CHIMERIC RECEPTOR THERAPY

REFERENCE TO SEQUENCE LISTING

[0001] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by refernece in its entirety. Said ASCII copy, created on January 10, 2022, is named 75594-348568_SL.txt and is 579,627 bytes in size.

BACKGROUND OF THE DISCLOSURE

[0002] Chimeric antigen receptor (CAR-T) cell and T cell receptor (TCR) therapies have recently undergone rapid development and have been shown to successfully direct killing of tumor cells. Such therapies are, for example, useful in treating autoimmune disorders and cancers. Indeed, several targets for such therapies have been identified to date, including but not limited to CD19, CD33, BCMA, CD44, α-Folate receptor, CAIX, CD30, ROR1, CEA, EGP-2, EGP-40, HER2, HER3, Folate-binding Protein, GD2, GD3, IL-13R-a2, KDR, EDB-F, mesothelin, CD22, EGFR, Folate receptor α, Mucins such as MUC1, MUC4 or MUC16, MAGE-A1, h5T4, PSMA, TAG-72, EGFR, CD20, EGFRvIII, CD123 or VEGF-R2. Among these, CD19, CD33, MUC1, MUC16, and ROR1 have shown particular promise as targets for immunotherapy.

[0003] CD19 is an attractive target for immunotherapy due to several factors. It is expressed on a variety of B cell lymphomas and leukemias and on normal B cells, but it is not found on hematopoietic stem cells, plasma cells, and other healthy tissues. In addition, CD19 has a broader expression profile than that of CD20, which is the target of monoclonal antibody therapies such as rituximab, and it is thought to be a better target for antibody-drug conjugates (ADC) compared with CD20, which suffers from inefficient internalization. CD19 has also been shown to be expressed in cases where monoclonal antibody treatment (e.g., rituximab) is ineffective due to CD20 downregulation or other factors. Additionally, because CD19-targeting agents have a mode of action that is distinct from that of anti-CD20 antibodies, they could complement existing monoclonal antibody regimens.

[0004] CD33 is an attractive target for immunotherapy due to its high expression in cancer and

minimal expression in healthy adult tissues. CD33 is overexpressed on myeloid leukemia and leukemic stem cells. CD33 is overexpressed in acute myeloid leukemia (AML), which is the most common acute leukemia in adults. 85 to 90% of AML patients show expression of CD33 on blast cells. CD33 is also overexpressed in myelodysplastic syndromes (MDS), which are cancerous conditions of the bone marrow generally found in adults in their 70s.

[0005] MUC1 is an attractive target for immunotherapy because it is overexpressed in breast cancer, and is absent or expressed at low levels in normal mammary glands. In addition, MUC1 is mostly aberrantly underglycosylated in cancer and the antigens on the cancer surface are different from those on normal cells. Therefore targeting MUC1 for cancer immunotherapy can exploit the differences between cancerous and normal cells.

[0006] MUC16 is an attractive target for immunotherapy due to its high expression in cancer and minimal expression in healthy adult tissues. MUC16 is aberrantly expressed in ovarian cancer, breast cancer, pancreatic cancer, endothermal cancer, and lung cancer. For example, MUC16 is overexpressed in over 80% of ovarian tumors, which is the most lethal of the gynecologic malignancies. Meanwhile, limited expression of MUC16 has been found on healthy tissue. The current standard of care for ovarian cancer is surgery, followed by chemotherapy with a combination of platinum agents and taxanes. However, recurrence of the disease occurs in most patients after initial treatment, resulting in a cycle of repeated surgeries and additional rounds of chemotherapy.

[0007] Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is an attractive target for immunotherapy due to its high expression in cancer and minimal expression in healthy adult tissues. ROR1 is aberrantly expressed in multiple hematological tumors, including chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), acute lymphoblastic leukemia (ALL), and diffuse B-cell lymphoma (DLBCL) and solid tumors, including breast adenocarcinomas encompassing triple negative breast cancer (TNBC), pancreatic cancer, ovarian cancer, and lung adenocarcinoma.

[0008] Although many patients have durable responses with CAR-T and TCR therapies, for some patients the anti-tumor effects of such therapies are either short-lived or ineffective. Another immunotherapy that has shown promise is immune checkpoint inhibition, which can prevent the

switching off of T cells and promote the activity of these cells. Examples of checkpoint inhibitor targets include but are not limited to PD1, PD-L1, CTLA-4, TIGIT, 4-1BB, PIK3IP1, CD27, CD28, CD40, CD70, CD122, CD137, OX40 (CD134), GITR, ICOS, A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA, IDO, KIR, LAG3, TIM-3, or VISTA. Among these, targeting CTLA4, PD-1, PD-L1, TIM3, TIGIT, LAG3, and/or PIK3IP1 has shown the most promise. One of the most studied checkpoint inhibition pathway is the PD-1/programmed death ligand 1 (PD-L1) pathway, which plays a vital role in how tumor cells evade immune response. Immunotherapy utilizing PD-1/PD-L1 blocking antibodies has been extensively evaluated in the clinic and has been shown to improve tumor regression across multiple malignancies, especially when administered in conjunction with CAR-T cells. However, checkpoint inhibitor blocking antibodies have not performed consistently across cancer types, may have limited access to the tumor microenvironment, require repeated administration, and may lose effectiveness over time. Genome editing is an alternate approach to eliminate PD-1 mediated CAR-T cell exhaustion, and has the advantage of restricting the PD-1 blockade to only the engineered CAR-T cells. However, gene editing adds complexity to the manufacturing process, which increases the turnaround time and cost of the cell therapy.

[0009] There is accordingly a continuing need in the art to obtain safer, more effective, less expensive therapies to antigen-associated diseases and conditions, including treatments that combine CAR-T and/or TCR therapy with systemic checkpoint inhibition.

[0010] There is also a need to devise ways of diversifying treatment regimens to provide a multi-pronged targeting of antigens in order to address complex *in vivo* biological issues such as loss of immunological surveillance, genetic alterations in tumor antigen composition and tumor heterogeneity (giving rise to cancer cell phenotypic differences).

SUMMARY OF THE DISCLOSURE

[0011] The present invention relates in part to a non-naturally occurring polynucleotide encoding a miRNA that inhibits the expression of an immune checkpoint protein. In certain embodiments, the miRNA targets CTLA4, PD-1, PD-L1, TIM3, TIGIT, LAG3, GITR, or PIK3IP1. In certain embodiments, the miRNA targets PD-1.

[0012] In certain embodiments, the polynucleotide comprises a nucleic acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 72–87 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 72–87. In certain such embodiments, the polynucleotide comprises a nucleic acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 72, 74, 76, 78, 80, 82, 84, and 86 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 72, 74, 76, 78, 80, 82, 704, 705, 709, and 710. In certain embodiments, the polynucleotide comprises a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 179 or 180 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 179 or 180. In certain embodiments, the polynucleotide comprises a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 267 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 267.

[0013] In certain embodiments, the polynucleotide further comprises: a) a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 292 or is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 291; and b) a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 292 or is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 292.

[0014] In certain embodiments, the polynucleotide further encodes a chimeric receptor. In certain embodiments, the chimeric receptor is a T-cell receptor or a chimeric antigen receptor.

[0015] In certain embodiments, the chimeric antigen receptor comprises an antigen-binding domain that binds to an epitope on CD19, CD33, MUC1, MUC16, or ROR1. In certain embodiments, the antigen-binding domain binds to an epitope on ROR1.

[0016] In certain embodiments, the antigen-binding domain comprises a variable light chain domain comprising the amino acid sequence of any one of SEQ ID NOs: 347, 351, 355, 359, 363, 367, 371, 375, 379, 383, 387, 391, 395, 399, 403, 407, 411, 415, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, and 463, or a functional fragment or variant thereof. In certain such embodiments, the variable light chain domain comprises the amino acid sequence of SEQ ID NO: 387 or a functional fragment or variant thereof.

[0017] In certain embodiments, the antigen-binding domain comprises a variable heavy chain domain comprising the amino acid sequence of any one of SEQ ID NOs: 349, 353, 357, 361, 365, 369, 373, 377, 381, 385, 389, 393, 397, 401, 405, 409, 413, 417, 421, 425, 429, 433, 437, 441, 445, 449, 453, 457, and 461, or a functional fragment or variant thereof. In certain such embodiments, the variable heavy chain domain comprises the amino acid sequence of SEQ ID NO: 349 or a functional fragment or variant thereof.

[0018] In certain embodiments, the chimeric antigen receptor comprises a spacer. In certain such embodiments, the spacer comprises a stalk region that is a CD8α hinge domain or a functional fragment or variant thereof. In certain such embodiments, the stalk region comprises the amino acid sequence of SEQ ID NO: 467 or a functional fragment or variant thereof. In certain embodiments, the spacer comprises a stalk extension region. In certain such embodiments, the stalk extension region comprises the amino acid sequence of SEQ ID NO: 473 or a functional fragment or variant thereof.

[0019] In certain embodiments, the chimeric antigen receptor further comprises a transmembrane domain. In certain such embodiments, the transmembrane domain comprises a CD8α transmembrane domain or a functional fragment or variant thereof. In certain such embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 475 or a functional fragment or variant thereof.

[0020] In certain embodiments, the chimeric antigen receptor further comprises an intracellular signaling domain. In certain such embodiments, the intracellular signaling domain comprises a CD3ζ signaling domain or a functional fragment or variant thereof. In certain such embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 479 or a functional fragment or variant thereof.

[0021] In certain embodiments, the intracellular signaling domain comprises a co-stimulatory domain. In certain such embodiments, the intracellular signaling domain comprises a CD28 signaling domain or a functional fragment or variant thereof. In certain embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 481 or a functional fragment or variant thereof.

[0022] In certain embodiments, the polynucleotide further encodes a cytokine. In certain embodiments, the cytokine is IL-15 or a functional fragment or variant thereof. In certain such embodiments, the cytokine comprises the amino acid sequence of SEQ ID NO: 519 or a functional fragment or variant thereof.

[0023] In certain embodiments, the IL-15, or functional fragment or variant thereof, is membrane bound. In certain such embodiments, the IL-15, or functional fragment or variant thereof, forms part of a fusion protein that also comprises IL-15Rα, or a functional fragment or variant thereof. In certain such embodiments, the fusion protein comprises the amino acid sequence of SEQ ID NO: 523 or a functional fragment or variant thereof. In certain embodiments, the fusion protein comprises the amino acid sequence of SEQ ID NO: 525 or a functional fragment or variant thereof.

[0024] In certain embodiments, the polynucleotide further encodes a cell tag. In certain embodiments, the cell tag comprises a truncated HER1, or a functional fragment or variant thereof. In certain embodiments, the truncated HER1 comprises a HER1 Domain III, or a functional fragment or variant thereof, and a truncated HER1 Domain IV, or a functional fragment or variant thereof. In certain such embodiments, the truncated HER1 comprises the amino acid sequence of SEQ ID NO: 565, or a functional fragment or variant thereof, and the amino acid sequence of SEQ ID NO: 567, or a functional fragment or variant thereof.

[0025] In certain embodiments, the cell tag further comprises a CD28 transmembrane domain or a functional fragment or variant thereof. In certain such embodiments, the cell tag comprises the amino acid sequence of SEQ ID NO: 571 or a functional fragment or variant thereof.

[0026] The present invention also relates to a vector comprising the aforementioned polynucleotide. The vector may be viral or non-viral. In certain embodiments, the vector comprises a *Sleeping Beauty* transposon.

[0027] The present invention further relates to a modified immune effector cell comprising the aforementioned polynucleotide. In certain embodiments, the cell is a T-cell.

[0028] The present invention additionally relates to compositions and kits comprising the aforementioned polynucleotide and/or cell.

[0029] A further aspect of the present invention is a method for treating a subject suffering from a disease or disorder, comprising administering the aforementioned cell to a subject in need thereof. The invention also relates to the use of the aforementioned cell in the manufacture of a medicament for the treatment of a disease or disorder. The disease or disorder may be one associated with the overexpression of an antigen, for example, CD19, CD33, ROR1, MUC1, or MUC16. In certain embodiments, the disease or disorder is one associated with the overexpression of ROR1.

[0030] In certain embodiments, the disease or disorder is cancer. In certain embodiments, the disease or disorder is chronic lymphocytic leukemia, mantle cell lymphoma, acute lymphoblastic leukemia, or diffuse large B-cell lymphoma, breast adenocarcinomas encompassing triple negative breast cancer, pancreatic cancer, ovarian cancer, or lung adenocarcinoma.

[0031] Another aspect of the present invention is a method for the detection of a disease or disorder associated with the overexpression of an antigen in a subject, the method comprising: a) contacting a sample from the subject with one or more of the antibodies, or antigen-binding fragments thereof; and b) detecting an increased level of binding of the antibody or fragment thereof to the sample as compared to such binding to a control sample lacking the disease, thereby detecting the disease in the subject.

[0032] A yet further aspect of the present invention is a method for the treatment of a disease or disorder, such as cancer and auto-immune disease or disorders, comprising the serial administration of cells, nucleic acids, viral vectors, or non-viral vectors comprising polynucleotides encoding chimeric antigen receptors selected from a collection of chimeric antigen receptors having different structural compositions and binding specificities for an array of antigen targets. In certain such embodiments, the method comprises a first administration of cells expressing one or more chimeric antigen receptors from the collection followed by a second administration of cells expressing one or more chimeric antigen receptors from the collection, wherein a period of time elapses between the first and second administrations.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] FIG. 1A is an exemplary depiction of a vector comprising a combination of one or more

checkpoint inhibitor miRNAs with a chimeric receptor (SD= Splice Donor; SA= Splice Acceptor). FIG. 1B is an exemplary depiction of hairpin and loop design of various pri- miRNAs including target miRNA and complementary sequences at various 5' or 3' positions. FIG. 1C is exemplary depiction of hairpin and loop design of various pri- miRNAs including its position in a transgene cassette.

[0034] FIG. 2 is a graph depicting PD1 relative RNA expression following transfection of various combinations of miRNA constructs in the presence or absence of MUC16-specific CAR. Constructs #1-8 as depicted on the X-axis are as schematically presented in Table 10.

[0035] FIG. 3A, 3B and 3C are graphs depicting normalized absolute transcript counts obtained from gene analysis of >700 genes using a Nanostring human gene panel code set with CD3/CD28 bead-stimulated CD33 CAR-T cells. In FIG. 3A, the Y-axis plots the transcript counts from CAR-T cells containing an intron coding for 2 checkpoint inhibitor miRNAs targeting PD-1 and TIGIT (CD33 CAR-mbIL15-HER1t + miRNA (PD-1 + TIGIT)), and the X-axis plots the transcripts from CAR-T cells only (not containing any checkpoint inhibitory miRNA). The circles denote the genes of interest. In FIG. 3B, the Y-axis plots the transcript counts from PD-1 miRNA containing CAR-T cells (CD33 CAR-mbIL15-HER1t + miRNA (PD-1 + PD-1)) and the X-axis plots the transcripts from CAR-T cells only. FIG. 3C plots the non-targeting miRNA control (CD33 CAR-mbIL15-HER1t + miRNA scrambled*) on the Y-axis and CAR-T cells without a miRNA containing intron on the X-axis. The circles denote the genes of interest and used to depict the on-target specificity of the checkpoint inhibitor miRNA designs. All three graphs were derived from one donor. * Scrambled controls are non-targeting miRNAs.

[0036] FIG. 4A-C are graphs depicting the normalized absolute transcript counts obtained from gene analysis of >700 genes using a Nanostring human gene panel code set with CD3/CD28 bead stimulated MUC16-specific CAR-T cells. In FIG. 4A, the Y-axis plots the transcript counts from CAR-T cells containing an intron coding for miRNAs targeting two different sequences within PD-1 and a sequence for TIGIT (MUC16CAR-mbIL15-HER1t (collectively also referred to as "MUC16CAR")+miRNA (PD-1/PD-1/TIGIT)), and the X-axis plots the transcripts from CAR-T cells without a miRNA-containing intron (MUC16CAR-mbIL15-HER1t). The black circles denote the genes of interest. In FIG. 4B, the X-axis is the same, and the Y-axis plots the transcript

counts from dual PD-1 targeting miRNA containing CAR-T cells (MUC16CAR-mbIL15-HER1t +miRNA (PD-1/PD-1)). FIG. 4C plots the non-targeting miRNA control (MUC16CAR-mbIL15-HER1t +miRNA (scrambled)) on the Y-axis and CAR-T cells without a miRNA-containing intron on the X-axis. All three graphs are from one donor.

[0037] FIG. 5A is a graph depicting the number of GFP+ K562 cells expressing MUC16 over time. The line with black circle filled dots at each time point denotes number GFP+ target cells in wells without CAR-T cells. The line with square open points denotes target cell counts in wells with MUC16 CAR-mbIL15-HER1t CAR-T cells without a miRNA-containing intron, (("with CAR-T cells")). The line with grey circle filled points denotes the target cell counts in wells with CAR-T cells containing a synthetic intron with dual PD-1 targeting miRNAs (MUC16 CAR-mbIL15-HER1t+ miRNA (PD-1/PD-1) ("with CAR-T + miRNA cells"). Data are from one donor, plotted is the mean + SD of triplicate wells. *** $P<0.001$ based on a 2-way ANOVA with Dunnett's Multiple Comparison post hoc test.

[0038] FIG. 5B is a graph depicting the number of GFP+ K562/MUC16+/PD-L1+/CD155+ cells over time. The line with square filled points at each time point denotes number GFP+ target cells only in wells. The line with open circle points denotes target cell counts in wells with MUC16-specific CAR-T cells without a miRNA-containing intron (MUC16 CAR-mbIL15-HER1t (with CAR-T cells)). The line with open circle filled points denotes the target cell counts in wells with CAR-T cells containing a synthetic intron with dual PD-1 and a TIGIT targeting miRNAs (MUC16CAR-mbIL15-HER1t +miRNA (PD-1/PD-1/TIGIT)(with CAR-T + miRNA cells)). Data are from one donor, plotted is the mean + SD of triplicate wells.

[0039] FIG. 6A-B depicts cytokine expression levels of IFN gamma and GM-CSF in MUC16 CAR-T cells with a combination of one or more checkpoint inhibitor miRNAs following co-culture with tumor target cells (K562/MUC16t). FIG. 6C-D depicts cytokine expression levels of IFN gamma and GM-CSF in MUC16 CAR-T cells with a combination of one or more checkpoint inhibitor miRNAs without co-culture with tumor target cells. Constructs # 1-11 as depicted on the X-axis are as schematically presented in Table 11.

[0040] FIG. 7 shows the tumor burden in mice treated with MUC16 CAR+mbIL-15+HER1t (shown as "MUC16 CAR") in combination with various miRNAs.

[0041] FIG. 8A demonstrates PD-1 levels in cell populations following gating hCD45/CD3+/HER1t+ expression in the blood of MUC16CAR+mbIL15+HER1t (CAR only) and MUC16CAR+mbIL15+HER1t+miRNA (PD1/PD-1) (CAR+miRNA (PD-1/PD-1)) treated mice. FIG. 8B shows PD-1 levels as measured by median fluorescent intensity (MFI) in CAR and CAR+miRNA (PD-1/PD-1) treated mice.

[0042] FIG. 9A and 9B demonstrates PD-1 and TIGIT MFI levels in cell populations following gating hCD45/CD3+/HER1t+ expression in the blood of various CAR and CAR+miRNA treated mice. Groups # 1-9 as depicted on the X-axis are as schematically presented in Table 12.

[0043] FIG. 10A demonstrates that the PD1 silencer module produces guide miRNAs and a corresponding decrease in PD1 mRNA expression in UltraCAR-T cells generated from 5 T cell donors. RT-qPCR results of PD1-targeting guide miRNAs are depicted.

[0044] FIG. 10B demonstrates that the PD1 silencer module produced guide miRNAs and a corresponding decrease in PD1 mRNA expression in ultraCAR-T cells generated from 5 T cell donors. RT-qPCR results of PD1 mRNA are depicted.

[0045] FIG. 11 demonstrates that the PD1 silencing module preferentially produced PD1-targeting guide miRNAs over non-targeting passenger miRNAs.

[0046] FIGs. 12A-E demonstrate that guide miRNAs are the predominant small RNA species originating from the PD1 silencer module.

[0047] FIG. 13 shows quantification of mature miRNAs mapping to the PD1 silencer module as a percentage of total small RNAseq reads.

[0048] FIG. 14 A and B shows differential gene expression in ROR1+PD1 silencer cells compared to ROR1 UltraCAR-T control cells.

[0049] FIGs. 15A-D show a comparison of predicted miRNA binding strength to transcript log fold change.

[0050] FIG. 16 provides an exemplary scheme for a genetic construct of the present disclosure.

[0051] FIG. 17 provides a schematic depiction of pathways and elements of the present disclosure.

[0052] FIG. 18 provides examples of pathways by which treatment regimens of the present disclosure can proceed.

[0053] FIG. 19 provides an indication of additional embodiments of the present disclosure.

[0054] FIG. 20 provides an indication of additional embodiments of the present disclosure.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0055] The following description and examples illustrate embodiments of the present disclosure in detail.

[0056] It is to be understood that the present disclosure 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 variations and modifications of the present disclosure, which are encompassed within the scope of the present invention.

[0057] 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.

[0058] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0059] Although various features of the disclosure 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 present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment.

[0060] 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. The terminology used herein is for the purpose of describing particular

embodiments only, and is not intended to be limiting.

I. Definitions

[0061] In this application, the use of the singular includes the plural unless specifically stated otherwise. As used in the specification, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0062] In this application, the use of "or" means "and/or" unless stated otherwise. The terms "and/or" and "any combination thereof" and their grammatical equivalents as used herein, can be used interchangeably. These terms can convey that any combination is specifically contemplated. Solely for illustrative purposes, the following phrases "A, B, and/or C" or "A, B, C, or any combination thereof" can mean "A individually; B individually; C individually; A and B; B and C; A and C; and A, B, and C." The term "or" can be used conjunctively or disjunctively, unless the context specifically refers to a disjunctive use.

[0063] Furthermore, use of the term "including" as well as other forms, such as "include," "includes," and "included," is not limiting.

[0064] 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 present disclosures.

[0065] As used in this specification and the 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 disclosure, and vice versa. Furthermore, compositions of the present disclosure can be used to achieve methods of the present disclosure.

[0066] The term "about" or "approximately" means within an acceptable error range for the

particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. For example, "about" can mean within 1 or more than 1 standard deviation, per the practice in the art. Alternatively, "about" can mean a range of up to 20%, up to 10%, up to 5%, or up to 1% of a given value. In another example, the amount "about 10" includes 10 and any amounts from 9 to 11. In yet another 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. Alternatively, particularly with respect to biological systems or processes, the term "about" can mean within an order of magnitude, preferably within 5-fold, and more preferably within 2-fold, of a value. Where particular values are described in the application and claims, unless otherwise stated the term "about" meaning within an acceptable error range for the particular value should be assumed.

[0067] A "therapeutically-effective amount" or "therapeutically-effective dose" refers to an amount or dose effective, for periods of time necessary, to achieve a desired therapeutic result. The amount can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the inventive nucleic acid sequences to elicit a desired response in the individual.

[0068] "Polynucleotide" or "oligonucleotide" refers to a polymeric form of nucleotides or nucleic acids 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.

[0069] Unless otherwise stated, nucleic acid sequences in the text of this specification are given, when read from left to right, in the 5′ to 3′ direction.

[0070] The terms "transfection," "transformation," "nucleofection," or "transduction" as used herein refer to the introduction of one or more exogenous polynucleotides into a host cell or organism by using physical, chemical, and/or electrical methods. The nucleic acid sequences and

vectors disclosed herein can be introduced into a cell or organism by any such methods, including, for example, by electroporation, calcium phosphate co-precipitation, strontium phosphate DNA co-precipitation, liposome mediated-transfection, DEAE dextran mediated-transfection, polycationic mediated-transfection, tungsten particle-facilitated microparticle bombardment, viral, and/or non-viral mediated transfection. In some cases, the method of introducing nucleic acids into the cell or organism involves the use of viral, retroviral, lentiviral, or transposon, or transposable element-mediated (e.g., *Sleeping Beauty*) vectors.

[0071] "Polypeptide," "peptide," and their grammatical equivalents as used herein refer to a polymer of amino acid residues. The polypeptide can optionally include glycosylation or other modifications typical for a given protein in a given cellular environment. Polypeptides and proteins disclosed herein (including functional fragments and functional variants thereof) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, α-amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, α-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, ornithine, α-aminocyclopentane carboxylic acid, α-aminocyclohexane carboxylic acid, α-aminocycloheptane carboxylic acid, α-(2-amino-2-norbornane)-carboxylic acid, α,γ-diaminobutyric acid, α,β-diaminopropionic acid, homophenylalanine, and α-tert-butylglycine. The present disclosure further contemplates that expression of polypeptides or proteins described herein in an engineered cell can be associated with post-translational modifications of one or more amino acids of the polypeptide or protein. Non-limiting examples of post-translational modifications include phosphorylation, acylation including acetylation and formylation, glycosylation (including N-linked and O-linked), amidation, hydroxylation, alkylation including methylation and ethylation, ubiquitylation, addition of pyrrolidone carboxylic acid, formation of disulfide bridges, sulfation, myristoylation, palmitoylation, isoprenylation, farnesylation, geranylation, glypiation, lipoylation and iodination.

[0072] The term "conservative amino acid substitution" or "conservative mutation" refers to the replacement of one amino acid by another amino acid with a common property. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous organisms (Schulz, G. E. and Schirmer, R. H., Principles of Protein Structure, Springer-Verlag, New York (1979)). According to such analyses, groups of amino acids can be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and Schirmer, R. H., *supra*). Examples of conservative mutations include amino acid substitutions of amino acids within the sub-groups below, for example, lysine for arginine and vice versa such that a positive charge can be maintained; glutamic acid for aspartic acid and vice versa such that a negative charge can be maintained; serine for threonine such that a free –OH can be maintained; and glutamine for asparagine such that a free $-NH_2$ can be maintained. Exemplary conservative amino acid substitutions are shown in the following chart:

Type of Amino Acid	**Substitutable Amino Acids**
Hydrophilic	Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser, Thr
Sulphydryl	Cys
Aliphatic	Val, Ile, Leu, Met
Basic	Lys, Arg, His
Aromatic	Phe, Tyr, Trp

An amino acid sequence that differs from a reference amino acid sequence by only conservative amino acid substitutions will be referred to herein as a "conservatively-substituted variant" of the reference sequence.

[0073] In some embodiments, the functional variants can comprise the amino acid sequence of the reference protein with at least one non-conservative amino acid substitution. The term "non-

conservative mutations" involve amino acid substitutions between different groups, for example, lysine for tryptophan, or phenylalanine for serine, etc. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with, or inhibit the biological activity of, the functional variant. The non-conservative amino acid substitution can enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the homologous parent protein. Amino acid substitutability is discussed in more detail, for example, in L. Y. Yampolsky and A. Stoltzfus, "The Exchangeability of Amino acids in Proteins," *Genetics* 2005 Aug.; 170(4):1459-1472.

[0074] The terms "identical" and its grammatical equivalents as used herein or "sequence identity" in the context of two nucleic acid sequences or amino acid sequences of polypeptides refer to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. A "comparison window," as used herein, refers to a segment of at least about 20 contiguous positions, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence can be compared to a reference sequence of the same number of contiguous positions after the two sequences are aligned optimally. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.*, 2:482 (1981); by the alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.*, 48:443 (1970); by the search for similarity method of Pearson and Lipman, *Proc. Nat. Acad. Sci U.S.A.*, 85:2444 (1988); by computerized implementations of these algorithms (including, but not limited to CLUSTAL in the PC/Gene program by Intelligentics, Mountain View Calif., GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wis., U.S.A.); the CLUSTAL program is well described by Higgins and Sharp, *Gene,* 73:237-244 (1988) and Higgins and Sharp, *CABIOS*, 5:151-153 (1989); Corpet et al., *Nucleic Acids Res.*, 16:10881-10890 (1988); Huang et al., *Computer Applications in the Biosciences*, 8:155-165 (1992); and Pearson et al., *Methods in Molecular Biology*, 24:307-331 (1994). Alignment is also often performed by inspection and manual alignment. In one class of embodiments, the polypeptides herein are at least 80%, 85%, 90%, 98% 99% or 100% identical to a reference polypeptide (*i.e.*, the full length thereof), 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 (*i.e.*, the full length thereof) 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 said 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.

[0075] For purposes of this specification and the claims, it is understood that the phrase "having at least 50% sequence identity with" a reference sequence, or referencing any range therein (*e.g.*, "at least 80% sequence identity with") encompasses the reference sequence itself. Thus, for example, a claim reciting "a nucleic acid having at least 80% sequence identity with SEQ ID NO: 0" encompasses SEQ ID NO: 0 itself.

[0076] The term "substantially identical" and its grammatical equivalents as applied to nucleic acid or amino acid sequences mean that a nucleic acid or amino acid sequence comprises a sequence that has at least 95% sequence identity with a reference sequence using the programs described above, *e.g.*, BLAST, using standard parameters.

[0077] "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. Methods for determining sequence identity percentages (*e.g.*, BLASTP and BLASTN using default parameters) are described herein and are generally available. 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 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.

[0078] Also contemplated and included herein are nucleic acid molecules that hybridize to the disclosed sequences. Hybridization conditions may be mild, moderate, or stringent, as is warranted.

[0079] Appropriate stringency conditions which promote DNA hybridization, for example, 6× sodium chloride/sodium citrate (SSC) at about 45° C, followed by a wash of 2×SSC at 50° C, are known or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. "Stringent hybridization conditions" are those that include a salt concentration of 1.0 M NaCl in 50% formamide, at a temperature of 37 ºC for 4 to 12 hours, followed by a wash in 0.1X SSC at 60-65 ºC.

[0080] As will be appreciated by the skilled practitioner, slight changes in nucleic acid sequence do not necessarily alter the amino acid sequence of the encoded polypeptide. This disclosure embraces the degeneracy of codon usage as would be understood by one of ordinary skill in the art. For example, as known in the art, different codons will code for the same amino acid as illustrated in the following chart.

Amino Acid	**Codons**
Ala/A	GCT, GCC, GCA, GCG
Arg/R	CGT, CGC, CGA, CGG, AGA, AGG
Asn/N	AAT, AAC
Asp/D	GAT, GAC
Cys/C	TGT, UGC
Gln/Q	CAA, CAG
Glu/E	GAA, GAG
Gly/G	GGT, GGC, GGA, GGG
His/H	CAT, CAC
Ile/I	ATT, ATC, ATA
Leu/L	TTA, TTG, CTT, CTC, CTA, CTG
Lys/K	AAA, AAG

Met/M	ATG
Phe/F	TTT, TTC
Pro/P	CCT, CCC, CCA, CCG
Ser/S	TCT, TCC, TCA, TCG, AGT, AGC
Thr/T	ACT, ACC, ACA, ACG
Trp/W	TGG
Tyr/Y	TAT, TAC
Val/V	GTT, GTC, GTA, GTG
START	ATG
STOP	TAG, TGA, TAA

As used herein, the phrase "codon degenerate variant" when used with reference to a nucleic acid sequence means a nucleic acid sequence that differs from the referenced sequence, but that encodes a polypeptide having the same amino acid sequence as that encoded by the referenced sequence.

[0081] Additionally, it will be appreciated by persons skilled in the art that partial sequences often work as effectively as full-length versions. The ways in which the nucleotide sequence can be varied or shortened are well known to persons skilled in the art, as are ways of testing the suitability or effectiveness of the altered genes. In certain embodiments, suitability and/or effectiveness of the altered gene may easily be tested by, for example, conventional gas chromatography. All such variations of the genes are therefore included as part of the present disclosure.

[0082] The term "isolated" and its grammatical equivalents as used herein refer to the removal of a nucleic acid from its natural environment. 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.

[0083] The term "purified" and its grammatical equivalents as used herein refer to a molecule or composition, whether removed from nature (including genomic DNA and mRNA) or synthesized (including cDNA) and/or amplified under laboratory conditions, that has been increased in purity, wherein "purity" is a relative term, not "absolute purity." For example, nucleic acids typically are mixed with an acceptable carrier or diluent when used for introduction into

cells. The term "substantially purified" and its grammatical equivalents as used herein refer to a nucleic acid sequence, polypeptide, protein or other compound that is essentially free, *i.e.*, is more than about 50% free of, more than about 70% free of, more than about 90% free of, the polynucleotides, proteins, polypeptides and other molecules that the nucleic acid, polypeptide, protein or other compound is naturally associated with.

[0084] "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.

[0085] "Transposon," "transposable element" or "TE" refers to 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 transposon. Class I transposons 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 transposon itself. The characteristics of retrotransposons are similar to retroviruses, such as HIV. The cut-and-paste transposition mechanism of class II transposons 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 transposon excision by transposase. Cut-and-paste transposons 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 transposons. Autonomous transposons can move by themselves while non-autonomous transposons require the presence of another transposon to move. This is often because non-

autonomous transposons lack transposase (for class II) or reverse transcriptase (for class I).

[0086] "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.

[0087] An "expression vector" or "vector" is any genetic element, *e.g.*, a plasmid, a mini-circle, a nanoplasmid, 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 that 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. 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. A vector also can comprise a selectable marker gene. In certain embodiments where nano plasmids are utilized, strains such as R6K that utilizes an antisense RNA selection marker (e.g. sucrose tolerance) can be used.

[0088] The term "selectable marker gene" 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.

[0089] The term "coding sequence" refers to a segment of a polynucleotide that encodes for protein or 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.

[0090] The term "operably linked" 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, that 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 and in the correct reading frame with respect to the transcription initiation site so as to allow 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, respectively, increase or decrease the transcription of the DNA sequence. Enhancers and silencers can be located upstream or downstream of 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 pre-protein 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.

[0091] The terms "induce," "induction" and their grammatical equivalents as used herein refer 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.

[0092] 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).

[0093] 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 or downstream of coding sequences or within 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).

[0094] The term "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. The term "promoter activity" and its grammatical equivalents as used herein refer 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.

[0095] "Inducible promoter" refers to a promoter, that 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. Non-limiting examples of inducible promoters include alcohol-regulated promoters, tetracycline-regulated promoters, steroid-regulated promoters, metal-regulated promoters, pathogenesis-regulated promoters, temperature-regulated promoters and light-regulated promoters. The inducible promoter can be part of a gene switch or genetic switch.

[0096] "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.

[0097] As used herein, the phrase "functional fragment" when used with reference to a polypeptide refers to a fragment of such polypeptide that possesses the primary function of the referenced polypeptide. For example, a functional fragment of a polypeptide that serves as a transmembrane domain is a fragment of that polypeptide that also serves as a transmembrane domain. In certain embodiments, the functional fragment of a polypeptide is shorter than the referenced polypeptide by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. When used with reference to a nucleic acid, the phrase "functional fragment" refers to a fragment of the referenced nucleic acid that encodes a polypeptide having the same primary function as the polypeptide encoded by the referenced nucleic acid.

[0098] As used herein, the phrase "functional variant" when used with reference to a polypeptide refers to a polypeptide that differs from the referenced polypeptide but possesses the primary function of the referenced polypeptide. For example, a functional variant of a polypeptide that serves as a transmembrane domain is a fragment of that polypeptide that also serves as a

transmembrane domain. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the referenced amino acid sequence and/or is a conservatively-substituted variant of the referenced sequence. When used with reference to a nucleic acid, the phrase "functional variant" refers to a nucleic acid that differs from the referenced nucleic acid but encodes a polypeptide having the same primary function as the polypeptide encoded by the referenced nucleic acid. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the referenced nucleic acid sequence, hybridizes under stringent hybridization conditions with the complement of the referenced nucleic acid sequence, or is a codon degenerate variant of the nucleic acid sequence.

[0099] The term "antibody," also known as immunoglobulin (Ig), as used herein can refer to a monoclonal or polyclonal antibody. The term "monoclonal antibodies," as used herein, refers to antibodies that are produced by a single clone of B-cells and bind to the same epitope. In contrast, "polyclonal antibodies" refer to a population of antibodies that is produced by different B-cells and bind to different epitopes of the same antigen. The antibodies can be from any animal origin. An antibody can be IgG (including IgGl, IgG2, IgG3, and IgG4), IgA (including IgA1 and IgA2), IgD, IgE, or IgM, and IgY. In some embodiments, the antibody can a single-chain whole antibody. An antibody typically consists of four polypeptides: two identical copies of a heavy (H) chain polypeptide and two identical copies of a light (L) chain polypeptide. Each of the heavy chains contains one N-terminal variable (V_H) region and three C-terminal constant (CH1, CH2 and CH3) regions, and each light chain contains one N-terminal variable (V_L) region and one C-terminal constant (C_L) region. The variable regions of each pair of light and heavy chains form the antigen-binding site of an antibody. The V_H and V_L regions have a similar general structure, with each region comprising four framework regions, whose sequences are relatively conserved. The framework regions are connected by three complementarity determining regions (CDRs). The three CDRs, known as CDR1, CDR2, and CDR3, form the "hypervariable region" of an antibody, which is responsible for antigen binding. These particular regions have been described by Kabat et al., J. Biol. Chem. 252, 6609-6616 (1977) and Kabat et al., Sequences of protein of immunological interest. (1991), by Chothia et al., J. Mol. Biol. 196:901-917 (1987), and by MacCallum et al., J. Mol. Biol. 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Preferably, the term "CDR" is

a CDR as defined by Kabat, based on sequence comparisons. CDRH1, CDRH2 and CDRH3 denote the heavy chain CDRs, and CDRL1, CDRL2 and CDRL3 denote the light chain CDRs.

[0100] The terms "fragment of an antibody," "antibody fragment," "fragment of an antibody," "antigen-binding portion" and their grammatical equivalents are used interchangeably herein to mean one or more fragments or portions of an antibody that retain the ability to specifically bind to an antigen (see, generally, Holliger et al., Nat. Biotech., 23(9):1126-1129 (2005)). The antibody fragment desirably comprises, for example, one or more CDRs, the variable region (or portions thereof), the constant region (or portions thereof), or combinations thereof. Non-limiting examples of antibody fragments include (1) a Fab fragment, which is a monovalent fragment consisting of the V_L, V_H, C_L, and CH1 domains; (2) a F(ab')2 fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the stalk region; (3) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody; (4) a single chain Fv (scFv), which is a monovalent molecule consisting of the two domains of the Fv fragment (*i.e.*, V_L and V_H) joined by a linker that enables the two domains to be synthesized as a single polypeptide chain (see, *e.g.*, Bird et al., Science, 242: 423-426 (1988); Huston et al., Proc. Natl. Acad. Sci. USA, 85: 5879-5883 (1988); and Osbourn et al., Nat. Biotechnol., 16: 778 (1998)) and (5) a diabody, which is a dimer of polypeptide chains, wherein each polypeptide chain comprises a V_H connected to a V_L by a peptide linker that is too short to allow pairing between the V_H and V_L on the same polypeptide chain, thereby driving the pairing between the complementary domains on different V_H-V_L polypeptide chains to generate a dimeric molecule having two functional antigen-binding sites. Antibody fragments are known in the art and are described in more detail in, *e.g.*, U.S. Patent 8,603,950.

[0101] The terms "antigen recognition moiety," "antigen recognition domain," "antigen-binding domain," and "antigen binding region" refer to a molecule or portion of a molecule that specifically binds to an antigen. In one embodiment, the antigen recognition moiety is an antibody, antibody like molecule or fragment thereof.

[0102] The term "proliferative disease" refers to a unifying concept in which excessive proliferation of cells and/or turnover of cellular matrix contributes significantly to the pathogenesis of the disease, including cancer. In some embodiments, the proliferative disease is cancer.

[0103] "Patient" or "subject" refers to a mammalian subject diagnosed with or suspected of having or developing a proliferative disorder such as cancer. In some embodiments, the term "patient" refers to a mammalian subject with a higher than average likelihood of developing a proliferative disorder such as cancer. Exemplary patients can be humans, apes, dogs, pigs, cattle, cats, horses, goats, sheep, rodents and other mammalians that can benefit from the therapies disclosed herein. Exemplary human patients can be male and/or female. "Patient in need thereof" or "subject in need thereof" means a patient diagnosed with or suspected of having a disease or disorder, for instance, but not restricted to cancer.

[0104] "Administering" refers to herein as providing one or more compositions described herein to a patient or a subject. By way of example and not limitation, composition administration, *e.g.*, injection, can be performed by intravenous (i.v.) injection, sub-cutaneous (s.c.) injection, intradermal (i.d.) injection, intraperitoneal (i.p.) injection, or intramuscular (i.m.) injection. One or more such routes can be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time. Alternatively, or concurrently, administration can be by the oral route. Additionally, administration can also be by surgical deposition of a bolus or pellet of cells, or positioning of a medical device.

[0105] As used herein, the terms "treatment," "treating," and their grammatical equivalents refer to obtaining a desired pharmacologic and/or physiologic effect. In some embodiments, the effect is therapeutic, *i.e.*, the effect partially or completely cures a disease and/or adverse symptom attributable to the disease. In some embodiments, the term "treating" can include "preventing" a disease or a condition.

[0106] As used herein, a "treatment interval" refers to a treatment cycle, for example, a course of administration of a therapeutic agent that can be repeated, e.g., on a regular schedule. In some embodiments, a dosage regimen can have one or more periods of no administration of the therapeutic agent in between treatment intervals.

[0107] The terms "administered in combination," "co-administration," "co-administering," and "co-providing" as used herein, mean that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, *e.g.*, the two or more treatments are delivered after the subject has been diagnosed with the disorder and before the

disorder has been cured or eliminated or treatment has ceased for other reasons. In some embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In some embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, e.g., an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In some embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[0108] In some embodiments, the first treatment and second treatment can be administered simultaneously (e.g., at the same time), in the same or in separate compositions, or sequentially. Sequential administration refers to administration of one treatment before (e.g., immediately before, less than 5, 10, 15, 30, 45, 60 minutes; 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 48, 72, 96 or more hours; 4, 5, 6, 7, 8, 9 or more days; 1, 2, 3, 4, 5, 6, 7, 8 or more weeks before) administration of an additional, e.g., secondary, treatment. The order of administration of the first and secondary treatment can also be reversed.

[0109] The terms "therapeutically effective amount," therapeutic amount," "immunologically effective amount," "anti-tumor effective amount," "tumor-inhibiting effective amount," and their grammatical equivalents refer to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. The therapeutically effective amount can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of a composition described herein to elicit a desired response in one or more subjects. The precise amount of the compositions of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject).

[0110] Alternatively, the pharmacologic and/or physiologic effect of administration of one or more compositions described herein to a patient or a subject of can be "prophylactic," *i.e.*, the effect completely or partially prevents a disease or symptom thereof. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired prophylactic result (*e.g.*, prevention of disease onset).

[0111] As used herein, the term "immune checkpoint protein" refers to a molecule that transmits a suppressive signal or has an immunosuppressive function. Examples of such immune checkpoint proteins include, but are not limited to, CTLA-4, PD-1, PD-L1 (programmed cell death-ligand 1), PD-L2 (programmed cell death-ligand 2), LAG-3 (Lymphocyte activation gene 3), TIM3 (T cell immunoglobulin and mucin-3), BTLA (B and T lymphocyte attenuator), B7H3, B7H4, CD160, CD39, CD70, CD73, A2aR (adenosine A2a receptor), KIR (killer inhibitory receptor), VISTA (V-domain Ig-containing suppressor of T cell activation), IDO1 (Indoleamine 2,3-dioxygenase), Arginase I, TIGIT (T cell immunoglobulin and ITIM domain), CD115, and the like (see, Nature Reviews Cancer, 12, p. 252-264, 2012 and Cancer Cell, 27, p. 450-461, 2015).

[0112] As used herein, terms used in the identification of biological moieties may include, or may not include, a dash " – " within the term. The presence or absence of a dash does not change the intended meaning or identification of the biological moiety. By way of illustration only, and without limitation to these biological moieties, each of the following paired terms (shown with/without a dash) indicate and identify the same biological entities: CCR-4/CCR4, CD-3/CD3, CD-4/CD4, CD-33/CD33, EGFR-2/EGFR2, FLT-1/FLT1, HER-1/HER1, HER-1t/HER1t, IL-12/IL12, IL-15/IL15, IL-15Rα/IL15Rα MUC-1/MUC1, MUC-16/MUC16, ROR-1/ROR1, ROR-1R/ROR1R, TGF-Beta/TGFBeta, VEGF-1/VEGF1, VEGF-R2/VEGFR2."

II. Combinations

[0113] In certain embodiments, miRNA(s) or polynucleotides encoding the miRNA(s) as described herein can be used in combination with a chimeric receptor or can further comprise a nucleic acid sequence encoding a chimeric receptor, respectively. In some cases, the chimeric receptor is a chimeric antigen receptor (CAR). In some instances, the CAR comprises a pattern-recognition receptor. In other cases, the chimeric receptor comprises an engineered T-cell receptor (TCR). The miRNA(s) can be used in combination with specific CARs, cytokines, and cell tags,

for example MUC16-specific CAR with a fusion protein comprising IL15 and IL-15Rα (mbIL15) and truncated HER1 (HER1t), MUC1-specific CAR with mbIL15 and HER1t, or CD33-specific CAR with mbIL15 and HER1t.

[0114] In certain embodiments, the genetic construct can include a 5' untranslated region (5'UTR) and/or a 3' untranslated region (3'UTR). In some embodiments, the sequences encoding the miRNA(s) can be located in the 5'UTR and/or the 3'UTR. In some embodiments, the 5'UTR and/or the 3'UTR do not contain an intron.

[0115] In certain embodiments, a nucleic acid sequence encoding a synthetic intron with checkpoint inhibitor miRNAs is inserted into the same genetic construct as that encoding the chimeric receptor (for example, in the portion of the construct corresponding to the 5'UTR of the mRNA encoding the chimeric receptor). An exemplary depiction of the order of polynucleotide sequences can be found in FIG. 1A. In one embodiment, the 5'UTR contains one or more mature miRNAs. In some embodiments, the one or more mature miRNA(s) can be directed to the same immune checkpoint protein target, for example PD-1. In certain other embodiments, the mature miRNAs can be directed to more than one immune checkpoint protein target, for example PD-1 and TIGIT, PD1 and CTLA4, or TIGIT and CTLA4.

III. miRNA(s)

[0116] As used herein, the terms "miR," "mir" and "miRNA" are used to refer to microRNA, a class of small non-coding RNA molecules that are capable of affecting the expression of a gene (the "target gene") by modulating the translation of messenger RNA transcribed therefrom (either increasing or decreasing the gene's expression) and/or destabilizing such messenger RNA.

[0117] The miRNAs can be non-naturally occurring. The terms "non-naturally occurring," "synthetic," and "artificial," as used to describe miRNA(s) herein, are used interchangeably and refer to an miRNA having a sequence that does not occur in nature. In some embodiments, a non-naturally occurring miRNA effectively mimics naturally-occurring miRNA. Non-naturally occurring miRNA can be designed such that the desired hairpins or loops of the corresponding naturally-occurring miRNA are maintained when a naturally-occurring mature miRNA sequence is replaced with a synthetic sequence designed to target a specific transcript. For example, the non-

naturally occurring miRNA can have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or greater sequence identity with naturally-occurring miRNA and/or can hybridize under stringent hybridization conditions with naturally-occurring miRNA. The term "miRNA," unless otherwise indicated, refers generically to the mature, pri-, and pre-forms of a particular microRNA and functional fragments and variants thereof.

[0118] The term "pri-miRNA" refers to the primary miRNA transcript containing at least one RNA hairpin. Exemplary depictions of pri-miRNA are illustrated in FIG. 1B. The RNA hairpin(s) are cleaved from the pri-miRNA in the cell nucleus to form one or more precursor miRNAs ("pre-miRNAs). This pre-miRNA is exported into the cytoplasm where the stem loop structure is cleaved to produce a double-stranded miRNA comprising a miRNA-5p strand from the former 5' arm of the hairpin loop and a miRNA-3p strand from the former 3' arm of the hairpin loop. The Argonaute protein then binds the double-stranded miRNA and one of the strands (either the miRNA-5p sequence or the miRNA-3p sequence) is released. The remaining bound strand become the "guide strand" whereas the released strand is known as the "passenger strand" and preferably degrades. The guide strand then goes on to interact with the messenger RNA derived from the target gene, thus affecting its translation.

[0119] Both the miRNA-5p and miRNA-3p strand sequences will be referred to herein as "mature miRNA" sequences. The remaining portions of the pri-miRNA (the portion thereof 5' to the miRNA-5p sequence, the portion thereof 3' to the miRNA-3p sequence, and the stem loop sequence in between the miRNA-5p and miRNA-3p sequences) will be collectively referred to as miRNA backbone sequences. The term "5' backbone sequence" will be used herein to refer to the backbone sequence that, in a pri-miRNA, is 5' of the miRNA-5p sequence. The term "3' backbone sequence" will be used herein to refer to the backbone sequence that, in a pri-miRNA, is 3' of the miRNA-3p sequence. The term "stem loop sequence" refers to the backbone sequence that, in a pri-miRNA, is between the miRNA-5p and miRNA-3p sequences.

[0120] The pri-miRNA of the present invention may be produced from naturally-occurring pri-miRNAs by removing the native mature miRNA sequences and replacing them with non-native

mature miRNA sequences wherein one of the sequences is capable of serving as a guide miRNA targeting a gene of interest. In the design of a non-naturally occurring pri-miRNA, backbone miRNA nucleic acid sequences can be derived from mouse, rat, or human miRNA sequences. As a non-limiting example, backbone miRNA sequences can be derived from miR150, miR206, miR204, miR17, miR16, miR30a, miR126, miR122, miR213, miR29b1 or miR133a1.

[0121] Examples of backbone sequences that may be used in the practice of the present invention include, but are not limited, to those encoded by the DNA sequences listed in Table 1 below. The symbols of "X" and "Y" in Table 1 indicate nucleic acid sequences encoding, respectively, the guide miRNA (which may be either miRNA-5p or miRNA-3p) and the passenger miRNA (which may be either miRNA-5p or miRNA-3p), whereas the symbol of "n" indicates the number of nucleotides in such sequences, for example 16-30, preferably 18-25. In some embodiments, n can be 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 or 35 nucleotides. In certain embodiments, the backbone miRNA sequences are those that are encoded by sequences that hybridize under stringent hybridization conditions with the complement of any one of the sequences listed in Table 1.

Table 1: Nucleic acids encoding miRNA backbone sequences

Synthetic miRNA	Nucleic acid sequences encoding 5' Backbone Sequence – X_n-Stem Loop Sequence –Y_n-3' Backbone Sequence
miR204	SEQ ID NO: 1-**X_n**-SEQ ID NO: 2-**Y_n**-SEQ ID NO: 3
miR206	SEQ ID NO: 4-**Y_n**-SEQ ID NO: 5-**X_n**-SEQ ID NO: 6
miR17	SEQ ID NO: 7-**X_n**-SEQ ID NO: 8-**Y_n**-SEQ ID NO: 9
miR150	SEQ ID NO: 10-**X_n**-SEQ ID NO: 11-**Y_n**-SEQ ID NO: 12
miR150	SEQ ID NO: 13-**Y_n**-SEQ ID NO: 14-**X_n**-SEQ ID NO: 15
miR16	SEQ ID NO: 16-**X_n**-SEQ ID NO: 17-**Y_n**-SEQ ID NO: 18
miR30a	SEQ ID NO: 19-**X_n(or Y_n)**-SEQ ID NO: 20-**Y_n(or X_n)**-SEQ ID NO: 21
miR126	SEQ ID NO: 22-**Y_n**-SEQ ID NO: 23-**X_n**-SEQ ID NO: 24
miR122	SEQ ID NO: 25-**X_n**-SEQ ID NO: 26-**Y_n**-SEQ ID NO: 27
miR214	SEQ ID NO: 28-**Y_n**-SEQ ID NO: 29-**X_n**-SEQ ID NO: 30
miR214	SEQ ID NO: 31-**X_n**-SEQ ID NO: 32-**Y_n**-SEQ ID NO: 33
miR29b1	SEQ ID NO: 34-**Y_n**-SEQ ID NO: 35-**X_n**-SEQ ID NO: 36
miR29b1	SEQ ID NO: 37-**X_n**-SEQ ID NO: 38-**Y_n**-SEQ ID NO: 39
miR133a1	SEQ ID NO: 40-**Y_n**-SEQ ID NO: 41-**X_n**-SEQ ID NO: 42
miR26a	SEQ ID NO: 43-**X_n**-SEQ ID NO: 44-**Y_n**-SEQ ID NO: 45
miR412	SEQ ID NO: 46-**X_n**-SEQ ID NO: 47-**Y_n**-SEQ ID NO: 48
miR-19	SEQ ID NO: 49-**X_n**-SEQ ID NO: 50-**Y_n**-SEQ ID NO: 51
miR-21	SEQ ID NO: 52-**X_n**-SEQ ID NO: 53-**Y_n**-SEQ ID NO: 54
miR-142	SEQ ID NO: 55-**X_n**-SEQ ID NO: 56-**Y_n**-SEQ ID NO: 57
miR-494	SEQ ID NO: 58-**X_n**-SEQ ID NO: 59-**Y_n**-SEQ ID NO: 60
miR-1915	SEQ ID NO: 61-**X_n**-SEQ ID NO: 62-**Y_n**-SEQ ID NO: 63

[0122] While the miRNA-5p and miRNA-3p sequences hybridize with each other, they are not necessarily exactly complementary. In the design of a non-naturally occurring miRNA, compensatory mutations can be made in the miRNA-5p and/or miRNA-3p sequences so as to maintain the RNA folding and free energy of the native miRNA. In certain embodiments, the

sequence encoding the miRNA-3p sequence has at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the complement to the sequence encoding the miRNA-5p sequence or is capable of hybridizing under stringent hybridization conditions with the sequence encoding the miRNA-5p sequence.

[0123] In certain embodiments, the target gene encodes a checkpoint inhibitor protein. Thus, the present invention relates in part to a polynucleotide encoding a non-naturally occurring miRNA that inhibits the expression of an immune checkpoint protein. In certain such embodiments, the target gene encodes CTLA4, PD-1, PD-L1, TIGIT, TIM3, LAG3, GITR, or PIK3IP1. Non-limiting examples of nucleic acid sequences encoding the guide miRNA targeting genes encoding such checkpoint inhibitors are listed in Table 2. Table 2 also lists the sequences encoding the passenger strand. As previous discussed, the guide and passenger strand are not necessarily complementary. It is contemplated that the passenger strand may also serve to target the messenger RNA associated with the target gene. It is also contemplated that sequences that sequences that hybridize under stringent hybridization conditions with the complments of the sequences listed in Table 2 may also be used. The mature miRNA sequences used may be combined with a specific pri-miRNA backbone. Table 2 also lists backbones that can be combined with the mature guide and passenger miRNAs listed therein.

Table 2: Nucleic acid sequences encoding mature miRNA sequences

miRNA Target	Backbone	DNA encoding guide miRNA	DNA encoding passenger miRNA
CTLA4	miR-204	(SEQ ID NO: 64)	(SEQ ID NO: 65)
CTLA4	miR-26a	(SEQ ID NO: 66)	(SEQ ID NO: 67)
CTLA4	miR-30a	(SEQ ID NO: 68)	(SEQ ID NO: 69)
CTLA4	miR-206	(SEQ ID NO: 70)	(SEQ ID NO: 71)
PD1	miR-204	(SEQ ID NO: 72)	(SEQ ID NO: 73)
PD1	miR-204	(SEQ ID NO: 704)	
PD1	miR-204	(SEQ ID NO: 705)	
PD1	miR-204		(SEQ ID NO: 706)
PD1	miR-204		(SEQ ID NO: 707)
PD1	miR-204		(SEQ ID NO: 708)
PD1	miR-206	(SEQ ID NO: 74)	(SEQ ID NO: 75)
PD1	miR-206	(SEQ ID NO: 709)	
PD1	miR-206	(SEQ ID NO: 710)	
PD1	miR-206		(SEQ ID NO: 711)
PD1	miR-206		(SEQ ID NO: 712)
PD1	miR-206		(SEQ ID NO: 713)
PD1	miR-30a	(SEQ ID NO: 76)	(SEQ ID NO: 77)
PD1	miR-412	(SEQ ID NO: 78)	(SEQ ID NO: 79)
PD1	miR122	(SEQ ID NO: 80)	(SEQ ID NO: 81)
PD1	miR-17	(SEQ ID NO: 82)	(SEQ ID NO: 83)
PD1	miR-150	(SEQ ID NO: 74)	(SEQ ID NO: 85)
PD1	miR-486	(SEQ ID NO: 76)	(SEQ ID NO: 87)
TIGIT	miR-17	(SEQ ID NO: 88)	(SEQ ID NO: 89)
TIGIT	miR-150	(SEQ ID NO: 90)	(SEQ ID NO: 91)
TIGIT	miR-204	(SEQ ID NO: 92)	(SEQ ID NO: 93)
TIGIT	miR29b1	(SEQ ID NO: 94)	(SEQ ID NO: 95)
TIGIT	miR214	(SEQ ID NO: 96)	(SEQ ID NO: 97)
TIGIT	miR-206	(SEQ ID NO: 98)	(SEQ ID NO: 99)

TIGIT	miR-204	(SEQ ID NO: 100)	(SEQ ID NO: 101)
TIGIT	miR-22	(SEQ ID NO: 102)	(SEQ ID NO: 103)
TIGIT	miR-16	(SEQ ID NO: 104)	(SEQ ID NO: 105)
TIGIT	miR-21	(SEQ ID NO: 106)	(SEQ ID NO: 107)
TIGIT	miR-494	(SEQ ID NO: 108)	(SEQ ID NO: 109)
TIGIT	miR-142	(SEQ ID NO: 110)	(SEQ ID NO: 111)
TIGIT	miR-19	(SEQ ID NO: 112)	(SEQ ID NO: 113)
TIGIT	miR-1915	(SEQ ID NO: 114)	(SEQ ID NO: 115)
TIGIT	miR-206	(SEQ ID NO: 116)	(SEQ ID NO: 117)
TIGIT	miR-204	(SEQ ID NO: 118)	(SEQ ID NO: 119)
TIGIT	miR-22	(SEQ ID NO: 120)	(SEQ ID NO: 121)
TIGIT	miR-142	(SEQ ID NO: 122)	(SEQ ID NO: 123)
TIGIT	miR-16	(SEQ ID NO: 124)	(SEQ ID NO: 125)
TIGIT	miR-206	(SEQ ID NO: 126)	(SEQ ID NO: 127)
TIGIT	miR-204	(SEQ ID NO: 128)	(SEQ ID NO: 129)
TIGIT	miR-21	(SEQ ID NO: 130)	(SEQ ID NO: 131)
TIGIT	miR-494	(SEQ ID NO: 132)	(SEQ ID NO: 133)
TIGIT	miR-19	(SEQ ID NO: 134)	(SEQ ID NO: 135)
TIGIT	miR-1915	(SEQ ID NO: 136)	(SEQ ID NO: 137)
TIGIT	miR-204	(SEQ ID NO: 138)	(SEQ ID NO: 139)
TIGIT	miR-206	(SEQ ID NO: 140)	(SEQ ID NO: 141)
TIGIT	miR-21	(SEQ ID NO: 142)	(SEQ ID NO: 143)
TIGIT	miR-22	(SEQ ID NO: 144)	(SEQ ID NO: 145)
TIM3	miR204	(SEQ ID NO: 146)	(SEQ ID NO: 147)
TIM3	miR206	(SEQ ID NO: 148)	(SEQ ID NO: 149)
TIM3	miR17	(SEQ ID NO: 150)	(SEQ ID NO: 151)
TIM3	miR126	(SEQ ID NO: 152)	(SEQ ID NO: 153)
TIM3	miR122	(SEQ ID NO: 154)	(SEQ ID NO: 155)
TIM3	miR214	(SEQ ID NO: 156)	(SEQ ID NO: 157)
LAG3	miR-30a	(SEQ ID NO: 158)	(SEQ ID NO: 159)

LAG3	miR122	(SEQ ID NO: 160)	(SEQ ID NO: 161)
GITR	miR206	(SEQ ID NO: 162)	(SEQ ID NO: 163)
GITR	miR29b1	(SEQ ID NO: 164)	(SEQ ID NO: 165)
PIK3IP1	miR206	(SEQ ID NO: 166)	(SEQ ID NO: 167)
PIK3IP1	miR126	(SEQ ID NO: 168)	(SEQ ID NO: 169)
PIK3IP1	miR30a	(SEQ ID NO: 170)	(SEQ ID NO: 171)

[0124] In certain embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80% sequence identity with any one of SEQ ID NOs: 64–83, 85, 87–171, and 704–713 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 64–83, 85, 87–171, and 704–713. In certain such embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 704, 705, 709, and 710 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 704, 705, 709, and 710.

[0125] In certain embodiments, the miRNA targets PD-1. In certain such embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 72–83, 85, 87, and 704–713 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 72–83, 85, 87, and 704–713. In certain embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 72, 74, 76, 78, 80, 82, 704, 705, 709, and 710 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 72, 74, 76, 78, 80, 82, 704, 705, 709, and 710.

[0126] In certain embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 72–75 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 72–75. In certain such embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 72 or 74 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 72 or 74.

[0127] In certain embodiments, the present invention relates to a polynucleotide comprising:

a) a sequence encoding a 5' miRNA backbone sequence;

b) a sequence encoding a guide miRNA sequence;

c) a sequence encoding a stem loop sequence;

d) a sequence encoding a passenger miRNA sequence; and

e) a sequence encoding a 3' backbone sequence.

[0128] In certain embodiments, the sequence encoding the guide miRNA sequence has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 704, 705, 709, and 710; or is capable of hybridizing under stringent hybridization conditions to the complement of any one of such sequences.

[0129] In certain embodiments, the sequence encoding the passenger miRNA sequence has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 706–708, and 711–713; or is capable of hybridizing under stringent hybridization conditions to the complement of any one of such

sequences.

[0130] In certain embodiments, the polynucleotide encoding a pri-miRNA encodes:

a) a guide miRNA sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 704, 705, 709, and 710, or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of such sequences; and

b) a passenger sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to, respectively, any one of SEQ ID NOs: 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 706–708, and 711–713, or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of such sequences.

[0131] In any of the foregoing embodiments, the sequences encoding the 5' miRNA backbone sequence, the stem loop sequence, and the 3' miRNA backbone sequence can each comprise:

SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, respectively;
SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6, respectively;
SEQ ID NO: 7, SEQ ID NO: 8, and SEQ ID NO: 9, respectively;
SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12, respectively;
SEQ ID NO: 13, SEQ ID NO: 14, and SEQ ID NO: 15, respectively;
SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18, respectively;
SEQ ID NO: 19, SEQ ID NO: 20, and SEQ ID NO: 21, respectively;
SEQ ID NO: 22, SEQ ID NO: 23, and SEQ ID NO: 24, respectively;
SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, respectively;
SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30, respectively;
SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33, respectively;
SEQ ID NO: 34, SEQ ID NO: 35, and SEQ ID NO: 36, respectively;

SEQ ID NO: 37, SEQ ID NO: 38, and SEQ ID NO: 39, respectively;
SEQ ID NO: 40, SEQ ID NO: 41, and SEQ ID NO: 42, respectively;
SEQ ID NO: 43, SEQ ID NO: 44, and SEQ ID NO: 45, respectively;
SEQ ID NO: 46, SEQ ID NO: 47, and SEQ ID NO: 48, respectively;
SEQ ID NO: 49, SEQ ID NO: 50, and SEQ ID NO: 51, respectively;
SEQ ID NO: 52, SEQ ID NO: 53, and SEQ ID NO: 54, respectively;
SEQ ID NO: 55, SEQ ID NO: 56, and SEQ ID NO: 57, respectively;
SEQ ID NO: 58, SEQ ID NO: 59, and SEQ ID NO: 60, respectively; or
SEQ ID NO: 61, SEQ ID NO: 62, and SEQ ID NO: 63, respectively;

or sequences having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with such sequences, or that are capable of hybridizing under stringent hybridization conditions to the complements of such sequences.

[0132] Nucleic acids encoding exemplary non-naturally occurring pri-miRNA sequences targeting specific checkpoint inhibitors are described in Table 3. In certain embodiments, the non-naturally occurring pri-miRNA sequence may be a sequence that is capable of hybridizing under stringent hybridization conditions with the complement of any one of the sequences listed in Table 3.

Table 3: Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences

miRNA Target	Encoded pri-miRNA(s)	DNA Sequence
CTLA4	miR204	(SEQ ID NO: 178)
PD1	miR204	(SEQ ID NO: 179)
PD1	miR206	(SEQ ID NO: 180)
TIGIT	miR17	(SEQ ID NO: 181)
TIGIT	miR150	(SEQ ID NO: 182)
TIGIT	miR204	(SEQ ID NO: 183)
TIGIT	miR-206	(SEQ ID NO: 184)
TIGIT	miR-204	(SEQ ID NO: 185)
TIGIT	miR-22	(SEQ ID NO: 186)

miRNA Target	Encoded pri-miRNA(s)	DNA Sequence
TIGIT	miR-16	(SEQ ID NO: 187)
TIGIT	miR-16	(SEQ ID NO: 188)
TIGIT	miR-21	(SEQ ID NO: 189)
TIGIT	miR-494	(SEQ ID NO: 190)
TIGIT	miR-142	(SEQ ID NO: 191)
TIGIT	miR-19	(SEQ ID NO: 192)
TIGIT	miR-1915	(SEQ ID NO: 193)
TIGIT	miR-206	(SEQ ID NO: 194)
TIGIT	miR-204	(SEQ ID NO: 195)
TIGIT	miR-22	(SEQ ID NO: 196)
TIGIT	miR-142	(SEQ ID NO: 197)
TIGIT	miR-16	(SEQ ID NO: 198)
TIGIT	miR-206	(SEQ ID NO: 199)
TIGIT	miR-204	(SEQ ID NO: 200)
TIGIT	miR-21	(SEQ ID NO: 201)
TIGIT	miR-494	(SEQ ID NO: 202)
TIGIT	miR-19	(SEQ ID NO: 203)
TIGIT	miR-1915	(SEQ ID NO: 204)
TIGIT	miR-204	(SEQ ID NO: 205)
TIGIT	miR-206	(SEQ ID NO: 206)
TIGIT	miR-21	(SEQ ID NO: 207)
TIGIT	miR-22	(SEQ ID NO: 208)
TIM3	miR204	(SEQ ID NO: 209)
TIM3	miR150	(SEQ ID NO: 210)
TIM3	miR30a	(SEQ ID NO: 211)
TIM3	miR206	(SEQ ID NO: 212)
TIM3	miR16	(SEQ ID NO: 213)
TIM3	miR17	(SEQ ID NO: 214)

miRNA Target	Encoded pri-miRNA(s)	DNA Sequence
TIM3	miR126	(SEQ ID NO: 215)
TIM3	miR122	(SEQ ID NO: 216)
TIM3	miR214	(SEQ ID NO: 217)
TIM3	miR29b1	(SEQ ID NO: 218)
TIM3	miR204	(SEQ ID NO: 219)
TIM3	miR133a1	(SEQ ID NO: 220)
LAG3	miR30a	(SEQ ID NO: 221)
LAG3	miR206	(SEQ ID NO: 222)
LAG3	miR204	(SEQ ID NO: 223)
LAG3	miR150	(SEQ ID NO: 224)
LAG3	miR17	(SEQ ID NO: 225)
LAG3	miR122	(SEQ ID NO: 226)
LAG3	miR126	(SEQ ID NO: 227)
GITR	miR30a	(SEQ ID NO: 228)
GITR	miR206	(SEQ ID NO: 229)
GITR	miR17	(SEQ ID NO: 230)
GITR	miR122	(SEQ ID NO: 231)
GITR	miR150	(SEQ ID NO: 232)
GITR	miR29	(SEQ ID NO: 233)
GITR	miR181a1	(SEQ ID NO: 234)
TIGIT	miR206	(SEQ ID NO: 235)
TIGIT	miR30a	(SEQ ID NO: 236)
TIGIT	miR133a1	(SEQ ID NO: 237)
TIGIT	miR122	(SEQ ID NO: 238)
TIGIT	miR29b1	(SEQ ID NO: 239)
TIGIT	miR214	(SEQ ID NO: 240)
PD1	miR30a	(SEQ ID NO: 241)
PD1	miR412	(SEQ ID NO: 242)

miRNA Target	Encoded pri-miRNA(s)	DNA Sequence
PD1	miR17	(SEQ ID NO: 243)
PD1	miR122	(SEQ ID NO: 244)
PD1	miR150	(SEQ ID NO: 245)
PD1	miR486	(SEQ ID NO: 246)
PD1	miR206	(SEQ ID NO: 247)
PD1	miR122	(SEQ ID NO: 248)
PD1	miR30a	(SEQ ID NO: 249)
CTLA4	miR206	(SEQ ID NO: 250)
CTLA4	miR26a	(SEQ ID NO: 251)
CTLA4	miR30a	(SEQ ID NO: 252)
PIK3IP1	miR206	(SEQ ID NO: 253)
PIK3IP1	miR126	(SEQ ID NO: 254)
PIK3IP1	miR30a	(SEQ ID NO: 255)
TCRa3'UTR	miR150	(SEQ ID NO: 256)
TCRa3'UTR	miR204	(SEQ ID NO: 257)
TCRa3'UTR	miR206	(SEQ ID NO: 258)
TCRa3'UTR	miR26a	(SEQ ID NO: 259)
TCRa3'UTR	miR150	(SEQ ID NO: 260)
TCRa3'UTR	miR16	(SEQ ID NO: 261)
TCRa3'UTR	miR206	(SEQ ID NO: 262)
TCRa3'UTR	miR26a	(SEQ ID NO: 263)

[0133] In certain such embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 178–263 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 178–263.

[0134] In certain embodiments, the miRNA targets PD-1. In certain such embodiments, the

present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 179, 180, and 241–249 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 179, 180, and 241–249. In certain embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NO: 179 or 180 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 179 or 180.

[0135] Certain embodiments of the present relate to a polynucleotide that comprises nucleic acid sequences encoding at least two pri-miRNAs. The two or more pri-miRNAs may contain guide miRNA sequences that target the same target gene or the various guide miRNAs may target different genes. In the case of multiple pri-miRNAs, each design may be based on a different native miRNA backbone to reduce the likelihood of misfolding of one miRNA with another. Table 4 provides examples of nucleic acid sequences encoding one or more pri-miRNAs.

Table 4: Non-naturally occurring miRNA sequences comprising two or more pri-miRNAs

miRNA Target	**miRNA backbone**	**DNA Sequence**
PD1	miR204 + miR206	(SEQ ID NO: 267)
PD1 + TIGIT	miR204 + miR150	(SEQ ID NO: 268)
PD1 + TIGIT	miR206 + miR150	(SEQ ID NO: 269)
TIGIT + PD1	miR17 + miR204	(SEQ ID NO: 270)
TIGIT + PD1	miR17 + miR206	(SEQ ID NO: 271)
TIGIT + PD1	miR204 + miR206	(SEQ ID NO: 272)

miRNA Target	**miRNA backbone**	**DNA Sequence**
TIGIT + PD1	miR150 + miR204	(SEQ ID NO: 273)
TIGIT + PD1	miR150 + miR206	(SEQ ID NO: 274)
TIGIT+PD1 +PD1	miR17+miR204+miR206	(SEQ ID NO: 275)
TIGIT+PD1 + PD1	miR17+miR204+miR206 extra spacing 1	(SEQ ID NO: 276)
TIGIT+PD1 +PD1	miR17+miR204+miR206 extra spacing 2	(SEQ ID NO: 277)
PD1+PD1+ TIGIT	miR204+miR206+miR17	(SEQ ID NO: 278)
PD1+PD1+ TIGIT	miR204+miR206+miR17 extra spacing 1	(SEQ ID NO: 279)
PD1+PD1+ TIGIT	miR204+miR206+miR17 extra spacing 2	(SEQ ID NO: 280)
PD1+CTLA4	miR204+miR26a	(SEQ ID NO: 281)
PD1+PD1	miR204+miR206	(SEQ ID NO: 282)
PD1+CTLA4	miR206+miR26a	(SEQ ID NO: 283)
PD1+CTLA4	miR206+miR204	(SEQ ID NO: 284)
TIGIT+CTLA4	miR17+miR26a	(SEQ ID NO: 285)

miRNA Target	**miRNA backbone**	**DNA Sequence**
TIGIT+CTLA4	miR17+miR204	(SEQ ID NO: 286)
TIGIT+PD1	miR17+miR204	(SEQ ID NO: 287)
TIGIT+PD1	miR17+206	(SEQ ID NO: 288)
TIGIT+CTLA4	miR204+miR26a	(SEQ ID NO: 289)
TIGIT+PD1	miR204+miR206	(SEQ ID NO: 290)

[0136] In certain such embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 267–290 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 267–290.

[0137] In certain such embodiments, the pri-miRNA comprises at least two pre-miRNAs that target PD-1. In certain embodiments, the present invention relates to a polynucleotide comprising a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NO: 267 or that is capable of hybridizing under stringent hybridization conditions to the complement of any one of SEQ ID NOs: 267.

[0138] In certain embodiments, the nucleic acid encoding the pri-miRNA(s) is contained in the same genetic construct as that comprising one or more genes encoding protein(s) of interest (*e.g.*, a chimeric antigen receptor, a cytokine, or a cell tag). In certain embodiments, such a genetic construct includes a nucleic acid sequence encoding a 5' untranslated region (5'UTR) directly upstream of the gene(s) of interest and the sequence encoding the miRNA is included in the 5'UTR. In certain embodiments, such a genetic construct includes a nucleic acid sequence encoding a 3' untranslated region (3'UTR) directly downstream of the gene(s) of interest and the sequence encoding the miRNA is included in the 3'UTR.

[0139] In embodiments wherein the sequence encoding the pri-miRNA is included in the sequence corresponding to the 5'UTR, the transcribed RNA can include additional sequences such as splice donor, branchpoint and/or acceptor site sequences. The inclusion of splice donor, branchpoint, and acceptor sites is important for splicing of the miRNAs from the transcribed RNA. Without splicing, the highly structured miRNA sequence is likely to impede ribosome scanning to the translation initiation sequence relating to the gene of interest. Examples of sequences encoding such splice donor/acceptor sites include SEQ ID NOs: 291 and 292, sequences having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with such sequences and sequences that are capable of hybridizing with the complement of such sequences under stringent hybridization conditions.

[0140] Thus, in certain embodiments, the polynucleotide of the present invention further comprises: a) a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 291 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 291; and b) a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 292 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 292.

IV. Chimeric Antigen Receptors (CARs)

[0141] In any of the foregoing embodiments, a polynucleotide of the present disclosure can further encode a chimeric receptor, such as a chimeric antigen receptor (CAR). Thus, a polynucleotide of the present disclosure can encode miRNA(s) and a CAR as described herein. In any of the embodiments of the present disclosure, a modified immune effector cell can comprise a chimeric receptor as described herein.

[0142] A CAR is an engineered receptor that grafts an exogenous specificity onto an immune effector cell. In some instances, a CAR comprises an extracellular domain (ectodomain) that comprises an antigen-binding domain, a transmembrane domain, and an intracellular (endodomain) domain. The intracellular domain comprises an intracellular signaling domain. In certain embodiments, the extracellular domain further comprises a spacer between the antigen-binding domain and the transmembrane domain.

A. Antigen-Binding Domain

[0143] An antigen-binding domain can comprise complementary determining regions of a monoclonal antibody and/or antigen binding fragments thereof. A complementarity determining region (CDR) is a short amino acid sequence found in the variable domains of antigen receptor (*e.g.*, immunoglobulin and T-cell receptor) proteins that bind an antigen and therefore provides the receptor with its specificity for that particular antigen. Each polypeptide chain of an antigen receptor can contain three CDRs (CDR1, CDR2, and CDR3).

[0144] In certain embodiments, the antigen-binding domain comprises an antibody, or functional fragment or variant thereof, that binds to a target antigen. The functional fragment or variant may comprise the variable domain of the heavy chain of an antibody (V_H) and/or the variable domain of the light chain of an antibody (V_L), or functional fragments or variants thereof. In certain embodiments, the antigen-binding domain comprises a Fv, Fab, Fab_2, Fab', $F(ab')_2$, or $F(ab')_3$ fragment of an antibody. In certain embodiments, the antigen-binding domain comprises a scFv, $sc(Fv)_2$, a dsFv, a diabody, a minibody, a nanobody, or binding fragments thereof. In certain embodiments, the antigen-binding domain further comprises an Fc fragment of an antibody, for example it may comprise an scFv linked with an Fc fragment.

[0145] In some embodiments, the CAR targets an antigen that is elevated in cancer cells, in autoimmune cells, or in cells that are infected by a virus, bacteria or parasite. Pathogens that may be targeted include, without limitation, Plasmodium, trypanosome, Aspergillus, Candida, Hepatitis A, Hepatitis B, Hepatitis C, HSV, HPV, RSV, EBV, CMV, JC virus, BK virus, or Ebola pathogens. Autoimmune diseases can include graft-versus-host disease, rheumatoid arthritis, lupus, celiac disease, Crohn's disease, Sjogren Syndrome, polymyalgia rheumatic, multiple sclerosis, neuromyelitis optica, ankylosing spondylitis, Type 1 diabetes, alopecia areata, vasculitis, temporal arteritis, bullous pemphigoid, psoriasis, pemphigus vulgaris, and autoimmune uveitis.

The pathogen recognized by a CAR may be essentially any kind of pathogen, but in some embodiments, the pathogen is a fungus, bacteria, or virus. Exemplary viral pathogens include those of the families of Adenoviridae, Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Respiratory Syncytial Virus (RSV), JC virus, BK virus, HPV, HSV, HHV family of viruses, Hepatitis family of viruses, *Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae,*

Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, and *Togaviridae*. Exemplary pathogenic viruses cause smallpox, influenza, mumps, measles, chickenpox, ebola, and rubella. Exemplary pathogenic fungi include *Candida, Aspergillus, Cryptococcus, Histoplasma, Pneumocystis,* and *Stachybotrys.* Exemplary pathogenic bacteria include *Streptococcus, Pseudomonas, Shigella, Campylobacter, Staphylococcus, Helicobacter, E. coli, Rickettsia, Bacillus, Bordetella, Chlamydia, Spirochetes*, and *Salmonella*. In some embodiments, the pathogen receptor Dectin-1 may be used to generate a CAR that recognizes the carbohydrate structure on the cell wall of fungi such as Aspergillus. In another embodiment, CARs can be made based on an antibody recognizing viral determinants (*e.g.*, the glycoproteins from CMV and Ebola) to interrupt viral infections and pathology.

[0146] In some embodiments, a CAR described herein comprises an antigen-binding domain that binds to an epitope on B7H4, BCMA, BTLA, CAIX, CA125, CCR4, CD3, CD4, CD5, CD7, CD16, CD19, CD20, CD22, CD24, CD25, CD28, CD30, CD33, CD38, CD40, CD44, CD44v6, CD44v7/v8, CD47, CD52, CD56, CD70, CD79b, CD80, CD81, CD86, CD123, CD133, CD137, CD138, CD151, CD171, CD174, CD276, CEA, CEACAM6, CLL-1, c-MET, CS1, CSPG4, CTLA-4, DLL3, EDB-F, EGFR, EGFR2, EGFRvIII, EGP-2, EGP-40, EphA2, FAP, FLT1, FLT4, Folate-binding Protein, Folate Receptor, Folate receptor α, α-Folate receptor, Frizzled, GD2, GD3, GHR, GHRHR, GITR, GPC3, Gp100, gp130, HBV antigens, HER1, HER2, HER3, HER4, HER1/HER3, h5T4, HPV antigens, HVEM, IGF1R, IgKAppa, IL-1-RAP, IL-2R, IL6R, IL-11Rα, IL-13R-a2, KDR, KRASG12V, LewisA, LewisY, L1-CAM, LIFRP, LRP5, LTPR, MAGE-A, MAGE-A1, MAGE-A10, MAGE-A3, MAGEA3/A6, MAGE-A4, MAGE-A6, MART-1, MCAM, mesothelin, PSCA, Mucins such as MUC1, MUC-4 or MUC16, NGFR, NKG2D, Notch-1-4, NY-ESO-1, O-acetylGD2, O-acetylGD3, OX40, P53, PD1, PDE10A, PD-L1, PD-L2, PMSA, PRAME, PSCA, PSMA, PTCH1, RANK, Robol, ROR1, ROR1R, ROR-2, TACI, TAG-72, TCRa, TCRp, TGF, TGFBeta, TGFBeta-II, TGFBR1, TGFBR2, Titin, TLR7, TLR9, TNFR1, TNFR2, TNFRSF4, TRBC1, TWEAK-R, VEGF, VEGF-R2, or WT-1.

[0147] In some embodiments, a CAR described herein comprises an antigen-binding domain that binds to an epitope on CD19, CD33, MUC1, MUC16, or ROR1. In some instances, a CAR described herein comprises an antigen-binding domain that binds to an epitope on CD19. In some cases, a CAR described herein comprises an antigen-binding domain that binds to an epitope on

CD33. In some instances, a CAR described herein comprises an antigen-binding domain that binds to an epitope on MUC1. In some instances, a CAR described herein comprises an antigen-binding domain that binds to an epitope on MUC16. In some instances, a CAR described herein comprises an antigen-binding domain that binds to an epitope on ROR1. In further embodiments, a CAR described herein comprises an autoantigen or an antigen binding domain that binds to an epitope on HLA-A2, myelin oligodendrocyte glycoprotein (MOG), factor VIII (FVIII), MAdCAM1, SDF1, or collagen type II.

[0148] Antigen binding can be assessed by flow cytometry or a cell based assay or any other equivalent assay. Cell based assays may utilize a cell type expressing antigen of interest on the surface to assess antigen-binding. An antigen or a fragment thereof expressed as a soluble protein can be utilized to assess antigen-binding using flow cytometry or similar assay. Improvements in antigen-binding may be indirectly assessed by functional measurement of antigen-binding domain or a chimeric receptor. For example, improved antigen-binding of a chimeric receptor or a CAR, as described herein, can be measured by increased specific cytotoxicity against target cells expressing the antigen.

[0149] Cell surface expression level of a polypeptide of the present disclosure can be assessed, for example, using a flow cytometry based assay. Improved expression of an antigen-binding polypeptide can be measured as percentage of analyzed cells expressing said antigen-binding polypeptide or alternatively as average density of said antigen-binding polypeptide on the surface of a cell. Additional suitable methods that can be used for assessing cell surface expression of the antigen-binding polypeptides described herein include western blotting or any other equivalent assay.

1. CD19-specific Antigen-Binding Domain

[0150] CD19 is a cell surface glycoprotein of the immunoglobulin superfamily and is found predominately in malignant B-lineage cells. In some instances, CD19 has also been detected in solid tumors such as pancreatic cancer, liver cancer, and prostate cancer.

[0151] In some embodiments, the CAR comprises a CD19-specific antigen-binding domain, in which the antigen-binding domain comprises a $F(ab')_2$, Fab', Fab, Fv, or scFv. In some

embodiments, the antigen-binding domain recognizes an epitope on CD19 that is also recognized by FMC63. In some embodiments the scFv and/or VH/VL domains is/are derived from FMC63. FMC63 generally refers to a mouse monoclonal IgGl antibody raised against Nalm-l and -16 cells expressing CD19 of human origin (Ling, N. R., el al. (1987). Leucocyte typing III. 302).

[0152] In some embodiments, the antigen-binding domain recognizes an epitope on CD19 that is also recognized by JCAR014, JCAR015, JCAR017, or 19-28z CAR (Juno Therapeutics).

[0153] In some embodiments, the antigen-binding domain comprises a scFv antigen-binding domain that recognizes an epitope on CD19 that is also recognized by JCAR014, JCAR015, JCAR017, or 19-28z CAR (Juno Therapeutics).

[0154] In some embodiments, the antigen-binding domain comprises an anti-CD19 antibody described in U.S. Patent Application Publication No. 2016/0152723. In some embodiments, the antigen-binding domain comprises an anti-CD19 antibody described in International Patent Application Publication No. WO2015/123642. In some embodiments, the antigen-binding domain comprises an anti-CD19 scFv derived from clone FMC63 (Nicholson et al., Construction and characterization of a functional CD19 specific single chain Fv fragment for immunotherapy of B lineage leukemia and lymphoma., Mol. Immunol. 34:1157-1165 (1997)).

[0155] In some embodiments, the antigen-binding domain recognizes an epitope on CD19 that is also recognized by KTE-C19 (Kite Pharma, Inc.).

[0156] In some embodiments, the antigen-binding domain comprises a scFv antigen-binding domain, and the antigen-binding domain recognizes an epitope on CD19 that is also recognized by KTE-C19.

[0157] In some embodiments, the antigen-binding domain comprises an anti-CD19 antibody described in International Patent Application Publication No. WO2015/187528 or fragment or variant thereof.

[0158] In some embodiments, the antigen-binding domain recognizes an epitope on CD19 that is also recognized by CTL019 (Novartis).

[0159] In some embodiments, the antigen-binding domain comprises a scFv antigen-binding

domain, and the antigen-binding domain recognizes an epitope on CD19 that is also recognized by CTL019.

[0160] In some embodiments, the antigen-binding domain recognizes an epitope on CD19 that is also recognized by UCART19 (Cellectis).

[0161] In some embodiments, the antigen-binding domain comprises a scFv antigen-binding domain, and the antigen-binding domain recognizes an epitope on CD19 that is also recognized by UCART19.

[0162] In some embodiments, the antigen-binding domain recognizes an epitope on CD19 that is also recognized by BPX-401 (Bellicum).

[0163] In some embodiments, the antigen-binding domain comprises a scFv antigen-binding domain, and the antigen-binding domain recognizes an epitope on CD19 that is also recognized by BPX-401.

[0164] In some cases, the antigen-binding domain recognizes an epitope on CD19 that is also recognized by blinatumomab (Amgen), coltuximabravtansine (ImmunoGen Inc./Sanofi-aventis), MOR208 (Morphosys AG/Xencor Inc.), MEDI-551 (Medimmune), denintuzumabmafodotin (Seattle Genetics), B4 (or DI-B4) (Merck Serono), taplitumomabpaptox (National Cancer Institute), XmAb 5871 (Amgen/Xencor, Inc.), MDX-1342 (Medarex) or AFM11 (Affimed).

[0165] In some embodiments, the antigen-binding domain comprises a $F(ab')_2$, Fab', Fab, Fv, or scFv, and the antigen-binding domain recognizes an epitope on CD19 that is also recognized by blinatumomab (Amgen), coltuximabravtansine (ImmunoGen Inc./Sanofi-aventis), MOR208 (Morphosys AG/Xencor Inc.), MEDI-551 (Medimmune), denintuzumabmafodotin (Seattle Genetics), B4 (or DI-B4) (Merck Serono), taplitumomabpaptox (National Cancer Institute), XmAb 5871 (Amgen/Xencor, Inc.), MDX-1342 (Medarex) or AFM11 (Affimed).

[0166] In some cases, the antigen-binding domain comprises a scFv antigen-binding domain, and the antigen-binding domain recognizes an epitope on CD19 that is also recognized by FMC63, blinatumomab (Amgen), coltuximabravtansine (ImmunoGen Inc./Sanofi-aventis), MOR208 (Morphosys AG/Xencor Inc.), MEDI-551 (Medimmune), denintuzumabmafodotin (Seattle

Genetics), B4 (or DI-B4) (Merck Serono), taplitumomabpaptox (National Cancer Institute), XmAb 5871 (Amgen/Xencor, Inc.), MDX-1342 (Medarex) or AFM11 (Affimed).

2. CD33-specific Antigen-Binding Domain

[0167] "CD33," is a 67kDa single pass transmembrane glycoprotein and is a member of the sialic acid-binding immunoglobulin-like lectins (Siglecs) super-family. CD33 is characterized by a V-set Ig-like domain responsible for sialic acid binding and a C2-set Ig-like domain in its extracellular domain. CD33 is expressed in myeloid lineage cells and has also been detected in lymphoid cells. Alternative splicing of CD33 mRNA leads to a shorter isoform (CD33m) lacking the V-set Ig-like domain as well as the disulfide bond linking the V- and C2-set Ig-like domains. In healthy subjects, CD33 is primarily expressed as a myeloid differentiation antigen found on normal multipotent myeloid precursors, unipotent colony-forming cells, monocytes and maturing granulocytes. CD33 is expressed on more than 80% of myeloid leukemia cells but not on normal hematopoietic stem cells or mature granulocytes. (Andrews, R. et al., The L4F3 antigen is expressed by unipotent and multipotent colony-forming cells but not by their precursors, *Blood,* 68(5):1030-5 (1986)). CD33 has been reported to be expressed on malignant myeloid cells, activated T cells and activated NK cells and is found on at least a subset of blasts in the vast majority of AML patients (Pollard, J. et al., Correlation of CD33 expression level with disease characteristics and response to gemtuzumab ozogamicin containing chemotherapy in childhood AML, *Blood,* 119(16):3705-11 (2012)). In addition to broad expression on AML blasts, CD33 may be expressed on stem cells underlying AML.

[0168] In some embodiments, the antigen-binding domain of a CAR described herein is specific to CD33 (CD33 CAR). The CD33-specific CAR, when expressed on the cell surface, redirects the specificity of T cells to human CD33. In some 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-CD33 antibody joined by a flexible linker, such as a glycine-serine linker or a Whitlow linker. In some embodiments, the scFv is M195, m2H12, DRB2, and/or My9-6. In some embodiments, the scFv is humanized, for example, hM195. In some embodiments, the antigen-binding domain may comprise VH and VL that are directionally linked, for example, from N to C terminus, VH-linker-

VL or VL-linker-VH. In some embodiments, the antigen-binding domain comprises a $F(ab')_2$, Fab', Fab, Fv, or scFv that binds CD33. In some embodiments, the antigen-binding region recognizes an epitope on CD33 that is also recognized by Lintuzumab (Seattle Genetics), BI 836858 (Boehringer Ingelheim).

[0169] In some embodiments, a CAR described herein comprises an antigen-binding domain comprising a VL polypeptide.

[0170] In certain embodiments, the antigen-binding domain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 293 (hM195 VL domain), SEQ ID NO: 296 (M2H12 VL domain), SEQ ID NO: 298 (DRB2 VL domain), SEQ ID NO: 300 (My9-6 VL domain) or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than any one of the aforementioned sequences by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 293, 296, 298, and 300, and/or is a conservatively-substituted variant of any one of such sequences.

[0171] In certain embodiments, the antigen-binding domain comprises a VL domain encoded by SEQ ID NO: 301 (nucleic acid encoding the VL domain for hM195). In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 301; hybridizes under stringent hybridization conditions with the complement of the nucleic acid sequence of SEQ ID NO: 301; or is a codon degenerate variant of SEQ ID NO: 301.

[0172] In some embodiments, a CAR described herein comprises an antigen-binding domain comprising a VH polypeptide.

[0173] In certain embodiments, the antigen-binding domain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 294 (hM195 VH domain), SEQ ID NO: 295 (M2H12 VH domain), SEQ ID NO: 297 (DRB2 VH domain), SEQ ID NO: 299 (My9-6 VH domain) or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than any one of the aforementioned sequences by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2,

or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 294, 295, 297, or 299, and/or is a conservatively-substituted variant of the amino acid sequence of any one of SEQ ID NOs: 294, 295, 297, or 299.

[0174] In certain embodiments, the antigen-binding domain comprises a VH domain encoded by SEQ ID NO: 302 (nucleic acid encoding the VH domain for hM195), or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 302; hybridizes under stringent hybridization conditions with the complement of the nucleic acid sequence of SEQ ID NO: 302; or is a codon degenerate variant of SEQ ID NO: 302.

[0175] In some embodiments, the antigen-binding domain can comprise VH and VL that are directionally linked, for example, from N to C terminus, VH-linker-VL or VL-linker-VH. Any linker as described herein can be used to link the VH and VL domains.

[0176] In certain embodiments, the antigen-binding domain comprises scFv. In certain such embodiments, the domain comprises the amino acid sequence of SEQ ID NO: 303, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than any one of the aforementioned sequence by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 303, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 303.

[0177] In certain embodiments, the antigen-binding domain is encoded by SEQ ID NO: 304, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 304; hybridizes under stringent hybridization conditions with the complement of the nucleic acid sequence of SEQ ID NO: 304; or is a codon degenerate variant of SEQ ID NO: 304.

3. MUC1-specific Antigen-Binding Domain

[0178] In one embodiment, the antigen-binding domain binds to an epitope on MUC1. In some

embodiments, the anti-MUC1 antibody fragment is a Fab, Fab_2, $(Fab')_2$, Fv, $(Fv)_2$, scFv, scFv-F_C, F_C, diabody, triabody, or minibody of the anti-MUC1. In some embodiments, the anti-MUC1 antibody fragment is a single-domain antibody of the anti-MUC1 antibody. In some embodiments, the single-domain antibody is a V_{NAR} or V_HH fragment of the anti-MUC1 antibody.

[0179] In some embodiments, a CAR described herein comprises an antigen-binding domain comprising a VL polypeptide of MUC1.

[0180] In certain embodiments, the antigen-binding domain comprises a VL domain comprising the amino acid sequence of any one of SEQ ID NOs: 310–314, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of any one of SEQ ID NOs: 310–314 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 310–314, and/or is a conservatively-substituted variant of the amino acid sequence of any one of SEQ ID NOs: 310–314.

[0181] In some embodiments, a CAR described herein comprises an antigen-binding domain comprising a VH polypeptide of MUC1.

[0182] In certain embodiments, the antigen-binding domain comprises a VH domain comprising the amino acid sequence of any one of SEQ ID NOs: 305–309, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of any one of SEQ ID NOs: 305–309 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 305–309, and/or is a conservatively-substituted variant of the amino acid sequence of any one of SEQ ID NOs: 305–309.

[0183] 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. Any linker as described herein can be used to link the VH and VL domains.

4. MUC16-specific Antigen-Binding Domain

[0184] In some embodiments, the antigen binding moiety of a CAR described herein is specific to MUC16 (MUC16 CAR). The MUC16-specific CAR, when expressed on the cell surface, redirects the specificity of T cells to human MUC16.

[0185] In certain embodiments, the antigen-binding domain comprises a VL domain comprising the amino acid sequence of any one of SEQ ID NOs: 329, 331, 333, 335, 337, 339, 341, 662, 664, 666, 668, 670, 688, 690, 692, 694, 696, 698, and 700, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of any one of the aforementioned sequences by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 329, 331, 333, 335, 337, 339, 341, 662, 664, 666, 668, 670, 688, 690, 692, 694, 696, 698, and 700; and/or is a conservatively-substituted variant of the amino acid sequence of any one of SEQ ID NOs: 329, 331, 333, 335, 337, 339, 341, 662, 664, 666, 668, 670, 688, 690, 692, 694, 696, 698, and 700.

[0186] In certain embodiments, the antigen-binding domain comprises a VL domain encoded by any one of SEQ ID NOs: 330, 332, 334, 336, 338, 340, 342, 663, 665, 667, 669, 671, 689, 691, 693, 695, 697, 699, and 701, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 330, 332, 334, 336, 338, 340, 342, 663, 665, 667, 669, 671, 689, 691, 693, 695, 697, 699, and 701; or hybridizes under stringent hybridization conditions with the complement of any one of SEQ ID NOs: 330, 332, 334, 336, 338, 340, 342, 663, 665, 667, 669, 671, 689, 691, 693, 695, 697, 699, and 701.

[0187] In certain embodiments, the antigen-binding domain comprises a VL domain comprising the amino acid sequence of SEQ ID NO: 692, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 692 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 692; and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 692.

[0188] In certain embodiments, the antigen-binding domain comprises a VL domain encoded by SEQ ID NO: 693, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 693; or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 693.

[0189] In certain embodiments, the antigen-binding domain comprises a VH domain comprising the amino acid sequence of any one of SEQ ID NOs: 315, 317, 319, 321, 323, 325, 327, 648, 650, 652, 654, 656, 658, 660, 672, 674, 676, 678, 680, 682, 684, and 686, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of any one of the aforementioned sequences by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 315, 317, 319, 321, 323, 325, 327, 648, 650, 652, 654, 656, 658, 660, 672, 674, 676, 678, 680, 682, 684, and 686; and/or is a conservatively-substituted variant of the amino acid sequence of any one of SEQ ID NOs: 315, 317, 319, 321, 323, 325, 327, 648, 650, 652, 654, 656, 658, 660, 672, 674, 676, 678, 680, 682, 684, and 686.

[0190] In certain embodiments, the antigen-binding domain comprises a VH domain encoded by any one of SEQ ID NOs: 316, 318, 320, 322, 324, 326, 328, 649, 651, 653, 655, 657, 659, 661, 673, 675, 677, 679, 681, 683, 685, and 687, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 316, 318, 320, 322, 324, 326, 328, 649, 651, 653, 655, 657, 659, 661, 673, 675, 677, 679, 681, 683, 685, and 687; or hybridizes under stringent hybridization conditions with the complement of any one of SEQ ID NOs: 316, 318, 320, 322, 324, 326, 328, 649, 651, 653, 655, 657, 659, 661, 673, 675, 677, 679, 681, 683, 685, and 687.

[0191] In certain embodiments, the antigen-binding domain comprises a VH domain comprising the amino acid sequence of SEQ ID NO: 676, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 676 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%

sequence identity with the amino acid sequence of SEQ ID NO: 676; and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 676.

[0192] In certain embodiments, the antigen-binding domain comprises a VH domain encoded by SEQ ID NO: 677, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 677; or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 677.

[0193] 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. Any linker as described herein can be used to link the VH and VL domains.

[0194] In some 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-MUC16 antibody joined by a flexible linker, such as a glycine-serine linker or a Whitlow linker. In certain embodiments, the scFv comprises the VH and VL from MUC16-3 and a linker. In certain such embodiments, the scFv comprises the amino acid sequence of SEQ ID NO: 343, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 343 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 343; and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 343.

[0195] In certain embodiments, the antigen-binding domain comprises a scFv encoded by SEQ ID NO: 344, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 344; or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 344.

5. ROR1-specific Antigen-Binding Domain

[0196] In some embodiments, a CAR described herein comprises an antigen-binding domain

comprising the V_L domain of an anti-ROR1 antibody.

[0197] In certain such embodiments, the antigen-binding domain may comprise an amino acid sequence of any one of SEQ ID NOs: 347, 351, 355, 359, 363, 367, 371, 375, 379, 383, 387, 391, 395, 399, 403, 407, 411, 415, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, and 463, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than any one of the aforementioned sequences by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 347, 351, 355, 359, 363, 367, 371, 375, 379, 383, 387, 391, 395, 399, 403, 407, 411, 415, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, and 463, and/or is a conservatively-substituted variant of the amino acid sequence of any one of SEQ ID NOs: 347, 351, 355, 359, 363, 367, 371, 375, 379, 383, 387, 391, 395, 399, 403, 407, 411, 415, 419, 423, 427, 431, 435, 439, 443, 447, 451, 455, 459, and 463.

[0198] In certain embodiments, the antigen-binding domain comprises a V_L domain encoded by any one of SEQ ID NOs: 348, 352, 356, 360, 364, 368, 372, 376, 380, 384, 388, 392, 396, 400, 404, 408, 412, 416, 420, 424, 428, 432, 436, 440, 444, 448, 452, 456, 460, and 464, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 348, 352, 356, 360, 364, 368, 372, 376, 380, 384, 388, 392, 396, 400, 404, 408, 412, 416, 420, 424, 428, 432, 436, 440, 444, 448, 452, 456, 460, and 464; hybridizes under stringent hybridization conditions with the complement of any one of SEQ ID NOs: 348, 352, 356, 360, 364, 368, 372, 376, 380, 384, 388, 392, 396, 400, 404, 408, 412, 416, 420, 424, 428, 432, 436, 440, 444, 448, 452, 456, 460, and 464; or is a codon degenerate variant of any one of SEQ ID NOs: 348, 352, 356, 360, 364, 368, 372, 376, 380, 384, 388, 392, 396, 400, 404, 408, 412, 416, 420, 424, 428, 432, 436, 440, 444, 448, 452, 456, 460, and 464.

[0199] In some embodiments, a CAR described herein comprises the V_H domain of an anti-ROR1 antibody.

[0200] In certain such embodiments, the antigen-binding domain may comprise an amino acid sequence of any one of SEQ ID NOs: 349, 353, 357, 361, 365, 369, 373, 377, 381, 385, 389, 393,

397, 401, 405, 409, 413, 417, 421, 425, 429, 433, 437, 441, 445, 449, 453, 457, and 461, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than any one of the aforementioned sequences by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 349, 353, 357, 361, 365, 369, 373, 377, 381, 385, 389, 393, 397, 401, 405, 409, 413, 417, 421, 425, 429, 433, 437, 441, 445, 449, 453, 457, and 461, and/or is a conservatively-substituted variant of the amino acid sequence of any one of SEQ ID NOs: 349, 353, 357, 361, 365, 369, 373, 377, 381, 385, 389, 393, 397, 401, 405, 409, 413, 417, 421, 425, 429, 433, 437, 441, 445, 449, 453, 457, and 461.

[0201] In certain embodiments, the antigen-binding domain comprises a V_H domain encoded by any one of SEQ ID NOs: 350, 354, 358, 362, 366, 370, 374, 378, 382, 386, 390, 394, 398, 402, 406, 410, 414, 418, 422, 426, 430, 434, 438, 442, 446, 450, 454, 458, and 462, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 350, 354, 358, 362, 366, 370, 374, 378, 382, 386, 390, 394, 398, 402, 406, 410, 414, 418, 422, 426, 430, 434, 438, 442, 446, 450, 454, 458, and 462; hybridizes under stringent hybridization conditions with the complement of any one of SEQ ID NOs: 350, 354, 358, 362, 366, 370, 374, 378, 382, 386, 390, 394, 398, 402, 406, 410, 414, 418, 422, 426, 430, 434, 438, 442, 446, 450, 454, 458, and 462; or is a codon degenerate variant of any one of SEQ ID NOs: 350, 354, 358, 362, 366, 370, 374, 378, 382, 386, 390, 394, 398, 402, 406, 410, 414, 418, 422, 426, 430, 434, 438, 442, 446, 450, 454, 458, and 462.

[0202] In certain embodiments, the antigen-binding domain comprises at least one CDR selected from those comprising the amino acid sequence of any one of the CDRs that bind ROR1 or a functional fragment or variant thereof.

[0203] In certain embodiments, the antigen-binding domain comprises the amino acid sequence of any one of SEQ ID NOs: 715–725, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the sequence of any one of the aforementioned sequences by at most 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-

terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 715–725, and/or is a conservatively-substituted variant of any one of SEQ ID NOs: 715–725.

[0204] In certain embodiments, the antigen-binding domain comprises a V_H domain comprising the amino acid sequences of SEQ ID NO: 715, SEQ ID NO: 716, and SEQ ID NO: 717.

[0205] In certain embodiments, the antigen-binding domain comprises a V_H domain comprising the amino acid sequences of SEQ ID NO: 718, SEQ ID NO: 719, and SEQ ID NO: 720.

[0206] In certain embodiments, the antigen-binding domain comprises a V_L domain comprising the amino acid sequences of SEQ ID NO: 721, SEQ ID NO: 722, and SEQ ID NO: 723.

[0207] In certain embodiments, the antigen-binding domain comprises a V_L domain comprising the amino acid sequences of SEQ ID NO: 724, SEQ ID NO: 725, and SEQ ID NO: 723.

[0208] In certain embodiments, the antigen-binding domain comprises both the aforementioned V_H and V_L domains.

[0209] In certain embodiments, the antigen-binding domain comprises a variable heavy chain domain comprising the amino acid sequence of SEQ ID NO: 349 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 349 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 349, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 349.

[0210] In certain embodiments, the antigen-binding domain comprises a V_H domain encoded by SEQ ID NO: 350, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 350; hybridizes under stringent hybridization conditions with the complement SEQ ID NO: 350; or is a codon degenerate variant of SEQ ID NO: 350.

[0211] In certain embodiments, the antigen-binding domain comprises a variable light chain domain comprising the amino acid sequence of SEQ ID NO: 387 or a functional fragment or

variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 387 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 387, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 387.

[0212] In certain embodiments, the antigen-binding domain comprises a V_L domain encoded by SEQ ID NO: 388, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 388; hybridizes under stringent hybridization conditions with the complement SEQ ID NO: 388; or is a codon degenerate variant of SEQ ID NO: 388.

[0213] In certain embodiments, the antigen-binding domain comprises a variable heavy chain domain comprising the amino acid sequence of SEQ ID NO: 349 or a functional fragment or variant thereof, wherein the variable heavy chain domain comprises, in N-terminal to C-terminal order, the sequences of SEQ ID NO: 715, SEQ ID NO: 716, and SEQ ID NO: 717. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 349 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 349, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 349.

[0214] In certain embodiments, the antigen-binding domain comprises a variable heavy chain domain comprising the amino acid sequence of SEQ ID NO: 349 or a functional fragment or variant thereof, wherein the variable heavy chain domain comprises, in N-terminal to C-terminal order, the sequences of SEQ ID NO: 718, SEQ ID NO: 719, and SEQ ID NO: 720. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 349 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 349, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 349.

[0215] In certain embodiments, the antigen-binding domain comprises a variable light chain

domain comprising the amino acid sequence of SEQ ID NO: 387 or a functional fragment or variant thereof, wherein the variable heavy chain domain comprises, in N-terminal to C-terminal order, the sequences of SEQ ID NO: 721, SEQ ID NO: 722, and SEQ ID NO: 723. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 387 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 387, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 387.

[0216] In certain embodiments, the antigen-binding domain comprises a variable light chain domain comprising the amino acid sequence of SEQ ID NO: 387 or a functional fragment or variant thereof, wherein the variable heavy chain domain comprises, in N-terminal to C-terminal order, the sequences of SEQ ID NO: 724, SEQ ID NO: 725, and SEQ ID NO: 723. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 387 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 387, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 387.

[0217] In certain embodiments, the antigen-binding domain comprises a variable heavy chain domain comprising the amino acid sequence of SEQ ID NO: 726 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 726 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 726, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 726.

[0218] In certain embodiments, the antigen-binding domain comprises a variable light chain domain comprising the amino acid sequence of SEQ ID NO: 727 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 727 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%,

97%, 98%, or 99% sequence identity with SEQ ID NO: 727, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 727.

[0219] In certain embodiments, the antigen-binding domain comprises a variable heavy chain domain comprising the amino acid sequence of SEQ ID NO: 728 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 728 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 728, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 728.

[0220] In certain embodiments, the antigen-binding domain comprises a variable light chain domain comprising the amino acid sequence of SEQ ID NO: 729 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 729 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 729, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 729.

[0221] In certain embodiments, the antigen-binding domain comprises a linker that links the V_H and V_L domains. In certain such embodiments, the linker comprises: (a) the amino acid sequence of SEQ ID NO: 424 ($(G_4S)_3$) or a conservatively-substituted variant thereof; or (b) an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 424. In certain such embodiments, the linker is encoded by SEQ ID NO: 425, hybridizes under stringent conditions to the complement of SEQ ID NO: 425, or is a codon degenerate version of SEQ ID NO: 425. Any linker as described herein can be used to link the VH and VL domains.

[0222] In certain embodiments, the antigen-binding domain comprises an scFv. In certain such embodiments, the domain comprises an amino acid sequence of SEQ ID NO: 465, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 465 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%,

95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 465, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 465.

[0223] In certain embodiments, the antigen-binding domain comprises an scFv encoded by SEQ ID NO: 466, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 466; hybridizes under stringent hybridization conditions with the complement SEQ ID NO: 466; or is a codon degenerate variant of SEQ ID NO: 466.

6. EGFRvIII-specific Antigen-Binding Domain

[0224] In another embodiment, a CAR described herein is a EGFRvIII-specific CAR. "EGFRvIII", "EGFR variant III", "EGFR type III mutant", "EGFR.D2-7" or "de2-7EGFR" is a mutated form of epidermal growth factor receptor (EGFR; ErbB-1; HER1), a transmembrane protein that is a receptor for members of the epidermal growth factor (EGF) family of extracellular protein ligands in human and non-human subjects. EGFRvIII is characterized by a deletion of exons 2-7 of the wild type EGFR gene, which results in an in-frame deletion of 267 amino acids in the extracellular domain of the full length wild type EGFR protein. EGFRvIII also contains a novel glycine residue inserted at the fusion junction compared to wild type EGFR. The truncated receptor EGFRvIII is unable to bind any known EGFR ligand; however, it shows constitutive tyrosine kinase activity. This constitutive activation is important to its pro-oncogenic effect. A kinase-deficient EGFRvIII is unable to confer a similar oncogenic advantage. EGFRvIII is highly expressed in glioblastoma (GBM) and can be detected in some other solid tumor types but not in normal tissues.

[0225] In some embodiments, the antigen binding moiety of a CAR described herein is specific to EGFRvIII (EGFRvIII CAR). The EGFRvIII-specific CAR, when expressed on the cell surface, redirects the specificity of T cells to human EGFRvIII. 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-EGFRvIII antibody joined by a flexible linker, such as a glycine-serine linker or a Whitlow linker. In some embodiments, the antigen binding moiety may comprise VH and VL that are directionally linked, for example, from N to C terminus, VH-linker-VL or VL-linker-VH.

B. Spacer

[0226] In some embodiments, a chimeric antigen receptor of the present disclosure further includes a spacer that is used to link the antigen-binding domain to the transmembrane domain. In some embodiments, the spacer is flexible enough to allow the antigen-binding domain to orient in different directions to facilitate antigen recognition.

[0227] In certain embodiments, a chimeric antigen receptor comprising a spacer has improved functional activity compared to an otherwise identical antigen-binding polypeptide lacking the spacer. In certain embodiments, a chimeric antigen receptor comprising a spacer has increased expression on a cell surface compared to an otherwise identical polypeptide lacking the spacer. In an embodiment, a chimeric antigen receptor comprising a spacer is a polypeptide that, were it not for the spacer, would not express on the cell membrane surface and/or would not be able to bind its target due to lack of proximity or steric hindrance.

[0228] In certain embodiments, the spacer comprises a stalk region, for example a hinge region from an antibody. In some instances, the stalk region comprises the hinge region from an IgG, for example IgG1. In alternative instances, the stalk region comprises the CH_2CH_3 region of immunoglobulin and, optionally, portions of CD3. In some cases, the stalk region comprises a CD8α hinge region (SEQ ID NO: 426), an IgG4-Fc 12 amino acid hinge region (SEQ ID NO: 631), or an IgG4 hinge region as described in WO2016/073755. The stalk region can be an extracellular portion of the CAR that links the antigen-binding domain to the cell surface and/or transmembrane region.

[0229] In some embodiments, the stalk region can be from about 20 to about 300 amino acids in length. In some cases, the 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. In some cases a stalk region can be less than 20 amino acids in length.

[0230] In some embodiments, the stalk region comprises a CD8α hinge domain, a CD28 hinge domain or a CTLA-4 hinge domain, or a functional fragment or variant thereof.

[0231] In certain embodiments, the stalk region comprises a CD8α hinge region, or a functional fragment or variant thereof. In certain such embodiments, the spacer comprises the amino acid sequence of SEQ ID NO: 426 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 467 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 467, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 467.

[0232] In certain embodiments, the CD8α hinge region, or functional fragment or variant thereof, is encoded by SEQ ID NO: 468, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 468, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 468, or is a codon degenerate variant of SEQ ID NO: 468.

[0233] In some embodiments, the stalk region can be capable of dimerizing with a homologous stalk region of a second CAR.

[0234] In certain embodiments, in addition to a stalk region, the spacer may comprise one or more stalk extension region(s). In certain embodiments, the stalk extension region is a polypeptide that is homologous to the stalk region. For example, it may comprise at least one amino acid residue substitution as compared with the stalk region. In some embodiments, the stalk region comprises a sequence with at least about 70%, 75%, 80%, 85%, 90%, 95% , 96%, 97%, 98%, or 99% identity to the stalk region to which it is attached, for example a CD8α hinge domain, a CD28 hinge domain, or a CTLA-4 hinge domain.

[0235] In some embodiments, the spacer comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 stalk extension regions.

[0236] In certain embodiments, the stalk region can be linked to the stalk extension region by way of a linker.

[0237] In certain embodiments, the 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.

[0238] In some embodiments, the stalk region comprises at least one dimerization site. In certain embodiments, the stalk region may 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 may contain mutations that eliminate dimerization sites altogether.

[0239] In certain embodiments, the stalk extension region has 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 cases, one or more dimerization site(s) can be membrane proximal. In other cases, one or more dimerization site(s) can be membrane distal.

[0240] In certain embodiments, the dimerization site is a cysteine residue capable of forming a disulfide bond. In certain embodiments, the stalk extension region is capable of forming fewer disulfide bond(s) as compared to a stalk region. For example, if a stalk region is capable of forming two disulfide bonds, a stalk extension region may be capable of forming one or no disulfide bonds. As another example, if a stalk region is capable of forming one disulfide bond, a stalk extension region may be capable of forming no such bonds.

[0241] Each of the stalk extension regions can be about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or greater amino acids in length.

[0242] In certain embodiments, the stalk extension region is homologous to the CD8α hinge region. In certain such embodiments, the stalk extension region comprises the amino acid sequence of SEQ ID NO: 469 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 469 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 469, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 469.

[0243] In certain embodiments, the stalk extension region is encoded by any one of SEQ ID NOs:

470–472, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 470–472, hybridizes under stringent hybridization conditions with the complement of any one of SEQ ID NOs: 470–472, or is a codon degenerate variant of any one of SEQ ID NOs: 470–472.

[0244] In certain embodiments, the spacer comprises a stalk region and 1 to 3 stalk extension regions. In certain such embodiments, the spacer comprises a stalk region and 2 stalk extension regions, for example a CD8α hinge region and 2 stalk extension regions wherein each stalk extension region is homologous to the CD8α hinge region.

[0245] In some embodiments, each of the stalk region and stalk extension region(s) can be derived from at least one of a CD8α 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 CD8α 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 CD8α 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.

[0246] In certain such embodiments, the spacer comprises the amino acid sequence of SEQ ID NO: 473 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 473 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 473, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 473.

[0247] In certain embodiments, the spacer is encoded by SEQ ID NO: 474, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 474, hybridizes under

stringent hybridization conditions with the complement of SEQ ID NO: 474, or is a codon degenerate variant of SEQ ID NO: 474.

C. Transmembrane Domain

[0248] The transmembrane domain can be derived from either a natural or a synthetic source. Where the source is natural, the domain can, for example, be derived from any membrane-bound or transmembrane protein. Suitable transmembrane domains include transmembrane domains from a TCR-alpha chain, a TCR-beta chain, a TCR-γ1 chain, a TCR-δ chain, a TCR-zeta chain, CD28, CD3 epsilon, CD3ζ, CD45, CD4, CD5, CD8α, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, ICOS, GITR, CD152 (CTLA-4), or CD154, or a functional fragment or variant thereof. 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. In some embodiments, the transmembrane domain comprises a CD8α transmembrane domain, a CD152 (CTLA-4), TCRγ1, TCRδ or a CD3ζ transmembrane domain.

[0249] Optionally, a short oligonucleotide or polypeptide linker, in some embodiments between 2 and 10 amino acids in length, may link the transmembrane domain with the intracellular signaling domain of a CAR. In some embodiments, the linker is a glycine-serine linker.

[0250] In some embodiments, the transmembrane domain comprises a CD8α transmembrane domain or a CD3ζ transmembrane domain, or a functional fragments or variants thereof.

[0251] In certain embodiments, the transmembrane domain comprises a CD8α transmembrane domain, or a functional fragment or variant thereof. In certain such embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 475 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 475 by at most 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 475, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 475.

[0252] In certain embodiments, the CD8α transmembrane domain, or functional fragment or

variant thereof, is encoded by SEQ ID NO: 476 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 476, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 476, or is a codon degenerate variant of SEQ ID NO: 476.

[0253] In certain embodiments, the transmembrane domain comprises a CD28 transmembrane domain, or a functional fragment or variant thereof. In certain such embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 477 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 477 by at most 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 477, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 477.

[0254] In certain embodiments, the CD28 transmembrane domain, or functional fragment or variant thereof, is encoded by SEQ ID NO: 478 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 478, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 478, or is a codon degenerate variant of SEQ ID NO: 438.

D. Intracellular Signaling Domain

[0255] The intracellular signaling domain of the CAR may be 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. 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. In some embodiments, the intracellular domain further comprises a signaling domain for T-cell activation.

[0256] In some embodiments, the intracellular cell signaling domain interacts with a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), or a regulatory T cell.

[0257] The intracellular domain can comprise an amino acid sequence derived from FCER1G, CD19, CD40, KIR3DL1, KIR3DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL1, KIR3DL2, KIR3DL3, SIRPA, FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, FCRL6, FCGR1A, FCGR2A, FCGR2B, FCGR3A, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, PILRB, NCR1, NCR2, NCR3, NKG2A, NKG2C, NKG2D, DAP12, FCER1G, DAP10, CD84, CD19, KIR3DL1, KIR3DL2, KIR2DL2, KIR2DL3, KIR2DL4, KIR2DL5, KIR3DL2, KIR3DL3, SIRPA, FCRL1, FCRL2, FCRL3, FCRL4, FCRL5, FCRL6, CD4, CD8A, CD8B, LAT, FCGR1A, FCGR2A, FCGR2B, FCGR3A, TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, NCR1, NCR2, NCR3, LY9, NKG2C, TCR zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD3ζ, CD5, CD22, CD79a, CD79b or CD66d, or a functional fragment or variant thereof. In some cases, the signaling domain for T-cell activation comprises a domain derived from CD3ζ, or a functional fragment or variant thereof.

[0258] In certain embodiments, the intracellular signaling domain comprises a CD3ζ domain, or a functional fragment or variant thereof. In certain such embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 479 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 479 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 479, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 479.

[0259] In certain embodiments, the CD3ζ domain, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 480 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 480, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 480, or is a codon degenerate variant of SEQ ID NO: 480.

[0260] The intracellular signaling domain can further comprise one or more co-stimulatory

domains. Exemplary co-stimulatory domains include, but are not limited to, CD8, CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, OX40 (CD134), and CD3-zeta co-stimulatory domains and functional fragments or variants thereof. In some instances, a CAR described herein comprises one or more, or two or more of co-stimulatory domains selected from CD8, CD27, CD28, 4-1BB (CD137), ICOS, DAP10, DAP12, and OX40 (CD134) co-stimulatory domains and functional fragments or variants thereof. In some instances, a CAR described herein comprises one or more, or two or more of co-stimulatory domains selected from CD27, CD28, 4-1BB (CD137), ICOS, and OX40 (CD134) co-stimulatory domains and functional fragments or variants thereof. In some instances, a CAR described herein comprises one or more, or two or more of co-stimulatory domains selected from CD8, CD28, 4-1BB (CD137), DAP10, and DAP12 co-stimulatory domains and functional fragments or variants thereof. In some instances, a CAR described herein comprises one or more, or two or more co-stimulatory domains selected from CD28 and 4-1BB (CD137) co-stimulatory domains and functional fragments or variants thereof. In some instances, a CAR described herein comprises CD28 and 4-1BB (CD137) co-stimulatory domains or their respective functional fragments or variants. In some instances, a CAR described herein comprises CD28 and OX40 (CD134) co-stimulatory domains or their respective functional fragments and variants. In some instances, a CAR described herein comprises CD8 and CD28 co-stimulatory domains or their respective functional fragments and variants. In some instances, a CAR described herein comprises a CD28 co-stimulatory domains or a functional fragment or variant thereof. In some instances, a CAR described herein comprises a 4-1BB (CD137) co-stimulatory domain or a functional fragment or variant thereof. In some instances, a CAR described herein comprises an OX40 (CD134) co-stimulatory domain or a functional fragment or variant thereof. In some instances, a CAR described herein comprises a CD8 co-stimulatory domain or a functional fragment or variant thereof. In some instances, the CAR described herein comprises a DAP10 co-stimulatory domain or a functional fragment or variant thereof. In some instances, the CAR described herein comprises a DAP12 co-stimulatory domain or a functional fragment or variant thereof.

[0261] In certain embodiments, the intracellular signaling domain comprises a CD28 co-stimulatory domain, or a functional fragment or variant thereof. In certain such embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 481 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter

than the amino acid sequence of SEQ ID NO: 481 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 481, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 481.

[0262] In certain embodiments, the CD28 co-stimulatory domain, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 482 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 482, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 482, or is a codon degenerate variant of SEQ ID NO: 442.

[0263] In certain embodiments, the intracellular signaling domain comprises a 4-1BB co-stimulatory domain, or a functional fragment or variant thereof. In certain such embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 483 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 483 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 483, and/or is a conservatively-substituted variant of SEQ ID NO: 483.

[0264] In certain embodiments, the 4-1BB co-stimulatory domain, or functional fragment or variant thereof, is encoded by SEQ ID NO: 484 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 484, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 484, or is a codon degenerate variant of SEQ ID NO: 484.

[0265] In certain embodiments, the intracellular signaling domain comprises a DAP10 co-stimulatory domain, or a functional fragment or variant thereof. In certain embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 485 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter

than the amino acid sequence of SEQ ID NO: 485 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 485, and/or is a conservatively-substituted variant of SEQ ID NO: 485.

[0266] In certain embodiments, the DAP10 co-stimulatory domain, or functional fragment or variant thereof, is encoded by the sequence of SEQ ID NO: 486 , or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 486, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 486, or is a codon degenerate variant of SEQ ID NO: 486.

[0267] In certain embodiments, the intracellular signaling domain comprises a DAP12 co-stimulatory domain, or a functional fragment or variant thereof. In certain embodiments, the intracellular signaling domain comprises the amino acid sequence of SEQ ID NO: 487 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 487 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 487, and/or is a conservatively-substituted variant of SEQ ID NO: 487.

[0268] In certain embodiments, the DAP12 co-stimulatory domain, or functional fragment or variant thereof, is encoded by the sequence of SEQ ID NO: 488, or functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 488, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 488, or is a codon degenerate variant of SEQ ID NO: 488.

[0269] In certain embodiments, the intracellular signaling domain comprises both a CD28 co-signaling domain and a 4-1BB co-signaling domain, or respective functional fragments or variants thereof, as described above.

E. Signal Peptide

[0270] In an embodiment, a signal peptide directs the nascent CAR protein into the endoplasmic reticulum. This is, for example, if the receptor is to be glycosylated and anchored in the cell membrane. Any eukaryotic signal peptide sequence is envisaged to be functional. Generally, the signal peptide natively attached to the protein or, in the case of a fusion protein, the component closest to the N-terminus is used (*e.g.*, in a scFv with the V_L component at closest to the N-terminus, the native signal of the light chain is used). In some embodiments, the signal peptide is native for GM-CSFRa (SEQ ID NO: 489) or IgK (SEQ ID NO: 491), IgE (SEQ ID NO: 493) or a functional fragment or variant thereof. Other signal peptides that can be used include those native to CD8α (SEQ ID NO: 495) and CD28. In some embodiments, the signal peptide is that native to Mouse Ig V_H region 3 (SEQ ID NO: 497), β2M signal peptide (SEQ ID NO: 499), Azurocidin (SEQ ID NO: 501), Human Serum Albumin signal peptide (SEQ ID NO: 503), A2M receptor associated protein signal peptide (SEQ ID NO: 505), IGHV3-23 (SEQ ID NO: 507), IGKV1-D33 (HuL1) (SEQ ID NO: 509), IGKV1-D33 (L14F) (HuH7) (SEQ ID NO: 511), or a functional fragment or variant thereof.

[0271] In certain embodiments, the CAR is linked to a GM-CSFRa signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the GM-CSFRa signal peptide has the amino acid sequence of SEQ ID NO: 489 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 489 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 489, and/or is a conservatively-substituted variant of SEQ ID NO: 489.

[0272] In certain embodiments, the GM-CSFRa signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 490 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 490, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 490, or is a codon degenerate variant of SEQ ID NO: 490.

[0273] In certain embodiments, the CAR is linked to an Igκ signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the Igκ signal peptide has the amino

acid sequence of SEQ ID NO: 491 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 491 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 491, and/or is a conservatively-substituted variant of SEQ ID NO: 491.

[0274] In certain embodiments, the Igκ signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 492 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 492, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 492, or is a codon degenerate variant of SEQ ID NO: 492.

[0275] In certain embodiments, the CAR is linked to an IgE signal peptide native to IgE ("IgE signal peptide"), or a functional fragment or variant thereof. In certain such embodiments, the IgE signal peptide the amino acid sequence of SEQ ID NO: 493 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 493 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 493, and/or is a conservatively-substituted variant of SEQ ID NO: 493.

[0276] In certain embodiments, the IgE signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 494 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 494, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 494, or is a codon degenerate variant of SEQ ID NO: 494.

[0277] In certain embodiments, the CAR is linked to an CD8α signal peptide native to CD8α, or a functional fragment or variant thereof. In certain such embodiments, the CD8α signal peptide comprises the amino acid sequence of SEQ ID NO: 495 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 495 by at most 15,

10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 495, and/or is a conservatively-substituted variant of SEQ ID NO: 495.

[0278] In certain embodiments, the CD8α signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 496 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 496, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 496 or is a codon degenerate variant of SEQ ID NO: 496.

[0279] In certain embodiments, the CAR is linked to a Mouse Ig VH region 3 signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the Mouse Ig VH region 3 signal peptide has the amino acid sequence of SEQ ID NO: 497 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 497 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 497, and/or is a conservatively-substituted variant of SEQ ID NO: 497.

[0280] In certain embodiments, the Mouse Ig VH region 3 signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 498 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 498, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 498, or is a codon degenerate variant of SEQ ID NO: 498.

[0281] In certain embodiments, the CAR is linked to a β2M signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the β2M signal peptide has the amino acid sequence of SEQ ID NO: 499 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 499 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the

functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 499, and/or is a conservatively-substituted variant of SEQ ID NO: 499.

[0282] In certain embodiments, the β2M signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 500 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 500, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 500, or is a codon degenerate variant of SEQ ID NO: 500.

[0283] In certain embodiments, the CAR is linked to an Azurocidin signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the Azurocidin signal peptide has the amino acid sequence of SEQ ID NO: 501 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 501 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 501, and/or is a conservatively-substituted variant of SEQ ID NO: 501.

[0284] In certain embodiments, the Azurocidin signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 502 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 502, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 502, or is a codon degenerate variant of SEQ ID NO: 502.

[0285] In certain embodiments, the CAR is linked to a human serum albumin signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the human serum albumin signal peptide has the amino acid sequence of SEQ ID NO: 503 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 503 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 503, and/or is a conservatively-substituted variant of SEQ ID NO: 503.

[0286] In certain embodiments, the human serum albumin signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 504 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 504, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 504, or is a codon degenerate variant of SEQ ID NO: 504.

[0287] In certain embodiments, the CAR is linked to an A2M receptor associated protein signal peptide, or a functional fragment or variant thereof. In certain such embodiments, A2M receptor associated protein signal peptide has the amino acid sequence of SEQ ID NO: 505 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 505 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 505, and/or is a conservatively-substituted variant of SEQ ID NO: 505.

[0288] In certain embodiments, the A2M receptor associated protein signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 506 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 506, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 506, or is a codon degenerate variant of SEQ ID NO: 506.

[0289] In certain embodiments, the CAR is linked to an IGHV3-23 signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the IGHV3-23 signal peptide has the amino acid sequence of SEQ ID NO: 507 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 507 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 507, and/or is a conservatively-substituted variant of SEQ ID NO: 507.

[0290] In certain embodiments, the IGHV3-23 signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 508 or a functional fragment or

variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 508, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 508, or is a codon degenerate variant of SEQ ID NO: 508.

[0291] In certain embodiments, the CAR is linked to an IGKV1-D33 signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the IGKV1-D33 signal peptide has the amino acid sequence of SEQ ID NO: 509 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 509 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 509, and/or is a conservatively-substituted variant of SEQ ID NO: 509.

[0292] In certain embodiments, the IGKV1-D33 signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 510 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 510, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 510, or is a codon degenerate variant of SEQ ID NO: 510.

[0293] In certain embodiments, the CAR is linked to an IGHV3-33 (L14F) signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the IGHV3-33 (L14F) signal peptide has the amino acid sequence of SEQ ID NO: 511 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 511 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 511, and/or is a conservatively-substituted variant of SEQ ID NO: 511.

[0294] In certain embodiments, the IGHV3-33 (L14F) signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 512 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%,

95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 512, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 512, or is a codon degenerate variant of SEQ ID NO: 512.

[0295] In certain embodiments, the CAR is linked to an TVB2 (T21A) signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the TVB2 (T21A) signal peptide has the amino acid sequence of SEQ ID NO: 513 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 513 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 513, and/or is a conservatively-substituted variant of SEQ ID NO: 513.

[0296] In certain embodiments, the TVB2 (T21A) signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 514 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 514, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 514, or is a codon degenerate variant of SEQ ID NO: 514.

[0297] In certain embodiments, the CAR is linked to an CD52 signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the CD52 signal peptide has the amino acid sequence of SEQ ID NO: 515 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 515 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 515, and/or is a conservatively-substituted variant of SEQ ID NO: 515.

[0298] In certain embodiments, the CD52 signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 516 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 516, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 516, or is a codon degenerate variant

of SEQ ID NO: 516.

[0299] In certain embodiments, the CAR is linked to an LNGFR signal peptide, or a functional fragment or variant thereof. In certain such embodiments, the LNGFR signal peptide has the amino acid sequence of SEQ ID NO: 517 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 517 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 517, and/or is a conservatively-substituted variant of SEQ ID NO: 517.

[0300] In certain embodiments, the LNGFR signal peptide, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 518 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 518, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 518, or is a codon degenerate variant of SEQ ID NO: 518.

F. Exemplary CAR Constructs

[0301] By way of example, but not limitation, the CAR can comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with any one of SEQ ID NOs: 591, 593, 595, 597, 599, 601, 603, 605, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, and 629 or a conservatively-substituted variant thereof. By way of further example, but not limitation, a polynucleotide encoding the CAR can comprise a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the sequence of any one of SEQ ID NOs: 592, 594, 596, 598, 600, 602, 604, 606, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, and 630; a sequence that hybridizes under stringent hybridization conditions with the complement of any one of such sequences; or a codon degenerate variant of any one of such sequences.

[0302] In certain embodiments, the CAR can comprise an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 623 or a conservatively-substituted variant thereof. In certain such embodiments, the polynucleotide

encoding the CAR can comprise a nucleic acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the sequence of SEQ ID NO: 624; a sequence that hybridizes under stringent hybridization conditions with the complement of any one of such sequences; or a codon degenerate variant of any one of such sequences.

[0303] CARs and CAR construction as well as compositions are also described, for example, in:

- Chimeric Antigen Receptor (CAR) T-Cell Therapies for Cancer: A Practical Guide, Edited by: Daniel W. Lee and Nirali N. Shah, 2020 (ISBN 978-0-323-66181-2; DOI https://doi.org/10.1016/C2017-0-04066-1);

- Second Generation Cell and Gene-based Therapies, Biological Advances, Clinical Outcomes and Strategies for Capitalisation, Editors-in-Chief: Alain A. Vertès, Devyn M. Smith, Nathan J. Dowden, 2020 (ISBN 978-0-12-812034-7; DOI https://doi.org/10.1016/C2016-0-02070-3);

- Basics of Chimeric Antigen Receptor (CAR) Immunotherapy, Author: Mumtaz Yaseen Balkhi, 2020 (ISBN 978-0-12-819573-4, DOI https://doi.org/10.1016/C2018-0-05356-6);

- Engineering and Design of Chimeric Antigen Receptors, Authors: Sonia Guedan, Hugo Calderon, Avery D. Posey, Jr., and Marcela V. Maus, *Molecular Therapy: Methods & Clinical Development,* Vol. 12, March (2019) (https://www.cell.com/molecular-therapy-family/methods/pdf/S2329-0501(18)30133-5.pdf);

- Chimeric Antigen Receptor T Cell Therapy Pipeline at a Glance: A Retrospective and Systematic Analysis from Clinicaltrials.Gov, Authors: Eider F Moreno Cortes, Caleb K Stein, Paula A Lengerke Diaz, Cesar A Ramirez-Segura, Januario E. Castro, MD, *Blood* (2019) 134 (Supplement_1): 5629 (https://doi.org/10.1182/blood-2019-132273);

- WO2020209934 (PCT/US2020/017794) - Novel chimeric antigen receptors and libraries (MIT);

- WO2020037142 (PCT/US2019/046691) - Compositions and methods for high-throughput activation screening to boost t cell effector function (Yale);

- WO2015123642 (PCT/US2015/016057) - Chimeric antigen receptors and methods of making (Univ. TX);

- WO2020014366 (PCT/US2019/041213) - Ror-1 specific chimeric antigen receptors and uses thereof (Precigen);

- WO2019236577 (PCT/US2019/035384) - Muc16 specific chimeric antigen receptors and uses thereof (Precigen)

- WO2019079486 (PCT/US2018/056334) - Polypeptide compositions comprising spacers (Precigen)

- WO2017214333 (PCT/US2017/036440) - Cd33 specific chimeric antigen receptors (Precigen)

V. Cytokine

[0304] In some embodiments, the modified immune effector cell of the present invention can comprise a cytokine. The cytokine may, for example, be encoded by the polynucleotide of the present disclosure. For example, the polynucleotide may encode the miRNA(s), CAR and cytokine, or the miRNA(s) and cytokine.

[0305] In some cases, the cytokine comprises at least one chemokine, interferon, interleukin, lymphokine, tumor necrosis factor, or variant or combination thereof. In certain embodimetns, the cytokine is an interferon, GM-CSF, G-CSF, M-CSF, LT-beta, TNF-alpha, growth factors, hGH, and/or a ligand of human Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, IFN-alpha, IFN-beta, or IFN-gamma.

[0306] In certain embodiments, the cytokine is an interleukin. In some cases the interleukin is 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, IL-35, or a functional variant or fragment thereof.

[0307] In certain embodiments, the cytokine may be IL-12, or a functional fragment or variant thereof. 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.

[0308] In certain embodiments, the cytokine may be IL-15, or a functional fragment or variant thereof. In certain embodiments, the IL-15, or functional fragment or variant thereof, is membrane-bound. Such may occur when IL-15, or a functional fragment or variant thereof, is bound to membrane-bound IL-15Rα, or a functional fragment or variant thereof. Thus, certain embodimetns of the present invention may involve a fusion protein comprising IL-15 and IL-15Rα, or their respective functional fragments or variants.

[0309] In certain embodiments, the IL-15, or functional fragment or variant thereof, comprises the amino acid sequence of SEQ ID NO: 519, or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 519 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 519, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 519.

[0310] In certain embodiments, the IL-15, or functional fragment or variant thereof, is encoded by a nucleic acid comprising the sequence of SEQ ID NO: 520, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 520, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 520, or is a codon degenerate variant of SEQ ID NO: 520.

[0311] In certain embodiments, the IL-15Rα, or functional fragment or variant thereof, comprises the amino acid sequence of SEQ ID NO: 521 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 521 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 521, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 521.

[0312] In certain embodiments, the IL-15Rα, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 522, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 522, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 522, or is a codon degenerate variant of SEQ ID NO: 522.

[0313] In certain embodiments, the IL-15, or functional fragment or variant thereof, is linked to the IL-15Rα, or functional fragment thereof by way of a linker.

[0314] In certain embodiments, the linker comprises the amino acid sequence of SEQ ID NO: 529 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than SEQ ID NO: 529 by at most 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 529, and/or is a conservatively-substituted variant of SEQ ID NO: 529.

[0315] In certain embodiments, the linker is encoded by a nucleic acid comprising SEQ ID NO: 530 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 530, hybridizes under stringent hybridization conditions with SEQ ID NO: 530, or is a codon degenerate variant of SEQ ID NO: 530.

[0316] In certain embodiments, the fusion protein comprising IL-15 and IL-15Rα comprises the amino acid sequence of SEQ ID NO: 523 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 523 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 523, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 523.

[0317] In certain embodiments, the fusion protein comprising IL-15 and IL-15Rα is encoded by a nucleic acid comprising SEQ ID NO: 524 or a functional fragment or variant thereof. In certain

embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 524, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 524, or is a codon degenerate variant of SEQ ID NO: 603.

[0318] In certain embodiments, the cytokine is linked to a signal peptide. Any signal for use in eukaryotic cells, including those described above for use with the CARs may be linked to the cytokine. In certain embodiments, the cytokine is linked to an IgE signal peptide.

[0319] In certain embodiments, the fusion protein comprising IL-15 and IL-15Rα comprises the amino acid sequence of SEQ ID NO: 525 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 525 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 525, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 525.

[0320] In certain embodiments, the fusion protein comprising IL-15 and IL-15Rα is encoded by a nucleic acid comprising SEQ ID NO: 526 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 526, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 526, or is a codon degenerate variant of SEQ ID NO: 526.

VI. Cell Tag

[0321] In some embodiments, the modified immune effector cell of the present invention can comprise a cell tag. The cell tag may, for example, be encoded by the polynucleotide of the present disclosure. For example, the polynucleotide may encode the miRNA(s), CAR and/or cytokine described herein as well as a cell tag. In some aspects, the cell tag is used as a kill switch, selection marker, a biomarker, or a combination thereof.

[0322] In certain embodiments, the cell tag is capable of being bound by a predetermined binding partner. In certain such embodiments, the cell tag is non-immunogenic. In certain such embodiments, the cell tag comprises a polypeptide that is truncated so that it is non-immunogenic.

[0323] In certain embodiments, the administration of the predetermined binding partner allows for depletion of infused CAR-T cells. For example, the administration of cetuximab or any antibody that recognizes HER1 allows for the elimination of cells expressing a cell tag comprising truncated non-immunogenic HER1. The truncation of the HER1 sequence eliminates the potential for EGF ligand binding, homo- and hetero- dimerization of EGFR, and/or EGFR-mediated signaling while keeping cetuximab-binding ability intact (Ferguson, K., 2008. A structure-based view of Epidermal Growth Factor Receptor regulation. *Annu Rev Biophys,* Volume 37, pp. 353-373).

[0324] In certain embodiments, the cell tag comprises at least one of a truncated non-immunogenic HER1 polypeptide, a truncated non-immunogenic LNGFR polypeptide, a truncated non-immunogenic CD20 polypeptide, or a truncated non-immunogenic CD52 polypeptide, or a functional fragment or variant thereof.

[0325] In certain embodiments, the cell tag comprises a truncated non-immunogenic HER1 polypeptide comprising a HER1 Domain III and a truncated HER1 Domain IV. Such domains and the nucleic acid sequences encoding the same include those described in WO 2018/226897.

[0326] In certain embodiments, the HER1 Domain III comprises the amino acid sequence of SEQ ID NO: 604 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 565 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 565, and/or is a conservatively-substituted variant the amino acid sequence of sequence of SEQ ID NO: 565.

[0327] In certain embodiments, the HER1 Domain III, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 566 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 566, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 566, or is a codon degenerate variant of SEQ ID NO: 566.

[0328] In certain embodiments, the truncated HER1 Domain IV comprises the amino acid sequence of SEQ ID NO: 567 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 567 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 567, and/or is a conservatively-substituted variant the amino acid sequence of sequence of SEQ ID NO: 567.

[0329] In certain embodiments, the truncated HER1 Domain IV, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 568 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 568, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 568, or is a codon degenerate variant of SEQ ID NO: 568.

[0330] In certain such embodiments, the truncated non-immunogenic HER1 comprises the amino acid sequence of SEQ ID NO: 569 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 569 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 569, and/or is a conservatively-substituted variant the amino acid sequence of sequence of SEQ ID NO: 569.

[0331] In certain embodiments, the truncated non-immunogenic HER1, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 570 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 570, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 570, or is a codon degenerate variant of SEQ ID NO: 570.

[0332] In certain embodiments, the cell tag comprises a truncated non-immunogenic CD20, or CD20t-1, or a functional fragment or variant thereof. In certain such embodiments, the cell tag comprises the amino acid sequence of SEQ ID NO: 573, SEQ ID NO: 575, or a functional fragment

or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequences of SEQ ID NO: 573 or SEQ ID NO: 575 by at most 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 573 or SEQ ID NO: 575, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 573 or SEQ ID NO: 575.

[0333] In certain embodiments, the truncated non-immunogenic CD20, or CD20t-1, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 574 or SEQ ID NO: 576 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 574 or SEQ ID NO: 576, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 574 or SEQ ID NO: 576, or is a codon degenerate variant of SEQ ID NO: 574 or SEQ ID NO: 576.

[0334] In certain embodiments, the cell tag further comprises a transmembrane domain. The transmembrane domain can be derived from either a natural or a synthetic source. Where the source is natural, the domain can, for example, be derived from any membrane-bound or transmembrane protein. Suitable transmembrane domains can include the transmembrane domain(s) of alpha, beta or zeta chain of the T-cell receptor; or a transmembrane domain from CD28, CD3 epsilon, CD3ζ, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 or CD154, or a functional fragment or variant thereof. 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.

[0335] In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain, or a functional fragment or variant thereof. In certain such embodiments, the transmembrane domain comprises the amino acid sequence of SEQ ID NO: 477 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 477 by at most 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at

least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 477, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 477.

[0336] In certain embodiments, the CD28 transmembrane domain, or functional fragment or variant thereof, is encoded by a nucleic acid comprising SEQ ID NO: 478, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 478, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 478, or is a codon degenerate variant of SEQ ID NO: 478.

[0337] In certain embodiments, the cell tag comprises a truncated HER1, or functional fragment or variant thereof, and a transmembrane domain, or a functional fragment or variant thereof. In some embodiments, the cell comprises the amino acid sequence of SEQ ID NO: 571 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 571 by at most 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 571, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 571.

[0338] In certain embodiments, the cell tag is encoded by a nucleic acid comprising SEQ ID NO: 572, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 572, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 572, or is a codon degenerate variant of SEQ ID NO: 572.

[0339] In certain embodiments, the cell tag is linked with a signal peptide. The signal peptide can be any signal peptide suitable for use in a eukaryotic cell including those described with respect to CARs herein. In certain embodiments, the signal peptide is a Igκ signal peptide comprising the amino acid sequence of SEQ ID NO: 491, or a functional fragment or variant thereof

VII. Genetic Construct

[0340] As shown in FIG. 16, an exemplary polynucleotide is provided which can be used as template for the expression of various genes and other regulatory elements in cells. It should be understood that various elements can be included or omitted in the polynucleotide and that different options are shown for various exemplary sites in the polynucleotide.

[0341] As shown, the polynucleotide can include an integration signal for attP/attB phage integration of the polynucleotide into a bacterial genome. The polynucleotide can further include a 5' homology arm or 5' terminal repeat and a 3' homology arm or 3' terminal repeat. The polynucleotide can further include insulators, boundary elements and S/MAR positioned 3' adjacent to the 5' homology arm or 5' terminal repeat and 5' adjacent to the 3' homology arm or 3' terminal repeat. Between the insulators, boundary elements, or S/MAR, the polynucleotide can include, from 5' to 3', a promoter which can include a silencer, enhancer, TF binding modules and a core promoter; a 5' untranslated region which can include stability modules, translation control elements, and intron-embedded elements such as miRNA encoding sequences; one or more genes which can include signal peptides, extracellular domains, transmembrane domains, signaling domains, antibody domains, peptide linkers, inteins and epitope tags; and a 3' untranslated region that can include stability modules, translation control, 3' end processing signals and a transcription terminator. It should be understood that the genes to be expressed can be separated by IRES, cleavage peptides, or ribosomal skipping peptides.

[0342] The CAR may be encoded in the same genetic construct with the miRNA, the cytokine, and/or the cell tag. An advantage of having two or more of such components expressed using one genetic construct is stoichiometric expression of such components.

A. Linkers

[0343] In certain embodiments, the polypeptides of the present invention (*e.g.*, the CAR, the cytokine, and the cell tag) are linked by linker polypeptide(s). The linkers may also be used to link domains of a polypeptide (*e.g.*, the VH and VL domains of a CAR, the truncated HER1 and transmembrane domains of the cell tag, and the IL-15 and IL-15Rα domains).

[0344] Linkers suitable in the present invention include flexible linkers, rigid linkers, and *in vivo* cleavable linkers. In some cases, the linker acts to link functional domains together (as in flexible

and rigid linkers) or to release a free functional domain *in vivo* as in *in vivo* cleavable linkers.

[0345] As noted, in some cases, the linker sequence may 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. 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 4 is a (G4S)4 linker as shown in SEQ ID NO: 608 or a conservatively substituted amino acid sequence thereof. Besides GS linkers, other flexible linkers can be utilized for recombinant fusion proteins. In some cases, flexible linkers 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.

[0346] 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 linker may be adjusted to allow for proper folding or to achieve optimal biological activity of the fusion proteins.

[0347] A rigid linker can be utilized to maintain a fixed distance between domains of a polypeptide. Examples of rigid linkers include Alpha helix-forming linkers, Pro-rich sequence, (XP)n, X-Pro backbone, A(EAAAK)nA (n = 2-5) (SEQ ID NO: 563) and functional fragments and variants thereof, 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.

[0348] A linker useful in the present invention can be cleavable in some cases. In other cases, the 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*.

[0349] 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 some cases, a cleavable linker can allow for targeted cleavage. For example, 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. A cleavable linker can comprise a hydrazone, peptides, a disulfide, or a 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. Examples of cleavable linkers include Furinlink, fmdv, and 2A linkers (*e.g.*, P2A, GSG-P2A, FP2A, T2A, and Furin-T2A), or functional fragments or variants thereof.

[0350] A fmdv linker may comprise the amino acid sequence of SEQ ID NO: 539 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 539 by at most 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 539, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 539.

[0351] In certain embodiments, the fmdv linker is encoded by a nucleic acid comprising SEQ ID NO: 540, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 540, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 540, or is a codon degenerate variant of SEQ ID NO: 540.

[0352] A P2A linker may comprise the amino acid sequence of SEQ ID NO: 547 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 547 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid

residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 547, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 547.

[0353] In certain embodiments, the P2A linker is encoded by a nucleic acid comprising SEQ ID NO: 548, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 548, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 548, or is a codon degenerate variant of SEQ ID NO: 548.

[0354] A GSG-P2A linker may comprise the amino acid sequence of SEQ ID NO: 549 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 549 by at most 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 549, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 549.

[0355] In certain embodiments, the GSG-P2A linker is encoded by a nucleic acid comprising SEQ ID NO: 550, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 550, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 550, or is a codon degenerate variant of SEQ ID NO: 550.

[0356] A FP2A linker may comprise the amino acid sequence of SEQ ID NO: 555 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 555 by at most 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 555, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 555.

[0357] In certain embodiments, the FP2A linker is encoded by a nucleic acid comprising SEQ ID NO: 556, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 556, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 556, or is a codon degenerate variant of SEQ ID NO: 556.

[0358] A T2A linker may comprise the amino acid sequence of SEQ ID NO: 541 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 541 by at most 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 541, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 541.

[0359] In certain embodiments, the T2A linker is encoded by a nucleic acid comprising SEQ ID NO: 475428, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 542, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 54, or is a codon degenerate variant of SEQ ID NO: 542.

[0360] In some embodiments, the linker comprises 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. Such linkers are called "Furin-T2A" linkers. In some cases, the 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, the polypeptide linker can include one or more GS linker sequences, for instance (GS)n, (SG)n, and (GSG)n, wherein n can be any number from zero to thirty.

[0361] A Furin-T2A linker may comprise the amino acid sequence of SEQ ID NO: 543 or 545 or a functional fragment or variant thereof. In certain embodiments, the functional fragment is shorter than the amino acid sequence of SEQ ID NO: 543 or 545 by at most 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid residues at the N- and/or C-terminus. In certain embodiments, the

functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of SEQ ID NO: 543 or 545, and/or is a conservatively-substituted variant of the amino acid sequence of SEQ ID NO: 543 or 545.

[0362] In certain embodiments, the Furin-T2A linker is encoded by a nucleic acid comprising SEQ ID NO: 544 or 546, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 544 or 546, hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 544 or 546, or is a codon degenerate variant of SEQ ID NO: 544 or 546.

[0363] A linker can be an engineered linker. For example, a linker can be designed to comprise chemical characteristics such as hydrophobicity. 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.

[0364] Further exemplary linkers are provided in the Sequence Listing.

[0365] In some cases, at least two linker sequences can be included in the same protein. For example, polypeptides of interest within a fusion protein can be separated by at least two linkers. In some cases, polypeptides can be separated by 2, 3, 4, 5, 6, 7, 8, 9, or up to 10 linkers.

[0366] The CAR, cell tag, and/or cytokine of the present invention may be expressed as a fusion protein. In such embodiments, such components may be linked together using a self-cleaving peptide, for example a 2A peptide.

[0367] In certain embodiments, the self-cleaving peptide is a T2A peptide, or a functional fragment or variant thereof.

[0368] In certain embodiments, the self-cleaving peptide is a Furin-T2A peptide, or a functional fragment or variant thereof.

In certain such embodiments, the CAR, the cytokine, and the cell tag are expressed as a fusion

protein with the CAR and the cytokine linked by a self-cleaving linker, for example one comprising Furin-T2A, and the cytokine and cell tag linked by a self-cleaving linker, for example one comprising T2A.

B. Promoters

[0369] The polynucleotide of the invention can be present in the construct in operable linkage with a promoter. Appropriate promoters can be selected based on the host cell and effect sought. Suitable promoters include constitutive and inducible promoters. The promoters can be tissue specific, such promoters being well known in the art.

[0370] Examples of constitutive promoters for use in the present invention include immediate early cytomegalovirus (CMV) promoter; human elongation growth factor 1 alpha 1 (hEF1A1); simian virus 40 (SV40) early promoter; mouse mammary tumor virus (MMTV); human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter; MoMuLV promoter; avian leukemia virus promoter; Epstein-Barr virus immediate early promoter; Rous sarcoma virus promoter; and human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter; and functional fragments and variants thereof.

[0371] In certain embodiments, the promoter is a hEF1A1 promoter. A hEF1A1 promoter may comprise the sequence of SEQ ID NO: 577, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 577, or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 577. In certain embodiments, the promoter is a CMV promoter. A CMV promoter may comprise the sequence of SEQ ID NO: 578, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 578, or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 578.

[0372] In contrast to constitutive promoters, the use of an inducible promoter provides a molecular switch capable of turning on the 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 a RHEOSWITCH® gene switch.

VIII. Vectors and Delivery Systems

[0373] In certain embodiments, the polynucleotide of the present invention can be delivered to a target cell by any suitable delivery system, including non-viral and viral delivery systems. In some embodiments, a vector can include a polynucleotide of the present disclosure encoding the miRNA, CAR, cytokine, cell tag, or any combination thereof.

[0374] In certain cases, the miRNA(s), CAR, cytokine, and/or cell tag are expressed in separate vectors. In other aspects, the miRNA(s), CAR, cytokine, and/or cell tag are expressed from one single vector. In certain cases, the CAR and the miRNA(s) are expressed in separate vectors. In other aspects, the miRNA(s), CAR and cytokine are expressed from one single vector. In specific cases, the vectors can be lentiviral vectors, retroviral vectors, *Sleeping Beauty* transposons or vectors containing sequences for serine recombinase mediated integration. In some aspects, the vector is a plasmid, a mini-circle DNA or a nanoplasmid.

[0375] In certain embodiments, where the vector is a plasmid, mini-circle DNA or a nanoplasmid, the plasmid, mini-circle DNA or nanoplasmid can further include a bacterial origin of replication. In certain embodiments, the bacterial origin of replication can be from a ColE1 plasmid. In certain embodiments, the bacterial origin of replication comprises the sequence of SEQ ID NO: 579, or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 579, or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 579.

[0376] In order to assess the expression of one or more miRNA(s) and 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.

[0377] 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.

[0378] In some 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.

A. Methods for Introducing Nucleic Acids into Cells

[0379] 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.

1. Physical Methods

[0380] 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 some 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.

a. Electroporation (EP) Buffers

[0381] Various buffers can be used for electroporation. The buffers disclosed herein were found to have improved properties, including enhanced transfection capabilities, notwithstanding that these buffers comprise fewer components as compared to other known electroporation buffers.

[0382] Table 4 provides differing amounts of monobasic and dibasic phosphate used as buffering agents. Table 5 provides buffers 1-20 which contain buffering agents and glucose. Table Z provides buffers 21-37 which contain buffering agents and mannitol. Table 7 provides pH, conductivity, and osmolality for Buffers 1, 2 and 3 compared to a control buffer (Mirus Bio™ Ingenio™ electroporation solution, Catalog No. MIR-50117; Mirus Bio LLC, Madison, WI, USA) ("Control 1").

Table 4: Differing Amounts of Monobasic and Dibasic Phosphate Used as a Buffering Agent

0.2 M NaH_2PO_4 (mL)	0.2 M Na_2HPO_4 (mL)	pH
92.0	8.0	5.8
90.0	10.0	5.9
87.7	12.3	6.0
85.5	15.0	6.1

81.5	19.5	6.2
77.5	22.5	6.3
73.5	26.5	6.4
68.5	31.5	6.5
62.5	37.5	6.6
56.5	43.5	6.7
51.0	49.0	6.8
45.0	55.0	6.9
39.0	61.0	7.0
33.0	67.0	7.1
28.0	72.0	7.2
23.0	77.0	7.3
19.0	81.0	7.4
16.0	84.0	7.5
13.0	87.0	7.6
10.5	89.5	7.7
8.5	91.5	7.8

Table 5: Buffers 1 through 20 – Buffering Agents and Glucose

Sample No.	Glucose (mM)	HEPES (mM)	Na_2HPO_4/NaH_2PO_4 (mM)	KCl (mM)	$MgCl_2$ (mM)	DMSO (%)
1	30	5	105	10	20	0
2	31	0	90	5	15	0
3	30	10	90	5	15	0
4	25	10	120	15	25	0
5	30	25	50	2	10.5	5
6	0	5	160	10	10.5	0
7	0	5	160	2	20	5
8	15	25	160	10	20	5

9	30	5	160	2	1	2.5
10	15	15	105	6	10.5	2.5
11	30	25	50	10	1	0
12	30	5	50	6	20	5
13	30	15	160	10	1	5
14	15	5	50	2	1	0
15	0	5	50	10	1	5
16	0	25	50	10	20	2.5
17	30	25	160	2	20	0
18	0	15	50	2	20	0
19	0	25	160	6	1	0
20	0	25	105	2	1	5

Table 6: Buffers 21 through 37 – Buffering Agents and Mannitol

Sample No.	Mannitol (mM)	HEPES (mM)	Na_2HPO_4/NaH_2PO_4 (mM)	KCl (mM)	$MgCl_2$ (mM)	DMSO (%)
21	5	25	160	6	1	0
22	150	25	50	2	10.5	5
23	5	15	50	2	20	0
24	150	25	50	10	1	0
25	5	25	105	2	1	5
26	77.5	5	50	2	1	0
27	150	5	160	2	1	2.5
28	150	15	160	10	1	5
29	5	5	50	10	1	5
30	150	25	160	2	20	0
31	150	5	105	10	20	0
32	77.5	15	105	6	10.5	2.5
33	77.5	25	160	10	20	5

34	150	5	50	6	20	5
35	5	25	50	10	20	2.5
36	5	5	160	10	10.5	0
37	5	5	160	2	20	5

Table 7: Composition, pH, Conductivity, and Osmolality of Buffers 1, 2, and 3 Compared to Control 1

Sample No.	Glucose (mM)	HEPES (mM)	Na2HPO4/NaH2PO4 (mM)	KCl (mM)	MgCl2 (mM)	pH	Conductivity (ms/cm)	osm (mOsm/kgH20)
1	30	5	105	10	20	7.0	14.3	340
2	31	0	90	5	15	7.1	11.6	280
3	30	10	90	5	15	7.1	12.8	292
Control 1	X	X				7.3	16.9	575

[0383] In some embodiments, the buffer comprises a solvent, such as water. In some embodiments, the water may be purified and/or sterilized. For example, the water may be subjected to deionization (*e.g.*, capacitive deionization or electrodeionization), reverse osmosis, carbon filtering, microfiltration, ultrafiltration, and/or ultraviolet sterilization. In some embodiments, the water is deionized. In some embodiments, the water is of a quality designated as "water for injection"; also known as "sterile water for injection." Water for injection is generally made by distillation or reverse osmosis. Water for injection is a sterile, nonpyrogenic, solute-free preparation of water, chemically designated "H_2O," and having a pH of between about 5.0 and about 7.0, preferably about 5.5.

[0384] In some embodiments, the solvent comprises between 0.1% and 99.9% by volume of the total buffer volume. For example, the solvent may comprise at least about 0.1%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or 99.1% by volume of the total buffer volume.

[0385] In some embodiments, the buffer comprises a solute, for example a sugar or an organic compound derived from sugar, for example a sugar alcohol. In embodiments wherein the buffer comprises a sugar, the sugar may comprise a monosaccharide, a disaccharide, and/or a polysaccharide. In some embodiments, the sugar comprises a monosaccharide, for example glucose, fructose, and/or galactose. In some embodiments, the sugar comprises a disaccharide, for example sucrose, lactose, and maltose. In some embodiments, the sugar comprises a polysaccharide, for example cellulose or starch. In embodiments wherein the buffer comprises a sugar alcohol, the sugar alcohol may comprise mannitol, sorbitol, xylitol, lactitol, isomalt, maltitol, and/or hydrogenated starch hydrolysates (HSH).

[0386] In some embodiments, the sugar is present in an amount less than about 50 millimolar (mM). For example, the sugar may be present in an amount less than about 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, or 5 mM. In some embodiments, the sugar is present in an amount that ranges between about 10 mM and about 50 mM, about 10 mM and about 40 mM, about 10 mM and about 20 mM, or about 25 mM and about 35 mM. In some embodiments, the sugar is present in an amount of about 25mM, 26mM, 27mM, 28mM, 29mM, 30mM, 31mM, 32mM, 33mM, 34mM, or 35mM.

[0387] In some embodiments, the sugar is glucose. In these embodiments, the glucose may be present in an amount less than about 50 millimolar (mM). For example, the glucose may be present in an amount less than about 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, or 5 mM. In some embodiments, the glucose is present in an amount that ranges between about 10 mM and about 50 mM, about 10 mM and about 40 mM, about 10 mM and about 20 mM, or about 25 mM and about 35 mM. In some embodiments, the glucose is present in an amount of about 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, or 35 mM. In certain embodiments, the glucose is present in an amount of about 30 mM or 31 mM.

[0388] In some embodiments, the sugar is mannitol. In these embodiments, the mannitol may be present in an amount less than about 50 millimolar (mM). For example, the mannitol may be present in an amount less than about 45 mM, 40 mM, 35 mM, 30 mM, 25 mM, 20 mM, 15 mM, 10 mM, or 5 mM. In some embodiments, the mannitol is present in an amount that ranges between about 10 mM and about 50 mM, about 10 mM and about 40 mM, about 10 mM and about 20 mM,

or about 25 mM and about 35 mM. In some embodiments, the mannitol is present in an amount of about 25 mM, 26 mM, 27 mM, 28 mM, 29 mM, 30 mM, 31 mM, 32 mM, 33 mM, 34 mM, or 35 mM.

[0389] In some embodiments, the EP buffer comprises one or more chloride salts, for example potassium chloride (KCl) and/or magnesium chloride ($MgCl_2$). In some embodiments, the buffer further comprises one or more buffering agents, for example, Na_2HPO_4, NaH_2PO_4, or Na_2HPO_4/ NaH_2PO_4. In some embodiments, the buffer further comprises one or more of HEPES and/or DMSO. In other embodiments, the buffer specifically excludes one or more buffering agents commonly found in commercial electroporation (EP) buffers. For example, in some embodiments, the buffer excludes one or both of DMSO and/or HEPES.

[0390] In some embodiments, the buffer comprises water (H_2O), glucose, KCl, $MgCl_2$, and Na_2HPO_4/NaH_2PO_4. In some embodiments, the buffer comprises water (H_2O), glucose, KCl, $MgCl_2$, Na_2HPO_4/NaH_2PO_4, and HEPES. In other embodiments, the buffer comprises water (H_2O), glucose, KCl, $MgCl_2$, Na_2HPO_4/NaH_2PO_4, HEPES, and DMSO.

[0391] In some embodiments, the buffer consists essentially of water (H_2O), glucose, KCl, $MgCl_2$, and Na_2HPO_4/NaH_2PO_4. In some embodiments, the buffer consists essentially of water (H_2O), glucose, KCl, $MgCl_2$, Na_2HPO_4/NaH_2PO_4, and HEPES. In other embodiments, the buffer consists essentially of water (H_2O), glucose, KCl, $MgCl_2$, Na_2HPO_4/NaH_2PO_4, HEPES, and DMSO.

[0392] In some embodiments, the buffer consists of water (H_2O), glucose, KCl, $MgCl_2$, and Na_2HPO_4/NaH_2PO_4. In some embodiments, the buffer consists of water (H_2O), glucose, KCl, $MgCl_2$, Na_2HPO_4/NaH_2PO_4, and HEPES. In other embodiments, the buffer consists of water (H_2O), glucose, KCl, $MgCl_2$, Na_2HPO_4/NaH_2PO_4, HEPES, and DMSO.

[0393] In some embodiments, the buffering agent has a pH ranging from about 6.0 to 8.0, 6.5 to 8.0, 7.0 to 8.0, 7.5 to 8.0, 6.0 to 7.5, 6.0 to 7.0, 6.0 to 6.5, 6.5 to 7.5, or 6.5 to 7.0. In some embodiments, the buffering agent has a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7., 7.8, 7.9, or 8.0.

[0394] In some embodiments, the buffer comprising the one or more buffering agents has a pH

ranging from about 6.0 to 8.0, 6.5 to 8.0, 7.0 to 8.0, 7.5 to 8.0, 6.0 to 7.5, 6.0 to 7.0, 6.0 to 6.5, 6.5 to 7.5, or 6.5 to 7.0. In some embodiments, the buffer has a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7., 7.8, 7.9, or 8.0.

[0395] In some embodiments, the buffer comprises one or both of Na_2HPO_4 and/or NaH_2PO_4. In embodiments wherein the buffer comprises both buffering agents, the ratio of the two (i.e., Na_2HPO_4/NaH_2PO_4) may be about 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 9:1, 8:1, 7:1, 6:1 5:1, 4:1, 3:1, 2:1, or 2:3. In some embodiments, the ratio of Na_2HPO_4/NaH_2PO_4 has a pH of 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, or 7.9.

[0396] In some embodiments, a mixture of Na_2HPO_4 and NaH_2PO_4 (also referred to "Na_2HPO_4/NaH_2PO_4" or "sodium phosphate") may be present in the buffer in an amount ranging from about 50 mM and 160 mM, 60 mM to 150 mM, 70 mM to 140 mM, 75 mM to 130 mM, 80 mM to 125 mM, 90 mM to 125 mM, 90 mM to 120 mM, 90 mM to 115 mM, or 90 mM to 105 mM. In some embodiments, the Na_2HPO_4/NaH_2PO_4 is present in an amount of at least 50 mM, 60 mM, 70 mM, 80 mM, 90 mM, or 100 mM. In some embodiments, the Na_2HPO_4/NaH_2PO_4 is present in an amount of 80 mM, 81 mM, 82 mM, 83 mM, 84 mM, 85 mM, 86 mM, 87 mM, 88 mM, 89 mM, 90 mM, 91 mM, 92 mM, 93 mM, 94 mM, 95 mM, 96 mM, 97 mM, 98 mM, 99 mM, 100 mM, 101 mM, 102 mM, 103 mM, 104 mM, 105 mM, 106 mM, 107 mM, 108 mM, 109 mM, 110 mM, 111 mM, 112 mM, 113 mM, 114 mM, 115 mM, 116 mM, 117 mM, 118 mM, 119 mM, or 120 mM. In certain embodiments, the Na_2HPO_4/NaH_2PO_4 is present in an amount of 90 mM or 105 mM.

[0397] In embodiments wherein the buffer comprises KCl, the KCl may be present in an amount less than about 30 mM. For example, the KCl may be present in an amount less than about 25 mM, 20 mM, 15 mM, 10 mM, or 5 mM. In some embodiments, the KCl is present in an amount that ranges between about 1 mM and about 30 mM, about 2 mM and about 25 mM, about 3 mM and about 20 mM, about 4 mM and about 15 mM, about 5 mM and about 10 mM, or about 5 mM to about 15 mM. In some embodiments, the KCl is present in an amount of about 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, or 15mM. In certain embodiments, the KCl is present in an amount of about 5 mM or 10 mM. In some embodiments, the KCl has a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5,

7.6, 7.7, 7.8, or 7.9.

[0398] In embodiments wherein the buffer comprises $MgCl_2$, the $MgCl_2$ may be present in an amount less than about 50 mM. For example, the $MgCl_2$ may be present in an amount less than about 45 mM, 35 mM, 30 mM 25 mM, 20 mM, 15 mM, 10 mM, or 5 mM. In some embodiments, the $MgCl_2$ is present in an amount that ranges between about 5 mM and about 50 mM, about 6 mM and about 45 mM, about 7 mM and about 40 mM, about 8 mM and about 35 mM, about 9 mM and about 30 mM, about 10 mM and about 25 mM, or about 15 mM and about 25 mM. In some embodiments, the $MgCl_2$ is present in an amount of about 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, 25mM, 26 mM, 27 mM, 28 mM, 29 mM, or 30 mM. In certain embodiments, the $MgCl_2$ is present in an amount of about 15 mM or 20 mM. In some embodiments, the $MgCl_2$ has a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, or 7.9.

[0399] In embodiments wherein the buffer comprises HEPES, the HEPES may be present in an amount less than about 30 mM. For example, the HEPES may be present in an amount less than about 25 mM, 20 mM, 15 mM, 10 mM, 5 mM, 4 mM, 3 mM, 2 mM, 1 mM, 0.5 mM, or 0.1 mM. In some embodiments, the HEPES is present in an amount that ranges between about 1 mM and about 30 mM, about 2 mM and about 25 mM, about 3 mM and about 20 mM, about 4 mM and about 15 mM, about 5 mM and about 10 mM. In some embodiments, the HEPES is present in an amount of about 0.1 mM, 0.5 mM, 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, 11 mM, 12 mM, 13 mM, 14 mM, or 15mM. In certain embodiments, the HEPES is present in an amount of 0 mM, 5 mM, or 10 mM. In some embodiments, the HEPES has a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, or 7.9.

[0400] In embodiments wherein the buffer comprises DMSO, the DMSO may be present in an amount equal to or less than 5%, 4%, 3%, 2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% by volume of the total buffer volume. In some embodiments, DMSO is present from about 0% to about 2.5% by volume of the total buffer volume. In some embodiments, DMSO is present in an amount ranging from about 0.1% to 5%, 1% to 5 %, 2% to 5%, 3% to 5%, or 4% to 5% by volume of the total buffer volume. In some embodiments, the DMSO has a pH of about 6.5,

6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, or 7.9. In other embodiments, DMSO is not included in the buffer at all.

[0401] In certain embodiments, the buffer comprises a sugar in an amount equal to or less than 50 mM; HEPES in an amount equal to or less than 25 mM; Na_2HPO_4/NaH_2PO_4 in an amount equal to or less than 160 mM; KCl in an amount equal to or less than 10 mM; $MgCl_2$ in an amount equal to or less than 20 mM; and DMSO in an amount equal to or less than 5% by volume of the total buffer volume. In some of these embodiments, the sugar may comprise a monosaccharide and/or a sugar alcohol. In some of these embodiments, the sugar is mannitol and/or glucose. In some of these embodiments, the sugar is glucose. In some embodiments, the buffer does not comprise DMSO.

[0402] In certain embodiments, the buffer comprises a sugar in an amount of at least about 15 mM; HEPES in an amount equal to or less than 25 mM; Na_2HPO_4/NaH_2PO_4 in an amount of at least about 90 mM; KCl in an amount of at least about 2 mM; $MgCl_2$ in an amount of at least 15 mM; and DMSO in an amount equal to or less than 5% by volume of the total buffer volume. In some of these embodiments, the sugar may comprise a monosaccharide and/or a sugar alcohol. In some of these embodiments, the sugar is mannitol and/or glucose. In some of these embodiments, the sugar is glucose. In some embodiments, the buffer does not comprise DMSO.

[0403] In certain embodiments, the buffer comprises a sugar in an amount ranging from about 15 mM to about 35 mM; KCl in an amount ranging from about 5 mM to about 10 mM; $MgCl_2$ in an amount ranging from about 10.5 mM to about 20 mM; Na_2HPO_4/NaH_2PO_4 in an amount ranging from about 90 mM to about 105 mM; HEPES in an amount equal to or less than 25 mM; and DMSO in an amount equal to or less than 5% by volume of the total buffer volume. In some of these embodiments, the sugar may comprise a monosaccharide and/or a sugar alcohol. In some of these embodiments, the sugar is mannitol and/or glucose. In some of these embodiments, the sugar is glucose. In some embodiments, the buffer does not comprise DMSO.

[0404] In certain embodiments, the buffer comprises glucose in an amount of about 15 mM; KCl in an amount of about 6 mM; $MgCl_2$ in an amount of about 10.5 mM Na_2HPO_4/NaH_2PO_4 in an amount of about 105 mM; HEPES in an amount ranging from about 15 mM; and DMSO in an amount of about 2.5% by volume of total buffer volume.

[0405] In certain embodiments, the buffer comprises glucose in an amount of about 30 mM; KCl in an amount of about 10 mM; $MgCl_2$ in an amount of about 20 mM; Na_2HPO_4/NaH_2PO_4 in an amount of about 105 mM; and HEPES in an amount of about 5 mM. In some embodiments, DMSO is specifically excluded from the buffer.

[0406] In certain embodiments, the buffer comprises glucose in an amount of about 31 mM; KCl in an amount of about 5 mM; and $MgCl_2$ in an amount of about 15 mM; and Na_2HPO_4/NaH_2PO_4 in an amount of about 90 mM. In some embodiments, one or more of HEPES and DMSO is/are specifically excluded from the buffer.

[0407] In certain embodiments, the buffer comprises glucose in an amount of about 30 mM; KCl in an amount of about 5 mM; and $MgCl_2$ in an amount of about 15 mM; Na_2HPO_4/NaH_2PO_4 in an amount of about 90 mM; and HEPES in an amount of about 10 mM. In some embodiments, DMSO is specifically excluded from the buffer.

[0408] In certain embodiments, the buffer comprises glucose in an amount of about 25 mM; KCl in an amount of about 15 mM; and $MgCl_2$ in an amount of about 25 mM; Na_2HPO_4/NaH_2PO_4 in an amount of about 120 mM; and HEPES in an amount of about 10 mM. In some embodiments, DMSO is specifically excluded from the buffer.

[0409] In certain embodiments, the pH of the buffer may be adjusted. In some embodiments, the buffer is adjusted to a pH of between 6.5 and 8. In some embodiments, the buffer is adjusted to a pH between about 7.0 and 7.6. In some embodiments, the buffer is adjusted to a pH between about 6.9 and 7.2, or between about 7.0 and 7.1. In some embodiments, the buffer is adjusted to a pH of about 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, or 8.0. In certain embodiments, the buffer is adjusted to a pH of about 7.0 or 7.1.

[0410] In certain embodiments, the conductivity of the buffer is between about 7.0 ms/cm to about 16.0 ms/cm, about 9.0 ms/cm to about 16.0 ms/cm, about 11.0 ms/cm to about 16.0 ms/cm, or about 13.0 ms/cm to about 16.0 ms/cm. In some embodiments, the conductivity of the buffer is between about 7.0 ms/cm to about 15.0 ms/cm, about 9.0 ms/cm to about 15.0 ms/cm, about 11.0 ms/cm to about 15.0 ms/cm, or about 13.0 ms/cm to about 15.0 ms/cm. In some embodiments, the conductivity of the buffer is about 7.0 ms/cm, about 7.1 ms/cm, about 7.2 ms/cm, about 7.3 ms/cm,

about 7.4 ms/cm, about 7.5 ms/cm, about 7.6 ms/cm, about 7.7 ms/cm, about 7.8 ms/cm, about 7.9 ms/cm, about 8.0 ms/cm, about 8.1 ms/cm, about 8.2 ms/cm, about 8.3 ms/cm, about 8.4 ms/cm, about 8.5 ms/cm, about 8.6 ms/cm, about 8.7 ms/cm, about 8.8 ms/cm, about 8.9 ms/cm, about 9.0 ms/cm, about 9.1 ms/cm, about 9.2 ms/cm, about 9.3 ms/cm, about 9.4 ms/cm, about 9.5 ms/cm, about 9.6 ms/cm, about 9.7 ms/cm, about 9.8 ms/cm, about 9.9 ms/cm, about 10.0 ms/cm, about 10.1 ms/cm, about 10.2 ms/cm, about 10.3 ms/cm, about 10.4 ms/cm, about 10.5 ms/cm, about 10.6 ms/cm, about 10.7 ms/cm, about 10.8 ms/cm, about 10.9 ms/cm, about 11.0 ms/cm, about 11.1 ms/cm, about 11.2 ms/cm, about 11.3 ms/cm, about 11.4 ms/cm, about 11.5 ms/cm, about 11.6 ms/cm, about 11.7 ms/cm, about 11.8 ms/cm, about 11.9 ms/cm, about 12.0 ms/cm, about 12.1 ms/cm, about 12.2 ms/cm, about 12.3 ms/cm, about 12.4 ms/cm, about 12.5 ms/cm, about 12.6 ms/cm, about 12.7 ms/cm, about 12.8 ms/cm, about 12.9 ms/cm, about 13.0 ms/cm, about 13.1 ms/cm, about 13.2 ms/cm, about 13.3 ms/cm, about 13.4 ms/cm, about 13.5 ms/cm, about 13.6 ms/cm, about 13.7 ms/cm, about 13.8 ms/cm, about 13.9 ms/cm, about 14.0 ms/cm, about 14.1 ms/cm, about 14.2 ms/cm, about 14.3 ms/cm, about 14.4 ms/cm, about 14.5 ms/cm, about 14.6 ms/cm, about 14.7 ms/cm, about 14.8 ms/cm, about 14.9 ms/cm, about 15.0 ms/cm, about 15.1 ms/cm, about 15.2 ms/cm, about 15.3 ms/cm, about 15.4 ms/cm, about 15.5 ms/cm, about 15.6 ms/cm, about 15.7 ms/cm, about 15.8 ms/cm, about 15.9 ms/cm, or about 16.0 ms/cm. In certain embodiments, the conductivity of the buffer is about 11.6, 12.8, or 14.3.

[0411] In some embodiments, the osmolality of the buffer is lower than the osmolality of the cells being transfected (*i.e.*, also known as "intracellular osmolality"). In some embodiments, the osmolality of the buffer ranges from about 250 mOsm/kg H_2O to about 1255 mOsm/kg H_2O, about 250 mOsm/kg H_2O to about 1100 mOsm/kg H_2O, about 250 mOsm/kg H_2O to about 900 mOsm/kg H_2O, about 250 mOsm/kg H_2O to about 700 mOsm/kg H_2O, about 250 mOsm/kg H_2O to about 500 mOsm/kg H_2O, about 250 mOsm/kg H_2O to about 400 mOsm/kg H_2O, or about 250 mOsm/kg H_2O to about 360 mOsm/kg H_2O. In some embodiments, the osmolality is about 360 mOsm/kg H_2O to about 1255 mOsm/kg H_2O, about 360 mOsm/kg H_2O to about 1100 mOsm/kg H_2O, about 360 mOsm/kg H_2O to about 900 mOsm/kg H_2O, about 360 mOsm/kg H_2O to about 700 mOsm/kg H_2O, about 360 mOsm/kg H_2O to about 500 mOsm/kg H_2O, about 360 mOsm/kg H_2O to about 400 mOsm/kg H_2O. In some embodiments, the osmolality is about 250 mOsm/kg H_2O, 255 mOsm/kg H_2O, 260 mOsm/kg H_2O, 270 mOsm/kg H_2O, 275 mOsm/kg H_2O, about 280 mOsm/kg H_2O, about 285 mOsm/kg H_2O, about 290 mOsm/kg H_2O, about 300 mOsm/kg H_2O, about 305

mOsm/kg H_2O, about 310 mOsm/kg H_2O, about 315 mOsm/kg H_2O, about 320 mOsm/kg H_2O, about 325 mOsm/kg H_2O, about 330 mOsm/kg H_2O, about 335 mOsm/kg H_2O, about 340 mOsm/kg H_2O, about 345 mOsm/kg H_2O, about 350 mOsm/kg H_2O, about 355 mOsm/kg H_2O, about 360 mOsm/kg H_2O, about 365 mOsm/kg H_2O, about 370 mOsm/kg H_2O, about 375 mOsm/kg H_2O, about 380 mOsm/kg H_2O, about 385 mOsm/kg H_2O, about 390 mOsm/kg H_2O, about 395 mOsm/kg H_2O, or about 400 mOsm/kg H_2O. In certain embodiments, the osmolality is about 280 mOsm/kg H_2O, about 292 mOsm/kg H_2O, about 340 mOsm/kg H_2O, or about 362 mOsm/kg H_2O.

[0412] In some embodiments, the buffer is selected from one or more of the exemplary buffers set forth in Tables 5 and 6. In certain embodiments, the buffer is selected from Buffer 1, Buffer 2, or Buffer 3.

[0413] In some embodiments, the buffer of the invention is used in conjunction with an UltraPorator™ electroporation apparatus and cartridge (or, cassette); *see*, PCT/US20/59984 (filed Nov-11-2020) and U.S. Patent Application Serial No. 17/095,028 (filed Nov-11-2020). This apparatus is designed to enable rapid manufacturing for a range of gene and cell therapies. UltraPorator™ is a high-throughput, semi-closed electroporation system for electroporation of large quantities of cells in a single operation. The UltraPorator™ system is an advancement over current electroporation devices by significantly reducing the processing time and contamination risk. For example, UltraPorator may be utilized as a scale-up and commercialization solution for decentralized chimeric antigen receptor (CAR) T-cell manufacturing, such as in the UltraCAR-T™ manufacturing of T-cells reprogrammed to target cancer antigens *in vivo*.

[0414] Buffers of the invention are surprisingly effective in producing high cell transfection efficiencies when electroporation is performed using the buffers in the UltraPorator™ electroporation apparatus and/or cartridge (or, cassette); *see*, PCT/US20/59984 (filed Nov-11-2020) and U.S. Patent Application Serial No. 17/095,028.

b. Methods Utilizing the EP Buffer and Recombinant Cells Produced Using Those Methods

[0415] In another aspect of the invention, a method is provided that utilizes the buffer according

to the invention to introduce biologically active material (e.g., DNA or RNA) into cells via electric current (i.e., electroporation). The method comprises forming a suspension by combining cells obtained from a human along with an exogenous biological material into the buffer of the invention, and then applying an electric current in the form of a voltage pulse to the suspension, thereby facilitating the introduction of the biological material into the cells.

[0416] In certain embodiments, the voltage pulse may have a field strength of up to 1 to 10 kV*cm−1 and a duration of 5 to 250 µs and a current density of at least 2 A*cm−2. In certain embodiments, the voltage pulse permits the biologically active material (e.g., DNA) to be transfected directly into the cell nucleus of animal and human cells. In certain embodiments, a current flow following the voltage pulse without interruption, having a current density of 2 to 14 A*cm−2, preferably up to 5 A*cm−2, and a duration of 1 to 100 ms, may also be applied.

[0417] Using the method according to the invention, the transfection of biologically active material into cells, including into the nucleus of animal cells, may be optimized. In this case, the biologically active material (e.g., nucleic acids, polypeptides, or the like) can be introduced into quiescent or dividing animal cells with a high efficiency.

[0418] In some embodiments, the cells are exposed to the buffer for less than 10 minutes. For example, the cells may be exposed to the buffer for less than 9 minutes, less than 8 minutes, less than 7 minutes, less than 6 minutes, less than 5 minutes, less than 4 minutes, less than 3 minutes, less than 2 minutes, or less than 1 minute.

[0419] In some embodiments, the method is used to introduce biologically active material into primary human blood cells, pluripotent precursor cells of human blood, as well as primary human fibroblasts and endothelial cells. In some embodiments, the cells are human blood cells, for example immune cells. In certain embodiments, the immune cells are neutrophils, eosinophils, basophils, mast cells, monocytes, macrophages, dendritic cells, natural killer cells, and lymphocytes (B cells and T cells), or some combination thereof. In some embodiments, the lymphocytes are T-cells. In certain embodiments, the cells are obtained from a patient.

[0420] In some embodiments, the biological material includes a nucleic acid, peptide, polypeptide, protein, enzyme, RNP, or some combination thereof. In some embodiments, the

biological material is heterologous to the cells. In some embodiments, the biological material is partially or fully synthetic.

[0421] In some embodiments, the nucleic acid is selected from DNA or RNA. In some embodiments, the DNA may comprise cDNA. In some embodiments, the RNA may comprise mRNA, tRNA, rtRNA, lncRNA, sRNA, or a combination thereof. In some embodiments, the nucleic acid is a recombinant nucleic acid. In some embodiments, the peptide comprises a polypeptide, protein, enzyme, antibody, antibody fragment, or combination thereof. In some embodiments, the peptide is recombinant.

[0422] Methods utilizing the buffer of the invention result in desirably high transfection yields, especially as compared to methods utilizing other electroporation buffers. In some embodiments, the transfection yield with a buffer of the invention is at least about 1.1 times that of the transfection yield with a control (prior art) buffer. For example, the transfection yield with a buffer of the invention may be about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 2.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0 times higher than that of a control (prior art) buffer. In some embodiments, the transfection yield with a buffer of the invention may be greater than 5 times than that of a control (prior art) buffer, such as 6, 7, 8, 9, or 10 times higher. In certain embodiments, the transfection yield with a buffer of the invention is 1.35, 1.41, 1.46, 1.97, 1.98, 2.05, 2.12, 2.40, or 2.44 times higher than that of a control (prior art) buffer.

[0423] Methods utilizing the buffer of the invention result in desirably high transfected cell recovery yields, especially as compared to methods utilizing other electroporation buffers. In some embodiments, the transfected cell recovery yield with a buffer of the invention is at least about 1.1 times that of the transfected cell recovery yield with a control (prior art) buffer. For example, the transfected cell recovery yield with a buffer of the invention may be about 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 2.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, or 5.0 higher than that of a control (prior art) buffer. In some embodiments, the transfected cell recovery yield with a buffer of the invention may be greater than 5 times than that of a control (prior art) buffer. In certain embodiments, the transfected cell recovery yield with a buffer of the invention is 1.53, 1.66, 1.72, 1.80, 2.06, 2.17,

2.23, 2.34, or 2.61 times higher than that of a control (prior art) buffer.

[0424] In another aspect of the invention, recombinant cells are provided. In some embodiments, recombinant immune cells are produced using the method of the invention. In certain embodiments, the recombinant immune cell is a modified T-cell. In some embodiments, the modified T-cell is a chimeric antigen receptor (CAR) T-cell. In some embodiments, the CAR-T cell is administered to a patient for therapeutic purposes.

c. Electroporation Apparatuses and Their Methods of Use

[0425] An exemplary electroporation apparatus comprises: one or more chambers configured to store the buffer and cells during an electroporation process; one or more pairs of electrodes configured to generate electric fields within the one or more chambers during the electroporation process, each electric field corresponding to one chamber; and a flow channel configured to transport the cells during a cell collection process after the electroporation process.

[0426] In some embodiments, the apparatus comprises one chamber, two chambers, three chambers, four chambers, five chambers, six chambers, seven chambers, eight chambers, nine chambers, ten chambers, or ten or more chambers. In certain embodiments, the apparatus utilizes continuous flow or a microfluidic system.

[0427] In some embodiments, the electroporation apparatus further comprises a pump for pumping a liquid medium from the flow channel into at least one of the chambers during a collection process, wherein the liquid medium is obtained at the inlet port. In some embodiments, the pump comprises a valve or valves connecting the one or more chambers to the flow channel. In some embodiments, the valve or valves are opened one at a time. In some embodiments, the valve or valves permit only one-directional flow of fluid. In some embodiments, each valve corresponds to one chamber. In some embodiments, each valve corresponding to the chamber valves is a pinch-valve or pinch-type valve. In some embodiments, each of the valves operates using a spring motion, a lever motion, or a piston motion.

[0428] In some embodiments, the one or more chambers comprises a given chamber; each electrode of the pair of electrodes is located on opposite sides of the given chamber; and each electrode of the pair of electrodes comprises both an interior portion inside the given chamber and

an exterior portion external to the given chamber.

[0429] In some embodiments, the electroporation apparatus further comprises: an inlet port; an outlet port; and one or more flanking flow channels connecting the inlet port and the outlet port to the flow channel.

[0430] In some embodiments, the electroporation apparatus further comprises: a pump for pumping a liquid medium from the flow channel into at least one of the chambers during a collection process, wherein the liquid medium is obtained at the inlet port.

[0431] In some embodiments, the electroporation apparatus further comprises: a surface comprising a one or more openings leading to the one or more chambers; and an airflow channel below the openings and connecting airflow between the chambers.

[0432] In some embodiments, the electroporation apparatus further comprises: a vent or air filter connecting the airflow channel to an exterior of the electroporation apparatus.

[0433] In some embodiments, the electroporation apparatus further comprises: a seal configured to cover the one or more openings. In some embodiments, each chamber in the electroporation apparatus comprises a shape which narrows toward the respective valve(s). In some embodiments, the electroporation apparatus further comprises a pair of electrodes wherein each electrode of the electrode pair is located on opposite sides of each chamber. The distance between the two electrodes in an electrode pair is referred to as the "gap distance" or "separation distance." This distance spans the width of the chamber.

[0434] In some embodiments, each of the one or more chambers comprises a gap distance of about 0.1 mm to about 20 mm, about 0.5 mm to about 10 mm, about 1 mm to about 7 mm, or about 1 mm to about 4 mm. In some embodiments, the gap distance is about 0.5 mm, 1.0 mm, 1.5 mm, 2.0 mm, 2.5 mm, 3.0 mm, 3.5 mm, 4.0 mm, 4.5 mm, 5.0 mm, 5.5 mm, 6.0 mm, 6.5 mm, 7.0 mm, 7.5 mm, or 8.0 mm. In some embodiments, a gap distance of about 2.5 mm, 2.6 mm, 2.7 mm, 2.8 mm, 2.9 mm, 3.0 mm, 3.1 mm, 3.2 mm, 3.3 mm, 3.4 mm, 3.5 mm, 3.6 mm, 3.7 mm, 3.8 mm, 3.9 mm, or 4.0 mm. In some embodiments, the gap distance is less than about 4 mm, less than about 3.5 mm, less than about 3.0 mm, less than about 2.5 mm, less than about 2.0 mm, less than about 1.5 mm, or less than about 1.0 mm. In some embodiments, a gap distance of less than about 4.0

mm improves the electroporation performance of the buffer provided herein.

[0435] In some embodiments, each electrode of the pair of electrodes of the electroporation apparatus comprises: an interior portion inside the given chamber; and an exterior portion external to the given chamber, wherein each pair of electrodes is configured to connect to an electric circuit. In some embodiments, the interior portion inside the given chamber has an elliptical face and comprises a gold coating.

[0436] In some embodiments, each chamber of the electroporation apparatus is configured to store a volume of at least about 50 μL, at least about 100 μL, at least about 150 at least about μL, at least about 200 μL, at least about 250 μL, at least about 300 μL, at least about 350 μL, at least about 400 μL, at least about 450 μL, at least about 150 μL, at least about 500 μL, at least about 550 μL, at least about 600 μL, at least about 650 μL, at least about 700 μL, at least about 750 μL, at least about 800 μL, at least about 850 μL, at least about 900 μL, at least about 950 μL, or at least about 1000 μL (1.0 mL).

[0437] In some embodiments, the chambers of the electroporation apparatus, in combination, are configured to store at least about 500 μL, at least about 1.0 mL, at least about 1.2 mL, at least about 1.4 mL, at least about 1.6 mL, at least about 1.8 mL, at least about 2.0 mL, at least about 2.2 mL, at least about 2.4 mL, at least about 2.6 mL, at least about 2.8 mL, at least about 3.0 mL, at least about 3.2 mL, at least about 3.4 mL, at least about 3.6 mL, at least about 3.8 mL, at least about 4.0 mL, at least about 4.2 mL, at least about 4.4 mL, at least about 4.6 mL, at least about 4.8 mL, at least about 5.0 mL, at least about 5.2 mL, at least about 5.4 mL, at least about 5.6 mL, at least about 5.8 mL, at least about 6.0 mL, at least about 6.2 mL, at least about 6.4 mL, at least about 6.6 mL, at least about 6.8 mL, or at least about 7.0 mL of cells in liquid suspension for electroporation.

[0438] In some embodiments, the cells involved in the electroporation process comprises a population selected from a group consisting of: at least $1x10^8$ cells, at least $2x10^8$ cells, at least $3x10^8$ cells, at least $4x10^8$ cells, at least $5x10^8$ cells, at least $6x10^8$ cells, at least $7x10^8$ cells, at least $8x10^8$ cells, at least $9x10^8$ cells, at least $1x10^9$ cells, at least $2x10^9$ cells, at least $3x10^9$ cells, at least $4x10^9$ cells, at least $5x10^9$ cells, at least $6x10^9$ cells, at least $7x10^9$ cells, at least $8x10^9$ cells, at least $9x10^9$ cells, at least $1x10^{10}$ cells, at least $2x10^{10}$ cells, at least $3x10^{10}$ cells, at least $4x10^{10}$ cells, at

least $5x10^{10}$ cells, at least $6x10^{10}$ cells, at least $7x10^{10}$ cells, at least $8x10^{10}$ cells, at least $9x10^{10}$ cells, at least $1x10^{11}$ cells, at least $2x10^{11}$ cells, at least $3x10^{11}$ cells, at least $4x10^{11}$ cells, at least $5x10^{11}$ cells, at least $6x10^{11}$ cells, at least $7x10^{11}$ cells, at least $8x10^{11}$ cells, at least $9x10^{11}$ cells, at least $1x10^{12}$ cells, at least $2x10^{12}$ cells, at least $3x10^{12}$ cells, at least $4x10^{12}$ cells, at least $5x10^{12}$ cells, at least $6x10^{12}$ cells, at least $7x10^{12}$ cells, at least $8x10^{12}$ cells, and at least $9x10^{12}$.

[0439] In some embodiments, the apparatus of the invention comprises an UltraPorator™ electroporation apparatus and cartridge (*see*, PCT/US20/59984 and U.S. Pat. App. Ser. No. 17/095,028). As noted above, the UltraPorator™ electroporation apparatus is designed to enable rapid manufacturing for a range of gene and cell therapies. The apparatus may be utilized as a scale-up and commercialization solution for decentralized CAR T-cell manufacturing, such as in the UltraCAR-T™ manufacturing of T-cells reprogrammed to target cancer antigens *in vivo*.

[0440] In some embodiments, the apparatus of the invention is used in a method of electroporation, the method comprising: executing an electroporation process by generating an electric field within a chamber using a pair of electrodes, wherein the chamber is configured to store the buffer and cells during the electroporation process; and executing a cell collection process by: opening a valve connected to the chamber; and transporting the buffer and cells to an outlet port using a flow channel connected to the valve, wherein the chamber, the electrode pair, the valve, the outlet port, and the flow channel are each located within an electroporation apparatus.

[0441] In some embodiments, the step of executing a cell collection process further comprises: pumping, through use of a pump, a liquid medium from the flow channel into the chamber, wherein the liquid medium is obtained at an inlet port, and wherein the inlet port and the outlet port are connected to the flow channel by a flanking flow channel within the electroporation apparatus.

[0442] In some embodiments, the cell collection process further comprises: draining the chamber into the flow channel, wherein pressure within the chamber is maintained via a vent or air filter connected to an air flow channel running between the chamber and another chamber.

[0443] In some embodiments, the method of electroporation further comprises: depositing the cells into an opening leading to the chamber holding the buffer; applying a seal to the opening; and connecting the electrode pair to at least one circuit by, for example, inserting the

electroporation apparatus into a docking station.

[0444] In some embodiments, the method utilizes one or more of the exemplary buffers set forth in Tables 5 and 6. In certain embodiments, the method utilizes Buffer 1, Buffer 2, or Buffer 3.

[0445] In some embodiments, the method is performed in an UltraPorator™ electroporation apparatus (*see*, PCT/US20/59984 and U.S. Pat. App. Ser. No. 17/095,028). In certain embodiments, the method is performed in an UltraPorator™ electroporation apparatus and utilizes one or more of the exemplary buffers set forth in Tables 5 and 6. In certain embodiments, the method is performed in an UltraPorator™ electroporation apparatus and utilizes Buffer 1, Buffer 2, or Buffer 3 (as set forth in Table 5).

d. Electroporation Systems

[0446] In another aspect of the invention, a system for electroporation is provided. In some embodiments, the system for electroporation comprises an electroporation apparatus, as described herein, and an electroporation buffer, as described herein. In some embodiments, the electroporation system comprises and UltraPorator™ electroporation apparatus and cartridge (*see*, PCT/US20/59984 and U.S. Pat. App. Ser. No. 17/095,028). As noted above, the UltraPorator™ electroporation apparatus is designed to enable rapid manufacturing for a range of gene and cell therapies. The device may be utilized as a scale-up and commercialization solution for decentralized CAR T-cell manufacturing, such as in the UltraCAR-T™ manufacturing of T-cells reprogrammed to target cancer antigens *in vivo*.

[0447] In some embodiments, the system for electroporation further comprises a buffer is from one or more of the exemplary buffers set forth in Tables 5 and 6. In certain embodiments, the system for electroporation comprises a buffer selected from Buffer 1, Buffer 2, or Buffer 3. It has been found that systems comprising an UltraPorator™ device and one of Buffers 1, 2, or 3 result in surprisingly high cell transfection efficiencies, as compared to systems comprising an UltraPorator™ device and a control buffer.

e. Kits for Electroporation

[0448] In another aspect of the invention, a kit for electroporation is provided. The kit may

include any of the buffers as described herein. In some embodiments, the kit comprises one or more of the exemplary buffers set forth in Tables 5 and 6. In certain embodiments, the kit comprises a buffer selected from Buffer 1, Buffer 2, or Buffer 3.

[0449] In some embodiments, the kit includes one or more containers filled with a buffer according to the invention and other suitable reagents and/or devices. For example, the kit may additionally comprise a vector comprising a nucleic acid of interest. In some embodiments, the kit may include a dropper, pipette, and/or cuvette. In some embodiments, the buffer may be packaged in aliquoted containers or as a stock solution.

In some embodiments, the kit further comprises packaging to safely transport the buffer and any additional reagents and/or devices. In some embodiments, the kit includes information about the contents of the buffer and any additional reagents. Further, the kit may comprise written materials, for example a user manual or answers to frequently asked questions.

2. Chemical Methods

[0450] Chemical methods 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).

3. Viral Vectors and Delivery Methods

[0451] 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.

[0452] 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.

[0453] 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)), adeno-associated virus based vectors, lentivirus-based vectors (*e.g.*, the lentiviral-based pLPI from Life Technologies (Carlsbad, Calif.)), retroviral vectors (*e.g.*,

the pFB-ERV plus pCFB-EGSH), and herpes virus-based vectors. 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).

[0454] In some embodiments, a lentiviral vector is provided comprising a backbone and a nucleic acid sequence encoding one or more miRNA(s) and a CAR. Optionally, the vector further comprises a nucleic acid encoding a cytokine and a cell tag such as truncated HER1, CD20t-1 or a full length CD20.

[0455] In some embodiments, the nucleic acid encoding one or more miRNA(s) and a 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 some embodiments, the vector encoding one or more miRNA(s) and a CAR also encodes a cytokine in a single construct. In some embodiments, the one or more miRNA(s) and a CAR and cytokine are encoded on two separate lentiviral vectors. In some embodiments, the cytokine is expressed with a cell tag. In some embodiments, one or more miRNA(s) and a CAR can be co-expressed with the cytokine and the cell tag from a single lentiviral vector. In further embodiments, one or more miRNA(s) and a CAR can be under the control of an inducible promoter. In another embodiment, the cytokine 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.

[0456] 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^{TM}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^{TM} 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.

[0457] In some embodiments, the cell tag gene is cloned into a lentiviral plasmid backbone in frame with the CAR gene. In other embodiments, the cell tag is cloned into a separate lentiviral vector.

4. Non-Viral Vectors and Delivery Systems

a. Sleeping Beauty Transposon System

[0458] A polynucleotide encoding one or more miRNA(s) alone or a polynucleotide encoding one or more miRNA(s) and a chimeric receptor, cytokine, and/or cell tag as described herein can be introduced into immune effector cells using non-viral based delivery systems, such as the "*Sleeping Beauty* (SB) Transposon System," which refers to a synthetic DNA transposon system for introducing DNA sequences into the chromosomes of vertebrates. An exemplary *Sleeping*

Beauty transposon system is described for example, in U.S. Patent Nos. 6,489,458 and 8,227,432. As used herein, the *Sleeping Beauty* transposon system can comprise *Sleeping Beauty* transposase polypeptides as well as derivatives, functional fragments, and variants thereof, and *Sleeping Beauty* transposon polynucleotides, derivatives, and functional variants and fragments thereof. In certain embodiments, the *Sleeping Beauty* transposase is encoded by an mRNA. In some embodiments, the mRNA encodes for a SB10, SB11, SB100x or SB110 transposase. In some embodiments, the mRNA comprises a cap and a poly-A tail.

[0459] 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. *Sleeping Beauty* transposons envisaged to be used as non-viral vectors for introduction of genes into genomes of vertebrate animals and for gene therapy.

[0460] Briefly, the *Sleeping Beauty* 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 involves two steps: (i) the electro-transfer of DNA plasmids expressing a *Sleeping Beauty* transposon (Jin et al., Gene Ther 18:849-56, (2011); Kebriaei et al., Hum Gene Ther 23:444-50, (2012)) and *Sleeping Beauty* 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.

[0461] In one embodiment, the *Sleeping Beauty* 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).

[0462] In some embodiments, one or more miRNA(s), a CAR, a cytokine, and a cell tag can be encoded by a single transposon DNA plasmid vector. In some embodiments, the one or more miRNA(s), CAR, cytokine, and cell tag can be encoded by different transposon DNA plasmid vectors. In further embodiments, one or more miRNA(s) and a CAR can be under the control of an inducible promoter. In another embodiment, the cytokine 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 further described below. In certain embodiments, the CAR, cytokine, and cell tag can be configured in one, two or more transposons.

[0463] In some embodiments, the MUC16, CD33, or ROR1-specific 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 Nos. 6,489,458; 8,227,432, 9,228,180, Wilson et al, "PiggyBac Transposon-mediated Gene Transfer in Human Cells," Molecular Therapy 15:139-145 (2007) and WO 2016/145146) 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).

[0464] In certain embodiments, the vector is a *Sleeping Beauty* plasmid that comprises a left transposon repeat region and a right transposon repeat region. In certain embodiments, the left transposon repeat region comprises a nucleic acid comprising SEQ ID NO: 580 or a functional

fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 580, or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 580. In certain embodiments, the right transposon repeat region comprises a nucleic acid comprising SEQ ID NO: 581 or a functional fragment or variant thereof. In certain embodiments, the functional variant has at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity with SEQ ID NO: 581, or hybridizes under stringent hybridization conditions with the complement of SEQ ID NO: 581.

b. Recombinase-Based Delivery Systems

[0465] In other embodiments, nucleic acids encoding one or more miRNA(s), a CAR, a cytokine, and/or a cell 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.

[0466] 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).

[0467] 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).

[0468] 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.

[0469] 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.)

[0470] 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.

[0471] 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

[0472] 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.

c. Other Non-Viral Delivery Systems

[0473] In other embodiments, nucleic acids encoding one or more miRNA(s), a CAR, a cytokine, and/or a cell tag, can be integrated into the immune effector cell's DNA through gene editing systems that utilize CRISPR, TALEN or Zinc-Finger nucleases.

[0474] 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.

IX. Engineered T-Cell Receptor (TCR)

[0475] In some embodiments, the chimeric receptor comprises an engineered T-cell receptor. The T cell receptor (TCR) is composed of two chains (αβ or γδ) that pair on the surface of the T cell to form a heterodimeric receptor. In some instances, the αβ TCR is expressed on most T cells

in the body and is known to be involved in the recognition of specific MHC-restricted antigens. Each α and β chain are composed of two domains: a constant domain (C) that anchors the protein to the cell membrane and is associated with invariant subunits of the CD3 signaling apparatus; and a variable domain (V) that confers antigen recognition through six loops, referred to as complementarity determining regions (CDRs). In some instances, each of the V domains comprises three CDRs; *e.g.*, CDR1, CDR2 and CDR3 with CDR3 as the hypervariable region. These CDRs interact with a complex formed between an antigenic peptide bound to a protein encoded by the major histocompatibility complex (pepMHC) (*e.g.*, HLA-A, HLA-B, HLA-C, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1, HLA-DRA, or HLA-DRB1 complex). In some instances, the constant domain further comprises a joining region that connects the constant domain to the variable domain. In some cases, the beta chain further comprises a short diversity region which makes up part of the joining region.

[0476] Both the α and β chains are highly variable, although the T-cell receptor α chain contains a constant (preserved region), *i.e.*, the Va24-Ja18 junction (amino acid sequence GSTLGR or a conservatively substituted amino acid sequence thereof).

[0477] In some cases, such TCR are reactive to specific tumor antigen, e.g. NY-ESO, Mage A3, Titin, MART-1, HPV, HBV, MAGE-A4, MAGE-A10, MAGE A3/A6, gp100, MAGE-A1, or PRAME. In other cases, such TCR are reactive to specific neoantigens expressed within a patient's tumor (i.e. patient-specific, somatic, non-synonymous mutations expressed by tumors). In some cases, engineered TCRs can be affinity-enhanced.

[0478] In some embodiments, a TCR is described using the International Immunogenetics (IMGT) TCR nomenclature, and links to the IMGT public database of TCR sequences. For example, there can be several types of alpha chain variable (Vα) regions and several types of beta chain variable (Vβ) regions distinguished by their framework, CDR1, CDR2, and CDR3 sequences. As such, a Vα type can be referred to in IMGT nomenclature by a unique TRAV number. For example, "TRAV21" defines a TCR Vα region having unique framework and CDR1 and CDR2 sequences, and a CDR3 sequence, which is partly defined by an amino acid sequence which is preserved from TCR to TCR but which also includes an amino acid sequence which varies from TCR to TCR. Similarly, "TRBV5-1" defines a TCR Vβ region having unique framework and

CDR1 and CDR2 sequences, but with only a partly defined CDR3 sequence.

[0479] In some cases, the beta chain diversity region is referred to in IMGT nomenclature by the abbreviation TRBD.

[0480] In some instances, the unique sequences defined by the IMGT nomenclature are widely known and accessible to those working in the TCR field. For example, they can be found in the IMGT public database and in "T cell Receptor Factsbook", (2001) LeFranc and LeFranc, Academic Press, ISBN 0-12-441352-8.

[0481] In some embodiments, an αβ heterodimeric TCR is, for example, transfected as full length chains having both cytoplasmic and transmembrane domains. In some cases, the TCRs contain an introduced disulfide bond between residues of the respective constant domains, as described, for example, in WO 2006/000830.

[0482] In some instances, TCRs described herein are in single chain format, for example see WO 2004/033685. Single chain formats include αβ TCR polypeptides of the Vα-L-Vβ, Vβ-L-Vα, Vα-Cα-L-Vβ, Vα-L-Vβ-Cβ, Vα-Cα-L-Vβ-Cβ types, wherein Vα and Vβ are TCR α and β variable regions respectively, Cα and Cβ are TCR α and β constant regions respectively, and L is a linker sequence. In certain embodiments single chain TCRs of the invention may have an introduced disulfide bond between residues of the respective constant domains, as described in WO 2004/033685.

[0483] In some embodiments, the TCR can be an αβ TCR. In some embodiments, the TCR can be a γδ TCR. It should be understood that a TCR of the present disclosure can bind to any of the antigen targets described herein.

[0484] In some embodiments, the TCR may be any one of the Vδ1, Vδ2, and Vδ1negVδ2neg TCR subsets. In some embodiments, the engineered cell may express any combination of a Vδ1, Vδ2, Vδ3, Vδ5, Vδ7, or Vδ8 TCR chain with a Vγ2, Vγ3, Vγ7, Vγ8, Vγ9, Vγ10, or Vγ11 TCR chain. In some embodiments, the engineered cell may have essentially identical genetic material. In one aspect, the engineered cell may not contain a chimeric antigen receptor.

[0485] The TCR described herein may be associated with a detectable label, a therapeutic agent

or a PK modifying moiety. Exemplary detectable labels for diagnostic purposes include, but are not limited to, fluorescent labels, radiolabels, enzymes, nucleic acid probes and contrast reagents.

[0486] In some cases, each chain of TCR disclosed herein, for example αβ or γδ, comprises a modified spacer region connecting the constant region of a TCR chain to the transmembrane region. In some cases, a spacer region of each chain of TCR disclosed herein comprises 1) a stalk region and 1 or more stalk extension region(s) adjacent to said stalk region. The stalk and stalk extension regions may, for example, be those as previously described for use in chimeric antigen receptors. In some embodiments, each chain of TCR disclosed herein incorporates a spacer that comprises a stalk region (s) and up to 20 stalk extension regions.

[0487] In some instances, the stalk region comprises the extracellular hinge region from TCRα or TCRβ chain or the stalk region comprises a sequence with at least 80% homology to the extracellular hinge region from TCRα or TCRβ chain. In alternative instances, the stalk region comprises any portion of extracellular region of TCRα or TCRβ constant region with at least 80% homology to the extracellular region of TCRα or TCRβ constant region respectively. For example, the stalk region can comprise a sequence with at least 80%, 85%, 90%, 95%, or greater than 95% homology to the any portion of extracellular region of TCRα or TCRβ constant region.

[0488] TCR chain heterodimers are formed by inter-chain disulfide bonds in extracellular hinge region of α and β chains. In some embodiments, the stalk region comprises a dimerization site. A dimerization site can comprise a disulfide bond formation site. A dimerization site can comprise cysteine residue(s). A stalk region can be capable of forming a disulfide bond. Such a disulfide bond can be formed at a disulfide bond forming site or a dimerization site. In some examples, the dimerization occurs between α and β chains of TCR.

[0489] In some embodiments, a stalk extension region is used to link the stalk region to the transmembrane region TCR α and β chains. In certain embodiments, a stalk extension region is used to link the stalk region to constant region of TCR α and β chains. In certain embodiments, the stalk region and the stalk extension region(s) can be connected via a linker.

[0490] In some instances, the stalk extension domain comprises a sequence that is partially homologous to the stalk region. In some instances, each of the stalk extension region comprises a

sequence that is homologous to the stalk region, except that the stalk extension region lacks the dimerization site of the stalk region. In some cases, each of the stalk extension region comprises a sequence identical to the stalk region. In other cases, each of the stalk extension regions comprise a sequence identical to the stalk region with at least one amino acid residue substitution relative to the stalk region. In some cases, each of the stalk extension region is not capable of forming a disulfide bond or is not capable of dimerization with a homologous stalk extension region.

[0491] In other embodiments, one stalk extension region can be connected to another stalk extension region via a linker. Examples of such linkers can include glycine-serine rich linkers.

[0492] In some embodiments, the addition of stalk extension region(s) prevents mispairing of transgenic TCR α and β chains with native TCR α and β chains expressed by T cells that are genetically modified.

[0493] In some embodiments, a modified immune effector cell of the present disclosure can comprise a TCR of the present disclosure and a cytokine of the present disclosure. In certain embodiments, the modified immune effector cell can comprise a TCR and a fusion protein comprising IL-15 and IL-15Rα, or a fusion protein comprising functional fragments or variants of such domains.

X. Immune Checkpoint Inhibitors

[0494] In some embodiments, an engineered cell of the present disclosure can include an immune checkpoint inhibitor. In certain embodiments, a polynucleotide of the present disclosure can further encode an immune checkpoint inhibitor. In certain embodiments, an engineered cell of the present disclosure can comprise such a polynucleotide.

[0495] In certain embodiments, the immune checkpoint inhibitor can be an antibody or a functional fragment or variant thereof. In certain embodiments, the immune checkpoint inhibitor can inhibit the activity of an immune checkpoint protein such as PD1, PD-L1, CTLA-4, TIGIT, 4-1BB, PIK3IP1, CD27, CD28, CD40, CD70, CD122, CD137, OX40 (CD134), GITR, ICOS, A2AR, B7-H3 (CD276), B7-H4 (VTCN1), BTLA, IDO, KIR, LAG3, TIM-3, or VISTA.

[0496] In some embodiments, the immune checkpoint inhibitor can be an anti-CTLA-4

antibody. The anti-CTLA-4 antibody (e.g., ipilimumab) has shown durable anti-tumor activities and prolonged survival in participants with advanced melanoma, resulting in its Food and Drug Administration (FDA) approval in 2011. See Hodi et al., Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. (2010) Aug 19; 363(8):711-23. In some embodiments, the one or more checkpoint inhibitors can be an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody can be a full-length atezolizumab (anti-PD-L1), avelumab (anti-PD-L1), durvalumab (anti-PD-L1), or a fragment or a variant thereof. In some embodiments, the one or more checkpoint inhibitors can be any one or more of CD27 inhibitor, CD28 inhibitor, CD40 inhibitor, CD122 inhibitor, CD137 inhibitor, OX40 (also known as CD134) inhibitor, GITR inhibitor, ICOS inhibitor, or any combination thereof. In some embodiments, the one or more checkpoint inhibitors can be any one or more of A2AR inhibitor, B7-H3 (also known as CD276) inhibitor, B7-H4 (also known as VTCN1) inhibitor, BTLA inhibitor, IDO inhibitor, KIR inhibitor, LAG3 inhibitor, TIM-3 inhibitor, VISTA inhibitor, or any combination thereof.

[0497] In some embodiments, the immune checkpoint inhibitor is an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-CD28 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, an anti-TIM3 antibody, an anti-GITR antibody, an anti-4-1BB antibody, or an anti-OX-40 antibody. In some embodiments, the additional therapeutic agent is an anti-TIGIT antibody. In some embodiments, the immune checkpoint inhibitor is an anti-PD-L1 antibody selected from the group consisting of: BMS935559 (MDX-1105), atexolizumab (MPDL3280A), durvalumab (MEDI4736), and avelumab (MSB0010718C). In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody selected from the group consisting of: ipilimumab (YERVOY) and tremelimumab. In some embodiments, the additional therapeutic agent is an anti-LAG-3 antibody selected from the group consisting of: BMS-986016 and LAG525. In some embodiments, the immune checkpoint inhibitor is an anti-OX-40 antibody selected from the group consisting of: MEDI6469, MEDI0562, and MOXR0916. In some embodiments, the additional therapeutic agent is an anti-4-1BB antibody selected from the group consisting of: PF-05082566.

[0498] In some embodiments, the engineered cell can include an immune checkpoint inhibitor comprising a PD1-binding moiety. In some embodiments, the PD1-binding moiety (referred to herein as an "anti-PD-1") is selected from an antibody identified in Table 8 (below), or a functional fragment or variant thereof.

Table 8: PD-1 Antibodies

Name	Also Known as	Company	Reference(s)
cemiplimab	Libtayo, cemiplimab, REGN2810	Regeneron, Sanofi	WO 2015/112800
pembrolizumab	Keytruda, MK-3475, SCH 900475, lambrolizumab	Merck (MSD), Schering-Plough	WO 2008/156712, U.S. 8,354,509, U.S. 8,952,136, U.S. 8,900,587
nivolumab	Opdivo, ONO-4538, MDX-1106, BMS-936558, 5C4	BMS, Medarex, Ono	U.S. 8,728,474, U.S. 8,779,105, U.S. 8,008,449, U.S. 9,067,999, US 9,073,994
toripalimab	JS001, JS-001, TAB001, triprizumab	Junmeng Biosciences, Shanghai Junshi, TopAlliance Bio	Si-Yang Liu et al., *J. Hematol. Oncol.* 10:136 (2017)
sintilimab	Tyvyt, IBI308	Adimab, Innovent, Lilly	WO 2017/024465, WO 2017/025016, WO 2017/132825, WO 2017/133540
LY3434172	-	Lilly, Zymeworks	ClinicalTrials.gov Identifier: NCT03936959
JTX-4014	-	Jounce Therapeutics Inc.	U.S. 2018/0118829; ClinicalTrials.gov Identifier: NCT03790488; Papdopolous et al., *Cancer Immunol Immunother* 70(3):763–772 (2021))
609 A	609A	3S Bio; Sunshine Guojian Pharma	ClinicalTrials.gov Identifier: NCT03998345

Sym021	-	Symphogen A/S	Gjetting et al., *mAbs* 11(4): 666-680 (2019
LZM009	-	Livzon Pharmaceutical Group	ClinicalTrials.gov Identifier: NCT03286296
budigalimab	ABBV-181, PR-1648817	Abbvie	Powderly et al., *Annals of Oncology* 29(8) (2018); ClinicalTrials.gov Identifier: NCT03000257
IB	IBI-318	Innovent, Lilly	ClinicalTrials.gov Identifier: NCT03875157
SCT-I10A	-	Sinocelltech Ltd.	ClinicalTrials.gov Identifier: NCT03821363
SG001	-	CSPC ZhongQi Pharmaceutical Technology Co., Ltd.	ClinicalTrials.gov Identifier: NCT03852823
AMP-224	GSK-2661380	Astra Zeneca, Glaxo Smith Kline	Floudas et al., *Clin. Colorectal Cancer* 18(4) (2019)
AMG 404	AMG404	Amgen	ClinicalTrials.gov Identifier: NCT03853109
AK112	-	Akesobio Australia Pty Ltd	ClinicalTrials.gov Identifier: NCT04047290
CS1003	-	CStone Pharma	Li et al., *Acta Pharmacologica Sinica* 42: 142–148 (2021)
MEDI0680	AMP-514	Astra Zeneca, Amplimmune, Medimmune	WO 2012/145493, WO 2014/194293
RO7121661	-	Roche	ClinicalTrials.gov Identifier: NCT03708328

F520	-	Shandong New Time Pharmaceutical Co.	ClinicalTrials.gov Identifier: NCT03657381
sasanlimab	PF-06801591, RN-888	Pfizer	Cho et al., *Annals of Oncology* 30(5) (2019)
BI 754091	BI754091	Boehringer Ingelheim	Kang et al., *J. Clin. Oncology* 38(15) (2020)
cetrelimab	JNJ-63723283	Janssen Biotech	Rutkowski et al., *J. Clin. Oncology 37:8* (2019)
HerinCAR-PD-1	-	Ningbo Cancer Hospital	ClinicalTrials.gov Identifier: NCT02873390
HX008	-	Taizhou Hanzhong Bio	Zhang et al, *mAbs* 12(1) (2020); ClinicalTrials.gov Identifier: NCT03704246
zimberelimab	WBP3055, GLS-010, AB122	Arcus, Guangzhou Gloria Bio, Harbin Gloria Pharma, WuXi Biologics	US 2019/0270815, Si-Yang Liu et al., *J. Hematol. Oncol.* 10:136 (2017)
retifanlimab	MGA012, INCMGA00012	Incyte, MacroGenics	WO 2017/19846
balstilimab	AGEN2034, AGEN-2034	Agenus, Ludwig Inst., Sloan-Kettering	WO 2017/040790
pidilizumab	CT-011, hBat-1, MDV9300	CureTech, Medivation, Teva	Rosenblatt et al., *J Immunother.* 34(5): 409–418 (2011)
teripalimab	-	Henan Cancer Hospital	ClinicalTrials.gov Identifier: NCT03985670
CBT-501	GB226, GB 226, Genolimzumab, Genormab	CBT Pharmaceuticals, Genor	ClinicalTrials.gov Identifier: NCT03053466.
BAT1306	-	Bio-Thera Solutions	Wu et al., *J. Clin. Oncol.*

			37(4) (2019)
tislelizumab	BGB-A317	BeiGene, Celgene	WO 2015/35606, US 2015/0079109
AK105	-	Akeso, HanX Bio	ClinicalTrials.gov Identifier: NCT03866967
spartalizumab	PDR001, BAP049	Dana-Farber, Novartis	WO 2015/112900; Lin et al., *Ann. of Oncology*, 29:8 (2018)
prolgolimab	BCD-100	Biocad	Kaplon et al., *mAbs* 10(2):183-203 (2018)
serplulimab	HLX10	Henlix Biotech	ClinicalTrials.gov Identifier: NCT04297995
dostarlimab	ANB011, TSR-042, ABT1, WBP-285	AnaptysBio, Tesaro	WO 2014/179664
camrelizumab	SHR-1210	Incyte, Jiangsu Hengrui, Shanghai Hengrui	WO 2015/085847; Si-Yang Liu et al., *J. Hematol. Oncol.* 10:136 (2017)
IBI319	IBI-319	Innovent Biologics (Suzhou) Co. Ltd., Lilly	ClinicalTrials.gov Identifier: NCT04708210
KY1043	-	Kymab	Van Krinks, Kymab poster no. P625, "KY1043, a novel PD-L1 IL-2 immunocytokine directed towards CD25, delivers potent anti-tumour activity *in vitro* and *in vivo*"
STI-1110	-	Sorrento Therapeutics	WO 2014/194302
CA05100948	Antibody 948	UCB Biopharma	U.S. 8,993,731

Nb97	MY2935, MY2626	Fudan University, Novamab	Xian et al., *Biochem. & Biophys. Res. Comm's* 519(3), 267-273 (2019)
ENUM 388D4	-	Enumeral	Scheuplein et al., *Immunology*, Abstract 4871 (2016)
hAb-10D3	hAb-10D3	BPS Bioscience	U.S. 10,759,859
Unknown	-	Isis Innovation	WO 2010/029434
ANB030	-	AnaptysBio Inc.	Grebinoski et al., *Current Opinion in Immunol.* 67: 1-9 (2020)
MCLA-134	-	Merus N.V.	WO 2019/009727
hAb21	-	Suzhou Stainwei Biotech Inc.	U.S. 2020/0277376
-	-	Ampsource	U.S. 2019/0367617
-	-	Reyoung (Suzhou) Biology Science & Technology Co.	U.S. 2019/0071501
-	-	Xencor, Inc.	WO 2018/071918
-	-	Crown Bioscience Inc.	WO 2016/014688
-	-	Beijing Hanmi	U.S. 2020/0299412
-	-	Beijing Hanmi	U.S. 2021/0032343
-	-	Beijing Hanmi	U.S. 2019/0367615
-	-	MabQuest	WO 2016/020856
-	-	Fuso Pharma, Hokkaido U.	WO 2018/034226
-	-	Eureka Therapeutics, Inc., Sloan-Kettering	WO 2016/210129
-	-	Sutro Biopharma, Inc.	WO 2016/077397
-	-	Y-Biologics	U.S. 2019/0248900

-	-	Harbour Biomed Ltd.	WO 2017/016497
-	-	Shanghai PharmaExplorer Co.	Liu et al., *Sci. Rep* 9(1) (2019)
-	-	Bio X Cell	Grasselly et al., *Front Immunol* 9:2100 (2018)
-	-	Tongji University	Cai et al., *Invest. New Drugs* 37(5):799-809 (2019)
-	-	Janssen Biotech	U.S. 2018/0355061
-	-	Shanghai Henlius Biotech, Inc.	U.S. 2019/0218295
-	-	Ultrahuman Nine Ltd.	WO 2019/170898
-	-	CytomX	WO 2017/011580
-	-	Nanjing Legend Biotech	WO 2017/133633
-	-	Aduro Biotech	U.S. 10,494,436
-	-	Sutro Biopharm	U.S. 10,822,414
-	-	Versitech Ltd.	U.S. 10,047,137
-	-	STCube & Co., University of Texas	WO 2016/160792
-	-	Tayu Huaxia Biotech Medivcal Group Co.	U.S. 2019/0144543
-	-	Hangzhou Sumgen Biotechnology	U.S. 2019/0016799
-	-	Jiangsu Hengrui Medicine Co, Shanghai Hengrui Pharmaceutical Co	WO 2015/085847
-	-	Salubris (Chengdu)	WO 2019/201169

		Biotech Co.	
-	-	Kadmon Corp.	U.S. 10,407,502
-	-	Augusta University Research Institute	WO 2019/051164
-	-	Abbvie Biotherapeutics Inc.	WO 2018/053106
-	-	Lyvgen Biopharma Holdings Ltd.	WO 2017/087599
-	-	Crescendo Biologics Ltd.	WO 2018/127711
-	-	Zhejiang Teruisi Pharmaceutics Inc.	U.S. 2019/0322747
-	-	Biosion Inc.	WO 2019/204132
-	-	Celgene Corp.	U.S. 2017/0088618
-	-	Asia Biotech Pte. Ltd.	WO 2017/058115
-	-	The Brigham and Women's Hospital	U.S. 10,934,352
-	-	Singapore Immunology Network (SIgN), Institute of Medical Biology, Agency for Science, Technology and Research (A*STAR)	Seidel et al., *Front. Oncol.* 28 (2018)
-	-	Chinese People Liberation Army General Hospital	Sun et al., *J Gastrointest Oncol*. 11(6): 1421–1430 (2020)
-	-	Immunomedics Inc.	U.S. 10,669,338
-	-	Xiangtan Tenghua Bio	WO 2018/162944

-	-	Beijing Dongfang Biotech	U.S. 2019/0144541
-	-	REMD Biotherapeutics	WO 2018/119474
-	-	Rinat Neuroscience Corp.	U.S. 10,155,037
-	-	Medimmune, Wyeth	WO 2004/056875
-	-	Fudan University	Yuan et al., *Invest. New Drugs* 39(1): 34-51 (2021)

XI. Gene Switch

[0499] 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.

[0500] In some embodiments, the polynucleotide or an additional polynucleotide encodes polypeptides for a gene switch system for ligand-inducible control of heterologous gene expression, wherein the gene switch polypeptides include: (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.

[0501] In some embodiments, the gene switch system comprises: (a) a first gene switch polypeptide comprising a transactivation domain; (b) a second gene switch polypeptide comprising

a DNA binding domain fused to a ligand binding domain; and (c) at least one heterologous polypeptide; wherein one of said first gene switch polypeptide, said second gene switch polypeptide and said heterologous polypeptide is connected by a linker to another one of said first gene switch polypeptide, said second gene switch polypeptide and said heterologous polypeptide, and wherein said polypeptide linker comprises a cleavable linker or ribosome skipping linker sequence.

[0502] The heterologous polypeptide may, for example, be a chimeric receptor (*e.g.*, a CAR), a cell tag, or a cytokine as described herein.

[0503] In certain embodiments, the gene switch system is of the type described in WO 2018/132494.

[0504] In some 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 other embodiments, the DNA binding domain comprises the amino acid sequence of SEQ ID NO: 638 or a functional fragment or variant thereof. 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: 632 or a functional fragment or variant thereof. 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 farnesol receptor, or a functional fragment or variant thereof. In some embodiments, at least one of the first nuclear receptor ligand binding domain, the second nuclear receptor ligand binding domain, and the ligand binding domain is derived from the Ecdysone Receptor polypeptide sequence of SEQ ID NOs: 640 or 642 or a functional fragment or variant thereof. In some embodiments, the nuclear receptor ligand binding domain is a RxR domain of SEQ ID NO: 634 or a functional fragment or variant thereof.

[0505] In some embodiments, the polynucleotide encodes 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, or a functional fragment or variant thereof. In other embodiments, the DNA binding domain is encoded by a nucleotide sequence as shown in SEQ ID NO: 639 or a functional fragment or variant thereof. In some embodiments, the polynucleotide encodes at least one of a VP16 transactivation domain and a B42 acidic activator transactivation domain. In other cases, the transactivation domain is encoded by a nucleotide sequence as shown in SEQ ID NO: 633 or a functional fragment or variant thereof. In some embodiments, at least one of the first nuclear receptor ligand binding domain, the second nuclear receptor ligand binding domain, and the ligand binding domain is encoded by SEQ ID NO: 641 or 643 or a functional fragment or variant thereof. In some embodiments, at least one of the first nuclear receptor ligand binding domain, the second nuclear receptor ligand binding domain, and the ligand binding domain is encoded by SEQ ID NO: 635 or a functional fragment or variant thereof.

[0506] In yet another embodiment, the first gene switch polypeptide comprises a GAL4 DBD, or a functional fragment or variant thereof, fused to an EcR nuclear receptor ligand binding domain, or a functional fragment or variant thereof, and the second gene switch polypeptide comprises a VP16 transactivation domain, or a functional fragment or variant thereof, fused to a retinoid receptor X (RXR) nuclear receptor ligand binding domain, or a functional fragment or variant thereof. 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: 531), SGSG linker (SEQ ID NO: 533), furinlink variants and derivatives thereof.

[0507] 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: 640 or 642 or a functional fragment or variant thereof. In some embodiments, the first gene switch polypeptide comprises a GAL4 DBD, or a functional fragment or variant thereof, fused to an EcR nuclear receptor ligand binding domain, or a functional fragment or variant thereof, and the second gene switch polypeptide comprises a VP16 transactivation domain, or a functional fragment or variant thereof, fused to a retinoid receptor X (RXR) nuclear receptor ligand binding domain, or a functional fragment or variant thereof. In some

embodiments, the Gal4 DBD, or a functional fragment or variant thereof, fused to the EcR nuclear receptor ligand binding domain, or a functional fragment or variant thereof, comprises an amino acid sequence as shown in SEQ ID NOs: 644 or 646, or a functional fragment or variant thereof, and the VP16 transactivation domain, or a functional fragment or variant thereof, fused to the retinoid receptor X (RXR) nuclear receptor ligand binding domain, or a functional fragment or variant thereof, comprises an amino acid sequence as shown in SEQ ID NO: 636, or a functional fragment or variant thereof.

[0508] In some cases, at least one of the first nuclear receptor ligand binding domain and the second nuclear receptor ligand binding domain are encoded by the amino acid sequences as shown in SEQ ID NOs: 641 or 643, or a functional fragment or variant thereof. In some embodiments, the Gal4 DBD, or a functional fragment or variant thereof, fused to the EcR nuclear receptor ligand binding domain, or a functional fragment or variant thereof, is encoded by the nucleotide sequence as shown in SEQ ID NOs: 645 or 647, or a functional fragment or variant thereof, and the VP16 transactivation domain, or a functional fragment or variant thereof, fused to the retinoid receptor X (RXR) nuclear receptor ligand binding domain, or a functional fragment or variant thereof, is encoded by the nucleotide sequence of SEQ ID NO: 637, or a functional fragment or variant thereof.

[0509] In any of the foregoing gene switch embodiments, the linker can be a cleavable linker, a ribosome skipping linker sequence or an IRES linker. In some cases, the linker is an IRES linker and is encoded by a nucleic acid comprising the sequence of SEQ ID NOs: 702 or 703 or a functional fragment or variant thereof. In other cases, the linker is a cleavable linker or ribosome skipping linker sequence. In some embodiments, 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, SGSG linker, furinlink linker variants and derivatives thereof. In other embodiments, the cleavable linker or said ribosome skipping linker sequence has a sequence as shown in any one of SEQ ID NOs: 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557 and 559 or is encoded by the sequence as shown in any one of SEQ ID NOs: 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, and 560.

[0510] In any of the gene switch embodiments, expression of at least one of the first gene switch

polypeptide, the second gene switch polypeptide, the antigen-binding polypeptide, and the heterologous polypeptide of any of the compositions as provided herein can be modulated by a promoter, where the promoter is a tissue-specific promoter or an EF1A promoter or functional fragment or variant thereof. In some cases, the promoter comprises the sequence of SEQ ID NOs: 58 and 59 of WO 2018/132494, or a functional fragment or variant thereof. In other cases, the promoter is a tissue-specific promoter comprising a T-cell-specific response element. In another case, the tissue-specific promoter comprises one or more NFAT response element(s). In yet another case, the NFAT response element has a sequence of any one of SEQ ID NOs: 51 to 57 of WO 2018/132494 or a functional fragment or variant thereof.

[0511] In an embodiment, expression of the at least one heterologous polypeptide is modified by an inducible promoter. In some embodiments, the inducible promoter has a sequence of any one of SEQ ID NOs: 40 to 64 of WO 2018/132494 or a functional fragment or variant thereof. In other embodiments, the inducible promoter is modulated by at least one of the first gene switch polypeptide and the second gene switch polypeptide.

[0512] It should be understood that any of the foregoing polynucleotides can be included in a vector as described herein.

[0513] Also provided herein is a method of regulating the expression of a heterologous gene in an effector cell, the method comprising: (a) introducing into the effector cell one or more polynucleotides encoding the polypeptides of the first and second gene switch polypeptides as described herein and the heterologous polypeptide; and (b) contacting the effector cell with a ligand in an amount sufficient to induce expression of the gene encoding the heterologous polypeptide.

[0514] In certain embodiments, the ligand in the method of regulating the expression of the heterologous gene in the effector cell as provided herein comprises at least one of: (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.

[0515] In some cases, the expression of the gene encoding the polypeptide in the effector cell as provided herein is reduced or eliminated in the absence of the ligand, as compared to the expression in the presence of the ligand. In certain cases, the expression of said heterologous polypeptide is restored by providing additional amounts of the ligand.

[0516] In some embodiments, the one or more expression cassettes of the gene switch system further comprise one or more of the following: (a) one or more recombinase attachment sites; and (b) a sequence encoding a serine recombinase. In other embodiments, the one or more expression

cassettes further comprise one or more of the following: (a) a non-inducible promoter; and (b) an inducible promoter.

[0517] In some embodiments, one of the first and second gene switch polypeptides can be connected to the heterologous polypeptide by a linker.

[0518] In some embodiments, the first and second gene switch polypeptides are connected by a polypeptide linker that is an IRES linker.

[0519] In some embodiments, the expression cassette can further comprise a second gene encoding a second heterologous polypeptide.

[0520] In some embodiments, the gene switch system as provided is for integrating a heterologous gene in a host cell, wherein upon contacting the host cell with the one or more expression cassettes in the presence of the serine recombinase and the one or more recombinase attachment sites, the heterologous gene is integrated in the host cell. In certain embodiments, the gene switch system further comprises a ligand, wherein the heterologous gene is expressed in the host cell upon contact of the host cell by the ligand. In certain embodiments, the one or more recombinase attachment sites can comprise a phage genomic recombination attachment site (attP) or a bacterial genomic recombination attachment site (attB). In some cases, the serine recombinase can be SF370.

[0521] In some cases, the expression cassette has the sequence of any one of SEQ ID NOs: 131 to 126 of WO 2018/132494 or a functional fragment or variant thereof.

[0522] In certain embodiments, the inducible promoter of the gene switch system can be activated by the transactivation domain. It should be understood that the gene switch system can be included in a single vector or in multiple vectors.

[0523] 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.

[0524] 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.

[0525] U.S. Patent 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. Patent 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.

[0526] 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. Patent 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 antibody with the TGF-β cytokine trap, 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.

[0527] 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.

[0528] 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).

[0529] 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/US52002/005235 (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); WO 2018/132494 (PCT/US2018/013196); and, U.S. Pat. No. 9,402,919.

[0530] As used herein, the term "ligand," as applied to ligand-activated ecdysone receptor-based gene switches are small molecules of varying solubility (such as diacylhydrazine compounds) having the capability of activating a gene switch to stimulate gene expression (*i.e.*, therein providing ligand inducible expression of polynucleotides (*e.g.*, mRNAs, miRNAs, *etc*) and/or polypeptides). Examples of such ligands include, but are not limited to those described in:WO 2004/072254 (PCT/US2004/003775); WO 2004/005478 (PCT/US2003/021149); WO 2005/017126 (PCT/US2004/005149); WO 2004/078924 (PCT/US2004/005912); WO

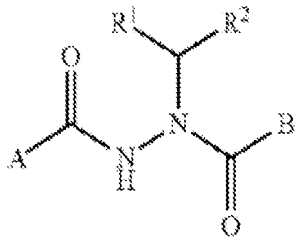
2008/153801 (PCT/US2008/006757); WO 2009/114201 (PCT/US2009/001639); WO 2013/036758 (PCT/US2012/054141); WO 2014/144380 (PCT/US2014/028768); and, WO 2016/044390 (PCT/US2015/050375).

[0531] Examples of ligands also include, without limitation, 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.: 6,258,603; 6,013,836; 5,117,057; 5,530,028; 5,378,726; 7,304,161; 7,851,220; 8,748,125; 9,272,986; 7,456,315; 7,563,928; 8,524,948; 9,102,648; 9,169,210; 9,255,273; and, 9,359,289; oxadiazolines as described in U.S. Patent Nos.: 8,669,072; and, 8,895,306; dibenzoylalkyl cyanohydrazines such as those disclosed in European Application No. 2,461,809; 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; amidoketones such as those described in U.S. Patent Nos.: 7,375,093; 8,129,355; and, 9,802,936; 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, famesol, bile acids, 1,1-biphosphonate esters, juvenile hormone III, and the like. Examples of diacylhydrazine ligands useful in the present invention include RG-115819 (3,5-Dimethyl-benzoic acid N-(1-ethyl-2,2-dimethyl-propyl)-N′-(2-methyl-3-methoxybenzoyl)-hydrazide), RG-115932 ((R)-3,5-Dimethyl-benzoic acid N-(1-tert-butyl-butyl)N′-(2-ethyl-3-methoxy-benzoyl)-hydrazide), and RG-115830 (3,5-Dimethyl-benzoic acid N-(1-tert-butyl-butyl)-N′-(2-ethyl-3-methoxy-benzoyl)-hydrazide). *See, e.g.*, WO 2008/153801 (PCT/US2008/006757); and, WO 2013/036758 (PCT/US2012/054141).

[0532] For example, a ligand for the ecdysone receptor-based gene switch may be selected from any suitable ligands. Both naturally occurring ecdysone or ecdysone analogs (e.g., 20-hydroxyecdysone, muristerone A, ponasterone A, ponasterone B, ponasterone C, 26-iodoponasterone A, inokosterone or 26-mesylinokosterone) and non-steroid inducers may be used as a ligand for gene switch of the present invention. U.S. Pat. No. 6,379,945, describes an insect steroid receptor isolated from Heliothis virescens ("HEcR") which is capable of acting as a gene

switch responsive to both steroid and certain non-steroidal inducers. Non-steroidal inducers have a distinct advantage over steroids, in this and many other systems which are responsive to both steroids and non-steroid inducers, for several reasons including, for example: lower manufacturing cost, metabolic stability, absence from insects, plants, or mammals, and environmental acceptability. U.S. Pat. No. 6,379,945 describes the utility of two dibenzoylhydrazines, 1,2-dibenzoyl-1-tert-butyl-hydrazine and tebufenozide (N-(4-ethylbenzoyl)-N′-(3,5-dimethylbenzoyl)-N′-tert-butyl-hydrazine) as ligands for an ecdysone-based gene switch. Also included in the present invention as a ligand are other dibenzoylhydrazines, such as those disclosed in U.S. Pat. No. 5,117,057. Use of tebufenozide as a chemical ligand for the ecdysone receptor from Drosophila melanogaster is also disclosed in U.S. Pat. No. 6,147,282. Additional, non-limiting examples of ecdysone ligands are 3,5-di-tert-butyl-4-hydroxy-N-isobutyl-benzamide, 8-O-acetylharpagide, a 1,2-diacyl hydrazine, an N′-substituted-N,N′-disubstituted hydrazine, a dibenzoylalkyl cyanohydrazine, an N-substituted-N-alkyl-N,N-diaroyl hydrazine, an N-substituted-N-acyl-N-alkyl, carbonyl hydrazine or an N-aroyl-N‘-alkylN’-aroyl hydrazine. (See U.S. Pat. No. 6,723,531).

[0533] In one embodiment, the ligand for an ecdysone-based gene switch system is a diacylhydrazine ligand or chiral diacylhydrazine ligand. The ligand used in the gene switch system may be compounds of Formula I

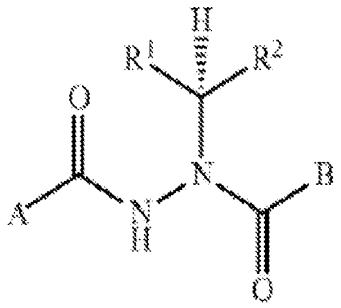


Formula I

wherein A is alkoxy, arylalkyloxy or aryloxy; B is optionally substituted aryl or optionally substituted heteroaryl; and R1 and R2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or

optionally substituted heteroaryl; or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

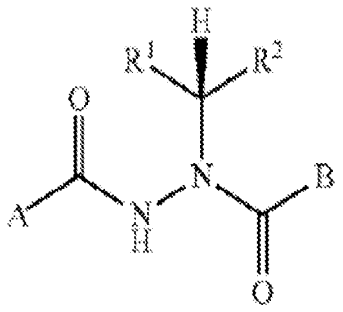
[0534] In another embodiment, the ligand may be enantiomerically enriched compounds of Formula II



Formula II

[0535] wherein A is alkoxy, arylalkyloxy, aryloxy, arylalkyl, optionally substituted aryl or optionally substituted heteroaryl; B is optionally substituted aryl or optionally substituted heteroaryl; and R1 and R2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl; with the proviso that R1 does not equal R2; wherein the absolute configuration at the asymmetric carbon atom bearing R1 and R2 is predominantly S; or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

[0536] In certain embodiments, the ligand may be enantiomerically enriched compounds of Formula III



Formula III

[0537] wherein A is alkoxy, arylalkyloxy, aryloxy, arylalkyl, optionally substituted aryl or optionally substituted heteroaryl; B is optionally substituted aryl or optionally substituted heteroaryl; and R1 and R2 are independently optionally substituted alkyl, arylalkyl, hydroxyalkyl, haloalkyl, optionally substituted cycloalkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heterocyclo, optionally substituted aryl or optionally substituted heteroaryl; with the proviso that R1 does not equal R2; wherein the absolute configuration at the asymmetric carbon atom bearing R1 and R2 is predominantly R; or pharmaceutically acceptable salts, hydrates, crystalline forms or amorphous forms thereof.

[0538] In one embodiment, a ligand may be (R)-3,5-dimethyl-benzoic acid N-(1-tertbutyl-butyl)-N′-(2-ethyl-3-methoxy-benzoyl)-hydrazide having an enantiomeric excess of at least 95% or a pharmaceutically acceptable salt, hydrate, crystalline form or amorphous form thereof.

[0539] The diacylhydrazine ligands of Formula I and chiral diacylhydrazine ligands of Formula II or III, when used with an ecdysone-based gene switch system, provide the means for external temporal regulation of expression of a therapeutic polypeptide or therapeutic polynucleotide of the present invention. See U.S. Patent Nos.: 8,076,517; 8,884,060; and, 9,598,355.

[0540] The ligands used in the present invention may form salts. The term "salt(s)" as used herein denotes acidic and/or basic salts formed with inorganic and/or organic acids and bases. In addition, when a compound of Formula I, II or III contains both a basic moiety and an acidic moiety, zwitterions ("inner salts") may be formed and are included within the term "salt(s)" as used herein. Pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts are

used, although other salts are also useful, e.g., in isolation or purification steps which may be employed during preparation. Salts of the compounds of Formula I, II or III may be formed, for example, by reacting a compound with an amount of acid or base, such as an equivalent amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

[0541] The ligands which contain a basic moiety may form salts with a variety of organic and inorganic acids. Exemplary acid addition salts include acetates (such as those formed with acetic acid or trihaloacetic acid, for example, trifluoroacetic acid), adipates, alginates, ascorbates, aspartates, benzoates, benzenesulfonates, bisulfates, borates, butyrates, citrates, camphorates, camphorsulfonates, cyclopentanepropionates, digluconates, dodecylsulfates, ethanesulfonates, fumarates, glucoheptanoates, glycerophosphates, hemisulfates, heptanoates, hexanoates, hydrochlorides (formed with hydrochloric acid), hydrobromides (formed with hydrogen bromide), hydroiodides, 2-hydroxyethanesulfonates, lactates, maleates (formed with maleic acid), methanesulfonates (formed with methanesulfonic acid), 2-naphthalenesulfonates, nicotinates, nitrates, oxalates, pectinates, persulfates, 3-phenylpropionates, phosphates, picrates, pivalates, propionates, salicylates, succinates, sulfates (such as those formed with sulfuric acid), sulfonates (such as those mentioned herein), tartrates, thiocyanates, toluenesulfonates such as tosylates, undecanoates, and the like.

[0542] The ligands which contain an acidic moiety may form salts with a variety of organic and inorganic bases. Exemplary basic salts include ammonium salts, alkali metal salts such as sodium, lithium, and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, salts with organic bases (for example, organic amines) such as benzathines, dicyclohexylamines, hydrabamines (formed with N,N-bis(dehydroabietyl)ethylenediamine), N-methyl-D-glucamines, N-methyl-D-glucamides, t-butyl amines, and salts with amino acids such as arginine, lysine and the like.

[0543] Non-limiting examples of the ligands for the inducible gene expression system also includes those utilizing the FK506 binding domain are FK506, Cyclosporin A, or Rapamycin. FK506, rapamycin, and their analogs are disclosed in U.S. Pat. Nos.: 6,649,595; 6,187,757; 7,276,498; and, 7,273,874.

[0544] In some embodiments, a diacylhydrazine ligand for inducible gene expression is administered at unit daily dose of about 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100 or 120 mg. In some embodiments, the diacylhydrazine ligand is administered at a unit daily dose of about 5 mg. In some embodiments, the diacylhydrazine ligand is administered at a unit daily dose of about 10 mg. In some embodiments, the diacylhydrazine ligand is administered at a unit daily dose of about 15 mg. In some embodiments, the diacylhydrazine ligand is administered daily at a unit daily dose of about 20 mg.

[0545] In some embodiments, the cytokine, cell tag and/or CAR 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 cases, the gene switch system used may be the one described in WO 2018/132494.

[0546] 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. In some cases, the gene switch system used may be the one described in WO 2018/132494.

[0547] In some embodiments, the modified immune effector cells as described herein 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 as described in WO2018/132494.

XII. Modified Immune Effector Cells

[0548] Provided herein are modified immune effector cells expressing one or more miRNAs, CARs, cytokines, and/or cell tags as described herein. In some embodiments, modified immune effector cells are modified T cells and/or natural killer (NK) 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. In certain aspects, the embodiments described herein include making and/or expanding the modified immune effector cells (*e.g.*, T-cells, Tregs, NK-cell or NK T-cells). Such may be accomplished by transfecting the cells with an expression vector containing a DNA (or RNA) construct encoding the one or more miRNAs, CARs, cytokines, and/or cell tags as described herein. It should be understood that the cells of the present disclosure can be human or animal cells.

[0549] 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. T helper 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.

[0550] 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.

[0551] 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.

[0552] 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.

[0553] 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 and Tc 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.

[0554] 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.

A. Immune Effector Cell Sources

[0555] In certain aspects, the embodiments described herein include methods of making and/or expanding the modified (antigen-specific redirected) immune effector cells (*e.g.*, T-cells, Tregs, NK-cell or NK T-cells) that comprise 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, CD34+ cord blood cells, iPS cell, T cell differentiated from iPS cell, immune effector cell or a precursor of these cells.

[0556] 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, cells 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. In some embodiments, the genetically modified cells are not cryopreserved.

[0557] 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 some 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.

[0558] 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.

[0559] 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.

[0560] 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.

[0561] 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.

[0562] 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.

[0563] 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.

[0564] 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.

[0565] 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.

B. Activation and Expansion of Immune Effector Cells

[0566] Whether prior to or after engineering of the immune effector cells to express a CAR described herein, the 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.

[0567] Generally, the immune effector 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 cells. In particular, 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 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)).

[0568] In certain embodiments, the primary stimulatory signal and the co-stimulatory signal for

the immune effector 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.

[0569] 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 some 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.

[0570] 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.

[0571] 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.

[0572] 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.

[0573] 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 may 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).

[0574] *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.

[0575] The procedure for *ex vivo* expansion of hematopoietic stem and progenitor cells is described in U.S. Pat. No. 5,199,942, 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 effector 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.

[0576] Effector 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.

[0577] 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.

[0578] 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. 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.

[0579] 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 MUC16, CD33, or ROR1 (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 have been tested 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).

[0580] 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 some embodiments, the population of CAR-T cells is cultured and/or stimulated for at least 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30 or more days. In some embodiments, the population of CAR-T cells is cultured and/or stimulated for at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more days. In some embodiments, the population of CAR expressing effector cells is cultured and/or stimulated for at least 7, 14, 21, 28, 35, 42, 49, 56, 63 or more 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.

[0581] 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 WO2014/190273. 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.

[0582] 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.

[0583] 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.

[0584] 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.

[0585] 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.

[0586] 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.

[0587] 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. For example, the target antigen can be an infectious disease antigen or a tumor-associated antigen (TAA).

C. Rapid Manufacturing

[0588] In one embodiment of the present disclosure, the immune effector cells described herein are modified at a point-of-care site. In one embodiment of the present disclosure, the immune effector cells described herein are modified at or near a point-of-care site. In some cases, modified immune effector cells are also referred to as engineered T cells. In some cases, the facility or treatment site is at a hospital, at a facility (*e.g.*, a medical facility), or at a treatment site 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. In one aspect, the elutriation step is eliminated completely.

[0589] In some cases, the immune effector cells are modified by engineering/introducing a one or more miRNA(s), 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 CAR. In another case, the cytokine can be IL-15 (for example, as part of a fusion protein with IL-15Rα) or IL-12. In yet another case, expression of cytokine 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 the cytokine.

[0590] 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 every 48 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.

[0591] 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.

[0592] In some embodiments, such immune effectors cells are modified by the constructs as described herein 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.

[0593] 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 some cases, the modified immune effector cells are place in PBS/EDTA buffer. In certain cases, the modified immune effector cells are placed in a buffer that includes IL-2 and IL-21 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.

[0594] 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.

[0595] 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.

[0596] In some embodiments, the subject is not lymphodepleted prior to infusion.

[0597] In one embodiment, the immune effector cells are modified by engineering/introducing one or more miRNA(s), 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 one or more miRNA(s), 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 one or more miRNA(s), 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.

[0598] 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 one or more miRNA(s), 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 one or more miRNA(s), 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.

[0599] 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 one or more miRNA(s), 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 one or more miRNA(s), 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.

[0600] 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 one or more miRNA(s), 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 one or more miRNA(s), 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.

XIII. Kits and Compositions

[0601] One aspect of the disclosure relates to kits and compositions that comprise: a CAR, a cytokine, a cell tag, and/or one or more miRNAs as described previously, or nucleic acids encoding the same. 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. In other

embodiments, the vectors can comprise sequences for serine recombinase-mediated integration.

[0602] 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 some embodiments, the kit includes IL-15, for example as part of a fusion protein with IL-15Rα.

[0603] 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.

[0604] 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.

[0605] For example, the container(s) include CAR-T cells (*e.g.*, MUC16-, CD33-, and ROR1-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.

[0606] 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.

[0607] 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.

[0608] The present invention relates also to pharmaceutical compositions comprising a modified immune effector cell as described above. In certain embodimetns, the composition comprising an immune effector cell (*e.g.*, T cell) modified with sequences comprising one or more miRNA(s), a CAR, one or more cell tags, and/or one or more cytokines and optionally, components of the gene switch system as described herein. In certain embodiments, the immune effector cell is modified with *Sleeping Beauty* transposon(s) and *Sleeping Beauty* transposase. For example, the *Sleeping Beauty* transposon or transposons can include one or more miRNA(s), a CAR, 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 modified T-cell can elicit a CAR-mediated T-cell response.

[0609] 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 modified T cells of the invention are used in the treatment of malignancies. In certain embodiments, the cells of the invention are used in the treatment of patients at risk for developing malignancies. Thus, the methods for the treatment or prevention of malignancies comprising administering to a subject in need thereof, a therapeutically effective amount of the modified T cells of the invention. In embodiments, the cells activated and expanded as described herein can be utilized in the treatment of malignancies.

[0610] 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.

[0611] Briefly, pharmaceutical compositions described herein can comprise a population comprising modified immune effector cells 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 some embodiments, compositions of the present invention are formulated for intravenous administration.

[0612] 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.

[0613] 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.

[0614] 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.

[0615] 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.

[0616] A "carrier" or "carrier materials" include any commonly used excipients in pharmaceutics and should be selected on the basis of compatibility with the polynucleotides, vectors, and/or cells disclosed herein, 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 & Wilkins 1999).

[0617] "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.

[0618] Combinations of one or more erosion facilitator with one or more diffusion facilitator can also be used in the present compositions.

[0619] 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.

[0620] "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.

[0621] "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.

[0622] "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.

[0623] "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.

[0624] "Stabilizers" include compounds such as any antioxidation agents, buffers, acids, preservatives and the like.

[0625] "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.

[0626] "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.

[0627] "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.

[0628] "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.

XIV. Methods of Treatment

[0629] The present invention also relates to a method of treating a disease or disorder comprising administering a modified immune effector cell of the present disclosure to a subject in need thereof. In certain embodiments, the cell is administered in a therapeutically effective amount.

[0630] The present invention also relates to the use of a modified immune effector cell of the present disclosure in the manufacture of a medicament for the treatment of a disease or disorder.

[0631] In some embodiments, the disease can be cancer. In certain aspects, the cancer can be hematological or a solid tumor. In other instances, the cancer is a hematologic malignancy. In some cases, the cancer is a metastatic cancer. In some cases, the cancer is a relapsed or refractory cancer. In certain aspects, the cancer is selected from B cell cancer, *e.g.*, multiple myeloma, Waldenstrom's macroglobulinemia, the heavy chain diseases, such as, for example, alpha chain disease, gamma chain disease, and mu chain disease, benign monoclonal gammopathy, and immunocytic amyloidosis, melanomas, breast cancer, lung cancer, bronchus cancer, colorectal cancer, prostate cancer (e.g., metastatic, hormone refractory prostate cancer), pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, cancer of hematological tissues, and the like. Other non-limiting examples of types of cancers that can be treated by the methods of the present disclosure include human sarcomas and carcinomas, *e.g.*, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, colorectal cancer, pancreatic cancer, breast cancer, beast adenocarcinomas *e.g.* triple negative breast cancer,

ovarian cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, liver cancer, hepatocellular carcinoma (HCC), choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, bone cancer, brain tumor, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute myeloid leukemia, acute lymphocytic leukemia, mantle cell lymphoma, acute lymphoblastic leukemia, and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); diffuse large B-cell lymphoma; and polycythemia vera, lymphoma (Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, and heavy chain disease.

[0632] In some instances, the cancer is a solid tumor. Exemplary solid tumors include, but are not limited to, anal cancer; appendix cancer; bile duct cancer (*i.e.*, cholangiocarcinoma); bladder cancer; brain tumor; breast cancer; cervical cancer; colon cancer; cancer of Unknown Primary (CUP); esophageal cancer; eye cancer; fallopian tube cancer; gastroenterological cancer; kidney cancer; liver cancer; lung cancer; medulloblastoma; melanoma; oral cancer; ovarian cancer; pancreatic cancer; parathyroid disease; penile cancer; pituitary tumor; prostate cancer; rectal cancer; skin cancer; stomach cancer; testicular cancer; throat cancer; thyroid cancer; uterine cancer; vaginal cancer; or vulvar cancer.

[0633] In some instances, the cancer is a hematologic malignancy. In some cases, a hematologic malignancy comprises a lymphoma, a leukemia, a myeloma, or a B-cell malignancy. In some cases, a hematologic malignancy comprises a lymphoma, a leukemia or a myeloma. In some instances, exemplary hematologic malignancies include chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, non-CLL/SLL lymphoma, prolymphocytic leukemia (PLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenstrom's macroglobulinemia, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt

high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis. In some embodiments, the hematologic malignancy comprises a myeloid leukemia. In some embodiments, the hematologic malignancy comprises acute myeloid leukemia (AML) or chronic myeloid leukemia (CML).

[0634] In some instances, disclosed herein are methods of administering to a subject having a hematologic malignancy selected from chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma (SLL), high risk CLL, non-CLL/SLL lymphoma, prolymphocytic leukemia (PLL), follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), Waldenstrom's macroglobulinemia, multiple myeloma, extranodal marginal zone B cell lymphoma, nodal marginal zone B cell lymphoma, Burkitt's lymphoma, non-Burkitt high grade B cell lymphoma, primary mediastinal B-cell lymphoma (PMBL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, plasmacytoma, mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, primary effusion lymphoma, or lymphomatoid granulomatosis a modified effector cell described herein. In some instances, disclosed herein are methods of administering to a subject having a hematologic malignancy selected from AML or CML a modified effector cell to the subject.

[0635] In still other embodiments, the epithelial cancer is non-small-cell lung cancer, nonpapillary renal cell carcinoma, cervical carcinoma, ovarian carcinoma (e.g., serous ovarian carcinoma), or breast carcinoma. The epithelial cancers can be characterized in various other ways including, but not limited to, serous, endometrioid, mucinous, clear cell, brenner, or undifferentiated. In some embodiments, the methods and compositions of the present disclosure are used in the treatment, diagnosis, and/or prognosis of lymphoma or its subtypes, including, but not limited to, mantle cell lymphoma.

[0636] In some embodiments, the disease or disorder is associated with the overexpression of an antigen. In certain embodiments, the antigen is CD19, CD33, ROR1, MUC1, or MUC16. In some

embodiments, the disease is ovarian cancer, a myelodysplastic syndrome (MDS).

[0637] In some embodiments, disclosed herein are methods of administering a modified effector cell comprising a polypeptide described herein to a subject having a disorder, for instance a cancer. In some cases, the cancer is a cancer associated with an expression of CD19, CD20, CD33, CD44, BCMA, CD123, EGFRvIII, α-Folate receptor, CAIX, CD30, ROR1, CEA, EGP-2, EGP-40, HER2, HER3, Folate-binding Protein, GD2, GD3, IL-13R-a2, KDR, EDB-F, mesothelin, GPC3, CSPG4, HER1/HER3, HER2, CD44v6, CD44v7/v8, CD20, CD174, CD138, L1-CAM, FAP, c-MET, PSCA, CS1, CD38, IL-11Rα, EphA2, CLL-1, CD22, EGFR, Folate receptor α, Mucins such as MUC1 or MUC16, MAGE-A1, h5T4, PSMA, CSPG4, TAG-72 or VEGF-R2.

[0638] In some embodiments, the disease is associated with the overexpression of MUC16. In certain such embodiments, the disease is ovarian cancer, breast cancer, pancreatic cancer, endometrial cancer, or lung cancer.

[0639] In some embodiments, the disease is associated with the overexpression of CD33. In certain such embodiments, the disease is acute myeloid leukemia (AML) or a myelodysplastic syndrome (MDS).

[0640] In some embodiments, the disease is associated with the overexpression of ROR1. In certain such embodiments, the disease involves a hematological tumor, for example chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), acute lymphoblastic leukemia (ALL), and diffuse large B-cell lymphoma (DLBCL). In certain such embodiments, the disease involves a solid tumor, for example breast adenocarcinomas encompassing triple negative breast cancer (TNBC), pancreatic cancer, ovarian cancer, and lung adenocarcinoma.

[0641] 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 embodiments, genetically modified T cells can be expanded and transferred into patients treated with or without preconditioning lymphodepletion according to well-known protocols. In some cases, the one or more transposons

encode one or more miRNA(s), 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 one or more miRNA(s), 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 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.

[0642] Studies conducted in patients with metastatic melanoma demonstrated that lymphodepletion prior to adoptive cell transfer dramatically improves the efficacy of therapy with *in vitro* expanded tumor-infiltrating lymphocytes (TILs). Muranski P, et al., "Increased intensity lymphodepletion and adoptive immunotherapy—how far can we go?" *Nat Clin Pract Oncol.* 2006;3(12):668-681. Lymphodepletion likely works by multiple mechanisms, including eliminating sinks for homeostatic cytokines, such as interleukin-2 (IL-2), IL-7, and IL-15, eradicating immunosuppressive elements, such as regulatory T cells and myeloid-derived suppressor cells, inducing costimulatory molecules and downregulating indoleamine 2,3-

dioxygenase in tumor cells, and promoting expansion, function, and persistence of adoptively transferred T cells. These experiences led to the use of lymphodepletion in clinical trials with CAR T-cell therapy. Kochenderfer et al showed an association between an increase in serum IL-15 levels post-lymphodepletion and clinical response after anti-CD19 CAR T-cell therapy. Kochenderfer JN, et al., "Lymphoma remissions caused by anti-CD19 chimeric antigen receptor T cells are associated with high serum interleukin-15 levels," *J Clin Oncol.* 2017; 35(16):1803-1813. Turtle and colleagues showed that the addition of fludarabine to cyclophosphamide (Cy/Flu) in the lymphodepleting regimen was associated with improved anti-CD19 CAR T-cell expansion and persistence and better clinical outcome compared with non-Flu lymphodepleting regimens in patients with non-Hodgkin lymphoma. Turtle CJ, et al., "Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells," *Sci Transl Med.* 2016; (8)355-355ra116. Other studies indicated that the magnitude of the CAR T-cell expansion in vivo with respect to both the peak and the area under the curve over the first month may be associated with response and/or durability. Neelapu SS, et al., "Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma," *N Engl J Med.* 2017;377(26):2531-2544.

[0643] In some embodiments, the subject is subjected to lymphodepletion before the step of administering the modified immune effector cells to the subject. As used herein, "lymphodepletion" involves methods that reduce the number of lymphocytes in a subject, for example by administration of a lymphodepletion agent. Examples of lymphodepletion include nonmyeloablative lymphodepleting chemotherapy, myeloablative lymphodepleting chemotherapy. Lymphodepletion can also be attained by partial body or whole body fractioned radiation therapy. A lymphodepletion agent can be a chemical compound or composition capable of decreasing the number of functional lymphocytes in a mammal when administered to the mammal. One example of such an agent is one or more chemotherapeutic agents. Such agents and dosages are known, and can be selected by a treating physician depending on the subject to be treated. Examples of lymphodepletion agents include, but are not limited to, fludarabine, cyclophosphamide, cladribine, denileukin diftitox, or combinations thereof. In some embodiments, the subject is not subjected to lymphodepletion before the step of administering the modified immune effector cells to the subject.

[0644] In some embodiments, lymphodepletion is performed by administration of cyclophosphamide at a dose of about 10 mg/kg to about 100 mg/kg, preferably about 40 mg/kg to about 80 mg/kg, for example about 60 mg/kg. In such embodiments, the cyclophosphamide can be administered concomitantly with fludarabine at a dose of about 10 mg/m^2 to about 50 mg/m^2, preferably about 20 mg/m^2 to about 40 mg/m^2, for example about 30 mg/m^2. In some embodiments, lymphodepletion is performed by administration of fludarabine at a dose of about 10 mg/m^2 to about 50 mg/m^2, preferably about 20 mg/m^2 to about 40 mg/m^2, for example about 30 mg/m^2. In such embodiments, the fludarabine can be administered concomitantly with cyclophosphamide at a dose of about 200 mg/m^2 to about 900 mg/m^2, preferably about 400 to about 600 mg/m^2, for example 500 mg/m^2.

[0645] In some embodiments, patients or subjects are not lymphodepleted prior to blood being withdrawn to produce the autologous modified immune effector cells.

[0646] In some embodiments, the modified immune effector cells are autologous to the subject. In some embodiments, the modified immune effector cells are allogeneic to the subject.

[0647] In some embodiments, an amount of modified immune effector cells that 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.

[0648] 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 immune effector 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.

[0649] 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.

[0650] 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).

[0651] 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.

[0652] 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.

[0653] 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.

[0654] 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.

[0655] 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.

[0656] 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 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 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 disclosed immune effector cells or the vectors described herein and the one or more additional therapeutic agents can be administered simultaneously.

[0657] An example of a therapeutic agents that can be included in or co-administered with the inventive host cells and/or the inventive vectors are interleukins, cytokines, interferons, adjuvants and chemotherapeutic agents. In some 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.

A. Modified Immune Effector Cell Doses

[0658] In some embodiments, an amount of modified immune effector cells administered comprises about 10^2 to about 10^9 modified effector cells/kg of the subject's body weight. In some embodiments, an amount of modified immune effector cells administered comprises about 10^3 to about 10^9 modified effector cells/kg of the subject's body weight. In some embodiments, an amount of modified immune effector cells administered comprises about 10^4 to about 10^9 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^5 to about 10^9 modified effector cells/kg of the subject's body weight. In some

cases, an amount of modified effector cells comprises about 10^5 to about 10^8 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^5 to about 10^7 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^6 to about 10^9 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^6 to about 10^8 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^7 to about 10^9 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^5 to about 10^6 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^6 to about 10^7 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^7 to about 10^8 modified effector cells/kg of the subject's body weight. In some cases, an amount of modified effector cells comprises about 10^8 to about 10^9 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^9 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^8 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^7 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^6 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^5 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^4 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^3 modified effector cells/kg of the subject's body weight. In some instances, an amount of modified effector cells comprises about 10^2 modified effector cells/kg of the subject's body weight.

[0659] In some embodiments, the modified immune effector cells are modified CAR-T cells. In some instances, the modified CAR-T cells further comprise one or more miRNA(s) as described herein. In some cases, an amount of modified CAR-T cells comprises about 10^4 to about 10^9 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^5 to about 10^9 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^5 to about 10^8

modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^5 to about 10^7 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^6 to about 10^9 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^6 to about 10^8 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^7 to about 10^9 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^5 to about 10^6 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^6 to about 10^7 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^7 to about 10^8 modified CAR-T cells/kg of the subject's body weight. In some cases, an amount of modified CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of modified CAR-T cells comprises about 10^9 modified CAR-T cells/kg of the subject's body weight. In some instances, an amount of modified CAR-T cells comprises about 10^8 modified CAR-T cells/kg of the subject's body weight. In some instances, an amount of modified CAR-T cells comprises about 10^7 modified CAR-T cells/kg of the subject's body weight. In some instances, an amount of modified CAR-T cells comprises about 10^6 modified CAR-T cells/kg of the subject's body weight. In some instances, an amount of modified CAR-T cells comprises about 10^5 modified CAR-T cells/kg of the subject's body weight.

[0660] In some embodiments, the modified CAR-T cells are CD19-specific CAR-T cells. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^6 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg of the subject's body weight. In some cases,

an amount of CD19-specific CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD19-specific CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^8 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^7 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^6 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD19-specific CAR-T cells comprises about 10^5 CAR-T cells/kg of the subject's body weight.

[0661] In some embodiments, the modified CAR-T cells are CD33-specific CAR-T cells. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^6 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of CD33-specific CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD33-specific CAR-T cells comprises about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD33-specific CAR-T cells comprises about 10^8 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD33-specific CAR-T cells comprises about 10^7 CAR-T cells/kg of the subject's body weight. In some instances, an amount of CD33-specific CAR-T cells comprises about 10^6 CAR-T cells/kg

of the subject's body weight. In some instances, an amount of CD33-specific CAR-T cells comprises about 10^5 CAR-T cells/kg of the subject's body weight.

[0662] In some embodiments, the modified CAR-T cells are MUC1-specific CAR-T cells. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^6 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC1-specific CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC1-specific CAR-T cells comprises about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC1-specific CAR-T cells comprises about 10^8 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC1-specific CAR-T cells comprises about 10^7 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC1-specific CAR-T cells comprises about 10^6 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC1-specific CAR-T cells comprises about 10^5 CAR-T cells/kg of the subject's body weight.

[0663] In some embodiments, the modified CAR-T cells are MUC16-specific CAR-T cells. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about

10^6 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of MUC16-specific CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC16-specific CAR-T cells comprises about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC16-specific CAR-T cells comprises about 10^8 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC16-specific CAR-T cells comprises about 10^7 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC16-specific CAR-T cells comprises about 10^6 CAR-T cells/kg of the subject's body weight. In some instances, an amount of MUC16-specific CAR-T cells comprises about 10^5 CAR-T cells/kg of the subject's body weight.

[0664] In some embodiments, the modified CAR-T cells are ROR1-specific CAR-T cells. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^5 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^5 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^5 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^6 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^6 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^7 to about 10^9 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^5 to about 10^6 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^6 to about 10^7 CAR-T cells/kg of the subject's body weight. In some cases, an amount of ROR1-specific CAR-T cells comprises about 10^7 to about 10^8 CAR-T cells/kg of the subject's body weight. In some cases, an

amount of ROR1-specific CAR-T cells comprises about 10^8 to about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of ROR1-specific CAR-T cells comprises about 10^9 CAR-T cells/kg of the subject's body weight. In some instances, an amount of ROR1-specific CAR-T cells comprises about 10^8 CAR-T cells/kg of the subject's body weight. In some instances, an amount of ROR1-specific CAR-T cells comprises about 10^7 CAR-T cells/kg of the subject's body weight. In some instances, an amount of ROR1-specific CAR-T cells comprises about 10^6 CAR-T cells/kg of the subject's body weight. In some instances, an amount of ROR1-specific CAR-T cells comprises about 10^5 CAR-T cells/kg of the subject's body weight.

[0665] In some embodiments, the modified T cells are engineered TCR T-cells. In some cases, an amount of engineered TCR T- cells comprises about 10^5 to about 10^9 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^5 to about 10^8 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^5 to about 10^7 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^6 to about 10^9 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^6 to about 10^8 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^7 to about 10^9 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^5 to about 10^6 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^6 to about 10^7 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^7 to about 10^8 TCR cells/kg of the subject's body weight. In some cases, an amount of engineered TCR cells comprises about 10^8 to about 10^9 TCR cells/kg of the subject's body weight. In some instances, an amount of engineered TCR cells comprises about 10^9 TCR cells/kg of the subject's body weight. In some instances, an amount of engineered TCR cells comprises about 10^8 TCR cells/kg of the subject's body weight. In some instances, an amount of engineered TCR cells comprises about 10^7 TCR cells/kg of the subject's body weight. In some instances, an amount of engineered TCR cells comprises about 10^6 TCR cells/kg of the subject's body weight. In some instances, an amount of engineered TCR cells comprises about 10^5 TCR cells/kg of the subject's body weight.

[0666] It is to be noted that dosage values may vary with the type and severity of the condition

to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[0667] In some embodiments, the cancer whose phenotype is determined by the method of the present disclosure is an epithelial cancer such as, but not limited to, bladder cancer, breast cancer, cervical cancer, colon cancer, gynecologic cancers, renal cancer, laryngeal cancer, lung cancer, oral cancer, head and neck cancer, ovarian cancer, pancreatic cancer, prostate cancer, or skin cancer. In other embodiments, the cancer is breast cancer, prostate cancer, lung cancer, or colon cancer.

B. Combination Therapies

[0668] In certain embodiments, the modified immune effector cells and compositions thereof described herein may be used, alone or with other therapies, to treat cancers that have about 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95%, 96%, 97%, 98%, or 99% average response rate to standard therapy (including but not limited to chemotherapy, chemotherapy and current clinical trial therapies). Such cancers include but are not limited to, Hodgkin's lymphoma, melanoma, non-small cell lung cancer (NSCLC), microsatellite instability (MSI)-high or mismatch repair (MMR)-deficient solid tumors, CSCC, RCC, CRC, melanoma, Merkel cell cancer, bladder cancer, RCC, hepatocellular carcinoma (HCC), head & neck cancer (H&N), cervical cancer, gastric cancer, small cell lung cancer (SCLC), endometrial cancer, mesothelioma, ovarian cancer, triple negative breast cancer (TNBC), breast cancer, colorectal cancer (CRC), pancreatic cancer, prostate cancer.

[0669] In some embodiments, the compositions described herein can be administered as a combination therapy with an additional therapeutic agent. In some embodiments, an additional therapeutic agent comprises a biological molecule, such as an antibody. For example, treatment can involve the combined administration of the modified immune effector cells as described herein with antibodies against tumor-associated antigens including, but not limited to, antibodies that bind EGFR, HER2/ErbB2, and/or VEGF. In certain embodiments, the additional therapeutic agent is

an antibody specific for a cancer stem cell marker. In certain embodiments, the additional therapeutic agent is an antibody that is an angiogenesis inhibitor (*e.g.*, an anti-VEGF or VEGF receptor antibody). In certain embodiments, the additional therapeutic agent is bevacizumab (AVASTIN), ramucirumab, trastuzumab (HERCEPTIN), pertuzumab (OMNITARG), panitumumab (VECTIBIX), nimotuzumab, zalutumumab, or cetuximab (ERBITUX). In some embodiments, an additional therapeutic agent comprises an agent such as a small molecule. For example, treatment can involve the combined administration of the modified immune effector cells as described herein with a small molecule that acts as an inhibitor against tumor-associated antigens including, but not limited to, EGFR, HER2 (ErbB2), and/or VEGF. In some embodiments, the modified immune effector cells as described herein administered in combination with a protein kinase inhibitor selected from the group consisting of: gefitinib (IRESSA), erlotinib (TARCEVA), sunitinib (SUTENT), lapatanib, vandetanib (ZACTIMA), AEE788, CI-1033, cediranib (RECENTIN), sorafenib (NEXAVAR), and pazopanib (GW786034B). In some embodiments, an additional therapeutic agent comprises an mTOR inhibitor. In another embodiment, the additional therapeutic agent is chemotherapy or other inhibitors that reduce the number of T_{REG} cells. In certain embodiments, the therapeutic agent is cyclophosphamide or an anti-CTLA4 antibody. In another embodiment, the additional therapeutic reduces the presence of myeloid-derived suppressor cells. In a further embodiment, the additional therapeutic is carbotaxol. In a further embodiment, the additional therapeutic agent is ibrutinib. In some embodiments, an additional therapeutic agent comprises an anti-PD-1 or anti-PDL1 inhibitor. In some embodiments, the method can further comprise one or more checkpoint inhibitors in combination with modified immune effector cells as described herein. In some embodiments, the additional checkpoint inhibitor can be an anti-CTLA-4 antibody. The anti-CTLA-4 antibody (*e.g.*, ipilimumab) has shown durable anti-tumor activities and prolonged survival in participants with advanced melanoma, resulting in its Food and Drug Administration (FDA) approval in 2011. See Hodi *et al.*, Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. (2010) Aug 19; 363(8):711-23. In some embodiments, the one or more checkpoint inhibitors can be an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody can be a full length atezolizumab (anti-PD-L1), avelumab (anti-PD-L1), durvalumab (anti-PD-L1), or a fragment or a variant thereof. In some embodiments, the one or more checkpoint inhibitors can be any one or more of CD27 inhibitor, CD28 inhibitor, CD40 inhibitor, CD122 inhibitor, CD137 inhibitor, OX40

(also known as CD134) inhibitor, GITR inhibitor, ICOS inhibitor, or any combination thereof. In some embodiments, the one or more checkpoint inhibitors can be any one or more of A2AR inhibitor, B7-H3 (also known as CD276) inhibitor, B7-H4 (also known as VTCN1) inhibitor, BTLA inhibitor, IDO inhibitor, KIR inhibitor, LAG3 inhibitor, TIM-3 inhibitor, VISTA inhibitor, or any combination thereof.

[0670] In certain embodiments, an additional therapeutic agent comprises a second immunotherapeutic agent. In some embodiments, the additional immunotherapeutic agent includes, but is not limited to, a colony stimulating factor, an interleukin, an antibody that blocks immunosuppressive functions (*e.g.*, an anti-CTLA-4 antibody, anti-CD28 antibody, anti-CD3 antibody, anti-PD-L1 antibody, anti-TIGIT antibody), an antibody that enhances immune cell functions (*e.g.*, an anti-GITR antibody, an anti-OX-40 antibody, an anti-CD40 antibody, or an anti-4-1BB antibody), a toll-like receptor (*e.g.*, TLR4, TLR7, TLR9), a soluble ligand (*e.g.*, GITRL, GITRL-Fc, OX-40L, OX-40L-Fc, CD40L, CD40L-Fc, 4-1BB ligand, or 4-1BB ligand-Fc), or a member of the B7 family (*e.g.*, CD80, CD86). In some embodiments, the additional immunotherapeutic agent targets CTLA-4, CD28, CD3, PD-L1, TIGIT, GITR, OX-40, CD-40, or 4-1BB.

[0671] In some embodiments, the additional therapeutic agent is an additional immune checkpoint inhibitor. As used herein, "an immune checkpoint inhibitor" is an agent that inhibits the activity of an immune checkpoint protein. In some embodiments, the additional immune checkpoint inhibitor is an anti-PD-L1 antibody, an anti-CTLA-4 antibody, an anti-CD28 antibody, an anti-TIGIT antibody, an anti-LAG3 antibody, an anti-TIM3 antibody, an anti-GITR antibody, an anti-4-1BB antibody, or an anti-OX-40 antibody. In some embodiments, the additional therapeutic agent is an anti-TIGIT antibody. In some embodiments, the additional therapeutic agent is an anti-PD-L1 antibody selected from the group consisting of: BMS935559 (MDX-1105), atexolizumab (MPDL3280A), durvalumab (MEDI4736), and avelumab (MSB0010718C). In some embodiments, the additional therapeutic agent is an anti-CTLA-4 antibody selected from the group consisting of: ipilimumab (YERVOY) and tremelimumab. In some embodiments, the additional therapeutic agent is an anti-LAG-3 antibody selected from the group consisting of: BMS-986016 and LAG525. In some embodiments, the additional therapeutic agent is an anti-OX-40 antibody selected from the group consisting of: MEDI6469, MEDI0562, and MOXR0916. In

some embodiments, the additional therapeutic agent is an anti-4-1BB antibody selected from the group consisting of: PF-05082566. In some embodiments, the modified immune effector cells as described herein can be administered in combination with a biologic molecule selected from the group consisting of: cytokines, adrenomedullin (AM), angiopoietin (Ang), BMPs, BDNF, EGF, erythropoietin (EPO), FGF, GDNF, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor (SCF), GDF9, HGF, HDGF, IGF, migration-stimulating factor, myostatin (GDF-8), NGF, neurotrophins, PDGF, thrombopoietin, TGF-α, TGF-β, TNF-α, VEGF, PlGF, gamma-IFN, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-12, IL-15,and IL-18.

XVI. Personalized Therapy

[0672] In an embodiment, the invention involves the detection of a disease or disorder associated with the overexpression of an antigen in a subject. The method of detection comprises: a) contacting a sample from a subject with one or more of the antibodies, or antigen-binding fragments thereof, that are described herein; and b) detecting an increased level of binding of the antibody or fragment thereof to the sample as compared to such binding to a control sample lacking the disease, thereby detecting the disease in the subject. In certain embodiments, the disease is cancer. In certain such embodiments, 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 detection of binding is immunohistochemical, for example by enzyme-linked immunosorbent assay (ELISA), fluorescence-activated cell sorting (FACS), Western blot, immunoprecipitation, and/or radiographic imaging.

[0673] In an embodiment of the invention, a collection approach is used to transform the personalized cell therapy landscape for patients, including cancer patients. The approach includes the use of a developed and validated collection of non-viral plasmids to target tumor-associated antigens. These design and manufacturing advantages include the use of UltraCAR-T™ technology for generation of genetically modified cells, coupled with the capabilities of the UltraPorator™ electroporation system. These methods, compounds and compositions allow therapies and treatment options to assist cancer centers and physicians deliver personalized, autologous genetically modified cell treatments with overnight manufacturing to cancer patients.

Based on the patient's cancer indication and/or biomarker profile, one or more non-viral plasmids may be selected from the library to build a personalized UltraCAR-T cell treatment. After initial treatment, this approach has potential to allow re-dosing of UltraCAR-T cells targeting the same or new tumor antigen(s) based on the treatment response and the changes in antigen expression of the patient's tumor. The combination of the advanced UltraVector™ DNA construction platform and the ease of overnight manufacturing gives this library approach an advantage over traditional T-cell therapies.

[0674] Provided herein is a method for the treatment of a disease or disorder comprising the serial administration of polynucleotides encoding a chimeric antigen receptor or a cell comprising the same. The disease or disorder may, for example, be a cancer or auto-immune disease or disorders. The polynucleotides may be present in a viral or non-viral vector. The chimeric antigen receptors selected from a collection of chimeric antigen receptors having different structural compositions and binding specificities for an array of antigen targets.

[0675] In certain embodiments, the collection of chimeric antigen receptors comprises a chimeric antigen receptor that targets at least one of the following antigens: BCMA, CAIX, CA125, CCR4, CD3, CD4, CD5, CD7, CD16, CD19, CD20, CD22, CD24, CD25, CD28, CD30, CD33, CD38, CD40, CD44, CD44v6, CD44v7/v8, CD47, CD52, CD56, CD70, CD79b, CD80, CD81, CD86, CD123, CD133, CD138, CD151, CD171, CD174, CD276, CEA, CEACAM6, CLL-1, c-MET, CS1, CSPG4, CTLA-4, DLL3, EDB-F, EGFR, EGFR2, EGFRvIII, EGP-2, EGP-40, EphA2, FAP, FLT1, FLT4, Folate-binding Protein, Folate Receptor, Folate receptor α, α-Folate receptor, Frizzled, GD2, GD3, GHR, GHRHR, GITR, GPC3, Gp100, gp130, HBV antigens, HER1, HER2, HER3, HER4, h5T4, HVEM, IGF1R, IgKAppa, IL-1-RAP, IL-2R, IL6R, IL-11Rα, IL-13R-a2, KDR, KRASG12V, LewisA, LewisY, L1-CAM, LIFRP, LRP5, LTPR, MAGE-A, MAGE-A1, MAGE-A10, MAGE-A3, MAGEA3/A6, MAGE-A4, MAGE-A6, MART-1, MCAM, mesothelin, PSCA, Mucins such as MUC1, MUC4 or MUC16, NGFR, NKG2D, Notch-1-4, NY-ESO-1, O-acetylGD2, O-acetylGD3, OX40, P53, PD1, PDE10A, PD-L1, PD-L2, PRAME, PSMA, PTCH1, RANK, Robol, ROR1, ROR1R, ROR-2, TACI, TAG-72, TCRa, TCRp, TGF, TGFBeta, TGFBeta-II, TGFBR1, TGFBR2, Titin, TLR7, TLR9, TNFR1, TNFR2, TNFRSF4, TRBC1, TWEAK-R, VEGF, VEGF-R2, and WT-1. In certain embodiments, the collection of chimeric antigen receptors comprises a chimeric antigen receptor that targets at least one of the

following antigens: B7H4, BCMA, BTLA, CA125, CAIX, CCR4, CD123, CD133, CD137, CD138, CD151, CD16, CD171, CD174, CD19, CD20, CD22, CD24, CD25, CD276, CD28, CD3, CD30, CD33, CD38, CD4, CD40, CD44, CD44v6, CD44v7/v8, CD47, CD5, CD52, CD56, CD7, CD70, CD79b, CD80, CD81, CD86, CEA, CEACAM6, CLL-1, c-MET, CS1, CSPG4, CTLA-4, DLL3, EDB-F, EGFR, EGFR2, EGFRvIII, EGP-2, EGP-40, EphA2, HER1, HER2, HER3, HER4, FAP, FLT1, FLT4, Folate receptor α, Folate-binding protein, folate receptor, Frizzled, GD2, GD3, GHR, GHRHR, GITR, Gp100, gp130, GPC3, h5T4, HBV antigens, HER1/HER3, HPV antigens, HVEM, IGF1R, IgKAppa, IL-11Rα, IL-13R-α2, IL-1-RAP, IL-2R, IL6R, KDR, KRASG12V, L1-CAM, LewisA, LewisY, LIFRP, LRP5, LTPR, MAGE-A, MAGE-A1, MAGE-A10, MAGE-A3, MAGEA3/A6, MAGE-A4, MAGE-A6, MART-1, MCAM, Mesothelin, mucins such as MUC1 and MUC16, NGFR, NKG2D, Notch 1-4, NY-ESO-1, O-acetylGD2, O-acetylGD3, OX40, P53, PD1, PDE10A, PD-L1, PD-L2, PMSA, PRAME, PSCA, PSMA, PTCH1, RANK, Robol, ROR1, ROR1R, ROR-2, TACI, TAG-72, TCRα, TCRp, TGF, TGFß, TGFß-II, TGFBR1, TGFBR2, Titin, TLR7, TLR9, TNFR1, TNFR2, TNFRSF4, TRBC1, TWEAK-R, VEGF, VEGF-R2, and WT-1.

[0676] In certain embodiments, the method comprises a first administration of cells expressing one or more chimeric antigen receptors from the collection followed by a second administration of cells expressing one or more chimeric antigen receptors from the collection, wherein a period of time elapses between the first and second administrations. In certain embodiments, the same one or more CARs in the first administration are administered again. In certain other embodiments, at least one of the CARs expressed by cells in the second administration is different from CARs in the first administration. In certain embodiments, the invention comprises a third, fourth, fifth, sixth, seventh, eighth, ninth, tenth or any additional number of rounds of administration of cells expressing CARs selected from the collection, wherein in each subsequent round of administration a different CAR is administered which was not administered in a previous round of treatment.

[0677] In certain embodiments, the dose of the cells is autologous. In certain embodiments, the dose of cells is allogenic. In certain embodiments, one dose of the cells is autologous and another dose is allogenic.

[0678] In certain embodiments, prior to a second or subsequent administration of a CAR or CARs, a period of time is allowed to elapse that is sufficient for biologic or therapeutic

activity of one or more CARs in a preceding administration to become diminished from peak biologic or therapeutic activity, become negligible or become undetectable. In certain embodiments, a subsequent administration of a CAR takes place at least one day following the previous administration of a CAR.

[0679] The polynucleotide encoding the CAR may be introduced into the cells of a subject by way of viral transduction, non-viral transfection or electroporation methods of delivery.

[0680] In certain embodiments, the polynucleotide encoding the CAR may be administered as part of a vector, such as those described herein. In certain embodiments of the invention, one or more polynucleotides encoding a CAR further comprises nucleotide sequences encoding a cytokine, a cell tag, and/or a gene switch, as described previously. In certain embodiments, the vector comprises the polynucleotide encoding the CAR and one or more polynucleotides encoding the cytokine, cell tag, and/or a gene switch.

[0681] In certain embodiments, the vector is a "regulatory approved vector," meaning that it has been approved by a Regulatory Authority, national, supra-national (*e.g.*, the U.S. Food and Drug Administration (FDA), the European Commission or the Council of the EU), regional, state or local regulatory agency, department, bureau, commission, council or other governmental entity, wherein such approval is necessary or sufficient for the manufacture, distribution, use or sale of a vector in a regulatory jurisdiction. In certain embodiments, such a "regulatory approved vector" means a vector which has been approved, at a minimum, for use in human clinical safety trials (sometimes referred to as "phase 1 clinical trial") in a regulatory jurisdiction. "Clinical safety" or "Phase 1" trial means a trial to assess at least safety, optionally to assess safety and dosages, for analysis of side effects associated with the test article, optionally to assess side effects in conjunction with varying dosages. In certain embodiments, "regulatory approved vector" means a vector which has been approved, at a minimum, for use in "Phase 2" studies. A "Phase 2" study is one in which the test article is administered to a larger group human subjects (as compared to a smaller Phase 1 group of subjects) for evaluation of a larger group of patients (typically up to a few hundred) with a disease, disorder or condition for which the test article is developed, to initially assess its effectiveness and to further study its safety. In certain embodiments, a Phase 2 study is to assess the optimal dose or doses of a test article to maximize benefits, while minimizing risks.

In certain embodiments, "regulatory approved vector" means a vector which has been approved, at a minimum, for use in "Phase 3" studies. A Phase 3 study (sometimes referred to as "pivotal trials") typically involves about 300 to 3,000 participants from a patient population for which the test article is intended to be used. Participants in a Phase 3 study are typically assigned to receive either the test article or a placebo (a substance that has no therapeutic effect). A Phase 3 study is intended to demonstrate whether or not a test article offers a treatment benefit to a specific population and to provide more detailed safety data, and to serve as the basis for product labeling in regard to diseases or conditions for which the test article may be used. In certain embodiments, "regulatory approved vector" means a vector which has been approved for commercial manufacturing, use or sale for treatment of a disease, disorder or condition in humans by a regulatory authority in the regulatory jurisdiction (*e.g.*, approval by U.S. FDA for manufacturing, use or sale in the United States of America). In certain embodiments, the "regulatory approved vector" is a regulatory approved lentivirus vector, a regulatory approved retroviral vector, or a regulatory approved non-viral vector. In some embodiments, the regulatory approved vector is a non-viral vector that is a *Sleeping Beauty* vector.

[0682] In certain embodiments, the regulatory authority governs approval of pharmaceuticals, biologics, or other medicines or medical treatments in a country selected from the United Arab Emirates, Antigua and Barbuda, Albania, Armenia, Angola, Austria, Australia, Azerbaijan, Bosnia and Herzegovina, Barbados, Belgium, Burkina Faso, Bulgaria, Bahrain, Benin, Brunei Darussalam, Brazil, Botswana, Belarus, Belize, Canada, Central African Republic, Congo, Switzerland, Côte d'Ivoire, Chile, Cameroon, China (People's Republic of China (PCR)), Colombia, Costa Rica, Cuba, Cyprus, Czech, Germany, Djibouti, Denmark, Dominica, Dominican Republic, Algeria, Ecuador, Estonia, Egypt, Spain, Finland, France, Gabon, United Kingdom, Grenada, Georgia, Ghana, Gambia, Guinea, Equatorial Guinea, Greece, Guatemala, Guinea-Bissau, Honduras, Croatia, Hungary, Indonesia, Ireland, Israel, India, Iran (Islamic Republic of), Iceland, Italy, Jordan, Japan, Kenya, Kyrgyzstan, Cambodia, Comoros, Saint Kitts and Nevis, Democratic People's Republic of Korea, Republic of Korea, Kuwait, Kazakhstan, Lao People's Democratic Republic, Saint Lucia, Liechtenstein, Sri Lanka, Liberia, Lesotho, Lithuania, Luxembourg, Latvia, Libya, Morocco, Monaco, Republic of Moldova, Montenegro, Madagascar, North Macedonia, Mali, Mongolia, Mauritania, Malta, Malawi, Mexico, Malaysia, Mozambique, Namibia, Niger, Nigeria, Nicaragua, Netherlands, Norway, New Zealand, Oman, Panama, Peru,

Papua New Guinea, Philippines, Poland, Portugal, Qatar, Romania, Serbia, Russian Federation, Rwanda, Saudi Arabia, Seychelles, Sudan, Sweden, Singapore, Slovenia, Slovakia, Sierra Leone, San Marino, Senegal, Sao Tome and Principe, El Salvador, Syrian Arab Republic, Eswatini, Chad, Togo, Thailand, Tajikistan, Turkmenistan, Tunisia, Turkey, Trinidad and Tobago, United Republic of Tanzania, Ukraine, Uganda, United States of America, Uzbekistan, Saint Vincent and the Genadines, Viet Nam, Samoa, South Africa, Zambia, Zimbabwe, Afghanistan, Andorra, ARIPO, Bangladesh, Bolivia, Cayman Islands, Ethiopia, Fiji, Gibraltar, Guam, Haiti, Iraq, Lebanon, Martinique, Micronesia, federated states of, Nauru, OAPI, Palestinian territory, occupied (Gaza), Pitcairn, Saint Barthélemy, Somalia, Taiwan, Tonga, Vanuatu, Yemen, Åland Islands, Anguilla, Aruba, Bermuda, Burundi, Congo (Democratic Republic of the), Eurasian Patent Organization, French Guiana, Greenland, Guernsey, Holy see (Vatican), Jamaica, Macau, Mauritius, Montserrat, Nepal, Pakistan, Palestinian territory, occupied (West Bank), Puerto Rico, Saint Helena, South Sudan, Timor-Leste, Tuvalu, Venezuela, American Samoa, Argentina, Bahamas, Bhutan, Cape Verde, Eritrea, European Union, French Polynesia, Guadeloupe, Guyana, Hong Kong, Kiribati, Maldives, Mayotte, Myanmar, New Caledonia, Palau, Paraguay, Réunion, Saint Pierre and Miquelon, Suriname, Tokelau, Uruguay, and Western Sahara..

[0683] In certain embodiments, the regulatory authority is selected from a multinational organization, such as the World Health Organization, the Pan-American Health Organization, the World Trade Organization (WTO), the International Conference on Harmonization (ICH), and the World Intellectual Property Organization (WIPO); and a national health authority, such as, in Asia and the Pacific, the Australian Government: Department of Health and Ageing, Australian Government: Therapeutic Goods Administration (TGA), Brunei: Ministry of Health, People's Republic of China: State Food and Drug Administration, People's Republic of China: Ministry of Health, People's Republic of China: National Medical Products Administration, People's Republic of China: National Institute for the Control of Pharmaceutical and Biological Products, People's Republic of China: Ministry of Agriculture, Fiji: Ministry of Health, Hong Kong: Department of Health, India: Ministry of Consumer Affairs, Food & Public Distribution, India: Central Drug Standards Control Organization (CDSCO), India: Ministry of Food and Consumer Affairs, Indonesia: Ministry of Health, Japan: Ministry of Health, Labor and Welfare, Japan: Pharmaceuticals and Medical Devices Evaluation Agency, Korea: Food and Drug Administration, Malaysia: Ministry of Health, Malaysia: National Pharmaceutical Regulatory Agency, New

Zealand: Ministry of Health, New Zealand: Medicines and Medical Devices Safety Authority, New Zealand: Food Safety Authority, Papua New Guinea: Department of Health, Philippines: Department of Health, Philippines: National Food Authority, Singapore: Ministry of Health, Singapore: Health Sciences Authority, Sri Lanka: Ministry of Health, Taiwan: Department of Health, Taiwan: National Laboratories of Foods and Drugs, Thailand: Ministry of Public Health, Thailand: Food and Drug Administration, Thailand: Ministry of Agriculture and Co-operatives, in Europe, the European Medicines Agency (EMA), European Commission Directorate General: Medicinal Products for Veterinary Use, Andorra: Ministry of Health and Welfare, Armenia: Ministry of Health, Armenia: Drug and Medical Technology Agency, Austria: Secretariat of Health, Belgium: Health, Food Chain Safety and Environment, Belgium: Pharmaceutical Inspectorate, Belgium: Federal Agency for the Safety of the Food Chain, Bulgaria: Ministry of Health, Bulgaria: Drug Agency, Croatia: Ministry of Health and Social Care, Czech Republic: Ministry of Health, Czech Republic: State Institute for Drug Control, Denmark: Ministry of Health, Denmark: Danish Medicines Agency, Denmark: Veterinary and Food Administration, Estonia: State Agency of Medicines, Finland: Ministry of Social Affairs and Health, Finnish Medicines Agency, France: Ministry of Health, France: National Agency for Veterinary Medicinal Products, Georgia: Ministry of Labor, Health and Social Affairs, Germany: Ministry of Health, Germany: Federal Institute for Drugs and Medical Devices, Germany: Robert Koch Institute, Germany: Paul Ehrlich Institute, Germany: Federal Institute for Risk Assessment, Germany: Ministry of Consumer Protection, Food and Agriculture, Greece: Ministry of Health and Social Solidarity, Greece: National Organization for Medicines, Hungary: Ministry of Health, Social and Family Affairs, Hungary: National Institute of Pharmacy, Iceland: Ministry for Health and Social Security, Icelandic Medicines Agency, Iceland: The Environment Agency, Ireland: Department of Health and Children, Irish Medicines Board, Italy: Ministry of Health, Italy: National Institute of Health, Latvia: State Agency of Medicines, Lithuania: Ministry of Health, Lithuania: State Medicines Control Agency, Luxembourg: Ministry of Health, Malta: Ministry of Health, Elderly and Community Care, Netherlands: Ministry of Health, Welfare and Sport, Netherlands: Medicines Evaluation Board, Netherlands: Inspectorate for Health Protection and Veterinary Public Health, Norway: Ministry of Health and Care Services, Norway: Norwegian Board of Health Supervision, Norway: Norwegian Medicines Agency, Norway: Ministry of Agriculture and Food, Poland: Ministry of Health and Social Security, Poland: Drug Institute, Portugal: Ministry

of Health, Portugal: National Authority of Medicines and Health Products, Romania: Ministry of Health and the Family, Russian Federation: Scientific Centre for Expert Evaluation of Medicinal Products, Russian Federation: State Institute of Drugs and Good Practices, San Marino, Ministry of Health and Social Security, National Insurance and Gender Equality (in Italian), Slovak Republic: Ministry of Health, Slovak Republic: State Institute for Drug Control, Slovak Republic: State Veterinary and Food Administration, Slovenia: Ministry of Health, Slovenia: Institute of Public Health, Spain: Ministry of Health and Consumption; Spanish Agency for Medicines and Health Products, Sweden: Medical Products Agency, Sweden: National Board of Health and Welfare, Sweden: National Food Administration, Switzerland: Federal Office of Public Health, Switzerland: Agency for Therapeutic Products, Switzerland: Federal Veterinary Office, Turkey: Ministry of Health (in Turkish), Ukraine: Ministry of Health, UK: Department of Health, UK: Health Protection Agency, UK: Medicines and Healthcare Products Regulatory Agency (MHRA), UK: National Institute for Biological Standards and Control, UK: Veterinary Medicines Directorate, in the Middle East, the Bahrain: Ministry of Health, Israel: Ministry of Health, Israel: Ministry of Industry, Trade and Labor, Jordan: Ministry of Health, Lebanon: Ministry of Public Health, Palestinian Authority: Ministry of Health, Saudi Arabia: Ministry of Health, United Arab Emirates: Ministry of Health, United Arab Emirates: Federal Department of Pharmacies, Yemen: Ministry of Public Health & Population, in Africa, the Benin: Ministry of Health, Botswana: Ministry of Health, Egypt: Ministry of Agriculture and Land Reclamation, Ghana: Ministry of Health, Ghana: Ministry of Food and Agriculture, Kenya: Ministry of Health, Maldives: Ministry of Health, Mauritius: Ministry of Health & Quality of Life, Mauritius: Ministry of Agro Industry and Food Security, Morocco: Ministry of Public Health, Namibia: Ministry of Health and Social Services, Senegal: Ministry of Health and Prevention, South Africa: Department of Health, Swaziland: Ministry of Health and Social Welfare, Tanzania: Ministry of Health, Tunisia: Ministry of Public Health, Tunisia: Office of Pharmacy and Medicines, Uganda: Ministry of Health, Zimbabwe: Ministry of Health and Child Welfare, and in the Americas, the Argentina: Ministry of Health, Argentina: National Administration of Drugs, Foods and Medical Technology, Belize: Ministry of Health, Bolivia: Ministry of Health and Social Welfare, Brazil: Ministry of Health, Brazil: National Health Surveillance Agency, Brazil: Fundacao Oswaldo Cruz, Canada: Health Canada, Canada: Health Products and Food Branch, Chile: Health Ministry, Chile: Institute of Public Health, Colombia: Ministry of Health, Colombia: INVIMA Instituto Nacional de Vigilancia

de Medicamentos y Alimentos, Costa Rica: Ministry of Health, Dominican Republic: Ministry of Agriculture, Ecuador: Ministry of Public Health, El Salvador: Ministry of Public Health and Social Assistance, El Salvador: Ministry of Agriculture, Guatemala: Ministry of Health, Guyana: Ministry of Health, Guyana: National Bureau of Standards, Jamaica: Ministry of Health, Mexico: Ministry of Health, Mexico: Federal Commission for the Protection Against Sanitary Risks, Netherlands Antilles: Department of Public Health and Environmental Protection, Nicaragua: Ministry of Health, Panama: Ministry of Health, Peru: Ministry of Health, Peru: General Directorate of Medicines, Supplies and Drugs, St. Lucia: Ministry of Agriculture, Lands Forestry and Fisheries, Trinidad and Tobago: Ministry of Health, Trinidad & Tobago: Bureau of Standards, United States of America (USA/U.S.): Food and Drug Administration (FDA), Uruguay: Ministry of Public Health, and the Venezuela: Ministry of Health and Social Development. Other exemplary regulatory authorities can also be found at https://iaocr.com/clinical-research-regulations/regulatory-authority-links/ and https://www.pda.org/scientific-and-regulatory-resources/global-regulatory-authority-websites.

[0684] In certain embodiments, cells expressing one or more CARs are isolated for continued *ex vivo* expansion and/or are cryopreserved for future use.

[0685] The present invention also relates to a composition comprising a chimeric antigen receptor as described above.

[0686] The present invention further relates to a kit comprising a first vector encoding a CAR as described above and a second vector encoding a CAR as described above. -

EXAMPLES

[0687] These Examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein. The following table includes abbreviations and special terms that apply to the Examples only. These abbreviations and special terms are not otherwise limiting, and neither replace nor narrow the broader definitions set forth above, which shall continue to apply to the claims.

Table 9: Abbreviations and Special Terms for Use in the Examples

Abbreviation of Special Term	Explanation
Artificial miRNA	A non-naturally occurring miRNA designed to target a specific gene
CAR-T	Chimeric Antigen Receptor T Cell
Guide miRNA	The mature miRNA processed from the pre-miRNA that is complementary to an intended target gene
LFC	Log2 fold change
Mature miRNA	The fully processed 21-23nt miRNA
mbIL15	A fusion protein comprising IL-15 and IL-15Rα, the protein comprising the amino acid sequence of SEQ ID NO: 525
miRNA	microRNA, a small RNA species generally 21-23nt in length that is complementary to a target mRNA transcript and reduces expression of the target transcript through either RNA degradation or inhibition of translation
Passenger miRNA	A mature miRNA that may be processed from the side opposite of the guide miRNA in the RNA stem-loop
PD1 Silencer Module	A genetic module encoded within the VVN-5351 transgene cassette designed to reduce expression of PD1 within ROR1+PD1 silencer cells. The module consists of a splice unit containing a non-coding RNA that is processed by internal cellular machinery into 2 unique microRNAs with homology to PD1 mRNA.
Pre-miRNA	Precursor miRNA, which is the intermediate miRNA species that has undergone the first processing step to remove the RNA stem-loop structure from the primary mRNA transcript. Pre-miRNA undergoes a second processing step in which the mature miRNA is cleaved and loaded into the RNA-induced silencing complex (RISC).
ROR1+PD1 silencer cells	ROR1 CAR-T cells expressing mbIL15 and HER1t with anti-PD-1 miRNAs

Pri-miRNA/pri-miRNA scaffold	The primary transcript encoding RNA stem-loop structures that are processed by the endogenous cellular machinery into precursor and then mature miRNA. The artificial pri-miRNA sequence and structures are based on endogenous human pri-miRNAs, herein referred to as a scaffold, with the natural guide and passenger strands replaced with a guide miRNA to target PD1 and a passenger miRNA to maintain the predicted folded RNA structure.
UTR	Untranslated region
VVN-5351	A DNA transposon plasmid encoding the ROR1 UltraCAR-T transgene cassette plus a dual PD1-targeting miRNA in the 5'UTR encoded within a synthetic splice unit.
VVN-5355	A DNA transposon plasmid encoding the ROR1 UltraCAR-T transgene cassette.

Example 1. PD1 Module Design

[0688] The PD1-silencing module of miRNA-expressing ROR1 UltraCAR-T cells encodes two artificial miRNAs designed to specifically reduce expression of PD-1 mRNA within UltraCAR-T cells while avoiding off-target silencing of other endogenous transcripts. The two artificial miRNAs of miRNA-expressing ROR1 UltraCAR-T cells are encoded within a dual primary miRNA (pri-miRNA) sequence placed within a 5' UTR splice unit of the UltraCAR-T transgene cassette (Figure 1B). The dual pri-miRNA forms stem-loop structures that are recognized and processed by cellular complexes to generate two unique 21-24 nucleotide mature guide miRNAs that are homologous to specific sequences within the PD1 target transcript. Interaction of the guide miRNAs with the PD1 target sequence is expected to trigger the silencing of PD1 by induction of RNA degradation or translational inhibition (Guo et al, 2010).

[0689] The guide miRNAs encoded within miRNA-expressing ROR1 UltraCAR-T cells were designed to be highly specific for the PD1 target transcript NM_005018.3 by implementation of an internally-designed computational workflow using a combination of twenty-one ranking parameters based on three validated rules-based siRNA prediction algorithms (Amarzguioui and

Prydz, 2004; Reynolds et al, 2004; Ui-Tei et al, 2004). Multi-level specificity profiling was performed against the human reference exome (RefSeq) and the activated T-cell transcriptome (Zhao et al, 2014) to ensure that the mature miRNAs are highly specific for the PD1 target gene. To further reduce risk of off-target silencing by the non-PD1 targeting passenger strand miRNAs, pri-miRNA scaffolds were selected that produce a high ratio of guide:passenger miRNA (Miniarikova et al, 2016). The PD1-targeting guide miRNA PD1_1843 was incorporated into a pri-miRNA scaffold based on human miRNA hsa-miR-204 (accession# MI0000284) while the PD1_2061 guide miRNA was incorporated into the hsa-miR-206 (accession #MI0000490) scaffold. Mutations were created on the passenger strand side of each pri-miRNA to assure the specific miRNA structure was maintained and that thermodynamic stability was not substantially altered. RNA structures were predicted using CLC Main Workbench software. The PD1 silencing module contains the miR204 PD1_1843 pri-miRNA placed directly upstream of miR206 PD1_2061 pri-miRNA within a synthetic splice unit in the 5' UTR of the CAR-T transgene expression cassette. The splice units were ordered as gBlock from IDT and can be cloned into *Sleeping Beauty* CAR vectors and can be cut using ClaI/NheI for cloning into the 5'UTR of any other *Sleeping Beauty* CAR vector.

Example 2. Reduction of PD1 transcript expression by miRNAs

[0690] Primary human T cells were transfected with the constructs listed in Table 10 and expanded *in vitro* with the use of AaPC cells expressing cognate antigen that were grown in large batches and then frozen into aliquots. The generated CAR-T cells were then further activated using anti-CD3/anti-CD28 beads at a bead:T cell ratio of 1:1 and 1 x 10^6 T cells/mL for 48hrs prior to harvesting the cells for RNA isolation. RNA isolation was performed according to manufacturer's recommended protocol (Qiagen) then subjected to RT-qPCR analysis using the SuperScript VILO Master Mix with ezDNase (Invitrogen) and TaqMan FAST Advanced master mix for qPCR to evaluate PD-1 expression levels using specific primer/probes (Human PD1: Hs00169472_m1; Human TIGIT: Hs00545087_m1; and Human ACTb: Hs99999903_m1). All samples were also normalized to beta-actin expression levels. The relative expression values are based upon ΔΔCT method by normalizing to construct #1 (MUC16 CAR-T cells only). Results are shown in Figure 2. Shown is the mean ± SD from 3 donors.

Table 10: Description of constructs 1-8 as utilized in FIG 2 sequences of miRNA are as described in Table 4)

Construct #	miRNA	Effector Genes
1	N/A	MUC16 CAR-mbIL15-HER1t
2	Scrambled 1 (SEQ ID NO: 586)	MUC16 CAR-mbIL15-HER1t
3	Scrambled 2 (SEQ ID NO: 582)	MUC16 CAR-mbIL15-HER1t
4	miR204 PD1 + miR206 PD1	MUC16 CAR-mbIL15-HER1t
5	miR17 TIGIT	MUC16 CAR-mbIL15-HER1t
6	miR150 TIGIT + miR206 PD1	MUC16 CAR-mbIL15-HER1t
7	miR204 PD1 + miR206 PD1 + miR17 TIGIT	MUC16 CAR-mbIL15-HER1t
8	N/A	MUC16 CAR+HER1t

[0691] The data demonstrates the specificity of PD-1 checkpoint inhibitor miRNA targeting the intended sequence. CAR constructs that expressed the scrambled miRNA (Constructs 2 and 3) along with the CAR construct that did not express mbIL-15 (construct #8) did not show any decrease in PD-1 expression whereas CAR constructs that contain PD-1 miRNA (construct 4) or a combination of PD-1 and TIGIT miRNA (constructs 6 and 7) did show a decrease in PD-1 expression. On the other hand, the CAR construct containing only a TIGIT miRNA sequence (construct 5) did not show any decrease in PD-1 mRNA expression levels further demonstrating the specificity in targeting.

Example 3. Downregulation of targeted mRNA

[0692] Primary human T cells were transfected with a vector encoding CD33-specific CAR or a vector encoding MUC16-specific CAR. The vectors comprise a synthetic intron containing miRNA sequences that target PD-1, PD-1 and/or TIGIT or a non-targeting scrambled control

miRNA. T cell cultures were *in vitro* expanded using antigen presenting cells with cognate tumor antigen that were K562 cells modified to express either CD33 or MUC16 plus other co-stimulatory molecules (based on "Clone 1") and at a ratio of 1:1 (AaPC:T cell). The generated CAR+ cells were then stimulated with anti-CD3/anti-CD28 beads (1:1 bead:T cell ratio) in the absence of cytokines for 48hrs. RNA was isolated using Qiagen kits (AllPrep Universal DNA/RNA/miRNA kit #80224) as per manufacturer's protocol and utilized for the Human PanCancer Immune gene set panel Kit from Nanostring. Briefly, the RNA was hybridized with capture and reporter probe sets and samples processed then hybridized to the slide using the nCounter Prep Station and transcript counts were generated by the nCounter Digital Analyzer according to the manufacturer's protocol. Data validation, QC and normalization were performed by nSolver software (Nanostring Technologies).

[0693] The distribution of the transcript count data on the graph would be a line (from lower left to top right corner) with the slope of 1 if the samples were identical. Data points falling off this line represent variations in transcript counts between the two samples. Counts that were found below the main line represent reduced expression whereas counts above the main line represent increased expression to the sample being compared. The transcript targeted by the miRNA, either PD-1 or TIGIT expressed in the CAR-T cells, specifically showed lower expression of the transcripts of their respective targeted genes compared to the CAR-T cells without the miRNA. See FIG. 3A-B. In addition, to further demonstrate the specificity of targeting, the CAR-T cells with the scrambled control miRNA had no alterations to the transcript levels of either PD-1 or TIGIT, as the data distribution in this graph remained close to the diagonal indicating very little variation in transcript levels. See FIG. 3C. Similar results were observed in T cells transfected with the MUC16 CAR vector comprising a synthetic intron containing miRNA sequences that target PD-1, PD-1 and/or TIGIT (see FIG. 4A-C).

Example 4. Enhancement of tumor cell cytotoxicity effect of miRNA MUC16 CAR-T cells

[0694] Primary human T cells were transfected with a vector encoding MUC16-specific CAR and miRNA sequences that target two sequences within the PD-1 transcript, or with a vector that encodes the MUC16-specific CAR but not an miRNA. CAR-T cells used in this assay were *in vitro* expanded, normalized for CAR expression then seeded in triplicates with GFP+ K562 cells

expressing MUC16 ("tumor cells") in 96 well plates at the 3:1 E:T ratios. The plate was loaded into the IncuCyteS3 instrument and 4 images per well were taken every 2hr for 7 days. The IncuCyte Software was used to analyze the data and normalize the GFP+ cell counts/image to the 0 hr time point.

[0695] The outgrowth assay determines the rate of target cell growth in the presence or absence of CAR-T cells over the course of 7 days in culture. The lower target cell count in cultures containing CAR-T cells indicate the CAR-T cytolytic activity. FIG. 5A depicts the difference between the tumor target cells only (black circle, filled) and the cells expressing the MUC16-specific CAR only (open square), demonstrating the killing capacity of the MUC16 CAR-T cells. The cells further expressing miRNA targeting PD-1 (grey circle filled) further demonstrates improved cytolytic activity, based upon the sustained low GFP+ counts over the time course evaluation, compared to the cells expressing MUC16 specific CAR only (open square). This data demonstrates the enhanced cytolytic activity of CAR-T cells containing PD-1 targeting miRNA.

[0696] A similar experiment was conducted utilizing GFP+ K562 cells expressing MUC16, PD-L1, and CD155. As shown in FIG. 5B, the difference between the tumor target cells only (square, filled) and cells expressing the CAR only (circle, open), demonstrates the killing capacity of the MUC16 CAR-T cells. The CAR-T cells incorporating miRNAs targeting both PD-1 and TIGIT (circle, filled) further demonstrates improved cytolytic activity, based upon the sustained low GFP+ counts over the time course evaluation, compared to the MUC16 CAR-T cells only (circle, filled). This data demonstrates the enhanced cytolytic activity of CAR-T cells containing 3 targeting miRNAs (2 for PD-1 and 1 for TIGIT) in a single construct.

Example 5. Improvement of Cytokine Expression

[0697] Primary human T cells were transfected with vectors encoding MUC16-specific CAR but not miRNA targeting sequences, and vectors encoding MUC16-specific CAR and single or combinations of miRNA targeting sequences of PD-1 and TIGIT (see Table 11). *In vitro* expanded CAR-T cells were *in vitro* expanded, normalized for CAR expression then seeded in triplicates in 96 well plates with K562 tumor target cells expressing truncated MUC16 9MUC16t) at an effector to target ratio of 1:1 or the CAR-T cells were cultured in media alone. Culture supernatants were collected after co-culturing for 3 days. Culture supernatants were collected and interferon-gamma

(IFNγ) and granulocyte/macrophage-colony stimulating factor (GM-CSF) was assessed by multiplex cytokine analysis (Luminex) according to manufacturer's protocol. Shown in FIG. 6 is the mean ± SD from duplicate wells.

[0698] For the CAR-T cell constructs cultured in media only had basal levels of IFNγ and GM-CSF detected. No cytokines were observed from the use of target cells or media only. The supernatants following co-culture of MUC16 CAR-T cells only (Vector 1) with the tumor cells provided a baseline value for expression levels of IFNγ and GM-CSF. With the inclusion of checkpoint inhibitor miRNA into the CAR constructs, improved cytokine expression may be observed, particularly through inhibition via the PD-1 pathway. In the constructs that contained dual PD-1 (Construct #3) or the combination of a single PD-1 and TIGIT miRNA (Construct #6) or dual PD-1 and a TIGIT miRNA (Constructs #10 and 11) provided higher levels of cytokine expression.

Table 11: Description of Constructs #1-11 in FIGS. 6A-D (sequences of miRNA are as described in Table 4)

Constructs	miRNA	Effector genes
1	N/A	MUC16 CAR-mbIL15-HER1t
2	Scrambled control 1	MUC16 CAR-mbIL15-HER1t
3	miR204 PD1+ miR206 PD1	MUC16 CAR-mbIL15-HER1t
4	miR17 TIGIT	MUC16 CAR-mbIL15-HER1t
5	miR17 TIGIT+ miR206 PD1	MUC16 CAR-mbIL15-HER1t
6	miR150 TIGIT+ miR206 PD1	MUC16 CAR-mbIL15-HER1t
7	miR17 TIGIT+ miR204 PD1+ miR206 PD1	MUC16 CAR-mbIL15-HER1t
8	miR17 TIGIT+ miR204 PD1+ miR206 PD1extended v1	MUC16 CAR-mbIL15-HER1t
9	miR17 TIGIT+ miR204 PD1+ miR206 PD1extended v2	MUC16 CAR-mbIL15-HER1t
10	miR204 PD1+ miR206 PD1+ miR17 TIGIT	MUC16 CAR-mbIL15-HER1t
11	miR204 PD1+ miR206 PD1+ miR17	MUC16 CAR-mbIL15-HER1t

	TIGITextended v1	

Example 6. Tumor burden in treated mice

[0699] Non-obese diabetic/severe combined immunodeficiency (NOD/SCID) gamma mice (NSG) mice were intraperitoneally implanted with fLUC-GFP+ SK-OV-3 tumor cells expressing MUCt on Day 0. Tumor burden was monitored in these mice throughout the study using an in vivo imaging system (IVIS) by luminescence with an IVIS Spectrum instrument (Perkin Elmer). IVIS data was analyzed using the Living Image Software (Version 4.1) based upon a defined region of interest to obtain total flux values (photons/sec). Prior to administration of the CAR-T cells, mice were randomized based upon tumor burden and body weight into the different groups then administered the test articles on Day 6 All CARs tested expressed the MUC16-specific CAR along with mbIL15 and HER1t and are referred to as MUC16 CAR. All test articles were normalized to a $0.5x10^6$ CAR-T cells/mouse and administered intraperitoneally. IVIS imaging was performed twice/week to monitor overall tumor burden in the mice. Data shown is the mean ±SEM from n=4-8 mice/group.

[0700] As shown in FIG. 7, mice given saline only (gray-filled circles), had continuous tumor growth as evidenced by the increasing total flux levels observed throughout the course of the study. Eventually, these mice succumbed to the tumor burden and were euthanized. Mice given the MUC16 CAR only (black-filled squares) were able to control tumor burden. CAR-T cells expressing the checkpoint inhibitor miRNA to PD-1 and TIGIT within the constructs (open squares and circles) were found to maintain anti-tumor activity, based upon the decrease in tumor flux values to background levels. In addition, the CAR-T cells expressing the checkpoint inhibitor miRNA to PD-1 and TIGIT showed a faster time frame and the rate of tumor burden decrease compared to the MUC16 CAR only construct.

Example 7. *In vivo* phenotyping experiment

[0701] SKOV-3/MUC16 tumor bearing mice were administered CAR-T cells (expressing MUC16-specific CAR, mbIL15 and HER1t) of either CAR only or CAR with PD-1/PD-1 miRNA on Study Day 6. Whole blood from mice were taken on Study Day 31 for phenotypic evaluation

by flow cytometry of the administered CAR-T cells. Briefly, cocktails of fluorescently conjugated antibodies were used to stain the whole blood samples then concurrently fixed along with red blood cells lysis using a one-step Fix/Lyse buffer. The fixed samples were read on the flow cytometer (BD LSRFortessaX-20) instrument. CAR-T cells were identified based upon gating of hCD45/CD3+/HER1t+ expression. In, FIG. 8A, the sample of CAR only (dotted line) shows high PD-1 expression, whereas the CAR with PD-1/PD-1 miRNA (solid line) shows a significant decrease in the level of PD-1 expression detected. To further quantify the reduced expression of PD-1 detected on the CAR+ miRNA (PD-1/PD-1) group, the median fluorescent intensity (MFI) was examined (FIG. 8B). The mean MFI of PD1 expression in mice given CAR-T cells (stripe bar) only was ~709 whereas the mean MFI of the CAR-T cells with a PD-1/PD-1 miRNA (solid bar) was reduced down to ~236. The mean ± SEM from 5-8 mice is shown.

Example 8. Specific PD-1 and TIGIT downregulation

[0702] SKOV-3 tumor bearing mice were administered CAR-T cells (expressing MUC16-specific CAR ("MUC16 CAR"), mbIL15 and HER1t) of either CAR only or CAR with different checkpoint miRNA inhibitors (PD-1 and TIGIT) on Study Day 6. See Table 12.

Table 12: Description of Groups #1-9 in FIGS. 9A-B

Group	CAR-T Cells
1	Saline control
2	MUC16 CAR/mbIL15/HER1t
3	MUC16 CAR/mbIL15/HER1t + anti-PD1
4	MUC16 CAR/mbIL15/HER1t +scrambled miRNA 2
5	MUC16 CAR/mbIL15/HER1t + miR206 PD1
6	MUC16 CAR/mbIL15/HER1t + miR17 TIGIT
7	MUC16 CAR/mbIL15/HER1t + miR204 PD1/miR206 PD1
8	MUC16 CAR/mbIL15/HER1t + miR150 TIGIT/miR206 PD-1
9	MUC16 CAR/mbIL15/HER1t + miR204 PD1/miR206 PD1/miR150 TIGIT

[0703] Whole blood from mice were taken on Study Day 45 (D45) for phenotypic evaluation by flow cytometry of the administered CAR-T cells. Briefly, cocktails of fluorescently conjugated antibodies, which included specific antibodies for human PD-1 and human TIGIT, were used to stain the whole blood samples then fixed using a one step Fix/Lyse buffer. The fixed samples were read on the flow cytometer (BD LSRFortessaX-20) instrument. CAR-T cells were identified in the mouse based upon gating of hCD45/CD3+/HER1t+ expression. To further evaluate the specificity of miRNA used in the CAR vector for the checkpoint inhibitor, the median fluorescent intensity (MFI) for expression of PD-1 and TIGIT was analyzed (FIG.9A and B). The MFI for PD-1 is shown on the left and the MFI for TIGIT is shown on the right for a quantitative assessment of the expression levels for the same set of vectors. As shown in FIG. 9A, reduced expression of PD-1 was seen on the CAR-T cells for the groups indicated with the down arrows (solid line for PD-1), which are constructs with a PD-1 miRNA (single, double and in combination with another miRNA checkpoint inhibitor), when compared to the CAR vector only. On the right side (downward dashed arrows) highlights cell populations with a reduced expression of TIGIT expression seen on the CAR-T cells. The downregulated expression of TIGIT corresponded to the samples that contained a miRNA for TIGIT (either as a single or in combination with other checkpoint miRNA inhibitors). The mean ± SEM from 5-8 mice is shown.

Example 9. Expression of PD1-targeting miRNAs in ROR1-targeted CAR-T cells.

[0704] Briefly, miRNA-expressing ROR1 UltraCAR-T cells or control ROR1 UltraCAR-T cells were generated from T cells from five donors. PanT cells from five healthy donors were transfected with an indicated transposon vector (VVN-5355 or VVN-5351) plus SB11 transposase vector, and expanded by weekly stimulations for 4 weeks (~35 days before adding beads) with ROR1 antigen presenting cells. Following a rest period of 7-8 days, UltraCAR-T cells were activated with CD3/CD28 dynabeads (1:1 bead: T cell ratio, T cells at 1 x 10^6 cells/mL) for 48 hours prior to RNA harvest, 7-8 days after the last AaPC stimulation. Expression of PD1-targeting guide miRNAs and the impact on PD1 mRNA expression were verified by RT-qPCR. Small RNAseq, which is an established method to identify the predicted and alternate miRNA sequences that may arise from a pri-miRNA (Borel et al, 2018; Miniarikova et al, 2016), was performed to compare expression levels of the PD1-targeting guide miRNAs to additional small RNA species, such as passenger miRNA, that may be generated from the PD1 silencer module. Small RNAseq was also

used to assess potential changes on global endogenous miRNA expression (Mueller et al, 2012). RNAseq analysis was performed to evaluate global transcript expression and to identify changes in molecular pathway signaling or off-target gene silencing attributed to the PD1 silencer. *In silico* miRNA target prediction was performed using the miRanda algorithm to identify most likely targets of miRNAs generated from the PD1 silencer module. Expression of the predicted target genes was evaluated by RNAseq. Details of each method is included in the sections below.

[0705] To characterize expression of miRNAs encoded by the PD1 silencer and impact on PD1 transcript levels, RT-qPCR and small RNAseq were performed. To characterize changes in specific genes or cellular pathways, RNAseq was performed.

[0706] Nucleic acids were purified from cell pellets using Qiagen's AllPrep DNA/RNA/miRNA Universal kit (Cat#80224) following the manufacturers protocol. Total RNA was eluted in 50uL nuclease-free water and concentration measured on a NanodropTM 2000 spectrophotometer.

[0707] To quantify the expression of PD1 guide and passenger miRNAs, total RNA was used as input for cDNA synthesis using Qiagen's miRCURY LNA RT Kit (#339340). Per the manufacturer's protocol, cDNA was diluted 1:60 with nuclease-free water and 3µL of the diluted cDNA was used as input for qPCR using miRCURY LNA SYBR Green PCR Kit (Qiagen # 339345) with custom miRCURY LNA primers specific to the two PD1 guide miRNAs. An endogenous miRNA, hsa-let-7a-5p, was quantified as a reference small RNA to allow for input normalization (Qiagen product #339306 with custom #YP00205727). Samples were run in a 384 well format on a QuantStudio 6 Flex instrument. Relative quantification (dCT) calculations were performed in Microsoft Excel and data graphed in GraphPad Prism 9. Calculations for dCT were performed on each technical replicate as follows:

dCT = CT(guide miRNA) – CT(hsa-let-7a)

ddCT= dCT(miRNACART replicate) – dCT(average of VVN-5355 technical replicates)

Fold Change = 2^-ddCT

[0708] The VVN-5355 ROR1 UltraCAR-T control sample serves as the reference control sample for comparison to miRNA expressing ROR1 UltraCAR-T cells within each donor set.

Average fold change and standard deviation of technical replicates was calculated and reported in Figure 10A.

[0709] To quantify and compare the production of guide and passenger miRNAs originating from the PD1 silencing module, RT-qPCR was performed as described above, except this second experiment included primer assays to detect the passenger miRNAs as well as an additional endogenous reference small RNA, RNU1A1. Expression calculations were performed to compare expression of each guide or passenger strand mature miRNA relative to the average of the endogenous control small RNAs as follows:

dCT = CT(mature miRNA) – CT(average of hsa-let-7a and RNU1A1)

Fold change=2^-dCT

[0710] Average fold change was calculated from three technical replicates. These values are plotted in Figure 11 with mean and standard deviation shown for the donor sample sets tested.

[0711] To quantify the expression of endogenous PD1 mRNA, cDNA synthesis was performed using a final RNA concentration of 5ng/µL using Invitrogen's SuperScript IV VILO Master Mix with ezDNase enzyme kit (#11766050). Multiplex Taqman qPCR was performed using Invitrogen's TaqMan MastAdvanced Master Mix (#4444963) with 1 microliter of cDNA and Invitrogen (ThermoFisher Scientific) Taqman assays. Human PD1 Taqman assay (Invitrogen# Hs00169472_m1) was FAM labeled and the internal normalizer gene, ACTb (Invitrogen# Hs99999903_m1), which was VIC-labelled. Samples were run in a 384 well format on a QuantStudio 6 Flex instrument. Relative quantification (dCT) calculations were performed in Microsoft Excel as described above and data graphed in GraphPad Prism 9.

Table 13: Test and Control Articles

Sample Name	Description	Designation
VVN-5355	CAR-T cells generated using *Sleeping Beauty* transposon plasmid VVN-5355, which contains a transgene cassette to express ROR1-specific CAR + mbIL-15 + HER1t.	Control

VVN-5351	CAR-T cells generated using *Sleeping Beauty* transposon plasmid VVN-5351, which contains a transgene cassette to express ROR1-specific CAR + mbIL-15 + HER1t plus a PD1 silencer module.	Test Article

[0712] The PD1 Silencer Module is designed to produce two mature guide miRNAs that bind to the PD1 transcript to silence PD1 expression. Expression of the two PD1-targeting guide miRNAs, referred to as PD1_1843 and PD1_2061, was confirmed in miRNA expressing ROR1 UltraCAR-T cells generated from multiple donors (Figure 10A). A corresponding reduction in PD1 mRNA expression was verified in the miRNA-expressing ROR1 UltraCAR-T cells from all donors tested (Figure 10B). This result demonstrated that the PD1 Silencer module produces the intended guide miRNAs and functions as designed.

[0713] To reduce the risk of silencing genes other than PD1, the PD1 silencer module was designed using pri-miRNA scaffolds that preferentially produce PD1-targeting guide miRNA over the non-targeting passenger miRNA. Both guide and passenger mature miRNAs were quantified from miRNA expressing ROR1 UltraCAR-T cells by RT-qPCR, which verified PD1 targeting guide miRNAs as the predominant species compared to non-targeting passenger strand miRNA (Figure 4). The strong processing preference for the PD1 targeted guide miRNA was confirmed by small RNAseq, with 99.7% of reads mapping to the PD1 Silencer Module matching the intended PD1 targeting guide miRNAs (Figures 12 A-E). Furthermore, the start and stop position of the miRNAs was as expected, with miRNAs of 21-23 nucleotides detected that had the same 5' end and variable length at the 3' end (Figures 12 A-E). The extremely low incidence of passenger strand miRNAs and lack of unexpected small RNAs generated by aberrant RNA processing substantially reduced the risk for off-target gene silencing.

[0714] To ensure that expression of the PD1 Silencer module does not overwhelm the internal cellular RNAi machinery, endogenous miRNA counts were compared from miRNA expressing ROR1 UltraCAR-T cells vs control ROR1 UltraCAR-T cells. Examination of the top twenty expressed endogenous miRNAs demonstrated no statistically significant changes in expression across the samples (Table 15). Furthermore, the mature miRNAs generated from the PD1 silencer

module accounted for approximately 4% of all quantified small RNAs (Figure 13). The data indicated that expression of miRNAs from the PD1 silencer module did not saturate the cellular RNAi machinery and had no detectable impact on global endogenous miRNA expression.

Table 14: Small RNAseq Comparison of Guide and Passenger miRNA Counts

Mature miRNA_Length (Sequence)	Normalized Mean	% Total
Guide PD1_204_21nt (TTCAGGAATGGGTTCCAAGGA; SEQ ID NO: 704)	486,432.3	88.2%
Guide PD1_204_22nt (TTCAGGAATGGGTTCCAAGGAT; SEQ ID NO: 72)	12,763.5	
Guide PD1_204_23nt (TTCAGGAATGGGTTCCAAGGATG; SEQ ID NO: 705)	59,759.4	
Passenger PD1_204_21nt (TCCTGGAAGCTATTCCTGACG; SEQ ID NO: 706)	380.6	0.1%
Passenger PD1_204_22nt (TCCTGGAAGCTATTCCTGACGT; SEQ ID NO: 707)	20.2	
Passenger PD1_204_23nt (TCCTGGAAGCTATTCCTGACGTT; SEQ ID NO: 708)	54.7	
Guide PD1_206_21nt (TATAATATAATAGAACCACAG; SEQ ID NO: 709)	3,752.7	11.5%
Guide PD1_206_22nt (TATAATATAATAGAACCACAGG; SEQ ID NO: 74)	49,181.3	
Guide PD1_206_23nt (TATAATATAATAGAACCACAGGA; SEQ ID NO: 710)	19,695.3	
Passenger PD1_206_21nt (TGTGGTTCTGTTATATCCATA; SEQ ID NO: 711)	1,581.0	0.3%
Passenger PD1_206_22nt (TGTGGTTCTGTTATATCCATAT; SEQ ID NO: 712)	3.1	

Passenger PD1_206_23nt (TGTGGTTCTGTTATATCCATATA; SEQ ID NO: 713)	13.2	

Table 15: Top 20 Expressed Endogenous Mature miRNAs Detected by Small RNAseq

Endogenous miRNA	Base Mean	Log2 Fold Change	P-value Adjusted
hsa-miR-181a-5p	796180.25	0.11	0.7948
hsa-miR-191-5p	421094.34	-0.19	0.5622
hsa-miR-21-5p	386727.78	0.03	0.9411
hsa-miR-92a-3p	368149.34	0.26	0.2298
hsa-miR-142-5p	345451.62	0.08	0.8549
hsa-miR-146b-5p	286127.09	0.24	0.5471
hsa-miR-16-5p	261883.66	0.00	0.9954
hsa-miR-146a-5p	229059.23	-0.27	0.4569
hsa-let-7f-5p	188491.61	0.16	0.6018
hsa-miR-22-3p	123453.58	-0.01	0.9899
hsa-miR-26a-5p	110714.77	0.19	0.5166
hsa-let-7a-5p	104253.31	0.00	0.9921
hsa-miR-155-5p	72533.08	-0.29	0.2298
hsa-miR-181b-5p	67527.35	0.12	0.7687
hsa-let-7i-5p	64631.72	0.30	0.2116
hsa-miR-21-3p	52519.27	0.25	0.5358
hsa-miR-186-5p	52479.56	-0.20	0.4189
hsa-miR-25-3p	50411.16	0.02	0.9696
hsa-miR-27a-3p	47911.68	-0.10	0.7818
hsa-let-7g-5p	47159.42	0.01	0.9811

Example 10: PD1 Silencer Module Specifically Reduces Expression of PD-1.

[0715] An *in silico* miRNA target prediction algorithm, miRanda (Betel et al, 2008; Betel et al, 2010), was used to predict the most likely target transcripts for the guide and passenger miRNAs generated from the PD1 silencer module. The algorithm assigned a score for each potential miRNA target gene, with higher scores indicating higher likelihood of silencing by the input miRNA sequence. A summary of the top ten hits for each mature miRNA generated from the PD1 silencing module is listed in Table 16. PD1 is the only gene with perfect homology to any of the guide and

passenger miRNAs and has the highest predicted miRanda score of any potential target gene. Expression of each predicted target gene was characterized from the RNAseq differential expression data set. PD1 was the most downregulated of all predicted target genes, with a log2 fold change (LFC) of -2.63 (~84% PD1 reduction in miRNA expressing ROR1 UltraCAR-T cells compared to control ROR1 CAR-T cells) and a highly significant adjusted p-value. The expression of other predicted target genes was unchanged; those genes with adjusted p-values below 0.05 had LFC in the range of -0.33 to 0.22, which is an expression decrease or increase of <20%. One exception is a weakly predicted target gene of PD1_2061 guide miRNA, HDAC9, which has a LFC of -1.51. HDAC9 is a transcriptional repressor that is mechanistically linked to PD1 expression through BCL6 (Xie et al, 2017; Gil et al, 2016). It is likely that HDAC9 is not directly targeted by PD1_2061 guide miRNA, and that the reduction in HDAC9 is an indirect effect of reduced PD1 expression.

Table 16: *In silico* Predicted miRNA Target Genes

miRNA	Target Gene	miRanda Score	free energy kcal/mol	% Identity to Target	Base Mean Count	log2 FoldChange	lfcSE	P value	Adjusted P Value
PD1_1843 miR204 Guide miRNA	**PD1**	**200**	**-41.9**	**100.0**	**84**	**-2.63**	**0.293**	**2.51E-19**	**2.64E-16**
	MIER3	184	-32.1	84.2	1073	0.01	0.063	0.9104	0.9688
	SLFN12L	182	-34.2	89.5	1404	-0.22	0.068	0.0015	**0.0134**
	FMO4	180	-28.5	84.2	15	-0.18	0.461	0.6975	NA
	GIGYF1	180	-33.5	84.2	2749	-0.07	0.046	0.1123	0.3211
	FAM83G	180	-31.5	84.2	171	0.33	0.144	0.0214	0.1026
	PPRC1	180	-31.2	84.2	2679	-0.18	0.045	0.0001	**0.0011**
	PRMT9	180	-29.1	84.2	335	0.09	0.100	0.3704	0.6432
	MGAT4B	179	-29.3	88.9	4480	-0.21	0.063	0.0007	**0.0076**
	BRWD1	179	-25.0	88.9	1816	0.05	0.052	0.3581	0.6328
PD1_1843 miR204 Passenger miRNA	DOCK9	179	-28.5	83.3	1239	-0.12	0.077	0.1240	0.3428
	NFYA	179	-27.5	83.3	1615	-0.02	0.052	0.7446	0.8911
	IFIT5	178	-30.4	89.5	379	-0.11	0.102	0.2629	0.5351
	OTUB2	178	-33.7	84.2	248	-0.32	0.131	0.0154	0.0805
	UBIAD1	177	-32.1	88.9	1064	-0.07	0.066	0.3085	0.5859

	MYO15A	176	-29.5	93.3	11	-0.25	0.510	0.6263	NA
	ADRB2	175	-27.1	83.3	62	0.61	0.264	0.0206	0.0997
	HIC2	175	-28.5	83.3	312	-0.01	0.102	0.8962	0.9621
	NECTIN3	175	-33.5	72.2	662	0.05	0.081	0.5587	0.7864
	TBC1D10C	175	-27.3	77.8	7562	0.03	0.041	0.4409	0.7056
PD1_2061 miR206 Guide miRNA	**PD1**	**200**	**-34.3**	**100.0**	**84**	**-2.63**	**0.293**	**2.51E-19**	**2.64E-16**
	LCOR	180	-16.7	84.2	1321	-0.19	0.064	0.0029	**0.0230**
	STARD4	179	-16.7	88.9	6871	-0.18	0.055	0.0013	**0.0120**
	UQCRC2	179	-19.1	81.0	4759	0.03	0.043	0.4641	0.7216
	EXD2	178	-17.5	88.2	58	0.18	0.265	0.5055	0.7511
	NOL8	177	-18.6	88.9	2190	-0.08	0.050	0.1007	0.2996
	ATP23	176	-18.6	84.2	318	0.22	0.100	0.0265	0.1201
	ELAPOR2	176	-20.6	84.2	70	-0.08	0.211	0.7139	0.8793
	HDAC9	176	-19.5	84.2	73	-1.51	0.280	6.77E-08	**3.53E-06**
	WDR47	176	-19.6	79.0	625	0.08	0.089	0.3892	0.6617
PD1_2061 miR206 Passenger miRNA	COG2	172	-18.9	93.3	1244	0.06	0.060	0.3399	0.6170
	NRG4	171	-25.6	77.8					
	AGBL3	170	-19.2	79.0	49	0.01	0.250	0.9811	0.9934
	PDK3	170	-20.9	100.0	605	0.18	0.081	0.0256	0.1169
	PDXK	170	-21.7	82.4	1786	0.22	0.058	0.0002	**0.0022**
	N4BP2	169	-25.1	77.8	2834	-0.33	0.051	1.86E-10	**1.95E-08**
	ZNF271P	169	-23.8	83.3	777	0.03	0.074	0.7197	0.8812
	NFKB1	168	-18.8	88.2	7889	-0.11	0.039	0.0036	**0.0269**
	XYLT1	168	-26.9	82.4	5281	0.08	0.051	0.1100	0.3177
	CNOT6	167	-20.1	72.2	2030	-0.24	0.052	0.0000	**0.0001**

[0716] It is expected that changes in PD1 expression will impact expression of other genes in downstream pathways. As expected, analysis of RNAseq data confirmed the differential expression of several genes in miRNA expressing ROR1 UltraCAR-T cells compared to the control ROR1 UltraCAR-T cells (Figures 14 A and B, Table 17). To elucidate direct versus indirect changes in gene expression, the differential expression (LFC) in miRNA expressing ROR1 UltraCAR-T cells compared to control ROR1 CAR-T cells was plotted against the predicted

binding potential (predicted free energy) of the PD1 miRNAs for genes, which were in silico predicted as potential PD1 miRNA targets (Figures 15 A-D). Genes with a highly negative free energy and a statistically significant reduction in expression are likely to be directly targeted by miRNA, while downregulated genes with a weak free energy are likely to be indirectly impacted by the miRNAs. PD1 is clearly separated from all other genes in the plots with a strong reduction in expression and high miRNA binding potential, which suggests a strong and preferential direct targeting of PD1, but not other genes, by the PD1 silencer miRNAs.

Table 17: Top 10 Down- and Up-Regulated Genes in ROR1+PD1 Silencer Cells Relative to Control ROR1 UltraCAR-T Cells

Ensembl_ID	Symbol	base Mean	Log2Fold Change	lfcSE	stat	pvalue	padj
ENSG00000188389	PDCD1	84.2	-2.6	0.3	-9.0	2.51E-19	2.64E-16
ENSG00000172020	GAP43	71.6	-2.2	0.4	-5.7	1.37E-08	9.21E-07
ENSG00000171951	SCG2	110.5	-2.2	0.4	-6.1	1.13E-09	9.89E-08
ENSG00000105974	CAV1	172.3	-2.1	0.5	-4.2	2.93E-05	5.78E-04
ENSG00000136193	SCRN1	37.4	-1.9	0.5	-3.8	0.000136	1.97E-03
ENSG00000130287	NCAN	206.1	-1.7	0.4	-4.4	1.23E-05	2.84E-04
ENSG00000156052	GNAQ	145.0	-1.7	0.2	-7.8	6.88E-15	2.18E-12
ENSG00000135898	GPR55	84.5	-1.6	0.3	-5.7	1.47E-08	9.48E-07
ENSG00000152495	CAMK4	856.5	-1.6	0.2	-7.5	9.08E-14	2.21E-11
ENSG00000102271	KLHL4	184.3	-1.6	0.3	-4.7	3.00E-06	8.90E-05
ENSG00000165025	SYK	120.2	1.6	0.4	4.0	6.65E-05	1.10E-03
ENSG00000276597	TRBV11-3	123.1	1.6	0.6	2.7	0.006913	4.44E-02
ENSG00000082781	ITGB5	329.1	1.7	0.3	5.7	1.45E-08	9.44E-07

ENSG00000244242	IFITM10	555.8	1.7	0.2	7.6	4.18E-14	1.10E-11
ENSG00000143842	SOX13	116.9	1.7	0.2	7.6	3.87E-14	1.06E-11
ENSG00000180549	FUT7	318.7	1.7	0.4	4.3	1.71E-05	3.71E-04
ENSG00000137474	MYO7A	1057.3	1.8	0.2	8.2	3.56E-16	1.62E-13
ENSG00000177508	IRX3	317.9	1.8	0.5	3.3	0.001046	1.03E-02
ENSG00000136689	IL1RN	242.1	1.9	0.5	4.0	5.65E-05	9.70E-04
ENSG00000189013	KIR2DL4	800.0	1.9	0.3	6.4	1.51E-10	1.62E-08

Example 11

[0717] In one instance a patient is diagnosed with a particular cancer. In this example the patient is diagnosed with breast cancer. The patient is identified to receive a particular chimeric antigen receptor therapy. The patient provides an initial blood sample for isolation of the desired cell type. In this example, the isolated cell type is a T-cell. In this example only a portion of the isolated T-cells are transfected with the desired initial CAR selected from the collection of CARs. The remaining T-cells are coded and stored in appropriate condition for potential future use by the patient. The remaining T-cells are transfected through an appropriate means. In this example, the transfection occurs through a non-viral means. In particular, the non-viral means utilizes a single sleeping beauty vector that encodes for at least the selected CAR, a T-cell expansion cytokine, and a termination switch. The patient is treated within 48 hours from the initial T-cell isolation.

[0718] Once the above round of treatment is complete the patient is followed in accordance with monitoring protocols. In the event there is an expansion of the cancer, in this example breast cancer, the patient is reevaluated. The patient may be redosed with the initial CAR, however, in the event the evaluation demonstrates that after the initial or potential redose there is antigen escape the protocol will call for a dosing of a new CAR from the CAR collection. In this instance, the patient's stored T-cells will be selected for use in the transfection of the newly selected CAR. The patient will be able to be redosed in a timely fashion. Further as the patient has already received each portion of the follow-on therapy except the newly selected CAR, the risk of toxicities and or

adverse events is reduced. Further the knowledge of previous T-cell expansion for this patient is informed by the levels of expansion from the initial CAR selection. This further allows for a potential change in the dosage size, be it greater or less than the initial dose.

Example 12

[0719] In one instance a patient is not diagnosed with a particular cancer, but rather a specific antigen has been identified as causing undesired proliferation of cells or expansion of an infective agent. The initial CAR is selected based on antigen being presented in the patient. The patient provides an initial blood sample for isolation of the desired cell type. In this example, the isolated cell type is a T-cell. In this example all of the isolated T-cells are transfected with the desired initial CAR selected from the collection of CARs. The T-cells are transfected through an appropriate means. In this example, the transfection occurs through a non-viral means. In particular, the non-viral means utilizes a single sleeping beauty vector that encodes for at least the selected CAR, a T-cell expansion cytokine, and a kill switch. The patient is treated within 48 hours from the initial T-cell isolation.

[0720] Once the above round of treatment is complete the patient is followed in accordance with monitoring protocols. Following further evaluation the patient demonstrates that there is antigen escape. The patient is then dosed with a new CAR from the CAR collection following collection of new T-cells. Further as the patient has already received each portion of the follow-on therapy except the newly selected CAR, the risk of toxicities and or adverse events is reduced. Further the knowledge of previous T-cell expansion for this patient is informed by the levels of expansion from the initial CAR selection. This further allows for a potential change in the dosage size, be it greater or less than the initial dose.

Example 13

[0721] The clinician develops a treatment plan for a patient that involves use of an initial CAR from the CAR collection. The treatment plan includes a dosing of different CARs from the collection in consecutive manner to prevent antigen escape.

Example 14

[0722] In one instance a patient is diagnosed with a particular cancer. In this example the patient is diagnosed with pancreatic cancer. The patient is identified to receive a particular chimeric antigen receptor therapy. The patient provides an initial blood sample for isolation of the desired cell type. In this example, the isolated cell type is a T-cell. In this example the isolated T-cell's are transfected with the desired initial CAR selected from the collection of CARs. The T-cells are transfected through a non-viral means utilizing a single sleeping beauty vector that encodes for at least the selected CAR, a T-cell expansion cytokine, and a kill switch. The patient is treated within 48 hours from the initial T-cell isolation.

[0723] Once the above round of treatment is complete the patient is followed in accordance with monitoring protocols. In this instance, the patient through reevaluation is noted to suffer antigen escape. In this instance the patient requires dosing with a new CAR. However, due to the condition of the patient a sufficient sample of autologous T-cell may not be obtained. As a result the method allows for the inclusion of allogenic T-cells to be incorporated to the therapy. The CAR from the library is transduced into the allogenic T-cells and the patient is dosed in a timely fashion. Further as the patient has already received each portion of the follow-on therapy, the risk of toxicities and or adverse events is reduced.

Example 15

[0724] In one instance a patient is not diagnosed with a particular cancer, but rather a specific antigen has been identified as causing undesired proliferation of cells or expansion of an infective agent. The initial CAR is selected based on antigen being presented in the patient. The patient is unable to provide an initial blood sample for isolation of the desired cell type due to the current health of the patient. As such, allogenic T-cells are transfected with the desired initial CAR selected from the collection of CARs. The T-cells are transfected through an appropriate means utilizing a single attsite vector that encodes for at least the selected CAR, a T-cell expansion cytokine, and a termination switch.

[0725] Once the above round of treatment is complete the patient is followed in accordance with monitoring protocols. Following further evaluation the patient demonstrates that there is antigen escape. The patient is then dosed with a new CAR using the same source of allogenic T-cells. Further as the patient has already received each portion of the follow-on therapy except the newly

selected CAR, the risk of toxicities and or adverse events is reduced. Further the knowledge of previous T-cell expansion for this patient is informed by the levels of expansion from the initial CAR selection. This further allows for a potential change in the dosage size, be it greater or less than the initial dose.

Example 16

[0726] The clinician develops a treatment plan for a patient that involves use of an initial CAR from the CAR collection. The treatment plan includes a dosing of different CARs from the collection in consecutive manner to prevent antigen escape. This treatment plan may include the use of both autologous and allogenic cells.

Example 17

[0727] In one instance a patient is diagnosed with a specific antigen has been identified as causing undesired proliferation of cells or expansion of an infective agent. The initial CAR is selected based on antigen being presented in the patient. The patient is unable to provide an initial blood sample for isolation of the desired cell type due to the current health of the patient. As such, allogenic cells are transfected with the desired initial CAR selected from the collection of CARs. The cells are transfected through an appropriate means utilizing a single sleeping beauty vector that encodes for at least the selected CAR and a termination switch.

[0728] Once the above round of treatment is complete the patient is followed in accordance with monitoring protocols. Following further evaluation the patient demonstrates that there is antigen escape. The patient is then dosed with a new CAR using a source of allogenic T-cells or if the patient show sufficient improvement autologous T-cells.

Example 18

[0729] In one instance a patient is not diagnosed with a particular cancer, but rather a specific antigen has been identified as causing undesired proliferation of cells or expansion of an infective agent. The initial CAR is selected based on antigen being presented in the patient. The patient provides an initial blood sample for isolation of T-cells. The T-cells are transfected with the desired initial CAR selected from the collection of CARs utilizing a single sleeping beauty vector that

encodes for the selected CAR, a T-cell expansion cytokine, and a kill switch. The patient is treated within 48 hours from the initial T-cell isolation.

[0730] Once the above round of treatment is complete the patient is followed in accordance with monitoring protocols. Following further evaluation the patient demonstrates that there is antigen escape. The patient is then dosed with the same CAR and a new CAR from the CAR collection.

Example 19

[0731] In one instance a patient is diagnosed with a particular cancer. In this example the patient is diagnosed with AML. The patient is identified to receive a particular chimeric antigen receptor therapy. The patient provides an initial blood sample for isolation of the desired cell type. The cells are transfected with the desired two different CARs selected from the collection of CARs. The cells are transfected through a non-viral means utilizing a single vector that encodes for both selected CARs and a kill switch.

[0732] Once the above round of treatment is complete the patient is followed in accordance with monitoring protocols. In this instance, the patient through reevaluation is noted to suffer antigen escape. In this instance the patient requires dosing with a new CAR. However, due to the condition of the patient a sufficient sample of autologous cell may not be obtained. As a result the method allows for the inclusion of allogenic T-cells to be incorporated to the therapy. The CAR from the library is transduced into the allogenic cells and the patient is dosed in a timely fashion.

Nucleic Acid Sequences Encoding Backbone miRNA Sequences			
Synthetic miRNA	**DNA encoding 5' backbone sequence**	**DNA encoding stem loop**	**DNA encoding 3' backbone sequence**
miR204	AGGAGGGTGGGGGTGG AGGCAAGCAGAGGACTT CCTGATCGCGTACCCATG GCTACAGTCTTTCTTCATG TGACTCGTGGAC (SEQ ID NO: 1)	GAGAATATATGAAGG (SEQ ID NO: 2)	GTTCAATTGTCATCACTG GCATCTTTTTTGATCATTG CACCATCATCAAATGCAT TGGGATAACCATGAC (SEQ ID NO: 3)
miR206	GATGCTACAAGTGGCCCA CTTCTGAGATGCGGGCTG CTTCTGGATGACACTGCT TCCCGAGG (SEQ ID NO: 4)	TATGGATTACTTTGCTA (SEQ ID NO: 5)	TTTCGGCAAGTGCCTCCT CGCTGGCCCCAGGGTACC ACCCGGAGCACAGGTTTG GTGACCTT (SEQ ID NO: 6)
miR17	TTAGCAGGAAAAAAGAG AACATCACCTTGTAAAAC TGAAGATTGTGACCAGTC AGAATAATGT (SEQ ID NO: 7)	TGATATGTGCAT (SEQ ID NO: 8)	GCATTATGGTGACAGCTG CCTCGGGAAGCCAAGTTG GGCTTTAAAGTGCAGGG CCTGCTGATGT (SEQ ID NO: 9)
miR150	AGGGACTGGGCCCACGG GGAGGCAGCGTCCCCGA GGCAGCAGCGGCAGCGG CGGCTCCTCTCCCCATGG CCCTG (SEQ ID NO: 10)	CTGGGCTCAG (SEQ ID NO: 11)	CAGGGACCTGGGGACCC CGGCACCGGCAGGCCCC AAGGGGTGAGGTGAGCG GGCATTGGGACCTCCCCT CCCTGTACTC (SEQ ID NO: 12)
miR150	AGGGACTGGGCCCACGG GGAGGCAGCGTCCCCGA GGCAGCAGCGGCAGCGG CGGCTCCTCTCCCCATGG CC (SEQ ID NO: 13)	TGCTGGGCTCAGACC (SEQ ID NO: 14)	GGACCTGGGGACCCCGG CACCGGCAGGCCCCAAG GGGTGAGGTGAGCGGGC ATTGGGACCTCCCCTCCC TGTACTC (SEQ ID NO: 15)
miR16	CTTCTGAAGAAAATATAT TTCTTTTTATTCATAGCTC TTATGATAGCAATGTCAG CAGTGCCT (SEQ ID NO: 16)	TTAAGATTCTAAAATTATC T (SEQ ID NO: 17)	AAGTAAGGTTGACCATAC TCTACAGTTGTGTTTTAAT GTATATTAATGTTACTAAT GTGTTTT (SEQ ID NO: 18)
miR30a	CAGGTTAACCCAACAGAA GGCTAAAGAAGGTATATT GCTGTTGACAGTGAGCG AC (SEQ ID NO: 19)	CTGTGAAGCCACAGATG GG (SEQ ID NO: 20)	TGCCTACTGCCTCGGACT TCAAGGGGCTACTTTAGG AGCAATTATCTTGTTTA (SEQ ID NO: 21)

miR126	CCGGGGTCCTGTCTGCATCCAGCGCAGCATTCTGGAAGACGCCACGCCTCCGCTGGCGACGG (SEQ ID NO: 22)	CTGTGACACTTCAAAC (SEQ ID NO: 23)	CCGTCCACGGCACCGCATCGAAAACGCCGCTGAGACCTCAGCCTTGACCTCCCTCAGCGTGG (SEQ ID NO: 24)
miR122	CAATGGTGGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTACAGAGTTTCCTTAGCAGAGCTG (SEQ ID NO: 25)	TGTCTAAACTAT (SEQ ID NO: 26)	TAGCTACTGCTAGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTGAGCAAGAAGGTTCATCTGATATCAGTCTTCTCAATCT (SEQ ID NO: 27)
miR214	ACAGGCTGATTGTATCTGTCTATGAGCAAAGGAAACCTGAAGGAACCAAGGGCCTGGCTGGACAGAGTTGTCATGTG (SEQ ID NO: 28)	GCAGAACATCCGCTCACCTGT (SEQ ID NO: 29)	CACATGACAACCCAGCCTGAATGACAACCAGCCATTGAAAGAAAGCAGCCCTCACACCATAGCATCTA (SEQ ID NO: 30)
miR214	ACAGGCTGATTGTATCTGTCTATGAGCAAAGGAAACCTGAAGGAACCAAGGGCCTGGCTGGACAGAGTTGTCATGTGTC (SEQ ID NO: 31)	AGAACATCCGCTCACCT (SEQ ID NO: 32)	CACATGACAACCCAGCCTGAATGACAACCAGCCATTGAAAGAAAGCAGCCCTCACACCATAGCATCTA (SEQ ID NO: 33)
miR29b1	GGGTTTATTGTAAGAGAGCATTATGAAGAAAAAAATAGATCATAAAGCTTCTTCAGG (SEQ ID NO: 34)	GATTTAAATAGTGATTGTC (SEQ ID NO: 35)	CTTGGGGGAGACCAGCTGCGCTGCACTACCAACAGCAAAAGAAGTGAATGGGACAGCT (SEQ ID NO: 36)
miR29b1	GGGTTTATTGTAAGAGAGCATTATGAAGAAAAAAATAGATCATAAAGCTTCTTCAGGAA (SEQ ID NO: 37)	TTTAAATAGTGATTG (SEQ ID NO: 38)	GTTCTTGGGGGAGACCAGCTGCGCTGCACTACCAACAGCAAAAGAAGTGAATGGGACAGCT (SEQ ID NO: 39)
miR133a1	TTTACCAATGAAAAGCATTTAACTGTTTTGGATTCCAAACTAGCAGCACTACAATGCTTTGCTA (SEQ ID NO: 40)	TCGCCTCTTCAATGGA (SEQ ID NO: 41)	TAGCTATGCATTGATTACTACGGGACAACCAACGTTTTCATTTGTGAATATCAATTACTTGCCA (SEQ ID NO: 42)

miR26a	TGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCG (SEQ ID NO: 43)	GTGCAGGTCCCAATG (SEQ ID NO: 44)	CGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC (SEQ ID NO: 45)
miR412	GTCTTGGAGGCTGGGGCACCTCGGGGAAGGACGCCGGCATCAGCACCATTCTGGGGTACGGGGATGGA (SEQ ID NO: 46)	GTTTCT (SEQ ID NO: 47)	GCCGTCCGTATCCGCTGCAGCCTGTGGGGCCTGCGGGCCGGGGAGCCGATCGCGCTTCAGCTCAGCGCCT (SEQ ID NO: 48)
miR-19	CCAATAATTCAAGCCAAGCAAGTATATAGGTGTTTTAATAGTTTTTGTTTGCAGTCCTCTG (SEQ ID NO: 49)	TACAAGAAGAATGTAGT (SEQ ID NO: 50)	TGGTGGCCTGCTATTTCCTTCAAATGAATGATTTTTACTAATTTTGTGTACTTTTATTGTG (SEQ ID NO: 51)
miR-21	TGTCTGCTTGTTTTGCCTACCATCGTGACATCTCCATGGCTGTACCACCTTGTCGGG (SEQ ID NO: 52)	CTGTTGAATCTCATGG (SEQ ID NO: 53)	TCTGACATTTTGGTATCTTTCATCTGACCATCCATATCCAATGTTCTCATTTAAACA (SEQ ID NO: 54)
miR-142	GGAGTCAGGAGGCCTGGGCAGCCTGAAGAGTACACGCCGACGGACAGACAGACAGTGCAGTCACC (SEQ ID NO: 55)	AACAGCACTGGAGGG (SEQ ID NO: 56)	GATGAGTGTACTGTGGGCTTCGGAGATCACGCCACTGCTGCCGCCCGCTGCCCGCCACCATCTTC (SEQ ID NO: 57)
miR-494	CAGTTCTGTTTTGATTTTTTTGTTTGTTTTTTGATCAGTGCTAATCTTCGATACTCGAAGGA (SEQ ID NO: 58)	TCTTTATTTATGA (SEQ ID NO: 59)	TTTTTTAGTATCAAATCCCACCCTGGAGGCACTTCCTGTTCCTGATGCAGCCTTCAGGGAGG (SEQ ID NO: 60)
miR-1915	CGGACCACGGTGTCCCCTTCTCTCCAGCTGGGGGTCTCGGGTCCTGGCGCTGAGAGGCCGC (SEQ ID NO: 61)	GTGCACCCGTG (SEQ ID NO: 62)	GCGGCCCTAGCGACCTGCGGCGGCGCCGGGAAAGCCCTGCCTCTGCAGCGGGTCCCAGGGGTC (SEQ ID NO: 63)

Nucleic acid sequences encoding mature miRNA sequences			
miRNA Target	**Backbone**	**DNA encoding guide miRNA**	**DNA encoding passenger miRNA**
CTLA4	miR-204	AATATAGTCTTCTCCCTCGCTT (SEQ ID NO: 64)	AAGCAGGGACAGGACTATATT (SEQ ID NO: 65)
CTLA4	miR-26a	AATATAGTCTTCTCCCTCGCTT (SEQ ID NO: 66)	GAGCGAGGGATAGGACTATACT (SEQ ID NO: 67)
CTLA4	miR-30a	AATATAGTCTTCTCCCTCGCTG (SEQ ID NO: 68)	CAGTGAGGGAAGACTATGTT (SEQ ID NO: 69)
CTLA4	miR-206	AATATAGTCTTCTCCCTCGCTG (SEQ ID NO: 70)	CAGCGAGGGAGAAGATTACCATT (SEQ ID NO: 71)
PD1	miR-204	TTCAGGAATGGGTTCCAAGGAT (SEQ ID NO: 72)	ATCCTGGAAGCTATTCCTGAC (SEQ ID NO: 73)
PD1	miR-206	TATAATATAATAGAACCACAGG (SEQ ID NO: 74)	CCTGTGGTTCTGTTATATCCATA (SEQ ID NO: 75)
PD1	miR-30a	TTCAGGAATGGGTTCCAAGGAG (SEQ ID NO: 76)	CTCCTTGGACCATTCCTGAA (SEQ ID NO: 77)
PD1	miR-412	TTCAGGAATGGGTTCCAAGGAATT (SEQ ID NO: 78)	AATGTCCTGAAGCCATTCATGGA (SEQ ID NO: 79)
PD1	miR122	TTCAGGAATGGGTTCCAAGGAG (SEQ ID NO: 80)	CTCCTTGGAACACATTCCTACA (SEQ ID NO: 81)
PD1	miR-17	TTCAGGAATGGGTTCCAAGGAAG (SEQ ID NO: 82)	CTTCTTGGTACACGTTCCTGCTCA (SEQ ID NO: 83)
PD1	miR-150	TATAATATAATAGAACCACAGG (SEQ ID NO: 74)	ACGTGTGGTTTATCCTGTTGTA (SEQ ID NO: 85)
PD1	miR-486	TATAATATAATAGAACCACAGG (SEQ ID NO: 74)	CCAGCTTGTGGTTCTATTATGTTATA (SEQ ID NO: 87)
TIGIT	miR-17	AGATCCACGTTACTCACCCTAG (SEQ ID NO: 88)	CTAGGGTGTGTCATGTGGATGAA (SEQ ID NO: 89)
TIGIT	miR-150	AGATCCACGTTACTCACCCGTG (SEQ ID NO: 90)	ACCCGGGTGATAATATGGATCT (SEQ ID NO: 91)

TIGIT	miR-204	AGATCCACGTTACTCACCCCCT (SEQ ID NO: 92)	AGGGGTGAGAAGCGTGGATCC (SEQ ID NO: 93)
TIGIT	miR29b1	AGATCCACGTTACTCACCCTTA (SEQ ID NO: 94)	TAGGTGAGTTACGTGGATCTGTT (SEQ ID NO: 95)
TIGIT	miR214	AGATCCACGTTACTCACCCTGC (SEQ ID NO: 96)	GTAGGGTGAGACTCGTGGATCT (SEQ ID NO: 97)
TIGIT	miR-206	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 98)	TCTGCGTAGCAGTCATCGCCGGT (SEQ ID NO: 99)
TIGIT	miR-204	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 100)	TCTGATGGCCGTCATCGTGGC (SEQ ID NO: 101)
TIGIT	miR-22	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 102)	TCTGTACAGCAGCATCGATGGT (SEQ ID NO: 103)
TIGIT	miR-16	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 104)	TGCGCAGCTGTTCATCGTGGT (SEQ ID NO: 105)
TIGIT	miR-21	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 106)	CTGCGAAGCAGCATCGTGGC (SEQ ID NO: 107)
TIGIT	miR-494	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 108)	TCTGCAACGCAGTCTCGCTGG (SEQ ID NO: 109)
TIGIT	miR-142	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 110)	GATGCACAGCAATCATCGTGGT (SEQ ID NO: 111)
TIGIT	miR-19	ACCACGATGACTGCTGTGCAGAT (SEQ ID NO: 112)	ATCTGCACAGCAGTTCGTGG (SEQ ID NO: 113)
TIGIT	miR-1915	ACCACGATGACTGCTGTGCAGA (SEQ ID NO: 114)	TCTGCCAATAGCTGTAATCGTGG (SEQ ID NO: 115)
TIGIT	miR-206	TATCGTTCACGGTCAGCGACTG (SEQ ID NO: 116)	CAGTCGTTGACTGTGGACTTATA (SEQ ID NO: 117)
TIGIT	miR-204	TATCGTTCACGGTCAGCGACTG (SEQ ID NO: 118)	CAGTGCTGAACGTGAACGATC (SEQ ID NO: 119)
TIGIT	miR-22	TATCGTTCACGGTCAGCGACTG (SEQ ID NO: 120)	TAGTCGCTGACTTGAACAGATA (SEQ ID NO: 121)

TIGIT	miR-142	TATCGTTCACGGTCAGCGACT (SEQ ID NO: 122)	TGTCGCTGACAGTGAACGATA (SEQ ID NO: 123)
TIGIT	miR-16	TATCGTTCACGGTCAGCGACTG (SEQ ID NO: 124)	GTCGCTGAGCGCTGAACGATG (SEQ ID NO: 125)
TIGIT	miR-206	TAACTCAGGACATTGAAGTAGT (SEQ ID NO: 126)	ACTACTTCAATGTCCTGACCTTA (SEQ ID NO: 127)
TIGIT	miR-204	TAACTCAGGACATTGAAGTAGT (SEQ ID NO: 128)	ACTATTCAGAGTCCTGAGTTC (SEQ ID NO: 129)
TIGIT	miR-21	TAACTCAGGACATTGAAGTAGT (SEQ ID NO: 130)	CTACTCCAATGCCTGAGTTG (SEQ ID NO: 131)
TIGIT	miR-494	TAACTCAGGACATTGAAGTAGT (SEQ ID NO: 132)	ACTACTGTAATGTCTGACGTT (SEQ ID NO: 133)
TIGIT	miR-19	TAACTCAGGACATTGAAGTAGTC (SEQ ID NO: 134)	GACTACTTCAATGTTGAGTT (SEQ ID NO: 135)
TIGIT	miR-1915	AGATCCACGTTACTCACCCTAG (SEQ ID NO: 136)	CTAGGCAGTGAGCAAAGTGGATC (SEQ ID NO: 137)
TIGIT	miR-204	AGATCCACGTTACTCACCCTAG (SEQ ID NO: 138)	CTAGGTGAGAAACGTGGATCG (SEQ ID NO: 139)
TIGIT	miR-206	AGATCCACGTTACTCACCCTAG (SEQ ID NO: 140)	CTAGGGTGAGTAACGTGGCCTCT (SEQ ID NO: 141)
TIGIT	miR-21	AGATCCACGTTACTCACCCTAG (SEQ ID NO: 142)	TAGGGCGAGTACGTGGATCG (SEQ ID NO: 143)
TIGIT	miR-22	AGATCCACGTTACTCACCCTAG (SEQ ID NO: 144)	CTAGGGTGAGTACGTGGCATCT (SEQ ID NO: 145)
TIM3	miR204	CATTATGCCTGGGATTTGGATC (SEQ ID NO: 146)	GATCAGATCGCAGGCATAATT (SEQ ID NO: 147)
TIM3	miR206	CATTATGCCTGGGATTTGGATC (SEQ ID NO: 148)	GATCCGGATCCTAGGTATCCATG (SEQ ID NO: 149)
TIM3	miR17	CATTATGCCTGGGATTTGGATCG (SEQ ID NO: 150)	CGGCCAGAACCAAGGCATAAGTA (SEQ ID NO: 151)

TIM3	miR126	CATTATGCCTGGGATTTGGATC (SEQ ID NO: 152)	ACTCCAAATCCCGTGCATAA TCG (SEQ ID NO: 153)
TIM3	miR122	CATTATGCCTGGGATTTGGATC (SEQ ID NO: 154)	GATTCGAATCCAAGGCATAT GG (SEQ ID NO: 155)
TIM3	miR214	TAATTCACATCCCTTTCATCAG (SEQ ID NO: 156)	ATGATGAAAGGACAGTGAA TTA (SEQ ID NO: 157)
LAG3	miR-30a	TAGTCGTTGGGTAAAGTCGCCA (SEQ ID NO: 158)	TGGTGACTTCTCAACGATTA (SEQ ID NO: 159)
LAG3	miR122	GTTGCTTTCCGCTAAGTGGTGA (SEQ ID NO: 160)	TCACCACTTAGAGGAAAGCC TC (SEQ ID NO: 161)
GITR	miR206	TTTGCAGTGGCCTTCGTGGCCC (SEQ ID NO: 162)	GGGCTATGAAGGCCATTGA GAAA (SEQ ID NO: 163)
GITR	miR29b1	AGCCTCCCGTCCTAAGACCCCAC (SEQ ID NO: 164)	GTGGGTCTTAGGTCGGGAG CTGCT (SEQ ID NO: 165)
PIK3IP1	miR206	TGAACGACCAGTGTTTAACCGG (SEQ ID NO: 166)	CCGGTTGAACATTGGTTGCC TCA (SEQ ID NO: 167)
PIK3IP1	miR126	TTCTCCTTGGAGTTCATCCGCG (SEQ ID NO: 168)	ATGAACTTAGAGGAGACG (SEQ ID NO: 169)
PIK3IP1	miR30a	TTCTCCTTGGAGTTCATCCGCG (SEQ ID NO: 170)	CGCGGATGATCCAAGGAGG A (SEQ ID NO: 171)

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
CTLA4	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACAATA TAGTCTTCTCCCTCGCTTGAGAATATATGAAGGAAGCAGGGAC AGGACTATATTGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 178)
PD1	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCA

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		GGAATGGGTTCCAAGGATGAGAATATATGAAGGATCCTGGAA GCTATTCCTGACGTTCAATTGTCATCACTGGCATCTTTTTTGATC ATTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 179)
PD1	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATAT GGATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCA AGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGT TTGGTGACCTT (SEQ ID NO: 180)
TIGIT	miR17	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATT GTGACCAGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTG ATATGTGCATCTAGGGTGTGTCATGTGGATGAAGCATTATGGT GACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGGG CCTGCTGATGT (SEQ ID NO: 181)
TIGIT	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAG CGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGAGATCCACGTTA CTCACCCGTGCTGGGCTCAGACCCGGGTGATAATATGGATCTC AGGGACCTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTGA GGTGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 182)
TIGIT	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACAGAT CCACGTTACTCACCCCCTGAGAATATATGAAGGAGGGGTGAGA AGCGTGGATCCGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 183)
TIGIT	miR-206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGTCTGCGTAGCAGTCATCGCCGGTTA TGGATTACTTTGCTAACCACGATGACTGCTGTGCAGATTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 184)
TIGIT	miR-204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACACCA CGATGACTGCTGTGCAGAGAGAATATATGAAGGTCTGATGGCC GTCATCGTGGCGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 185)

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
TIGIT	miR-22	CATTTTCCCTCCCTTTCCCTTAGGAGCCTGTTCCTCTCACGCCCTC ACCTGGCTGAGCCGCATCTGTACAGCAGCATCGATGGTTATGTC CTGACCCAGCTAACCACGATGACTGCTGTGCAGATGCCCTCTGC CCCTGGCTTCGAGGAGGAGGAGGAGCTGCTTTCCCCATCATCT GGAAGGTG (SEQ ID NO: 186)
TIGIT	miR-16	cttcTGAAGAAAATATATTTCTTTTTATTCATAGCTCTTATGATAG CAATGTCAGCAGTGCCTACCACGATGACTGCTGTGCAGATTAA GATTCTAAAATTATCTTGCGCAGCTGTTCATCGTGGTAGTAAGG TTGACCATACTCTACAGTTGTGTTTTAATGTATATTAATGTTACT AATGTGTTTT (SEQ ID NO: 187)
TIGIT	miR-16	cttcTGAAGAAAATATATTTCTTTTTATTCATAGCTCTTATGATAG CAATGTCAGCAGTGCCTACCACGATGACTGCTGTGCAGATTAA GATTCTAAAATTATCTTGCGCAGCTGTTCATCGTGGTAAGTAAG GTTGACCATACTCTACAGTTGTGTTTTAATGTATATTAATGTTAC TAATGTGTTTT (SEQ ID NO: 188)
TIGIT	miR-21	TGTCTGCTTGTTTTGCCTACCATCGTGACATCTCCATGGCTGTAC CACCTTGTCGGGACCACGATGACTGCTGTGCAGACTGTTGAATC TCATGGCTGCGAAGCAGCATCGTGGCTCTGACATTTTGGTATCT TTCATCTGACCATCCATATCCAATGTTCTCATTTAAACA (SEQ ID NO: 189)
TIGIT	miR-494	CAGTTCTGTTTTGATTTTTTTTGTTTGTTTTTTGATCAGTGCTAAT CTTCGATACTCGAAGGATCTGCAACGCAGTCTCGCTGGTCTTTA TTTATGAACCACGATGACTGCTGTGCAGATTTTTTAGTATCAAA TCCCACCCTGGAGGCACTTCCTGTTCCTGATGCAGCCTTCAGGG AGG (SEQ ID NO: 190)
TIGIT	miR-142	GGAGTCAGGAGGCCTGGGCAGCCTGAAGAGTACACGCCGACG GACAGACAGACAGTGCAGTCACCACCACGATGACTGCTGTGCA GAACAGCACTGGAGGGATGCACAGCAATCATCGTGGTGATGA GTGTACTGTGGGCTTCGGAGATCACGCCACTGCTGCCGCCCGC TGCCCGCCACCATCTTC (SEQ ID NO: 191)
TIGIT	miR-19	CCAATAATTCAAGCCAAGCAAGTATATAGGTGTTTTAATAGTTT TTGTTTGCAGTCCTCTGATCTGCACAGCAGTTCGTGGTACAAGA AGAATGTAGTACCACGATGACTGCTGTGCAGATTGGTGGCCTG CTATTTCCTTCAAATGAATGATTTTTACTAATTTTGTGTACTTTTA TTGTG

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		(SEQ ID NO: 192)
TIGIT	miR-1915	CGGACCACGGTGTCCCCTTCTCTCCAGCTGGGGGTCTCGGGTCC TGGCGCTGAGAGGCCGCACCACGATGACTGCTGTGCAGAGTGC ACCCGTGTCTGCCAATAGCTGTAATCGTGGGCGGCCCTAGCGA CCTGCGGCGGCGCCGGGAAAGCCCTGCCTCTGCAGCGGGTCCC AGGGGTC (SEQ ID NO: 193)
TIGIT	miR-206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCAGTCGTTGACTGTGGACTTATATA TGGATTACTTTGCTATATCGTTCACGGTCAGCGACTGTTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 194)
TIGIT	miR-204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTATC GTTCACGGTCAGCGACTGGAGAATATATGAAGGCAGTGCTGAA CGTGAACGATCGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 195)
TIGIT	miR-22	CATTTTCCCTCCCTTTCCCTTAGGAGCCTGTTCCTCTCACGCCCTC ACCTGGCTGAGCCGCATAGTCGCTGACTTGAACAGATATATGTC CTGACCCAGCTATATCGTTCACGGTCAGCGACTGTGCCCTCTGC CCCTGGCTTCGAGGAGGAGGAGGAGCTGCTTTCCCCATCATCT GGAAGGTG (SEQ ID NO: 196)
TIGIT	miR-142	GGAGTCAGGAGGCCTGGGCAGCCTGAAGAGTACACGCCGACG GACAGACAGACAGTGCAGTCACCTATCGTTCACGGTCAGCGAC TAACAGCACTGGAGGGTGTCGCTGACAGTGAACGATAGATGA GTGTACTGTGGGCTTCGGAGATCACGCCACTGCTGCCGCCCGC TGCCCGCCACCATCTTC (SEQ ID NO: 197)
TIGIT	miR-16	cttcTGAAGAAAATATATTTCTTTTTATTCATAGCTCTTATGATAG CAATGTCAGCAGTGCCTTATCGTTCACGGTCAGCGACTGTTAAG ATTCTAAAATTATCTGTCGCTGAGCGCTGAACGATGAAGTAAG GTTGACCATACTCTACAGTTGTGTTTTAATGTATATTAATGTTAC TAATGTGTTTT (SEQ ID NO: 198)
TIGIT	miR-206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGACTACTTCAATGTCCTGACCTTATAT GGATTACTTTGCTATAACTCAGGACATTGAAGTAGTTTTCGGCA

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		AGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGT TTGGTGACCTT (SEQ ID NO: 199)
TIGIT	miR-204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTAAC TCAGGACATTGAAGTAGTGAGAATATATGAAGGACTATTCAGA GTCCTGAGTTCGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 200)
TIGIT	miR-21	TGTCTGCTTGTTTTGCCTACCATCGTGACATCTCCATGGCTGTAC CACCTTGTCGGGTAACTCAGGACATTGAAGTAGTCTGTTGAATC TCATGGCTACTCCAATGCCTGAGTTGTCTGACATTTTGGTATCTT TCATCTGACCATCCATATCCAATGTTCTCATTTAAACA (SEQ ID NO: 201)
TIGIT	miR-494	CAGTTCTGTTTTGATTTTTTTTGTTTGTTTTTTGATCAGTGCTAAT CTTCGATACTCGAAGGAACTACTGTAATGTCTGACGTTTCTTTAT TTATGATAACTCAGGACATTGAAGTAGTTTTTTTAGTATCAAATC CCACCCTGGAGGCACTTCCTGTTCCTGATGCAGCCTTCAGGGAG G (SEQ ID NO: 202)
TIGIT	miR-19	CCAATAATTCAAGCCAAGCAAGTATATAGGTGTTTTAATAGTTT TTGTTTGCAGTCCTCTGGACTACTTCAATGTTGAGTTTACAAGA AGAATGTAGTTAACTCAGGACATTGAAGTAGTCTGGTGGCCTG CTATTTCCTTCAAATGAATGATTTTTACTAATTTTGTGTACTTTTA TTGTG (SEQ ID NO: 203)
TIGIT	miR-1915	CGGACCACGGTGTCCCCTTCTCTCCAGCTGGGGGTCTCGGGTCC TGGCGCTGAGAGGCCGCAGATCCACGTTACTCACCCTAGGTGC ACCCGTGCTAGGCAGTGAGCAAAGTGGATCGCGGCCCTAGCGA CCTGCGGCGGCGCCGGGAAAGCCCTGCCTCTGCAGCGGGTCCC AGGGGTC (SEQ ID NO: 204)
TIGIT	miR-204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACAGAT CCACGTTACTCACCCTAGGAGAATATATGAAGGCTAGGTGAGA AACGTGGATCGGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 205)
TIGIT	miR-206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCTAGGGTGAGTAACGTGGCCTCTT

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		ATGGATTACTTTGCTAAGATCCACGTTACTCACCCTAGTTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 206)
TIGIT	miR-21	TGTCTGCTTGTTTTGCCTACCATCGTGACATCTCCATGGCTGTAC CACCTTGTCGGGAGATCCACGTTACTCACCCTAGCTGTTGAATC TCATGGTAGGGCGAGTACGTGGATCGTCTGACATTTTGGTATCT TTCATCTGACCATCCATATCCAATGTTCTCATTTAAACA (SEQ ID NO: 207)
TIGIT	miR-22	CATTTTCCCTCCCTTTCCCTTAGGAGCCTGTTCCTCTCACGCCCTC ACCTGGCTGAGCCGCACTAGGGTGAGTACGTGGCATCTTATGT CCTGACCCAGCTAAGATCCACGTTACTCACCCTAGTGCCCTCTG CCCCTGGCTTCGAGGAGGAGGAGGAGCTGCTTTCCCCATCATC TGGAAGGTG (SEQ ID NO: 208)
TIM3	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACCATT ATGCCTGGGATTTGGATCGAGAATATATGAAGGGATCAGATCG CAGGCATAATTGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 209)
TIM3	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAG CGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGCATTATGCCTGG GATTTGGATCCTGGGCTCAGACCTCCAAATCCACACATAGTGCA GGGACCTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTGAG GTGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 210)
TIM3	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACCATTATGCCTGGGATTTGGATCCTGTGAAGC CACAGATGGGGATCCAGATCAGGCATAGTGGCTGCCTACTGCC TCGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 211)
TIM3	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGGATCCGGATCCTAGGTATCCATGTA TGGATTACTTTGCTACATTATGCCTGGGATTTGGATCTTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 212)
TIM3	miR16	CTTCTGAAGAAAATATATTTCTTTTTATTCATAGCTCTTATGATA GCAATGTCAGCAGTGCCTCATTATGCCTGGGATTTGGATCTTAA

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		GATTCTAAAATTATCTTCCGAATCACATGGCATAATGAAGTAAG GTTGACCATACTCTACAGTTGTGTTTTAATGTATATTAATGTTAC TAATGTGTTTT (SEQ ID NO: 213)
TIM3	miR17	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATT GTGACCAGTCAGAATAATGTCATTATGCCTGGGATTTGGATCGT GATATGTGCATCGGCCAGAACCAAGGCATAAGTAGCATTATGG TGACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGG GCCTGCTGATGT (SEQ ID NO: 214)
TIM3	miR126	CCGGGGTCCTGTCTGCATCCAGCGCAGCATTCTGGAAGACGCC ACGCCTCCGCTGGCGACGGACTCCAAATCCCGTGCATAATCGCT GTGACACTTCAAACCATTATGCCTGGGATTTGGATCCCGTCCAC GGCACCGCATCGAAAACGCCGCTGAGACCTCAGCCTTGACCTC CCTCAGCGTGG (SEQ ID NO: 215)
TIM3	miR122	CAATGGTGGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTAC AGAGTTTCCTTAGCAGAGCTGCATTATGCCTGGGATTTGGATCT GTCTAAACTATGATTCGAATCCAAGGCATATGGTAGCTACTGCT AGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTGA GCAAGAAGGTTCATCTGATATCAGTCTTCTCAATCT (SEQ ID NO: 216)
TIM3	miR214	ACAGGCTGATTGTATCTGTCTATGAGCAAAGGAAACCTGAAGG AACCAAGGGCCTGGCTGGACAGAGTTGTCATGTGATGATGAAA GGACAGTGAATTAGCAGAACATCCGCTCACCTGTTAATTCACAT CCCTTTCATCAGCACATGACAACCCAGCCTGAATGACAACCAGC CATTGAAAGAAAGCAGCCCTCACACCATAGCATCTA (SEQ ID NO: 217)
TIM3	miR29b1	GGGTTTATTGTAAGAGAGCATTATGAAGAAAAAAATAGATCAT AAAGCTTCTTCAGGACGATGGAAGGGTTGTGAACGTTAGATTT AAATAGTGATTGTCTAATTCACATCCCTTTCATCAGTCTTGGGG GAGACCAGCTGCGCTGCACTACCAACAGCAAAAGAAGTGAATG GGACAGCT (SEQ ID NO: 218)
TIM3	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTAATT CACATCCCTTTCATCAGGAGAATATATGAAGGCTGAGGAAGAG GTGTGAATTCGTTCAATTGTCATCACTGGCATCTTTTTTGATCAT TGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 219)

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
TIM3	miR133a1	TTTACCAATGAAAAGCATTTAACTGTTTTGGATTCCAAACTAGC AGCACTACAATGCTTTGCTAGTGATGAATAGGCTGCGAATTATC GCCTCTTCAATGGATAATTCACATCCCTTTCATCAGTAGCTATGC ATTGATTACTACGGGACAACCAACGTTTTCATTTGTGAATATCA ATTACTTGCCA (SEQ ID NO: 220)
LAG3	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACTAGTCGTTGGGTAAAGTCGCCACTGTGAAGC CACAGATGGGTGGTGACTTCTCAACGATTAGCTGCCTACTGCCT CGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 221)
LAG3	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGTGGCGATTTTACCCAATGCTCTATA TGGATTACTTTGCTATAGTCGTTGGGTAAAGTCGCCATTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 222)
LAG3	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTAGT CGTTGGGTAAAGTCGCCAGAGAATATATGAAGGTGGCACTTTC CTCAACGACTCGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 223)
LAG3	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAG CGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGTAGTCGTTGGG TAAAGTCGCCACTGGGCTCAGACCGCGACTTACCACACGACTAC AGGGACCTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTGA GGTGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 224)
LAG3	miR17	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATT GTGACCAGTCAGAATAATGTGTTGCTTTCCGCTAAGTGGTGAGT GATATGTGCATCTCCCACTCAGAGGAAAGCACTAGCATTATGGT GACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGGG CCTGCTGATGT (SEQ ID NO: 225)
LAG3	miR122	CAATGGTGGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTAC AGAGTTTCCTTAGCAGAGCTGGTTGCTTTCCGCTAAGTGGTGAT GTCTAAACTATTCACCACTTAGAGGAAAGCCTCTAGCTACTGCT AGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTGA GCAAGAAGGTTCATCTGATATCAGTCTTCTCAATCT

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		(SEQ ID NO: 226)
LAG3	miR126	CCGGGGTCCTGTCTGCATCCAGCGCAGCATTCTGGAAGACGCC ACGCCTCCGCTGGCGACGGGAACCACTTAGCGCGAAGCAAGGC TGTGACACTTCAAACGTTGCTTTCCGCTAAGTGGTGACCGTCCA CGGCACCGCATCGAAAACGCCGCTGAGACCTCAGCCTTGACCT CCCTCAGCGTGG (SEQ ID NO: 227)
GITR	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACTTTGCAGTGGCCTTCGTGGCCCCTGTGAAGC CACAGATGGGGGGTCACGAGCCACTGCGAAGCTGCCTACTGCC TCGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 228)
GITR	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGGGGCTATGAAGGCCATTGAGAAAT ATGGATTACTTTGCTATTTGCAGTGGCCTTCGTAGCCCTTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 229)
GITR	miR17	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATT GTGACCAGTCAGAATAATGTTTTGCAGTGGCCTTCGTGGCCCGT GATATGTGCATCGGTCACGTAGACTACTGCACTCGCATTATGGT GACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGGG CCTGCTGATGT (SEQ ID NO: 230)
GITR	miR122	CAATGGTGGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTAC AGAGTTTCCTTAGCAGAGCTGTTTGCAGTGGCCTTCGTGGCCCT GTCTAAACTATGGGTCACGAAGACCACTGCCCATAGCTACTGCT AGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTGA GCAAGAAGGTTCATCTGATATCAGTCTTCTCAATCT (SEQ ID NO: 231)
GITR	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAG CGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGAGCCTCCCGTCC TAAGACCCCACTGGGCTCAGACAGGGTCTTGGAAAGGAGGCTC AGGGACCTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTGA GGTGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 232)
GITR	miR29	GGGTTTATTGTAAGAGAGCATTATGAAGAAAAAAATAGATCAT AAAGCTTCTTCAGGGTGGGTCTTAGGTCGGGAGCTGCTGATTT AAATAGTGATTGTCAGCCTCCCGTCCTAAGACCCCACCTTGGGG GAGACCAGCTGCGCTGCACTACCAACAGCAAAAGAAGTGAATG

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		GGACAGCT (SEQ ID NO: 233)
GITR	miR181a1	TCTCCCATCCCCTTCAGATACTTACAGATACTGTAAAGTGAGTA GAATTCTGAGTTTTGAGGTTGCTTCAGTGAGCCTCCCGTCCTAA GACCCCACTTGGAATTAAAATCAAGTGGGTCTTGACGGGTGGC ACCCTATGGCTAACCATCATCTACTCCATGGTGCTCAGAATTCG CTGAAGACAGGAAACCAAAGGTGGACACACCAGG (SEQ ID NO: 234)
TIGIT	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCCAGGGTGAGTAACGTGGCCTCTT ATGGATTACTTTGCTAAGATCCACGTTACTCACCCTGGTTTCGG CAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAG GTTTGGTGACCTT (SEQ ID NO: 235)
TIGIT	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACAGATCCACGTTACTCACCCAAGCTGTGAAGC CACAGATGGGCTTGGGTGAGACGTGGATCTGCTGCCTACTGCC TCGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 236)
TIGIT	miR133a1	TTTACCAATGAAAAGCATTTAACTGTTTTGGATTCCAAACTAGC AGCACTACAATGCTTTGCTAGAGGGGTGTATACCGCGGATCTTC GCCTCTTCAATGGAAGATCCACGTTACTCACCCCTGTAGCTATG CATTGATTACTACGGGACAACCAACGTTTTCATTTGTGAATATC AATTACTTGCCA (SEQ ID NO: 237)
TIGIT	miR122	CAATGGTGGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTAC AGAGTTTCCTTAGCAGAGCTGAGATCCACGTTACTCACCCTTGT GTCTAAACTATCAAGGGTGAGTCACGTGGAGATTAGCTACTGC TAGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTG AGCAAGAAGGTTCATCTGATATCAGTCTTCTCAATCT (SEQ ID NO: 238)
TIGIT	miR29b1	GGGTTTATTGTAAGAGAGCATTATGAAGAAAAAAATAGATCAT AAAGCTTCTTCAGGAAAGATCCACGTTACTCACCCTTAGATTTA AATAGTGATTGTCTAGGTGAGTTACGTGGATCTGTTCTTGGGG GAGACCAGCTGCGCTGCACTACCAACAGCAAAAGAAGTGAATG GGACAGCT (SEQ ID NO: 239)
TIGIT	miR214	ACAGGCTGATTGTATCTGTCTATGAGCAAAGGAAACCTGAAGG AACCAAGGGCCTGGCTGGACAGAGTTGTCATGTGTCAGATCCA CGTTACTCACCCTGCAGAACATCCGCTCACCTGTAGGGTGAGAC

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		TCGTGGATCTGTCACATGACAACCCAGCCTGAATGACAACCAGC CATTGAAAGAAAGCAGCCCTCACACCATAGCATCTA (SEQ ID NO: 240)
PD1	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACTTCAGGAATGGGTTCCAAGGAGCTGTGAAGC CACAGATGGGCTCCTTGGACCATTCCTGAAGCTGCCTACTGCCT CGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 241)
PD1	miR412	GTCTTGGAGGCTGGGGCACCTCGGGGAAGGACGCCGGCATCA GCACCATTCTGGGGTACGGGGATGGATTCAGGAATGGGTTCCA AGGAATTGTTTCTAATGTCCTGAAGCCATTCATGGAGCCGTCCG TATCCGCTGCAGCCTGTGGGGCCTGCGGGCCGGGGAGCCGATC GCGCTTCAGCTCAGCGCCT (SEQ ID NO: 242)
PD1	miR17	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATT GTGACCAGTCAGAATAATGTTTCAGGAATGGGTTCCAAGGAAG TGATATGTGCATCTTCTTGGTACACGTTCCTGCTCACATTATGGT GACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGGG CCTGCTGATGT (SEQ ID NO: 243)
PD1	miR122	CAATGGTGGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTAC AGAGTTTCCTTAGCAGAGCTGTTCAGGAATGGGTTCCAAGGAG TGTCTAAACTATCTCCTTGGAACACATTCCTACATAGCTACTGCT AGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTGA GCAAGAAGGTTCATCTGATATCAGTCTTCTCAATCT (SEQ ID NO: 244)
PD1	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAG CGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGTATAATATAATA GAACCACAGGCTGGGCTCAGACGTGTGGTTTATCCTGTTGTAC AGGGACCTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTGA GGTGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 245)
PD1	miR486	TGTGGTGCTGGGGGCTTCAGCGGCCGGCTCTGATCTCCATCCTC CCTGGGGCATATAATATAATAGAACCACAGGGCCCTTCATGCTG CCCAGCTTGTGGTTCTATTATGTTATAACTCGGGGTGGGAGTCA GCAGGAGGTGAGGGGGCATGGTGGCCCCAGTGCAGC (SEQ ID NO: 246)
PD1	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCGTGCTGAACTGGTATCGAAATGT ATGGATTACTTTGCTACATGCGGTACCAGTTTAGCACGTTTCGG

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		CAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAG GTTTGGTGACCTT (SEQ ID NO: 247)
PD1	miR122	CAATGGTGGAATGTGGAGGTGAAGTTAACACCTTCGTGGCTAC AGAGTTTCCTTAGCAGAGCTGCATGCGGTACCAGTTTAGCACGT GTCTAAACTATCGTGCTAGACTTGTACTGTCAGTAGCTACTGCT AGGCAATCCTTCCCTCGATAAATGTCTTGGCATCGTTTGCTTTGA GCAAGAAGGTTCATCTGATATCAGTCTTCTCAATCT (SEQ ID NO: 248)
PD1	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACCATGCGGTACCAGTTTAGCACGCTGTGAAGC CACAGATGGGCGTGCTAGAGGTGTCGCATGGCTGCCTACTGCC TCGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 249)
CTLA4	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCAGCGAGGGAGAAGATTACCATTT ATGGATTACTTTGCTAAATATAGTCTTCTCCCTCGCTGTTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 250)
CTLA4	miR26a	TGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGC GAAGGCCGTGGCCTCGAATATAGTCTTCTCCCTCGCTTGTGCAG GTCCCAATGGAGCGAGGGATAGGACTATACTCGGGGACGCGG GCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCA GAGGC (SEQ ID NO: 251)
CTLA4	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACAATATAGTCTTCTCCCTCGCTGCTGTGAAGCC ACAGATGGGCAGTGAGGGAAGACTATGTTGCTGCCTACTGCCT CGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 252)
PIK3IP1	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCCGGTTGAACATTGGTTGCCTCATA TGGATTACTTTGCTATGAACGACCAGTGTTTAACCGGTTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 253)
PIK3IP1	miR126	CCGGGGTCCTGTCTGCATCCAGCGCAGCATTCTGGAAGACGCC ACGCCTCCGCTGGCGACGGGACGGATGAACTTAGAGGAGACG CTGTGACACTTCAAACTTCTCCTTGGAGTTCATCCGCGCCGTCCA

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		CGGCACCGCATCGAAAACGCCGCTGAGACCTCAGCCTTGACCT CCCTCAGCGTGG (SEQ ID NO: 254)
PIK3IP1	miR30a	CAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTTG ACAGTGAGCGACTTCTCCTTGGAGTTCATCCGCGCTGTGAAGCC ACAGATGGGCGCGGATGATCCAAGGAGGAGCTGCCTACTGCCT CGGACTTCAAGGGGCTACTTTAGGAGCAATTATCTTGTTTA (SEQ ID NO: 255)
TCRa3'UTR	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAG CGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGATACACATCAGA ATCCTTACTGCTGGGCTCAGACCGTAAGGATCTCCTGTGTATCA GGGACCTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTGAG GCGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 256)
TCRa3'UTR	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGC GTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACATAC ACATCAGAATCCTTACTTGAGAATATATGAAGGAGGTAGGATA CTGATGTGTACGTTCAATTGTCATCACTGGCATCTTTTTTGATCA TTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 257)
TCRa3'UTR	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCGGTAAGGATTCTGATGTAATATTA TGGATTACTTTGCTAATACACATCAGAATCCTTACTGTTTCGGCA AGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGT TTGGTGACCTT (SEQ ID NO: 258)
TCRa3'UTR	miR26a	TGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGC GAAGGCCGTGGCCTCGATACACATCAGAATCCTTACTTGTGCAG GTCCCAATGGAGTAAGGATCCTGATGTGTCTCGGGGACGCGGG CCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAG AGGC (SEQ ID NO: 259)
TCRa3'UTR	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAG CGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGTTGTTGAAGGC GTTTGCACATGCTGGGCTCAGACCTGTGCAATGCGATCAATAG CAGGGACCTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTG AGGCGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 260)
TCRa3'UTR	miR16	CTTCTGAAGAAAATATATTTCTTTTTATTCATAGCTCTTATGATA GCAATGTCAGCAGTGCCTTTGTTGAAGGCGTTTGCACATGTTAA

Nucleic acid sequences encoding non-naturally occurring pri-miRNA sequences		
miRNA Target	**Encoded pri-miRNA(s)**	**DNA Sequence**
		GATTCTAAAATTATCTTGTGCGGAAGCACTTCAATAAAAGTAAG GTTGACCATACTCTACAGTTGTGTTTTAATGTATATTAATGTTAC TAATGTGTTTT (SEQ ID NO: 261)
TCRa3'UTR	miR206	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGG ATGACACTGCTTCCCGAGGCGTGTGTGAACGCCTTCACATAATA TGGATTACTTTGCTATTGTTGAAGGCGTTTGCACATGTTTCGGC AAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTT (SEQ ID NO: 262)
TCRa3'UTR	miR26a	TGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGC GAAGGCCGTGGCCTCGTTGTTGAAGGCGTTTGCACATTGTGCA GGTCCCAATGGGTGTGCAAAGGTCTTCAATCACGGGGACGCGG GCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCA GAGGC (SEQ ID NO: 263)

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
PD1	miR204 + miR206	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCC ATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCC AAGGATGAGAATATATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATT GTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGG ATAACCATGACGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTT CTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATG GATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCT CCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTT (SEQ ID NO: 267)
PD1 + TIGIT	miR204 + miR150	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCC ATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCC AAGGATGAGAATATATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATT GTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGG

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		ATAACCATGACAGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGC AGCAGCGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGAGATCCACGTTA CTCACCCGTGCTGGGCTCAGACCCGGGTGATAATATGGATCTCAGGGAC CTGGGGACCCCGGCACCGGCAGGCCCCAAGGGGTGAGGTGAGCGGGCA TTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 268)
PD1 + TIGIT	miR206 + miR150	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGGATGACA CTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATGGATTACTTTGC TATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCTCCTCGCTGGCC CCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTTAGGGACTGGGCCC ACGGGGAGGCAGCGTCCCCGAGGCAGCAGCGGCAGCGGCGGCTCCTCT CCCCATGGCCCTGAGATCCACGTTACTCACCCGTGCTGGGCTCAGACCCG GGTGATAATATGGATCTCAGGGACCTGGGGACCCCGGCACCGGCAGGC CCCAAGGGGTGAGGTGAGCGGGCATTGGGACCTCCCCTCCCTGTACTC (SEQ ID NO: 269)
TIGIT + PD1	miR17 + miR204	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACC AGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCT AGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAG CCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTAGGAGGGTGGGG GTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCT TTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCCAAGGATGAGAATA TATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATTGTCATCACTGGCA TCTTTTTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 270)
TIGIT + PD1	miR17 + miR206	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACC AGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCT AGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAG CCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTGATGCTACAAGTG GCCCACTTCTGAGATGCGGGCTGCTTCTGGATGACACTGCTTCCCGAGGC CTGTGGTTCTGTTATATCCATATATGGATTACTTTGCTATATAATATAATA GAACCACAGGTTTCGGCAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCC GGAGCACAGGTTTGGTGACCTT

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		(SEQ ID NO: 271)
TIGIT + PD1	miR204 + miR206	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCC ATGGCTACAGTCTTTCTTCATGTGACTCGTGGACAGATCCACGTTACTCAC CCCCTGAGAATATATGAAGGAGGGGTGAGAAGCGTGGATCCGTTCAATT GTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGG ATAACCATGACGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTT CTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATG GATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCT CCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTT (SEQ ID NO: 272)
TIGIT + PD1	miR150 + miR204	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAGCGGCA GCGGCGGCTCCTCTCCCCATGGCCCTGAGATCCACGTTACTCACCCGTGC TGGGCTCAGACCCGGGTGATAATATGGATCTCAGGGACCTGGGGACCCC GGCACCGGCAGGCCCCAAGGGGTGAGGTGAGCGGGCATTGGGACCTCC CCTCCCTGTACTCAGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCT GATCGCGTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCA GGAATGGGTTCCAAGGATGAGAATATATGAAGGATCCTGGAAGCTATTC CTGACGTTCAATTGTCATCACTGGCATCTTTTTTGATCATTGCACCATCAT CAAATGCATTGGGATAACCATGAC (SEQ ID NO: 273)
TIGIT + PD1	miR150 + miR206	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGCAGCGGCA GCGGCGGCTCCTCTCCCCATGGCCCTGAGATCCACGTTACTCACCCGTGC TGGGCTCAGACCCGGGTGATAATATGGATCTCAGGGACCTGGGGACCCC GGCACCGGCAGGCCCCAAGGGGTGAGGTGAGCGGGCATTGGGACCTCC CCTCCCTGTACTCGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGC TTCTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATAT GGATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTGCC TCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTT (SEQ ID NO: 274)
TIGIT+PD1+ PD1	miR17+miR204 +miR206	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACC AGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCT AGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAG

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		CCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTAGGAGGGTGGGG GTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCT TTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCCAAGGATGAGAATA TATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATTGTCATCACTGGCA TCTTTTTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATGACGA TGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGGATGACACTG CTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATGGATTACTTTGCTAT ATAATATAATAGAACCACAGGTTTCGGCAAGTGCCTCCTCGCTGGCCCCA GGGTACCACCCGGAGCACAGGTTTGGTGACCTT (SEQ ID NO: 275)
TIGIT+PD1+ PD1	miR17+miR204 +miR206 extra spacing 1	TTAGGATGAGTTGAGATCCCAGTGATCTTCTCGCTAAGAGTTTCCTGCCT GGGCAAGGAGGAAATTAGCAGGAAAAAAGAGAACATCACCTTGTAAAA CTGAAGATTGTGACCAGTCAGAATAATGTAGATCCACGTTACTCACCCTA GTGATATGTGCATCTAGGGTGTGTCATGTGGATGAAGCATTATGGTGAC AGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGAT GTAGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTAC CCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCAGGAATGGGTT CCAAGGATGAGAATATATGAAGGATCCTGGAAGCTATTCCTGACGTTCA ATTGTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATT GGGATAACCATGACGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCT GCTTCTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATAT ATGGATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTG CCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTT CTTCCTCATCAGGGCTTTGTGCCAGCAAATGACTCCCTCACCAAGGAAGC AAGAGCCTCTGAATCCCATCTGGGCTCTTCCTGAACACCCCTATCTCCCCC TCT (SEQ ID NO: 276)
TIGIT+PD1+ PD1	miR17+miR204 +miR206 extra spacing 2	TTAGGGATTATGCTGAATTTGTATGGTTTATAGTTGTTAGAGTTTGAGGT GTTAATTCTAATTATCTATTTCAAATTTTAGCAGGAAAAAAGAGAACATC ACCTTGTAAAACTGAAGATTGTGACCAGTCAGAATAATGTAGATCCACGT TACTCACCCTAGTGATATGTGCATCTAGGGTGTGTCATGTGGATGAAGCA TTATGGTGACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGG GCCTGCTGATGTAGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCT GATCGCGTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCA GGAATGGGTTCCAAGGATGAGAATATATGAAGGATCCTGGAAGCTATTC

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		CTGACGTTCAATTGTCATCACTGGCATCTTTTTTGATCATTGCACCATCAT CAAATGCATTGGGATAACCATGACGATGCTACAAGTGGCCCACTTCTGA GATGCGGGCTGCTTCTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGT TATATCCATATATGGATTACTTTGCTATATAATATAATAGAACCACAGGTT TCGGCAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGT TTGGTGACCTTCTTCCTCATCAGGGCTTTGTGCCAGCAAATGACTCCCTCA CCAAGGAAGCAAGAGCCTCTGAATCCCATCTGGGCTCTTCCTGAACACCC CTATCTCCCCCTCT (SEQ ID NO: 277)
PD1+PD1+ TIGIT	miR204+miR206+miR17	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCC ATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCC AAGGATGAGAATATATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATT GTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGG ATAACCATGACGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTT CTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATG GATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCT CCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTTTTA GCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACCAGT CAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCTAGG GTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAGCCA AGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGT (SEQ ID NO: 278)
PD1+PD1+ TIGIT	miR204+miR206+miR17 extra spacing 1	TTAGGATGAGTTGAGATCCCAGTGATCTTCTCGCTAAGAGTTTCCTGCCT GGGCAAGGAGGAAAAGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTT CCTGATCGCGTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTT CAGGAATGGGTTCCAAGGATGAGAATATATGAAGGATCCTGGAAGCTAT TCCTGACGTTCAATTGTCATCACTGGCATCTTTTTTGATCATTGCACCATC ATCAAATGCATTGGGATAACCATGACGATGCTACAAGTGGCCCACTTCTG AGATGCGGGCTGCTTCTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTG TTATATCCATATATGGATTACTTTGCTATATAATATAATAGAACCACAGGT TTCGGCAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGG TTTGGTGACCTTTTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGA AGATTGTGACCAGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGA TATGTGCATCTAGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCT GCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTCT

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		TCCTCATCAGGGCTTTGTGCCAGCAAATGACTCCCTCACCAAGGAAGCAAGAGCCTCTGAATCCCATCTGGGCTCTTCCTGAACACCCCTATCTCCCCCTCT (SEQ ID NO: 279)
PD1+PD1+ TIGIT	miR204+miR206+miR17 extra spacing 2	TGGAGAGGAGGGTGGGGGTGGAGGCAAGCAGAGGACCTCCTGATCATGTACCCATAGGACAGGGTGATGGAAAGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCCAAGGATGAGAATATATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATTGTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATGACGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATGGATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTTTTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACCAGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCTAGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAGCCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTCTTCCTCATCAGGGCTTTGTGCCAGCAAATGACTCCCTCACCAAGGAAGCAAGAGCCTCTGAATCCCATCTGGGCTCTTCCTGAACACCCCTATCTCCCCCTCT (SEQ ID NO: 280)
PD1+CTLA4	miR204+miR26a	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCCAAGGATGAGAATATATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATTGTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATGACTGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGAATATAGTCTTCTCCCTCGCTTGTGCAGGTCCCAATGGAGCGAGGGATAGGACTATACTCGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC (SEQ ID NO: 281)
PD1+PD1	miR204+miR206	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCCAAGGATGAGAATATATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATT

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		GTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGG ATAACCATGACGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTT CTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATG GATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCT CCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTT (SEQ ID NO: 282)
PD1+CTLA4	miR206+miR26a	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGGATGACA CTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATGGATTACTTTGC TATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCTCCTCGCTGGCC CCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTTTGAAGCCACAGGA GCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGAAT ATAGTCTTCTCCCTCGCTTGTGCAGGTCCCAATGGAGCGAGGGATAGGA CTATACTCGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCC CCCTCTCTGCCAGAGGC (SEQ ID NO: 283)
PD1+CTLA4	miR206+miR204	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCTGGATGACA CTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATGGATTACTTTGC TATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCTCCTCGCTGGCC CCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTTAGGAGGGTGGGG GTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCT TTCTTCATGTGACTCGTGGACAATATAGTCTTCTCCCTCGCTTGAGAATAT ATGAAGGAAGCAGGGACAGGACTATATTGTTCAATTGTCATCACTGGCA TCTTTTTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 284)
TIGIT+CTLA4	miR17+miR26a	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACC AGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCT AGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAG CCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTTGAAGCCACAGGA GCCAAGAGCAGGAGGACCAAGGCCCTGGCGAAGGCCGTGGCCTCGAAT ATAGTCTTCTCCCTCGCTTGTGCAGGTCCCAATGGAGCGAGGGATAGGA CTATACTCGGGGACGCGGGCCTGGACGCCGGCATCCGGGCTCAGGACCC CCCTCTCTGCCAGAGGC

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		(SEQ ID NO: 285)
TIGIT+CTLA 4	miR17+miR204	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACC AGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCT AGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAG CCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTAGGAGGGTGGGG GTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCT TTCTTCATGTGACTCGTGGACAATATAGTCTTCTCCCTCGCTTGAGAATAT ATGAAGGAAGCAGGGACAGGACTATATTGTTCAATTGTCATCACTGGCA TCTTTTTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 286)
TIGIT+PD1	miR17+miR204	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACC AGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCT AGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAG CCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTAGGAGGGTGGGG GTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCCATGGCTACAGTCT TTCTTCATGTGACTCGTGGACTTCAGGAATGGGTTCCAAGGATGAGAATA TATGAAGGATCCTGGAAGCTATTCCTGACGTTCAATTGTCATCACTGGCA TCTTTTTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATGAC (SEQ ID NO: 287)
TIGIT+PD1	miR17+206	TTAGCAGGAAAAAAGAGAACATCACCTTGTAAAACTGAAGATTGTGACC AGTCAGAATAATGTAGATCCACGTTACTCACCCTAGTGATATGTGCATCT AGGGTGTGTCATGTGGATGAAGCATTATGGTGACAGCTGCCTCGGGAAG CCAAGTTGGGCTTTAAAGTGCAGGGCCTGCTGATGTGATGCTACAAGTG GCCCACTTCTGAGATGCGGGCTGCTTCTGGATGACACTGCTTCCCGAGGC CTGTGGTTCTGTTATATCCATATATGGATTACTTTGCTATATAATATAATA GAACCACAGGTTTCGGCAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCC GGAGCACAGGTTTGGTGACCTT (SEQ ID NO: 288)
TIGIT+CTLA 4	miR204+miR26 a	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCC ATGGCTACAGTCTTTCTTCATGTGACTCGTGGACAGATCCACGTTACTCAC CCCCTGAGAATATATGAAGGAGGGGTGAGAAGCGTGGATCCGTTCAATT GTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGG

Non-naturally occurring miRNA sequences (two or more pri-miRNAs)		
miRNA Target	**miRNA backbone**	**DNA Sequence**
		ATAACCATGACTGAAGCCACAGGAGCCAAGAGCAGGAGGACCAAGGCC CTGGCGAAGGCCGTGGCCTCGAATATAGTCTTCTCCCTCGCTTGTGCAGG TCCCAATGGAGCGAGGGATAGGACTATACTCGGGGACGCGGGCCTGGA CGCCGGCATCCGGGCTCAGGACCCCCCTCTCTGCCAGAGGC (SEQ ID NO: 289)
TIGIT+PD1	miR204+miR206	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATCGCGTACCC ATGGCTACAGTCTTTCTTCATGTGACTCGTGGACAGATCCACGTTACTCAC CCCCTGAGAATATATGAAGGAGGGGTGAGAAGCGTGGATCCGTTCAATT GTCATCACTGGCATCTTTTTTGATCATTGCACCATCATCAAATGCATTGGG ATAACCATGACGATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTT CTGGATGACACTGCTTCCCGAGGCCTGTGGTTCTGTTATATCCATATATG GATTACTTTGCTATATAATATAATAGAACCACAGGTTTCGGCAAGTGCCT CCTCGCTGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTT (SEQ ID NO: 290)

Splice donor and splice acceptor site sequences

5' side of intron (SEQ ID NO: 291)	AGGTAAGAGTCGATCG (SEQ ID NO: 291)
3' side of intron (SEQ ID NO: 292)	ACGCGTTACTAACTGGTACCTCTTTTTTTTTTTTGATATCCTGCAGGC (SEQ ID NO: 292)

Additional miRNA Sequences

Mature miRNA_Length (Sequence)	SEQ ID NO:
Guide PD1_204_21nt (TTCAGGAATGGGTTCCAAGGA)	704
Guide PD1_204_23nt (TTCAGGAATGGGTTCCAAGGATG)	705
Passenger PD1_204_21nt (TCCTGGAAGCTATTCCTGACG)	706
Passenger PD1_204_22nt (TCCTGGAAGCTATTCCTGACGT)	707

Passenger PD1_204_23nt (TCCTGGAAGCTATTCCTGACGTT)	708
Guide PD1_206_21nt (TATAATATAATAGAACCACAG)	709
Guide PD1_206_23nt (TATAATATAATAGAACCACAGGA)	710
Passenger PD1_206_21nt (TGTGGTTCTGTTATATCCATA)	711
Passenger PD1_206_22nt (TGTGGTTCTGTTATATCCATAT)	712
Passenger PD1_206_23nt (TGTGGTTCTGTTATATCCATATA)	713

Exemplary anti-CD33 VH and VL Sequences

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
hM195 VL	293	DIQMTQSPSSLSASVG DRVTITCRASESVDNY GISFMNWFQQKPGKA PKLLIYAASNQGSGVPS RFSGSGSGTDFTLTISSL QPDDFATYYCQQSKEV PWTFGQGTKVEIK	301	GACATTCAGATGACCCAGTCTCCGAGCTCTCTGTC CGCATCAGTAGGAGACAGGGTCACCATCACATGC AGAGCCAGCGAAAGTGTCGACAATTATGGCATTA GCTTTATGAACTGGTTCCAACAGAAACCCGGGAA GGCTCCTAAGCTTCTGATTTACGCTGCATCCAACC AAGGCTCCGGGGTACCCTCTCGCTTCTCAGGCAG TGGATCTGGGACAGACTTCACTCTCACCATTTCAT CTCTGCAGCCTGATGACTTCGCAACCTATTACTGT CAGCAAAGTAAGGAGGTTCCGTGGACGTTCGGTC AAGGGACCAAGGTGGAGATCAAA
hM195 VH	294	QVQLVQSGAEVKKPGS SVKVSCKASGYTFTDY NMHWVRQAPGQGLE WIGYIYPYNGGTGYNQ KFKSKATITADESTNTA YMELSSLRSEDTAVYYC ARGRPAMDYWGQGT LVTVSS	302	CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGTG AAGAAGCCTGGGAGCTCAGTGAAGGTTTCCTGCA AAGCTTCTGGCTACACCTTCACTGACTACAACATG CACTGGGTGAGGCAGGCTCCTGGCCAAGGCCTG GAATGGATTGGATATATTTATCCTTACAATGGTG GTACCGGCTACAACCAGAAGTTCAAGAGCAAGG CCACAATTACAGCAGACGAGAGTACTAACACAGC CTACATGGAACTCTCCAGCCTGAGGTCTGAGGAC ACTGCAGTCTATTACTGCGCAAGAGGGCGCCCCG CTATGGACTACTGGGGCCAAGGGACTCTGGTCAC TGTCTCTTCA
M2H12 VH	295	QVQLQQSGPELVRPGT FVKISCKASGYTFTNYDI NWVNQRPGQGLEWI GWIYPGDGSTKYNEKF KAKATLTADKSSSTAYL		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		QLNNLTSENSAVYFCA SGYEDAMDYWGQGT SVTVSS		
M2H12 VL	296	DIKMTQSPSSMYASLG ERVIINCKASQDINSYLS WFQQKPGKSPKTLIYR ANRLVDGVPSRFSGSG SGQDYSLTISSLEYEDM GIYYCLQYDEFPLTFGA GTKLELKR		
DRB2 VH	297	EVKLQESGPELVKPGAS VKMSCKASGYKFTDYV VHWLKQKPGQGLEWI GYINPYNDGTKYNEKF KGKATLTSDKSSSTAY MEVSSLTSEDSAVYYC ARDYRYEVYGMDYWG QGTSVTVSS		
DRB2 VL	298	DIVLTQSPTIMSASPGE RVTMTCTASSSVNYIH WYQQKSGDSPLRWIF DTSKVASGVPARFSGS GSGTSYSLTISTMEAED AATYYCQQWRSYPLTF GDGTRLELKRADAAPT VS		
My9-6 VH	299	QVQLQQPGAEVVKPG ASVKMSCKASGYTFTS YYIHWIKQTPGQGLEW VGVIYPGNDDISYNQK FKGKATLTADKSSTTAY MQLSSLTSEDSAVYYC AREVRLRYFDVWGAG TTVTVSS		
My9-6 VL	300	NIMLTQSPSSLAVSAG EKVTMSCKSSQSVFFSS SQKNYLAWYQQIPGQ SPKLLIYWASTRESGVP DRFTGSGSGTDFTLTIS SVQSEDLAIYYCHQYLS		

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		SRTFGGGTKLEIKR		
hM195 scFv	303	DIQMTQSPSSLSA SVGDRVTITCRAS ESVDNYGISFMN WFQQKPGKAPKL LIYAASNQGSGVP SRFSGSGSGTDFT LTISSLQPDDFATY YCQQSKEVPWTF GQGTKVEIKGGGG SGGGGSGGGGSQ VQLVQSGAEVKKP GSSVKVSCKASGY TFTDYNMHWVRQ APGQGLEWIGYIY PYNGGTGYNQKF KSKATITADESTNT AYMELSSLRSEDT AVYYCARGRPAM DYWGQGTLVTVS S	304	GACATTCAGATGACCCAGTCTCCGAGCTCTCTGTC CGCATCAGTAGGAGACAGGGTCACCATCACATGC AGAGCCAGCGAAAGTGTCGACAATTATGGCATTA GCTTTATGAACTGGTTCCAACAGAAACCCGGGAA GGCTCCTAAGCTTCTGATTTACGCTGCATCCAACC AAGGCTCCGGGGTACCCTCTCGCTTCTCAGGCAG TGGATCTGGGACAGACTTCACTCTCACCATTTCAT CTCTGCAGCCTGATGACTTCGCAACCTATTACTGT CAGCAAAGTAAGGAGGTTCCGTGGACGTTCGGTC AAGGGACCAAGGTGGAGATCAAAGGTGGCGGTG GCTCGGGCGGTGGTGGGTCGGGTGGCGGCGGAT CTCAGGTTCAGCTGGTGCAGTCTGGAGCTGAGGT GAAGAAGCCTGGGAGCTCAGTGAAGGTTTCCTGC AAAGCTTCTGGCTACACCTTCACTGACTACAACAT GCACTGGGTGAGGCAGGCTCCTGGCCAAGGCCT GGAATGGATTGGATATATTTATCCTTACAATGGT GGTACCGGCTACAACCAGAAGTTCAAGAGCAAG GCCACAATTACAGCAGACGAGAGTACTAACACAG CCTACATGGAACTCTCCAGCCTGAGGTCTGAGGA CACTGCAGTCTATTACTGCGCAAGAGGGCGCCCC GCTATGGACTACTGGGGCCAAGGGACTCTGGTCA CTGTCTCTTCA

Exemplary anti-MUC1 VH and VL Sequences

Name	SEQ ID NO	Amino Acid Sequence
Anti-MUC1 VH1	305	QVQLVQSGAEVKKPGSSVKVSCKASGYAFSNFWMNWVRQAPGQGLEWMGQI YPGDGDTNYNGKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARSYYRSAWFA YWGQGTLVTVSS
Anti-MUC1 VH2	306	QVQLVQSGAEVKKPGASVKVSCKASGYAFSNFWMNWVRQAPGQGLEWMGQI YPGDGDTNYNGKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARSYYRSAWF AYWGQGTLVTVSS
Anti-MUC1 VH3	307	QVQLVQSGAEVKKPGASVKVSCKASGYAFSNFWMNWVRQAPGQGLEWMGQI YPGDGDTNYNGKFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARSYYRSAWF AYWGQGTLVTVSS
Anti-MUC1 VH4	308	QVQLVQSGAEVKKPGATVKISCKVSGYAFSNFWMNWVQQAPGKGLEWMGQIY PGDGDTNYNGKFKGRVTITADTSTDTAYMELSSLRSEDTAVYYCARSYYRSAWFAY WGQGTLVTVSR

Name	SEQ ID NO	Amino Acid Sequence
Anti-MUC1 VH5	309	EVQLVQSGAEVKKPGESLKISCKGSGYAFSNFWMNWVRQMPGKGLEWMGQIY PGDGDTNYNGKFKGQVTISADKSISTAYLQWSSLKASDTAMYYCARSYYRSAWFA YWGQGTLVTVSL
Anti-MUC1 VL1	310	EIVLTQSPDFQSVTPKEKVTITCRASQSIGTSIHWYQQKPDQSPKLLIKYASESISGVP SRFSGSGSGTDFTLTINSLEAEDAATYYCQQSNNWPLTFGQGTKVEIK
Anti-MUC1 VL2	311	EIVMTQSPATLSVSPGERATLSCRASQSIGTSIHWYQQKPGQAPRLLIYYASESISGI PARFSGSGSGTEFTLTISSLQSEDFAVYYCQQSNNWPLTFGGGTKVEIK
Anti-MUC1 VL3	312	EIVLTQSPATLSLSPGERATLSCRASQSIGTSIHWYQQKPGQAPRLLIYYASESISGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYCQQSNNWPLTFGGGTKVEIK
Anti-MUC1 VL4	313	AIQLTQSPSSLSASVGDRVTITCRASQSIGTSIHWYQQKPGKAPKLLIYYASESISGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQSNNWPLTFGGGTKVEIK
Anti-MUC1 VL5	314	DIVMTQSPSLLSASTGDRVTISCRASQSIGTSIHWYQQKPGKAPELLIYYASESISGV PSRFSGSGSGTDFTLTISCLQSEDFATYYCQQSNNWPLTFGQGTKVEIK

Exemplary anti-MUC16 Sequences

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Anti-MUC16 VH1	315	QVQLQESGPGLVKPS QTLSLTCTVSGYSIVSH YYWSWIRQHPGKGLE WIGYISSDGSNYYNPSL KSLVTISVDTSKNQFSL KLSSVTAADTAVYYCV RGVDYWGQGTMVTV SS	316	CAGGTGCAACTGCAGGAATCAGGTCCAGGCTTG GTCAAGCCATCGCAGACTCTTAGTCTGACATGCA CCGTGAGTGGCTATAGCATCGTGTCGCACTATTA TTGGTCTTGGATCAGGCAGCATCCAGGAAAGGG ACTGGAGTGGATCGGGTACATTAGCAGCGATGG GAGCAACTATTACAACCCATCTCTGAAGTCCCTGG TAACTATTAGCGTGGATACAAGCAAAAATCAGTT TTCATTAAAGCTCTCTTCAGTGACCGCAGCTGATA CCGCCGTCTATTATTGCGTGCGGGGGGTGGACTA CTGGGGTCAGGGCACCATGGTTACTGTGTCATCA
Anti-MUC16 VH2	317	QVQLQESGPGLVKPSD TLSLTCAVSGYSIVSHY YWGWIRQPPGKGLE WIGYISSDGSNYYNPSL KSRVTMSVDTSKNQFS LKLSSVTAVDTAVYYCV RGVDYWGQGTMVTV SS	318	CAGGTACAGCTGCAGGAGAGTGGCCCTGGTTTA GTAAAGCCATCAGATACACTTTCACTTACCTGCGC CGTGTCTGGTTATTCTATCGTGAGCCACTATTACT GGGGATGGATCCGCCAGCCCCCTGGCAAAGGTCT TGAGTGGATTGGCTATATAAGTTCGGATGGCAGT AACTATTACAATCCTTCTCTGAAGAGCCGTGTCAC TATGAGCGTGGACACTAGCAAAAACCAGTTCAGC CTGAAGCTGTCCTCCGTCACCGCCGTAGACACCG CTGTCTACTATTGTGTTAGGGGGGTGGACTACTG GGGCCAAGGCACCATGGTCACGGTGAGCAGC
Anti-	319	EVQLVESGGGLVQPG	320	

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
MUC16 VH3		GSLRLSCAASGYSIVSH YYMSWVRQAPGKGLE WVSVISSDGSNYYADS VKGRFTISRDNSKNTLY LQMNSLRAEDTAVYYC VRGVDYWGQGTLVTV SS		
Anti-MUC16 VH4	321	EVQLVESGGGLVQPG RSLRLSCAASGYSIVSH YYMHWVRQAPGKGL EWVSAISSDGSNEYAD SVEGRFTISRDNAKNSL YLQMNSLRAEDTAVYY CVRGVDYWGQGTLVT VSS	322	GAGGTGCAGCTCGTCGAGTCCGGAGGCGGTCTG GTGCAACCCGGCCGTTCTTTGCGGCTGAGTTGCG CTGCCAGTGGGTATAGCATCGTGAGTCACTATTA CATGCATTGGGTTCGTCAAGCCCCTGGCAAGGGA CTAGAGTGGGTGTCCGCCATCTCCTCAGACGGTA GTAATGAGTACGCGGACAGCGTAGAGGGTAGAT TCACCATTTCTCGGGACAATGCCAAAAATAGTCTA TACCTCCAAATGAATTCCCTTAGGGCCGAAGACA CTGCCGTGTACTACTGTGTTCGGGGCGTGGACTA CTGGGGGCAGGGGACATTGGTGACTGTGAGCTC A
Anti-MUC16 VH5	323	QVQLQESGPGLVKPS QTLSLTCTVSGYSIVSH YYWSWIRQHPGKGLE WIGYISSDGSNEYNPSL KSLVTISVDTSKNQFSL KLSSVTAADTAVYFCV RGVDYWGQGTMVTV SS	324	CAGGTCCAACTGCAGGAATCTGGCCCCGGACTGG TTAAACCATCTCAGACACTCTCCCTGACCTGCACC GTGTCTGGATACAGCATCGTTTCTCATTATTACTG GTCATGGATTAGGCAGCATCCCGGAAAAGGGCTT GAATGGATTGGCTACATCTCCTCCGACGGCTCCA ATGAGTACAACCCATCACTTAAATCTCTGGTCACG ATAAGCGTAGACACATCTAAAAATCAGTTCTCATT AAAGCTCAGCTCTGTTACAGCTGCCGACACCGCT GTGTACTTCTGTGTGCGAGGGGTTGACTACTGGG GGCAGGGCACAATGGTGACAGTGTCTTCC
Anti-MUC16 VH6	325	QVQLVQSGAEVKKPG SSVKVSCKASGYSIVSH YYISWVRQAPGQGLE WMGGISSDGSNNYA QKFQGRVTITADESTS TAYMELSSLRS EDTAVYYCVRGVDYW GQGTLVTVSS	326	CAGGTTCAACTGGTTCAGTCCGGAGCCGAGGTCA AAAAGCCTGGATCCTCTGTGAAGGTGTCATGTAA GGCTTCTGGCTACAGCATCGTCTCACATTATTACA TATCTTGGGTGCGACAGGCCCCCGGCCAGGGGCT CGAGTGGATGGGAGGTATTTCCTCCGACGGGAG TAACAATTACGCTCAGAAATTTCAGGGCCGGGTG ACCATTACCGCCGACGAAAGTACAAGCACCGCTT ATATGGAATTAAGCTCTTTAAGATCAGAGGACAC GGCTGTGTACTACTGTGTAAGGGGCGTGGATTAC TGGGGTCAGGGGACGCTCGTCACCGTCTCGAGC
Anti-MUC16	327	QVQLQESGPGLVKPSE TLSLTCTVSGYSIVSHYY	328	CAGGTCCAGCTCCAGGAATCCGGCCCAGGGTTGG TGAAGCCTTCGGAGACCCTGTCTCTGACATGCAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
VH7		WSWIRQPPGKGLEWIGYISSDGSNNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCVRGVDYWGQGTTVTVSS		AGTCAGCGGCTATAGTATCGTCTCCCACTATTATTGGTCTTGGATTCGGCAACCTCCAGGCAAGGGGTTAGAATGGATTGGATACATCTCAAGCGATGGGTCCAATAACTACAACCCAAGTCTCAAAAGTAGAGTGACTATCTCTGTGGATACCAGTAAAAACCAGTTTTCACTCAAGTTGAGTTCCGTCACCGCCGCCGACACAGCCGTTTACTACTGTGTTCGGGGAGTGGACTACTGGGGCCAAGGTACCACGGTTACCGTGAGCAGC
Anti-MUC16 VH8	648	QVQLQESGPGLVKPSDTLSLTCAVSGYSIVSHYYWHWIRQPPGKGLEWMGYISSDGSNDFNPSLKTRITISRDTSKNQFSLKLSSVTAVDTAVYYCVRGVDYWGQGTLVTVSS	649	CAGGTGCAGCTGCAGGAGAGCGGCCCCGGCCTGGTGAAGCCCAGCGACACCCTGAGCCTGACCTGCGCCGTGAGCGGCTACAGCATCGTGAGCCACTACTACTGGCACTGGATCAGACAGCCCCCCGGCAAGGGCCTGGAGTGGATGGGCTACATCAGCAGCGACGGCAGCAACGACTTCAACCCCAGCCTGAAGACCAGAATCACCATCAGCAGAGACACCAGCAAGAACCAGTTCAGCCTGAAGCTGAGCAGCGTGACCGCCGTGGACACCGCCGTGTACTACTGCGTGAGAGGCGTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC
Anti-MUC16 VH9	650	QVQLQESGPGLVKPSQTLSLTCAVYGYSIVSHYYWSWIRQPPGKGLEWIGEISSDGSNNYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCVRGVDYWGQGTLVTVSS	651	CAGGTGCAGCTGCAGGAGAGCGGCCCCGGCCTGGTGAAGCCCAGCCAGACCCTGAGCCTGACCTGCGCCGTGTACGGCTACAGCATCGTGAGCCACTACTACTGGAGCTGGATCAGACAGCCCCCCGGCAAGGGCCTGGAGTGGATCGGCGAGATCAGCAGCGACGGCAGCAACAACTACAACCCCAGCCTGAAGAGCAGAGTGACCATCAGCGTGGACACCAGCAAGAACCAGTTCAGCCTGAAGCTGAGCAGCGTGACCGCCGCCGACACCGCCGTGTACTACTGCGTGAGAGGCGTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC
Anti-MUC16 VH10	652	QVQLQESGPGLVKPSETLSLTCAVSGYSIVSHYYWGWIRQPPGKGLEWIGSISSDGSNYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCVRGVDYWGQGTLVTVSS	653	CAGGTGCAGCTGCAGGAGAGCGGCCCCGGCCTGGTGAAGCCCAGCGAGACCCTGAGCCTGACCTGCGCCGTGAGCGGCTACAGCATCGTGAGCCACTACTACTGGGGCTGGATCAGACAGCCCCCCGGCAAGGGCCTGGAGTGGATCGGCAGCATCAGCAGCGACGGCAGCAACTACTACAACCCCAGCCTGAAGAGCAGAGTGACCATCAGCGTGGACACCAGCAAGAACCAGTTCAGCCTGAAGCTGAGCAGCGTGACCGCCGCCGACACCGCCGTGTACTACTGCGTGAGAGGCGTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Anti-MUC16 VH11	654	QVQLVESGGGVVQPGRSLRLSCAASGYSIVSHYYWNWVRQAPGKGLEWVAYISSDGSNEYNPSLKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYCVRGVDYWGQGTTVTVSS	655	CAGGTGCAGCTGGTGGAGAGCGGCGGCGGCGTGGTGCAGCCCGGCAGAAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTACAGCATCGTGAGCCACTACTACTGGAACTGGGTGAGACAGGCCCCCGGCAAGGGCCTGGAGTGGGTGGCCTACATCAGCAGCGACGGCAGCAACGAGTACAACCCCAGCCTGAAGAACAGATTCACCATCAGCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGTGAGAGGCGTGGACTACTGGGGCCAGGGCACCACCGTGACCGTGAGCAGC
Anti-MUC16 VH12	656	QVQLQESGPGLVKPSQTLSLTCTVSGYSIVSHYYWNWIRQHPGKGLEWIGYISSDGSNEYNPSLKNLVTISVDTSKNQFSLKLSSVTAADTAVYYCVRGVDYWGQGTMVTVSS	657	CAGGTGCAGCTGCAGGAGAGCGGCCCCGGCCTGGTGAAGCCCAGCCAGACCCTGAGCCTGACCTGCACCGTGAGCGGCTACAGCATCGTGAGCCACTACTACTGGAACTGGATCAGACAGCACCCCGGCAAGGGCCTGGAGTGGATCGGCTACATCAGCAGCGACGGCAGCAACGAGTACAACCCCAGCCTGAAGAACCTGGTGACCATCAGCGTGGACACCAGCAAGAACCAGTTCAGCCTGAAGCTGAGCAGCGTGACCGCCGCCGACACCGCCGTGTACTACTGCGTGAGAGGCGTGGACTACTGGGGCCAGGGCACCATGGTGACCGTGAGCAGC
Anti-MUC16 VH13	658	QVQLQESGPGLVKPSDTLSLTCAVSGYSIVSHYYWNWIRQPPGKGLEWIGYISSDGSNEYNPSLKNRVTMSVDTSKNQFSLKLSSVTAVDTAVYYCVRGVDYWGQGTMVTVSS	659	CAGGTGCAGCTGCAGGAGAGCGGCCCCGGCCTGGTGAAGCCCAGCGACACCCTGAGCCTGACCTGCGCCGTGAGCGGCTACAGCATCGTGAGCCACTACTACTGGAACTGGATCAGACAGCCCCCCGGCAAGGGCCTGGAGTGGATCGGCTACATCAGCAGCGACGGCAGCAACGAGTACAACCCCAGCCTGAAGAACAGAGTGACCATGAGCGTGGACACCAGCAAGAACCAGTTCAGCCTGAAGCTGAGCAGCGTGACCGCCGTGGACACCGCCGTGTACTACTGCGTGAGAGGCGTGGACTACTGGGGCCAGGGCACCATGGTGACCGTGAGCAGC
Anti-MUC16 VH14	660	EVQLLESGGGLVQPGGSLRLSCAASGYSIVSHYYWNWVRQAPGKGLEWVSYISSDGSNEYNPSLKNRFTISRDNSKNTLYLQMNSLRAEDTAVYYC	661	GAGGTGCAGCTGCTGGAGAGCGGCGGCGGCCTGGTGCAGCCCGGCGGCAGCCTGAGACTGAGCTGCGCCGCCAGCGGCTACAGCATCGTGAGCCACTACTACTGGAACTGGGTGAGACAGGCCCCCGGCAAGGGCCTGGAGTGGGTGAGCTACATCAGCAGCGACGGCAGCAACGAGTACAACCCCAGCCTGAAGAACAGATTCACCATCAGCAGAGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		VRGVDYWGQGTLVTVSS		GACACCGCCGTGTACTACTGCGTGAGAGGCGTGGACTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC
Anti-MUC16 VH	672	VKLQESGGGFVKPGGSLKVSCAASGFTFSSYAMSWVRLSPEMRLEWVATISSAGGYIFYSDSVQGRFTISRDNAKNTLHLQMGSLRSGDTAMYYCARQGFGNYGDYYAMDYWGQGTTVTVSS	673	GTGAAGCTGCAAGAGTCCGGCGGAGGCTTTGTGAAGCCTGGCGGCTCTCTGAAAGTGTCCTGTGCCGCCAGCGGCTTCACCTTTAGCAGCTACGCCATGAGCTGGGTCCGACTGAGCCCTGAGATGAGACTGGAATGGGTCGCCACCATCAGTAGCGGCAGGCGGCTACATCTTCTACAGCGACTCTGTGCAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACACCCTGCACCTCCAGATGGGCAGTCTGAGAAGCGGCGATACCGCCATGTACTACTGCGCCAGACAAGGCTTCGGCAACTACGGCGACTACTATGCCATGGATTACTGGGGCCAGGGCACCACCGTGACAGTCTCTTCT
Anti-MUC16 VH	674	VKLEESGGGFVKPGGSLKISCAASGFTFRNYAMSWVRLSPEMRLEWVATISSAGGYIFYSDSVQGRFTISRDNAKNTHLQMGSLRSGDTAMYYCARQGFGNYGDYYAMDYWGQGTTVTVSS	675	GTGAAGCTGGAAGAGTCCGGCGGAGGCTTTGTGAAGCCTGGCGGAAGCCTGAAGATCAGCTGTGCCGCCAGCGGCTTCACCTTCAGAAACTACGCCATGAGCTGGGTCCGACTGAGCCCCGAGATGAGACTGGAATGGGTCGCCACAATCAGCAGCGGCAGGCGGCTACATCTTCTACAGCGATAGCGTGCAGGGCAGATTCACCATCAGCCGGGACAACGCCAAGAACACCCTGCACCTCCAGATGGGCAGTCTGAGATCTGGCGACACCGCCATGTACTACTGGCCAGACAAGGCTTCGGCAACTACGGCGACTACTATGCCATGGATTACTGGGGCCAGGGCACCACCGTGACAGTCTCTTCT
Anti-MUC16 VH	676	DVQLLESGPGLVRPSQSLSLTCSVTGYSIVSHYYWNWIRQFPGNKLEWMGYISSDGSNEYNPSLKNRISISLDTSKNQFFLKFDFVTTADTATYFCVRGVDYWGQGTTLTVSS	677	GACGTGCAACTTCTGGAGAGCGGGCCAGGGCTAGTCAGGCCCTCCCAGTCGCTTTCACTGACTTGCAGTGTGACCGGTTACTCTATTGTGAGTCACTACTATTGGAACTGGATTCGGCAGTTCCCAGGCAACAAACTGGAATGGATGGGGTACATATCTTCCGATGGCTCGAATGAATATAACCCATCATTGAAAAATCGTATTTCCATCAGTCTGGATACGAGTAAAAACCAGTTTTTCCTCAAATTCGATTTCGTGACTACAGCAGATACTGCCACATACTTCTGTGTACGAGGTGTCGATTATTGGGGACAGGGCACAACG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CTGACCGTAAGTTCT
Anti-MUC16 VH	678	DVQLQESGPGLVNPSQSLSLTCTVTGYSITNDYAWNWIRQFPGNKLEWMGYINYSGYTTYNPSLKSRISITRDTSKNQFFLHLNSVTTEDTATYYCARWDGGLTYWGQGTLVTVSA	679	GACGTTCAGCTGCAAGAGTCTGGCCCTGGCCTGGTCAATCCTAGCCAGAGCCTGAGCCTGACATGTACCGTGACCGGCTACAGCATCACCAACGACTACGCCTGGAACTGGATCAGACAGTTCCCCGGCAACAAGCTGGAATGGATGGGCTACATCAACTACAGCGGCTACACCACCTACAATCCCAGCCTGAAGTCCCGGATCTCCATCACCAGAGACACCAGCAAGAACCAGTTCTTCCTGCACCTGAACAGCGTGACCACCGAGGATACCGCCACCTACTACTGCGCTAGATGGGATGGCGGCCTGACATATTGGGGCCAGGGAACACTGGTCACCGTGTCTGCT
Anti-MUC16 VH	680	EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGYINYSGYTTYNPSLKSRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARWDGGLTYWGQGTLVTVSS	681	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGCCTGGTGCAGCCCGGCGGCAGCCTGAGGCTGAGCTGCGCCGCCAGCGGCTACAGCATCACCAACGACTACGCCTGGAACTGGGTGAGGCAGGCCCCCGGCAAGGGCCTGGAGTGGGTGGGCTACATCAACTACAGCGGCTACACCACCTACAACCCCAGCCTGAAGAGCAGGTTCACCATCAGCAGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGTGGGACGGCGGCCTGACCTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC
Anti-MUC16 VH	682	EVQLVESGGGLVQPGGSLRLSCAASGYSITNDYAWNWVRQAPGKGLEWVGYINYSGYTTYNPSLKSRFTISRDNSKNTFYLQMNSLRAEDTAVYYCARWDGGLTYWGQGTLVTVSS	683	GAGGTGCAGCTGGTGGAGAGCGGCGGCGGCCTGGTGCAGCCCGGCGGCAGCCTGAGGCTGAGCTGCGCCGCCAGCGGCTACAGCATCACCAACGACTACGCCTGGAACTGGGTGAGGCAGGCCCCCGGCAAGGGCCTGGAGTGGGTGGGCTACATCAACTACAGCGGCTACACCACCTACAACCCCAGCCTGAAGAGCAGGTTCACCATCAGCAGGGACAACAGCAAGAACACCTTCTACCTGCAGATGAACAGCCTGAGGGCCGAGGACACCGCCGTGTACTACTGCGCCAGGTGGGACGGCGGCCTGACCTACTGGGGCCAGGGCACCCTGGTGACCGTGAGCAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
Anti-MUC16 VH	684	EVQLQQSGAELVKPG ASVKLSCTASGFNIKD TYMHWVKQRPEQGL EWIGRVDPANGNTK YDPKFQGKATLTADT SSNTAYLQLSSLTSEDT AVYFCVRDYYGHTYG FAFCDQGTTLTVSA	685	GAGGTTCAGCTGCAGCAGTCTGGCGCCGAACTT GTGAAACCTGGCGCCTCTGTGAAGCTGAGCTGT ACCGCCAGCGGCTTCAACATCAAGGACACCTAC ATGCACTGGGTCAAGCAGAGGCCTGAGCAGGG CCTCGAATGGATCGGAAGAGTGGATCCCGCCA ACGGCAACACCAAATACGACCCCAAGTTCCAGG GCAAAGCCACACTGACCGCCGACACCTCTAGCA ACACAGCCTACCTGCAGCTGTCCAGCCTGACCTC TGAAGATACCGCCGTGTACTTCTGCGTGCGGGA CTACTACGGCCATACCTACGGCTTCGCCTTCTGC GACCAAGGCACAACCCTGACAGTGTCTGCT
Anti-MUC16 VH	686	EVQLVESGGGLVQPG GSLRLSCAASGFNIKD TYMHWVRQAPGKGL EWVGRVDPANGNTK YDPKFQGRFTISADTS KNTAYLQMNSLRAED TAVYYCVRDYYGHTY GFAFWGQGTLVTVSS	687	GAGGTGCAGCTGGTTGAATCTGGCGGAGGACT GGTTCAGCCTGGCGGATCTCTGAGACTGTCTTG TGCCGCCAGCGGCTTCAACATCAAGGACACCTA CATGCACTGGGTCCGACAGGCCCCTGGCAAAGG ACTTGAGTGGGTTGGAAGAGTGGACCCCGCCAA CGGCAACACCAAATACGACCCCAAGTTCCAGGG CAGATTCACCATCAGCGCCGACACCAGCAAGAA CACCGCCTACCTGCAGATGAACAGCCTGAGAG CCGAGGACACCGCCGTGTACTATTGCGTGCG GGATTACTACGGCCATACCTACGGCTTCGCCT TTTGGGGCCAGGGCACACTGGTTACCGTTAGCT CT
Anti-MUC16 VL1	329	DIQMTQSPSSLSASVG DRVTITCQASRDINNFL NWYQQKPGKAPKLLIY RANNLETGVPSRFSGS GSGTDFTFTISSLQPED IATYFCLQYGDLYTFGG GTKVEIK	330	GACATACAGATGACTCAGAGCCCCTCCTCACTCTC GGCATCAGTCGGCGACAGGGTCACAATTACCTGT CAGGCTTCTCGCGACATTAATAACTTCCTGAATTG GTATCAGCAAAAGCCCGGGAAGGCCCCTAAGCT GTTGATTTATAGAGCAAATAATCTCGAAACCGGC GTGCCCAGTAGGTTTAGCGGGTCCGGGAGCGGA ACAGACTTCACATTCACCATTTCTAGTTTGCAGCC CGAAGACATTGCTACATATTTTTGCCTGCAGTACG GGGATCTCTACACTTTCGGGGGCGGAACAAAGGT TGAGATAAAA
Anti-MUC16 VL2	331	DIQMTQSPSSLSASVG DRVTITCQASRDINNFL NWYQQKPGKAPKLLIY RANNLETGVPSRFSGS GSGTDFTFTISSLQPED	332	GATATTCAAATGACGCAGTCACCCTCATCGCTCTC TGCGTCAGTAGGGGATCGTGTCACGATAACCTGT CAAGCATCAAGGGACATCAACAACTTCCTCAACT GGTACCAACAGAAGCCTGGCAAGGCACCTAAACT CCTGATCTACCGGGCTAACAACCTAGAAACCGGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		IATYYCLQYGDLYTFGG GTKVEIK		GTTCCGAGCCGATTCAGTGGGTCTGGAAGCGGG ACAGACTTTACGTTCACTATTAGTTCGCTACAGCC CGAAGACATTGCGACATATTACTGTCTTCAGTATG GGGATTTGTATACCTTTGGGGGAGGCACCAAGGT AGAGATAAAG
Anti-MUC16 VL3	333	DIQMTQSPSSLSASVG DRVTITCRASRDINNFL GWYQQKPGKAPKRLI YRANSLQSGVPSRFSG SGSGTEFTLTISSLQPE DFATYYCLQYGDLYTF GQGTKVEIK	334	GACATCCAGATGACTCAGAGCCCGTCTTCTCTATC CGCAAGTGTAGGCGATCGTGTCACCATCACATGC CGGGCTTCCCGGGATATCAACAACTTCCTTGGGT GGTATCAGCAGAAGCCCGGAAAAGCCCCCAAAC GGCTCATCTACAGAGCGAATTCCCTGCAGTCAGG TGTCCCCAGTAGGTTCAGCGGATCAGGCTCGGGG ACCGAATTCACTCTGACCATTAGCTCACTGCAGCC TGAGGATTTCGCTACTTACTATTGCCTGCAATACG GCGATCTGTACACTTTCGGGCAGGGCACCAAGGT GGAAATAAAA
Anti-MUC16 VL4	335	EIVLTQSPGTLSLSPGE RATLSCRASRDINNFLA WYQQKPGQAPRLLIYR ANSRATGIPDRFSGSG SGTDFTLTISRLEPEDF AVYYCLQYGDLYTFGQ GTKVEIK	336	GAAATCGTACTGACCCAGTCTCCCGGAACCCTGA GTCTCTCACCCGGCGAGCGCGCAACACTGTCGTG TAGGGCCAGTAGGGACATAAATAACTTCCTAGCC TGGTACCAACAAAAACCGGGTCAGGCTCCAAGAC TGTTGATCTATAGAGCTAACTCCAGGGCCACCGG CATCCCAGACCGATTCTCAGGCTCCGGATCTGGA ACCGACTTCACGCTCACCATTAGCCGACTAGAACC TGAGGACTTTGCTGTATACTATTGCCTGCAGTACG GCGACCTGTATACCTTTGGACAGGGTACCAAGGT CGAGATCAAG
Anti-MUC16 VL5	337	EIVLTQSPATLSLSPGE RATLSCRASRDINNFLA WYQQKPGQAPRLLIYR ANNRATGIPARFSGSG PGTDFTLTISSLEPEDFA VYYCLQYGDLYTFGGG TKVEIK	338	GAGATCGTACTTACGCAGAGCCCAGCAACTCTGT CTCTGTCCCCCGGAGAACGGGCCACCCTGTCGTG CCGGGCCAGCCGTGATATTAATAATTTCCTGGCCT GGTATCAACAAAAACCGGGGCAGGCTCCTCGACT GTTGATCTACCGGGCCAACAATAGAGCAACTGGT ATCCCTGCTCGCTTCTCCGGCAGTGGGCCAGGTA CAGACTTCACCCTGACTATTTCGTCACTCGAACCA GAAGACTTTGCCGTGTATTATTGCTTACAATACGG GGATCTGTACACTTTCGGAGGAGGAACTAAGGTC GAAATTAAG
Anti-MUC16 VL6	339	EIVLTQSPDFQSVTPKE KVTITCRASRDINNFLH WYQQKPDQSPKLLIKR ANQSFSGVPSRFSGSG SGTDFTLTINSLEAEDA	340	GAGATCGTGCTGACCCAGAGCCCCGACTTCCAGA GCGTGACCCCCAAGGAGAAGGTGACCATCACCTG CAGAGCCAGCAGAGACATCAACAACTTCCTGCAC TGGTACCAGCAGAAGCCCGACCAGAGCCCCAAG CTGCTGATCAAGAGAGCCAACCAGAGCTTCAGCG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		ATYYCLQYGDLYTFGQ GTKVEIK		GCGTGCCCAGCAGATTCAGCGGCAGCGGCAGCG GCACCGACTTCACCCTGACCATCAACAGCCTGGA GGCCGAGGACGCCGCCACCTACTACTGCCTGCAG TACGGCGACCTGTACACCTTCGGCCAGGGCACCA AGGTGGAGATCAAG
Anti-MUC16 VL7	341	DIQMTQSPSSLSASVG DRVTITCRASRDINNFL AWFQQKPGKAPKSLIY RANSLQSGVPSRFSGS GSGTDFTLTISSLQPED FATYYCLQYGDLYTFG GGTKVEIK	342	GACATCCAGATGACCCAGAGCCCCAGCAGCCTGA GCGCCAGCGTGGGCGACAGAGTGACCATCACCT GCAGAGCCAGCAGAGACATCAACAACTTCCTGGC CTGGTTCCAGCAGAAGCCCGGCAAGGCCCCCAAG AGCCTGATCTACAGAGCCAACAGCCTGCAGAGCG GCGTGCCCAGCAGATTCAGCGGCAGCGGCAGCG GCACCGACTTCACCCTGACCATCAGCAGCCTGCA GCCCGAGGACTTCGCCACCTACTACTGCCTGCAG TACGGCGACCTGTACACCTTCGGCGGCGGCACCA AGGTGGAGATCAAG
Anti-MUC16 VL8	662	DIQMTQSPSSLSASVG DRVTITCRASRDINNFL AWYQQKPGKAPKLLL YRANRLESGVPSRFSG SGSGTDYTLTISSLQPE DFATYYCLQYGDLYTF GGGTKVEIK	663	GACATCCAGATGACCCAGAGCCCCAGCAGCCTGA GCGCCAGCGTGGGCGACAGAGTGACCATCACCT GCAGAGCCAGCAGAGACATCAACAACTTCCTGGC CTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAA GCTGCTGCTGTACAGAGCCAACAGACTGGAGAG CGGCGTGCCCAGCAGATTCAGCGGCAGCGGCAG CGGCACCGACTACACCCTGACCATCAGCAGCCTG CAGCCCGAGGACTTCGCCACCTACTACTGCCTGC AGTACGGCGACCTGTACACCTTCGGCGGCGGCAC CAAGGTGGAGATCAAG
Anti-MUC16 VL9	664	DIQMTQSPSSLSASVG DRVTITCKASRDINNFL SWYQQKPGKAPKLLIY RANRLVDGVPSRFSGS GSGTDFTFTISSLQPED IATYYCLQYGDLYTFGG GTKVEIK	665	GACATCCAGATGACCCAGAGCCCCAGCAGCCTGA GCGCCAGCGTGGGCGACAGAGTGACCATCACCT GCAAGGCCAGCAGAGACATCAACAACTTCCTGAG CTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAA GCTGCTGATCTACAGAGCCAACAGACTGGTGGAC GGCGTGCCCAGCAGATTCAGCGGCAGCGGCAGC GGCACCGACTTCACCTTCACCATCAGCAGCCTGCA GCCCGAGGACATCGCCACCTACTACTGCCTGCAG TACGGCGACCTGTACACCTTCGGCGGCGGCACCA AGGTGGAGATCAAG
Anti-MUC16 VL10	666	DIQMTQSPSSLSASVG DRVTITCKASRDINNFL SWYQQKPGKAPKLLIY RANRLVDGVPSRFSGS GSGTDFTLTISSLQPED	667	GACATCCAGATGACCCAGAGCCCCAGCAGCCTGA GCGCCAGCGTGGGCGACAGAGTGACCATCACCT GCAAGGCCAGCAGAGACATCAACAACTTCCTGAG CTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAA GCTGCTGATCTACAGAGCCAACAGACTGGTGGAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		FATYYCLQYGDLYTFG QGTKVEIK		GGCGTGCCCAGCAGATTCAGCGGCAGCGGCAGC GGCACCGACTTCACCCTGACCATCAGCAGCCTGC AGCCCGAGGACTTCGCCACCTACTACTGCCTGCA GTACGGCGACCTGTACACCTTCGGCCAGGGCACC AAGGTGGAGATCAAG
Anti-MUC16 VL11	668	EIVLTQSPGTLSLSPGE RATLSCKASRDINNFLS WYQQKPGQAPRLLIYR ANRLVDGIPDRFSGSG SGTDFTLTISRLEPEDF AVYYCLQYGDLYTFGQ GTKVEIK	669	GAGATCGTGCTGACCCAGAGCCCCGGCACCCTGA GCCTGAGCCCCGGCGAGAGAGCCACCCTGAGCT GCAAGGCCAGCAGAGACATCAACAACTTCCTGAG CTGGTACCAGCAGAAGCCCGGCCAGGCCCCCAG ACTGCTGATCTACAGAGCCAACAGACTGGTGGAC GGCATCCCCGACAGATTCAGCGGCAGCGGCAGC GGCACCGACTTCACCCTGACCATCAGCAGACTGG AGCCCGAGGACTTCGCCGTGTACTACTGCCTGCA GTACGGCGACCTGTACACCTTCGGCCAGGGCACC AAGGTGGAGATCAAG
Anti-MUC16 VL12	670	EIVLTQSPATLSLSPGE RATLSCKASRDINNFLS WYQQKPGQAPRLLIYR ANRLVDGIPARFSGSG SGTDFTLTISSLEPEDFA VYYCLQYGDLYTFGQG TKVEIK	671	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGA GCCTGAGCCCCGGCGAGAGAGCCACCCTGAGCT GCAAGGCCAGCAGAGACATCAACAACTTCCTGAG CTGGTACCAGCAGAAGCCCGGCCAGGCCCCCAG ACTGCTGATCTACAGAGCCAACAGACTGGTGGAC GGCATCCCCGCCAGATTCAGCGGCAGCGGCAGC GGCACCGACTTCACCCTGACCATCAGCAGCCTGG AGCCCGAGGACTTCGCCGTGTACTACTGCCTGCA GTACGGCGACCTGTACACCTTCGGCCAGGGCACC AAGGTGGAGATCAAG
Anti-MUC16 VL	688	DIELTQSPSSLAVSAGE KVTMSCKSSQSLLNSR TRKNQLAWYQQKPG QSPELLIYWASTRQSG VPDRFTGSGSGTDFTL TISSVQAEDLAVYYCQ QSYNLLTFGPGTKLEV KR	689	GACATCGAGCTGACACAGAGCCCATCTAGCCTG GCTGTGTCTGCCGGCGAGAAAGTGACCATGAG CTGCAAGAGCAGCCAGAGCCTGCTGAACAGCC GGACCAGAAAGAATCAGCTGGCCTGGTATCAGC AGAAGCCCGGCCAATCTCCTGAGCTGCTGATCT ACTGGGCCAGCACAAGACAGAGCGGCGTGCC CGATAGATTCACAGGATCTGGCAGCGGCACCG ACTTCACCCTGACAATCAGTTCTGTGCAGGCCG AGGACCTGGCCGTG TACTACTGTCAGCAGAGCTACAACCTGCTGACCT TCGGACCCGGCACCAAGCTGGAAGTGAAGAGA
Anti-MUC16 VL	690	DIELTQSPSSLAVSAGE KVTMSCKSSQSLLNSR TRKNQLAWYQQKTG	691	GACATCGAGCTGACACAGAGCCCATCTAGCCTG GCTGTGTCTGCCGGCGAGAAAGTGACCATGA GCTGCAAGAGCAGCCAGAGCCTGCTGAACAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		QSPELLIYWASTRQSG VPDRFTGSGSGTDFTL TISSVQAEDLAVYYCQ QSYNLLTFGPGTKLEIK R		CGGACCAGAAAGAATCAGCTGGCCTGGTATCA GCAGAAAACCGGACAGAGCCCCGAGCTGCTG ATCTACTGGGCCAGCACAAGACAGAGCGGCGTG CCCGATAGATTCACAGGATCTGGCAGCGGCACC GACTTCACCCTGACAATCAGTTCTGTGCAGGCC GAGGACCTGGCCGTGTACTACTGTCAGCAGAGC TACAACCTGCTGACCTTCGGACCCGGCACCAAG CTGGAAATCAAGAGA
Anti-MUC16 VL	692	DIKMAQSPSSVNASL GERVTITCKASRDINN FLSWFHQKPGKSPKTL IYRANRLVDGVPSRFS GSGSGQDYSFTISSLEY EDVGIYYCLQYGDLYTF GGGTKLEIK	693	GACATCAAGATGGCTCAGTCCCCTTCTAGCGT GAATGCTTCGCTAGGGGAGCGTGTGACCATCAC ATGTAAAGCATCACGCGACATAAATAATTTCCTT TCCTGGTTTCATCAGAAACCGGGCAAGTCGCCTA AGACGCTGATTTACAGAGCAAATCGGTTGGTAG ATGGAGTGCCAAGCAGATTCAGCGGGAGCGG AAGTGGACAGGATTATAGCTTCACTATTTCATC CCTGGAATACGAGGACGTAGGTATCTATTATTG CCTCCAGTATGGCGATCTTTACACATTTGGTGGG GGGACTAAGCTGGAGATTAAG
Anti-MUC16 VL	694	DIQMTQSSSFLSVSLG GRVTITCKASDLIHN WLAWYQQKPGNAPR LLISGATSLETGVPSRF SGSGSGNDYTLSIASLQ TEDAATYYCQQYWTT PFTFGSGTKLEIK	695	GACATCCAGATGACCCAGAGCAGCAGCTTCCTG AGCGTGTCCCTTGGCGGCAGAGTGACCATCAC CTGTAAAGCCAGCGACCTGATCCACAACTGGCT GGCCTGGTATCAGCAGAAGCCTGGCAACGCTCC CAGACTGCTGATTAGCGGCGCCACCTCTCTGGA AACAGGCGTGCCAAGCAGATTTTCCGGCAGCGG CTCCGGCAACGACTACACACTGTCTATTGCCAG CCTGCAGACCGAGGATGCCGCCACCTATTACTG CCAGCAGTACTGGACCACACCTTTCACCTTTG GCAGCGGCACCAAGCTGGAAATCAAG
Anti-MUC16 VL	696	DIQMTQSPSSLSASV GDRVTITCKASDLIHN WLAWYQQKPGKAPK LLISGATSLETGVPSRFS GSGSGTDFTLTISSLQP EDFATYYCQQYWTTPF TFGQGTKVEIKR	697	GACATCCAGATGACCCAGAGCCCCAGCAGCCTGA GCGCCAGCGTGGGCGACAGGGTGACCATCACCT GCAAGGCCAGCGACCTGATCCACAACTGGCTGGC CTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAA GCTGCTGATCAGCGGCGCCACCAGCCTGGAGACC GGCGTGCCCAGCAGGTTCAGCGGCAGCGGCAGC GGCACCGACTTCACCCTGACCATCAGCAGCCTG CAGCCCGAGGACTTCGCCACCTACTACTGCCAG CAGTACTGGACCACCCCCTTCACCTTCGGCCAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GGCACCAAGGTGGAGATCAAGAGG
Anti-MUC16 VL	698	DIVLTQSPAIMSASLG ERVTMTCTASSSVSS SYLHWYQQKPGSSP KLWIYSTSNLASGVPG RFSGSGSGTSYSLTIS SMEAEDAATYYCH QYHRSPYTFGGGTKV EIKR	699	GACATCGTGCTGACACAGAGCCCTGCCATCATG TCTGCCAGCCTCGGCGAGCGAGTGACCATGACA TGTACAGCCAGCAGCAGCGTGTCCAGCAGCTAC CTGCATTGGTATCAGCAGAAGCCCGGCAGCAGC CCCAAGCTGTGGATCTACAGCACAAGCAATCTG GCCAGCGGCGTGCCAGGCAGATTTTCTGGTTC TGGCAGCGGCACCAGCTACAGCCTGACAATCAG CAGCATGGAAGCCGAGGATGCCGCCACCTAC TACTGCCACCAGTACCACAGAAGCCCCTACACC TTTGGCGGAGGCACCAAGGTGGAAATCAAGC GG
Anti-MUC16 VL	700	DIQMTQSPSSLSASV GDRVTITCTASSSVSSS YLHWYQQKPGKAPKL LIYSTSNLASGVPSRFS GSGSGTDFTLTISSLQ PEDFATYYCHQYHRSP YTFGQGTKVEIKR	701	GACATCCAGATGACACAGAGCCCTAGCAGCCTG TCTGCCAGCGTGGGAGACAGAGTGACCATCACC TGTACAGCCAGCAGCAGCGTGTCCAGCAGCTAC CTGCATTGGTATCAGCAGAAGCCCGGCAAGGCC CCTAAGCTGCTGATCTACAGCACCAGCAATCTGG CCAGCGGCGTGCCAAGCAGATTTTCTGGCTCT GGCAGCGGCACCGACTTCACCCTGACCATATCT AGCCTGCAGCCTGAGGACTTCGCCACCTACTAC TGCCACCAGTACCACAGAAGCCCCTACACCTTT GGCCAGGGCACCAAGGTGGAAATCAAGCGG
Anti-MUC16 scFv	343	DIKMAQSPSSVNA SLGERVTITCKASR DINNFLSWFHQKP GKSPKTLIYRANRL VDGVPSRFSGSGS GQDYSFTISSLEYE DVGIYYCLQYGDL YTFGGGTKLEIKG GGGSGGGGSGGG GSDVQLLESGPGL VRPSQSLSLTCSVT GYSIVSHYYWNWI RQFPGNKLEWMG YISSDGSNEYNPSL KNRISISLDTSKNQ FFLKFDFVTTADT	344	GACATCAAGATGGCTCAGTCCCCTTCTAGCGTGA ATGCTTCGCTAGGGGAGCGTGTGACCATCACATG TAAAGCATCACGCGACATAAATAATTTCCTTTCCT GGTTTCATCAGAAACCGGGCAAGTCGCCTAAGAC GCTGATTTACAGAGCAAATCGGTTGGTAGATGGA GTGCCAAGCAGATTCAGCGGGAGCGGAAGTGGA CAGGATTATAGCTTCACTATTTCATCCCTGGAATA CGAGGACGTAGGTATCTATTATTGCCTCCAGTAT GGCGATCTTTACACATTTGGTGGGGGGACTAAGC TGGAGATTAAG GGCGGAGGCGGAAGCGGAGGCGGAGGCTCCGG CGGAGGCGGAAGCGACGTGCAACTTCTGGAGAG CGGGCCAGGGCTAGTCAGGCCCTCCCAGTCGCTT TCACTGACTTGCAGTGTGACCGGTTACTCTATTGT GAGTCACTACTATTGGAACTGGATTCGGCAGTTC CCAGGCAACAAACTGGAATGGATGGGGTACATA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		ATYFCVRGVDYW GQGTTLTVSS		TCTTCCGATGGCTCGAATGAATATAACCCATCATT GAAAAATCGTATTTCCATCAGTCTGGATACGAGT AAAAACCAGTTTTTCCTCAAATTCGATTTCGTGAC TACAGCAGATACTGCCACATACTTCTGTGTACGA GGTGTCGATTATTGGGGACAGGGCACAACGCTG ACCGTAAGTTCT

Exemplary anti-ROR1 Variable Heavy (VH) and Variable Light (VL) Sequences

		Amino acid sequence		Nucleotide sequence
hROR1 VH_04	345	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSAISRGGTTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCG RYDYDGYYAMDYWGQGTLVTVSS	346	GAGGTGCAGCTCGTGGAAT CCGGCGGTGGCCTGGTGCA GCCGGGCGGCAGTCTTCGA CTCTCCTGTGCGGCGTCAG GCTTTACGTTCAGTTCTTAT GCCATGAGCTGGGTGAGGC AAGCTCCCGGTAAGGGACT GGAGTGGGTCTCTGCTATC AGCCGGGGAGGTACGACCT ACTACGCTGACTCCGTAAAA GGAAGATTTACCATAAGTC GTGACAATTCCAAAAACACT CTATACTTACAGATGAACTC GCTCAGGGCCGAAGATACC GCAGTCTACTATTGTGGGA GATACGATTACGACGGCTA CTATGCTATGGATTATTGGG GTCAGGGTACGCTCGTGAC GGTGTCCTCC
hROR1 VL_04	347	DIQMTQSPSSLSASVGDRVTITCQA SPDINSYLNWYQQKPGKAPKLLIYR ANNLETGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIK	348	GATATTCAAATGACGCAAA GTCCCAGCAGCCTCTCCGCC TCCGTTGGAGACAGGGTGA CTATTACATGCCAAGCCAGC CCCGATATTAATAGCTACTT AAATTGGTATCAGCAGAAA CCTGGGAAGGCACCTAAAC TTCTCATCTACCGCGCTAAC AATCTGGAGACCGGCGTGC CGTCTAGATTTTCCGGCTCT GGATCAGGGACCGATTTTA CTCTGACAATTAGTTCCCTG CAACCCGAAGACATCGCCA CTTATTATTGCCTGCAATAT GATGAGTTTCCTTACACATT TGGTCAGGGAACTAAACTA GAGATTAAG
hROR1 VH_05	349	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSSISRGGTTYYPDSVKGRFTISRDN	350	GAAGTGCAACTGGTCGAGT CTGGGGGCGGCCTTGTGCA ACCTGGAGGCAGCCTTCGA

		SKNTLYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS		CTCAGTTGCGCCGCGTCTG GTTTTACCTTCTCCTCTTACG CGATGAGCTGGGTTCGCCA GGCCCCCGGCAAGGGACTT GAGTGGGTTAGTTCGATCT CCCGCGGAGGCACCACATA TTATCCTGACTCGGTTAAGG GACGCTTCACTATCTCTAGG GACAATTCAAAGAACACAC TGTATCTCCAAATGAACTCC TTGCGGGCCGAGGACACTG CTGTGTATTATTGCGGACG ATACGACTACGATGGGTAT TACGCCATGGATTACTGGG GGCAAGGTACACTGGTCAC TGTGAGTTCG
hROR1 VL_05	351	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIK	352	GATATTCAGATGACCCAGTC ACCTTCGAGTCTGAGCGCA TCCGTGGGCGACAGAGTGA CCATTACCTGTAAGGCCAGC CCGGACATTAACAGCTACCT ATCGTGGTATCAGCAAAAG CCTGGTAAGGCCCCTAAACT CCTTATCTACAGGGCTAATA GGTTGGTAGACGGGGTGCC TAGCCGGTTCTCTGGTTCCG GCAGCGGTACGGACTTTAC TCTGACCATAAGCTCTCTGC AACCAGAAGACATCGCAAC ATACTACTGTTTACAATACG ACGAATTTCCTTATACCTTT GGCCAGGGGACCAAGTTAG AGATCAAG
hROR1 VH_06	353	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAIIWVRQAPGKGLEWV ARISRGGTTRYADSVKGRFTISADT SKETAYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS	354	GAGGTTCAGCTGGTCGAGT CCGGGGGAGGCTTAGTGCA GCCAGGAGGCAGTCTGCGG CTCTCTTGCGCTGCAAGTGG CTTCACATTCAGTTCATACG CAATCATCTGGGTTCGACA GGCTCCTGGTAAGGGCCTC GAATGGGTCGCAAGGATAT CACGAGGTGGAACCACTAG ATACGCAGACTCTGTTAAG GGCAGGTTCACAATTAGCG

				CGGATACCTCCAAGGAGAC TGCTTATTTACAGATGAACT CTCTGAGAGCCGAGGACAC TGCTGTTTACTACTGCGGCC GATACGATTACGACGGATA TTACGCAATGGATTACTGG GGCCAGGGCACGCTGGTGA CAGTTTCATCG
hROR1 VL_06	355	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIK	356	GATATCCAGATGACTCAGA GTCCCAGTAGCCTGTCGGC AAGCGTCGGAGATCGGGTC ACAATTACCTGCAAAGCTA GTCCTGATATTAATTCTTAC TTGTCCTGGTATCAGCAGA AGCCTGGTAAGGCCCCTAA GTTGCTCATCTATCGGGCTA ACCGGCTGGTGGACGGTGT TCCCTCTAGATTCTCAGGGA GTGGAAGCGGCACTGACTT CACCCTGACTATATCGAGCC TTCAGCCAGAGGACATTGC CACATACTACTGTCTGCAAT ATGATGAATTTCCATATACA TTCGGACAAGGTACAAAGT TAGAAATTAAG
hROR1 VH_07	357	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAIIWVRQAPGKGLEWV ARISRGGTTRYADSVKGRFTISADT SKETAYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS	358	GAAGTCCAACTGGTGGAGT CTGGCGGGGGCTTGGTGCA GCCCGGTGGCTCCCTTAGG CTGTCTTGCGCTGCCAGCG GGTTCACATTCAGCTCCTAT GCGATTATATGGGTCCGAC AGGCACCCGGCAAGGGATT GGAGTGGGTGGCTCGCATC AGCAGAGGCGGCACTACTC GTTACGCCGACTCCGTGAA AGGCAGATTCACCATCAGT GCAGACACATCCAAGGAAA CCGCATATCTTCAGATGAAT AGCCTGCGAGCGGAGGATA CCGCCGTCTATTATTGCGGA CGCTATGATTACGACGGTT ATTATGCTATGGACTACTGG GGCCAGGGCACACTTGTGA CCGTCAGTAGC

hROR1 VL_07	359	DIQMTQSPSSLSASVGDRVTITCRA SPDINSYVAWYQQKPGKAPKLLIYR ANFLESGVPSRFSGSRSGTDFTLTIS SLQPEDFATYYCLQYDEFPYTFGQG TKVEIK	360	GACATTCAAATGACGCAAA GCCCTAGTAGCTTGTCAGCT TCTGTGGGGGACCGTGTCA CAATCACTTGTCGGGCCTCT CCAGATATAAACTCCTACGT TGCTTGGTATCAGCAGAAG CCCGGAAAGGCTCCGAAAT TGTTGATTTATCGCGCTAAT TTCTTAGAGTCAGGAGTGC CCAGCCGGTTCTCAGGGTC TCGCTCTGGAACCGACTTCA CACTCACTATTTCTAGCCTA CAGCCTGAGGATTTTGCAA CTTACTACTGTCTACAGTAC GACGAGTTTCCGTACACTTT CGGACAGGGGACCAAGGT GGAGATCAAG
hROR1 VH_08	361	QVQLVQSGGGLVKPGGSLRLSCAA SGFTFSSYAMSWVRQIPGKGLEW VSSISRGGTTYYPDSVKGRFTISRDN VKNTLYLQMSSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTMVTVSS	362	CAAGTACAGCTCGTGCAGA GCGGCGGTGGCCTGGTGAA GCCAGGAGGTAGTCTTAGA CTGAGCTGTGCGGCTTCTG GTTTCACGTTCAGCAGTTAT GCTATGTCCTGGGTTAGGC AAATCCCCGGCAAAGGATT GGAGTGGGTTAGCAGTATC TCAAGGGGGGGAACCACAT ATTATCCTGACTCTGTCAAA GGACGGTTTACAATCAGCC GCGATAACGTTAAAAATAC CCTCTACCTCCAGATGTCTT CGCTCCGCGCTGAAGATAC AGCGGTTTACTACTGTGGC AGATACGACTACGACGGTT ATTACGCCATGGACTACTG GGGACAGGGAACTATGGTC ACAGTTAGCTCT
hROR1 VL_08	363	DIKMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKTLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQYEDMAIYYCLQYDEFPYTFGD GTKVEIK	364	GACATCAAAATGACGCAGT CACCTAGTAGCCTCTCCGCC TCGGTTGGCGATCGGGTAA CCATTACCTGCAAAGCATCT CCAGACATAAATAGTTATCT TAGTTGGTATCAACAGAAA CCTGGCAAAGCTCCTAAGA CCCTCATCTACCGCGCTAAC

				CGCCTCGTGGATGGTGTTC CAAGTCGGTTCTCAGGAAG CGGCAGTGGCACAGACTTT ACACTGACAATTAGTTCCCT CCAGTATGAGGATATGGCC ATATATTACTGCCTTCAGTA TGATGAGTTTCCATACACAT TCGGAGACGGTACAAAGGT GGAGATCAAG
hROR1 VH_09	365	QVSLRESGGGLVQPGRSLRLSCTAS GFTFSSYAMTWVRQAPGKGLEWV ASISRGGTTHFADSVKGRFTISRDN SNNTLYLQMDNVRDEDTAIYYCGR YDYDGYYAMDYWGRGTLVTVSS	366	CAAGTGAGCCTCCGGGAGA GTGGGGGCGGTCTGGTCCA ACCAGGACGGTCACTGCGG CTGTCATGCACTGCCAGCG GCTTCACATTTAGCTCTTAC GCCATGACTTGGGTCCGCC AAGCTCCCGGTAAGGGACT GGAGTGGGTGGCCAGCATT AGCAGGGGTGGTACAACCC ACTTCGCGGATTCAGTTAA GGGGAGATTCACTATCTCC AGGGATAATTCCAACAACA CGCTGTACCTTCAGATGGAT AACGTGAGAGACGAGGATA CCGCGATATACTACTGTGG CCGCTATGACTACGATGGTT ATTATGCTATGGATTACTGG GGGCGGGGCACCCTGGTG ACTGTGTCCTCG
hROR1 VL_09	367	DIVMTQSPSSLSASVGDRVTITCRA SPDINSYLAWYQQKPGKAPKLLIYR ANSLQSGVPSRFSGSGSGTEFTLTIS SLQPEDFATYYCLQYDEFPYTFGQG TKLEMK	368	GATATCGTGATGACACAGT CACCTAGCTCCCTGAGCGCA AGCGTGGGGGATAGGGTT ACCATAACTTGCAGGGCCA GTCCCGACATCAATAGTTAT TTGGCCTGGTATCAACAGA AGCCTGGGAAGGCACCTAA GTTGCTTATTTATAGGGCTA ACTCGTTACAGAGCGGTGT GCCAAGTCGGTTCTCAGGC TCAGGGTCCGGGACCGAGT TCACCCTGACCATCAGTAGC TTGCAGCCAGAAGATTTTG CCACCTACTACTGTCTTCAA TACGATGAGTTTCCTTACAC

				TTTTGGACAGGGCACCAAA CTAGAGATGAAG
hROR1 VH_10	369	QVQLVESGGGVVQPGRSLRLSCAA SGFTFSSYAMNWVRQAPAKGLEW VAIISRGGTQYYADSVKGRFTISRD NSKNTLYLQMNGLRAEDTAVYYCG RYDYDGYYAMDYWGQGTLVTVSS	370	CAGGTTCAACTGGTAGAAT CCGGCGGAGGTGTAGTGCA GCCTGGAAGGTCATTACGG TTAAGTTGCGCCGCCTCCG GGTTCACATTTAGCAGCTAT GCTATGAACTGGGTGCGCC AGGCCCCTGCGAAAGGACT CGAATGGGTTGCCATCATC AGCCGAGGAGGCACACAGT ATTATGCCGATTCTGTGAAG GGTCGTTTTACTATTTCCAG AGACAACAGTAAAAATACG CTGTACCTGCAAATGAACG GATTGAGGGCTGAGGATAC CGCCGTGTACTACTGTGGA CGCTACGACTATGATGGGT ACTACGCGATGGACTATTG GGGGCAAGGAACCCTTGTA ACCGTTAGTTCA
hROR1 VL_10	371	EIVLTQSPDFQSVTPKEKVTITCRAS PDINSYLSWYQQKPDQSPKLLIKRA NQSFSGVPSRFSGSGSGTDFTLTIN SLEAEDAAAYYCLQYDEFPYTFGPG TKVDIK	372	GAGATCGTTTTGACACAGA GCCCCGATTTCCAGAGCGT CACGCCCAAGGAGAAGGTC ACCATCACCTGCCGAGCCA GCCCCGACATCAACAGTTAT CTTTCATGGTATCAACAGAA ACCTGATCAGAGCCCTAAG CTGCTGATTAAGCGCGCCA ACCAGAGCTTCTCAGGGGT TCCTTCACGGTTTTCCGGGT CAGGCAGCGGGACTGACTT CACGTTGACCATTAACTCTT TGGAGGCTGAGGATGCTGC TGCCTATTACTGCCTTCAGT ACGACGAGTTCCCCTATACA TTTGGTCCTGGAACAAAAG TGGATATAAAG
hROR1 VH_11	373	QVQLVQSGAEVKKPGASVKVSCKA SGFTFSSYAMHWVRQAPGQGLE WMGNISRGGTTNYAEKFKNRVTM TRDTSISTAYMELSRLRSDDTAVYY CGRYDYDGYYAMDYWGQGTLVT VSS	374	CAGGTGCAGCTCGTCCAGA GCGGAGCCGAAGTGAAGA AGCCGGGAGCATCAGTGAA AGTTTCCTGCAAAGCAAGT GGCTTCACTTTCAGCAGTTA CGCGATGCACTGGGTGCGG

				CAGGCACCAGGTCAGGGAC TGGAATGGATGGGGAACAT CTCTCGCGGCGGAACAACC AATTACGCAGAGAAGTTTA AGAATCGCGTTACGATGAC CAGAGACACTTCTATTAGTA CAGCCTATATGGAGTTGTC GCGTCTGAGAAGCGACGAT ACCGCTGTCTACTATTGCGG CCGGTACGATTATGACGGC TACTATGCAATGGATTACTG GGGACAGGGCACACTTGTG ACAGTGTCTAGT
hROR1 VL_11	375	DIVMTQSPLSLPVTPGEPASISCRSS PDINSYLEWYLQKPGQSPQLLIYRA NDRFSGVPDRFSGSGSGTDFTLKIS RVEAEDVGVYYCLQYDEFPYTFGQ GTKVEIK	376	GACATTGTGATGACTCAGT CTCCACTCAGCCTGCCTGTC ACGCCCGGCGAACCCGCTT CTATCTCTTGTAGGAGTAGC CCTGATATCAACAGCTACCT CGAATGGTATCTCCAGAAA CCTGGTCAGAGCCCCCAGC TCTTGATCTATAGAGCAAAC GACAGGTTCTCTGGCGTGC CTGATAGGTTTTCCGGTAGT GGCAGCGGAACCGACTTCA CACTTAAGATTTCAAGGGTC GAGGCCGAGGACGTGGGG GTGTATTACTGCTTACAGTA CGATGAGTTTCCGTATACAT TCGGGCAAGGCACAAAGGT GGAAATTAAG
hROR1 VH_12	377	EVQLVESGGGLVQPGGSLRLSCTG SGFTFSSYAMHWLRQVPGEGLEW VSGISRGGTIDYADSVKGRFTISRD DAKKTLSLQMNSLRAEDTAVYYCG RYDYDGYYAMDYWGQGTMVTVS S	378	GAAGTGCAACTGGTCGAAA GTGGAGGGGGACTAGTGC AGCCCGGAGGGTCACTGAG GCTATCATGCACCGGCTCTG GTTTTACTTTTTCCAGCTAT GCCATGCACTGGCTCAGAC AGGTTCCGGGGGAAGGACT GGAGTGGGTTAGCGGAATC TCCAGAGGCGGAACTATTG ACTACGCAGACAGCGTGAA AGGTAGGTTTACCATCAGC AGGGACGATGCTAAAAAGA CCCTGTCACTTCAAATGAAT AGCCTGAGAGCTGAGGATA

				CGGCCGTGTATTACTGTGG ACGCTATGACTACGATGGA TATTACGCAATGGACTACTG GGGCCAGGGAACAATGGT GACCGTCTCAAGC
hROR1 VL_12	379	EIVLTQSPATLSVSPGERATLSCRAS PDINSYLAWYQQKPGQAPRLLFSR ANNRATGIPARFTGSGSGTDFTLTI SSLEPEDFAIYYCLQYDEFPYTFGQG TKVEIK	380	GAGATCGTCCTGACCCAGA GCCCAGCTACTTTGTCAGTT TCGCCAGGCGAGCGGGCCA CACTGAGCTGTAGGGCTTC TCCTGATATCAATTCTTACC TGGCCTGGTATCAACAGAA ACCGGGACAGGCCCCTCGC CTGCTGTTCTCCCGCGCCAA CAATAGGGCGACTGGCATA CCAGCTCGGTTTACTGGGA GTGGGTCAGGCACTGATTT CACGCTTACAATCAGTAGCC TGGAGCCCGAAGACTTCGC CATCTACTACTGTTTACAAT ACGATGAGTTCCCCTATACC TTCGGCCAAGGGACCAAGG TGGAGATCAAG
hROR1 VH_13	381	EVQLVESGGGVVQPGRSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTQYYADSVKGRFTISRD NSKNTLYLQMNGLRAEDTAVYYCG RYDYDGYYAMDYWGQGTLVTVSS	382	GAAGTGCAGCTAGTAGAAA GTGGTGGTGGGGTCGTGCA GCCAGGCCGCTCGCTCAGG CTGTCTTGCGCTGCGAGTG GTTTCACATTCTCTTCATAC GCCATGAGCTGGGTGAGAC AGGCTCCCGGCAAGGGCCT CGAATGGGTCGCATCTATA AGCAGAGGCGGAACCCAGT ACTACGCTGACAGTGTGAA GGGTCGCTTTACAATCTCAC GGGACAACAGTAAAAACAC CCTCTATCTACAGATGAATG GCTTGCGAGCTGAAGACAC GGCTGTGTATTATTGCGGG CGCTATGACTATGATGGTTA CTACGCTATGGATTACTGG GGCCAGGGCACCCTGGTTA CTGTTTCATCA
hROR1 VL_13	383	EIVLTQSPDFQSVTPKEKVTITCRAS PDINSYLPWYQQKPDQSPKLLIKRA NQSFSGVPSRFSGSGSGTDFTLTIN	384	GAAATAGTCCTGACCCAGA GCCCAGACTTCCAGTCCGT GACCCCTAAGGAGAAGGTT

		SLEAEDAAAYYCLQYDEFPYTFGPG TKVDIK		ACTATCACTTGCAGGGCAA GCCCTGACATAAATTCATAC CTGCCATGGTATCAGCAGA AGCCAGACCAGTCGCCGAA GCTATTAATCAAACGCGCCA ACCAGTCTTTTAGCGGCGTA CCATCCCGATTCTCAGGTTC GGGGTCCGGGACCGATTTC ACACTCACGATAAACTCCCT TGAGGCAGAGGATGCAGC GGCTTACTACTGTTTACAGT ACGACGAGTTTCCATATACG TTCGGCCCCGGCACGAAGG TAGATATCAAG
hROR1 VH_14	385	EVQLVESGGGLVQPGGSLRLSCAT SGFTFSSYAMSWMRQAPGKGLE WVASISRGGTTYYADSVKGRFTISV DKSKNTLYLQMNSLRAEDTAVYYC GRYDYDGYYAMDYWGQGTLVTVS S	386	GAAGTGCAGCTGGTGGAGT CTGGCGGCGGTCTGGTGCA GCCCGGCGGCTCTCTGCGC CTCTCCTGTGCCACCTCTGG TTTTACATTCTCCTCCTACGC TATGTCCTGGATGCGGCAA GCCCCCGGCAAGGGCCTAG AGTGGGTCGCCTCAATCAG CAGGGGCGGGACGACTTAT TATGCCGATTCAGTTAAGG GGAGATTCACAATTTCCGT GGATAAATCCAAGAATACC TTATACCTCCAGATGAACTC TCTGCGGGCCGAAGATACG GCCGTATATTATTGTGGGA GGTATGACTACGACGGATA TTACGCCATGGATTATTGG GGGCAGGGGACACTTGTTA CAGTGAGTTCC
hROR1 VL_14	387	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLNWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDYTLTI SSLQPEDFATYYCLQYDEFPYTFGA GTKVEIK	388	GATATACAGATGACACAGA GCCCTTCAAGTTTATCTGCA AGCGTCGGCGATCGTGTTA CAATAACTTGCAAGGCATCT CCCGACATCAATTCCTACCT CAACTGGTATCAGCAGAAG CCTGGGAAGGCTCCTAAGC TGCTTATTTACAGAGCAAAT CGCCTGGTGGACGGCGTGC CCAGTCGGTTTTCCGGGTCT GGGAGCGGAACGGATTACA

				CACTGACCATCTCAAGCCTG CAACCCGAAGACTTCGCTAC ATATTACTGCCTTCAGTATG ATGAGTTCCCATATACCTTC GGCGCTGGGACCAAGGTG GAGATAAAG
hROR1 VH_14-1	389	EVQLVESGGGLVQPGGSLRLSCASS GFTFSSYAMSWRRQAPGKGLEWV AGISRGGTTSYADSVKGRFTISSDD SKNTLYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS	390	GAGGTCCAGCTCGTCGAAT CTGGCGGAGGTTTAGTGCA ACCAGGCGGGTCGCTCCGA TTAAGTTGTGCGTCCAGTG GCTTCACCTTCTCCAGCTAC GCCATGTCGTGGAGGCGAC AGGCTCCTGGCAAAGGCTT GGAGTGGGTTGCTGGTATC TCCCGAGGAGGCACCACTA GTTACGCTGACAGTGTAAA AGGACGTTTCACTATTTCCT CTGACGACAGCAAGAACAC ACTCTATCTGCAAATGAATA GTCTCCGTGCTGAGGACAC AGCCGTGTATTATTGCGGG CGGTATGATTACGACGGCT ACTACGCTATGGACTACTG GGGCCAGGGAACTCTGGTC ACTGTGAGCTCT
hROR1 VL_14-1	391	DIQMTQSPSSLSASVGDRVTITCRA SPDINSYLSWYQQKPGKAPKLLIYR ANTLESGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCLQYDEFPYTFGQG TKIEIK	392	GATATACAGATGACTCAAA GTCCTAGCTCCTTGAGCGCC TCAGTGGGAGATCGGGTCA CTATAACTTGTAGAGCCTCA CCAGATATAAACTCCTATCT CTCTTGGTATCAGCAGAAG CCCGGCAAAGCACCAAAGC TCTTGATCTATAGAGCTAAT ACGCTAGAGAGCGGAGTGC CTTCACGGTTTTCTGGTTCC GGGAGCGGAACCGACTTTA CCCTTACAATTTCTAGCCTC CAGCCAGAGGACTTCGCAA CTTACTATTGTCTCCAGTAT GATGAATTTCCTTACACCTT CGGCCAAGGGACCAAGATC GAGATAAAG
hROR1 VH_14-2	393	EVQLVESGGGLVQPGGSLRLSCASS GFTFSSYAMSWVRQAPGKGLEWV	394	GAGGTGCAGCTCGTTGAGT CCGGTGGGGGGCTGGTGC

		AGISRGGTTSYADSVKGRFTISADT SKNTLYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS		AGCCTGGCGGGTCTCTCCG CCTCTCTTGTGCCTCCTCCG GCTTTACCTTCAGCAGCTAT GCTATGTCATGGGTGCGGC AGGCACCAGGCAAAGGTCT GGAATGGGTCGCTGGGATC AGTAGAGGCGGCACAACCT CCTATGCCGACAGCGTTAA GGGGAGGTTCACAATCTCG GCTGATACAAGCAAGAACA CTCTGTATCTCCAAATGAAC AGTCTCCGGGCAGAGGACA CCGCGGTCTATTACTGCGG CCGGTACGACTACGACGGG TACTACGCAATGGACTATTG GGGACAGGGAACTCTGGTT ACTGTCAGCTCT
hROR1 VL_14-2	395	DIQMTQSPSSLSASVGDRVTITCRA SPDINSYLSWYQQKPGKAPKLLIYR ANTLESGVPSRFSGSGSGTDFTLTIS SLQPEDFATYYCLQYDEFPYTFGTG TKLEIK	396	GATATCCAGATGACTCAAA GCCCATCTTCTCTCAGCGCA AGCGTGGGTGACCGAGTGA CCATCACCTGCCGGGCGTCT CCTGATATCAACTCATACCT GTCCTGGTATCAGCAGAAG CCCGGAAAGGCCCCTAAGC TGCTGATCTACCGCGCAAAT ACACTGGAGAGCGGGGTCC CAAGCAGATTCAGTGGGTC CGGCAGTGGTACGGACTTT ACTCTGACCATCAGCTCCCT GCAACCGGAGGACTTTGCT ACTTATTACTGTCTCCAGTA CGACGAGTTCCCATACACTT TCGGAACAGGCACTAAGCT GGAGATCAAA
hROR1 VH_14-3	397	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCG RYDYDGYYAMDYWGQGTLVTVSS	398	GAGGTTCAACTTGTGGAAT CCGGCGGCGGGTTAGTCCA GCCCGGCGGGAGCTTGCGG CTGTCCTGCGCCGCCTCTGG ATTCACTTTTAGCTCCTATG CTATGTCTTGGGTAAGGCA GGCCCCTGGTAAAGGACTA GAGTGGGTGGCCTCGATCT CCCGTGGTGGCACTACATA CTACGCCGACTCCGTTAAAG

				GCCGGTTTACCATCTCCCGT GACAACTCTAAAAATACTTT GTACCTGCAAATGAACTCCC TGCGGGCAGAAGACACAGC CGTGTACTATTGCGGGCGT TACGATTACGACGGATATTA CGCAATGGACTACTGGGGC CAGGGCACACTGGTCACCG TGAGCAGC
hROR1 VL_14-3	399	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLNWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGG GTKVEIK	400	GATATACAAATGACTCAGTC CCCTAGTAGCCTTAGTGCTA GTGTGGGAGACAGAGTGAC CATCACCTGCAAAGCATCTC CTGATATCAATTCCTACCTT AACTGGTATCAACAGAAGC CTGGCAAAGCTCCAAAGCT CCTGATTTATCGCGCGAACA GATTGGTCGATGGGGTCCC TTCCAGATTCAGCGGCTCA GGGTCAGGGACCGATTTCA CCCTCACAATTAGTTCACTT CAGCCCGAGGACATCGCCA CGTATTATTGCCTTCAGTAC GATGAGTTCCCTTACACCTT TGGCGGGGGAACTAAAGTC GAAATTAAG
hROR1 VH_14-4	401	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTLVTVSS	402	GAAGTGCAGCTTGTGGAGT CAGGAGGAGGGCTAGTTCA GCCAGGCGGCTCTCTGAGA CTATCTTGTGCTGCCTCCGG CTTCACATTTAGCTCTTATG CAATGTCCTGGGTCCGCCA GGCCCCTGGTAAAGGCCTG GAATGGGTTGCTTCTATCTC TAGAGGCGGAACCACTTAC TACCCTGATTCAGTGAAGG GGAGATTCACAATTAGTAG GGACAACGTGCGGAACATC CTCTACCTACAGATGTCAAG TTTACGCAGTGAGGACACT GCGATGTATTACTGCGGTC GATACGATTATGATGGATA TTATGCAATGGATTATTGG

				GGCCAGGGCACTCTGGTCA CAGTATCTTCC
hROR1 VL_14-4	403	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLNWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDYTLTI SSLQPEDFATYYCLQYDEFPYTFGA GTKVEIK	404	GACATCCAGATGACCCAAT CACCATCGAGTCTTAGTGCA TCCGTTGGGGATAGAGTGA CAATCACTTGTAAGGCATCC CCGGACATCAACTCATATCT TAATTGGTATCAGCAAAAG CCGGGCAAGGCCCCTAAGC TCCTGATTTATAGGGCCAAC CGCCTTGTGGATGGAGTCC CCTCCCGCTTTAGTGGAAGC GGCTCTGGCACAGACTACA CCCTGACTATCAGCTCCTTG CAGCCTGAGGATTTTGCTAC CTACTACTGTCTTCAGTACG ATGAATTTCCATACACTTTC GGTGCTGGGACAAAAGTGG AGATCAAA
hROR1 VH_14-5	405	EVQLVESGGGLVQPGGSLRLSCAT SGFTFSSYAMSWMRKAPGKGLEY VASISRGGTTYYADSVKGRFTISVDK SKNTLYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS	406	GAAGTCCAGCTGGTTGAGT CTGGCGGAGGCCTCGTGCA GCCCGGTGGTTCCTTGCGA CTGTCATGCGCTACCAGCG GGTTCACATTCAGCTCTTAT GCAATGTCCTGGATGCGGA AGGCACCGGGTAAGGGCCT GGAGTATGTGGCCTCAATC TCCCGAGGAGGCACCACAT ACTATGCCGATTCTGTGAAA GGCCGATTCACCATTTCTGT GGATAAGTCTAAAAACACT CTCTACCTCCAGATGAACTC CCTACGTGCCGAAGACACA GCCGTGTATTATTGCGGGC GATACGATTATGACGGTTA TTATGCGATGGATTACTGG GGTCAAGGCACACTGGTAA CAGTGTCTTCC
hROR1 VL_14-5	407	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLNWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDYTLTI SSLQPEDFATYYCLQYDEFPYTFGA GTKVEIK	408	GATATTCAGATGACACAATC ACCTAGCTCACTGTCAGCGA GCGTCGGTGACCGGGTTAC TATCACATGCAAAGCCTCAC CCGATATCAATTCATACCTT AACTGGTATCAACAAAAAC

				CAGGAAAGGCTCCAAAGCT GCTAATTTATCGGGCCAATC GGTTGGTGGATGGCGTCCC GTCGAGGTTTAGTGGCTCC GGGAGCGGGACAGACTAC ACTCTTACAATTTCTTCTCTC CAGCCAGAGGACTTCGCAA CCTACTACTGCTTGCAGTAC GATGAATTTCCATATACCTT CGGCGCAGGGACAAAAGT GGAAATCAAA
hROR1 VH_15	409	EVQLVESGGGLVQPGGSLRLSCVTS GFTFSSYAMSWVRQAPGKGLEWV ASISRGGTTYYSDSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCGRY DYDGYYAMDYWGQGTLVTVSS	410	GAGGTGCAGCTTGTAGAAA GCGGGGGGGGCCTGGTGC AACCTGGCGGGTCCCTGCG GCTTAGTTGCGTTACGAGC GGATTTACATTTTCCAGTTA TGCCATGTCTTGGGTGAGA CAAGCCCCCGGTAAGGGTC TGGAGTGGGTGGCAAGCAT TAGCCGAGGCGGCACTACA TACTACAGTGATAGTGTGA AAGGCCGTTTCACAATCAGT AGAGATAATTCTAAAAACA CCCTGTACTTGCAGATGAAC AGCCTGCGCGCCGAGGATA CAGCCGTGTACTACTGTGG AAGATACGACTACGATGGA TATTATGCGATGGATTACTG GGGACAGGGAACCCTTGTC ACCGTTTCCTCT
hROR1 VL_15	411	DIVLTQSPATLSLSPGERATLSCKAS PDINSYMNWYQQKPGQAPRLLISR ANRLVDGVPARFSGSGSGTDFTLTI SSLEPEDFAVYYCLQYDEFPYTFGQ GTKVEIK	412	GACATAGTGTTGACGCAGT CCCCTGCCACCCTGAGCCTG AGCCCCGGAGAGCGAGCAA CGTTAAGTTGCAAGGCCAG TCCAGATATTAACTCATACA TGAATTGGTATCAACAGAA ACCAGGCCAGGCTCCTAGA CTTCTCATATCTCGGGCAAA TCGACTGGTGGATGGAGTA CCCGCAAGATTCAGCGGCA GCGGCAGCGGAACGGATTT CACGCTCACCATCTCTTCCC TTGAGCCTGAGGACTTTGC AGTCTATTATTGCTTGCAGT

				ATGATGAGTTCCCCTACACA TTCGGGCAAGGCACAAAAG TGGAAATTAAG
hROR1 VH_16	413	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTTYYDPKFQDRATISAD NSKNTAYLQMNSLRAEDTAVYYCG RYDYDGYYAMDYWGQGTLVTVSS	414	GAGGTGCAGCTGGTGGAG AGCGGAGGGGGCCTTGTCC AACCAGGAGGTAGCCTCAG GCTGTCTTGCGCTGCCTCAG GATTTACTTTTTCATCCTAC GCAATGAGCTGGGTGCGGC AAGCCCCAGGGAAGGGATT AGAATGGGTTGCCAGCATT TCTAGGGGGGGGACGACCT ACTACGATCCGAAGTTTCAG GATCGCGCCACTATCTCAGC CGATAACTCCAAGAATACT GCCTACTTACAGATGAACA GCCTGCGGGCCGAAGACAC GGCCGTCTACTATTGCGGC CGATATGATTACGACGGCT ATTACGCCATGGATTACTG GGGGCAAGGGACTCTGGTC ACAGTGAGCTCT
hROR1 VL_16	415	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLNWYQQKPGKAPKVLIYR ANRLVDGVPSRFSGSGSGTDYTLTI SSLQPEDFATYYCLQYDEFPYTFGQ GTKVEIK	416	GATATTCAGATGACCCAGTC GCCCAGCAGTCTCTCGGCCT CAGTGGGCGACCGGGTCAC TATCACTTGCAAAGCAAGTC CTGATATAAACTCCTATCTT AATTGGTATCAGCAGAAGC CCGGCAAGGCACCTAAGGT TCTGATATATCGCGCAAATC GGCTCGTGGATGGAGTACC CAGCCGATTTTCCGGCAGC GGCTCAGGCACTGACTACA CACTGACAATCAGCAGCTT GCAGCCTGAAGATTTCGCC ACATACTATTGTCTACAGTA CGACGAGTTCCCTTATACAT TCGGCCAGGGGACCAAGGT CGAGATCAAG
hROR1 VH_17	417	EVQLVESGGGLVQPGGSLRLSCTG SGFTFSSYAMSWLRQVPGEGLEW VSSISRGGTTDYADSVKGRFTISRD DAKKTLSLQMNSLRAEDTAVYYCG	418	GAGGTCCAACTCGTGGAGA GCGGAGGGGGGCTAGTGC AACCAGGTGGCTCCCTCCG CTTGTCCTGTACGGGCTCG GGGTTCACATTTTCATCCTA

		RYDYDGYYAMDYWGQGTMVTVSS		TGCCATGAGCTGGCTGAGACAGGTGCCTGGCGAGGGCCTGGAATGGGTGTCTAGTATCAGCAGAGGGGGTACAACTGATTACGCAGATTCCGTCAAGGGACGTTTTACCATCTCAAGAGACGATGCCAAGAAGACATTATCACTCCAAATGAACTCACTGAGGGCCGAGGACACCGCTGTGTACTATTGTGGGAGATACGACTACGACGGATACTATGCCATGGACTATTGGGGACAAGGCACGATGGTGACGGTATCTAGC
hROR1 VL_17	419	EIVLTQSPATLSVSPGERATLSCKASPDINSYLAWYQQKPGQAPRLLFSRANRLVDGIPARFTGSGSGTDFTLTISSLEPEDFAIYYCLQYDEFPYTFGQGTKVEIK	420	GAGATAGTGCTAACCCAGTCTCCCGCAACCCTGTCTGTGTCCCCCGGAGAGCGCGCTACTCTGAGCTGCAAAGCCAGCCCGGACATTAATTCCTACCTTGCCTGGTATCAGCAGAAGCCTGGACAGGCCCCAAGATTGCTCTTTTCACGCGCCAACCGCCTGGTAGATGGTATTCCAGCTAGGTTTACGGGCTCAGGCAGCGGAACAGACTTCACTCTCACTATTAGCTCATTGGAGCCTGAGGACTTTGCAATTTACTATTGTCTTCAGTACGACGAGTTCCCATATACTTTCGGCCAGGGCACAAAAGTAGAGATCAAG
hROR1 VH_18	421	EVQLVESGGGLVQPGGSLRLSCSASGFTFSSYAMSWVRQVPGKGLVWISSISRGGTTYYADSVRGRFIISRDNAKNTLYLEMNNLRGEDTAVYYCARYDYDGYYAMDYWGQGTLVTVSS	422	GAGGTTCAACTCGTGGAGTCTGGAGGCGGGCTAGTGCAGCCTGGCGGCTCCCTGCGACTGTCTTGCAGCGCATCAGGCTTTACATTCAGTTCTTATGCCATGAGCTGGGTGAGGCAGGTGCCCGGCAAGGGTCTGGTGTGGATCAGCTCAATCTCCAGGGGCGGGACTACATATTACGCCGATTCGGTCAGGGGTCGTTTTATCATTAGCAGGGATAATGCCAAGAACACCTTGTATTTGGAGATGAAC

				AACCTAAGAGGCGAAGACA CCGCTGTGTACTATTGTGCC CGTTACGACTACGATGGGT ACTACGCCATGGACTATTG GGGCCAGGGAACCTTGGTG ACTGTGTCAAGT
hROR1 VL_18	423	DIQLTQSPDSLAVSLGERATINCKAS PDINSYLSWYQQRPGQPPRLLIHR ANRLVDGVPDRFSGSGFGTDFTLTI TSLQAEDVAIYYCLQYDEFPYTFGQ GTKLEIK	424	GACATACAGTTGACTCAGTC ACCGGATTCGCTGGCAGTT TCGCTGGGTGAGAGAGCAA CCATCAACTGCAAAGCATCT CCCGATATCAACTCTTATCT GTCTTGGTATCAGCAGCGT CCGGGACAACCCCCTAGGC TGCTTATTCACCGAGCCAAC AGGCTGGTGGACGGGGTG CCAGACCGCTTCTCGGGAT CAGGATTTGGAACCGATTTT ACCCTAACAATTACTAGTCT CCAAGCGGAAGACGTGGCG ATCTATTATTGTCTACAATA TGACGAGTTCCCCTACACCT TCGGCCAGGGCACGAAGTT GGAGATCAAG
hROR1 VH_19	425	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTTYYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCAR YDYDGYYAMDYWGQGTLVTVSS	426	GAGGTCCAGCTCGTCGAAT CCGGTGGAGGGCTAGTTCA GCCAGGCGGCTCATTGCGT TTGTCTTGTGCCGCCTCCGG TTTCACATTCTCTTCTTACGC TATGTCCTGGGTCCGACAA GCCCCAGGAAAAGGCTTGG AATGGGTGGCCAGTATCAG TAGAGGTGGGACTACATAT TATGCCGACTCCGTGAAGG GCAGATTCACCATCTCAGCT GACACCAGTAAGAACACTG CCTACCTACAGATGAACAG CCTTCGGGCCGAGGACACC GCTGTGTATTACTGTGCCCG GTACGATTATGATGGATATT ATGCTATGGACTATTGGGG TCAGGGGACCTTGGTGACC GTCTCTAGC
hROR1 VL_19	427	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKLLIYR	428	GACATTCAGATGACTCAATC GCCGAGTTCTCTTAGCGCTT

		ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCLQYDEFPYTFGQ GTKVEIK		CTGTTGGGGACCGGGTGAC AATCACATGCAAGGCCTCTC CCGATATAAACTCCTATCTA AGCTGGTATCAGCAGAAGC CAGGGAAGGCCCCCAAGTT GTTAATCTATCGCGCCAACA GACTGGTGGATGGGGTGCC CTCTCGATTCTCCGGGAGTG GCAGTGGGACTGATTTTAC ACTGACCATTTCCTCATTGC AGCCCGAAGACTTCGCTAC CTATTACTGCTTGCAGTACG ATGAGTTCCCATATACATTC GGTCAGGGGACTAAAGTGG AGATAAAA
hROR1 VH_20	429	EVQLLESGGGLVQPGGSLRLSCAAS GFTFSSYAMSWVRQAPGKGLEWV SSISRGGTTYYADSVKGRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARY DYDGYYAMDYWGQGTLVTVSS	430	GAGGTACAGCTGCTGGAAT CTGGTGGGGGGCTGGTCCA GCCAGGGGGGTCACTACGA CTGAGCTGCGCTGCCTCCG GTTTTACATTCAGCAGCTAT GCAATGTCATGGGTCAGAC AGGCACCAGGTAAAGGCCT CGAATGGGTATCCTCCATCT CACGTGGTGGGACCACTTA CTATGCCGATAGTGTGAAG GGCAGGTTCACGATCTCAA GAGATAATTCAAAGAATAC ACTCTATCTACAAATGAACA GTTTAAGGGCCGAGGACAC CGCTGTTTACTATTGTGCCA GATATGACTACGACGGTTA TTATGCTATGGATTACTGGG GACAAGGAACGCTGGTAAC TGTTAGCTCT
hROR1 VL_20	431	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGEAPKLLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCLQYDEFPYTFGQ GTKVEIK	432	GACATCCAAATGACCCAGT CGCCTTCCTCCTTGTCTGCA TCTGTCGGAGATCGGGTGA CGATCACTTGCAAAGCGAG TCCAGACATCAACTCATATC TGTCCTGGTATCAGCAGAA GCCGGGAGAGGCACCTAAG CTCCTGATCTACAGAGCAAA CAGATTAGTGGATGGTGTG CCCTCACGGTTTTCTGGCTC

				CGGGTCCGGCACCGATTTC ACCTTGACCATCTCATCCCT ACAGCCCGAGGATTTCGCT ACTTACTATTGCTTACAGTA TGATGAGTTTCCATACACCT TCGGTCAAGGCACCAAGGT TGAGATTAAG
hROR1 VH_21	433	EVQLLETGGGLVKPGGSLRLSCAAS GFTFSSYAMSWIRQAPGKGLEWV ASISRGGTTYYGDSVKGRFTISRDH AKNSLYLQMNSLRVEDTAVYYCVR YDYDGYYAMDYWGLGTLVTVSS	434	GAAGTTCAACTGCTTGAGA CCGGAGGCGGCCTGGTAAA ACCTGGGGGCTCACTGAGG CTGAGTTGTGCCGCTTCTGG GTTCACCTTTTCATCCTATG CGATGTCATGGATACGGCA GGCTCCTGGGAAGGGGCTT GAGTGGGTTGCATCAATTT CACGAGGTGGGACAACTTA TTATGGGGATTCCGTTAAA GGTAGATTTACGATCTCTAG AGACCATGCCAAAAATTCTC TCTATCTCCAGATGAATAGT CTTAGGGTGGAGGACACCG CTGTGTACTACTGTGTCCGG TACGACTATGATGGGTACT ATGCTATGGACTATTGGGG GCTCGGCACTCTGGTCACT GTTAGCTCT
hROR1 VL_21	435	AIRMTQSPSFLSASVGDRVTITCKA SPDINSYLSWYQQRPGKAPKLLIYR ANRLVDGVPSRFSGGGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIK	436	GCCATCCGCATGACACAATC TCCCTCCTTCCTTTCTGCCAG TGTCGGGGACAGAGTGACT ATCACATGCAAAGCCAGCC CAGATATTAATTCGTACCTG TCTTGGTATCAGCAGAGGC CCGGCAAGGCACCAAAGCT GTTGATATATCGGGCCAAC CGCTTAGTGGACGGTGTCC CCTCTCGATTCAGCGGAGG CGGTAGCGGGACGGACTTT ACACTGACCATCTCCAGTCT CCAACCCGAGGATATTGCC ACTTACTATTGTCTTCAGTA TGACGAGTTCCCCTACACAT TTGGACAGGGCACCAAGCT AGAAATTAAG

hROR1 VH_22	437	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTTYYAESLEGRFTISRDD SKNSLYLQMNSLKTEDTAVYYCARY DYDGYYAMDYWGQGTLVTVSS	438	GAGGTTCAGCTGGTGGAGT CTGGTGGGGGGCTCGTACA GCCGGGTGGCTCCCTAAGG CTGAGTTGCGCTGCCTCAG GCTTTACCTTCTCAAGCTAC GCGATGTCCTGGGTGAGAC AGGCCCCTGGCAAAGGACT GGAGTGGGTGGCAAGCATT AGCCGGGGCGGAACTACCT ATTACGCTGAGTCGTTAGA GGGGCGGTTTACTATCTCC AGAGACGATTCAAAGAACT CGTTATACTTGCAGATGAAC AGCCTCAAGACCGAGGACA CCGCCGTGTACTACTGCGCC CGGTACGACTATGACGGGT ACTATGCTATGGATTATTGG GGACAAGGCACCCTCGTGA CCGTCTCTAGC
hROR1 VL_22	439	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKTLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCLQYDEFPYTFGQ GTKLEIK	440	GACATCCAGATGACACAGT CCCCTTCTTCACTTTCCGCTT CTGTGGGCGACAGGGTGAC GATCACGTGTAAGGCCTCG CCAGACATTAATTCGTACTT ATCGTGGTATCAGCAGAAA CCGGGTAAAGCTCCGAAGA CTCTGATCTATAGAGCAAAT AGGCTCGTAGACGGTGTCC CATCTAGATTTAGTGGGAG CGGCAGCGGAACCGACTTC ACTCTCACCATCTCATCCCT GCAACCGGAGGATTTCGCT ACTTACTATTGCTTGCAGTA TGACGAGTTTCCATATACGT TTGGTCAGGGAACCAAATT AGAGATCAAA
hROR1 VH_23	441	QVTLRESGPALVKPTQTLTLTCAAS GFTFSSYAMSWIRQPPGKALEWLA SISRGGTTYYNPSLKDRLTISKDTSA NQVVLKVTNMDPADTATYYCARY DYDGYYAMDYWGQGTTVTVSS	442	CAGGTAACACTCCGAGAGA GTGGGCCAGCTCTCGTGAA GCCCACGCAGACTTTAACAC TAACGTGTGCGGCAAGCGG CTTTACATTTTCGAGCTACG CGATGAGCTGGATAAGGCA ACCTCCTGGGAAGGCGTTG GAGTGGTTGGCCTCAATTA

				GCCGGGGTGGCACCACTTA CTACAATCCTAGTCTTAAGG ACAGACTTACTATTTCAAAA GATACGTCCGCCAACCAGG TGGTACTGAAGGTCACAAA TATGGACCCAGCTGACACT GCTACTTACTACTGCGCCCG GTACGATTACGATGGTTACT ACGCTATGGATTACTGGGG TCAAGGAACCACAGTGACC GTCAGTTCA
hROR1 VL_23	443	DIQMTQSPSTLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTAFTLTI SSLQPDDFATYYCLQYDEFPYTFGG GTKVEIK	444	GATATCCAGATGACGCAGT CCCCTTCAACCCTCAGTGCC AGCGTTGGTGACCGGGTTA CTATCACCTGTAAGGCTAGT CCCGATATAAATTCCTATTT GTCTTGGTATCAGCAGAAG CCAGGCAAGGCTCCTAAGC TGCTCATCTACCGGGCTAAC AGGTTAGTTGACGGTGTGC CCTCCCGATTTTCCGGCAGT GGCAGCGGGACCGCTTTCA CTCTTACAATCTCATCTCTTC AACCGGACGACTTCGCTAC GTACTACTGCCTCCAATATG ATGAGTTTCCATACACATTC GGAGGAGGCACAAAAGTC GAAATCAAG
hROR1 VH_24	445	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSAISRGGTTYYADSVKGRFTISADT SKETAYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS	446	GAAGTCCAGCTGGTGGAGT CCGGCGGAGGCTTGGTTCA GCCCGGAGGATCTTTGCGA CTGTCTTGCGCCGCCAGCG GTTTCACTTTCAGCAGCTAT GCCATGAGTTGGGTTAGAC AAGCTCCCGGCAAGGGGCT GGAATGGGTTAGTGCTATT AGCCGGGGAGGGACAACA TATTACGCTGACTCTGTCAA AGGCCGATTCACCATCTCTG CTGACACGAGCAAAGAAAC CGCCTACCTCCAAATGAACA GCCTGCGAGCTGAGGACAC TGCCGTCTACTATTGTGGTC GATATGATTATGATGGGTA

				CTATGCAATGGACTATTGG GGGCAGGGCACACTGGTGA CCGTGAGCTCT
hROR1 VL_24	447	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIK	448	GATATTCAGATGACGCAGA GTCCCTCCTCCCTATCTGCC TCTGTTGGAGATCGAGTCA CCATTACGTGTAAAGCGTCT CCCGATATCAACAGCTACCT CTCTTGGTATCAGCAGAAAC CAGGGAAGGCCCCCAAGCT GCTGATCTATAGAGCTAATC GCTTAGTGGATGGAGTGCC AAGCAGGTTCTCCGGGTCC GGCAGTGGAACCGATTTCA CCTTGACAATAAGTAGCTTG CAACCTGAGGATATTGCAA CATACTACTGTCTACAGTAC GACGAGTTCCCCTACACCTT CGGCCAAGGGACAAAGCTG GAGATTAAG
hROR1 VH_25	449	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSAISRGGTTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCG RYDYDGYYAMDYWGQGTLVTVSS	450	GAAGTGCAGCTCGTGGAGA GCGGCGGCGGTCTGGTACA GCCAGGGGGGTCACTGCGT CTCTCATGTGCTGCGAGTG GCTTTACGTTCTCTTCCTAC GCTATGTCCTGGGTCAGGC AGGCACCGGGGAAGGGCTT AGAGTGGGTTAGTGCAATC TCTAGGGGCGGTACAACCT ACTATGCCGACTCTGTCAAG GGCAGGTTTACAATTTCAA GAGATAATTCTAAGAATACT CTTTACCTACAGATGAATAG CTTGCGGGCGGAAGACACA GCAGTCTATTATTGTGGCCG CTATGACTACGACGGATACT ATGCCATGGACTACTGGGG CCAAGGCACTTTGGTCACG GTGAGCTCT
hROR1 VL_25	451	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIK	452	GACATCCAGATGACCCAGA GCCCTAGTTCATTGTCTGCC AGTGTGGGGGATAGGGTC ACTATCACGTGTAAGGCTTC CCCTGACATCAATTCATACC

				TGTCATGGTATCAGCAGAA GCCTGGAAAAGCCCCTAAA CTGCTGATCTACCGCGCGA ATAGGCTTGTGGACGGCGT TCCAAGCCGCTTCTCTGGCT CTGGATCAGGGACCGACTT CACCCTCACGATCTCCAGCC TCCAACCCGAGGATATCGC CACCTATTATTGCCTTCAGT ACGATGAGTTCCCCTATACA TTCGGCCAGGGGACAAAGC TGGAAATCAAA
hROR1 VH_26	453	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSAISRGGTTYYADSVKGRFTISADT SKETAYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS	454	GAGGTCCAGCTCGTCGAGT CGGGTGGGGGCTTGGTGCA ACCCGGTGGCAGTTTGCGC CTGAGCTGCGCCGCGAGCG GGTTCACTTTCAGTTCGTAT GCCATGAGTTGGGTGCGAC AAGCGCCCGGCAAAGGACT GGAGTGGGTGTCAGCCATT AGCCGGGGCGGTACTACCT ACTATGCGGACTCGGTCAA GGGAAGATTCACCATCAGC GCTGATACCAGTAAGGAAA CCGCTTATCTTCAGATGAAC TCCCTGCGTGCCGAGGATA CAGCAGTCTACTATTGCGG GCGCTACGATTATGACGGA TATTATGCCATGGATTACTG GGGGCAGGGCACTCTGGTC ACAGTCAGCTCT
hROR1 VL_26	455	DIQMTQSPSSLSASVGDRVTITCQA SPDINSYLNWYQQKPGKAPKLLIYR ANNLETGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIK	456	GATATTCAGATGACGCAGT CTCCCTCTTCCCTGAGCGCC TCCGTCGGCGATAGAGTTA CGATCACCTGTCAGGCCAG CCCAGATATCAACTCCTATC TGAATTGGTATCAGCAAAA GCCTGGGAAGGCTCCCAAG TTGCTGATCTACAGAGCCAA TAACTTAGAGACTGGCGTG CCGTCTCGGTTCAGCGGGT CCGGCAGTGGAACCGACTT TACACTGACCATTTCCAGCC TCCAACCTGAGGATATCGCC

				ACATATTATTGTCTCCAGTA TGACGAGTTCCCTTACACAT TTGGTCAAGGAACTAAACT GGAAATCAAA
hROR1 VH_27	457	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSAISRGGTTYYADSVKGRFTISADT SKETAYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSS	458	GAGGTGCAGCTGGTCGAAA GTGGAGGCGGACTCGTGCA GCCCGGCGGTAGTCTGCGA TTGAGCTGTGCCGCGTCCG GCTTTACTTTCTCATCTTACG CTATGAGTTGGGTCCGCCA GGCCCCAGGCAAAGGACTG GAGTGGGTATCAGCCATCA GTAGGGGGGGAACTACCTA TTACGCAGATTCTGTGAAG GGACGCTTCACCATCAGCG CGGACACTAGCAAGGAGAC TGCCTACCTGCAAATGAATA GTCTGAGAGCCGAGGATAC CGCCGTGTACTATTGTGGC AGGTATGACTACGATGGCT ATTATGCTATGGATTACTGG GGCCAGGGGACGTTAGTGA CAGTAAGCTCT
hROR1 VL_27	459	DIQMTQSPSSLSASVGDRVTITCRA SPDINSYVAWYQQKPGKAPKLLIYR ANFLESGVPSRFSGSRSGTDFTLTIS SLQPEDFATYYCLQYDEFPYTFGQG TKVEIK	460	GATATTCAGATGACCCAATC CCCTTCTTCTCTGAGCGCTT CTGTGGGCGATAGAGTTAC AATAACCTGTCGGGCGTCC CCAGACATTAACTCTTATGT AGCATGGTATCAGCAAAAG CCTGGAAAGGCACCAAAGT TACTGATCTACCGGGCCAAT TTTCTGGAGTCGGGCGTGC CCTCACGATTTAGCGGTAG CAGATCAGGCACAGACTTT ACTCTGACCATTAGCTCTCT GCAACCCGAGGACTTCGCC ACCTACTACTGTTTGCAGTA TGACGAGTTTCCATACACTT TTGGTCAAGGAACCAAAGT CGAAATCAAA
hROR1 VH_28	461	QIQLVQSGAEVKKPGASVKVSCAA SGFTFSSYAMSWVRQAPGKSFKW MGSISRGGTTYYSADFKGRFAITKD	462	CAGATACAGCTGGTGCAGT CTGGTGCCGAGGTTAAAAA GCCCGGAGCCTCGGTTAAA GTGAGTTGTGCGGCAAGCG

		TSASTAYMELSSLRSEDTAVYYCAR YDYDGYYAMDYWGQGTLVTVSS		GATTCACGTTCAGTTCCTAC GCTATGTCCTGGGTGCGGC AGGCTCCTGGCAAGTCATTT AAGTGGATGGGGTCGATCT CACGGGGTGGAACCACCTA TTACTCTGCCGACTTCAAGG GGAGATTTGCGATTACAAA AGATACAAGCGCCTCTACG GCCTACATGGAGTTAAGTA GCCTTAGAAGCGAAGACAC GGCGGTGTACTACTGCGCC AGATATGACTATGACGGCT ACTACGCCATGGACTACTG GGGCCAGGGCACACTGGTT ACAGTCAGCTCT
hROR1 VL_28	463	DIVMTQSPDSLAVSLGERATISCKA SPDINSYLSWYQQKPGQPPKLLIYR ANRLVDGVPDRFSGSGSRTDFTLTI SSLQAEDVAVYYCLQYDEFPYTFGQ GTKVEIK	464	GATATCGTGATGACACAAA GCCCAGACAGTCTGGCAGT GTCCCTCGGCGAGCGCGCT ACCATCTCATGCAAAGCTAG TCCCGACATCAATTCCTATC TGTCCTGGTATCAGCAAAA ACCAGGCCAACCCCCCAAG CTGCTTATCTATCGGGCTAA CCGATTAGTCGATGGGGTG CCAGATAGATTTTCAGGCTC TGGTTCCCGGACAGATTTTA CTCTCACGATCTCCTCACTA CAGGCAGAAGATGTTGCAG TGTATTACTGCCTGCAATAC GACGAGTTCCCCTACACCTT CGGCCAAGGCACGAAAGTG GAGATCAAG
hROR scFv	465	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSSISRGGTTYYPDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSSG GGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCKASPDINSYLN WYQQKPGKAPKLLIYRANRLVDGV PSRFSGSGSGTDYTLTISSLQPEDFA TYYCLQYDEFPYTFGAGTKVEIK	466	GAAGTGCAACTGGTCGAGT CTGGGGGCGGCCTTGTGCA ACCTGGAGGCAGCCTTCGA CTCAGTTGCGCCGCGTCTG GTTTTACCTTCTCCTCTTACG CGATGAGCTGGGTTCGCCA GGCCCCCGGCAAGGGACTT GAGTGGGTTAGTTCGATCT CCCGCGGAGGCACCACATA TTATCCTGACTCGGTTAAGG GACGCTTCACTATCTCTAGG GACAATTCAAAGAACACAC

				TGTATCTCCAAATGAACTCC TTGCGGGCCGAGGACACTG CTGTGTATTATTGCGGACG ATACGACTACGATGGGTAT TACGCCATGGATTACTGGG GGCAAGGTACACTGGTCAC TGTGAGTTCG GGGGGCGGCGGAAGTGGT GGAGGGGGAAGTGGTGGA GGAGGAAGCGATATACAGA TGACACAGAGCCCTTCAAG TTTATCTGCAAGCGTCGGC GATCGTGTTACAATAACTTG CAAGGCATCTCCCGACATCA ATTCCTACCTCAACTGGTAT CAGCAGAAGCCTGGGAAG GCTCCTAAGCTGCTTATTTA CAGAGCAAATCGCCTGGTG GACGGCGTGCCCAGTCGGT TTTCCGGGTCTGGGAGCGG AACGGATTACACACTGACC ATCTCAAGCCTGCAACCCGA AGACTTCGCTACATATTACT GCCTTCAGTATGATGAGTTC CCATATACCTTCGGCGCTGG GACCAAGGTGGAGATAAAG

Anti-ROR1 CDRs

		SEQ ID NO
IMGT Method		
VH-CDR-1	GFTFSSYA	715
VH-CDR-2	ISRGGTT	716
VH-CDR-3	GRYDYDGYYAMDY	717
Kabat Method		
VH-CDR-1	SYAMS	718
VH-CDR-2	AISRGGTTYYADSVKG	719
VH-CDR-3	YDYDGYYAMDY	720
IMGT Method		
VL-CDR-1	PDINSY	721
VL-CDR-2	RAN	722
VL-CDR-3	LQYDEFPYT	723

Kabat Method		
VL-CDR-1	RASPDINSYLS	724
VL-CDR-2	RANTLES	725
VL-CDR-3	LQYDEFPYT	723

Portions of ROR1-specific antigen binding domain

Portion of VH Domain	GFTFSSYAMSWVRQAPGKGLEWVSSISRGGTTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCGRYDYDGYYAMDY	SEQ ID NO: 726
Portion of VL Domain	ASPDINSYLNWYQQKPGKAPKLLIYRANRLVDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCLQYDEFPYT	SEQ ID NO: 727
Portion of VH Domain	SYAMSWVRQAPGKGLEWVSSISRGGTTYYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCGRYDYDGYYAMDY	SEQ ID NO: 728
Portion of VL Domain	PDINSYLNWYQQKPGKAPKLLIYRANRLVDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCLQYDEFPYT	SEQ ID NO: 729

Exemplary Spacer Sequences

CD8α hinge	467	KPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	468	AAGCCCACCACCACCCCTGCCCCTAGACCTCCAACCCCAGCCCCTACAATCGCCAGCCAGCCCCTGAGCCTGAGGCCCGAAGCCTGTAGACCTGCCGCTGGCGGAGCCGTGCACACCAGAGGCCTGGATTTCGCCTGCGAC
CD8α hinge-homologous stalk extension region	469	KPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASD	470	AAGCCTACCACCACCCCCGCACCTCGTCCTCCAACCCCTGCACCTACGATTGCCAGTCAGCCTCTTTCACTGCGGCCTGAGGCCAGCAGACCAGCTGCCGGCGGTGCCGTCCATACAAGAGGACTGGACTTCGCGTCCGAT

CD8α hinge-homologous stalk extension region	469	KPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASD	471	AAACCTACTACCACTCCAGCCCCAAGGCCCCCAACCCCAGCACCGACTATCGCATCACAGCCTTTGTCACTGCGTCCTGAAGCCAGCCGGCCAGCTGCAGGGGGGGCCGTCCACACAAGGGGACTCGACTTTGCGAGTGAT
CD8α hinge-homologous stalk extension region	469	KPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASD	472	AAACCTACTACAACTCCTGCCCCCGGCCTCCTACACCAGCTCCTACTATCGCCTCCCAGCCACTCAGTCTCAGACCCGAGGCTTCTAGGCCAGCGGCCGGAGGCGCGGTCCACACCCGCGGGCTGGACTTTGCATCCGAT
CD8α hinge and 2 CD8α-homologous stalk extension regions	473	KPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDKPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD	474	AAGCCTACCACCACCCCCGCACCTCGTCCTCCAACCCCTGCACCTACGATTGCCAGTCAGCCTCTTTCACTGCGGCCTGAGGCCAGCAGACCAGCTGCCGGCGGTGCCGTCCATACAAGAGGACTGGACTTCGCGTCCGATAAACCTACTACCACTCCAGCCCCAAGGCCCCCAACCCCAGCACCGACTATCGCATCACAGCCTTTGTCACTGCGTCCTGAAGCCAGCCGGCCAGCTGCAGGGGGGGCCGTCCACACAAGGGGACTCGACTTTGCGAGTGATAAGCCCACCACCACCCCTGCCCCTAGACCTCCAACCCCAGCCCCTACAATCGCCAGCCAGCCCCTGAGCCTGAGGCCCGAAGCCTGTAGACCTGCCGCTGGCGGAGCCGTGCACACCAGAGGCCTGGATTTCGCCTGCGAC

Exemplary Transmembrane Domain Sequences

CD8α Transmembrane Domain	475	IYIWAPLAGTCGVLLLSLVITLYCNHRN	476	ATCTACATCTGGGCCCCTCTGGCCGGCACCTGTGGCGTGCTGCTGCTGAGCCTGGTCATCACCCTGTACTGCAACCACCGGAAT
CD28 Transmembrane Domain	477	FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS	478	TTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGC

Exemplary Intracellular Signaling Domain Sequences

CD3ς signaling domain	479	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	480	CGGGTGAAGTTCAGCCGGAGCGCCGACGCCCCTGCCTACCAGCAGGGCCAGAACCAGCTGTACAACGAGCTGAACCTGGGCCGGAGGGAGGAGTACGACGTGCTGGACAAGCGGAGAGGCCGGGACCCTGAGATGGGCGGCAAGCCCCGGAGAAAGAACCCTCAGGAGGGCCTGTATAACGAACTGCAGAAAGACAAGATGGCCGAGGCCTACAGCGAGATCGGCATGAAGGGCGAGCGGCGGAGGGGCAAGGGCCACGACGGCCTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTACGACGCCCTGCACATGCAGGCCCTGCCCCCCAGA
CD28 co-stimulatory domain	481	RSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS	482	AGGAGCAAGCGGAGCAGAGGCGGCCACAGCGACTACATGAACATGACCCCCCGGAGGCCTGGCCCCACCCGGAAGCACTACCAGCCCTACGCCCCTCCCAGGGACTTCGCCGCCTACCGGAGC

4-1BB co-stimulatory domain	483	KRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEGGCEL	484	AAGAGAGGCCGGAAGAAA CTGCTGTACATCTTCAAGCA GCCCTTCATGCGGCCCGTG CAGACCACCCAGGAAGAGG ACGGCTGCAGCTGCCGGTT CCCCGAGGAAGAGGAAGG CGGCTGCGAACTG
DNAX-activation protein 10 (DAP10) co-stimulatory domain	485	LCARPRRSPAQEDGKVYINMPGRG	486	CTGTGCGCACGCCCACGCC GCAGCCCCGCCCAAGAAGA TGGCAAAGTCTACATCAAC ATGCCAGGCAGGGGC
DNAX-activation protein 12 (DAP12) co-stimulatory	487	YFLGRLVPRGRGAAEAATRKQRITE TESPYQELQGQRSDVYSDLNTQRP YYK	488	TACTTCCTGGGCCGGCTGG TCCCTCGGGGGCGAGGGGC TGCGGAGGCAGCGACCCG GAAACAGCGTATCACTGAG ACCGAGTCGCCTTATCAGG AGCTCCAGGGTCAGAGGTC GGATGTCTACAGCGACCTC AACACACAGAGGCCGTATT ACAAA

Exemplary Signal Peptide Sequences

GM-CSFRa signal peptide	489	MLLLVTSLLLCELPHPAFLLIP	490	ATGCTGCTGCTGGTGACCA GCCTGCTGCTGTGTGAGCT GCCCCACCCCGCCTTTCTGC TGATCCCC
Ig Kappa signal peptide	491	MRLPAQLLGLLMLWVPGSSG	492	ATGAGGCTCCCTGCTCAGCT CCTGGGGCTGCTAATGCTCT GGGTCCCAGGATCCAGTGG G
Immuno-globulin E signal peptide	493	MDWTWILFLVAAATRVHS	494	ATGGATTGGACCTGGATTCT GTTTCTGGTGGCCGCTGCCA CAAGAGTGCACAGC
CD8α signal peptide	495	MALPVTALLLPLALLLHAARP	496	ATGGCGCTGCCCGTGACCG CCTTGCTCCTGCCGCTGGCC TTGCTGCTCCACGCCGCCAG GCCG

Mouse Ig VH region 3 signal peptide	497	MGWSCIILFLVATATGVHS	498	ATGGGCTGGTCCTGCATCATCCTGTTTCTGGTGGCTACCGCCACCGGCGTGCACAGC
β2M signal peptide	499	MSRSVALAVLALLSLSGLEA	500	ATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCTGGAGGCT
Azurocidin signal peptide	501	MTRLTVLALLAGLLASSRA	502	ATGACCCGGCTGACAGTCCTGGCCCTGCTGGCTGGTCTGCTGGCGTCCTCGAGGGCC
Human Serum Albumin signal peptide	503	MKWVTFISLLFLFSSAYS	504	ATGAAGTGGGTAACCTTTATTTCCCTTCTTTTTCTCTTTAGCTCGGCTTATTCC
A2M receptor associated protein signal peptide	505	MGKNKLLHPSLVLLLLVLLPTDA	506	ATGGGGAAGAACAAACTCCTTCATCCAAGTCTGGTTCTTCTCCTCTTGGTCCTCCTGCCCACAGACGCC
IGHV3-23 signal peptide	507	MEFGLSWLFLVAILKGVQC	508	ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGT
IGKV1-D33 (HuL1)signal peptide	509	MDMRVPAQLLGLLLLWLSGARC	510	ATGGACATGAGGGTCCCTGCTCAGCTCCTGGGGCTCCTGCTGCTCTGGCTCTCAGGTGCCAGATGT
IGHV3-33(L14F) (HuH7)signal peptide	511	MEFGLSWVFLVALFRGVQC	512	ATGGAGTTTGGGCTGAGCTGGGTTTTCCTCGTTGCTCTTTTTAGAGGTGTCCAGTGT
TVB2 (T21A) signal peptide	513	MGTSLLCWMALCLLGADHADA	514	ATGGGCACCAGCCTCCTCTGCTGGATGGCCCTGTGTCTCCTGGGGGCAGATCACGCAGATGCT
CD52 signal peptide	515	MKRFLFLLLTISLLVMVQIQTGLS	516	ATGAAGCGCTTCCTCTTCCTCCTACTCACCATCAGCCTCCTGGTTATGGTACAGATACAAACTGGACTCTCA
Low-affinity nerve growth factor receptor (LNGFR, TNFRSF16) signal peptide	517	MGAGATGRAMDGPRLLLLLLLGVSLGGA	518	ATGGGGGCAGGTGCCACCGGCCGCGCCATGGACGGGCCGCGCCTGCTGCTGTTGCTGCTTCTGGGGGTGTCCCTTGGAGGTGCC

Exemplary Cytokine Sequences

IL-15	519	NWVNVISDLKKIEDLIQSMHIDATL YTESDVHPSCKVTAMKCFLLELQVI SLESGDASIHDTVENLIILANNSLSS NGNVTESGCKECEELEEKNIKEFLQ SFVHIVQMFINTS	520	AACTGGGTGAATGTGATCA GCGACCTGAAGAAGATCGA GGATCTGATCCAGAGCATG CACATTGATGCCACCCTGTA CACAGAATCTGATGTGCAC CCTAGCTGTAAAGTGACCG CCATGAAGTGTTTTCTGCTG GAGCTGCAGGTGATTTCTCT GGAAAGCGGAGATGCCTCT ATCCACGACACAGTGGAGA ATCTGATCATCCTGGCCAAC AATAGCCTGAGCAGCAATG GCAATGTGACAGAGTCTGG CTGTAAGGAGTGTGAGGAG CTGGAGGAGAAGAACATCA AGGAGTTTCTGCAGAGCTT TGTGCACATCGTGCAGATG TTCATCAATACAAGC
IL-15Rα	521	ITCPPPMSVEHADIWVKSYSLYSRE RYICNSGFKRKAGTSSLTECVLNKA TNVAHWTTPSLKCIRDPALVHQRP APPSTVTTAGVTPQPESLSPSGKEP AASSPSSNNTAATTAAIVPGSQLM PSKSPSTGTTEISSHESSHGTPSQTT AKNWELTASASHQPPGVYPQGHS DTTVAISTSTVLLCGLSAVSLLACYLK SRQTPPLASVEMEAMEALPVTWG TSSRDEDLENCSHHL	522	ATTACATGCCCTCCTCCAAT GTCTGTGGAGCACGCCGAT ATTTGGGTGAAGTCCTACA GCCTGTACAGCAGAGAGAG ATACATCTGCAACAGCGGC TTTAAGAGAAAGGCCGGCA CCTCTTCTCTGACAGAGTGC GTGCTGAATAAGGCCACAA ATGTGGCCCACTGGACAAC ACCTAGCCTGAAGTGCATTA GAGATCCTGCCCTGGTCCA CCAGAGGCCTGCCCCTCCAT CTACAGTGACAACAGCCGG AGTGACACCTCAGCCTGAA TCTCTGAGCCCTTCTGGAAA AGAACCTGCCGCCAGCTCTC CTAGCTCTAATAATACCGCC GCCACAACAGCCGCCATTG TGCCTGGATCTCAGCTGAT GCCTAGCAAGTCTCCTAGCA CAGGCACAACAGAGATCAG CAGCCACGAATCTTCTCACG GAACACCTTCTCAGACCACC GCCAAGAATTGGGAGCTGA

				CAGCCTCTGCCTCTCACCAG CCTCCAGGAGTGTATCCTCA GGGCCACTCTGATACAACA GTGGCCATCAGCACATCTAC AGTGCTGCTGTGTGGACTG TCTGCCGTGTCTCTGCTGGC CTGTTACCTGAAGTCTAGAC AGACACCTCCTCTGGCCTCT GTGGAGATGGAGGCCATG GAAGCCCTGCCTGTGACAT GGGGAACAAGCAGCAGAG ATGAGGACCTGGAGAATTG TTCTCACCACCTG
mbIL15	523	NWVNVISDLKKIEDLIQSMHIDATL YTESDVHPSCKVTAMKCFLLELQVI SLESGDASIHDTVENLIILANNSLSS NGNVTESGCKECEELEEKNIKEFLQ SFVHIVQMFINTSSGGGSGGGGSG GGGSGGGGSGGGSLQITCPPPMS VEHADIWVKSYSLYSRERYICNSGF KRKAGTSSLTECVLNKATNVAHWT TPSLKCIRDPALVHQRPAPPSTVTT AGVTPQPESLSPSGKEPAASSPSSN NTAATTAAIVPGSQLMPSKSPSTGT TEISSHESSHGTPSQTTAKNWELTA SASHQPPGVYPQGHSDTTVAISTST VLLCGLSAVSLLACYLKSRQTPPLAS VEMEAMEALPVTWGTSSRDEDLE NCSHHL	524	AACTGGGTGAATGTGATCA GCGACCTGAAGAAGATCGA GGATCTGATCCAGAGCATG CACATTGATGCCACCCTGTA CACAGAATCTGATGTGCAC CCTAGCTGTAAAGTGACCG CCATGAAGTGTTTTCTGCTG GAGCTGCAGGTGATTTCTCT GGAAAGCGGAGATGCCTCT ATCCACGACACAGTGGAGA ATCTGATCATCCTGGCCAAC AATAGCCTGAGCAGCAATG GCAATGTGACAGAGTCTGG CTGTAAGGAGTGTGAGGAG CTGGAGGAGAAGAACATCA AGGAGTTTCTGCAGAGCTT TGTGCACATCGTGCAGATG TTCATCAATACAAGCTCTGG CGGAGGATCTGGAGGAGG CGGATCTGGAGGAGGAGG CAGTGGAGGCGGAGGATCT GGCGGAGGATCTCTGCAGA TTACATGCCCTCCTCCAATG TCTGTGGAGCACGCCGATA TTTGGGTGAAGTCCTACAG CCTGTACAGCAGAGAGAGA TACATCTGCAACAGCGGCTT TAAGAGAAAGGCCGGCACC TCTTCTCTGACAGAGTGCGT GCTGAATAAGGCCACAAAT GTGGCCCACTGGACAACAC

				CTAGCCTGAAGTGCATTAG AGATCCTGCCCTGGTCCACC AGAGGCCTGCCCCTCCATCT ACAGTGACAACAGCCGGAG TGACACCTCAGCCTGAATCT CTGAGCCCTTCTGGAAAAG AACCTGCCGCCAGCTCTCCT AGCTCTAATAATACCGCCGC CACAACAGCCGCCATTGTG CCTGGATCTCAGCTGATGCC TAGCAAGTCTCCTAGCACA GGCACAACAGAGATCAGCA GCCACGAATCTTCTCACGGA ACACCTTCTCAGACCACCGC CAAGAATTGGGAGCTGACA GCCTCTGCCTCTCACCAGCC TCCAGGAGTGTATCCTCAG GGCCACTCTGATACAACAG TGGCCATCAGCACATCTACA GTGCTGCTGTGTGGACTGT CTGCCGTGTCTCTGCTGGCC TGTTACCTGAAGTCTAGACA GACACCTCCTCTGGCCTCTG TGGAGATGGAGGCCATGGA AGCCCTGCCTGTGACATGG GGAACAAGCAGCAGAGAT GAGGACCTGGAGAATTGTT CTCACCACCTG
mbIL15 + IgE signal Peptide	525	MDWTWILFLVAAATRVHSNWVN VISDLKKIEDLIQSMHIDATLYTESD VHPSCKVTAMKCFLLELQVISLESG DASIHDTVENLIILANNSLSSNGNVT ESGCKECEELEEKNIKEFLQSFVHIV QMFINTSSGGGSGGGGSGGGGSG GGGSGGGSLQITCPPPMSVEHADI WVKSYSLYSRERYICNSGFKRKAGT SSLTECVLNKATNVAHWTTPSLKCI RDPALVHQRPAPPSTVTTAGVTPQ PESLSPSGKEPAASSPSSNNTAATT AAIVPGSQLMPSKSPSTGTTEISSHE SSHGTPSQTTAKNWELTASASHQP PGVYPQGHSDTTVAISTSTVLLCGL SAVSLLACYLKSRQTPPLASVEMEA MEALPVTWGTSSRDEDLENCSHHL	526	ATGGATTGGACCTGGATTC TGTTTCTGGTGGCCGCTGCC ACAAGAGTGCACAGCAACT GGGTGAATGTGATCAGCGA CCTGAAGAAGATCGAGGAT CTGATCCAGAGCATGCACA TTGATGCCACCCTGTACACA GAATCTGATGTGCACCCTA GCTGTAAAGTGACCGCCAT GAAGTGTTTTCTGCTGGAG CTGCAGGTGATTTCTCTGGA AAGCGGAGATGCCTCTATC CACGACACAGTGGAGAATC TGATCATCCTGGCCAACAAT AGCCTGAGCAGCAATGGCA ATGTGACAGAGTCTGGCTG

				TAAGGAGTGTGAGGAGCTG GAGGAGAAGAACATCAAG GAGTTTCTGCAGAGCTTTGT GCACATCGTGCAGATGTTC ATCAATACAAGCTCTGGCG GAGGATCTGGAGGAGGCG GATCTGGAGGAGGAGGCA GTGGAGGCGGAGGATCTG GCGGAGGATCTCTGCAGAT TACATGCCCTCCTCCAATGT CTGTGGAGCACGCCGATAT TTGGGTGAAGTCCTACAGC CTGTACAGCAGAGAGAGAT ACATCTGCAACAGCGGCTTT AAGAGAAAGGCCGGCACCT CTTCTCTGACAGAGTGCGT GCTGAATAAGGCCACAAAT GTGGCCCACTGGACAACAC CTAGCCTGAAGTGCATTAG AGATCCTGCCCTGGTCCACC AGAGGCCTGCCCCTCCATCT ACAGTGACAACAGCCGGAG TGACACCTCAGCCTGAATCT CTGAGCCCTTCTGGAAAAG AACCTGCCGCCAGCTCTCCT AGCTCTAATAATACCGCCGC CACAACAGCCGCCATTGTG CCTGGATCTCAGCTGATGCC TAGCAAGTCTCCTAGCACA GGCACAACAGAGATCAGCA GCCACGAATCTTCTCACGGA ACACCTTCTCAGACCACCGC CAAGAATTGGGAGCTGACA GCCTCTGCCTCTCACCAGCC TCCAGGAGTGTATCCTCAG GGCCACTCTGATACAACAG TGGCCATCAGCACATCTACA GTGCTGCTGTGTGGACTGT CTGCCGTGTCTCTGCTGGCC TGTTACCTGAAGTCTAGACA GACACCTCCTCTGGCCTCTG TGGAGATGGAGGCCATGGA AGCCCTGCCTGTGACATGG GGAACAAGCAGCAGAGAT

				GAGGACCTGGAGAATTGTT CTCACCACCTG

Exemplary Linker Sequences

Linker Name	SEQ ID NO	Amino Acids Sequence	SEQ ID NO	Polynucleotide Sequence
Whitlow Linker	527	GSTSGSGKPGSGEGSTKG	528	GGCAGCACCTCCGGCAGCGG CAAGCCTGGCAGCGGCGAGG GCAGCACCAAGGGC
Linker	529	SGGGSGGGGSGGGGSGGGGSGG GSLQ	530	TCTGGCGGAGGATCTGGAGG AGGCGGATCTGGAGGAGGAG GCAGTGGAGGCGGAGGATCT GGCGGAGGATCTCTGCAG
GSG linker	531	GSG	532	GGAAGCGGA
SGSG linker	533	SGSG	534	AGTGGCAGCGGC
(G4S)3 linker	535	GGGGSGGGGSGGGGS	536	GGTGGCGGTGGCTCGGGCGG TGGTGGGTCGGGTGGCGGCG GATCT
Furin cleavage site/ Furinlink1	537	RAKR	538	CGTGCAAAGCGT
Fmdv	539	RAKRAPVKQTLNFDLLKLAGDVESN PGP	540	AGAGCCAAGAGGGCACCGGT GAAACAGACTTTGAATTTTGA CCTTCTGAAGTTGGCAGGAGA CGTTGAGTCCAACCCTGGGCC C
Thosea asigna virus 2A region (T2A)	541	EGRGSLLTCGDVEENPGP	542	GAGGGCAGAGGAAGTCTGCT AACATGCGGTGACGTCGAGG AGAATCCTGGACCT
Furin-GSG-T2A	543	RAKRGSGEGRGSLLTCGDVEENPG P	544	AGAGCTAAGAGGGGAAGCGG AGAGGGCAGAGGAAGTCTGC TAACATGCGGTGACGTCGAGG AGAATCCTGGACCT
Furin-SGSG-T2A	545	RAKRSGSGEGRGSLLTCGDVEENP GP	546	AGGGCCAAGAGGAGTGGCAG CGGCGAGGGCAGAGGAAGTC TTCTAACATGCGGTGACGTGG AGGAGAATCCCGGCCCT
Porcine teschovirus-1 2A region (P2A)	547	ATNFSLLKQAGDVEENPGP	548	GCAACGAACTTCTCTCTCCTAA AACAGGCTGGTGATGTGGAG GAGAATCCTGGTCCA
GSG-P2A	549	GSGATNFSLLKQAGDVEENPGP	550	GGAAGCGGAGCTACTAACTTC AGCCTGCTGAAGCAGGCTGG AGACGTGGAGGAGAACCCTG GACCT
Equine rhinitis A	551	QCTNYALLKLAGDVESNPGP	552	CAGTGTACTAATTATGCTCTCT

Linker Name	SEQ ID NO	Amino Acids Sequence	SEQ ID NO	Polynucleotide Sequence
virus 2A region (E2A)				TGAAATTGGCTGGAGATGTTG AGAGCAACCCTGGACCT
Foot-and-mouth disease virus 2A region (F2A)	553	VKQTLNFDLLKLAGDVESNPGP	554	GTCAAACAGACCCTAAACTTT GATCTGCTAAAACTGGCCGGG GATGTGGAAAGTAATCCCGGC CCC
FP2A	555	RAKRAPVKQGSGATNFSLLKQAGD VEENPGP	556	CGTGCAAAGCGTGCACCGGTG AAACAGGGAAGCGGAGCTAC TAACTTCAGCCTGCTGAAGCA GGCTGGAGACGTGGAGGAGA ACCCTGGACCT
Linker-GSG	557	APVKQGSG	558	GCACCGGTGAAACAGGGAAG CGGA
Linker	559	GGGGSGGGSGGGGSGGGGS	560	GGTGGCGGTGGCTCGGGCGG TGGTGGGTCGGGTGGCGGCG GATCTGGTGGCGGTGGCTCG
Linker	561	APVKQ	562	GCACCGGTGAAACAG
Linker	563	A(EAAAK)nA (n = 2-5)	564	

Exemplary Cell Tag Sequences

	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Polynucleotide Sequence
HER1 Domain III	565	RKVCNGIGIGEFKDSLSINATNIKHF KNCTSISGDLHILPVAFRGDSFTHTP PLDPQELD ILKTVKEITGFLLIQAWPENRTDLHA FENLEIIRGRTKQHGQFSLAVVSLNI TSLGLRSL KEISDGDVIISGNKNLCYANTINWK KLFGTSGQKTKIISNRGENSCKATG Q	566	CGCAAAGTGTGTAACGGAA TAGGTATTGGTGAATTTAA AGACTCACTCTCCATAAATG CTACGAATATTAAACACTTC AAAAACTGCACCTCCATCAG TGGCGATCTCCACATCCTGC CGGTGGCATTTAGGGGTGA CTCCTTCACACATACTCCTC CTCTGGATCCACAGGAACT GGATATTCTGAAAACCGTA AAGGAAATCACAGGGTTTT TGCTGATTCAGGCTTGGCCT GAAAACAGGACGGACCTCC ATGCCTTTGAGAACCTAGA AATCATACGCGGCAGGACC AAGCAACATGGTCAGTTTTC TCTTGCAGTCGTCAGCCTGA ACATAACATCCTTGGGATTA

				CGCTCCCTCAAGGAGATAA GTGATGGAGATGTGATAAT TTCAGGAAACAAAAATTTGT GCTATGCAAATACAATAAA CTGGAAAAAACTGTTTGGG ACCTCCGGTCAGAAAACCA AAATTATAAGCAACAGAGG TGAAAACAGCTGCAAGGCC ACAGGCCAG
HER1 truncated Domain IV	567	VCHALCSPEGCWGPEPRDCVS	568	GTCTGCCATGCCTTGTGCTC CCCCGAGGGCTGCTGGGGC CCGGAGCCCAGGGACTGCG TCTCT
HER1t	569	RKVCNGIGIGEFKDSLSINATNIKHF KNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWP ENRTDLHAFENLEIIRGRTKQHGQF SLAVVSLNITSLGLRSLKEISDGDVIIS GNKNLCYANTINWKKLFGTSGQKT KIISNRGENSCKATGQVCHALCSPE GCWGPEPRDCVSCRNVSRGRECV DKCNLLEGEPREFVENSECIQCHPE CLPQAMNITCTGRGPDNCIQCAHY IDGPHCVKTCPAGVMGENNTLVW KYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGPKIPSIATGMVGALLL LLVVALGIGLFM	570	CGCAAAGTGTGTAACGGAA TAGGTATTGGTGAATTTAA AGACTCACTCTCCATAAATG CTACGAATATTAAACACTTC AAAAACTGCACCTCCATCAG TGGCGATCTCCACATCCTGC CGGTGGCATTTAGGGGTGA CTCCTTCACACATACTCCTC CTCTGGATCCACAGGAACT GGATATTCTGAAAACCGTA AAGGAAATCACAGGGTTTT TGCTGATTCAGGCTTGGCCT GAAAACAGGACGGACCTCC ATGCCTTTGAGAACCTAGA AATCATACGCGGCAGGACC AAGCAACATGGTCAGTTTTC TCTTGCAGTCGTCAGCCTGA ACATAACATCCTTGGGATTA CGCTCCCTCAAGGAGATAA GTGATGGAGATGTGATAAT TTCAGGAAACAAAAATTTGT GCTATGCAAATACAATAAA CTGGAAAAAACTGTTTGGG ACCTCCGGTCAGAAAACCA AAATTATAAGCAACAGAGG TGAAAACAGCTGCAAGGCC ACAGGCCAGGTCTGCCATG CCTTGTGCTCCCCCGAGGG CTGCTGGGGCCCGGAGCCC

				AGGGACTGCGTCTCTTGCC GGAATGTCAGCCGAGGCAG GGAATGCGTGGACAAGTGC AACCTTCTGGAGGGTGAGC CAAGGGAGTTTGTGGAGAA CTCTGAGTGCATACAGTGC CACCCAGAGTGCCTGCCTCA GGCCATGAACATCACCTGC ACAGGACGGGGACCAGAC AACTGTATCCAGTGTGCCCA CTACATTGACGGCCCCCACT GCGTCAAGACCTGCCCGGC AGGAGTCATGGGAGAAAAC AACACCCTGGTCTGGAAGT ACGCAGACGCCGGCCATGT GTGCCACCTGTGCCATCCAA ACTGCACCTACGGATGCACT GGGCCAGGTCTTGAAGGCT GTCCAACGAATGGGCCTAA GATCCCGTCCATCGCCACTG GGATGGTGGGGGCCCTCCT CTTGCTGCTGGTGGTGGCC CTGGGGATCGGCCTCTTCAT G
HER1t- 1	571	RKVCNGIGIGEFKDSLSINATNIKHF KNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWP ENRTDLHAFENLEIIRGRTKQHGQF SLAVVSLNITSLGLRSLKEISDGDVIIS GNKNLCYANTINWKKLFGTSGQKT KIISNRGENSCKATGQVCHALCSPE GCWGPEPRDCVSGGGGSGGGSG GGGSGGGGSFWVLVVVGGVLACY SLLVTVAFIIFWVRSKRS	572	CGCAAAGTGTGTAACGGAA TAGGTATTGGTGAATTTAA AGACTCACTCTCCATAAATG CTACGAATATTAAACACTTC AAAAACTGCACCTCCATCAG TGGCGATCTCCACATCCTGC CGGTGGCATTTAGGGGTGA CTCCTTCACACATACTCCTC CTCTGGATCCACAGGAACT GGATATTCTGAAAACCGTA AAGGAAATCACAGGGTTTT TGCTGATTCAGGCTTGGCCT GAAAACAGGACGGACCTCC ATGCCTTTGAGAACCTAGA AATCATACGCGGCAGGACC AAGCAACATGGTCAGTTTTC TCTTGCAGTCGTCAGCCTGA ACATAACATCCTTGGGATTA CGCTCCCTCAAGGAGATAA GTGATGGAGATGTGATAAT

				TTCAGGAAACAAAAATTTGT GCTATGCAAATACAATAAA CTGGAAAAAACTGTTTGGG ACCTCCGGTCAGAAAACCA AAATTATAAGCAACAGAGG TGAAAACAGCTGCAAGGCC ACAGGCCAGGTCTGCCATG CCTTGTGCTCCCCCGAGGG CTGCTGGGGCCCGGAGCCC AGGGACTGCGTCTCTGGTG GCGGTGGCTCGGGCGGTG GTGGGTCGGGTGGCGGCG GATCTGGTGGCGGTGGCTC GTTTTGGGTGCTGGTGGTG GTTGGTGGAGTCCTGGCTT GCTATAGCTTGCTAGTAACA GTGGCCTTTATTATTTTCTG GGTGAGGAGTAAGAGGAG CTAA
FL CD20	573	MTTPRNSVNGTFPAEPMKGPIAM QSGPKPLFRRMSSLVGPTQSFFMR ESKTLGAVQIMNGLFHIALGGLLMI PAGIYAPICVTVWYPLWGGIMYIIS GSLLAATEKNSRKCLVKGKMIMNS LSLFAAISGMILSIMDILNIKISHFLK MESLNFIRAHTPYINIYNCEPANPSE KNSPSTQYCYSIQSLFLGILSVMLIFA FFQELVIAGIVENEWKRTCSRPKSNI VLLSAEEKKEQTIEIKEEVVGLTETSS QPKNEEDIEIIPIQEEEEEETETNFPE PPQDQESSPIENDSSP	574	ATGACAACACCCAGAAATT CAGTAAATGGGACTTTCCC GGCAGAGCCAATGAAAGGC CCTATTGCTATGCAATCTGG TCCAAAACCACTCTTCAGGA GGATGTCTTCACTGGTGGG CCCCACGCAAAGCTTCTTCA TGAGGGAATCTAAGACTTT GGGGGCTGTCCAGATTATG AATGGGCTCTTCCACATTGC CCTGGGGGGTCTTCTGATG ATCCCAGCAGGGATCTATG CACCCATCTGTGTGACTGTG TGGTACCCTCTCTGGGGAG GCATTATGTATATTATTTCC GGATCACTCCTGGCAGCAA CGGAGAAAAACTCCAGGAA GTGTTTGGTCAAAGGAAAA ATGATAATGAATTCATTGAG CCTCTTTGCTGCCATTTCTG GAATGATTCTTTCAATCATG GACATACTTAATATTAAAAT TTCCCATTTTTTAAAAATGG AGAGTCTGAATTTTATTAGA GCTCACACACCATATATTAA

				CATATACAACTGTGAACCA GCTAATCCCTCTGAGAAAA ACTCCCCATCTACCCAATAC TGTTACAGCATACAATCTCT GTTCTTGGGCATTTTGTCAG TGATGCTGATCTTTGCCTTC TTCCAGGAACTTGTAATAGC TGGCATCGTTGAGAATGAA TGGAAAAGAACGTGCTCCA GACCCAAATCTAACATAGTT CTCCTGTCAGCAGAAGAAA AAAAAGAACAGACTATTGA AATAAAAGAAGAAGTGGTT GGGCTAACTGAAACATCTTC CCAACCAAAGAATGAAGAA GACATTGAAATTATTCCAAT CCAAGAAGAGGAAGAAGA AGAAACAGAGACGAACTTT CCAGAACCTCCCCAAGATCA GGAATCCTCACCAATAGAA AATGACAGCTCTCCT
CD20t-1	575	MTTPRNSVNGTFPAEPMKGPIAM QSGPKPLFRRMSSLVGPTQSFFMR ESKTLGAVQIMNGLFHIALGGLLMI PAGIYAPICVTVWYPLWGGIMYIIS GSLLAATEKNSRKCLVKGKMIMNS LSLFAAISGMILSIMDILNIKISHFLK MESLNFIRAHTPYINIYNCEPANPSE KNSPSTQYCYSIQSLFLGILSVMLIFA FFQELVIAGIVENEWKRTCSRPKSNI VLLSAEEKKEQTIEIKEEVVGLTETSS QPKNEEDIE	576	ATGACCACACCACGGAACT CTGTGAATGGCACCTTCCCA GCAGAGCCAATGAAGGGAC CAATCGCAATGCAGAGCGG ACCCAAGCCTCTGTTTCGGA GAATGAGCTCCCTGGTGGG CCCAACCCAGTCCTTCTTTA TGAGAGAGTCTAAGACACT GGGCGCCGTGCAGATCATG AACGGACTGTTCCACATCGC CCTGGGAGGACTGCTGATG ATCCCAGCCGGCATCTACGC CCCTATCTGCGTGACCGTGT GGTACCCTCTGTGGGGCGG CATCATGTATATCATCTCCG GCTCTCTGCTGGCCGCCACA GAGAAGAACAGCAGGAAG TGTCTGGTGAAGGGCAAGA TGATCATGAATAGCCTGTCC CTGTTTGCCGCCATCTCTGG CATGATCCTGAGCATCATG GACATCCTGAACATCAAGA TCAGCCACTTCCTGAAGATG

				GAGAGCCTGAACTTCATCA GAGCCCACACCCCTTACATC AACATCTATAATTGCGAGCC TGCCAACCCATCCGAGAAG AATTCTCCAAGCACACAGTA CTGTTATTCCATCCAGTCTC TGTTCCTGGGCATCCTGTCT GTGATGCTGATCTTTGCCTT CTTTCAGGAGCTGGTCATC GCCGGCATCGTGGAGAACG AGTGGAAGAGGACCTGCAG CCGCCCCAAGTCCAATATCG TGCTGCTGTCCGCCGAGGA GAAGAAGGAGCAGACAATC GAGATCAAGGAGGAGGTG GTGGGCCTGACCGAGACAT CTAGCCAGCCTAAGAATGA GGAGGATATCGAG

Exemplary Vector Sequences

Human EF1A1 Promoter			577	GCCGCAATAAAATATCTTTA TTTTCATTACATCTGTGTGTT GGTTTTTTGTGTGAATCGTA ACTAACATACGCTCTCCATC AAAACAAAACGAAACAAAA CAAACTAGCAAAATAGGCT GTCCCCAGTGCAAGTGCAG GTGCCAGAACATTTCTCTAT CGAAGGATCTGCGATCGCT CCGGTGCCCGTCAGTGGGC AGAGCGCACATCGCCCACA GTCCCCGAGAAGTTGGGGG GAGGGGTCGGCAATTGAAC CGGTGCCTAGAGAAGGTGG CGCGGGGTAAACTGGGAAA GTGATGTCGTGTACTGGCTC CGCCTTTTTCCCGAGGGTGG GGGAGAACCGTATATAAGT GCAGTAGTCGCCGTGAACG TTCTTTTTCGCAACGGGTTT GCCGCCAGAACACAG

Human CMV immediate Early Promoter			578	GTGATGCGGTTTTGGCAGT ACATCAATGGGCGTGGATA GCGGTTTGACTCACGGGGA TTTCCAAGTCTCCACCCCATT GACGTCAATGGGAGTTTGT TTTGGCACCAAAATCAACGG GACTTTCCAAAATGTCGTAA CAACTCCGCCCCATTGACGC AAATGGGCGGTAGGCGTGT ACGGTGGGAGGTCTATATA AGCAGAGCTC
ColE1 ORI			579	TGTGAGCAAAAGGCCAGCA AAAGGCCAGGAACCGTAAA AAGGCCGCGTTGCTGGCGT TTTTCCATAGGCTCCGCCCC CCTGACGAGCATCACAAAA ATCGACGCTCAAGTCAGAG GTGGCGAAACCCGACAGGA CTATAAAGATACCAGGCGTT TCCCCCTGGAAGCTCCCTCG TGCGCTCTCCTGTTCCGACC CTGCCGCTTACCGGATACCT GTCCGCCTTTCTCCCTTCGG GAAGCGTGGCGCTTTCTCAT AGCTCACGCTGTAGGTATCT CAGTTCGGTGTAGGTCGTTC GCTCCAAGCTGGGCTGTGT GCACGAACCCCCCGTTCAGC CCGACCGCTGCGCCTTATCC GGTAACTATCGTCTTGAGTC CAACCCGGTAAGACACGAC TTATCGCCACTGGCAGCAGC CACTGGTAACAGGATTAGC AGAGCGAGGTATGTAGGCG GTGCTACAGAGTTCTTGAA GTGGTGGCCTAACTACGGC TACACTAGAAGAACAGTATT TGGTATCTGCGCTCTGCTGA AGCCAGTTACCTTCGGAAAA AGAGTTGGTAGCTCTTGATC CGGCAAACAAACCACCGCT GGTAGCGGTGGTTTTTTTGT TTGCAAGCAGCAGATTACG CGCAGAAAAAAAGGATCTC

				AAGAAGATCCTTTGATCTTT TCTACGGGG
Left Transposon Repeat Region			580	CTACAGTTGA AGTCGGAAGT TTACATACAC TTAAGTTGGA GTCATTAAAA CTCGTTTTTC AACTACTCCA CAAATTTCTT GTTAACAAAC AATAGTTTTG GCAAGTCAGT TAGGACATCT ACTTTGTGCA TGACACAAGT CATTTTTCCA ACAATTGTTT ACAGACAGAT TATTTCACTT ATAATTCACT GTATCACAAT TCCAGTGGGT CAGAAGTTTA CATACACTAA GTTGACTGTG CCTTTAAACA GCTTGGAAAA TTCCAGAAAA TGATGTCATG GCTTTAG
Right Transposon Repeat Region			581	GTGGAAGGCT ACTCGAAATG TTTGACCCAA GTTAAACAAT TTAAAGGCAA TGCTACCAAA TACTAATTGA GTGTATGTTA ACTTCTGACC CACTGGGAAT GTGATGAAAG AAATAAAAGC TGAAATGAAT CATTCTCTCT ACTATTATTC TGATATTTCA CATTCTTAAA ATAAAGTGGT GATCCTAACT GACCTTAAGA CAGGGAATCT TTACTCGGAT TAAATGTCAG GAATTGTGAA AAAGTGAGTT TAAATGTATT TGGCTAAGGT

				GTATGTAAAC TTCCGACTTC AACTGTAGG

Exemplary control sequences

miRNA Target	SEQ ID NO	miRNA backbone	DNA sequence of Pri-miRNA
Scrambled control 2	582	miR206, shorter arms	GATGCTACAAGTGGCCCACTTCTGAGATGCGGGCTGCTTCT GGATGACACTGCTTCCCGAGGCATTTCGCCCTATCTGCAAGT ACTATGGATTACTTTGCTAGTGGTGTAGATAGGGTGAAATG TTTCGGCAAGTGCCTCCTCGCTGGCCCCAGGGTACCACCCGG AGCACAGGTTTGGTGACCTT
neg. ctl c.elegans cel-miR-67	583	miR150	AGGGACTGGGCCCACGGGGAGGCAGCGTCCCCGAGGCAGC AGCGGCAGCGGCGGCTCCTCTCCCCATGGCCCTGTCACAACC TCCTAGAAAGAGTACTGGGCTCAGACCCTCTTTCAGGCCGTT GTGACAGGGACCTGGGGACCCCGGCACCGGCAGGCCCCAA GGGGTGAGGTGAGCGGGCATTGGGACCTCCCCTCCCTGTAC TC
neg. ctl c.elegans cel-miR-67	584	miR204	AGGAGGGTGGGGGTGGAGGCAAGCAGAGGACTTCCTGATC GCGTACCCATGGCTACAGTCTTTCTTCATGTGACTCGTGGAC TTGTACTACACAAAAGTACTGTGAGAATATATGAAGGACAG GCTTTAGTGTAGTATGCGTTCAATTGTCATCACTGGCATCTTT TTTGATCATTGCACCATCATCAAATGCATTGGGATAACCATG AC
210bp stuffer	585	-	GGTAAGTCATGACTCCCTACAATGGACATGATCATAGTAGGT ACTATAAGGCACCTAGCTATACCCTCCTATAGAGAGTTTGAG TCTATTGTAGCAAGTCTTTCTTTTAGGGCCTAGGACTCTTCCC ACTCTCTTCCATCGCCTAAGCTCTAACTCTTCCTTGTAGTAGA AATAAGTCACTTCTAAGGCTGGGACCCTCCTAGACCCTAA
Scrambled control 1	586	miR206	TTAGGATGAGTTGAGATCCCAGTGATCTTCTCGCTAAGAGTT TCCTGCCTGGGCAAGGAGGAAAGATGCTACAAGTGGCCCAC TTCTGAGATGCGGGCTGCTTCTGGATGACACTGCTTCCCGAG GCATTTCGCCCTATCTGCAAGTACTATGGATTACTTTGCTAGT GGTGTAGATAGGGTGAAATGTTTCGGCAAGTGCCTCCTCGC TGGCCCCAGGGTACCACCCGGAGCACAGGTTTGGTGACCTT CTTCCTCATCAGGGCTTTGTGCCAGCAAATGACTCCCTCACC AAGGAAGCAAGAGCCTCTGAATCCCATCTGGGCTCTTCCTGA ACACCCCTATCTCCCCCTCT

Table 14: Additional Sequences

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
CAR Sequences				
Human ROR1	587	PLLALLAALLLAARGAAAQETELSVS AELVPTSSWNISSELNKDSYLTLDEP MNNITTSLGQTAELHCKVSGNPPP TIRWFKNDAPVVQEPRRLSFRSTIY GSRLRIRNLDTTDTGYFQCVATNG KEVVSSTGVLFVKFGPPPTASPGYS DEYEEDGFCQPYRGIACARFIGNRT VYMESLHMQGEIENQITAAFTMIG TSSHLSDKCSQFAIPSLCHYAFPYCD ETSSVPKPRDLCRDECEILENVLCQT EYIFARSNPMILMRLKLPNCEDLPQ PESPEAANCIRIGIPMADPINKNHK CYNSTGVDYRGTVSVTKSGRQCQP WNSQYPHTHTFTALRFPELNGGHS YCRNPGNQKEAPWCFTLDENFKS DLCDIPACDSKDSKEKNKMEILYILV PSVAIPLAIALLFFFICVCRNNQKSSS APVQRQPKHVRGQNVEMSMLNA YKPKSKAKELPLSAVRFMEELGECA FGKIYKGHLYLPGMDHAQLVAIKTL KDYNNPQQWTEFQQEASLMAEL HHPNIVCLLGAVTQEQPVCMLFEYI NQGDLHEFLIMRSPHSDVGCSSDE DGTVKSSLDHGDFLHIAIQIAAGME YLSSHFFVHKDLAARNILIGEQLHVK ISDLGLSREIYSADYYRVQSKSLLPIR WMPPEAIMYGKFSSDSDIWSFGV VLWEIFSFGLQPYYGFSNQEVIEMV RKRQLLPCSEDCPPRMYSLMTECW NEIPSRRPRFKDIHVRLRSWEGLSS HTSSTTPSGGNATTQTTSLSASPVS NLSNPRYPNYMFPSQGITPQGQIA GFIGPPIPQNQRFIPINGYPIPPGYA AFPAAHYQPTGPPRVIQHCPPPKS RSPSSASGSTSTGHVTSLPSSGSNQ EANIPLLPHMSIPNHPGGMGITVF GNKSQKPYKIDSKQASLLGDANIHG HTESMISAEL	588	ATGCACCGGCCGCGCCGCC GCGGGACGCGCCCGCCGCT CCTGGCGCTGCTGGCCGCG CTGCTGCTGGCCGCACGCG GGGCTGCTGCCCAAGAAAC AGAGCTGTCAGTCAGTGCT GAATTAGTGCCTACCTCATC ATGGAACATCTCAAGTGAA CTCAACAAAGATTCTTACCT GACCCTCGATGAACCAATG AATAACATCACCACGTCTCT GGGCCAGACAGCAGAACTG CACTGCAAAGTCTCTGGGA ATCCACCTCCCACCATCCGC TGGTTCAAAAATGATGCTCC TGTGGTCCAGGAGCCCCGG AGGCTCTCCTTTCGGTCCAC CATCTATGGCTCTCGGCTGC GGATTAGAAACCTCGACAC CACAGACACAGGCTACTTCC AGTGCGTGGCAACAAACGG CAAGGAGGTGGTTTCTTCC ACTGGAGTCTTGTTTGTCAA GTTTGGCCCCCCTCCCACTG CAAGTCCAGGATACTCAGA TGAGTATGAAGAAGATGGA TTCTGTCAGCCATACAGAG GGATTGCATGTGCAAGATT TATTGGCAACCGCACCGTCT ATATGGAGTCTTTGCACATG CAAGGGGAAATAGAAAATC AGATCACAGCTGCCTTCACT ATGATTGGCACTTCCAGTCA CTTATCTGATAAGTGTTCTC AGTTCGCCATTCCTTCCCTG TGCCACTATGCCTTCCCGTA CTGCGATGAAACTTCATCCG TCCCAAAGCCCCGTGACTTG TGTCGCGATGAATGTGAAA TCCTGGAGAATGTCCTGTGT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CAAACAGAGTACATTTTTGC AAGATCAAATCCCATGATTC TGATGAGGCTGAAACTGCC AAACTGTGAAGATCTCCCCC AGCCAGAGAGCCCAGAAGC TGCGAACTGTATCCGGATT GGAATTCCCATGGCAGATC CTATAAATAAAAATCACAA GTGTTATAACAGCACAGGT GTGGACTACCGGGGGACCG TCAGTGTGACCAAATCAGG GCGCCAGTGCCAGCCATGG AATTCCCAGTATCCCCACAC ACACACTTTCACCGCCCTTC GTTTCCCAGAGCTGAATGG AGGCCATTCCTACTGCCGCA ACCCAGGGAATCAAAAGGA AGCTCCCTGGTGCTTCACCT TGGATGAAAACTTTAAGTCT GATCTGTGTGACATCCCAG CGTGCGATTCAAAGGATTC CAAGGAGAAGAATAAAATG GAAATCCTGTACATACTAGT GCCAAGTGTGGCCATTCCC CTGGCCATTGCTTTACTCTT CTTCTTCATTTGCGTCTGTC GGAATAACCAGAAGTCATC GTCGGCACCAGTCCAGAGG CAACCAAAACACGTCAGAG GTCAAAATGTAGAGATGTC AATGCTGAATGCATATAAA CCCAAGAGCAAGGCTAAAG AGCTACCTCTTTCTGCTGTA CGCTTTATGGAAGAATTGG GTGAGTGTGCCTTTGGAAA AATCTATAAAGGCCATCTCT ATCTCCCAGGCATGGACCAT GCTCAGCTGGTTGCTATCAA GACCTTGAAAGACTATAAC AACCCCCAGCAATGGACGG AATTTCAACAAGAAGCCTCC CTAATGGCAGAACTGCACC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCCCAATATTGTCTGCCTT CTAGGTGCCGTCACTCAGG AACAACCTGTGTGCATGCTT TTTGAGTATATTAATCAGGG GGATCTCCATGAGTTCCTCA TCATGAGATCCCCACACTCT GATGTTGGCTGCAGCAGTG ATGAAGATGGGACTGTGAA ATCCAGCCTGGACCACGGA GATTTTCTGCACATTGCAAT TCAGATTGCAGCTGGCATG GAATACCTGTCTAGTCACTT CTTTGTCCACAAGGACCTTG CAGCTCGCAATATTTTAATC GGAGAGCAACTTCATGTAA AGATTTCAGACTTGGGGCT TTCCAGAGAAATTTACTCCG CTGATTACTACAGGGTCCA GAGTAAGTCCTTGCTGCCC ATTCGCTGGATGCCCCCTGA AGCCATCATGTATGGCAAA TTCTCTTCTGATTCAGATAT CTGGTCCTTTGGGGTTGTCT TGTGGGAGATTTTCAGTTTT GGACTCCAGCCATATTATG GATTCAGTAACCAGGAAGT GATTGAGATGGTGAGAAAA CGGCAGCTCTTACCATGCTC TGAAGACTGCCCACCCAGA ATGTACAGCCTCATGACAG AGTGCTGGAATGAGATTCC TTCTAGGAGACCAAGATTTA AAGATATTCACGTCCGGCTT CGGTCCTGGGAGGGACTCT CAAGTCACACAAGCTCTACT ACTCCTTCAGGGGGAAATG CCACCACACAGACAACCTCC CTCAGTGCCAGCCCAGTGA GTAATCTCAGTAACCCCAGA TATCCTAATTACATGTTCCC GAGCCAGGGTATTACACCA CAGGGCCAGATTGCTGGTT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				TCATTGGCCCGCCAATACCT CAGAACCAGCGATTCATTCC CATCAATGGATACCCAATAC CTCCTGGATATGCAGCGTTT CCAGCTGCCCACTACCAGCC AACAGGTCCTCCCAGAGTG ATTCAGCACTGCCCACCTCC CAAGAGTCGGTCCCCAAGC AGTGCCAGTGGGTCGACTA GCACTGGCCATGTGACTAG CTTGCCCTCATCAGGATCCA ATCAGGAAGCAAATATTCCT TTACTACCACACATGTCAAT TCCAAATCATCCTGGTGGAA TGGGTATCACCGTTTTTGGC AACAAATCTCAAAAACCCTA CAAAATTGACTCAAAGCAA GCATCTTTACTAGGAGACG CCAATATTCATGGACACACC GAATCTATGATTTCTGCAGA ACTG
Human ROR1 (1-437)	589	MHRPRRRGTRPPLLALLAALLLAAR GAAAQETELSVSAELVPTSSWNISS ELNKDSYLTLDEPMNNITTSLGQTA ELHCKVSGNPPPTIRWFKNDAPVV QEPRRLSFRSTIYGSRLRIRNLDTTD TGYFQCVATNGKEVVSSTGVLFVK FGPPPTASPGYSDEYEEDGFCQPYR GIACARFIGNRTVYMESLHMQGEI ENQITAAFTMIGTSSHLSDKCSQFA IPSLCHYAFPYCDETSSVPKPRDLCR DECEILENVLCQTEYIFARSNPMIL MRLKLPNCEDLPQPESPEAANCIRI GIPMADPINKNHKCYNSTGVDYRG TVSVTKSGRQCQPWNSQYPHTHT FTALRFPELNGGHSYCRNPGNQKE APWCFTLDENFKSDLCDIPACDSKD SKEKNKMEILYILVPSVAIPLAIALLF FFICVCRNNQKSSSA	590	ATGCACCGGCCGCGCCGCC GCGGGACGCGCCCGCCGCT CCTGGCGCTGCTGGCCGCG CTGCTGCTGGCCGCACGCG GGGCTGCTGCCCAAGAAAC AGAGCTGTCAGTCAGTGCT GAATTAGTGCCTACCTCATC ATGGAACATCTCAAGTGAA CTCAACAAAGATTCTTACCT GACCCTCGATGAACCAATG AATAACATCACCACGTCTCT GGGCCAGACAGCAGAACTG CACTGCAAAGTCTCTGGGA ATCCACCTCCCACCATCCGC TGGTTCAAAAATGATGCTCC TGTGGTCCAGGAGCCCCGG AGGCTCTCCTTTCGGTCCAC CATCTATGGCTCTCGGCTGC GGATTAGAAACCTCGACAC CACAGACACAGGCTACTTCC AGTGCGTGGCAACAAACGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CAAGGAGGTGGTTTCTTCC ACTGGAGTCTTGTTTGTCAA GTTTGGCCCCCCTCCCACTG CAAGTCCAGGATACTCAGA TGAGTATGAAGAAGATGGA TTCTGTCAGCCATACAGAG GGATTGCATGTGCAAGATT TATTGGCAACCGCACCGTCT ATATGGAGTCTTTGCACATG CAAGGGGAAATAGAAAATC AGATCACAGCTGCCTTCACT ATGATTGGCACTTCCAGTCA CTTATCTGATAAGTGTTCTC AGTTCGCCATTCCTTCCCTG TGCCACTATGCCTTCCCGTA CTGCGATGAAACTTCATCCG TCCCAAAGCCCCGTGACTTG TGTCGCGATGAATGTGAAA TCCTGGAGAATGTCCTGTGT CAAACAGAGTACATTTTTGC AAGATCAAATCCCATGATTC TGATGAGGCTGAAACTGCC AAACTGTGAAGATCTCCCCC AGCCAGAGAGCCCAGAAGC TGCGAACTGTATCCGGATT GGAATTCCCATGGCAGATC CTATAAATAAAAATCACAA GTGTTATAACAGCACAGGT GTGGACTACCGGGGGACCG TCAGTGTGACCAAATCAGG GCGCCAGTGCCAGCCATGG AATTCCCAGTATCCCCACAC ACACACTTTCACCGCCCTTC GTTTCCCAGAGCTGAATGG AGGCCATTCCTACTGCCGCA ACCCAGGGAATCAAAAGGA AGCTCCCTGGTGCTTCACCT TGGATGAAAACTTTAAGTCT GATCTGTGTGACATCCCAG CGTGCGATTCAAAGGATTC CAAGGAGAAGAATAAAATG GAAATCCTGTACATACTAGT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCCAAGTGTGGCCATTCCC CTGGCCATTGCTTTACTCTT CTTCTTCATTTGCGTCTGTC GGAATAACCAGAAGTCATC GTCGGCA
Murine ROR1 (VL-VH). IgG4 Fc-CD28m-Z	591	DIKMTQSPSSMYASLGERVTITCKA SPDINSYLSWFQQKPGKSPKTLIYR ANRLVDGVPSRFSGGGSGQDYSLT INSLEYEDMGIYYCLQYDEFPYTFG GGTKLEMKGSTSGSGKPGSGEGST KGEVKLVESGGGLVKPGGSLKLSCA ASGFTFSSYAMSWVRQIPEKRLEW VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTSVTVSS ESKYGPPCPPCPAPEFEGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKP REEQFQSTYRVVSVLTVLHQDWLN GKEYKCKVSNKGLPSSIEKTISKAKG QPREPQVYTLPPSQEEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSRLTVDKS RWQEGNVFSCSVMHEALHNHYT QKSLSLSLGKMFWVLVVVGGVLAC YSLLVTVAFIIFWVRSKRSRGGHSD YMNMTPRRPGPTRKHYQPYAPPR DFAAYRSRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGR DPEMGGKPRRKNPQEGLYNELQK DKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPP R	592	GACATCAAGATGACCCAGA GCCCCAGCTCTATGTACGCC AGCCTGGGCGAGCGCGTGA CCATCACATGCAAGGCCAG CCCCGACATCAACAGCTACC TGTCCTGGTTCCAGCAGAA GCCCGGCAAGAGCCCCAAG ACCCTGATCTACCGGGCCA ACCGGCTGGTGGACGGCGT GCCAAGCAGATTTTCCGGC GGAGGCAGCGGCCAGGAC TACAGCCTGACCATCAACA GCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAGGGCA GCACCTCCGGCAGCGGCAA GCCTGGCAGCGGCGAGGG CAGCACCAAGGGCGAAGTG AAGCTGGTGGAAAGCGGC GGAGGCCTGGTGAAACCTG GCGGCAGCCTGAAGCTGAG CTGCGCCGCCAGCGGCTTC ACCTTCAGCAGCTACGCCAT GAGCTGGGTCCGACAGATC CCCGAGAAGCGGCTGGAAT GGGTGGCCAGCATCAGCAG GGGCGGCACCACCTACTAC CCCGACAGCGTGAAGGGCC GGTTCACCATCAGCCGGGA CAACGTGCGGAACATCCTG TACCTGCAGATGAGCAGCC TGCGGAGCGAGGACACCGC CATGTACTACTGCGGCAGA TACGACTACGACGGCTACT ACGCCATGGATTACTGGGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCAGGGCACCAGCGTGACC GTGTCTAGCGAGAGCAAGT ACGGCCCTCCCTGCCCCCCT TGCCCTGCCCCCGAGTTCGA GGGCGGACCCAGCGTGTTC CTGTTCCCCCCCAAGCCCAA GGACACCCTGATGATCAGC CGGACCCCCGAGGTGACCT GTGTGGTGGTGGACGTGTC CCAGGAGGACCCCGAGGTC CAGTTCAACTGGTACGTGG ACGGCGTGGAGGTGCACAA CGCCAAGACCAAGCCCCGG GAGGAGCAGTTCCAGAGCA CCTACCGGGTGGTGTCCGT GCTGACCGTGCTGCACCAG GACTGGCTGAACGGCAAGG AATACAAGTGTAAGGTGTC CAACAAGGGCCTGCCCAGC AGCATCGAGAAAACCATCA GCAAGGCCAAGGGCCAGCC TCGGGAGCCCCAGGTGTAC ACCCTGCCCCCTAGCCAAGA GGAGATGACCAAGAATCAG GTGTCCCTGACCTGCCTGGT GAAGGGCTTCTACCCCAGC GACATCGCCGTGGAGTGGG AGAGCAACGGCCAGCCCGA GAACAACTACAAGACCACC CCCCCTGTGCTGGACAGCG ACGGCAGCTTCTTCCTGTAC AGCAGGCTGACCGTGGACA AGAGCCGGTGGCAGGAGG GCAACGTCTTTAGCTGCTCC GTGATGCACGAGGCCCTGC ACAACCACTACACCCAGAA GAGCCTGTCCCTGAGCCTG GGCAAGATGTTCTGGGTGC TGGTCGTGGTGGGTGGCGT GCTGGCCTGCTACAGCCTG CTGGTGACAGTGGCCTTCA TCATCTTTTGGGTGAGGAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CAAGCGGAGCAGAGGCGG CCACAGCGACTACATGAAC ATGACCCCCCGGAGGCCTG GCCCCACCCGGAAGCACTA CCAGCCCTACGCCCCTCCCA GGGACTTCGCCGCCTACCG GAGCCGGGTGAAGTTCAGC CGGAGCGCCGACGCCCCTG CCTACCAGCAGGGCCAGAA CCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAG GAGTACGACGTGCTGGACA AGCGGAGAGGCCGGGACC CTGAGATGGGCGGCAAGCC CCGGAGAAAGAACCCTCAG GAGGGCCTGTATAACGAAC TGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATC GGCATGAAGGGCGAGCGG CGGAGGGGCAAGGGCCAC GACGGCCTGTACCAGGGCC TGAGCACCGCCACCAAGGA TACCTACGACGCCCTGCACA TGCAGGCCCTGCCCCCCAG A
Murine ROR1 (VL-VH). IgG4 Fcm-CD28m-Z	593	DIKMTQSPSSMYASLGERVTITCKA SPDINSYLSWFQQKPGKSPKTLIYR ANRLVDGVPSRFSGGGSGQDYSLT INSLEYEDMGIYYCLQYDEFPYTFG GGTKLEMKGSTSGSGKPGSGEGST KGEVKLVESGGGLVKPGGSLKLSCA ASGFTFSSYAMSWVRQIPEKRLEW VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTSVTVSS QGTSVTVSSESKYGPPCPPCPAPEF LGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSQEDPEVQFNWYVDGVE VHNAKTKPREEQFNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKGLPSS IEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEW	594	GACATCAAGATGACCCAGA GCCCCAGCTCTATGTACGCC AGCCTGGGCGAGCGCGTGA CCATCACATGCAAGGCCAG CCCCGACATCAACAGCTACC TGTCCTGGTTCCAGCAGAA GCCCGGCAAGAGCCCCAAG ACCCTGATCTACCGGGCCA ACCGGCTGGTGGACGGCGT GCCAAGCAGATTTTCCGGC GGAGGCAGCGGCCAGGAC TACAGCCTGACCATCAACA GCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAGGGCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		ESNGQPENNYKTTPPVLDSDGSFFL YSRLTVDKSRWQEGNVFSCSVMH EALHNHYTQKSLSLSLGKMFWVLV VVGGVLACYSLLVTVAFIIFWVRSK RSRGGHSDYMNMTPRRPGPTRKH YQPYAPPRDFAAYRSRVKFSRSAD APAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDA LHMQALPPR		GCACCAGCGGCAGCGGCAA GCCTGGAAGCGGCGAGGG CTCCACCAAGGGCGAAGTG AAGCTGGTGGAAAGCGGC GGAGGCCTGGTGAAACCTG GCGGCAGCCTGAAGCTGAG CTGCGCCGCCAGCGGCTTC ACCTTCAGCAGCTACGCCAT GAGCTGGGTCCGACAGATC CCCGAGAAGCGGCTGGAAT GGGTGGCCAGCATCAGCAG GGGCGGCACCACCTACTAC CCCGACAGCGTGAAGGGCC GGTTCACCATCAGCCGGGA CAACGTGCGGAACATCCTG TACCTGCAGATGAGCAGCC TGCGGAGCGAGGACACCGC CATGTACTACTGCGGCAGA TACGACTACGACGGCTACT ACGCCATGGATTACTGGGG CCAGGGCACCAGCGTGACC GTGTCTAGCCAGGGAACCT CCGTGACAGTGTCCAGCGA GTCCAAATATGGTCCCCCAT GCCCACCATGCCCAGCACCT GAGTTCCTGGGGGGACCAT CAGTCTTCCTGTTCCCCCCA AAACCCAAGGACACTCTCAT GATCTCCCGGACCCCTGAG GTCACGTGCGTGGTGGTGG ACGTGAGCCAGGAAGACCC CGAGGTCCAGTTCAACTGG TACGTGGATGGCGTGGAGG TGCATAATGCCAAGACAAA GCCCCGGGAGGAGCAGTTC AATAGCACCTACCGGGTGG TGTCCGTGCTGACCGTGCT GCACCAGGACTGGCTGAAC GGCAAGGAATACAAGTGTA AGGTGTCCAACAAGGGCCT GCCCAGCAGCATCGAGAAA ACCATCAGCAAGGCCAAGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCCAGCCTCGGGAGCCCCA GGTGTACACCCTGCCCCCTA GCCAAGAGGAGATGACCAA GAATCAGGTGTCCCTGACC TGCCTGGTGAAGGGCTTCT ACCCCAGCGACATCGCCGT GGAGTGGGAGAGCAACGG CCAGCCCGAGAACAACTAC AAGACCACCCCCCCTGTGCT GGACAGCGACGGCAGCTTC TTCCTGTACAGCAGGCTGA CCGTGGACAAGAGCCGGTG GCAGGAGGGCAACGTCTTT AGCTGCTCCGTGATGCACG AGGCCCTGCACAACCACTA CACCCAGAAGAGCCTGTCC CTGAGCCTGGGCAAGATGT TCTGGGTGCTGGTCGTGGT GGGTGGCGTGCTGGCCTGC TACAGCCTGCTGGTGACAG TGGCCTTCATCATCTTTTGG GTGAGGAGCAAGCGGAGC AGAGGCGGCCACAGCGACT ACATGAACATGACCCCCCG GAGGCCTGGCCCCACCCGG AAGCACTACCAGCCCTACG CCCCTCCCAGGGACTTCGCC GCCTACCGGAGCCGGGTGA AGTTCAGCCGGAGCGCCGA CGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACA ACGAGCTGAACCTGGGCCG GAGGGAGGAGTACGACGT GCTGGACAAGCGGAGAGG CCGGGACCCTGAGATGGGC GGCAAGCCCCGGAGAAAG AACCCTCAGGAGGGCCTGT ATAACGAACTGCAGAAAGA CAAGATGGCCGAGGCCTAC AGCGAGATCGGCATGAAGG GCGAGCGGCGGAGGGGCA AGGGCCACGACGGCCTGTA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCAGGGCCTGAGCACCGCC ACCAAGGATACCTACGACG CCCTGCACATGCAGGCCCT GCCCCCCAGA
Murine ROR1 (VL-VH). CD8α. CD28z	595	DIKMTQSPSSMYASLGERVTITCKA SPDINSYLSWFQQKPGKSPKTLIYR ANRLVDGVPSRFSGGGSGQDYSLT INSLEYEDMGIYYCLQYDEFPYTFG GGTKLEMKGSTSGSGKPGSGEGST KGEVKLVESGGGLVKPGGSLKLSCA ASGFTFSSYAMSWVRQIPEKRLEW VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTSVTVSS KPTTTPAPRPPTPAPTIASQPLSLRP EACRPAAGGAVHTRGLDFACDIYI WAPLAGTCGVLLLSLVITLYCNHRN RSKRSRGGHSDYMNMTPRRPGPT RKHYQPYAPPRDFAAYRSRVKFSRS ADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPRRKN PQEGLYNELQKDKMAEAYSEIGMK GERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR	596	GACATCAAGATGACCCAGA GCCCCAGCTCTATGTACGCC AGCCTGGGCGAGCGCGTGA CCATCACATGCAAGGCCAG CCCCGACATCAACAGCTACC TGTCCTGGTTCCAGCAGAA GCCCGGCAAGAGCCCCAAG ACCCTGATCTACCGGGCCA ACCGGCTGGTGGACGGCGT GCCAAGCAGATTTTCCGGC GGAGGCAGCGGCCAGGAC TACAGCCTGACCATCAACA GCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAGGGCA GCACCTCCGGCAGCGGCAA GCCTGGCAGCGGCGAGGG CAGCACCAAGGGCGAAGTG AAGCTGGTGGAAAGCGGC GGAGGCCTGGTGAAACCTG GCGGCAGCCTGAAGCTGAG CTGCGCCGCCAGCGGCTTC ACCTTCAGCAGCTACGCCAT GAGCTGGGTCCGACAGATC CCCGAGAAGCGGCTGGAAT GGGTGGCCAGCATCAGCAG GGGCGGCACCACCTACTAC CCCGACAGCGTGAAGGGCC GGTTCACCATCAGCCGGGA CAACGTGCGGAACATCCTG TACCTGCAGATGAGCAGCC TGCGGAGCGAGGACACCGC CATGTACTACTGCGGCAGA TACGACTACGACGGCTACT ACGCCATGGATTACTGGGG CCAGGGCACCAGCGTGACC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GTGTCTAGCAAGCCCACCA CCACCCCTGCCCCTAGACCT CCAACCCCAGCCCCTACAAT CGCCAGCCAGCCCCTGAGC CTGAGGCCCGAAGCCTGTA GACCTGCCGCTGGCGGAGC CGTGCACACCAGAGGCCTG GATTTCGCCTGCGACATCTA CATCTGGGCCCCTCTGGCC GGCACCTGTGGCGTGCTGC TGCTGAGCCTGGTCATCACC CTGTACTGCAACCACCGGA ATAGGAGCAAGCGGAGCA GAGGCGGCCACAGCGACTA CATGAACATGACCCCCGG AGGCCTGGCCCCACCCGGA AGCACTACCAGCCCTACGCC CCTCCCAGGGACTTCGCCG CCTACCGGAGCCGGGTGAA GTTCAGCCGGAGCGCCGAC GCCCCTGCCTACCAGCAGG GCCAGAACCAGCTGTACAA CGAGCTGAACCTGGGCCGG AGGGAGGAGTACGACGTG CTGGACAAGCGGAGAGGCC GGGACCCTGAGATGGGCG GCAAGCCCCGGAGAAAGAA CCCTCAGGAGGGCCTGTAT AACGAACTGCAGAAAGACA AGATGGCCGAGGCCTACAG CGAGATCGGCATGAAGGGC GAGCGGCGGAGGGGCAAG GGCCACGACGGCCTGTACC AGGGCCTGAGCACCGCCAC CAAGGATACCTACGACGCC CTGCACATGCAGGCCCTGC CCCCCAGA
Murine ROR1 (VL-VH). CD8α(2x).CD28z	597	DIKMTQSPSSMYASLGERVTITCKA SPDINSYLSWFQQKPGKSPKTLIYR ANRLVDGVPSRFSGGGSGQDYSLT INSLEYEDMGIYYCLQYDEFPYTFG GGTKLEMKGSTSGSGKPGSGEGST	598	GACATCAAGATGACCCAGA GCCCCAGCTCTATGTACGCC AGCCTGGGCGAGCGCGTGA CCATCACATGCAAGGCCAG CCCCGACATCAACAGCTACC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		KGEVKLVESGGGLVKPGGSLKLSCA ASGFTFSSYAMSWVRQIPEKRLEW VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTSVTVSS KPTTTPAPRPPTPAPTIASQPLSLRP EASRPAAGGAVHTRGLDFASDKPT TTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAP LAGTCGVLLLSLVITLYCNHRNRSKR SRGGHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNLGRREEYD VLDKRRGRDPEMGGKPRRKNPQE GLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDAL HMQALPPR		TGTCCTGGTTCCAGCAGAA GCCCGGCAAGAGCCCCAAG ACCCTGATCTACCGGGCCA ACCGGCTGGTGGACGGCGT GCCAAGCAGATTTTCCGGC GGAGGCAGCGGCCAGGAC TACAGCCTGACCATCAACA GCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAGGGCA GCACCTCCGGCAGCGGCAA GCCTGGCAGCGGCGAGGG CAGCACCAAGGGCGAAGTG AAGCTGGTGGAAAGCGGC GGAGGCCTGGTGAAACCTG GCGGCAGCCTGAAGCTGAG CTGCGCCGCCAGCGGCTTC ACCTTCAGCAGCTACGCCAT GAGCTGGGTCCGACAGATC CCCGAGAAGCGGCTGGAAT GGGTGGCCAGCATCAGCAG GGGCGGCACCACCTACTAC CCCGACAGCGTGAAGGGCC GGTTCACCATCAGCCGGGA CAACGTGCGGAACATCCTG TACCTGCAGATGAGCAGCC TGCGGAGCGAGGACACCGC CATGTACTACTGCGGCAGA TACGACTACGACGGCTACT ACGCCATGGATTACTGGGG CCAGGGCACCAGCGTGACC GTGTCTAGCAAACCTACTAC AACTCCTGCCCCCCGGCCTC CTACACCAGCTCCTACTATC GCCTCCCAGCCACTCAGTCT CAGACCCGAGGCTTCTAGG CCAGCGGCCGGAGGCGCG GTCCACACCCGCGGGCTGG ACTTTGCATCCGATAAGCCC ACCACCACCCCTGCCCCTAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCTCCAACCCCAGCCCCTA CAATCGCCAGCCAGCCCCT GAGCCTGAGGCCCGAAGCC TGTAGACCTGCCGCTGGCG GAGCCGTGCACACCAGAGG CCTGGATTTCGCCTGCGACA TCTACATCTGGGCCCCTCTG GCCGGCACCTGTGGCGTGC TGCTGCTGAGCCTGGTCATC ACCCTGTACTGCAACCACCG GAATAGGAGCAAGCGGAG CAGAGGCGGCCACAGCGAC TACATGAACATGACCCCCCG GAGGCCTGGCCCCACCCGG AAGCACTACCAGCCCTACG CCCCTCCCAGGGACTTCGCC GCCTACCGGAGCCGGGTGA AGTTCAGCCGGAGCGCCGA CGCCCCTGCCTACCAGCAG GGCCAGAACCAGCTGTACA ACGAGCTGAACCTGGGCCG GAGGGAGGAGTACGACGT GCTGGACAAGCGGAGAGG CCGGGACCCTGAGATGGGC GGCAAGCCCCGGAGAAAG AACCCTCAGGAGGGCCTGT ATAACGAACTGCAGAAAGA CAAGATGGCCGAGGCCTAC AGCGAGATCGGCATGAAGG GCGAGCGGCGGAGGGGCA AGGGCCACGACGGCCTGTA CCAGGGCCTGAGCACCGCC ACCAAGGATACCTACGACG CCCTGCACATGCAGGCCCT GCCCCCCAGA
Murine ROR1 (VL-VH). CD8a(3x).CD28z	599	DIKMTQSPSSMYASLGERVTITCKA SPDINSYLSWFQQKPGKSPKTLIYR ANRLVDGVPSRFSGGGSGQDYSLT INSLEYEDMGIYYCLQYDEFPYTFG GGTKLEMKGSTSGSGKPGSGEGST KGEVKLVESGGGLVKPGGSLKLSCA ASGFTFSSYAMSWVRQIPEKRLEW	600	GACATCAAGATGACCCAGA GCCCCAGCTCTATGTACGCC AGCCTGGGCGAGCGCGTGA CCATCACATGCAAGGCCAG CCCCGACATCAACAGCTACC TGTCCTGGTTCCAGCAGAA GCCCGGCAAGAGCCCCAAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTSVTVSS KPTTTPAPRPPTPAPTIASQPLSLRP EASRPAAGGAVHTRGLDFASDKPT TTPAPRPPTPAPTIASQPLSLRPEAS RPAAGGAVHTRGLDFASDKPTTTP APRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDIYIWAPLAG TCGVLLLSLVITLYCNHRNRSKRSRG GHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALH MQALPPR		ACCCTGATCTACCGGGCCA ACCGGCTGGTGGACGGCGT GCCAAGCAGATTTTCCGGC GGAGGCAGCGGCCAGGAC TACAGCCTGACCATCAACA GCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAGGGCA GCACCTCCGGCAGCGGCAA GCCTGGCAGCGGCGAGGG CAGCACCAAGGGCGAAGTG AAGCTGGTGGAAAGCGGC GGAGGCCTGGTGAAACCTG GCGGCAGCCTGAAGCTGAG CTGCGCCGCCAGCGGCTTC ACCTTCAGCAGCTACGCCAT GAGCTGGGTCCGACAGATC CCCGAGAAGCGGCTGGAAT GGGTGGCCAGCATCAGCAG GGGCGGCACCACCTACTAC CCCGACAGCGTGAAGGGCC GGTTCACCATCAGCCGGGA CAACGTGCGGAACATCCTG TACCTGCAGATGAGCAGCC TGCGGAGCGAGGACACCGC CATGTACTACTGCGGCAGA TACGACTACGACGGCTACT ACGCCATGGATTACTGGGG CCAGGGCACCAGCGTGACC GTGTCTAGCAAGCCTACCAC CACCCCCGCACCTCGTCCTC CAACCCCTGCACCTACGATT GCCAGTCAGCCTCTTTCACT GCGGCCTGAGGCCAGCAGA CCAGCTGCCGGCGGTGCCG TCCATACAAGAGGACTGGA CTTCGCGTCCGATAAACCTA CTACCACTCCAGCCCCAAGG CCCCCAACCCCAGCACCGAC TATCGCATCACAGCCTTTGT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CACTGCGTCCTGAAGCCAG CCGGCCAGCTGCAGGGGG GGCCGTCCACACAAGGGGA CTCGACTTTGCGAGTGATA AGCCCACCACCACCCCTGCC CCTAGACCTCCAACCCCAGC CCCTACAATCGCCAGCCAGC CCCTGAGCCTGAGGCCCGA AGCCTGTAGACCTGCCGCT GGCGGAGCCGTGCACACCA GAGGCCTGGATTTCGCCTG CGACATCTACATCTGGGCCC CTCTGGCCGGCACCTGTGG CGTGCTGCTGCTGAGCCTG GTCATCACCCTGTACTGCAA CCACCGGAATAGGAGCAAG CGGAGCAGAGGCGGCCAC AGCGACTACATGAACATGA CCCCCCGGAGGCCTGGCCC CACCCGGAAGCACTACCAG CCCTACGCCCCTCCCAGGGA CTTCGCCGCCTACCGGAGC CGGGTGAAGTTCAGCCGGA GCGCCGACGCCCCTGCCTA CCAGCAGGGCCAGAACCAG CTGTACAACGAGCTGAACC TGGGCCGGAGGGAGGAGT ACGACGTGCTGGACAAGCG GAGAGGCCGGGACCCTGA GATGGGCGGCAAGCCCCG GAGAAAGAACCCTCAGGAG GGCCTGTATAACGAACTGC AGAAAGACAAGATGGCCGA GGCCTACAGCGAGATCGGC ATGAAGGGCGAGCGGCGG AGGGGCAAGGGCCACGAC GGCCTGTACCAGGGCCTGA GCACCGCCACCAAGGATAC CTACGACGCCCTGCACATGC AGGCCCTGCCCCCCAGA
Murine ROR1 (VL-VH).	601	DIKMTQSPSSMYASLGERVTITCKA SPDINSYLSWFQQKPGKSPKTLIYR	602	GACATCAAGATGACCCAGA GCCCCAGCTCTATGTACGCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
CD8a(4x).CD28z		ANRLVDGVPSRFSGGGSGQDYSLTINSLEYEDMGIYYCLQYDEFPYTFGGGTKLEMKGSTSGSGKPGSGEGSTKGEVKLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQIPEKRLEWVASISRGGTTYYPDSVKGRFTISRDNVRNILYLQMSSLRSEDTAMYYCGRYDYDGYYAMDYWGQGTSVTVSSKPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDKPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDKPTTTPAPRPPTPAPTIASQPLSLRPEASRPAAGGAVHTRGLDFASDKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRSKRSRGGHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR		AGCCTGGGCGAGCGCGTGACCATCACATGCAAGGCCAGCCCCGACATCAACAGCTACCTGTCCTGGTTCCAGCAGAAGCCCGGCAAGAGCCCCAAGACCCTGATCTACCGGGCCAACCGGCTGGTGGACGGCGTGCCAAGCAGATTTTCCGGCGGAGGCAGCGGCCAGGACTACAGCCTGACCATCAACAGCCTGGAATACGAGGACATGGGCATCTACTACTGCCTGCAGTACGACGAGTTCCCCTACACCTTCGGAGGCGGCACCAAGCTGGAAATGAAGGGCAGCACCTCCGGCAGCGGCAAGCCTGGCAGCGGCGAGGGCAGCACCAAGGGCGAAGTGAAGCTGGTGGAAAGCGGCGGAGGCCTGGTGAAACCTGGCGGCAGCCTGAAGCTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGCCATGAGCTGGGTCCGACAGATCCCCGAGAAGCGGCTGGAATGGGTGGCCAGCATCAGCAGGGGCGGCACCACCTACTACCCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACGTGCGGAACATCCTGTACCTGCAGATGAGCAGCCTGCGGAGCGAGGACACCGCCATGTACTACTGCGGCAGATACGACTACGACGGCTACTACGCCATGGATTACTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGCAAGCCTACCACCACCCCCGCACCTCGTCCTCCAACCCCTGCACCTACGATTGCCAGTCAGCCTCTTTCACTGCGGCCTGAGGCCAGCAGACCAGCTGCCGGCGGTGCCG	

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				TCCATACAAGAGGACTGGA CTTCGCGTCCGATAAACCTA CTACCACTCCAGCCCCAAGG CCCCCAACCCCAGCACCGAC TATCGCATCACAGCCTTTGT CACTGCGTCCTGAAGCCAG CCGGCCAGCTGCAGGGGG GGCCGTCCACACAAGGGGA CTCGACTTTGCGAGTGATA AACCTACTACAACTCCTGCC CCCCGGCCTCCTACACCAGC TCCTACTATCGCCTCCCAGC CACTCAGTCTCAGACCCGA GGCTTCTAGGCCAGCGGCC GGAGGCGCGGTCCACACCC GCGGGCTGGACTTTGCATC CGATAAGCCCACCACCACCC CTGCCCCTAGACCTCCAACC CCAGCCCCTACAATCGCCAG CCAGCCCCTGAGCCTGAGG CCCGAAGCCTGTAGACCTG CCGCTGGCGGAGCCGTGCA CACCAGAGGCCTGGATTTC GCCTGCGACATCTACATCTG GGCCCCTCTGGCCGGCACC TGTGGCGTGCTGCTGCTGA GCCTGGTCATCACCCTGTAC TGCAACCACCGGAATAGGA GCAAGCGGAGCAGAGGCG GCCACAGCGACTACATGAA CATGACCCCCCGGAGGCCT GGCCCCACCCGGAAGCACT ACCAGCCCTACGCCCCTCCC AGGGACTTCGCCGCCTACC GGAGCCGGGTGAAGTTCAG CCGGAGCGCCGACGCCCCT GCCTACCAGCAGGGCCAGA ACCAGCTGTACAACGAGCT GAACCTGGGCCGGAGGGA GGAGTACGACGTGCTGGAC AAGCGGAGAGGCCGGGAC CCTGAGATGGGCGGCAAGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCCGGAGAAAGAACCCTCA GGAGGGCCTGTATAACGAA CTGCAGAAAGACAAGATGG CCGAGGCCTACAGCGAGAT CGGCATGAAGGGCGAGCG GCGGAGGGGCAAGGGCCA CGACGGCCTGTACCAGGGC CTGAGCACCGCCACCAAGG ATACCTACGACGCCCTGCAC ATGCAGGCCCTGCCCCCA GA
Murine ROR1 (VL-VH). LNGFR ECD. CD8TM. CD28z	603	DIKMTQSPSSMYASLGERVTITCKA SPDINSYLSWFQQKPGKSPKTLIYR ANRLVDGVPSRFSGGGSGQDYSLT INSLEYEDMGIYYCLQYDEFPYTFG GGTKLEMKGSTSGSGKPGSGEGST KGEVKLVESGGGLVKPGGSLKLSCA ASGFTFSSYAMSWVRQIPEKRLEW VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTSVTVSS KEACPTGLYTHSGECCKACNLGEG VAQPCGANQTVCEPCLDSVTFSDV VSATEPCKPCTECVGLQSMSAPCV EADDAVCRCAYGYYQDETTGRCEA CRVCEAGSGLVFSCQDKQNTVCEE CPDGTYSDEANHVDPCLPCTVCED TERQLRECTRWADAECEEIPGRWI TRSTPPEGSDSTAPSTQEPEAPPEQ DLIASTVAGVVTTVMGSSQPVVTR GTTDNIYIWAPLAGTCGVLLLSLVIT LYCNHRNRSKRSRGGHSDYMNMT PRRPGPTRKHYQPYAPPRDFAAYR SRVKFSRSADAPAYQQGQNQLYN ELNLGRREEYDVLDKRRGRDPEMG GKPRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR	604	GACATCAAGATGACCCAGA GCCCCAGCTCTATGTACGCC AGCCTGGGCGAGCGCGTGA CCATCACATGCAAGGCCAG CCCCGACATCAACAGCTACC TGTCCTGGTTCCAGCAGAA GCCCGGCAAGAGCCCCAAG ACCCTGATCTACCGGGCCA ACCGGCTGGTGGACGGCGT GCCAAGCAGATTTTCCGGC GGAGGCAGCGGCCAGGAC TACAGCCTGACCATCAACA GCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAGGGCA GCACCTCCGGCAGCGGCAA GCCTGGCAGCGGCGAGGG CAGCACCAAGGGCGAAGTG AAGCTGGTGGAAAGCGGC GGAGGCCTGGTGAAACCTG GCGGCAGCCTGAAGCTGAG CTGCGCCGCCAGCGGCTTC ACCTTCAGCAGCTACGCCAT GAGCTGGGTCCGACAGATC CCCGAGAAGCGGCTGGAAT GGGTGGCCAGCATCAGCAG GGGCGGCACCACCTACTAC CCCGACAGCGTGAAGGGCC GGTTCACCATCAGCCGGGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CAACGTGCGGAACATCCTG TACCTGCAGATGAGCAGCC TGCGGAGCGAGGACACCGC CATGTACTACTGCGGCAGA TACGACTACGACGGCTACT ACGCCATGGATTACTGGGG CCAGGGCACCAGCGTGACC GTGTCTAGCAAGGAGGCAT GCCCCACAGGCCTGTACAC ACACAGCGGTGAGTGCTGC AAAGCCTGCAACCTGGGCG AGGGTGTGGCCCAGCCTTG TGGAGCCAACCAGACCGTG TGTGAGCCCTGCCTGGACA GCGTGACGTTCTCCGACGT GGTGAGCGCGACCGAGCC GTGCAAGCCGTGCACCGAG TGCGTGGGGCTCCAGAGCA TGTCGGCGCCGTGCGTGGA GGCCGACGACGCCGTGTGC CGCTGCGCCTACGGCTACT ACCAGGATGAGACGACTGG GCGCTGCGAGGCGTGCCGC GTGTGCGAGGCGGGCTCG GGCCTCGTGTTCTCCTGCCA GGACAAGCAGAACACCGTG TGCGAGGAGTGCCCCGACG GCACGTATTCCGACGAGGC CAACCACGTGGACCCGTGC CTGCCCTGCACCGTGTGCG AGGACACCGAGCGCCAGCT CCGCGAGTGCACACGCTGG GCCGACGCCGAGTGCGAG GAGATCCCTGGCCGTTGGA TTACACGGTCCACACCCCCA GAGGGCTCGGACAGCACAG CCCCCAGCACCCAGGAGCC TGAGGCACCTCCAGAACAA GACCTCATAGCCAGCACGG TGGCAGGTGTGGTGACCAC AGTGATGGGCAGCTCCCAG CCCGTGGTGACCCGAGGCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCACCGACAACATCTACATC TGGGCCCCTCTGGCCGGCA CCTGTGGCGTGCTGCTGCT GAGCCTGGTCATCACCCTGT ACTGCAACCACCGGAATAG GAGCAAGCGGAGCAGAGG CGGCCACAGCGACTACATG AACATGACCCCCCGGAGGC CTGGCCCCACCCGGAAGCA CTACCAGCCCTACGCCCCTC CCAGGGACTTCGCCGCCTA CCGGAGCCGGGTGAAGTTC AGCCGGAGCGCCGACGCCC CTGCCTACCAGCAGGGCCA GAACCAGCTGTACAACGAG CTGAACCTGGGCCGGAGGG AGGAGTACGACGTGCTGGA CAAGCGGAGAGGCCGGGA CCCTGAGATGGGCGGCAAG CCCCGGAGAAAGAACCCTC AGGAGGGCCTGTATAACGA ACTGCAGAAAGACAAGATG GCCGAGGCCTACAGCGAGA TCGGCATGAAGGGCGAGCG GCGGAGGGGCAAGGGCCA CGACGGCCTGTACCAGGGC CTGAGCACCGCCACCAAGG ATACCTACGACGCCCTGCAC ATGCAGGCCCTGCCCCCCA GA
Murine ROR1 (VH-VL). CD8a(3x).41BBz	605	EVKLVESGGGLVKPGGSLKLSCAAS GFTFSSYAMSWVRQIPEKRLEWVA SISRGGTTYYPDSVKGRFTISRDNVR NILYLQMSSLRSEDTAMYYCGRYD YDGYYAMDYWGQGTSVTVSSGST SGSGKPGSGEGSTKGDIKMTQSPS SMYASLGERVTITCKASPDINSYLS WFQQKPGKSPKTLIYRANRLVDGV PSRFSGGGSGQDYSLTINSLEYEDM GIYYCLQYDEFPYTFGGGTKLEMKK PTTTPAPRPPTPAPTIASQPLSLRPE ASRPAAGGAVHTRGLDFASDKPTT	606	GAAGTGAAGCTGGTGGAAA GCGGCGGAGGCCTGGTGA AACCTGGCGGCAGCCTGAA GCTGAGCTGCGCCGCCAGC GGCTTCACCTTCAGCAGCTA CGCCATGAGCTGGGTCCGA CAGATCCCCGAGAAGCGGC TGGAATGGGTGGCCAGCAT CAGCAGGGGCGGCACCACC TACTACCCCGACAGCGTGA AGGGCCGGTTCACCATCAG CCGGGACAACGTGCGGAAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		TPAPRPPTPAPTIASQPLSLRPEASR PAAGGAVHTRGLDFASDKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNKRGRKKLL YIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCELRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQ ALPPR		ATCCTGTACCTGCAGATGA GCAGCCTGCGGAGCGAGG ACACCGCCATGTACTACTGC GGCAGATACGACTACGACG GCTACTACGCCATGGATTAC TGGGGCCAGGGCACCAGC GTGACCGTGTCTAGCGGCA GCACCTCCGGCAGCGGCAA GCCTGGCAGCGGCGAGGG CAGCACCAAGGGCGACATC AAGATGACCCAGAGCCCCA GCTCTATGTACGCCAGCCTG GGCGAGCGCGTGACCATCA CATGCAAGGCCAGCCCCGA CATCAACAGCTACCTGTCCT GGTTCCAGCAGAAGCCCGG CAAGAGCCCCAAGACCCTG ATCTACCGGGCCAACCGGC TGGTGGACGGCGTGCCAAG CAGATTTTCCGGCGGAGGC AGCGGCCAGGACTACAGCC TGACCATCAACAGCCTGGA ATACGAGGACATGGGCATC TACTACTGCCTGCAGTACGA CGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGA AATGAAGAAGCCTACCACC ACCCCCGCACCTCGTCCTCC AACCCCTGCACCTACGATTG CCAGTCAGCCTCTTTCACTG CGGCCTGAGGCCAGCAGAC CAGCTGCCGGCGGTGCCGT CCATACAAGAGGACTGGAC TTCGCGTCCGATAAACCTAC TACCACTCCAGCCCCAAGGC CCCCAACCCCAGCACCGACT ATCGCATCACAGCCTTTGTC ACTGCGTCCTGAAGCCAGC CGGCCAGCTGCAGGGGGG GCCGTCCACACAAGGGGAC TCGACTTTGCGAGTGATAA GCCCACCACCACCCCTGCCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CTAGACCTCCAACCCCAGCC CCTACAATCGCCAGCCAGCC CCTGAGCCTGAGGCCCGAA GCCTGTAGACCTGCCGCTG GCGGAGCCGTGCACACCAG AGGCCTGGATTTCGCCTGC GACATCTACATCTGGGCCCC TCTGGCCGGCACCTGTGGC GTGCTGCTGCTGAGCCTGG TCATCACCCTGTACTGCAAC CACCGGAATAAGAGAGGCC GGAAGAAACTGCTGTACAT CTTCAAGCAGCCCTTCATGC GGCCCGTGCAGACCACCCA GGAAGAGGACGGCTGCAG CTGCCGGTTCCCCGAGGAA GAGGAAGGCGGCTGCGAA CTGCGGGTGAAGTTCAGCC GGAGCGCCGACGCCCCTGC CTACCAGCAGGGCCAGAAC CAGCTGTACAACGAGCTGA ACCTGGGCCGGAGGGAGG AGTACGACGTGCTGGACAA GCGGAGAGGCCGGGACCC TGAGATGGGCGGCAAGCCC CGGAGAAAGAACCCTCAGG AGGGCCTGTATAACGAACT GCAGAAAGACAAGATGGCC GAGGCCTACAGCGAGATCG GCATGAAGGGCGAGCGGC GGAGGGGCAAGGGCCACG ACGGCCTGTACCAGGGCCT GAGCACCGCCACCAAGGAT ACCTACGACGCCCTGCACAT GCAGGCCCTGCCCCCCAGA
Murine ROR1 (VH-VL). IgG4 Fcm. CD8aTM. 41BBz	607	EVKLVESGGGLVKPGGSLKLSCAAS GFTFSSYAMSWVRQIPEKRLEWVA SISRGGTTYYPDSVKGRFTISRDNVR NILYLQMSSLRSEDTAMYYCGRYD YDGYYAMDYWGQGTSVTVSSGST SGSGKPGSGEGSTKGDIKMTQSPS SMYASLGERVTITCKASPDINSYLS	608	GAAGTGAAGCTGGTGGAAA GCGGCGGAGGCCTGGTGA AACCTGGCGGCAGCCTGAA GCTGAGCTGCGCCGCCAGC GGCTTCACCTTCAGCAGCTA CGCCATGAGCTGGGTCCGA CAGATCCCCGAGAAGCGGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		WFQQKPGKSPKTLIYRANRLVDGV PSRFSGGGSGQDYSLTINSLEYEDM GIYYCLQYDEFPYTFGGGTKLEMKE SKYGPPCPPCPAPEFEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSQE DPEVQFNWYVDGVEVHNAKTKPR EEQFQSTYRVVSVLTVLHQDWLNG KEYKCKVSNKGLPSSIEKTISKAKGQ PREPQVYTLPPSQEEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSRLTVDKSR WQEGNVFSCSVMHEALHNHYTQ KSLSLSLGKMIYIWAPLAGTCGVLLL SLVITLYCNHRNKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEG GCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRGKGHDGL YQGLSTATKDTYDALHMQALPPR		TGGAATGGGTGGCCAGCAT CAGCAGGGGCGGCACCACC TACTACCCCGACAGCGTGA AGGGCCGGTTCACCATCAG CCGGGACAACGTGCGGAAC ATCCTGTACCTGCAGATGA GCAGCCTGCGGAGCGAGG ACACCGCCATGTACTACTGC GGCAGATACGACTACGACG GCTACTACGCCATGGATTAC TGGGGCCAGGGCACCAGC GTGACCGTGTCTAGCGGCA GCACCTCCGGCAGCGGCAA GCCTGGCAGCGGCGAGGG CAGCACCAAGGGCGACATC AAGATGACCCAGAGCCCCA GCTCTATGTACGCCAGCCTG GGCGAGCGCGTGACCATCA CATGCAAGGCCAGCCCCGA CATCAACAGCTACCTGTCCT GGTTCCAGCAGAAGCCCGG CAAGAGCCCCAAGACCCTG ATCTACCGGGCCAACCGGC TGGTGGACGGCGTGCCAAG CAGATTTTCCGGCGGAGGC AGCGGCCAGGACTACAGCC TGACCATCAACAGCCTGGA ATACGAGGACATGGGCATC TACTACTGCCTGCAGTACGA CGAGTTCCCCTACACCTTCG GAGGCGGCACCAAGCTGGA AATGAAGGAGAGCAAGTAC GGCCCTCCCTGCCCCCCTTG CCCTGCCCCCGAGTTCGAG GGCGGACCCAGCGTGTTCC TGTTCCCCCCCAAGCCCAAG GACACCCTGATGATCAGCC GGACCCCCGAGGTGACCTG TGTGGTGGTGGACGTGTCC CAGGAGGACCCCGAGGTCC AGTTCAACTGGTACGTGGA CGGCGTGGAGGTGCACAAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCCAAGACCAAGCCCCGGG AGGAGCAGTTCCAGAGCAC CTACCGGGTGGTGTCCGTG CTGACCGTGCTGCACCAGG ACTGGCTGAACGGCAAGGA ATACAAGTGTAAGGTGTCC AACAAGGGCCTGCCCAGCA GCATCGAGAAAACCATCAG CAAGGCCAAGGGCCAGCCT CGGGAGCCCCAGGTGTACA CCCTGCCCCCTAGCCAAGA GGAGATGACCAAGAATCAG GTGTCCCTGACCTGCCTGGT GAAGGGCTTCTACCCCAGC GACATCGCCGTGGAGTGGG AGAGCAACGGCCAGCCCGA GAACAACTACAAGACCACC CCCCCTGTGCTGGACAGCG ACGGCAGCTTCTTCCTGTAC AGCAGGCTGACCGTGGACA AGAGCCGGTGGCAGGAGG GCAACGTCTTTAGCTGCTCC GTGATGCACGAGGCCCTGC ACAACCACTACACCCAGAA GAGCCTGTCCCTGAGCCTG GGCAAGATGATCTACATCT GGGCCCCTCTGGCCGGCAC CTGTGGCGTGCTGCTGCTG AGCCTGGTCATCACCCTGTA CTGCAACCACCGGAATAAG AGAGGCCGGAAGAAACTGC TGTACATCTTCAAGCAGCCC TTCATGCGGCCCGTGCAGA CCACCCAGGAAGAGGACGG CTGCAGCTGCCGGTTCCCC GAGGAAGAGGAAGGCGGC TGCGAACTGCGGGTGAAGT TCAGCCGGAGCGCCGACGC CCCTGCCTACCAGCAGGGC CAGAACCAGCTGTACAACG AGCTGAACCTGGGCCGGAG GGAGGAGTACGACGTGCTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GACAAGCGGAGAGGCCGG GACCCTGAGATGGGCGGCA AGCCCCGGAGAAAGAACCC TCAGGAGGGCCTGTATAAC GAACTGCAGAAAGACAAGA TGGCCGAGGCCTACAGCGA GATCGGCATGAAGGGCGA GCGGCGGAGGGGCAAGGG CCACGACGGCCTGTACCAG GGCCTGAGCACCGCCACCA AGGATACCTACGACGCCCT GCACATGCAGGCCCTGCCC CCCAGA
Murine ROR1_v2 VL	609	DVQITQSPSSLYASLGERVTITCKAS PDINSYLSWFQQKPGKSPKTLIYRA NRLVDGVPSRFSGGGSGQDYSLTI NSLEYEDMGIYYCLQYDEFPYTFGG GTKLEMK	610	GACGTGCAGATCACCCAGA GCCCCAGCAGCCTGTATGC CAGCCTGGGCGAGAGAGTG ACCATTACCTGCAAGGCCA GCCCCGACATCAACAGCTA CCTGAGCTGGTTCCAGCAG AAGCCCGGCAAGAGCCCCA AGACCCTGATCTACCGGGC CAACAGACTGGTGGATGGC GTGCCCAGCAGATTCAGCG GCGGAGGCTCTGGCCAGGA CTACAGCCTGACCATCAACT CCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAG
Murine ROR1_v2 VH	611	EVKLVESGGGLVKPGGSLKLSCAAS GFTFSSYAMSWVRQIPEKRLEWVA SISRGGTTYYPDSVKGRFTISRDNVR NILYLQMSSLRSEDTAMYYCGRYD YDGYYAMDYWGQGTSVTVSS	612	GAAGTGAAGCTGGTGGAAT CTGGCGGCGGACTCGTGAA GCCTGGCGGCTCTCTGAAG CTGTCTTGTGCCGCCAGCG GCTTCACCTTCAGCAGCTAC GCCATGAGCTGGGTGCGGC AGATCCCCGAGAAGCGGCT GGAATGGGTGGCCAGCATC AGCAGAGGCGGAACCACCT ACTACCCCGACTCTGTGAAG GGCCGGTTCACCATCAGCC GGGACAACGTGCGGAACAT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCTGTACCTGCAGATGAGC AGCCTGCGGAGCGAGGACA CCGCCATGTACTACTGTGGC AGATACGACTACGACGGCT ACTATGCCATGGATTACTG GGGCCAGGGCACCAGCGT GACCGTGTCATCT
Murine ROR1_v2 (VL-VH). CD8a(3x).CD28z	613	DVQITQSPSSLYASLGERVTITCKAS PDINSYLSWFQQKPGKSPKTLIYRA NRLVDGVPSRFSGGGSGQDYSLTI NSLEYEDMGIYYCLQYDEFPYTFGG GTKLEMKGSTSGSGKPGSGEGSTK GEVKLVESGGGLVKPGGSLKLSCAA SGFTFSSYAMSWVRQIPEKRLEWV ASISRGGTTYYPDSVKGRFTISRDN VRNILYLQMSSLRSEDTAMYYCGR YDYDGYYAMDYWGQGTSVTVSSK PTTTPAPRPPTPAPTIASQPLSLRPE ASRPAAGGAVHTRGLDFASDKPTT TPAPRPPTPAPTIASQPLSLRPEASR PAAGGAVHTRGLDFASDKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRSKRSRG GHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALH MQALPPR	614	GACGTGCAGATCACCCAGA GCCCCAGCAGCCTGTATGC CAGCCTGGGCGAGAGAGTG ACCATTACCTGCAAGGCCA GCCCCGACATCAACAGCTA CCTGAGCTGGTTCCAGCAG AAGCCCGGCAAGAGCCCCA AGACCCTGATCTACCGGGC CAACAGACTGGTGGATGGC GTGCCCAGCAGATTCAGCG GCGGAGGCTCTGGCCAGGA CTACAGCCTGACCATCAACT CCCTGGAATACGAGGACAT GGGCATCTACTACTGCCTGC AGTACGACGAGTTCCCCTAC ACCTTCGGAGGCGGCACCA AGCTGGAAATGAAGGGCA GCACAAGCGGCAGCGGCAA GCCTGGATCTGGCGAGGGA AGCACCAAGGGCGAAGTGA AGCTGGTGGAATCTGGCGG CGGACTCGTGAAGCCTGGC GGCTCTCTGAAGCTGTCTTG TGCCGCCAGCGGCTTCACCT TCAGCAGCTACGCCATGAG CTGGGTGCGGCAGATCCCC GAGAAGCGGCTGGAATGG GTGGCCAGCATCAGCAGAG GCGGAACCACCTACTACCCC GACTCTGTGAAGGGCCGGT TCACCATCAGCCGGGACAA CGTGCGGAACATCCTGTAC CTGCAGATGAGCAGCCTGC GGAGCGAGGACACCGCCAT GTACTACTGTGGCAGATAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GACTACGACGGCTACTATG CCATGGATTACTGGGGCCA GGGCACCAGCGTGACCGTG TCATCTAAGCCTACCACCAC CCCCGCACCTCGTCCTCCAA CCCCTGCACCTACGATTGCC AGTCAGCCTCTTTCACTGCG GCCTGAGGCCAGCAGACCA GCTGCCGGCGGTGCCGTCC ATACAAGAGGACTGGACTT CGCGTCCGATAAACCTACTA CCACTCCAGCCCCAAGGCCC CCAACCCCAGCACCGACTAT CGCATCACAGCCTTTGTCAC TGCGTCCTGAAGCCAGCCG GCCAGCTGCAGGGGGGGC CGTCCACACAAGGGGACTC GACTTTGCGAGTGATAAGC CCACCACCACCCCTGCCCCT AGACCTCCAACCCCAGCCCC TACAATCGCCAGCCAGCCCC TGAGCCTGAGGCCCGAAGC CTGTAGACCTGCCGCTGGC GGAGCCGTGCACACCAGAG GCCTGGATTTCGCCTGCGA CATCTACATCTGGGCCCCTC TGGCCGGCACCTGTGGCGT GCTGCTGCTGAGCCTGGTC ATCACCCTGTACTGCAACCA CCGGAATAGGAGCAAGCG GAGCAGAGGCGGCCACAG CGACTACATGAACATGACC CCCCGGAGGCCTGGCCCCA CCCGGAAGCACTACCAGCC CTACGCCCCTCCCAGGGACT TCGCCGCCTACCGGAGCCG GGTGAAGTTCAGCCGGAGC GCCGACGCCCCTGCCTACCA GCAGGGCCAGAACCAGCTG TACAACGAGCTGAACCTGG GCCGGAGGGAGGAGTACG ACGTGCTGGACAAGCGGAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				AGGCCGGGACCCTGAGATG GGCGGCAAGCCCCGGAGA AAGAACCCTCAGGAGGGCC TGTATAACGAACTGCAGAA AGACAAGATGGCCGAGGCC TACAGCGAGATCGGCATGA AGGGCGAGCGGCGGAGGG GCAAGGGCCACGACGGCCT GTACCAGGGCCTGAGCACC GCCACCAAGGATACCTACG ACGCCCTGCACATGCAGGC CCTGCCCCCCAGA
hROR1 (VH-VL) _14. CD8a(3x).CD28z	615	EVQLVESGGGLVQPGGSLRLSCAT SGFTFSSYAMSWMRQAPGKGLE WVASISRGGTTYYADSVKGRFTISV DKSKNTLYLQMNSLRAEDTAVYYC GRYDYDGYYAMDYWGQGTLVTVS SGGGGSGGGGSGGGGSDIQMTQ SPSSLSASVGDRVTITCKASPDINSY LNWYQQKPGKAPKLLIYRANRLVD GVPSRFSGSGSGTDYTLTISSLQPED FATYYCLQYDEFPYTFGAGTKVEIKK PTTTPAPRPPTPAPTIASQPLSLRPE ASRPAAGGAVHTRGLDFASDKPTT TPAPRPPTPAPTIASQPLSLRPEASR PAAGGAVHTRGLDFASDKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRSKRSRG GHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALH MQALPPR	616	GAAGTGCAGCTGGTGGAGT CTGGCGGCGGTCTGGTGCA GCCCGGCGGCTCTCTGCGC CTCTCCTGTGCCACCTCTGG TTTTACATTCTCCTCCTACGC TATGTCCTGGATGCGGCAA GCCCCCGGCAAGGGCCTAG AGTGGGTCGCCTCAATCAG CAGGGGCGGGACGACTTAT TATGCCGATTCAGTTAAGG GGAGATTCACAATTTCCGT GGATAAATCCAAGAATACC TTATACCTCCAGATGAACTC TCTGCGGGCCGAAGATACG GCCGTATATTATTGTGGGA GGTATGACTACGACGGATA TTACGCCATGGATTATTGG GGGCAGGGGACACTTGTTA CAGTGAGTTCCGGTGGTGG GGGGTCTGGAGGCGGGGG CAGTGGAGGCGGAGGGTC TGATATACAGATGACACAG AGCCCTTCAAGTTTATCTGC AAGCGTCGGCGATCGTGTT ACAATAACTTGCAAGGCAT CTCCCGACATCAATTCCTAC CTCAACTGGTATCAGCAGA AGCCTGGGAAGGCTCCTAA GCTGCTTATTTACAGAGCAA ATCGCCTGGTGGACGGCGT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCCCAGTCGGTTTTCCGGG TCTGGGAGCGGAACGGATT ACACACTGACCATCTCAAGC CTGCAACCCGAAGACTTCG CTACATATTACTGCCTTCAG TATGATGAGTTCCCATATAC CTTCGGCGCTGGGACCAAG GTGGAGATAAAGAAGCCTA CCACCACCCCCGCACCTCGT CCTCCAACCCCTGCACCTAC GATTGCCAGTCAGCCTCTTT CACTGCGGCCTGAGGCCAG CAGACCAGCTGCCGGCGGT GCCGTCCATACAAGAGGAC TGGACTTCGCGTCCGATAA ACCTACTACCACTCCAGCCC CAAGGCCCCCAACCCCAGC ACCGACTATCGCATCACAGC CTTTGTCACTGCGTCCTGAA GCCAGCCGGCCAGCTGCAG GGGGGGCCGTCCACACAAG GGGACTCGACTTTGCGAGT GATAAGCCCACCACCACCCC TGCCCCTAGACCTCCAACCC CAGCCCCTACAATCGCCAGC CAGCCCCTGAGCCTGAGGC CCGAAGCCTGTAGACCTGC CGCTGGCGGAGCCGTGCAC ACCAGAGGCCTGGATTTCG CCTGCGACATCTACATCTGG GCCCCTCTGGCCGGCACCT GTGGCGTGCTGCTGCTGAG CCTGGTCATCACCCTGTACT GCAACCACCGGAATAGGAG CAAGCGGAGCAGAGGCGG CCACAGCGACTACATGAAC ATGACCCCCCGGAGGCCTG GCCCCACCCGGAAGCACTA CCAGCCCTACGCCCCTCCCA GGGACTTCGCCGCCTACCG GAGCCGGGTGAAGTTCAGC CGGAGCGCCGACGCCCCTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCTACCAGCAGGGCCAGAA CCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAG GAGTACGACGTGCTGGACA AGCGGAGAGGCCGGGACC CTGAGATGGGCGGCAAGCC CCGGAGAAAGAACCCTCAG GAGGGCCTGTATAACGAAC TGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATC GGCATGAAGGGCGAGCGG CGGAGGGGCAAGGGCCAC GACGGCCTGTACCAGGGCC TGAGCACCGCCACCAAGGA TACCTACGACGCCCTGCACA TGCAGGCCCTGCCCCCCAG A
hROR1 (VL-VH) _05. CD8a(3x).CD2 8z	617	DIQMTQSPSSLSASVGDRVTITCKA SPDINSYLSWYQQKPGKAPKLLIYR ANRLVDGVPSRFSGSGSGTDFTLTI SSLQPEDIATYYCLQYDEFPYTFGQ GTKLEIKGGGGSGGGGSGGGGSEV QLVESGGGLVQPGGSLRLSCAASG FTFSSYAMSWVRQAPGKGLEWVS SISRGGTTYYPDSVKGRFTISRDNSK NTLYLQMNSLRAEDTAVYYCGRYD YDGYYAMDYWGQGTLVTVSSKPT TTPAPRPPTPAPTIASQPLSLRPEAS RPAAGGAVHTRGLDFASDKPTTTP APRPPTPAPTIASQPLSLRPEASRPA AGGAVHTRGLDFASDKPTTTPAPR PPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCNHRNRSKRSRGG HSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRSRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQ ALPPR	618	GATATTCAGATGACCCAGTC ACCTTCGAGTCTGAGCGCA TCCGTGGGCGACAGAGTGA CCATTACCTGTAAGGCCAGC CCGGACATTAACAGCTACCT ATCGTGGTATCAGCAAAAG CCTGGTAAGGCCCCTAAACT CCTTATCTACAGGGCTAATA GGTTGGTAGACGGGGTGCC TAGCCGGTTCTCTGGTTCCG GCAGCGGTACGGACTTTAC TCTGACCATAAGCTCTCTGC AACCAGAAGACATCGCAAC ATACTACTGTTTACAATACG ACGAATTTCCTTATACCTTT GGCCAGGGGACCAAGTTAG AGATCAAGGGGGGCGGCG GAAGTGGTGGAGGGGGAA GTGGTGGAGGAGGAAGCG AAGTGCAACTGGTCGAGTC TGGGGGCGGCCTTGTGCAA CCTGGAGGCAGCCTTCGAC TCAGTTGCGCCGCGTCTGG TTTTACCTTCTCCTCTTACGC GATGAGCTGGGTTCGCCAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCCCCCGGCAAGGGACTTG AGTGGGTTAGTTCGATCTCC CGCGGAGGCACCACATATT ATCCTGACTCGGTTAAGGG ACGCTTCACTATCTCTAGGG ACAATTCAAAGAACACACT GTATCTCCAAATGAACTCCT TGCGGGCCGAGGACACTGC TGTGTATTATTGCGGACGAT ACGACTACGATGGGTATTA CGCCATGGATTACTGGGGG CAAGGTACACTGGTCACTG TGAGTTCGAAGCCTACCACC ACCCCCGCACCTCGTCCTCC AACCCCTGCACCTACGATTG CCAGTCAGCCTCTTTCACTG CGGCCTGAGGCCAGCAGAC CAGCTGCCGGCGGTGCCGT CCATACAAGAGGACTGGAC TTCGCGTCCGATAAACCTAC TACCACTCCAGCCCCAAGGC CCCCAACCCCAGCACCGACT ATCGCATCACAGCCTTTGTC ACTGCGTCCTGAAGCCAGC CGGCCAGCTGCAGGGGGG GCCGTCCACACAAGGGGAC TCGACTTTGCGAGTGATAA GCCCACCACCACCCCTGCCC CTAGACCTCCAACCCCAGCC CCTACAATCGCCAGCCAGCC CCTGAGCCTGAGGCCCGAA GCCTGTAGACCTGCCGCTG GCGGAGCCGTGCACACCAG AGGCCTGGATTTCGCCTGC GACATCTACATCTGGGCCCC TCTGGCCGGCACCTGTGGC GTGCTGCTGCTGAGCCTGG TCATCACCCTGTACTGCAAC CACCGGAATAGGAGCAAGC GGAGCAGAGGCGGCCACA GCGACTACATGAACATGAC CCCCCGGAGGCCTGGCCCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCCGGAAGCACTACCAGC CCTACGCCCCTCCCAGGGAC TTCGCCGCCTACCGGAGCC GGGTGAAGTTCAGCCGGAG CGCCGACGCCCCTGCCTACC AGCAGGGCCAGAACCAGCT GTACAACGAGCTGAACCTG GGCCGGAGGGAGGAGTAC GACGTGCTGGACAAGCGGA GAGGCCGGGACCCTGAGAT GGGCGGCAAGCCCCGGAG AAAGAACCCTCAGGAGGGC CTGTATAACGAACTGCAGA AAGACAAGATGGCCGAGGC CTACAGCGAGATCGGCATG AAGGGCGAGCGGCGGAGG GGCAAGGGCCACGACGGCC TGTACCAGGGCCTGAGCAC CGCCACCAAGGATACCTAC GACGCCCTGCACATGCAGG CCCTGCCCCCCAGATGA
hROR1 (VH-VL) _14-3. CD8a(3x).CD28z	619	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTTYYADSVKGRFTISRD NSKNTLYLQMNSLRAEDTAVYYCG RYDYDGYYAMDYWGQGTLVTVSS GGGGSGGGGSGGGGSDIQMTQS PSSLSASVGDRVTITCKASPDINSYL NWYQQKPGKAPKLLIYRANRLVDG VPSRFSGSGSGTDFTLTISSLQPEDI ATYYCLQYDEFPYTFGGGTKVEIKK PTTTPAPRPPTPAPTIASQPLSLRPE ASRPAAGGAVHTRGLDFASDKPTT TPAPRPPTPAPTIASQPLSLRPEASR PAAGGAVHTRGLDFASDKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRSKRSRG GHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLY	620	GAGGTTCAACTTGTGGAAT CCGGCGGCGGGTTAGTCCA GCCCGGCGGGAGCTTGCGG CTGTCCTGCGCCGCCTCTGG ATTCACTTTTAGCTCCTATG CTATGTCTTGGGTAAGGCA GGCCCCTGGTAAAGGACTA GAGTGGGTGGCCTCGATCT CCCGTGGTGGCACTACATA CTACGCCGACTCCGTTAAAG GCCGGTTTACCATCTCCCGT GACAACTCTAAAAATACTTT GTACCTGCAAATGAACTCCC TGCGGGCAGAAGACACAGC CGTGTACTATTGCGGGCGT TACGATTACGACGGATATTA CGCAATGGACTACTGGGGC CAGGGCACACTGGTCACCG TGAGCAGCGGGGGCGGAG GAAGTGGAGGAGGCGGTA GTGGTGGGGGAGGAAGCG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		NELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALH MQALPPR		ATATACAAATGACTCAGTCC CCTAGTAGCCTTAGTGCTAG TGTGGGAGACAGAGTGACC ATCACCTGCAAAGCATCTCC TGATATCAATTCCTACCTTA ACTGGTATCAACAGAAGCC TGGCAAAGCTCCAAAGCTC CTGATTTATCGCGCGAACA GATTGGTCGATGGGGTCCC TTCCAGATTCAGCGGCTCA GGGTCAGGGACCGATTTCA CCCTCACAATTAGTTCACTT CAGCCCGAGGACATCGCCA CGTATTATTGCCTTCAGTAC GATGAGTTCCCTTACACCTT TGGCGGGGGAACTAAAGTC GAAATTAAGAAGCCTACCA CCACCCCCGCACCTCGTCCT CCAACCCCTGCACCTACGAT TGCCAGTCAGCCTCTTTCAC TGCGGCCTGAGGCCAGCAG ACCAGCTGCCGGCGGTGCC GTCCATACAAGAGGACTGG ACTTCGCGTCCGATAAACCT ACTACCACTCCAGCCCCAAG GCCCCCAACCCCAGCACCG ACTATCGCATCACAGCCTTT GTCACTGCGTCCTGAAGCC AGCCGGCCAGCTGCAGGG GGGGCCGTCCACACAAGGG GACTCGACTTTGCGAGTGA TAAGCCCACCACCACCCCTG CCCCTAGACCTCCAACCCCA GCCCCTACAATCGCCAGCCA GCCCCTGAGCCTGAGGCCC GAAGCCTGTAGACCTGCCG CTGGCGGAGCCGTGCACAC CAGAGGCCTGGATTTCGCC TGCGACATCTACATCTGGG CCCCTCTGGCCGGCACCTGT GGCGTGCTGCTGCTGAGCC TGGTCATCACCCTGTACTGC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				AACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCC ACAGCGACTACATGAACAT GACCCCCCGGAGGCCTGGC CCCACCCGGAAGCACTACC AGCCCTACGCCCCTCCCAGG GACTTCGCCGCCTACCGGA GCCGGGTGAAGTTCAGCCG GAGCGCCGACGCCCCTGCC TACCAGCAGGGCCAGAACC AGCTGTACAACGAGCTGAA CCTGGGCCGGAGGGAGGA GTACGACGTGCTGGACAAG CGGAGAGGCCGGGACCCT GAGATGGGCGGCAAGCCCC GGAGAAAGAACCCTCAGGA GGGCCTGTATAACGAACTG CAGAAAGACAAGATGGCCG AGGCCTACAGCGAGATCGG CATGAAGGGCGAGCGGCG GAGGGGCAAGGGCCACGA CGGCCTGTACCAGGGCCTG AGCACCGCCACCAAGGATA CCTACGACGCCCTGCACATG CAGGCCCTGCCCCCCAGA
hROR1 (VH-VL) _14-4. CD8a(3x).CD28z	621	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VASISRGGTTYYPDSVKGRFTISRD NVRNILYLQMSSLRSEDTAMYYCG RYDYDGYYAMDYWGQGTLVTVSS GGGGSGGGGSGGGGSDIQMTQS PSSLSASVGDRVTITCKASPDINSYL NWYQQKPGKAPKLLIYRANRLVDG VPSRFSGSGSGTDYTLTISSLQPEDF ATYYCLQYDEFPYTFGAGTKVEIKK PTTTPAPRPPTPAPTIASQPLSLRPE ASRPAAGGAVHTRGLDFASDKPTT TPAPRPPTPAPTIASQPLSLRPEASR PAAGGAVHTRGLDFASDKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRSKRSRG	622	GAAGTGCAGCTTGTGGAGT CAGGAGGAGGGCTAGTTCA GCCAGGCGGCTCTCTGAGA CTATCTTGTGCTGCCTCCGG CTTCACATTTAGCTCTTATG CAATGTCCTGGGTCCGCCA GGCCCCTGGTAAAGGCCTG GAATGGGTTGCTTCTATCTC TAGAGGCGGAACCACTTAC TACCCTGATTCAGTGAAGG GGAGATTCACAATTAGTAG GGACAACGTGCGGAACATC CTCTACCTACAGATGTCAAG TTTACGCAGTGAGGACACT GCGATGTATTACTGCGGTC GATACGATTATGATGGATA TTATGCAATGGATTATTGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		GHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALH MQALPPR		GGCCAGGGCACTCTGGTCA CAGTATCTTCCGGCGGCGG TGGTTCTGGCGGTGGTGGA AGCGGAGGGGGGGGGTCC GACATCCAGATGACCCAAT CACCATCGAGTCTTAGTGCA TCCGTTGGGGATAGAGTGA CAATCACTTGTAAGGCATCC CCGGACATCAACTCATATCT TAATTGGTATCAGCAAAAG CCGGGCAAGGCCCCTAAGC TCCTGATTTATAGGGCCAAC CGCCTTGTGGATGGAGTCC CCTCCCGCTTTAGTGGAAGC GGCTCTGGCACAGACTACA CCCTGACTATCAGCTCCTTG CAGCCTGAGGATTTTGCTAC CTACTACTGTCTTCAGTACG ATGAATTTCCATACACTTTC GGTGCTGGGACAAAAGTGG AGATCAAAAAGCCTACCAC CACCCCCGCACCTCGTCCTC CAACCCCTGCACCTACGATT GCCAGTCAGCCTCTTTCACT GCGGCCTGAGGCCAGCAGA CCAGCTGCCGGCGGTGCCG TCCATACAAGAGGACTGGA CTTCGCGTCCGATAAACCTA CTACCACTCCAGCCCCAAGG CCCCCAACCCCAGCACCGAC TATCGCATCACAGCCTTTGT CACTGCGTCCTGAAGCCAG CCGGCCAGCTGCAGGGGG GGCCGTCCACACAAGGGGA CTCGACTTTGCGAGTGATA AGCCCACCACCACCCCTGCC CCTAGACCTCCAACCCCAGC CCCTACAATCGCCAGCCAGC CCCTGAGCCTGAGGCCCGA AGCCTGTAGACCTGCCGCT GGCGGAGCCGTGCACACCA GAGGCCTGGATTTCGCCTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CGACATCTACATCTGGGCCC CTCTGGCCGGCACCTGTGG CGTGCTGCTGCTGAGCCTG GTCATCACCCTGTACTGCAA CCACCGGAATAGGAGCAAG CGGAGCAGAGGCGGCCAC AGCGACTACATGAACATGA CCCCCCGGAGGCCTGGCCC CACCCGGAAGCACTACCAG CCCTACGCCCCTCCCAGGGA CTTCGCCGCCTACCGGAGC CGGGTGAAGTTCAGCCGGA GCGCCGACGCCCCTGCCTA CCAGCAGGGCCAGAACCAG CTGTACAACGAGCTGAACC TGGGCCGGAGGGAGGAGT ACGACGTGCTGGACAAGCG GAGAGGCCGGGACCCTGA GATGGGCGGCAAGCCCCG GAGAAAGAACCCTCAGGAG GGCCTGTATAACGAACTGC AGAAAGACAAGATGGCCGA GGCCTACAGCGAGATCGGC ATGAAGGGCGAGCGGCGG AGGGGCAAGGGCCACGAC GGCCTGTACCAGGGCCTGA GCACCGCCACCAAGGATAC CTACGACGCCCTGCACATGC AGGCCCTGCCCCCCAGA
hROR1 (VH_5-VL_14).CD8a(3x).CD28z	623	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSSISRGGTTYYPDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSSG GGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCKASPDINSYLN WYQQKPGKAPKLLIYRANRLVDGV PSRFSGSGSGTDYTLTISSLQPEDFA TYYCLQYDEFPYTFGAGTKVEIKKPT TTPAPRPPTPAPTIASQPLSLRPEAS RPAAGGAVHTRGLDFASDKPTTTP APRPPTPAPTIASQPLSLRPEASRPA	624	GAAGTGCAACTGGTCGAGT CTGGGGGCGGCCTTGTGCA ACCTGGAGGCAGCCTTCGA CTCAGTTGCGCCGCGTCTG GTTTTACCTTCTCCTCTTACG CGATGAGCTGGGTTCGCCA GGCCCCCGGCAAGGGACTT GAGTGGGTTAGTTCGATCT CCCGCGGAGGCACCACATA TTATCCTGACTCGGTTAAGG GACGCTTCACTATCTCTAGG GACAATTCAAAGAACACAC TGTATCTCCAAATGAACTCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		AGGAVHTRGLDFASDKPTTTPAPR PPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCNHRNRSKRSRGG HSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRSRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQ ALPPR		TTGCGGGCCGAGGACACTG CTGTGTATTATTGCGGACG ATACGACTACGATGGGTAT TACGCCATGGATTACTGGG GGCAAGGTACACTGGTCAC TGTGAGTTCGGGGGGCGGC GGAAGTGGTGGAGGGGGA AGTGGTGGAGGAGGAAGC GATATACAGATGACACAGA GCCCTTCAAGTTTATCTGCA AGCGTCGGCGATCGTGTTA CAATAACTTGCAAGGCATCT CCCGACATCAATTCCTACCT CAACTGGTATCAGCAGAAG CCTGGGAAGGCTCCTAAGC TGCTTATTTACAGAGCAAAT CGCCTGGTGGACGGCGTGC CCAGTCGGTTTTCCGGGTCT GGGAGCGGAACGGATTACA CACTGACCATCTCAAGCCTG CAACCCGAAGACTTCGCTAC ATATTACTGCCTTCAGTATG ATGAGTTCCCATATACCTTC GGCGCTGGGACCAAGGTG GAGATAAAGAAGCCTACCA CCACCCCCGCACCTCGTCCT CCAACCCCTGCACCTACGAT TGCCAGTCAGCCTCTTTCAC TGCGGCCTGAGGCCAGCAG ACCAGCTGCCGGCGGTGCC GTCCATACAAGAGGACTGG ACTTCGCGTCCGATAAACCT ACTACCACTCCAGCCCCAAG GCCCCCAACCCCAGCACCG ACTATCGCATCACAGCCTTT GTCACTGCGTCCTGAAGCC AGCCGGCCAGCTGCAGGG GGGGCCGTCCACACAAGGG GACTCGACTTTGCGAGTGA TAAGCCCACCACCACCCCTG CCCCTAGACCTCCAACCCCA GCCCCTACAATCGCCAGCCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GCCCCTGAGCCTGAGGCCC GAAGCCTGTAGACCTGCCG CTGGCGGAGCCGTGCACAC CAGAGGCCTGGATTTCGCC TGCGACATCTACATCTGGG CCCCTCTGGCCGGCACCTGT GGCGTGCTGCTGCTGAGCC TGGTCATCACCCTGTACTGC AACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCC ACAGCGACTACATGAACAT GACCCCCCGGAGGCCTGGC CCCACCCGGAAGCACTACC AGCCCTACGCCCCTCCCAGG GACTTCGCCGCCTACCGGA GCCGGGTGAAGTTCAGCCG GAGCGCCGACGCCCCTGCC TACCAGCAGGGCCAGAACC AGCTGTACAACGAGCTGAA CCTGGGCCGGAGGGAGGA GTACGACGTGCTGGACAAG CGGAGAGGCCGGGACCCT GAGATGGGCGGCAAGCCCC GGAGAAAGAACCCTCAGGA GGGCCTGTATAACGAACTG CAGAAAGACAAGATGGCCG AGGCCTACAGCGAGATCGG CATGAAGGGCGAGCGGCG GAGGGGCAAGGGCCACGA CGGCCTGTACCAGGGCCTG AGCACCGCCACCAAGGATA CCTACGACGCCCTGCACATG CAGGCCCTGCCCCCCAGA
hROR1 (VH_5-VL_16).CD8a(3x).CD28z	625	EVQLVESGGGLVQPGGSLRLSCAA SGFTFSSYAMSWVRQAPGKGLEW VSSISRGGTTYYPDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCGR YDYDGYYAMDYWGQGTLVTVSSG GGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCKASPDINSYLN WYQQKPGKAPKVLIYRANRLVDG VPSRFSGSGSGTDYTLTISSLQPEDF	626	GAAGTGCAACTGGTCGAGT CTGGGGGCGGCCTTGTGCA ACCTGGAGGCAGCCTTCGA CTCAGTTGCGCCGCGTCTG GTTTTACCTTCTCCTCTTACG CGATGAGCTGGGTTCGCCA GGCCCCCGGCAAGGGACTT GAGTGGGTTAGTTCGATCT CCCGCGGAGGCACCACATA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		ATYYCLQYDEFPYTFGQGTKVEIKK PTTTPAPRPPTPAPTIASQPLSLRPE ASRPAAGGAVHTRGLDFASDKPTT TPAPRPPTPAPTIASQPLSLRPEASR PAAGGAVHTRGLDFASDKPTTTPA PRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGT CGVLLLSLVITLYCNHRNRSKRSRG GHSDYMNMTPRRPGPTRKHYQPY APPRDFAAYRSRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRR GKGHDGLYQGLSTATKDTYDALH MQALPPR		TTATCCTGACTCGGTTAAGG GACGCTTCACTATCTCTAGG GACAATTCAAAGAACACAC TGTATCTCCAAATGAACTCC TTGCGGGCCGAGGACACTG CTGTGTATTATTGCGGACG ATACGACTACGATGGGTAT TACGCCATGGATTACTGGG GGCAAGGTACACTGGTCAC TGTGAGTTCGGGGGGCGGC GGAAGTGGTGGAGGGGGA AGTGGTGGAGGAGGAAGC GATATTCAGATGACCCAGTC GCCCAGCAGTCTCTCGGCCT CAGTGGGCGACCGGGTCAC TATCACTTGCAAAGCAAGTC CTGATATAAACTCCTATCTT AATTGGTATCAGCAGAAGC CCGGCAAGGCACCTAAGGT TCTGATATATCGCGCAAATC GGCTCGTGGATGGAGTACC CAGCCGATTTTCCGGCAGC GGCTCAGGCACTGACTACA CACTGACAATCAGCAGCTT GCAGCCTGAAGATTTCGCC ACATACTATTGTCTACAGTA CGACGAGTTCCCTTATACAT TCGGCCAGGGGACCAAGGT CGAGATCAAGAAGCCTACC ACCACCCCCGCACCTCGTCC TCCAACCCCTGCACCTACGA TTGCCAGTCAGCCTCTTTCA CTGCGGCCTGAGGCCAGCA GACCAGCTGCCGGCGGTGC CGTCCATACAAGAGGACTG GACTTCGCGTCCGATAAACC TACTACCACTCCAGCCCCAA GGCCCCCAACCCCAGCACC GACTATCGCATCACAGCCTT TGTCACTGCGTCCTGAAGCC AGCCGGCCAGCTGCAGGG GGGGCCGTCCACACAAGGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GACTCGACTTTGCGAGTGA TAAGCCCACCACCACCCCTG CCCCTAGACCTCCAACCCCA GCCCCTACAATCGCCAGCCA GCCCCTGAGCCTGAGGCCC GAAGCCTGTAGACCTGCCG CTGGCGGAGCCGTGCACAC CAGAGGCCTGGATTTCGCC TGCGACATCTACATCTGGG CCCCTCTGGCCGGCACCTGT GGCGTGCTGCTGCTGAGCC TGGTCATCACCCTGTACTGC AACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCC ACAGCGACTACATGAACAT GACCCCCCGGAGGCCTGGC CCCACCCGGAAGCACTACC AGCCCTACGCCCCTCCCAGG GACTTCGCCGCCTACCGGA GCCGGGTGAAGTTCAGCCG GAGCGCCGACGCCCCTGCC TACCAGCAGGGCCAGAACC AGCTGTACAACGAGCTGAA CCTGGGCCGGAGGGAGGA GTACGACGTGCTGGACAAG CGGAGAGGCCGGGACCCT GAGATGGGCGGCAAGCCCC GGAGAAAGAACCCTCAGGA GGGCCTGTATAACGAACTG CAGAAAGACAAGATGGCCG AGGCCTACAGCGAGATCGG CATGAAGGGCGAGCGGCG GAGGGGCAAGGGCCACGA CGGCCTGTACCAGGGCCTG AGCACCGCCACCAAGGATA CCTACGACGCCCTGCACATG CAGGCCCTGCCCCCCAGA
hROR1 (VH_18-VL_04). CD8a(3x).CD28z	627	EVQLVESGGGLVQPGGSLRLSCSAS GFTFSSYAMSWVRQVPGKGLVWI SSISRGGTTYYADSVRGRFIISRDNA KNTLYLEMNNLRGEDTAVYYCARY DYDGYYAMDYWGQGTLVTVSSG	628	GAGGTTCAACTCGTGGAGT CTGGAGGCGGGCTAGTGCA GCCTGGCGGCTCCCTGCGA CTGTCTTGCAGCGCATCAG GCTTTACATTCAGTTCTTAT

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		GGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCQASPDINSYLN WYQQKPGKAPKLLIYRANNLETGV PSRFSGSGSGTDFTLTISSLQPEDIA TYYCLQYDEFPYTFGQGTKLEIKKPT TTPAPRPPTPAPTIASQPLSLRPEAS RPAAGGAVHTRGLDFASDKPTTTP APRPPTPAPTIASQPLSLRPEASRPA AGGAVHTRGLDFASDKPTTTPAPR PPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCNHRNRSKRSRGG HSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRSRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQ ALPPR		GCCATGAGCTGGGTGAGGC AGGTGCCCGGCAAGGGTCT GGTGTGGATCAGCTCAATC TCCAGGGGCGGGACTACAT ATTACGCCGATTCGGTCAG GGGTCGTTTTATCATTAGCA GGGATAATGCCAAGAACAC CTTGTATTTGGAGATGAAC AACCTAAGAGGCGAAGACA CCGCTGTGTACTATTGTGCC CGTTACGACTACGATGGGT ACTACGCCATGGACTATTG GGGCCAGGGAACCTTGGTG ACTGTGTCAAGTGGCGGGG GCGGCAGCGGAGGCGGTG GCAGCGGAGGCGGCGGTT CTGATATTCAAATGACGCAA AGTCCCAGCAGCCTCTCCGC CTCCGTTGGAGACAGGGTG ACTATTACATGCCAAGCCAG CCCCGATATTAATAGCTACT TAAATTGGTATCAGCAGAA ACCTGGGAAGGCACCTAAA CTTCTCATCTACCGCGCTAA CAATCTGGAGACCGGCGTG CCGTCTAGATTTTCCGGCTC TGGATCAGGGACCGATTTT ACTCTGACAATTAGTTCCCT GCAACCCGAAGACATCGCC ACTTATTATTGCCTGCAATA TGATGAGTTTCCTTACACAT TTGGTCAGGGAACTAAACT AGAGATTAAGAAGCCTACC ACCACCCCCGCACCTCGTCC TCCAACCCCTGCACCTACGA TTGCCAGTCAGCCTCTTTCA CTGCGGCCTGAGGCCAGCA GACCAGCTGCCGGCGGTGC CGTCCATACAAGAGGACTG GACTTCGCGTCCGATAAACC TACTACCACTCCAGCCCCAA GGCCCCCAACCCCAGCACC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GACTATCGCATCACAGCCTT TGTCACTGCGTCCTGAAGCC AGCCGGCCAGCTGCAGGG GGGGCCGTCCACACAAGGG GACTCGACTTTGCGAGTGA TAAGCCCACCACCACCCCTG CCCCTAGACCTCCAACCCCA GCCCCTACAATCGCCAGCCA GCCCCTGAGCCTGAGGCCC GAAGCCTGTAGACCTGCCG CTGGCGGAGCCGTGCACAC CAGAGGCCTGGATTTCGCC TGCGACATCTACATCTGGG CCCCTCTGGCCGGCACCTGT GGCGTGCTGCTGCTGAGCC TGGTCATCACCCTGTACTGC AACCACCGGAATAGGAGCA AGCGGAGCAGAGGCGGCC ACAGCGACTACATGAACAT GACCCCCCGGAGGCCTGGC CCCACCCGGAAGCACTACC AGCCCTACGCCCCTCCCAGG GACTTCGCCGCCTACCGGA GCCGGGTGAAGTTCAGCCG GAGCGCCGACGCCCCTGCC TACCAGCAGGGCCAGAACC AGCTGTACAACGAGCTGAA CCTGGGCCGGAGGGAGGA GTACGACGTGCTGGACAAG CGGAGAGGCCGGGACCCT GAGATGGGCGGCAAGCCCC GGAGAAAGAACCCTCAGGA GGGCCTGTATAACGAACTG CAGAAAGACAAGATGGCCG AGGCCTACAGCGAGATCGG CATGAAGGGCGAGCGGCG GAGGGGCAAGGGCCACGA CGGCCTGTACCAGGGCCTG AGCACCGCCACCAAGGATA CCTACGACGCCCTGCACATG CAGGCCCTGCCCCCCAGA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
hROR1 (VH_18-VL_14). CD8a(3x).CD28z	629	EVQLVESGGGLVQPGGSLRLSCSAS GFTFSSYAMSWVRQVPGKGLVWI SSISRGGTTYYADSVRGRFIISRDNA KNTLYLEMNNLRGEDTAVYYCARY DYDGYYAMDYWGQGTLVTVSSG GGGSGGGGSGGGGSDIQMTQSPS SLSASVGDRVTITCKASPDINSYLN WYQQKPGKAPKLLIYRANRLVDGV PSRFSGSGSGTDYTLTISSLQPEDFA TYYCLQYDEFPYTFGAGTKVEIKKPT TTPAPRPPTPAPTIASQPLSLRPEAS RPAAGGAVHTRGLDFASDKPTTTP APRPPTPAPTIASQPLSLRPEASRPA AGGAVHTRGLDFASDKPTTTPAPR PPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTC GVLLLSLVITLYCNHRNRSKRSRGG HSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRSRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKR RGRDPEMGGKPRRKNPQEGLYNE LQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQ ALPPR	630	GAGGTTCAACTCGTGGAGT CTGGAGGCGGGCTAGTGCA GCCTGGCGGCTCCCTGCGA CTGTCTTGCAGCGCATCAG GCTTTACATTCAGTTCTTAT GCCATGAGCTGGGTGAGGC AGGTGCCCGGCAAGGGTCT GGTGTGGATCAGCTCAATC TCCAGGGGCGGGACTACAT ATTACGCCGATTCGGTCAG GGGTCGTTTTATCATTAGCA GGGATAATGCCAAGAACAC CTTGTATTTGGAGATGAAC AACCTAAGAGGCGAAGACA CCGCTGTGTACTATTGTGCC CGTTACGACTACGATGGGT ACTACGCCATGGACTATTG GGGCCAGGGAACCTTGGTG ACTGTGTCAAGTGGCGGGG GCGGCAGCGGAGGCGGTG GCAGCGGAGGCGGCGGTT CTGATATACAGATGACACA GAGCCCTTCAAGTTTATCTG CAAGCGTCGGCGATCGTGT TACAATAACTTGCAAGGCAT CTCCCGACATCAATTCCTAC CTCAACTGGTATCAGCAGA AGCCTGGGAAGGCTCCTAA GCTGCTTATTTACAGAGCAA ATCGCCTGGTGGACGGCGT GCCCAGTCGGTTTTCCGGG TCTGGGAGCGGAACGGATT ACACACTGACCATCTCAAGC CTGCAACCCGAAGACTTCG CTACATATTACTGCCTTCAG TATGATGAGTTCCCATATAC CTTCGGCGCTGGGACCAAG GTGGAGATAAAGAAGCCTA CCACCACCCCCGCACCTCGT CCTCCAACCCCTGCACCTAC GATTGCCAGTCAGCCTCTTT CACTGCGGCCTGAGGCCAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CAGACCAGCTGCCGGCGGT GCCGTCCATACAAGAGGAC TGGACTTCGCGTCCGATAA ACCTACTACCACTCCAGCCC CAAGGCCCCCAACCCCAGC ACCGACTATCGCATCACAGC CTTTGTCACTGCGTCCTGAA GCCAGCCGGCCAGCTGCAG GGGGGGCCGTCCACACAAG GGGACTCGACTTTGCGAGT GATAAGCCCACCACCACCCC TGCCCCTAGACCTCCAACCC CAGCCCCTACAATCGCCAGC CAGCCCCTGAGCCTGAGGC CCGAAGCCTGTAGACCTGC CGCTGGCGGAGCCGTGCAC ACCAGAGGCCTGGATTTCG CCTGCGACATCTACATCTGG GCCCCTCTGGCCGGCACCT GTGGCGTGCTGCTGCTGAG CCTGGTCATCACCCTGTACT GCAACCACCGGAATAGGAG CAAGCGGAGCAGAGGCGG CCACAGCGACTACATGAAC ATGACCCCCCGGAGGCCTG GCCCCACCCGGAAGCACTA CCAGCCCTACGCCCCTCCCA GGGACTTCGCCGCCTACCG GAGCCGGGTGAAGTTCAGC CGGAGCGCCGACGCCCCTG CCTACCAGCAGGGCCAGAA CCAGCTGTACAACGAGCTG AACCTGGGCCGGAGGGAG GAGTACGACGTGCTGGACA AGCGGAGAGGCCGGGACC CTGAGATGGGCGGCAAGCC CCGGAGAAAGAACCCTCAG GAGGGCCTGTATAACGAAC TGCAGAAAGACAAGATGGC CGAGGCCTACAGCGAGATC GGCATGAAGGGCGAGCGG CGGAGGGGCAAGGGCCAC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GACGGCCTGTACCAGGGCC TGAGCACCGCCACCAAGGA TACCTACGACGCCCTGCACA TGCAGGCCCTGCCCCCCAG A
IgG4-Fc 12 amino acid hinge region	631	ESKYGPPCPPCP		
RTS-COMPONENTS				
VP16 activation domain	632	GPKKKRKVAPPTDVSLGDELHLDG EDVAMAHADALDDFDLDMLGDG DSPGPGFTPHDSAPYGALDMADF EFEQMFTDALGIDEYGG	633	GGCCCCAAGAAGAAAAGG AAGGTGGCCCCCCCCACCG ACGTGAGCCTGGGCGACGA GCTGCACCTGGACGGCGAG GACGTGGCCATGGCCCACG CCGACGCCCTGGACGACTT CGACCTGGACATGCTGGGC GACGGCGACAGCCCCGGCC CCGGCTTCACCCCCCACGAC AGCGCCCCCTACGGCGCCC TGGACATGGCCGACTTCGA GTTCGAGCAGATGTTCACC GACGCCCTGGGCATCGACG AGTACGGCGGC
Retinoid x receptor (RxR)	634	EMPVDRILEAELAVEQKSDQGVEG PGGTGGSGSSPNDPVTNICQAADK QLFTLVEWAKRIPHFSSLPLDDQVIL LRAGWNELLIASFSHRSIDVRDGILL ATGLHVHRNSAHSAGVGAIFDRVL TELVSKMRDMRMDKTELGCLRAII LFNPEVRGLKSAQEVELLREKVYAA LEEYTRTTHPDEPGRFAKLLLRLPSL RSIGLKCLEHLFFFRLIGDVPIDTFLM EMLESPSDS	635	GAGATGCCCGTGGACAGGA TTCTGGAGGCCGAACTCGC CGTGGAGCAGAAAAGCGAC CAGGGCGTGGAGGGCCCC GGCGGAACCGGCGGCAGC GGCAGCAGCCCCAACGACC CCGTGACCAACATCTGCCA GGCCGCCGACAAGCAGCTG TTCACCCTGGTGGAGTGGG CCAAGAGGATTCCCCACTTC AGCAGCCTGCCCCTGGACG ACCAGGTGATCCTGCTGAG GGCCGGATGGAACGAGCTG CTGATCGCCAGCTTCAGCCA CAGGAGCATCGACGTGAGG GACGGCATCCTGCTGGCCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CCGGCCTGCACGTCCATAG GAACAGCGCCCACAGCGCC GGAGTGGGCGCCATCTTCG ACAGGGTGCTGACCGAGCT GGTGAGCAAGATGAGGGA CATGAGGATGGACAAGACC GAGCTGGGCTGCCTGAGGG CCATCATCCTGTTCAACCCC GAGGTGAGGGGCCTGAAA AGCGCCCAGGAGGTGGAG CTGCTGAGGGAGAAGGTGT ACGCCGCCCTGGAGGAGTA CACCAGGACCACCCACCCC GACGAGCCCGGCAGATTCG CCAAGCTGCTGCTGAGGCT GCCCAGCCTGAGGAGCATC GGCCTGAAGTGCCTGGAGC ACCTGTTCTTCTTCAGGCTG ATCGGCGACGTGCCCATCG ACACCTTCCTGATGGAGAT GCTGGAGAGCCCCAGCGAC AGC
VP16-linker-RxR	636	GPKKKRKVAPPTDVSLGDELHLDG EDVAMAHADALDDFDLDMLGDG DSPGPGFTPHDSAPYGALDMADF EFEQMFTDALGIDEYGGEFEMPVD RILEAELAVEQKSDQGVEGPGGTG GSGSSPNDPVTNICQAADKQLFTL VEWAKRIPHFSSLPLDDQVILLRAG WNELLIASFSHRSIDVRDGILLATGL HVHRNSAHSAGVGAIFDRVLTELV SKMRDMRMDKTELGCLRAIILFNP EVRGLKSAQEVELLREKVYAALEEY TRTTHPDEPGRFAKLLLRLPSLRSIG LKCLEHLFFFRLIGDVPIDTFLMEML ESPSDS	637	GGCCCCAAGAAGAAAAGG AAGGTGGCCCCCCCCACCG ACGTGAGCCTGGGCGACGA GCTGCACCTGGACGGCGAG GACGTGGCCATGGCCCACG CCGACGCCCTGGACGACTT CGACCTGGACATGCTGGGC GACGGCGACAGCCCCGGCC CCGGCTTCACCCCCCACGAC AGCGCCCCCTACGGCGCCC TGGACATGGCCGACTTCGA GTTCGAGCAGATGTTCACC GACGCCCTGGGCATCGACG AGTACGGCGGCGAATTCGA GATGCCCGTGGACAGGATT CTGGAGGCCGAACTCGCCG TGGAGCAGAAAAGCGACCA GGGCGTGGAGGGCCCCGG CGGAACCGGCGGCAGCGG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				CAGCAGCCCCAACGACCCC GTGACCAACATCTGCCAGG CCGCCGACAAGCAGCTGTT CACCCTGGTGGAGTGGGCC AAGAGGATTCCCCACTTCA GCAGCCTGCCCCTGGACGA CCAGGTGATCCTGCTGAGG GCCGGATGGAACGAGCTGC TGATCGCCAGCTTCAGCCAC AGGAGCATCGACGTGAGG GACGGCATCCTGCTGGCCA CCGGCCTGCACGTCCATAG GAACAGCGCCCACAGCGCC GGAGTGGGCGCCATCTTCG ACAGGGTGCTGACCGAGCT GGTGAGCAAGATGAGGGA CATGAGGATGGACAAGACC GAGCTGGGCTGCCTGAGGG CCATCATCCTGTTCAACCCC GAGGTGAGGGGCCTGAAA AGCGCCCAGGAGGTGGAG CTGCTGAGGGAGAAGGTGT ACGCCGCCCTGGAGGAGTA CACCAGGACCACCCACCCC GACGAGCCCGGCAGATTCG CCAAGCTGCTGCTGAGGCT GCCCAGCCTGAGGAGCATC GGCCTGAAGTGCCTGGAGC ACCTGTTCTTCTTCAGGCTG ATCGGCGACGTGCCCATCG ACACCTTCCTGATGGAGAT GCTGGAGAGCCCCAGCGAC AGC
GAL4 DNA Binding Domain	638	MKLLSSIEQACDICRLKKLKCSKEKP KCAKCLKNNWECRYSPKTKRSPLTR AHLTEVESRLERLEQLFLLIFPREDLD MILKMDSLQDIKALLTGLFVQDNV NKDAVTDRLASVETDMPLTLRQHR ISATSSSEESSNKGQRQLTVSPEF	639	ATGAAGCTGCTGAGCAGCA TCGAGCAGGCTTGCGACAT CTGCAGGCTGAAGAAGCTG AAGTGCAGCAAGGAGAAG CCCAAGTGCGCCAAGTGCC TGAAGAACAACTGGGAGTG CAGATACAGCCCCAAGACC AAGAGGAGCCCCCTGACCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				GGGCCCACCTGACCGAGGT GGAGAGCAGGCTGGAGAG GCTGGAGCAGCTGTTCCTG CTGATCTTCCCCAGGGAGG ACCTGGACATGATCCTGAA GATGGACAGCCTGCAAGAC ATCAAGGCCCTGCTGACCG GCCTGTTCGTGCAGGACAA CGTGAACAAGGACGCCGTG ACCGACAGGCTGGCCAGCG TGGAGACCGACATGCCCCT GACCCTGAGGCAGCACAGG ATCAGCGCCACCAGCAGCA GCGAGGAGAGCAGCAACA AGGGCCAGAGGCAGCTGAC CGTGAGCCCCGAGTTT
Ecdysone Receptor Ligand Binding Domain – VY variant (EcR)	640	IRPECVVPETQCAMKRKEKKAQKE KDKLPVSTTTVDDHMPPIMQCEPP PPEAARIHEVVPRFLSDKLLVTNRQ KNIPQLTANQQFLIARLIWYQDGYE QPSDEDLKRITQTWQQADDENEE SDTPFRQITEMTILTVQLIVEFAKGL PGFAKISQPDQITLLKACSSEVMML RVARRYDAASDSILFANNQAYTRD NYRKAGMAEVIEDLLHFCRCMYS MALDNIHYALLTAVVIFSDRPGLEQ PQLVEEIQRYYLNTLRIYILNQLSGS ARSSVIYGKILSILSELRTLGMQNSN MCISLKLKNRKLPPFLEEIWDVAD MSHTQPPPILESPTNL	641	ATCAGGCCCGAGTGCGTGG TGCCCGAGACCCAGTGCGC CATGAAAAGGAAGGAGAA GAAGGCCCAGAAGGAGAA GGACAAGCTGCCCGTGAGC ACCACCACCGTCGATGACC ACATGCCCCCCATCATGCAG TGCGAGCCCCCCCCCCCGA GGCCGCCAGGATTCACGAG GTCGTGCCCAGGTTCCTGA GCGACAAGCTGCTGGTGAC CAACAGGCAGAAGAACATC CCCCAGCTGACCGCCAACC AGCAGTTCCTGATCGCCAG GCTGATCTGGTATCAGGAC GGCTACGAGCAGCCCAGCG ACGAGGACCTGAAAAGGAT CACCCAGACCTGGCAGCAG GCCGACGACGAGAACGAG GAGAGCGACACCCCCTTCA GGCAGATCACCGAGATGAC CATCCTGACCGTGCAGCTG ATCGTGGAGTTCGCCAAGG GCCTGCCCGGATTCGCCAA GATCAGCCAGCCCGACCAG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ATCACCCTGCTGAAGGCTT GCAGCAGCGAGGTGATGAT GCTGAGGGTGGCCAGGAG GTACGACGCCGCCAGCGAC AGCATCCTGTTCGCCAACAA CCAGGCTTACACCAGGGAC AACTACAGGAAGGCTGGCA TGGCCGAGGTGATCGAGGA CCTCCTGCACTTCTGCAGAT GTATGTACAGCATGGCCCT GGACAACATCCACTACGCC CTGCTGACCGCCGTGGTGA TCTTCAGCGACAGGCCCGG CCTGGAGCAGCCCCAGCTG GTGGAGGAGATCCAGAGGT ACTACCTGAACACCCTGAG GATCTACATCCTGAACCAGC TGAGCGGCAGCGCCAGGA GCAGCGTGATCTACGGCAA GATCCTGAGCATCCTGAGC GAGCTGAGGACCCTGGGAA TGCAGAACAGCAATATGTG TATCAGCCTGAAGCTGAAG AACAGGAAGCTGCCCCCCT TCCTGGAGGAGATTTGGGA CGTGGCCGACATGAGCCAC ACCCAGCCCCCCCCCATCCT GGAGAGCCCCACCAACCTG
Ecdysone Receptor Ligand Binding Domain – VY variant (EcR)	642	RPECVVPETQCAMKRKEKKAQKEK DKLPVSTTTVDDHMPPIMQCEPPP PEAARIHEVVPRFLSDKLLVTNRQK NIPQLTANQQFLIARLIWYQDGYE QPSDEDLKRITQTWQQADDENEE SDTPFRQITEMTILTVQLIVEFAKGL PGFAKISQPDQITLLKACSSEVMML RVARRYDAASDSILFANNQAYTRD NYRKAGMAEVIEDLLHFCRCMYS MALDNIHYALLTAVVIFSDRPGLEQ PQLVEEIQRYYLNTLRIYILNQLSGS ARSSVIYGKILSILSELRTLGMQNSN MCISLKLKNRKLPPFLEEIWDVAD MSHTQPPPILESPTNL	643	CGGCCTGAGTGCGTAGTAC CCGAGACTCAGTGCGCCAT GAAGCGGAAAGAGAAGAA AGCACAGAAGGAGAAGGA CAAACTGCCTGTCAGCACG ACGACGGTGGACGACCACA TGCCGCCCATTATGCAGTGT GAACCTCCACCTCCTGAAGC AGCAAGGATTCACGAAGTG GTCCCAAGGTTTCTCTCCGA CAAGCTGTTGGTGACAAAC CGGCAGAAAAACATCCCCC AGTTGACAGCCAACCAGCA GTTCCTTATCGCCAGGCTCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				TCTGGTACCAGGACGGGTA CGAGCAGCCTTCTGATGAA GATTTGAAGAGGATTACGC AGACGTGGCAGCAAGCGG ACGATGAAAACGAAGAGTC GGACACTCCCTTCCGCCAGA TCACAGAGATGACTATCCTC ACGGTCCAACTTATCGTGG AGTTCGCGAAGGGATTGCC AGGGTTCGCCAAGATCTCG CAGCCTGATCAAATTACGCT GCTTAAGGCTTGCTCAAGT GAGGTAATGATGCTCCGAG TCGCGCGACGATACGATGC GGCCTCAGACAGTATTCTGT TCGCGAACAACCAAGCGTA CACTCGCGACAACTACCGC AAGGCTGGCATGGCCGAGG TCATCGAGGATCTACTGCAC TTCTGCCGGTGCATGTACTC TATGGCGTTGGACAACATC CATTACGCGCTGCTCACGG CTGTCGTCATCTTTTCTGAC CGGCCAGGGTTGGAGCAGC CGCAACTGGTGGAAGAGAT CCAGCGGTACTACCTGAAT ACGCTCCGCATCTATATCCT GAACCAGCTGAGCGGGTCG GCGCGTTCGTCCGTCATATA CGGCAAGATCCTCTCAATCC TCTCTGAGCTACGCACGCTC GGCATGCAAAACTCCAACA TGTGCATCTCCCTCAAGCTC AAGAACAGAAAGCTGCCGC CTTTCCTCGAGGAGATCTG GGATGTGGCGGACATGTCG CACACCCAACCGCCGCCTAT CCTCGAGTCCCCCACGAATC TCTAG
GAL4-Linker-EcR	644	MKLLSSIEQACDICRLKKLKCSKEKP KCAKCLKNNWECRYSPKTKRSPLTR AHLTEVESRLERLEQLFLLIFPREDLD	645	ATGAAGCTACTGTCTTCTAT CGAACAAGCATGCGATATT TGCCGACTTAAAAAGCTCA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		MILKMDSLQDIKALLTGLFVQDNV NKDAVTDRLASVETDMPLTLRQHR ISATSSSEESSNKGQRQLTVSPEFPG IRPECVVPETQCAMKRKEKKAQKE KDKLPVSTTTVDDHMPPIMQCEPP PPEAARIHEVVPRFLSDKLLVTNRQ KNIPQLTANQQFLIARLIWYQDGYE QPSDEDLKRITQTWQQADDENEE SDTPFRQITEMTILTVQLIVEFAKGL PGFAKISQPDQITLLKACSSEVMML RVARRYDAASDSILFANNQAYTRD NYRKAGMAEVIEDLLHFCRCMYS MALDNIHYALLTAVVIFSDRPGLEQ PQLVEEIQRYYLNTLRIYILNQLSGS ARSSVIYGKILSILSELRTLGMQNSN MCISLKLKNRKLPPFLEEIWDVAD MSHTQPPPILESPTNL		AGTGCTCCAAAGAAAAACC GAAGTGCGCCAAGTGTCTG AAGAACAACTGGGAGTGTC GCTACTCTCCCAAAACCAAA AGGTCTCCGCTGACTAGGG CACATCTGACAGAAGTGGA ATCAAGGCTAGAAAGACTG GAACAGCTATTTCTACTGAT TTTTCCTCGAGAAGACCTTG ACATGATTTTGAAAATGGAT TCTTTACAGGATATAAAAGC ATTGTTAACAGGATTATTTG TACAAGATAATGTGAATAA AGATGCCGTCACAGATAGA TTGGCTTCAGTGGAGACTG ATATGCCTCTAACATTGAGA CAGCATAGAATAAGTGCGA CATCATCATCGGAAGAGAG TAGTAACAAAGGTCAAAGA CAGTTGACTGTATCGCCGG AATTCCCGGGGATCCGGCC TGAGTGCGTAGTACCCGAG ACTCAGTGCGCCATGAAGC GGAAAGAGAAGAAAGCAC AGAAGGAGAAGGACAAAC TGCCTGTCAGCACGACGAC GGTGGACGACCACATGCCG CCCATTATGCAGTGTGAACC TCCACCTCCTGAAGCAGCAA GGATTCACGAAGTGGTCCC AAGGTTTCTCTCCGACAAGC TGTTGGTGACAAACCGGCA GAAAAACATCCCCCAGTTG ACAGCCAACCAGCAGTTCCT TATCGCCAGGCTCATCTGGT ACCAGGACGGGTACGAGCA GCCTTCTGATGAAGATTTGA AGAGGATTACGCAGACGTG GCAGCAAGCGGACGATGAA AACGAAGAGTCGGACACTC CCTTCCGCCAGATCACAGA GATGACTATCCTCACGGTCC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				AACTTATCGTGGAGTTCGC GAAGGGATTGCCAGGGTTC GCCAAGATCTCGCAGCCTG ATCAAATTACGCTGCTTAAG GCTTGCTCAAGTGAGGTAA TGATGCTCCGAGTCGCGCG ACGATACGATGCGGCCTCA GACAGTATTCTGTTCGCGA ACAACCAAGCGTACACTCG CGACAACTACCGCAAGGCT GGCATGGCCGAGGTCATCG AGGATCTACTGCACTTCTGC CGGTGCATGTACTCTATGG CGTTGGACAACATCCATTAC GCGCTGCTCACGGCTGTCG TCATCTTTTCTGACCGGCCA GGGTTGGAGCAGCCGCAAC TGGTGGAAGAGATCCAGCG GTACTACCTGAATACGCTCC GCATCTATATCCTGAACCAG CTGAGCGGGTCGGCGCGTT CGTCCGTCATATACGGCAA GATCCTCTCAATCCTCTCTG AGCTACGCACGCTCGGCAT GCAAAACTCCAACATGTGC ATCTCCCTCAAGCTCAAGAA CAGAAAGCTGCCGCCTTTCC TCGAGGAGATCTGGGATGT GGCGGACATGTCGCACACC CAACCGCCGCCTATCCTCGA GTCCCCCACGAATCTCTAG
GAL4-Linker-EcR	646	MKLLSSIEQACDICRLKKLKCSKEKP KCAKCLKNNWECRYSPKTKRSPLTR AHLTEVESRLERLEQLFLLIFPREDLD MILKMDSLQDIKALLTGLFVQDNV NKDAVTDRLASVETDMPLTLRQHR ISATSSSEESSNKGQRQLTVSPEFPG RPECVVPETQCAMKRKEKKAQKEK DKLPVSTTTVDDHMPPIMQCEPPP PEAARIHEVVPRFLSDKLLVTNRQK NIPQLTANQQFLIARLIWYQDGYE QPSDEDLKRITQTWQQADDENEE	647	ATGAAGCTGCTGAGCAGCA TCGAGCAGGCTTGCGACAT CTGCAGGCTGAAGAAGCTG AAGTGCAGCAAGGAGAAG CCCAAGTGCGCCAAGTGCC TGAAGAACAACTGGGAGTG CAGATACAGCCCCAAGACC AAGAGGAGCCCCCTGACCA GGGCCCACCTGACCGAGGT GGAGAGCAGGCTGGAGAG GCTGGAGCAGCTGTTCCTG

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
		SDTPFRQITEMTILTVQLIVEFAKGL PGFAKISQPDQITLLKACSSEVMML RVARRYDAASDSILFANNQAYTRD NYRKAGMAEVIEDLLHFCRCMYS MALDNIHYALLTAVVIFSDRPGLEQ PQLVEEIQRYYLNTLRIYILNQLSGS ARSSVIYGKILSILSELRTLGMQNSN MCISLKLKNRKLPPFLEEIWDVAD MSHTQPPPILESPTNL		CTGATCTTCCCCAGGGAGG ACCTGGACATGATCCTGAA GATGGACAGCCTGCAAGAC ATCAAGGCCCTGCTGACCG GCCTGTTCGTGCAGGACAA CGTGAACAAGGACGCCGTG ACCGACAGGCTGGCCAGCG TGGAGACCGACATGCCCCT GACCCTGAGGCAGCACAGG ATCAGCGCCACCAGCAGCA GCGAGGAGAGCAGCAACA AGGGCCAGAGGCAGCTGAC CGTGAGCCCCGAGTTTCCC GGGCGGCCTGAGTGCGTAG TACCCGAGACTCAGTGCGC CATGAAGCGGAAAGAGAA GAAAGCACAGAAGGAGAA GGACAAACTGCCTGTCAGC ACGACGACGGTGGACGACC ACATGCCGCCCATTATGCAG TGTGAACCTCCACCTCCTGA AGCAGCAAGGATTCACGAA GTGGTCCCAAGGTTTCTCTC CGACAAGCTGTTGGTGACA AACCGGCAGAAAAACATCC CCCAGTTGACAGCCAACCA GCAGTTCCTTATCGCCAGGC TCATCTGGTACCAGGACGG GTACGAGCAGCCTTCTGAT GAAGATTTGAAGAGGATTA CGCAGACGTGGCAGCAAGC GGACGATGAAAACGAAGA GTCGGACACTCCCTTCCGCC AGATCACAGAGATGACTAT CCTCACGGTCCAACTTATCG TGGAGTTCGCGAAGGGATT GCCAGGGTTCGCCAAGATC TCGCAGCCTGATCAAATTAC GCTGCTTAAGGCTTGCTCAA GTGAGGTAATGATGCTCCG AGTCGCGCGACGATACGAT GCGGCCTCAGACAGTATTC

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				TGTTCGCGAACAACCAAGC GTACACTCGCGACAACTACC GCAAGGCTGGCATGGCCGA GGTCATCGAGGATCTACTG CACTTCTGCCGGTGCATGTA CTCTATGGCGTTGGACAAC ATCCATTACGCGCTGCTCAC GGCTGTCGTCATCTTTTCTG ACCGGCCAGGGTTGGAGCA GCCGCAACTGGTGGAAGAG ATCCAGCGGTACTACCTGA ATACGCTCCGCATCTATATC CTGAACCAGCTGAGCGGGT CGGCGCGTTCGTCCGTCAT ATACGGCAAGATCCTCTCAA TCCTCTCTGAGCTACGCACG CTCGGCATGCAAAACTCCA ACATGTGCATCTCCCTCAAG CTCAAGAACAGAAAGCTGC CGCCTTTCCTCGAGGAGATC TGGGATGTGGCGGACATGT CGCACACCCAACCGCCGCCT ATCCTCGAGTCCCCCACGAA TCTCTAG
EMCV IRES			702	CCCCCTCTCCCTCCCCCCCCC CTAACGTTACTGGCCGAAG CCGCTTGGAATAAGGCCGG TGTGCGTTTGTCTATATGTT ATTTTCCACCATATTGCCGT CTTTTGGCAATGTGAGGGC CCGGAAACCTGGCCCTGTC TTCTTGACGAGCATTCCTAG GGGTCTTTCCCCTCTCGCCA AAGGAATGCAAGGTCTGTT GAATGTCGTGAAGGAAGCA GTTCCTCTGGAAGCTTCTTG AAGACAAACAACGTCTGTA GCGACCCTTTGCAGGCAGC GGAACCCCCCACCTGGCGA CAGGTGCCTCTGCGGCCAA AAGCCACGTGTATAAGATA CACCTGCAAAGGCGGCACA

Name	SEQ ID NO	Amino Acid Sequence	SEQ ID NO	Nucleotide Sequence
				ACCCCAGTGCCACGTTGTG AGTTGGATAGTTGTGGAAA GAGTCAAATGGCTCTCCTCA AGCGTATTCAACAAGGGGC TGAAGGATGCCCAGAAGGT ACCCCATTGTATGGGATCTG ATCTGGGGCCTCGGTGCAC ATGCTTTACATGTGTTTAGT CGAGGTTAAAAAACGTCTA GGCCCCCCGAACCACGGGG ACGTGGTTTTCCTTTGAAAA ACACGATC
2xRbm3 IRES			703	ACTAGTTTTATAATTTCTTCT TCCAGAATTTCTGACATTTT ATAATTTCTTCTTCCAGAAG ACTCACAACCTC

CLAIMS

1. A non-naturally occurring polynucleotide encoding: (a) a miRNA that inhibits the expression of an immune checkpoint protein; and (b) a chimeric receptor.

2. The polynucleotide of claim 1, wherein the miRNA inhibits the expression of PD-1.

3. The polynucleotide of claim 2, comprising a nucleic acid sequence having at least 80% sequence identity with SEQ ID NO: 267 or that is capable of hybridizing under stringent hybridization conditions to the complement of SEQ ID NO: 267.

4. The polynucleotide of claim 1, wherein the chimeric receptor is a T-cell receptor.

5. The polynucleotide of claim 1, wherein the chimeric receptor is a chimeric antigen receptor.

6. The polynucleotide of claim 5, wherein the chimeric antigen receptor comprises an antigen-binding domain that binds to an epitope on ROR1.

7. The polynucleotide of claim 6, wherein the chimeric antigen receptor comprises the amino acid sequence of SEQ ID NO: 465, or a functional fragment or variant thereof.

8. The polynucleotide of claim 5, wherein the chimeric antigen receptor comprises a spacer comprising a stalk region comprising the amino acid sequence of SEQ ID NO: 467, or a functional fragment or variant thereof, and a stalk extension region comprising the amino acid sequence of SEQ ID NO: 473, or a functional fragment or variant thereof.

9. The polynucleotide of claim 1, further encoding a cytokine.

10. The polynucleotide of claim 9, wherein the cytokine is IL-15, or functional fragment or variant thereof, and is membrane bound.

11. The polynucleotide of claim 10, encoding a fusion protein comprising IL-15, or a functional fragment or variant thereof, and IL-15Rα, or a functional fragment or variant thereof.

12. The polynucleotide of claim 11, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 523 or a functional fragment or variant thereof.

13. The polynucleotide of claim 1, further encoding a cell tag.

14. The polynucleotide of claim 13, wherein the truncated HER1 comprises a HER1 Domain III, or a functional fragment or variant thereof, and a truncated HER1 Domain IV, or a functional fragment or variant thereof.

15. The polynucleotide of claim 14, wherein the cell tag further comprises a CD28 transmembrane domain or a functional fragment or variant thereof.

16. A vector comprising the polynucleotide of any one of claims 1–15.

17. The vector of claim 16, comprising a *Sleeping Beauty* transposon.

18. A modified immune effector cell comprising the polynucleotide of any one of claims 1–15.

19. A composition comprising the polynucleotide of any one of claims 1–15 or the cell of claim 18.

20. A kit comprising the polynucleotide of any one of claims 1–15 or the cell of claim 18.

21. A method of treating a subject suffering from a disease or disorder, comprising administering the cell of claim 18 to a subject in need thereof.

22. The use of a modified immune effector cell of claim 18 in the manufacture of a medicament for the treatment of a disease or disorder.

23. A method for the detection of a disease or disorder associated with the overexpression of an antigen in a subject, the method comprising: a) contacting a sample from the subject with one or more of the antibodies, or antigen-binding fragments thereof; and b) detecting an increased level of binding of the antibody or fragment thereof to the sample as compared to such binding to a control sample lacking the disease, thereby detecting the disease in the subject.

24. A method for the treatment of a disease or disorder comprising the serial administration of polynucleotides encoding a chimeric antigen receptor or a cell comprising the same, wherein the encoded chimeric antigen receptors are selected from a collection of chimeric antigen receptors having different structural compositions and binding specificities for an array of antigen targets.

25. The method of claim 23, comprising a first administration of cells expressing one or more chimeric antigen receptors from the collection followed by a second administration of cells expressing one or more chimeric antigen receptors from the collection, wherein a period of time elapses between the first and second administrations.