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(54) Title: IMMUNE CELL STIMULATORY SEQUENCES

(57) Abstract: The present disclosure provides, among other things, methods and compositions useful in the engineering of chimeric antigen receptors (CARs) and/or natural killer (NK) cells. The present disclosure provides, among other things, sequences for use in stimulatory domains, such as CAR stimulatory domains that are engineered to promote expansion, persistence, and/or function of NK cells. The present disclosure further provides combinations of sequences for use in stimulatory domains, such as CAR stimulatory domains that are engineered to promote expansion, persistence, and/or function of NK cells.

IMMUNE CELL STIMULATORY SEQUENCES

CROSS-REFERENCE TO RELATED APPLICATION

[1] This application claims the benefit of U.S. Provisional Application No.: 63/440,838, filed January 24, 2023, the content of which is hereby incorporated by reference in its entirety.

BACKGROUND

[2] Natural killer (NK) cells are immune cells that can participate in efficient clearance of target cells. Natural functions of NK cells include, among other things, participation in immune responses against tumors and infections. NK cells can be engineered to express chimeric antigen receptors (CARs). Engineered NK cells have been used, e.g., in tumor immunotherapy.

SUMMARY

[3] The present disclosure provides, among other things, methods and compositions useful in the engineering of chimeric antigen receptors (CARs) and/or natural killer (NK) cells. The present disclosure provides, among other things, sequences for use in stimulatory domains, such as CAR stimulatory domains that are engineered to promote expansion, persistence, and/or function of NK cells. The present disclosure further provides combinations of sequences for use in stimulatory domains, such as CAR stimulatory domains that are engineered to promote expansion, persistence, and/or function of NK cells.

[4] Without wishing to be bound by any particular scientific theory, the present disclosure is based in part on the observation that CAR stimulatory domains known in the art can include sequences and combinations of sequences that were not developed for use in NK cells and/or are not satisfactory for use in NK cells. The present disclosure includes the recognition that sequences of the present disclosure for use in stimulatory domains, and combinations thereof, provide unexpected advantages in engineered NK cells, including without limitation unexpectedly advantageous expansion, persistence, and/or function of engineered NK cells. Furthermore, it is recognized that sequences demonstrating enhanced function in NK cells could enhance function in other cell types in which these specific sequences have not yet been tested.

[5] In at least one aspect, the present disclosure provides a chimeric antigen receptor (CAR) including an antigen-binding domain, a transmembrane domain, and at least a first stimulatory sequence, wherein the stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity with a sequence selected from SEQ ID NOs: 1-17 (see, e.g., Table 1). In at least one aspect, the present disclosure provides a chimeric antigen receptor (CAR) including an antigen-binding domain, a transmembrane domain, and a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the stimulatory region has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 18-219 (see, e.g., Tables 2 and 3). In at least one aspect, the present disclosure provides a chimeric antigen receptor (CAR) including an antigen-binding domain, a transmembrane domain, and a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 220-421 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 422-623, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 2 or Table 3. In at least one aspect, the present disclosure provides a chimeric antigen receptor (CAR) including an antigen-binding domain, a transmembrane domain, and a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 220-310 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 422-512, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 2. In at least one aspect, the present disclosure provides a chimeric antigen receptor (CAR) including an antigen-binding domain, a transmembrane domain, and a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 311-421 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 513-623, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of or Table 3. In at least one aspect, the present disclosure provides a

chimeric antigen receptor (CAR) including an antigen-binding domain, a transmembrane domain, and a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a first domain sequence of Table 2 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a second domain sequence of Table 2, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 2. In at least one aspect, the present disclosure provides a chimeric antigen receptor (CAR) including an antigen-binding domain, a transmembrane domain, and a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a first domain sequence of Table 3 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a second domain sequence of Table 3, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 3. In various embodiments, the stimulatory region includes a linker positioned between the first stimulatory sequence and the second stimulatory sequence, optionally wherein the linker is a flexible linker and/or wherein the amino acids between the first stimulatory sequence and the second stimulatory sequence consist or consist essentially of the linker. In various embodiments, an exemplary linker can have or include the amino acid sequence GS.

[6] In at least one aspect, the present disclosure provides a stimulatory region including at least a first stimulatory sequence, wherein the stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity with a sequence selected from SEQ ID NOs: 1-17. In at least one aspect, the present disclosure provides a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the stimulatory region has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with both the first and second stimulatory a sequences selected from SEQ ID NOs: 18-219. In at least one aspect, the present disclosure provides a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 220-421 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 422-623, optionally wherein the first stimulatory sequence and second stimulatory sequence are each

present in a row of Table 2 or Table 3. In at least one aspect, the present disclosure provides a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 220-310 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 422-512, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 2. In at least one aspect, the present disclosure provides a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 311-421 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 513-623, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 3. In at least one aspect, the present disclosure provides a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a first domain sequence of Table 2 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a second domain sequence of Table 2, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 2. In at least one aspect, the present disclosure provides a stimulatory region including a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with first domain sequence of Table 3 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a second domain sequence of Table 3, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 3. In various embodiments, the stimulatory region includes a linker positioned between the first stimulatory sequence and the second stimulatory sequence, optionally wherein the linker is a flexible linker and/or wherein the amino acids between the first stimulatory sequence and the second stimulatory sequence consist or consist essentially of the linker. In various embodiments, an exemplary linker can have or include the amino acid sequence GS. In various embodiments, the stimulatory region is operably linked with an

antigen-binding domain. In various embodiments, the stimulatory region is operably linked with a transmembrane domain.

[7] In at least one aspect, the present disclosure provides an engineered immune cell including a chimeric antigen receptor (CAR) of the present disclosure and/or a stimulatory region of the present disclosure. In various embodiments, the cell is an NK cell. In various embodiments, the cell is a CD56+ cell. In various embodiments, the CD56+ cell is differentiated from an induced pluripotent stem cell (iPSC), embryonic stem cell (ESC), or CD34+ progenitor cell (HSPC).

[8] In at least one aspect, the present disclosure provides a method of producing an engineered immune cell, the method including contacting the immune cell with a nucleic acid encoding a chimeric antigen receptor (CAR) of the present disclosure and/or a stimulatory region of the present disclosure. In various embodiments, the cell is an NK cell. In various embodiments, the cell is a CD56+ cell. In various embodiments, the CD56+ cell is differentiated from an induced pluripotent stem cell (iPSC), embryonic stem cell (ESC), or CD34+ progenitor cell. In various embodiments, the contacting includes viral delivery of the nucleic acid to the cell. In various embodiments, the contacting includes non-viral delivery of the nucleic acid to the cell.

[9] In at least one aspect, the present disclosure provides a method of treating cancer in a subject in need thereof, the method including administering to the subject an engineered immune cell of the present disclosure. In various embodiments, the cancer is a solid tumor. In various embodiments, the solid tumor is of a cancer selected from colorectal cancer, ovarian cancer, non small cell lung cancer, glioblastoma, triple negative breast cancer, hepatocellular carcinoma, prostate cancer, melanoma, small cell lung cancer, head and neck cancer, and pancreatic cancer. In various embodiments, the cancer is a liquid cancer. In various embodiments, the liquid cancer is selected from acute myeloid leukemia (AML), multiple myeloma, acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), and mantle cell lymphoma (MCL). In various embodiments, the cancer expresses a biomarker selected from Her2, EGFR, CD19, BCMA, Muc1, CD20, Mesothelin, GPC3, Ror1, MAGE-A4, PRAME, NY-ESO-1, and PSA. In various embodiments, the administration is intravenous. In various embodiments, the administration is peri-tumoral. In various embodiments, the administration is intra-tumoral.

DEFINITIONS

[10] ***A, An, The, Or*:** As used herein, "a", "an", and "the" refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" discloses embodiments of exactly one element and embodiments including more than one element. As used herein, the terms "or" and "and/or", as conjunctions in a list of at least two elements, encompass and disclose embodiments in which the listed elements are included in the alternative, together, or in any combination.

[11] ***About*:** As used herein, term "about", when used in reference to a value, refers to a value that is similar, in context to the referenced value. In general, those skilled in the art, familiar with the context, will appreciate the relevant degree of variance encompassed by "about" in that context. For example, in some embodiments, the term "about" may encompass a range of values that within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less of the referenced value.

[12] ***Administration*:** As used herein, the term "administration" typically refers to administration of a composition to a subject or system to achieve delivery of an agent that is, or is included in, the composition.

[13] ***Amino acid*:** in its broadest sense, as used herein, refers to any compound and/or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N–C(H)(R)–COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a non-natural amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. "Standard amino acid" refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. "Nonstandard amino acid" refers to any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or obtained from a natural source. In some embodiments, an amino acid, including a carboxy- and/or amino-terminal amino acid in a polypeptide, can contain a structural modification as compared with a typical or canonical amino acid structure. For example, in some embodiments, an amino acid can be modified by methylation, amidation, acetylation, pegylation, glycosylation, phosphorylation, and/or substitution (e.g., of the amino group, the carboxylic acid group, one or more protons, and/or the hydroxyl group) as compared with the general structure. In some embodiments, such modification can, for example, alter the circulating half-life of a polypeptide containing the modified amino acid as compared with one containing an otherwise identical unmodified amino acid. In some embodiments, such modification does not significantly alter a relevant activity of a polypeptide containing the modified amino acid, as compared with one containing an otherwise identical unmodified amino acid. As will be clear from context, in some

embodiments, the term "amino acid" can be used to refer to a free amino acid; in some embodiments it can be used to refer to an amino acid residue of a polypeptide.

[14] ***Antibody*:** As used herein, the term "antibody" refers to a polypeptide that includes one or more canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular antigen (e.g., a heavy chain variable domain, a light chain variable domain, and/or one or more CDRs). Thus, the term antibody includes, without limitation, human antibodies, non-human antibodies, synthetic and/or engineered antibodies, fragments thereof, and agents including the same. Antibodies can be naturally occurring immunoglobulins (e.g., generated by an organism reacting to an antigen). Synthetic, non-naturally occurring, or engineered antibodies can be produced by recombinant engineering, chemical synthesis, or other artificial systems or methodologies known to those of skill in the art.

[15] As is well known in the art, typical human immunoglobulins are approximately 150 kD tetrameric agents that include two identical heavy (H) chain polypeptides (about 50 kD each) and two identical light (L) chain polypeptides (about 25 kD each) that associate with each other to form a structure commonly referred to as a "Y-shaped" structure. Typically, each heavy chain includes a heavy chain variable domain (VH) and a heavy chain constant domain (CH). The heavy chain constant domain includes three CH domains: CH1, CH2 and CH3. A short region, known as the "switch", connects the heavy chain variable and constant regions. The "hinge" connects CH2 and CH3 domains to the rest of the immunoglobulin. Each light chain includes a light chain variable domain (VL) and a light chain constant domain (CL), separated from one another by another "switch." Each variable domain contains three hypervariable loops known as "complement determining regions" (CDR1, CDR2, and CDR3) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). In each VH and VL, the three CDRs and four FRs arearranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of a heavy and/or a light chain are typically understood to provide a binding moiety that can interact with an antigen. Constant domains can mediate binding of an antibody to various immune system cells (*e.g.*, effector cells and/or cells that mediate cytotoxicity), receptors, and elements of the complement system. Heavy and light chains are linked to one another by a single disulfide bond, and two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. When natural immunoglobulins fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure.

[16] In some embodiments, an antibody is a polyclonal, monoclonal, monospecific, or multispecific antibody (e.g., a bispecific antibody). In some embodiments, an antibody includes at

least one light chain monomer or dimer, at least one heavy chain monomer or dimer, at least one heavy chain-light chain dimer, or a tetramer that includes two heavy chain monomers and two light chain monomers. Moreover, the term "antibody" can include (unless otherwise stated or clear from context) any art-known constructs or formats utilizing antibody structural and/or functional features including without limitation intrabodies, domain antibodies, antibody mimetics, Zybodies®, Fab fragments, Fab' fragments, F(ab')2 fragments, Fd' fragments, Fd fragments, isolated CDRs or sets thereof, single chain antibodies, single-chain Fvs (scFvs), disulfide-linked Fvs (sdFv), polypeptide-Fc fusions, single domain antibodies (e.g., shark single domain antibodies such as IgNAR or fragments thereof), cameloid antibodies, camelized antibodies, masked antibodies (e.g., Probodies®), affybodies, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-anti-Id antibodies), Small Modular ImmunoPharmaceuticals ("SMIPsTM"), single chain or Tandem diabodies (TandAb®), VHHs, Anticalins®, Nanobodies® minibodies, BiTE®s, ankyrin repeat proteins or DARPINs®, Avimers®, DARTs, TCR-like antibodies,, Adnectins®, Affilins®, Trans-bodies®, Affibodies®, TrimerX®, MicroProteins, Fynomers®, Centyrins®, and KALBITOR®s, CARs, engineered TCRs, and antigen-binding fragments of any of the above.

[17] In various embodiments, an antibody includes one or more structural elements recognized by those skilled in the art as a complementarity determining region (CDR) or variable domain. In some embodiments, an antibody can be a covalently modified ("conjugated") antibody (e.g., an antibody that includes a polypeptide including one or more canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular antigen, where the polypeptide is covalently linked with one or more of a therapeutic agent, a detectable moiety, another polypeptide, a glycan, or a polyethylene glycol molecule). In some embodiments, antibody sequence elements are humanized, primatized, chimeric, etc., as is known in the art.

[18] An antibody including a heavy chain constant domain can be, without limitation, an antibody of any known class, including but not limited to, IgA, secretory IgA, IgG, IgE and IgM, based on heavy chain constant domain amino acid sequence (*e.g.*, alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ)). IgG subclasses are also well known to those in the art and include but are not limited to human IgG1, IgG2, IgG3 and IgG4. "Isotype" refers to the Ab class or subclass (*e.g.*, IgM or IgG1) that is encoded by the heavy chain constant region genes. As used herein, a "light chain" can be of a distinct type, *e.g.*, kappa (κ) or lambda (λ), based on the amino acid sequence of the light chain constant domain. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human immunoglobulins. Naturally-produced immunoglobulins are glycosylated, typically on the CH2 domain. As is known in the art, affinity and/or other binding attributes of Fc regions for Fc receptors can be modulated through glycosylation or other modification. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, antibodies

produced and/or utilized in accordance with the present invention include glycosylated Fc domains, including Fc domains with modified or engineered such glycosylation.

[19] ***Antibody fragment***: As used herein, an "antibody fragment" refers to a portion of an antibody or antibody agent as described herein, and typically refers to a portion that includes an antigen-binding portion or variable region thereof. An antibody fragment can be produced by any means. For example, in some embodiments, an antibody fragment can be enzymatically or chemically produced by fragmentation of an intact antibody or antibody agent. Alternatively, in some embodiments, an antibody fragment can be recombinantly produced (i.e., by expression of an engineered nucleic acid sequence. In some embodiments, an antibody fragment can be wholly or partially synthetically produced. In some embodiments, an antibody fragment (particularly an antigen-binding antibody fragment) can have a length of at least about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190 amino acids or more, in some embodiments at least about 200 amino acids.

[20] ***Between or From*:** As used herein, the term "between" refers to content that falls between indicated upper and lower, or first and second, boundaries (or "bounds"), inclusive of the boundaries. Similarly, the term "from", when used in the context of a range of values, indicates that the range includes content that falls between indicated upper and lower, or first and second, boundaries, inclusive of the boundaries.

[21] ***Cancer:*** As used herein, the term "cancer" refers to a disease, disorder, or condition in which cells exhibit relatively abnormal, uncontrolled, and/or autonomous growth, so that they display an abnormally elevated proliferation rate and/or aberrant growth phenotype characterized by a significant loss of control of cell proliferation. In some embodiments, a cancer can include one or more tumors. In some embodiments, a cancer can be or include cells that are precancerous (e.g., benign), malignant, pre-metastatic, metastatic, and/or non-metastatic. In some embodiments, a cancer can be or include a solid tumor. In some embodiments, a cancer can be or include a hematologic tumor.

[22] ***Chimeric antigen receptor*:** As used herein, "Chimeric antigen receptor" or "CAR" refers to an engineered protein that includes (i) an extracellular domain that includes a moiety that binds a target antigen; (ii) a transmembrane domain; and (iii) an intracellular signaling domain that sends activating signals when the CAR is stimulated by binding of the extracellular binding moiety with a target antigen. A T cell that has been genetically engineered to express a chimeric antigen receptors may be referred to as a CAR T cell. Thus, for example, when certain CARs are expressed by a T cell, binding of the CAR extracellular binding moiety with a target antigen can activate the T cell. CARs are also known as artificial T cell receptors, chimeric T cell receptors or chimeric immunoreceptors.

[23] ***Domain:*** The term "domain" as used herein refers to a section or portion of an entity. In some embodiments, a "domain" is associated with a particular structural and/or functional feature of the entity so that, when the domain is physically separated from the rest of its parent entity, it substantially or entirely retains the particular structural and/or functional feature. Alternatively or

additionally, a domain may be or include a portion of an entity that, when separated from that (parent) entity and linked with a different (recipient) entity, substantially retains and/or imparts on the recipient entity one or more structural and/or functional features that characterized it in the parent entity. In some embodiments, a domain is a section or portion of a molecule (e.g., a small molecule, carbohydrate, lipid, nucleic acid, or polypeptide). In some embodiments, a domain is a section of a polypeptide; in some such embodiments, a domain is characterized by a particular structural element (e.g., a particular amino acid sequence or sequence motif, 〈-helix character, β-sheet character, coiled-coil character, random coil character, etc.), and/or by a particular functional feature (e.g., binding activity, enzymatic activity, folding activity, signaling activity, etc.). In some embodiments, a domain is or includes a characteristic portion or characteristic sequence element.

[24] ***Engineered*:** As used herein, the term "engineered" refers to the aspect of having been manipulated by the hand of man. For example, a polynucleotide is considered to be "engineered" when two or more sequences, that are not linked together in that order in nature, are manipulated by the hand of man to be linked to one another in the engineered polynucleotide. Those of skill in the art will appreciate that an "engineered" nucleic acid or amino acid sequence can be a recombinant nucleic acid or amino acid sequence. In some embodiments, an engineered polynucleotide includes a coding sequence and/or a regulatory sequence that is found in nature operably linked with a first sequence but is not found in nature operably linked with a second sequence, which is in the engineered polynucleotide and operably linked in with the second sequence by the hand of man. In some embodiments, a cell or organism is considered to be "engineered" if it has been manipulated so that its genetic information is altered (e.g., new genetic material not previously present has been introduced, for example by transformation, mating, somatic hybridization, transfection, transduction, or other mechanism, or previously present genetic material is altered or removed, for example by substitution, deletion, or mating). As is common practice and is understood by those of skill in the art, progeny or copies, perfect or imperfect, of an engineered polynucleotide or cell are typically still referred to as "engineered" even though the direct manipulation was of a prior entity.

[25] ***Operably linked*:** As used herein, "operably linked" refers to the association of at least a first element and a second element such that the component elements are in a relationship permitting them to function in their intended manner. For example, a nucleic acid sequence or amino acid sequence is operably linked with another sequence if it modifies the expression, structure, or activity of the linked sequence, e.g., in an intended manner. For example, a nucleic acid regulatory sequence is "*operably linked*" to a nucleic acid coding sequence if the regulatory sequence and coding sequence are associated in a manner that permits control of expression of the coding sequence by the regulatory sequence. In some embodiments, an "*operably linked*" regulatory sequence is directly or indirectly covalently associated with a coding sequence (e.g., in a single nucleic acid). In some embodiments, a regulatory sequence controls expression of a coding sequence in *trans* and inclusion of the regulatory

sequence in the same nucleic acid as the coding sequence is not a requirement of operable linkage. In many cases, two amino acid sequences are operably linked if they are expressed as a single polypeptide.

[26] ***Polypeptide:*** As used herein, "polypeptide" refers to any polymeric chain of amino acids. In some embodiments, a polypeptide has an amino acid sequence that occurs in nature. In some embodiments, a polypeptide has an amino acid sequence that does not occur in nature. In some embodiments, a polypeptide has an amino acid sequence that is engineered in that it is designed and/or produced through action of the hand of man. In some embodiments, a polypeptide may be or include of natural amino acids, non-natural amino acids, or both. In some embodiments, a polypeptide may be or include only natural amino acids or only non-natural amino acids. In some embodiments, a polypeptide can include D-amino acids, L-amino acids, or both. In some embodiments, a polypeptide may include only L-amino acids. In some embodiments, a polypeptide may include one or more pendant groups or other modifications, e.g., one or more amino acid side chains, e.g., at the polypeptide's N-terminus, at the polypeptide's C-terminus, at non-terminal amino acids, or at any combination thereof. In some embodiments, such pendant groups or modifications may be selected from acetylation, amidation, lipidation, methylation, phosphorylation, glycosylation, glycation, sulfation, mannosylation, nitrosylation, acylation, palmitoylation, prenylation, pegylation, etc., including combinations thereof. In some embodiments, a polypeptide may be cyclic, and/or may include a cyclic portion.

[27] In some embodiments, the term "polypeptide" may be appended to a name of a reference polypeptide, activity, or structure to indicate a class of polypeptides that share a relevant activity or structure. For such classes, the present specification provides and/or those skilled in the art will be aware of exemplary polypeptides within the class whose amino acid sequences and/or functions are known. In some embodiments, a member of a polypeptide class or family shows significant sequence homology or identity with, shares a common sequence motif (e.g., a characteristic sequence element) with, and/or shares a common activity (in some embodiments at a comparable level or within a designated range) with a reference polypeptide of the class. For example, in some embodiments, a member polypeptide shows an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40%, and is often greater than about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (e.g., a conserved region that can in some embodiments be or include a characteristic sequence element) that shows very high sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and in some instances up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids. In some embodiments, a relevant polypeptide can be or include a fragment of a parent polypeptide. In some embodiments, a

useful polypeptide may be or include a plurality of fragments, each of which is found in the same parent polypeptide in a different spatial arrangement relative to one another than is found in the polypeptide of interest (e.g., fragments that are directly linked in the parent may be spatially separated in the polypeptide of interest or vice versa, and/or fragments may be present in a different order in the polypeptide of interest than in the parent), so that the polypeptide of interest is a derivative of its parent polypeptide.

[28] ***Subject:*** As used herein, the term "subject" refers to an organism, typically a mammal (e.g., a human, rat, or mouse). In some embodiments, a subject is suffering from a disease, disorder or condition. In some embodiments, a subject is susceptible to a disease, disorder, or condition. In some embodiments, a subject displays one or more symptoms or characteristics of a disease, disorder or condition. In some embodiments, a subject is not suffering from a disease, disorder or condition. In some embodiments, a subject does not display any symptom or characteristic of a disease, disorder, or condition. In some embodiments, a subject has one or more features characteristic of susceptibility to or risk of a disease, disorder, or condition. In some embodiments, a subject is a subject that has been tested for a disease, disorder, or condition, and/or to whom therapy has been administered. In some instances, a human subject can be interchangeably referred to as a "patient" or "individual." A subject administered an agent associated with treatment of a disease, disorder, or condition with which the subject is associated can be referred to as a subject in need of the agent, i.e., as a subject in need thereof.

[29] ***Therapeutically effective amount:*** As used herein, "therapeutically effective amount" refers to an amount that produces the desired effect for which it is administered. In some embodiments, the term refers to an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, and/or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence and/or severity of, and/or delays onset of, one or more symptoms of the disease, disorder, and/or condition. Those of ordinary skill in the art will appreciate that a therapeutically effective amount does not necessarily achieve successful treatment in every particular treated individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine, etc.). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount of a particular agent or therapy may be formulated and/or administered in a single dose. In some embodiments, a therapeutically effective agent may be formulated and/or administered in a plurality of doses, for example, as part of a dosing regimen.

[30] ***Treatment***: As used herein, the term "treatment" (also "treat" or "treating") refers to administration of a therapy that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, and/or reduces incidence of one or more symptoms, features, and/or causes of a particular disease, disorder, or condition, or is administered for the purpose of achieving any such result. In some embodiments, such treatment can be of a subject who does not exhibit signs of the relevant disease, disorder, or condition and/or of a subject who exhibits only early signs of the disease, disorder, or condition. Alternatively or additionally, such treatment can be of a subject who exhibits one or more established signs of the relevant disease, disorder and/or condition. In some embodiments, treatment can be of a subject who has been diagnosed as suffering from the relevant disease, disorder, and/or condition. In some embodiments, treatment can be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, or condition.

BRIEF DESCRIPTION OF THE DRAWINGS

[31] Fig. 1 is a schematic of an exemplary general architecture of CARs.

[32] Fig. 2 describes an exemplary CAR architecture in which CARs that include combinations of distinct stimulatory sequences derived from different full protein domains are expressed in NK cells. NK cells are subsequently subjected to a serial restimulation assay.

[33] Fig. 3 is a graph displaying data from an example in which CARs that include combinations of distinct stimulatory sequences derived from different combinations of full protein domains are expressed in NK cells that are subsequently subjected to a serial restimulation assay. The chart depicts the log2 fold-change of NK cell numbers for NK cells expressing different CAR designs at day 11 versus day 0 after daily stimulation with Raji target cells at a 1:1 NK:Raji ratio.

[34] Fig. 4 is a graph displaying data from an example in which CARs that include combinations of distinct stimulatory sequences derived from different combinations of full protein domains are expressed in NK cells that are subsequently subjected to a serial restimulation assay. The chart depicts the log2 fold-change of NK cell numbers for NK cells expressing different CAR designs at day 11 versus day 0 after daily stimulation with Raji target cells at a 1:1 NK:Raji ratio in low IL-2 conditions.

[35] Fig. 5 is a graph displaying data from an example in which CARs that include combinations of distinct stimulatory sequences derived from different combinations of full protein domains are expressed in NK cells that are subsequently subjected to a serial

restimulation assay. The chart depicts the log2 fold-change of NK cell numbers for NK cells expressing different CAR designs at day 11 versus day 0 after stimulation with Raji target cells at a 1:1 NK:Raji ratio every 2-3 days.

[36] Fig. 6 is a schematic of CAR architecture for an example in which CARs that include combinations of distinct stimulatory sequences derived from different combinations of full protein domains are expressed in NK cells that are subsequently subjected to a serial restimulation assay.

[37] Fig. 7 is a graph displaying data from an example in which CARs that include combinations of distinct stimulatory sequences derived from different combinations of protein signaling motifs are expressed in NK cells that are subsequently subjected to a serial restimulation assay. The chart depicts the log2 fold-change of NK cell numbers for NK cells expressing different CAR designs at day 11 versus day 0 after daily stimulation with Raji target cells at a 1:1 NK:Raji ratio.

[38] Fig. 8 is a graph displaying data from an example in which CARs that include combinations of distinct stimulatory sequences derived from different combinations of protein signaling motifs are expressed in NK cells that are subsequently subjected to a serial restimulation assay. The chart depicts the log2 fold-change of NK cell numbers for NK cells expressing different CAR designs at day 11 versus day 0 after daily stimulation with Raji target cells at a 1:1 NK:Raji ratio in low IL-2 conditions.

[39] Fig. 9 is a graph displaying data from an example in which CARs that include combinations of distinct stimulatory sequences derived from different combinations of protein signaling motifs are expressed in NK cells that are subsequently subjected to a serial restimulation assay. The chart depicts the log2 fold-change of NK cell numbers for NK cells expressing different CAR designs at day 11 versus day 0 after stimulation with Raji target cells at a 1:1 NK:Raji ratio every 2-3 days.

[40] Fig. 10 is a graph displaying serial restimulation NK cell number data from an arrayed validation of the Example 1 pooled screening workflow. Different shading depicts whether that particular stimulatory sequences was positively or negatively enriched in the pooled assay.

[41] Fig. 11 is a graph of spheroid killing by different CAR-NK variants in Low-vs-Normal IL-2 conditions.

[42] Fig. 12 is a tabular depiction of killing scores from an acute cytotoxicity assay of different CAR-NK variants against different target cells and at different effector:target (E:T) ratios.

[43] Fig. 13 is representative IVIS imaging of remaining tumor burden in a Raji xenograft model after treatment with an industry benchmark or novel CAR-NK design.

DETAILED DESCRIPTION

[44] The present disclosure provides, among other things, sequences for use in stimulatory domains of CARs (which can be referred to herein as "stimulatory sequences"). In various embodiments, the present disclosure provides sequences for use in stimulatory domains of CARs that are particularly useful in engineering of NK cells. Accordingly, the present disclosure includes NK cells engineered to express CARs (CAR-NK cells) including stimulatory sequences of the present disclosure.

Chimeric Antigen Receptors

[45] CARs are engineered proteins designed to redirect and amplify the response of immune cells against cells expressing specific antigen targets. CARs generally include three modules: an extracellular binding domain, a transmembrane domain, and one or more intracellular stimulatory sequences (see, e.g., Figs. 1 and 6). As those of skill in the art will appreciate, extracellular binding domains, transmembrane domains, and intracellular stimulatory sequence(s) are modular at least in that sequences of each can be independently engineered and/or that a functional CAR can be produced by independent selection of sequences for each. Accordingly, although an extracellular binding domain, a transmembrane domain, and intracellular stimulatory sequence(s) of a CAR function cooperatively, those of skill in the art appreciate that each is an independently engineered and independently useful component.

[46] An extracellular binding domain can be or include a binding domain such as an antibody or antibody fragment, that specifically binds an antigen. For example, an extracellular domain can be an scFv or nanobody that specifically binds a given antigen target.

[47] Transmembrane domains within a CAR molecule can serve to connect the extracellular component and intracellular component through the cell membrane. The transmembrane domain can anchor the expressed molecule in a cell's membrane. CAR transmembrane domains can be derived from transmembrane domains of proteins such as

CD28, CD27, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22; CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

[48] Broadly, intracellular stimulatory sequences determine the signaling consequences of antigen binding. The first intracellular stimulatory sequences were designed to model TCR signaling in T cells, and subsequent generations (e.g., including multiple stimulatory sequences) have likewise been developed for use in T cells. First generation CARs utilized the cytoplasmic region of CD3 as a stimulatory sequence. Second generation CARs utilized CD3 in combination with cluster of differentiation 28 (CD28) or 4-1BB (CD137), while third generation CARs have utilized CD3 in combination with CD28 and 4-IBB within intracellular effector domains.

[49] By contrast, the exploration of stimulatory sequences for use in NK cells has been shallow..

CAR-T and CAR-NK cells

[50] Without wishing to be bound by any particular scientific theory, CARs function by tying an antigen-binding event to specific signaling activity in the immune cell. CARs in T cells can be viewed as synthetic TCRs, where the predominant signaling driven by the CAR falls along the canonical T cell activation pathways. For example, CAR designs featuring the CD3z domain activate T cells via PLCg-dependent signaling that drives Jun and Stat3-mediated transcriptional activation. Inclusion of co-stimulatory domains such as 4-1BB and CD28 drive additional pro-survival signaling through the PI3K and Jnk pathways.

[51] CARs can also be expressed in NK cells (canonically CD56+, CD3-). In the mammalian immune system, NK cells activate by summing signals across a wide range of primary and supporting activating receptors such as CD16, NKG2D, NKp46, and 2B4. These native receptors represent functional and signaling information that is distinct from that of canonical TCR signaling in T cells. Thus, the present disclosure includes the recognition that there exists a breadth of potential NK-specific CAR designs that incorporate features of NK cell activation, including features that fall outside of canonical NK activation pathways.

Stimulatory Sequences

[52] Without wishing to be bound by any particular scientific theory, binding of a target antigen initiates downstream CAR signaling events through recruitment of adapter and second messenger proteins to stimulatory sequences (including, e.g., domains and motifs

associated with stimulatory activity). Downstream CAR signaling events can cause activation of cells in which they occur, where activation can include one or more of differentiation, proliferation and/or activation or other effector functions. Canonical CAR designs (e.g., for use in CAR-T cells) feature stimulatory sequences that activate Src family protein tyrosine kinases. For example, such stimulatory sequences are found in the cytoplasmic tails of various CD3 chains in T cells, as well as in those of NK receptors such as CD16.

[53] Several alternative stimulatory domains have been explored in NK cells, including CD28, 2B4, and OX40. However, the present disclosure includes the recognition that there is a need for further, alternative, and/or improved stimulatory sequences for use in NK cells (e.g., NK-CAR cells), and that certain such sequences can be advantageously selected and/or derived from NK-native stimulatory domains. The present disclosure includes the recognition that such stimulatory sequences and combinations thereof could provide an increased diversity in CAR signaling and functional outcomes (e.g., in CARs and/or for NK-CAR cells), and/or drive enhanced stimulation.

[54] The present disclosure discloses stimulatory sequences and combinations thereof that are, e.g., particularly useful in engineering of CARs for use in NK cells, and production of CAR-NK cells.

[55] The present disclosure includes the discovery that certain stimulatory sequences identified herein as useful in CARs and/or NK-CAR cells are unexpectedly characterized by (e.g., having, or derived from domains having) certain shared features and/or biological functions. The present disclosure further includes the discovery that combinations of stimulatory sequences that include a first stimulatory sequence characterized by a first feature and/or biological function and a second stimulatory sequence characterized by a second feature and/or biological function can be particularly advantageous.

[56] The present disclosure describes two categories of stimulatory sequences: those incorporating one or more full protein domains, and those incorporating one or more individual signaling motifs. Among stimulatory sequences representing one or more protein domains provided herein, various such domains are characterized by a certain biological function when present in cells, and combinations of full stimulatory sequence domains having certain such biological functions give rise to unexpectedly advantageous properties, e.g., for NK cell activation. CD40 (e.g. included in SEQ ID NOs 21, 22, 30) is essential for mediating a broad variety of immune and inflammatory responses via NFkB signaling. 4-

1BB (e.g. included in SEQ ID NOs 22, 27, 33) signaling results in increased NFkB pathway activation. DAP10 (e.g. included in SEQ ID NOs 23, 37, 51) is involved in JAK3/STAT5a and PI3K signaling. CD27 transduces signals that lead to the activation of NFkB and MAPK8/JNK. CD16 (e.g. included in SEQ ID NOs 44, 56, 59) domains contain immunomodulatory tyrosine activating motifs (ITAMs) and are a canonical route of NK cell activation. FCER1G (e.g. included in SEQ ID NOs 79, 94, 107) activation domains also contain activating motifs and have been used as alternatives to CD3z in CAR designs.

[57] Among the stimulatory sequences including one or more signaling motifs, again each motif represents a downstream functional effect in the cell, the combination of which gives rise to the specific overall functionality. Motifs derived from OX40 (e.g. included in SEQ ID NOs 109, 110, 166) and CD40 (e.g. included in SEQ ID NOs 128, 133, 137) are involved in canonical NFkB signaling. Those derived from TANK (e.g. included in SEQ ID NOs 114, 117, 119) can activate NK cells through non-canonical NFkB signaling.

Table 1: Stimulatory sequence domains/motifs

Seq ID #	Protein that sequences are derived from	Category	Sequence	Motif ID (if applicable)
1	NKp30	Full Domain	GSTVYYQGKCLTWKGPRRQLPAV VPAPLPPPCGSSAHLLPPVPGG	NA
2	NKp46	Full Domain	RKRTRERASRASTWEGRRRLNTQT L	NA
3	Fn14	Full Domain	RRCRRREKFTTPIEETGGEGCPAVA LIQ	NA
4	NKp44	Full Domain	WWGDIWWKTMMELRSLDTQKAT CHLQQVTDLPWTSVSSPVEREILY HTVARTKISDDDDEHTL	NA
5	RANK	Full Domain	CYRKKGKALTANLWHWINEACGR LSGDKESSGDSCVSTHTANFGQQG ACEGVLLLTLEEKTFPEDMCYPDQ GGVCQGTCVGGGPYAQGEDARM LSLVSKTEIEEDSFRQMPTEDEYM DRPSQPTDQLLFLTEPGSKSTPPFSE PLEVGENDSLSQCFTGTQSTVGSES CNCTEPLCRTDWTPMSSENYLQKE VDSGHCPHWAASPSPNWADVCTG CRNPPGEDCEPLVGSPKRGPLPQC AYGMGLPPEEEASRTEARDQPEDG	NA

			ADGRLPSSARAGAGSGSSPGGQSP ASGNVTGNSNSTFISSGQVMNFKG DIIVVYVSQTSQEGAAAAAEPMGR PVQEETLARRDSFAGNGPRFPDPC GGPEGLREPEKASRPVQEQGGAKA	
6	CRTAM	Full Domain	IMKLRKAHVIWKKENEVSEHTLES YRSRSNNEETSSEEKNGQSSHPMR CMNYITKLYSEAKTKRKENVQHS KLEEKHIQVPESIV	NA
7	TANK	Motif	TETQCSVPIQCTDKTDKQTETQCS VPIQCTDKTDKQ	TANK_HUMAN_TANK_180
8	CADH5	Motif	LGTDSSDDVDYDFLNDWGDVDYD FLNDWGDWGPRFK	CADH5_HUMAN_ITIM_755
9	KI3L2	Motif	KTPLTDTTSVYTELPNAETSVYTEL PNAEEPRSKVV	KI3L2_HUMAN_ITIM_428
10	CXCR6	Motif	LVKDIGCCLPYLGVSHQWCLPYLG VSHQWWKSSEDN	CXCR6_HUMAN_ITIM_311
11	FCG2A	Motif	DYETADGGYMTLNPRAPTDDDKN IYLTLPPNDHVNS	FCG2A_HUMAN_ITAM_288304
12	FCG2A	Motif	NDYETADDGGYMTLNPRADGGY MTLNPRAAPTDDDK	FCG2A_HUMAN_ITAM_288
13	FCG2A	Motif	APTDDDKKNIYLTLPPNDKNIYLTL PPNDDHVNSNN	FCG2A_HUMAN_ITAM_304
14	LIRB4	Motif	HDEDPQAAVTYAKVKHSRAVTYA KVKHSRRPRREMA	LIRB4_HUMAN_ITIM_360
15	LIRB4	Motif	ASEAPQDDVTYAQLHSFTDVTYA QLHSFTTLRQKAT	LIRB4_HUMAN_ITIM_412
16	PECA1	Motif	KEPLNSDDVQYTEVQVSSDVQYTE VQVSSSAESHKD	PECA1_HUMAN_ITIM_690
17	PECA1	Motif	MGKKDTEETVYSEVRKAVETVYS EVRKAVVPDAVES	PECA1_HUMAN_ITSM_713

Table 2: Stimulatory sequences derived from one or more full protein domains

SEQ ID NO	**Protein that first domain sequence is derived from**	**SEQ ID NO of first dom. seq.**	**first domain sequence**	**Linker**	**Protein that second domain sequence is derived from**	**SEQ ID NO of second dom. seq.**	**second domain sequence**

18	4-1BB	220	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	Fn14	422	RRCRRREKFTTPIEETGG EGCPAVALIQ
19	Fn14	221	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	CD27	423	QRRKYRSNKGESPVEPA EPCHYSCPREEEGSTIPI QEDYRKPEPACSP
20	Fn14	222	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	NKp30	424	GSTVYYQGKCLTWKGP RRQLPAVVPAPLPPPCG SSAHLLPPVPGG
21	CD40	223	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	FcgRIIIa	425	KTNIRSSTRDWKDHKFK WRKDPQDK
22	CD40	224	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	4-1BB	426	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL
23	CD27	225	QRRKYRSNKGESPV EPAEPCHYSCPREEE GSTIPIQEDYRKPEP ACSP	GS	DAP10	427	LCARPRRSPAQEDGKV YINMPGRG
24	ICOS	226	CWLTKKKYSSSVH DPNGEYMFMRAVN TAKKSRLTDVTL	GS	CD40	428	KKVAKKPTNKAPHPKQ EPQEINFPDDLPGSNTA APVQETLHGCQPVTQE DGKESRISVQERQ
25	NKp46	227	RKRTRERASRASTW EGRRRLNTQTL	GS	Fn14	429	RRCRRREKFTTPIEETGG EGCPAVALIQ
26	NKp44	228	WWGDIWWKTMME LRSLDTQKATCHLQ QVTDLPWTSVSSPV EREILYHTVARTKIS DDDDEHTL	GS	Fn14	430	RRCRRREKFTTPIEETGG EGCPAVALIQ

27	NKp30	229	GSTVYYQGKCLTW KGPRRQLPAVVPAP LPPPCGSSAHLLPPV PGG	GS	4-1BB	431	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL
28	CD40	230	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	NKp30	432	GSTVYYQGKCLTWKGP RRQLPAVVPAPLPPPCG SSAHLLPPVPGG
29	CD40	231	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	FCER1G	433	RLKIQVRKAAITSYEKS DGVYTGLSTRNQETYET LKHEKPPQ
30	Fn14	232	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	CD40	434	KKVAKKPTNKAPHPKQ EPQEINFPDDLPGSNTA APVQETLHGCQPVTQE DGKESRISVQERQ
31	NKp44	233	WWGDIWWKTMME LRSLDTQKATCHLQ QVTDLPWTSVSSPV EREILYHTVARTKIS DDDDEHTL	GS	ICOS	435	CWLTKKKYSSSVHDPN GEYMFMRAVNTAKKSR LTDVTL
32	CD27	234	QRRKYRSNKGESPV EPAEPCHYSCPREEE GSTIPIQEDYRKPEP ACSP	GS	FCER1G	436	RLKIQVRKAAITSYEKS DGVYTGLSTRNQETYET LKHEKPPQ
33	4-1BB	235	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	CD40	437	KKVAKKPTNKAPHPKQ EPQEINFPDDLPGSNTA APVQETLHGCQPVTQE DGKESRISVQERQ
34	Fn14	236	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	FcgRIIIa	438	KTNIRSSTRDWKDHKFK WRKDPQDK

35	4-1BB	237	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	2B4	439	WRRKRKEKQSETSPKEF LTIYEDVKDLKTRRNHE QEQTFPGGGSTIYSMIQS QSSAPTSQEPAYTLYSLI QPSRKSGSRKRNHSPSFN STIYEVIGKSQPKAQNPA RLSRKELENFDVYS
36	Fn14	238	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	4-1BB	440	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL
37	CD40	239	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	DAP10	441	LCARPRRSPAQEDGKV YINMPGRG
38	RANK	240	CYRKKGKALTANL WHWINEACGRLSG DKESSGDSCVSTHT ANFGQQGACEGVL LLTLEEKTFPEDMC YPDQGGVCQGTCV GGGPYAQGEDARM LSLVSKTEIEEDSFR QMPTEDEYMDRPS QPTDQLLFLTEPGS KSTPPFSEPLEVGEN DSLSQCFTGTQSTV GSESCNCTEPLCRT DWTPMSSENYLQK EVDSGHCPHWAAS PSPNWADVCTGCR NPPGEDCEPLVGSP KRGPLPQCAYGMG LPPEEEASRTEARD QPEDGADGRLPSSA RAGAGSGSSPGGQS PASGNVTGNSNSTFI	GS	CD28	442	RSKRSRLLHSDYMNMT PRRPGPTRKHYQPYAPP RDFAAYRS

			SSGQVMNFKGDIIV VYVSQTSQEGAAA AAEPMGRPVQEETL ARRDSFAGNGPRFP DPCGGPEGLREPEK ASRPVQEQGGAKA				
39	NKp46	241	RKRTRERASRASTW EGRRRLNTQTL	GS	DAP10	443	LCARPRRSPAQEDGKV YINMPGRG
40	RANK	242	CYRKKGKALTANL WHWINEACGRLSG DKESSGDSCVSTHT ANFGQQGACEGVL LLTLEEKTFPEDMC YPDQGGVCQGTCV GGGPYAQGEDARM LSLVSKTEIEEDSFR QMPTEDEYMDRPS QPTDQLLFLTEPGS KSTPPFSEPLEVGEN DSLSQCFTGTQSTV GSESCNCTEPLCRT DWTPMSSENYLQK EVDSGHCPHWAAS PSPNWADVCTGCR NPPGEDCEPLVGSP KRGPLPQCAYGMG LPPEEEASRTEARD QPEDGADGRLPSSA RAGAGSGSSPGGQS PASGNVTGNSNSTFI SSGQVMNFKGDIIV VYVSQTSQEGAAA AAEPMGRPVQEETL ARRDSFAGNGPRFP DPCGGPEGLREPEK ASRPVQEQGGAKA	GS	NKp30	444	GSTVYYQGKCLTWKGP RRQLPAVVPAPLPPPCG SSAHLLPPVPGG

41	Fn14	243	RRCRRREKFTTPIEETGGEGCPAVALIQ	GS	FCER1G	445	RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
42	DAP12	244	YFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK	GS	Fn14	446	RRCRRREKFTTPIEETGGEGCPAVALIQ
43	CD40	245	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ	GS	DAP12	447	YFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK
44	FcgRIIIa	246	KTNIRSSTRDWKDHKFKWRKDPQDK	GS	CD40	448	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
45	Fn14	247	RRCRRREKFTTPIEETGGEGCPAVALIQ	GS	Fn14	449	RRCRRREKFTTPIEETGGEGCPAVALIQ
46	4-1BB	248	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL	GS	NKp30	450	GSTVYYQGKCLTWKGPRRQLPAVVPAPLPPPCGSSAHLLPPVPGG
47	DAP12	249	YFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK	GS	ICOS	451	CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL
48	NKp44	250	WWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDDDEHTL	GS	NKp46	452	RKRTRERASRASTWEGRRRLNTQTL
49	CD40	251	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHG	GS	NKp44	453	WWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDDDEHTL

			CQPVTQEDGKESRISVQERQ				
50	Fn14	252	RRCRRREKFTTPIEETGGEGCPAVALIQ	GS	DAP12	454	YFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK
51	DAP10	253	LCARPRRSPAQEDGKVYINMPGRG	GS	BAFFR	455	SWRRRQRRLRGASSAEAPDGDKDAPEPLDKVIILSPGISDATAPAWPPPGEDPGTTPPGHSVPVPATELGSTELVTTKTAGPEQQ
52	CD27	254	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP	GS	CD40	456	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
53	CD40	255	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ	GS	CD40	457	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
54	Fn14	256	RRCRRREKFTTPIEETGGEGCPAVALIQ	GS	DAP10	458	LCARPRRSPAQEDGKVYINMPGRG
55	blank	257		GS	CD40	459	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
56	SLAMF7	258	WFLKRERQEEYIEEKKRVDICRETPNICPHSGENTEYDTIPHTNRTILKEDPANTVYSTVEIPKKMENPHSLLTMPDTPRLFAYENVI	GS	FcgRIIIa	460	KTNIRSSTRDWKDHKFKWRKDPQDK

57	Fn14	259	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS		461	
58	CD40	260	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	SLAMF7	462	WFLKRERQEEYIEEKKR VDICRETPNICPHSGENT EYDTIPHTNRTILKEDPA NTVYSTVEIPKKMENPH SLLTMPDTPRLFAYENVI
59	CD2	261	KRKKQRSRRNDEEL ETRAHRVATEERGR KPHQIPASTPQNPAT SQHPPPPPGHRSQAP SHRPPPPGHRVQHQ PQKRPPAPSGTQVH QQKGPPLPRPRVQP KPPHGAAENSLSPSS N	GS	FcgRIIIa	463	KTNIRSSTRDWKDHKFK WRKDPQDK
60	CRTAM	262	IMKLRKAHVIWKKE NEVSEHTLESYRSR SNNEETSSEEKNGQ SSHPMRCMNYITKL YSEAKTKRKENVQ HSKLEEKHIQVPESI V	GS	FcgRIIIa	464	KTNIRSSTRDWKDHKFK WRKDPQDK
61	blank	263		GS	BAFFR	465	SWRRRQRRLRGASSAEA PDGDKDAPEPLDKVIILS PGISDATAPAWPPPGEDP GTTPPGHSVPVPATELGS TELVTTKTAGPEQQ
62	Fn14	264	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	SLAMF7	466	WFLKRERQEEYIEEKKR VDICRETPNICPHSGENT EYDTIPHTNRTILKEDPA NTVYSTVEIPKKMENPH SLLTMPDTPRLFAYENVI

63	4-1BB	265	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	DAP10	467	LCARPRRSPAQEDGKV YINMPGRG
64	DAP10	266	LCARPRRSPAQEDG KVYINMPGRG	GS	CD40	468	KKVAKKPTNKAPHPKQ EPQEINFPDDLPGSNTA APVQETLHGCQPVTQE DGKESRISVQERQ
65	ICOS	267	CWLTKKKYSSSVH DPNGEYMFMRAVN TAKKSRLTDVTL	GS	CD28	469	RSKRSRLLHSDYMNMT PRRPGPTRKHYQPYAPP RDFAAYRS
66	blank	268		GS	Fn14	470	RRCRRREKFTTPIEETGG EGCPAVALIQ
67	blank	269		GS	NKp65	471	DLKFWHKKMDFSQNV NVSSLSGHNYLCPNDW LLNEGKCYWFSTSFKT WKESQRDCTQLQAHLL VIQNLDELEFIQNSLKPG HFGWIGLYVTFQGNLW MWIDEHFLVPELFSVIG PTDDRSCAVITGNWVYS EDCSSTFKGICQRDAILT HNGTSGV
68	Fn14	270	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	2B4	472	WRRKRKEKQSETSPKEF LTIYEDVKDLKTRRNHE QEQTFPGGGSTIYSMIQS QSSAPTSQEPAYTLYSLI QPSRKSGSRKRNHSPSFN STIYEVIGKSQPKAQNPA RLSRKELENFDVYS
69	CD27	271	QRRKYRSNKGESPV EPAEPCHYSCPREEE GSTIPIQEDYRKPEP ACSP	GS	SLAMF6	473	LRKRRDSLSLSTQRTQGP AESARNLEYVSVSPTNN TVYASVTHSNRETEIWTP RENDTITIYSTINHSKESK PTFSRATALDNVV

70	4-1BB	272	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	CD28	474	RSKRSRLLHSDYMNMT PRRPGPTRKHYQPYAPP RDFAAYRS
71	FcgRIIIa	273	KTNIRSSTRDWKDH KFKWRKDPQDK	GS	SLAMF6	475	LRKRRDSLSLSTQRTQGP AESARNLEYVSVSPTNN TVYASVTHSNRETEIWTP RENDTITIYSTINHSKESK PTFSRATALDNVV
72	4-1BB	274	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	CD27	476	QRRKYRSNKGESPVEPA EPCHYSCPREEEGSTIPI QEDYRKPEPACSP
73	CD3zeta	275	RVKFSRSADAPAYQ QGQNQLYNELNLG RREEYDVLDKRRG RDPEMGGKPRRKN PQEGLYNELQKDK MAEAYSEIGMKGER RRGKGHDGLYQGL STATKDTYDALHM QALPPR	GS	CD3zeta	477	RVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKM AEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTY DALHMQALPPR
74	NKp44	276	WWGDIWWKTMME LRSLDTQKATCHLQ QVTDLPWTSVSSPV EREILYHTVARTKIS DDDDEHTL	GS	SLAMF6	478	LRKRRDSLSLSTQRTQGP AESARNLEYVSVSPTNN TVYASVTHSNRETEIWTP RENDTITIYSTINHSKESK PTFSRATALDNVV
75	DAP10	277	LCARPRRSPAQEDG KVYINMPGRG	GS	4-1BB	479	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL
76	FcgRIIIa	278	KTNIRSSTRDWKDH KFKWRKDPQDK	GS	4-1BB	480	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL
77	SLAMF7	279	WFLKRERQEEYIEE KKRVDICRETPNICP HSGENTEYDTIPHT NRTILKEDPANTVY	GS	CD28	481	RSKRSRLLHSDYMNMT PRRPGPTRKHYQPYAPP RDFAAYRS

			STVEIPKKMENPHS LLTMPDTPRLFAYE NVI				
78	Fn14	280	RRCRRREKFTTPIEE TGGEGCPAVALIQ	GS	CRTAM	482	IMKLRKAHVIWKKENEV SEHTLESYRSRSNNEETS SEEKNGQSSHPMRCMNY ITKLYSEAKTKRKENVQ HSKLEEKHIQVPESIV
79	NKp44	281	WWGDIWWKTMME LRSLDTQKATCHLQ QVTDLPWTSVSSPV EREILYHTVARTKIS DDDDEHTL	GS	FCER1G	483	RLKIQVRKAAITSYEKS DGVYTGLSTRNQETYET LKHEKPPQ
80	SLAMF6	282	LRKRRDSLSLSTQR TQGPAESARNLEYV SVSPTNNTVYASVT HSNRETEIWTPREN DTITIYSTINHSKESK PTFSRATALDNVV	GS	CD27	484	QRRKYRSNKGESPVEPA EPCHYSCPREEEGSTIPI QEDYRKPEPACSP
81	FcgRIIIa	283	KTNIRSSTRDWKDH KFKWRKDPQDK	GS	CD27	485	QRRKYRSNKGESPVEPA EPCHYSCPREEEGSTIPI QEDYRKPEPACSP
82	blank	284		GS	4-1BB	486	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL
83	2B4	285	WRRKRKEKQSETSP KEFLTIYEDVKDLK TRRNHEQEQTFPGG GSTIYSMIQSQSSAP TSQEPAYTLYSLIQP SRKSGSRKRNHSPS FNSTIYEVIGKSQPK AQNPARLSRKELEN FDVYS	GS	NKp46	487	RKRTRERASRASTWEG RRRLNTQTL

84	DAP10	286	LCARPRRSPAQEDG KVYINMPGRG	GS	Fn14	488	RRCRRREKFTTPIEETGG EGCPAVALIQ
85	ICOS	287	CWLTKKKYSSSVH DPNGEYMFMRAVN TAKKSRLTDVTL	GS	Fn14	489	RRCRRREKFTTPIEETGG EGCPAVALIQ
86	NKp46	288	RKRTRERASRASTW EGRRRLNTQTL	GS	NKp44	490	WWGDIWWKTMMELRS LDTQKATCHLQQVTDL PWTSVSSPVEREILYHT VARTKISDDDDEHTL
87	RANK	289	CYRKKGKALTANL WHWINEACGRLSG DKESSGDSCVSTHT ANFGQQGACEGVL LLTLEEKTFPEDMC YPDQGGVCQGTCV GGGPYAQGEDARM LSLVSKTEIEEDSFR QMPTEDEYMDRPS QPTDQLLFLTEPGS KSTPPFSEPLEVGEN DSLSQCFTGTQSTV GSESCNCTEPLCRT DWTPMSSENYLQK EVDSGHCPHWAAS PSPNWADVCTGCR NPPGEDCEPLVGSP KRGPLPQCAYGMG LPPEEEASRTEARD QPEDGADGRLPSSA RAGAGSGSSPGGQS PASGNVTGNSNSTFI SSGQVMNFKGDIIV VYVSQTSQEGAAA AAEPMGRPVQEETL ARRDSFAGNGPRFP DPCGGPEGLREPEK ASRPVQEQGGAKA	GS	4-1BB	491	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL

88	CD27	290	QRRKYRSNKGESPV EPAEPCHYSCPREEE GSTIPIQEDYRKPEP ACSP	GS	SLAMF7	492	WFLKRERQEEYIEEKKR VDICRETPNICPHSGENT EYDTIPHTNRTILKEDPA NTVYSTVEIPKKMENPH SLLTMPDTPRLFAYENVI
89	CD27	291	QRRKYRSNKGESPV EPAEPCHYSCPREEE GSTIPIQEDYRKPEP ACSP	GS	Fn14	493	RRCRRREKFTTPIEETGG EGCPAVALIQ
90	4-1BB	292	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	DNAM1	494	NRRRRRERRDLFTESW DTQKAPNNYRSPISTSQ PTNQSMDDTREDIYVN YPTFSRRPKTRV
91	FcgRIIIa	293	KTNIRSSTRDWKDH KFKWRKDPQDK	GS	CD3zeta	495	RVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRR KNPQEGLYNELQKDKM AEAYSEIGMKGERRRGK GHDGLYQGLSTATKDTY DALHMQALPPR
92	4-1BB	294	KRGRKKLLYIFKQP FMRPVQTTQEEDGC SCRFPEEEEGGCEL	GS	DAP12	496	YFLGRLVPRGRGAAEA ATRKQRITETESPYQEL QGQRSDVYSDLNTQRP YYK
93	DNAM1	295	NRRRRRERRDLFTE SWDTQKAPNNYRS PISTSQPTNQSMDDT REDIYVNYPTFSRRP KTRV	GS	ICOS	497	CWLTKKKYSSSVHDPN GEYMFMRAVNTAKKSR LTDVTL
94	OX40	296	QVSHRYPRIQSIKVQ FTEYKKEKGFILTSQ KEDEIMKVQNNSVII NCDGFYLISLKGYF SQEVNISLHYQKDE EPLFQLKKVRSVNS	GS	FCER1G	498	RLKIQVRKAAITSYEKS DGVYTGLSTRNQETYET LKHEKPPQ

			LMVASLTYKDKVY LNVTTDNTSLDDFH VNGGELILIHQNPGE FCVL				
95	FcgRIIIa	297	KTNIRSSTRDWKDH KFKWRKDPQDK	GS		499	
96	CD40	298	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	CD28	500	RSKRSRLLHSDYMNMT PRRPGPTRKHYQPYAPP RDFAAYRS
97	blank	299		GS	CD27	501	QRRKYRSNKGESPVEPA EPCHYSCPREEEGSTIPI QEDYRKPEPACSP
98	NKp30	300	GSTVYYQGKCLTW KGPRRQLPAVVPAP LPPPCGSSAHLLPPV PGG	GS	NKp44	502	WWGDIWWKTMMELRS LDTQKATCHLQQVTDL PWTSVSSPVEREILYHT VARTKISDDDDEHTL
99	CD27	301	QRRKYRSNKGESPV EPAEPCHYSCPREEE GSTIPIQEDYRKPEP ACSP	GS	CD84	503	RLFKRRQGRIFPEGSCLN TFTKNPYAASKKTIYTYI MASRNTQPAESRIYDEIL QSKVLPSKEEPVNTVYSE VQFADKMGKASTQDSK PPGTSSYEIVI
100	CD40	302	KKVAKKPTNKAPH PKQEPQEINFPDDLP GSNTAAPVQETLHG CQPVTQEDGKESRI SVQERQ	GS	NKp46	504	RKRTRERASRASTWEG RRRLNTQTL
101	CD2	303	KRKKQRSRRNDEEL ETRAHRVATEERGR KPHQIPASTPQNPAT SQHPPPPPGHRSQAP SHRPPPPGHRVQHQ PQKRPPAPSGTQVH	GS	Fn14	505	RRCRRREKFTTPIEETGG EGCPAVALIQ

			QQKGPPLPRPRVQP KPPHGAAENSLSPSS N				
102	NKp46	304	RKRTRERASRASTW EGRRRLNTQTL	GS	CD40	506	KKVAKKPTNKAPHPKQ EPQEINFPDDLPGSNTA APVQETLHGCQPVTQE DGKESRISVQERQ
103	CRTAM	305	IMKLRKAHVIWKKE NEVSEHTLESYRSR SNNEETSSEEKNGQ SSHPMRCMNYITKL YSEAKTKRKENVQ HSKLEEKHIQVPESI V	GS	CD84	507	RLFKRRQGRIFPEGSCLN TFTKNPYAASKKTIYTYI MASRNTQPAESRIYDEIL QSKVLPSKEEPVNTVYSE VQFADKMGKASTQDSK PPGTSSYEIVI
104	CD27	306	QRRKYRSNKGESPV EPAEPCHYSCPREEE GSTIPIQEDYRKPEP ACSP	GS	2B4	508	WRRKRKEKQSETSPKEF LTIYEDVKDLKTRRNHE QEQTFPGGGSTIYSMIQS QSSAPTSQEPAYTLYSLI QPSRKSGSRKRNHSPSFN STIYEVIGKSQPKAQNPA RLSRKELENFDVYS
105	NKp46	307	RKRTRERASRASTW EGRRRLNTQTL	GS	4-1BB	509	KRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPE EEEGGCEL
106	LMP1	308	YYHGQRHSDEHHH DDSLPHPQQATDDS GHESDSNSNEGRHH LLVSGAGDGPPLCS QNLGAPGGGPDNG PQDPDNTDDNGPQ DPDNTDDNGPHDPL PQDPDNTDDNGPQ DPDNTDDNGPHDPL PHSPSDSAGNDGGP PQLTEEVENKGGDQ GPPLMTDGGGGHS	GS	LMP1	510	YYHGQRHSDEHHHDDS LPHPQQATDDSGHESDS NSNEGRHHLLVSGAGDG PPLCSQNLGAPGGGPDN GPQDPDNTDDNGPQDPD NTDDNGPHDPLPQDPDN TDDNGPQDPDNTDDNGP HDPLPHSPSDSAGNDGG PPQLTEEVENKGGDQGP PLMTDGGGGHSHDSGH GGGDPHLPTLLLGSSGSG GDDDDPHGPVQLSYYD

			HDSGHGGGDPHLPTLLLGSSGSGGDDDDPHGPVQLSYYD				
107	CD84	309	RLFKRRQGRIFPEGSCLNTFTKNPYAASKKTIYTYIMASRNTQPAESRIYDEILQSKVLPSKEEPVNTVYSEVQFADKMGKASTQDSKPPGTSSYEIVI	GS	FCER1G	511	RLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
108	CD27	310	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP	GS	4-1BB	512	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

Table 3: Stimulatory sequences derived from one or more protein signaling motifs

SEQ ID NO	seq_name	Protein that first domain sequence is derived from	SEQ ID NO	first domain sequence	Linker	Protein that second domain sequence is derived from	SEQ ID NO	second domain sequence
109	PECA1_HUMAN_ITSM_713__TNR4_HUMAN_Ox40_262	PECA1	311	LGKKDTEETVYSEVRKAVETVYSEVRKAVVPDAVES	GS	Ox40	513	GGGSFRTPIQEEQADAHSGGGSFRTPIQEEQADAHS
110	TNR4_HUMAN_Ox40_262__PECA1_HUMAN_ITIM_690	Ox40	312	GGGSFRTPIQEEQADAHSGGGSFRTPIQEEQADAHS	GS	PECA1	514	KEPLNSDDVQYTEVQVSSDVQYTEVQVSSSAESHKD
111	KI3L2_HUMAN_ITIM_428__CD	KI3L2	313	KTPLTDTTSVYTELPNAETSVYTE	GS	CD3Z	515	KGERRRGKGHDGLYQGLSTA

	3Z_HUMAN_ITAM_142153			LPNAEEPRSKVV				TKDTYDALHMQALPPR
112	CD3Z_HUMAN_ITAM_83__LMP1_EBVB9_LMP1_203	CD3Z	314	ELNLGRRREEYDVLDKRRREEYDVLDKRRRGRDPEM	GS	EBVB9	516	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH
113	TYOBP_HUMAN_ITAM_102__CD3Z_HUMAN_ITAM_64	TYOBP	315	YQELQGQRSSDVYSDLNTQRSDVYSDLNTQRRPYYK	GS	CD3Z	517	FSRSADAAPAYQQGQNQLAPAYQQGQNQLLYNELNL
114	TNR9_HUMAN_CD137_234__TANK_HUMAN_TANK_180	CD137	316	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR	GS	TANK	518	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ
115	TNR9_HUMAN_CD137_234__TYOBP_HUMAN_ITAM_91	CD137	317	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR	GS	TYOBP	519	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD
116	LMP1_EBVB9_LMP1_203__CD3Z_HUMAN_ITAM_6483	EBVB9	318	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH	GS	CD3Z	520	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
117	CD3Z_HUMAN_ITAM_142153__TANK_HUMAN_TANK_180	CD3Z	319	KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	GS	TANK	521	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ
118	spacer__CD244_HUMAN_ITSM_271	CD244	320	TSPKEFLLTIYEDVKDLKLTIYEDVKDLKKTRRNHE	GS		522	
119	CADH5_HUMAN_ITIM_755__TANK_HUMAN_TANK_180	CADH5	321	LGTDSSDDVDYDFLNDWGDVDYDFLNDWGDWGPRFK	GS	TANK	523	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ

120	TNR5_HUMAN_CD40_250__TNR9_HUMAN_CD137_234	CD40	322	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP	GS	CD137	524	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR
121	LIRB4_HUMAN_ITIM_412__CD3E_HUMAN_ITAM_188199	LIRB4	323	ASEAPQDDVTYAQLHSFTDVTYAQLHSFTTLRQKAT	GS	CD3E	525	QRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI
122	LMP1_EBVB9_LMP1_203	EBVB9	324	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH	GS		526	
123	KI3L2_HUMAN_ITIM_428__FCERG_HUMAN_ITAM_6576	KI3L2	325	KTPLTDTTSVYTELPNAETSVYTELPNAEEPRSKVV	GS	FCERG	527	RKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
124	CD3E_HUMAN_ITAM_188__CD3E_HUMAN_ITAM_199	CD3E	326	RPPPVPNNPDYEPIRKGQNPDYEPIRKGQQRDLYSG	GS	CD3E	528	NPDYEPIRKGQRRDLYSGLNQRRRDLYSGLNQRRRI
125	CD3Z_HUMAN_ITAM_64__FCERG_HUMAN_ITAM_6576	CD3Z	327	FSRSADAAPAYQQGQNQLAPAYQQGQNQLLYNELNL	GS	FCERG	529	RKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
126	IL6RB_HUMAN_ITIM_759__spacer	IL6RB	328	SQNTSSTTVQYSTVVHSGTVQYSTVVHSGGYRHQVP	GS		530	
127	CD3Z_HUMAN_ITAM_111__TANK_HUMAN_TANK_180	CD3Z	329	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS	GS	TANK	531	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ
128	TNR9_HUMAN_CD137_234__T	CD137	330	PFMRPVQTTQEEDGCSCRPFMR	GS	CD40	532	APHPKQEPQEINFPDDLPAPHP

	NR5_HUMAN_CD40_233			PVQTTQEEDGCSCR				KQEPQEINFPDDLP
129	TANK_HUMAN_TANK_180__FCG2A_HUMAN_ITAM_288	TANK	331	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ	GS	FCG2A	533	NDYETADDGGYMTLNPRADGGYMTLNPRAAPTDDDK
130	TNR9_HUMAN_CD137_234__CD3Z_HUMAN_ITAM_6483	CD137	332	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR	GS	CD3Z	534	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
131	FCG2A_HUMAN_ITAM_288__KI2L3_HUMAN_ITIM_303	FCG2A	333	NDYETADDGGYMTLNPRADGGYMTLNPRAAPTDDDK	GS	KI2L3	535	DEQDPQEEVTYAQLNHCVEVTYAQLNHCVVFTQRKI
132	LMP1_EBVB9_LMP1_203__FCG2A_HUMAN_ITAM_304	EBVB9	334	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH	GS	FCG2A	536	APTDDDKKNIYLTLPPNDKNIYLTLPPNDDHVNSNN
133	TNR5_HUMAN_CD40_250__TYOBP_HUMAN_ITAM_91	CD40	335	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP	GS	TYOBP	537	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD
134	LIRB4_HUMAN_ITIM_412	LIRB4	336	ASEAPQDDVTYAQLHSFTDVTYAQLHSFTTLRQKAT	GS		538	
135	TNR5_HUMAN_CD40_250__CD3Z_HUMAN_ITAM_6483	CD40	337	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP	GS	CD3Z	539	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
136	CD244_HUMAN_ITSM_317__CD3E_HUMAN_ITAM_199	CD244	338	TSQEPAYYTLYSLIQPSRYTLYSLIQPSRRKSGSRK	GS	CD3E	540	NPDYEPIRKGQRRDLYSGLNQRRRDLYSGLNQRRRI

137	TNR5_HUMAN_CD40_250__LMP1_EBVB9_LMP1_203	CD40	339	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP	GS	EBVB9	541	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH
138	KI2L3_HUMAN_ITIM_303__TANK_HUMAN_TANK_180	KI2L3	340	DEQDPQEEVTYAQLNHCVEVTYAQLNHCVVFTQRKI	GS	TANK	542	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ
139	CD244_HUMAN_ITSM_271__TNR5_HUMAN_CD40_250	CD244	341	TSPKEFLLTIYEDVKDLKLTIYEDVKDLKKTRRNHE	GS	CD40	543	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP
140	CD3Z_HUMAN_ITAM_142__CD244_HUMAN_ITSM_342	CD3Z	342	RRGKGHDDGLYQGLSTATDGLYQGLSTATTKDTYDA	GS	CD244	544	HSPSFNSSTIYEVIGKSQSTIYEVIGKSQQPKAQNP
141	LMP1_EBVB9_LMP1_203__CD3Z_HUMAN_ITAM_142153	EBVB9	343	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH	GS	CD3Z	545	KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
142	CD3E_HUMAN_ITAM_199__TYOBP_HUMAN_ITAM_91102	CD3E	344	NPDYEPIRKGQRRDLYSGLNQRRRDLYSGLNQRRRI	GS	TYOBP	546	ATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK
143	TNR9_HUMAN_CD137_246__TYOBP_HUMAN_ITAM_91	CD137	345	EDGCSCRFPEEEEGGCELEDGCSCRFPEEEEGGCEL	GS	TYOBP	547	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD
144	CD3Z_HUMAN_ITAM_142153__LMP1_EBVB9_LMP1_203	CD3Z	346	KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	GS	EBVB9	548	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH
145	CD3Z_HUMAN_ITAM_142__T	CD3Z	347	RRGKGHDDGLYQGLSTATDGL	GS	TYOBP	549	YQELQGQRSSDVYSDLNTQR

	YOBP_HUMAN_ITAM_102			YQGLSTATTKDTYDA				SDVYSDLNTQRRPYYK
146	TGFR1_HUMAN_TGFBR1_482__TNR5_HUMAN_CD40_233	TGFBR1	348	WYANGAARLTALRIKKTLWYANGAARLTALRIKKTL	GS	CD40	550	APHPKQEPQEINFPDDLPAPHPKQEPQEINFPDDLP
147	TANK_HUMAN_TANK_180__CD3Z_HUMAN_ITAM_111	TANK	349	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ	GS	CD3Z	551	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS
148	CADH5_HUMAN_ITIM_755__CD3E_HUMAN_ITAM_188199	CADH5	350	LGTDSSDDVDYDFLNDWGDVDYDFLNDWGDWGPRFK	GS	CD3E	552	QRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI
149	LIRB4_HUMAN_ITIM_360__CD3Z_HUMAN_ITAM_153	LIRB4	351	HDEDPQAAVTYAKVKHSRAVTYAKVKHSRRPRREMA	GS	CD3Z	553	YQGLSTATKKDTYDALHMQAKDTYDALHMQAALPPR
150	CD3E_HUMAN_ITAM_188199	CD3E	352	QRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI	GS		554	
151	CD3Z_HUMAN_ITAM_123142__LMP1_EBVB9_LMP1_203	CD3Z	353	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT	GS	EBVB9	555	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH
152	TYOBP_HUMAN_ITAM_91__PDCD1_HUMAN_ITSM_248	TYOBP	354	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD	GS	PDCD1	556	VPCVPEQQTEYATIVFPSQTEYATIVFPSSGMGTSS
153	TNR9_HUMAN_CD137_246__LMP1_EBVB9_LMP1_203	CD137	355	EDGCSCRFPEEEEGGCELEDGCSCRFPEEEEGGCEL	GS	EBVB9	557	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH

154	CD3Z_HUMAN_ITAM_6483__FCG2A_HUMAN_ITAM_304	CD3Z	356	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD	GS	FCG2A	558	APTDDDKKNIYLTLPPNDKNIYLTLPPNDDHVNSNN
155	PILRA_HUMAN_ITIM_269__CD3Z_HUMAN_ITAM_123142	PILRA	357	LNPKDDGGIVYASLALSSGIVYASLALSSSSTSPRA	GS	CD3Z	559	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
156	TNR9_HUMAN_CD137_234__LMP1_EBVB9_LMP1_203	CD137	358	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR	GS	EBVB9	560	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH
157	TNR5_HUMAN_CD40_233__LMP1_EBVB9_LMP1_203	CD40	359	APHPKQEPQEINFPDDLPAPHPKQEPQEINFPDDLP	GS	EBVB9	561	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH
158	CD3Z_HUMAN_ITAM_142153__PDCD1_HUMAN_ITSM_248	CD3Z	360	KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	GS	PDCD1	562	VPCVPEQQTEYATIVFPSQTEYATIVFPSSGMGTSS
159	TNR9_HUMAN_CD137_246	CD137	361	EDGCSCRFPEEEEGGCELEDGCSCRFPEEEEGGCEL	GS		563	
160	CD3Z_HUMAN_ITAM_64__PDCD1_HUMAN_ITIM_223	CD3Z	362	FSRSADAAPAYQQGQNQLAPAYQQGQNQLLYNELNL	GS	PDCD1	564	SAVPVFSSVDYGELDFQWSVDYGELDFQWWREKTPE
161	CD3Z_HUMAN_ITAM_64__CD3Z_HUMAN_ITAM_123142	CD3Z	363	FSRSADAAPAYQQGQNQLAPAYQQGQNQLLYNELNL	GS	CD3Z	565	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
162	TNR5_HUMAN_CD40_250__TN	CD40	364	PGSNTAAPVQETLHGCQPPGSN	GS	CD137	566	EDGCSCRFPEEEEGGCELEDG

	R9_HUMAN_CD137_246			TAAPVQETLHGCQP				CSCRFPEEEEGGCEL
163	KI2L4_HUMAN_ITIM_300__PDCD1_HUMAN_ITSM_248	KI2L4	365	DEQDPQEEVTYAQLDHCIEVTYAQLDHCIIFTQRKI	GS	PDCD1	567	VPCVPEQQTEYATIVFPSQTEYATIVFPSSGMGTSS
164	IL6RB_HUMAN_ITIM_759__TNR9_HUMAN_CD137_234	IL6RB	366	SQNTSSTTVQYSTVVHSGTVQYSTVVHSGGYRHQVP	GS	CD137	568	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR
165	LIRB4_HUMAN_ITIM_412__NCTR2_HUMAN_ITIM_259	LIRB4	367	ASEAPQDDVTYAQLHSFTDVTYAQLHSFTTLRQKAT	GS	NCTR2	569	SSPVEREEILYHTVARTKEILYHTVARTKKISDDDD
166	TNR4_HUMAN_Ox40_262__spacer	Ox40	368	GGGSFRTPIQEEQADAHSGGGSFRTPIQEEQADAHS	GS		570	
167	CD3E_HUMAN_ITAM_199__TNR9_HUMAN_CD137_246	CD3E	369	NPDYEPIRKGQRRDLYSGLNQRRRDLYSGLNQRRRI	GS	CD137	571	EDGCSCRFPEEEEGGCELEDGCSCRFPEEEEGGCEL
168	LIRB4_HUMAN_ITIM_360__CD3Z_HUMAN_ITAM_111	LIRB4	370	HDEDPQAAVTYAKVKHSRAVTYAKVKHSRRPRREMA	GS	CD3Z	572	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS
169	FCG2A_HUMAN_ITAM_304__FCERG_HUMAN_ITAM_76	FCG2A	371	APTDDDKKNIYLTLPPNDKNIYLTLPPNDDHVNSNN	GS	FCERG	573	VYTGLSTRNQQETYETLKHEKQETYETLKHEKKPPQ
170	NCTR2_HUMAN_ITIM_259__TYOBP_HUMAN_ITAM_102	NCTR2	372	SSPVEREEILYHTVARTKEILYHTVARTKKISDDDD	GS	TYOBP	574	YQELQGQRSSDVYSDLNTQRSDVYSDLNTQRRPYYK

171	KI2L3_HUMAN_ITIM_303__CD3Z_HUMAN_ITAM_111	KI2L3	373	DEQDPQEEVTYAQLNHCVEVTYAQLNHCVVFTQRKI	GS	CD3Z	575	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS
172	TNR5_HUMAN_CD40_233__CD3Z_HUMAN_ITAM_111	CD40	374	APHPKQEPQEINFPDDLPAPHPKQEPQEINFPDDLP	GS	CD3Z	576	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS
173	FCG2A_HUMAN_ITAM_288__CD3Z_HUMAN_ITAM_6483	FCG2A	375	NDYETADDGGYMTLNPRADGGYMTLNPRAAPTDDDK	GS	CD3Z	577	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
174	TYOBP_HUMAN_ITAM_91__CD3Z_HUMAN_ITAM_153	TYOBP	376	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD	GS	CD3Z	578	YQGLSTATKKDTYDALHMQAKDTYDALHMQAALPPR
175	CD3Z_HUMAN_ITAM_142153__CD3Z_HUMAN_ITAM_142	CD3Z	377	KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	GS	CD3Z	579	RRGKGHDDGLYQGLSTATDGLYQGLSTATTKDTYDA
176	TNR5_HUMAN_CD40_250__CD3Z_HUMAN_ITAM_153	CD40	378	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP	GS	CD3Z	580	YQGLSTATKKDTYDALHMQAKDTYDALHMQAALPPR
177	TNR4_HUMAN_Ox40_262__CD3Z_HUMAN_ITAM_6483	Ox40	379	GGGSFRTPIQEEQADAHSGGGSFRTPIQEEQADAHS	GS	CD3Z	581	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
178	FCG2A_HUMAN_ITAM_304__TNR9_HUMAN_CD137_246	FCG2A	380	APTDDDKKNIYLTLPPNDKNIYLTLPPNDDHVNSNN	GS	CD137	582	EDGCSCRFPEEEEGGCELEDGCSCRFPEEEEGGCEL
179	TNR4_HUMAN_Ox40_262__TY	Ox40	381	GGGSFRTPIQEEQADAHSGGGSF	GS	TYOBP	583	QRITETEESPYQELQGQRESPY

	OBP_HUMAN_ITAM_91			RTPIQEEQADAHS				QELQGQRRSDVYSD
180	TYOBP_HUMAN_ITAM_91__TNR5_HUMAN_CD40_233	TYOBP	382	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD	GS	CD40	584	APHPKQEPQEINFPDDLPAPHPKQEPQEINFPDDLP
181	TNR9_HUMAN_CD137_234__spacer	CD137	383	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR	GS		585	
182	TANK_HUMAN_TANK_180__CD244_HUMAN_ITSM_271	TANK	384	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ	GS	CD244	586	TSPKEFLLTIYEDVKDLKLTIYEDVKDLKKTRRNHE
183	CD3Z_HUMAN_ITAM_6483__TYOBP_HUMAN_ITAM_91	CD3Z	385	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD	GS	TYOBP	587	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD
184	CD3Z_HUMAN_ITAM_123142__KI3L2_HUMAN_ITIM_428	CD3Z	386	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT	GS	KI3L2	588	KTPLTDTTSVYTELPNAETSVYTELPNAEEPRSKVV
185	KI2L3_HUMAN_ITIM_303__CD3Z_HUMAN_ITAM_6483	KI2L3	387	DEQDPQEEVTYAQLNHCVEVTYAQLNHCVVFTQRKI	GS	CD3Z	589	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
186	CD244_HUMAN_ITSM_342__CD3Z_HUMAN_ITAM_6483	CD244	388	HSPSFNSSTIYEVIGKSQSTIYEVIGKSQQPKAQNP	GS	CD3Z	590	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
187	TYOBP_HUMAN_ITAM_91__CD3Z_HUMAN_ITAM_123142	TYOBP	389	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD	GS	CD3Z	591	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT

188	CD3Z_HUMAN_ITAM_64__LMP1_EBVB9_LMP1_203	CD3Z	390	FSRSADAAPAYQQGQNQLAPAYQQGQNQLLYNELNL	GS	EBVB9	592	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH
189	PECA1_HUMAN_ITIM_690__FCERG_HUMAN_ITAM_65	PECA1	391	KEPLNSDDVQYTEVQVSSDVQYTEVQVSSSAESHKD	GS	FCERG	593	TSYEKSDDGVYTGLSTRNDGVYTGLSTRNNQETYET
190	LMP1_EBVB9_LMP1_203__CD3Z_HUMAN_ITAM_111	EBVB9	392	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH	GS	CD3Z	594	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS
191	CD3Z_HUMAN_ITAM_123142__KI3L1_HUMAN_ITIM_428	CD3Z	393	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT	GS	KI3L1	595	KTPPTDTTILYTELPNAKTILYTELPNAKKPRSKVV
192	FCERG_HUMAN_ITAM_6576	FCERG	394	RKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ	GS		596	
193	TNR4_HUMAN_Ox40_262__FCG2A_HUMAN_ITAM_304	Ox40	395	GGGSFRTPIQEEQADAHSGGGSFRTPIQEEQADAHS	GS	FCG2A	597	APTDDDKKNIYLTLPPNDKNIYLTLPPNDDHVNSNN
194	CD244_HUMAN_ITSM_271__TYOBP_HUMAN_ITAM_91	CD244	396	TSPKEFLLTIYEDVKDLKLTIYEDVKDLKKTRRNHE	GS	TYOBP	598	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD
195	TNR5_HUMAN_CD40_233__CD244_HUMAN_ITSM_342	CD40	397	APHPKQEPQEINFPDDLPAPHPKQEPQEINFPDDLP	GS	CD244	599	HSPSFNSSTIYEVIGKSQSTIYEVIGKSQQPKAQNP
196	TNR4_HUMAN_Ox40_262__CD	Ox40	398	GGGSFRTPIQEEQADAHSGGGSF	GS	CD3Z	600	FSRSADAAPAYQQGQNQLAPA

	3Z_HUMAN_ITAM_64			RTPIQEEQADAHS				YQQGQNQLLYNELNL
197	TYOBP_HUMAN_ITAM_91102__spacer	TYOBP	399	ATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK	GS		601	
198	CD3Z_HUMAN_ITAM_111__CD3Z_HUMAN_ITAM_64	CD3Z	400	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS	GS	CD3Z	602	FSRSADAAPAYQQGQNQLAPAYQQGQNQLLYNELNL
199	CD3Z_HUMAN_ITAM_123__CD3Z_HUMAN_ITAM_142153	CD3Z	401	LQKDKMAAEAYSEIGMKGAEAYSEIGMKGGERRRGK	GS	CD3Z	603	KGERRRGKGHDGLYQGLSATKDTYDALHMQALPPR
200	CD3Z_HUMAN_ITAM_142153__TNR11_HUMAN_RANK_344	CD3Z	402	KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR	GS	RANK	604	EDSFRQMPTEDEYMDRPSEDSFRQMPTEDEYMDRPS
201	TNR5_HUMAN_CD40_250__TYOBP_HUMAN_ITAM_102	CD40	403	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP	GS	TYOBP	605	YQELQGQRSSDVYSDLNTQRSDVYSDLNTQRRPYYK
202	FCG2A_HUMAN_ITAM_288304__CD3Z_HUMAN_ITAM_111	FCG2A	404	DYETADGGYMTLNPRAPTDDDKNIYLTLPPNDHVNS	GS	CD3Z	606	RRKNPQEEGLYNELQKDKEGLYNELQKDKKMAEAYS
203	CD3Z_HUMAN_ITAM_123142__CD3Z_HUMAN_ITAM_83	CD3Z	405	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT	GS	CD3Z	607	ELNLGRRREEYDVLDKRRREEYDVLDKRRRGRDPEM
204	LIRB4_HUMAN_ITIM_360__KI3L1_HUMAN_ITIM_428	LIRB4	406	HDEDPQAAVTYAKVKHSRAVTYAKVKHSRRPRREMA	GS	KI3L1	608	KTPPTDTTILYTELPNAKTILYTELPNAKKPRSKVV

205	TNR4_HUMAN_Ox40_262__CD244_HUMAN_ITSM_342	Ox40	407	GGGSFRTPIQEEQADAHSGGGSFRTPIQEEQADAHS	GS	CD244	609	HSPSFNSSTIYEVIGKSQSTIYEVIGKSQQPKAQNP
206	TNR5_HUMAN_CD40_250__CD244_HUMAN_ITSM_317	CD40	408	PGSNTAAPVQETLHGCQPPGSNTAAPVQETLHGCQP	GS	CD244	610	TSQEPAYYTLYSLIQPSRYTLYSLIQPSRRKSGSRK
207	FCERG_HUMAN_ITAM_65__CD3Z_HUMAN_ITAM_6483	FCERG	409	TSYEKSDDGVYTGLSTRNDGVYTGLSTRNNQETYET	GS	CD3Z	611	SADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRD
208	FCG2A_HUMAN_ITAM_304__TYOBP_HUMAN_ITAM_91	FCG2A	410	APTDDDKKNIYLTLPPNDKNIYLTLPPNDDHVNSNN	GS	TYOBP	612	QRITETEESPYQELQGQRESPYQELQGQRRSDVYSD
209	KI2L4_HUMAN_ITIM_300__CD3Z_HUMAN_ITAM_153	KI2L4	411	DEQDPQEEVTYAQLDHCIEVTYAQLDHCIIFTQRKI	GS	CD3Z	613	YQGLSTATKKDTYDALHMQAKDTYDALHMQAALPPR
210	FCG2A_HUMAN_ITAM_288304__TYOBP_HUMAN_ITAM_91102	FCG2A	412	DYETADGGYMTLNPRAPTDDDKNIYLTLPPNDHVNS	GS	TYOBP	614	ATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK
211	LMP1_EBVB9_LMP1_203__KI3L2_HUMAN_ITIM_428	EBVB9	413	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH	GS	KI3L2	615	KTPLTDTTSVYTELPNAETSVYTELPNAEEPRSKVV
212	CD3E_HUMAN_ITAM_188__TNR9_HUMAN_CD137_246	CD3E	414	RPPPVPNNPDYEPIRKGQNPDYEPIRKGQQRDLYSG	GS	CD137	616	EDGCSCRFPEEEEGGCELEDGCSCRFPEEEEGGCEL

213	KI2L3_HUMAN_ITIM_303__CD244_HUMAN_ITSM_342	KI2L3	415	DEQDPQEEVTYAQLNHCVEVTYAQLNHCVVFTQRKI	GS	CD244	617	HSPSFNSSTIYEVIGKSQSTIYEVIGKSQQPKAQNP
214	FCG2A_HUMAN_ITAM_288__TGFR1_HUMAN_TGFBR1_482	FCG2A	416	NDYETADDGGYMTLNPRADGGYMTLNPRAAPTDDDK	GS	TGFR1	618	WYANGAARLTALRIKKTLWYANGAARLTALRIKKTL
215	TANK_HUMAN_TANK_180__TNR9_HUMAN_CD137_234	TANK	417	TETQCSVPIQCTDKTDKQTETQCSVPIQCTDKTDKQ	GS	CD137	619	PFMRPVQTTQEEDGCSCRPFMRPVQTTQEEDGCSCR
216	CD3Z_HUMAN_ITAM_123142__spacer	CD3Z	418	DKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT	GS		620	
217	CD3E_HUMAN_ITAM_188199__TNR5_HUMAN_CD40_233	CD3E	419	QRGQNKERPPPVPNPDYEPIRKGQRDLYSGLNQRRI	GS	CD40	621	APHPKQEPQEINFPDDLPAPHPKQEPQEINFPDDLP
218	LMP1_EBVB9_LMP1_203__KI3L1_HUMAN_ITIM_428	EBVB9	420	HHDDSLPHPQQATDDSGHHHDDSLPHPQQATDDSGH	GS	KI3L1	622	KTPPTDTTILYTELPNAKTILYTELPNAKKPRSKVV
219	CD244_HUMAN_ITSM_317__FCG2A_HUMAN_ITAM_288304	CD244	421	TSQEPAYYTLYSLIQPSRYTLYSLIQPSRRKSGSRK	GS	FCG2A	623	DYETADGGYMTLNPRAPTDDDKNIYLTLPPNDHVNS

[58] For the avoidance of doubt, Tables 2 and 3 provide the sequences of stimulatory regions (SEQ ID NOs: 18-219) that include a first stimulatory domain sequence (SEQ ID NOs: 220-421) and a second stimulatory domain sequence (SEQ ID NOs: 422-623). Each of the stimulatory regions according to SEQ ID NOs: 18-219 consists of, from N terminus to C

terminus, (1) the indicated first domain sequence, (2) the GS linker, and (3) the indicated second domain sequence.

[59] The present disclosure includes the recognition that stimulatory regions that include a first stimulatory domain sequence (SEQ ID NOs: 220-421) and a second stimulatory domain sequence (SEQ ID NOs: 422-623), e.g., in combinations as set forth in rows of Tables 2 and 3, do not require a linker to function in the manner provided herein. The present inventors have discovered that stimulatory regions without a linker (i.e., where the sequence of a first stimulatory domain sequence of the present disclosure is directly joined to a second stimulatory domain sequence of the present disclosure, e.g., in a combination set forth in a row of Table 2 or 3) are useful and advantageous for use as disclosed herein. The present inventors have further discovered that stimulatory regions that include a linker between a first stimulatory domain sequence of the present disclosure and a second stimulatory domain sequence of the present disclosure can demonstrate further increased stimulatory activity (e.g., when included in a TCR and/or NK cell) as compared to a reference sequence without such a linker. Without wishing to be bound by any particular scientific theory, separating multiple stimulatory sequences on the same receptor using short, flexible peptide linkers could potentially limit steric hindrance effects that might otherwise hamper the downstream function driven by each sequence.

[60] Linkers of the present disclosure include sequences that are useful to connect different elements to one another. For example, those of ordinary skill in the art appreciate that a polypeptide whose structure includes two or more functional or organizational domains (e.g., first and second stimulatory domain sequences) can include a stretch of amino acids between such domains that links them to one another. In some embodiments, a polypeptide including a linker element can have an overall structure of the general form S1-L-S2, wherein S1 and S2 may be the same or different and represent two domains associated with one another by the linker. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide. A variety of different linker elements that can appropriately be used when engineering polypeptides (e.g., fusion polypeptides) known in the art (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2: 1 121-1123).

[61] In some embodiments, a polypeptide linker can be, be at least, and/or be about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100, or more, amino acids in length. In

certain embodiments, a polypeptide linker can be be, be at least, or be about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length. In some embodiments, a polypeptide linker can have a length that is within a range having a lower bound selected from 1, 2, 3, 4, or 5 amino acids and an upper bound selected from 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 amino acids. In some embodiments, a polypeptide linker can have a length that 2 to 15 amino acids, 2 to 10 amino acids, 2 to 5 amino acids, 2 to 4 amino acids, or 2 or 3 amino acids. In certain embodiments, amino acids of a linker include, consist of, or consist essentially of amino acids selected from one or both of glycine and serine. The present disclosure exemplifies, without limitation, use of flexible linkers, such as linkers that include, consist of, or consist essentially of the minimal flexible linker GS.

Applications

[62] CAR designs disclosed herein could drive diverse and useful cell states in engineered cell therapies. Enhancing the signaling space captured by CAR designs is a strategy for enhancing therapeutically relevant cell characteristics in an antigen-binding dependent manner. For example, transmembrane domains that modulate CAR presentation levels on the surface of immune cells could be used to tune the sensitivity of the immune cell response to different levels of antigen. Alternatively, stimulatory domains could be used not only to activate the cell, but also to stimulate signaling pathways that increase cell metabolic fitness in suppressive tumor microenvironments. Such domains could be largely derived from native receptor sequences, or manipulated at the level of individual protein binding motifs.

[63] While certain signaling domains have been characterized previously, such as CD40, CD28, 4-1BB, and DAP10, specific stimulatory sequences featuring combinations of stimulatory sequences drive potentially therapeutically useful cell states in ways that are challenging to predict *a priori*.

[64] Fn14-related domains as core stimulatory domains represent a strategy to improve NK cell expansion and survival in addition to cytotoxicity, owing to previous descriptions of Fn14 driving non-canonical NFkB signaling in NK cells. The observed function of Fn14-derived domains in combination with 4-1BB and CD40 domains demonstrates the ability to layer novel signaling cascades that drive pro-fitness effects.

EXAMPLES

[65] The present Examples demonstrate that compositions and methods of the present disclosure can impart enhanced functionality in immune cells. These Examples focus on the ability to drive enhanced proliferation of CAR-NK cells upon repeated antigen exposure, which addresses a commonly cited barrier to CAR-NK function therapeutically.

Example 1: Functional assessment of large stimulatory sequences by serial restimulation assay

[66] The present Example demonstrates that specific stimulatory sequences representing individual or combinations of full protein domains such as SEQ ID NOs: 1-6 and18-108 drive enhanced cell proliferation of NK cells, as compared to a reference, in the context of an 11 day serial restimulation with Raji tumor cells. In the present Example, full CAR designs included a CD19 binder, CD28 transmembrane domain, and stimulatory sequences representing pairwise combinations of full protein domains (Fig. 2).

[67] In these experiments, NK cells were transduced with different CD19-targeted CARs including different stimulatory sequences or combinations thereof, and co-cultured with cells from the Raji CD19+ tumor cell line in low-IL-2 conditions to create a tumor-mimicking metabolic stress. NK cells killed the Raji cells, after which additional target cells were added to the cultures. Each day, the NK cells were counted in order to gauge the degree of NK expansion in conditions of daily antigen refresh (Fig. 3), daily antigen refresh and low supportive cytokine (Fig. 4), and periodic antigen refresh (Fig. 5). SEQ ID NOs: 1-6 and 18-108 demonstrated positive expansion of at least two-fold after 11 days in at least one of the culture conditions. Notably, screening experiments identifying these sequences additionally included 2500 large stimulatory sequences that did not demonstrate positive expansion. Such effects demonstrate therapeutic utility at least in that metabolically sustainable serial killing of tumor cells enhances the therapeutic area-under-the-curve of NK cell therapies, which is broadly advantageous in therapeutic contexts and particularly advantageous for treatment of solid tumors.

Example 2: Functional assessment of small stimulatory sequences by serial restimulation assay

[68] In this Example, CAR designs featured specific stimulatory sequences representing combinations of individual signaling motifs incorporated into a CAR with a CD19 binder and

CD28 transmembrane domain. This overall structure is employed to demonstrate the utility of stimulatory sequences based on signaling motifs.

[69] In these experiments, NK cells were transduced with different CAR designs targeting CD19 and co-cultured with cells from the Raji CD19+ tumor cell line in low-IL-2 conditions to create a tumor-mimicking metabolic stress. NK cells killed the Raji cells, after which additional target cells were added to the cultures. Each day, the NK cells were counted in order to gauge the degree of NK expansion in conditions of daily antigen refresh (Fig. 7), daily antigen refresh and low supportive cytokine (Fig. 8), and periodic antigen refresh (Fig. 9). SEQ ID NOs: 7-17 and 109-219 demonstrated positive expansion after 11 days in at least one of the culture conditions. Notably, screening experiments identifying these sequences additionally included 2800 small stimulatory sequences that did not demonstrate positive expansion. Such effects represent potential therapeutic utility in that metabolically sustainable serial killing of tumor cells enhances the therapeutic area-under-the-curve of NK cell therapies, which is broadly advantageous in therapeutic contexts and particularly advantageous for treatment of in solid tumors.

Materials and Methods for Examples 1 and 2

[70] *Stimulatory Sequence Cloning Strategy*

[71] A nested Golden Gate cloning strategy was used to create strings of stimulatory sequences in a CAR backbone. Golden Gate cloning sites for PaqCl and Esp3l Type IIS restriction enzymes, as well as stop codons, were included in the backbone. Cloning of stimulatory sequences was performed serially, first utilizing synthesized gene fragments in the insertion at the PaqCl site, followed by insertion of a second synthesized gene fragment in the Esp31 site.

[72] *Lentivirus Production*

[73] The above transfer plasmid encoding the CAR was co-transfected along with Rev, envelope and gag/pol encoding plasmids into Takara Lenti-X 293 cells. After three days of incubation, at 37C, the supernatant was harvested and concentrated 100X using Lenti-X. Virus was subsequently titrated in NK cells by staining for CAR and observing transduction efficiency by flow cytometry.

[74] *NK Cell Transduction*

[75] NK cells were seeded in NK culture media containing IL-2 and 10ug/mL polybrene before adding virus at the desired concentration. Plates were spun at 1200xg for 30 min at

32C, then resuspended by pipetting up and down. After incubation at 37C for 1 hr, plates were again spun at 1200xg for 10 min at 32C before discarding the transduction media and adding fresh NK media. Cells were subsequently expanded using K562 feeder cells.

[76] *NK Serial Restimulation Experiment*

[77] On Day 0, transduced CAR-NK cells were co-cultured with Raji tumor cells at a 1:1 ratio in basal NK media containing human serum AB and 5u/mL IL-2. Each subsequent day, NK and Raji cells were counted, and new Raji cells were added to reset the 1:1 NK:Raji ratio. NK cell counts at each timepoint represent the degree of NK expansion in continuous antigen-exposure conditions.

Example 3: Functional assessment of stimulatory sequences of the present disclosure in an arrayed serial restimulation assay

[78] The present Example represents confirmatory data of the pooled serial restimulation assay in Example 1. Select stimulatory sequences representing combinations of full domains (drawn from SEQ ID Nos: 18-108) were included in CAR designs including a CD19 antigen-binding domain and CD28 transmembrane domain. The present Example provides an arrayed experiment in which CAR-NK cells including various distinct stimulatory sequences of the present disclosure are individually prepared and tested in isolated culture conditions, furthering supporting results reported in Example 1. The present Example confirms functional persistence of CAR-NK cells that include stimulatory sequences of the present disclosure.

[79] In these experiments, NK cells were transduced with CD19-targeted CARs including distinct stimulatory sequence combinations, and co-cultured with cells from the Raji CD19+ tumor cell line. The present experiment was carried out in low-IL-2 conditions (5 IU/mL) representative of low-cytokine conditions that can characterize some instances of clinical disease, and therefore provide additional support for clinical utility evidenced in normal-IL-2 conditions (500 IU/mL). NK cells killed the Raji cells, after which additional target cells were added to the cultures. Each day, the NK cells were counted. From Example 1, a set of specific stimulatory sequences were selected. The present Example quantified CAR-NK cell expansion over the restimulation assay, and rank-ordered variants by NK cell number (Figure 10). The present Example included CAR constructs found to be positively enriched in Example 1, as well as certain control CAR constructs that were negatively enriched.

[80] The present Example demonstrated that sequences that had been positively enriched from the Example 1 assay performed well in the present arrayed experiment, while the sequences that had been negatively enriched from the Example 1 assay were among the lowest ranking variants, further supporting the results reported in Example 1. Accordingly, the present Example validated and confirmed results reported in Example 1 and throughout the present specification.

Example 4: Functional assessment of stimulatory sequences as compared to industry benchmark in a spheroid killing assay

[81] The present Example demonstrated that stimulatory sequences as disclosed herein can modulate and improve a CAR-NK cell's response to a lack of cytokine stimulation. NK cells require supportive cytokines such as IL-2 for fitness, and other cell therapy developers have envisioned strategies for augmenting cytokine signaling in NK cells.

[82] In these experiments, stimulatory sequences of the present disclosure were included in CARs that featured a CD19 antigen-binding domain and CD28 transmembrane domain. CAR-NK cells were co-cultured with fluorescently-labeled Raji target cell spheroids and spheroids were imaged over the course of 20 hours to quantify target cell killing. As an industry benchmark for comparison, an OX40-CD3z control stimulatory sequence that represents the CAR from an established NK cell industry player was included. Additionally, experiments were performed in both low-IL-2 and normal IL-2 conditions to test the ability of the cells to rescue a lack of cytokine signaling in the presence of different CAR variants.

[83] Figure 11 shows that in normal IL-2 conditions, spheroid killing is achieved across the CAR variants, with performance comparable to or superior to the industry benchmark. However, in low IL-2 conditions, data demonstrated outperformance of presently disclosed stimulatory sequences as compared to the industry benchmark, suggesting that presently disclosed stimulatory sequences can rescue a lack of cytokine signaling. These data demonstrate that the superiority of stimulatory sequences disclosed herein, and CARs including such sequences, is further revealed by superior efficacy and/or sensitivity under low IL-2 conditions, and, e.g., to a greater extent than may be evident under normal or high IL-2 laboratory assay conditions. Such a capability has particular utility for application areas including autoimmune disease and cancer (e.g., lupus and certain solid tumor indications such as colon and pancreatic cancer), in which a dearth of supportive cytokines (including in

particular IL-2 and cytokines with similar effects on CAR cell therapy) could potentially limit cell therapy function.

Example 5: Functional assessment of stimulatory sequences of the present disclosure as compared to industry benchmark in an acute cytotoxicity assay

[84] The present Example demonstrated that stimulatory sequences disclosed herein can enhance the cytotoxicity of CAR NK cells across diverse target cells, irrespective of effector-to-target (E:T) ratios. In these experiments, stimulatory sequences of the present disclosure were included in CARs that featured a CD19 antigen-binding domain and CD28 transmembrane domain. CAR-NK cells were co-cultured with either the Raji tumor cell line or primary B cells from human donors. The primary B cell targets have particular utility for autoimmune applications. After 4 hours, target cell killing was quantified by flow cytometry and normalized to input cell counts.

[85] Figure 12 depicts normalized killing scores across a set of sequence variants for the different cell types and E:T ratios. As in Example 4, the industry benchmark OX40-CD3z stimulatory sequence was included for comparison. Data demonstrate that across the different assay variations, including diverse cell types and diverse E:T ratios, presently disclosed stimulatory sequences were found perform in accordance with Example 1 and further found to out-perform the industry benchmark.

Example 6: In vivo assessment of a stimulatory sequence of the present disclosure as compared to industry benchmark in a xenograft killing experiment

[86] The present Example provides *in vivo* data demonstrating the utility of CAR NK cells including a representative stimulatory sequence of the present disclosure. In particular, the present Example demonstrated the ability of one particular stimulatory sequence (Fn14_CRTAM, SEQ ID NO: 78) to eliminate a CD19+ xenograft *in vivo*, with comparison to an industry benchmark. Luciferase-expressing Raji cells were injected into NSG mice to create an *in vivo* target cell burden. Two days later, CAR-NK cells featuring either the Fn14_CRTAM CAR or the industry benchmark OX40_CD3z CAR were injected intravenously. At Days 12 and 19, IVIS imaging was performed to visualize the remaining tumor burden. Figure 13 demonstrated that tumor dominated the vehicle control mice, where as the industry benchmark showed strong clearance. Notably, the representative Fn14_CRTAM CAR demonstrated even greater clearance than the clinically-validated

industry benchmark, further establishing the utility and superiority of stimulatory sequences and CARs disclosed herein.

Example 7: Table of SEQ ID NOs: 18-219

Table 4: Table of SEQ ID NOs: 18-219

SEQ ID NO	Sequence
18	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSRRCRRREKFTTPIEETG GEGCPAVALIQ
19	RRCRRREKFTTPIEETGGEGCPAVALIQGSQRRKYRSNKGESPVEPAEPCHYSCPREEEGS TIPIQEDYRKPEPACSP
20	RRCRRREKFTTPIEETGGEGCPAVALIQGSGSTVYYQGKCLTWKGPRRQLPAVVPAPLPP PCGSSAHLLPPVPGG
21	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSKTNIRSSTRDWKDHKFKWRKDPQDK
22	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
23	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSLCARPRRSPAQE DGKVYINMPGRG
24	CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTLGSKKVAKKPTNKAPHPKQEP QEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
25	RKRTRERASRASTWEGRRRLNTQTLGSRRCRRREKFTTPIEETGGEGCPAVALIQ
26	WWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDD DEHTLGSRRCRRREKFTTPIEETGGEGCPAVALIQ
27	GSTVYYQGKCLTWKGPRRQLPAVVPAPLPPPCGSSAHLLPPVPGGGSKRGRKKLLYIFKQ PFMRPVQTTQEEDGCSCRFPEEEEGGCEL
28	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSGSTVYYQGKCLTWKGPRRQLPAVVPAPLPPPCGSSAHLLPPVPGG
29	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
30	RRCRRREKFTTPIEETGGEGCPAVALIQGSKKVAKKPTNKAPHPKQEPQEINFPDDLPGSN TAAPVQETLHGCQPVTQEDGKESRISVQERQ
31	WWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDD DEHTLGSCWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL
32	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSRLKIQVRKAAIT SYEKSDGVYTGLSTRNQETYETLKHEKPPQ
33	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSKKVAKKPTNKAPHPK QEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
34	RRCRRREKFTTPIEETGGEGCPAVALIQGSKTNIRSSTRDWKDHKFKWRKDPQDK
35	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSWRRKRKEKQSETSPKE FLTIYEDVKDLKTRRNHEQEQTFPGGGSTIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGS RKRNHSPSFNSTIYEVIGKSQPKAQNPARLSRKELENFDVYS
36	RRCRRREKFTTPIEETGGEGCPAVALIQGSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCEL
37	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSLCARPRRSPAQEDGKVYINMPGRG
38	CYRKKGKALTANLWHWINEACGRLSGDKESSGDSCVSTHTANFGQQGACEGVLLLTLE EKTFPEDMCYPDQGGVCQGTCVGGGPYAQGEDARMLSLVSKTEIEEDSFRQMPTEDEY MDRPSQPTDQLLFLTEPGSKSTPPFSEPLEVGENDSLSQCFTGTQSTVGSESCNCTEPLCRT DWTPMSSENYLQKEVDSGHCPHWAASPSPNWADVCTGCRNPPGEDCEPLVGSPKRGPL PQCAYGMGLPPEEEASRTEARDQPEDGADGRLPSSARAGAGSGSSPGGQSPASGNVTGN SNSTFISSGQVMNFKGDIIVVYVSQTSQEGAAAAAEPMGRPVQEETLARRDSFAGNGPRF PDPCGGPEGLREPEKASRPVQEQGGAKAGSRSKRSRLLHSDYMNMTPRRPGPTRKHYQP YAPPRDFAAYRS
39	RKRTRERASRASTWEGRRRLNTQTLGSLCARPRRSPAQEDGKVYINMPGRG

40	CYRKKGKALTANLWHWINEACGRLSGDKESSGDSCVSTHTANFGQQGACEGVLLLTLE EKTFPEDMCYPDQGGVCQGTCVGGGPYAQGEDARMLSLVSKTEIEEDSFRQMPTEDEY MDRPSQPTDQLLFLTEPGSKSTPPFSEPLEVGENDSLSQCFTGTQSTVGSESCNCTEPLCRT DWTPMSSENYLQKEVDSGHCPHWAASPSPNWADVCTGCRNPPGEDCEPLVGSPKRGPL PQCAYGMGLPPEEEASRTEARDQPEDGADGRLPSSARAGAGSGSSPGGQSPASGNVTGN SNSTFISSGQVMNFKGDIIVVYVSQTSQEGAAAAAEPMGRPVQEETLARRDSFAGNGPRF PDPCGGPEGLREPEKASRPVQEQGGAKAGSGSTVYYQGKCLTWKGPRRQLPAVVPAPLP PPCGSSAHLLPPVPGG
41	RRCRRREKFTTPIEETGGEGCPAVALIQGSRLKIQVRKAAITSYEKSDGVYTGLSTRNQET YETLKHEKPPQ
42	YFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYKGSRRCRR REKFTTPIEETGGEGCPAVALIQ
43	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSYFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK
44	KTNIRSSTRDWKDHKFKWRKDPQDKGSKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNT AAPVQETLHGCQPVTQEDGKESRISVQERQ
45	RRCRRREKFTTPIEETGGEGCPAVALIQGSRRCRRREKFTTPIEETGGEGCPAVALIQ
46	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSGSTVYYQGKCLTWKG PRRQLPAVVPAPLPPPCGSSAHLLPPVPGG
47	YFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYKGSCWLTK KKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL
48	WWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDD DEHTLGSRKRTRERASRASTWEGRRRLNTQTL
49	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSWWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKI SDDDDEHTL
50	RRCRRREKFTTPIEETGGEGCPAVALIQGSYFLGRLVPRGRGAAEAATRKQRITETESPYQ ELQGQRSDVYSDLNTQRPYYK
51	LCARPRRSPAQEDGKVYINMPGRGGSSWRRRQRRLRGASSAEAPDGDKDAPEPLDKVIIL SPGISDATAPAWPPPGEDPGTTPPGHSVPVPATELGSTELVTTKTAGPEQQ
52	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSKKVAKKPTNKA PHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQERQ
53	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRI SVQERQ
54	RRCRRREKFTTPIEETGGEGCPAVALIQGSLCARPRRSPAQEDGKVYINMPGRG
55	GSKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISV QERQ
56	WFLKRERQEEYIEEKKRVDICRETPNICPHSGENTEYDTIPHTNRTILKEDPANTVYSTVEI PKKMENPHSLLTMPDTPRLFAYENVIGSKTNIRSSTRDWKDHKFKWRKDPQDK
57	RRCRRREKFTTPIEETGGEGCPAVALIQGS
58	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSWFLKRERQEEYIEEKKRVDICRETPNICPHSGENTEYDTIPHTNRTILKEDPANTVYS TVEIPKKMENPHSLLTMPDTPRLFAYENVI
59	KRKKQRSRRNDEELETRAHRVATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSH RPPPPGHRVQHQPQKRPPAPSGTQVHQQKGPPLPRPRVQPKPPHGAAENSLSPSSNGSKT NIRSSTRDWKDHKFKWRKDPQDK
60	IMKLRKAHVIWKKENEVSEHTLESYRSRSNNEETSSEEKNGQSSHPMRCMNYITKLYSEA KTKRKENVQHSKLEEKHIQVPESIVGSKTNIRSSTRDWKDHKFKWRKDPQDK
61	GSSWRRRQRRLRGASSAEAPDGDKDAPEPLDKVIILSPGISDATAPAWPPPGEDPGTTPPG HSVPVPATELGSTELVTTKTAGPEQQ
62	RRCRRREKFTTPIEETGGEGCPAVALIQGSWFLKRERQEEYIEEKKRVDICRETPNICPHSG ENTEYDTIPHTNRTILKEDPANTVYSTVEIPKKMENPHSLLTMPDTPRLFAYENVI
63	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSLCARPRRSPAQEDGKV YINMPGRG
64	LCARPRRSPAQEDGKVYINMPGRGGSKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTA APVQETLHGCQPVTQEDGKESRISVQERQ
65	CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTLGSRSKRSRLLHSDYMNMTPR RPGPTRKHYQPYAPPRDFAAYRS

66	GSRRCRRREKFTTPIEETGGEGCPAVALIQ
67	GSDLKFWHKKMDFSQNVNVSSLSGHNYLCPNDWLLNEGKCYWFSTSFKTWKESQRDC TQLQAHLLVIQNLDELEFIQNSLKPGHFGWIGLYVTFQGNLWMWIDEHFLVPELFSVIGPT DDRSCAVITGNWVYSEDCSSTFKGICQRDAILTHNGTSGV
68	RRCRRREKFTTPIEETGGEGCPAVALIQGSWRRKRKEKQSETSPKEFLTIYEDVKDLKTRR NHEQEQTFPGGGSTIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRKRNHSPSFNSTIYE VIGKSQPKAQNPARLSRKELENFDVYS
69	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSLRKRRDSLSLST QRTQGPAESARNLEYVSVSPTNNTVYASVTHSNRETEIWTPRENDTITIYSTINHSKESKPT FSRATALDNVV
70	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSRSKRSRLLHSDYMNMT PRRPGPTRKHYQPYAPPRDFAAYRS
71	KTNIRSSTRDWKDHKFKWRKDPQDKGSLRKRRDSLSLSTQRTQGPAESARNLEYVSVSP TNNTVYASVTHSNRETEIWTPRENDTITIYSTINHSKESKPTFSRATALDNVV
72	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSQRRKYRSNKGESPVEP AEPCHYSCPREEEGSTIPIQEDYRKPEPACSP
73	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGL YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGSR VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLY NELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
74	WWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDD DEHTLGSLRKRRDSLSLSTQRTQGPAESARNLEYVSVSPTNNTVYASVTHSNRETEIWTP RENDTITIYSTINHSKESKPTFSRATALDNVV
75	LCARPRRSPAQEDGKVYINMPGRGGSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPE EEEGGCEL
76	KTNIRSSTRDWKDHKFKWRKDPQDKGSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCEL
77	WFLKRERQEEYIEEKKRVDICRETPNICPHSGENTEYDTIPHTNRTILKEDPANTVYSTVEI PKKMENPHSLLTMPDTPRLFAYENVIGSRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA PPRDFAAYRS
78	RRCRRREKFTTPIEETGGEGCPAVALIQGSIMKLRKAHVIWKKENEVSEHTLESYRSRSNN EETSSEEKNGQSSHPMRCMNYITKLYSEAKTKRKENVQHSKLEEKHIQVPESIV
79	WWGDIWWKTMMELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDD DEHTLGSRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
80	LRKRRDSLSLSTQRTQGPAESARNLEYVSVSPTNNTVYASVTHSNRETEIWTPRENDTITI YSTINHSKESKPTFSRATALDNVVGSQRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQ EDYRKPEPACSP
81	KTNIRSSTRDWKDHKFKWRKDPQDKGSQRRKYRSNKGESPVEPAEPCHYSCPREEEGSTI PIQEDYRKPEPACSP
82	GSKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
83	WRRKRKEKQSETSPKEFLTIYEDVKDLKTRRNHEQEQTFPGGGSTIYSMIQSQSSAPTSQE PAYTLYSLIQPSRKSGSRKRNHSPSFNSTIYEVIGKSQPKAQNPARLSRKELENFDVYSGSR KRTRERASRASTWEGRRRLNTQTL
84	LCARPRRSPAQEDGKVYINMPGRGGSRRCRRREKFTTPIEETGGEGCPAVALIQ
85	CWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTLGSRRCRRREKFTTPIEETGGE GCPAVALIQ
86	RKRTRERASRASTWEGRRRLNTQTLGSWWGDIWWKTMMELRSLDTQKATCHLQQVTD LPWTSVSSPVEREILYHTVARTKISDDDDEHTL
87	CYRKKGKALTANLWHWINEACGRLSGDKESSGDSCVSTHTANFGQQGACEGVLLLTLE EKTFPEDMCYPDQGGVCQGTCVGGGPYAQGEDARMLSLVSKTEIEEDSFRQMPTEDEY MDRPSQPTDQLLFLTEPGSKSTPPFSEPLEVGENDSLSQCFTGTQSTVGSESCNCTEPLCRT DWTPMSSENYLQKEVDSGHCPHWAASPSPNWADVCTGCRNPPGEDCEPLVGSPKRGPL PQCAYGMGLPPEEEASRTEARDQPEDGADGRLPSSARAGAGSGSSPGGQSPASGNVTGN SNSTFISSGQVMNFKGDIIVVYVSQTSQEGAAAAAEPMGRPVQEETLARRDSFAGNGPRF PDPCGGPEGLREPEKASRPVQEQGGAKAGSKRGRKKLLYIFKQPFMRPVQTTQEEDGCS CRFPEEEEGGCEL
88	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSWFLKRERQEEYI EEKKRVDICRETPNICPHSGENTEYDTIPHTNRTILKEDPANTVYSTVEIPKKMENPHSLLT MPDTPRLFAYENVI

89	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSRRCRRREKFTTP IEETGGEGCPAVALIQ
90	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSNRRRRRERRDLFTESW DTQKAPNNYRSPISTSQPTNQSMDDTREDIYVNYPTFSRRPKTRV
91	KTNIRSSTRDWKDHKFKWRKDPQDKGSRVKFSRSADAPAYQQGQNQLYNELNLGRREE YDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDG LYQGLSTATKDTYDALHMQALPPR
92	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELGSYFLGRLVPRGRGAAEA ATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK
93	NRRRRRERRDLFTESWDTQKAPNNYRSPISTSQPTNQSMDDTREDIYVNYPTFSRRPKTR VGSCWLTKKKYSSSVHDPNGEYMFMRAVNTAKKSRLTDVTL
94	QVSHRYPRIQSIKVQFTEYKKEKGFILTSQKEDEIMKVQNNSVIINCDGFYLISLKGYFSQE VNISLHYQKDEEPLFQLKKVRSVNSLMVASLTYKDKVYLNVTTDNTSLDDFHVNGGELI LIHQNPGEFCVLGSRLKIQVRKAAITSYEKSDGVYTGLSTRNQETYETLKHEKPPQ
95	KTNIRSSTRDWKDHKFKWRKDPQDKGS
96	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
97	GSQRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSP
98	GSTVYYQGKCLTWKGPRRQLPAVVPAPLPPPCGSSAHLLPPVPGGGSWWGDIWWKTM MELRSLDTQKATCHLQQVTDLPWTSVSSPVEREILYHTVARTKISDDDDEHTL
99	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSRLFKRRQGRIFP EGSCLNTFTKNPYAASKKTIYTYIMASRNTQPAESRIYDEILQSKVLPSKEEPVNTVYSEV QFADKMGKASTQDSKPPGTSSYEIVI
100	KKVAKKPTNKAPHPKQEPQEINFPDDLPGSNTAAPVQETLHGCQPVTQEDGKESRISVQE RQGSRKRTRERASRASTWEGRRRLNTQTL
101	KRKKQRSRRNDEELETRAHRVATEERGRKPHQIPASTPQNPATSQHPPPPPGHRSQAPSH RPPPPGHRVQHQPQKRPPAPSGTQVHQQKGPPLPRPRVQPKPPHGAAENSLSPSSNGSRR CRRREKFTTPIEETGGEGCPAVALIQ
102	RKRTRERASRASTWEGRRRLNTQTLGSKKVAKKPTNKAPHPKQEPQEINFPDDLPGSNT AAPVQETLHGCQPVTQEDGKESRISVQERQ
103	IMKLRKAHVIWKKENEVSEHTLESYRSRSNNEETSSEEKNGQSSHPMRCMNYITKLYSEA KTKRKENVQHSKLEEKHIQVPESIVGSRLFKRRQGRIFPEGSCLNTFTKNPYAASKKTIYT YIMASRNTQPAESRIYDEILQSKVLPSKEEPVNTVYSEVQFADKMGKASTQDSKPPGTSSY EIVI
104	QRRKYRSNKGESPVEPAEPCHYSCPREEEGSTIPIQEDYRKPEPACSPGSWRRKRKEKQSE TSPKEFLTIYEDVKDLKTRRNHEQEQTFPGGGSTIYSMIQSQSSAPTSQEPAYTLYSLIQPS RKSGSRKRNHSPSFNSTIYEVIGKSQPKAQNPARLSRKELENFDVYS
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219	TSQEPAYYTLYSLIQPSRYTLYSLIQPSRRKSGSRKGSDYETADGGYMTLNPRAPTDDDK NIYLTLPPNDHVNS

OTHER EMBODIMENTS

[87] It will be appreciated that the scope of the present disclosure is to be defined by that which may be understood from the disclosure and claims rather than by the specific embodiments that have been presented by way of example. Elements described with respect to one aspect or embodiment of the present disclosure are also contemplated with respect to other aspects or embodiments of the present disclosure. For example, elements of claims that depend directly or indirectly from a certain independent claim presented herein serve as support for those elements being presented in additional dependent claims of one or more other independent claims. Throughout the description, where compositions or methods are described as having, including, or comprising specific elements, compositions that consist essentially of, consist of, or do not comprise the recited elements are likewise hereby disclosed. All references cited herein are hereby incorporated by reference.

CLAIMS

What is claimed is:

1. A chimeric antigen receptor (CAR) comprising an antigen-binding domain, a transmembrane domain, and at least a first stimulatory sequence, wherein the stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity with a sequence selected from SEQ ID NOs: 1-17.

2. A chimeric antigen receptor (CAR) comprising an antigen-binding domain, a transmembrane domain, and a stimulatory region comprising a first stimulatory sequence and a second stimulatory sequence, wherein the stimulatory region has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 18-219.

3. A chimeric antigen receptor (CAR) comprising an antigen-binding domain, a transmembrane domain, and a stimulatory region comprising a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 220-421 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 422-623, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 2 or Table 3.

4. The CAR of claim 2, wherein the stimulatory region comprises a linker positioned between the first stimulatory sequence and the second stimulatory sequence, optionally wherein the linker is a flexible linker and/or wherein the amino acids between the first stimulatory sequence and the second stimulatory sequence consist or consist essentially of the linker.

5. A stimulatory region comprising at least a first stimulatory sequence, wherein the stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity with a sequence selected from SEQ ID NOs: 1-17.

6. A stimulatory region comprising a first stimulatory sequence and a second stimulatory sequence, wherein the stimulatory region has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with both the first and second stimulatory a sequences selected from SEQ ID NOs: 18-219.

7. A stimulatory region comprising a first stimulatory sequence and a second stimulatory sequence, wherein the first stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 220-421 and the second stimulatory sequence has at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with a sequence selected from SEQ ID NOs: 422-623, optionally wherein the first stimulatory sequence and second stimulatory sequence are each present in a row of Table 2 or Table 3.

8. The stimulatory region of claim 7, wherein the stimulatory region comprises a linker positioned between the first stimulatory sequence and the second stimulatory sequence, optionally wherein the linker is a flexible linker and/or wherein the amino acids between the first stimulatory sequence and the second stimulatory sequence consist or consist essentially of the linker.

9. The stimulatory region of any one of claims 5-8, wherein the stimulatory region is operably linked with an antigen-binding domain.

10. The stimulatory region of any one of claims 5-9, wherein the stimulatory region is operably linked with a transmembrane domain.

11. An engineered immune cell comprising a chimeric antigen receptor (CAR) according to any one of claims 1-4 or a stimulatory region according to any one of claims 5-10.

12. The engineered immune cell of claim 11, wherein the cell is an NK cell.

13. The engineered immune cell of claim 11 or 12, wherein the cell is a CD56+ cell.

14. The engineered immune cell of claim 13, wherein the CD56+ cell is differentiated from an induced pluripotent stem cell (iPSC), embryonic stem cell (ESC), or CD34+ progenitor cell (HSPC).

15. A method of producing an engineered immune cell, the method comprising contacting the immune cell with a nucleic acid encoding a chimeric antigen receptor (CAR) according to any one of claims 1-4 or a stimulatory region according to any one of claims 5-10.

16. The method of claim 15, wherein the cell is an NK cell.

17. The method of claim 15 or 16, wherein the cell is a CD56+ cell.

18. The method of claim 17, wherein the CD56+ cell is differentiated from an induced pluripotent stem cell (iPSC), embryonic stem cell (ESC), or CD34+ progenitor cell.

19. The method of any one of claims 15-18, wherein the contacting comprises viral delivery of the nucleic acid to the cell.

20. The method of any one of claims 15-18, wherein the contacting comprises non-viral delivery of the nucleic acid to the cell.

21. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an engineered immune cell according to any one of claims 11-14.

22. The method of claim 21, wherein the cancer is a solid tumor.

23. The method of claim 22, wherein the solid tumor is of a cancer selected from colorectal cancer, ovarian cancer, non small cell lung cancer, glioblastoma, triple negative breast cancer, hepatocellular carcinoma, prostate cancer, melanoma, small cell lung cancer, head and neck cancer, and pancreatic cancer.

24. The method of claim 21, wherein the cancer is a liquid cancer.

25. The method of claim 24, wherein the liquid cancer is selected from acute myeloid leukemia (AML), multiple myeloma, acute lymphocytic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), and mantle cell lymphoma (MCL).

26. The method of any one of claims 21-25, wherein the cancer expresses a biomarker selected from Her2, EGFR, CD19, BCMA, Muc1, CD20, Mesothelin, GPC3, Ror1, MAGE-A4, PRAME, NY-ESO-1, and PSA.

26. The method of any one of claims 21-26, wherein the administration is intravenous.

27. The method of any one of claims 21-26, wherein the administration is peri-tumoral.

28. The method of any one of claims 21-26, wherein the administration is intra-tumoral.

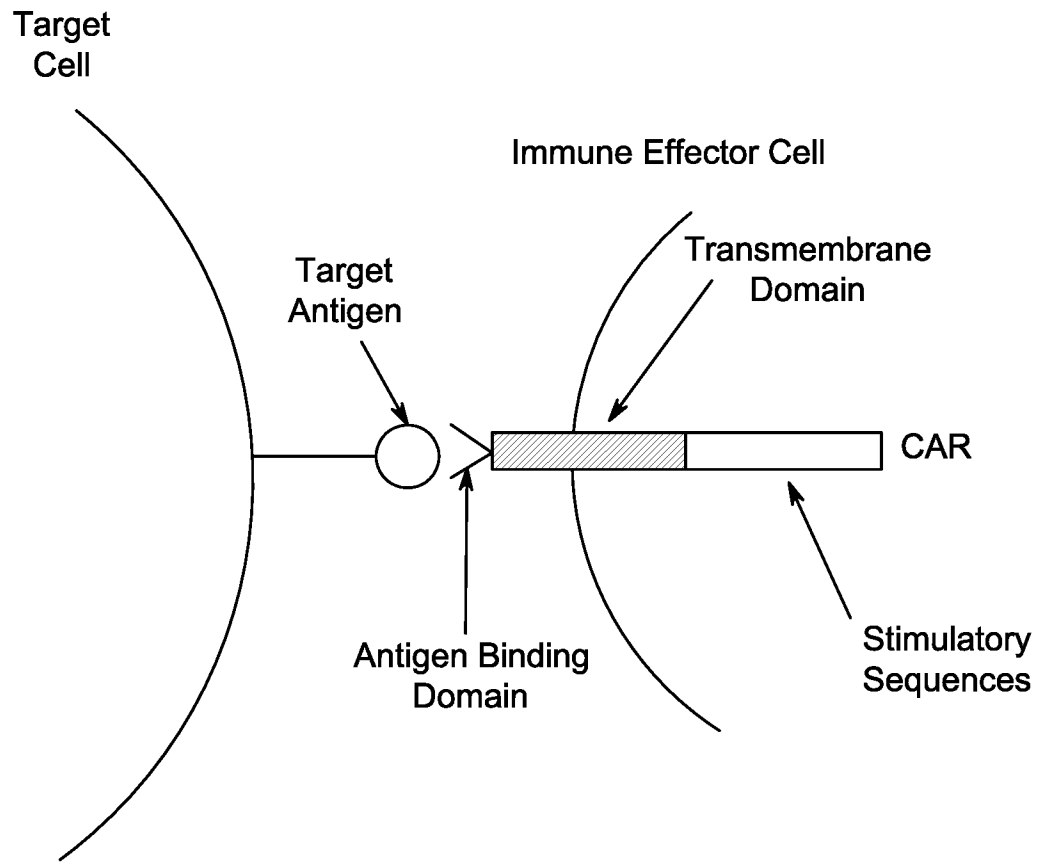
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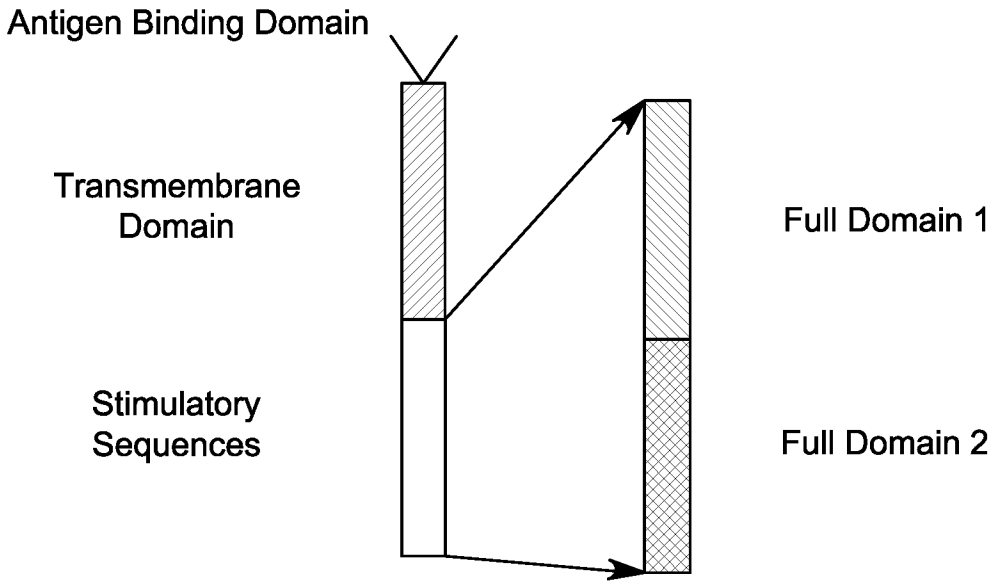
FIG. 1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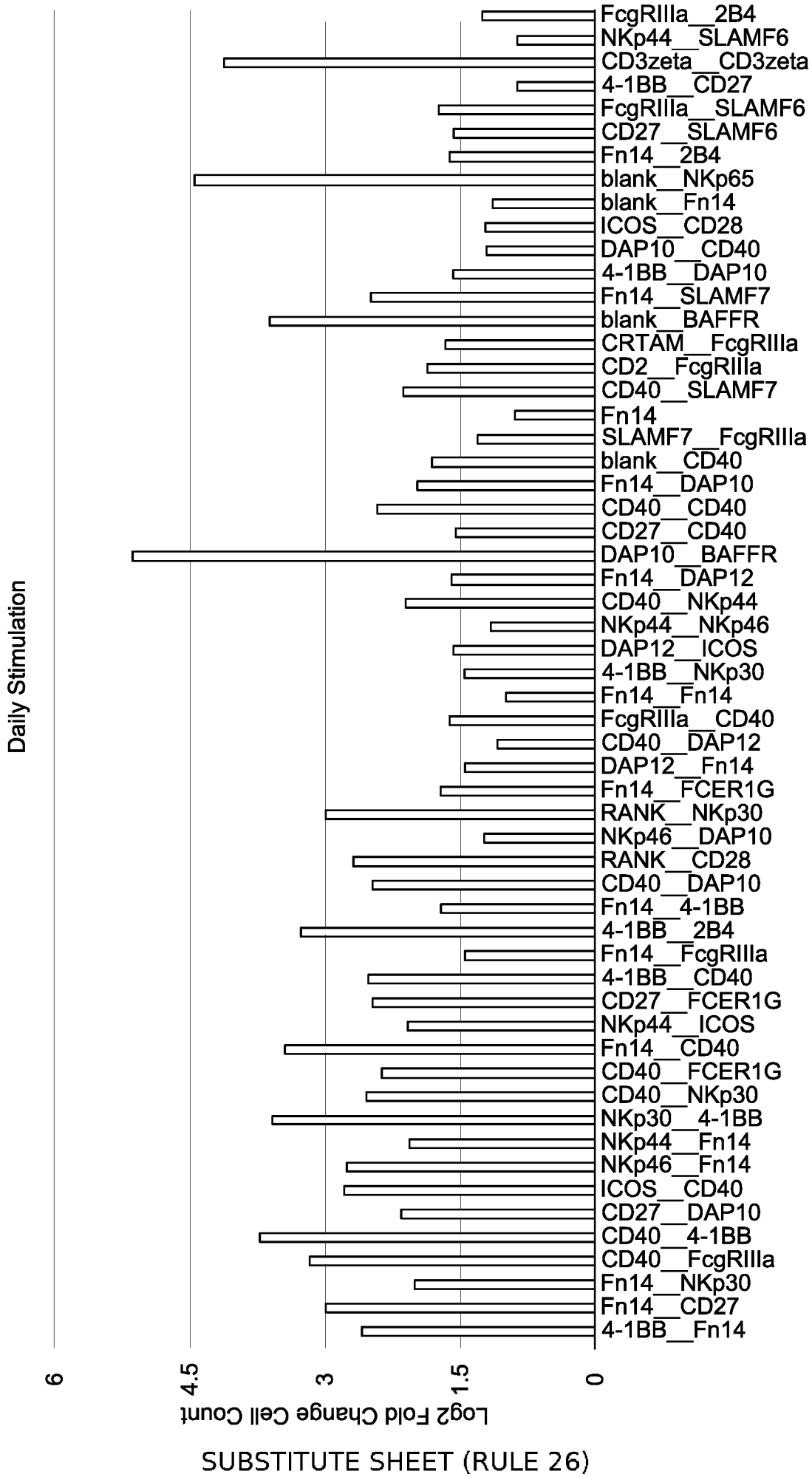
Full Domain 1
Full Domain 2

FIG. 2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Fn14__DAP10
CD40__CD40
CD27__CD40
DAP10__BAFFR
Fn14__DAP12
CD40__NKp44
NKp44__NKp46
DAP12__ICOS
4-1BB__NKp30
Fn14__Fn14
FcgRIIIa__CD40
CD40__DAP12
DAP12__Fn14
Fn14__FCER1G
RANK__NKp30
NKp46__DAP10
RANK__CD28
CD40__DAP10
Fn14__4-1BB
4-1BB__2B4
Fn14__FcgRIIIa
4-1BB__CD40
CD27__FCER1G
NKp44__ICOS
Fn14__CD40
CD40__FCER1G
CD40__NKp30
NKp30__4-1BB
NKp44__Fn14
NKp46__Fn14
ICOS__CD40
CD27__DAP10
CD40__4-1BB
CD40__FcgRIIIa
Fn14__NKp30
Fn14__CD27
4-1BB__Fn14
Daily Stimulation
6
4.5
3
1.5
0
Log2 Fold Change Cell Count

FIG. 3

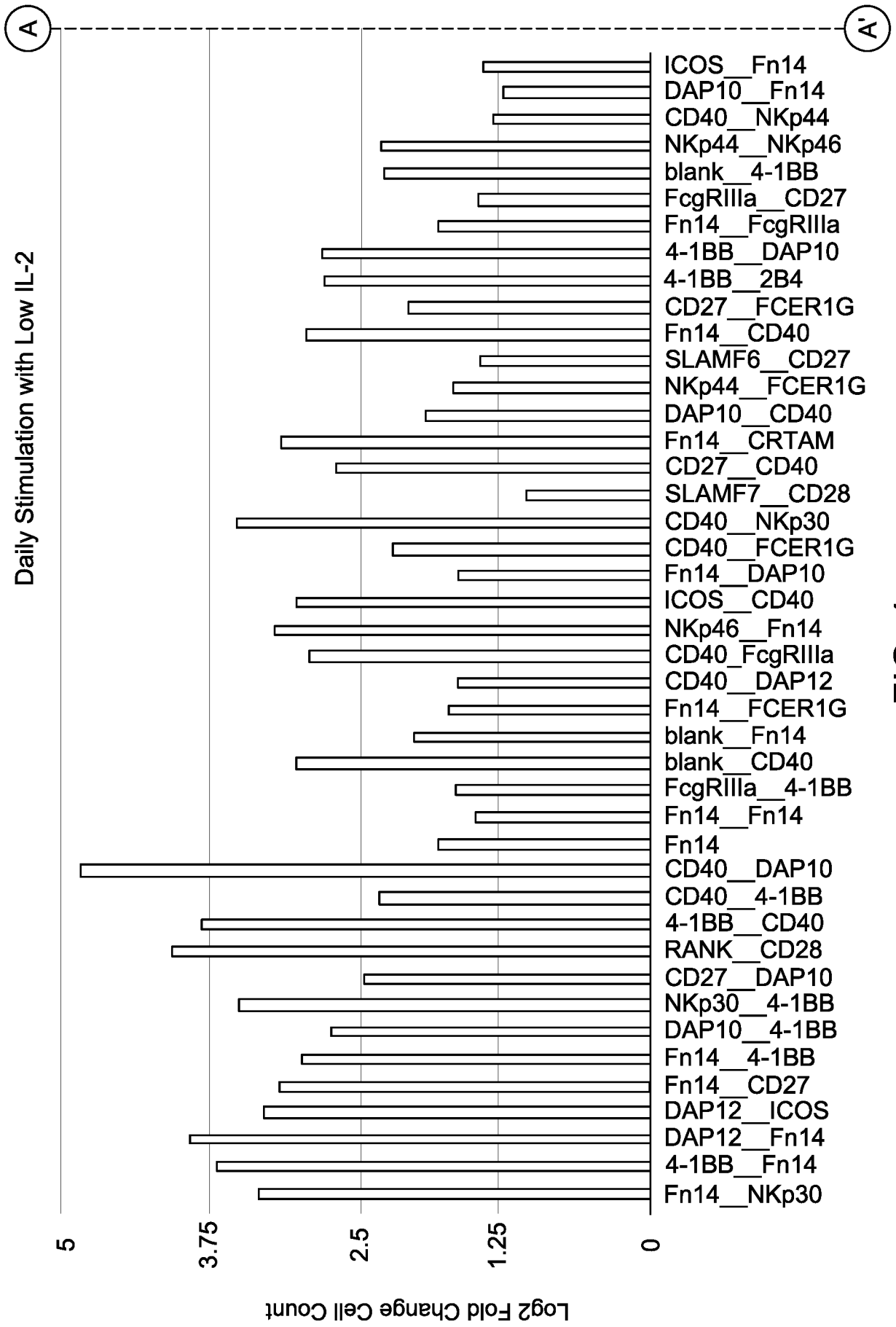
A
A'
Daily Stimulation with Low IL-2
ICOS__Fn14
DAP10__Fn14
CD40__NKp44
NKp44__NKp46
blank__4-1BB
FcgRIIIa__CD27
Fn14__FcgRIIIa
4-1BB__DAP10
4-1BB__2B4
CD27__FCER1G
Fn14__CD40
SLAMF6__CD27
NKp44__FCER1G
DAP10__CD40
Fn14__CRTAM
CD27__CD40
SLAMF7__CD28
CD40__NKp30
CD40__FCER1G
Fn14__DAP10
ICOS__CD40
NKp46__Fn14
CD40_FcgRIIIa
CD40__DAP12
Fn14__FCER1G
blank__Fn14
blank__CD40
FcgRIIIa__4-1BB
Fn14__Fn14
Fn14
CD40__DAP10
CD40__4-1BB
4-1BB__CD40
RANK__CD28
CD27__DAP10
NKp30__4-1BB
DAP10__4-1BB
Fn14__4-1BB
Fn14__CD27
DAP12__ICOS
DAP12__Fn14
4-1BB__Fn14
Fn14__NKp30
5
3.75
2.5
1.25
0
Log2 Fold Change Cell Count

FIG. 4

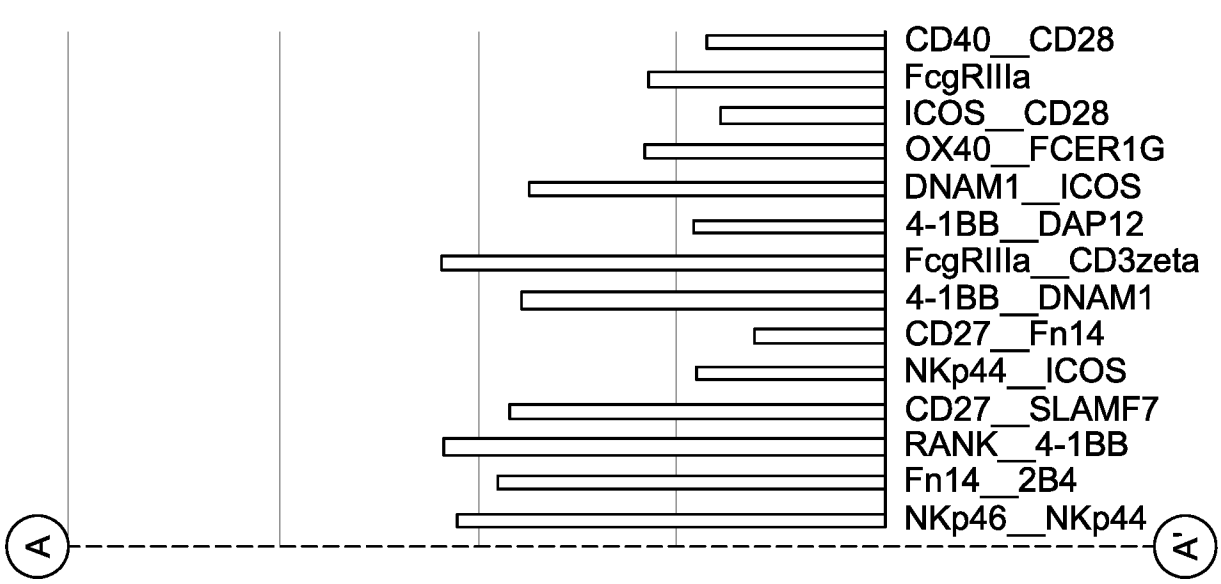

FIG. 4 (Continued)

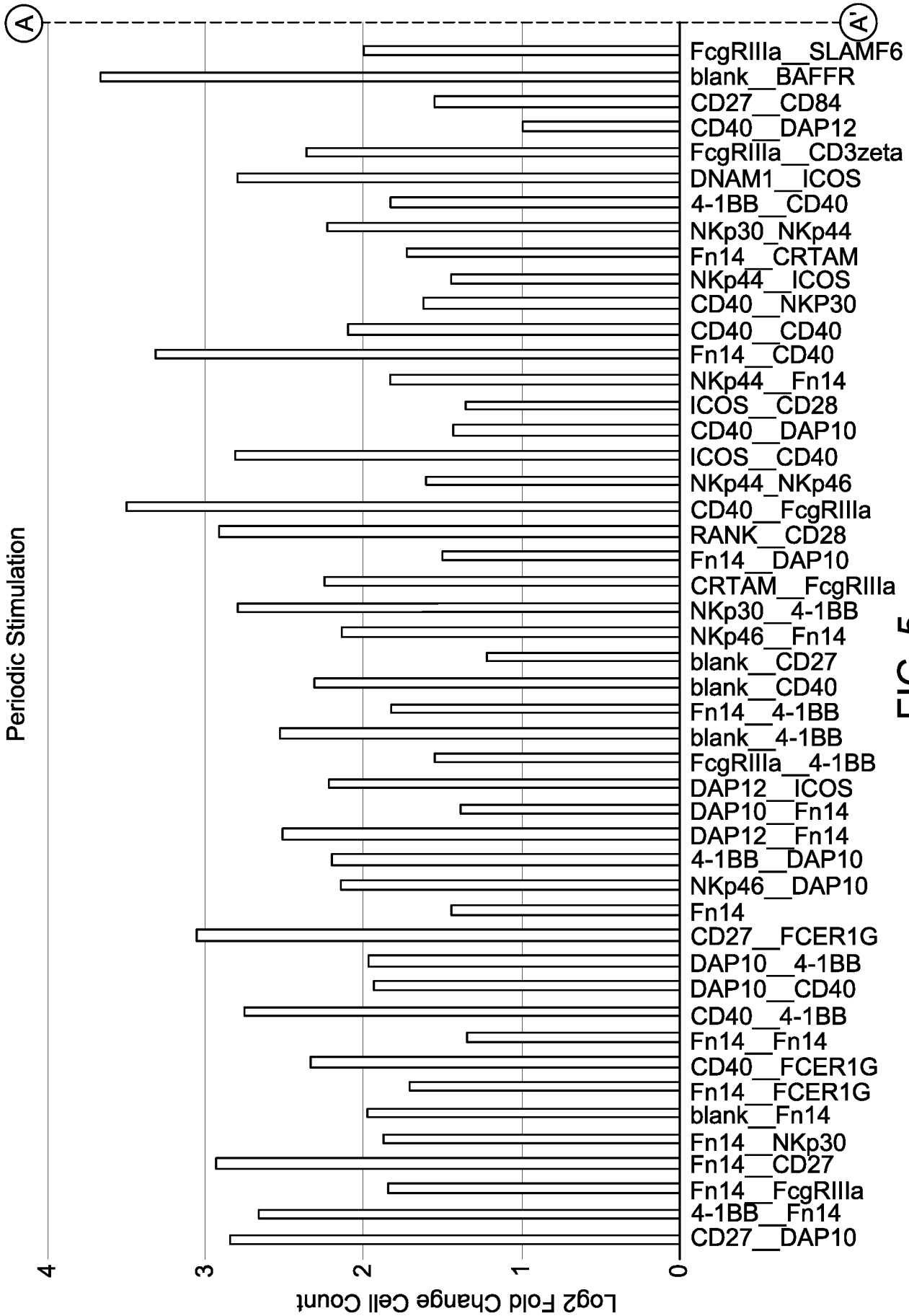
A
A'
FcgRIIIa__SLAMF6
blank__BAFFR
CD27__CD84
CD40__DAP12
FcgRIIIa__CD3zeta
DNAM1__ICOS
4-1BB__CD40
NKp30_NKp44
Fn14__CRTAM
NKp44__ICOS
CD40__NKP30
CD40__CD40
Fn14__CD40
NKp44__Fn14
ICOS__CD28
CD40__DAP10
ICOS__CD40
NKp44_NKp46
CD40__FcgRIIIa
RANK__CD28
Fn14__DAP10
CRTAM__FcgRIIIa
NKp30__4-1BB
NKp46__Fn14
blank__CD27
blank__CD40
Fn14__4-1BB
blank__4-1BB
FcgRIIIa__4-1BB
DAP12__ICOS
DAP10__Fn14
DAP12__Fn14
4-1BB__DAP10
NKp46__DAP10
Fn14
CD27__FCER1G
DAP10__4-1BB
DAP10__CD40
CD40__4-1BB
Fn14__Fn14
CD40__FCER1G
Fn14__FCER1G
blank__Fn14
Fn14__NKp30
Fn14__CD27
Fn14__FcgRIIIa
4-1BB__Fn14
CD27__DAP10
Periodic Stimulation
4
3
2
1
0
Log2 Fold Change Cell Count

FIG. 5

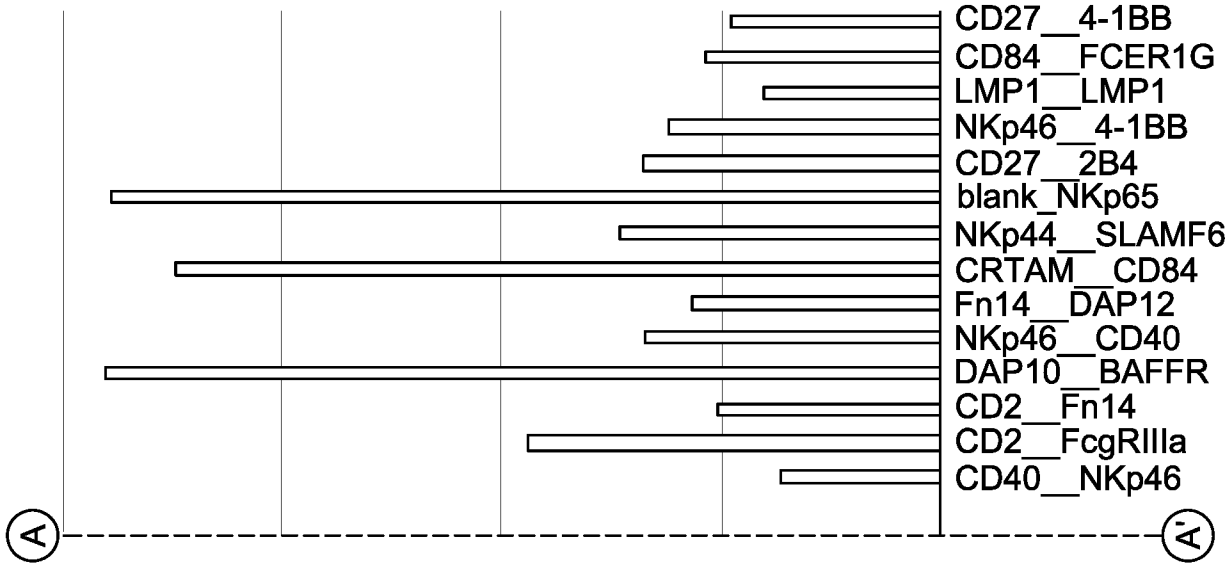

FIG. 5 (Continued)

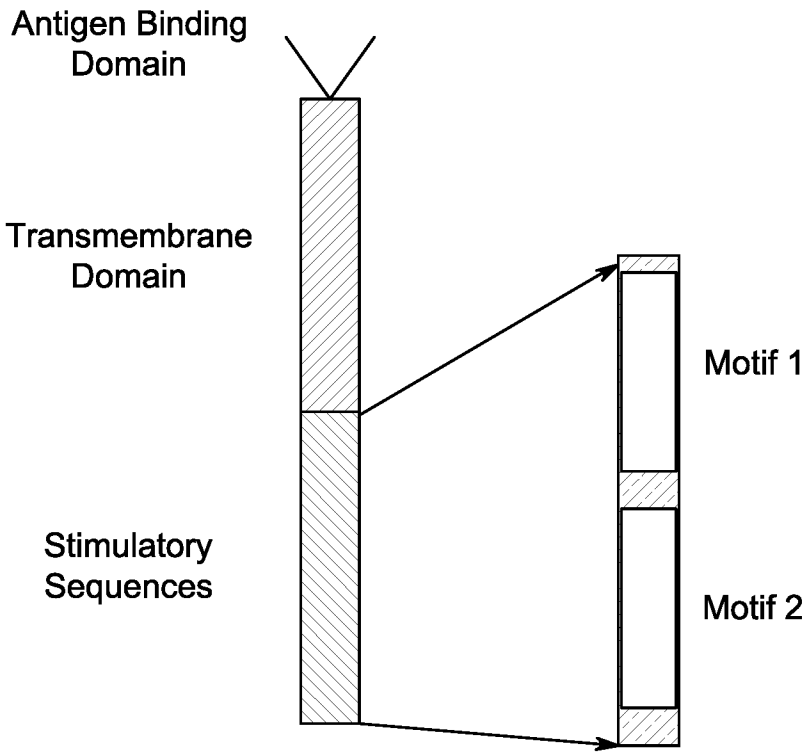
Antigen Binding Domain
Transmembrane Domain
Stimulatory Sequences
Motif 1
Motif 2

FIG. 6

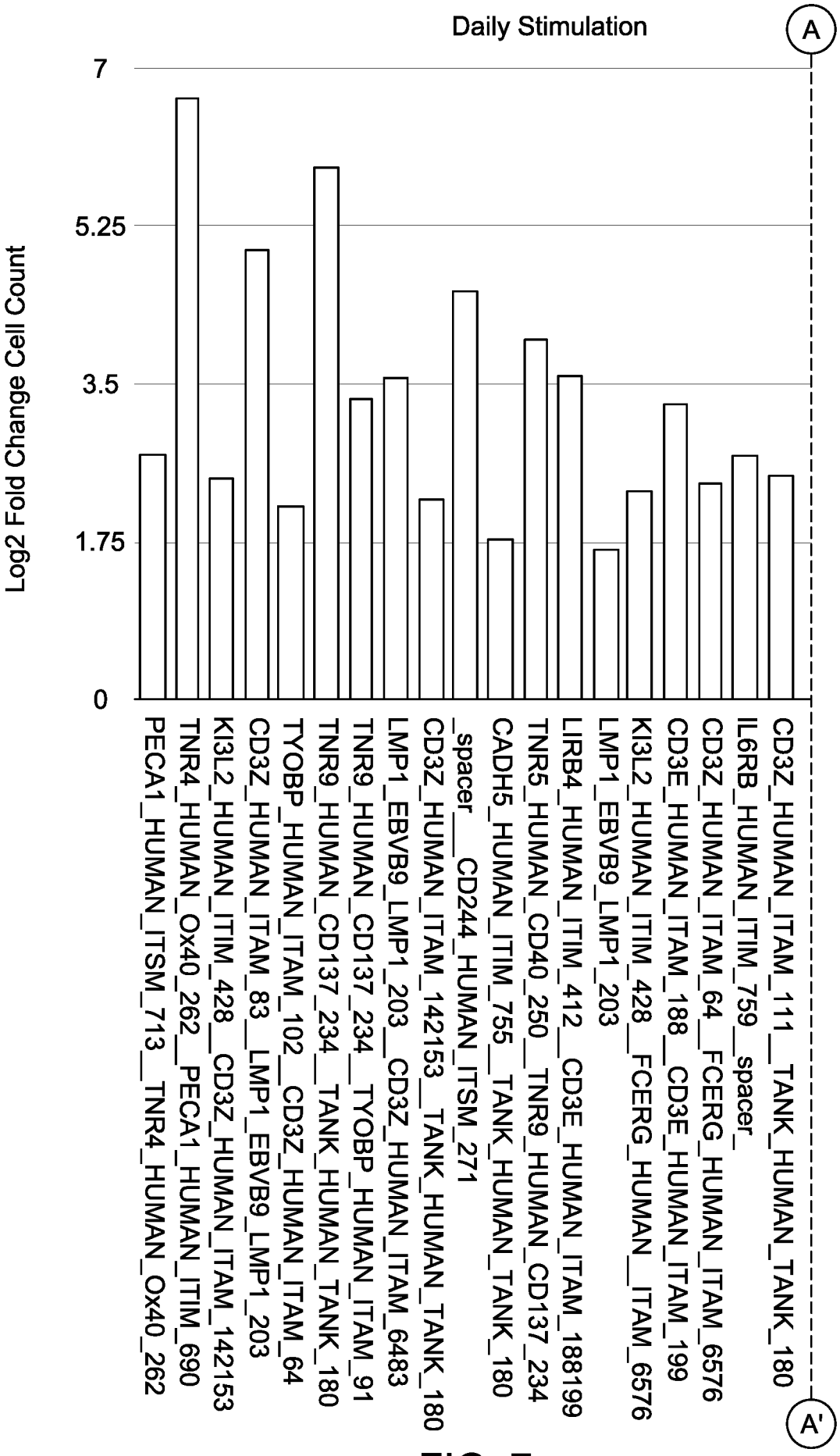
Daily Stimulation
A
A'
Log2 Fold Change Cell Count
7
5.25
3.5
1.75
0
CD3Z_HUMAN_ITAM_111__TANK_HUMAN_TANK_180
IL6RB_HUMAN_ITIM_759__spacer_
CD3Z_HUMAN_ITAM_64__FCERG_HUMAN_ITAM_6576
CD3E_HUMAN_ITAM_188__CD3E_HUMAN_ITAM_199
KI3L2_HUMAN_ITIM_428__FCERG_HUMAN__ITAM_6576
LMP1_EBVB9_LMP1_203
LIRB4_HUMAN_ITIM_412__CD3E_HUMAN_ITAM_188199
TNR5_HUMAN_CD40_250__TNR9_HUMAN_CD137_234
CADH5_HUMAN_ITIM_755__TANK_HUMAN_TANK_180
_spacer___CD244_HUMAN_ITSM_271
CD3Z_HUMAN_ITAM_142153__TANK_HUMAN_TANK_180
LMP1_EBVB9_LMP1_203__CD3Z_HUMAN_ITAM_6483
TNR9_HUMAN_CD137_234__TYOBP_HUMAN_ITAM_91
TNR9_HUMAN_CD137_234__TANK_HUMAN_TANK_180
TYOBP_HUMAN_ITAM_102__CD3Z_HUMAN_ITAM_64
CD3Z_HUMAN_ITAM_83__LMP1_EBVB9_LMP1_203
KI3L2_HUMAN_ITIM_428__CD3Z_HUMAN_ITAM_142153
TNR4_HUMAN_Ox40_262__PECA1_HUMAN_ITIM_690
PECA1_HUMAN_ITSM_713__TNR4_HUMAN_Ox40_262

FIG. 7

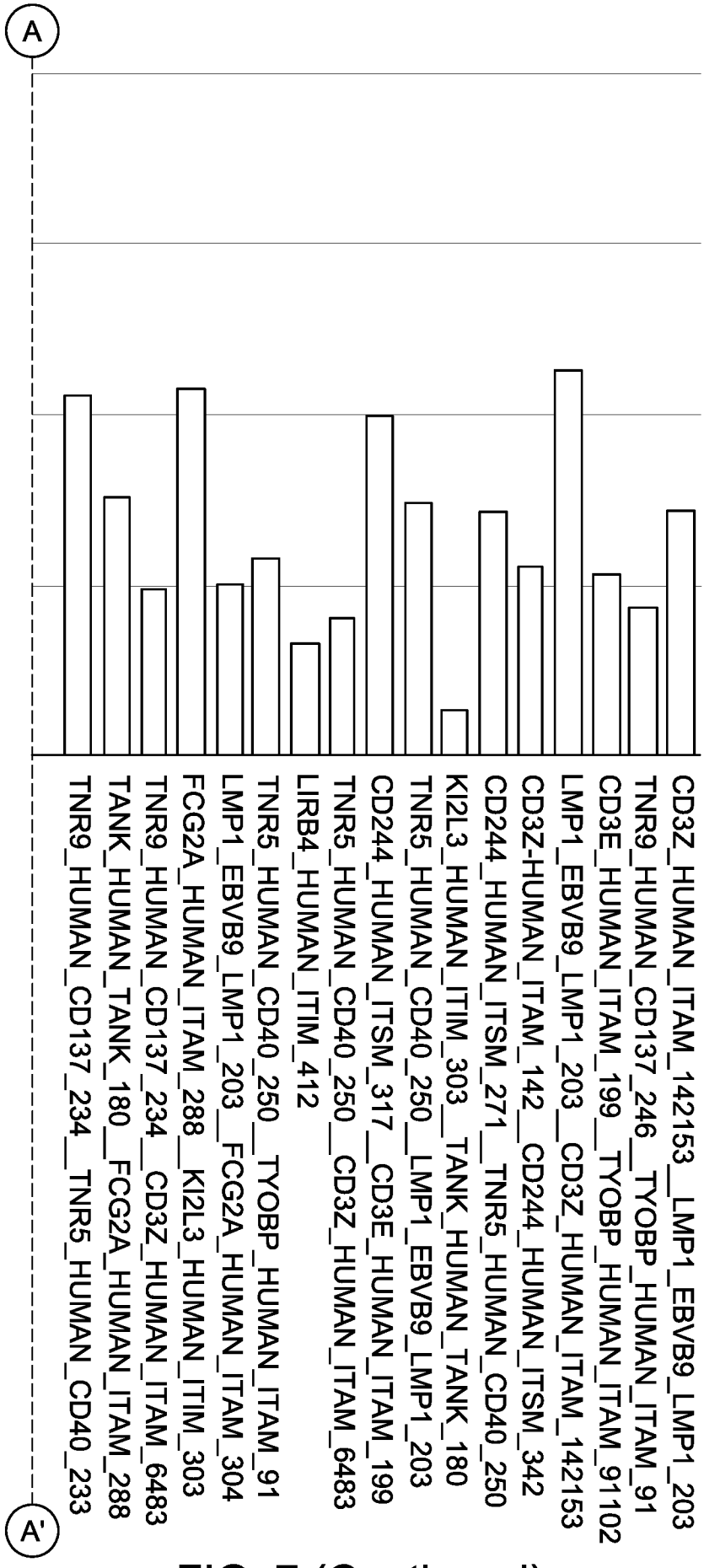

FIG. 7 (Continued)

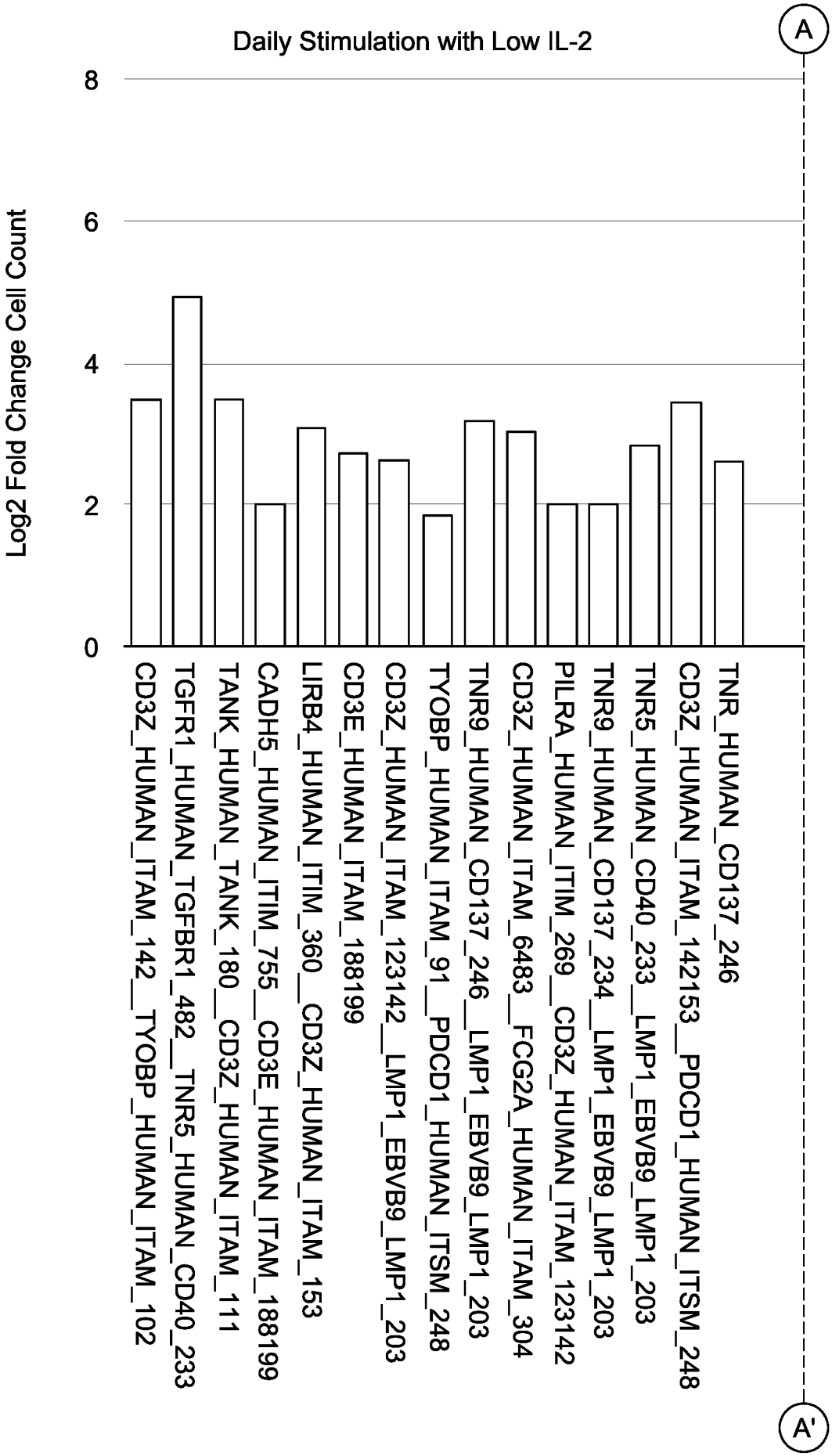
Daily Stimulation with Low IL-2
Log2 Fold Change Cell Count
0
2
4
6
8
A
A'
CD3Z_HUMAN_ITAM_142__TYOBP_HUMAN_ITAM_102
TGFR1_HUMAN_TGFBR1_482__TNR5_HUMAN_CD40_233
TANK_HUMAN_TANK_180__CD3Z_HUMAN_ITAM_111
CADH5_HUMAN_ITIM_755__CD3E_HUMAN_ITAM_188199
LIRB4_HUMAN_ITIM_360__CD3Z_HUMAN_ITAM_153
CD3E_HUMAN_ITAM_188199
CD3Z_HUMAN_ITAM_123142__LMP1_EBVB9_LMP1_203
TYOBP_HUMAN_ITAM_91__PDCD1_HUMAN_ITSM_248
TNR9_HUMAN_CD137_246__LMP1_EBVB9_LMP1_203
CD3Z_HUMAN_ITAM_6483__FCG2A_HUMAN_ITAM_304
PILRA_HUMAN_ITIM_269__CD3Z_HUMAN_ITAM_123142
TNR9_HUMAN_CD137_234__LMP1_EBVB9_LMP1_203
TNR5_HUMAN_CD40_233__LMP1_EBVB9_LMP1_203
CD3Z_HUMAN_ITAM_142153__PDCD1_HUMAN_ITSM_248
TNR_HUMAN_CD137_246

FIG. 8

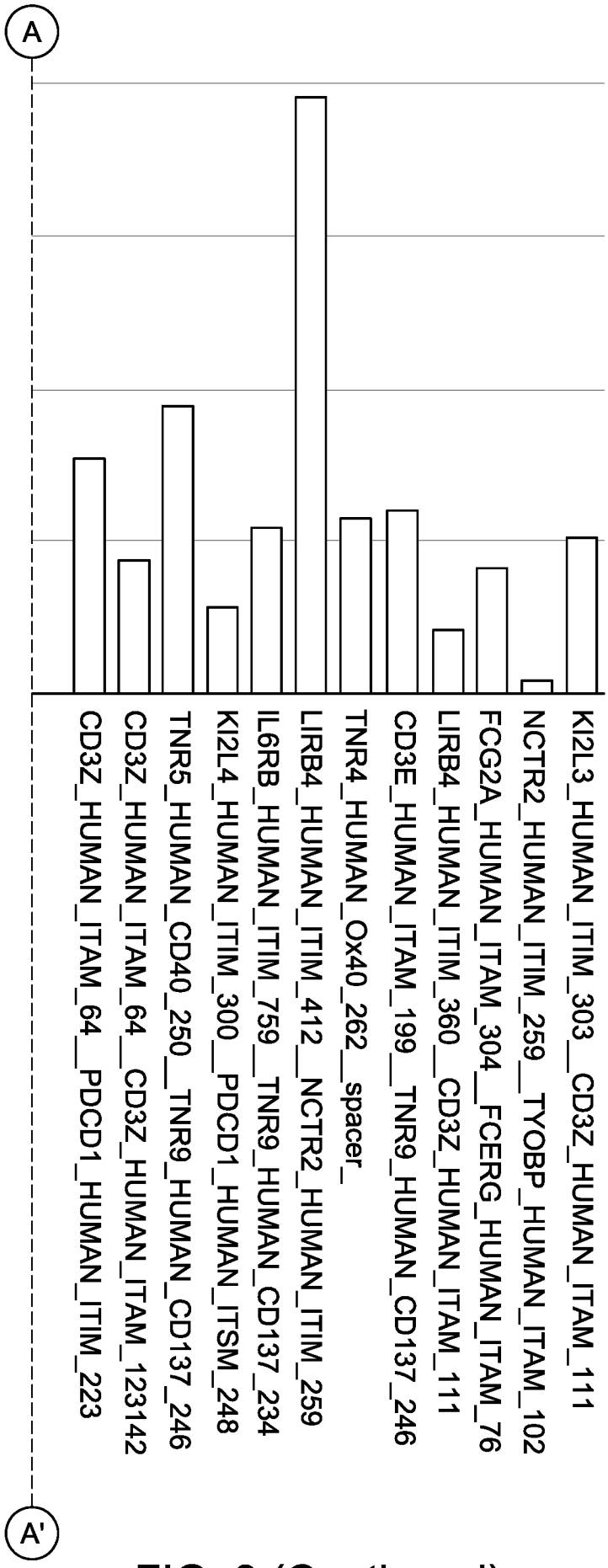

FIG. 8 (Continued)

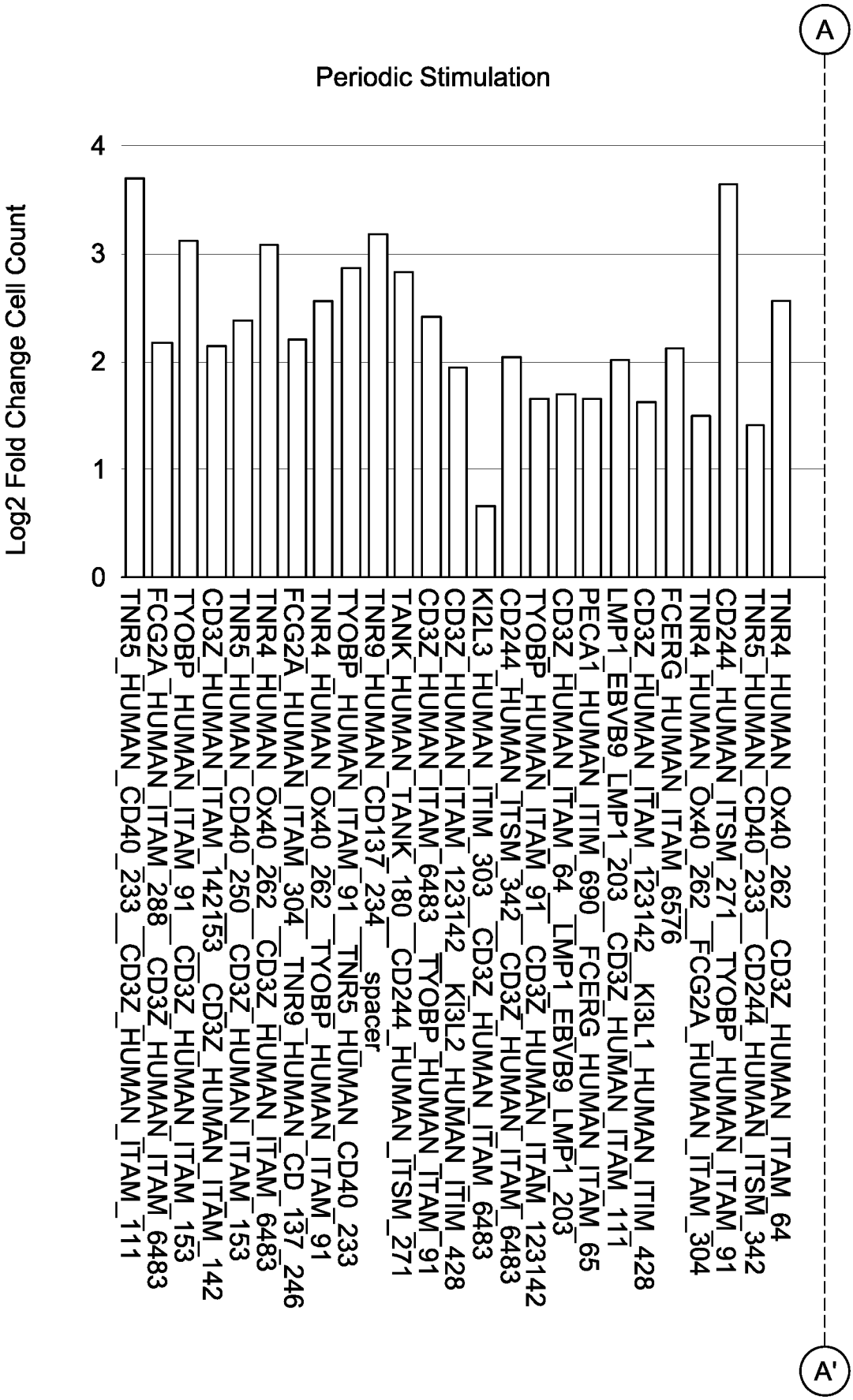
A
Periodic Stimulation
Log2 Fold Change Cell Count
4
3
2
1
0
TNR4_HUMAN_Ox40_262__CD3Z_HUMAN_ITAM_64
TNR5_HUMAN_CD40_233__CD244_HUMAN_ITSM_342
CD244_HUMAN_ITSM_271__TYOBP_HUMAN_ITAM_91
TNR4_HUMAN_Ox40_262__FCG2A_HUMAN_ITAM_304
FCERG_HUMAN_ITAM_6576
CD3Z_HUMAN_ITAM_123142__KI3L1_HUMAN_ITIM_428
LMP1_EBVB9_LMP1_203__CD3Z_HUMAN_ITAM_111
PECA1_HUMAN_ITIM_690__FCERG_HUMAN_ITAM_65
CD3Z_HUMAN_ITAM_64__LMP1_EBVB9_LMP1_203
TYOBP_HUMAN_ITAM_91__CD3Z_HUMAN_ITAM_123142
CD244_HUMAN_ITSM_342__CD3Z_HUMAN_ITAM_6483
KI2L3_HUMAN_ITIM_303__CD3Z_HUMAN_ITAM_6483
CD3Z_HUMAN_ITAM_123142__KI3L2_HUMAN_ITIM_428
CD3Z_HUMAN_ITAM_6483__TYOBP_HUMAN_ITAM_91
TANK_HUMAN_TANK_180__CD244_HUMAN_ITSM_271
TNR9_HUMAN_CD137_234__spacer
TYOBP_HUMAN_ITAM_91__TNR5_HUMAN_CD40_233
TNR4_HUMAN_Ox40_262__TYOBP_HUMAN_ITAM_91
FCG2A_HUMAN_ITAM_304__TNR9_HUMAN_CD_137_246
TNR4_HUMAN_Ox40_262__CD3Z_HUMAN_ITAM_6483
TNR5_HUMAN_CD40_250__CD3Z_HUMAN_ITAM_153
CD3Z_HUMAN_ITAM_142153__CD3Z_HUMAN_ITAM_142
TYOBP_HUMAN_ITAM_91__CD3Z_HUMAN_ITAM_153
FCG2A_HUMAN_ITAM_288__CD3Z_HUMAN_ITAM_6483
TNR5_HUMAN_CD40_233__CD3Z_HUMAN_ITAM_111
A'

FIG. 9

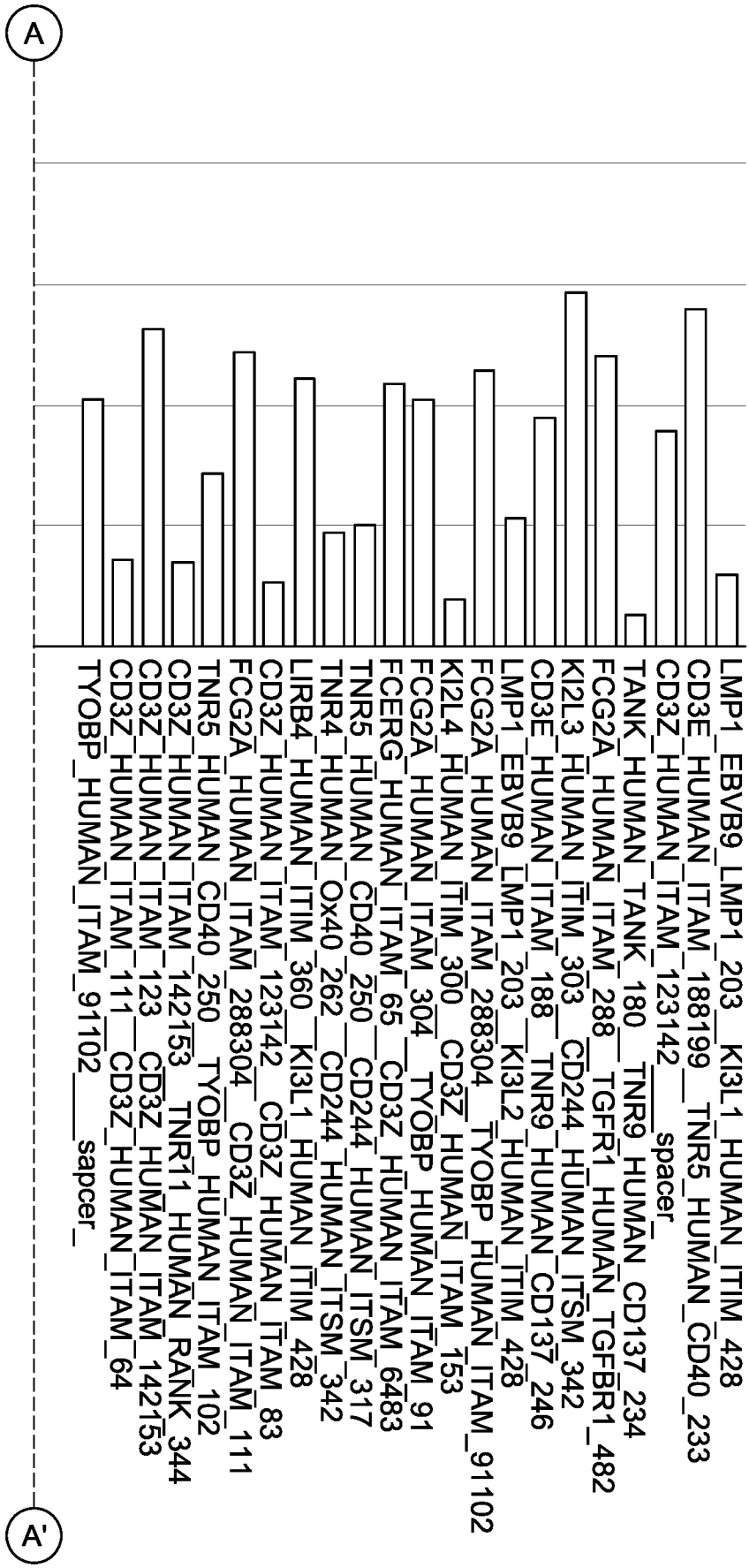

FIG. 9 (Continued)

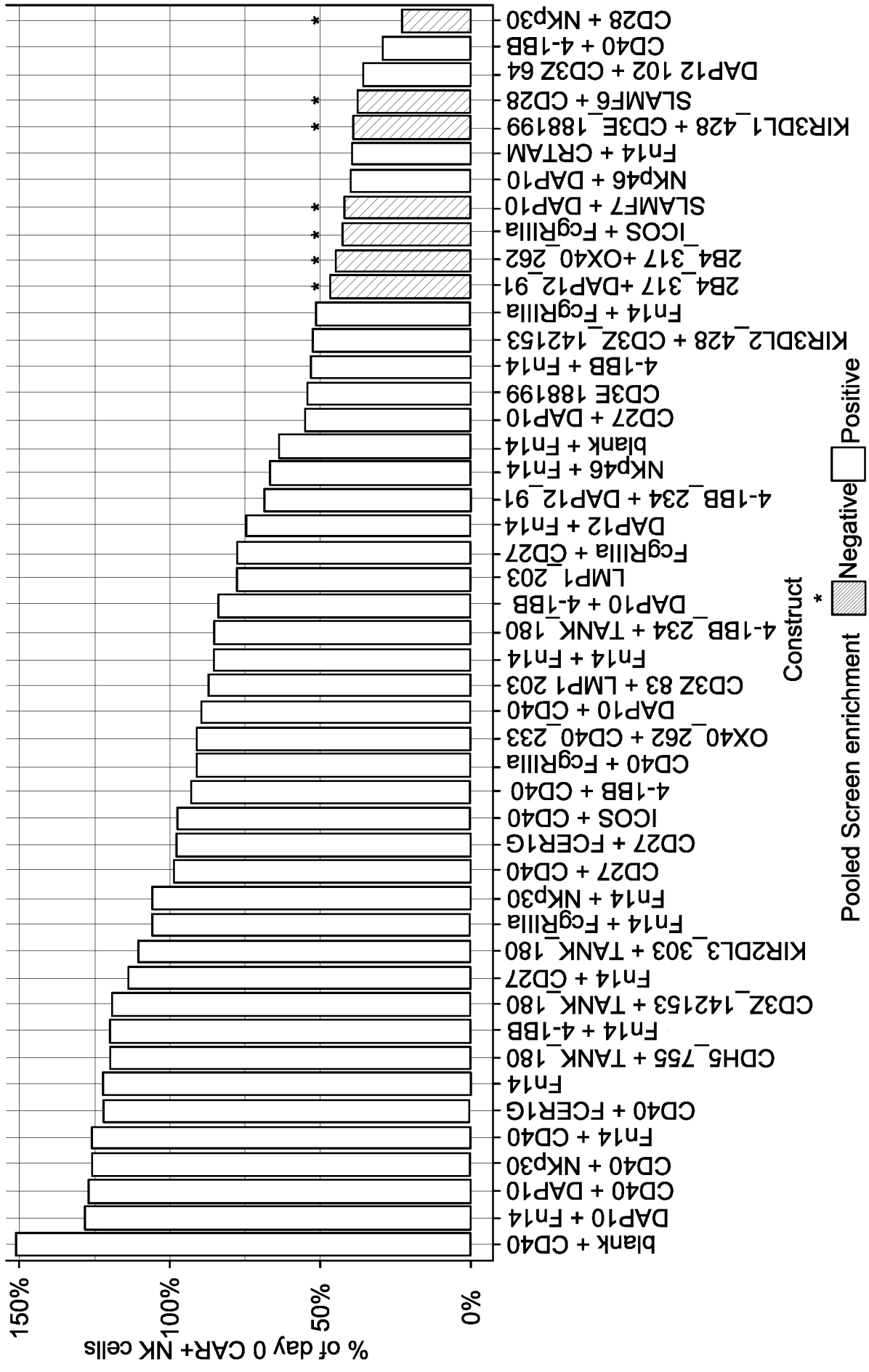
% of day 0 CAR+ NK cells
150%
100%
50%
0%
blank + CD40
DAP10 + Fn14
CD40 + DAP10
CD40 + NKp30
Fn14 + CD40
CD40 + FCER1G
Fn14
CDH5_755 + TANK_180
Fn14 + 4-1BB
CD3Z_142153 + TANK_180
Fn14 + CD27
KIR2DL3_303 + TANK_180
Fn14 + FcgRIIIa
Fn14 + NKp30
CD27 + CD40
CD27 + FCER1G
ICOS + CD40
4-1BB + CD40
CD40 + FcgRIIIa
OX40_262 + CD40_233
DAP10 + CD40
CD3Z 83 + LMP1 203
Fn14 + Fn14
4-1BB_234 + TANK_180
_DAP10 + 4-1BB
LMP1_203
FcgRIIIa + CD27
DAP12 + Fn14
4-1BB_234 + DAP12_91
NKp46 + Fn14
blank + Fn14
CD27 + DAP10
CD3E 188199
4-1BB + Fn14
KIR3DL2_428 + CD3Z_142153
Fn14 + FcgRIIIa
2B4_317 +DAP12_91
2B4_317 +OX40_262
_ICOS + FcgRIIIa
SLAMF7 + DAP10
NKp46 + DAP10
Fn14 + CRTAM
KIR3DL1_428 + CD3E_188199
SLAMF6 + CD28
DAP12 102 + CD3Z 64
CD40 + 4-1BB
CD28 + NKp30
Construct
*
Pooled Screen enrichment
Negative
Positive

FIG. 10

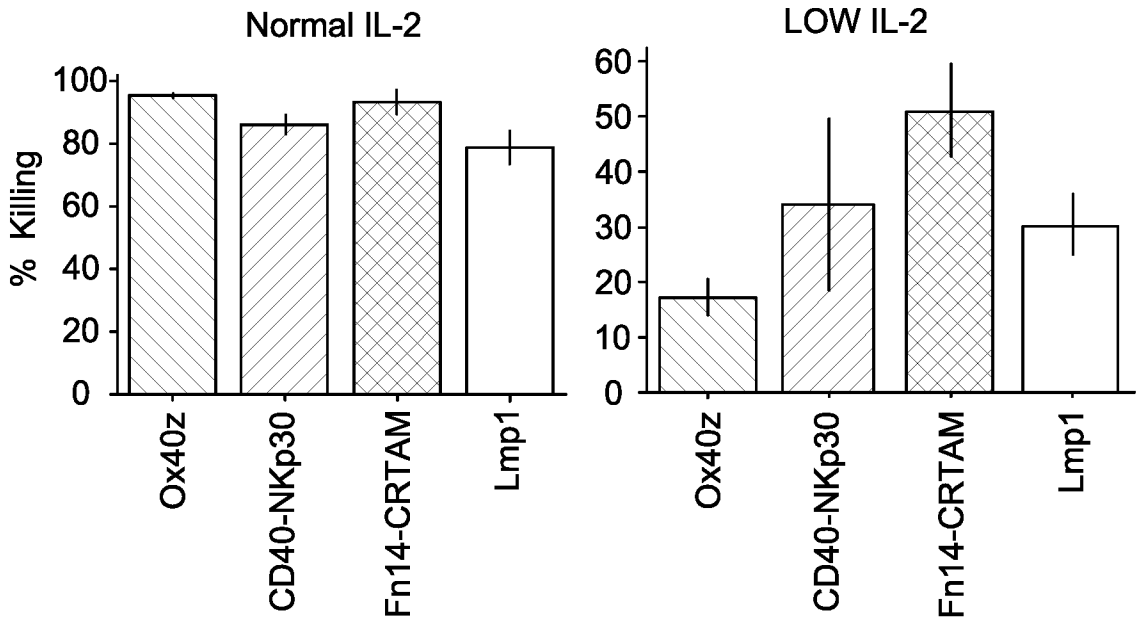
Normal IL-2
% Killing
100
80
60
40
20
0
Ox40z
CD40-NKp30
Fn14-CRTAM
Lmp1
LOW IL-2
60
50
40
30
20
10
0
Ox40z
CD40-NKp30
Fn14-CRTAM
Lmp1

FIG. 11

		NormalizedCytotoxIndex									
		Donor 3_Raji			Donor 3_B cell			Donor 4_B cell			
ID	Description	2:1	1:1	1:2	2:1	1:1	1:2	2:1	1:1	1:2
1	Fn14_CRTAM	5.2	4.1	2.8	1.8	1.2	0.8	4.4	3.6	3.0
2	CD3Z_142153_TANK_180	7.0	5.4	3.5	2.1	1.8	1.5	6.1	6.0	3.5
3	CD40_NKp30	7.6	5.9	4.1	2.7	2.6	1.7	7.6	7.0	5.8
4	LMP1_203	6.5	6.0	4.7	3.1	3.5	2.6	6.8	6.4	5.5
11	Benchmark: OX40z (Nkarta Design)	5.3	4.1	2.4	2.8	1.8	1.5	6.0	4.3	2.1

FIG. 12

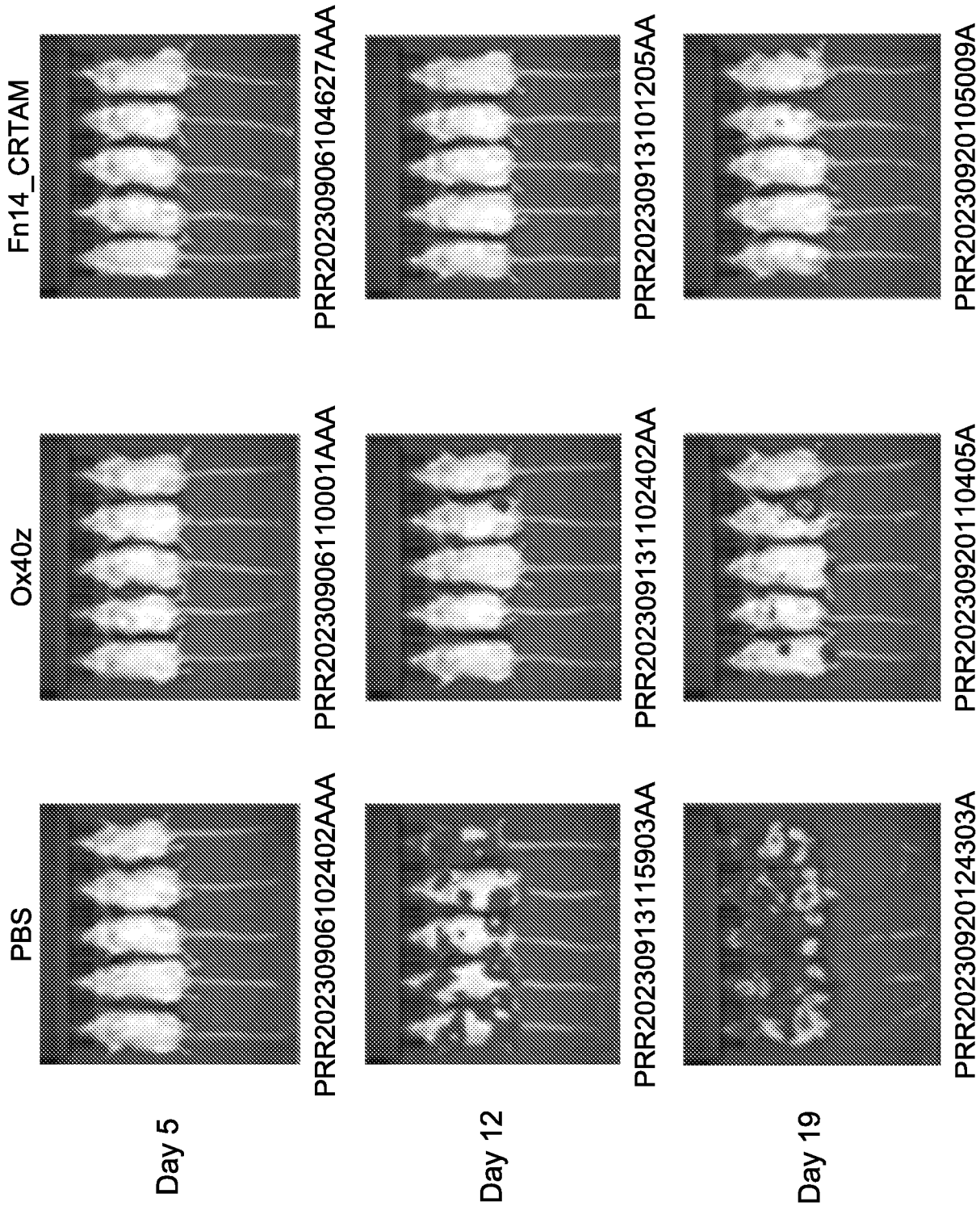
PBS
Ox40z
Fn14_CRTAM
Day 5
Day 12
Day 19
PRR20230906102402AAA
PRR20230906110001AAA
PRR20230906104627AAA
PRR20230913115903AA
PRR20230913102402AA
PRR20230913101205AA
PRR20230920124303A
PRR20230920110405A
PRR20230920105009A

FIG. 13