMCISLKLKNR	282	CGGCCTGAGTGCGTAGTACCCGAGACT CAGTGCGCCATGAAGCGGAAAGAGAA GAAAGCACAGAAGGAGAAGGACAAAC TGCCTGTCAGCACGACGACGGTGGACG ACCACATGCCGCCCATTATGCAGTGTG AACCTCCACCTCCTGAAGCAGCAAGGA TTCACGAAGTGGTCCCAAGGTTTCTCT CCGACAAGCTGTTGGTGACAAACCGGC AGAAAAACATCCCCCAGTTGACAGCCA ACCAGCAGTTCCTTATCGCCAGGCTCA TCTGGTACCAGGACGGGTACGAGCAGC CTTCTGATGAAGATTTGAAGAGGATTA CGCAGACGTGGCAGCAAGCGGACGAT GAAAACGAAGAGTCGGACACTCCCTTC CGCCAGATCACAGAGATGACTATCCTC ACGGTCCAACTTATCGTGGAGTTCGCG AAGGGATTGCCAGGGTTCGCCAAGATC TCGCAGCCTGATCAAATTACGCTGCTT AAGGCTTGCTCAAGTGAGGTAATGATG CTCCGAGTCGCGCGACGATACGATGCG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		KLPPFLEEIWDVADM SHTQPPPILESPTNL		GCCTCAGACAGTATTCTGTTCGCGAAC AACCAAGCGTACACTCGCGACAACTAC CGCAAGGCTGGCATGGCCGAGGTCATC GAGGATCTACTGCACTTCTGCCGGTGC ATGTACTCTATGGCGTTGGACAACATC CATTACGCGCTGCTCACGGCTGTCGTC ATCTTTTCTGACCGGCCAGGGTTGGAG CAGCCGCAACTGGTGGAAGAGATCCA GCGGTACTACCTGAATACGCTCCGCAT CTATATCCTGAACCAGCTGAGCGGGTC GGCGCGTTCGTCCGTCATATACGGCAA GATCCTCTCAATCCTCTCTGAGCTACGC ACGCTCGGCATGCAAAACTCCAACATG TGCATCTCCCTCAAGCTCAAGAACAGA AAGCTGCCGCCTTTCCTCGAGGAGATC TGGGATGTGGCGGACATGTCGCACACC CAACCGCCGCCTATCCTCGAGTCCCCC ACGAATCTCTAG
GAL4-Linker-EcR	137	MKLLSSIEQACDICRL KKLKCSKEKPKCAKC LKNNWECRYSPKTKR SPLTRAHLTEVESRLE RLEQLFLLIFPREDLD MILKMDSLQDIKALL TGLFVQDNVNKDAV TDRLASVETDMPLTL RQHRISATSSSEESSN KGQRQLTVSPEFPGIR PECVVPETQCAMKRK EKKAQKEKDKLPVST TTVDDHMPPIMQCEP PPPEAARIHEVVPRFL SDKLLVTNRQKNIPQ LTANQQFLIARLIWY QDGYEQPSDEDLKRI TQTWQQADDENEES DTPFRQITEMTILTVQ LIVEFAKGLPGFAKIS QPDQITLLKACSSEV MMLRVARRYDAASD SILFANNQAYTRDNY RKAGMAEVIEDLLHF CRCMYSMALDNIHY ALLTAVVIFSDRPGLE QPQLVEEIQRYYLNT LRIYILNQLSGSARSS VIYGKILSILSELRTLG MQNSNMCISLKLKNR KLPPFLEEIWDVADM SHTQPPPILESPTNL	283	ATGAAGCTACTGTCTTCTATCGAACAA GCATGCGATATTTGCCGACTTAAAAAG CTCAAGTGCTCCAAAGAAAAACCGAA GTGCGCCAAGTGTCTGAAGAACAACTG GGAGTGTCGCTACTCTCCCAAAACCAA AAGGTCTCCGCTGACTAGGGCACATCT GACAGAAGTGGAATCAAGGCTAGAAA GACTGGAACAGCTATTTCTACTGATTTT TCCTCGAGAAGACCTTGACATGATTTT GAAAATGGATTCTTTACAGGATATAAA AGCATTGTTAACAGGATTATTTGTACA AGATAATGTGAATAAAGATGCCGTCAC AGATAGATTGGCTTCAGTGGAGACTGA TATGCCTCTAACATTGAGACAGCATAG AATAAGTGCGACATCATCATCGGAAGA GAGTAGTAACAAAGGTCAAAGACAGT TGACTGTATCGCCGGAATTCCCGGGGA TCCGGCCTGAGTGCGTAGTACCCGAGA CTCAGTGCGCCATGAAGCGGAAAGAG AAGAAAGCACAGAAGGAGAAGGACAA ACTGCCTGTCAGCACGACGACGGTGGA CGACCACATGCCGCCCATTATGCAGTG TGAACCTCCACCTCCTGAAGCAGCAAG GATTCACGAAGTGGTCCCAAGGTTTCT CTCCGACAAGCTGTTGGTGACAAACCG GCAGAAAAACATCCCCCAGTTGACAGC CAACCAGCAGTTCCTTATCGCCAGGCT CATCTGGTACCAGGACGGGTACGAGCA GCCTTCTGATGAAGATTTGAAGAGGAT TACGCAGACGTGGCAGCAAGCGGACG ATGAAAACGAAGAGTCGGACACTCCCT TCCGCCAGATCACAGAGATGACTATCC TCACGGTCCAACTTATCGTGGAGTTCG CGAAGGGATTGCCAGGGTTCGCCAAGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				TCTCGCAGCCTGATCAAATTACGCTGC TTAAGGCTTGCTCAAGTGAGGTAATGA TGCTCCGAGTCGCGCGACGATACGATG CGGCCTCAGACAGTATTCTGTTCGCGA ACAACCAAGCGTACACTCGCGACAACT ACCGCAAGGCTGGCATGGCCGAGGTCA TCGAGGATCTACTGCACTTCTGCCGGT GCATGTACTCTATGGCGTTGGACAACA TCCATTACGCGCTGCTCACGGCTGTCG TCATCTTTTCTGACCGGCCAGGGTTGG AGCAGCCGCAACTGGTGGAAGAGATC CAGCGGTACTACCTGAATACGCTCCGC ATCTATATCCTGAACCAGCTGAGCGGG TCGGCGCGTTCGTCCGTCATATACGGC AAGATCCTCTCAATCCTCTCTGAGCTA CGCACGCTCGGCATGCAAAACTCCAAC ATGTGCATCTCCCTCAAGCTCAAGAAC AGAAAGCTGCCGCCTTTCCTCGAGGAG ATCTGGGATGTGGCGGACATGTCGCAC ACCCAACCGCCGCCTATCCTCGAGTCC CCCACGAATCTCTAG
GAL4-Linker-EcR	138	MKLLSSIEQACDICRL KKLKCSKEKPKCAKC LKNNWECRYSPKTKR SPLTRAHLTEVESRLE RLEQLFLLIFPREDLD MILKMDSLQDIKALL TGLFVQDNVNKDAV TDRLASVETDMPLTL RQHRISATSSSEESSN KGQRQLTVSPEFPGR PECVVPETQCAMKRK EKKAQKEKDKLPVST TTVDDHMPPIMQCEP PPPEAARIHEVVPRFL SDKLLVTNRQKNIPQ LTANQQFLIARLIWY QDGYEQPSDEDLKRI TQTWQQADDENEES DTPFRQITEMTILTVQ LIVEFAKGLPGFAKIS QPDQITLLKACSSEV MMLRVARRYDAASD SILFANNQAYTRDNY RKAGMAEVIEDLLHF CRCMYSMALDNIHY ALLTAVVIFSDRPGLE QPQLVEEIQRYYLNT LRIYILNQLSGSARSS VIYGKILSILSELRTLG MQNSNMCISLKLKNR	284	ATGAAGCTGCTGAGCAGCATCGAGCAG GCTTGCGACATCTGCAGGCTGAAGAAG CTGAAGTGCAGCAAGGAGAAGCCCAA GTGCGCCAAGTGCCTGAAGAACAACTG GGAGTGCAGATACAGCCCCAAGACCA AGAGGAGCCCCCTGACCAGGGCCCACC TGACCGAGGTGGAGAGCAGGCTGGAG AGGCTGGAGCAGCTGTTCCTGCTGATC TTCCCCAGGGAGGACCTGGACATGATC CTGAAGATGGACAGCCTGCAAGACATC AAGGCCCTGCTGACCGGCCTGTTCGTG CAGGACAACGTGAACAAGGACGCCGT GACCGACAGGCTGGCCAGCGTGGAGA CCGACATGCCCCTGACCCTGAGGCAGC ACAGGATCAGCGCCACCAGCAGCAGC GAGGAGAGCAGCAACAAGGGCCAGAG GCAGCTGACCGTGAGCCCCGAGTTTCC CGGGCGGCCTGAGTGCGTAGTACCCGA GACTCAGTGCGCCATGAAGCGGAAAG AGAAGAAAGCACAGAAGGAGAAGGAC AAACTGCCTGTCAGCACGACGACGGTG GACGACCACATGCCGCCCATTATGCAG TGTGAACCTCCACCTCCTGAAGCAGCA AGGATTCACGAAGTGGTCCCAAGGTTT CTCTCCGACAAGCTGTTGGTGACAAAC CGGCAGAAAAACATCCCCCAGTTGACA GCCAACCAGCAGTTCCTTATCGCCAGG CTCATCTGGTACCAGGACGGGTACGAG CAGCCTTCTGATGAAGATTTGAAGAGG ATTACGCAGACGTGGCAGCAAGCGGA CGATGAAAACGAAGAGTCGGACACTC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		KLPPFLEEIWDVADM SHTQPPPILESPTNL		CCTTCCGCCAGATCACAGAGATGACTA TCCTCACGGTCCAACTTATCGTGGAGT TCGCGAAGGGATTGCCAGGGTTCGCCA AGATCTCGCAGCCTGATCAAATTACGC TGCTTAAGGCTTGCTCAAGTGAGGTAA TGATGCTCCGAGTCGCGCGACGATACG ATGCGGCCTCAGACAGTATTCTGTTCG CGAACAACCAAGCGTACACTCGCGACA ACTACCGCAAGGCTGGCATGGCCGAGG TCATCGAGGATCTACTGCACTTCTGCC GGTGCATGTACTCTATGGCGTTGGACA ACATCCATTACGCGCTGCTCACGGCTG TCGTCATCTTTTCTGACCGGCCAGGGTT GGAGCAGCCGCAACTGGTGGAAGAGA TCCAGCGGTACTACCTGAATACGCTCC GCATCTATATCCTGAACCAGCTGAGCG GGTCGGCGCGTTCGTCCGTCATATACG GCAAGATCCTCTCAATCCTCTCTGAGC TACGCACGCTCGGCATGCAAAACTCCA ACATGTGCATCTCCCTCAAGCTCAAGA ACAGAAAGCTGCCGCCTTTCCTCGAGG AGATCTGGGATGTGGCGGACATGTCGC ACACCCAACCGCCGCCTATCCTCGAGT CCCCCACGAATCTCTAG

Name	SEQ ID NO	Nucleotide Sequence
Human EEF1A1 promoter variant	139	GAGCGTGCGTGAGGCTCCGGTGCCCGTCAGTGGGCAGAGCGC ACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGGTCGG CGATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTG GGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGT GGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTT CTTTTTCGCAACGGGTTTGCCGCCAGAACACAG
UBC promoter	140	GGCCTCCGCGCCGGGTTTTGGCGCCTCCCGCGGGCGCCCCCCT CCTCACGGCGAGCGCTGCCACGTCAGACGAAGGGCGCAGCGA GCGTCCTGATCCTTCCGCCCGGACGCTCAGGACAGCGGCCCGC TGCTCATAAGACTCGGCCTTAGAACCCCAGTATCAGCAGAAGG ACATTTTAGGACGGGACTTGGGTGACTCTAGGGCACTGGTTTT CTTTCCAGAGAGCGGAACAGGCGAGGAAAAGTAGTCCCTTCT CGGCGATTCTGCGGAGGGATCTCCGTGGGGCGGTGAACGCCG ATGATTATATAAGGACGCGCCGGGTGTGGCACAGCTAGTTCCG TCGCAGCCGGGATTTGGGTCGCGGTTCTTGTTTGTGGATCGCT GTGATCGTCACTTGGTGAGTAGCGGGCTGCTGGGCTGGGTACG TGCGCTCGGGGTTGGCGAGTGTGTTTTGTGAAGTTTTTTAGGC ACCTTTTGAAATGTAATCATTTGGGTCAATATGTAATTTTCAGT GTTAGACTAGTAAATTGTCCGCTAAATTCTGGCCGTTTTTGGCT TTTTTGTTAGACG
6 site GAL4-inducible proximal factor binding element (PFB)	141	ATTGTTCGGAGCAGTGCGGCGCGTTTAGCGGAGTACTGTCCTC CGATATTAATCGGGGCAGACTATTCCGGGGTTTACCGGCGCAC TCTCGCCCGAACTTCACCGGCGGTCTTTCGTCCGTGCTTTATCG GGGCGGATCACTCCGAAC

Name	SEQ ID NO	Nucleotide Sequence
Synthetic minimal promoter 1 [Inducible Promoter]	142	AGGTCTATATAAGCAGAGCTCGTTTAGTGAACCCTCATTCTGG AGACGGATCCCGAGCCGAGTGTTTTGACCTCCATAGAA
Synthetic 5' UTR based on RPL6	143	CAGCCGCTAAATCCAAGGTAAGGTCAGAAGA
SV40e polyA	144	AACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATA GCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCT AGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATGTCTG G
Bidirectional aCA polyA [bidirectional polyA]	145	ATCGATTAATCTAGCGGCCCTAGACGAGCAGACATGATAAGA TACATTGATGAGTTTGGACAAACCACAACTAGAATGCAGTGA AAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATT TGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAAT TGCATTCATTTTATGTTTCAGGTTCAGGGGGAGATGTGGGAGG TTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTAAAATCCG ATAAGCGTACCTAGAGGC
2xRbm3 IRES	146	ACTAGTTTTATAATTTCTTCTTCCAGAATTTCTGACATTTTATA ATTTCTTCTTCCAGAAGACTCACAACCTC
EMCV IRES	147	CCCCCTCTCCCTCCCCCCCCCCTAACGTTACTGGCCGAAGCCG CTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCA CCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGG CCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCG CCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAG TTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGAC CCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTC TGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCG GCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAA GAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAA GGATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGG CCTCGGTGCACATGCTTTACATGTGTTTAGTCGAGGTTAAAAA ACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGA AAAACACGATC

CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises
    (a) a ROR-1 antigen binding domain;
    (b) a spacer;
    (c) a transmembrane domain;
    (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and
    (e) a CD3 zeta signaling domain.

2. The isolated nucleic acid of claim 1, wherein the CAR comprises at least one of:
    (a) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 3-14; or
    (b) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 75-82.

3. The isolated nucleic acid of claim 1, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15-74.

4. The isolated nucleic acid of claim 1, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73.

5. The isolated nucleic acid of claim 1, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

6. The isolated nucleic acid of claim 1, wherein the costimulatory signaling domain comprises 4-1BB.

7. The isolated nucleic acid of claim 1, wherein the costimulatory signaling domain comprises CD28.

8. The isolated nucleic acid of claim 1, wherein the CD3 zeta signaling domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 93.

9. The isolated nuclei acid of claim 1, further comprising a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NOs: 94-108.

10. The isolated nucleic acid of any of claims 1-9, further comprising a cell tag.

11. The isolated nucleic acid claim 10, wherein the cell tag is a truncated epidermal growth factor receptor.

12. The isolated nucleic acid of claim 11, wherein the truncated epidermal growth factor receptor is HER1t and comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 109.

13. The isolated nucleic acid of claim 11, wherein the truncated epidermal growth factor receptor is HER1t-1 and comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with the amino acid sequence of SEQ ID NO: 110.

14. The isolated nucleic acid of claim 1, further comprising an additional polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

15. A vector comprising a backbone and a nucleic acid sequence encoding:
    (1) a cell tag;
    (2) a cytokine; and
    (3) a chimeric antigen receptor (CAR), wherein the CAR comprises

(a) a ROR-1 antigen binding domain;

(b) a spacer;

(c) a transmembrane domain;

(d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and

(e) a CD3 zeta signaling domain.

16. The vector of claim 15, wherein the vector is a lentivirus vector, a retroviral vector, or a non-viral vector.

17. The vector of claim 15, wherein the cell tag is a truncated epidermal growth factor receptor.

18. The vector of claim 17, wherein the truncated epidermal growth factor receptor is a HER1t, HER1t-1 or a functional variant thereof.

19. The vector of claim 15, wherein the cytokine is IL-15.

20. The vector of claim 19, wherein the IL-15 is a membrane bound IL-15.

21. The vector of any of claims 15-20, further comprising a nucleotide sequence encoding a self-cleaving *Thosea asigna* virus (T2A) peptide.

22. The vector of any of claims 15-21, wherein the backbone is Sleeping Beauty transposon DNA plasmid.

23. The vector of claim 15, wherein the CAR comprises at least one of:

(a) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 3-14; or

(b) a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity with at least one of amino acid sequences as shown in SEQ ID NOs: 75-82.

24. The vector of claim 15, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 15-74.

25. The vector of claim 15, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at

least one of amino acid sequences as shown in SEQ ID NOs: 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, and 73.

26. The vector of claim 15, wherein the ROR-1 antigen binding domain comprises a polypeptide having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 99% or 100% identify with at least one of amino acid sequences as shown in SEQ ID NOs: 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, and 74.

27. The vector of claim 16, wherein the non-viral vector is a Sleeping Beauty transposon.

28. The vector of claim 15, further comprising an additional vector comprising a polynucleotide encoding gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

29. An immune effector cell comprising the isolated nucleic acid of any one of claims 1-14.

30. An immune effector cell comprising the vector of any one of claims 15-28.

31. An immune effector cell comprising (1) a cell tag; (2) a cytokine; and (3) a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a ROR-1 antigen binding domain; (b) a spacer; (c) a transmembrane domain; (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both; and (e) a CD3 zeta signaling domain.

32. The immune effector cell of claim 31, wherein the cytokine is IL-15.

33. The immune effector cell of claim 32, wherein the IL-15 is a membrane bound IL-15.

34. The immune effector cell of claim 31, wherein the cell tag is a truncated epidermal growth factor receptor.

35. The immune effector cell of claim 34, wherein the truncated epidermal growth factor receptor is a HER1t, HER1t-1 or a functional variant thereof.

36. The immune effector cell of any one of claims 31-35, wherein the cell is a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), or a regulatory T cell.

37. The immune effector cell of claim 31, wherein the CAR comprises at least one of a polypeptide having amino acid sequences as shown in SEQ ID NOs: 3-14 or 75-82.

38. The immune effector cell of claim 31, wherein the CAR comprises at least one of a polypeptide having amino acid sequences as shown in SEQ ID NOs: 15-74.

39. The immune effector cell of claim 31, further comprising gene switch polypeptides for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides comprise: (a) a first gene switch polypeptide that comprises a DNA binding domain fused to a first nuclear receptor ligand binding domain; and (b) a second gene switch polypeptide that comprises a transactivation domain fused to a second nuclear receptor ligand binding domain; wherein the first gene switch polypeptide and the second gene switch polypeptide are connected by a linker.

40. A method for stimulating a T cell-mediated immune response to a target cell population or tissue in a human subject in need thereof, comprising administering to the human subject an effective amount of a cell genetically modified to express a CAR, wherein the CAR comprises
    (a) a ROR-1 antigen binding domain;
    (b) a spacer;
    (c) a transmembrane domain;
    (d) a costimulatory signaling domain comprising 4-1BB or CD28, or both;
    (e) a CD3 zeta signaling domain; and
    (f) a truncated epidermal growth factor receptor (HER1t).

41. The method of claim 40, wherein the human has been diagnosed with at least one of lung cancer, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, adrenal cancer, melanoma, uterine cancer, testicular cancer, or bladder cancer.

42. The method of claim 40, wherein the human has been diagnosed with non-Hodgkin's lymphoma, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), multiple myeloma (MM), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML).

43. A method of treating cancer in a subject in need thereof comprising administering to the subject one or more doses of an effective amount of engineered T-cells, wherein the engineered T-cells comprise ROR-1 CAR and membrane bound IL-15.

44. The method of claim 43, wherein a first dose of an effective amount of engineered T-cells is administered intraperitoneally or intravenously.

45. The method of claim 43, wherein the cancer is non-Hodgkin's lymphoma, acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), multiple myeloma (MM), acute myeloid leukemia (AML), or chronic myeloid leukemia (CML).

46. The method of claim 43, wherein the cancer is lung cancer, breast cancer, pancreatic cancer, ovarian cancer, prostate cancer, adrenal cancer, melanoma, uterine cancer, testicular cancer, or bladder cancer.

47. The method of claim 43, wherein the ROR-1 CAR is encoded by any one of sequences as shown in SEQ ID NOs: 3-14, 75-82, or 15-74.

48. The method of claim 43, wherein the effective amount of engineered T-cells is at least 10^2 cells/kg, 10^4 cells/kg, or 10^5 cells/kg.