

5 This application claims priority to and the benefit of U.S. Provisional Application No. 63/163,171, filed on March 19, 2021 and U.S. Provisional Application No. 63/133,494, filed on January 4, 2021, the contents of each of which are hereby incorporated by reference in their entirety.

While adoptive cell therapies show efficacy in cancer treatment, the effectiveness of these therapies can be further improved through genetic engineering of T cells for better expansion and persistence. T cells require functionally non-overlapping co-stimulatory signals from CD28 family and tumor necrosis factor receptor (TNFR) family along with antigen triggered TCR signaling to promote full-fledged activation and persistent proliferation. In developing gene-engineered T cell therapeutics, there is a need to introduce chimeric T cell co-stimulatory molecules that can be locally activated upon T cell engaging with pathological antigens to potentially augment T cell activation for increased therapeutic efficacy. Currently, the second generation of chimeric co-stimulatory molecules incorporating one co-stimulatory signaling domain from proteins of either CD28 family or TNFR family has been widely adopted in CAR T cell therapy. Chimeric antigen receptors (CARs) integrating one co-stimulatory signaling domain augment T cell function through both activating and co-stimulatory signals, thus resulting in enhanced anti-tumor potency and T cell persistence. Given that the capacity for T cell to expand depends on the structural design, the current second generation co-stimulatory proteins may not be optimal for induction of a durable tumor remissions. Thus, there is a desired effort to develop third-generation chimeric molecules combining two co-stimulatory signaling domains from CD28 family and TNFR family members to further enhance T cell therapeutic potential, capitalizing on non-overlapping functions of the two families of co-stimulatory molecules. In addition, such third-generation chimeric co-stimulatory molecules can be integrated into TCR T therapy where T cell activation remains suboptimal due to insufficient co-stimulatory signals during activation of exogenously expressed TCRs by antigens. However, existing recombinant DNA strategies often suffer from reduced cell surface expression of the chimeric

proteins combining two co-stimulatory signaling domains, preventing realization of the functional potential of the chimeric proteins. The present application addresses such needs.

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

SUMMARY

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

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

The chimeric co-stimulatory intracellular domains provided herein comprise: (a) a
20 first signaling domain that is based on the intracellular signaling domain of a CD28 protein, ICOS protein or a combination thereof; and (b) at least a second signaling domain that comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain. In some embodiments, the mutant CD137 (4-1BB) intracellular domain or the mutant CD134 (OX-40) intracellular domain comprises a deletion, an insertion or a
25 substitution of one or more amino acids in the membrane proximal portion of the CD137 or CD134 intracellular domain. Without being bound by theory, in some embodiments, the one or more amino acids in the membrane proximal portion can be ubiquitination sites involved in the ubiquitination and degradation of the CD137 or CD134 protein.

Also disclosed herein is a functionally optimized intracellular co-stimulatory domain
30 for use in novel adoptive cell therapy, optionally in combination with cell-intrinsic immune checkpoint inhibitory receptors or immune-stimulatory receptors or portions thereof, developed to treat human diseases and disorders, including hematological and solid tumors. Also disclosed herein is a functionally optimized intracellular co-stimulatory domain for use

in combination with a T cell receptor (TCR), e.g. an endogenous TCR or an affinity enhanced TCR targeting a tumor-associated antigen. Optionally, the intracellular co-stimulatory domain is used in combination with a second component (e.g., a cell surface receptor or portion thereof) that directs migration of an immune cell to bind to a target tissue or cell or induces activation and/or proliferation of an immune cell, such as a PD-1 switch receptor (PD-1 based co-stimulatory molecule), that can increase T cell functionality in tumors, such as a PD-L1/PD-L2-expressing tumor. Also disclosed herein is a therapy that utilizes the PD-1 checkpoint blockade in a cell-intrinsic fashion, which simultaneously minimizes autoimmune side effects and provides increased on-tumor functionality. The present application discloses recombinant T cell co-stimulatory receptors (RTCRs) based on T cell co-receptors or chimeric antigen receptors (CARs) comprising a functionally optimized intracellular co-stimulatory domain of the present application. The present application also discloses T cell co-receptors comprising a functionally optimized intracellular co-stimulatory domain and a PD-1 extracellular domain (i.e., PD-1 switch receptors or PD-1 based co-stimulatory molecules). The present application also discloses CD19 and B cell maturation Ag (BCMA) based CARs comprising a functionally optimized intracellular co-stimulatory domain that promotes CD19 and BCMA binding mediated T cell activation, proliferation, and tumor killing. The RTCRs disclosed in the present application can be used for evaluation of checkpoint targets, safety screening, and for development of pre-clinical animal models to evaluate the effectiveness of the combination of the functionally optimized intracellular co-stimulatory domain of the present application with any TCRs or CARs. Additional cell-intrinsic immune checkpoint inhibitors with the efficacious TCRs are also developed.

The present disclosure provides a recombinant T cell co-stimulatory receptor (RTCR), comprising: (a) an extracellular domain; (b) a transmembrane domain; and (c) a chimeric intracellular domain comprising a first and at least a second signal transduction domains, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least second signal transduction domain comprises a mutant intracellular signaling domain of a tumor necrosis factor receptor (TNFR) family protein.

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

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

- 5 The present disclosure also provides a modified T lymphocyte (T cell), comprising: (a) a modification of an endogenous sequence encoding a T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR; and (b) a recombinant T cell co-stimulatory receptor (RTCR) disclosed herein.

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

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

- 20 The present disclosure provides a method of producing a plurality of modified T cells, wherein the method comprises: a) providing a plurality of primary T cells disclosed herein; b) providing a composition comprising the RTCR disclosed herein, the nucleic acid encoding the RTCR disclosed herein, or the vector comprising the nucleic acid encoding the RTCR disclosed herein; and c) introducing into the plurality of primary T cells of (a) the
25 composition of (b), to produce a plurality of modified T cells under conditions that stably express the RTCR within the plurality of modified T cells. In some embodiments, the method of producing a plurality of modified T cells disclosed herein, further comprises a step of modifying an endogenous sequence encoding an endogenous T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the endogenous
30 TCR. In some embodiments, the method of producing a plurality of modified T cells disclosed herein, further comprises a step of modifying an endogenous sequence, wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I). In some embodiments, the method of producing a plurality of modified T cells disclosed herein, further comprises: d) maintaining

the plurality of modified T cells in a suitable cell culture media; and e) either: i) cryopreserving the plurality of modified T cells in a suitable cell freezing media; or ii) preparing the plurality of modified T cells for administering to a subject suffering from a disease or disorder.

5 The present disclosure also provides a method of treating a disease or disorder, comprising administering to a subject in need thereof a therapeutically effective number of the cell comprising the nucleic acid encoding or the vector comprising the nucleic acid encoding the RTCR disclosed herein, a therapeutically effective number of any one of the modified T cell disclosed herein, a therapeutically effective amount of any one of the
10 compositions disclosed herein, or a therapeutically effective number of the plurality of modified T cells produced by the method disclosed herein.

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

 The present disclosure also provides a chimeric co-stimulatory intracellular protein (CIP) comprising a first and at least a second signal transduction domains, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least
20 second signal transduction domain comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain.

 Throughout the specification the term “comprising,” or variations such as “comprises” or “comprise,” will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other
25 element, integer or step, or group of elements, integers or steps.

 Throughout the specification the term “signal domain”, “signaling domain”, and “signal transduction domain”, are used interchangeably, unless the context dictates otherwise.

 While the disclosure has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the
30 disclosure, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

 The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published

foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

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DESCRIPTION OF THE FIGURES

FIG. 1 depicts alignment of the intracellular tails of TNF receptor superfamily members that are used in the T cell co-stimulatory molecules of the present application. Membrane-proximal poly-basic regions are italicized. Potential PI3K binding sites are bold and underlined. TRAF1/2 binding motifs: major motif Px(Q/E)E and minor motif Px(Q/E)x,
10 are underlined. Potential ubiquitination sites are in bold.

FIGs. 2A-2B depict the modular design of 2nd and 3rd generation co-stimulatory molecules. FIG. 2A depicts modular design of co-stimulatory molecules denoting the signal peptide, extracellular domain, transmembrane domain, and intracellular signaling domain.
15 FIG. 2B depicts structures and sequences of first signal transduction domains: ICOS, CD28 and ICOS intracellular domain with a portion of CD28 domain inserted. Regions and known binding partners of the ICOS and CD28 intracellular domain with specific binding function are indicated. The amino acid/nucleic acid sequences of the co-stimulatory molecules and the intracellular domains are as indicated.

20 FIG. 3 depicts the combinations of extracellular effector domains and intracellular signaling domains of the present application.

FIGs. 4A-4B depict that deletion of the N-terminal section of the 4-1BB signaling domain, including the polybasic domain and lysine residues, rescues the expression of the co-stimulatory molecules. FIG. 4A depicts expression of human PD1 and huEGFRt on the
25 surface of T-lymphocytes following lentiviral transduction with the indicated construct. FIG. 4B depicts the normalized PD1 surface expression on huEGFRt-expressing cells expressing different co-stimulatory molecules with ICOS or CD28 based chimeric intracellular domains comprising wild type or truncated 4-1BB domains or OX-40 domains, as indicated. The amino acid/nucleic acid sequences of the chimeric intracellular domains are as indicated.

30 FIGs. 5A-5D depict cytokine production and proliferation of T cells expressing different co-stimulatory molecules with ICOS based chimeric intracellular domains comprising wild type or truncated 4-1BB or OX-40 domains. FIGs. 5A-5C depict that antibody-mediated crosslinking of co-stimulatory molecules increases T cell cytokine

production *in-vitro*. IL-2 (FIG. 5 A), TNF (FIG. 5 B), and IFN γ (FIG. 5 C) were measured by bead-based multiplex assay in culture supernatants following 18hr stimulation of T cells transduced with the indicated constructs with plate-bound anti-PD1 [2 μ g/mL] and the indicated amount of plate-bound anti-CD3. The x-axis indicates the different co-stimulatory molecules and the y-axis indicates the amount of cytokine produced expressed as absorbance unit (A.U.). FIG. 5D depicts proliferation of T cells stimulated with the indicated plate-bound antibodies for 96hrs. The amino acid/nucleic acid sequences of the chimeric intracellular domains are as indicated.

FIGs. 6A-6C depict that PD-L1 engagement of co-stimulatory molecules increases T cell cytokine production *in-vitro*. FIG. 6A depicts IL-2 (upper panel), IFN γ (middle panel), and TNF (lower panel) measured by bead-based multiplex assay in culture supernatants following 18hr stimulation of T cells transduced with the indicated constructs. The x-axis indicates amount of anti-CD3 antibody (μ g/ml) and the y-axis indicates cytokine production as percentage of control. FIGs. 6B-6C depict IL-2 (upper panels), IFN γ (middle panel), and TNF (lower panel) production by T cells transduced with the indicated co-stimulatory molecules comprising CD28 intracellular domain (FIG. 6B, upper, middle and lower panels) and ICOS intracellular domain (FIG. 6C, upper, middle and lower panels), respectively, and stimulated with the indicated concentration of soluble anti-CD3 antibody in the presence of K562 cells expressing HLA-A2 (left panels) or HLA-A2 and PD-L1(right panels). The x-axis indicates amount of anti-CD3 antibody (μ g/ml) and the y-axis indicates cytokine production as percentage of control. The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

FIGs. 7A-7C depict that PD-L1 engagement of co-stimulatory molecules increases T cell cytotoxicity and proliferation *in-vitro*. T cells expressing PD-1 constructs, as indicated, and K562 cells were mixed and stimulated as in FIG. 6. FIGs. 7A and 7B depict the number of remaining K562 cells (upper panel) and number of T cells (lower panel) evaluated by flow cytometry, after 96 hours of stimulation with the indicated concentration of soluble anti-CD3 antibody in the presence of K562 cells expressing HLA-A2 (left panels) or HLA-A2 and PD-L1 (right panels). FIG. 7C depicts proliferation of T cells expressing the various PD1 constructs co-cultured with K562 cells expressing HLA-A2 (indicated by "X") or HLA-A2 and PD-L1 (indicated by "*") and 0.3 μ g/ml of anti-CD3, as measured by shift in Cell Trace violet dilution as indicated on x-axis. FIG. 7D is a graph depicting target cell (K562) numbers remaining evaluated by flow cytometry, after 96 hours post stimulation with T cells

expressing co-stimulatory molecules, in presence of increasing amounts anti-CD3 antibody ($\mu\text{g/ml}$), as indicated. FIG. 7E is a graph depicting number of T cells evaluated by flow cytometry, after 96 hours post stimulation with T cells expressing co-stimulatory molecules, in presence of increasing amounts anti-CD3 antibody ($\mu\text{g/ml}$), as indicated. The x-axis indicates amount of CD3 antibody ($\mu\text{g/ml}$) and the y-axis indicates number of cells. The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

FIG. 8 depicts that engagement of co-stimulatory molecules increases T cell proliferation *in-vitro*. T cells were stimulated for 96 hrs on plate-bound antibodies with 2 $\mu\text{g/mL}$ anti-PD1 and the concentration of anti-CD3 [mg/mL] (indicated by “*”) or only anti-CD3 (indicated by “X”), as indicated on y-axis, and proliferation of T cells expressing the various PD1 constructs as measured by shift in crystal violet tracing as indicated on x-axis. The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

FIGs. 9A-9C depict the effect of mutation of the polybasic and lysine residues on expression or function of co-stimulatory molecules incorporating ICOS and 4-1BB signaling domains. FIG. 9A is a series of flow cytometry plots depicting proliferation of T-cells expressing either a wild type PD1 receptor (indicated by “*”) or the different PD1 based co-stimulatory molecules (indicated by “X”), as indicated by labeling at top of each plot. T-cells expressing endogenous PD-1 were used as control (line with no indication). FIG. 9B is a graph depicting PD-1 expression (expressed as a fold increase from endogenous levels) from the FACS plots in FIG. 9A. FIG. 9C are graphs depicting cytokine production (IL-2, left panels; IFN γ , middle panels; and TNF, right panels) (y-axis) by T cells expressing different co-stimulatory molecules, as indicated, responding to K562 cells (top row) and K562-PDL1 expressing cells (middle row), when stimulated with the indicated concentration of anti-CD3 (x-axis). The difference between the level of cytokine production between T cells responding to K562 cells and K562-PDL1 expressing cells, is depicted in the graphs in the bottom row. FIG. 9D are graphs depicting proliferation of T cells 96hr post culturing with K562 cells (left graph) or K562 cells expressing PD-L1 (middle graph), when stimulated with the indicated concentration of anti-CD3 (x-axis). The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

FIGs. 10A-10D depicts the expression and function of co-stimulatory molecules incorporating ICOS and OX-40 signaling domains. FIG. 10A is a series of flow cytometry plots depicting proliferation of T-cells expressing either a wild type PD1 receptor (indicated by “*”) or the different PD1-switch receptors (PD1 based costimulatory molecules)

(indicated by "X"), as indicated by labeling at top of each plot. T-cells expressing endogenous PD-1 were used as control (no indication). FIG. 10B is a graph depicting PD-1 expression (fold of endogenous expression) from the FACS plots in FIG. 10A. FIG. 10C are graphs depicting cytokine production (IL-2, left panels; IFN γ , middle panels; and TNF, right panels) (y-axis) by T cells expressing different co-stimulatory molecules, as indicated, responding to K562 cells (top row) and K562-PDL1 expressing cells (middle row), when stimulated with the indicated concentration of anti-CD3 (x-axis). The difference between the level of cytokine production between T cells responding to K562 cells and K562-PDL1 expressing cells, is depicted in the graphs in the bottom row. FIG. 10D are graphs depicting T cell proliferation 96hr post culturing with K562 cells (left graph) or K562 cells expressing PD-L1 (middle graph), when stimulated with the indicated concentration of anti-CD3 (x-axis). The difference between T cell proliferation in the presence or absence of PD-L1 on the target cells is depicted in the right-most graph. The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

FIGs. 11A-11B depict that engagement of co-stimulatory molecules increases T cell conjugation with PD-L1 expressing cells. FIG. 11A depicts flow cytometry gating strategy of a 30-minute conjugation of CFSE-labelled T cells with CTV-labelled K562 targets. FIG. 11B depicts quantification of results from two experiments shown in FIG. 11A and normalized to control conjugations. The amino acid/nucleic acid sequences of the co-stimulatory molecules, with and without a signaling peptide, are as indicated.

FIGs. 12A-12C depict increase in T cell proliferation and function upon engagement of co-stimulatory molecules with PD-L1 expressing cells. FIG. 12A are flow cytometry plots depicting surface expression of PD-1 and TCR β chain, in T cells expressing either a wild type HLA-A2/NY-ESO-1 specific TCRs or mutant NY-ESO TCR as indicated, with (lower middle and right plots) and without (upper middle and right plots) a co-stimulatory molecule construct comprising an ICOS_4-1BB(truncated) signaling domain (PD-1_ICOS_BBt), as indicated, when co-cultured with K562 cells), 72hrs after lentiviral transfection. FIG. 12B are graphs depicting IL-2 (top graph) and IFN γ (bottom graph) production T cells expressing NY-ESO-1/PD1 based co-stimulatory molecule combinations, as indicated, when co-cultured with A375-tumor cells that express HLA-A2 and antigen, at T cell: A375 cell ratio as indicated in x-axis. FIG. 12C is a graph depicting dose dependent killing of A375 cells by T cells expressing the indicated wild type NY-ESO TCR or mutant, high affinity (HA) NY-ESO TCR, as indicated with/without a co-stimulatory molecules construct comprising an

ICOS_4-1BB(truncated) signaling domain (PD-1_ICOS_BBt), as indicated. The x-axis depicts the dose (T cell: A375 cell ratio) and percentage of total input A375 cells surviving. The amino acid/nucleic acid sequences of the co-stimulatory molecules, with and without a signaling peptide, are as indicated.

5 FIGs. 13A-13B depict that mutations of 3rd generation tails increase surface expression of CD-19 CAR receptors on transduced primary T cells. FIG. 13A depicts histograms of CD-19Fc binding to the untransduced T cells (marked by x) or T cells transduced with the indicated constructs (marked by *). FIG. 13B depicts MFI measurements of histograms shown in FIG. 13A, normalized to FMC63scFV_BB_Z. The amino
10 acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

 FIGs. 14A-14C depict that modified 3rd generation tails increase cytokine production and tumor killing. In-vitro killing of CD19-positive cells by CAR-transduced primary T cells is shown. FIG. 14A depicts residual cell number of B cell line (Nalm6 cells), after a 96-hr co-culture with CAR-T cells expressing CD28-based (left panel) and ICOS-based (right panel)
15 2nd generation and 3rd generation receptors. FIG. 14B depicts residual cell number of B cell line (Raji cells), after a 96-hr co-culture with CAR-T cells expressing CD28-based (left panel) and ICOS-based (right panel) 2nd generation and 3rd-generation receptors. The y-axis depicts number of remaining CD19-positive cells corresponding to the ratio of T cells to CD19-positive cell indicated on x-axis. FIG. 14C depicts 18hr-IFN γ production, as indicated
20 on the y-axis, by T cells expressing 2nd generation and 3rd generation, CD28-based receptors (left panel) and ICOS-based receptors (right panel), in response to incubation with CD19-positive B cells for 18 hours, at T cell: target cell ratio, as indicated on x-axis. The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

 FIG. 15A-15E depict modified 3rd generation signaling domains increase CD19-CAR
25 function in-vitro, compared to original 3rd generation signaling domains. FIG. 15A is a graph depicting cumulative T cell numbers (indicative of T cell proliferation) (y-axis) of T-cells expressing: a) CD28-based 2nd and 3rd generation receptors (left panel); and b) ICOS-based 2nd-and 3rd-generation receptors (right panel), as indicated, over repeated stimulations with Nalm6 B cells, as indicated on x-axis. FIG. 15B is a graph depicting cumulative T cell
30 numbers (indicative of T cell proliferation) (y-axis) of T-cells expressing: a) CD28-based 2nd and 3rd generation receptors (left panel); and b) ICOS-based 2nd and 3rd generation receptors (right panel), as indicated, over repeated stimulations with RAJI B cells, as indicated on x-axis. FIG. 15C is a graph depicting cumulative target cell (Nalm6) numbers (indicative of

target cell killing) (y-axis) of T-cells expressing: a) CD28-based 2nd and 3rd generation receptors (left panel); and b) ICOS-based 2nd and 3rd generation receptors (right panel), as indicated, over repeated stimulations with Nalm6 B cells, as indicated on x-axis. FIG. 15D is a graph depicting cumulative target cell (Raji) numbers (indicative of target cell killing) (y-axis) of T-cells expressing: a) CD28-based 2nd and 3rd generation receptors (left panel); and b) ICOS-based 2nd and 3rd generation receptors (right panel), as indicated, over repeated stimulations with Raji B cells, as indicated on x-axis. FIG. 15E is a series of flow cytometry plots depicting Tim3 and PD-1 expression on CAR-T cells, as indicated, at time zero or after 5 consecutive stimulations, with RAJI B cell targets. The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

FIGs. 16A-16E depicts modified 3rd-generation signaling domains increase BCMA-CAR function in-vitro, compared to original 3rd-generation sequences. FIG. 16A depicts flow cytometry histograms of BCMA-Fc binding to untransduced T cells (indicated by "X") or T cells transduced with the indicated BCMA CAR-T receptor comprising CD28-based and ICOS based- 2nd generation and 3rd generation co-stimulatory molecules (indicated by "*"), as indicated. FIG. 16 B is a graph depicting BCMA-Fc binding (MFI) (x-axis) by from the transduced T cells of the FACS plots in FIG. 16A. FIG. 16C is a set of graphs depicting cumulative T cell numbers (indicative of T cell proliferation) (y-axis) of T-cells expressing: a) CD28-based 2nd and 3rd generation receptors (left panel); and b) ICOS-based 2nd and 3rd generation receptors (right panel), as indicated, over repeated stimulations with RPMI-8226 multiple myeloma target cells. FIG. 16D is a graph depicting cumulative target cell (RPMI-8226 cells) numbers (indicative of target cell killing) (y-axis) of T-cells expressing: a) CD28-based 2nd and 3rd generation receptors (left panel); and b) ICOS-based 2nd and 3rd generation receptors (right panel), as indicated, over repeated stimulations with RPMI-8226 multiple myeloma target cells, as indicated on x-axis. FIG. 16E is a set of graphs depicting cytokine production (IL-2, left panel, TNF, middle panel and IFN γ , right panel) (y-axis) with CAR-T cells transduced with the indicated BCMA CAR constructs comprising CD28-based or ICOS-based co-stimulatory molecules, as indicated, incubated at the indicated effector to target ratio indicated on x-axis, for 18hrs. The amino acid/nucleic acid sequences of the co-stimulatory molecules are as indicated.

DETAILED DESCRIPTION

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

The CD28 family proteins have a single extracellular immunoglobulin variable-like (IgV) domain followed by a short cytoplasmic tail. Members of the CD28 family proteins include CD28, CD28H, inducible costimulator (ICOS), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4, CD152), program death-1 (PD-1), and B- and T-lymphocyte attenuator (BTLA). CD28, CD28H and ICOS are co-stimulatory proteins that are expressed on T cells that promote activation, high levels of cytokine/chemokine expression, resistance to apoptosis, and proliferation of T cells.

The Tumor Necrosis Factor Receptor (TNFR) family proteins includes TNFR1 (tumor necrosis factor receptor 1 / TNFRSF1A), TNFR2 (tumor necrosis factor receptor 2 / TNFRSF1B), lymphotoxin β receptor / TNFRSF3, OX40 / TNFRSF4, CD40 / TNFRSF5, Fas / TNFRSF6, decoy receptor 3 / TNFRSF6B, CD27 / TNFRSF7, CD30 / TNFRSF8, 4-1BB / TNFRSF9, DR4 (death receptor 4 / TNFRSF10A), DR5 (death receptor 5 / TNFRSF10B), decoy receptor 1 / TNFRSF10C, decoy receptor 2 / TNFRSF10D, RANK (receptor activator of NF-kappa B / TNFRSF11A), OPG (osteoprotegerin / TNFRSF11B), DR3 (death receptor 3 / TNFRSF25), TWEAK receptor / TNFRSF12A, TACI / TNFRSF13B, BAFF-R (BAFF receptor / TNFRSF13C), HVEM (herpes virus entry mediator / TNFRSF14), nerve growth factor receptor / TNFRSF16, BCMA (B cell maturation antigen / TNFRSF17, GITR (glucocorticoid-induced TNF receptor / TNFRSF18), TAJ (toxicity and JNK inducer / TNFRSF19), RELT / TNFRSF19L, DR6 (death receptor 6 / TNFRSF21), TNFRSF22, TNFRSF23, ectodysplasin A2 isoform receptor / TNFRSF27 and ectodysplasin 1-anhidrotic receptor. Interactions between tumor necrosis factor superfamily (TNFSF) ligands and TNF receptor superfamily (TNFRSF) receptors provide the co-stimulatory signals that control the survival, proliferation, differentiation, and effector function of immune cells. Depending upon the specific intracellular signal induced by TNFRSF members, they can be categorized into three groups – death domain (DD)-containing receptors, decoy receptors, and TNF receptor-associated factor (TRAF)-binding receptors. Some TNFRSFs such as TNFR-1, Fas, DR3, DR4, DR5, and DR6, contain their own DDs and/or interact with other

cytoplasmic DD-containing adaptor molecules. Some other TNFRSFs, such as TNFR-2, CD27, CD30, CD40, glucocorticoid-induced TNFR family-related gene (GITR), Fn1, lymphotoxin beta-receptor (LT β R), OX40, receptor activator of NF- κ B (RANK), and XEDAR, lack a DD and contain motifs with four to six amino acids called TRAF-interacting motifs (TIMs) which recruits TRAF proteins. TRAF proteins are adaptor molecules that activate multiple downstream signaling pathways such as NF- κ B, Janus kinase (JNK), ERK, p38MAPK, and PI3K that help in cell survival, proliferation, and cytokine production. In some embodiments, the first signaling domain that is based on the intracellular signaling domain of a CD28 family protein is selected from a CD28 protein, ICOS protein or a combination thereof. In some embodiments, the at least second signaling domain is based on a mutant of the intracellular signaling domain of a TNFR family protein is selected from CD137 (4-1BB) and CD134 (OX-40).

Provided herein are novel chimeric co-stimulatory intracellular domains based on the third-generation co-stimulatory domains of the present application. Reduced surface expression is a major hindrance in the development of chimeric co-stimulatory proteins for therapeutic purposes. The present disclosure provides novel chimeric co-stimulatory intracellular domains generated through mutations in the third-generation co-stimulatory domains of the present application that are both highly expressed and highly functional compared to the current second-generation and third-generation chimeric receptors that are effective in inducing costimulation. The chimeric co-stimulatory intracellular domains provided herein comprise: (a) a first signaling domain that is based on the intracellular signaling domain of a CD28 protein, ICOS protein or a combination thereof; and (b) at least a second signaling domain that is a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain. In some embodiments, the mutant CD137 (4-1BB) intracellular domain or the mutant CD134 (OX-40) intracellular domain comprises a deletion, an insertion or a substitution of one or more amino acids in the membrane proximal portion of the CD137 or CD134 intracellular domain. In some embodiments, the one or more amino acids in the membrane proximal portion are ubiquitination sites involved in the ubiquitination and degradation of the CD137 or CD134 protein. In some embodiments, the mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain comprises substitution or deletion of one or more lysine residues in the membrane proximal portion of the CD137 or CD134 intracellular domain. In some embodiments, the lysine residues are ubiquitination sites involved in the ubiquitination and degradation of the CD137 or CD134 protein. In some embodiments, the chimeric co-stimulatory intracellular domains provided

herein further comprise a third signaling domain. In some embodiments, the third signaling domain can be based on a CD3 ζ signaling domain.

In some embodiments, the novel co-stimulatory intracellular domain of the present application can be combined or fused in frame with the extracellular domain of any known co-stimulatory protein, a cell intrinsic immune checkpoint inhibitor, a chimeric antigen receptor, an antibody or a portion thereof, a ligand or a receptor thereof, a cytokine or a receptor thereof, a chemokine or a receptor thereof or a complement receptor, to form a functional recombinant T cell co-stimulatory receptor (RTCR). In some embodiments, the RTCR can be expressed in a cell in combination with another T cell receptor (TCR), chimeric antigen receptor or co-stimulatory protein. A RTCR comprising the novel co-stimulatory intracellular domain disclosed herein, when co-expressed with a TCR in a T cell, significantly increases the cell surface expression of the RTCR, and/or cell proliferation, activation, persistence, cytokine production and/or effector function of the T cell, as compared to a second-generation co-stimulatory receptor.

A highly efficacious adoptive cell therapeutic targeting a shared and safe tumor associated antigen and comprising a cell-intrinsic inhibitor of T cell exhaustion able to withstand the suppressive tumor microenvironment is described in the present application. An exemplary chimeric molecule expressing the extracellular domain of PD-1 and a functionally optimized chimeric intracellular co-stimulatory domain are disclosed herein. Modified T cells expressing the chimeric molecule of the present disclosure are generated to show the efficacy of the chimeric molecule in enhancing T cell stimulation, activation and proliferation. Both molecules are expressed on the same T cell, creating a TCR-T product that responds robustly to tumor cells expressing both the cognate MHC/peptide complex and high levels of PD-L1/PD-L2.

For exemplification, a cell-intrinsic inhibitor of T cell exhaustion is developed by co-expression of third generation chimeric PD-1 receptors combined with T cell receptors targeting tumor associated or specific antigens to enhance the efficacy of T cell mediated killing of tumor cells. The 3rd generation chimeric receptors disclosed herein can be used in combination with any endogenous or modified T cell receptors as well as with chimeric artificial receptors (CARs). The results herein show that the 3rd-generation co-stimulatory molecules disclosed herein produces T cells with high physiological avidity and persistent proliferative potential, while negating negative signaling by PD-1, delivering instead co-stimulatory signals in a PD-L1 rich environment. The novel co-stimulatory molecule can be

co-expressed with a tumor associated antigen (TAA) specific TCR and used to target PD-L1/PD-L2 and the TAA expressing tumors. This demonstrates that the synergistic effect between the TCR activation and co-stimulatory molecule significantly increases the therapeutic window and generate a potentially more effective candidate for clinical investigation. In the disclosure described below, the design of the third-generation chimeric proteins is systematically optimized, to further validate *in vitro* the improved anti-cancer effectiveness, and to investigate the *in vivo* anti-tumor efficacy.

Co-stimulatory molecules incorporating the extracellular domains of PD-1 with the intracellular domains of CD28, ICOS, CD134, and CD137 alone and in various combinations are generated. These sequences are optimized for surface expression and functionality by incorporating key mutations/deletions within the signaling domain of the chimeric receptors, focusing on the junction between CD28 and TNF-receptor family signaling domains. The functionality of these receptors is tested based on surface expression, *in-vitro* signaling, *in-vitro* T cell conjugation, cytokine production, proliferation, and cytotoxicity using a combination of soluble and plate-bound antibody stimulations and K562 target cells expressing PD-L1 or A375 tumor cells.

The disclosure herein provides an approach in which the TCR-T product co-expresses a chimeric co-stimulatory molecule alongside a recombinant TAA-specific TCR or an endogenous TCR. This approach allows for the targeting of the tumor associated antigen with simultaneous antagonization of checkpoint inhibition and delivery of co-stimulatory signals to the transfused T cell product. This approach not only results in a much-improved product, but also help to develop a universal function-boosting platform for additional TCR-T products.

The key technical challenge hindering the clinical adoption of 3rd-generation CARs combining the two domains has been the abnormally low expression of chimeric proteins at the cell surface and associated diminished functionality (Zhao, 2015, Guedan, 2018). This has held true in co-stimulatory molecules where the combination of the CD28 and CD137 signaling domains resulted in a poorly expressed and non-functional receptor (Ankri, 2013). Disclosed herein is a switch receptor/co-stimulatory molecule based on the ICOS/CD137 signaling domain and optimized for surface expression that is a significant improvement over past trials using the CD28 signaling domain alone, mediating both increased effector function and persistence of adoptively transferred cells. Results described herein show that the 3rd-generation co-stimulatory molecule disclosed herein produces T cells with high physiological avidity and persistent proliferation potential, while negating negative signaling by PD-1,

delivering instead a co-stimulatory signal in a PD-L1 rich environment. The novel switch receptor/co-stimulatory molecule disclosed herein can be co-expressed with an endogenous TCR or a TAA specific TCR and used to target PD-L1/PD-L2 expressing tumors. This demonstrates that the synergistic effect between the TCR activation and co-stimulatory molecule significantly increases the therapeutic window for a potentially more effective candidate for clinical investigation.

Tumor associated antigens and tumor specific antigens allow for the immunological targeting of the tumor with relatively minimal risk of off-tumor, on-target side effects. Tumor cells can upregulate these antigens which can then be targeted by the human immune response or ACT. The disclosure herein combines a co-stimulatory molecule based on 3rd-generation CARs that exhibits superior functionality to CD28-based receptors with a new affinity enhanced TCR targeting TAAs to generate a TCR-T product that resists the suppressive function of the TME.

The present disclosure provides a recombinant T cell co-stimulatory receptor (RTCR), comprising: (a) an extracellular domain; (b) a transmembrane domain; and (c) a chimeric intracellular domain comprising a first and at least a second signal transduction domains, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least second signal transduction domain comprises a mutant intracellular signaling domain of a tumor necrosis factor receptor (TNFR) family protein.

In some embodiments, the mutant intracellular signaling domain of a TNFR family protein is any one of a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain.

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

Unless indicated otherwise, the terms “co-stimulatory molecule”, “costimulatory molecule”, “co stimulatory molecule”, “co-stimulatory protein”, “costimulatory protein”, “co stimulatory protein”, “co-stimulatory receptor”, “costimulatory receptor” “co stimulatory receptor” and “switch receptor” are used interchangeably, to refer to the recombinant T cell co-stimulatory receptors (RTCRs) comprising the novel chimeric co-stimulatory intracellular domains of the present application. These terms may be used in combination with terms such

as “recombinant T cell”, “recombinant”, “chimeric T cell”, and “chimeric”, to refer to the RTCRs of the present application.

As described herein, “a recombinant T cell co-stimulatory receptor” or “switch receptor” of the present disclosure is a “costimulatory molecule” “co-stimulatory receptor” or
5 “co-stimulatory protein” generated by operably linking an extracellular domain to an intracellular chimeric intracellular protein of the present disclosure.

“CD137” as described herein is a member of the tumor necrosis factor (TNF) receptor family, and also referred to as 4-1BB, CD137, tumor necrosis factor receptor superfamily member 9 (TNFRSF9) and induced by lymphocyte activation (ILA). As described herein, the
10 terms “CD137”, “4-1BB”, “4-1BB wt”, “4-1BB wild type”, “BB”, “BB wt” and “BB wild type” are used interchangeably throughout, for example, when describing constructs or co-stimulatory molecules of the present application, unless otherwise indicated.

In some embodiments, the CD137 intracellular domain can be from a mammalian CD137. In some embodiments, the mammalian CD137 can be a human CD137, a mouse
15 CD137, a rat CD137 or a monkey CD137. In some embodiments, the CD137 intracellular domain can be from a human CD137, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the human CD137 amino acid sequence according to GenBank Accession Nos: U03397, AAA62478, NP_001552, Q07011, AAH06196 and XP_006710681. In some embodiments, the CD137 intracellular domain can be from a mouse
20 CD137, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the mouse CD137 amino acid sequence according to GenBank Accession Nos: NP_001070977.1, NP_001070976.1, NP_035742.1, NP_033430.1, P20334.1, XP_011248530.1, XP_011248530.1, ABI30213.1, BAE32724.1 and AAH28507.1. In some embodiments, the CD137 intracellular domain can be from a rat CD137, or an isoform or a
25 variant thereof, comprising an amino acid sequence identical to any one of the rat CD137 amino acid sequence according to GenBank Accession Nos: NP_852049.1, NP_001020944.1, BAD99404.1, XP_008762504.1, XP_006239534.1, EDL81196.1, AAH97483.1, EHB16663.1, EHB16663.1, KFO38282.1, XP_010618177.1, XP_029414155.1, XP_029414154.1, XP_021099219.1 and XP_012888584.1. In some
30 embodiments, the CD137 intracellular domain can be from a monkey CD137, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the monkey CD137 amino acid sequence according to GenBank Accession Nos: ABY47575.1, ABI30212.1, ABY47577.1, ABY47576.1 and ABY47578.1.

In some embodiments, the CD137 intracellular domain, as described herein, comprises an amino acid sequence starting from the amino acid position 214 to the last amino acid at the C-terminal end of the amino acid sequence of the human CD137 protein, described herein. In some embodiments, the CD137 intracellular domain, as described herein,
5 comprises an amino acid sequence starting from the amino acid position 215 to the last amino acid at the C-terminal end of the amino acid sequence of the mouse CD137 protein, described herein.

In some embodiments, the mutant CD137 intracellular domain described herein is from any one of the CD137 proteins as described herein, comprising one or more mutation(s),
10 wherein the mutation can be addition/insertion, deletion/truncation or substitution/replacement of one or more amino acids within the amino acid sequence of the CD137 protein. In some embodiments, the mutant CD137 intracellular domain described herein is any one of the CD137 intracellular domain sequences, as described herein, comprising one or more mutation(s), wherein the mutation can be addition/insertion,
15 deletion/truncation or substitution/replacement of one or more amino acids within the amino acid sequence of the CD137 intracellular domain. In some embodiments, the mutant CD137 intracellular domain described herein is a CD137 intracellular domain as described herein, comprising a deletion or substitution of one or more amino acids within the amino acid sequence of the CD137 intracellular domain that can be targets for ubiquitination. In some
20 embodiments, the mutant CD137 intracellular domain described herein is a CD137 protein as described herein, comprising a deletion or substitution, of one or more lysine residues within the amino acid sequence of the CD137 intracellular domain that can be targets for ubiquitination. In some embodiments, the mutant CD137 intracellular domain described herein is a CD137 protein as described herein, comprising a deletion or substitution, of one,
25 two, three or four lysine residues within the amino acid sequence of the CD137 intracellular domain that can be targets for ubiquitination. In some embodiments, the lysine residues within the amino acid sequence of the CD137 intracellular domain described herein, that can be deleted or substituted are at amino acid positions 214, 218, 219 and/or 225 of the CD137 intracellular domain.

30 In some embodiments, the mutant CD137 intracellular domain can be a truncated CD137 intracellular domain. A truncated CD137 intracellular domain as described herein can be any one of the CD137 proteins described herein, in which a continuous stretch of more than one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty-five, fifty, hundred, two hundred or more amino acids are deleted

from the N-terminus the CD137 protein as described herein. A truncated CD137 intracellular domain as described herein can be any one of the CD137 intracellular domain sequences described herein, in which a continuous stretch of more than one, two, three, four, five, six, seven, eight, nine, ten or more amino acids are deleted from the N-terminus the CD137 intracellular domain as described herein. In some embodiments, the amino acids deleted from the N-terminus the CD137 intracellular domain includes one or more proximal polybasic amino acids of the CD137 intracellular domain.

In some embodiments, the mutant CD137 intracellular domain can be a truncated CD137 intracellular domain. In some embodiments, the truncated CD137 intracellular domain comprises an amino acid sequence according to amino acid position 13 to amino acid position 42 of the CD137 intracellular domain, of the present disclosure. In some embodiments, the truncated CD137 intracellular domain comprises a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the truncated CD137 intracellular domain comprises a deletion of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the truncated CD137 intracellular domain comprises a deletion of amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the CD137 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 1.

In some embodiments, the truncated CD137 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 3. In some embodiments, the truncated CD137 intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 3.

A truncated CD137 intracellular domain as described herein, is referred to as “truncated CD137”, “CD137t”, “truncated 4-1BB”, “4-1BBt”, “truncated BB” or “BBt” interchangeably throughout, for example, when describing constructs or co-stimulatory molecules of the present application, unless otherwise indicated. In some embodiments, the mutant CD137 intracellular domain comprises a deletion of one, two, three or four lysine residue(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the mutant CD137 intracellular domain comprises one or more lysine mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of

the present disclosure. In some embodiments, the mutant CD137 intracellular domain comprises one or more lysine mutation(s) at amino acid positions selected from amino acid positions 1, 5, 6 and 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the one or more lysine mutation(s) are lysine to alanine mutations. In some embodiments, the CD137 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 1.

In some embodiments, the mutant CD137 intracellular domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, 3, 4, 5 and 6 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the mutant CD137 intracellular domain comprising one or more proximal basic amino acid mutation(s), of the present disclosure, further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the lysine mutation is a lysine to alanine mutation. In some embodiments, the CD137 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 1.

“CD134” as described herein is a member of the tumor necrosis factor (TNF) receptor family, and also referred to as OX-40, ACT35, IMD16, TXGP1L and tumor necrosis factor receptor superfamily member 4 (TNFRSF4). As described herein, the terms “CD134”, “OX-40”, “OX40”, “OX-40 wild type”, “OX-40 wt”, “OX40 wild type”, “OX40 wt”, “40”, “40 wild type” and “40wt” are used interchangeably throughout, for example, when describing constructs or co-stimulatory molecules of the present application, unless otherwise indicated.

In some embodiments, the CD134 intracellular domain can be from a mammalian CD134. In some embodiments, the mammalian CD134 can be a human CD134, a mouse CD134, a rat CD134 or a monkey CD134. In some embodiments, the CD134 intracellular domain can be from a human CD134, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the human CD134 amino acid sequence according to GenBank Accession Nos: NP_003318, AAI05071, AAI05073, XP_016857721.1, XP_016857720.1, XP_011540377.1, XP_011540379.1, XP_011540378.1, XP_011540376.1,

P43489.1, NP_001284491.1, NP_003317.1, EAW56278.1 and CAB96543.1. In some embodiments, the CD134 intracellular domain can be from a mouse CD134, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the mouse CD134 amino acid sequence according to GenBank Accession Nos: NP_035789.1, AAI39267.1, AAI39240.1, NP_033478.1, XP_006538787.3, P47741.1, EDL15067.1, CAA79772.1, CAA59476.1, XP_021017102.2, and XP_021056714.1. In some embodiments, the CD134 intracellular domain can be from a rat CD134, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the rat CD134 amino acid sequence according to GenBank Accession Nos: NP_035789.1, NP_037181.1, P15725.1, EDL81353.1, CAB96543.1, and CAA34897.1. In some embodiments, the CD134 intracellular domain can be from a monkey CD134, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the monkey CD134 amino acid sequence according to GenBank Accession Nos: XP_010375483.1, XP_001090870.1, XP_021523144.1, XP_017750744.1, XP_003939714.1, XP_026313229.1, XP_026313228.1, XP_003890998.2, XP_025242473.1, XP_011768627.1, XP_005545179.1, XP_011886513.1, XP_011886512.1, XP_011857387.1 and XP_011811769.1.

In some embodiments, the CD134 intracellular domain, as described herein, comprises an amino acid sequence starting from amino acid position 241 to the last amino acid at the C-terminal end of the amino acid sequence of any one of the human CD134 protein, described herein. In some embodiments, the CD134 intracellular domain, as described herein, comprises an amino acid sequence starting from the amino acid position 236 to the last amino acid at the C-terminal of the amino acid sequence of the mouse CD134 protein, described herein.

In some embodiments, the mutant CD134 intracellular domain described herein is from any one of the CD134 proteins as described herein, comprising one or more mutation(s), wherein the mutation can be addition/insertion, deletion/truncation or substitution/replacement of one or more amino acids within the amino acid sequence of the CD134 protein. In some embodiments, the mutant CD134 intracellular domain described herein, is any one of the CD134 intracellular domain sequences as described herein, comprising one or more mutation(s), wherein the mutation can be addition/insertion, deletion/truncation or substitution/replacement of one or more amino acids within the amino acid sequence of the CD134 intracellular domain. In some embodiments, the mutant CD134 intracellular domain described herein is a CD134 intracellular domain as described herein, comprising a deletion or substitution of one or more amino acids within the amino acid

sequence of the CD134 intracellular domain that can be targets for ubiquitination. In some embodiments, the mutant CD134 intracellular domain described herein is a CD134 protein as described herein, comprising a deletion or substitution, of one or more lysine residues within the amino acid sequence of the CD134 intracellular domain that can be targets for ubiquitination. In some embodiments, the mutant CD134 intracellular domain described herein is a CD134 protein as described herein, comprising a deletion or substitution, of one or two lysine residues within the amino acid sequence of the CD134 intracellular domain that can be targets for ubiquitination. In some embodiments, the lysine residues within the amino acid sequence of the CD134 intracellular domain described herein, that can be deleted or substituted are at amino acid positions 252 and/or 276 of the CD134 intracellular domain.

In some embodiments, the mutant CD134 intracellular domain can be a truncated CD134 intracellular domain. A truncated CD134 intracellular domain as described herein can be any one of the CD134 proteins described herein, in which a continuous stretch of more than one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty-five, fifty, hundred, two hundred or more amino acids are deleted from the N-terminus the CD137 protein as described herein. A truncated CD134 intracellular domain as described herein can be any one of the CD134 intracellular domain sequences described herein, in which a continuous stretch of more than one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids are deleted from the N-terminus the CD134 intracellular domain as described herein. In some embodiments, the amino acids deleted from the N-terminus the CD134 intracellular domain includes one or more proximal polybasic amino acids of the CD134 intracellular domain.

In some embodiments, the truncated CD134 intracellular domain comprises an amino acid sequence according to amino acid position 15 to amino acid position 37 of a CD134 intracellular domain, of the present disclosure. In some embodiments, the truncated CD134 intracellular domain comprises a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments, the truncated CD134 intracellular domain comprises a deletion of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments, the truncated CD137 intracellular domain comprises a deletion of amino acid position 1 to amino acid position 14 of the N-terminus of

the CD134 intracellular domain, of the present disclosure. In some embodiments, the CD134 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 4.

In some embodiments, the mutant CD134 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 6. In some embodiments, the mutant CD134 intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 6.

A truncated CD134 intracellular domain as described herein, is referred to as “truncated CD134”, “CD134t”, “truncated OX-40”, “truncated OX40”, “OX-40t”, “OX40t” and “40t” are used interchangeably throughout, for example, when describing constructs or co-stimulatory molecules of the present application, unless otherwise indicated.

In some embodiments, the mutant CD134 intracellular domain comprises a deletion of a lysine residue from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments, the mutant CD134 intracellular domain comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments, the lysine mutation is a lysine to alanine mutation. In some embodiments, the CD134 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 4.

In some embodiments, the mutant CD134 intracellular domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments, the mutant CD134 intracellular domain comprises one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments, the mutant CD134 intracellular domain comprises one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, and 5 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments, the mutant CD137 intracellular domain further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments, the CD134 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 4.

Table 1. Amino acid sequences of second signal transduction domains of RTCR (CD137/4-1BB and CD134/OX-40 intracellular signaling domain).

Human CD137/4-1BB (SEQ ID NO: 1)	<i>KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF</i> <u>PEEE</u> EGGCCE L
Mouse CD137/4-1BB (SEQ ID NO: 2)	<i>RKKFPHIFKQPFKKTGAAQEEDACSCRC</i> <u>PQEEE</u> EGGGGGY EL
truncated/mutated CD137/4-1BB (SEQ ID NO: 3)	<i>QPFMRPVQTTQEEDGCSCRF</i> <u>PEEE</u> EGGCCEL
Human CD134/OX-40 (SEQ ID NO: 4)	<i>RRDQRL</i> <u>PPDAH</u> <u>KPP</u> GGGSFRT <u>PIQEE</u> QADAHSTLAKI
Mouse CD134 (SEQ ID NO: 5)	<i>RKAWRL</i> <u>PNTPK</u> PCWGNFRT <u>PIQEE</u> HTDAHFTLAKI
truncated/mutated CD134/OX-40 (SEQ ID NO: 6)	<i>GGGSFRT</i> <u>PIQEE</u> QADAHSTLA
Human CD27 (SEQ ID NO: 7)	<i>QRRKYRSNKGES</i> <u>PVEPA</u> EPCCHYSC <u>REEE</u> GST <u>PIQED</u> YRKPE EPACSP
Human GITR (SEQ ID NO: 8)	<i>QLGLHIWQLRSQCMWP</i> <u>RET</u> QLLLEVPPSTEDARSCQF <u>PEE</u> <u>ERGERSAEEK</u> GRLGLDWV

Membrane-proximal poly-basic regions are italicized. Potential PI3K binding sites are bold and underlined. TRAF1/2 binding motifs, major motif P $\times$ (Q/E)E and minor motifs P $\times$ (Q/E) $\times$, are highlighted in underlined. Potential ubiquitination sites are in bold.

In some embodiments, the chimeric intracellular domain comprises a first signal transduction domain derived from a protein of the CD28 family. In some embodiments, the first signal transduction domain derived from any one of CD28, CD28H, ICOS or a combination thereof.

In some embodiments, the chimeric intracellular domain comprises a first signal transduction domain derived from ICOS protein.

10 The “ICOS protein” as described herein is an inducible T cell co-stimulatory protein, also referred to as AILIM, CD278, CCLP, CRP-1, H4, Ly115 and CVID1. In some embodiments, the ICOS intracellular domain can be from a mammalian ICOS. In some embodiments, the mammalian ICOS can be a human ICOS, a mouse ICOS, a rat ICOS or a monkey ICOS. In some embodiments, the ICOS intracellular domain can be from a human

ICOS, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the human ICOS amino acid sequence according to GenBank Accession Nos: AAH28006.1, NP_036224.1, AIC51287.1, AIC60036.1, NP_036224.1, Q9Y6W8.1, EAW70357.1, EAW70356.1, EAW70355.1, AAL40934.1, AAL40933.1, CAC06612.1, AAX93073.1, AAM00909.1, AAH28210.1 and CAD59742.1. In some embodiments, the ICOS intracellular domain can be from a mouse ICOS, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the mouse ICOS amino acid sequence according to GenBank Accession Nos: NP_059508.2, Q9WVS0.2, EDL00161.1, CAM13242.1, CAM13241.1, CAB71153.1, AAG48732.1, AAH34852.1, XP_006496203.1, XP_006496202.1, XP_006496201.1, ACX50464.1, ACX50463.1, AAH28006.1, XP_021052880.1, XP_029334968.1 and XP_021030282.1. In some embodiments, the ICOS intracellular domain can be from a rat ICOS, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the rat ICOS amino acid sequence according to GenBank Accession Nos: NP_072132.1, Q9R1T7.1, XP_008765358.1, XP_006245100.1, XP_006245099.1, EDL98922.1, EDL98921.1, XP_038940099.1, XP_032755449.1, XP_017457364.1, XP_006256324.1, XP_006256323.1, XP_006256322.1, XP_029425757.1, XP_029425757.1, XP_021119236.1, XP_012929934.1, XP_012867370.1 and XP_012867363.1. In some embodiments, the ICOS intracellular domain can be from a monkey ICOS, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the monkey ICOS amino acid sequence according to GenBank Accession Nos: XP_007964137.1, NP_001253918.1, XP_010350939.1, XP_012301785.1, XP_012301784.1, XP_017739861.1, XP_010334714.1, XP_003925677.1, AFH29328.1, XP_008997520.1, XP_023075107.1, XP_023075099.1, XP_021779593.1, XP_003907887.1, XP_025260988.1, XP_025260987.1, XP_025260986.1, XP_011716287.1, XP_011716285.1, XP_005574075.1, XP_011903009.1, XP_011805288.1, XP_011805287.1, XP_011847867.1, XP_011847866.1, XP_017392362.1, XP_033086489.1, XP_032134414.1, XP_032134413.1, and XP_017802331.1.

In some embodiments, the human ICOS intracellular domain as described herein, comprises an amino acid sequence from amino acid position 133 to the last amino acid at the C-terminus of the amino acid sequence of the human ICOS protein, described herein. In some embodiments, the human ICOS intracellular domain as described herein, comprises an amino acid sequence from an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids N-terminus to the amino acid position 133, to the last amino acid at the C-terminus of the amino acid sequence of the human ICOS protein, described

herein. In some embodiments, the human ICOS intracellular domain as described herein, comprises an amino acid sequence from amino acid position 133 to an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids N-terminus to the last amino acid at the C-terminus of the amino acid sequence of the human ICOS protein, described herein. In some embodiments, the human ICOS intracellular domain as described herein, comprises an amino acid sequence from an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids N-terminus to the amino acid position 133, to an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids N-terminus to the last amino acid at the C-terminus of the amino acid sequence of the human ICOS protein, described herein.

In some embodiments, the human ICOS(28) intracellular domain as described herein, comprises a portion of the ICOS domain amino acid sequence from amino acid position 133 to amino acid position 183, and a portion of the ICOS domain amino acid sequence from amino acid position 184 to the last amino acid at the C-terminus of the amino acid sequence of the human ICOS protein, described herein. In some embodiments, the human ICOS(28) intracellular domain as described herein, comprises a portion of the ICOS domain amino acid sequence from an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids N-terminus to the amino acid position 133, to amino acid position 183 of the human ICOS protein, described herein. In some embodiments, the human ICOS(28) intracellular domain as described herein, comprises a portion of the ICOS domain amino acid sequence from amino acid position 133, to an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids C-terminus to the amino acid position 183 of the human ICOS protein, described herein. In some embodiments, the human ICOS(28) intracellular domain as described herein, comprises a portion of the ICOS domain amino acid sequence from an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids N-terminus to the amino acid position 133, to an amino acid position at one, two, three, four, five, six, seven, eight, nine, ten or more amino acids C-terminus to the amino acid position 183 of the human ICOS protein, described herein.

The “CD28 protein”, also referred to as Tp44, is a constitutively expressed receptor for CD80 (B7.1) and CD86 (B7.2) proteins on naïve T cells and is important for T cell activation. In some embodiments, the CD28 intracellular domain can be from a mammalian CD28. In some embodiments, the mammalian CD28 can be a human CD28, a mouse CD28, a rat CD28 or a monkey CD28. In some embodiments, the CD28 intracellular domain can be from a human CD28, or an isoform or a variant thereof, comprising an amino acid sequence

identical to any one of the human CD28 amino acid sequence according to GenBank Accession Nos: P10747.1, NP_001230007.1, NP_001230006.1, NP_006130.1, EAW70350.1, EAW70349.1, EAW70348.1, EAW70347.1, AIC48451.1, CAC29237.1, AAA51945.1, AAA51944.1, AAL40931.1, AAF33794.1, AAF33793.1, AAF33792.1, 5 XP_011510499.1, XP_011510497.1, XP_011510496.1, AAI12086.1, AAH93698.1, ABK41938.1, AAY24123.1, CAD57003.1 and AAA60581. In some embodiments, the CD28 intracellular domain can be from a mouse CD28, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the mouse CD28 amino acid sequence according to GenBank Accession Nos: AAA37396.1, NP_031668.3, P31041.2, 10 AAH64058.1, EDL00156.1, CAM13249.1, XP_036012281.1, XP_021054806.1, XP_021027481.1, XP_036015651.1, and XP_030104805. In some embodiments, the CD28 intracellular domain can be from a rat CD28, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the rat CD28 amino acid sequence according to GenBank Accession Nos: CAA39003.1, NP_037253.2, P31042.1, XP_008765300.1, 15 EDL98926.1, XP_032755445.1, XP_034354910.1, XP_019061859.2, XP_008844474.1, XP_004851403.1 and XP_012865504.1. In some embodiments, the CD28 intracellular domain can be from a monkey CD28, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the monkey CD28 amino acid sequence according to GenBank Accession Nos: ABH06891.1, ABH08508.1, ABH06892.1, ABH08509.1, 20 ABQ09493.1, NP_001274262.1, NP_001036106.2, ABG77998.1, ABG77997.1 and XP_014966207.1.

The “CD28H protein”, also referred to CD28 homolog, transmembrane and immunoglobulin domain-containing protein 2, has co-stimulatory activity in T cells by binding to B7H7. CD28H was initially described as a molecule involved in cell-cell 25 interaction, cell migration, and angiogenesis of epithelial and endothelial cells (7, 8). CD28H has a single extracellular immunoglobulin domain followed by a transmembrane domain and a 110 amino acid-long cytoplasmic region. In some embodiments, the CD28 intracellular domain can be from a mammalian CD28H. In some embodiments, the mammalian CD28 can be a human CD28H, a mouse CD28H, a rat CD28H or a monkey CD28H. In some 30 embodiments, the CD28H intracellular domain can be from a human CD28H, or an isoform or a variant thereof, comprising an amino acid sequence identical to any one of the human CD28H amino acid sequence according to GenBank Accession Nos: NP_001295161.1, NP_001162597.1, Q96BF3.2, XP_024307127.1 and XP_016881773.1.

In some embodiments, the human CD28 intracellular domain as described herein, comprises an amino acid sequence from amino acid position 145 to the last amino acid at the C-terminus of the amino acid sequence of the human CD28 protein, described herein. In some embodiments, a portion of the human CD28 intracellular domain as described herein, can comprise an amino acid sequence from about amino acid position 195 to about amino acid position 212 of the amino acid sequence of the human CD28 protein, described herein. In some embodiments, a portion of the human CD28 intracellular domain as described herein, can comprises an amino acid sequence from one, two, three, four, five, six, seven, eight, nine or 10 or more amino acid amino acid position N-terminus to amino acid position 195 to one, two, three, four, five, six, seven, eight, nine or 10 or more amino acid amino acid position C-terminus amino acid position 220 of the amino acid sequence of the human CD28 protein, described herein.

In some embodiments, the first signal transduction domain derived from ICOS comprises an amino acid sequence according to SEQ ID NO: 9. In some embodiments, the first signal transduction domain derived from ICOS comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 9.

In some embodiments, the chimeric intracellular domain comprises a first signal transduction domain comprising a portion of a CD28 intracellular domain combined with an ICOS protein (ICOS (28) domain) according to SEQ ID NO: 9. In some embodiments, the ICOS (28) domain comprises the portion of CD28 intracellular domain inserted N-terminal to the PI-3K binding site of the ICOS protein according to SEQ ID NO: 9. In some embodiments, the ICOS (28) domain comprises the portion of CD28 inserted at 1, 2, 3, 4 or 5 amino acid position N-terminal to the PI-3K binding site of the ICOS protein according to SEQ ID NO: 9. In some embodiments, the ICOS(28) domain comprises the portion of CD28 inserted C-terminal to the PI-3K binding site of the ICOS protein according to SEQ ID NO: 9. In some embodiments, the ICOS(28) domain comprises the portion of CD28 inserted at 1, 2, 3, 4 or 5 amino acid position C-terminal to the PI-3K binding site of the ICOS protein according to SEQ ID NO: 9.

In some embodiments, the portion of CD28 is inserted at any amino acid position before amino acid position 48 within an ICOS protein of amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted at any amino acid position between amino acid position 1 and amino acid position 48, within an ICOS protein of amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of

CD28 is inserted between the amino acid position 47 and amino acid position 48 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted between the amino acid position 46 and amino acid position 47 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9. In some
5 embodiments, the portion of CD28 is inserted between the amino acid position 45 and amino acid position 46 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted between the amino acid position 44 and amino acid position 45 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted between the amino
10 acid position 43 and amino acid position 44 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9.

 In some embodiments, the portion of CD28 is inserted at any position after amino acid position 51 within an ICOS protein of amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted at any amino acid position between
15 amino acid position 51 and amino acid position 67, within an ICOS protein of amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted between the amino acid position 51 and amino acid position 52 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted between the amino acid position 53 and amino acid position 54 of an ICOS
20 protein with the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted between the amino acid position 54 and amino acid position 55 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9. In some embodiments, the portion of CD28 is inserted between the amino acid position 56 and amino acid position 57 of an ICOS protein with the amino acid sequence according to SEQ ID NO:
25 9. In some embodiments, the portion of CD28 is inserted between the amino acid position 57 and amino acid position 58 of an ICOS protein with the amino acid sequence according to SEQ ID NO: 9.

 In some embodiments, the portion of CD28 of the ICOS(28) domain disclosed herein comprises an amino acid sequence according to amino acid position 51 to amino acid position
30 68 of a CD28 signaling domain according to SEQ ID NO: 10. In some embodiments, the portion of CD28 of the ICOS(28) domain disclosed herein comprises an amino acid sequence according to amino acid position 51 to amino acid position 76 of a full length CD28 signaling domain according to SEQ ID NO: 10. In some embodiments, the portion of CD28 of the ICOS(28) domain disclosed herein comprises an amino acid sequence according to amino

acid position 45 to amino acid position 68 of a CD28 signaling domain according to SEQ ID NO: 10. In some embodiments, the portion of CD28 inserted within the ICOS(28) domain comprises a PRRP motif. In some embodiments, the portion of CD28 inserted within the ICOS(28) domain comprises an amino acid sequence according to SEQ ID NO: 11. In some
5 embodiments, the portion of CD28 inserted within the ICOS(28) domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 11.

 In some embodiments, the ICOS(28) domain comprises an amino acid sequence according to SEQ ID NO: 12. In some embodiments, the ICOS(28) comprises an amino acid
10 sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 12.

 In some embodiments, the chimeric intracellular domain comprises a first signal transduction domain derived from CD28. In some embodiments, the first signal transduction domain derived from CD28 comprises an amino acid sequence according to SEQ ID NO: 10. In some
15 embodiments, the first signal transduction domain derived from CD28 comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 10.

 A signal transduction domain derived from CD28 as described herein, is referred to as “CD28” or “28”, interchangeably throughout.

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Table 2. Amino acid sequences of first intracellular signaling domains.

ICOS signaling domain (SEQ ID NO: 9) (Other name: ICOS): Stalk (<u>underlined</u>), TM (regular font), intracellular domain (IC) (bold) and PI-3K binding site (bold and underlined)
<u>SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMEFMRAV</u> NTAKKSRLTDVTL
CD28 Transmembrane_CD28 Signaling Domain (Other names: CD28 or 28) (SEQ ID NO: 10): CD28 Stalk (<u>underlined</u>), TM (regular font), intracellular domain (IC) (bold), PI3K regulatory subunit binding, GRB2, GADS association domain (bold and dotted-underlined), ITK interaction site (bold and double-underlined), GRB2, GADS, LCK interaction site (bold and dash-underlined)
<u>LFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRL</u> <u>LHSDYMNMT</u> <u>PRR</u> <u>PGPTRKHYPYAPPRDFAAYRS</u>

CD28 fragment (Other names: mini-CD28 or (28)) (SEQ ID NO: 11): PRRP motif in bold
TPRRPGPTRKHYPYAPP
ICOS (28) domain (Other name: ICOS (mini-CD28)) (SEQ ID NO: 12): intracellular domain (italicized), TBK1 binding site (TRAF-like motif) (italicized and dotted-underlined), PI-3K regulatory subunit binding site (italicized and underlined), CD28 portion (bold), distal domain (italicized and dash-underlined)
SQLCCQLKFWLPIGCAAFVVVCILGCILICWLT <u>TKKKYSSSVHDPNGEYMFMT</u> TPRRPG P <u>TRKHYPYAPPRAVNTAKKSRLTDVTL</u>

In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 13. In some embodiments, the chimeric intracellular domain

5 comprises an amino acid sequence according to any one of SEQ ID NOs: 14-17. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to any one of SEQ ID NOs: 14-17. In some embodiments, the chimeric intracellular domain

10 comprises an amino acid sequence according to SEQ ID NO: 14. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 14. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 15. In some embodiments, the chimeric intracellular domain

15 comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 15. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 16. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 16. In some embodiments, the chimeric intracellular domain

20 comprises an amino acid sequence according to SEQ ID NO: 17. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 17.

In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to any one of SEQ ID NOs: 120-129. In some embodiments, the chimeric

25 intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%,

70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to any one of SEQ ID NOs: 120-129. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 120. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 120. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 121. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 121. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 122. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 122. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 123. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 123. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 124. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 124. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 125. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 125. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 126. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 126. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 127. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 127. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 128. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 128. In

some embodiments, the chimeric intracellular domain comprises an amino acid sequence according to SEQ ID NO: 129. In some embodiments, the chimeric intracellular domain comprises an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 129.

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Table 3. Amino acid sequences of chimeric intracellular domains of RTCR.

ICOS Transmembrane_ICOS Signaling Domain_4-1BB Signaling Domain (other names: ICOS-4-1BB (CD137) intracellular domain, ICOS-137; ICOS_137; ICOS137; ICOS_BB; ICOS-BB; ICOSBB; ICOSBBwt; ICOS_BBwt or ICOS_BB wild type) (SEQ ID NO: 13): ICOS sequence underlined and 4-1BB (BB) domain in normal font <u>SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMR</u><u>AVN</u> <u>TAKKSRLTDVTLKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL</u>
ICOS Transmembrane_ICOS Signaling Domain_Truncated 4-1BB Signaling Domain (other names: ICOS-truncated 4-1BB (CD137) intracellular domain; ICOS_137t; ICOS137t; ICOS-137t; ICOS_BBt; ICOSBBt or ICOS-BBt) (SEQ ID NO: 14): ICOS sequence underlined and mutated/truncated 4-1BB (BBt) domain in normal font <u>SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMR</u><u>AVN</u> <u>TAKKSRLTDVTLQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL</u>
ICOS Transmembrane_ICOS Signaling Domain_Truncated OX-40 Signaling Domain (other names: ICOS-truncated OX-40 (CD134) intracellular domain, ICOS_OX40t; ICOS-OX40t; ICOS_40t; ICOS40t or ICOS-40t (SEQ ID NO: 15): ICOS sequence underlined and mutated/truncated OX-40 (OX40t, 40t) domain in normal font <u>SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMR</u><u>AVN</u> <u>TAKKSRLTDVTLGGGSFRTPIQEEQADAHSTLA</u>
ICOS Transmembrane_ICOS Signaling Domain (mini-CD28)_Truncated 4-1BB Signaling Domain (other names: ICOS(28)-truncated 4-1BB (BBt) intracellular domain, ICOS(28)_BBt; ICOS(28)BBt; ICOS(28)-BBt; ICOS(28)_4-1BBt; ICOS(28)4-1BBt or ICOS(28)-4-1BBt) (SEQ ID NO: 16): ICOS sequence underlined, CD28 portion in bold and BBt domain in normal font <u>SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMF</u><u>MT</u><u>PRRP</u> <u>GPTRKHYQPYAPPRAVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCSCRFPEEEEE</u> <u>GCEL</u>

ICOS Transmembrane_ICOS Signaling Domain (mini-CD28)_Truncated OX-40 Signaling Domain (other names: ICOS(28)-truncated OX-40; ICOS(28)-OX40t; ICOS(28)_OX40t; ICOS(28)OX40t; ICOS (28)-40t; ICOS (28)_40t or ICOS(28)40t) (CD134) intracellular domain (SEQ ID NO: 17): ICOS sequence underlined, CD28 portion in bold and OX40 (OX-40t, 40t) domain in normal font <u>SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMTTPRRP</u> GPTRKHYQPYAPPRAVNTAKKSRLTDVTLGGGSFRTPIQEEQADAHSTLA
ICOS(28) (shortened ICOS(28)) (SEQ ID NO: 109): ICOS Transmembrane_ICOS Signaling Domain (mini-CD28) CWLTKKKYSSSVHDPNGEYMFMTTPRRPGPTRKHYQPYAPPRAVNTAKKSRLTDV TL
CD28 Transmembrane_CD28 Signaling Domain_4-1BB Signaling Domain (other names: CD28_BBwt signaling domain; CD28_BB; CD28BB; CD28-BB; 28_BBwt; 28BB; 28_BB or 28-BB) (SEQ NO: 120): CD28 Transmembrane domain_CD28 Signaling Domain_4-1BB Signaling Domain LFPGPSKPFWVLVVVGGVLACYSLLVTVAFIHFWVRSKRSRLLHSDYMNMTTPRRP GPTRKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL
CD28 Transmembrane_CD28 Signaling Domain_truncated 4-1BB Signaling Domain (other names: CD28_BBt signaling domain; CD28BBt; CD28-BBt; 28_BBt; 28BB; or 28-BBt) (SEQ ID NO: 121): LFPGPSKPFWVLVVVGGVLACYSLLVTVAFIHFWVRSKRSRLLHSDYMNMTTPRRP GPTRKHYQPYAPPRDFAAYRSQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
CD28 Transmembrane_CD28 Signaling Domain_truncated OX-40 Signaling Domain (other names: CD28_OX40t signaling domain; 28-OX40t; 28-40t; 28_OX40t; 28_40t; 28OX40t or 2840t) (SEQ ID NO: 122): LFPGPSKPFWVLVVVGGVLACYSLLVTVAFIHFWVRSKRSRLLHSDYMNMTTPRRP GPTRKHYQPYAPPRDFAAYRSGGSFRTPIQEEQADAHSTLA
Transmembrane_ICOS Signaling Domain_4-1BB Signaling Domain with mutated polybasic region (other names: ICOS_BB(xPB); ICOSBB(xPB) or ICOS-BB(xPB)) (SEQ ID NO: 123): ICOS SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVN TAKKSRLTDVTLAAGAAALLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

ICOS Transmembrane_ICOS Signaling Domain_4-IBB Signaling Domain with mutated lysines (Other names: ICOS_BB(xUB); ICOS-BB(xUB) or ICOSBB(xUB)) ((SEQ ID NO: 124): SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLKRGRKKLLYIFAQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL
ICOS Transmembrane_ICOS Signaling Domain_4-IBB Signaling Domain with mutated lysines and polybasic regions (Other names: ICOS_BB(xPB xUB); ICOS-BB(xPB xUB) or ICOSBB(xPB xUB)) (SEQ ID NO: 125): SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLAAGAAALLYIFAQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL
ICOS Transmembrane_ICOS Signaling Domain_OX-40 Signaling Domain (Other names: ICOS_OX40wt; ICOS-40; ICOS_40 wild type; ICOS_40wt; ICOS_40wt; ICOS-40wt; ICOS-OX40wt; ICOSOX40wt; ICOS40 or ICOS40wt) (SEQ ID NO: 126): SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLRRDQRLPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKI
ICOS Transmembrane_ICOS Signaling Domain_OX-40 Signaling Domain with mutated polybasic region (other names: ICOS_OX40(xPB); ICOS_40(xPB); ICOS-40(xPB); ICOS40(xPB); ICOS-OX40(xPB); or ICOSOX40(xPB)) (SEQ ID NO: 127): SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLAADQALPPDAHKKPPGGGSFRTPIQEEQADAHSTLAKI
ICOS Transmembrane_ICOS Signaling Domain_OX-40 Signaling Domain with mutated lysine residues (other names: ICOS_OX40(xUB) ICOS_40(xUB); ICOS-40(xUB); ICOS40(xUB); ICOS-OX40(xUB); or ICOSOX40(xUB)) (SEQ ID NO: 128): SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLRRDQRLPPDAHAPPGGGSFRTPIQEEQADAHSTLAAI
ICOS Transmembrane_ICOS Signaling Domain_OX-40 Signaling Domain with mutated lysine residues and polybasic regions (other names: ICOS_OX40(xPBxUB) ICOS_40(xPBxUB); ICOS-40(xPBxUB); ICOS40(xPBxUB); ICOS-OX40(xPBxUB); or ICOSOX40(xPBxUB)) (SEQ ID NO: 129): SQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLAADQALPPDAHAPPGGGSFRTPIQEEQADAHSTLAAI
ICOS intracellular domain (SEQ ID NO: 147) CWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL

CD28 intracellular domain (SEQ ID NO: 193)

RSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

In some embodiments, the chimeric intracellular domain further comprises a third signal transduction domain. In some embodiments, the third signal transduction domain is derived from any one of a CD3 signaling domain, a CD2 signaling domain, or an interleukin 2 receptor binding (IL-2RB) protein signaling domain. In some embodiments, the CD3 signaling domain is derived from a CD3 ζ or a CD3 ϵ domain or a combination thereof.

In some embodiments, the chimeric intracellular domain further comprises a third signal transduction domain derived from a CD3 ζ protein. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a CD3 ζ protein of amino acid sequence according to SEQ ID NO: 18. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a CD3 ζ comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to according to SEQ ID NOs: 18.

Human CD3 ζ full length (CD3 ζ full length) (SEQ ID NO: 18)

MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a CD3 signaling domain comprising an amino acid sequence according to any one of SEQ ID NOs: 45, 46, 47 and 48. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a CD3 ζ comprising an amino acid sequence according to SEQ ID NO: 45. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a CD3 ζ comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 45. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a truncated CD3 ζ comprising an amino acid sequence according to SEQ ID NO: 46. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a truncated CD3 ζ comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 46. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a truncated CD3 ϵ comprising an amino acid sequence

according to SEQ ID NO: 47. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a truncated CD3ε comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 47. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a combination of a CD3ε and a truncated CD3ζ domains (CD3ζε domain). In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a CD3ζε comprising an amino acid sequence according to SEQ ID NO: 48. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a CD3ζε comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 48.

In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a mutant CD2 signaling domain. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a truncated CD2 signaling domain. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a truncated CD2 signaling domain comprising an amino acid sequence according to SEQ ID NO: 49. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is a truncated CD2 signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NOs: 49.

In some embodiments, the third signal transduction domain of the chimeric intracellular domain is an IL-2RB protein signaling domain comprising an amino acid sequence according to SEQ ID NO: 50. In some embodiments, the third signal transduction domain of the chimeric intracellular domain is an IL-2RB protein signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NOs: 50.

In some embodiments, the chimeric intracellular domain further comprises a fourth signal transduction domain. In some embodiments, the fourth signal transduction domain is derived from any one of a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-2RB) protein signaling domain or a combination thereof, wherein the third and the fourth signal transduction domain are not identical.

In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a CD3 signaling domain comprising an amino acid sequence according to any one of SEQ ID NOs: 45, 46, 47 and 48. In some embodiments, the fourth

signal transduction domain of the chimeric intracellular domain is a CD3 ζ comprising an amino acid sequence according to SEQ ID NO: 45. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a CD3 ζ comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 45. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a truncated CD3 ζ comprising an amino acid sequence according to SEQ ID NO: 46. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a truncated CD3 ζ comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 46. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a truncated CD3 ϵ comprising an amino acid sequence according to SEQ ID NO: 47. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a truncated CD3 ϵ comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 47. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a combination of a CD3 ϵ and a truncated CD3 ζ domains (CD3 $\zeta\epsilon$ domain). In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a CD3 $\zeta\epsilon$ comprising an amino acid sequence according to SEQ ID NO: 48. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a CD3 $\zeta\epsilon$ comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 48.

In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a mutant CD2 signaling domain. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a truncated CD2 signaling domain. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a truncated CD2 signaling domain comprising an amino acid sequence according to SEQ ID NO: 49. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is a truncated CD2 signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NOs: 49.

In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is an IL-2RB protein signaling domain comprising an amino acid

sequence according to SEQ ID NO: 50. In some embodiments, the fourth signal transduction domain of the chimeric intracellular domain is an IL-2RB protein signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NOs: 50.

5 The terms T cell, T-cell, t cell, t-cell, and T lymphocyte can be used interchangeably in the present disclosure.

 In some embodiments, the extracellular domain comprises a protein or a portion thereof that binds to a target to induce activation and/or proliferation of an immune cell. In some embodiments, the extracellular domain comprises any one of: a) a component of a T
10 cell Receptor (TCR) complex; b) a component of a chimeric antigen receptor (CAR); c) a component of a T cell co-receptor, wherein the T cell co-receptor is a T cell co-stimulatory protein or T cell inhibitory protein; d) a ligand that binds to a cell surface receptor or a component thereof; e) a component of a cytokine receptor; e) a component of a chemokine receptor; g) a component of an integrin receptor; h) a component of an endothelial cell
15 surface protein receptor or a fragment thereof; i) a component of a neuronal guidance protein receptor; and f) a component of a complement receptor. In some embodiments, the component of the T cell co-receptor or the CAR is a component of PD1, CD28, CD2, OX-40, ICOS, CTLA-4, CD28, CD3, CD4, CD8, CD40L, Lag-3, Tim-3, or TIGIT, or a combination thereof. In some embodiments, the ligand or component of the T cell co-receptor or CAR
20 binds to CD19, B cell maturation Ag (BCMA), PD-L1, PD-L2, IL-10, a proliferation-inducing ligand (APRIL), BAFF, OX-40L, ICOS-L, B7-1, B7-2, CD40, CD58, CD59, nectin, CD155, or CD112, or a combination thereof. In some embodiments, the cytokine receptor binds to IL-10, IL-27, TGF- β , IL-12, IL-1, IL-2, IL-4, IL-5, IFN- γ , or IFN- α/β , or a combination thereof. In some embodiments, the component of the complement receptor is a
25 component of a single C3aR, C5aR, CD46/MCP, CD55, CD97, or DAF, or a combination thereof.

 In some embodiments, the extracellular domain comprises an amino acid sequence of a component of any one of: a) a chemokine receptor; b) a cytokine receptor; c) a ligand for a cell surface receptor; d) an integrin receptor; e) a cell adhesion molecule or a receptor thereof;
30 f) an endothelial cell surface protein receptor or a fragment thereof; g) a complement receptor; and h) a neuronal guidance protein receptor. In some embodiments, the extracellular domain comprises an amino acid sequence of a component of any one of epithelial growth factor receptor (EGFR), vascular-endothelial growth factor (VEGFR), chemokine receptor

(CCR) 4, CCR5, CCR7, CCR10, Lymphocyte function-associated antigen-1 (LFA-1), leukocyte-specific $\beta 2$ integrins ($\alpha L\beta 2$, $\alpha M\beta 2$, $\alpha X\beta 2$, $\alpha D\beta 2$), $\beta 7$ integrins ($\alpha 4\beta 7$ and $\alpha E\beta 7$), extracellular matrix (ECM)-binding $\beta 1$ integrins ($\alpha 1$ – $\alpha 6\beta 1$), L-selectin, or sialyl Lewis^x.

In some embodiments, the extracellular domain is a protein, a peptide, a glycoprotein, an antibody or a fragment thereof. In some embodiments, the antibody or fragment thereof is a Fab fragment, a F(ab)₂ fragment, a diabody, a nanobody, a sdAb, Fv, a VHH fragment, or a single chain Fv fragment.

In some embodiments, the extracellular domains comprises two or more binding sites for targeting two or more non-identical target antigens. In some embodiments, the extracellular domains comprises two or more binding sites for targeting two or more non-identical sites on a target antigen. In some embodiments, the extracellular domain comprises two antigen binding domains or fragments of a bispecific antibody. In some embodiments, the extracellular domain comprises a F(ab)₂ fragment of a bispecific antibody. In some embodiments, the extracellular domain comprises two or more antigen binding domains or fragments of a multi-specific antibody.

In some embodiments, the extracellular domain binds to a target that is a tumor antigen, a pathogen associated protein, or an antigen associated with the disease or disorder that is a cancer, an autoimmune disease or disorder, an infectious disease, an inflammatory disease, a renal disease or disorder, a lung disease or disorder, a liver disease or disorder, a cardiovascular disease or disorder, a neurodegenerative disorder or disorder, or a metabolic disorder or disorder.

In some embodiments, the tumor antigen is any one of a tumor associated antigen (TAA), a tumor secreted antigen (TSA) or an unconventional antigen (UCA). In some embodiments, the TAA is any one of a cancer germline antigen (CGA), a Human endogenous retroviruses (HERVs), tissue differentiation antigen (TDA) and overexpressed tumor antigen. In some embodiments, the TSA is derived from any one of a mosaic single nucleotide variations (mSNVs), a insertion-deletion mutations (INDELs), gene fusions and viral oncoproteins. In some embodiments, the UCA is derived from non-coding regions of the genome or from coding regions of the genome. In some embodiments, the UCA is derived from aberrant transcription, translation, or post-translational modifications.

In some embodiments, the TAA is associated with a solid tumor or cancer or a hematologic cancer. In some embodiments, the TAA is associated with a solid tumor or

cancer is selected from a sarcoma, a carcinoma or a lymphoma that manifests as, leads to, or is associated with a solid tumor.

In some embodiments, the TAA is associated with a sarcoma that is a soft tissue sarcoma or a bone sarcoma (osteosarcoma). In some embodiments, the TAA is associated
5 with a sarcoma selected from vesicular rhabdomyosarcoma, vesicular soft tissue sarcoma, ameloblastoma, angiosarcoma, chondrosarcoma, chordoma, bright tissue sarcoma, dedifferentiated liposarcoma, Hyperplastic small round cell tumor of connective tissue, embryonic rhabdomyosarcoma, epithelioid fibrosarcoma, epithelioid hemangioendothelioma, epithelioid sarcoma, sensitive neuroblastoma (esthesioneuroblastoma), Ewing sarcoma,
10 extrarenal rhabdomyosarcoma, extraosseous myxoid chondrosarcoma, extraosseous osteosarcoma, fibrosarcoma, giant cell tumor, hemangiopericytoma, infantile fibrosarcoma, inflammatory myofibroblastoma, Kaposi sarcoma, bone smooth muscle sarcoma, liposarcoma, osteosarcoma, malignant fibrous histiocytoma (MFH), malignant fibrous histiocytoma (MFH), malignant mesenchymal tumor, malignant peripheral nerve sheath
15 tumor, mesenchymal chondrosarcoma, myxoid liposarcoma, myxoid inflammatory fibroblastic sarcoma, multiple tumors with perivascular epithelioid cell differentiation, osteosarcoma, extraperiosteal osteosarcoma, tumors with perivascular epithelial cell differentiation, periosteum osteosarcoma, polymorphic liposarcoma, polymorphic rhabdomyosarcoma, PNET / extraosseous Ewing's tumor, rhabdomyosarcoma, small cell
20 osteosarcoma, single fibroids, synovial sarcoma or capillary dilated osteosarcoma.

In some embodiments, the TAA is associated with a carcinoma selected from basal cell carcinoma, squamous cell carcinoma, renal cell carcinoma, ductal carcinoma in situ (DCIS), invasive ductal carcinoma or adenocarcinoma. In some embodiments, the TAA is associated with a carcinoma selected from adenosquamous carcinoma, anaplastic carcinoma,
25 large cell carcinoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma or small cell carcinoma.

In some embodiments, the TAA is associated with a solid tumor or cancer selected from anal cancer, appendix cancer, cholangiocarcinoma (i.e., biliary tract cancer), breast cancer, bladder cancer, brain tumor, breast cancer, cervical cancer, colon cancer, colorectal
30 cancer, colon polyp, unidentified primary cancer (cup), esophagus cancer, eye cancer, tubal cancer, kidney cancer, liver cancer, lung cancer, medulloblastoma, melanoma, oral cancer, ovarian cancer, prostate cancer, pancreatic cancer, gastric cancer, testicular cancer, laryngeal cancer, thyroid cancer, uterine cancer, vaginal cancer, or vulvar cancer.

In some embodiments, the breast cancer is an invasive breast duct cancer, carcinoma in situ of the duct, invasive lobular carcinoma or lobular carcinoma in situ. In some embodiments, the pancreatic cancer is adenocarcinoma or islet cell carcinoma. In some embodiments, the colorectal cancer is adenocarcinoma. In some embodiments, colonic polyps are associated with familial adenomatous polyposis. In some embodiments, the bladder cancer is transitional cell bladder cancer, squamous cell bladder cancer, or adenocarcinoma. In some embodiments, the lung cancer is non-small cell lung cancer. In some embodiments, the non-small cell lung cancer is adenocarcinoma, squamous cell lung cancer, or large cell lung cancer. In some embodiments, the non-small cell lung cancer is large cell lung cancer. In some embodiments, the lung cancer is small cell lung cancer. In some embodiments, the prostate cancer is adenocarcinoma or small cell carcinoma. In some embodiments, the ovarian cancer is epithelial ovarian cancer. In some embodiments, the cholangiocarcinoma is proximal cholangiocarcinoma or distal cholangiocarcinoma.

In some embodiments, the TAA is associated with any one of the hematological cancer selected from a leukemia, a myeloma or a lymphoma. In some embodiments, the TAA is associated with a leukemia selected from acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, a B cell, T cell or FAB ALL, acute myeloid leukemia (AML), acute myelogenous leukemia, chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, acute promyelocytic leukemia (APL), mixed-lineage leukemia (MLL) or myelodysplastic syndrome (MDS).

In some embodiments, the TAA is associated with a myeloma that is a multiple myeloma. In some embodiments, the TAA is associated with a multiple myeloma selected from the hyperdiploid (HMM) or the non-hyperdiploid or hypodiploid subtypes of multiple myeloma. In some embodiments, the TAA is associated with a multiple myeloma selected from light chain myeloma, non-secretory myeloma, solitary plasmacytoma, extramedullary plasmacytoma, monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), immunoglobulin D (IgD) myeloma or, immunoglobulin E (IgE) myeloma.

In some embodiments, the TAA is associated with a lymphoma that is a Hodgkin's lymphoma or a non-Hodgkin's lymphoma. In some embodiments, the TAA is associated with a non-Hodgkin's lymphoma. In some embodiments, the TAA is associated with a non-Hodgkin's lymphoma selected from a Small lymphocytic lymphoma (SLL), Lymphoplasmacytic lymphoma, Diffuse large cell lymphoma, Follicle center cell lymphoma, Burkitt's lymphoma, Burkitt-like lymphoma, Mantle cell lymphoma or Marginal zone B-cell

lymphoma. In some embodiments, the TAA is associated with a lymphoma that is a Hodgkin's lymphoma. In some embodiments, the TAA is associated with a Hodgkin's lymphoma selected from nodular sclerosis classical Hodgkin lymphoma, lymphocyte-rich classical Hodgkin lymphoma or lymphocyte-depleted classical Hodgkin lymphoma.

- 5 In some embodiments, the TAA is associated with a cancer that is any one of acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, B cell, T cell or FAB ALL, acute myeloid leukemia (AML), acute myelogenous leukemia, chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), Hodgkin's lymphoma, Hodgkin's disease, non-Hodgkin's
- 10 lymphoma, multiple myeloma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, bladder cancer, breast cancer, colorectal cancer, endometrial cancer, head cancer, neck cancer, hereditary nonpolyposis cancer, liver cancer, lung cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, testicular cancer,
- 15 adenocarcinomas, sarcomas, malignant melanoma, and hemangioma.

- In some embodiments, the extracellular domain binds to a TAA selected from kallikrein 4, papillomavirus binding factor (PBF), preferentially expressed antigen of melanoma (PRAME), Wilms' tumor-I (WTI), Hydroxysteroid Dehydrogenase Like I (HSDLI), mesothelin, cancer testis antigen (NY-ES0-1), carcinoembryonic antigen (CEA),
- 20 p53, human epidermal growth factor receptor 2/neuro receptor tyrosine kinase (Her2/Neu), carcinoma-associated epithelial cell adhesion molecule (EpCAM), ovarian and uterine carcinoma antigen (CAI25), folate receptor a, sperm protein 17, tumor-associated differentially expressed gene-12 (TADG-12), mucin-16 (MUC-16), LI cell adhesion molecule (LICAM), mannan-MUC-1, Human endogenous retrovirus K (HERV-K-MEL),
- 25 Kita-kyushu lung cancer antigen-I (KK-LC-1), human cancer/testis antigen (KM-HN-1), cancer testis antigen (LAGE-I), melanoma antigen-A1 (MAGE-A1), Sperm surface zona pellucida binding protein (Spl 7), Synovial Sarcoma, X Breakpoint 4 (SSX-4), Transient axonal glycoprotein-1 (TAG-I), Transient axonal glycoprotein-2 (TAG-2), Enabled Homolog (ENAH), mammoglobin-A, NY-BR-I, Breast Cancer Antigen, (BAGE-1), B melanoma
- 30 antigen, melanoma antigen-A1 (MAGE-A1), melanoma antigen-A2 (MAGE-A2), mucin k, synovial sarcoma, X breakpoint 2 (SSX-2), Taxol-resistance-associated gene-3 (TRAG-3), Avian Myelocytomatosis Viral Oncogene (c-myc), cyclin B 1, mucin I (MUC I), p62, survivin, lymphocyte common antigen (CD45), Dickkopf/WNT Signaling Pathway Inhibitor I (DKKI), telomerase, Kirsten rat sarcoma viral oncogene homolog (K-ras), G250, intestinal

carboxyl esterase, alpha-fetoprotein, Macrophage Colony-Stimulating Factor (M-CSF), Prostate-specific membrane antigen (PSMA), caspase 5 (CASP-5), Cytochrome C Oxidase Assembly Factor I Homolog (COA-1), O-linked β -N-acetylglucosamine transferase (OGT), Osteosarcoma Amplified 9, Endoplasmic Reticulum Lectin (OS-9), Transforming Growth

5 Factor Beta Receptor 2 (TGF-betaRII), murine leukemia glycoprotein 70 (gp70), Calcitonin Related Polypeptide Alpha (CALCA), Programmed cell death 1 ligand 1 (CD274), Mouse Double Minute 2 Homolog (mdm-2), alpha-actinin-4, elongation factor 2, Malic Enzyme 1 (MEI), Nuclear Transcription Factor Y Subunit C (NFYC), G Antigen 1,3 (GAGE-1,3), melanoma antigen-A6 (MAGE-A6), cancer testis antigen XAGE-1b, six transmembrane

10 epithelial antigen of the prostate 1 (STEAP1), PAP, prostate specific antigen (PSA), Fibroblast Growth Factor 5 (FGF5), heat shock protein hsp70-2, melanoma antigen-A9 (MAGE-A9), Arg-specific ADP-ribosyltransferase family C (ARTC1), B-Raf Proto-Oncogene (B-RAF), Serine/Threonine Kinase, beta-catenin, Cell Division Cycle 27 homolog (Cdc27), cyclin dependent kinase 4 (CDK4), cyclin dependent kinase 12 (CDK12), Cyclin

15 Dependent Kinase Inhibitor 2A (CDKN2A), Casein Kinase 1 Alpha 1 (CSNK1A1), Fibronectin 1 (FNI), Gruh1 Anest Specific 7 (GAS7), Glycoprotein nonmetastatic melanoma protein B (GPNMB), HAUS Augmin Like Complex Subunit 3 (HAUS3), LDLR-fucosyltransferase, Melanoma Antigen Recognized By T cells 2 (MART2), myostatin (MSTN), Melanoma Associated Antigen (Mutated) 1 (MUM-1-2-3), Poly(A) polymerase

20 gamma (neo-PAP), myosin class I, Protein phosphatase 1 regulatory subunit 3B (PPP1R3B), Peroxiredoxin-5 (PRDX5), Receptor-type tyrosine-protein phosphatase kappa (PTPRK), Transforming protein N-Ras (N-ras), retinoblastoma-associated factor 600 (RBAF600), sirtuin-2 (SIRT2), SNRPD1, triosephosphate isomerase, Ocular Albinism Type 1 Protein (OAI), member RAS oncogene family (RAB38), Tyrosinase related protein 1-2 (TRP-1-2),

25 Melanoma Antigen Gp75 (gp75), tyrosinase, Melan-A (MART-1), Glycoprotein 100 melanoma antigen (gp100), N-acetylglucosaminyltransferase V gene (GnTVf), Lymphocyte Antigen 6 Complex Locus K (LY6K), melanoma antigen-A10 (MAGE-A10), melanoma antigen-A12 (MAGE-A12), melanoma antigen-C2 (MAGE-C2), melanoma antigen NA88-A, Taxol-resistant-associated protein 3 (TRAG-3), BDZ binding kinase (pbk), caspase 8 (CASP-

30 8), sarcoma antigen 1 (SAGE), Breakpoint Cluster Region-Abelson oncogene (BCR-ABL), fusion protein in leukemia, deK-can, Elongation Factor Tu GTP Binding Domain Containing 2 (EFTUD2), ETS Variant gene 6/acute myeloid leukemia fusion protein (ETV6-AML1), FMS-like tyrosine kinase-3 internal tandem duplications (FLT3-ITD), cyclin-A1, Fibronectin

Type III Domain Containing 3B (FDNC3B,) promyelocytic leukemia/retinoic acid receptor alpha fusion protein (pml-RARalpha), melanoma antigen-C1 (MAGE-C1), membrane protein alternative spliced isoform (D393-CD20), melanoma antigen-A4 (MAGE-A4), or melanoma antigen-A3 (MAGE-A3).

- 5 In some embodiments, the autoimmune condition or disorder is any one of Type 1 Diabetes, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), multiple sclerosis (MS), celiac disease, sjögren syndrome, polymyalgia rheumatica, ankylosing spondylitis, alopecia areata, vasculitis and temporal arteritis. In some embodiments, the tumor associated antigen (TAA) associated with the autoimmune condition or disorder is derived from any one
- 10 of Carboxypeptidase H, Chromogranin A, Glutamate decarboxylase, Imogen-38, Insulin, Insulinoma antigen-2 and 2 β , Islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP), Proinsulin, α -enolase, Aquaporin-4, β -arrestin, Myelin basic protein, Myelin oligodendrocytic glycoprotein, Proteolipid protein, S100- β , Citrullinated protein, Collagen II, Heat shock proteins, Human cartilage glycoprotein, Double-stranded DNA, La antigen,
- 15 Nucleosomal histones and ribonucleoproteins (snRNP), Phospholipid- β -2 glycoprotein I complex, Poly(ADP-ribose) polymerase, and Sm antigens of U-1 small ribonucleoprotein complex.

- In some embodiments, the pathogen associated antigen is an antigen from a bacterial, a fungal or a parasitic protein or fragment thereof. In some embodiments, the pathogen
- 20 associated antigen is associated with HIV infection, human Cytomegalovirus infection, Hepatitis B infection, Hepatitis C infection, Ebola virus infection, Dengue, Yellow fever, Listeriosis, Tuberculosis, Cholera, Malaria, Leishmaniasis, or Trypanosoma infection, or a combination thereof.

- In some embodiments, the neurodegenerative disorder or condition is any one of
- 25 Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD) and PD-related disorders, Prion disease, Motor neurone diseases (MND), Huntington's disease (HD), Spinocerebellar ataxia (SCA) or Spinal muscular atrophy (SMA). In some embodiments, the antigen associated with the neurodegenerative disorder or condition is any one of Amyloid β (Ab), tau, alpha-synuclein (α -syn), mHTT or prion PrP^{Sc} or a combination thereof.

- 30 In some embodiments, the extracellular domain binds to a target with a binding affinity of 1 fM to 100 μ M. In some embodiments, the extracellular domain binds to a target with a binding affinity of 1 pM to 100 μ M. In some embodiments, the extracellular domain binds to a target with a binding affinity of 1 pM to 10 pM. In some embodiments, the

extracellular domain binds to a target with a binding affinity of 10 pM to 50 pM. In some embodiments, the extracellular domain binds to a target with a binding affinity of 10 pM to 100 pM. In some embodiments, the extracellular domain binds to a target with a binding affinity of 100 pM to 500 pM. In some embodiments, the extracellular domain binds to a target with a binding affinity of 500 pM to 1 nM. In some embodiments, the extracellular domain binds to a target with a binding affinity of 1 nM to 10 nM. In some embodiments, the extracellular domain binds to a target with a binding affinity of 10 nM to 100 nM. In some embodiments, the extracellular domain binds to a target with a binding affinity of 100 nM to 500 nM. In some embodiments, the extracellular domain binds to a target with a binding affinity of 500 nM to 1 μ M. In some embodiments, the extracellular domain binds to a target with a binding affinity of 1 μ M to 10 μ M. In some embodiments, the extracellular domain binds to a target with a binding affinity of 1 μ M to 5 μ M. In some embodiments, the extracellular domain binds to a target with a binding affinity of 5 μ M to 7.5 μ M. In some embodiments, the extracellular domain binds to a target with a binding affinity of 7.5 μ M to 10 μ M.

In some embodiments, the extracellular domain comprises a signal peptide at the N-terminus. In some embodiments, the signal peptide can be derived from a surface expressing protein or a secretory protein. In some embodiments, the signal peptide can be derived from Preprolactin, HIV pre-Env, HCV polyprotein, CB virus polyprotein, Pestivirus polyprotein, Precalreticulin, pre-VSV-G, HLA class I histocompatibility antigen or PD-1 signal peptide (PD-1 SP), interleukin 12 (IL12), GM-CSF or CD8 alpha chain (CD8a). In some embodiments, the signal peptide is PD-1 signal peptide (PD-1 SP). In some embodiments, the signal peptide is a HLA class I histocompatibility antigen or a portion thereof. In some embodiments, the extracellular domain is derived from PD1. In some embodiments, the extracellular domain comprises the amino acid sequence from position 1 to 163 of the amino acid sequence according to any one of SEQ ID NOs: 19-21. In some embodiments, the extracellular domain comprises the amino acid sequence from position 1 to 163 of the amino acid sequence according to SEQ ID NOs: 19. In some embodiments, the extracellular domain comprises the amino acid sequence from position 1 to 163 of the amino acid sequence according to SEQ ID NOs: 20. In some embodiments, the extracellular domain comprises the amino acid sequence from position 1 to 163 of the amino acid sequence according to SEQ ID NOs: 21.

In some embodiments, the extracellular domain comprises the amino acid sequence according to any one of SEQ ID NOs: 22-23. In some embodiments, the extracellular domain comprises the amino acid sequence according to SEQ ID NOs: 22. In some embodiments, the extracellular domain comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NOs: 22. In some embodiments, the extracellular domain comprises the amino acid sequence according to SEQ ID NOs: 23. In some embodiments, the extracellular domain comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NOs: 23.

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Table 4. Amino acid sequences of PD1 extracellular domains of RTCR.

PD1 (PD1 Signal Peptide_PD1 Extracellular_PD1 Transmembrane_PD1 Intracellular) (other names: PD1_wt (human-wild type); PD1:WT; PD-1; PD-1 wt; PD-1 wild type; PD1; PD1wt or PD1 wild type) (SEQ ID NO: 19): Signal Peptide (<i>italicized</i>), Extracellular domain (IG-like V domain in bold and stalk in bold and <u>underlined</u>), Trans Membrane (<u>underlined</u>) and Intracellular domain in double underline <i>MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCS</i> FSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFH MSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRV<u>TERRAEVPTAHPS</u><u>SP</u> <u>RPAGQFOTLVVGVVGGLLGSLVLLVWVLAVICSR</u><u>AARGTIGARRTGOPLKEDPS</u> <u>AVPVFSVDYGELDFQWREKTPEPPVPCVPEOTEYATIVFPSGMGTSSPARRGSADG</u> <u>PRSAOPLRPEDGHCSWPL</u>
PD1 Signal Peptide_PD1 Extracellular_PD1 Transmembrane (Other name: PD-1 truncated) (SEQ ID NO: 20): PD1 Signal Peptide (<i>italicized</i>), Extracellular domain (IG-like V domain in bold and stalk in bold and <u>underlined</u>), Trans Membrane (<u>underlined</u>) and Intracellular tail in double underline <i>MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCS</i> SFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFH MSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRV<u>TERRAEVPTAHPS</u><u>SP</u> <u>RPAGQFOTLVVGVVGGLLGSLVLLVWVLAVICSR</u>
HLA-A2 Signal Peptide_PD1 Extracellular_PD1 Transmembrane (PD1_TLs; HLASP-Truncated, PD1-TLs, PD1:TLs) (SEQ ID NO: 21): HLA-A2 Signal Peptide (<i>italicized</i>),

Extracellular domain (IG-like V domain in bold and stalk in bold and underlined), Trans Membrane (underlined) and Intracellular tail in double underline <i>MAVMAPRTLVL</i><u>LLSGALALTQTWAF</u><u>LDSPDRPWNPPTFSPALLVVTEGDNATFTCS</u> <u>FSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFH</u> <u>MSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRV</u><u>TERRAEVPTAHPSPSP</u> <u>RPAGQFOTLVVGVVGGLLGSLVLLVWVLAVICSR</u>
PD-1 (extracellular domain) (SEQ ID NO: 22): Signal Peptide (italicized), Extracellular domain (IG-like V domain in bold and stalk in bold and underlined) <i>MQIPQAPWPVVWAVLQLGWRPGWF</i><u>LDSPDRPWNPPTFSPALLVVTEGDNATFTC</u> <u>SFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFH</u> <u>MSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRV</u><u>TERRAEVPTAHPSPSP</u> <u>RPA</u>
PD-1 (HLA A2-Signal Peptide extracellular domain) (other name: PD-1 (HLA A2-SP extracellular domain) (SEQ ID NO: 23): Signal Peptide (italicized), Extracellular domain (IG-like V domain in bold and stalk in bold and underlined) <i>MAVMAPRTLVL</i><u>LLSGALALTQTWAF</u><u>LDSPDRPWNPPTFSPALLVVTEGDNATFTCS</u> <u>FSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFH</u> <u>MSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRV</u><u>TERRAEVPTAHPSPSP</u> <u>RP</u>

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to any one of SEQ ID NOs: 24-44 and 130-132.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 24. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 24.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 25. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 25.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 26. In some embodiments, the RTCR disclosed herein comprises

the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 26.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 27. In some embodiments, the RTCR disclosed herein comprises
5 the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 27.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 28. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,
10 95%, 97% or 99% identity to SEQ ID NO: 28.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 29. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 29.

15 In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 30. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 30.

In some embodiments, the extracellular domain of the RTCR disclosed herein
20 comprises the amino acid sequence according to SEQ ID NO: 31. In some embodiments, the extracellular domain of the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 31.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence
25 according to SEQ ID NO: 32. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 32.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 33. In some embodiments, the RTCR disclosed herein comprises
30 the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 33.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 34. In some embodiments, the RTCR disclosed herein comprises

the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 34.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 35. In some embodiments, the RTCR disclosed herein comprises
5 the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 35.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 36. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,
10 95%, 97% or 99% identity to SEQ ID NO: 36.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 37. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 37.

15 In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 38. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 38.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 39. In some embodiments, the RTCR disclosed herein comprises
20 the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 39.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 40. In some embodiments, the RTCR disclosed herein comprises
25 the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 40.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 41. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%,
30 95%, 97% or 99% identity to SEQ ID NO: 41.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 42. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 42.

In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 43. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 43.

5 In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 44. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 44.

10 In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 130. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 130.

15 In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 131. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 131.

20 In some embodiments, the RTCR disclosed herein comprises the amino acid sequence according to SEQ ID NO: 132. In some embodiments, the RTCR disclosed herein comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 132.

Table 5. Amino acid sequences of PD1 based recombinant T cell co-stimulatory receptor (RTCR).

HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling Domain (Other names: PD-1-CD28 Domain Swap; HLA A2-SP-PD-1_28; HLA A2-SP-PD-1_CD28 DS; HLA A2-SP-PD-1_CD28; PD1_CD28 or PD1:CD28 or PD1_28) (SEQ ID NO: 24): HLA-A2 Signal Peptide (<i>italicized</i>), PD 1 extracellular domain (IG-like V domain in bold and stalk in bold and <u>underlined</u>), CD28 Transmembrane (<u>underlined</u>) and Intracellular domain in <u>double underline</u>; and CD28 signal domain: Stalk (<u>underlined</u> and <i>italicized</i>), transmembrane domain (<u>double underlined</u>), intracellular domain (IC) (<u>dashed underlined</u>) (SEQ ID NO: 10)
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*MAVMAPRTLVL*LLSGALALTQTWAFLDSPDRPWNPPPTFSPALLVVTEGDNATFTCS
FSNTSESVFLNWYRMSPSNQTDKLAAP**PEDRSQPGQDCRFRVTQLPNGRDFH**
MSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRV**TERRAEVPTAHPSPSP**
RPALFPGPSKPFWVLVVVGVLACYSLLVTVAFIIFWVR**SKRSRL**LHSDYMN**MTP**
RRPGPTRKHYQPYAPPRDFAAYRS

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling
 Domain (Other names: PD-1-ICOS Domain Swap; HLASP-PD-1-ICOS DS; HLA A2-SP-
 PD-1_ICOS; PD-1-ICOS; PD-1:ICOS; PD1_ICOS) (SEQ ID NO: 25): HLA-A2 Signal
 Peptide (italicized), PD 1 extracellular domain (IG-like V domain in bold and stalk in bold
 and underlined), ICOS Transmembrane (underlined) and Intracellular domain in double
 underline; and ICOS domain: Stalk (underlined and italicized), transmembrane domain
 (double underlined), intracellular domain (IC) (dashed underlined)

*MAVMAPRTLVL*LLSGALALTQTWAFLDSPDRPWNPPPTFSPALLVVTEGDNATFTCS
FSNTSESVFLNWYRMSPSNQTDKLAAP**PEDRSQPGQDCRFRVTQLPNGRDFH**
MSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRV**TERRAEVPTAHPSPSP**
RPASQLCCOLKEFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMF**MRA**
YNTAKKSRLTDVTL

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling
 Domain_4-1BB Signaling Domain (other names: PD1 HLASP-ICOS-4-1BB; HLA A2-SP-
 PD-1_ICOS_BBwt; HLA A2-SP-PD-1_ICOS_BB; HLA A2-SP-PD-1_ICOS_CD137;
 HLA A2-SP-PD-1_ICOS_CD137 wt; PD1_ICOS_BBwt; PD1:ICOSBBwt; PD1:ICOSBB)
 (SEQ ID NO: 26): PD 1 extracellular domain in regular font; ICOS domain underlined; and
 4-1BB domain (CD137 signaling domain, wild type) in bold

*MAVMAPRTLVL*LLSGALALTQTWAFLDSPDRPWNPPPTFSPALLVVTEGDNATFTC
SFSNTSESVFLNWYRMSPSNQTDKLAAP**PEDRSQPGQDCRFRVTQLPNGRDFHMS**
VVRARRNDSGTYLCGAISLAPKAQIKESLRAELRV**TERRAEVPTAHPSPSPRPASQL**
CCOLKEFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMF**MRAVNTAK**
KSRLTDVTLKRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling
 Domain_Truncated 4-1BB Signaling Domain
 (Other names: PD1 HLASP-ICOS-truncated 4-1BB; HLA A2-SP-PD-1-ICOS_BBt; HLA
 A2-SP-PD-1_ICOS_BBt; HLA A2-SP-PD-1_ICOS_truncated CD137; HLA A2-SP-PD-
 1_ICOS_truncated CD137 wt; PD1_ICOS_BBt; PD1:ICOSBBt) (SEQ ID NO: 27): PD 1

extracellular domain in regular font; ICOS domain underlined; and truncated 4-1BB (truncated CD137 signaling domain in bold
MAVMAPRTLVL LLSGALALTQTWAF L DSPDRPWN PPTFSPALLVVTEGDNATFTC SFSNTSESFVLNWYRMSPSNQTDKLA A FPEDRSQPGQDCRFRVTQLPNGRDFHMS VVRARRNDSGTYL CGAISLAPKAQIKESLRAELRV TERRAEVPTAHPSPSRPASQL <u>CCOLKFWLP</u>IGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAK <u>KSRLTDVTLQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL</u>
HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling Domain_Truncated OX-40 Signaling Domain (Other names: PD1 HLASP-ICOS -truncated OX40; HLA A2-SP-PD-1_ICOS_OX40t, HLA A2-SP-PD-1_ICOS_40t; HLA A2-SP-PD-1_ICOS_truncated CD134; PD1_ICOS_OX40t; PD1:ICOS40t) (SEQ ID NO: 28): PD 1 extracellular domain in regular font; ICOS domain underlined; and truncated OX40 (truncated CD134 signaling domain) in bold
MAVMAPRTLVL LLSGALALTQTWAF L DSPDRPWN PPTFSPALLVVTEGDNATFTC SFSNTSESFVLNWYRMSPSNQTDKLA A FPEDRSQPGQDCRFRVTQLPNGRDFHMS VVRARRNDSGTYL CGAISLAPKAQIKESLRAELRV TERRAEVPTAHPSPSRPASQL <u>CCOLKFWLP</u>IGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMRVNTAK <u>KSRLTDVTLGGGSFRTPIQEEQADAHSTLA</u>
HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling Domain (mini-CD28)_Truncated 4-1BB Signaling Domain (Other names: PD1_ICOS(28)_BBt; PD1_ICOS(28)_truncated CD137; PD1:ICOS(28)-BBt or PD1:ICOS(28)BBt) (SEQ ID NO: 29): PD 1 extracellular domain in regular font; ICOS (28) domain: ICOS portions are underlined and inserted CD28 portion bold and underline; and truncated CD137 domain in bold
MAVMAPRTLVL LLSGALALTQTWAF L DSPDRPWN PPTFSPALLVVTEGDNATFTC SFSNTSESFVLNWYRMSPSNQTDKLA A FPEDRSQPGQDCRFRVTQLPNGRDFHMS VVRARRNDSGTYL CGAISLAPKAQIKESLRAELRV TERRAEVPTAHPSPSRPASQL <u>CCOLKFWLP</u>IGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMT<u>PRRPGP</u> <u>TRKHYOPYAPPRAVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL</u>

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling Domain (mini-CD28)_Truncated OX-40 Signaling Domain (Other names: HLASP-ICOS DS-CD28(PRRP)-mutated CD134; PD1_ICOS(28)_OX40t; PD1_ICOS(28)_40t; PD1_ICOS(28)_truncated CD134; PD1:ICOS(28)-OX40t; PD1:ICOS(28)OX40t; PD1:ICOS(28)-40t or PD1:ICOS(28)40t) (SEQ ID NO: 30): PD 1 extracellular domain in regular font; ICOS (28) domain: ICOS portions are underlined and inserted CD28 portion bold and underline; and truncated CD134 domain in bold
MAVMAPRTLVLVLLSGALALTQTWAFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPASQLCCOLKFWLPIGCAAFVVVCILGCILICWLTKKKYSSSVHDPNGEYMFMT <u>PRRPGP</u> <u>TRKHYPYAPPRAVNTAKKSRLTDVT</u> <u>GGGSFRTPIQEEQADAHSTLA</u>
PD1 Extracellular (without HLA A2 Signal Peptide) (SEQ ID NO: 31)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPA
PD1 extracellular domain without signal peptide-CD28 domain swap (DS) (Other name: PD1_28) (SEQ ID NO: 32)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPALFPGPSKPFWVLVVGGVLACYSLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS
PD1 extracellular domain without signal peptide-CD28 DS-CD137 domain (Other name: PD1_28_BBwt) (SEQ ID NO: 33)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPALFPGPSKPFWVLVVGGVLACYSLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL
PD1 extracellular domain without signal peptide-CD28 DS- truncated CD137 domain (Other name: PD1_28_BBt) (SEQ ID NO: 34)

FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSPRPALFPGPSKPFWVLVVVGGVLACYSLLVT VAFHFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSQPFMRP VQTTQEEDGCSCRFPEEEEEGGCEL
PD1 extracellular domain without signal peptide-CD28 DS- truncated CD134 domain (Other name: PD1_28_OX40t) (SEQ ID NO: 35)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSPRPALFPGPSKPFWVLVVVGGVLACYSLLVT VAFHFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSGGGSFRT PIQEEQADAHSTLA
PD1 extracellular domain without signal peptide-ICOS DS (Other name: PD1_ICOS) (SEQ ID NO: 36)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSPRPASQLCCQLKFWLPICCAAFVVCILGCILI CWLTKKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTL
PD1 extracellular domain without signal peptide-ICOS DS- CD137 (Other name: PD1_ICOS_BBwt) (SEQ ID NO: 37)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSPRPASQLCCQLKFWLPICCAAFVVCILGCILI CWLTKKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLKRGRKKLLYIFKQPFM RPVQTTQEEDGCSCRFPEEEEEGGCEL
PD1 extracellular domain without signal peptide-ICOS DS-truncated CD137 (Other name: PD1_ICOS_BBt) (SEQ ID NO: 38)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSPRPASQLCCQLKFWLPICCAAFVVCILGCILI CWLTKKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGC SCRFPEEEEEGGCEL

PD1 extracellular domain without signal peptide-ICOS DS-truncated CD134 (Other name : PD1_ICOS_OX40t (SEQ ID NO: 39)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSRPASQLCCQLKFWLPIGCAAFVVVCILGCILI CWLTKKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLGGGSFRTPIQEEQADA HSTLA
PD1 extracellular domain without signal peptide-ICOS DS-CD28(PRRP)-truncated CD137) (Other name: PD1_ICOS(28)_BBt (SEQ ID NO: 40)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSRPASQLCCQLKFWLPIGCAAFVVVCILGCILI CWLTKKKKYSSSVHDPNGEYMFMTPRRPGPTRKHYQPYAPPRAVNTAKKSRLTDV TLQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL
PD1 extracellular domain without signal peptide-ICOS DS-CD28(PRRP)-truncated CD134 (Other name: PD1_ICOS(28)_OX40t) (SEQ ID NO: 41)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSRPASQLCCQLKFWLPIGCAAFVVVCILGCILI CWLTKKKKYSSSVHDPNGEYMFMTPRRPGPTRKHYQPYAPPRAVNTAKKSRLTDV TLGGGSFRTPIQEEQADAHSTLA
PD1 extracellular domain without signal peptide-ICOS DS-truncated CD137 domain-truncated CD2 signaling domain (Other name: PD1_ICOS_BBt:CD2t) (SEQ ID NO: 42)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSRPASQLCCQLKFWLPIGCAAFVVVCILGCILI CWLTKKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGC SCRFPEEEEEGGCELQNPATSQHPPPPGHRSQAPSHRPPPPGHRVQHQPQKRPPAPS GTQVHQKGPPLPRPRVQPKPPHGAAENSLSPSSN
PD1 extracellular domain without signal peptide-ICOS DS-truncated CD137 domain-truncated CD2 signaling domain (Other name: PD1_ICOS_BBt:IL2RB(YLRO)) (SEQ ID NO: 43)

FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSPRPASQLCCQLKFWLPIGCAAFVVCILGCILI CWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGC SCRFPEEEEGGCELNCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSP FPSSSFSPGGLAPEISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPTHLVSYLRQ WVVIPLPLSSPGPQAS
PD1 extracellular domain without signal peptide-ICOS DS-truncated CD137 domain- truncated CD2 signaling domain- IL-2 receptor binding (IL2RB)(YLRQ) protein (Other name: PD1_ICOS_BBt_CD2t_IL2RB(YLRQ) (SEQ ID NO: 44)
FLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKL AAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQI KESLRAELRVTERRAEVPTAHPSPSPRPASQLCCQLKFWLPIGCAAFVVCILGCILI CWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGC SCRFPEEEEGGCELQNPATSQHPPPPGHRSQAPSHRPPPPGHRVQHQPQRPPAPS GTQVHQKGPPLPRPRVQPKPPHGAAENSLSPSSNNCRNTGPWLKKVLKCNTPDP SKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEISPLEVLERDKVTQLLPLNTDA YLSLQELQGQDPTHLVSYLRQWVVIPLPLSSPGPQAS
HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling Domain_4-1BB Signaling Domain (Other names: PD1_28_BBwt; PD1_28_BB; PD1_CD28_BB; PD1_CD28_BB wt; PD1_CD28_CD137; PD1:28BB; PD1:28BB wt; PD1:28-BB or PD1:28-BB wt) (SEQ ID NO: 130)
MAVMAPRTLVLVLLSGALALTQTWAFLDSPDRPWNPPTFSPALLVVTEGDNATFTC SFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMS VVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPALFP GPSKPFVVLVVGGVLACYLLVTVAFIHFVVRSKRSRLHSDYMNMTPRRPGPT RKHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEG GCEL
HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling Domain_Truncated 4-1BB Signaling Domain (Other names: PD1_28_BBt; PD1_CD28_BBt; PD1_CD28_truncated CD137; PD1:28BBt; or PD1:28-BBt) (SEQ ID NO: 131)

MAVMAPRTLVLVLLSGALALTQTWAFDSDPDRPWNPPTFSPALLVVTEGDNATFTC SFSNTSESFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMS VVRARRNDSGTLYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPALFP GPSKPFWVLVVVGGVLACYLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPT RKHYQPYAPPRDFAAYRSQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL
HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling Domain_Truncated OX-40 Signaling Domain (Other names: PD1_28_OX40t; PD1_28_40t; PD1_CD28_OX40t; PD1_CD28_40t; PD-1_CD28_truncated CD134; PD1:2840t; PD1:28OX40t; PD1:20-OX40t or PD1:28-40t) (SEQ ID NO: 132): PD1 Extracellular (with HLA-A2 Signal Peptide)_CD28 Transmembrane_CD28 Signaling Domain_Truncated OX-40 Signaling Domain
MAVMAPRTLVLVLLSGALALTQTWAFDSDPDRPWNPPTFSPALLVVTEGDNATFTC SFSNTSESFVLNWYRMSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMS VVRARRNDSGTLYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPALFP GPSKPFWVLVVVGGVLACYLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPT RKHYQPYAPPRDFAAYRSGGGSFRTPIQEEQADAHSTLA

Table 6: Amino acid sequences of third and fourth signaling domains of RTCR.

Human CD3 ζ intracellular signaling domain (Other names: CD3Z (intracellular Signaling Domain); CD3Z) (SEQ ID NO: 45) RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKQRRK NPQEGLYNELOKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR
Human CD3 Z signaling domain truncated (CD3 Z truncated domain) (Other names: Human CD3 ζ signaling domain truncated Z; CD3 ζ truncated domain; CD3Zt or Zt) (SEQ ID NO: 46) RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGK
Human CD3 E signaling domain truncated (CD3 E truncated domain) (Other name: Human CD3 ϵ signaling domain truncated; CD3 ϵ truncated domain; CD3Et or Et (SEQ ID NO: 47) PVTRGAGAGGRQRGQNKERPPVPNPDIYPIRKGGQRDLYSGLNQRR

Human CD3 ZE signaling domain (CD3 ZE domain) (Other name: Human CD3 ζε signaling domain; CD3 ζε domain; CD3ZE or ZE) (SEQ ID NO: 48)
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPVTRG AGAGGRQRGQNKERPPPVNPDYEPKRGQRDLYSGLNQRRRI
CD2 truncated Signaling Domain (Other name: CD2 or 2) (SEQ ID NO: 49)
QNPATSQHPPPPGHRQAPSHRPPPPGHRVQHQPQKRPPAPSGTQVHQQKGPPLP RP RVQPKPPHGA AENSLSPSSN
IL-2 receptor binding (IL2RB) protein Signaling Domain (YLRQ shown in bold) (Other name: IL2RB(YLRQ)) (SEQ ID NO: 50)
NCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPFPSSSFSPGGLAPEI SPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPTHLVSYLRQWVVIPPLSSPGPQ AS

In some embodiments, the extracellular domain is derived from CD19 binding protein. In some embodiments, the CD19 binding protein is a CD19 binding chimeric antigen receptor (CAR). In some embodiments, the extracellular domain comprises the amino acid sequence according to SEQ ID NO: 51. In some embodiments, the extracellular domain comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 51.

In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to any one of SEQ ID NOs: 52-69. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 52. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 52.

In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 53. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 53.

In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 54. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 54.

In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 55. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 55.

5 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 56. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 56.

10 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 57. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 57.

15 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 58. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 58.

20 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 59. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 59.

In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 60. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 60.

25 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 61. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 61.

30 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to any one of SEQ ID NO: 62. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 62.

In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 63. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 63.

5 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 64. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 64.

10 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 65. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 65.

15 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 66. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 66.

20 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 67. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 67.

In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 68. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 68.

25 In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 69. In some embodiments, the CD19 binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 69.

Table 7: Amino acid sequences related to CD19 CAR based RTCR.

CD19 binding extracellular domain, FMC63scFV (Other name: CD19) (SEQ ID NO: 51): CD8a leader/signal peptide (bold, SEQ ID NO: 117) and CD8a Hinge (underlined, SEQ ID NO: 118) [FMC63 scFV (CD8a Leader_Light Chain_Linkers_Heavy Chain_CD8a Hinge)]
MALPVTALLLPLALLLHAARPD IQMTQTSSLSASLGDRVTISCRASQDISKYLN WYQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQ GNTLPYTFGGGTKLEITGGGGSGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTV SGVSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFL KMNSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSTVVSSTTTTPAPRPPTPAPTI <u>ASOPLSLRPEACRPAAGGAVHTRGLDFAC</u>
CD19 (FMC63 scFV)_CD8a Transmembrane_4-1BB Signaling_CD3Z chimeric antigen receptor (CAR) (Other names: FMC63scFV_BB_Z; CD19_BB_Z; CD19_BBwt_Z; CD19_CD137_Z; CD19-BBZ; or CD19:BBZ) (SEQ ID NO: 52): CD19 binding extracellular domain (underlined)-CD137 intracellular domain-CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPD</u> IQMTQTSSLSASLGDRVTISCRASQDISKYLNW <u>YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN</u> <u>TLPYTFGGGTKLEITGGGGSGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIROPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSTVVSSTTTTPAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGR</u> KKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQN QLYNELNLGRREEYDVLDRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_CD3Z CAR (Other names: FMC63scFV_28_Z; CD19_28_Z; CD19_CD28_Z; CD19_28Z or CD19-28Z or CD19:28Z) (SEQ ID NO: 53): CD19 binding extracellular domain (underlined)-CD28 DS- CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPD</u> IQMTQTSSLSASLGDRVTISCRASQDISKYLNW <u>YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN</u> <u>TLPYTFGGGTKLEITGGGGSGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIROPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLQTDDTAIYYCAKHYYYGGSYAMDYWGQGTSTVVSSTTTTPAPRPPTPAPTAS</u>

<u>QPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLVTV</u> <u>AFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSA</u> <u>DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYN</u> <u>ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR</u>
CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_4-1BB Signaling_CD3Z CAR (Other names: FMC63scFV_28_BBwt_Z; CD19_CD28_BB_Z; CD19_28_BBwt_Z; CD19-28BBwt_Z; CD19-28BBZ; or CD19:28BBZ) (SEQ ID NO: 54): [CD19 binding extracellular domain (underlined)-CD28 DS-CD137 intracellular domain-CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPDIOQTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDATYFCQOQN</u> <u>TLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIROPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLOTDDTAIYYCAKHYYYGGSYAMDYWGOGTSVTVSSTTTAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLVTV</u> <u>AFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSKRGRKKL</u> <u>LYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLY</u> <u>NELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEI</u> <u>GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR</u>
CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_4-1BB truncated Signaling_CD3Z CAR (Other names: FMC63scFV_28_BBt_Z; CD19_CD28_BBt_Z; CD19_28_BBt_Z; CD19-28BBt_Z; CD19-28BBtZ or CD19:28BBtZ) (SEQ ID NO: 55): [] CD19 binding extracellular domain (underlined)-CD28 DS-truncated CD137 intracellular domain-CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPDIOQTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDATYFCQOQN</u> <u>TLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIROPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLOTDDTAIYYCAKHYYYGGSYAMDYWGOGTSVTVSSTTTAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLVTV</u> <u>AFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSQPFMRPV</u> <u>QTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEY</u>

DVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTYDALHMQALPPR
CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_OX-40 Truncated Signaling_CD3Z CAR (Other names: FMC63scFV_28_OX40t_Z; CD19_CD28_OX40t_Z; CD19_28_OX40t_Z; CD19_28_OX40tZ; CD19_28_40t_Z; CD19_28_40tZ; CD19-2840tZ or CD19:2840tZ) (SEQ ID NO: 56): CD19 binding extracellular domain (underlined)-CD28 DS-truncated CD134 intracellular domain- CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPDQMTQTTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN</u> <u>TLPYTFGGGTKEITGGGGSGGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG</u> <u>YSLPDYGVSWIROPPRKGLEWLGVWGSSETTYYNSALKSRLTHKDNSKSOVFLKM</u> <u>NSLQTDDBTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSTTTTPAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLVTV</u> AFIIFWVRSKRSRLHSDYMNMTPRPGPTRKHYPYAPPRDFAAYRSGGGSFRTP IQEEQADAHSTLARVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGL STATKDTYDALHMQALPPR
CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_CD3Z CAR (Other names: FMC63scFV_ICOS_Z; CD19_ICOS_Z; CD19_ICOSZ; CD19-ICOSZ or CD19:ICOSZ) (SEQ ID NO: 57): [(CD19 binding extracellular domain (underlined)- ICOS DS - CD3ζ signaling domain)
<u>MALPVTALLLPLALLLHAARPDQMTQTTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN</u> <u>TLPYTFGGGTKEITGGGGSGGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG</u> <u>YSLPDYGVSWIROPPRKGLEWLGVWGSSETTYYNSALKSRLTHKDNSKSOVFLKM</u> <u>NSLQTDDBTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSTTTTPAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPIGCAAFVVVCILGCILIC</u> WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_4-1BB Signaling_CD3Z CAR (Other names: FMC63scFV_ICOS_BBwt_Z; CD19_ICOS_BB_Z; CD19_ICOS_BBwt_Z; CD19-ICOSBBwt_Z; CD19-ICOSBBZ or CD19:ICOSBBZ) (SEQ ID NO: 58): [] CD19 binding extracellular domain (underlined)- ICOS DS -CD137 intracellular domain-CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQOQN</u> <u>TLPYTFGGGTKEITGGGSGGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLOTDDTAIYYCAKHYYYGGSYAMDYWGOGTSVTVSSTTTTPAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPIGCAAFVVVCILGCILIC</u> WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRRE EYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRR GKGHDLGYQGLSTATKDTYDALHMQALPPR
CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_4-1BB Truncated Signaling_CD3Z CAR (Other names: FMC63scFV_ICOS_BBt_Z; CD19_ICOS_BBt_Z; CD19-ICOSBBtZ or CD19:ICOSBBtZ) (SEQ ID NO: 59): [] CD19 binding extracellular domain (underlined)- ICOS DS -truncated CD137 intracellular domain-CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQOQN</u> <u>TLPYTFGGGTKEITGGGSGGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLOTDDTAIYYCAKHYYYGGSYAMDYWGOGTSVTVSSTTTTPAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPIGCAAFVVVCILGCILIC</u> WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCS CRFPEEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDLGYQGL STATKDTYDALHMQALPPR

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_OX-40 Truncated Signaling_CD3Z CAR (Other names: FMC63scFV_ICOS_OX40t_Z; CD19_ICOS_OX40t_Z; CD19_ICOS_40t_Z; CD19_ICOS_OX40tZ; CD19_ICOS_40tZ; CD19-ICOS40tZ or CD19:ICOS40tZ) (SEQ ID NO: 60): CD19 binding extracellular domain (underlined)- ICOS DS –truncated CD134 intracellular domain-CD3ζ signaling domain
<u>MALPVTALLLPLALLLHAARPDQMTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQGN</u> <u>TLPYTFGGGTKEITGGGSGGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVWGSSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLQTD DTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSTTTAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPIGCAAFVVVCILGCILIC</u> WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLGGGSFRTPIQEEQADAH STLARVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPKQ RRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYD ALHMQALPPR
CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling (mini CD28 Signaling)_CD3Z CAR (Other names: FMC63scFV_ICOS(28)_Z; CD19_ICOS(28)_Z; CD19-ICOS(28)_Z; CD19-ICOS(28)Z or CD19:ICOS(28)Z) (SEQ ID NO: 61): [] CD19 binding extracellular domain (underlined)- ICOS DS-CD28(PRRP)-CD3ζ signaling domain)
<u>MALPVTALLLPLALLLHAARPDQMTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQQKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQGN</u> <u>TLPYTFGGGTKEITGGGSGGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVWGSSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLQTD DTAIYYCAKHYYYGGSYAMDYWGQTSVTVSSTTTAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPIGCAAFVVVCILGCILIC</u> WLTKKKYSSSVHDPNGEYMFMTPRRPGPTRKHYPYAPPRAVNTAKKSRLTDVT LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPKQRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRRGK GHDGLYQGLSTATKDTYDALH MQALPPR
CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling (mini CD28 Signaling)_4-1BB Truncated Signaling_CD3Z CAR (Other names: FMC63scFV_ICOS(28)_BBt_Z;

CD19_ICOS(28)_BBt_Z; CD19_ICOS(28)_BBtZ; CD19-ICOS(28)BBtZ or
 CD19:ICOS(28)BBtZ) (SEQ ID NO: 62): (CD19 binding extracellular domain
 (underlined)- ICOS DS-CD28(PRRP)-truncated CD137 intracellular domain-
 CD3ζ signaling domain)

MALPVTALLLPLALLLHAARPDQMTQTSSLSASLGDRVTISCRASQDISKYLNW
YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN
TLPYTFGGGKTLEITGGGGSGGGGSGGGGSEVKLOESGPGLVAPSOQLSVTCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVWGSSETTYYN SALKSRLTIHKDNSKSQVFLKM
NSLQTD DTAIYYCAKHYYYGGSYAMDYWGQGT SVTVSSTTTPAPRPPTPAPTAS
QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICAAAFVVVCILGCILIC
 WLTKKKYSSSVHDPNGEYMFMTPRRPGPTRKHYPYAPPRAVNTAKKSRLTDVT
 LQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL
 NLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGM
 KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling (mini CD28

Signaling)_OX-40 Truncated Signaling_CD3Z CAR (Other names:

FMC63scFV_ICOS(28)_OX40t_Z CD19_ICOS(28)_OX40t_Z;

CD19_ICOS(28)_OX40tZ; CD19_ICOS(28)_40t_Z; CD19_ICOS(28)_40tZ; CD19-
 ICOS(28)40tZ or CD19:ICOS(28)40tZ) (SEQ ID NO: 63): CD19 binding extracellular
 domain (underlined)- ICOS DS-CD28(PRRP)-truncated CD134 intracellular domain-
 CD3ζ signaling domain

MALPVTALLLPLALLLHAARPDQMTQTSSLSASLGDRVTISCRASQDISKYLNW
YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQQGN
TLPYTFGGGKTLEITGGGGSGGGGSGGGGSEVKLOESGPGLVAPSOQLSVTCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVWGSSETTYYN SALKSRLTIHKDNSKSQVFLKM
NSLQTD DTAIYYCAKHYYYGGSYAMDYWGQGT SVTVSSTTTPAPRPPTPAPTAS
QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICAAAFVVVCILGCILIC
 WLTKKKYSSSVHDPNGEYMFMTPRRPGPTRKHYPYAPPRAVNTAKKSRLTDVT
 LGGGSFRTPIQEEQADAHSTLARVKFSRSADAPAYQQGQNQLYNELNLGRREEYD
 VLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG
 HDGLYQGLSTATKDTYDALHMQALPPR

CD19_ICOSBBt_Zt CAR (CD19 binding extracellular domain- ICOS DS-truncated CD137 intracellular domain- CD3Ztruncated domain) CAR (Other names: FMC63scFV_ICOS_BBt_Zt; CD19_ICOS_BBt_Zt; CD19-ICOSBBtZt or CD19:ICOSBBtZt) (SEQ ID NO: 64); CD19 binding extracellular domain (underlined)- ICOS DS-truncated CD137 intracellular domain- CD3ζ truncated domain
<u>MALPVTALLLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEOEDIATYFCOQGN</u> <u>TLPYTFGGGTKEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLOTDDTAIYYCAKHYYYGGSYAMDYWGOGTSVTVSSTTTTPAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICAAAFVVVCILGCILIC</u> WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCS CRFPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGR DPEMGGK
CD19_ICOSBBt_ZE CAR (CD19 binding extracellular domain- ICOS DS -truncated CD137 intracellular domain- CD3ZE domain) (Other names: FMC63scFV_ICOS_BBt_ZE; CD19 CD19-ICOSBBtZE_ICOS_BBt_ZE; or CD19:ICOSBBtZE) (SEQ ID NO: 65); CD19 binding extracellular domain (underlined)- ICOS DS -truncated CD137 intracellular domain- CD3ζε domain
<u>MALPVTALLLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASQDISKYLNW</u> <u>YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEOEDIATYFCOQGN</u> <u>TLPYTFGGGTKEITGGGSGGGGSGGGGSEVKLQESGPGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYN SALKSRLTIHKDNSKSQVFLKM</u> <u>NSLOTDDTAIYYCAKHYYYGGSYAMDYWGOGTSVTVSSTTTTPAPRPPTPAPTAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICAAAFVVVCILGCILIC</u> WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCS CRFPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGR DPEMGGKPVTRGAGAGGRQGRGQNKERPPPVNPDYEPKRGQRDL YSGLNQRR
CD19_ICOSBBt:CD2t_Z CAR (CD19 binding extracellular domain- ICOS DS -truncated CD137 intracellular domain- CD2 truncated Signaling Domain -CD3 domain) CAR (Other names: FMC63scFV_ICOS_BBt_CD2tZ; CD19_ICOS_BBt_CD2t_Z; CD19_ICOS_BBt_CD2tZ; CD19_ICOS_BBtCD2tZ; CD19-ICOSBBtCD2tZ or CD19:ICOSBBtCD2tZ) (SEQ ID NO: 66). CD19 binding extracellular domain

(underlined)- ICOS DS -truncated CD137 intracellular domain- CD2 truncated Signaling Domain_-CD3ζ domain
<u>MALPVTALLLPLALLLHAARPD</u> <u>IQMTQT</u> <u>TSSLSASLGDRVTISCRASODISKYLNW</u> <u>YQQKPDGTVKLLIYHTSRLHSGVPSRFS</u> <u>SGSGTDYSLTISNLEQEDIATYFCQOQN</u> <u>TLPYTFGGG</u> <u>TKLEITGGGGSGGGGSGGGGSEVKLOESG</u> <u>PGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVWGS</u> <u>SETTYNSALKSRLTIKD</u> <u>NSKSQVFLKM</u> <u>NSLQTD</u> <u>DDTAIYYCAKHYYYGGSYAMDYWGQ</u> <u>TSVTVSSTTT</u> <u>PAPRPPTPAPT</u> <u>IAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFAC</u> <u>SQLCCQLKFWLPIGCAAFVVVCILGCILIC</u> <u>WLT</u> <u>TKKKYSSSVHDPNGEYMFMR</u> <u>AVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCS</u> <u>CRFPEEEEEGGCE</u> <u>LQNPATSQHPPPPGHR</u> <u>SQAPSHRPPPGHRVQHQPQKRPPAPSG</u> <u>TQVHQKQGPPLPRPRVQPKPPHGA</u> <u>ENSLSPSSNRVKFSRSADAPAYQQGQNQLY</u> <u>NELNLGRREEYDVL</u> <u>DKRRGRDPEMGGK</u> <u>PQRRKNPQEGLYNELQKDKMAEAYSEI</u> <u>GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR</u>
CD19_ICOSBBt:CD2t_ZE CAR (CD19 binding extracellular domain- ICOS DS - truncated CD137 intracellular domain- CD2 truncated Signaling Domain -CD3ZE domain) (Other names: FMC63scFV_ICOS_BBt_CD2tZE CAR CD19_ICOS_BBt_CD2tZE; CD19_ICOS_BBt_CD2t_ZE; CD19_ICOS_BBt_2tZE; CD19_ICOS_BBt_2t_ZE; CD19- ICOSBBtCD2tZE; CD19-ICOSBBt2tZE; CD19:ICOSBBtCD2tZE or CD19:ICOSBBt2tZE) (SEQ ID NO: 67): CD19 binding extracellular domain (underlined)- ICOS DS -truncated CD137 intracellular domain- CD2 truncated Signaling Domain_- CD3ζ domain
<u>MALPVTALLLPLALLLHAARPD</u> <u>IQMTQT</u> <u>TSSLSASLGDRVTISCRASODISKYLNW</u> <u>YQQKPDGTVKLLIYHTSRLHSGVPSRFS</u> <u>SGSGTDYSLTISNLEQEDIATYFCQOQN</u> <u>TLPYTFGGG</u> <u>TKLEITGGGGSGGGGSGGGGSEVKLOESG</u> <u>PGLVAPSQSLSVTCTVSG</u> <u>VSLPDYGVSWIRQPPRKGLEWLGVWGS</u> <u>SETTYNSALKSRLTIKD</u> <u>NSKSQVFLKM</u> <u>NSLQTD</u> <u>DDTAIYYCAKHYYYGGSYAMDYWGQ</u> <u>TSVTVSSTTT</u> <u>PAPRPPTPAPT</u> <u>IAS</u> <u>QPLSLRPEACRPAAGGAVHTRGLDFAC</u> <u>SQLCCQLKFWLPIGCAAFVVVCILGCILIC</u> <u>WLT</u> <u>TKKKYSSSVHDPNGEYMFMR</u> <u>AVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCS</u> <u>CRFPEEEEEGGCE</u> <u>LQNPATSQHPPPPGHR</u> <u>SQAPSHRPPPGHRVQHQPQKRPPAPSG</u> <u>TQVHQKQGPPLPRPRVQPKPPHGA</u> <u>ENSLSPSSNRVKFSRSADAPAYQQGQNQLY</u> <u>NELNLGRREEYDVL</u> <u>DKRRGRDPEMGGK</u> <u>PVTRGAGAGGRQ</u> <u>RGQNKERPPPVNPD</u> <u>YEP</u> <u>IRK</u> <u>GQ</u> <u>RDLYSGLNYRHQRRI</u>

CD19_ICOSBBt:IL2RB(YLRQ) _Z CAR (CD19 binding extracellular domain- ICOS DS -truncated CD137 intracellular domain- IL2RB(YLRQ) domain -CD3Z domain) (Other names: FMC63scFV_ICOS_BBt_IL2RB(YLRQ) Z CAR

CD19_ICOS_BBt_IL2RB(YLRQ)Z; CD19_ICOS_BBt_IL2RB(YLRQ)_Z;

CD19_ICOS_BBtIL2RB(YLRQ)Z; CD19-ICOSBBtIL2RB(YLRQ)Z or

CD19:ICOSBBtIL2RB(YLRQ)Z) (SEQ ID NO: 68): CD19 binding extracellular domain (underlined)- ICOS DS -truncated CD137 intracellular domain- IL2RB(YLRQ) domain_-CD3ζ domain)

MALPVTALLLPLALLLHAARPDQM TQTSSLSASLGDRVTISCRASQDISKYLNW
YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEOEDIA TYFCQOQN
TLPYTFGGGT KLEITGGGGSGGGSGGGGSEVKLOESGPGLVAPSQSLSVTCTVSG
VSLPDYGVSWIROPPRKGLEWLGVWGSSETTYNSALKSRLTIKDNSKSQVFLKM
NSLOTDDTAIYYCAKHYYYGGSYAMDYWGOGTSVTVSSTTTPAPRPPTPAPTAS
QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPIGCAAFVVVCILGCILIC
 WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCS
 CRFPEEEEGGCELNCRNTGPWLKKVLKNTDPDSKFFSQLSSEHGGDVQKWLSSPF
 PSSSFSPGGLAPEISPLEVLERDKVTQLPLNTDAYLSLQELQGQDPHTLV RVKFSR
 SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEG
 YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAYRHQALPP
 R

CD19_ICOSBBt:IL2RB(YLRQ) _ZE CAR (CD19 binding extracellular domain- ICOS DS -truncated CD137 intracellular domain- IL2RB(YLRQ) domain -CD3ZE domain) CAR

(Other names: FMC63scFV_ICOS_BBt_IL2RB(YLRQ) ZE

CD19_ICOS_BBt_IL2RB(YLRQ) ZE; CD19_ICOS_BBtIL2RB(YLRQ)ZE; CD19-

ICOSBBtIL2RB(YLRQ)ZE or CD19:ICOSBBtIL2RB(YLRQ)ZE) (SEQ ID NO: 69):

CD19 binding extracellular domain (underlined)- ICOS DS -truncated CD137 intracellular domain- IL2RB(YLRQ) domain_-CD3ζε domain

MALPVTALLLPLALLLHAARPDIQMTQTSSLSASLGDRVTISCRASODISKYLNW
YQOKPDGTVKLLIYHTSRLHSGVPSRFSGSGSGTDYSLTISNLEQEDIATYFCQOQN
TLPYTFGGGTKLEITGGGGSGGGGSGGGGSEVKLOESGPGLVAPSQSLSVCTVSG
VSLPDYGVSWIRQPPRKGLEWLGVIWGSETTYYNALKSRLTIKDNSKSOVFLKM
NSLOTDDTAIYYCAKHYYYGGSYAMDYWGQGTSTVTSSTTTPAPRPPTPAPTAS
QPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICAAAFVVVCILGCILIC
WLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEEDGCS
CRFEEEEEGGCELNCRNTGPWLKKVLKCNTPDPSKFFSQLSSEHGGDVQKWLSSPF
PSSSFSPGGLAPEISPLEVLERDKVTQLLPLNTDAYLSLQELQGQDPHTLVRVKFSR
SADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGKPVTRGAGAGGR
QRGQNKERPPVPNPDYPIRKQORDLYSGLNYRHQRRRI

In some embodiments, the extracellular domain comprises a hinge region. In some
 embodiments, the hinge region is derived from CD8, PD-1, CD28, ICOS, or IgG. In some
 embodiments, the transmembrane domain of the RTCR disclosed herein, is derived from
 5 CD8, PD1, CD28, ICOS, or IgG.

The present disclosure also provides a nucleic acid encoding the RTCR disclosed
 herein. In some embodiments, the nucleic acid encoding the RTCR disclosed herein is
 according to SEQ ID NO: 75-86 and 92-110. In some embodiments, the nucleic acid
 disclosed herein comprises a nucleic acid sequence encoding a chimeric intracellular domain.
 10 In some embodiments, the RTCR disclosed herein is for expression in a T cell, wherein the T
 cell co-expresses at least one of the endogenous co-stimulatory molecules CD28, CD2, OX-
 40, ICOS, CD28, CD3, CD4, CD8 and CD40L or a combination thereof.

The present disclosure also provides a vector comprising the nucleic acid disclosed
 herein. In some embodiments, the vector disclosed herein is any one of a viral vector, a
 15 plasmid, a cosmid, a yeast artificial chromosome, a bacterial artificial chromosome or a
 transposon/transposase system. In some embodiments, the viral vector is an adeno-viral
 vector or a lentiviral vector. In some embodiments, the vector is a lentiviral vector.

The present disclosure also provides a cell comprising the nucleic acid or the vector
 disclosed herein. In some embodiments, the cell disclosed herein is a modified T cell. In
 20 some embodiments, the modified T cell is an allogenic T cell. In some embodiments, the
 modified T cell is an autologous T cell. In some embodiments, the modified T cell is any one

of a naïve T cell, an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}), a central memory T cell (T_{CM}) and a regulatory T cell (T_{reg}).

In some embodiments, the extracellular domain is a B cell maturation Ag (BCMA) binding protein. In some embodiments, the BCMA binding protein is a BCMA specific T cell receptor (TCR). In some embodiments, the BCMA binding protein is a BCMA specific chimeric antigen receptor (CAR). In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to any one of: SEQ ID NOs: 137-146.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 137. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 137.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 138. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 138.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 139. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 139.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 140. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 140.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 141. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 141.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 142. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO:

5 142.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 143. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO:

10 143.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 144. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO:

15 144.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 145. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO:

20 145.

In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to SEQ ID NO: 146. In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO:

25 146.

In some embodiments, the extracellular domain is a B cell maturation Ag (BCMA) binding protein. In some embodiments, the BCMA binding protein is a BCMA specific T cell receptor (TCR). In some embodiments, the BCMA binding protein is a BCMA specific chimeric antigen receptor (CAR). In some embodiments, the BCMA binding chimeric antigen receptor comprises the amino acid sequence according to any one of: SEQ ID NOs: 141, 142, 145 and 146.

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Table 8: Amino acid sequences of BCMA specific chimeric antigen receptors (CAR) and BCMA specific CAR-based RTCR.

11-D5-3 scFv (CD8a Signal Peptide_11-D5-3 scFv, mouse_CD8a Hinge_BB_Z) (SEQ ID NO: 133): CD8a Signal Peptide (bold, SEQ ID NO: 117)_11-D5-3 scFv, mouse (italics)_CD8a Hinge (bold, SEQ ID NO: 118)_BB_Z)
MALPVTALLLPLALLLHAARPDIVLTQSPPSLAMSLGKRATISCRASESVTILGSHLIH <i>WYQQKPGQPPTLLIQLASNVQTGVPARFSGSGSRITDFTLTIDPVEEDDVAVYYCQSRIT</i> <i>PRTFGGGTKLEIKGSTSGSGKPGSGEGSTKGQIQLVQSGPELKKPGETVKISKASGYTF</i> <i>TDYSINWVKRAPGKGLKWMGWINTETREPAYAYDFRGRFAFSLETSASTAYLQINNLKY</i> <i>EDTATYFCALDYSYAMDYWGQGTSTVTSSTTTTPAPRPPTPAPTIASQPLSLRPEACR</i> PAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQP FMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGE RRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
FHVH33 HVV (CD8a Signal Peptide_FHVH33 HVV_CD8a Hinge_BB_Z) (SEQ ID NO: 134): (CD8a Signal Peptide (bold, SEQ ID NO: 117)_FHVH33 HVV (italicized)_CD8a Hinge (bold, SEQ ID NO: 118)_BB_Z)
MALPVTALLLPLALLLHAARPEVOLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS <i>WVRQAPGKGLEWVSSISGSGDYIYADSVKGRFTISRDISKNTLYLQMNSLRAEDTAVYY</i> <i>CAKEGTGANSSLADYRGQGTLVTVSSFPVFLPAKPTTTTPAPRPPTPAPTIASQPLSL</i> RPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNKRGR RKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPQRRKNPQEGLYNELQKDKMAE AYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
BCAR003 HVV (CD8a Signal Peptide_BCAR003 HVV_CD8a Hinge_BB_Z) (SEQ ID NO: 135): CD8a Signal Peptide (Bold)_BCAR003 HVV (italicized)_CD8a Hinge (Bold)_BB_Z

MALPVTALLLPLALLLHAARPQVKLEESGGGLVQAGRSLRLSCAASEHTFSSHVMG <i>WFRQAPGKERESVAVIGWRDISTSYADSVKGRFTISRDNAKKTLYLQMNSLKPEDTAVYY</i> <i>CAARRIDAADFDSWGQGTQVTVSSGGGGSGGGSGGGSGGGSGGGSSAVQLVES</i> <i>GGGLVQAGDSLRLTCTASGRAFSYFMAWFRQAPGKEREFVAGIAWSGGSTAYADSVK</i> <i>GRFTISRDNAKNTVYLQMNSLKSEDTAVYYCASRGIEVEEFGAWGQGTQVTVSSSTTTT</i> PAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCG VLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRV KFSRSADAPAYQQQNQLYNELNLGRREEYDVLDKRRGRDPGMPGPKPQRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA LPPR
GS15022 HVV (CD8a Signal Peptide_GS15022 HVV_CD8a Hinge_BB_Z) (SEQ ID NO: 136): CD8a Signal Peptide (bold, SEQ ID NO: 117)_GS15022 HVV (italicized)_CD8a Hinge (bold, SEQ ID NO: 118)_BB_Z
MALPVTALLLPLALLLHAARPQVKLEESGGGLVQAGRSLRLSCAASEHTFSSHVMG <i>WFRQAPGKERESVAVIGWRDISTSYADSVKGRFTISRDNAKKTLYLQMNSLKPEDTAVYY</i> <i>CAARRIDAADFDSWGQGTQVTVSSGGGGSGGGSGGGSGGGSEVQLVESGGGLVQAGGSL</i> <i>RLSCAASGRFTMGWFRQAPGKEREFVAAISLPTLAYAESVKGRFTISRDNAKNTVVL</i> <i>QMNSLKPEDTALYYCAADRKSVMISIRPDYWGQGTQVTVSSSTTTTPAPRPPTPAPTIA</i> SQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKR GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDKRRGRDPGMPGPKPQRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
BCMAsdAb1 HVV (CD8a Signal Peptide_anti-BCMAsdAb1 HHV_CD8a Hinge) (SEQ ID NO: 137): CD8a Signal Peptide (bold, SEQ ID NO: 117)_anti-BCMAsdAb1 HHV (italicized)_CD8a Hinge (bold, SEQ ID NO: 118)
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRFTSTYFMA <i>WFRQPPGKGLIYVGGIRWSDGVPHYADSVKGRFTISRDNAKNTVYLQMNSLRAEDTAV</i> <i>YFCASRGADGSDFGSYGQGTQVTVSSSTTTTPAPRPPTPAPTIASQPLSLRPEACRPA</i> AGGAVHTRGLDFAC
BCMAsdAb1 HVV_CD8a Transmembrane_4-1BB Signaling_CD3Z (Other names: BCMAsdAb1_BB_Z or BCMAsdAb1-BBZ) (SEQ ID NO: 138): BCMAsdAb1 HVV (bold, SEQ ID NO: 137)_CD8a Transmembrane_4-1BB Signaling_CD3Z

MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTEFST YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGVLLLSLVITLYCKR GRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQG QNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMA EAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
BCMAsdAb1 HVV_CD28 Transmembrane_CD28 Signaling_CD3Z (Other name: BCMAsdAb1_28_Z; BCMAsdAb1-28Z) (SEQ ID NO: 139): BCMAsdAb1 HVV (bold, SEQ ID NO: 137)_CD28 Transmembrane_CD28 Signaling_CD3Z
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTEFST YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLV TVAFHFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSR SADAPAYQQGQNQLYNELNLGRREEYDVLDRRRGRDPEMGGKPQRRKNPQEGL YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPP R
BCMAsdAb1 HVV_CD28 Transmembrane_CD28 Signaling_4-1BB Signaling_CD3Z (Other names: BCMAsdAb1_28_BBwt_Z; BCMAsdAb1_28_BB_Z; BCMAsdAb1_28_BBZ; BCMAsdAb1-28_BBz; or BCMAsdAb1-28BBZ) (SEQ ID NO: 140): BCMAsdAb1 HVV (bold, SEQ ID NO: 137)_CD28 Transmembrane_CD28 Signaling_4-1BB Signaling_CD3Z
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTEFST YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLV TVAFHFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSKRGRK KLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQ LYNELNLGRREEYDVLDRRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAY SEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

BCMAsdAb1 HVV_CD28 Transmembrane_CD28 Signaling_4-1BB truncated Signaling_CD3Z (Other names: BCMAsdAb1_28_BBt_Z; BCMAsdAb1_28_BBtZ; BCMAsdAb1-28_BBtZ; or BCMAsdAb1-28BBtZ) (SEQ ID NO: 141): BCMAsdAb1 HVV (bold, SEQ ID NO: 137)_CD28 Transmembrane_CD28 Signaling_4-1BB truncated Signaling_CD3Z
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTEFT YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLV TVAFHFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSQPFMRP VQTTQEEDGCSCRFPEEEEGGCELRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRG KGHDGLYQGLSTATKDTYDALHMQALPPR
BCMAsdAb1 HVV_CD28 Transmembrane_CD28 Signaling_OX-40 Truncated Signaling_CD3Z (Other names: BCMAsdAb1_28_OX40t_Z; BCMAsdAb1_28_OX40tZ; BCMAsdAb1_28_40t_Z; BCMAsdAb1_28_40tZ BCMAsdAb1-28_40tZ; or BCMAsdAb1-2840tZ) (SEQ ID NO: 142): BCMAsdAb1 HVV (bold, SEQ ID NO: 137)_CD28 Transmembrane_CD28 Signaling_OX-40 Truncated Signaling_CD3Z
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTEFT YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACLFPGPSKPFWVLVVVGGVLACYSLLV TVAFHFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRSGGGSFR TPIQEEQADAHSTLARVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQG LSTATKDTYDALHMQALPPR
BCMAsdAb1 HVV_ICOS Transmembrane_ICOS Signaling_CD3Z (Other names: BCMAsdAb1_ICOS_Z; BCMAsdAb1_ICOSZ; or BCMAsdAb1-ICOSZ) (SEQ ID NO: 143): BCMAsdAb1 HVV (bold, SEQ ID NO: 137)_ICOS Transmembrane_ICOS Signaling_CD3Z

MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTFST YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICCAAFVVVCILGCI LICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLRVKFSRSADAPAYQ QQQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDK MAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
BCMAsdAb1 HVV _ICOS Transmembrane _ICOS Signaling_4-1BB Signaling_CD3Z (Other names: BCMAsdAb1 _ICOS_BBwt_Z; BCMAsdAb1 _ICOS_BB_Z; BCMAsdAb1 _ICOS_BBZ; BCMAsdAb1-28_BBZ; or BCMAsdAb1-ICOSBBZ) (SEQ ID NO: 144): BCMAsdAb1 HVV (bold, SEQ ID NO: 137) _ICOS Transmembrane _ICOS Signaling_4-1BB Signaling_CD3Z
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTFST YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICCAAFVVVCILGCI LICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLKRGRKKLLYIFKQPF MRPVQTTQEEDGCSCRFPEEEEEGGCEL RVKFSRSADAPAYQQQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGER RRGKGHDGLYQGLSTATKDTYDALHMQALPPR
BCMAsdAb1 HVV _ICOS Transmembrane _ICOS Signaling_4-1BB Truncated Signaling_CD3Z (Other names: BCMAsdAb1 _ICOS_BBt_Z; BCMAsdAb1 _ICOS_BBtZ; BCMAsdAb1-ICOS_BBtZ; or BCMAsdAb1-ICOSBBtz) (SEQ ID NO: 145): BCMAsdAb1 HVV (bold, SEQ ID NO: 137) _ICOS Transmembrane _ICOS Signaling_4-1BB Truncated Signaling_CD3Z
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRTFST YFMAWFRQPPGKGLEIVGGIRWSDGVPHYADSVKGRFTISRDNANTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPICCAAFVVVCILGCI LICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLQPFMRPVQTTQEED GCSCRFPEEEEEGGCEL RVKFSRSADAPAYQQQNQLYNELNLGRREEYDVLDKRR GRDPEMGGKPQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQ GLSTATKDTYDALHMQALPPR

BCMAsdAb1 HVV_ICOS Transmembrane_ICOS Signaling_OX-40 Truncated Signaling_CD3Z (Other names: BCMAsdAb1_ICOS_OX40t_Z; BCMAsdAb1_ICOS_OX40tZ; BCMAsdAb1_ICOS40t_Z; BCMAsdAb1_ICOS_40tZ; BCMAsdAb1-ICOS_40tZ; or BCMAsdAb1-ICOS40tZ) (SEQ ID NO: 146); BCMAsdAb1 HVV (bold, SEQ ID NO: 137)_Transmembrane_ICOS Signaling_OX-40 Truncated Signaling_CD3Z
MALPVTALLLPLALLLHAARPEVQLQASGGGLAQPGGSLRLSCAASGRFTST YFMAWFRQPPGKGLEYYGGIRWSDGVPHYADSVKGRFTISRDNKNTVYLQ MNSLRAEDTAVYFCASRGADGSDFGSYGQGTQVTVSSTTTPAPRPPTPAPTIA SQPLSLRPEACRPAAGGAVHTRGLDFACSQLCCQLKFWLPIGCAAFVVVCILGCI LICWLTKKKYSSSVHDPNGEYMFMRVNTAKKSRLTDVTLGGGSFRTPIQEEQAD AHSTLARVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGK PQRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR

Table 9: Nucleic acid sequences of intracellular signaling domains, extracellular domains of RTCRs and RTCRs.

CD2 truncated Signaling Domain (SEQ ID NO: 70) CAGAATCCTGCCACCTCTCAGCACCTCCACCTCCACCTGGACACAGATCTCAG GCCCCATCTCACAGACCTCCACCACCTGGTCATCGGGTGCAGCATCAGCCCCA GAAAAGACCTCCTGCTCCTAGCGGAACACAGGTGCACCAGCAAAAGGGACCTC CACTGCCTAGACCTAGAGTGCAGCCTAAGCCTCCTCATGGCGCTGCCGAGAAT AGCCTGTCTCCTAGCAGCAAC
IL2RB(YLRQ) Signaling Domain (SEQ ID NO: 71) AATTGCAGAAACACAGGCCCTGGCTGAAGAAAGTGCTGAAGTGCAACACCCC TGATCCGAGCAAGTTCTTTAGCCAGCTGAGCAGCGAGCATGGCGGCGACGTTC AGAAATGGCTGTCTAGCCCATTTCTTAGCAGCAGCTTCAGCCCTGGTGGACTG GCCCCTGAGATTAGCCCTCTGGAAGTGCTGGAACGGGACAAAGTGACCCAGCT GCTGCCCCTGAATACCGACGCTTACCTGAGCCTGCAAGAGCTGCAAGGACAGG ACCCTACACACCTGGTGTCTACCTGAGACAGTGGGTCGTGATCCCTCCACCTC TCTCTAGTCCTGGACCTCAGGCCTCT
PD1 (PD1 Signal Peptide_PD1 Extracellular_PD1 Transmembrane_PD1 Intracellular) PD-1 (Other name:PD1-wt or PD1 (SEQ ID NO: 72)

ATGCAGATTCCITCAAGCTCCTTGGCCTGTCGTGTGGGCCGTTCTGCAACTTGGG
TGGCGGCCTGGCTGGTTCCTGGACTCTCCTGACAGACCCTGGAATCCTCCAACA
TTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGT
AGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCC
CAGCAACCAGACCGACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCG
GCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCAC
ATGTCGTGTCGTCCGGGCCAGAAGAAACGACAGCGGCACATACTGTGCGGGCGC
CATTTCTCTGGCCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGA
GAGTGACAGAAAGACGGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCA
AGACCTGCCGGCCAGTTCCAGACACTGGTCGTGGGAGTTGTTGGCGGACTGCT
GGGATCTCTGGTGTCTGCTTGTGTTGGGTGCTCGCCGTGATCTGTAGCAGAGCCGC
CAGAGGAACAATCGGCGCCAGAAGGACAGGCCAGCCTCTGAAAGAGGATCCC
TCTGCTGTCCCCGTGTTACGCGTGGACTATGGCGAGCTGGATTTCCAGTGGCGG
GAAAAGACACCCGAGCCTCCAGTGCCTTGTGTGCCTGAGCAGACAGAGTACGC
CACCATCGTGTTCCTAGCGGCATGGGCACATCTAGCCCTGCCAGAAGAGGAT
CTGCCGACGGACCTAGATCTGCCCAGCCTCTCAGACCTGAGGATGGCCACTGT
TCTTGGCCTCTT

PD1 Extracellular (Other name: PD1) (SEQ ID NO: 73)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCCG
GCCAGAAGAAACGACAGCGGCACATACTGTGCGGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCT

PD-1 (extracellular domain without signal peptide)-CD28 domain swap (DS) (SEQ ID NO: 74)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCCG

GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCTCTGTTC
CCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCC
TGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAG
CGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACGGCCCCG
ACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGCCGCCT
ACAGATCT

PD-1 (extracellular domain without signal peptide)-CD28 DS-CD137 domain (SEQ ID
NO: 75)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTTCGG
GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCTCTGTTC
CCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCC
TGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAG
CGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACGGCCCCG
ACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGCCGCCT
ACAGATCCAAGCGGGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTC
ATGCGGCCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGTCTCGGTTCCC
CGAGGAAGAAGAAGGCGGTTGCGAACTG

PD-1 (extracellular domain without signal peptide)-CD28 DS- truncated CD137 domain
(SEQ ID NO: 76)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTTCGG
GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC

TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCTCTGTTC
CCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCC
TGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAG
CGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACGGCCCCG
ACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGCCGCCT
ACAGATCCCAGCCTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGC
TGCTCCTGTCCGTTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTT

PD-1 (extracellular domain without signal peptide)-CD28 DS- truncated CD134 domain
(SEQ ID NO: 77)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCCG
GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCTCTGTTC
CCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTGCTGGCC
TGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGAAGCAAG
CGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACGGCCCCG
ACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCGCCGCCT
ACAGATCTGGCGGCGGAAGCTTCAGAACCCTATCCAAGAGGAACAGGCCGA
CGCTCACTCTACACTGGCT

PD-1 (extracellular domain without signal peptide)-ICOS DS (SEQ ID NO: 78)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCCG
GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA

GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT
TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA
GCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCGTGAAC
ACCGCCAAGAAGTCCAGACTGACCGACGTGACACTT

PD-1 (extracellular domain without signal peptide)-ICOS DS-truncated CD137 (SEQ ID
NO: 79)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCGG
GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA
GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT
TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA
GCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCGTGAAC
ACCGCCAAGAAGTCCAGACTGACCGACGTGACCCTGAAGCGGGGCAGAAAGA
AACTGCTGTACATCTTCAAGCAGCCCTTCATGCGGCCCCGTGCAGACCACACAA
GAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAGAAGAAGGCGGGCTGCG
AACTT

PD-1 (extracellular domain without signal peptide)-ICOS DS-truncated CD137 (SEQ ID
NO: 80)

TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG
CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCGG
GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA
GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT
TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA

GCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCGTGAAC ACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTCAGCCTTTCATGAGGCC CGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGCAGATTCCCCGAGGAA GAGGAAGGCGGTTGCGAACTT
PD-1 (extracellular domain without signal peptide)-ICOS DS-truncated CD134 (SEQ ID NO: 81) TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGG GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA GCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCGTGAAC ACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTGGCGGGCGGAAGCTTTAG AACCCTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCT
PD1 (extracellular domain without signal peptide)-ICOS DS-CD28(PRRP)-truncated CD137) (SEQ ID NO: 82) TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGG GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA GCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGACCCCTAGAAGG CCTGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGCCGTGAA

CACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTCAGCCTTTCATGAGGC CCGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGCAGATTCCCCGAGGAA GAGGAAGGCGGTTGCGAACTT
PD1 (extracellular domain without signal peptide)-ICOS DS-CD28(PRRP)-truncated CD134) (SEQ ID NO: 83) TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCCGCCAGGACTGCCGG TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCGG GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA GCAGCTCCGTGCACGACCCCCAACGGCGAGTACATGTTTCATGACCCCTAGAAGG CCTGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGCCGTGAA CACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTGCGCGCGGAAGCTTTA GAACCCCTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCT
PD1.ICOSBB1:CD2t (PD1 extracellular domain without signal peptide)-ICOS DS- truncated CD137 domain-truncated CD2 signaling domain) (SEQ ID NO: 84) TTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCTACATTCAGCCCCGCTCTG CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCCGCCAGGACTGCCGG TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGTTCGG GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA GCAGCTCCGTGCACGACCCCCAACGGCGAGTACATGTTTCATGAGAGCCGTGAAC ACCGCCAAGAAGTCCAGACTGACCGACGTGACCCCTCCAGCCTTTCATGAGGCC

TGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGTCGGTTCCTCCGAGGAAG
 AGGAAGGCGGTTGCGAACTCCAGAATCCTGCCACCTCTCAGCACCTCCACCT
 CCACCTGGACACAGATCTCAGGCCCCATCTCACAGACCTCCACCACCTGGTCAT
 CGGGTGCAGCATCAGCCCCAGAAAAGACCTCCTGCTCCTAGCGGAACACAGGT
 GCACCAGCAAAAGGGACCTCCACTGCCTAGACCTAGAGTGCAGCCTAAGCCTC
 CTCATGGCGCTGCCGAGAATAGCCTGTCTCCTAGCAGCAAC

PD1 extracellular domain without signal peptide-ICOS DS-truncated CD134 domain-
 truncated CD2 signaling domain (SEQ ID NO: 85)

TTTCTGGACAGCCCCGACAGACCTGGAATCCTCCTACATTCAGCCCCGCTCTG
 CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
 CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG
 ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG
 TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCGTGCTTCGG
 GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
 TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
 CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCCAGCCA
 GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT
 TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA
 GCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCGTGAAC
 ACCGCCAAGAAGTCCAGACTGACCGACGTGACCCTCCAGCCTTTCATGAGGCC
 TGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGTCGGTTCCTCCGAGGAAG
 AGGAAGGCGGCTGCGAACTGAATTGCAGAAACACAGGCCCCCTGGCTGAAGAA
 AGTGCTGAAGTGCAACACCCCTGATCCGAGCAAGTTCTTTAGCCAGCTGAGCA
 GCGAGCATGGCGGCGACGTTCAAGAAATGGCTGTCTAGCCCATTTCTTAGCAGC
 AGCTTCAGCCCTGGTGGACTGGCCCCTGAGATTAGCCCTCTGGAAGTGCTGGA
 ACGGGACAAAGTGACCCAGCTGCTGCCCCTGAATACCGACGCTTACCTGAGCC
 TGCAAGAGCTGCAAGGACAGGACCTACACACCTGGTGTCTACCTGAGACAG
 TGGGTCTGTATCCCTCCACCTCTCTCTAGTCCTGGACCTCAGGCCTCT

PD1 extracellular domain without signal peptide)-ICOS DS-truncated CD137 domain-
 truncated CD2 signaling domain- IL-2 receptor binding (IL2RB)(YLRQ) (SEQ ID NO: 86)

TTTCTGGACAGCCCCGACAGACCTGGAATCCTCCTACATTCAGCCCCGCTCTG
 CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC
 CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG

ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCCGCCAGGACTGCCGG
TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTCGG
GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC
TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA
CGGGCCGAAGTGCCACAGCTCACCTTTCACCTTCTCCAAGACCTGCCAGCCA
GCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTCGTGGT
TGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAAGTACA
GCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCGTGAAC
ACCGCCAAGAAGTCCAGACTGACCGACGTGACCTCCAGCCTTTCATGAGGCC
TGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGTCTGGTTCCCCGAGGAAG
AGGAAGGCGGTTGCGAACTCCAGAATCCTGCCACCTCTCAGCACCTCCACCT
CCACCTGGACACAGATCTCAGGCCCCATCTCACAGACCTCCACCACCTGGTCAT
CGGGTGCAGCATCAGCCCCAGAAAAGACCTCCTGCTCCTAGCGGAACACAGGT
GCACCAGCAAAAGGGACCTCCACTGCCTAGACCTAGAGTGCAGCCTAAGCCTC
CTCATGGCGCTGCCGAGAATAGCCTGTCTCCTAGCAGCAACAACCTGCCGCAAC
ACAGGCCCTGGCTGAAGAAAGTGCTGAAGTGCAACACCCCTGATCCGAGCAA
GTTCTTTAGCCAGCTGAGCAGCGAGCATGGCGGCGACGTTTCAGAAATGGCTGT
CTAGCCCATTTCCAAGCAGCAGCTTCAGCCCTGGTGGACTGGCCCCTGAGATTA
GCCCTCTGGAAGTGCTGGAACGGGACAAAGTGACCCAGCTGCTGCCCCTGAAT
ACCGACGCTTACCTGAGCCTGCAAGAGCTGCAAGGACAGGACCCTACACACCT
GGTGTCTACCTGAGACAGTGGGTCTGTATCCACCTCCTTTGAGCAGTCCAGG
ACCTCAGGCCTCT

CD3Z (intracellular Signaling Domain (Human CD3 ζ signaling domain) (SEQ ID NO:
87)

AGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAA
CCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTG
GACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAA
AGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGCCGA
GGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACAC
GATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCT
GCACATGCAGGCCCTGCCTCCAAGA

Human CD3 Z signaling domain truncated (CD3 Z truncated domain) (Other name:

Human CD3 ζ signaling domain truncated; CD3 ζ truncated domain) (SEQ ID NO: 88)

AGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAA
CCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTG
GACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAA

Human CD3 E signaling domain truncated (CD3 E truncated domain (Human CD3
ε signaling domain truncated (CD3 ε truncated domain) (SEQ ID NO: 89)

CCTGTGACTAGAGGTGCTGGTGCTGGCGGCAGACAGAGAGGCCAGAACAAAG
AAAGACCTCCTCCTGTGCCTAATCCTGACTACGAGCCCATCCGGAAGGGCCAG
AGAGATCTGTACAGCGGCCTGAACCAGCGGAGAATC

Human CD3 ZE signaling domain (CD3 ZE domain) (Human CD3 ζε signaling domain
(CD3 ζε domain) (SEQ ID NO: 90)

AGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAA
CCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTG
GACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCTGTGACTAGAG
GTGCTGGTGCTGGCGGCAGACAGAGAGGCCAGAACAAAGAAAGACCTCCTCC
TGTGCCTAATCCTGACTACGAGCCCATCCGGAAGGGCCAGAGAGATCTGTACA
GCGGCCTGAACCAGCGGAGAATC

FMC63 scFV (CD8a Leader_Light Chain_Linkers_Heavy Chain_CD8a Hinge) (CD19 binding extracellular domain) (SEQ ID NO: 91)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCCTGC

CD19 (FMC63 scFV)_CD8a Transmembrane_4-1BB Signaling_CD3Z (Other names:

FMC63scFV_BB_Z chimeric antigen receptor (CAR), CD19_BB_Z) (SEQ ID NO: 92)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT

GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTTCCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTCGCCTGCGACATCTACATCTGGGCCCCCTCTGGCTGGAACATG
TGGCGTGCTGCTGCTGAGCCTGGTCATCACCTGTATTGCAAGCGGGGCAGAA
AGAAACTGCTCTACATCTTCAAGCAGCCCTTCATGCGGCCCCGTGCAGACCACA
CAAGAGGAAGATGGCTGCTCCTGTTCGGTTCCCCGAGGAAGAAGAAGGCGGCT
GCGAGCTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAG
GGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACG
ACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCA
GCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAG
ATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCA
AGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTAT
GATGCCCTGCACATGCAGGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_CD3Z (Other names:
FMC63scFV_28_Z CAR, CD19_28_Z) (SEQ ID NO: 93)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTTCCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT

ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCCTGCCTGTTTCCCGGACCTAGCAAGCCTTTCTGGGTGCT
CGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGCCTT
CATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACA
TGAACATGACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTAC
GCTCCTCCTAGAGACTTCGCCGCTACAGATCTAGAGTGAAGTTCAGCAGATC
CGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGA
ACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGA
TCCTGAAATGGGCGGCAAGCCCCAGCGGAGAAAAGAATCCTCAAGAGGGCCTG
TATAATGAGCTGCAAAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAA
TGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCT
GAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTC
CAAGATGA

CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_4-1BB Signaling_CD3Z
(Other names: FMC63scFV_28_BBwt_Z CAR, CD19_28_BBwt_Z) (SEQ ID NO: 94)
ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG

TCTCTGAGGCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCCTGTTTCCCGGACCTAGCAAGCCTTTCTGGGTGCT
CGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGCCTT
CATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACA
TGAACATGACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTAC
GCTCCTCCTAGAGACTTCGCCGCTACAGATCCAAGCGGGGCAGAAAGAAGCT
GCTGTACATCTTCAAGCAGCCCTTCATGCGGCCCGTGACAGACCACACAAGAGG
AAGATGGCTGCTCCTGTCGGTTCCCCGAGGAAGAAGAAGGCGGTTGCGAACTG
AGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAA
CCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTG
GACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAA
AGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGCCGA
GGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGGACAC
GATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCT
GCACATGCAGGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_4-1BB truncated
Signaling_CD3Z (Other names: FMC63scFV_28_BBt_Z CAR, CD19_28_BBt_Z (SEQ
ID NO: 95)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA

ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCCTGTTTCCCGGACCTAGCAAGCCTTTCTGGGTGCT
CGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGCCTT
CATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACA
TGAACATGACCCCTAGACGGCCCCGGACCAACCAGAAAGCACTACCAGCCTTAC
GCTCCTCCTAGAGACTTCGCCGCCTACAGATCCCAGCCTTTCATGAGGCCTGTG
CAGACCACACAAGAAGAGGACGGCTGCTCCTGTTCGGTTCCCCGAGGAAGAGG
AAGGCGGTTGCGAACTTAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCC
TATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAG
AAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGG
CAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAA
AAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCA
GAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAA
GGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_CD28 Transmembrane_CD28 Signaling_OX-40 Truncated
Signaling_CD3Z (Other names: FMC63scFV_28_OX40t_Z CAR, CD19_28_OX40t_Z)
(SEQ ID NO: 96)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA

ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCCTGTTTCCCGGACCTAGCAAGCCTTTCTGGGTGCT
CGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGCCTT
CATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACA
TGAACATGACCCCTAGACGGCCCCGGACCAACCAGAAAGCACTACCAGCCTTAC
GCTCCTCCTAGAGACTTCGCCGCCTACAGATCTGGCGGCGGAAGCTTCAGAAC
CCCTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCTAGAGTGAAGT
TCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTAC
AACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGGA
GAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAAAGAATCCTCA
AGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGCCGAGGCCTACAGC
GAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGT
ACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCAG
GCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_CD3Z (Other names:

FMC63scFV_ICOS_Z CAR, CD19_ICOS_Z (SEQ ID NO: 97)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG

TCTCTGAGGCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGCTGCC
TATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACACTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGG
GCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGA
CGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAG
CGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGA
TGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAA
GGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATG
ATGCCCTGCACATGCAGGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_4-1BB Signaling_CD3Z
(Other names: FMC63scFV_ICOS_BBwt_Z CAR, CD19_ICOS_BBwt_Z) (SEQ ID NO:
98)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGCTGCC

TATTGGCTGCGCCGCCTTCGIGGTTGIGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTGAAGCGGGGCAGAAAGAAGCTGCTGTATATCTTCAAGCAGCCCTTCA
TGCGGCCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGTCTGGTTCCCC
GAGGAAGAAGAAGGCGGTTGCGAACTGAGAGTGAAGTTCAGCAGATCCGCCG
ACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTG
GGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTG
AAATGGGCGGCAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAA
TGAGCTGCAAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAG
GGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCA
CCGCCACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA
TGA

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_4-1BB Truncated

Signaling_CD3Z (Other names: FMC63scFV_ICOS_BBt_Z CAR, CD19_ICOS_BBt_Z
(SEQ ID NO: 99))

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG

AGGACTGGATTTCGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGCTGCC
TATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTCCAGCCTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGCT
GCTCCTGTGCGTTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTTAGAGTGAAG
TTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTA
CAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGG
AGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAAAAGAATCCTC
AAGAGGGCCTGTATAATGAGCTGCAAAAAGGACAAGATGGCCGAGGCCTACAG
CGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTG
TACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCA
GGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling_OX-40 Truncated
Signaling_CD3Z (Other names: FMC63scFV_ICOS_OX40t_Z CAR,
CD19_ICOS_OX40t_Z (SEQ ID NO: 100)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG

AGGACTGGATTTCGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGCTGCC
TATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACACTCGGCGGAGGCAGCTTTAGAACCCCTATCCAAGAGGAACAGGCCGAC
GCTCACTCTACACTGGCTAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGC
CTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGA
GAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCG
GCAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCA
AAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGC
AGAAGAGGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCA
AGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling (mini CD28

Signaling)_CD3Z (Other names: FMC63scFV_ICOS(28)_Z CAR, CD19_ICOS(28)_Z
(SEQ ID NO: 101)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTCGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGCTGCC

TATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGACCCCTAGACGGCCCGGACCTACCAGAAAGCACTACCAGCC
TTACGCTCCTCCTCGGGCCGTGAACACAGCCAAGAAAAGCAGACTGACCGACG
TGACCCTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAG
GGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACG
ACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCA
GCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAG
ATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCA
AGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTAT
GATGCCCTGCACATGCAGGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling (mini CD28 Signaling)_4-
1BB Truncated Signaling_CD3Z (Other names: FMC63scFV_ICOS(28)_BBt_Z CAR,
CD19_ICOS(28)_BBt_Z) (SEQ ID NO: 102)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGGCGGAGGAAGCGGAGGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGCTGCC
TATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG

CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGCGAG
TACATGTTTCATGACCCCTAGACGGCCCGGACCTACCAGAAAGCACTACCAGCC
TTACGCTCCTCCTCGGGCCGTGAACACAGCCAAGAAAAAGCAGACTGACCGACG
TGACCCTCCAGCCTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGC
TGCTCCTGTCCGTTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTTAGAGTGAA
GTTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGGCCAAAACCAGCTGT
ACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCG
GAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAAAGAATCCT
CAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGCCGAGGCCTACA
GCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACT
GTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGC
AGGCCCTGCCTCCAAGATGA

CD19 (FMC63 scFV)_ICOS Transmembrane_ICOS Signaling (mini CD28

Signaling)_OX-40 Truncated Signaling_CD3Z (Other names:

FMC63scFV_ICOS(28)_40t_Z CAR, CD19_ICOS(28)_40t_Z) (SEQ ID NO: 103)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGCTGCC

TATTGGCTGCGCCGCCTTCGIGGTTGIGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGACCCCTAGACGGCCCGGACCTACCAGAAAGCACTACCAGCC
TTACGCTCCTCCTCGGGCCGTGAACACAGCCAAGAAAAAGCAGACTGACCGACG
TGACACTCGGCGGAGGCAGCTTTAGAACCCCTATCCAAGAGGAACAGGCCGAC
GCTCACTCTACACTGGCTAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGC
CTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGA
GAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCG
GCAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCA
AAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGC
AGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCA
AGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGATGA

CD19_ICOSBBt_Zt CAR (CD19 binding extracellular domain- ICOS DS-truncated
CD137 intracellular domain- CD3Ztruncated domain) (Other names:

FMC63scFV_ICOS_BBt_Zt CAR, CD19_ICOS_BBt_Zt (SEQ ID NO: 104)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTGCCTGCAGCCAGCTGTGCTGTCAGCTGAAGTTCTGGCTGCC

TATCGGCTGCGCCGCCTTTGIGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTCCAGCCTTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGCT
GCTCCTGTGCGTTCCCCGAGGAAGAGGAAGGCGGTTGCGAGCTGAGAGTGAAG
TTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTA
CAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGG
AGAGGCAGAGATCCTGAAATGGGCGGCAAATGA

CD19_ICOSBBt_ZE CAR (CD19 binding extracellular domain- ICOS DS -truncated
CD137 intracellular domain- CD3ZE domain) (Other names: FMC63scFV_ICOS_BBt_ZE
CAR, CD19_ICOS_BBt_ZE (SEQ ID NO: 105)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTTCCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCAGCCAGCTGTGCTGTCAGCTGAAGTTCTGGCTGCC
TATCGGCTGCGCCGCCTTTGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTCCAGCCTTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGCT

GCTCCTGTCGGTITCCCCGAGGAAGAGGAAGGCGGTIGCGAGCTGAGAGTGAAG
TTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTA
CAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGG
AGAGGCAGAGATCCTGAAATGGGCGGCAAGCCTGTGACTAGAGGTGCTGGTG
CTGGCGGCAGACAGAGAGGCCAGAACAAAGAAAGACCTCCTCCTGTGCCTAAT
CCTGACTACGAGCCCATCCGGAAGGGCCAGAGAGATCTGTACAGCGGCCTGAA
CCAGCGGAGAATCTGA

CD19_ICOSBBt:CD2t_Z CAR (CD19 binding extracellular domain- ICOS DS -truncated
CD137 intracellular domain- CD2 truncated Signaling Domain -CD3 domain) (Other
names: FMC63scFV_ICOS_BBt_CD2tZ CAR, CD19_ICOS_BBt_CD2tZ (SEQ ID NO:
106)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTTCCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCAGCCAGCTGTGCTGTCAGCTGAAGTTCTGGCTGCC
TATCGGCTGCGCCGCCTTTGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTCCAGCCTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGCT

GCTCCTGTCGGTTCCTCCGAGGAAGAGGAAGGCGGTTGCGAACTCCAGAATCCT
GCCACCTCTCAGCACCTCCACCTCCACCTGGACACAGATCTCAGGCTCCTAGC
CACAGACCTCCACCACCTGGTCATAGAGTGCAGCACCAGCCTCAGAAGAGGCC
TCCTGCTCCTTCTGGAACACAGGTGCACCAGCAAAAGGGCCCTCCACTGCCTA
GACCTAGGGTGCAGCCTAAACCTCCTCATGGCGCCGCTGAGAACTCTCTGAGC
CCCAGCAGCAACAGAGTGAAGTTCAGCAGATCCGCGGACGCTCCTGCCTATCA
GCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAG
TACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGC
CCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGA
CAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGA
GGAAAGGGACACGACGGACTGTACCAGGGCCTGAGCACCGCCACAAAGGATA
CCTATGACGCCCTGCACATGCAGGCCCTGCCTCCAAGATGA

CD19_ICOSBBt:CD2t_ZE CAR (CD19 binding extracellular domain- ICOS DS -
truncated CD137 intracellular domain- CD2 truncated Signaling Domain -CD3ZE domain)
(Other names: FMC63scFV_ICOS_BBt_CD2tZE CAR, CD19_ICOS_BBt_CD2tZE)
(SEQ ID NO: 107)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG

AGGACTGGATTTCGCCTGCAGCCAGCTGTGCTGTCAGCTGAAGTTCTGGCTGCC
TATCGGCTGCGCCGCCTTTGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTCCAGCCTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGCT
GCTCCTGTGCGTTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTCCAGAATCCT
GCCACCTCTCAGCACCTCCACCTCCACCTGGACACAGATCTCAGGCTCCTAGC
CACAGACCTCCACCACCTGGTCATAGAGTGCAGCACCAGCCTCAGAAGAGGCC
TCCTGCTCCTTCTGGAACACAGGTGCACCAGCAAAAGGGCCCTCCACTGCCTA
GACCTAGGGTGCAGCCTAAACCTCCTCATGGCGCCGCTGAGAACTCTCTGAGC
CCCAGCAGCAACAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCA
GCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAG
TACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGC
CTGTGACTAGAGGTGCTGGCGCTGGTGGAAGGCAGAGAGGCCAGAACAAAGA
ACGGCCTCCTCCTGTGCCTAATCCTGACTACGAGCCCATCCGGAAGGGCCAGA
GAGATCTGTACAGCGGCCTGAACTACAGACACCAGCGGAGAATCTGA

CD19_ICOSBBt:IL2RB(YLRQ)_ZCAR (CD19 binding extracellular domain- ICOS DS -
truncated CD137 intracellular domain- IL2RB(YLRQ) domain -CD3Z domain) (Other

names: FMC63scFV_ICOS_BBt_IL2RB(YLRQ)Z CAR,

CD19_ICOS_BBt_IL2RB(YLRQ)Z (SEQ ID NO: 108)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT
GGATCTGAAGTGAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTCTGAAGATGAACAGCCTCCAGAC

CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCAGCCAGCTGTGCTGTCAGCTGAAGTTCTGGCTGCC
TATCGGCTGCGCCGCCTTTGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTCCAGCCTTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGCT
GCTCCTGTGCGTTCCCCGAGGAAGAGGAAGGCGGCTGCGAACTGAATTGCAGA
AACACAGGCCCCCTGGCTGAAGAAAGTGCTGAAGTGCAACACCCCTGATCCGAG
CAAGTTTTTTCAGCCAGCTGAGCAGCGAGCATGGCGGCGACGTTTCAGAAATGGC
TGTCTAGCCCATTTCCTAGCTCCAGCTTCAGCCCTGGTGGACTGGCCCCCTGAGA
TTAGCCCTCTGGAAGTGCTGGAACGGGACAAAGTGACCCAGCTGCTGCCCCCTG
AATACCGACGCCTACCTGAGCCTGCAAGAGCTGCAAGGCCAGGATCCTACACA
CCTCGTGCGCGTGAAGTTCAGCAGATCCGCTGATGCCCCCTGCCTATCAGCAGG
GCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGA
CGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAG
CGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGA
TGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAA
GGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTACG
ATGCCTACAGACACCAGGCTCTGCCACCTAGATGA

CD19_ICOSBBt:IL2RB(YLRQ) _ZE CAR (CD19 binding extracellular domain- ICOS DS
-truncated CD137 intracellular domain- IL2RB(YLRQ) domain -CD3ZE domain (Other
names: FMC63scFV_ICOS_BBt_IL2RB(YLRQ) _ZE CAR) (SEQ ID NO: 110)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCCAGATGACCCAGACAACCAGCAGCCTGTCTGCCAGCCT
GGGCGATAGAGTGACCATCAGCTGTAGAGCCAGCCAGGACATCAGCAAGTAC
CTGAACTGGTATCAGCAGAAACCCGACGGCACCGTGAAGCTGCTGATCTACCA
CACCAGCAGACTGCACAGCGGCGTGCCAAGCAGATTTTCTGGCAGCGGCTCTG
GCACCGACTACAGCCTGACCATCTCCAACCTGGAACAAGAGGATATCGCTACC
TACTTCTGCCAGCAAGGCAACACCCTGCCTTACACCTTTGGCGGAGGCACCAA
GCTGGAAATCACAGGCGGCGGAGGAAGCGGAGGCGGAGGATCTGGTGGTGGT

GGATCTGAAGTGAAACTGCAAGAGTCTGGCCCTGGCCTGGTGGCCCCATCTCA
ATCTCTGAGCGTGACCTGTACCGTCAGCGGAGTGTCCCTGCCTGATTATGGCGT
GTCCTGGATCCGGCAGCCTCCTAGAAAAGGCCTGGAATGGCTGGGCGTGATCT
GGGGCAGCGAGACAACCTACTACAACAGCGCCCTGAAGTCCCGGCTGACCATC
ATCAAGGACAACCTCCAAGAGCCAGGTGTTTCCTGAAGATGAACAGCCTCCAGAC
CGACGACACCGCCATCTACTATTGCGCCAAGCACTACTACTACGGCGGCAGCT
ACGCCATGGATTATTGGGGCCAGGGCACCAGCGTGACCGTGTCTAGTACAACA
ACCCCTGCTCCTCGGCCTCCTACACCAGCTCCTACAATTGCCAGCCAGCCACTG
TCTCTGAGGCCCCGAAGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAG
AGGACTGGATTTTCGCTGCAGCCAGCTGTGCTGTCAGCTGAAGTTCTGGCTGCC
TATCGGCTGCGCCGCCTTTGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTG
CTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGACGACCCCAACGGCGAG
TACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGT
GACCCTCCAGCCTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACGGCT
GCTCCTGTGCGTTCCCCGAGGAAGAGGAAGGCGGCTGCGAACTGAATTGCAGA
AACACAGGGCCCCTGGCTGAAGAAAGTGCTGAAGTGCAACACCCCTGATCCGAG
CAAGTTTTTTCAGCCAGCTGAGCAGCGAGCATGGCGGCGACGTTTCAGAAATGGC
TGTCTAGCCCATTTCCTAGCTCCAGCTTCAGCCCTGGTGGACTGGCCCCCTGAGA
TTAGCCCTCTGGAAGTGCTGGAACGGGACAAAGTGACCCAGCTGCTGCCCCCTG
AATACCGACGCCTACCTGAGCCTGCAAGAGCTGCAAGGCCAGGATCCTACACA
CCTCGTGCGCGTGAAGTTCAGCAGATCCGCTGATGCCCCTGCCTATCAGCAGG
GCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGA
CGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCTGTG
ACTAGAGGTGCTGGTGCTGGCGGCAGACAGAGAGGCCAGAACAAAGAAAGAC
CTCCTCCTGTGCCTAATCCTGACTACGAGCCCATCCGGAAGGGCCAGAGAGAT
CTGTACAGCGGCCTGAACTACAGACACCAGCGGAGAATCTGA

CD28_BB signaling domain: CD28 signaling domain_4-1BB Signaling Domain (SEQ NO:
151)

CTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTG
CTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGA
AGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACG
GCCCCGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCG
CCGCCTACAGATCCAAGCGGGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAG

CCCTTCATGCGGCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGTCG GTTCCCCGAGGAAGAAGAAGGCGGTTGCGAACTG
CD28 Signaling Domain_truncated 4-1BB Signaling Domain (Other name: CD28_BBt): (SEQ ID NO: 152) CTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTG CTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGA AGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACG GCCCCGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCG CCGCCTACAGATCCCAGCCTTTTCATGAGGCCTGTGCAGACCACACAAGAAGAG GACGGCTGCTCCTGTCGGTTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTT
CD28 Signaling Domain_truncated OX-40 Signaling Domain (Other name: CD28_OX40t): (SEQ ID NO: 153) CTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTG CTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGA AGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACG GCCCCGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCG CCGCCTACAGATCTGGCGGCGGAAGCTTCAGAACCCCTATCCAAGAGGAACAG GCCGACGCTCACTCTACACTGGCT
ICOS Transmembrane_ICOS Signaling Domain (SEQ ID NO: 154) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTT
ICOS Transmembrane_ICOS Signaling Domain_4-1BB Signaling Domain (SEQ ID NO: 155) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACCCTGAAGCGGGGCAG AAAGAACTGCTGTACATCTTCAAGCAGCCCTTCATGCGGCCCCGTGCAGACCA CACAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAGAAGAAGGCGG CTGCGAACTT

ICOS Transmembrane_ICOS Signaling Domain_Truncated 4-1BB Signaling Domain (SEQ ID NO: 156) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTCAGCCTTTCATG AGGCCCCGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGCAGATTCCCCGA GGAAGAGGAAGGCGGTTGCGAACTT
ICOS Transmembrane_ICOS Signaling Domain_Truncated OX-40 Signaling Domain (ICOS_OX40t) (SEQ ID NO: 157) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTGCGCGCGGAAG CTTTAGAACCCCTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCT
ICOS Transmembrane_ICOS Signaling Domain (mini-CD28)_Truncated 4-1BB Signaling Domain (ICOS(28)_BBt) (SEQ ID NO: 158) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGACCCCTA GAAGGCCTGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGCC GTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTCAGCCTTTCAT GAGGCCCGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGCAGATTCCCCG AGGAAGAGGAAGGCGGTTGCGAACTT
ICOS(28)_truncated OX-40 (CD134) intracellular domain (ICOS(28)_OX40t) (SEQ ID NO: 159) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGACCCCTA GAAGGCCTGGACCTACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGCC GTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTGCGCGCGGAA GCTTTAGAACCCCTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCT

ICOS Transmembrane_ICOS Signaling Domain_4-1BB Signaling Domain with mutated polybasic region (ICOS_BB(xPB)) (SEQ ID NO: 160)

AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC
GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA
GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG
TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACCCTGGCTGCCGGCGCT
GCAGCTCTGCTGTACATCTTCAAGCAGCCCTTCATGCGGCCCGTGCAGACCACA
CAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAGAAGAAGGCGGCT
GCGAACTT

ICOS Transmembrane_ICOS Signaling Domain_4-1BB Signaling Domain with mutated lysines (ICOS_BB(xUb)) (SEQ ID NO: 161)

AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC
GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA
GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG
TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACCCTGAAGCGGGGCAG
AAAGAACTGCTGTACATCTTCGCACAGCCCTTCATGCGGCCCGTGCAGACCA
CACAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAGAAGAAGGCGG
CTGCGAACTT

ICOS Transmembrane_ICOS Signaling Domain_4-1BB Signaling Domain with mutated lysines and polybasic regions (ICOS_BB(xPBxUb)) (SEQ ID NO: 162)

AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC
GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA
GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG
TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACCCTGGCTGCCGGCGCT
GCAGCTCTGCTGTACATCTTCGCACAGCCCTTCATGCGGCCCGTGCAGACCACA
CAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAGAAGAAGGCGGCT
GCGAACTT

ICOS Transmembrane_ICOS Signaling Domain_Wild Type OX-40 Signaling Domain (ICOS_40) (SEQ ID NO: 163)

AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC
GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA
GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG
TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTAGGAGAGACCA

CGGTCTGCCACCAGATGCACATAAGCCACCTGGCGGCGGAAGCTTTAGAACCC CTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCTAAAATC
ICOS Transmembrane_ICOS Signaling Domain_OX-40 Signaling Domain with mutated polybasic region (ICOS_40 (xPB)) (SEQ ID NO: 164) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTGCCGCGGACCAG GCCCTGCCACCAGATGCACATAAGCCACCTGGCGGCGGAAGCTTTAGAACCCC TATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCTAAAATC
ICOS Transmembrane_ICOS Signaling Domain_OX-40 Signaling Domain with mutated lysine residues (ICOS_40 (xUb)): (SEQ ID NO: 165) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTAGGAGAGACCA GCGTCTGCCACCAGATGCACATGCACCACCTGGCGGCGGAAGCTTTAGAACCC CTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCTGCAATC
ICOS Transmembrane_ICOS Signaling Domain_OX-40 Signaling Domain with mutated lysine residues and polybasic regions (ICOS_40 (xPBxUb)): (SEQ ID NO: 166) AGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGCTGTGCCGCCTTC GTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTGACCAAGAAAAA GTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTTTCATGAGAGCCG TGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTGCCGCGGACCAG GCCCTGCCACCAGATGCACATGCACCACCTGGCGGCGGAAGCTTTAGAACCCC TATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCTGCAATC
PD1 Extracellular (SEQ ID NO: 167) TTCTGGACTCTCTGACAGACCCTGGAATCCTCCAACATTCAGCCCCGCTCTG CTGGTGGTTACCGAGGGCGATAATGCCACCTTCACCTGTAGCTTCAGCAACAC CAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAGCCCCAGCAACCAGACCG ACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGCCCGGCCAGGACTGCCGG TTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTCCACATGTCTGTCTGTCGCGG GCCAGAAGAAACGACAGCGGCACATATCTGTGCGGCGCCATTTCTCTGGCCCC

TAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGCTGAGAGTGACAGAAAGA CGGGCCGAAGTGCCACAGCTCACCTTCACCTTCTCCAAGACCTGCC
HLA-A2 Signal Peptide_PD1 Extracellular_PD1 Transmembrane (PD1 (HLASP- Truncated; PD1_TLs) (SEQ ID NO: 168) ATGGCTGTGATGGCTCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC TGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCTGAGGATAGATCTCAGC CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG CGCCATTTCTCTGGCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC TGAGAGTGACAGAAAGACGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT CCAAGACCTGCCGGCCAGTTCAGACACTGGTCGTGGGAGTTGTTGGCGGCCT GCTGGGATCTCTGGTTCTGCTTGTGTTGGTGCTCGCCGTGATCTGCTCTAGA
HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling Domain (HLASP CD28 DS or PD1_CD28 or PD1_28) (SEQ ID NO: 169) ATGGCTGTGATGGCTCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC TGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCTGAGGATAGATCTCAGC CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG CGCCATTTCTCTGGCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC TGAGAGTGACAGAAAGACGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT CCAAGACCTGCTCTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTT GTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATC TTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACAT GACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTC CTAGAGACTTCGCCGCCTACAGATCT

HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling
Domain_ 4-1BB Signaling Domain (PD1_28_BBwt) (SEQ ID NO: 170)

ATGGCTGTGATGGCCCCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT
CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT
ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC
TG TAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG
CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGC
CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC
CACATGTCTGTCGTTTCGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG
CGCCATTTCTCTGGCCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC
TGAGAGTGACAGAAAGACGGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT
CCAAGACCTGCTCTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTT
GTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATC
TTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACAT
GACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTC
CTAGAGACTTCGCCGCCTACAGATCCAAGCGGGGCAGAAAGAAGCTGCTGTAC
ATCTTCAAGCAGCCCTTCATGCGGCCCGTGCGAGACCACACAAGAGGAAGATGG
CTGCTCCTGTGCGGTTCCCCGAGGAAGAAGAAGGCGGTTGCGAACTG

HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling
Domain_ Truncated 4-1BB Signaling Domain (PD1_28_BBt) (SEQ ID NO: 171)

ATGGCTGTGATGGCCCCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT
CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT
ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC
TG TAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG
CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGC
CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC
CACATGTCTGTCGTTTCGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG
CGCCATTTCTCTGGCCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC
TGAGAGTGACAGAAAGACGGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT
CCAAGACCTGCTCTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTT
GTTGGCGGCGTGCTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATC
TTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACAT
GACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTC

CTAGAGACTTCGCCGCCTACAGATCCCAGCCTTTCATGAGGCCTGTGCAGACC
ACACAAGAAGAGGACGGCTGCTCCTGTCTGGTTCCCCGAGGAAGAGGAAGGCG
GTTGCGAACTT

HLA-A2 Signal Peptide_PD1 Extracellular_CD28 Transmembrane_CD28 Signaling
Domain_Truncated OX-40 Signaling Domain (PD1_28_OX40t) (SEQ ID NO: 172)

ATGGCTGTGATGGCCCCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT
CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT
ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC
TGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG
CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGC
CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCCAACGGCCGGGACTTC
CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG
CGCCATTTCTCTGGCCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC
TGAGAGTGACAGAAAGACGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT
CCAAGACCTGCTCTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTT
GTGCGCGGTGCTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATC
TTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACAT
GACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTC
CTAGAGACTTCGCCGCCTACAGATCTGGCGGCGGAAGCTTCAGAACCCCTATC
CAAGAGGAACAGGCCGACGCTCACTCTACACTGGCT

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS intracellular domain (PD1_ICOS) (SEQ
ID NO: 173)

ATGGCTGTGATGGCTCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT
CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT
ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC
TGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG
CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGC
CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCCAACGGCCGGGACTTC
CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG
CGCCATTTCTCTGGCCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC
TGAGAGTGACAGAAAGACGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT
CCAAGACCTGCCAGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGC
TGTGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTG

ACCAAGAAAAAGTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTT CATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTT
HLA-A2 Signal Peptide_PD1 Extracellular_ICOS DS_CD137 (PD1_ICOS_BBwt) (SEQ ID NO: 174) ATGGCTGTGATGGCTCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC TG TAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCTGAGGATAGATCTCAGC CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG CGCCATTTCTCTGGCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC TGAGAGTGACAGAAAGACGGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT CCAAGACCTGCCAGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGC TGTGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTG ACCAAGAAAAAGTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTT CATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACCTGA AGCGGGGCAGAAAGAAACTGCTGTACATCTTCAAGCAGCCCTTCATGCGGCCC GTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGCAGATTCCCCGAGGAAG AAGAAGGCGGCTGCGAACTT
HLA-A2 Signal Peptide_PD1 Extracellular_ICOS_truncated CD137 (PD1_ICOS_BBt) (SEQ ID NO: 175) ATGGCTGTGATGGCTCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC TG TAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCTGAGGATAGATCTCAGC CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG CGCCATTTCTCTGGCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC TGAGAGTGACAGAAAGACGGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT CCAAGACCTGCCAGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGC TGTGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTG

ACCAAGAAAAAGTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTT
CATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTC
AGCCTTTCATGAGGCCCCGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGC
AGATTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTT

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS _truncated CD134 (PD1_ICOS_OX40t)
(SEQ ID NO: 176)

ATGGCTGTGATGGCTCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT
CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT
ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC
TGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG
CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGC
CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC
CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG
CGCCATTTCTCTGGCCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC
TGAGAGTGACAGAAAGACGGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT
CCAAGACCTGCCAGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGC
TGTGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTG
ACCAAGAAAAAGTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTT
CATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTTG
GCGGCGGAAGCTTTAGAACCCCTATCCAAGAGGAACAGGCCGACGCTCACTCT
ACACTGGCT

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling
Domain (mini-CD28)_Truncated 4-1BB Signaling (PD1_ICOS(28)_BBt) (SEQ ID NO:
177)

ATGGCTGTGATGGCTCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCT
CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT
ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC
TGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG
CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCCTGAGGATAGATCTCAGC
CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC
CACATGTCTGTCGTTCTGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG
CGCCATTTCTCTGGCCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC
TGAGAGTGACAGAAAGACGGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT

CCAAGACCTGCCAGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGC
TGTGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTG
ACCAAGAAAAAGTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTT
CATGACCCCTAGAAGGCCTGGACCTACCAGAAAGCACTACCAGCCTTACGCTC
CTCCTAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTT
CAGCCTTTTCATGAGGCCCGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTG
CAGATTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTT

HLA-A2 Signal Peptide_PD1 Extracellular_ICOS Transmembrane_ICOS Signaling
Domain (mini-CD28)_Truncated OX-40 Signaling Domain (PD1 (HLASP-ICOS DS-
CD28(PRRP)-mutated CD134) (PD1_ICOS_OX40t, PD1:ICOS40t) (SEQ ID NO: 178)
ATGGCTGTGATGGCTCCTAGAACAACCTGGTGCTGCTGCTGTCTGGTGCCCTGGCT
CTGACTCAGACATGGGCCTTTCTGGACAGCCCCGACAGACCCTGGAATCCTCCT
ACATTCAGCCCCGCTCTGCTGGTGGTTACCGAGGGCGATAATGCCACCTTCACC
TGTAGCTTCAGCAACACCAGCGAGAGCTTCGTGCTGAACTGGTACAGAATGAG
CCCCAGCAACCAGACCGACAAGCTGGCCGCCTTTCTGAGGATAGATCTCAGC
CCGGCCAGGACTGCCGGTTCAGAGTTACACAGCTGCCCAACGGCCGGGACTTC
CACATGTCTGTCGTTTCGGGCCAGAAGAAACGACAGCGGCACATATCTGTGCGG
CGCCATTTCTCTGGCCCCTAAGGCTCAGATCAAAGAGAGCCTGAGAGCCGAGC
TGAGAGTGACAGAAAGACGGGCCGAAGTGCCACAGCTCACCTTCACCTTCT
CCAAGACCTGCCAGCCAGCTGTGCTGCCAGCTGAAGTTTTGGCTGCCTATCGGC
TGTGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGATCTGCTGGCTG
ACCAAGAAAAAGTACAGCAGCTCCGTGCACGACCCCAACGGCGAGTACATGTT
CATGACCCCTAGAAGGCCTGGACCTACCAGAAAGCACTACCAGCCTTACGCTC
CTCCTAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGACGTGACACTT
GGCGGCGGAAGCTTTAGAACCCTATCCAAGAGGAACAGGCCGACGCTCACTC
TAACTGGCT

11-D5-3 scFv (CD8a Signal Peptide_11-D5-3 scFv, mouse_CD8a Hinge_BBZ) (SEQ ID
NO: 179)
ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCCAGACCTGACATCGTGCTGACACAGTCTCCACCTAGCCTGGCCATGAGCCT
GGGAAAGAGAGCCACCATCAGCTGTAGAGCCAGCGAGAGCGTGACAATCCTG
GGCTCTCACCTGATCCACTGGTATCAGCAGAAGCCCGGCCAGCCTCCTAACT

GCTGATTTCAGCTGGCCTCCAATGTGTCAGACAGGCGTGCCAGCCAGATTTTCTG
GCAGCGGCAGCAGAAACCGACTTCACCCCTGACAATCGACCCCGTGGAAGAGGA
CGATGTGGCCGTGTACTACTGCCTCCAGAGCCGGACAATCCCCAGAACATTTG
GCGGAGGCACCAAGCTGGAAATCAAGGGCAGCACAAGCGGCTCTGGCAAGCC
TGGATCTGGCGAGGGATCTACCAAGGGACAGATCCAGCTGGTGCAGTCTGGCC
CCGAGCTGAAGAAACCTGGCGAGACAGTGAAGATCAGCTGCAAGGCCAGCGG
CTACACCTTCACCGACTACAGCATCAACTGGGTCAAGAGAGCCCCCTGGCAAGG
GCCTGAAATGGATGGGCTGGATCAACACCGAAACCAGAGAGCCCGCCTACGCC
TACGACTTCAGAGGCAGATTTCGCCTTCAGCCTGGAAACCAGCGCCAGCACAGC
CTACCTCCAGATCAACAACCTGAAGTACGAGGACACCGCCACCTACTTTTGCG
CCCTGGATTACAGCTACGCCATGGACTATTGGGGCCAGGGCACAAGCGTGACC
GTGTCCTCTACAACAACCCCTGCTCCTCGGCCTCCAACACCAGCTCCTACAATT
GCCTCTCAGCCCCCTGTCTCTGAGGCCCGAAGCTTGTAGACCTGCTGCTGGCGGA
GCCGTGCATACAAGAGGACTGGATTTTCGCCTGCGACATCTACATCTGGGCCCC
TCTGGCTGGAACATGTGGCGTGCTGCTGCTGAGCCTGGTCATCACCTGTACTG
CAAGCGGGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCATGCGGC
CCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGTGCGTTCCCTGAGGAA
GAAGAAGGCGGCTGCGAGCTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTC
CTGCCTATCAGCAGGGACAGAATCAGCTCTACAACGAGCTGAACCTGGGGCGC
AGAGAAGAGTACGACGTGCTGGACAAGAGAAGAGGCAGGGACCCCTGAGATGG
GCGGAAAGCCCCAGAGAAGAAAGAACCCTCAAGAGGGCCTGTACAATGAGCT
GCAAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAA
CGCAGAAGAGGAAAGGGCCACGACGGACTGTATCAGGGCCTGAGCACAGCCA
CCAAGGACACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA

FHVVH33 HVV (CD8a Signal Peptide_FHVVH33 HVV_CD8a Hinge_BBZ) (SEQ ID NO:
180)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAGGTGCAGCTGCTTGAATCTGGCGGAGGACTGGTTCAGCCTGG
CGGATCTCTGAGACTGTCTTGTGCCGCCAGCGGCTTCACCTTTAGCAGCTACGC
CATGAGCTGGGTCCGACAGGCTCCTGGCAAAGGCCTTGAATGGGTGTCCAGCA
TCAGCGGCAGCGGCGACTACATCTACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACATCAGCAAGAACACCCTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGACACCGCCGTGTACTACTGTGCCAAAGAAGGCACCGGCGCC

AATAGCAGCCTGGCCGATTATAGAGGCCAGGGCACACTGGTACCGTGTCCAG
TTTCGTGCCTGTGTTCTGCTGCCAAGCCTACCACAACACCCGCTCCTAGACC
TCCAACACCAGCTCCAACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCAGAAG
CTTGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTCGCC
TGCGACATCTACATCTGGGCCCCCTCTGGCTGGAACATGTGGCGTGCTGCTGCTG
AGCCTGGTCATCACACTGTACTGCAACCACCGGAACAAGCGGGGCAGAAAGA
AGCTGCTGTACATCTTTAAGCAGCCCTTCATGCGGCCCGTGCAGACCACACAA
GAGGAAGATGGCTGCTCCTGTCGGTTCCCCGAGGAAGAAGAAGGCGGCTGCG
AGCTGAGAGTGAAGTTCAGCAGATCCGCAGACGCCCCCTGCCTATCAGCAGGGA
CAGAACCAAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACG
TGCTGGACAAGCGGAGAGGCAGAGATCCTGAGATGGGCGGAAAGCCCCAGCG
GAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATG
GCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGG
GACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGAT
GCCCTGCACATGCAGGCCCTGCCTCCAAGA

BCAR003 HVV (CD8a Signal Peptide_BCAR003 HVV_CD8a Hinge_BBZ) (SEQ ID
NO: 181)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCCCAAGTGAAGCTGGAAGAGTCTGGCGGAGGACTGGTGCAGGCTG
GCAGATCTCTGAGACTGTCTTGTGCCGCCAGCGAGCACACCTTTAGCTCTCACG
TGATGGGCTGGTTCAGACAGGCCCTGGCAAAGAAAGGGAAAGCGTGGCCGTT
ATCGGCTGGCGGGATATCAGCACAAAGCTACGCCGATAGCGTGAAGGGCAGATT
CACCATCAGCCGGGACAACGCCAAGAAAACCCTGTACCTCCAGATGAACAGCC
TGAAGCCTGAGGACACCGCCGTGTACTACTGCGCCGCCAGAAGAATTGACGCC
GCCGACTTTGATTCTTGGGGCCAGGGAACCCAAGTGACCGTTTCTAGCGGAGG
CGGTGGAAGTGGCGGCGGTGGATCAGGTGGTGGTGGATCTGGTGGCGGAGGA
AGCGGCGGAGGCGGATCTGCTGTTTCACTTGTGAATCAGGTGGCGGCCTGGT
TCAGGCCGGGGATTCTCTTAGACTGACCTGCACAGCCAGCGGCAGAGCCTTCA
GCACCTACTTCATGGCCTGGTTTCGGCAGGCTCCCGGAAAAGAACGGGAATTT
GTGGCCGGAATCGCTTGGAGCGGAGGCTCTACAGCCTATGCCGATTCCGTGAA
AGGCCGTTTACCATCAGCAGAGATAATGCCAAAAACACGGTGTACCTGCAAA
TGAATCTCTGAAGTCCGAGGATACGGCCGTCTATTACTGTGCCAGCAGAGGC
ATCGAGGTGGAAGAGTTTGGAGCCTGGGGACAGGGCACACAAGTCACAGTGT

CTAGCACCAGCACCACCACACCAGCTCCTAGACCTCCAACCTCTGCTCCTACAA
TCGCCAGCCAGCCTCTGTCTCTGAGGCCAGAAGCTTGTAGACCTGCTGCTGGCG
GAGCCGTGCATACAAGAGGACTGGATTTCGCCTGCGACATCTACATCTGGGCC
CCTCTGGCTGGAACATGTGGCGTGCTGCTGCTGAGCCTGGTCATCACCTGTAC
TGCAAGCGGGGCAGAAAGAAGCTGCTGTACATCTTCAAGCAGCCCTTCATGCG
GCCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGTCGGTTCCCCGAGG
AAGAAGAAGGCGGCTGCGAGCTGAGAGTGAAGTTCAGCAGAAGTGCCGACGC
TCCCGCCTATCAGCAGGGACAGAACCAGCTGTACAACGAGCTGAACCTGGGGA
GAAGAGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAGAT
GGGCGGAAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAG
CTGCAAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCG
AGCGCAGAAGAGGCAAGGGACACGATGGACTGTATCAGGGCCTGAGCACCGC
CACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA

GSI5022 HVV (CD8a Signal Peptide_GSI5022 HVV_CD8a Hinge_BBZ) (SEQ ID NO:
182)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCCCAAGTGAAGCTGGAAGAGTCTGGCGGAGGACTGGTGCAGGCTG
GCAGATCTCTGAGACTGTCTTGTGCCGCCAGCGAGCACACCTTTAGCTCTCACG
TGATGGGCTGGTTCAGACAGGCCCTGGCAAAGAAAGGGAAAGCGTGGCCGTT
ATCGGCTGGCGGGATATCAGCACAAGCTACGCCGATAGCGTGAAGGGCAGATT
CACCATCAGCCGGGACAACGCCAAGAAAACCTGTACCTCCAGATGAACAGCC
TGAAGCCTGAGGACACCGCCGTGTACTACTGCGCCGCCAGAAGAATTGACGCC
GCCGACTTTGATTCTTGGGGCCAGGGAACCCAAGTGACCGTTTCTAGCGGAGG
CGGTGGAAGTGGCGGCGGTGGATCAGGTGGTGGTGGATCTGAAGTGCAGCTGG
TGGAATCAGGCGGCGGTCTTGTTCAGCCGGTGGTTCCTGAGACTGAGCTGT
GCCGCTTCCGGCAGAACCTTTACCATGGGATGGTTTtaggcaggctccagggaa
AGAACGCGAGTTCGTGGCCGCCATTTCTCTGTCTCCAACACTGGCCTACTACGC
CGAGTCCGTGAAAGGCCGGTTCACAATCTCCAGAGATAATGCCAAGAACACCG
TGGTGCTGCAAATGAACTCCCTGAAGCCAGAAGATACAGCCCTGTATTACTGT
GCCGCCGACCGGAAGTCCGTGATGAGCATCAGACCTGACTACTGGGGACAGGG
CACACAAGTCACAGTGTCCAGCACCAGCACCACAACACCCGCTCCTAGACCTC
CAACACCAGCTCCAACAATCGCCAGCCAGCCTCTGTCTCTGAGGCCAGAAGCT
TGTAGACCTGCTGCTGGCGGAGCCGTGCATACAAGAGGACTGGATTTCGCCTG

CGACATCTACATCTGGGCCCCCTCTGGCTGGAACATGTGGCGTGCTGCTGCTGAG
CCTGGTCATCACCTGTACTGCAAGCGGGGCAGAAAGAAGCTGCTGTACATCT
TCAAGCAGCCCTTCATGCGGCCCCGTGCAGACCACACAAGAGGAAGATGGCTGC
TCCTGTTCGGTTCCCCGAGGAAGAAGAAGGCGGCTGCGAGCTGAGAGTGAAGTT
CAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGACAGAACCAGCTGTACA
ACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGATAAGCGGAG
AGGCAGAGATCCTGAGATGGGCGGAAAGCCCCAGCGGAGAAAGAATCCTCAA
GAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGCCGAGGCCTACAGCG
AGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGTA
TCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCAGG
CCCTGCCTCCAAGA

BCMAsdAb1 HHV (CD8a Signal Peptide_anti-BCMAsdAb1 HHV_CD8a Hinge) (SEQ
ID NO: 183)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATAACCGCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTTCGCCTGC

BCMAsdAb1 HHV_CD8a Transmembrane_4-1BB Signaling_CD3Z (BCMAsdAb1 HHV
_BB_z) (SEQ ID NO: 184)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATAACCGCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG

GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTTCGCCTGCGACATCTACATCTGGGCCCCCTCTGGCTGGAA
CATGTGGCGTGCTGCTGCTGAGCCTGGTCATCACCCCTGTATTGCAAGCGGGGC
AGAAAGAAACTGCTCTACATCTTCAAGCAGCCCTTCATGCGGCCCGTGCAGAC
CACACAAGAGGAAGATGGCTGCTCCTGTTCGGTTCCCCGAGGAAGAAGAAGGC
GGCTGCGAGCTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCA
GCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAG
TACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGC
CCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGA
CAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGA
GGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATA
CCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA

BCMAsdAb1 HHV __CD28 Transmembrane__CD28 Signaling__CD3Z (BCMAsdAb1 HHV
__28_Z) (SEQ ID NO: 185)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATAACGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTTCGCCTGCCTGTTTCCCGACCTAGCAAGCCTTTCTGGGT
GCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGC
CTTCATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACT
ACATGAACATGACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCT
TACGCTCCTCCTAGAGACTTCGCCGCCTACAGATCTAGAGTGAAGTTCAGCAG
ATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTACAACGAGC

TGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAG
AGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGC
CTGTATAATGAGCTGCAAAAGGACAAGATGGCCGAGGCCCTACAGCGAGATCG
GAATGAAGGGCGAGCGCAGAAGAGGGCAAGGGACACGATGGACTGTACCAGGG
CCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGC
CTCCAAGA

BCMAsdAb1 HHV _CD28 Transmembrane_CD28 Signaling_4-1BB Signaling_CD3Z

(BCMAsdAb1 HHV _28_BBwt_z) (SEQ ID NO: 186)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATACCGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTGCCTGCCTGTTTCCCGGACCTAGCAAGCCTTTCTGGGT
GCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGC
CTTCATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACT
ACATGAACATGACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCT
TACGCTCCTCCTAGAGACTTCGCCGCCTACAGATCCAAGCGGGGAGAGAAAGAA
GCTGCTGTACATCTTCAAGCAGCCCTTCATGCGGCCCGTGACAGACCACACAAG
AGGAAGATGGCTGCTCCTGTTCGGTTCCCGAGGAAGAAGAAGGCGGTTGCGAA
CTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCA
AAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTG
CTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGA
GAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGC
CGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGA
CACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGC
CCTGCACATGCAGGCCCTGCCTCCAAGA

BCMAsdAb1 HHV _CD28 Transmembrane_CD28 Signaling_4-1BB truncated

Signaling_CD3Z BCMAsdAb1 HHV _28_BBt_z (SEQ ID NO: 187)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATACCGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTGCCTGCCTGTTTCCCGGACCTAGCAAGCCTTTCTGGGT
GCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGC
CTTCATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACT
ACATGAACATGACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCT
TACGCTCCTCCTAGAGACTTCGCCGCCTACAGATCCCAGCCTTTCATGAGGCCT
GTGCAGACCACACAAGAAGAGGACGGCTGCTCCTGTCTGGTTCCCCGAGGAAGA
GGAAGGCGGTTGCGAACTTAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTG
CCTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAG
AGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGC
GGCAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGC
AAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCG
CAGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACC
AAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA

BCMAsdAb1 HHV _CD28 Transmembrane_CD28 Signaling_OX-40 Truncated

Signaling_CD3Z (BCMAsdAb1 HHV _28_OX40t_z) (SEQ ID NO: 188)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC

ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATACCGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTGCCTGCCTGTTTCCCGGACCTAGCAAGCCTTTCTGGGT
GCTCGTTGTTGTTGGCGGCGTGCTGGCCTGTTACAGCCTGCTGGTTACCGTGGC
CTTCATCATCTTTTGGGTCCGAAGCAAGCGGAGCCGGCTGCTGCACAGCGACT
ACATGAACATGACCCCTAGACGGCCCGGACCAACCAGAAAGCACTACCAGCCT
TACGCTCCTCCTAGAGACTTCGCCGCCTACAGATCTGGCGGCGGAAGCTTCAG
AACCCCTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCTAGAGTGA
AGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTG
TACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGC
GGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAAAGAATCC
TCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGCCGAGGCCTAC
AGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGA
CTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACAT
GCAGGCCCTGCCTCCAAGA

BCMAsdAb1 HHV _ICOS Transmembrane_ICOS Signaling_CD3Z (BCMAsdAb1 HHV
_ICOS_Z) (SEQ ID NO: 189)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATACCGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGC
TGCCTATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGA

TCTGCTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGC
GAGTACATGTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGA
CGTGACACTGAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGC
AGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTA
CGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCC
CAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACA
AGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGG
CAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCT
ATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA

BCMAsdAb1 HHV _ICOS Transmembrane _ICOS Signaling_4-1BB Signaling_CD3Z

(BCMAsdAb1 HHV _ICOS_BBwt_z) (SEQ ID NO: 190)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATACCGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTGCCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGC
TGCCTATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGA
TCTGCTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGC
GAGTACATGTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGA
CGTGACCTGAAGCGGGGCAGAAAGAAGCTGCTGTATATCTTCAAGCAGCCCT
TCATGCGGCCCGTGCAGACCACACAAGAGGAAGATGGCTGCTCCTGTCGGTTC
CCCGAGGAAGAAGAAGGCGGTTGCGAACTGAGAGTGAAGTTCAGCAGATCCG
CCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAAC
CTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATC
CTGAAATGGGCGGCAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTA
TAATGAGCTGCAAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATG
AAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGA

GCACCGCCACCAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCA
AGA

BCMAsdAb1_ICOS Transmembrane_ICOS Signaling_4-1BB Truncated Signaling_CD3Z
(BCMAsdAb1 HHV_ICOS_BBt_z) (SEQ ID NO: 191)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG
CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATACCGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTGCGCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGC
TGCCTATTGGCTGCGCCGCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGA
TCTGCTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGC
GAGTACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGA
CGTGACCCTCCAGCCTTTCATGAGGCCTGTGCAGACCACACAAGAAGAGGACG
GCTGCTCCTGTGCGTTCCCCGAGGAAGAGGAAGGCGGTTGCGAACTTAGAGTG
AAGTTCAGCAGATCCGCCGACGCTCCTGCCTATCAGCAGGGCCAAAACCAGCT
GTACAACGAGCTGAACCTGGGGAGAAGAGAAGAGTACGACGTGCTGGACAAG
CGGAGAGGCAGAGATCCTGAAATGGGCGGCAAGCCCCAGCGGAGAAAGAATC
CTCAAGAGGGCCTGTATAATGAGCTGCAAAAGGACAAGATGGCCGAGGCCTA
CAGCGAGATCGGAATGAAGGGCGAGCGCAGAAGAGGCAAGGGACACGATGG
ACTGTACCAGGGCCTGAGCACCGCCACCAAGGATACCTATGATGCCCTGCACA
TGCAGGCCCTGCCTCCAAGA

BCMAsdAb1_ICOS Transmembrane_ICOS Signaling_OX-40 Truncated Signaling_CD3Z
(BCMAsdAb1 HHV_ICOS_OX40t_z) (SEQ ID NO: 192)

ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCC
GCTAGACCTGAAGTGCAGTTGCAGGCTTCTGGCGGAGGACTTGCTCAACCTGG

CGGAAGCCTGAGACTGTCTTGTGCCGCCTCTGGCAGAACCTTCAGCACCTACTT
CATGGCCTGGTTCAGACAGCCTCCTGGCAAAGGCCTGGAATACGTTGGCGGAA
TCCGTTGGAGTGATGGCGTGCCACACTACGCCGATAGCGTGAAGGGCAGATTC
ACCATCAGCCGGGACAACGCCAAGAACACCGTGTACCTCCAGATGAACAGCCT
GAGAGCCGAGGATACCGCCGTGTACTTCTGTGCCAGCAGAGGAATCGCCGACG
GCAGCGATTTTGGCTCTTATGGCCAGGGCACCCAAGTGACCGTGTCCAGCACA
ACAACCCCTGCTCCTAGACCTCCTACACCAGCTCCTACAATCGCCAGCCAGCCT
CTGTCTCTGAGGCCAGAGGCTTGTAGACCTGCTGCTGGCGGAGCCGTGCATAC
AAGAGGACTGGATTTTCGCTGCAGCCAGCTGTGCTGCCAGCTGAAGTTCTGGC
TGCCTATTGGCTGCGCCGCCCTTCGTGGTTGTGTGTATCCTGGGCTGCATCCTGA
TCTGCTGGCTGACCAAGAAAAAGTACAGCAGCAGCGTGCACGACCCCAACGGC
GAGTACATGTTTCATGAGAGCCGTGAACACCGCCAAGAAGTCCAGACTGACCGA
CGTGACACTCGGCGGAGGCAGCTTTAGAACCCCTATCCAAGAGGAACAGGCCG
ACGCTCACTCTACACTGGCTAGAGTGAAGTTCAGCAGATCCGCCGACGCTCCT
GCCTATCAGCAGGGCCAAAACCAGCTGTACAACGAGCTGAACCTGGGGAGAA
GAGAAGAGTACGACGTGCTGGACAAGCGGAGAGGCAGAGATCCTGAAATGGG
CGGCAAGCCCCAGCGGAGAAAGAATCCTCAAGAGGGCCTGTATAATGAGCTG
CAAAAGGACAAGATGGCCGAGGCCTACAGCGAGATCGGAATGAAGGGCGAGC
GCAGAAGAGGCAAGGGACACGATGGACTGTACCAGGGCCTGAGCACCGCCAC
CAAGGATACCTATGATGCCCTGCACATGCAGGCCCTGCCTCCAAGA

CD28 signaling domain: CD28 transmembrane_CD28 signaling domain (SEQ ID NO:
194)

CTGTTCCCCGGACCTAGCAAGCCCTTTTGGGTGCTCGTTGTTGTTGGCGGCGTG
CTGGCCTGTTATAGCCTGCTGGTTACCGTGGCCTTCATCATCTTTTGGGTCCGA
AGCAAGCGGAGCCGGCTGCTGCACAGCGACTACATGAACATGACCCCTAGACG
GCCCCGACCAACCAGAAAGCACTACCAGCCTTACGCTCCTCCTAGAGACTTCG
CCGCCTACAGATCT

Human CD137/4-1BB (SEQ ID NO: 195)

AAGCGGGGCAGAAAGAAACTGCTGTACATCTTCAAGCAGCCCTTCATGCGGCC
CGTGACAGCACACAAGAGGAAGATGGCTGCTCCTGCAGATTCCTCGAGGAAG
AAGAAGGCGGCTGCGAACTT

Human CD134/OX40 (SEQ ID NO: 196)

AGGAGAGACCAGCGTCTGCCACCAGATGCACATAAGCCACCTGGCGGCGGAA GCTTTAGAACCCCTATCCAAGAGGAACAGGCCGACGCTCACTCTACACTGGCT AAAATC
truncated/mutated CD137/4-1BB (SEQ ID NO: 197) CAGCCTTTTCATGAGGCCCCGTGCAGACCACACAAGAAGAGGACGGCTGCTCCTG CAGATTCCTCCGAGGAAGAGGAAGGCCGTTGCGAACTT
Truncated/mutated CD134/OX40 (SEQ ID NO: 198) GGCGGCGGAAGCTTTAGAACCCCTATCCAAGAGGAACAGGCCGACGCTCACTC TACACTGGCT

In some embodiments, the cell disclosed herein further comprises a sequence encoding an artificial antigen receptor, a therapeutic polypeptide, an immune cell modulatory protein, or a combination thereof. In some embodiments, the artificial antigen receptor comprises a chimeric antigen receptor (CAR). In some embodiments, the artificial antigen receptor comprises a recombinant T cell receptor (rTCR). In some embodiments, the artificial antigen receptor comprises an enhanced affinity TCR. In some embodiments, the artificial antigen receptor binds to a tumor associated antigen (TAA), a pathogen associated protein, or an antigen associated with the disease or disorder is a cancer, an autoimmune disease or disorder, an infectious disease, an inflammatory disease, a renal disease or disorder, a lung disease or disorder, a liver disease or disorder a neurodegenerative disorder or disorder, or a metabolic disorder or disorder.

In some embodiments, the artificial antigen receptor binds to a TAA associated with a solid tumor or a hematologic cancer. In some embodiments, artificial antigen receptor binds to a TAA associated with a cancer selected from any one of leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, B cell, T cell or FAB ALL, acute myeloid leukemia (AML), acute myelogenous leukemia, chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, bladder cancer, breast cancer, colorectal cancer, endometrial cancer, head cancer, neck cancer, hereditary nonpolyposis cancer, Hodgkin's lymphoma, liver cancer, lung cancer, non-small cell lung cancer, ovarian

cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, testicular cancer, adenocarcinomas, sarcomas, malignant melanoma, and hemangioma.

In some embodiments, the artificial antigen receptor binds to a TAA selected from kallikrein 4, papillomavirus binding factor (PBF), preferentially expressed antigen of melanoma (PRAME), Wilms' tumor-I (WTI), Hydroxysteroid Dehydrogenase Like I (HSDLI), mesothelin, cancer testis antigen (NY-ES0-1), carcinoembryonic antigen (CEA), p53, human epidermal growth factor receptor 2/neuro receptor tyrosine kinase (Her2/Neu), carcinoma-associated epithelial cell adhesion molecule (EpCAM), ovarian and uterine carcinoma antigen (CAI25), folate receptor a, sperm protein 17, tumor-associated differentially expressed gene-12 (TADG-12), mucin-16 (MUC-16), LI cell adhesion molecule (LICAM), mannan-MUC-1, Human endogenous retrovirus K (HERV-K-MEL), Kita-kyushu lung cancer antigen-I (KK-LC-1), human cancer/testis antigen (KM-HN-1), cancer testis antigen (LAGE-I), melanoma antigen-A1 (MAGE-A1), Sperm surface zona pellucida binding protein (Spl 7), Synovial Sarcoma, X Breakpoint 4 (SSX-4), Transient axonal glycoprotein-1 (TAG-I), Transient axonal glycoprotein-2 (TAG-2), Enabled Homolog (ENAH), mammoglobin-A, NY-BR-I, Breast Cancer Antigen, (BAGE-I), B melanoma antigen, melanoma antigen-A1 (MAGE-A1), melanoma antigen-A2 (MAGE-A2), mucin k, synovial sarcoma, X breakpoint 2 (SSX-2), Taxol-resistance-associated gene-3 (TRAG-3), Avian Myelocytomatosis Viral Oncogene (c-myc), cyclin B 1, mucin I (MUC I), p62, survivin, lymphocyte common antigen (CD45), Dickkopf/WNT Signaling Pathway Inhibitor I (DKKI), telomerase, Kirsten rat sarcoma viral oncogene homolog (K-ras), G250, intestinal carboxyl esterase, alpha-fetoprotein, Macrophage Colony-Stimulating Factor (M-CSF), Prostate-specific membrane antigen (PSMA), caspase 5 (CASP-5), Cytochrome C Oxidase Assembly Factor I Homolog (COA-1), O-linked β - N-acetylglucosamine transferase (OGT), Osteosarcoma Amplified 9, Endoplasmic Reticulum Lectin (OS-9), Transforming Growth Factor Beta Receptor 2 (TGF-betaRII), murine leukemia glycoprotein 70 (gp70), Calcitonin Related Polypeptide Alpha (CALCA), Programmed cell death 1 ligand 1 (CD274), Mouse Double Minute 2 Homolog (mdm-2), alpha-actinin-4, elongation factor 2, Malic Enzyme 1 (MEI), Nuclear Transcription Factor Y Subunit C (NFYC), G Antigen 1,3 (GAGE-1,3), melanoma antigen-A6 (MAGE-A6), cancer testis antigen XAGE-Ib, six transmembrane epithelial antigen of the prostate 1 (STEAPI), PAP, prostate specific antigen (PSA), Fibroblast Growth Factor 5 (FGF5), heat shock protein hsp70-2, melanoma antigen-A9 (MAGE-A9), Arg-specific ADP-ribosyltransferase family C (ARTCI), B-Raf Proto-

Oncogene (B-RAF), Serine/Threonine Kinase, beta-catenin, Cell Division Cycle 27 homolog (Cdc27), cyclin dependent kinase 4 (CDK4), cyclin dependent kinase 12 (CDK12), Cyclin Dependent Kinase Inhibitor 2A (CDKN2A), Casein Kinase 1 Alpha 1 (CSNK1A1), Fibronectin 1 (FNI), Gruwih Anest Specific 7 (GAS7), Glycoprotein nonmetastatic melanoma protein B (GPNMB), HAUS Augmin Like Complex Subunit 3 (HAUS3), LDLR-fucosyltransferase, Melanoma Antigen Recognized By T cells 2 (MART2), myostatin (MSTN), Melanoma Associated Antigen (Mutated) 1 (MUM-1-2-3), Poly(A) polymerase gamma (neo-PAP), myosin class I, Protein phosphatase 1 regulatory subunit 3B (PPP1R3B), Peroxiredoxin-5 (PRDX5), Receptor-type tyrosine-protein phosphatase kappa (PTPRK), Transforming protein N-Ras (N-ras), retinoblastoma-associated factor 600 (RBAF600), sirtuin-2 (SIRT2), SNRPDI, triosephosphate isomerase, Ocular Albinism Type 1 Protein (OAI), member RAS oncogene family (RAB38), Tyrosinase related protein 1-2 (TRP-1-2), Melanoma Antigen Gp75 (gp75), tyrosinase, Melan-A (MART-1), Glycoprotein 100 melanoma antigen (gp100), N-acetylglucosaminyltransferase V gene (GnTVf), Lymphocyte Antigen 6 Complex Locus K (LY6K), melanoma antigen-A10 (MAGE-A10), melanoma antigen-A12 (MAGE-A12), melanoma antigen-C2 (MAGE-C2), melanoma antigen NA88-A, Taxol-resistant-associated protein 3 (TRAG-3), BDZ binding kinase (pbk), caspase 8 (CASP-8), sarcoma antigen 1 (SAGE), Breakpoint Cluster Region-Abelson oncogene (BCR-ABL), fusion protein in leukemia, dek-can, Elongation Factor Tu GTP Binding Domain Containing 2 (EFTUD2), ETS Variant gene 6/acute myeloid leukemia fusion protein (ETV6-AML1), FMS-like tyrosine kinase-3 internal tandem duplications (FLT3-ITD), cyclin-A1, Fibronectin Type III Domain Containing 3B (FDNC3B,) promyelocytic leukemia/retinoic acid receptor alpha fusion protein (pml-RARalpha), melanoma antigen-C1 (MAGE-C1), membrane protein alternative spliced isoform (D393-CD20), melanoma antigen-A4 (MAGE-A4), and melanoma antigen-A3 (MAGE-A3).

In some embodiments, the artificial antigen receptor binds to an antigen associated with an autoimmune condition or disorder selected from any one of Type 1 Diabetes, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or multiple sclerosis (MS). In some embodiments, the artificial antigen receptor binds to an antigen associated with an autoimmune condition or disorder selected from any one of Carboxypeptidase H, Chromogranin A, Glutamate decarboxylase, Imogen-38, Insulin, Insulinoma antigen-2 and 2 β , Islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP), Proinsulin, α -enolase, Aquaporin-4, β -arrestin, Myelin basic protein, Myelin oligodendrocytic glycoprotein, Proteolipid protein, S100- β , Citrullinated protein, Collagen II, Heat shock

proteins, Human cartilage glycoprotein, Double-stranded DNA, La antigen, Nucleosomal histones and ribonucleoproteins (snRNP), Phospholipid- β -2 glycoprotein I complex, Poly(ADP-ribose) polymerase, Sm antigens of U-1 small ribonucleoprotein complex.

In some embodiments, the artificial antigen receptor binds to a pathogen associated antigen from a bacterial, a fungal or a parasitic protein or fragment thereof. In some
5 embodiments, the artificial antigen receptor binds to an antigen associated with HIV infection, human Cytomegalovirus infection, Hepatitis B infection, Hepatitis C infection, Ebola virus infection, Dengue, Yellow fever, Listeriosis, Tuberculosis, Cholera, Malaria, Leishmaniasis, or Trypanosoma infection, or a combination thereof.

10 In some embodiments, the artificial antigen receptor binds to an antigen associated with a neurodegenerative disorder or condition selected from Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD) and PD-related disorders, Prion disease, Motor neurone diseases (MND), Huntington's disease (HD), Spinocerebellar ataxia (SCA) or Spinal muscular atrophy (SMA). In some embodiments, the antigen associated with the
15 neurodegenerative disorder or condition is any one of Amyloid β ($A\beta$), tau, alpha-synuclein (α -syn), mHTT or prion PrP^{Sc} or a combination thereof.

In some embodiments, the therapeutic polypeptide is a cytokine, a cytokine receptor, a chemokine, a chemokine receptor, an immunogenic polypeptide, or a cell surface protein that binds to a target on the surface of another cell. In some embodiments, the immune cell
20 modulatory protein is a cytokine, a chemokine, a transcription factor, a protein kinase, a protease, a component or an adaptor protein of a cell signaling pathway.

In some embodiments, the cell disclosed herein expresses the RTCR disclosed herein. In some embodiments, the cell disclosed herein expresses the RTCR disclosed herein stably or transiently. In some embodiments, the cell disclosed herein expresses the RTCR disclosed
25 herein stably. In some embodiments, the cell disclosed herein expresses the RTCR disclosed herein transiently.

In some embodiments, the cell disclosed herein co-expresses at least one of the endogenous co-stimulatory molecules CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8 and CD40L or a combination thereof.

30 The present disclosure also provides a modified T lymphocyte (T cell), comprising: (a) a modification of an endogenous sequence encoding a T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the TCR or ; and (b) a recombinant T cell co-stimulatory receptor (RTCR) disclosed herein. In some embodiments,

the modification of an endogenous sequence encoding a T cell Receptor (TCR) is done using a nucleic acid modifying system. In some embodiments, the nucleic acid modifying system is one or more of a CRISPR/Cas protein, a Transcription Activator-Like Effector Nuclease (TALEN), a Zinc Finger Nuclease (ZFN), and an endonuclease. In some embodiments, the modification of an endogenous sequence encoding a T cell Receptor (TCR) is done by nonhomologous end joining repair. In some embodiments, the nonhomologous end joining repair is generated by zinc finger nuclease, introduced into the cell by physical means, viral vector, or non-viral vector. In some embodiments, the nonhomologous end joining repair is generated by TALE nuclease, introduced into the cell by physical means, viral vector, or non-viral vector. In some embodiments, the modification of an endogenous sequence encoding a T cell Receptor (TCR) reduces or eliminates a level of expression of the alpha chain of the TCR. In some embodiments, the modification of an endogenous sequence encoding a T cell Receptor (TCR) reduces or eliminates a level of expression of beta chain of the TCR. In some embodiments, the modification of an endogenous sequence encoding a T cell Receptor (TCR) reduces or eliminates a level of expression of both the alpha chain and the beta chain TCR alpha chain.

In some embodiments, the modified T cell disclosed herein co-expresses at least one of the endogenous co-stimulatory molecules CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8 and CD40L or a combination thereof.

In some embodiments, the method disclosed herein further comprises a modification of an endogenous sequence encoding a component of major histocompatibility complex (MHC) class I (MHC-I), wherein the modification reduces or eliminates a level of expression or activity of the MHC-I. In some embodiments, the modification reduces or eliminates the expression or activity of β 2-microglobulin.

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

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

disclosure also provides a composition comprising a population of cells, wherein the population comprises a plurality of the modified T cell disclosed herein.

The present disclosure also provides a method of producing a plurality of modified T cells, wherein the method comprises: a) providing a plurality of primary T cells disclosed herein; b) providing a composition comprising the RTCR disclosed herein, the nucleic acid encoding the RTCR disclosed herein, or the vector comprising the nucleic acid encoding the RTCR disclosed herein; and c) introducing into the plurality of primary T cells of (a) the composition of (b), to produce a plurality of modified T cells under conditions that stably express the RTCR within the plurality of modified T cells. In some embodiments, the method of producing a plurality of modified T cells disclosed herein, further comprises a step of modifying an endogenous sequence encoding an endogenous T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the endogenous TCR. In some embodiments, the method of producing a plurality of modified T cells disclosed herein, further comprises a step of modifying an endogenous sequence, wherein the modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I).

In some embodiments, the modifying an endogenous sequence encoding a T cell Receptor (TCR) is done using a nucleic acid modifying system. In some embodiments, the modifying an endogenous sequence that reduces or eliminates a level of expression or activity of is done using a nucleic acid modifying system. In some embodiments, the nucleic acid modifying system is a one or more of a CRISPR/Cas protein, a Transcription Activator-Like Effector Nuclease (TALEN), a Zinc Finger Nuclease (ZFN), and an endonuclease. In some embodiments, the modifying an endogenous sequence is done by nonhomologous end joining repair. In some embodiments, the nonhomologous end joining repair is generated by zinc finger nuclease, introduced into the cell by physical means, viral vector, or non-viral vector. In some embodiments, the nonhomologous end joining repair is generated by TALE nuclease, introduced into the cell by physical means, viral vector, or non-viral vector. In some embodiments, the modifying an endogenous sequence encoding a T cell Receptor (TCR) reduces or eliminates a level of expression of the alpha chain of the TCR. In some embodiments, the modifying an endogenous sequence encoding a T cell Receptor (TCR) reduces or eliminates a level of expression of beta chain of the TCR. In some embodiments, the modifying an endogenous sequence encoding a T cell Receptor (TCR) reduces or eliminates a level of expression of both the alpha chain and the beta chain TCR alpha chain.

In some embodiments, the modifying an endogenous sequence that reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I), wherein the modifying of an endogenous sequence reduces or eliminates a level of expression or activity of the MHC-I. In some embodiments, the modifying of an endogenous sequence reduces or eliminates the expression or activity of β 2-macroglobulin.

In some embodiments, the method of producing a plurality of modified T cells disclosed herein, further comprises: d) maintaining or expanding the plurality of modified T cells in a suitable cell culture media; and e) either: i) cryopreserving the plurality of modified T cells in a suitable cell freezing media; or ii) preparing the plurality of modified T cells for administering to a subject suffering from a disease or disorder.

The compositions comprising the cells or modified T cells of the disclosure, and the plurality of modified T cells produced by the methods of the disclosure, intended for administration to a subject may be required to meet one or more "release criteria" that indicate that the composition is safe and efficacious for formulation as a pharmaceutical product and/or administration to a subject. Release criteria may include a requirement that a composition of the disclosure (e.g., a cell or modified T cell of the disclosure) comprises a particular percentage of cells or modified T cells expressing the RTCR of the disclosure on their cell surface. The expansion process should be continued until a specific criterion has been met (e.g., achieving a certain total number of cells or modified T cells of the disclosure or a certain percentage of total number of cells or modified T cells expressing the RTCR of the disclosure).

Certain criterion signal a point at which the expansion process should end. For example, cells should be formulated, reactivated, or cryopreserved once they reach a cell size of 300fL (otherwise, cells reaching a size above this threshold may start to die). Cryopreservation immediately once a population of cells reaches an average cell size of less than 300 fL may yield better cell recovery upon thawing and culture because the cells haven't yet reached a fully quiescent state prior to cryopreservation (a fully quiescent size is approximately 180 fL). Prior to expansion, T cells of the disclosure may have a cell size of about 180 fL, but may more than quadruple their cell size to approximately 900 fL at 3 days post-expansion. Over the next 6-12 days, the population of T cells will slowly decrease cell size to full quiescence at 180 fL.

A process for preparing a cell population for formulation may include, but is not limited to the steps of, concentrating the cells of the cell population, washing the cells, and/or

further selection of the cells via drug resistance or magnetic bead sorting against a particular surface-expressed marker. A process for preparing a cell population for formulation may further include a sorting step to ensure the safety and purity of the final product. For example, if a tumor cell from a patient has been used to stimulate a modified T cell of the disclosure or that have been modified in order to stimulate a modified T cell of the disclosure that is being prepared for formulation, it is critical that no tumor cells from the patient are included in the final product.

In some embodiments, the cell disclosed herein, or the modified T cell disclosed herein, expresses on the cell surface the RTCR comprising a mutant CD137 or a mutant CD134 intracellular signaling domain disclosed herein, at a level that is at least about 2X, 3X, 4X, 5X, 6X, 7X, 8X, 9X, 10X or 20X, more as compared to the level of expression of a co-stimulatory molecule comprising a wild type CD137 or a wild type CD134 intracellular domain, respectively.

Pharmaceutical composition or formulation

In some embodiments, the compositions disclosed herein, and the population of modified T cells produced using the methods disclosed herein, is in the form of a pharmaceutical formulation (or composition). In some embodiments, the pharmaceutical formulation disclosed herein comprises a pharmaceutically acceptable carrier. A pharmaceutical formulation of the disclosure may be distributed into bags for infusion, cryopreservation, and/or storage.

A pharmaceutical formulation of the disclosure may be cryopreserved using a standard protocol and, optionally, an infusible cryopreservation medium. For example, a DMSO free cryopreservant (e.g. CryoSOfree™ DMSO-free Cryopreservation Medium) may be used to reduce freezing-related toxicity. A cryopreserved pharmaceutical formulation of the disclosure may be stored for infusion to a patient at a later date. An effective treatment may require multiple administrations of a pharmaceutical formulation of the disclosure and, therefore, pharmaceutical formulations may be packaged in pre-aliquoted “doses” that may be stored frozen but separated for thawing of individual doses.

A pharmaceutical formulation of the disclosure may be stored at room temperature. An effective treatment may require multiple administrations of a pharmaceutical formulation of the disclosure and, therefore, pharmaceutical formulations may be packaged in pre-aliquoted “doses” that may be stored together but separated for administration of individual doses.

A pharmaceutical formulation of the disclosure may be archived for subsequent re-expansion and/or selection for generation of additional doses to the same patient in the case of an allogenic therapy who may need an administration at a future date following, for example, a remission and relapse of a condition.

5 As noted above, the disclosure provides for stable formulations, which preferably comprise a phosphate buffer with saline or a chosen salt, as well as preserved solutions and formulations containing a preservative as well as multi-use preserved formulations suitable for pharmaceutical or veterinary use, comprising at least one modified cell in a pharmaceutically acceptable formulation. Preserved formulations contain at least one known
10 preservative or optionally selected from the group consisting of at least one phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (e.g., hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, polymers, or mixtures thereof in an aqueous diluent. Any
15 suitable concentration or mixture can be used as known in the art, such as about 0.0015%, or any range, value, or fraction therein. Non-limiting examples include, no preservative, about 0.1-2% m-cresol (e.g., 0.2, 0.3, 0.4, 0.5, 0.9, 1.0%), about 0.1-3% benzyl alcohol (e.g., 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), about 0.001-0.5% thimerosal (e.g., 0.005, 0.01), about 0.001-2.0% phenol (e.g., 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (e.g.,
20 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

As noted above, the disclosure provides an article of manufacture, comprising packaging material and at least one vial comprising a solution of at least one modified cell with the prescribed buffers and/or preservatives, optionally in an aqueous diluent, wherein
25 said packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater.

The articles of manufacture of the present disclosure are useful for administration over a period ranging from immediate to twenty-four hours or greater. Accordingly, the presently claimed articles of manufacture offer significant advantages to the patient.
30 Formulations of the disclosure can optionally be safely stored at temperatures of from about 2° C. to about 40° C. and retain the biological activity of the protein for extended periods of time, thus allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater.

The products of the present disclosure include packaging material. The packaging material provides, in addition to the information required by the regulatory agencies, the conditions under which the product can be used.

The present disclosure also provided a method of treating a disease or disorder,
5 comprising administering to a subject in need thereof a therapeutically effective number of the cell comprising the nucleic acid encoding or the vector comprising the nucleic acid encoding the RTCR disclosed herein, a therapeutically effective number of any one of the modified T cell disclosed herein, a therapeutically effective amount of any one of the compositions disclosed herein, or a therapeutically effective number of the plurality of
10 modified T cells produced by the method disclosed herein.

In some embodiments, the subject is a mammal. In some embodiments, the mammal is any one of a human, a primate, a rodent, a canine, a feline, an ungulate, an equine and a porcine. In some embodiments, the mammal is a human. In some embodiments, the disease or disorder is any one of a cancer, an autoimmune disorder, an infectious disease, an
15 inflammatory disease or condition, a renal disease or disorder, a lung disease or disorder, a liver disease or disorder, a cardiovascular system disease or disorder, a neurodegenerative disorder or condition, or a metabolic disorder or condition. In some embodiments, the cancer is a solid tumor or a hematologic cancer. In some embodiments, the infectious disease is caused by a bacteria, a virus, a fungi, a protozoa, or a parasite. In some embodiments, the
20 neurodegenerative disorder or condition is any one of Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD) and PD-related disorders, Prion disease, Motor neurone diseases (MND), Huntington's disease (HD), Spinocerebellar ataxia (SCA) or Spinal muscular atrophy (SMA).

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

The present disclosure also provides a chimeric co-stimulatory intracellular protein
30 (CIP) comprising a first and at least a second signal transduction domains, wherein the first and the at least second signal transduction domains are non-identical; and wherein the at least second signal transduction domain comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain.

In some embodiments of the CIP disclosed herein, the mutant intracellular signaling domain of a TNFR family protein is any one of a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain. In some embodiments, the CIP further comprises a transmembrane domain. In some embodiments of the CIP disclosed
5 herein, the mutant CD137 intracellular domain is a truncated CD137 intracellular domain.

In some embodiments of the CIP disclosed herein, the truncated CD137 intracellular domain comprises an amino acid sequence according to amino acid position 13 to amino acid position 42 of the CD137 intracellular domain, of the present disclosure. In some
10 embodiments of the CIP disclosed herein, the truncated CD137 intracellular domain comprises a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from the N-terminus the CD137 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the truncated CD137 intracellular domain comprises a deletion of one, two, three, four, five, six, seven, eight, nine,
15 ten or more amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the CD137 intracellular domain of the present disclosure comprises an amino acid sequence according to SEQ ID NO: 1.

In some embodiments of the CIP disclosed herein, the truncated CD137 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 3.

20 In some embodiments of the CIP disclosed herein, the mutant CD137 intracellular domain comprises a deletion of one, two, three or four lysine residue(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the mutant CD137 intracellular domain comprises one or more lysine mutation(s) from amino acid position 1 to
25 amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the mutant CD137 intracellular domain comprises one or more lysine mutation(s) at amino acid positions selected from amino acid positions 1, 5, 6 and 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure.

30 In some embodiments of the CIP disclosed herein, the mutant CD137 intracellular domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) from

amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, 3, 4, 5 and 6 of the N-terminus of the CD137 intracellular domain, of the present disclosure. In some
5 embodiments of the CIP disclosed herein, the mutant CD137 intracellular domain further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain, of the present disclosure.

10 In some embodiments of the CIP disclosed herein, the mutant CD134 intracellular domain is a truncated CD134 intracellular domain. In some embodiments of the CIP disclosed herein, the truncated CD134 intracellular domain comprises an amino acid sequence according to amino acid position 15 to amino acid position 37 of the CD134 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the truncated CD134 intracellular domain comprise a deletion of a continuous stretch
15 of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the truncated CD134 intracellular domain comprises a deletion of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from amino acid position 1 to amino acid
20 position 14 of the N-terminus of the CD134 intracellular domain, of the present disclosure.

 In some embodiments of the CIP disclosed herein, the truncated CD134 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 6.

 In some embodiments of the CIP disclosed herein, the mutant CD134 intracellular domain comprises a deletion of a lysine residue from amino acid position 1 to amino acid
25 position 14 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the mutant CD134 intracellular domain comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD134 intracellular domain, of the present disclosure.

 In some embodiments of the CIP disclosed herein, the mutant CD134 intracellular
30 domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain, of the present disclosure. In some embodiments of the CIP disclosed herein, the mutant CD134 intracellular domain comprises one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular

domain. In some embodiments of the CIP disclosed herein, the mutant CD134 intracellular domain comprises one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, and 5 of the N-terminus of the CD134 intracellular domain. In some embodiments of the CIP disclosed herein, the mutant CD134 intracellular domain further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD134 intracellular domain

In some embodiments, the CIP disclosed herein comprises a first signal transduction domain derived from a protein of the CD28 family. In some embodiments, the CIP disclosed herein comprises a first signal transduction domain derived from any one of CD28, CD28H, ICOS or a combination thereof.

In some embodiments, the CIP disclosed herein comprises a first signal transduction domain derived from ICOS. In some embodiments, the first signal transduction domain derived from ICOS comprises an amino acid sequence according to SEQ ID NO: 9.

In some embodiments, the CIP disclosed herein comprises a first signal transduction domain comprising a portion of a CD28 intracellular domain combined with an ICOS domain according to SEQ ID NO: 9. In some embodiments of the CIP disclosed herein, the first signal transduction domain comprises an amino acid sequence according to any one of SEQ ID NOs: 12 or 109. In some embodiments, the CIP disclosed herein comprises a first signal transduction domain derived from CD28. In some embodiments of the CIP disclosed herein, the first signal transduction domain derived from CD28 comprises an amino acid sequence according to SEQ ID NO: 10. In some embodiments of the CIP disclosed herein, the first signal transduction domain derived from CD28 comprises an amino acid sequence according to any one of SEQ ID NOs: 121-122. In some embodiments, the CIP comprises an amino acid sequence according to any one of SEQ ID NOs: 14-17.

In some embodiments, the CIP disclosed herein further comprises a third signal transduction domain. In some embodiments, the CIP disclosed herein further comprises a third signal transduction domain derived from any one of a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-2RB) protein signaling domain or a combination thereof. In some embodiments, the CD3 signaling domain of the CIP disclosed herein is derived from a CD3 ζ or a CD3 ϵ domain or a combination thereof. In some embodiments, the CD3 signaling domain of the CIP disclosed herein is a CD3 domain comprising an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47 and 48.

In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having according to SEQ ID NO: 18. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to according to SEQ ID NO: 18. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having according to SEQ ID NO: 45. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to according to SEQ ID NO: 45. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a truncated CD3 ζ domain comprising an amino acid sequence having according to SEQ ID NO: 46. In some embodiments, the third signal transduction domain of the CIP disclosed herein, the third signal transduction domain of the CIP disclosed herein is a truncated CD3 ζ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to according to SEQ ID NO: 46. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 ϵ domain comprising an amino acid sequence according to SEQ ID NO: 47. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 ϵ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 47. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a combination of a CD3 ϵ and a truncated CD3 ζ domains (CD3 $\zeta\epsilon$ domain). In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 $\zeta\epsilon$ domain comprising an amino acid sequence according to SEQ ID NO: 48. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD3 $\zeta\epsilon$ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 48.

In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD2 signaling domain. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a mutant CD2 signaling domain. In some embodiments, the mutant CD2 signaling domain is a truncated CD2 signaling domain. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD2

signaling domain comprising an amino acid sequence according to SEQ ID NO: 49. In some embodiments, the third signal transduction domain of the CIP disclosed herein is a CD2 signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 49.

5 In some embodiments, the third signal transduction domain of the CIP disclosed herein, is an IL-2RB protein signaling domain comprising an amino acid sequence according to SEQ ID NO: 50. In some embodiments, the third signal transduction domain of the CIP disclosed herein is an IL-2RB protein signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99%
10 identity to SEQ ID NO: 50.

In some embodiments, the CIP disclosed herein further comprises a fourth signal transduction domain. In some embodiments, the CIP disclosed herein further comprises a fourth signal transduction domain derived from any one of a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-2RB) protein signaling domain or a
15 combination thereof, wherein the third and the fourth signal transduction domain are not identical. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein, is derived from a CD3 ζ or a CD3 ϵ domain or a combination thereof. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 domain comprising an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46,
20 47 and 48.

In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having according to SEQ ID NO: 18. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having at least 50%, 55%, 60%,
25 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to according to SEQ ID NO: 18. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having according to SEQ ID NO: 45. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 ζ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%,
30 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to according to SEQ ID NO: 45. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a truncated CD3 ζ domain comprising an amino acid sequence having according to SEQ ID NO: 46. In some embodiments, the third signal transduction domain of the CIP disclosed

herein, the fourth signal transduction domain of the CIP disclosed herein is a truncated CD3 ζ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to according to SEQ ID NO: 46. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 ϵ domain comprising an amino acid sequence according to SEQ ID NO: 47. In some
5 embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 ϵ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 47. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a combination of a CD3 ϵ and
10 a truncated CD3 ζ domains (CD3 $\zeta\epsilon$ domain). In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 $\zeta\epsilon$ domain comprising an amino acid sequence according to SEQ ID NOs: 48. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD3 $\zeta\epsilon$ domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%
15 or 99% identity to SEQ ID NO: 48.

In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD2 signaling domain. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a mutant CD2 signaling domain. In some embodiments, the mutant CD2 signaling domain is a truncated CD2 signaling domain. In some
20 embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD2 signaling domain comprising an amino acid sequence according to SEQ ID NO: 49. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is a CD2 signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99% identity to SEQ ID NO: 49.

25 In some embodiments, the fourth signal transduction domain of the CIP disclosed herein, is an IL-2RB protein signaling domain comprising an amino acid sequence according to SEQ ID NO: 50. In some embodiments, the fourth signal transduction domain of the CIP disclosed herein is an IL-2RB protein signaling domain comprising an amino acid sequence having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97% or 99%
30 identity to SEQ ID NO: 50.

In some embodiments, the CIP disclosed herein is for expression in a T cell, wherein the T cell co-expresses at least one of the endogenous co-stimulatory molecules CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8 and CD40L or a combination thereof.

In some embodiments, the CIP disclosed herein, is co-expressed with a T cell receptor (TCR) in a T cell. In some embodiments, the TCR is an endogenous TCR. In some embodiments, the TCR is an artificial TCR. In some embodiments, the artificial TCR is an affinity enhanced TCR. In some embodiments, the CIP when co-expressed with a TCR in a T cell provides a second activation signal for inducing activation and proliferation of the T cell, wherein the first activation signal is provided by antigen binding by the TCR.

In some embodiments, the CIP disclosed herein, is expressed in a T cell as a component of an artificial receptor for a target. In some embodiments, the artificial receptor is a chimeric antigen receptor (CAR), a receptor for a ligand or a component thereof, an antibody or a fragment thereof. In some embodiments, the CIP disclosed herein, is expressed as a component of a CAR. In some embodiments, the CIP disclosed herein, is expressed as a component of an antibody or a fragment thereof. In some embodiments, the antibody or a fragment thereof is a Fab fragment, a F(ab)₂ fragment, a diabody, a nanobody, a sdAb, a Fv, a VHH fragment, or a single chain Fv fragment. In some embodiments, the CIP expressed as a component of an artificial receptor in a T cell, as disclosed herein induces activation and proliferation of the T cell upon target binding by the artificial receptor.

The term “about” or “approximately” can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. In some embodiments, “about” or “approximately” can be understood as within 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. In some embodiments, “about” or “approximately” can be understood as within 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. In some embodiments, “about” or “approximately” can be understood as within 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value.

The following examples are provided to better illustrate the present disclosure and are not to be interpreted as limiting the scope of the disclosure. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the disclosure. One skilled in the art may develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the disclosure.

Examples

30 Materials and Methods

Media and Cell Lines

DMEM was supplemented with Penn/Strep/Glutamine, 20mM HEPES, 10µg/mL Gentamycin and 10% FBS to make complete DMEM. RPMI was supplemented with Penn/Strep/Glutamine, 20mM HEPES, 10µg/mL Gentamycin, 10% FBS, and 50uM 2-ME to

make complete RPMI. T cell growth media was made by supplementing complete RPMI with 50 ng/ml IL2, 10 ng/ml IL7, and 10ng/mL IL15 (PeproTech). X-Vivo15 was supplemented with 1% Human Serum, 20mM HEPES, Penn/Strep/Glutamine, and 10µg/mL Gentamycin to make Cytokine Media. Human PBMCs were purchased from iSpecimen and cultured in complete RPMI. 293FT were purchased from Invitrogen. K562 and A375 cells were purchased from ATCC and cultured in complete DMEM.

Plasmids and Cloning

A lentiviral plasmid containing the PGK promoter driving a truncated human EGFR receptor (huEGFRt) followed by the MSCV promoter driving GFP and a subsequent WPRE sequence was ordered from vector builder. Co-stimulatory molecules followed by a P2A sequence were ordered as a single gene block (Invitrogen) and placed in frame with the huEGFRt sequence using NEB builder homology-based recombination. CAR and TCR sequences were constructed from 3 gene block fragments (Invitrogen) and cloned with NEB builder downstream of the MSCV promoter following GFP excision. PD-L1_P2A and HLA-A2 were cloned in frame with the huEGFRt and in place of GFP, respectively.

P2A amino acid sequence (SEQ ID NO: 111)

GSGATNFSLLKQAGDVEENPGP

Human EGFRt amino acid sequence (Other name: huEGFRt (AA112)) (SEQ ID NO: 112)

MLLLVTSLLLCELPHPAFLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSIGDHLIL
PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIHRGRTK
QHGQFSLAVVSLNITSLGLRSLKEISDGDVHISGNKNLCYANTINWKKLFGTSGQKTKI
ISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGREGVDKCNLLEGEPR
EFVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCAPAGVMGENN
TLVWKYADAGHVCHLCHPNCTYGCTGPGLEGCTNGPKIPSIATGMVGAALLLLLVV
ALGIGLFM

HLA-A2 signal peptide (SEQ ID NO: 113)

MAVMAPRTLVLVLLSGALALTQTWA

huGMCSF Signal Peptide, amino acid sequence (SEQ ID NO: 119)

MLLLVTSLLLCELPHPAFLIP

P2A nucleic acid sequence (SEQ ID NO: 114)

GGATCCGGCGCCACCAATTTTCAGCCTGCTGAAACAGGCTGGCGACGTGGAAGAG
AACCCTGGACCT

Human EGFRt nucleic acid sequence (SEQ ID NO: 115)

ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTTTCT
GCTGATCCCCAGAAAAGTGTGCAACGGCATCGGCATCGGAGAGTTCAAGGACAG
CCTGAGCATCAACGCCACCAACATCAAGCACTTCAAGAACTGCACCAGCATCAG
CGGCGACCTGCACATTCTGCCTGTGGCCTTTAGAGGCGACAGCTTCACCCACACA
5 CCTCCACTGGACCCTCAAGAGCTGGACATCCTGAAAACCGTGAAAGAGATCACC
GGATTTCTGTTGATCCAGGCTTGGCCCGAGAACCGGACAGATCTGCACGCCTTCG
AGAACCTGGAAATCATCAGAGGCCGGAACCAAGCAGCACGGCCAGTTTTCTCTGG
CTGTGGTGTCCCTGAACATCACCAGCCTGGGCCTGAGAAGCCTGAAAGAAATCA
GCGACGGCGACGTGATCATCTCCGGCAACAAGAACCTGTGCTACGCCAACACCA
10 TCAACTGGAAGAAGCTGTTTCGGCACCAGCGGCCAGAAAACAAAGATCATCAGCA
ACCGGGGCGGAGAACAGCTGCAAGGCTACAGGCCAAGTGTGCCACGCTCTGTGTA
GCCCTGAAGGCTGTTGGGGACCCGAGCCTAGAGATTGCGTGTCTGTGCGAATGT
GTCCCGGGGCGAGAGAATGCGTGGACAAGTGCAATCTGCTGGAAGGCGAGCCCCG
CGAGTTCGTGGAAAACAGCGAGTGCATCCAGTGTCACCCCGAGTGTCTGCCCCA
15 GGCCATGAACATTACCTGTACCGGCAGAGGCCCGACAACCTGTATTCAAGTGCGCC
CACTACATCGACGGCCCTCACTGCGTGAAAACATGTCCTGCTGGCGTGATGGGAG
AGAACAACACCCCTCGTGTGGAAGTATGCCGACGCCGGACATGTGTGCCACCTGT
GTCACCCTAATTGCACCTACGGCTGTACAGGCCCTGGCCTGGAAGGCTGTCCAAC
AAACGGACCTAAGATCCCCCTCTATCGCCACCGGCATGGTTGGAGCCCTGCTGCTT
20 CTGCTGGTGGTGGCCCTTGGAATCGGCCTGTTTCATGTGA

HLA-A2 signal peptide nucleic acid sequence (SEQ ID NO: 116)

ATGGCTGTGATGGCCCCTAGAACACTGGTGCTGCTGCTGTCTGGTGCCCTGGCTC
TGACTCAGACATGGGCC

CD8a signal peptide nucleic acid sequence (SEQ ID NO: 148)

25 ATGGCTCTGCCTGTGACAGCTCTGCTGCTGCCTCTGGCTCTGCTTCTGCATGCCGC
TAGACCT

CD8a Hinge (SEQ ID NO: 149)

ACCACCACCCCCGCCCCAGACCCCCACCCCCGCCCCACCATCGCCAGCCAGC
CCCTGAGCCTGAGACCCGAGGCCTGCAGACCCGCCGCCGGCGGCCGTGCACA
30 CCAGAGGCCTGGACTTCGCCTGC

huGMCSF Signal Peptide, nucleic acid sequence (SEQ ID NO: 150)

ATGCTGCTGCTGGTTACATCTCTGCTGCTGTGCGAGCTGCCCCATCCTGCCTTTCT
GCTGATCCCC

Lentiviral Production and Preparation of Retronectin Plates

VSV pseudotyped lentivirus was produced in 6 well plates. In brief, 293FT were seeded the night before or the day of at 0.9×10^6 or 1.4×10^6 cells/well, respectively. Once the cells had adhered and reached at least 80% confluency a mix of lentiviral plasmid, packaging vector (psPAX2) and VSV-G envelope expressing plasmid (PMD2.G) were transfected using lipofectamine 3000 (Invitrogen), according to the manufacturer's protocol. After 18hrs, the media was replaced with 3mLs of fresh DMEM. Viral supernatants were harvested 48hrs following changing the media and spun down at 1500RPM to remove 293FT cell/debris. Retronectin was coated on 24 well non-tissue culture treated plates at $20 \mu\text{g}/\text{well}$ in PBS/- for 2hrs at 37°C or overnight at 4°C . Retronectin was removed and washed once with PBS prior to addition of lentiviral SN (2mLs). The plate was then spun at 1500G for 90 minutes at 32°C to concentrate viral particles onto the retronectin. Lentiviral SN was removed immediately prior to transduction of primary T cells or tumor cells. Alternatively, T cells were transduced with polybrene at $8 \mu\text{g}/\text{mL}$ with a spinfection of 800G for 2hrs at 32°C .

T Cell Culture, Transduction, and Isolation

Human PBMCs were activated in T cell growth media with CD3/28 microbeads (Invitrogen) in complete RPMI ($100 \mu\text{l}$ beads/ 50×10^6 PBMCs). 48hrs after activation, activated PBMCs were transferred to Lentiviral-coated Retronectin plates for 48hrs before being transferred to 6 well plates containing fresh T cell growth media. After an additional 24hrs in culture cell transduction was determined by flow cytometry and transduced cells were enriched based on huEGFRt expression. To isolate cells based on EGFR expression, T cells cultures were collected and activation beads removed. Cells were then stained in 1:100 anti-EGFR-APC antibody in MACS buffer at 4°C for 30minutes. Cells were then washed and incubated with anti-APC microbeads (Miltenyi) for 15-30minutes at 4°C . Unbound microbeads were then removed by centrifugation and huEGFRt cells were isolated by positive selection on mini-macs columns. Cells were eluted from the mini-MACS columns and put back into culture in T cell growth media and used within 2 weeks for experiments. To create stable cell lines, cells were collected and transduced as with primary T cells. EGFR selection was performed twice, two weeks apart.

T cell Stimulation

In the case where T cells were stimulated with plate bound antibodies, maxisorp Flat-bottom plates (Invitrogen) were coated with the indicated amount of anti-human CD3 antibody (HIT3a-Biolegend) in PBS/- for 2hrs at 37°C . Plates were washed twice in basal RPMI before use. For K562 stimulation K562 cells were collected and resuspended in Cytokine media and aliquoted to U-bottom plates. Similarly, A375 cells were plated 1 day

prior to the addition of T cells in DMEM in 96 well flat-bottom plates. The media was exchanged prior to the addition of cognate T cells. Following EGFR+ selection, T cells were collected, counted, and resuspended at the appropriate concentration in Cytokine media and distributed to antibody or APC-bearing wells. For K562 experiments anti-CD3
5 (HIT3a/Biolegend) was added at the indicated dose following 1-2hrs of K562/T cell interaction at 37°C. In the case where T cell proliferation was to be tracked, T cells were labelled with Violet Tracking Dye (CTV) according to Biolegend's protocol prior to the addition to stimulatory plates. Supernatant was collected at 18-36hr post stimulation to assess cytokine secretion and proliferation/ T cell killing was assessed following 96hrs of
10 stimulation.

Cytokine Multiplex Assay

Following collection of T cell supernatants cytokines were measured with the Legendplex Multi-Analyte Flow Assay Kit for human Th or Th1 cytokines (Biolegend). Manufacturer's protocol was followed with the following exceptions: 75uL T cell SN was used
15 to measure cytokines and 2uL of each reagent was used/well. Secreted cytokines were measured by flow cytometry and the values were normalized to the maximal response of the control group in order to combine and analyze multiple experiments and normalize for variability between experiments and donors.

Conjugation Assays

To assess conjugation of T cells to target cells, T cells were labelled with CFSE
20 (Biolegend) and K562_HLA-A2 or K562_HLA-A2_PD-L1 cells were labelled with CTV according to manufacturer's protocols. T cells and APCs were mixed in a 1:2 ratio and briefly centrifuged in a 1.5mL eppendorf tube to encourage conjugation. Cell pellets were incubated at 37°C for 30minutes and then cell pellets were gently resuspended by repeat pipetting (20x)
25 with a p200 and a cut-off pipette tip and assessed immediately by flow cytometry.

Flow Cytometry

Cells were collected and washed in MACS Buffer (PBS-/-, 1%FBS, 1mM EDTA) before being stained in MACS buffer containing relevant antibodies. Anti-EGFR-APC, anti-mouse TCRbeta-FITC, anti-human PD1-PE, anti-CD3 APC-Cy7, anti-CD8 PE-Cy7 were all
30 purchased from biolegend. Following addition of antibodies, cells were stained for 30-60 minutes at 4°C. For the detection of CD-19 CAR expression cells were incubated with CD-19Fc recombinant protein in MACS buffer at 1µg/mL for 30minutes at RT. Cells were then washed and incubated with anti-human FC antibody at 1:100 dilution. Cells were then

washed 3X in MACS buffer and analyzed on an Acea NovoCyt flow cytometer. Cells were collected at constant volume, allowing for accurate cell counts to be obtained.

Example 1: Design of co-stimulatory molecules comprising chimeric intracellular signaling domains

5 The disclosure herein provides the design of the co-stimulatory molecules comprising intracellular signaling domains comprising or derived from CD137/4-1BB or CD134/OX-40 receptors as depicted in FIG. 1. Examination of the sequence of the CD137 family of cytoplasmic tails (FIG. 1) showed a common membrane-proximal polybasic domain as well as several lysine residues that could serve as ubiquitination sites, as well as the TRAF binding domain that serves to activate the NF- κ B signaling pathway following receptor ligation. Without being bound by the theories, the conserved lysine residues may function as ubiquitination sites that could control the ubiquitination and degradation CD134/CD137 and that the disrupted location of the CD137 or CD134 cytoplasmic tail in the potential CD28/ICOS-CD137 CAR or CD28/ICOS-CD134 CAR receptors, respectively, could be affecting the localization or half-life of the resulting molecule, through either the poly-basic domain or the conserved lysine residues. New fusion domains of ICOS/CD28 intracellular domain and the cytoplasmic domains of CD137 or OX-40 lacking the polybasic sequence and the conserved lysine residues, as well as their wild-type (WT) counterparts were generated (FIGs. 2A and 2B). A portion of the cytoplasmic domain of CD28 responsible for the binding of Lck and Vav3 to possible enhance stimulation was also included. The extracellular domain of PD-1 was used, creating either dominant-negative (DN) version by omitting the intracellular tail or an inhibitory-switch receptor that would change a negative regulatory signal into a positive one, thus providing a cell-intrinsic PD-1 blockade. The cytoplasmic tail, i.e., intracellular co-stimulatory domain, described herein can be expanded through the use of cytoplasmic tails of other signaling proteins of interest to create new CAR receptors or different inhibitory-switch receptors, or express other immune-modulatory extracellular domains, as detailed in FIG. 3.

Example 2: Generation and testing of the in-vitro functionality of checkpoint co-stimulatory molecules

30 The disclosure herein provides the design of the co-stimulatory molecules and validation of their effect on function of a high affinity TCR. The co-stimulatory molecules described herein were designed as depicted in FIGs. 2A and 2B. In all recombinant receptors, the PD1 signal peptide (SP) was exchanged for the signal peptide from HLA-A2, which increases the surface expression of the receptor. As controls, the PD1-WT and a truncated

PD-1 lacking the ITIM-containing intracellular tail (TLs) were included. Two second-generation receptors, containing the transmembrane and intracellular domains of CD28 or ICOS were included as 2nd-generation control receptors. Additionally, fifteen 3rd-generation receptors containing the transmembrane and intracellular domains derived from ICOS and/or CD28 linked to an intracellular signaling domain of a TNF-Receptor super family member were generated. The first contained the intracellular domain of wild type CD137/4-1BB (HLA A2-SP_PD1_ICOS_BB: SEQ ID NO: 26), while the second and third contained either the CD137/4-1BB domain or the CD134/OX-40 intracellular domain with key mutations incorporated to increase surface expression (HLA A2-SP_PD1_ICOS_BBt: SEQ ID NO: 27 and HLA A2-SP_PD1_ICOS_OX40t: SEQ ID NO: 28, respectively). Further 3rd generation receptors described herein contain a chimeric intracellular domain comprising a portion of CD28 intracellular domain inserted within an ICOS intracellular domain that is further linked to either the mutated CD137 (ICOS4BBt) or mutated CD134/OX40 (ICOS-OX40t) domains (HLA A2-SP_PD1_ICOS(28)_BBt: SEQ ID NO: 29 and HLA A2-SP_PD1_ICOS(28)_OX40t: SEQ ID NO: 30). Two more 3rd generation receptors were created as described herein containing a CD28 intracellular domain linked to either mutated CD137 (28_BBt) or mutated CD134/OX40 (28_OX40t) domains (HLA A2-SP_PD1_28_BBt: SEQ ID NO: 131 and HLA A2-SP_PD1_28_OX40t: SEQ ID NO: 132) (FIG. 2A). These vectors were cloned into a lentiviral vector and fused by a self-cleaving peptide to a truncated huEGFR receptor (huEGFRt) for tracking transduced cells and magnetic selection. When these receptors were expressed by lentiviral transduction into primary T cells, each receptor expresses significantly over endogenous PD-1 levels (FIG. 4A). While the 2nd generation co-stimulatory molecules were well expressed, the inclusion of the CD137 (4-1BB) intracellular domain resulted in a considerable decrease in surface expression of the recombinant receptor. The disclosure herein shows that inclusion of the mutated intracellular domains, which maintain the TRAF-binding domains, rescues the surface expression of these optimized 3rd generation receptors. The surface expression of huEGFRt and co-stimulatory molecules demonstrates the increased expression of the truncated CD137 (4-1BB) design (ICOS_BBt) compared to the non-truncated version (ICOS_BBwt). Similar mutations in the cytoplasmic domain of CD134 (OX-40) also resulted in high surface expression of the co-stimulatory molecules (FIG. 4B).

Following transduction, T cells were isolated based on the expression of huEGFRt, to >90% purity, and used in restimulation experiments. The results disclosed herein demonstrate that, *in-vitro*, engagement of co-stimulatory molecule enhanced T cell cytokine production

and proliferation, especially at lower doses of anti-CD3 antibody (FIGs. 5A-5D). To make a more physiological system, either HLA-A2 alone or HLA-A2 alongside PD-L1 were overexpressed on K562 cells and incubated with the co-stimulatory receptor-transduced T cells and the indicated dose of anti-CD3. Incubation with K562: PD-L1 cells reduced the amount of secreted cytokine, especially with T cells overexpressing PD1_WT (Fig. 6A). While expression of either PD1_TLs or PD1_ICOS_BBwt did little to affect the secretion of IL-2, TNF, or IFN γ , the expression of PD1_28 or PD1_ICOS increased effector cytokine secretion 3-4 fold over GFP control in the presence of PD-L1 expressing K562 cells. Notably, both PD1_ICOS_BBt and PD1_ICOS_OX40t co-stimulatory molecules further improved on this effect, increasing effector cytokine secretion 2 to 3-fold over PD1_ICOS expressing cells in the presence of PD-L1 (FIG. 6C). The expression of PD1_28, PD1_28_BBt and PD1_28_OX40t resulted in comparable effector cytokine secretion (FIG. 6B). None of the constructs were constitutively active and had minimal effect on cytokine secretion in the absence of PD-L1 or anti-CD3, indicating the necessity of both PD-1 and antigen to initiate a T cell response. Fitting with the cytokine data, T cells expressing a) PD1_28, PD1_28_BBt and PD1_28_OX40t (FIG. 7A, lower panels, and FIG. 7C), and b) PD1_ICOS_BBt and PD1_ICOS_OX40t (FIG. 7B, lower panels, and FIG. 7C), proliferated best in response to K562 cells expressing PD-L1 and were best able to kill PD-L1 expressing K562 cells (FIGs. 7A-7B, upper panels). Fitting with the cytokine data, T cells expressing PD1_ICOS_BBt and PD1_ICOS_OX40t proliferated best in response to K562 cells expressing PD-L1 and were best able to kill PD-L1 expressing K562 cells (FIG. 7C-7E). Again, this response required both anti-CD3 and PD-L1 expression. The co-stimulatory molecules demonstrated co-stimulatory ability as their expression increased T cell proliferation when cells were stimulated on 96-well plates coated with anti-CD3 and anti-PD1 (Fig. 8).

The effect of the receptors with mutation of the polybasic and lysine residues is less than the PD1_ICOS_BBt co-stimulatory molecule, in terms of both surface expression of the co-stimulatory molecule (FIGs. 9A-9B), effector cytokine production in response to stimulation with anti-CD3 antibody (FIG. 9C), and T cell proliferation in response to stimulation with target cells expressing PD-L1 (FIG. 9D). The PD1_ICOS_OX40t receptor (with truncated OX40 intracellular domain) had an effect comparable to that of the wild type PD1_ICOS_OX40wt receptor (comprising wild type OX40 intracellular domain), in terms of both surface expression of the co-stimulatory molecule (FIGs. 10A-10B), effector cytokine

production in response to stimulation with anti-CD3 antibody (FIG. 10C), and T cell proliferation in response to stimulation with target cells expressing PD-L1 (FIG. 10D).

Further, the ICOS-based co-stimulatory molecules encouraged T cell: PD-L1 expressing (PD-L1+) target cell interaction in a flow-based conjugation assay, suggesting that
 5 these receptors encourage prolonged T cell – APC interactions while scanning for cognate antigen, a useful property when scanning for low-abundance antigen in the TME (FIGs. 11A-11B).

The disclosure herein shows that the co-stimulatory molecules based on the modified
 3rd-generation intracellular signaling domain disclosed herein are superior to currently
 10 existing PD1_28 co-stimulatory molecules in enhancing T cell effector function when responding to a PD-L1+ target cell. This includes increased T cell proliferation, cytokine secretion, and target cell killing. The 3rd-generation intracellular signaling domain disclosed herein can be successfully combined with TCR-T therapy targeting TAAs.

Example 3: In-vitro preclinical studies

15 Described herein are T cells expressing specific HLA-A2/NY-ESO specific TCRs and co-stimulatory molecules comprising ICOS and mutated CD137 signaling domains, that increase expression of the co-stimulatory molecule on T cell surface (FIG. 12A), effector cytokine production (FIG. 12B), and killing of target cells expressing NY-ESO (FIG. 12C), as compared to T cells expressing the specific HLA-A2/NY-ESO specific TCRs alone.

20 Described herein are CD-19 CAR constructs comprising the modified 3rd-generation intracellular signaling domains disclosed herein. The CD-19 (FMC63scFV) CARs with 3rd-generation intracellular signaling constructs described herein include constructs comprising the intracellular chimeric domains: CD28-CD137-CD3ζ (28_BBwt_z), CD28-CD137mutant-CD3ζ (28_BBt_z), CD28-CD134mutant -CD3ζ (28_OX40t_z), ICOS-
 25 CD137-CD3ζ (ICOS_BB_z), ICOS-CD137mutant-CD3ζ (ICOS_BBt_z), and ICOS-CD134mutant -CD3ζ (ICOS_OX40t_z). Also, provided are CD-19 CARs with a 3rd-generation intracellular signaling construct with a portion of CD28 inserted within the ICOS domain: ICOS(28)-CD137-CD3ζ (ICOS(28)_BBwt_z), ICOS(28)-CD137mutant-CD3ζ (ICOS(28)_BBt_z), and ICOS(28) -CD134mutant -CD3ζ (ICOS(28) _OX40t_z). Second-
 30 generation constructs comprising CD137- CD3ζ (BBwt_z), CD28- CD3ζ (28_z) and ICOS-CD3ζ (ICOS_z) are used as controls. Similar to the study described herein using the PD-1 3rd-generation intracellular signaling constructs, the CD19 CAR constructs with the CD137 and CD134 mutants domains showed higher expression as compared to the corresponding

constructs with wild type CD137 and CD134 domains (28_BBwt_z and ICOS_BBwt_z, respectively) (FIGs. 13A-13B). In-Vitro studies described herein show increased killing of CD19 expressing cells (CD19+) (FIGs. 14A-14B and FIGs. 15C-15D), increased effector cytokine production (FIG. 14C, right panels) and increased T cell proliferation and
5 persistence (FIGs. 15A-15B and 15E), by primary T cells transduced with the 3rd generation CD28 based and ICOS based CD19 CARs.

The disclosure also shows that expression of CD28 based receptors comprising a mutated CD134/CD137 signaling domains and ICOS based receptors comprising a mutated CD134/CD137 signaling domains, increased binding of BCMA specific T cells (BCMA CAR
10 T cells,) to the target antigen (BCMA-Fc) (FIGs. 16A-16B). Also, the disclosure shows that expression of the CD28 based receptors comprising a mutated CD134/CD137 signaling domains and ICOS based receptors comprising a mutated CD134/CD137 signaling domains, increased proliferation and effector cytokine production (FIGs. 16C and 16E), and target cell
15 killing (FIG. 16D) by the BCMA specific T cells in response to myeloma cell line expressing BCMA.

CLAIMS

What is claimed is:

1. A recombinant T cell co-stimulatory receptor (RTCR), comprising:
 - (a) an extracellular domain;
 - 5 (b) a transmembrane domain; and
 - (c) a chimeric intracellular domain comprising a first and at least a second signal transduction domains,
wherein the first and the at least second signal transduction domains are non-identical; and
 - 10 wherein the at least second signal transduction domain comprises a mutant CD137 (4-1BB) intracellular domain or a mutant CD134 (OX-40) intracellular domain .
- 15 2. The RTCR of claim 1, wherein the mutant CD137 intracellular domain is a truncated CD137 intracellular domain.
3. The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises an amino acid sequence according to amino acid position 13 to amino acid position 42 of the
20 CD137 intracellular domain.
4. The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten or
25 more amino acids from the N-terminus of the CD137 intracellular domain.
5. The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises a deletion of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from
amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular
30 domain.
6. The RTCR of claim 2, wherein the truncated CD137 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 3.

7. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises a deletion of one, two, three or four lysine residue(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain.
- 5 8. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises one or more lysine mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain.
9. The RTCR of claim 8, wherein the mutant CD137 intracellular domain comprises one
10 or more lysine mutation(s) at amino acid positions selected from amino acid positions 1, 5, 6 and 12 of the N-terminus of the CD137 intracellular domain.
10. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid
15 position 12 of the N-terminus of the CD137 intracellular domain.
11. The RTCR of claim 1, wherein the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain.
20
12. The RTCR of claim 11, wherein the mutant CD137 intracellular domain comprises one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, 3, 4, 5 and 6 of the N-terminus of the CD137 intracellular domain.
- 25 13. The RTCR of claim 12, wherein the mutant CD137 intracellular domain further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain.
14. The RTCR of claim 1, wherein the mutant CD134 intracellular domain is a truncated
30 CD134 intracellular domain.
15. The RTCR of claim 14, wherein the truncated CD134 intracellular domain comprises an amino acid sequence according to amino acid position 15 to amino acid position 37 of the CD134 intracellular domain.

16. The RTCR of claim 14, wherein the truncated CD134 intracellular domain comprise a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from the N-terminus of the CD134 intracellular domain.
17. The RTCR of claim 14, wherein the truncated CD134 intracellular domain comprises a deletion of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.
18. The RTCR of claim 14, wherein the truncated CD134 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 6.
19. The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises a deletion of a lysine residue from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.
20. The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD134 intracellular domain.
21. The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.
22. The RTCR of claim 1, wherein the mutant CD134 intracellular domain comprises one or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.
23. The RTCR of claim 22, wherein the mutant CD134 intracellular domain comprises one or more proximal basic amino acid mutation(s) at amino acid positions selected from amino acid positions 1, 2, and 5 of the N-terminus of the CD134 intracellular domain.

24. The RTCR of claim 23, wherein the mutant CD137 intracellular domain further comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD137 intracellular domain.
- 5 25. The RTCR of any one of claims 1-24, wherein the chimeric intracellular domain comprises a first signal transduction domain derived from a protein of the CD28 family.
26. The RTCR of claim 25, wherein the first signal transduction domain is derived from CD28, CD28H, ICOS or a combination thereof.
- 10 27. The RTCR of claim 26, wherein the first signal transduction domain is derived from ICOS.
28. The RTCR of claim 19, wherein the first signal transduction domain derived from
15 ICOS comprises an amino acid sequence according to SEQ ID NO: 9.
29. The RTCR of any one of claims 1-24, wherein the chimeric intracellular domain comprises a first signal transduction domain comprising a portion of a CD28 intracellular domain combined with an ICOS domain according to SEQ ID NO: 9.
- 20 30. The RTCR of claim 29, wherein the first signal transduction domain comprises an amino acid sequence according to any one of SEQ ID NOs: 12 and 109.
31. The RTCR of any one of claims 1-24, wherein the chimeric intracellular domain
25 comprises a first signal transduction domain derived from CD28.
32. The RTCR of claim 31, wherein the first signal transduction domain derived from CD28 comprises an amino acid sequence according to SEQ ID NO: 10.
- 30 33. The RTCR of any one of claims 31-32, wherein the first signal transduction domain comprises an amino acid sequence according to any one of SEQ ID NOs: 121-122.
34. The RTCR of any one of claims 1-30, wherein the chimeric intracellular domain comprises an amino acid sequence according to any one of SEQ ID NOs: 14-17.

35. The RTCR of any one of claims 1-34, wherein the chimeric intracellular domain further comprises a third signal transduction domain.
- 5 36. The RTCR of claim 35, wherein the third signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-2RB) protein signaling domain or a combination thereof .
37. The RTCR of claim 36, wherein the CD3 signaling domain is derived form a CD3 ζ or
10 a CD3 ϵ domain or a combination thereof.
38. The RTCR of claim 37, wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47 and 48.
- 15 39. The RTCR of claim 36, wherein the CD2 signaling domain is a mutant CD2 signaling domain.
40. The RTCR of claim 39, wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain.
20
41. The RTCR of any one of claims 39-40, wherein the CD2 signaling domain comprises an amino acid sequence according to SEQ ID NO: 49.
42. The RTCR of claim 36, wherein the IL-2RB protein signaling domain comprises an
25 amino acid sequence according to SEQ ID NO: 50.
43. The RTCR of any one of claims 1-42, wherein the chimeric intracellular domain further comprises a fourth signal transduction domain.
- 30 44. The RTCR of claim 43, wherein the fourth signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-2RB) protein signaling domain or a combination thereof, wherein the third and the fourth signal transduction domain are not identical.

45. The RTCR of claim 44, wherein the CD3 signaling domain is derived from a CD3 ζ or a CD3 ϵ domain or a combination thereof.
- 5 46. The RTCR of claim 45, wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47 and 48.
47. The RTCR of claim 44, wherein the CD2 signaling domain is a mutant CD2 signaling domain.
- 10 48. The RTCR of claim 47, wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain.
49. The RTCR of any one of claims 47-48, wherein the CD2 signaling domain comprises an amino acid sequence according to SEQ ID NO: 49.
- 15 50. The RTCR of claim 44, wherein the IL-2RB protein signaling domain comprises an amino acid sequence according to SEQ ID NO: 50.
- 20 51. The RTCR of any one of claims 1-50, wherein the extracellular domain comprises a protein or a portion thereof that induces activation and/or proliferation of an immune cell.
52. The RTCR of claims 51, wherein the extracellular domain comprises any one of:
- 25 a) a component of a T cell Receptor (TCR) complex;
- b) a component of a chimeric antigen receptor (CAR);
- c) a component of a T cell co-receptor, wherein the T cell co-receptor is a T cell co-stimulatory protein or T cell inhibitory protein;
- d) a ligand that binds to a cell surface receptor or a component thereof;
- e) a component of a cytokine receptor;
- 30 f) a component of a chemokine receptor;
- g) a component of an integrin receptor;
- h) a component of an endothelial cell surface protein receptor or a fragment thereof;

- i) a component of a neuronal guidance protein receptor; and
- j) a component of a complement receptor.

53. The RTCR of claims 52, wherein the component of the T cell co-receptor or the CAR
5 is a component of PD1, CD28, CD2, OX-40, ICOS, CTLA-4, CD28, CD3, CD4, CD8,
CD40L, Lag-3, Tim-3, or TIGIT, or a combination thereof.

54. The RTCR of claims 52, wherein the component of T cell co-receptor or the CAR
binds to CD19, B cell maturation Ag (BCMA), PD-L1, PD-L2, IL-10, a proliferation-
10 inducing ligand (APRIL), BAFF, OX-40L, ICOS-L, B7-1, B7-2, CD40, CD58, CD59, nectin,
CD155, or CD112, or a combination thereof.

55. The RTCR of claim 52, wherein the cytokine receptor binds to IL-10, IL-27, TGF- β ,
IL-12, IL-1, IL-2, IL-4, IL-5, IFN- γ , or IFN- α/β , or a combination thereof.
15

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

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

25

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

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

60. The RTCR of any one of claims 51-54 and 58-59, wherein the extracellular domain binds to a target selected from a tumor antigen, a pathogen associated protein, and an antigen associated with an autoimmune, an inflammatory, a metabolic, or a neurodegenerative condition or disorder.
- 5
61. The RTCR of claim 60, wherein the tumor antigen is a tumor associated antigen (TAA), a tumor secreted antigen (TSA) or an unconventional antigen (UCA).
62. The RTCR of any one of claims 51-61, wherein the extracellular domain binds to a target with a binding affinity of 1 fM to 100 μ M.
- 10
63. The RTCR of claim 62, wherein the extracellular domain binds to a target with a binding affinity of 1 μ M to 10 μ M.
- 15
64. The RTCR of any one of claims 1-63, wherein the extracellular domain comprises a signal peptide at the N-terminus, wherein the signal peptide is derived from a surface or a secretory protein.
65. The RTCR of claim 64, wherein the signal peptide is PD-1 signal peptide (PD-1 SP).
- 20
66. The RTCR of claim 64, wherein the signal peptide is a HLA class I histocompatibility antigen or a portion thereof.
67. The RTCR of any one of claims 1-66, wherein the extracellular domain comprises a hinge region.
- 25
68. The RTCR of claim 68, wherein the hinge region is derived from CD8, PD-1, CD28, ICOS, or IgG.
- 30
69. The RTCR of any one of claims 1-68, wherein the transmembrane domain is derived from CD8, PD1, CD28, COS, or IgG.

70. The RTCR of any one of claims 1-69, wherein the RTCR is for expression in a T cell, wherein the T cell co-expresses at least one of the endogenous co-stimulatory molecules selected from CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, and CD40L, and a combination thereof.
- 5 71. A nucleic acid encoding the RTCR of any one of claims 1-70.
72. A vector comprising the nucleic acid of claim 71.
- 10 73. A cell comprising the nucleic acid of claim 71 or the vector of claim 72.
74. The cell of claim 73, wherein the cell is a modified T cell.
75. The cell of claim 73, wherein the cell is a modified natural killer T cell (NK-T cell).
- 15 76. The cell of claim 74, wherein the modified T cell is an allogenic T cell.
77. The cell of claim 74, wherein the modified T cell is an autologous T cell.
- 20 78. The cell of claim 74, wherein the modified T cell is a naïve T cell, an early memory T cell, a stem cell-like T cell, a stem memory T cell (T_{SCM}), a central memory T cell (T_{CM}), or a regulatory T cell (T_{reg}).
- 25 79. The cell of any one of claims 73-78, wherein the cell further comprises a sequence encoding an artificial antigen receptor, a therapeutic polypeptide, or an immune cell modulatory protein, or a combination thereof.
80. The cell of claim 79, wherein the artificial antigen receptor comprises a chimeric antigen receptor (CAR).
- 30 81. The cell of any one of claims 73-80, wherein the cell expresses the RTCR of any one of claims 1-70 either transiently or stably.

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

5 83. A modified T lymphocyte (T cell), comprising:

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

10 (b) a recombinant T cell co-stimulatory receptor (RTCR) according to any one of claims 1-70.

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

85. The modified T cell of any one of claims 83-84, wherein the modified T cell co-expresses with the RTCR at least one of the endogenous co-stimulatory molecules CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8 and CD40L or a combination thereof.
20

86. A composition comprising the RTCR of any one of claims 1-70.

87. A composition comprising the nucleic acid of claim 71.

25 88. A composition comprising the vector of claim 72.

89. A composition comprising the cell of any one of claims 73-82.

90. A composition comprising the modified T cell of any one of claims 83-85.
30

91. A composition comprising a population of cells, wherein the population comprises a plurality of the cell of any one of claims 73-82 or wherein the population comprises a plurality of the modified T cell of any one of claims 83-85.

92. A method of producing a plurality of modified T cells, wherein the method comprises:
a) providing a plurality of primary T cells;
b) providing a composition comprising the RTCR of any one of claims 1-70, the nucleic acid of claim 71, or the vector of claim 72; and
5 c) introducing into the plurality of primary T cells of (a) the composition of (b), to produce a plurality of modified T cells under conditions that stably express the RTCR within the plurality of modified T cells.
93. The method of claim 92, wherein the method further comprises a step of modifying an
10 endogenous sequence encoding an endogenous T cell Receptor (TCR), wherein the modification reduces or eliminates a level of expression or activity of the endogenous TCR.
94. The method of any one of claims 92-93, wherein the method further comprises a step of modifying an endogenous sequence of the plurality of primary T cells, wherein the
15 modification reduces or eliminates a level of expression or activity of a major histocompatibility complex (MHC) class I (MHC-I).
95. The method of any one of claims 92-94, wherein the method further comprises:
d) maintaining or expanding the plurality of modified T cells in a suitable cell culture
20 media; and
e) either:
i) cryopreserving the plurality of modified T cells in a suitable cell freezing media; or
ii) preparing the plurality of modified T cells for administration to a subject in
25 need thereof.
96. A method of treating a disease or disorder, comprising administering to a subject in need thereof a therapeutically effective number of the cell of any one of claims 73-82, a therapeutically effective number of the modified T cell of any one of claims 83-85, a
30 therapeutically effective amount of the composition of any one of claims 86-91, or a therapeutically effective number of the plurality of modified T cells produced by the method of any one of claims 92-95.
97. The method of claim 96, wherein the subject is a mammal.

98. The method of claim 97, wherein the mammal is a human.
99. The method of any one of claims 96-98, wherein the disease or disorder is a cancer,
5 an autoimmune disease or disorder, an infectious disease, an inflammatory disease, a renal disease or disorder, a lung disease or disorder, a liver disease or disorder, a cardiovascular system disease or disorder, a neurodegenerative disease or disorder, or a metabolic disease or disorder.
100. The method of claim 99, wherein the cancer is a solid tumor or a hematologic cancer.
101. The method of claim 100, wherein the solid cancer is a sarcoma, a carcinoma or a melanoma.
102. The method of claim 100, wherein the hematological cancer is a leukemia, a lymphoma or a myeloma.
103. The method of claim 99, wherein the cancer is selected from acute leukemia, acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, B cell, T cell or FAB ALL,
20 acute myeloid leukemia (AML), acute myelogenous leukemia, chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodysplastic syndrome (MDS), Hodgkin's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, multiple myeloma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, bladder
25 cancer, breast cancer, colorectal cancer, endometrial cancer, head cancer, neck cancer, hereditary nonpolyposis cancer, liver cancer, lung cancer, non-small cell lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, testicular cancer, adenocarcinomas, sarcomas, malignant melanoma, and hemangioma.
104. The method of claim 99, wherein the infectious disease is caused by a bacteria, a virus, a fungus, a protozoa, or a parasite.
105. The method of claim 99, wherein the neurodegenerative disorder is Alzheimer's disease (AD) and other dementias, Parkinson's disease (PD) and PD-related disorders, prion

disease, Motor neuron diseases (MND), Huntington's disease (HD), Spinocerebellar ataxia (SCA), or Spinal muscular atrophy (SMA).

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

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

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

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

110. The CIP of claim 108, wherein the mutant CD137 intracellular domain is a truncated
CD137 intracellular domain.

25 111. The CIP of claim 110, wherein the truncated CD137 intracellular domain comprises
an amino acid sequence according to amino acid position 13 to amino acid position 42 of the
CD137 intracellular domain.

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

113. The CIP of claim 110, wherein the truncated CD137 intracellular domain comprises a
deletion of one, two, three, four, five, six, seven, eight, nine, ten or more amino acids from

amino acid position 1 to amino acid position 12 of the N-terminus of the CD137 intracellular domain.

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

115. The CIP of claim 108, wherein the mutant CD137 intracellular domain comprises a
deletion of one, two, three or four lysine residue(s) from amino acid position 1 to amino acid
position 12 of the N-terminus of the CD137 intracellular domain.
10

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

117. The CIP of claim 116, wherein the mutant CD137 intracellular domain comprises one
or more lysine mutation(s) at amino acid positions selected from amino acid positions 1, 5, 6
and 12 of the N-terminus of the CD137 intracellular domain.
15

118. The CIP of claim 108, wherein the mutant CD137 intracellular domain comprises a
deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid
position 12 of the N-terminus of the CD137 intracellular domain.
20

119. The CIP of claim 108, wherein the mutant CD137 intracellular domain comprises one
or more proximal basic amino acid mutation(s) from amino acid position 1 to amino acid
position 12 of the N-terminus of the CD137 intracellular domain.
25

120. The CIP of claim 119, wherein the mutant CD137 intracellular domain comprises one
or more proximal basic amino acid mutation(s) at amino acid positions selected from amino
acid positions 1, 2, 3, 4, 5 and 6 of the N-terminus of the CD137 intracellular domain.
30

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

122. The CIP of claim 108, wherein the mutant CD134 intracellular domain is a truncated CD134 intracellular domain.
- 5 123. The CIP of claim 122, wherein the truncated CD134 intracellular domain comprises an amino acid sequence according to amino acid position 15 to amino acid position 37 of the CD134 intracellular domain.
- 10 124. The CIP of claim 122, wherein the truncated CD134 intracellular domain comprise a deletion of a continuous stretch of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from the N-terminus of the CD134 intracellular domain.
- 15 125. The CIP of claim 122, wherein the truncated CD134 intracellular domain comprises a deletion of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen or more amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.
- 20 126. The CIP of claim 122, wherein the truncated CD134 intracellular domain comprises an amino acid sequence according to SEQ ID NO: 6.
- 25 127. The CIP of claim 108, wherein the mutant CD134 intracellular domain comprises a deletion of a lysine residue from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.
128. The CIP of claim 127, wherein the mutant CD134 intracellular domain comprises a lysine mutation at amino acid position 12 of the N-terminus of the CD134 intracellular domain.
- 30 129. The CIP of claim 108, wherein the mutant CD134 intracellular domain comprises a deletion of one or more proximal basic amino acids from amino acid position 1 to amino acid position 14 of the N-terminus of the CD134 intracellular domain.

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

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

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

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

15

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

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

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

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

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

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

140. The CIP of claim 139, wherein the first signal transduction domain derived from CD28 comprises an amino acid sequence according to SEQ ID NO: 10
141. The CIP of any one of claims 139-140, wherein the first signal transduction domain
5 comprises an amino acid sequence according to any one of SEQ ID NOs: 121-122.
142. The CIP of any one of claims 107-138, wherein the CIP comprises an amino acid sequence according to any one of SEQ ID NOs: 14-17.
- 10 143. The CIP of any one of claims 107-142, wherein the CIP further comprises a third signal transduction domain.
144. The CIP of claim 143, wherein the third signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-
15 2RB) protein signaling domain or a combination thereof.
145. The CIP of claim 144, wherein the CD3 signaling domain is derived from a CD3 ζ or a CD3 ϵ domain or a combination thereof.
- 20 146. The CIP of claim 145, wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47 and 48.
147. The CIP of claim 144, wherein the CD2 signaling domain is a mutant CD2 signaling domain.
25
148. The CIP of claim 147, wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain.
149. The CIP of any one of claims 147-148, wherein the CD2 signaling domain comprises
30 an amino acid sequence according to SEQ ID NO: 49.
150. The CIP of claim 144, wherein the IL-2RB protein signaling domain comprises an amino acid sequence according to SEQ ID NO: 50.

151. The CIP of any one of claims 107-150, wherein the chimeric intracellular domain further comprises a fourth signal transduction domain.
- 5 152. The CIP of claim 135, wherein the fourth signal transduction domain is derived from a CD3 signaling domain, a CD2 signaling domain or an interleukin 2 receptor binding (IL-2RB) protein signaling domain or a combination thereof, wherein the third and the fourth signal transduction domain are not identical.
- 10 153. The CIP of claim 152, wherein the CD3 signaling domain is derived from a CD3 ζ or a CD3 ϵ domain or a combination thereof.
154. The CIP of claim 153, wherein the CD3 signaling domain comprises an amino acid sequence according to any one of SEQ ID NOs: 18, 45, 46, 47 and 48.
- 15 155. The CIP of claim 152, wherein the CD2 signaling domain is a mutant CD2 signaling domain.
156. The CIP of claim 155, wherein the mutant CD2 signaling domain is a truncated CD2 signaling domain.
- 20 157. The CIP of any one of claims 155-156, wherein the CD2 signaling domain comprises an amino acid sequence according to SEQ ID NO: 49.
- 25 158. The CIP of claim 152, wherein the IL-2RB protein signaling domain comprises an amino acid sequence according to SEQ ID NO: 50.
- 30 159. The CIP of any one of claims 107-158, wherein the CIP is for expression in a T cell, wherein the T cell co-expresses at least one of the endogenous co-stimulatory molecules selected from CD28, CD2, OX-40, ICOS, CD28, CD3, CD4, CD8, and CD40L, and a combination thereof.