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(54) Title: T CELL RECEPTORS SPECIFIC FOR MESOTHELIN AND THEIR USE IN IMMUNOTHERAPY

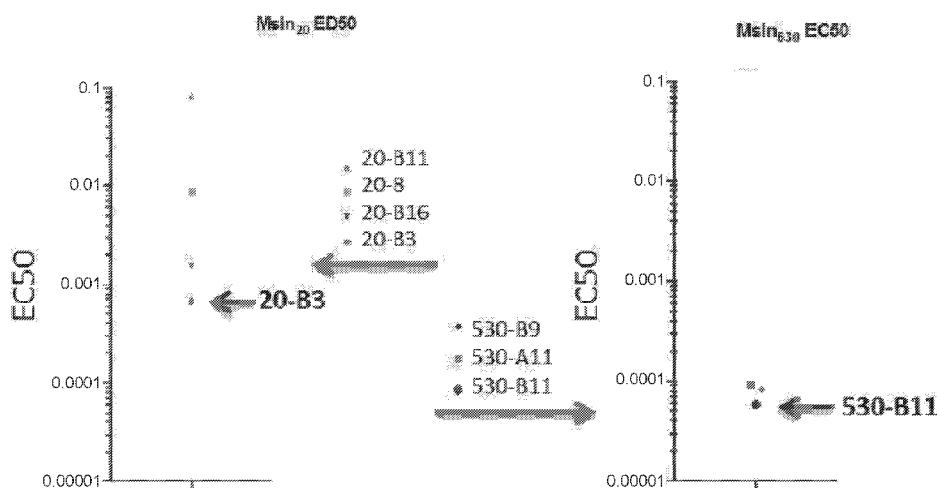


FIG. 6C

(57) Abstract: Binding proteins specific for Msln20-28 or Msln530-538 peptides are provided herein. Polynucleotides encoding the binding proteins, as well as compositions and recombinant host cells comprising the binding proteins or polynucleotides are also provided. The compositions and recombinant host cells may be used to treat a subject having mesothelioma, pancreatic cancer, ovarian cancer, lung cancer, a cancer wherein an Msln20-28 peptide is expressed on a tumor cell of the cancer, or a cancer wherein an Msln530-538 peptide is expressed on a tumor cell of the cancer.



GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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IMMUNOTHERAPY TARGETING MESOTHELIN

STATEMENT REGARDING SEQUENCE LISTING

[0001] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy, and is hereby incorporated by reference into the
5 specification. The name of the text file containing the Sequence Listing is 360056_475WO_SEQUENCE_LISTING.txt. The text file is 115 KB, was created on November 8, 2019, and is being submitted electronically via EFS-Web.

TECHNICAL FIELD

[0002] The present disclosure relates to the field of biomedicine and,
10 specifically, to methods and compositions useful for use in treating diseases or disorders in which cells express mesothelin, such as in cancer therapy. In particular, embodiments of the present disclosure relate to methods and compositions of TCRs with high affinity against tumor-associated antigen mesothelin, T cells expressing such high affinity antigen specific TCRs, nucleic acids encoding the same, and methods of
15 use for carrying out cellular immunotherapy including engineered T cells.

BACKGROUND

[0003] Adoptive transfer of tumor-specific T-cells is an appealing strategy to eliminate existing tumors and requires the establishment of a robust population of antigen-specific T cells *in vivo* to eliminate existing tumor and prevent recurrences (*see*
20 Stromnes, et al., Immunol. Rev. 257: 145, 2014). Although transfer of tumor-specific CD8⁺ cytotoxic T lymphocytes (CTLs) is safe and can mediate direct anti-tumor activity in select patients (*see* Chapuis et al., Cancer Res. 72:LB-136, 2012; Chapuis et al., Sci. Transl. Med. 5: 174ral27, 2013; and Chapuis et al., Proc. Natl. Acad. Sci. U.S.A. 09:4592, 2012), the variability in the avidity of the CTLs isolated from each
25 patient or donor limits the anti-tumor efficacy in clinical trials (*see* Chapuis et al., 2013). Since TCR affinity is an important determinant of CTL avidity (*see* Zoete et al., Frontiers Immunol. 4:26%, 2013), strategies have been developed to redirect the antigen specificity of donor or patient T cells using high affinity TCR α/β genes isolated

from a well-characterized T cell clone specific for a tumor-specific antigen (*see* Stromnes et al., Immunol. Rev. 257: 145, 2014 and Robbins et al., J. Clin. Oncol. 29:917, 2011).

[0004] Such high affinity self/tumor-reactive T cells are rare, since T cells that
 5 express self/tumor-reactive TCRs are subject to central and peripheral tolerance (*see* Stone and Kranz, Frontiers Immunol. 4:244, 2013), with relative TCR affinities varying widely between donors. Therefore, many matched donors must be screened to identify a sufficiently high-affinity tumor-specific T cell clone from which a TCR α/β gene
 10 therapy construct can be generated. For example, isolation of a naturally elicited Wilms' Tumor antigen 1 (WT1)-specific TCR with high functional avidity for a single HLA-allele required screening of hundreds of WT-specific T cell lines representing thousands of individual T cell clones from the peripheral repertoires of greater than 75 normal donors, a very time and labor intensive process (*see* Chapuis et al., 2013; Schmitt et al., Hum. Gene Ther. 20:1240, 2009; and Ho et al., J. Immunol. Methods
 15 310:40, 2006).

[0005] There is a need for alternative antigen-specific immunotherapies directed against various cancers, such as solid tumors. Presently disclosed embodiments address these needs and provide other related advantages.

BRIEF DESCRIPTION OF THE DRAWINGS

20 The embodiments disclosed herein will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings.

[0006] FIG. 1A depicts identification and selection of TCRs specific for Msln₂₀ (SEQ ID NO:31) based on the fold-enrichment of TCRs to peptide:HLA tetramer
 25 binding. A TCR selected for further studies is circled.

[0007] FIG. 1B depicts identification and selection of TCRs specific for Msln₅₃₀ (SEQ ID NO:32) based on the fold-enrichment of tetramer from a pool of TCRs with binding to a Msln₅₃₀:HLA tetramer. TCRs that were selected for further studies are circled.

[0008] FIG. 2 depicts tetramer binding by Msln₅₃₀-specific TCRs (ranked by affinity, based on tetramer binding) in assays to determine whether or not cell-expressed TCR was able to detect the Msln₅₃₀ peptide:HLA complex in the presence or absence of the CD8 co-receptor. CD8-independent binding correlates with high affinity of the respective T cell clone.

[0009] FIGS. 3A-3C show further functional testing of Msln-specific TCRs. (A) Representative data of a T cell clone evaluated for antigen-driven activation based on a reporter cell line expressing the Nur77-tdTomato transgene, as measured by flow cytometry. Nur77 is an indicator of antigen receptor signaling in human T cells (*see, e.g.,* Ashouri and Weiss, *J. Immunol.* 198(2):657-658 (2017)). In this assay, the T cell clone was incubated with T2 target cells that were pulsed with increasing concentrations of peptide, as indicated. (B) Nur77 reporter activity of TCRs, as ranked according to sensitivity to T2 cells pulsed with peptide at the indicated concentration. (C) Avidity ranking of TCRs, based on the EC₅₀ of peptide for Nur77 reporter activity. "B11" (also referred-to herein as 11B) had the lowest EC₅₀ of the tested TCR clones.

[0010] FIG. 4 shows functional evaluation of TCR clones in response to peptide, based on Nur77 tomato reporter activity. Several TCRs, including "B9" and "A11" (also referred to herein as "11A"), confer high antigen specificity despite exhibiting lower tetramer binding. TCRs are ranked (left-to right and top-to-bottom) according to tetramer binding.

[0011] FIGS. 5A-5C show functional evaluation of TCRs heterologously expressed in primary CD8⁺ T cells. CD8⁺ T cells were purified from donor PBMCs and lentivirally transduced with each TCR. After 8 days, cells that sorted high for tetramer-positive were further sorted and further expanded for 8-10 days.

[0012] FIGS. 6A-6C show characterization of primary CD8⁺ T cells that were transduced with the indicated Msln-specific TCR and assessed for functional activity upon incubation with peptide-pulsed T2 cells, as measured by interferon-gamma production (A, B). TCRs were ranked by the EC₅₀, based on the amount of peptide pulsed into T2 cells (C).

[0013] FIGS. 7A-7D show specific lysis of two representative Msln-positive tumor cell lines ((A, B) MDA-MB-468 and (C, D) MDA-MB-231) by CD8⁺ T cells

transduced with the indicated Msln₅₃₀-specific TCR or with a Msln₂₀-specific TCR and in the presence or absence of exogenous IFN- γ .

[0014] FIG. 8 relates to an alanine mutagenesis scanning experiment to assess which amino acids of the target Msln₂₀ peptide (SEQ ID NO:31) are essential for effective binding and killing by exemplary Msln₂₀-specific TCRs. A series of variant peptides were generated in which an alanine was substituted for each successive position along the peptide of SEQ ID NO:31, and each variant peptide was assessed for IFN- γ production by CD8⁺ T cells expressing the indicated Msln₂₀-specific TCR. These data show that positions 3-6 of SEQ ID NO:31 are essential for TCR binding. In the consensus sequence SEQ ID NO:60, an "X" indicates that a substitution mutation of the indicated residue in SEQ ID NO:31 to alanine does not impact or substantially impact functional binding of the TCR to its cognate peptide target.

[0015] FIGS. 9A-9D show results from alanine mutagenesis scanning experiments using TCRs specific for Msln₂₀ (SEQ ID NO:31) or Msln₅₃₀ (SEQ ID NO:32). The x-axis shows the percent of IFN- γ + T cells in response to each alanine-substituted peptide. The y-axis shows the sequence of the tested peptide, wherein an X indicates that this residue is not required for TCR specificity, as indicated by near normal functional activity as compared to the wild-type peptide.

[0016] FIG. 10 shows human peptides (SEQ ID NOs: 63-77) that were investigated for potential cross-reactivity with the target Msln₅₃₀ peptide (SEQ ID NO:32), based on the specificity of the TCR as determined by alanine scanning experiments. The genes encoding the indicated peptides are shown at the left of the table. Consensus sequences containing the essential residues identified by alanine scanning were input into prediction algorithms, as described in Example 8.

[0017] FIGS. 11A and 11B depict analysis of synthesized peptides with potential homology to Msln₅₃₀ in the human proteome. As described in Example 9, the functional activity of two Msln₅₃₀-specific TCRs (FIG. 11A, FIG. 11B) upon incubation with T2 cells pulsed with a high dose (10 μ M) of peptide was measured by IFN- γ . (B) For peptides that showed cross-reactivity with a tested TCR, a dose-dependent titration was performed to determine the EC₅₀. TCRs were ranked by the EC₅₀ based on the amount of peptide pulsed into T2 cells. Peptide #10 is from a gene called *EHF*. This

gene encodes a protein that belongs to an ETS transcription factor subfamily characterized by epithelial-specific expression. ETS acts as a transcriptional repressor, and may be involved in epithelial differentiation and carcinogenesis.

[0018] FIGS. 12A-12I depict experiments investigating the potential for
 5 alloreactivity of T cells expressing an exemplary Msln-specific TCR of the present disclosure (Meso20-3B, Meso530-11A, or Meso530-11B) by targeting diverse donor-derived lymphoblastoid cell lines (LCLs) in the presence of absence of wild-type peptide.

[0019] FIGS. 13A-13H depict additional analysis of alloreactivity by targeting
 10 diverse donor-derived LCLs to assess no cross-reactivity to other HLA subtypes, as described in Example 10. As indicated for cell lines FAH and GIM, Meso530-11B indicates potential reactivity in the presence of peptide (as highlighted in the table) for non HLA-A2 alleles.

DETAILED DESCRIPTION

[0020] In some aspects, the present disclosure provides binding proteins that
 15 comprise a TCR alpha chain variable domain ($V\alpha$) and a TCR beta chain variable domain ($V\beta$) and are capable of specifically binding to a Msln₂₀₋₂₈ or Msln₅₃₀₋₅₃₈ epitope and/or peptide (Msln₂₀₋₂₈ (SLLFLLFSL; SEQ ID NO: 31)) and Msln₅₃₀₋₅₃₈ (SEQ ID NO:32 (VLPLTVAEV)) are also referred to herein as Msln₂₀ and M₅₃₀, respectively);
 20 *e.g.*, in a peptide:HLA complex. In any of the presently disclosed embodiments, a Msln-specific binding protein is capable of binding to a Msln peptide:HLA complex, wherein the Msln peptide comprises the amino acid sequence set forth in SEQ ID NO:31 or 32 and wherein the HLA is or comprises HLA-A2, such as HLA-A*02:01.

[0021] In certain embodiments, a Msln-specific ₂₀₋₂₈-specific binding protein
 25 comprises: (a) a TCR $V\alpha$ comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:33 or 35, and a TCR $V\beta$, wherein optionally the TCR $V\beta$ has at least about 85% (*i.e.*, at least about 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the amino acid sequence set forth in SEQ ID NO:95 or 97; (b) a TCR $V\beta$ comprising a CDR3 amino acid sequence as set forth in SEQ ID
 30 NO:34 or 36, and (b) a TCR $V\alpha$, wherein optionally the TCR $V\alpha$ has at least about 85%

(*i.e.*, at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the amino acid sequence set forth in SEQ ID NO:96 or 98; or (c) a TCR V α comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:33 or 35, wherein optionally the TCR V α has at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98 and a TCR V β comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:34 or 36, wherein optionally the TCR V β has at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95 or 97.

[0022] Unless specifically indicated otherwise, as used herein, a sequence identity of "at least about" an indicated percentage includes the indicated percentage $\pm$ 20% thereof, and every integer and non-integer percentage above the specific percentage. Accordingly, "at least about 85%" identity to the referenced sequence (*e.g.*, any one of SEQ ID NOs:1-123) includes about 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the referenced sequence, and also includes all non-integer percentages in between two integer percentages (*e.g.*, 92.5%, 99.1%, *etc.*).

[0023] In certain embodiments, a Msln₂₀₋₂₈-specific binding protein comprises a CDR3 α amino acid sequence as set forth in SEQ ID NO:33 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:34. In further embodiments, the binding protein comprises a CDR1 α amino acid sequence as set forth in SEQ ID NO:80, a CDR2 α amino acid sequence as set forth in SEQ ID NO:81 or 118, a CDR1 β amino acid sequence as set forth in any one of SEQ ID NOs:78, 82, 83, or 84, and a CDR2 β amino acid sequence as set forth in SEQ ID NO:79. In certain embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95, wherein optionally there are no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0024] In certain embodiments, the Msln₂₀₋₂₈-specific binding protein comprises (i) a TCR V β comprising (a) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBV12-4*01 (*e.g.*, to a TRBV12-4*01-encoded amino acid sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50,

55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 108 contiguous amino acids in length); and/or (b) an amino acid sequence having at least about 85% identity an amino acid sequence encoded by TRBJ2-7*01 (*e.g.*, to a TRBJ2-7*01-encoded amino acid sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids long);

5 and/or (ii) TCR V α comprising (a) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAV1-1*01 (*e.g.*, to a TRAV1-1*01-encoded amino acid sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 107 contiguous amino acids in length) and/or (b) an amino acid sequence having at least about 85% identity to an amino acid

10 sequence encoded by TRAJ3*01 (*e.g.*, to a TRAJ3*01-encoded amino acid sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids long).

[0025] In any of the presently disclosed embodiments, a TCR V β may include an amino acid sequence that is at least about 85% identical to an amino acid sequence

15 encoded by TRBD1*01 or TRBD2*02.

[0026] Amino acid sequences encoded by these and other TCR genes are known and can be found at, for example, imgt.org, which provides gene tables and nucleotide and amino acid sequences for human TRAV, TRBV, TRAJ, TRBJ, TRBD, TRAC, and TRBD alleles.

20 **[0027]** In certain embodiments, a Msln₂₀₋₂₈-specific binding protein comprises a CDR3 α amino acid sequence as set forth in SEQ ID NO:36 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:35. In some embodiments, the binding protein further comprises a CDR1 α amino acid sequence as set forth SEQ ID NO:85, a CDR2 α amino acid sequence as set forth in SEQ ID NO:86 or 119, a CDR1 β amino acid

25 sequence as set forth in any one of SEQ ID NOs: 82, 83, or 84, and a CDR2 β amino acid sequence as set forth in SEQ ID NO:79. In certain embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:98, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID

30 NO:97, wherein there are optionally no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0028] In certain embodiments, the Msln₂₀₋₂₈-specific binding protein comprises a TCR V α comprising (a) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAV12-3*01 (*e.g.*, to a TRAV12-3*01-encoded amino acid sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 108 contiguous amino acids in length) and/or (b) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRA29*01 (*e.g.*, to a TRAJ29*01-encoded amino acid sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids long).

[0029] In certain embodiments, alanine mutagenesis of any one or more of residues 1, 2, 7, 8, or 9 of SEQ ID NO:31 does not abrogate or does not substantially impair binding by a Msln₂₀₋₂₈-specific binding protein. In certain embodiments, a Msln₂₀₋₂₈-specific binding protein is capable of binding to a peptide comprising or consisting of the consensus amino acid sequence set forth in SEQ ID NO:60; *e.g.*, in a peptide:HLA complex as disclosed herein.

[0030] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises: (a) a TCR V α comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:37 or 39, and a TCR V β , wherein the TCR V β optionally has at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:99 or 101; (b) a TCR V β comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:34 or 36, and (b) a TCR V α , wherein the TCR V α optionally has at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:100 or 102; or (c) a TCR V α comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:37 or 39 and a TCR V β comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:38 or 40.

[0031] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises a CDR3 α amino acid sequence as set forth in SEQ ID NO:37 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:38. In some embodiments, the binding protein further comprises a CDR1 α amino acid sequence as set forth SEQ ID NO:89, a CDR2 α amino acid sequence as set forth in SEQ ID NO:90, a CDR1 β amino acid sequence as set forth in any one of SEQ ID NOs: 83 or 87, and a CDR2 β amino acid sequence as set forth in SEQ ID NO:88. In certain embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in

SEQ ID NO:100, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:99, wherein optionally there are no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0032] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises
5 a CDR3 α amino acid sequence as set forth in SEQ ID NO:39 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:40. In some embodiments, the binding protein further comprises a CDR1 α amino acid sequence as set forth SEQ ID NO:93, a CDR2 α amino acid sequence as set forth in SEQ ID NO:94, a CDR1 β amino acid sequence as set forth in any one of SEQ ID NOs: 83, 84, or 91, and a CDR2 β amino acid sequence
10 as set forth in SEQ ID NO:92. In certain embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:102, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101, wherein there are optionally no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0033] In certain embodiments, the Msln₅₃₀₋₅₃₈-specific binding protein
15 comprises a TCR V β comprising an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBJ2-3*01 (e.g., to a TRBJ2-3*01-encoded amino acid sequence that is at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 15 contiguous amino acids in length).

[0034] In certain embodiments, alanine mutagenesis of any one or more of
20 residues 3, 5, 6, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair binding by a Msln₅₃₀₋₅₃₈-specific binding protein. In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein is capable of binding to a peptide comprising or consisting of the consensus amino acid sequence set forth in SEQ ID NO:61; e.g., in a
25 peptide:HLA complex as disclosed herein.

[0035] In certain embodiments, alanine mutagenesis of any one or more of
residues 1, 5, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair
binding by a Msln₅₃₀₋₅₃₈-specific binding protein. In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein is capable of binding to a peptide comprising or consisting
30 of the consensus amino acid sequence set forth in SEQ ID NO:62; e.g., in a peptide:HLA complex as disclosed herein.

[0036] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein of the present disclosure does not bind, or does not specifically bind relative to Msln₅₃₀₋₅₃₈, to a peptide:HLA complex wherein the peptide comprises or consists of the amino acid sequence set forth in any one or more of SEQ ID NOs:63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, and 77, and wherein the HLA optionally comprises an HLA-A2, such as HLA-A:02*01.

[0037] In any of the presently disclosed embodiments, a Msln-specific binding protein (*i.e.*, Msln₂₀₋₂₈-specific binding protein, Msln₅₃₀₋₅₃₈-specific binding protein) is capable of binding to a Msln peptide:HLA complex as disclosed herein in the absence of, or independent of, CD8. In certain embodiments, a binding protein (*e.g.*, when expressed on the cell surface of a human T cell) has a Msln peptide EC₅₀ of about 9 μM, about 8 μM, about 7 μM, about 6 μM, about 5 μM, about 4 μM, about 3 μM, about 2 μM, about 1 μM, about 0.9 μM, about 0.8 μM, about 0.7 μM, about 0.6 μM, about 0.5 μM, about 0.4 μM, about 0.3 μM, about 0.2 μM, or less.

[0038] Msln-specific binding proteins are non-alloreactive against various human HLA types in the absence of a Msln peptide antigen. In certain embodiments, an immune cell (*e.g.*, a T cell) expressing a Msln-specific binding protein of this disclosure does not produce IFN-γ and/or does not exhibit activation (*e.g.*, CD8 expression, CD3 expression, Nur77 expression) and/or cytotoxic activity (*e.g.*, specific killing, production and release of a perforin and/or a granzyme) when contacted with a cell expressing: (i) HLA-C6:02:01; (ii) HLA-B13:01:01 without HLA-B13:02:01; (iii) HLA-A3; (iv) HLA-A29; (v) HLA-B40; (vi) HLA-B44; (vii) HLA-C3; (viii) HLA-C16; (ix) HLA-A1; (x) HLA-24; (xi) HLA-B7; (xii) HLA-B57; (xiii) HLA-C7; (xiv) HLA-A11; (xv) HLA-B15; (xvi) HLA-C4; (xvii) HLA-C12; (xviii) HLA-B8; (xix) HLA-B49; (xx) HLA-B51; (xxi) HLA-C15; (xxii) HLA-A30; (xxiii) HLA-A68; (xxiv) HLA-C2; (xxv) HLA-A32; (xxvi) HLA-A33; (xxvii) HLA-B55; (xxviii) HLA-C1; (xxvix) HLA-C5; (xxix) HLA-B8; (xxx) HLA-B35; or (xxxi) any combination of (i)-(xxx), when in the absence of a Msln peptide as provided herein.

[0039] Compositions and recombinant host cells (*e.g.*, immune cells, such as a T cell) including, encoding, and/or expressing the binding proteins are also provided. In any of the presently disclosed embodiments, a binding protein is capable of expression

on a cell surface by a host T cell. In any of the presently disclosed embodiments, binding by a Msln-specific binding protein that is expressed on the surface of an immune cell (*e.g.*, a T cell) to a Msln peptide:HLA complex activates the immune cell, wherein activation is optionally determined by Nur77 expression and/or activity.

5 **[0040]** The presently disclosed binding proteins are highly sensitive for a cognate Mlsn peptide antigen. In certain embodiments, Nur77 expression is increased when the immune cell is in the presence of about 10^{-2} μ M peptide, about 10^{-1} μ M peptide, about 1 μ M peptide, or about 10^1 μ M peptide, wherein the peptide is optionally presented by an antigen presenting cell; *i.e.*, in a peptide:HLA complex.

10 **[0041]** In another aspect, polynucleotides are provided that encode a mesothelin-specific binding protein as described herein. In certain embodiments, a binding protein-encoding polynucleotide comprises a polynucleotide having at least about 50% sequence identity (*i.e.*, at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%,
15 or more) to the polynucleotide sequence set forth in any one of SEQ ID NOS: 1-5, 9-13, 17-21, 25-27, and 120. Vectors that contain a polynucleotide are also provided.

[0042] Presently disclosed binding proteins, and recombinant host cells, and related compositions may be used to treat a subject having a disease or disorder associated with mesothelin expression and/or activity, such as for example, a cancer. In
20 certain embodiments, the cancer is a solid cancer. In certain embodiments, the solid cancer is or comprises biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological
25 tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, mesothelioma, malignant melanoma, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or
30 uterine cancer. In certain embodiments, the presently disclosed compositions and recombinant host cells may be used to treat a cancer wherein an Msln₂₀₋₂₈ peptide is

expressed on a tumor cell of the cancer, or a cancer wherein an MslN₅₃₀₋₅₃₈ peptide is expressed on a tumor cell of the cancer, such as, for example, mesothelioma, pancreatic cancer, ovarian cancer, or lung cancer.

[0043] Also provided herein are polynucleotides that encode a binding protein
5 as provided herein, vectors that comprise a binding-protein-encoding polynucleotide, and host cells that comprise a vector.

[0044] Prior to setting forth this disclosure in more detail, it may be helpful to an understanding thereof to provide definitions of certain terms to be used herein. Unless specifically defined otherwise, the technical terms, as used herein, have their
10 normal meaning as understood in the art. Additional definitions are set forth throughout this disclosure.

[0045] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and
15 one hundredth of an integer), unless otherwise indicated. Also, any number range recited herein relating to any physical feature, such as polymer subunits, size or thickness, is to be understood to include any integer within the recited range, unless otherwise indicated. "About," as used herein, when referring to a measurable value, range, or structure, is meant to encompass variations of $\pm 20\%$, $\pm 10\%$, $\pm 5\%$, $\pm 1\%$, or
20 $\pm 0.1\%$ from the specified value, unless otherwise indicated.

[0046] It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (e.g., "or") should be understood to mean any one, all, or any combination of the alternatives. As used herein, the terms "include," "have," and "comprise" are used synonymously, which
25 terms and variants thereof are intended to be construed as non-limiting.

[0047] "Optional" or "optionally" means that the subsequently described element, component, event, or circumstance may or may not occur, and that the description includes instances in which the element, component, event, or circumstance occurs and instances in which they do not.

[0048] In addition, it should be understood that the individual constructs, or
30 groups of constructs, derived from the various combinations of the structures and

subunits described herein, are disclosed by the present application to the same extent as if each construct or group of constructs was set forth individually. Thus, selection of particular structures or particular subunits is within the scope of the present disclosure.

[0049] The term "consisting essentially of" is not equivalent to "comprising" and refers to the specified materials or steps of a claim, or to those that do not materially affect the basic characteristics of a claimed subject matter. For example, a protein domain, region, or module (e.g., a binding domain, hinge region, or linker) or a protein (which may have one or more domains, regions, or modules) "consists essentially of" a particular amino acid sequence when the amino acid sequence of a domain, region, module, or protein includes extensions, deletions, mutations, or a combination thereof (e.g., amino acids at the amino- or carboxy-terminus or between domains) that, in combination, contribute to at most 20% (e.g., at most 15%, 10%, 8%, 6%, 5%, 4%, 3%, 2% or 1%) of the length of a domain, region, module, or protein and do not substantially affect (i.e., do not reduce the activity by more than 50%, such as no more than 40%, 30%, 25%, 20%, 15%, 10%, 5%, or 1%) the activity of the domain(s), region(s), module(s), or protein (e.g., the target binding affinity of a binding protein).

[0050] As used herein, "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α -carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refer to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that function in a manner similar to a naturally occurring amino acid.

[0051] As used herein, "mutation" refers to a change in the sequence of a nucleic acid molecule or polypeptide molecule as compared to a reference or wild-type

nucleic acid molecule or polypeptide molecule, respectively. A mutation can result in several different types of change in sequence, including substitution, insertion or deletion of nucleotide(s) or amino acid(s). In certain embodiments, a mutation is a substitution of one or three codons or amino acids, a deletion of one to about 5 codons or amino acids, or a combination thereof.

[0052] A "conservative substitution" refers to amino acid substitutions that do not significantly affect or alter binding characteristics of a particular protein. Generally, conservative substitutions are ones in which a substituted amino acid residue is replaced with an amino acid residue having a similar side chain. Conservative substitutions include a substitution found in one of the following groups: Group 1: Alanine (Ala or A), Glycine (Gly or G), Serine (Ser or S), Threonine (Thr or T); Group 2: Aspartic acid (Asp or D), Glutamic acid (Glu or Z); Group 3: Asparagine (Asn or N), Glutamine (Gln or Q); Group 4: Arginine (Arg or R), Lysine (Lys or K), Histidine (His or H); Group 5: Isoleucine (Ile or I), Leucine (Leu or L), Methionine (Met or M), Valine (Val or V); and Group 6: Phenylalanine (Phe or F), Tyrosine (Tyr or Y), Tryptophan (Trp or W). Additionally or alternatively, amino acids can be grouped into conservative substitution groups by similar function, chemical structure, or composition (e.g., acidic, basic, aliphatic, aromatic, or sulfur-containing). For example, an aliphatic grouping may include, for purposes of substitution, Gly, Ala, Val, Leu, and Ile. Other conservative substitutions groups include: sulfur-containing: Met and Cysteine (Cys or C); acidic: Asp, Glu, Asn, and Gln; small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, and Gly; polar, negatively charged residues and their amides: Asp, Asn, Glu, and Gln; polar, positively charged residues: His, Arg, and Lys; large aliphatic, nonpolar residues: Met, Leu, Ile, Val, and Cys; and large aromatic residues: Phe, Tyr, and Trp. Additional information can be found in Creighton (1984) *Proteins*, W.H. Freeman and Company. Variant proteins, peptides, polypeptides, and amino acid sequences of the present disclosure can, in certain embodiments, comprise one or more conservative substitutions relative to a reference amino acid sequence.

[0053] As used herein, "protein" or "polypeptide" refers to a polymer of amino acid residues. Proteins apply to naturally occurring amino acid polymers, as well as to amino acid polymers in which one or more amino acid residue is an artificial chemical

mimetic of a corresponding naturally occurring amino acid and non-naturally occurring amino acid polymers.

[0054] As used herein, "fusion protein" refers to a protein that, in a single chain, has at least two distinct domains or motifs, wherein the domains or motifs are not naturally found together (e.g., in the specified arrangement, order, or number, or at all) in a protein. In certain embodiments, a fusion protein comprises at least two distinct domains or motifs that are not naturally found together in a single peptide or polypeptide. A polynucleotide encoding a fusion protein may be constructed using PCR, recombinantly engineered, or the like, or such fusion proteins can be synthesized.

10 A fusion protein may further contain other components, such as a tag, a linker, or a transduction marker. In certain embodiments, a fusion protein expressed or produced by a host cell (e.g., a T cell) locates to the cell surface, where the fusion protein is anchored to the cell membrane (e.g., via a transmembrane domain) and comprises an extracellular portion or component (e.g., containing a binding domain and, in certain

15 embodiments, a linker, a spacer, or both) and an intracellular portion or component.

[0055] "Junction amino acids" or "junction amino acid residues" refer to one or more (e.g., about 2–10) amino acid residues between two adjacent motifs, regions, or domains of a polypeptide, such as between a binding domain and an adjacent constant domain or between a TCR chain and an adjacent self-cleaving peptide. Junction amino acids may result from the construct design of a fusion protein (e.g., amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a fusion protein).

20

[0056] "Nucleic acid molecule" or "polynucleotide" refers to a polymeric compound including covalently linked nucleotides, which can be made up of natural subunits (e.g., purine or pyrimidine bases) or non-natural subunits (e.g., morpholine ring). Purine bases include adenine, guanine, hypoxanthine, and xanthine, and pyrimidine bases include uracil, thymine, and cytosine. Nucleic acid molecules include polyribonucleic acid (RNA), polydeoxyribonucleic acid (DNA), which includes cDNA, genomic DNA, and synthetic DNA, either of which may be single or double-stranded.

25

30 If single-stranded, the nucleic acid molecule may be the coding strand or non-coding (anti-sense strand). A nucleic acid molecule encoding an amino acid sequence includes

all nucleotide sequences that encode the same amino acid sequence. Some versions of the nucleotide sequences may also include intron(s) to the extent that the intron(s) would be removed through co- or post-transcriptional mechanisms. In other words, different nucleotide sequences may encode the same amino acid sequence as the result of the redundancy or degeneracy of the genetic code, or by splicing.

5 **[0057]** Variants of nucleic acid molecules of this disclosure are also contemplated. Variant nucleic acid molecules are at least 70%, 75%, 80%, 85%, 90%, and are preferably 95%, 96%, 97%, 98%, 99%, or 99.9% identical a nucleic acid molecule of a defined or reference polynucleotide as described herein, or that hybridize
10 to a polynucleotide under stringent hybridization conditions of 0.015M sodium chloride, 0.0015M sodium citrate at about 65-68°C or 0.015M sodium chloride, 0.0015M sodium citrate, and 50% formamide at about 42°C. Nucleic acid molecule variants retain the capacity to encode a fusion protein or a binding domain thereof having a functionality described herein, such as specifically binding a target molecule.

15 **[0058]** "Percent sequence identity" refers to a relationship between two or more sequences, as determined by comparing the sequences. Preferred methods to determine sequence identity are designed to give the best match between the sequences being compared. For example, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic
20 acid sequence for optimal alignment). Further, non-homologous sequences may be disregarded for comparison purposes. The percent sequence identity referenced herein is calculated over the length of the reference sequence, unless indicated otherwise. Methods to determine sequence identity and similarity can be found in publicly available computer programs. Sequence alignments and percent identity calculations
25 may be performed using a BLAST program (e.g., BLAST 2.0, BLASTP, BLASTN, or BLASTX). The mathematical algorithm used in the BLAST programs can be found in Altschul et al., Nucleic Acids Res. 25:3389-3402, 1997. Within the context of this disclosure, it will be understood that where sequence analysis software is used for analysis, the results of the analysis are based on the "default values" of the program
30 referenced. "Default values" mean any set of values or parameters which originally load with the software when first initialized.

[0059] As understood in the art, "similarity" between two polypeptides is determined by comparing the amino acid sequence and conserved amino acid substitutes thereto of the polypeptide to the sequence of a second polypeptide (e.g., using GENEWORKS™, Align, Clustal™, the BLAST algorithm, or the like). In 5 certain embodiments, the BLAST algorithm is preferred.

[0060] The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally occurring nucleic acid or polypeptide present in a living animal is not isolated, but the same nucleic acid or polypeptide, separated from some or all of the co-existing 10 materials in the natural system, is isolated. Such nucleic acid could be part of a vector and/or such nucleic acid or polypeptide could be part of a composition (e.g., a cell lysate), and still be isolated in that such vector or composition is not part of the natural environment for the nucleic acid or polypeptide. The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and 15 following the coding region ("leader and trailer") as well as intervening sequences (introns) between individual coding segments (exons).

[0061] In some contexts, the term "variant" as used herein, refers to at least one fragment of the full length sequence referred to, more specifically one or more amino acid or nucleic acid sequence which is, relative to the full-length sequence, truncated at 20 one or both termini by one or more amino acids. Such a fragment includes or encodes for a peptide having at least 6, 7, 8, 10, 12, 15, 20, 25, 50, 75, 100, 150, or 200 successive amino acids of the original sequence or a variant thereof. The total length of the variant may be at least 6, 7, 8, 9, 10, 11, 12, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or more amino acids.

25 [0062] In some embodiments, the term "variant" relates not only to at least one fragment, but also to a polypeptide or a fragment thereof including amino acid sequences that are at least 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% identical to the reference amino acid sequence referred to or the fragment thereof, wherein amino acids other than those 30 essential for the biological activity or the fold or structure of the polypeptide are deleted or substituted, one or more such essential amino acids are replaced in a conservative

manner, and/or amino acids are added such that the biological activity of the polypeptide is preserved. The state of the art includes various methods that may be used to align two given nucleic acid or amino acid sequences and to calculate the degree of identity (see, e.g., Arthur Lesk (2008), Introduction to bioinformatics, Oxford University Press, 2008, 3rd edition). In some embodiments, the Clustal W software can be used using default settings (Larkin, M. A., et al. (2007). Clustal W and Clustal X version 2.0. Bioinformatics, 23, 2947–2948).

[0063] In certain embodiments, variants may, in addition, include chemical modifications, for example, isotopic labels or covalent modifications such as glycosylation, phosphorylation, acetylation, decarboxylation, citrullination, hydroxylation and the like. Methods for modifying polypeptides are known and in general will be employed so as not to abolish or substantially diminish a desired activity of the polypeptide.

[0064] In an embodiment, the term "variant" of a nucleic acid molecule includes nucleic acids the complementary strand of which hybridizes, for example, under stringent conditions, to the reference or wild type nucleic acid. Stringency of hybridization reactions is readily determinable by one of ordinary skill in the art, and in general is an empirical calculation dependent on probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes less so. Hybridization generally depends on the ability of denatured DNA to reanneal to complementary strands present in an environment below their melting temperature: the higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which may be used. As a result, higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperature less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel, F. M. (1995), Current Protocols in Molecular Biology. John Wiley & Sons, Inc. Moreover, the person skilled in the art may follow the instructions given in the manual Boehringer Mannheim GmbH (1993) The DIG System Users Guide for Filter Hybridization, Boehringer Mannheim GmbH, Mannheim, Germany and in Liebl, W., Ehrmann, M., Ludwig, W., and Schleifer, K. H. (1991) International Journal of Systematic Bacteriology 41: 255-260 on

how to identify DNA sequences by means of hybridization. In an embodiment, stringent conditions are applied for any hybridization, i.e., hybridization occurs only if the probe is 70% or more identical to the target sequence. Probes having a lower degree of identity with respect to the target sequence may hybridize, but such hybrids
5 are unstable and will be removed in a washing step under stringent conditions, for example, lowering the concentration of salt to $2\times$ SSC or, optionally and subsequently, to $0.5\times$ SSC, while the temperature is, for example, about 50°C – 68°C , about 52°C – 68°C , about 54°C – 68°C , about 56°C – 68°C , about 58°C – 68°C , about 60°C – 68°C , about 62°C – 68°C , about 64°C – 68°C , or about 66°C – 68°C . In an embodiment, the
10 temperature is about 64°C – 68°C or about 66°C – 68°C . It is possible to adjust the concentration of salt to $0.2\times$ SSC or even $0.1\times$ SSC. Nucleic acid sequences having a degree of identity with respect to the reference or wild type sequence of at least 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5% may be isolated. In an embodiment, the term variant of a nucleic acid sequence, as used herein,
15 refers to any nucleic acid sequence that encodes the same amino acid sequence and variants thereof as the reference nucleic acid sequence, in line with the degeneracy of the genetic code.

[0065] A "functional variant" refers to a polypeptide or polynucleotide that is structurally similar or substantially structurally similar to a parent or reference
20 compound of this disclosure, but differs, in some contexts slightly, in composition (e.g., one base, atom or functional group is different, added, or removed; or one or more amino acids are mutated, inserted, or deleted), such that the polypeptide or encoded polypeptide is capable of performing at least one function of the encoded parent polypeptide with at least 50% efficiency, preferably at least 55%, 60%, 70%, 75%,
25 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% level of activity of the parent polypeptide. In other words, a functional variant of a polypeptide or encoded polypeptide of this disclosure has "similar binding," "similar affinity" or "similar activity" when the functional variant displays no more than a 50% reduction in performance in a selected assay as compared to the parent or reference polypeptide,
30 such as an assay for measuring binding affinity (e.g., Biacore® or tetramer staining measuring an association (K_a) or a dissociation (K_D) constant), avidity, or activation of

a host cell. As used herein, a "functional portion" or "functional fragment" refers to a polypeptide or polynucleotide that comprises only a domain, motif, portion or fragment of a parent or reference compound, and the polypeptide or encoded polypeptide retains at least 50% activity associated with the domain, portion or fragment of the parent or reference compound, preferably at least 55%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 100% level of activity of the parent polypeptide, or provides a biological benefit (e.g., effector function).

[0066] A "functional portion" or "functional fragment" of a polypeptide or encoded polypeptide of this disclosure has "similar binding" or "similar activity" when the functional portion or fragment displays no more than a 50% reduction in performance in a selected assay as compared to the parent or reference polypeptide (preferably no more than 20% or 10%, or no more than a log difference as compared to the parent or reference with regard to affinity), such as an assay for measuring binding affinity or measuring effector function (e.g., cytokine release). In certain embodiments, a functional portion refers to a "signaling portion" of an effector molecule, effector domain, costimulatory molecule, or costimulatory domain.

[0067] An "altered domain" or "altered protein" refers to a motif, region, domain, peptide, polypeptide, or protein with a non-identical sequence identity to a wild type motif, region, domain, peptide, polypeptide, or protein (e.g., a wild type TCR α chain, TCR β chain, TCR α constant domain, or TCR β constant domain) of at least 85% (e.g., 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9%).

[0068] As used herein, "heterologous" or "non-endogenous" or "exogenous" refers to any gene, protein, compound, nucleic acid molecule, or activity that is not native to a host cell or a subject, or any gene, protein, compound, nucleic acid molecule, or activity native to a host cell or a subject that has been altered. Heterologous, non-endogenous, or exogenous includes genes, proteins, compounds, or nucleic acid molecules that have been mutated or otherwise altered such that the structure, activity, or both is different as between the native and altered genes, proteins, compounds, or nucleic acid molecules. In certain embodiments, heterologous, non-endogenous, or exogenous genes, proteins, or nucleic acid molecules (e.g., receptors, ligands, etc.) may

not be endogenous to a host cell or a subject, but instead nucleic acids encoding such genes, proteins, or nucleic acid molecules may have been added to a host cell by conjugation, transformation, transfection, electroporation, or the like, wherein the added nucleic acid molecule may integrate into a host cell genome or can exist as extra-
5 chromosomal genetic material (e.g., as a plasmid or other self-replicating vector). It will be appreciated that in the case of a host cell that comprises a heterologous polynucleotide, the polynucleotide is "heterologous" to progeny of the host cell, whether or not the progeny were themselves manipulated (e.g., transduced) to contain the polynucleotide.

10 **[0069]** The term "homologous" or "homolog" refers to a gene, protein, compound, nucleic acid molecule, or activity found in or derived from a host cell, species, or strain. For example, a heterologous or exogenous polynucleotide or gene encoding a polypeptide may be homologous to a native polynucleotide or gene and encode a homologous polypeptide or activity, but the polynucleotide or polypeptide
15 may have an altered structure, sequence, expression level, or any combination thereof. A non-endogenous polynucleotide or gene, as well as the encoded polypeptide or activity, may be from the same species, a different species, or a combination thereof.

[0070] As used herein, the term "endogenous" or "native" refers to a polynucleotide, gene, protein, compound, molecule, or activity that is normally present
20 in a host cell or a subject.

[0071] The term "expression", as used herein, refers to the process by which a polypeptide is produced based on the encoding sequence of a nucleic acid molecule, such as a gene. The process may include transcription, post-transcriptional control, post-transcriptional modification, translation, post-translational control, post-
25 translational modification, or any combination thereof. An expressed nucleic acid molecule is typically operably linked to an expression control sequence (e.g., a promoter).

[0072] The term "operably linked" refers to the association of two or more nucleic acid molecules on a single nucleic acid fragment so that the function of one is
30 affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., the

coding sequence is under the transcriptional control of the promoter). "Unlinked" means that the associated genetic elements are not closely associated with one another and the function of one does not affect the other.

[0073] The term "introduced" in the context of inserting a nucleic acid molecule into a cell, means "transfection", or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid molecule into a eukaryotic or prokaryotic cell wherein the nucleic acid molecule may be incorporated into the genome of a cell (e.g., chromosome, plasmid, plastid, or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA). As used herein, the term "engineered," "recombinant" or "non-natural" or "modified" refers to an organism, microorganism, cell, nucleic acid molecule, or vector that includes at least one genetic alteration or has been modified by introduction of an exogenous nucleic acid molecule, wherein such alterations or modifications are introduced by genetic engineering (i.e., human intervention). Genetic alterations include, for example, modifications introducing expressible nucleic acid molecules encoding proteins, fusion proteins or enzymes, or other nucleic acid molecule additions, deletions, substitutions or other functional disruption of a cell's genetic material. Additional modifications include, for example, non-coding regulatory regions in which the modifications alter expression of a polynucleotide, gene or operon; e.g., such that expression of an endogenous nucleic acid molecule or gene is controlled, deregulated, or constitutive, where such alterations or modifications may be introduced by genetic engineering. Genetic alterations may include, for example, modifications introducing nucleic acid molecules (which may include an expression control element, such as a promoter) encoding one or more proteins or enzymes, or other nucleic acid molecule additions, deletions, substitutions, or other functional disruption of or addition to a cell's genetic material. Exemplary modifications include those in coding regions or functional fragments thereof of heterologous or homologous polypeptides from a reference or parent molecule.

[0074] As described herein, more than one heterologous nucleic acid molecule can be introduced into a host cell as separate nucleic acid molecules, as a plurality of individually controlled genes, as a polycistronic nucleic acid molecule, as a single

nucleic acid molecule encoding a fusion protein, or any combination thereof. When two or more heterologous nucleic acid molecules are introduced into a host cell, it is understood that the two or more heterologous nucleic acid molecules can be introduced as a single nucleic acid molecule (e.g., on a single vector), on separate vectors,

5 integrated into the host chromosome at a single site or multiple sites, or any combination thereof. The number of referenced heterologous nucleic acid molecules or protein activities refers to the number of encoding nucleic acid molecules or the number of protein activities, not the number of separate nucleic acid molecules introduced into a host cell.

10 **[0075]** The term "construct" refers to any polynucleotide that contains a recombinant nucleic acid molecule. A construct may be present in a vector (e.g., a bacterial vector, a viral vector) or may be integrated into a genome. A "vector" is a nucleic acid molecule that is capable of transporting another nucleic acid molecule. Vectors may be, for example, plasmids, cosmids, viruses, a RNA vector or a linear or
15 circular DNA or RNA molecule that may include chromosomal, non-chromosomal, semi-synthetic or synthetic nucleic acid molecules. Vectors of the present disclosure also include transposon systems (e.g., Sleeping Beauty, see, e.g., Geurts et al., Mol. Ther. 8:108, 2003; Mátés et al., Nat. Genet. 41:753, 2009). Exemplary vectors are those capable of autonomous replication (episomal vector), capable of delivering a
20 polynucleotide to a cell genome (e.g., viral vector), or capable of expressing nucleic acid molecules to which they are linked (expression vectors).

[0076] As used herein, the term "host" refers to a cell (e.g., an immune system cell as described herein) or microorganism targeted for genetic modification with a heterologous nucleic acid molecule to produce a polypeptide of interest. In certain
25 embodiments, a host cell may optionally already possess or be modified to include other genetic modifications that confer desired properties related or unrelated to, e.g., biosynthesis of the heterologous protein (e.g., inclusion of a detectable marker; deleted, altered or truncated endogenous TCR; or increased co-stimulatory factor expression).

[0077] A "binding domain" (also referred to as a "binding region" or "binding moiety"), as used herein, refers to a molecule, such as a peptide, oligopeptide,
30 polypeptide, or protein that possesses the ability to specifically and non-covalently

associate, unite, or combine with a target molecule (*e.g.*, Msln₂₀₋₂₈ peptide (SEQ ID NO:31) or Msln₅₃₀₋₅₃₈ peptide (SEQ ID NO:32), in certain embodiments, in a complex with an HLA molecule). A binding domain includes any naturally occurring, synthetic, semisynthetic, or recombinantly produced binding partner for a biological molecule or other target of interest. In some embodiments, the binding domain is an antigen-binding domain, such as an antibody or TCR or functional binding domain or antigen-binding fragment thereof. Exemplary binding domains include single chain antibody variable regions (*e.g.*, single domain antibodies, sFv, scFv, and Fab), receptor ectodomains (*e.g.*, TNF- α), ligands (*e.g.*, cytokines and chemokines), antigen-binding regions of TCRs, such as single chain TCRs (scTCRs), synthetic polypeptides selected for the specific ability to bind to a biological molecule, aptamers, or single domain antibodies (*e.g.*, camelid or fish derived single domain antibodies; *see, e.g.*, Arbabi-Ghahroudi M (2017) Front. Immunol. 8:1589).

[0078] The term "variable region" or "variable domain" refers to the domain of a TCR α -chain or β -chain (or γ -chain and δ -chain for $\gamma\delta$ TCRs), or of an antibody heavy or light chain, that is involved in binding to antigen (*i.e.*, contains amino acids and/or other structures that contact antigen and result in binding). The variable domains of the α -chain and β -chain (V α and V β , respectively) of a native TCR generally have similar structures, with each domain comprising four generally conserved framework regions (FRs) and three CDRs. Variable domains of antibody heavy (V_H) and light (V_L) chains each also generally comprise four generally conserved framework regions (FRs) and three CDRs. In both TCRs and antibodies, framework regions separate CDRs and CDRs are situated between framework regions (*i.e.*, in primary structure).

[0079] The variable domains of the α -chain and β -chain (V α and V β , respectively) of a native TCR generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three CDRs. The V α domain is encoded by two separate DNA segments, the variable gene segment and the joining gene segment (V-J); the V β domain is encoded by three 5 separate DNA segments, the variable gene segment, the diversity gene segment, and the joining gene segment (V-D-J). Human TCR V, D, and J alleles, including the nucleotide and encoded amino acid sequences thereof, are known in the art. A single V α or V β domain may be sufficient to

confer antigen-binding specificity. Furthermore, TCRs that bind a particular antigen may be isolated using a $V\alpha$ or $V\beta$ domain from a TCR that binds the antigen to screen a library of complementary $V\alpha$ or $V\beta$ domains, respectively.

[0080] The terms "complementarity determining region," and "CDR," are synonymous with "hypervariable region" or "HVR," and are known in the art to refer to sequences of amino acids within TCR or antibody variable regions, which, in general, confer antigen specificity and/or binding affinity and are separated from one another in primary structure by framework sequence. In some cases, framework amino acids can also contribute to binding, *e.g.*, may also contact the antigen or antigen-containing molecule. In general, there are three CDRs in each variable region (*i.e.*, three CDRs in each of the $TCR\alpha$ -chain and β -chain variable regions; 3 CDRs in each of the antibody heavy chain and light chain variable regions). In the case of TCRs, CDR3 is thought to be the main CDR responsible for recognizing processed antigen. CDR1 and CDR2 mainly, or in some cases exclusively, interact with the MHC. Variable domain sequences can be aligned to a numbering scheme (*e.g.*, Kabat, EU, International Immunogenetics Information System (IMGT) and Aho), which can allow equivalent residue positions to be annotated and for different molecules to be compared using Antigen receptor Numbering And Receptor Classification (ANARCI) software tool (2016, Bioinformatics 15:298-300). In certain embodiments herein, CDRs are numbered according to the IMGT numbering system.

[0081] As used herein, "specifically binds" refers to an association or union of a binding domain, or of a protein comprising the same, to a target molecule with an affinity or K_a (*i.e.*, an equilibrium association constant of a particular binding interaction with units of $1/M$) equal to or greater than $10^5 M^{-1}$, while not significantly associating or uniting with any other molecules or components in a sample. Binding domains (or fusion proteins thereof) may be classified as "high affinity" binding domains (or fusion proteins thereof) or "low affinity" binding domains (or fusion proteins thereof). "High affinity" binding domains refer to those binding domains with a K_a of at least $10^7 M^{-1}$, at least $10^8 M^{-1}$, at least $10^9 M^{-1}$, at least $10^{10} M^{-1}$, at least $10^{11} M^{-1}$, at least $10^{12} M^{-1}$, or at least $10^{13} M^{-1}$. "Low affinity" binding domains refer to those binding domains with a K_a of up to $10^7 M^{-1}$, up to $10^6 M^{-1}$, or up to $10^5 M^{-1}$. Alternatively, affinity may be

defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (*e.g.*, 10^{-5} M to 10^{-13} M). In certain embodiments, a binding domain may have "enhanced affinity," which refers to a selected or engineered binding domain with stronger binding to a target antigen than a wild type (or parent) binding domain.

- 5 For example, enhanced affinity may be due to a K_a (equilibrium association constant) for the target antigen that is higher than the wild type binding domain, or due to a K_d for the target antigen that is less than that of the wild type binding domain, or due to an off-rate (K_{off}) for the target antigen that is less than that of the wild type binding domain. A variety of assays are known for identifying binding domains of the present disclosure
- 10 that specifically bind a particular target, as well as determining binding domain or fusion protein affinities, such as western blot, ELISA, and BIACORE[®] analysis (*see also, e.g.*, Scatchard, et al., Ann. N. Y. Acad. Sci. 57:660, 1949; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

- [0082] Assays for assessing affinity or apparent affinity or relative affinity are
- 15 known. In certain examples, apparent affinity for a TCR is measured by assessing binding to various concentrations of tetramers, for example, by flow cytometry using labeled tetramers. In some examples, apparent K_d of a TCR is measured using 2-fold dilutions of labeled tetramers (*i.e.*, peptide:MHC tetramers) at a range of concentrations, followed by determination of binding curves by non-linear regression,
- 20 apparent K_d being determined as the concentration of ligand that yielded half-maximal binding. In certain embodiments, a Msln₂₀₋₂₈- or Msln₅₃₀₋₅₃₈-specific binding protein includes a Msln₂₀₋₂₈- or Msln₅₃₀₋₅₃₈-specific immunoglobulin superfamily binding protein or binding portion thereof, respectively.

- [0083] "MHC-peptide tetramer staining" refers to an assay used to detect
- 25 antigen-specific T cells, which features a tetramer of MHC molecules, each including an identical peptide having an amino acid sequence that is cognate (*e.g.*, identical or related to) at least one epitope (*e.g.*, Msln₂₀₋₂₈ or Msln₅₃₀₋₅₃₈), wherein the complex is capable of binding TCRs specific for the cognate epitope. Each of the MHC molecules may be tagged with a biotin molecule. Biotinylated MHC/peptides are
- 30 tetramerized by the addition of streptavidin, which can be fluorescently labeled. The tetramer may be detected by flow cytometry via the fluorescent label. In certain

embodiments, an MHC-peptide tetramer assay is used to detect or select enhanced affinity TCRs of the instant disclosure. Levels of cytokines may be determined according to methods described herein and practiced in the art, including for example, ELISA, ELISpot, intracellular cytokine staining, and flow cytometry and combinations thereof (e.g., intracellular cytokine staining and flow cytometry). Immune cell proliferation and clonal expansion resulting from an antigen-specific elicitation or stimulation of an immune response may be determined by isolating lymphocytes, such as circulating lymphocytes in samples of peripheral blood cells or cells from lymph nodes, stimulating the cells with antigen, and measuring cytokine production, cell proliferation, and/or cell viability, such as by incorporation of tritiated thymidine or non-radioactive assays, such as MTT assays and the like. The effect of an immunogen described herein on the balance between a Th1 immune response and a Th2 immune response may be examined, for example, by determining levels of Th1 cytokines, such as IFN- γ , IL-12, IL-2, and TNF- β , and Type 2 cytokines, such as IL-4, IL-5, IL-9, IL-10, and IL-13.

[0084] "Antigen" or "Ag" as used herein refers to an immunogenic molecule that provokes an immune response. This immune response may involve antibody production, activation of specific immunologically-competent cells (e.g., T cells), or both. An antigen (immunogenic molecule) may be, for example, a peptide, glycopeptide, polypeptide, glycopolypeptide, polynucleotide, polysaccharide, lipid or the like. It is readily apparent that an antigen can be synthesized, produced recombinantly, or derived from a biological sample. Exemplary biological samples that can contain one or more antigens include tissue samples, tumor samples, cells, biological fluids, or combinations thereof. Antigens can be chemically synthesized or produced by cells that have been modified or genetically engineered to express an antigen.

[0085] The term "epitope" or "antigenic epitope" includes any molecule, structure, amino acid sequence or protein determinant that is recognized and specifically bound by a cognate binding molecule, such as an immunoglobulin, T cell receptor (TCR), chimeric antigen receptor, or other binding molecule, domain or protein. Epitopic determinants generally contain chemically active surface groupings of

molecules, such as amino acids or sugar side chains, and can have specific three dimensional structural characteristics, as well as specific charge characteristics.

[0086] "Msln₂₀₋₂₈" and "Msln₂₀₋₂₈ peptide," and "Msln₂₀ peptide" as used herein, refer to a peptide comprising or consisting of mesothelin amino acids 20–28 of SEQ ID NO:50 (human mesothelin, isoform 1); *i.e.*, SLLFLLFSL (SEQ ID NO:31), which peptide can associate with HLA-A*201.

[0087] "Msln₅₃₀₋₅₃₈" and "Msln₅₃₀₋₅₃₈ peptide," "Msln₅₃₀ peptide" as used herein, refer to a peptide comprising or consisting of mesothelin amino acids 530–538 of SEQ ID NO:50; *e.g.*, VLPLTVAEV (SEQ ID NO:32), which peptide can associate with HLA-A*201.

[0088] The term "Msln₂₀₋₂₈-specific binding protein" refers to a protein or polypeptide that specifically binds to and/or that is specific for and/or that has or confers high avidity for a Msln₂₀₋₂₈ peptide. In some embodiments, a protein or polypeptide binds to Msln₂₀₋₂₈, such as a Msln₂₀₋₂₈ peptide complexed with an MHC or HLA molecule, *e.g.*, on a cell surface, with a, or at least about a, particular affinity. A Msln₂₀₋₂₈-specific binding protein may bind to a Msln₂₀₋₂₈ peptide, a variant thereof, or a fragment thereof. For example, the Msln₂₀₋₂₈-specific binding protein may bind to an amino acid sequence of SEQ ID NO:31 (SLLFLLFSL), or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:31. In certain embodiments, a Msln₂₀₋₂₈-specific binding protein binds a Msln₂₀₋₂₈-peptide:HLA complex (or Msln₂₀₋₂₈-derived peptide:MHC complex) with an affinity that is about the same as, at least about the same as, or is greater than at or about the affinity exhibited by an exemplary Msln₂₀₋₂₈-specific binding protein provided herein, such as any of the Msln₂₀₋₂₈-specific TCRs provided herein, for example, as measured by the same assay. In certain embodiments, a Msln₂₀₋₂₈-specific binding protein can bind to an Msln₂₀₋₂₈ epitope as provided herein; *e.g.*, a consensus epitope sequence according to SEQ ID NO:60. As disclosed herein, a Msln-specific binding protein does not bind, or does not substantially bind, to a non-Msln human protein or peptide having high sequence homology or identity to SEQ ID NO:60.

[0089] The term "Msln₅₃₀₋₅₃₈-specific binding protein" refers to a protein or polypeptide that specifically binds to and/or that is specific for and/or that has or

confers high avidity for a Msln₅₃₀₋₅₃₈ peptide. In some embodiments, a protein or polypeptide binds to Msln₅₃₀₋₅₃₈, such as a Msln₅₃₀₋₅₃₈ peptide is complexed with an MHC or HLA molecule, *e.g.*, on a cell surface, with a, or at least about a, particular affinity. A Msln₅₃₀₋₅₃₈-specific binding protein may bind to a Msln₅₃₀₋₅₃₈ peptide, a variant thereof, or a fragment thereof. For example, the Msln₅₃₀₋₅₃₈-specific binding protein may bind to an amino acid sequence of SEQ ID NO:32 (VLPLTVAEV), or an amino acid sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:32. In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein binds a Msln₅₃₀₋₅₃₈-peptide:HLA complex (or Msln₅₃₀₋₅₃₈-derived peptide:MHC complex) with an affinity that is about the same as, at least about the same as, or is greater than at or about the affinity exhibited by an exemplary Msln₅₃₀₋₅₃₈ specific binding protein provided herein, such as any of the Msln₅₃₀₋₅₃₈-specific TCRs provided herein, for example, as measured by the same assay. In certain embodiments, a Msln₅₃₀₋₅₃₈ -specific binding protein can bind to an Msln₅₃₀₋₅₃₈ epitope as provided herein; *e.g.*, a consensus epitope sequence according to SEQ ID NO:61 or 62. As disclosed herein, a Msln-specific binding protein does not bind, or does not substantially bind, to a non-Msln human protein or peptide having high sequence homology or identity to SEQ ID NO:61 or 62.

[0090] A target molecule, which is specifically bound by a binding domain of the present disclosure, may be found on or in association with a cell of interest ("target cell"). Exemplary target cells include a cancer cell, a cell associated with an autoimmune disease or disorder or with an inflammatory disease or disorder, and an infectious organism or cell (*e.g.*, bacteria, virus, or virus-infected cell). A cell of an infectious organism, such as a mammalian parasite, is also contemplated as a target cell.

[0091] The term "functional avidity" refers to a biological measure or activation threshold of an in vitro immune cell (*e.g.*, T cell, NK cell, NK-T cell) response to a given concentration of a ligand, wherein the biological measures can include cytokine production (*e.g.*, IFN γ production, IL-2 production, etc.), cytotoxic activity, and proliferation. For example, T cells that biologically (immunologically) respond in vitro to a low antigen dose by producing cytokines, being cytotoxic, or proliferating are considered to have high functional avidity, while T cells having lower functional

avidity require higher amounts of antigen before an immune response, similar to the high-avidity T cells, is elicited. It will be understood that functional avidity is different from affinity and avidity. Affinity refers to the strength of any given bond between a binding protein and its antigen/ligand. Some binding proteins are multivalent and bind to multiple antigens – in this case, the strength of the overall connection is the avidity.

[0092] Numerous correlations exist between the functional avidity and the effectiveness of an immune response. Some ex vivo studies have shown that distinct T cell functions (e.g., proliferation, cytokines production, etc.) can be triggered at different thresholds (see, e.g., Betts et al., J. Immunol. 172:6407, 2004; Langenkamp et al., Eur. J. Immunol. 32:2046, 2002). Factors that affect functional avidity include (a) the affinity of a TCR for the pMHC-complex, that is, the strength of the interaction between the TCR and pMHC (Cawthon et al., J. Immunol. 167:2577, 2001), (b) expression levels of the TCR and the CD4 or CD8 co receptors, and (c) the distribution and composition of signaling molecules (Viola and Lanzavecchia, Science 273:104, 1996), as well as expression levels of molecules that attenuate T cell function and TCR signaling.

[0093] The concentration of antigen needed to induce a half-maximum response between the baseline and maximum response after a specified exposure time is referred to as the "half maximal effective concentration" or "EC50". The EC50 value is generally presented as a molar (moles/liter) amount, but it is often converted into a logarithmic value as follows – $\log_{10}(\text{EC}_{50})$. For example, if the EC50 equals 1 μM (10^{-6} M), the $\log_{10}(\text{EC}_{50})$ value is -6. Another value used is pEC50, which is defined as the negative logarithm of the EC50 ($-\log_{10}(\text{EC}_{50})$). In the above example, the EC50 equaling 1 μM has a pEC50 value of 6. In certain embodiments, the functional avidity of the binding proteins of this disclosure will be a measure of its ability to promote $\text{IFN}\gamma$ production by T cells, which can be measured using assays described herein. "High functional avidity" TCRs or binding domains thereof refer to those TCRs or binding domains thereof having a EC50 of at least 10^{-4} M, at least about 10^{-5} M, or at least about 10^{-6} M.

[0094] In certain embodiments, mesothelin-specific binding proteins or domains as described herein may be expressed by a host T cell and can be functionally

characterized according to any of a large number of art accepted methodologies for assaying T cell activity, including determination of T cell binding, activation or induction and also including determination of T cell responses that are antigen-specific. In certain embodiments, the binding protein is capable of promoting an antigen-specific

5 T cell response against human mesothelin in a class I HLA-restricted manner. In further embodiments, the class I HLA-restricted response is transporter-associated with antigen processing (TAP)-independent. In certain embodiments, the antigen-specific T cell response comprises at least one of a CD4⁺ helper T lymphocyte (Th) response and a CD8⁺ cytotoxic T lymphocyte (CTL) response. In related embodiments, the CTL

10 response is directed against a mesothelin-overexpressing cell. Further examples of methodologies for assaying T cell activity include determination of T cell proliferation, T cell cytokine release, antigen-specific T cell stimulation, MHC restricted T cell stimulation, CTL activity (e.g., by detecting ⁵¹Cr release from pre-loaded target cells), changes in T cell phenotypic marker expression, and other measures of T-cell functions.

15 Procedures for performing these and similar assays are may be found, for example, in Lefkovits (Immunology Methods Manual: The Comprehensive Sourcebook of Techniques, 1998). See also Current Protocols in Immunology; Weir, Handbook of Experimental Immunology, Blackwell Scientific, Boston, MA (1986); Mishell and Shigii (eds.) Selected Methods in Cellular Immunology, Freeman Publishing, San

20 Francisco, CA (1979); Green and Reed, Science 281:1309 (1998) and references cited therein).

[0095] As used herein, an "immune system cell" refers to any cell of the immune system that originates from a hematopoietic stem cell in the bone marrow, which gives rise to two major lineages, a myeloid progenitor cell (which gives rise to

25 myeloid cells such as monocytes, macrophages, dendritic cells, megakaryocytes, and granulocytes) and a lymphoid progenitor cell (which gives rise to lymphoid cells such as T cells, B cells, and natural killer (NK) cells). Exemplary immune system cells include a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a regulatory T cell, a stem cell memory T cell, a natural killer cell (e.g., a NK cell or a

30 NK-T cell), a B cell, and a dendritic cell. Macrophages and dendritic cells may be referred to as "antigen presenting cells" or "APCs," which are specialized cells that can

activate T cells when a major histocompatibility complex (MHC) receptor on the surface of the APC complexed with a peptide interacts with a TCR on the surface of a T cell.

[0096] A "T cell" or "T lymphocyte" is an immune system cell that matures in the thymus and produces TCRs. T cells can be naïve (not exposed to antigen; increased expression of CD62L, CCR7, CD28, CD3, CD127, and CD45RA, and decreased expression of CD45RO as compared to T_{CM}), memory T cells (T_M) (antigen-experienced and long-lived), and effector cells (antigen-experienced, cytotoxic). T_M can be further divided into subsets of central memory T cells (T_{CM}, increased expression of CD62L, CCR7, CD28, CD127, CD45RO, and CD95, and decreased expression of CD54RA as compared to naïve T cells) and effector memory T cells (T_{EM}, decreased expression of CD62L, CCR7, CD28, CD45RA, and increased expression of CD127 as compared to naïve T cells or T_{CM}).

[0097] Effector T cells (T_E) refer to antigen-experienced CD8⁺ cytotoxic T lymphocytes that have decreased expression of CD62L, CCR7, CD28, and are positive for granzyme and perforin as compared to T_{CM}. Other exemplary T cells include regulatory T cells, such as CD4⁺ CD25⁺ (Foxp3⁺) regulatory T cells and Treg17 cells, as well as Tr1, Th3, CD8⁺CD28⁻, and Qa-1 restricted T cells.

[0098] Helper T cells (T_H) are CD4⁺ cells that influence the activity of other immune cells by releasing cytokines. CD4⁺ T cells can activate and suppress an adaptive immune response, and which of those two functions is induced will depend on presence of other cells and signals. T cells can be collected using known techniques, and the various subpopulations or combinations thereof can be enriched or depleted by known techniques, such as by affinity binding to antibodies, flow cytometry, or immunomagnetic selection.

[0099] "Cells of T cell lineage" refer to cells that show at least one phenotypic characteristic of a T cell, or a precursor or progenitor thereof that distinguishes the cells from other lymphoid cells, and cells of the erythroid or myeloid lineages. Such phenotypic characteristics can include expression of one or more proteins specific for T cells (*e.g.*, CD3⁺, CD4⁺, CD8⁺), or a physiological, morphological, functional, or immunological feature specific for a T cell. For example, cells of the T cell lineage

may be progenitor or precursor cells committed to the T cell lineage; CD25⁺ immature and inactivated T cells; cells that have undergone CD4 or CD8 lineage commitment; thymocyte progenitor cells that are CD4⁺CD8⁺ double positive; single positive CD4⁺ or CD8⁺; TCRαβ or TCR γδ; or mature and functional or activated T cells.

- 5 **[0100]** A "hematopoietic progenitor cell" is a cell derived from hematopoietic stem cells (HSCs) or fetal tissue that is capable of further differentiation into mature cell types (*e.g.*, cells of the T cell lineage). In certain embodiments, CD24^{lo} Lin⁻ CD117⁺ hematopoietic progenitor cells are useful. As defined herein, hematopoietic progenitor cells may include embryonic stem cells, which are capable of further
- 10 differentiation to cells of the T cell lineage. Hematopoietic progenitor cells may be from various animal species, including human, mouse, rat, or other mammals. A "thymocyte progenitor cell" or "thymocyte" is a hematopoietic progenitor cell present in the thymus.

- [0101]** "Hematopoietic stem cells" or "HSCs" refer to undifferentiated
- 15 hematopoietic cells that are capable of self-renewal either *in vivo*, essentially unlimited propagation *in vitro*, and capable of differentiation to other cell types including cells of the T cell lineage. HSCs may be isolated, for example, but not limited to, from fetal liver, bone marrow, and cord blood.

- [0102]** "Embryonic stem cells," "ES cells," or "ESCs" refer to undifferentiated
- 20 embryonic stem cells that have the ability to integrate into and become part of the germ line of a developing embryo. Embryonic stem cells are capable of differentiating into hematopoietic progenitor cells and any tissue or organ. Embryonic stem cells that are suitable for use herein include cells from the J1 ES cell line, 129J ES cell line, murine stem cell line D3 (American Type Culture Collection), the R1 or E14K cell lines
- 25 derived from 129/Sv mice, cell lines derived from Balb/c and C57B1/6 mice, and human embryonic stem cells (*e.g.*, from WICELL[®] Research Institute, WI; or ES cell International, Melbourne, Australia).

- [0103]** The term "T cell receptor" (TCR) refers to an immunoglobulin superfamily member (having a variable binding domain, a constant domain, a
- 30 transmembrane region, and a short cytoplasmic tail; *see, e.g.*, Janeway, et al., Immunobiology: The Immune System in Health and Disease, 3rd Ed., Current Biology

Publications, p. 4:33, 1997) capable of specifically binding to an antigen peptide bound to a MHC receptor. A TCR can be found on the surface of a cell or in soluble form and generally is comprised of a heterodimer having α and β chains (also known as TCR α and TCR β , respectively), or γ and δ chains (also known as TCR γ and TCR δ , respectively). Like immunoglobulins, the extracellular portion of TCR chains (*e.g.*, α -chain and β -chain) contain two immunoglobulin domains, a variable domain (*e.g.*, α -chain variable domain or V α , β -chain variable domain or V β ; typically amino acids 1 to 116 based on Kabat numbering (Kabat, et al., "Sequences of Proteins of Immunological Interest," US Dept. Health and Human Services, Public Health Service National Institutes of Health, 1991, 5th ed.)) at the N-terminus, and one constant domain (*e.g.*, α -chain constant domain or C α , typically amino acids 117 to 259 based on Kabat, β -chain constant domain or C β , typically amino acids 117 to 295 based on Kabat) adjacent to the cell membrane. Also like immunoglobulins, the variable domains contain complementary determining regions (CDRs) separated by framework regions (FRs) (*see, e.g.*, Jores, et al., Proc. Nat'l Acad. Sci. U.S.A. 57:9138, 1990; Chothia, et al., EMBO J. 7:3745, 1988; *see also* Lefranc, et al., Dev. Comp. Immunol. 27:55, 2003). In certain embodiments, a TCR is found on the surface of T cells (or T lymphocytes) and associates with the CD3 complex. The source of a TCR as used in the present disclosure may be from various animal species, such as a human, mouse, rat, cat, dog, goat, horse, or other mammal.

[0104] "CD3" is a multi-protein complex of six chains (*see*, Borst J, et al., J Biol Chem, 258(8):5135-41, 1983 and Janeway, et al., p. 172 and 178, 1999 *supra*). In mammals, the complex includes a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, and a homodimer of CD3 ζ chains. The CD3 γ , CD3 δ , and CD3 ϵ chains are related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3 γ , CD3 δ , and CD3 ϵ chains are negatively charged, which is a characteristic that is thought to allow these chains to associate with positively charged regions of TCR chains. The intracellular tails of the CD3 γ , CD3 δ , and CD3 ϵ chains each contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM, whereas each CD3 ζ chain has three. Without being bound by any one theory, it is believed the ITAMs are

important for the signaling capacity of a TCR complex. CD3 as used in the present disclosure may be from various animal species, including human, mouse, rat, or other mammals.

- [0105]** As used herein, "TCR complex" refers to a complex formed by the association of CD3 with TCR. For example, a TCR complex can be composed of a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, a homodimer of CD3 ζ chains, a TCR α chain, and a TCR β chain. Alternatively, a TCR complex can be composed of a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, a homodimer of CD3 ζ chains, a TCR γ chain, and a TCR δ chain.
- [0106]** A "component of a TCR complex," as used herein, refers to a TCR chain (*i.e.*, TCR α , TCR β , TCR γ , or TCR δ), a CD3 chain (*i.e.*, CD3 γ , CD3 δ , CD3 ϵ , or CD3 ζ), or a complex formed by two or more TCR chains or CD3 chains (*e.g.*, a complex of TCR α and TCR β , a complex of TCR γ and TCR δ , a complex of CD3 ϵ and CD3 δ , a complex of CD3 γ and CD3 ϵ , or a sub-TCR complex of TCR α , TCR β , CD3 γ , CD3 δ , and two CD3 ϵ chains).

- [0107]** "Major histocompatibility complex" (MHC) refers to glycoproteins that deliver peptide antigens to a cell surface. MHC class I molecules are heterodimers having a membrane spanning α chain (with three α domains) and a non-covalently associated β 2 microglobulin. MHC class II molecules are composed of two transmembrane glycoproteins, α and β , both of which span the membrane. Each chain has two domains. MHC class I molecules deliver peptides originating in the cytosol to the cell surface, where a peptide:MHC complex is recognized by CD8⁺ T cells. MHC class II molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by CD4⁺ T cells. Human MHC is referred to as human leukocyte antigen (HLA).

MESOTHELIN-SPECIFIC BINDING PROTEINS

- [0108]** In certain aspects, the present disclosure provides binding proteins that are capable of specifically binding to a mesothelin peptide antigen as described herein (*e.g.*, a peptide comprising, consisting, or consisting essentially of the amino acid sequence set forth in SEQ ID NO:31 or SEQ ID NO:32). Binding proteins herein include a TCR alpha chain variable domain (V α) and a TCR beta chain variable domain

(V β). In any of the presently disclosed embodiments, a mesothelin-specific binding protein is capable of specifically binding to a mesothelin peptide:HLA complex, such as a mesothelin peptide:HLA-A*02:01 complex.

- [0109]** In certain embodiments, a Msln-530-538-specific binding protein is provided that comprises: (a) a TCR V α comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:37 or 39, and a TCR V β , wherein the TCR V β optionally comprises an amino acid sequence having at least about 85% (i.e., at least about 86%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the amino acid sequence set forth in SEQ ID NO:99 or 101; (b) a TCR V β comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:38 or 40, and (b) a TCR V α , wherein the TCR V α optionally comprises an amino acid sequence having at least about 85% (i.e., at least about 86%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the amino acid sequence set forth in SEQ ID NO:100 or 102; or (c) a TCR V α comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:37 or 39, wherein the TCR V α optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:100 or 102, and a TCR V β comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:38 or 40, wherein the TCR V β optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:99 or 101.

- [0110]** In any of the embodiments described herein, an encoded polypeptide of this disclosure (e.g., TCR variable domain or TCR chain) can comprise a "signal peptide" (also known as a leader sequence, leader peptide, or transit peptide). Signal peptides target newly synthesized polypeptides to an appropriate location inside or outside the cell. A signal peptide may be removed from the polypeptide during or once localization or secretion is completed. Polypeptides that have a signal peptide are referred to herein as a "pre-protein" and polypeptides having their signal peptide removed are referred to herein as "mature" proteins or polypeptides. Representative signal peptides include those amino acid sequences from position 1 to position 19 any one of SEQ ID NOs: 6, 14, 22, 28, or 29; from position 1 to 17 of SEQ ID NO: 7; from

position 1 to 22 of SEQ ID NO: 15; from position 1 to 21 of SEQ ID NO: 23.

Exemplary mature polypeptide sequences are provided in SEQ ID NOs:95-119.

[0111] In any of the presently disclosed embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein can comprise a TCR V α comprising an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:100 or 102 and/or a TCR V β comprising an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:99 or 101. In certain embodiments, a binding protein comprises a variant of a referenced TCR variable domain sequence, provided that at least three or four of the CDRs of the binding protein have no change in sequence according to a referenced TCR variable domain sequence, wherein the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof.

[0112] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises a CDR3 α amino acid sequence as set forth in SEQ ID NO:39 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:40. In some embodiments, the binding protein further comprises a CDR1 α amino acid sequence as set forth SEQ ID NO:93, a CDR2 α amino acid sequence as set forth in SEQ ID NO:94, a CDR1 β amino acid sequence as set forth in any one of SEQ ID NOs: 83, 84, or 91, and/or a CDR2 β amino acid sequence as set forth in SEQ ID NO:92. In further embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:102, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101, wherein there are optionally no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0113] In certain embodiments, the Msln₅₃₀₋₅₃₈-specific binding protein comprises a TCR V β comprising an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBJ2-3*01 (e.g., to a TRBJ2-3*01-encoded amino acid sequence that is at least about 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 15 contiguous amino acids in length), and/or an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAV21*01 or TRAV21*02 (e.g., to a TRAV21*01 or TRAV21*02-encoded sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 107 amino

acids in length), and/or an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBV5-4*01 (e.g., to a TRBV5-4*01-encoded sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 108 amino acids in length), and/or an amino acid sequence
 5 having at least about 85% identity to an amino acid sequence encoded by TRAJ57*01 (e.g., to a TRAJ57*01-encoded sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length).

[0114] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises a TCR V α comprising or consisting of the amino acid sequence set forth in SEQ ID
 10 NO:102 and a TCR V β comprising or consisting of the amino acid sequence set forth in SEQ ID NO:101.

[0115] In other embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises a CDR3 α amino acid sequence as set forth in SEQ ID NO:37 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:38. In some embodiments, the binding protein
 15 further comprises a CDR1 α amino acid sequence as set forth SEQ ID NO:89, a CDR2 α amino acid sequence as set forth in SEQ ID NO:90, a CDR1 β amino acid sequence as set forth in any one of SEQ ID NOs: 83 or 87, and a CDR2 β amino acid sequence as set forth in SEQ ID NO:88. In certain embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in
 20 SEQ ID NO:100, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:99, wherein there are optionally no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0116] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises an amino acid sequence having at least about 85% identity to an amino acid sequence
 25 encoded by TRAV4-1*01 (e.g., to a TRAV4-1*01-encoded sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 108 amino acids in length), and/or an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAJ18*01 (e.g., to a TRAJ18*01-encoded sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,
 30 19, 20, or 21 amino acids in length) and/or an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBJ1-1*01 (e.g., to a TRBJ1-

1*01-encoded sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in length), and/or an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBJ2-3*01 (e.g., to a TRBJ2-3*01-encoded sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids in length).

[0117] In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein comprises a TCR V α comprising or consisting of the amino acid sequence set forth in SEQ ID NO:100 and a TCR V β comprising or consisting of the amino acid sequence set forth in SEQ ID NO:99.

10 **[0118]** In certain embodiments, alanine mutagenesis of any one or more of residues 3, 5, 6, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair binding by a Msln₅₃₀₋₅₃₈-specific binding protein. In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein is capable of binding to a peptide comprising or consisting of the consensus amino acid sequence set forth in SEQ ID NO:61; e.g., in a peptide:HLA complex as disclosed herein.

[0119] In certain embodiments, alanine mutagenesis of any one or more of residues 1, 5, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair binding by a Msln₅₃₀₋₅₃₈-specific binding protein. In certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein is capable of binding to a peptide comprising or consisting of the consensus amino acid sequence set forth in SEQ ID NO:62; e.g., in a peptide:HLA complex as disclosed herein.

[0120] Presently disclosed Msln-specific binding proteins advantageously present low to no risk of alloreactivity against non-Msln targets; e.g., that are expressed in healthy tissue. Briefly, the present disclosure shows that Msln-specific binding proteins do not react, or do not substantially react, with human proteins with sequence homology to a Msln peptide antigen as provided herein. Thus, the binding proteins are highly specific for Msln peptide antigens.

[0121] For example, in certain embodiments, a Msln₅₃₀₋₅₃₈-specific binding protein of the present disclosure does not bind, or does not specifically bind relative to binding to Msln₅₃₀₋₅₃₈, to a peptide:HLA complex wherein the peptide comprises or consists of the amino acid sequence set forth in any one or more of SEQ ID NOs:63, 64,

65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, and 77, and wherein the HLA optionally comprises an HLA-A2, such as HLA-A:02*01.

[0122] In certain embodiments, a Msln₂₀₋₂₈-specific binding protein is provided that comprises: (a) a TCR V α comprising a CDR3 amino acid sequence as set forth in
5 SEQ ID NO:33 or 35, and a TCR V β , wherein the TCR V β optionally has at least about 85% (i.e., at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the amino acid sequence set forth in SEQ ID NO:95 or 97; (b) a TCR V β comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:34 or 36, and (b) a TCR V α , wherein the TCR V α optionally has at least
10 about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98; or (c) a TCR V α comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:33 or 35 and a TCR V β comprising a CDR3 amino acid sequence as set forth in SEQ ID NO:34 or 36, wherein the TCR V α optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95 or 97,
15 and wherein the TCR V β optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98.

[0123] In any of the presently disclosed embodiments, a Msln₂₀₋₂₈-specific binding protein can comprise a TCR V α comprising an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98
20 and/or a TCR V β comprising an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95 or 97. In certain embodiments, a binding protein comprises a variant of a referenced TCR variable domain sequence, provided that at least three or four of the CDRs of the binding protein have no change in sequence according to a referenced TCR variable domain sequence, wherein the CDRs
25 that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof.

[0124] In certain embodiments, a Msln₂₀₋₂₈-specific binding protein specific binding protein comprises a CDR3 α amino acid sequence as set forth in SEQ ID NO:33 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:34. In some
30 embodiments, the binding protein further comprises a CDR1 α amino acid sequence as set forth SEQ ID NO:80, a CDR2 α amino acid sequence as set forth in SEQ ID NO:81

or 118, a CDR1 β amino acid sequence as set forth in any one of SEQ ID NOs: 78, 83, or 84, and a CDR2 β amino acid sequence as set forth in SEQ ID NO:79. In certain embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95, wherein there are optionally no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0125] In certain embodiments, the Msln₂₀₋₂₈-specific binding protein comprises (i) a TCR V β comprising (a) an amino acid sequence having at least about 85% identity (i.e., at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) to an amino acid sequence encoded by TRBV12-4*01 (e.g., to a TRBV12-4*01-encoded amino acid sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 108 contiguous amino acids in length); and/or (b) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBJ2-7*01 (e.g., to a TRBJ2-7*01-encoded amino acid sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 amino acids long); and/or (ii) TCR V α comprising (a) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAV1-1*01 (e.g., to a TRAV1-1*01-encoded amino acid sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 107 contiguous amino acids in length) and/or (b) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAJ3*01 (e.g., to a TRAJ3*01-encoded amino acid sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids long), and/or (c) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBJ2-3*01 (e.g., to a TRBJ2-3*01-encoded sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids in length).

[0126] In certain embodiments, a Msln₂₀₋₂₈-specific binding protein comprises a TCR V α comprising or consisting of the amino acid sequence set forth in SEQ ID NO:96, and a TCR V β comprising or consisting of the amino acid sequence set forth in SEQ ID NO:95.

[0127] In certain embodiments, a Msln₂₀₋₂₈-specific binding protein comprises a CDR3 α amino acid sequence as set forth in SEQ ID NO:35 and a CDR3 β amino acid sequence as set forth in SEQ ID NO:36. In some embodiments, the binding protein further comprises a CDR1 α amino acid sequence as set forth in SEQ ID NO:85, a CDR2 α amino acid sequence as set forth in SEQ ID NO:86 or 119, a CDR1 β amino acid sequence as set forth in any one of SEQ ID NOs:82, 83, or 84, and a CDR2 β amino acid sequence as set forth in SEQ ID NO:79. In certain embodiments, the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:98, and/or the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:97, wherein there are optionally no changes in CDR1 α , CDR2 α , CDR1 β , and/or CDR2 β .

[0128] In certain embodiments, the Msln₂₀₋₂₈-specific binding protein comprises a TCR V α comprising (a) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAV12-3*01 (e.g., to a TRAV12-3*01-encoded amino acid sequence that is at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, or 108 contiguous amino acids in length) and/or (b) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRAJ29*01 (e.g., to a TRAJ29*01-encoded amino acid sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acids long), and/or (c) an amino acid sequence having at least about 85% identity to an amino acid sequence encoded by TRBJ2-3*01 (e.g., to a TRBJ2-3*01-encoded sequence that is at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids in length).

[0129] In certain embodiments, a Msln₂₀₋₂₈-specific binding protein comprises a TCR V α comprising or consisting of the amino acid sequence set forth in SEQ ID NO:98 and a TCR V β comprising or consisting of the amino acid sequence set forth in SEQ ID NO:97.

[0130] In certain embodiments, alanine mutagenesis of any one or more of residues 1, 2, 7, 8, or 9 of SEQ ID NO:31 does not abrogate or does not substantially impair binding by a Msln₂₀₋₂₈-specific binding protein. In certain embodiments, a Msln₂₀₋₂₈-specific binding protein is capable of binding to a peptide comprising or

consisting of the consensus amino acid sequence set forth in SEQ ID NO:60; e.g., in a peptide:HLA complex as disclosed herein.

[0131] In any of the presently disclosed embodiments, a Msln-specific binding protein is capable of binding to a Msln peptide:HLA complex, wherein the Msln peptide comprises the amino acid sequence set forth in SEQ ID NO:31 or 32 and wherein the HLA is or comprises HLA-A2, such as HLA-A*02:01.

[0132] In any of the presently disclosed embodiments, an immune cell (e.g., a T cell) expressing a Msln-specific binding protein of this disclosure does not produce IFN- γ and/or does not exhibit activation (e.g., CD8 expression, CD3 expression, Nur77 expression) and/or cytotoxic activity (e.g., specific killing, production and release of a perforin and/or a granzyme) when contacted with a cell expressing: (i) HLA-C6:02:01; (ii) HLA-B13:01:01 without HLA-B13:02:01; (iii) HLA-A3; (iv) HLA-A29; (v) HLA-B40; (vi) HLA-B44; (vii) HLA-C3; (viii) HLA-C16; (ix) HLA-A1; (x) HLA-24; (xi) HLA-B7; (xii) HLA-B57; (xiii) HLA-C7; (xiv) HLA-A11; (xv) HLA-B15; (xvi) HLA-C4; (xvii) HLA-C12; (xviii) HLA-B8; (xix) HLA-B49; (xx) HLA-B51; (xxi) HLA-C15; (xxii) HLA-A30; (xxiii) HLA-A68; (xxiv) HLA-C2; (xxv) HLA-A32; (xxvi) HLA-A33; (xxvii) HLA-B55; (xxviii) HLA-C1; (xxvix) HLA-C5; (xxix) HLA-B8; (xxx) HLA-B35; or (xxxi) any combination of (i)-(xxx), when in the absence of a Msln peptide as provided herein.

[0133] In any of the presently disclosed embodiments, a Msln-specific binding protein, when expressed on the surface of a host cell, is capable of binding to a Msln peptide:HLA complex as disclosed herein in the absence of, or independent of, CD8.

[0134] In certain embodiments, a binding protein according to the present disclosure has a Msln peptide EC₅₀ of about 9 μ M, about 8 μ M, about 7 μ M, about 6 μ M, about 5 μ M, about 4 μ M, about 3 μ M, about 2 μ M, about 1 μ M, about 0.9 μ M, about 0.8 μ M, about 0.7 μ M, about 0.6 μ M, about 0.5 μ M, about 0.4 μ M, about 0.3 μ M, about 0.2 μ M, or less.

[0135] In any of the presently disclosed embodiments, a Msln-specific binding protein is capable of more efficiently associating with a CD3 protein, and/or has increased expression at a cell surface relative to an endogenous TCR, when the Msln-specific binding protein is expressed in a host T cell or NK-T cell.

[0136] In certain embodiments, the binding protein is a TCR, a single chain TCR (scTCR), or a CAR.

[0137] In some embodiments, the binding protein is a TCR. In certain embodiments, the binding protein comprises a TCR V β , a TCR C β , a TCR V α , and a TCR C α , wherein the V β and the C β together comprise a TCR β chain (TCR β), and wherein the V α and the C α together comprise a TCR α chain (TCR α), and wherein the TCR β and the TCR α are capable of associating to form a dimer. In further embodiments, a TCR C β comprises a cysteine amino acid in place of a native serine at amino acid position 57 (e.g., GV(S $\rightarrow$ C)TD) and a TCR C α comprises a cysteine amino acid in place of a native threonine at amino acid position 48 (e.g., DK(T $\rightarrow$ C)VL; see, e.g., Cohen et al., Cancer Res. 67(8):3898-3903 (2007)).

[0138] In further embodiments, a Msln-specific binding protein is a TCR comprising a TCR β and a TCR α , wherein the TCR β and the TCR α respectively comprise an amino acid sequence having at least about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity, or more, to the amino acid sequences set forth in SEQ ID NOs: (i) 103 or 6 (TCR β) and 104 or 7 (TCR α); (ii) 105 or 14 (TCR β) and 106 or 15 (TCR α); (iii) 107 or 22 (TCR β) and 108 or 23 (TCR α); or (iv) 109 or 28 (TCR β) and 110 or 29 (TCR α).

[0139] In certain embodiments, the binding protein is a soluble TCR, optionally including or coupled to a cytotoxic and/or detectable element or agent. (*see, e.g.,* Walseng *et al.*, *PLoS One* doi:10.1371/journal.pone.0119559 (2015)). Methods useful for isolating and purifying recombinantly produced soluble TCR, by way of example, may include obtaining supernatants from suitable host cell/vector systems that secrete the recombinant soluble TCR into culture media and then concentrating the media using a commercially available filter. Following concentration, the concentrate may be applied to a single suitable purification matrix or to a series of suitable matrices, such as an affinity matrix or an ion exchange resin. One or more reverse phase HPLC steps may be employed to further purify a recombinant polypeptide. These purification methods may also be employed when isolating an immunogen from its natural environment. Methods for large scale production of one or more of the isolated/recombinant soluble TCR described herein include batch cell culture, which is

monitored and controlled to maintain appropriate culture conditions. Purification of the soluble TCR may be performed according to methods described herein and known in the art and that comport with laws and guidelines of domestic and foreign regulatory agencies.

5 **[0140]** In some embodiments, two or more distinct polypeptide domains or sequences are connected by a linker (e.g., a TCRV α and a TCRV β in the context of a scTCR or a CAR). A "linker" refers to an amino acid sequence that connects two proteins, polypeptides, peptides, domains, regions, or motifs and may provide a spacer function compatible with interaction of the two sub-binding domains so that the
10 resulting polypeptide retains a specific binding affinity (e.g., scTCR) to a target molecule or retains signaling activity (e.g., TCR complex). In certain embodiments, a linker is comprised of about two to about 35 amino acids, about four to about 20 amino acids, about eight to about 15 amino acids, about 15 to about 25 amino acids, or another suitable number of amino acids. In general, a linker is preferably chemically inert,
15 flexible, and non-immunogenic or minimally immunogenic. Linker sequences can be repeated so as to achieve a desired length to, for example, facilitate a desired protein interaction by or between linked domains. Exemplary linkers (including glycine-serine linkers) and properties of linkers are discussed in, for example, Chen *et al.*, *Adv. Drug Deliv Rev*, 65(10):1357-1369 (2013), and in van Rosmalen *et al.*, *Biochemistry*
20 56(60):6565-6574 (2017), which linker amino acid sequences and design properties are incorporated herein by reference.

[0141] In particular embodiments, a Msln-specific binding protein is or comprises a scTCR (e.g., single chain $\alpha\beta$ TCR proteins such as V α -L-V β , V β -L-V α , V α -C α -L-V α , or V α -L-V β -C β , wherein V α and V β are TCR α and β variable domains
25 respectively, C α and C β are TCR α and β constant domains, respectively, and L is a linker).

[0142] In certain embodiments, a Msln-specific binding protein is or comprises a CAR. "Chimeric antigen receptor" (CAR) refers to a fusion protein of the present disclosure engineered to contain two or more naturally occurring (or engineered) amino
30 acid sequences linked together in a way that does not occur naturally or does not occur naturally in a host cell, which fusion protein can function as a receptor when present on

a surface of a cell. CARs of the present disclosure include an extracellular portion comprising an antigen-binding domain (*e.g.*, obtained or derived from an immunoglobulin or immunoglobulin-like molecule, such as a scFv or scTCR derived from an antibody or TCR specific for a cancer antigen, or an antigen-binding domain
5 derived or obtained from a killer immunoreceptor from an NK cell, or from another protein (natural, recombinant, or synthetic) that has, or is engineered to possess, the ability to specifically bind to an antigen) linked to a transmembrane domain and one or more intracellular signaling domains (optionally containing co-stimulatory domain(s)) (*see, e.g.*, Sadelain *et al.*, *Cancer Discov.*, 3(4):388 (2013); *see also* Harris and Kranz, 10 *Trends Pharmacol. Sci.*, 37(3):220 (2016); Stone *et al.*, *Cancer Immunol. Immunother.*, 63(11):1163 (2014)). In certain embodiments, a binding protein comprises a CAR comprising an antigen-specific TCR binding domain (*see, e.g.*, Walseng *et al.*, *Scientific Reports* 7:10713, 2017; the TCR CAR constructs and methods of which are hereby incorporated by reference in their entirety).

15 **POLYNUCLEOTIDES, VECTORS, AND HOST CELLS**

[0143] Also provided herein are polynucleotides that encode a Msln-specific binding protein, or a portion thereof, (*e.g.*, TCR variable domain) of this disclosure. It will be appreciated by those of ordinary skill in the art that, due to the degeneracy of the genetic code, there are numerous nucleotide sequences that encode a binding protein or
20 portion thereof, as described herein. Some such polynucleotides can bear limited or minimal sequence identity to the nucleotide sequence of a native, original, or identified polynucleotide sequence. Nonetheless, polynucleotides that vary due to differences in codon usage are expressly contemplated by the present disclosure.

[0144] In certain embodiments, sequences that have been codon-optimized for
25 expression in a host cell, such as a mammalian cell, are specifically contemplated. Codon optimization can be performed using known techniques and tools, *e.g.*, using the GenScript® OptimumGene™ tool. Codon-optimized sequences include sequences that are at least partially codon-optimized (*i.e.*, one or more codon is optimized for expression in the host cell) and those that are fully codon-optimized. Codon
30 optimization for expression in certain immune host cells is described in, for example, Scholten *et al.*, *Clin. Immunol.* 119:135, 2006.

[0145] Exemplary polynucleotide sequences encoding TCR chains of the present disclosure are provided in SEQ ID NOs:1-4, 9-12, 17-20, 25, and 26.

Accordingly, in certain embodiments, a polynucleotide encoding a Msln-specific binding protein comprises a polynucleotide having at least about 50% (*i.e.*, at least
5 about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the polynucleotide sequence set forth in any one of SEQ ID NOs:1-4, 9-12, 17-20, 25, and 26.

[0146] In certain embodiments, a TCR α chain-encoding polynucleotide and a TCR β chain-encoding polynucleotide are provided that have at least about 50% identity
10 to the polynucleotide sequences set forth in SEQ ID NOs: (i) 1 and 3, respectively; (ii) 2 and 4, respectively; (iii) 9 and 11, respectively; (iv) 10 and 12, respectively; (v) 17 and 19, respectively; (vi) 18 and 20, respectively; or (vii) 25 and 26, respectively.

[0147] In certain embodiments, a polynucleotide encoding two or more components or portions of a binding protein or TCR of the present disclosure comprises
15 the two or more coding sequences operatively associated in a single open reading frame. Such an arrangement can advantageously allow coordinated expression of desired gene products, such as, for example, contemporaneous expression of alpha and beta chains of a TCR, such that they are produced in about a 1:1 ratio. In certain embodiments, two or more substituent gene products of a binding protein of this
20 disclosure, such as a TCR (e.g., alpha and beta chains), are expressed as separate molecules and associate post-translationally. In further embodiments, two or more substituent gene products of a binding protein of this disclosure are expressed as a single peptide with the parts separated by a cleavable or removable segment.

[0148] For instance, self-cleaving peptides (also referred to as "ribosomal skip
25 elements") are useful for expression of separable polypeptides encoded by a single polynucleotide or vector are known in the art and include, for example, a P2A peptide encoded by a polynucleotide having the nucleotide sequence shown in any one of SEQ ID NOS:41-46, a Thosaasigna virus 2A (T2A) peptide, such as a peptide encoded by a polynucleotide having the nucleotide sequence shown in SEQ ID NO:47, an Equine
30 rhinitis A virus (ERAV) 2A (E2A) peptide, such as a peptide encoded by a polynucleotide having the nucleotide sequence shown in SEQ ID NO:48, and a Foot-

and-Mouth disease virus 2A (F2A) peptide, such as a peptide encoded by a polynucleotide having the nucleotide sequence shown in SEQ ID NO:49. Exemplary amino acid sequences of self-cleaving peptides are provided in SEQ ID NOs:113-117.

[0149] Exemplary polynucleotides encoding a Msln-specific TCR of the present disclosure, wherein a polynucleotide encoding a self-cleaving peptide is disposed between a polynucleotide encoding a TCR β chain and a polynucleotide encoding a TCR α chain, include those that encode an amino acid sequence as set forth in any one of SEQ ID NOs:8, 16, 24, and 30. Exemplary such polynucleotides have a polynucleotide sequence as set forth in any one of SEQ ID NOs:5, 13, 21, 27, and 120; in certain embodiments, a polynucleotide is provided that has at least about 50% identity to the polynucleotide sequence as set forth in any one of SEQ ID NOs:5, 13, 21, 27, and 120.

[0150] In further embodiments, a binding protein is expressed as part of a transgene construct that encodes, and/or a host immune cell containing the binding-protein-encoding polynucleotide can further encode: one or more additional accessory protein, such as a safety switch protein; a tag, a selection marker; a CD8 co receptor β chain; a CD8 co-receptor α chain or both; or any combination thereof. Polynucleotides and transgene constructs useful for encoding and expressing binding proteins and accessory components (e.g., one or more of a safety switch protein, a selection marker, CD8 co-receptor β -chain, or a CD8 co-receptor α -chain) are described in published PCT application no. WO 2018/058002, the polynucleotides, transgene constructs, and accessory components, including the nucleotide and amino acid sequences thereof, of which are hereby incorporated by reference. It will be understood that any or all of a binding protein of the present disclosure, a safety switch protein, a tag, a selection marker, a CD8 co-receptor β chain, or a CD8 co-receptor α -chain may be encoded by a single nucleic acid molecule or may be encoded by polynucleotide sequences that are, or are present on, separate nucleic acid molecules.

[0151] Exemplary safety switch proteins include, for example, a truncated EGF receptor polypeptide (huEGFRt) that is devoid of extracellular N terminal ligand binding domains and intracellular receptor tyrosine kinase activity, but that retains its native amino acid sequence, has type I transmembrane cell surface localization, and has

a conformationally intact binding epitope for pharmaceutical-grade anti-EGFR monoclonal antibody, cetuximab (Erbiximab) tEGF receptor (tEGFr; Wang et al., Blood 118:1255-1263, 2011); a caspase polypeptide (e.g., iCasp9; Straathof et al., Blood 105:4247-4254, 2005; Di Stasi et al., N. Engl. J. Med. 365:1673-1683, 2011; Zhou and
 5 Brenner, Exp. Hematol. pii:S0301-472X(16)30513-6. doi:10.1016/j.exphem.2016.07.011), RQR8 (Philip et al., Blood 124:1277-1287, 2014); a 10-amino-acid tag derived from the human c-myc protein (Myc) (Kieback et al., Proc. Natl. Acad. Sci. USA 105:623-628, 2008); and a marker/safety switch polypeptide, such as RQR (CD20 + CD34; Philip et al., 2014).

10 **[0152]** Other accessory components useful for recombinant host cells of the present disclosure comprise a tag or selection marker that allows the cells to be identified, sorted, isolated, enriched, or tracked. For example, marked cells having desired characteristics (e.g., an antigen-specific TCR and a safety switch protein) can be sorted away from unmarked cells in a sample and more efficiently activated and
 15 expanded for inclusion in a product of desired purity.

[0153] As used herein, the term "selection marker" comprises a nucleic acid construct (and the encoded gene product) that confers an identifiable change to a cell permitting detection and positive selection of immune cells transduced with a polynucleotide comprising a selection marker. RQR is a selection marker that
 20 comprises a major extracellular loop of CD20 and two minimal CD34 binding sites. In some embodiments, an RQR-encoding polynucleotide comprises a polynucleotide that encodes the 16-amino-acid CD34 minimal epitope. In some embodiments, the CD34 minimal epitope is incorporated at the amino terminal position of a CD8 co-receptor stalk domain (Q8). In further embodiments, the CD34 minimal binding site sequence
 25 can be combined with a target epitope for CD20 to form a compact marker/suicide gene for T cells (RQR8) (Philip et al., 2014, incorporated by reference herein). This construct allows for the selection of immune cells expressing the construct, with for example, CD34 specific antibody bound to magnetic beads (Miltenyi) and that utilizes clinically accepted pharmaceutical antibody, rituximab, that allows for the selective
 30 deletion of a transgene expressing engineered T cell (Philip et al., 2014).

[0154] Further exemplary selection markers also include several truncated type I transmembrane proteins normally not expressed on T cells: the truncated low-affinity nerve growth factor, truncated CD19, and truncated CD34 (see for example, Di Stasi et al., N. Engl. J. Med. 365:1673-1683, 2011; Mavilio et al., Blood 83:1988-1997, 1994; Fehse et al., Mol. Ther. 1:448-456, 2000; each incorporated herein in their entirety). A useful feature of CD19 and CD34 is the availability of the off-the-shelf Miltenyi CliniMACs™ selection system that can target these markers for clinical-grade sorting. However, CD19 and CD34 are relatively large surface proteins that may tax the vector packaging capacity and transcriptional efficiency of an integrating vector. Surface markers containing the extracellular, non-signaling domains or various proteins (e.g., CD19, CD34, LNGFR) also can be employed. Any selection marker may be employed and should be acceptable for Good Manufacturing Practices. In certain embodiments, selection markers are expressed with a polynucleotide that encodes a gene product of interest (e.g., a binding protein of the present disclosure, such as a TCR or CAR).

Further examples of selection markers include, for example, reporters such as GFP, EGFP, β -gal or chloramphenicol acetyltransferase (CAT). In certain embodiments, a selection marker, such as, for example, CD34 is expressed by a cell and the CD34 can be used to select enrich for, or isolate (e.g., by immunomagnetic selection) the transduced cells of interest for use in the methods described herein. As used herein, a CD34 marker is distinguished from an anti-CD34 antibody, or, for example, a scFv, TCR, or other antigen recognition moiety that binds to CD34.

[0155] In certain embodiments, a selection marker comprises an RQR polypeptide, a truncated low-affinity nerve growth factor (tNGFR), a truncated CD19 (tCD19), a truncated CD34 (tCD34), or any combination thereof.

[0156] In practicing various embodiments of the present disclosure, standard techniques may be used for recombinant DNA, peptide, and oligonucleotide synthesis; immunoassays; tissue culture; and transformation (e.g., electroporation and lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. These and related techniques and procedures may be generally performed according to conventional methods well-known in the art and as described in various

- general and more specific references in microbiology, molecular biology, biochemistry, molecular genetics, cell biology, virology, and immunology techniques that are cited and discussed throughout the present specification (see, e.g., Sambrook, et al, Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Current Protocols in Molecular Biology (John Wiley and Sons, updated July 2008); Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience; Glover, DNA Cloning: A Practical Approach, vol. I & II (IRL Press, Oxford Univ. Press USA, 1985); Current Protocols in Immunology (Edited by: John E. Coligan, Ada M. Kruisbeek, David H. Margulies, Ethan M. Shevach, Warren Strober 2001 John Wiley & Sons, NY, NY); Real-Time PCR: Current Technology and Applications, Edited by Julie Logan, Kirstin Edwards and Nick Saunders, 2009, Caister Academic Press, Norfolk, UK; Anand, Techniques for the Analysis of Complex Genomes, (Academic Press, New York, 1992); Guthrie and Fink, Guide to Yeast Genetics and Molecular Biology (Academic Press, New York, 1991); Oligonucleotide Synthesis (N. Gait, Ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, Eds., 1985); Transcription and Translation (B. Hames & S. Higgins, Eds., 1984); Animal Cell Culture (R. Freshney, Ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984); Next-Generation Genome Sequencing (Janitz, 2008 Wiley-VCH); PCR Protocols (Methods in Molecular Biology) (Park, Ed., 3rd Edition, 2010 Humana Press); Immobilized Cells And Enzymes (IRL Press, 1986); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller and M. P. Calos eds., 1987, Cold Spring Harbor Laboratory); Harlow and Lane, Antibodies, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1998); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (D. M. Weir and CC Blackwell, eds., 1986); Roitt, Essential Immunology, 6th Edition, (Blackwell Scientific Publications, Oxford, 1988); Embryonic Stem Cells: Methods and Protocols (Methods in Molecular Biology) (Kurstad Turksen, Ed., 2002); Embryonic Stem Cell Protocols: Volume I: Isolation and Characterization (Methods in Molecular Biology) (Kurstad Turksen, Ed., 2006);

Embryonic Stem Cell Protocols: Volume II: Differentiation Models (Methods in Molecular Biology) (Kurstad Turksen, Ed., 2006); Human Embryonic Stem Cell Protocols (Methods in Molecular Biology) (Kurstad Turksen Ed., 2006); Mesenchymal Stem Cells: Methods and Protocols (Methods in Molecular Biology) (Darwin J. Prockop, Donald G. Phinney, and Bruce A. Bunnell Eds., 2008); Hematopoietic Stem Cell Protocols (Methods in Molecular Medicine) (Christopher A. Klug, and Craig T. Jordan Eds., 2001); and Hematopoietic Stem Cell Protocols (Methods in Molecular Biology) (Kevin D. Bunting Ed., 2008) Neural Stem Cells: Methods and Protocols (Methods in Molecular Biology) (Leslie P. Weiner Ed., 2008)).

10 **[0157]** Also provided are vectors that comprise a polynucleotide according to the present disclosure. Any suitable expression vector, including an exemplary expression vector as disclosed herein, may be used. Furthermore, the expression vector may be configured to or capable of delivering the polynucleotide to a host cell.

15 **[0158]** A typical vector may include a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked, or which is capable of replication in a host organism. As discussed herein, some examples of vectors include plasmids, viral vectors, cosmids, and others.

20 **[0159]** Some vectors may be capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors), whereas other vectors may be integrated into the genome of a host cell upon introduction into the host cell and thereby replicate along with the host genome. Additionally, some vectors are capable of directing the expression of genes to which they are operatively linked (these vectors may be referred to as "expression vectors"). According to related embodiments, it is further understood
25 that, if one or more agents (*e.g.*, polynucleotides encoding a Msln-specific binding protein, or a variant thereof, as described herein) is co-administered to a subject, that each agent may reside in separate or the same vectors, and multiple vectors (each containing a different agent or the same agent) may be introduced to a cell or cell population or administered to a subject.

30 **[0160]** As used herein, "expression vector" refers to a DNA construct containing a nucleic acid molecule that is operably linked to a suitable control sequence capable of

effecting the expression of the nucleic acid molecule in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites, and sequences which control termination of transcription and translation. The vector
5 may be a plasmid, a phage particle, a virus, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may, in some instances, integrate into the genome itself. In the present specification, "plasmid," "expression plasmid," "virus" and "vector" are often used interchangeably.

10 **[0161]** In certain embodiments, a viral vector is used to introduce a non-endogenous nucleic acid sequence encoding a polypeptide specific for a target. A viral vector may be a retroviral vector or a lentiviral vector. A viral vector may also include nucleic acid sequences encoding transduction marker.

[0162] Viral vectors suitable for use with the compositions of the instant
15 disclosure include those identified for human gene therapy applications (see Pfeifer and Verma, Ann. Rev. Genomics Hum. Genet. 2: 177, 2001). Suitable viral vectors include vectors based on RNA viruses, such as retrovirus-derived vectors, e.g., Moloney murine leukemia virus (MLV)-derived vectors, and include more complex retrovirus-derived vectors, e.g., lentivirus-derived vectors. HIV-1-derived vectors belong to this category.

20 **[0163]** Viral vectors include retrovirus, adenovirus, parvovirus (e.g., adeno-associated viruses), coronavirus, negative strand RNA viruses such as orthomyxovirus (e.g., influenza virus), rhabdovirus (e.g., rabies and vesicular stomatitis virus), paramyxovirus (e.g., measles and Sendai), positive strand RNA viruses such as picornavirus and alphavirus, and double-stranded DNA viruses including adenovirus,
25 herpesvirus (e.g., Herpes Simplex virus types 1 and 2, Epstein-Barr virus, and cytomegalovirus), and poxvirus (e.g., vaccinia, fowlpox, and canarypox). Other viruses include, but are not limited to, Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus. Examples of retroviruses include avian leukosis-sarcoma, mammalian C-type, B-type viruses, D-type viruses, HTLV-BLV
30 group, lentivirus, and spumavirus (Coffin, J. M., Retroviridae: The viruses and their

replication, In Fundamental Virology, Third Edition, B. N. Fields, et al., Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

[0164] "Retroviruses" are viruses having an RNA genome, which is reverse-transcribed into DNA using a reverse transcriptase enzyme, the reverse-transcribed DNA is then incorporated into the host cell genome. "Gammaretrovirus" refers to a genus of the retroviridae family. Examples of gammaretroviruses include mouse stem cell virus, murine leukemia virus, feline leukemia virus, feline sarcoma virus, and avian reticuloendotheliosis viruses.

[0165] "Lentiviral vector," as used herein, refers to HIV-based lentiviral vectors for gene delivery, which can be integrative or non-integrative, have relatively large packaging capacity, and can transduce a range of different cell types. Lentiviral vectors are usually generated following transient transfection of three or more plasmids (packaging, envelope, and transfer) into producer cells. Like HIV, lentiviral vectors enter the target cell through the interaction of viral surface glycoproteins with receptors on the cell surface. On entry, the viral RNA undergoes reverse transcription, which is mediated by the viral reverse transcriptase complex. The product of reverse transcription is a double-stranded linear viral DNA, which is the substrate for viral integration into the DNA of infected cells. "Lentivirus" refers to a genus of retroviruses that are capable of infecting dividing and non-dividing cells. Several examples of lentiviruses include HIV (human immunodeficiency virus: including HIV type 1, and HIV type 2); equine infectious anemia virus; feline immunodeficiency virus (FIV); bovine immune deficiency virus (BIV); and simian immunodeficiency virus (SIV). Other examples include lentivirus vectors derived from HIV-2, FIV, equine infectious anemia virus, SIV, and Maedi-Visna virus (ovine lentivirus).

[0166] Methods of using retroviral and lentiviral viral vectors and packaging cells for transducing mammalian host cells with viral particles containing chimeric antigen receptor transgenes are known in the art and have been previously described, for example, in U.S. Patent No. 8,119,772; Walchli, et al., PLoS One 6:327930, 2011; Zhao, et al., J. Immunol. 174:4415, 2005; Engels, et al., Hum. Gene Ther. 14: 1155, 2003; Frecha, et al., Mol. Ther. 75: 1748, 2010; and Verhoeven, et al., Methods Mol.

Biol. 506:91, 2009. Retroviral and lentiviral vector constructs and expression systems are also commercially available.

5 **[0167]** In certain embodiments, the viral vector can be a gammaretrovirus, e.g., Moloney murine leukemia virus (MLV)-derived vectors. In other embodiments, the viral vector can be a more complex retrovirus-derived vector, e.g., a lentivirus-derived vector. HIV-1-derived vectors belong to this category. Other examples include lentivirus vectors derived from HIV-2, FIV, equine infectious anemia virus, SIV, and Maedi-Visna virus (ovine lentivirus). Methods of using retroviral and lentiviral viral vectors and packaging cells for transducing mammalian host cells with viral particles
10 containing TCR or CAR transgenes are known in the art and have been previously described, for example, in: U.S. Patent 8,119,772; Walchli et al., PLoS One 6:327930, 2011; Zhao et al., J. Immunol. 174:4415, 2005; Engels et al., Hum. Gene Ther. 14:1155, 2003; Frecha et al., Mol. Ther. 18:1748, 2010; and Verhoeyen et al., Methods Mol. Biol. 506:97, 2009. Retroviral and lentiviral vector constructs and expression systems
15 are also commercially available. Other viral vectors also can be used for polynucleotide delivery including DNA viral vectors, including, for example adenovirus-based vectors and adeno-associated virus (AAV)-based vectors; vectors derived from herpes simplex viruses (HSVs), including amplicon vectors, replication-defective HSV and attenuated HSV (Krisky et al., Gene Ther. 5:1517, 1998).

20 **[0168]** Other vectors developed for gene therapy uses can also be used with the compositions and methods of this disclosure. Such vectors include those derived from baculoviruses and α -viruses. (Jolly, D J. 1999. Emerging Viral Vectors. pp 209-40 in Friedmann T. ed. The Development of Human Gene Therapy. New York: Cold Spring Harbor Lab), or plasmid vectors (such as Sleeping Beauty or other transposon vectors).

25 **[0169]** When a viral vector genome comprises a plurality of polynucleotides to be expressed in a host cell as separate transcripts, the viral vector may also comprise additional sequences between the two (or more) transcripts allowing for bicistronic or multicistronic expression. Examples of such sequences used in viral vectors include internal ribosome entry sites (IRES), furin cleavage sites, viral 2A peptide, or any
30 combination thereof.

[0170] In certain embodiments, the polynucleotide encoding a Msln-specific binding protein may be operatively linked to one or more certain elements of a vector. For example, polynucleotide sequences that are needed to effect the expression and processing of coding sequences to which they are ligated may be operatively linked.

5 Expression control sequences may include appropriate transcription initiation, termination, promoter, and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequences); sequences that enhance protein stability; and possibly sequences that enhance protein
10 secretion. Expression control sequences may be operatively linked if they are contiguous with the gene of interest and expression control sequences that act in trans or at a distance to control the gene of interest. In some embodiments, a viral or plasmid vector further includes a transduction marker (e.g., green fluorescent protein, tEGFR, tCD19, tNGFR, etc.).

15 [0171] In certain embodiments, a vector is capable of delivering a polynucleotide construct to a host cell (e.g., a hematopoietic progenitor cell or a human immune system cell). In specific embodiments, a vector is capable of delivering a construct to human immune system cell, such as, for example, a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a dendritic
20 cell, or any combination thereof. In further embodiments, a vector is capable of delivering a construct to a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof. In some embodiments, a vector that encodes a construct of the present disclosure may further comprise a polynucleotide that encodes a nuclease that can be used to perform a chromosomal
25 knockout in a host cell (e.g., a CRISPR-Cas endonuclease or another endonuclease as disclosed herein) or that can be used to deliver a therapeutic transgene or portion thereof to a host cell in a gene therapy replacement or gene repair therapy. Alternatively, a nuclease used for a chromosomal knockout or a gene replacement or gene repair therapy can be delivered to a host cell independent of a vector that encodes
30 a construct of this disclosure.

[0172] Construction of an expression vector that is used for recombinantly producing a Msln-specific binding protein can be accomplished by using any suitable molecular biology engineering techniques known in the art, including the use of restriction endonuclease digestion, ligation, transformation, plasmid purification, and DNA sequencing, for example as described in Sambrook, et al. (1989 and 2001 editions; Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, NY) and Ausubel, et al. (Current Protocols in Molecular Biology (2003)). To obtain efficient transcription and translation, a polynucleotide in each recombinant expression construct includes at least one appropriate expression control sequence (also called a regulatory sequence), such as a leader sequence and particularly a promoter operably (i.e., operatively) linked to the nucleotide sequence encoding the protein or peptide of interest.

[0173] In certain embodiments, nucleic acid molecules encoding a binding protein specific for a Msln₂₀₋₂₈ or Msln₅₃₀₋₅₃₈ peptide are used to transfect/transduce a host cell (e.g., T cells) for use in adoptive transfer therapy. Advances in TCR sequencing have been described (e.g., Robins, et al, 2009 Blood 114:4099; Robins, et al, 2010 Sci. Translat. Med. 2:47ra64, PMID: 20811043; Robins, et al. 2011 (Sept. 10) J. Imm. Meth. Epub ahead of print, PMID: 21945395; and Warren, et al., 2011 Genome Res. 21:790) and may be employed in the course of practicing the embodiments according to the present disclosure.

[0174] Similarly, methods for transfecting/transducing T cells with desired nucleic acids have been described (e.g., US 2004/0087025) as have adoptive transfer procedures using T cells of desired antigen-specificity (e.g., Schmitt, et al., Hum. Gen. 20: 1240, 2009; Dossett, et al., Mol. Ther. 77:742, 2009; Till et al, Blood 112:2261, 2008; Wang, et al., Hum. Gene Ther. 18:112, 2007; Kuball et al, Blood 109:2331, 2007; US 2011/0243972; US 2011/0189141; and Leen, et al., Ann. Rev. Immunol. 25:243, 2007), such that adaptation of these methodologies to the presently disclosed embodiments is contemplated, based on the teachings herein, including those directed to binding proteins specific for a Msln₂₀₋₂₈ (SEQ ID NO:31) or Msln₅₃₀₋₅₃₈ (SEQ ID NO:32) peptide complexed with an HLA receptor.

[0175] The recombinant expression vectors may include, for example, lymphoid tissue-specific transcriptional regulatory elements (TRE) such as a B lymphocyte, T lymphocyte, or dendritic cell specific TRE. Lymphoid tissue specific TRE are known in the art (*see, e.g.*, Thompson, et al., Mol. Cell. Biol. 72:1043, (1992); Todd et al, J. Exp. Med. 177: 1663, (1993); and Penix, et al., J. Exp. Med. 775: 1483, (1993)).

[0176] Also provided are recombinant (e.g., modified) host cells that encode (e.g., comprise a heterologous polynucleotide encoding) and/or express a Msln-specific binding protein as disclosed herein. In some embodiments, the host cell may be a hematopoietic progenitor cell or an immune system cell as disclosed herein, such as a human immune system cell. In any of the presently disclosed embodiments, the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural kill T cell, a macrophage, a dendritic cell, or any combination thereof. Additionally, the T cell may be a naïve T cell, a central memory T cell, an effector memory T cell, a stem cell memory T cell, or any combination thereof. In certain embodiments, the host cell is modified to comprise or contain the heterologous polynucleotide using a vector as disclosed herein.

[0177] The recombinant host cell may be allogeneic, syngeneic, or autologous (e.g., to a subject that receives the host cell for a therapy). In certain embodiments wherein the host cell encodes an endogenous TCR, the heterologous binding protein or TCR expressed by the T cell is capable of more efficiently associating with a CD3 protein as compared to an endogenous TCR. In some embodiments, the Msln-specific binding protein expressed by a host T cell is able to associate with the CD3 complex and shows functional surface expression and immune activity, e.g., production of cytokines and/or killing of antigen-expressing target cells. In certain embodiments, the Msln-specific binding protein may have higher cell surface expression as compared to an endogenous TCR.

[0178] In any of the presently disclosed embodiments, a host cell, such as a host immune cell, can comprise a chromosomal gene knockout of an endogenous immune cell protein, such as, for example, PD-1, TIM3, LAG3, CTLA4, TIGIT, an HLA component, or a TCR component, or any combination thereof. As used herein, the term "chromosomal gene knockout" refers to a genetic alteration or introduced inhibitory

agent in a host cell that prevents (e.g., reduces, delays, suppresses, or abrogates) production, by the host cell, of a functionally active endogenous polypeptide product.

Alterations resulting in a chromosomal gene knockout can include, for example, introduced nonsense mutations (including the formation of premature stop codons),

- 5 missense mutations, gene deletion, and strand breaks, as well as the heterologous expression of inhibitory nucleic acid molecules that inhibit endogenous gene expression in the host cell.

[0179] A chromosomal gene knockout can be confirmed directly by DNA sequencing of the host immune cell following use of the knockout procedure or agent.

- 10 Chromosomal gene knockouts can also be inferred from the absence of gene expression (e.g., the absence of an mRNA or polypeptide product encoded by the gene) following the knockout.

[0180] In certain embodiments, a chromosomal gene knock-out or gene knock-in is made by chromosomal editing of a host cell. Chromosomal editing can be

- 15 performed using, for example, endonucleases. As used herein "endonuclease" refers to an enzyme capable of catalyzing cleavage of a phosphodiester bond within a polynucleotide chain. In certain embodiments, an endonuclease is capable of cleaving a targeted gene thereby inactivating or "knocking out" the targeted gene. An endonuclease may be a naturally occurring, recombinant, genetically modified, or
- 20 fusion endonuclease. The nucleic acid strand breaks caused by the endonuclease are commonly repaired through the distinct mechanisms of homologous recombination or non-homologous end joining (NHEJ). During homologous recombination, a donor nucleic acid molecule may be used for a donor gene "knock-in", for target gene "knock-out", and optionally to inactivate a target gene through a donor gene knock in or target
- 25 gene knock out event. NHEJ is an error-prone repair process that often results in changes to the DNA sequence at the site of the cleavage, e.g., a substitution, deletion, or addition of at least one nucleotide. NHEJ may be used to "knock-out" a target gene. Examples of endonucleases include zinc finger nucleases, TALE-nucleases, CRISPR-Cas nucleases, meganucleases, and megaTALs.

- 30 **[0181]** As used herein, a "zinc finger nuclease" (ZFN) refers to a fusion protein comprising a zinc finger DNA-binding domain fused to a non-specific DNA cleavage

domain, such as a FokI endonuclease. Each zinc finger motif of about 30 amino acids binds to about 3 base pairs of DNA, and amino acids at certain residues can be changed to alter triplet sequence specificity (see, e.g., Desjarlais et al., Proc. Natl. Acad. Sci. 90:2256-2260, 1993; Wolfe et al., J. Mol. Biol. 285:1917-1934, 1999). Multiple zinc

5 finger motifs can be linked in tandem to create binding specificity to desired DNA sequences, such as regions having a length ranging from about 9 to about 18 base pairs. By way of background, ZFNs mediate genome editing by catalyzing the formation of a site-specific DNA double strand break (DSB) in the genome, and targeted integration of a transgene comprising flanking sequences homologous to the genome at the site of

10 DSB is facilitated by homology directed repair. Alternatively, a DSB generated by a ZFN can result in knock out of target gene via repair by non-homologous end joining (NHEJ), which is an error-prone cellular repair pathway that results in the insertion or deletion of nucleotides at the cleavage site. In certain embodiments, a gene knockout comprises an insertion, a deletion, a mutation or a combination thereof, made using a

15 ZFN molecule.

[0182] As used herein, a "transcription activator-like effector nuclease" (TALEN) refers to a fusion protein comprising a TALE DNA-binding domain and a DNA cleavage domain, such as a FokI endonuclease. A "TALE DNA binding domain" or "TALE" is composed of one or more TALE repeat domains/units, each generally

20 having a highly conserved 33-35 amino acid sequence with divergent 12th and 13th amino acids. The TALE repeat domains are involved in binding of the TALE to a target DNA sequence. The divergent amino acid residues, referred to as the Repeat Variable Diresidue (RVD), correlate with specific nucleotide recognition. The natural (canonical) code for DNA recognition of these TALEs has been determined such that

25 an HD (histine-aspartic acid) sequence at positions 12 and 13 of the TALE leads to the TALE binding to cytosine (C), NG (asparagine-glycine) binds to a T nucleotide, NI (asparagine-isoleucine) to A, NN (asparagine-asparagine) binds to a G or A nucleotide, and NG (asparagine-glycine) binds to a T nucleotide. Non-canonical (atypical) RVDs are also known (see, e.g., U.S. Patent Publication No. US 2011/0301073, which atypical

30 RVDs are incorporated by reference herein in their entirety). TALENs can be used to direct site-specific double-strand breaks (DSB) in the genome of T cells. Non-

homologous end joining (NHEJ) ligates DNA from both sides of a double-strand break in which there is little or no sequence overlap for annealing, thereby introducing errors that knock out gene expression. Alternatively, homology directed repair can introduce a transgene at the site of DSB providing homologous flanking sequences are present in the transgene. In certain embodiments, a gene knockout comprises an insertion, a deletion, a mutation or a combination thereof, and made using a TALEN molecule.

[0183] As used herein, a "clustered regularly interspaced short palindromic repeats/Cas" (CRISPR/Cas) nuclease system refers to a system that employs a CRISPR RNA (crRNA)-guided Cas nuclease to recognize target sites within a genome (known as protospacers) via base-pairing complementarity and then to cleave the DNA if a short, conserved protospacer associated motif (PAM) immediately follows 3' of the complementary target sequence. CRISPR/Cas systems are classified into three types (i.e., type I, type II, and type III) based on the sequence and structure of the Cas nucleases. The crRNA-guided surveillance complexes in types I and III need multiple Cas subunits. Type II system, the most studied, comprises at least three components: an RNA-guided Cas9 nuclease, a crRNA, and a trans-acting crRNA (tracrRNA). The tracrRNA comprises a duplex forming region. A crRNA and a tracrRNA form a duplex that is capable of interacting with a Cas9 nuclease and guiding the Cas9/crRNA:tracrRNA complex to a specific site on the target DNA via Watson-Crick base-pairing between the spacer on the crRNA and the protospacer on the target DNA upstream from a PAM. Cas9 nuclease cleaves a double-stranded break within a region defined by the crRNA spacer. Repair by NHEJ results in insertions and/or deletions which disrupt expression of the targeted locus. Alternatively, a transgene with homologous flanking sequences can be introduced at the site of DSB via homology directed repair. The crRNA and tracrRNA can be engineered into a single guide RNA (sgRNA or gRNA) (see, e.g., Jinek et al., Science 337:816-21, 2012). Further, the region of the guide RNA complementary to the target site can be altered or programmed to target a desired sequence (Xie et al., PLOS One 9:e100448, 2014; U.S. Pat. Appl. Pub. No. US 2014/0068797, U.S. Pat. Appl. Pub. No. US 2014/0186843; U.S. Pat. No. 8,697,359, and PCT Publication No. WO 2015/071474; each of which is incorporated by reference). In certain embodiments, a gene knockout comprises an insertion, a

deletion, a mutation or a combination thereof, and made using a CRISPR/Cas nuclease system.

[0184] Exemplary gRNA sequences and methods of using the same to knock out endogenous genes that encode immune cell proteins include those described in Ren et al., Clin. Cancer Res. 23(9):2255-2266 (2017), the gRNAs, CAS9 DNAs, vectors, and gene knockout techniques of which are hereby incorporated by reference in their entirety.

[0185] As used herein, a "meganuclease," also referred to as a "homing endonuclease," refers to an endodeoxyribonuclease characterized by a large recognition site (double stranded DNA sequences of about 12 to about 40 base pairs).

Meganucleases can be divided into five families based on sequence and structure motifs: LAGLIDADG (SEQ ID NO:121), GIY-YIG (SEQ ID NO:122), HNH, His-Cys box and PD-(D/E)XK (SEQ ID NO:123). Exemplary meganucleases include I-SceI, I-CeuI, PI-PspI, PI-Sce, I-SceIV, I-CsmI, I-PanI, I-SceII, I-PpoI, I-SceIII, I-CreI, I-TevI, I-TevII and I-TevIII, whose recognition sequences are known (see, e.g., U.S. Patent Nos. 5,420,032 and 6,833,252; Belfort et al., Nucleic Acids Res. 25:3379-3388, 1997; Dujon et al., Gene 82:115-118, 1989; Perler et al., Nucleic Acids Res. 22:1125-1127, 1994; Jasin, Trends Genet. 12:224-228, 1996; Gimble et al., J. Mol. Biol. 263:163-180, 1996; Argast et al., J. Mol. Biol. 280:345-353, 1998).

[0186] In certain embodiments, naturally-occurring meganucleases may be used to promote site-specific genome modification of a target selected from PD-1, LAG3, TIM3, CTLA4, TIGIT, an HLA-encoding gene, or a TCR component-encoding gene.

[0187] In other embodiments, an engineered meganuclease having a novel binding specificity for a target gene is used for site-specific genome modification (see, e.g., Porteus et al., Nat. Biotechnol. 23:967-73, 2005; Sussman et al., J. Mol. Biol. 342:31-41, 2004; Epinat et al., Nucleic Acids Res. 31:2952-62, 2003; Chevalier et al., Molec. Cell 10:895-905, 2002; Ashworth et al., Nature 441:656-659, 2006; Paques et al., Curr. Gene Ther. 7:49-66, 2007; U.S. Patent Publication Nos. US 2007/0117128; US 2006/0206949; US 2006/0153826; US 2006/0078552; and US 2004/0002092). In further embodiments, a chromosomal gene knockout is generated using a homing endonuclease that has been modified with modular DNA binding domains of TALENs

to make a fusion protein known as a megaTAL. MegaTALs can be utilized to not only knock-out one or more target genes, but to also introduce (knock in) heterologous or exogenous polynucleotides when used in combination with an exogenous donor template encoding a polypeptide of interest.

- 5 **[0188]** In certain embodiments, a chromosomal gene knockout comprises an inhibitory nucleic acid molecule that is introduced into a host cell (e.g., an immune cell) comprising a heterologous polynucleotide encoding an antigen-specific receptor that specifically binds to a tumor associated antigen, wherein the inhibitory nucleic acid molecule encodes a target-specific inhibitor and wherein the encoded target-specific
- 10 inhibitor inhibits endogenous gene expression (i.e., of PD-1, TIM3, LAG3, CTLA4, TIGIT, an HLA component, or a TCR component, or any combination thereof) in the host immune cell.

- [0189]** In certain embodiments, a binding protein of interest may be knocked-in to a host cell; e.g., using any of the presently disclosed techniques or reagents useful for
- 15 knocking a polynucleotide of interest into a host cell. In some embodiments, a polynucleotide encoding a binding protein is knocked-in to a host cell and does not integrate into an endogenous chromosome, such as in the cell nucleus. In some embodiments, a polynucleotide encoding a binding protein is knocked-in to a host cell at an endogenous gene locus, optionally disrupting a coding sequence of the
- 20 endogenous locus. In certain embodiments, a polynucleotide encoding a binding protein is knocked-in to an endogenous TCR locus, thereby knocking-out endogenous TCR and knocking-in the protein of interest. *See, e.g., Eyquem et al., Nature* 543(7643):113–117 (2017).

- [0190]** In some embodiments, a polynucleotide encoding a mesothelin-specific
- 25 binding protein (e.g., a polypeptide comprising, consisting, or consisting essentially of the amino acid sequence set forth in any one or more of SEQ ID Nos:6-8, 14-16, 22-24, 28-40, 78-110, 118, 119) is knocked-in to a host cell. Binding proteins herein include a TCR alpha chain variable domain (V α) and a TCR beta chain variable domain (V β). In any of the presently disclosed embodiments, a mesothelin-specific binding protein is
- 30 capable of specifically binding to a mesothelin peptide:HLA complex, such as a mesothelin peptide:HLA-A*02:01 complex.

[0191] In some embodiments, gene knock-in may be used to introduce a polynucleotide encoding a binding protein that is capable of specifically binding to a mesothelin peptide antigen as described herein (*e.g.*, a peptide comprising, consisting, or consisting essentially of an amino acid sequence having at least about 85% (*i.e.*, at least about 86%, 85%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the amino acid sequence set forth in any one or more of SEQ ID Nos:6-8, 14-16, 22-24, 28-40, 78-110, 118, or 119).

[0192] In certain embodiments, a polynucleotide encoding a mesothelin-specific binding protein (*e.g.*, a TCR) is knocked-in to a host cell, and the host cell further comprises a polynucleotide encoding a different binding protein. In some embodiments, the different binding protein is heterologous to the host cell. In other embodiments, the different binding protein is endogenous to the host cell. In certain embodiments, the polynucleotide encoding the different binding protein is knocked-in to the host cell. In certain embodiments, the different binding protein is a binding protein specific for a different antigen (*e.g.*, a different Msln antigen, or an antigen from a different protein or target, such as, for example, BCMA, CA19-9, BRAF, CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR, FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM, MAGE-A (*e.g.*, including MAGE-A1, MAGE-A3, and MAGE-A4), KRAS, HER2, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor- or pathogen- associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, LT β R, LIFR β , LRP5, MUC1, OSMR β , TCR α , TCR β , CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD79a, CD79b, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, HA1-H, Robo1, α -fetoprotein (AFP), Frizzled, OX40, PRAME, and SSX-2. or the like). For example, a host cell can comprise a knocked-in polynucleotide encoding a binding protein that specifically binds to a Msln antigen:HLA complex and a (*e.g.*, knocked-in) polynucleotide encoding a binding protein (*e.g.*, a TCR or a CAR) that specifically binds to a CA19-9 antigen.

[0193] In certain embodiments, a host immune cell encoding and/or expressing a Msln-specific binding protein of the present disclosure is capable of preferentially migrating to or localizing in vivo in a target tissue that expresses a cognate Msln antigen, such as a tumor, but is present at a statistically significant reduced amount in non-adjacent tissue of the same type. By way of illustration, a host immune cell may be present in a lung tumor (e.g., as determined using deep sequencing for the TCR V-region of the encoded binding protein), but is present at a lower level, or not at all, in tissue of the same lung that is not adjacent to the tumor. In some embodiments, non-adjacent tissue comprises or refers to tissue that is removed from a diseased or malignant tissue by at least 3 cm.

[0194] In certain embodiments, a host cell is enriched in a composition of cells, such as may be administered to a subject. As used herein, "enriched" or "depleted" with respect to amounts of cell types in a mixture refers to an increase in the number of the "enriched" type, a decrease in the number of the "depleted" cells, or both, in a mixture of cells resulting from one or more enriching or depleting processes or steps. Thus, depending upon the source of an original population of cells subjected to an enriching process, a mixture or composition may contain 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more (in number or count) of the "enriched" cells. Cells subjected to a depleting process can result in a mixture or composition containing 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% percent or less (in number or count) of the "depleted" cells. In certain embodiments, amounts of a certain cell type in a mixture will be enriched and amounts of a different cell type will be depleted, such as enriching for CD4⁺ cells while depleting CD8⁺ cells, or enriching for CD62L⁺ cells while depleting CD62L[−] cells, or combinations thereof.

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[0195] In another aspect, the present disclosure provides methods treating a subject in need thereof (*i.e.*, having or suspected of having a disease or disorder associated with a mesothelin antigen by administering to the subject an effective amount of a composition (*e.g.*, binding protein, recombinant host cell, polynucleotide, vector, or related composition) as described herein. Also provided are such

compositions for use in treating such a disease, or for the manufacture of a medicament for the treatment of such a disease. Such diseases include various forms of proliferative or hyperproliferative disorders, such as solid cancers and hematological malignancies.

[0196] "Treat" or "treatment" or "ameliorate" refers to medical management of a disease, disorder, or condition of a subject (*e.g.*, a human or non-human mammal, such as a primate, horse, cat, dog, goat, mouse, or rat). In general, an appropriate dose or treatment regimen comprising a host cell expressing a binding protein of the present disclosure, and optionally an adjuvant, is administered in an amount sufficient to elicit a therapeutic or prophylactic benefit. Therapeutic or prophylactic/preventive benefit includes improved clinical outcome; lessening or alleviation of symptoms associated with a disease; decreased occurrence of symptoms; improved quality of life; longer disease-free status; diminishment of extent of disease; stabilization of disease state; delay of disease progression; remission; survival; prolonged survival; or any combination thereof.

[0197] As used herein, the terms "adoptive immune therapy" or "adoptive immunotherapy" and "adoptive cell therapy" refer to administration of naturally occurring or genetically engineered, disease-antigen-specific immune cells (*e.g.*, T cells). Adoptive cellular immunotherapy may be autologous (immune cells are from the recipient), allogeneic (immune cells are from a donor of the same species) or syngeneic (immune cells are from a donor genetically identical to the recipient).

[0198] A "therapeutically effective amount" or "effective amount" of a binding protein or host cell of this disclosure, refers to an amount of binding proteins or host cells sufficient to result in a therapeutic effect, including improved clinical outcome; lessening or alleviation of symptoms associated with a disease; decreased occurrence of symptoms; improved quality of life; longer disease-free status; diminishment of extent of disease; stabilization of disease state; delay of disease progression; remission; survival; or prolonged survival in a statistically significant manner. When referring to an individual active ingredient or a cell expressing a single active ingredient, administered alone, a therapeutically effective amount refers to the effects of that ingredient or cell expressing that ingredient alone. When referring to a combination, a therapeutically effective amount refers to the combined amounts of active ingredients or

combined adjunctive active ingredient with a cell expressing an active ingredient that results in a therapeutic effect, whether administered serially or simultaneously. A combination may also be a cell expressing more than one active ingredient, such as two different binding proteins that specifically bind an antigen, or a fusion protein of the present disclosure.

[0199] As used herein, "statistically significant" refers to a p-value of 0.050 or less when calculated using the Student's t-test and indicates that it is unlikely that a particular event or result being measured has arisen by chance.

[0200] As used herein, "hyperproliferative disorder" refers to excessive growth or proliferation as compared to a normal or undiseased cell. Exemplary hyperproliferative disorders include tumors, cancers, neoplastic tissue, carcinoma, sarcoma, malignant cells, pre malignant cells, as well as non-neoplastic or non-malignant hyperproliferative disorders (e.g., adenoma, fibroma, lipoma, leiomyoma, hemangioma, fibrosis, restenosis, as well as autoimmune diseases such as rheumatoid arthritis, osteoarthritis, psoriasis, inflammatory bowel disease, or the like). Certain diseases that involve abnormal or excessive growth that occurs more slowly than in the context of a hyperproliferative disease can be referred to as "proliferative diseases", and include certain tumors, cancers, neoplastic tissue, carcinoma, sarcoma, malignant cells, pre malignant cells, as well as non-neoplastic or non-malignant disorders.

[0201] Furthermore, "cancer" may refer to any accelerated proliferation of cells, including solid tumors, ascites tumors, blood or lymph or other malignancies; connective tissue malignancies; metastatic disease; minimal residual disease following transplantation of organs or stem cells; multi-drug resistant cancers, primary or secondary malignancies, angiogenesis related to malignancy, or other forms of cancer.

[0202] The presently disclosed binding proteins, host cells, polynucleotides, vectors, and compositions are useful to treat or manufacture a medicament for the treatment a cancer wherein a Msln₂₀₋₂₈ peptide is expressed on a tumor cell of the cancer, and/or wherein a Msln₅₃₀₋₅₃₈ peptide is expressed on a tumor cell of the cancer; exemplary cancers for treatment include mesothelioma, pancreatic cancer, ovarian cancer, and lung cancer.

[0203] In certain embodiments, the presently disclosed binding proteins, host cells, polynucleotides, vectors, and compositions are useful for treating and/or in the manufacture of a medicament for treating a cancer, such as a solid cancer or a hematological malignancy. In certain embodiments, the solid cancer is selected from or
 5 comprises biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, mesothelioma,
 10 malignant melanoma, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or uterine cancer.

[0204] In certain embodiments, a cancer treatable according to the presently disclosed methods and uses comprises a carcinoma, a sarcoma, a glioma, a lymphoma, a leukemia, a myeloma, or any combination thereof. In certain embodiments, cancer comprises a cancer of the head or neck, melanoma, pancreatic cancer, cholangiocarcinoma, hepatocellular cancer, breast cancer including triple-negative
 20 breast cancer (TNBC), gastric cancer, non-small-cell lung cancer, prostate cancer, esophageal cancer, mesothelioma, small-cell lung cancer, colorectal cancer, glioblastoma, or any combination thereof. In certain embodiments, a cancer comprises Askin's tumor, sarcoma botryoides, chondrosarcoma, Ewing's sarcoma, PNET, malignant hemangioendothelioma, malignant schwannoma, osteosarcoma, alveolar soft
 25 part sarcoma, angiosarcoma, cystosarcoma phyllodes, dermatofibrosarcoma protuberans (DFSP), desmoid tumor, desmoplastic small round cell tumor, epithelioid sarcoma, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, fibrosarcoma, gastrointestinal stromal tumor (GIST), hemangiopericytoma, hemangiosarcoma, Kaposi's sarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma,
 30 undifferentiated pleomorphic sarcoma, malignant peripheral nerve sheath tumor (MPNST), neurofibrosarcoma, rhabdomyosarcoma, synovial sarcoma, undifferentiated

pleomorphic sarcoma, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, linitis plastica, vipoma, cholangiocarcinoma, hepatocellular carcinoma, adenoid cystic carcinoma, renal cell carcinoma, Grawitz tumor, ependymoma, astrocytoma, oligodendroglioma, brainstem glioma, optic nerve glioma, a mixed glioma, Hodgkin's lymphoma, a B-cell lymphoma, non-Hodgkin's lymphoma (NHL), Burkitt's lymphoma, small lymphocytic lymphoma (SLL), diffuse large B-cell lymphoma, follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, and mantle cell lymphoma, Waldenström's macroglobulinemia, CD37+ dendritic cell lymphoma, lymphoplasmacytic lymphoma, splenic marginal zone lymphoma, extra-nodal marginal zone B-cell lymphoma of mucosa-associated (MALT) lymphoid tissue, nodal marginal zone B-cell lymphoma, mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, primary effusion lymphoma, adult T-cell lymphoma, extranodal NK/T-cell lymphoma, nasal type, enteropathy-associated T-cell lymphoma, hepatosplenic T-cell lymphoma, blastic NK cell lymphoma, Sezary syndrome, angioimmunoblastic T cell lymphoma, anaplastic large cell lymphoma, or any combination thereof.

[0205] In certain embodiments, the cancer comprises a solid tumor. In some embodiments, the solid tumor is a sarcoma or a carcinoma. In certain embodiments, the solid tumor is selected from: chondrosarcoma; fibrosarcoma (fibroblastic sarcoma); Dermatofibrosarcoma protuberans (DFSP); osteosarcoma; rhabdomyosarcoma; Ewing's sarcoma; a gastrointestinal stromal tumor; Leiomyosarcoma; angiosarcoma (vascular sarcoma); Kaposi's sarcoma; liposarcoma; pleomorphic sarcoma; or synovial sarcoma.

[0206] In certain embodiments, the solid tumor is selected from a lung carcinoma (e.g., Adenocarcinoma, Squamous Cell Carcinoma (Epidermoid Carcinoma); Squamous cell carcinoma; Adenocarcinoma; Adenosquamous carcinoma; anaplastic carcinoma; Large cell carcinoma; Small cell carcinoma; a breast carcinoma (e.g., Ductal Carcinoma in situ (non-invasive), Lobular carcinoma in situ (non-invasive), Invasive Ductal Carcinoma, Invasive lobular carcinoma, Non-invasive Carcinoma); a liver carcinoma (e.g., Hepatocellular Carcinoma, Cholangiocarcinomas or Bile Duct Cancer); Large-cell undifferentiated carcinoma, Bronchioalveolar carcinoma); an ovarian carcinoma (e.g., Surface epithelial-stromal tumor (Adenocarcinoma) or ovarian

epithelial carcinoma (which includes serous tumor, endometrioid tumor and mucinous cystadenocarcinoma), Epidermoid (Squamous cell carcinoma), Embryonal carcinoma and choriocarcinoma (germ cell tumors)); a kidney carcinoma (e.g., Renal adenocarcinoma, hypernephroma, Transitional cell carcinoma (renal pelvis), Squamous cell carcinoma, Bellini duct carcinoma, Clear cell adenocarcinoma, Transitional cell carcinoma, Carcinoid tumor of the renal pelvis); an adrenal carcinoma (e.g., Adrenocortical carcinoma), a carcinoma of the testis (e.g., Germ cell carcinoma (Seminoma, Choriocarcinoma, Embryonal carcinoma, Teratocarcinoma), Serous carcinoma); Gastric carcinoma (e.g., Adenocarcinoma); an intestinal carcinoma (e.g., Adenocarcinoma of the duodenum); a colorectal carcinoma; or a skin carcinoma (e.g., Basal cell carcinoma, Squamous cell carcinoma). In certain embodiments, the solid tumor is an ovarian carcinoma, an ovarian epithelial carcinoma, a cervical adenocarcinoma or small cell carcinoma, a pancreatic carcinoma, a colorectal carcinoma (e.g., an adenocarcinoma or squamous cell carcinoma), a lung carcinoma, a breast ductal carcinoma, or an adenocarcinoma of the prostate.

[0207] In any of the presently disclosed embodiments, the host cell is an allogeneic cell, a syngeneic cell, or an autologous cell. Subjects that can be treated by the present invention are, in general, human and other primate subjects, such as monkeys and apes for veterinary medicine purposes. In any of the aforementioned embodiments, the subject may be a human subject. The subjects can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. Cells according to the present disclosure may be administered in a manner appropriate to the disease, condition, or disorder to be treated as determined by persons skilled in the medical art. In any of the above embodiments, a cell comprising a fusion protein as described herein is administered intravenously, intraperitoneally, intratumorally, into the bone marrow, into a lymph node, or into the cerebrospinal fluid so as to encounter the tagged cells to be ablated. An appropriate dose, suitable duration, and frequency of administration of the compositions will be determined by such factors as a condition of the patient; size, type, and severity of the disease, condition, or disorder; the undesired type or level or activity of the tagged cells, the particular form of the active ingredient; and the method of administration.

[0208] In general, an appropriate dosage and treatment regimen provides the active molecules or cells in an amount sufficient to provide a benefit. Such a response can be monitored by establishing an improved clinical outcome (e.g., more frequent remissions, complete or partial, or longer disease-free survival) in treated subjects as compared to non-treated subjects. Increases in preexisting immune responses to a tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which are routine.

[0209] For prophylactic use, a dose should be sufficient to prevent, delay the onset of, or diminish the severity of a disease associated with disease or disorder. Prophylactic benefit of the immunogenic compositions administered according to the methods described herein can be determined by performing pre-clinical (including in vitro and in vivo animal studies) and clinical studies and analyzing data obtained therefrom by appropriate statistical, biological, and clinical methods and techniques, all of which can readily be practiced by a person skilled in the art.

[0210] In the case of an adoptive cell therapy, an effective dose is an amount of host cells encoding or expressing a Msln-specific binding protein used in adoptive transfer that is capable of producing a clinically desirable result (i.e., a sufficient amount to induce or enhance a specific T cell immune response against cells expressing an Msln-specific antigen response, e.g., a cytotoxic T cell response, in a statistically significant manner) in a treated human or non-human mammal. In particular embodiments, T cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof.

[0211] Also contemplated are pharmaceutical compositions (compositions) that comprise a Msln-specific binding protein, host (i.e., modified) immune cell, polynucleotide, or vector as disclosed herein and a pharmaceutically acceptable carrier, diluents, or excipient. The term "pharmaceutically acceptable excipient or carrier" or "physiologically acceptable excipient or carrier" refer to biologically compatible vehicles, e.g., physiological saline, which are described in greater detail herein, that are suitable for administration to a human or other non-human mammalian subject and generally recognized as safe or not causing a serious adverse event. Suitable excipients

include water, saline, dextrose, glycerol, or the like and combinations thereof. In embodiments, compositions comprising fusion proteins or host cells as disclosed herein further comprise a suitable infusion media. Suitable infusion media can be any isotonic medium formulation, typically normal saline, Normosol R (Abbott) or Plasma-Lyte A (Baxter), 5% dextrose in water, Ringer's lactate can be utilized. An infusion medium can be supplemented with human serum albumin or other human serum components.

[0212] Pharmaceutical compositions may be administered in a manner appropriate to the disease or condition to be treated (or prevented) as determined by persons skilled in the medical art. An appropriate dose and a suitable duration and frequency of administration of the compositions will be determined by such factors as the health condition of the patient, size of the patient (i.e., weight, mass, or body area), the type and severity of the patient's condition, the particular form of the active ingredient, and the method of administration. In general, an appropriate dose and treatment regimen provide the composition(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit (such as described herein, including an improved clinical outcome, such as more frequent complete or partial remissions, or longer disease-free and/or overall survival, or a lessening of symptom severity).

[0213] Also provided herein are unit doses that comprise an effective amount of a modified immune cell or of a composition comprising the modified immune cell. In certain embodiments, a unit dose comprises (i) a composition comprising at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% modified CD4⁺ T cells, combined with (ii) a composition comprising at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% modified CD8⁺ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells (i.e., has less than about 50%, less than about 40%, less than about 30%, less than about 20%, less than about 10%, less than about 5%, or less than about 1% the population of naïve T cells present in a unit dose as compared to a patient sample having a comparable number of PBMCs).

[0214] In some embodiments, a unit dose comprises (i) a composition comprising at least about 50% modified CD4⁺ T cells, combined with (ii) a composition comprising at least about 50% modified CD8⁺ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In

5 further embodiments, a unit dose comprises (i) a composition comprising at least about 60% modified CD4⁺ T cells, combined with (ii) a composition comprising at least about 60% modified CD8⁺ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In still further embodiments, a unit dose comprises (i) a composition comprising at least about 70% engineered CD4⁺ T

10 cells, combined with (ii) a composition comprising at least about 70% engineered CD8⁺ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In some embodiments, a unit dose comprises (i) a composition comprising at least about 80% modified CD4⁺ T cells, combined with (ii) a composition comprising at least about 80% modified CD8⁺ T cells, in about a 1:1

15 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In some embodiments, a unit dose comprises (i) a composition comprising at least about 85% modified CD4⁺ T cells, combined with (ii) a composition comprising at least about 85% modified CD8⁺ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells. In some embodiments, a unit dose

20 comprises (i) a composition comprising at least about 90% modified CD4⁺ T cells, combined with (ii) a composition comprising at least about 90% modified CD8⁺ T cells, in about a 1:1 ratio, wherein the unit dose contains a reduced amount or substantially no naïve T cells.

[0215] The amount of cells in a composition or unit dose is at least one cell (for

25 example, one recombinant CD8⁺ T cell subpopulation (*e.g.*, optionally comprising memory and/or naïve CD8⁺ T cells); one recombinant CD4⁺ T cell subpopulation (*e.g.*, optionally comprising memory and/or naïve CD4⁺ T cells)) or is more typically greater than 10² cells, for example, up to 10⁴, up to 10⁵, up to 10⁶, up to 10⁷, up to 10⁸, up to 10⁹, or more than 10¹⁰ cells. In certain embodiments, the cells are administered in a

30 range from about 10⁴ to about 10¹⁰ cells/m², preferably in a range of about 10⁵ to about 10⁹ cells/m². In some embodiments, an administered dose comprises up to about 3.3 x

10^5 cells/kg. In some embodiments, an administered dose comprises up to about 1×10^6 cells/kg. In some embodiments, an administered dose comprises up to about 3.3×10^6 cells/kg. In some embodiments, an administered dose comprises up to about 1×10^7 cells/kg. In certain embodiments, a recombinant host cell is administered to a subject at
 5 a dose comprising up to about 5×10^4 cells/kg, 5×10^5 cells/kg, 5×10^6 cells/kg, or up to about 5×10^7 cells/kg. In certain embodiments, a recombinant host cell is administered to a subject at a dose comprising at least about 5×10^4 cells/kg, 5×10^5 cells/kg, 5×10^6 cells/kg, or up to about 5×10^7 cells/kg. The number of cells will depend upon the ultimate use for which the composition is intended as well the type of
 10 cells included therein. For example, cells modified to express or encode a binding protein will comprise a cell population containing at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more of such cells. For uses provided herein, cells are generally in a volume of a liter or less, 500 mls or less, 250 mls or less, or 100 mls or less. In embodiments, the density of the desired cells is
 15 typically greater than 10^4 cells/ml and generally is greater than 10^7 cells/ml, generally 10^8 cells/ml or greater. The cells may be administered as a single infusion or in multiple infusions over a range of time. In certain embodiments, a clinically relevant number of cells can be apportioned into multiple infusions that cumulatively equal or exceed 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , or 10^{11} cells. In certain embodiments, a unit dose of the
 20 cells can be co-administered with (*e.g.*, simultaneously or contemporaneously with) hematopoietic stem cells from an allogeneic donor. In some embodiments, one or more of the cells comprised in the unit dose is autologous to the subject.

[0216] It will be appreciated that a unit dose, composition, or treatment regimen of the present disclosure may comprise a Msln-specific binding protein or recombinant
 25 host cell as described herein, and also comprise an (*e.g.*, modified) immune cell expressing a binding protein specific for a different antigen (*e.g.*, a different Msln antigen, or an antigen from a different protein or target, such as, for example, BCMA, CA19-9, BRAF, CD3, CEACAM6, c-Met, EGFR, EGFRvIII, ErbB2, ErbB3, ErbB4, EphA2, IGF1R, GD2, O-acetyl GD2, O-acetyl GD3, GHRHR, GHR, FLT1, KDR,
 30 FLT4, CD44v6, CD151, CA125, CEA, CTLA-4, GITR, BTLA, TGFBR2, TGFBR1, IL6R, gp130, Lewis A, Lewis Y, TNFR1, TNFR2, PD1, PD-L1, PD-L2, HVEM,

MAGE-A (e.g., including MAGE-A1, MAGE-A3, and MAGE-A4), KRAS, HER2, NY-ESO-1, PSMA, RANK, ROR1, TNFRSF4, CD40, CD137, TWEAK-R, HLA, tumor- or pathogen- associated peptide bound to HLA, hTERT peptide bound to HLA, tyrosinase peptide bound to HLA, LT β R, LIFR β , LRP5, MUC1, OSMR β , TCR α ,
 5 TCR β , CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD79a, CD79b, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, WT-1, HA1-H, Robo1, α -fetoprotein (AFP), Frizzled, OX40, PRAME, and SSX-2. or the like). For example, a unit dose or therapeutic regimen can comprise modified CD4+ T cells expressing a binding protein that specifically binds to a Msln antigen:HLA
 10 complex and modified CD4+ T cells (and/or modified CD8+ T cells) expressing a binding protein (e.g., a TCR or a CAR) that specifically binds to a CA19-9 antigen.

[0217] In any of the embodiments described herein, a unit dose comprises equal, or approximately equal, numbers of engineered CD45RA- CD3+ CD8+ and modified CD45RA- CD3+ CD4+ TM cells.

15 **[0218]** The pharmaceutical compositions described herein may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers may be frozen to preserve the stability of the formulation until infusion into the patient.

[0219] As used herein, administration of a composition refers to delivering the same to a subject, regardless of the route or mode of delivery, such as, for example,
 20 intravenous, oral vaginal, rectal, subcutaneous, or the like. Administration may be effected continuously or intermittently, and parenterally. Administration may be for treating a subject already confirmed as having a recognized condition, disease or disease state, or for treating a subject susceptible to or at risk of developing such a condition, disease or disease state. Co-administration with an adjunctive therapy may
 25 include simultaneous and/or sequential delivery of multiple agents in any order and on any dosing schedule (e.g., recombinant host cells with one or more cytokines; immunosuppressive therapy such as calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof).

[0220] If the subject composition is administered parenterally, the composition
 30 may also include sterile aqueous or oleaginous solution or suspension. Suitable non-toxic parenterally acceptable diluents or solvents include water, Ringer's solution,

isotonic salt solution, 1,3-butanediol, ethanol, propylene glycol, or polyethylene glycols in mixtures with water. Aqueous solutions or suspensions may further include one or more buffering agents, such as sodium acetate, sodium citrate, sodium borate, or sodium tartrate. Of course, any material used in preparing any dosage unit formulation should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations. Dosage unit form, as used herein, refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit may contain a predetermined quantity of recombinant cells or active compound calculated to produce the desired therapeutic effect in association with an appropriate pharmaceutical carrier.

[0221] In certain embodiments, a plurality of doses of a composition described herein (e.g., a recombinant host cell) is administered to the subject, which may be administered at intervals between administrations of about two to about four weeks.

[0222] Treatment or prevention methods of this disclosure may be administered to a subject as part of a treatment course or regimen, which may comprise additional treatments prior to, or after, administration of the instantly disclosed unit doses, cells, or compositions. For example, in certain embodiments, a subject receiving a unit dose of the (e.g., a recombinant host cell is receiving or had previously received a hematopoietic cell transplant (HCT; including myeloablative and non-myeloablative HCT). Techniques and regimens for performing HCT are known in the art and can comprise transplantation of any suitable donor cell, such as a cell derived from umbilical cord blood, bone marrow, or peripheral blood, a hematopoietic stem cell, a mobilized stem cell, or a cell from amniotic fluid. Accordingly, in certain embodiments, a recombinant host cell of the present disclosure can be administered with or shortly after hematopoietic stem cells in a modified HCT therapy. In some embodiments, the HCT comprises a donor hematopoietic cell comprising a chromosomal knockout of a gene that encodes an HLA component, a chromosomal knockout of a gene that encodes a TCR component, or both.

[0223] The level of a CTL immune response may be determined by any one of numerous immunological methods described herein and routinely practiced in the art.

The level of a CTL immune response may be determined prior to and following administration of any one of the herein described Msln-specific binding proteins (or a host cell encoding and/or expressing the same) or immunogenic compositions.

Cytotoxicity assays for determining CTL activity may be performed using any one of several techniques and methods routinely practiced in the art (see, e.g., Henkart, et al., "Cytotoxic T-Lymphocytes" in Fundamental Immunology, Paul (ed.) (2003 Lippincott Williams & Wilkins, Philadelphia, PA), pages 1127–50, and references cited therein).

[0224] Antigen-specific T cell responses are typically determined by comparisons of observed T cell responses according to any of the herein described T cell functional parameters (e.g., proliferation, cytokine release, CTL activity, altered cell surface marker phenotype, etc.) that may be made between T cells that are exposed to a cognate antigen in an appropriate context (e.g., the antigen used to prime or activate the T cells, when presented by immunocompatible antigen-presenting cells) and T cells from the same source population that are exposed instead to a structurally distinct or irrelevant control antigen. A response to the cognate antigen that is greater, with statistical significance, than the response to the control antigen signifies antigen-specificity.

[0225] A biological sample may be obtained from a subject for determining the presence and level of an immune response to a Msln peptide as described herein. A "biological sample" as used herein may be a blood sample (from which serum or plasma may be prepared), biopsy specimen, body fluids (e.g., lung lavage, ascites, mucosal washings, synovial fluid, etc.), bone marrow, lymph nodes, tissue explant, organ culture, or any other tissue or cell preparation from the subject or a biological source. Biological samples may also be obtained from the subject prior to receiving any immunogenic composition, which biological sample is useful as a control for establishing baseline (i.e., pre-immunization) data.

[0226] In some embodiments, the subject receiving the subject composition has previously received chemotherapy, such as a lymphodepleting chemotherapy. In further embodiments, the lymphodepleting chemotherapy comprises cyclophosphamide, fludarabine, anti-thymocyte globulin, oxaliplatin, or a combination thereof. In some embodiments, the subject composition has previously received radiation therapy,

immunotherapy comprising a cytokine, an antibody, an antibody-drug conjugate, or Fc fusion protein, antisense nucleotide therapy, gene therapy, a vaccine, or surgery, or any combination thereof.

[0227] Methods and uses according to this disclosure may further include
5 administering one or more additional agents to treat the disease or disorder in a combination therapy. For example, in certain embodiments, a combination therapy comprises administering a composition (e.g., any one or more binding protein, modified host cell encoding and/or expressing the same, polynucleotide, vector) with (concurrently, simultaneously, or sequentially) an immune checkpoint inhibitor. In
10 some embodiments, a combination therapy comprises administering a composition of the present disclosure with an agonist of a stimulatory immune checkpoint agent. In further embodiments, a combination therapy comprises administering a composition of the present disclosure with a secondary therapy, such as chemotherapeutic agent, a radiation therapy, a surgery, an antibody, antibody drug conjugate, a cytokine, an
15 antisense therapy, a gene therapy, a vaccine, or any combination thereof.

[0228] As used herein, the term "immune suppression agent" or "immunosuppression agent" refers to one or more cells, proteins, molecules, compounds or complexes providing inhibitory signals to assist in controlling or suppressing an immune response. For example, immune suppression agents include
20 those molecules that partially or totally block immune stimulation; decrease, prevent or delay immune activation; or increase, activate, or up regulate immune suppression. Exemplary immunosuppression agents to target (e.g., with an immune checkpoint inhibitor) include PD-1, PD-L1, PD-L2, LAG3, CTLA4, B7-H3, B7-H4, CD244/2B4, HVEM, BTLA, CD160, TIM3, GAL9, KIR, PVR1G (CD112R), PVRL2, adenosine,
25 A2aR, immunosuppressive cytokines (e.g., IL-10, IL-4, IL-1RA, IL-35), IDO, arginase, VISTA, TIGIT, LAIR1, CEACAM-1, CEACAM-3, CEACAM-5, Treg cells, or any combination thereof.

[0229] An immune suppression agent inhibitor (also referred to as an immune checkpoint inhibitor) may be a compound, an antibody, an antibody fragment or fusion
30 polypeptide (e.g., Fc fusion, such as CTLA4-Fc or LAG3-Fc), an antisense molecule, a ribozyme or RNAi molecule, or a low molecular weight organic molecule. In any of

the embodiments disclosed herein, a method may comprise a composition of the present disclosure with one or more inhibitor of any one of the following immune suppression components, singly or in any combination.

- [0230]** Accordingly, in certain embodiments, treatment methods according to
- 5 the present disclosure may further include administering a PD-1 inhibitor to the subject. The PD-1 inhibitor may include nivolumab (OPDIVO®); pembrolizumab (KEYTRUDA®); ipilimumab + nivolumab (YERVOY® + OPDIVO®); cemiplimab; IBI-308; nivolumab + relatlimab; BCD-100; camrelizumab; JS-001; spartalizumab; tislelizumab; AGEN-2034; BGBA-333 + tislelizumab; CBT-501; dostarlimab;
- 10 durvalumab + MEDI-0680; JNJ-3283; pazopanib hydrochloride + pembrolizumab; pidilizumab; REGN-1979 + cemiplimab; ABBV-181; ADUS-100 + spartalizumab; AK-104; AK-105; AMP-224; BAT-1306; BI-754091; CC-90006; cemiplimab + REGN-3767; CS-1003; GLS-010; LZM-009; MEDI-5752; MGD-013; PF-06801591; Sym-021; tislelizumab + pamiparib; XmAb-20717; AK-112; ALPN-202; AM-0001; an
- 15 antibody to antagonize PD-1 for Alzheimer's disease; BH-2922; BH-2941; BH-2950; BH-2954; a biologic to antagonize CTLA-4 and PD-1 for solid tumor; a bispecific monoclonal antibody to target PD-1 and LAG-3 for oncology; BLSM-101; CB-201; CB-213; CBT-103; CBT-107; a cellular immunotherapy + PD-1 inhibitor; CX-188; HAB-21; HEISCOIII-003; IKT-202; JTX-4014; MCLA-134; MD-402; mDX-400;
- 20 MGD-019; a monoclonal antibody to antagonize PDCD1 for oncology; a monoclonal antibody to antagonize PD-1 for oncology; an oncolytic virus to inhibit PD-1 for oncology; OT-2; PD-1 antagonist + ropeginterferon alfa-2b; PEGMP-7; PRS-332; RXI-762; STIA-1110; TSR-075; a vaccine to target HER2 and PD-1 for oncology; a vaccine to target PD-1 for oncology and autoimmune disorders; XmAb-23104; an antisense
- 25 oligonucleotide to inhibit PD-1 for oncology; AT-16201; a bispecific monoclonal antibody to inhibit PD-1 for oncology; IMM-1802; monoclonal antibodies to antagonize PD-1 and CTLA-4 for solid tumor and hematological tumor; nivolumab biosimilar; a recombinant protein to agonize CD278 and CD28 and antagonize PD-1 for oncology; a recombinant protein to agonize PD-1 for autoimmune disorders and inflammatory
- 30 disorders; SNA-01; SSI-361; YBL-006; AK-103; JY-034; AUR-012; BGB-108; drug to inhibit PD-1, Gal-9, and TIM-3 for solid tumor; ENUM-244C8; ENUM-388D4; MEDI-

0680; monoclonal antibodies to antagonize PD-1 for metastatic melanoma and metastatic lung cancer; a monoclonal antibody to inhibit PD-1 for oncology; monoclonal antibodies to target CTLA-4 and PD-1 for oncology; a monoclonal antibody to antagonize PD-1 for NSCLC; monoclonal antibodies to inhibit PD-1 and
5 TIM-3 for oncology; a monoclonal antibody to inhibit PD-1 for oncology; a recombinant protein to inhibit PD-1 and VEGF-A for hematological malignancies and solid tumor; a small molecule to antagonize PD-1 for oncology; Sym-016; inebilizumab + MEDI-0680; a vaccine to target PDL-1 and IDO for metastatic melanoma; an anti-PD-1 monoclonal antibody + a cellular immunotherapy for glioblastoma; an antibody to
10 antagonize PD-1 for oncology; monoclonal antibodies to inhibit PD-1/PD-L1 for hematological malignancies and bacterial infections; a monoclonal antibody to inhibit PD-1 for HIV; and/or a small molecule to inhibit PD-1 for solid tumor.

[0231] In certain embodiments, a composition of the present disclosure is used in combination with a LAG3 inhibitor, such as LAG525, IMP321, IMP701, 9H12,
15 BMS-986016, or any combination thereof.

[0232] In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of CTLA4. In particular embodiments, a composition is used in combination with a CTLA4 specific antibody or binding fragment thereof, such as ipilimumab, tremelimumab, CTLA4-Ig fusion proteins (e.g., abatacept,
20 belatacept), or any combination thereof.

[0233] In certain embodiments, a composition of the present disclosure is used in combination with a B7-H3 specific antibody or binding fragment thereof, such as enoblituzumab (MGA271), 376.96, or both. A B7-H4 antibody binding fragment may be a scFv or fusion protein thereof, as described in, for example, Dangaj et al., Cancer
25 Res. 73:4820, 2013, as well as those described in U.S. Patent No. 9,574,000 and PCT Patent Publication Nos. WO /201640724A1 and WO 2013/025779A1.

[0234] In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of CD244.

[0235] In certain embodiments, a composition of the present disclosure is used
30 in combination with an inhibitor of BLTA, HVEM, CD160, or any combination thereof.

Anti CD-160 antibodies are described in, for example, PCT Publication No. WO 2010/084158.

[0236] In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of TIM3.

5 **[0237]** In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of Gal9.

[0238] In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of adenosine signaling, such as a decoy adenosine receptor.

10 **[0239]** In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of A2aR.

[0240] In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of KIR, such as lirilumab (BMS-986015).

[0241] In certain embodiments, a composition of the present disclosure is used
15 in combination with an inhibitor of an inhibitory cytokine (typically, a cytokine other than TGF β) or Treg development or activity.

[0242] In certain embodiments, a composition of the present disclosure is used in combination with an IDO inhibitor, such as levo-1-methyl tryptophan, epacadostat (INCB024360; Liu et al., Blood 115:3520-30, 2010), ebselen (Terentis et al., Biochem.
20 49:591-600, 2010), indoximod, NLG919 (Mautino et al., American Association for Cancer Research 104th Annual Meeting 2013; Apr 6-10, 2013), 1-methyl-tryptophan (1-MT)-tira-pazamine, or any combination thereof.

[0243] In certain embodiments, a composition of the present disclosure is used in combination with an arginase inhibitor, such as N(omega)-Nitro-L-arginine methyl
25 ester (L-NAME), N-omega-hydroxy-nor-l-arginine (nor-NOHA), L-NOHA, 2(S)-amino-6-borono-hexanoic acid (ABH), S-(2-boronoethyl)-L-cysteine (BEC), or any combination thereof.

[0244] In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of VISTA, such as CA-170 (Curis, Lexington, Mass.).

30 **[0245]** In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of TIGIT such as, for example, COM902 (Compugen,

Toronto, Ontario Canada), an inhibitor of CD155, such as, for example, COM701 (Compugen), or both.

[0246] In certain embodiments, a composition of the present disclosure is used in combination with an inhibitor of PVRIG, PVRL2, or both. Anti-PVRIG antibodies
5 are described in, for example, PCT Publication No. WO 2016/134333. Anti-PVRL2 antibodies are described in, for example, PCT Publication No. WO 2017/021526.

[0247] In certain embodiments, a composition of the present disclosure is used in combination with a LAIR1 inhibitor.

[0248] In certain embodiments, a composition of the present disclosure n is used
10 in combination with an inhibitor of CEACAM-1, CEACAM-3, CEACAM-5, or any combination thereof.

[0249] In certain embodiments, a composition of the present disclosure is used in combination with an agent that increases the activity (i.e., is an agonist) of a stimulatory immune checkpoint molecule. For example a composition can be used in
15 combination with a CD137 (4-1BB) agonist (such as, for example, urelumab), a CD134 (OX-40) agonist (such as, for example, MEDI6469, MEDI6383, or MEDI0562), lenalidomide, pomalidomide, a CD27 agonist (such as, for example, CDX-1127), a CD28 agonist (such as, for example, TGN1412, CD80, or CD86), a CD40 agonist (such as, for example, CP-870,893, rhuCD40L, or SGN-40), a CD122 agonist (such as, for
20 example, IL-2) an agonist of GITR (such as, for example, humanized monoclonal antibodies described in PCT Patent Publication No. WO 2016/054638), an agonist of ICOS (CD278) (such as, for example, GSK3359609, mAb 88.2, JTX-2011, Icos 145-1, Icos 314-8, or any combination thereof). In any of the embodiments disclosed herein, a method may comprise administering a composition of the present disclosure with one or
25 more agonist of a stimulatory immune checkpoint molecule, including any of the foregoing, singly or in any combination.

[0250] In certain embodiments, a combination therapy comprises a composition of the present disclosure and a secondary therapy comprising one or more of: an antibody or antigen binding-fragment thereof that is specific for a cancer antigen
30 expressed by the non-inflamed solid tumor, a radiation treatment, a surgery, a chemotherapeutic agent, a cytokine, RNAi, or any combination thereof.

[0251] In certain embodiments, a combination therapy method comprises administering a composition of the present disclosure and further administering a radiation treatment or a surgery. Radiation therapy is well-known in the art and includes X-ray therapies, such as gamma-irradiation, and radiopharmaceutical therapies. Surgeries and surgical techniques appropriate to treating a given cancer in a subject are well-known to those of ordinary skill in the art.

[0252] Cytokines useful for promoting immune anticancer or antitumor response include, for example, IFN- α , IL-2, IL-3, IL-4, IL-10, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-21, IL-24, and GM-CSF, singly or in any combination with a composition of the present disclosure. In further embodiments, a cytokine is administered sequentially, provided that the subject was administered the Msln-specific composition at least three or four times before cytokine administration. In certain embodiments, the cytokine is administered subcutaneously. In some embodiments, the subject may have received or is further receiving an immunosuppressive therapy, such as calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof. In yet further embodiments, the subject being treated has received a non-myeloablative or a myeloablative hematopoietic cell transplant, wherein the treatment may be administered at least two to at least three months after the non-myeloablative hematopoietic cell transplant.

[0253] In certain embodiments, a combination therapy method comprises administering a composition of the present disclosure according to the present disclosure and further administering a chemotherapeutic agent. A chemotherapeutic agent includes, but is not limited to, an inhibitor of chromatin function, a topoisomerase inhibitor, a microtubule inhibiting drug, a DNA damaging agent, an antimetabolite (such as folate antagonists, pyrimidine analogs, purine analogs, and sugar-modified analogs), a DNA synthesis inhibitor, a DNA interactive agent (such as an intercalating agent), and a DNA repair inhibitor. Illustrative chemotherapeutic agents include, without limitation, the following groups: anti-metabolites/anti-cancer agents, such as pyrimidine analogs (5-fluorouracil, floxuridine, capecitabine, gemcitabine and cytarabine) and purine analogs, folate antagonists and related inhibitors (mercaptapurine, thioguanine, pentostatin and 2- chlorodeoxyadenosine (cladribine));

antiproliferative/antimitotic agents including natural products such as vinca alkaloids (vinblastine, vincristine, and vinorelbine), microtubule disruptors such as taxane (paclitaxel, docetaxel), vincristin, vinblastin, nocodazole, epothilones and navelbine, epidipodophyllotoxins (etoposide, teniposide), DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, Cytosan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchloroethamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, temozolamide, teniposide, triethylenethiophosphoramide and etoposide (VP 16)); antibiotics such as dactinomycin (actinomycin D), daunorubicin, doxorubicin (adriamycin), idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin) and mitomycin; enzymes (L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine); antiplatelet agents; antiproliferative/antimitotic alkylating agents such as nitrogen mustards (mechlorethamine, cyclophosphamide and analogs, melphalan, chlorambucil), ethylenimines and methylmelamines (hexamethylmelamine and thiotepa), alkyl sulfonates -busulfan, nitrosoureas (carmustine (BCNU) and analogs, streptozocin), trazenes— dacarbazine (DTIC); antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate); platinum coordination complexes (cisplatin, carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide; hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, nilutamide) and aromatase inhibitors (letrozole, anastrozole); anticoagulants (heparin, synthetic heparin salts and other inhibitors of thrombin); fibrinolytic agents (such as tissue plasminogen activator, streptokinase and urokinase), aspirin, dipyridamole, ticlopidine, clopidogrel, abciximab; antimigratory agents; antisecretory agents (breveldin); immunosuppressives (cyclosporine, tacrolimus (FK-506), sirolimus (rapamycin), azathioprine, mycophenolate mofetil); anti-angiogenic compounds (TNP470, genistein) and growth factor inhibitors (vascular endothelial growth factor (VEGF) inhibitors, fibroblast growth factor (FGF) inhibitors); angiotensin receptor blocker; nitric oxide donors; anti-sense oligonucleotides; antibodies (trastuzumab, rituximab); chimeric antigen receptors; cell cycle inhibitors and

differentiation inducers (tretinoin); mTOR inhibitors, topoisomerase inhibitors (doxorubicin (adriamycin), amsacrine, camptothecin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin, irinotecan (CPT-11) and mitoxantrone, topotecan, irinotecan), corticosteroids (cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisone, and prednisolone); growth factor signal transduction kinase inhibitors; mitochondrial dysfunction inducers, toxins such as Cholera toxin, ricin, Pseudomonas exotoxin, Bordetella pertussis adenylate cyclase toxin, or diphtheria toxin, and caspase activators; and chromatin disruptors.

[0254] In some embodiments, therapy further comprises administering a T cell based vaccine may be used (see, e.g., PCT Publication No. WO 2017/192924, of which the T cell vaccines, immunogenicity enhancers, transposon expression constructs, and related methods are incorporated by reference in their entireties entirety). In certain embodiments, a vaccine composition comprises a liposomal RNA preparation (see, e.g., Kreiter, et al, Nature 520: 692, 2015, which preparations and methods of making the same are incorporated by reference herein in their entireties). In certain embodiments, an vaccine composition is used to prepare a peptide-pulsed dendritic cell or other antigen-presenting cell, which may be performed ex vivo, in vitro, or in vivo.

[0255] The present disclosure also provides a method for preparing antigen-pulsed antigen-presenting cells. In some embodiments, the methods comprise contacting in vitro, under conditions and for a time sufficient for antigen processing and presentation by antigen-presenting cells to take place, (i) a population of antigen-presenting cells that are immunocompatible with a subject, and (ii) a polynucleotide, peptide, immunogenic composition, and/or an expression vector as described herein, thereby obtaining antigen-pulsed antigen-presenting cells capable of eliciting an antigen-specific T-cell response to a Msln peptide as described herein. The method may further include contacting the antigen-pulsed antigen-presenting cells with one or a plurality of immunocompatible T cells under conditions and for a time sufficient to generate Msln-specific T cells.

[0256] In certain embodiments, the method further comprises transfecting or transducing a population of immune cells in vitro or ex vivo with a polynucleotide comprising the binding protein-encoding nucleic acid sequence so-determined, thereby

obtaining a population of engineered Msln-specific immune cells, optionally in an amount effective to adoptively transfer or confer an antigen-specific T-cell response to a Msln antigen when the cells are administered to a subject.

[0257] In some embodiments, immune cell lines may be generated as described by Ho, et al. (see 2006 J Immunol Methods 310 (1-2):40-52)). For example, dendritic cells (DCs) may be derived from a plastic adherent fraction of PBMCs by culture over two days (days -2 to 0) in DC media (CELLGENIX™, Freiburg, Germany) supplemented with GM-CSF (800 U/ml) and IL-4 (1000 U/ml). On day -1, maturation cytokines TNF α (1100 U/ml), IL-1 β (2000 U/ml), IL-6 (1000 U/ml) and PGE2 (1 μ g/ml) can be added. On day 0, DCs can be harvested, washed, and pulsed with peptide (single peptides at 10 μ g/ml or peptide pools at 2 μ g/ml) over 2 to 4 hours in serum-free DC media. CD8 T cells can be isolated from PBMCs using anti-CD8 microbeads (MILTENYI BIOTECH™, Auburn, Calif.) and stimulated with DCs at an effector target (E:T) ratio of 1:5 to 1:10 in the presence of IL-21 (30 ng/ml). On day 3, IL-2 (12.5 U/ml), IL-7 (5 ng/ml), and IL-15 (5 ng/ml) can be added. Cells may be restimulated between days 10 and 14 using the plastic adherent fraction of irradiated autologous PBMCs as antigen presenting cells (APCs) after being peptide-pulsed for two hours and in the presence of IL-21. After restimulation, cells can be supplemented from day 1 on with IL-2 (25 U/ml), IL-7 (5 ng/ml), and IL-15 (5 ng/ml). T-cell clones can be generated by plating cells at limiting dilution and expanding with TM-LCLs coated with OKT3 (ORTHO BIOTECH™, Bridgewater, N.J.) and allogeneic PBMCs as feeders (REP protocol) as described (see Ho, et al., 2006 J Immunol Methods 310 (1-2):40-52).

[0258] The present disclosure provides, among other embodiments, the following embodiments.

[0259] In one embodiment, there is a binding protein comprising a T cell receptor (TCR) α -chain variable domain (V_α) and a TCR β -chain variable domain (V_β), wherein: (a) the V_α comprises the CDR3 amino acid sequence set forth in SEQ ID NO:39 or 37, and the V_β optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101 or 99; (b) the V_β comprises the CDR3 amino acid sequence set forth in SEQ ID NO:40 or SEQ ID

NO:38, and the V_{α} optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:102 or 100; and/or (c) the V_{α} comprises the CDR3 amino acid sequence set forth in SEQ ID NO:39 or 37, and the V_{β} comprises the CDR3 amino acid sequence set forth in SEQ ID NO:40 or 38,

5 wherein the binding protein is capable of specifically binding to a mesothelin (Msln) peptide:HLA complex. In an embodiment, (i) the V_{α} of (a), (b), and/or (c) of a binding protein as disclosed herein comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:102 or 100, provided that at least three or four of the CDRs have no change in sequence, wherein the CDRs that do

10 have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof; and/or (ii) the V_{β} of (a), (b), and/or (c) of a binding protein as disclosed herein comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101 or 99, provided that at least three or four of the CDRs have no change in sequence, wherein

15 the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof. Further, the binding protein referred to with respect to an embodiment may comprise: (a) the CDR1 α amino acid sequence set forth in SEQ ID NO:93; (b) the CDR2 α amino acid sequence set forth in SEQ ID NO:94; (c) the CDR3 α amino acid sequence set forth in

20 SEQ ID NO:39; (d) a CDR1 β amino acid sequence set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:84, further optionally as set forth in SEQ ID NO:91; (e) the CDR2 β amino acid sequence set forth in SEQ ID NO:92; and (f) the CDR3 β amino acid sequence set forth in SEQ ID NO:40. Still further, the binding protein referred to with respect to an embodiment may comprise an amino acid

25 sequence having at least 85% identity to an amino acid sequence encoded by: (a) TRBJ2-3*01; (b) TRAV21*01 or TRAV21*02; (c) TRBV5-4*01; (d) TRAJ57*01; and/or (e) TRBD1*01 or TRBD2*02. Still further, the binding protein referred to with respect to an embodiment may comprise a V_{α} comprising an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID

30 NO:102, and a V_{β} comprising an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101. In some instances, an

embodiment may comprise a binding protein wherein the V α comprises or consists of the amino acid sequence set forth in SEQ ID NO:102, and wherein the V β comprises or consists of the amino acid sequence set forth in SEQ ID NO:101. Still further, the binding protein in an embodiment may comprise a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:110 or 29, and/or wherein the TCR β comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:109 or 28. Some embodiments may comprise a binding protein wherein the TCR α comprises or consists of the amino acid sequence set forth in SEQ ID NO:110 or 29, and wherein the TCR β comprises or consists of the amino acid sequence set forth in SEQ ID NO:109 or 28. In further embodiments, the binding protein comprises: (a) a CDR1 α amino acid sequence as set forth in SEQ ID NO:89; (b) a CDR2 α amino acid sequence as set forth in SEQ ID NO:90; (c) a CDR3 α amino acid sequence as set forth in SEQ ID NO:37; (d) a CDR1 β amino acid sequence as set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:87; (e) a CDR2 β amino acid sequence as set forth in SEQ ID NO:88; and (f) a CDR3 β amino acid sequence as set forth in SEQ ID NO:38. In an embodiment, the binding protein may comprise an amino acid sequence having at least 85% identity to an amino acid sequence encoded by: (a) TRBJ1-1*01 or TRBJ2-3*01; (b) TRAV4-1*01; (c) TRAJ18*01; and/or (d) TRBD1*01 or TRBD2*02. In an embodiment, the binding protein may comprise a V α that comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:100 and the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:99. In an embodiment, the binding protein may comprise a V α that comprises or consists of the amino acid sequence set forth in SEQ ID NO:100, and wherein the V β comprises or consists of the amino acid sequence set forth in SEQ ID NO:99. In some embodiments the binding protein comprises a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:108 or 23, and/or wherein the TCR β comprises or consists of an amino

acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:107 or 22. In some embodiments the binding protein comprises a TCR α that comprises or consists of the amino acid sequence set forth in SEQ ID NO:108 or 23, and wherein the TCR β comprises or consists of the amino acid sequence set forth in SEQ ID NO:107 or 22. In some embodiments, the binding protein comprises a binding protein that is capable specifically binding to a SEQ ID NO:32:human leukocyte antigen (HLA) complex, and in some such instances the HLA comprises HLA-A*201. In some embodiments, alanine mutagenesis of any one or more of residues 3, 5, 6, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair binding by the binding protein to the Msln peptide:HLA complex. In some embodiments the binding protein is capable of binding to a peptide:HLA complex wherein the peptide comprises or consists of the consensus amino acid sequence set forth in SEQ ID NO:61. In some embodiments, alanine mutagenesis of any one or more of residues 1, 5, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair binding by the binding protein to the Msln peptide:HLA complex. In some embodiments, the binding protein is capable of binding to a peptide:HLA complex wherein the peptide comprises or consists of the consensus amino acid sequence set forth in SEQ ID NO:62. In some embodiments, the binding protein does not bind to, or does not specifically bind to, a peptide:HLA complex, wherein the peptide comprises or consists of the amino acid sequence set forth in any one or more of SEQ ID NOs:63-77, and wherein the HLA is optionally HLA-A:02*01.

[0260] In an embodiment, a binding protein comprises a T cell receptor (TCR) α -chain variable domain (V_α) and a TCR β -chain variable domain (V_β), wherein: (a) the V_α comprises the CDR3 amino acid sequence set forth in SEQ ID NO:33 or 35, and the V_β optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95 or 97; (b) the V_β comprises the CDR3 amino acid sequence set forth in SEQ ID NO: 34 or 36, and the V_α optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98; and/or (c) the V_α comprises the CDR3 amino acid sequence shown in SEQ ID NO:33 or 35, and the V_β comprises the CDR3 amino acid sequence shown in SEQ ID NO:40 or 38, wherein the binding protein is

capable of specifically binding to a mesothelin (Msln) peptide:HLA complex. In an embodiment, (i) the V_α of (a), (b), and/or (c) comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98, provided that at least three or four of the CDRs have no change in sequence, wherein

5 the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof; and/or (ii) the V_β of (a), (b), and/or (c) comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95 or 97, provided that at least three or four of the CDRs have no change in sequence, wherein the CDRs that do

10 have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof. In an embodiment, the binding protein comprises: (a) the CDR1 α amino acid sequence set forth in SEQ ID NO:80; (b) the CDR2 α amino acid sequence set forth in SEQ ID NO:81 or 118; (c) the CDR3 α amino acid sequence set forth in SEQ ID NO:33; (d) a CDR1 β amino acid

15 sequence as set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:84, further optionally as set forth in SEQ ID NO:78; (e) the CDR2 β amino acid sequence set forth in SEQ ID NO:79; and (f) the CDR3 β amino acid sequence set forth in SEQ ID NO:34. In an embodiment, the binding protein comprises an amino acid sequence having at least 85% identity to an amino acid sequence encoded by: (a) TRBJ2-7*01

20 or TRBJ2-3*01; (b) TRAV1-1*01; (c) TRBV12-4*01; (d) TRAJ3*01; and/or (e) TRBD1*01 or TRBD2*02. In an embodiment, the binding protein comprises a V_α that comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96, and a V_β that comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID

25 NO:95. In an embodiment, the binding protein comprises a V_α that comprises or consists of the amino acid sequence set forth in SEQ ID NO:96, and a V_β that comprises or consists of the amino acid sequence set forth in SEQ ID NO:95. In an embodiment, the binding protein comprises a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists of an amino acid sequence having at

30 least about 85% identity to the amino acid sequence set forth in SEQ ID NO:104 or 7, and/or wherein the TCR β comprises or consists of an amino acid sequence having at

- least about 85% identity to the amino acid sequence set forth in SEQ ID NO:103 or 6. In an embodiment, the binding protein comprises a TCR α that comprises or consists of the amino acid sequence set forth in SEQ ID NO:104 or 7, and wherein the TCR β comprises or consists of the amino acid sequence set forth in SEQ ID NO:103 or 106.
- 5 In an embodiment, the binding protein comprises: (a) the CDR1 α amino acid sequence set forth in SEQ ID NO:85; (b) the CDR2 α amino acid sequence set forth in SEQ ID NO:86 or 119; (c) the CDR3 α amino acid sequence set forth in SEQ ID NO:35; (d) a CDR1 β amino acid sequence set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:84, further optionally as set forth in SEQ ID NO:82; (e) the CDR2 β amino
- 10 acid sequence set forth in SEQ ID NO:79; and (f) the CDR3 β amino acid sequence set forth in SEQ ID NO:36. In an embodiment, the binding protein comprises an amino acid sequence having at least 85% identity to an amino acid sequence encoded by: (a) TRBJ2-3*01; (b) TRAV12-3*01; (c) TRBV12-3*01; (d) TRAJ29*01; and/or (e) TRBD1*01 or TRBD2*02. In an embodiment, the binding protein comprises a V α that
- 15 comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:98 and a V β that comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:97. In an embodiment, the binding protein comprises a V α that comprises or consists the amino acid sequence set forth in SEQ ID NO:98, and a V β that comprises
- 20 or consists the amino acid sequence set forth in SEQ ID NO:97. In an embodiment, the binding protein comprises a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:106 or 15, and/or wherein the TCR β comprises or consists of an amino acid sequence having at least about 85%
- 25 identity to the amino acid sequence set forth in SEQ ID NO:105 or 14. In an embodiment, the binding protein comprises a TCR α that comprises or consists the amino acid sequence set forth in SEQ ID NO:106 or 15, and a TCR β that comprises or consists of the amino acid sequence set forth in SEQ ID NO:105 or 14. In an embodiment, the binding protein is capable specifically binding to a SEQ ID
- 30 NO:31:human leukocyte antigen (HLA) complex, and wherein the HLA is optionally HLA-A*201. In an embodiment, the binding protein is or comprises a TCR, wherein

the TCR is optionally soluble, an antigen-binding fragment of a TCR, a scTCR, or CAR. In an embodiment, the binding protein is human, humanized, or chimeric. In an embodiment, the binding protein is capable of binding to the mesothelin:HLA complex in the absence of, or independent of, CD8.

5 **[0261]** In an embodiment, the binding protein has a Msln peptide EC50 of about 9 μ M, about 8 μ M, about 7 μ M, about 6 μ M, about 5 μ M, about 4 μ M, about 3 μ M, about 2 μ M, about 1 μ M, about 0.9 μ M, about 0.8 μ M, about 0.7 μ M, about 0.6 μ M, about 0.5 μ M, about 0.4 μ M, about 0.3 μ M, about 0.2 μ M, or less.

10 **[0262]** In an embodiment, a composition is provided that comprises a binding protein described herein and a pharmaceutically acceptable carrier, diluent, or excipient.

15 **[0263]** In an embodiment, a polynucleotide encodes a binding protein described herein. In some embodiments, the polynucleotide is codon optimized for expression in a host cell, wherein the host cell is optionally a human immune system cell, preferably a T cell. Also, in some embodiments, the polynucleotide has at least about 50% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs:1-4, 9-12, 17-20, 25, and 26. In some embodiments, the polynucleotide comprises a TCR α chain-encoding polynucleotide and a TCR β chain-encoding polynucleotide that have at least about 50% identity to the polynucleotide sequences set forth in SEQ ID NOs: (i) 1 and 3, respectively; (ii) 2 and 4, respectively; (iii) 9 and 11, respectively; (iv) 10 and 12, 20 respectively; (v) 17 and 19, respectively; (vi) 18 and 20, respectively; or (vii) 25 and 26, respectively. In some embodiments, the polynucleotide comprises a polynucleotide that encodes a self-cleaving peptide disposed between a TCR β chain-encoding polynucleotide and a TCR α chain-encoding polynucleotide. In some embodiments, the encoded polypeptide comprises the amino acid sequence as set forth in any one of SEQ 25 ID NOs:8, 16, 24, and 30. In some embodiments, the polynucleotide encoding the binding protein has at least about 50% identity to the polynucleotide sequence as set forth in any one of SEQ ID NOs:5, 13, 21, 27, and 120. In particular embodiments, a polynucleotide encoding a binding protein comprises or consists of the polynucleotide sequence set forth in SEQ ID NO:120.

30 **[0264]** In some embodiments, an expression vector is provided that comprises a polynucleotide described herein operably linked to an expression control sequence. In

some embodiments, the expression vector is capable of delivering the polynucleotide to a host cell. In some embodiments, the host cell is a hematopoietic progenitor cell or a human immune system cell. In some embodiments, the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a dendritic cell, or any combination thereof. In some embodiments, the immune system cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof. In some embodiments, the expression vector, is a viral vector. In some embodiments, the viral vector is a lentiviral vector or a γ -retroviral vector.

- 10 **[0265]** In an embodiment, a recombinant host cell comprises a heterologous polynucleotide encoding a binding protein as described herein and/or an expression vector as described herein, wherein the recombinant host cell is capable of expressing on its cell surface the encoded binding protein. In some embodiments, the recombinant host cell is a hematopoietic progenitor cell or a human immune system cell. In some
- 15 embodiments, the recombinant host cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a dendritic cell, or any combination thereof. In some embodiments, the recombinant host cell is a T cell. In some embodiments, the recombinant host cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T
- 20 cell, or any combination thereof. In some embodiments, the recombinant host cell is a T cell or a NK-T cell encoding an endogenous TCR, and wherein the mesothelin-specific binding protein encoded by the heterologous polynucleotide is capable of more efficiently associating with a CD3 protein as compared to the endogenous TCR. In some embodiments, *Nur77* expression is increased in the recombinant host cell when
- 25 the host cell is in the presence of the Msln peptide bound by the encoded binding protein at a concentration of about 10^{-2} μ M peptide, about 10^{-1} μ M peptide, about 1 μ M peptide, or about 10^1 μ M peptide, wherein the peptide is optionally presented to the host cell by an antigen presenting cell. In some embodiments, the recombinant host cell is one wherein the recombinant host cell does not produce IFN- γ and/or does not exhibit
- 30 activation and/or cytotoxic activity when contacted with a cell expressing: (i) HLA-C6:02:01; (ii) HLA-B13:01:01 without HLA-B13:02:01; (iii) HLA-A3; (iv)

HLA-A29; (v) HLA-B40; (vi) HLA-B44; (vii) HLA-C3; (viii) HLA-C16; (ix) HLA-A1; (x) HLA-24; (xi) HLA-B7; (xii) HLA-B57; (xiii) HLA-C7; (xiv) HLA-A11; (xv) HLA-B15; (xvi) HLA-C4; (xvii) HLA-C12; (xviii) HLA-B8; (xix) HLA-B49; (xx) HLA-B51; (xxi) HLA-C15; (xxii) HLA-A30; (xxiii) HLA-A68; (xxiv) HLA-C2; (xxv) HLA-A32; (xxvi) HLA-A33; (xxvii) HLA-B55; (xxviii) HLA-C1; (xxvix) HLA-C5; (xxix) HLA-B8; (xxx) HLA-B35; or (xxxi) any combination of (i)-(xxx), provided that the mesothelin peptide bound by the encoded binding protein is not present. In some embodiments, the recombinant host cell comprises T cell or a NK-T cell encoding an endogenous TCR, wherein the binding protein encoded by the heterologous

10 polynucleotide has higher cell surface expression as compared to the endogenous TCR.

[0266] In an embodiment, a cell composition is provided that comprises a recombinant host cell described herein and a pharmaceutically acceptable carrier, excipient, or diluent.

[0267] In an embodiment, a unit dose is provided that comprises an effective

15 amount of a recombinant host cell or a cell composition described herein.

[0268] In an embodiment, a method of treating a disease or disorder associated with mesothelin expression and/or activity in a subject is provided, wherein the method comprises: administering to the subject an effective amount of the binding protein described herein, the recombinant host cell described herein, the composition described

20 herein, or the unit dose of described herein. In an embodiment, the method comprises a method of treating a disease or disorder associated with mesothelin expression and/or activity in a subject, wherein the disease or disorder is a hyperproliferative disease or a proliferative disease. In an embodiment, the method comprises a method of treating a disease or disorder associated with mesothelin expression and/or activity in a subject,

25 wherein the disease or disorder is a cancer and, optionally, the cancer is a solid cancer or a hematological malignancy. In an embodiment, the method comprises a method of treating a disease or disorder associated with mesothelin expression and/or activity in a subject, wherein the disease or disorder is one of biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, colon cancer,

30 colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma,

glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, mesothelioma, malignant melanoma, osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or uterine cancer. In an embodiment, the method comprises a method of treating a disease or disorder associated with mesothelin expression and/or activity in a subject, wherein the disease or disorder is one of pancreatic cancer, ovarian cancer, breast cancer, gastric cancer, colorectal cancer, mesothelioma, or lung cancer. In an embodiment, the binding protein, host cell, composition, or unit dose is administered parenterally or intravenously. In an embodiment, the method comprises administering a plurality of doses of the binding protein, host cell, composition, or unit dose to the subject and, optionally, the plurality of doses are administered at intervals between administrations of about two to about four weeks. In an embodiment, method further comprises administering a cytokine to the subject. In an embodiment, the method comprises administering IL-2, IL-15, IL-21, or any combination thereof. In an embodiment, the method comprises a subject that is further receiving or has received an immune checkpoint inhibitor, an agonist of a stimulatory immune checkpoint agent, radiation therapy, an antibody, an antibody-drug conjugate, an Fc fusion protein, an antisense nucleotide therapy, a gene therapy, a vaccine, a surgery, a chemotherapy, or any combination thereof.

[0269] In an embodiment, the binding protein described herein, the composition described herein, the polynucleotide described herein, the expression vector described herein, the recombinant host cell described herein, the cell composition described herein, or the unit dose described herein, is for use in the treatment of a disease or disorder characterized by mesothelin expression and/or activity.

[0270] In an embodiment, the binding protein described herein, the composition described herein, the polynucleotide described herein, the expression vector described herein, the recombinant host cell described herein, the cell composition described herein, or the unit dose described herein, is for use in adoptive immunotherapy of a disease or disorder characterized by mesothelin expression and/or activity.

[0271] In an embodiment, the binding protein described herein, the composition described herein, the polynucleotide described herein, the expression vector described herein, the recombinant host cell described herein, the cell composition described herein, or the unit dose described herein, is for use in the manufacture of a medicament
5 for treating a disease or disorder characterized by mesothelin expression and/or activity.

[0272] In an embodiment, the binding protein, composition, polynucleotide, expression vector, recombinant host cell, cell composition, or unit dose for use described herein, wherein the disease or disorder characterized by mesothelin expression and/or activity is mesothelioma, pancreatic cancer, ovarian cancer, lung
10 cancer, a cancer wherein an Msln₂₀₋₂₈ peptide is expressed on a tumor cell of the cancer, or a cancer wherein an Msln₅₃₀₋₅₃₈ peptide is expressed on a tumor cell of the cancer.

[0273] In an embodiment, the binding protein, composition, polynucleotide, expression vector, recombinant host cell, cell composition, or unit dose for use described herein, wherein the disease or disorder characterized by mesothelin
15 expression and/or activity is pancreatic cancer, ovarian cancer, breast cancer, gastric cancer, colorectal cancer, mesothelioma, or lung cancer.

[0274] In an embodiment, an isolated polynucleotide is provided that encodes a binding protein that is capable of specifically binding to a SEQ ID NO:32:HLA-A:02*01 complex, wherein the polynucleotide comprises or consists of the
20 polynucleotide sequence set forth in SEQ ID NO:120. In an embodiment, an expression vector comprising the polynucleotide that encodes a binding protein that is capable of specifically binding to a SEQ ID NO:32:HLA-A:02*01 complex is provided, wherein the polynucleotide comprises or consists of the polynucleotide sequence set forth in SEQ ID NO:120 operably linked to an expression control sequence. In such an
25 embodiment, the expression vector, the expression vector is capable of delivering the polynucleotide to a host cell. In such an embodiment, the host cell is a hematopoietic progenitor cell or a human immune system cell. In such an embodiment, the expression vector immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a
30 dendritic cell, or any combination thereof. In such an embodiment, the expression vector immune system cell is a naïve T cell, a central memory T cell, a stem cell

memory T cell, an effector memory T cell, or any combination thereof. The expression vector of any one of claims 86-90, wherein the expression vector is a viral vector. In such an embodiment, the expression vector is a viral vector that is a lentiviral vector or a γ -retroviral vector.

- 5 **[0275]** In an embodiment, is a recombinant host cell comprising the polynucleotide described herein and/or the expression vector described herein, wherein the recombinant host cell is capable of expressing on its cell surface the encoded binding protein. In such an embodiment, the recombinant host cell is a hematopoietic progenitor cell or a human immune system cell. In such an embodiment, recombinant
- 10 host cell is an immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a dendritic cell, or any combination thereof. In such an embodiment, the recombinant host cell is a T cell. In such an embodiment, the recombinant host cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T
- 15 cell, or any combination thereof.

EXAMPLES

EXAMPLE 1

IDENTIFICATION AND SELECTION OF TCRs SPECIFIC FOR MSLN₂₀ OR MSLN₅₃₀.

- [0276]** Exemplary TCR clones specific for Msln₂₀ or Msln₅₃₀ are shown in
- 20 FIGS. 1A and 1B, respectively, and the enrichment score of each TCR clonotype in the next-generation sequencing (NGS) based method for TCR isolation presented herein is compared to T cell frequency. The enrichment score on the y-axis correlates with the magnitude of binding to peptide:HLA tetramer. All TCRs were synthesized and tested for function when expressed in reporter T cell lines and primary CD8⁺ PBMCs. The
- 25 TCRs with the highest functional avidity when transferred into recipient CD8⁺ T cells are encircled. These data show that the TCRs identified as having the highest functional activity did not have the highest magnitude of tetramer binding and were rare in the peptide-expanded T cell populations. Without being bound by theory, this may be due in part to decreased TCR surface expression by these highly avid clonotypes.

More than 100 TCR constructs were synthesized and evaluated (Msln₂₀ = 60 TCRs synthesized; Msln₅₃₀ = 42 TCRs synthesized). Mesothelin-specific T cell lines were generated from 18 donors. Cells were stained with titrated concentrations of tetramer, sorted, and analyzed by single-cell TCR sequencing (~8 sorting experiments in total).

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EXAMPLE 2

TETRAMER BINDING BY MSLN₅₃₀ TCRS

[0277] Msln₅₃₀-specific TCR coding constructs were lentivirally transduced into CD8⁺ Jurkat T cells that lack endogenous TCR α/β chains (Jurkat76 – dark gray plots) or Jurkat76 cells transduced to express CD8 $\alpha\beta$ (Jurkat76-CD8 $\alpha\beta$ – light gray plots) (*see* FIG. 2). In the absence of TCR α/β chains, CD3 cannot be expressed at the cell surface. Therefore, CD3 expression is a proxy for TCR surface expression in these cells, allowing tetramer binding to be assessed relative to TCR surface expression. With reference to FIG. 2, TCRs are presented in order of tetramer binding relative to CD3 expression, and TCRs above the indicated line are considered to be CD8-independent, characteristic of high affinity.

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EXAMPLE 3

EVALUATION OF ANTIGEN-SPECIFIC T CELL RESPONSES

[0278] The four TCRs that exhibited the highest level of tetramer binding were evaluated for antigen-specific function using a reporter Jurkat T cell line that has a tdTomato transgene knocked into the *Nur77* locus. T2 target cells were pulsed with titrated concentrations of peptide and TCR-expressing T cells were assessed for tdTomato expression, as indicated in FIG. 3A. The percentage of tdTomato-positive cells detected at each peptide concentration was plotted and fit to a dose-response curve by non-linear regression (FIG. 3B). The calculated EC₅₀ for each TCR was plotted and TCR B11 (also referred to herein as 11B) was identified as the most-avid TCR (*see* arrows in FIGS. 3B and 3C). The two TCRs with the highest level of tetramer binding (A16 and A3, also referred to as 16A and 3A, respectively) were found to have lower antigen sensitivity, and are also indicated by arrows in FIG. 3B.

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EXAMPLE 4**FUNCTIONAL EVALUATION OF MSLN₅₃₀-SPECIFIC TCRs**

[0279] Since function did not correlate with tetramer binding for the top four tetramer binders, all selected Msln₅₃₀-specific TCRs were assessed for tdTomato expression in response to a lower concentration of peptide (0.1 μ m) as a proxy for antigen sensitivity (*see* FIG. 4). Two TCRs that bound tetramer at lower levels (B9 and A11) were found to mediate high-level tdTomato expression in response to antigen. Data from these and other TCRs is shown in boxes in FIG. 4; these were included in the set of TCRs for further study. Several TCRs, including B9 and A11, confer high antigen-specific activity despite lower tetramer binding. In FIG. 4, the TCRs are presented in rank order of tetramer binding.

EXAMPLE 5**FUNCTIONAL EVALUATION OF SELECTED TCRs**

[0280] With reference to FIGS. 5A-5C, CD8⁺ T cells were purified from donor PBMCs and lentivirally transduced with TCRs specific for Msln₅₃₀ or Msln₂₀ (which were selected through a similar process to that described in Examples 3 and 4). After 8 days, tetramer^{hi} cells were sorted and further expanded for 8–10 days. Transduced T cells were stained with tetramer and CD8 to confirm purity and uniform high level CD8 expression.

[0281] As shown in FIGS. 6A-6C, TCR-transduced effector CD8⁺ T cells specific for Msln₂₀ (A) or Msln₅₃₀ (B) were incubated with peptide-pulsed T2 target cells and dose-dependent IFN- γ production was assessed by flow cytometric analysis of intracellular IFN- γ (effector cells = TCR-transduced primary CD8⁺ T cells (sorted); targets = peptide-pulsed T2 cells). The percentage of IFN- γ -positive cells detected at each peptide concentration was plotted and fit to a dose-response curve by non-linear regression. The calculated EC₅₀ for each TCR was plotted. In FIG. 6C, the most avid TCR specific for each epitope ("20-B3" and "530-B11", respectively) is indicated with an arrow.

EXAMPLE 6**TUMOR CELL KILLING BY TCR-TRANSDUCED CD8⁺ T CELLS**

[0282] Two tumor cell lines that express Msln, MDA-MB-231 and MDA-MB-468, were targeted by titrated ratios of sort-purified Msln-specific TCR-transduced CD8⁺ T cells using a chromium release assay measuring specific tumor cell lysis (*see* FIGS. 7A-7C). Results for TCR Msln₅₃₀-B11, the highest avidity TCR identified, are indicated with an arrow.

EXAMPLE 7**EPITOPE ANALYSIS BY ALANINE SCANNING OF MSLN₂₀ AND MSLN₅₃₀ PEPTIDES**

[0283] FIG. 8 demonstrates an epitope analysis assay in which each successive amino acid of the Msln target peptide sequence was replaced by an alanine and TCR-transduced T cells were incubated with HLA-A2⁺ target cells pulsed with the variant peptide. Representative data of IFN- γ production in response to each variant peptide by a Msln₂₀-specific TCR is shown at the bottom of the figure.

[0284] Results of the alanine scan assay, showing the percent IFN- γ ⁺ T cells in response to each alanine-substituted peptide for each of the four tested TCRs are shown in FIGS. 9A-9D. The essential residues are identified by their one-letter amino acid code and the non-essential residues are indicated by an X.

EXAMPLE 8**ANALYSIS FOR EPITOPES HOMOLOGOUS TO MSLN₅₃₀ IN THE HUMAN PROTEOME**

[0285] Human peptides predicted to have potential cross-reactivity with Msln-specific TCRs were identified using the ScanProsite tool by searching the human proteome for the indicated consensus epitope motif of each of the indicated Msln₅₃₀-specific TCRs (A11 and B11), as illustrated in FIG. 10. Resulting peptides were analyzed for HLA-A2 binding using three different prediction algorithms: SITHPATHI, PanMHCnet, and IEDB. The recommended cutoff for each approach is listed in parenthesis next to the name of the algorithm. Potential cross-reacting peptides are as indicated in the figure key, and were synthesized for further analysis.

EXAMPLE 9**ANALYSIS OF SYNTHESIZED PEPTIDES WITH POTENTIAL HOMOLGY TO MSLN₅₃₀ IN THE HUMAN PROTEOME**

[0286] T2 target cells were pulsed with peptides with potential for cross-reactivity with Msln₅₃₀-A11 and -B11 TCRs, and were incubated with T cells that were transduced to express those TCRs and sorted for purity (*see* FIGS. 11A-11B; effector T cells = tetramer-sorted TCR-transduced CD8⁺ T cells; target cells = T2 cells pulsed with 10 μ M peptide). A high dose of peptide (10 μ M) was used in order to detect potential reactivities. The percentage of IFN- γ -positive TCR-transduced T cells is shown in FIG. 11A for Msln₅₃₀-11A and Msln₅₃₀-11B. The response with 10 μ M of the wildtype Msln₅₃₀ peptide, and the maximal response obtained with a non-specific T cell activation cocktail are shown on the right side of the graph. Only one peptide (#10) elicited a low level (<20%) response from TCR Msln₅₃₀-11B-transduced T cells at 10 μ M peptide. The graph in FIG. 11B shows a dose-response curve for Msln₅₃₀-11B transduced T cell reactivity to the Msln₅₃₀ peptide versus several potential cross-reactive peptides, including peptide #10, to determine reactivity at physiological levels. The percent IFN- γ ⁺ data was fit to dose-response curves by non-linear regression, and EC50 values were calculated and are shown below the graph. These data show that the Msln₅₃₀-11B EC50 for peptide #10 is more than 3000 $\times$ higher than for that of Msln₅₃₀; therefore, Msln₅₃₀-11B has much greater specificity for Msln₅₃₀ than for peptide #10.

EXAMPLE 10**ANALYSIS OF ALLOREACTIVITY BY TARGETING DIVERSE DONOR-DERIVED LCLS**

[0287] In order to determine potential alloreactivity of T cells expressing Msln_{20-3B} or Msln₅₃₀-11A or -11B, TCR-transduced T cells were cultured with allogeneic LCLs that naturally express diverse HLA alleles, including many of the more common alleles. The LCL lines and corresponding HLA allele expression are listed in the table in FIG. 12A. For each cell line (FIGS. 12B-12I), the percentage of IFN- γ expression is shown when the T cells and LCL cells were co-cultured in the presence or absence of added Msln₅₃₀ peptide (which is presented by the transduced T cells when the LCL cell line lacks HLA-A2 expression).

- [0288]** Further analysis of T cell targeting of diverse LCL cell lines is shown in FIGS. 13A-13H. In order to determine potential alloreactivity of T cells expressing Msln_{20-3B} or Msln_{530-11A} or -11B, TCR-transduced T cells were cultured with allogeneic LCLs that naturally express diverse HLA alleles, including many of the more common alleles. The LCL lines and corresponding HLA allele expression are listed in the table in FIG. 13A. For each cell line, the percentage IFN- γ expression is shown following co-culture of target and effector cells in the presence or absence of added Msln₅₃₀ peptide (which is presented by the transduced T cells when the LCL cell lines lack HLA-A2 expression). This second set of LCLs include several lines that express HLA-C6 and HLA-B13, which exhibit linkage disequilibrium and are commonly found together. Several of these LCLs elicited a response from Msln_{530-11B}-transduced T cells. These data show that HLA-B13:02:01 is the alloreactive allele, since only cells that express HLA-B13:02:01 elicit a response, while cells expressing HLA-C6:02:01 or HLA-B13:01:01 without HLA-B13:02:01 do not elicit a response.
- [0289]** Table 1 shows the frequency of HLA-B13:02:01 and HLA-A2:01:01 co-expression in different populations. Some alloreactivity specific to HLA-B13:02:01 was detected. However, given the small haplotype frequency within the population, it is a rare event for a patient to present with an allele that is cross-reactive.

Table 1: HLA A2:01/B13:02 Haplotype Frequencies

European Americans	0.845% (B13:02)	(29.6% A2:01)
African Americans	0.177% (B13:02)	(12.5% A2:01)
Asians and Pacific Islanders	0.110% (B13:02)	(9.5% A2:01)
Hispanics	0.129% (B13:02)	(19.4% A2:01)

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- [0290]** The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, including U.S. Provisional Patent Application No. 62/758,397, filed November 9, 2018, are incorporated herein by reference, in their entirety. Aspects of the embodiments can

be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should
5 not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

CLAIMS

1. A binding protein comprising a T cell receptor (TCR) α -chain variable domain (V_α) and a TCR β -chain variable domain (V_β), wherein:

(a) the V_α comprises the CDR3 amino acid sequence set forth in SEQ ID NO:39 or 37, and the V_β optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101 or 99;

(b) the V_β comprises the CDR3 amino acid sequence set forth in SEQ ID NO:40 or SEQ ID NO:38, and the V_α optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:102 or 100; and/or

(c) the V_α comprises the CDR3 amino acid sequence set forth in SEQ ID NO:39 or 37, and the V_β comprises the CDR3 amino acid sequence set forth in SEQ ID NO:40 or 38,

wherein the binding protein is capable of specifically binding to a mesothelin (Msln) peptide:HLA complex.

2. The binding protein of claim 1, wherein:

(i) the V_α of (a), (b), and/or (c) comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:102 or 100, provided that at least three or four of the CDRs have no change in sequence, wherein the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof; and/or

(ii) the V_β of (a), (b), and/or (c) comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101 or 99, provided that at least three or four of the CDRs have no change in sequence, wherein the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof.

3. The binding protein of claim 1 or 2, comprising:

(a) the CDR1 α amino acid sequence set forth in SEQ ID NO:93;

(b) the CDR2 α amino acid sequence set forth in SEQ ID NO:94;

- (c) the CDR3 α amino acid sequence set forth in SEQ ID NO:39;
- (d) a CDR1 β amino acid sequence set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:84, further optionally as set forth in SEQ ID NO:91;
- (e) the CDR2 β amino acid sequence set forth in SEQ ID NO:92; and
- (f) the CDR3 β amino acid sequence set forth in SEQ ID NO:40.

4. The binding protein of any one of claims 1-3, comprising an amino acid sequence having at least 85% identity to an amino acid sequence encoded by:

- (a) TRBJ2-3*01;
- (b) TRAV21*01 or TRAV21*02;
- (c) TRBV5-4*01;
- (d) TRAJ57*01; and/or
- (e) TRBD1*01 or TRBD2*02.

5. The binding protein of any one of claims 1-4, wherein the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:102, and wherein the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:101.

6. The binding protein of claim 5, wherein the V α comprises or consists the amino acid sequence set forth in SEQ ID NO:102, and wherein the V β comprises or consists the amino acid sequence set forth in SEQ ID NO:101.

7. The binding protein of any one of claims 1-6, comprising a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:110 or 29, and/or wherein the TCR β comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:109 or 28.

8. The binding protein of claim 7, wherein the TCR α comprises or consists the amino acid sequence set forth in SEQ ID NO:110 or 29, and wherein the TCR β comprises or consists of the amino acid sequence set forth in SEQ ID NO:109 or 28.

9. The binding protein of claim 1 or 2, comprising:

- (a) a CDR1 α amino acid sequence as set forth in SEQ ID NO:89;
- (b) a CDR2 α amino acid sequence as set forth in SEQ ID NO:90;
- (c) a CDR3 α amino acid sequence as set forth in SEQ ID NO:37;
- (d) a CDR1 β amino acid sequence as set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:87;
- (e) a CDR2 β amino acid sequence as set forth in SEQ ID NO:88; and
- (f) a CDR3 β amino acid sequence as set forth in SEQ ID NO:38.

10. The binding protein of any one of claims 1, 2, or 9, comprising an amino acid sequence having at least 85% identity to an amino acid sequence encoded by:

- (a) TRBJ1-1*01 or TRBJ2-3*01;
- (b) TRAV4-1*01;
- (c) TRAJ18*01; and/or
- (d) TRBD1*01 or TRBD2*02

11. The binding protein of any one of claims 1, 2, 9, or 10, wherein the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:100 and the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:99.

12. The binding protein of claim 11, wherein the V α comprises or consists the amino acid sequence set forth in SEQ ID NO:100, and wherein the V β comprises or consists the amino acid sequence set forth in SEQ ID NO:99.

13. The binding protein of any one of claims 1, 2, or 9-12, comprising a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set

forth in SEQ ID NO:108 or 23, and/or wherein the TCR β comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:107 or 22.

14. The binding protein of claim 13, wherein the TCR α comprises or consists the amino acid sequence set forth in SEQ ID NO:108 or 23, and wherein the TCR β comprises or consists of the amino acid sequence set forth in SEQ ID NO:107 or 22.

15. The binding protein of any one of claims 1-14, wherein the binding protein is capable specifically binding to a SEQ ID NO:32:human leukocyte antigen (HLA) complex.

16. The binding protein of any one of claims 1-15, wherein the HLA comprises HLA-A*201.

17. The binding protein of claim 15 or 16, wherein alanine mutagenesis of any one or more of residues 3, 5, 6, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair binding by the binding protein to the Msln peptide:HLA complex.

18. The binding protein of any one of claims 15-17, wherein the binding protein is capable of binding to a peptide:HLA complex wherein the peptide comprises or consists of the consensus amino acid sequence set forth in SEQ ID NO:61.

19. The binding protein of any one of claims 15-18, wherein alanine mutagenesis of any one or more of residues 1, 5, or 9 of SEQ ID NO:32 does not abrogate or does not substantially impair binding by the binding protein to the Msln peptide:HLA complex.

20. The binding protein of any one of claims 15-19, wherein the binding protein is capable of binding to a peptide:HLA complex wherein the peptide comprises or consists of the consensus amino acid sequence set forth in SEQ ID NO:62.

21. The binding protein of one of claims 1-20, wherein the binding protein does not bind to or does not specifically bind to a peptide:HLA complex wherein the peptide comprises or consists of the amino acid sequence set forth in any one or more of SEQ ID NOs:63- 77, and wherein the HLA is optionally HLA-A:02*01.

22. A binding protein comprising a T cell receptor (TCR) α -chain variable domain (V_α) and a TCR β -chain variable domain (V_β), wherein:

(a) the V_α comprises the CDR3 amino acid sequence set forth in SEQ ID NO:33 or 35, and the V_β optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95 or 97;

(b) the V_β comprises the CDR3 amino acid sequence set forth in SEQ ID NO: 34 or 36, and the V_α optionally comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98; and/or

(c) the V_α comprises the CDR3 amino acid sequence shown in SEQ ID NO:33 or 35, and the V_β comprises the CDR3 amino acid sequence shown in SEQ ID NO:40 or 38,

wherein the binding protein is capable of specifically binding to a mesothelin (Msln) peptide:HLA complex.

23. The binding protein of claim 22, wherein:

(i) the V_α of (a), (b), and/or (c) comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96 or 98, provided that at least three or four of the CDRs have no change in sequence, wherein the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof; and/or

(ii) the V_β of (a), (b), and/or (c) comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95 or 97, provided that at least three or four of the CDRs have no change in sequence, wherein the CDRs that do have sequence changes have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof.

24. The binding protein of claim 22 or 23, comprising:
- (a) the CDR1 α amino acid sequence set forth in SEQ ID NO:80;
 - (b) the CDR2 α amino acid sequence set forth in SEQ ID NO:81 or 118;
 - (c) the CDR3 α amino acid sequence set forth in SEQ ID NO:33;
 - (d) a CDR1 β amino acid sequence as set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:84, further optionally as set forth in SEQ ID NO:78;
 - (e) the CDR2 β amino acid sequence set forth in SEQ ID NO:79; and
 - (f) the CDR3 β amino acid sequence set forth in SEQ ID NO:34.
25. The binding protein of any one of claims 22-24, comprising an amino acid sequence having at least 85% identity to an amino acid sequence encoded by:
- (a) TRBJ2-7*01 or TRBJ2-3*01;
 - (b) TRAV1-1*01;
 - (c) TRBV12-4*01;
 - (d) TRAJ3*01; and/or
 - (e) TRBD1*01 or TRBD2*02.
26. The binding protein of any one of claims 22-25, wherein the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:96, and wherein the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:95.
27. The binding protein of claim 26, wherein the V α comprises or consists the amino acid sequence set forth in SEQ ID NO:96, and wherein the V β comprises or consists the amino acid sequence set forth in SEQ ID NO:95.
28. The binding protein of any one of claims 22-27, comprising a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:104 or 7, and/or wherein the TCR β comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:103 or 6.

29. The binding protein of claim 28, wherein the TCR α comprises or consists the amino acid sequence set forth in SEQ ID NO:104 or 7, and wherein the TCR β comprises or consists of the amino acid sequence set forth in SEQ ID NO:103 or 106.

30. The binding protein of claim 22 or 23, comprising:

- (a) the CDR1 α amino acid sequence set forth in SEQ ID NO:85;
- (b) the CDR2 α amino acid sequence set forth in SEQ ID NO:86 or 119;
- (c) the CDR3 α amino acid sequence set forth in SEQ ID NO:35;
- (d) a CDR1 β amino acid sequence set forth in SEQ ID NO:83, optionally as set forth in SEQ ID NO:84, further optionally as set forth in SEQ ID NO:82;
- (e) the CDR2 β amino acid sequence set forth in SEQ ID NO:79; and
- (f) the CDR3 β amino acid sequence set forth in SEQ ID NO:36.

31. The binding protein of any one of claims 22, 23, or 30, comprising an amino acid sequence having at least 85% identity to an amino acid sequence encoded by:

- (a) TRBJ2-3*01;
- (b) TRAV12-3*01;
- (c) TRBV12-3*01;
- (d) TRAJ29*01; and/or
- (e) TRBD1*01 or TRBD2*02

32. The binding protein of any one of claims 22, 23, 30, or 31, wherein the V α comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:98 and the V β comprises an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:97.

33. The binding protein of claim 32, wherein the V α comprises or consists the amino acid sequence set forth in SEQ ID NO:98, and wherein the V β comprises or consists the amino acid sequence set forth in SEQ ID NO:97.

34. The binding protein of any one of claims 22, 23, or 30-33, comprising a TCR α chain (TCR α) and a TCR β chain (TCR β), wherein the TCR α comprises or consists

of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:106 or 15, and/or wherein the TCR β comprises or consists of an amino acid sequence having at least about 85% identity to the amino acid sequence set forth in SEQ ID NO:105 or 14.

35. The binding protein of claim 34, wherein the TCR α comprises or consists the amino acid sequence set forth in SEQ ID NO:106 or 15, and wherein the TCR β comprises or consists of the amino acid sequence set forth in SEQ ID NO:105 or 14.

36. The binding protein of any one of claims 22-35, wherein the binding protein is capable specifically binding to a SEQ ID NO:31:human leukocyte antigen (HLA) complex.

37. The binding protein of any one of claims 22-35, wherein the HLA comprises HLA-A*201.

38. The binding protein of any one of claims 1-35, wherein the binding protein is or comprises a TCR, wherein the TCR is optionally soluble, an antigen-binding fragment of a TCR, a scTCR, or CAR.

39. The binding protein of any one of claims 1-38, wherein the binding protein is human, humanized, or chimeric.

40. The binding protein of any one of claims 1-38, wherein the binding protein is capable of binding to the mesothelin:HLA complex in the absence of, or independent of, CD8.

41. The binding protein of any one of claims 1-40, wherein the binding protein has a Msln peptide EC₅₀ of about 9 μ M, about 8 μ M, about 7 μ M, about 6 μ M, about 5 μ M, about 4 μ M, about 3 μ M, about 2 μ M, about 1 μ M, about 0.9 μ M, about 0.8 μ M, about 0.7 μ M, about 0.6 μ M, about 0.5 μ M, about 0.4 μ M, about 0.3 μ M, about 0.2 μ M, or less.

42. A composition comprising the binding protein of any one of claims 1–41 and a pharmaceutically acceptable carrier, diluent, or excipient.

43. A polynucleotide encoding the binding protein of any one of claims 1–41.

44. The polynucleotide of claim 43, wherein the polynucleotide is codon optimized for expression in a host cell, wherein the host cell is optionally a human immune system cell, preferably a T cell.

45. The polynucleotide of claim 43 or 44, comprising a polynucleotide that has at least about 50% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs:1-4, 9-12, 17-20, 25, and 26.

46. The polynucleotide of any one of claims 43-45, comprising a TCR α chain-encoding polynucleotide and a TCR β chain-encoding polynucleotide that have at least about 50% identity to the polynucleotide sequences set forth in SEQ ID NOs:

- (i) 1 and 3, respectively;
- (ii) 2 and 4, respectively;
- (iii) 9 and 11, respectively;
- (iv) 10 and 12, respectively;
- (v) 17 and 19, respectively;
- (vi) 18 and 20, respectively; or
- (vii) 25 and 26, respectively.

47. The polynucleotide of any one of claims 43-46, comprising a polynucleotide that encodes a self-cleaving peptide disposed between a TCR β chain-encoding polynucleotide and a TCR α chain-encoding polynucleotide.

48. The polynucleotide of claim 47, wherein the encoded polypeptide comprises the amino acid sequence as set forth in any one of SEQ ID NOs:8, 16, 24, and 30.

49. The polynucleotide of claim 47 or 48, wherein the polynucleotide encoding the binding protein has at least about 50% identity to the polynucleotide sequence as set forth in any one of SEQ ID NOs:5, 13, 21, 27, and 120.

50. An expression vector, comprising the polynucleotide of any one of claims 43-49 operably linked to an expression control sequence.

51. The expression vector of claim 50, wherein the expression vector is capable of delivering the polynucleotide to a host cell.

52. The expression vector of claim 51, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

53. The expression vector of claim 52, wherein the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a dendritic cell, or any combination thereof.

54. The expression vector of claim 53, wherein the T cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof.

55. The expression vector of any one of claims 50-54, wherein the expression vector is a viral vector.

56. The expression vector of claim 55, wherein the viral vector is a lentiviral vector or a γ -retroviral vector.

57. A recombinant host cell comprising a heterologous polynucleotide of any one of claims 43-49 and/or the expression vector of any one of claims 50-56, wherein the recombinant host cell is capable of expressing on its cell surface the encoded binding protein.

58. The recombinant host cell claim 57, wherein the recombinant host cell is a hematopoietic progenitor cell or a human immune system cell.

59. The recombinant host cell of claim 58, wherein the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a dendritic cell, or any combination thereof.

60. The recombinant host cell of claim 58 or 59, wherein the immune system cell is a T cell.

61. The recombinant host cell of claim 60, wherein the T cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof.

62. The recombinant host cell of any one of claims 57-61, wherein the host cell is a T cell or a NK-T cell encoding an endogenous TCR, and wherein the binding protein is capable of more efficiently associating with a CD3 protein as compared to the endogenous TCR.

63. The recombinant host cell of any one of claims 57-62, wherein *Nur77* expression in the host cell is increased when the host cell is in the presence of the Msln peptide bound by the binding protein at a concentration of about 10⁻² μ M peptide, about 10⁻¹ μ M peptide, about 1 μ M peptide, or about 10¹ μ M peptide, wherein the peptide is optionally presented to the host cell by an antigen presenting cell.

64. The recombinant host cell of any one of claims 57-63, wherein the recombinant host cell does not produce IFN- γ and/or does not exhibit activation and/or cytotoxic activity when contacted with a cell expressing:

- (i) HLA-C6:02:01;
- (ii) HLA-B13:01:01 without HLA-B13:02:01;
- (iii) HLA-A3;

- (iv) HLA-A29;
 - (v) HLA-B40;
 - (vi) HLA-B44;
 - (vii) HLA-C3;
 - (viii) HLA-C16;
 - (ix) HLA-A1;
 - (x) HLA-24;
 - (xi) HLA-B7;
 - (xii) HLA-B57;
 - (xiii) HLA-C7;
 - (xiv) HLA-A11;
 - (xv) HLA-B15;
 - (xvi) HLA-C4;
 - (xvii) HLA-C12;
 - (xviii) HLA-B8;
 - (xix) HLA-B49;
 - (xx) HLA-B51;
 - (xxi) HLA-C15;
 - (xxii) HLA-A30;
 - (xxiii) HLA-A68;
 - (xxiv) HLA-C2;
 - (xxv) HLA-A32;
 - (xxvi) HLA-A33;
 - (xxvii) HLA-B55;
 - (xxviii) HLA-C1;
 - (xxvix) HLA-C5;
 - (xxix) HLA-B8;
 - (xxx) HLA-B35; or
 - (xxxi) any combination of (i)-(xxx),
- provided that the mesothelin peptide bound by the encoded binding protein is not present.

65. The recombinant host cell of any one of claims 57-64, wherein the host cell is a T cell or a NK-T cell encoding an endogenous TCR, wherein the binding protein encoded by the heterologous polynucleotide has higher cell surface expression as compared to the endogenous TCR.

66. A cell composition, comprising a recombinant host cell of any one of claims 57-65, and a pharmaceutically acceptable carrier, excipient, or diluent.

67. A unit dose, comprising the recombinant host cell of any one of claims 57-65 or the composition of claim 66.

68. A method of treating a disease or disorder associated with mesothelin expression and/or activity in a subject, the method comprising:

administering to the subject an effective amount of the binding protein of any one of claims 1-41, the recombinant host cell of any one of claims 57-65, the composition of claim 66, or the unit dose of claim 67.

69. The method of claim 68, disease or disorder associated with mesothelin expression and/or activity is a hyperproliferative disease or a proliferative disease.

70. The method of claim 68 or 69, wherein the disease or disorder associated with mesothelin expression and/or activity is a cancer.

71. The method of claim 70, wherein the cancer is a solid cancer or a hematological malignancy.

72. The method of claim 70 or 71, wherein the cancer comprises biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, lung cancer, mesothelioma, malignant melanoma,

osteosarcoma, ovarian cancer, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, or uterine cancer.

73. The method of claim 70 or 71, wherein the cancer comprises pancreatic cancer, ovarian cancer, breast cancer, gastric cancer, colorectal cancer, mesothelioma, or lung cancer.

74. The method of any one of claims 68-73, wherein the binding protein, host cell, composition, or unit dose is administered parenterally or intravenously.

75. The method of any one of claims 68-74, wherein the method comprises administering a plurality of doses of the binding protein, host cell, composition, or unit dose to the subject.

76. The method of claim 75, wherein the plurality of doses are administered at intervals between administrations of about two to about four weeks.

77. The method of any one of claims 68-76, wherein the method further comprises administering a cytokine to the subject.

78. The method of claim 77, wherein the cytokine comprises IL-2, IL-15, IL-21, or any combination thereof.

79. The method of any one of claims 78, wherein the subject is further receiving or has received an immune checkpoint inhibitor, an agonist of a stimulatory immune checkpoint agent, radiation therapy, an antibody, an antibody-drug conjugate, an Fc fusion protein, an antisense nucleotide therapy, a gene therapy, a vaccine, a surgery, a chemotherapy, or any combination thereof.

80. The binding protein of any one of claims 1–41, the composition of claim 42, the polynucleotide of any one of claims 43–49, the expression vector of any one of claims 50–56, the recombinant host cell of any one of claims 57–65, the cell composition of claim 66, or the unit dose of claim 67, for use in the treatment of a disease or disorder characterized by mesothelin expression and/or activity.

81. The binding protein of any one of claims 1–41, the composition of claim 42, the polynucleotide of any one of claims 43–49, the expression vector of any one of claims 50–56, the recombinant host cell of any one of claims 57–65, the cell composition of claim 66, or the unit dose of claim 67, for use in adoptive immunotherapy of a disease or disorder characterized by mesothelin expression and/or activity.

82. The binding protein of any one of claims 1–41, the composition of claim 42, the polynucleotide of any one of claims 43–49, the expression vector of any one of claims 50–56, the recombinant host cell of any one of claims 57–65, the cell composition of claim 66, or the unit dose of claim 67, for use in the manufacture of a medicament for treating a disease or disorder characterized by mesothelin expression and/or activity.

83. The binding protein, composition, polynucleotide, expression vector, recombinant host cell, cell composition, or unit dose for use according to any one of claims 80–82, wherein the disease or disorder characterized by mesothelin expression and/or activity is mesothelioma, pancreatic cancer, ovarian cancer, lung cancer, a cancer wherein an Msln₂₀₋₂₈ peptide is expressed on a tumor cell of the cancer, or a cancer wherein an Msln₅₃₀₋₅₃₈ peptide is expressed on a tumor cell of the cancer.

84. The binding protein, composition, polynucleotide, expression vector, recombinant host cell, cell composition, or unit dose for use according to any one of claims 80–83, wherein the disease or disorder characterized by mesothelin expression and/or activity is pancreatic cancer, ovarian cancer, breast cancer, gastric cancer, colorectal cancer, mesothelioma, or lung cancer.

85. An isolated polynucleotide that encodes a binding protein that is capable of specifically binding to a SEQ ID NO:32:HLA-A:02*01 complex, wherein the polynucleotide comprises or consists of the polynucleotide sequence set forth in SEQ ID NO:120.

86. An expression vector, comprising the polynucleotide of claim 85 operably linked to an expression control sequence.

87. The expression vector of claim 86, wherein the expression vector is capable of delivering the polynucleotide to a host cell.

88. The expression vector of claim 87, wherein the host cell is a hematopoietic progenitor cell or a human immune system cell.

89. The expression vector of claim 88, wherein the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a dendritic cell, or any combination thereof.

90. The expression vector of claim 89, wherein the T cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof.

91. The expression vector of any one of claims 86-90, wherein the expression vector is a viral vector.

92. The expression vector of claim 91, wherein the viral vector is a lentiviral vector or a γ -retroviral vector.

93. A recombinant host cell comprising the polynucleotide of claim 85 and/or the expression vector of any one of claims 86-92, wherein the recombinant host cell is capable of expressing on its cell surface the encoded binding protein.

94. The recombinant host cell claim 83, wherein the recombinant host cell is a hematopoietic progenitor cell or a human immune system cell.

95. The recombinant host cell of claim 94, wherein the immune system cell is a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁻ CD8⁻ double negative T cell, a $\gamma\delta$ T cell, a natural killer cell, a natural killer T cell, a macrophage, a dendritic cell, or any combination thereof.

96. The recombinant host cell of claim 94 or 95, wherein the immune system cell is a T cell.

97. The recombinant host cell of claim 96, wherein the T cell is a naïve T cell, a central memory T cell, a stem cell memory T cell, an effector memory T cell, or any combination thereof.

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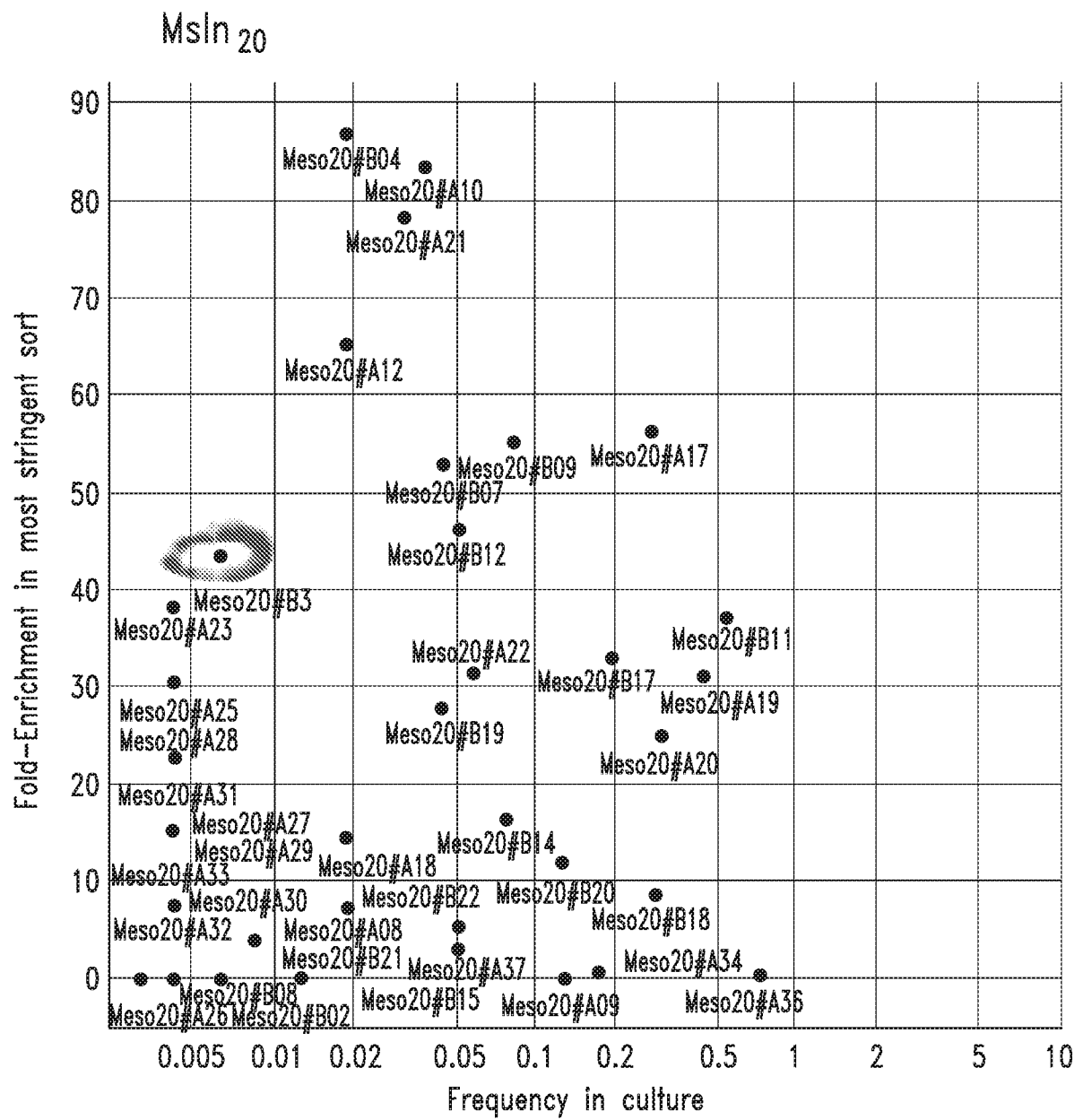


FIG. 1A

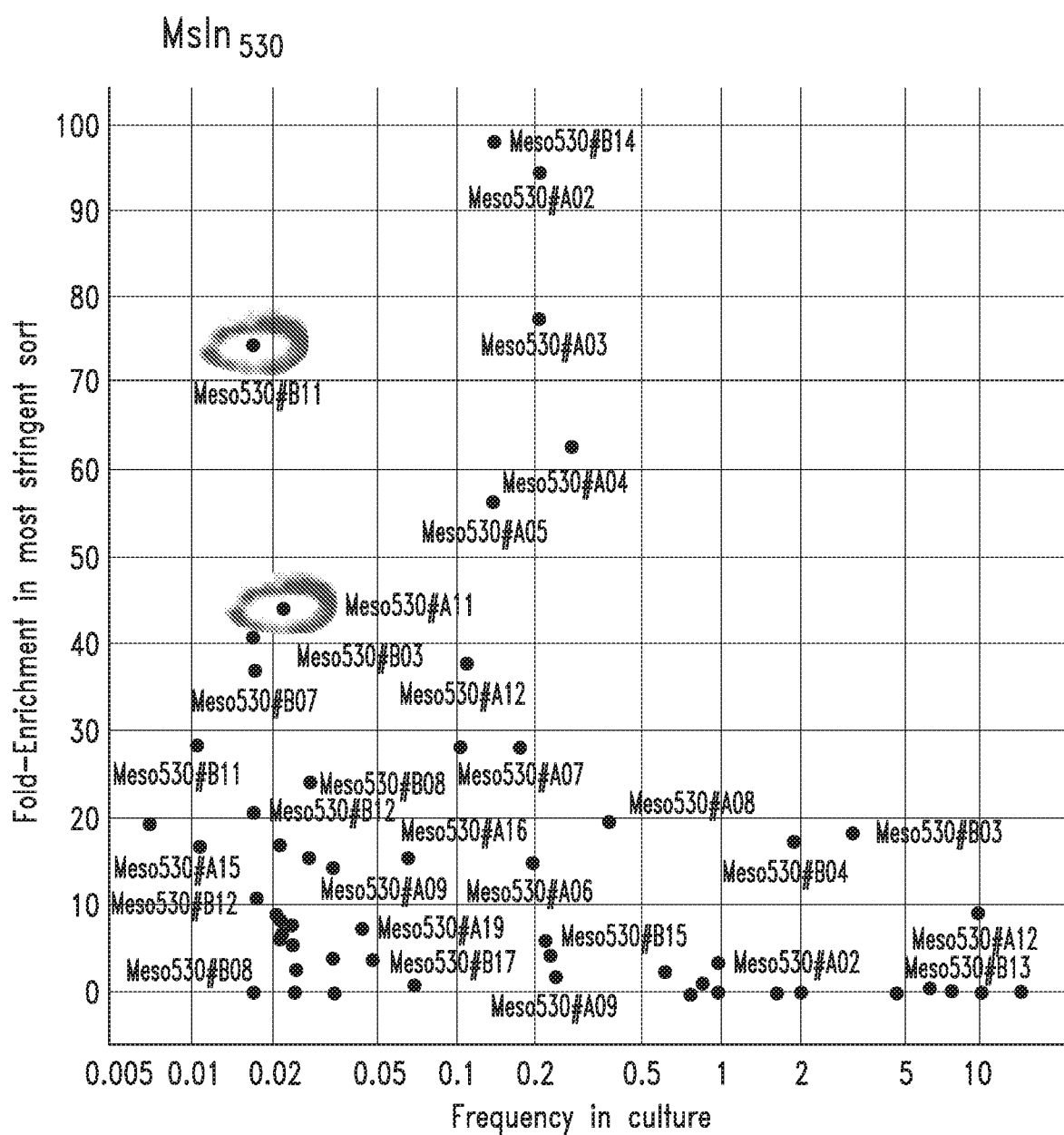


FIG. 1B

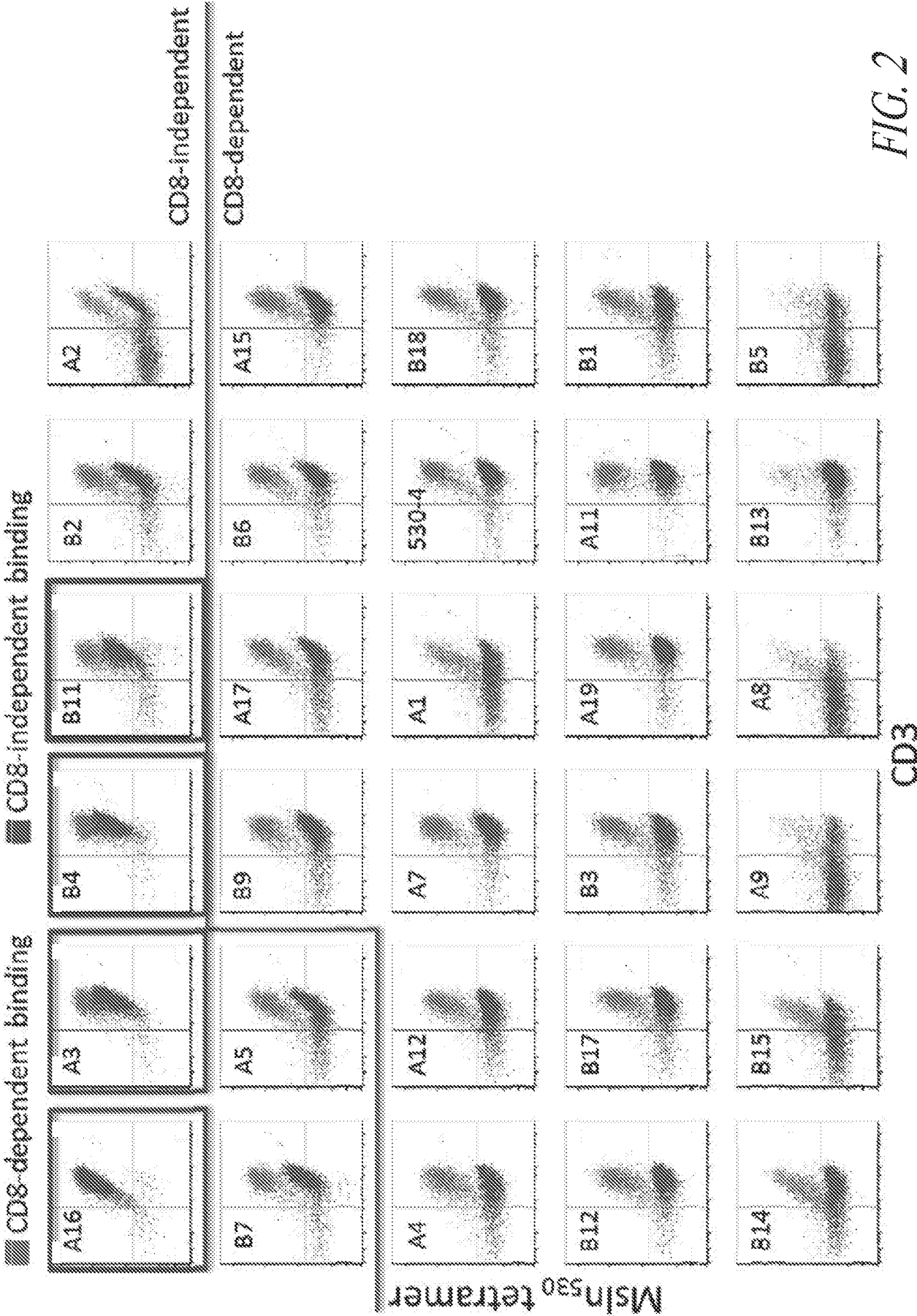


FIG. 2

Concentration of peptide pulsed onto T2 target cells.

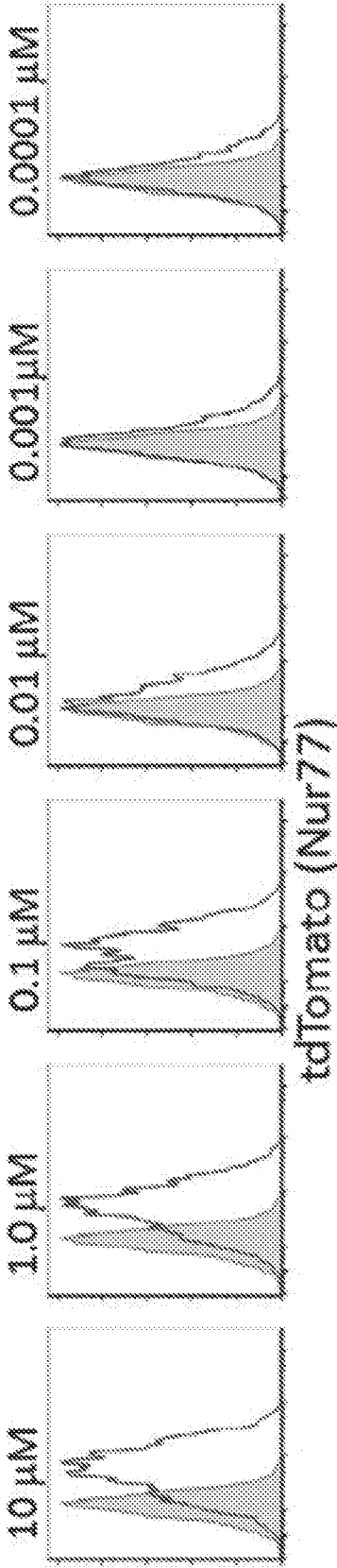


FIG. 3A

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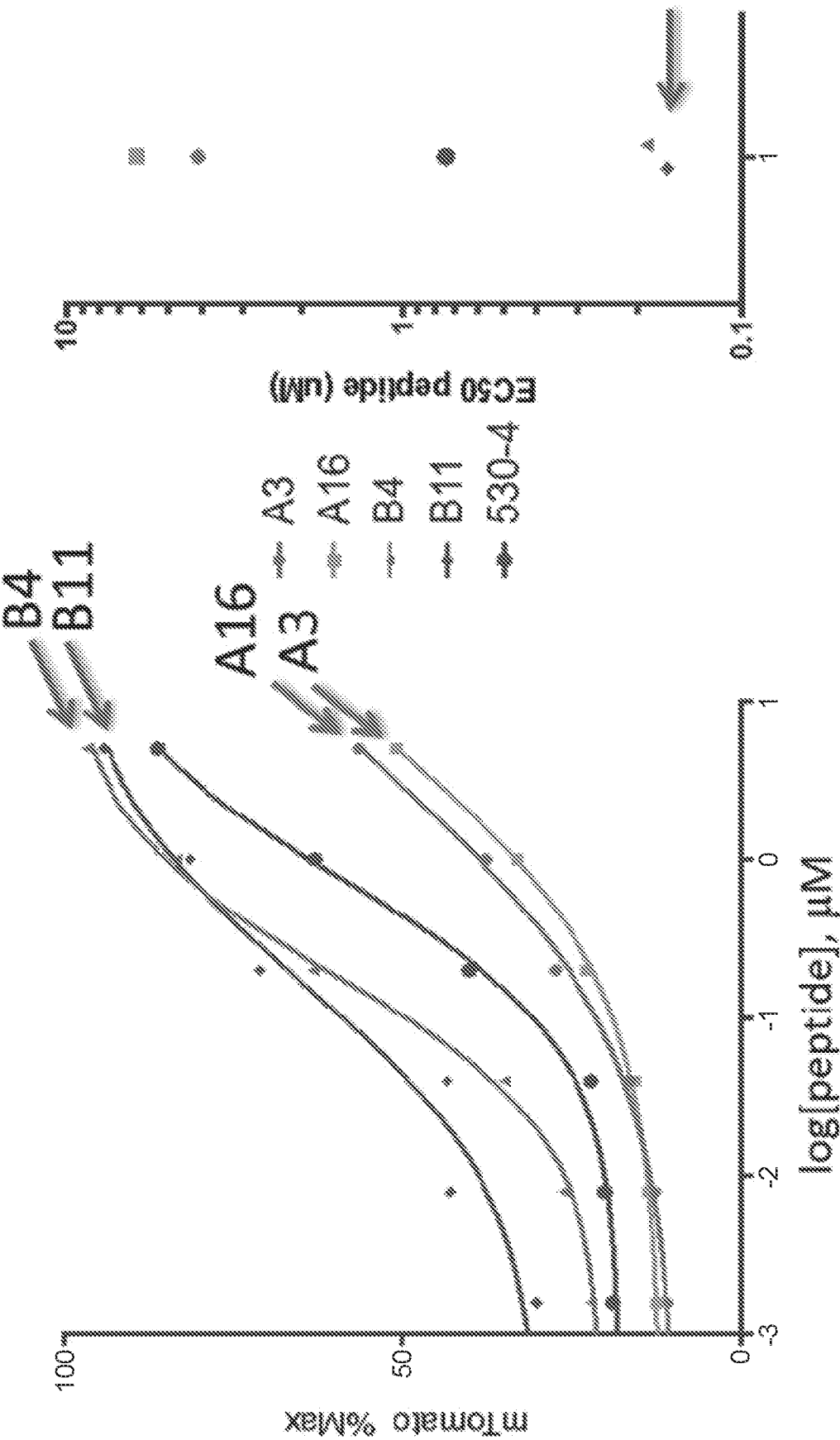


FIG. 3C

FIG. 3B

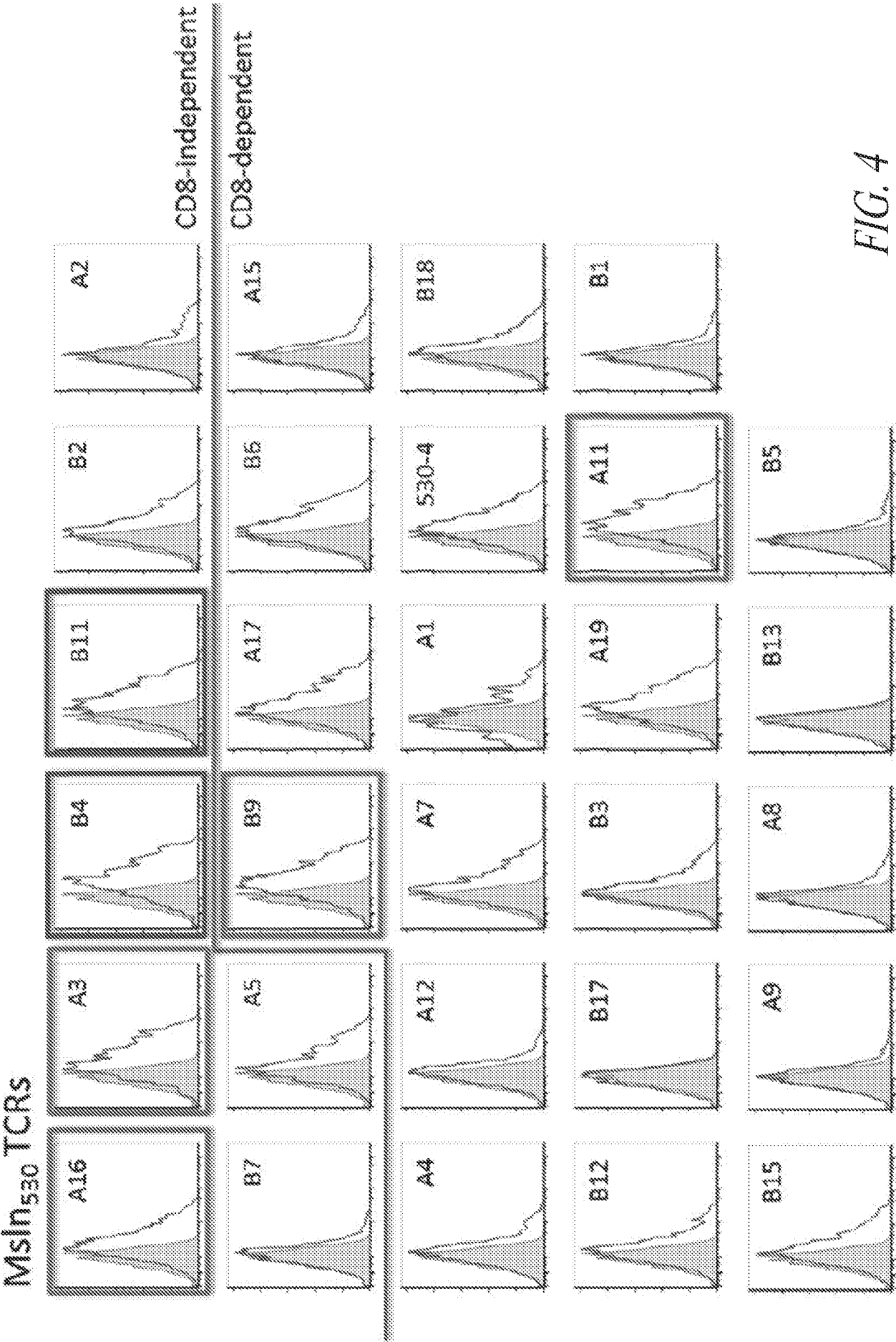
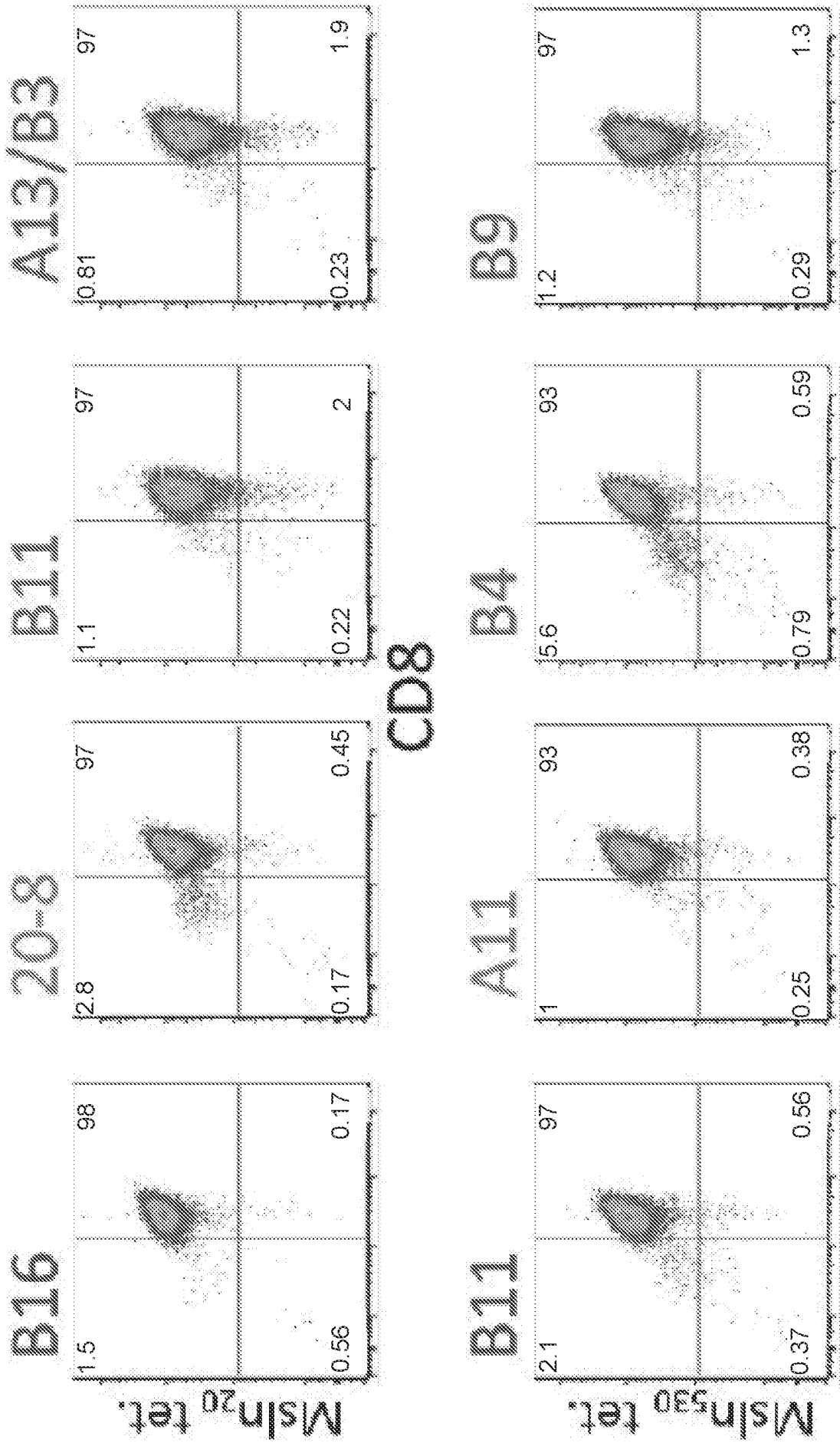


FIG. 4

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CD8
FIG. 5A

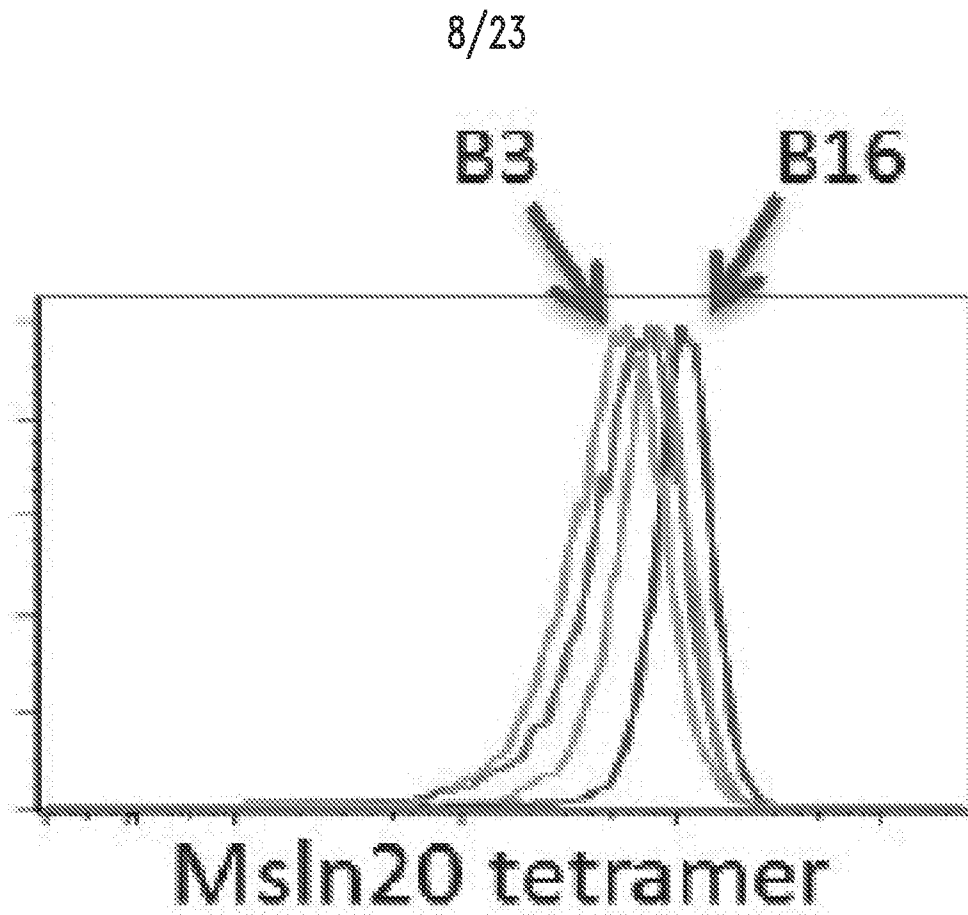


FIG. 5B

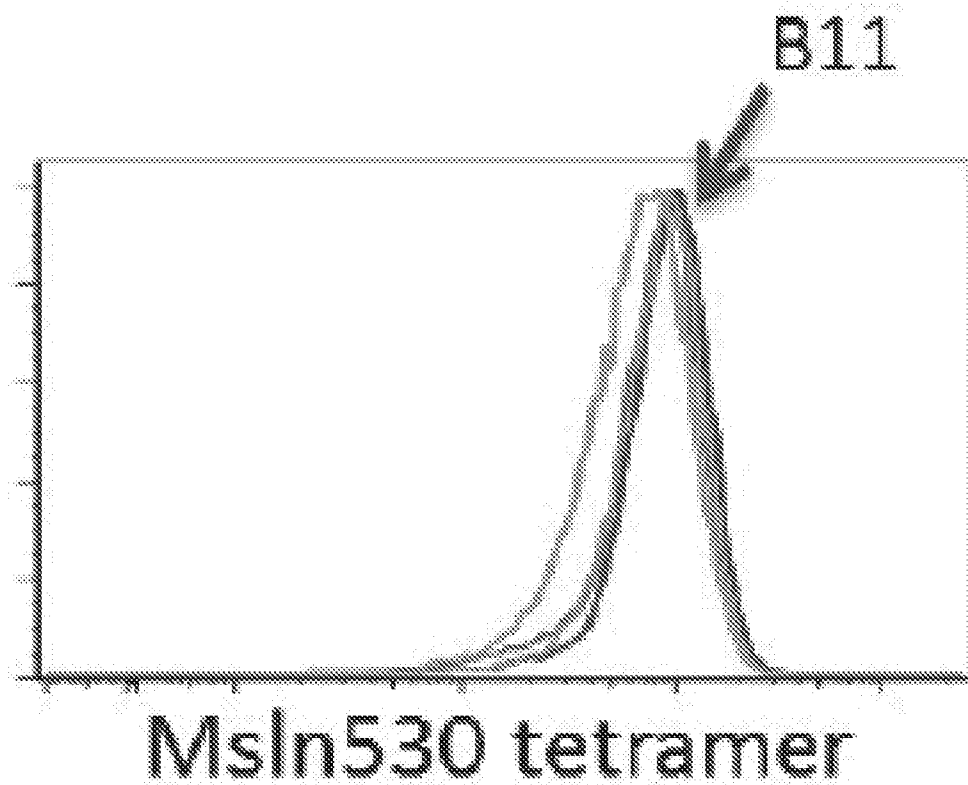


FIG. 5C

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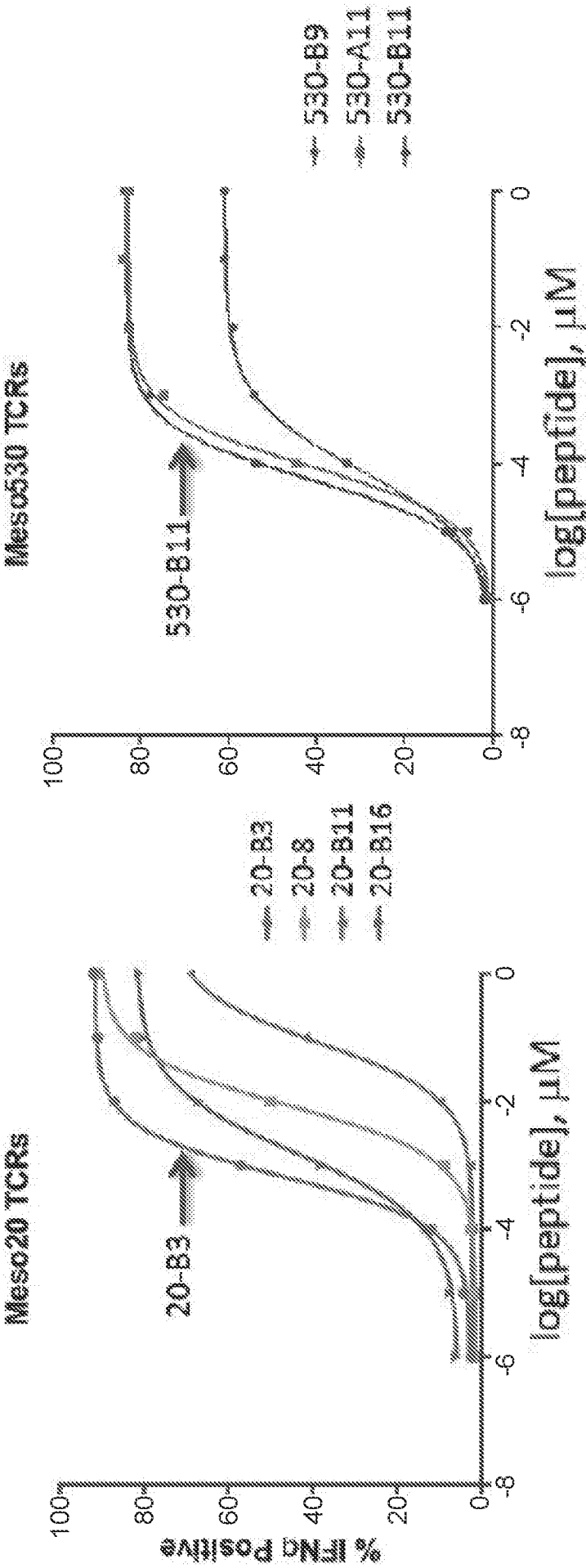


FIG. 6A

FIG. 6B

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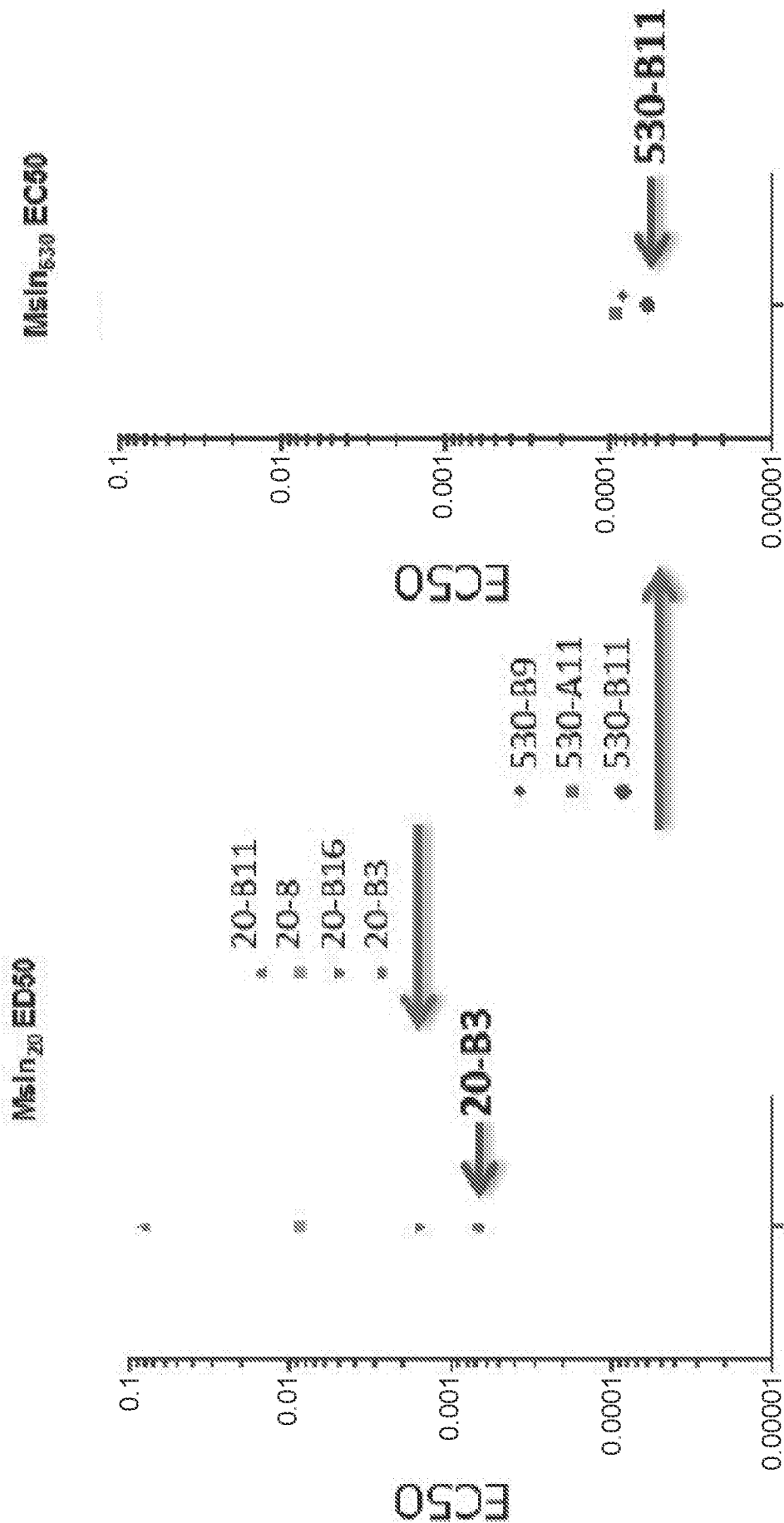


FIG. 6C

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- MsIn530-B11
- MsIn530-A11
- MsIn20-B3

MDA-MB-468

No IFN γ

Plus IFN γ

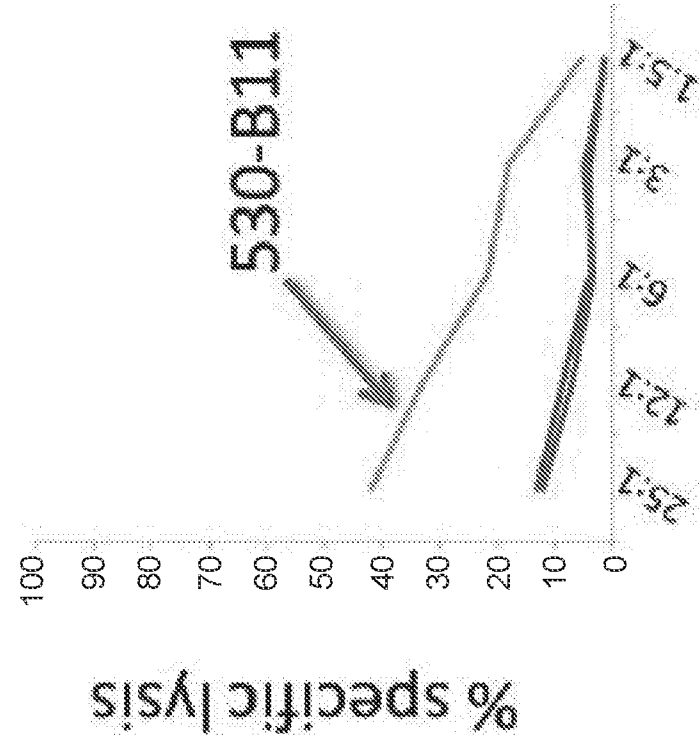


FIG. 7A

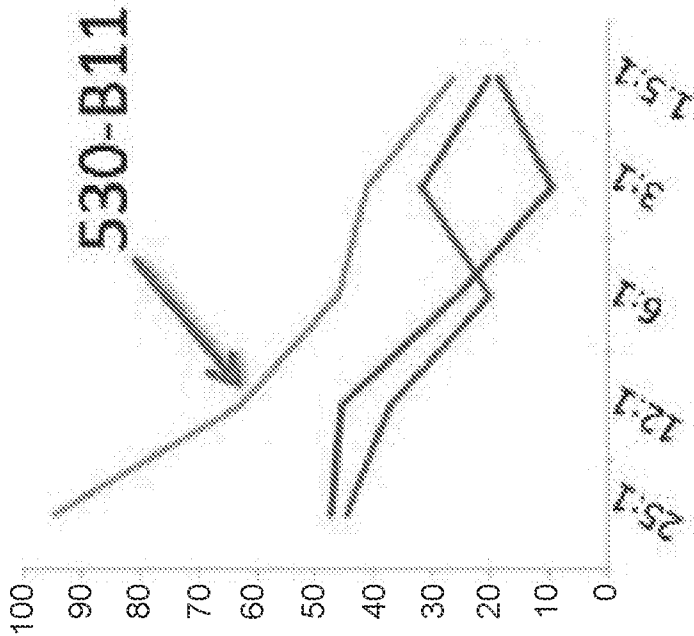


FIG. 7B

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- Ms/n530-B11
- Ms/n530-A11
- Ms/n20-B3

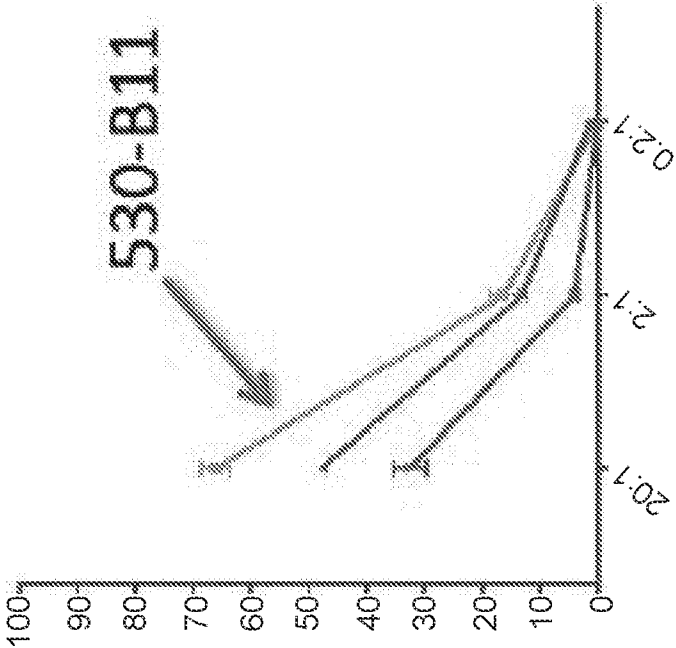


FIG. 7D

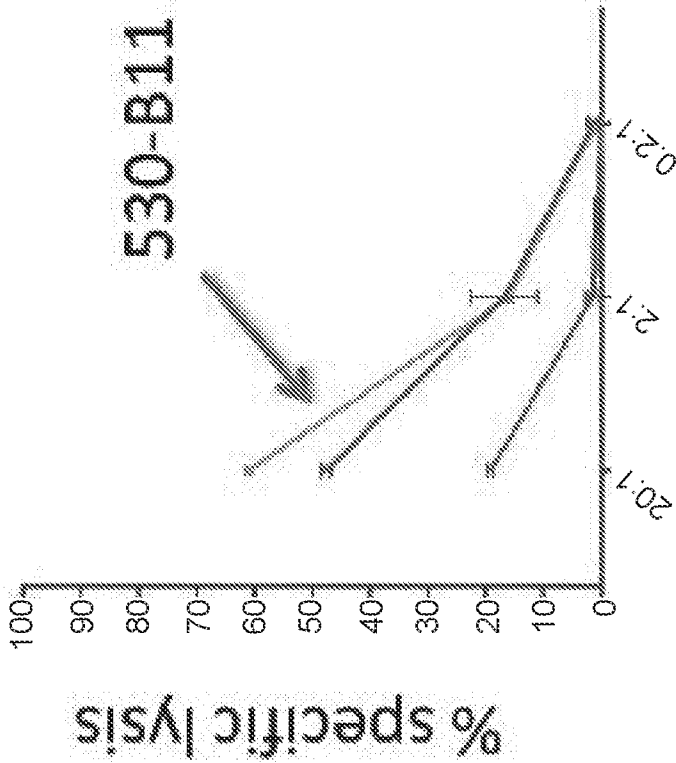


FIG. 7C

MDA-MB-231

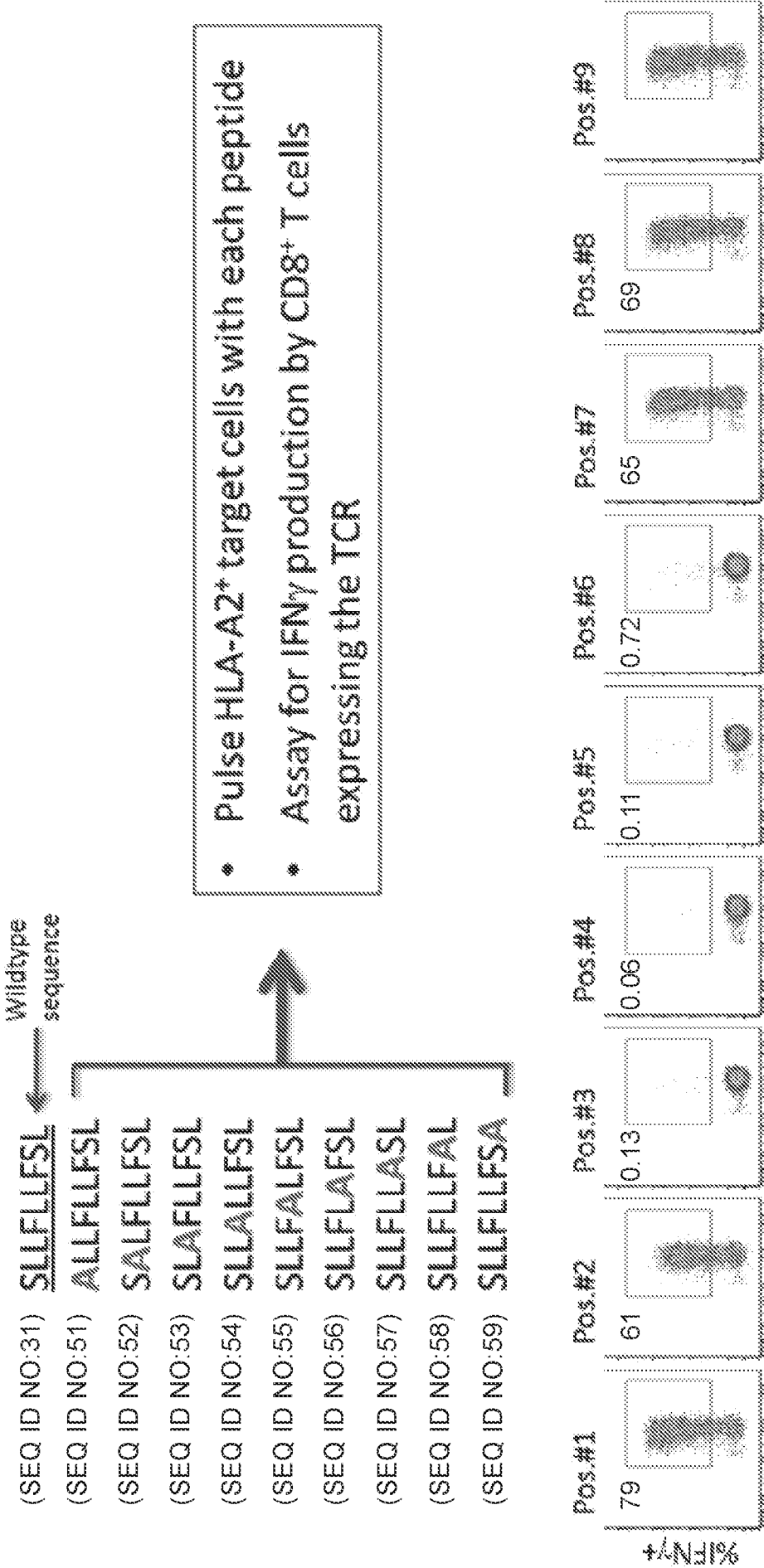


FIG. 8

MSLN₅₃₀ TCRs

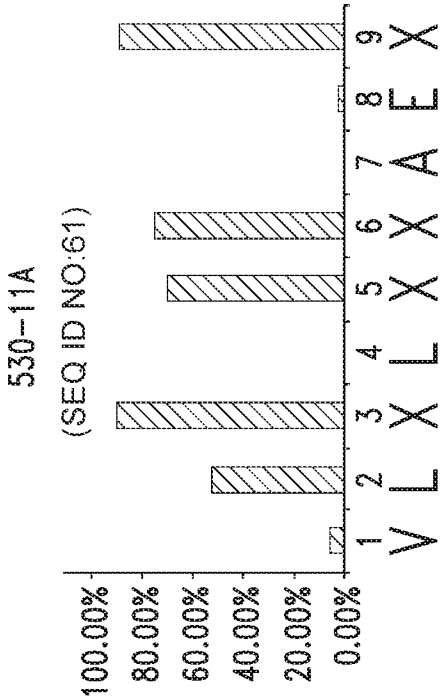


FIG. 9B

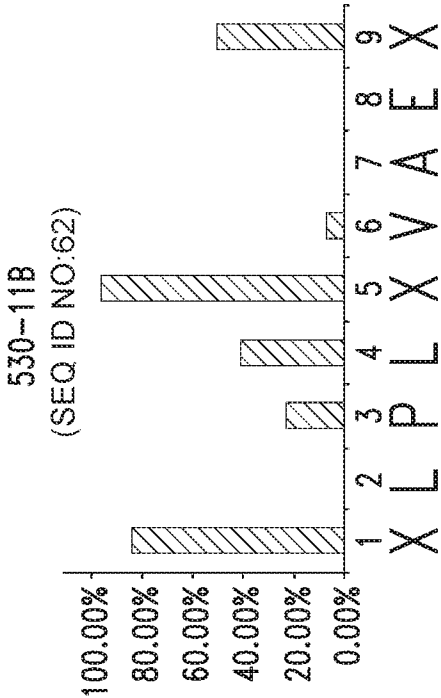


FIG. 9C

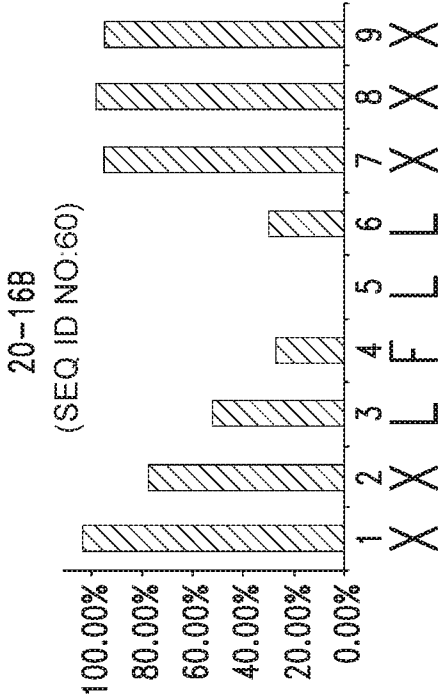
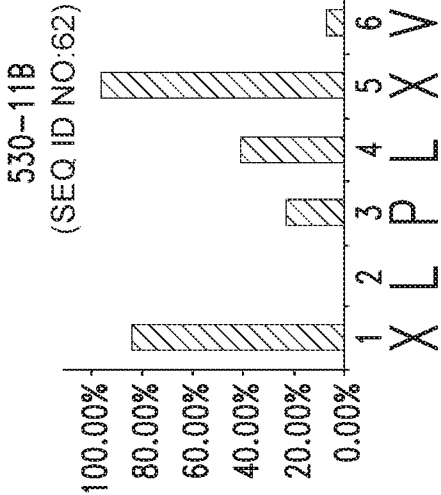


FIG. 9D



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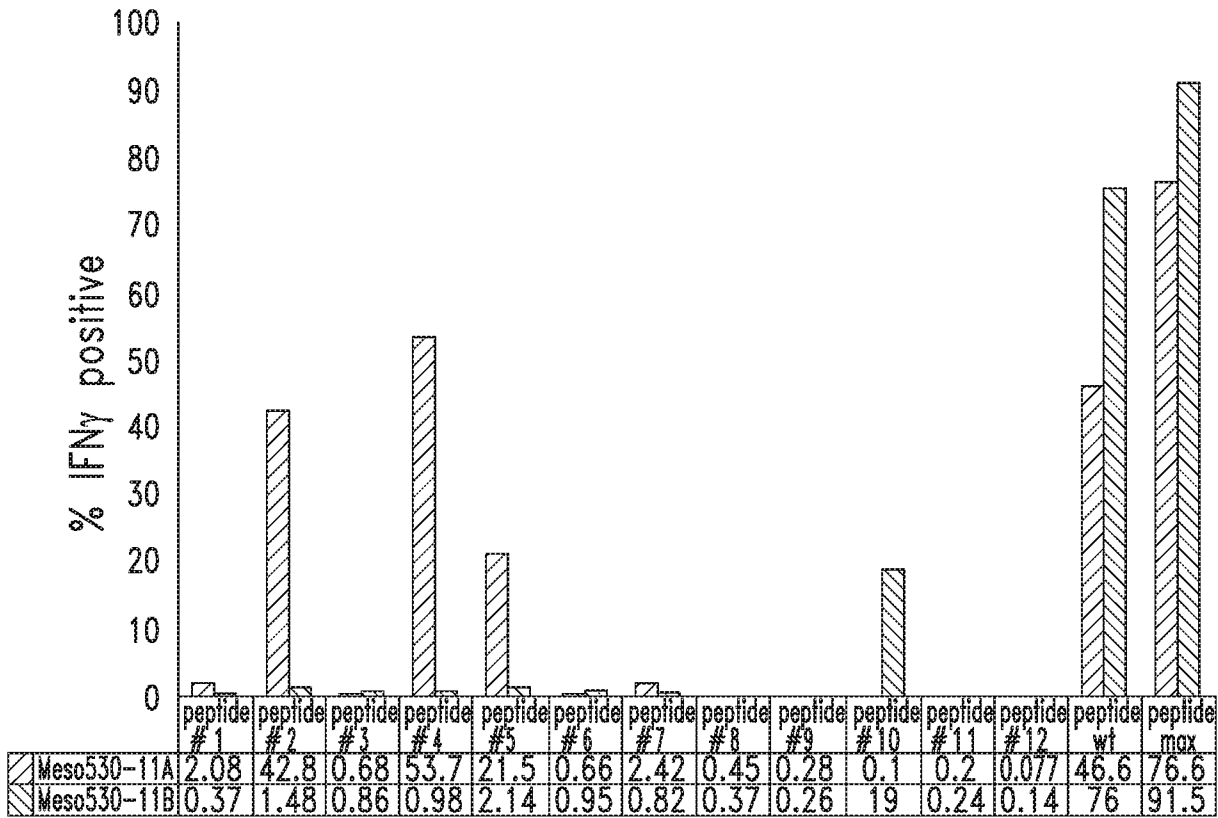


FIG. 11A

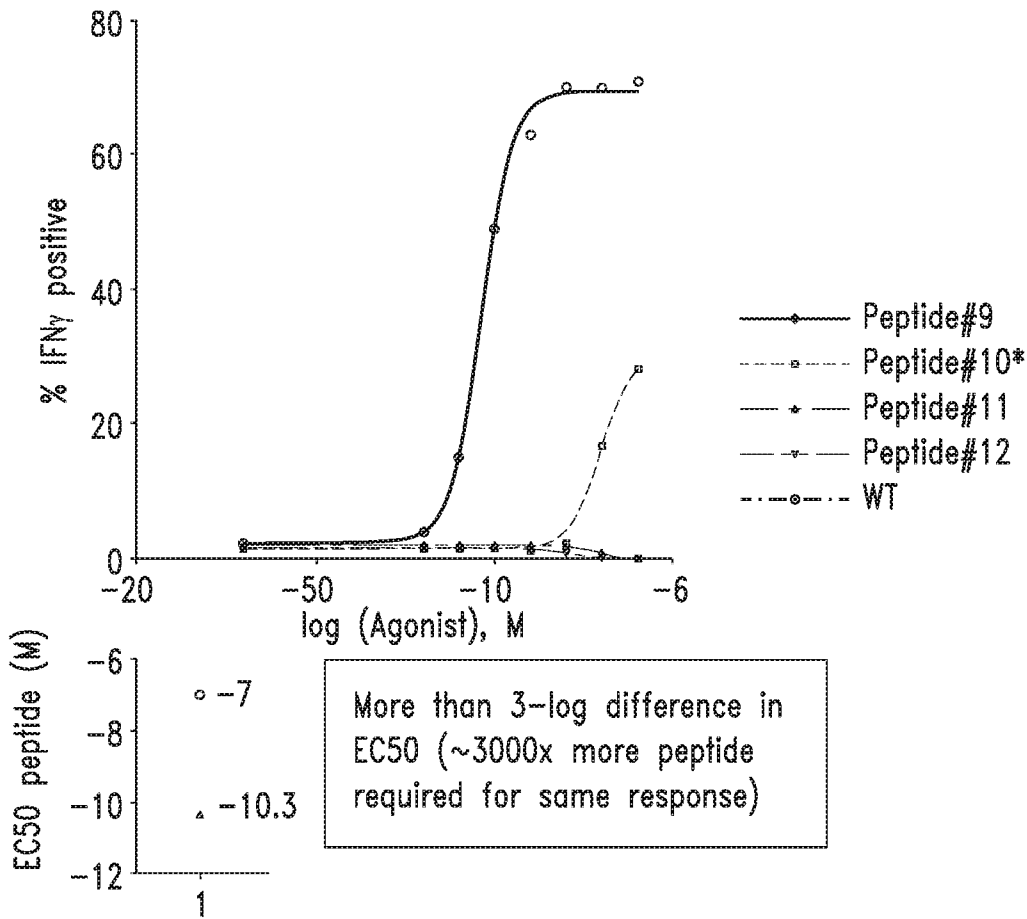
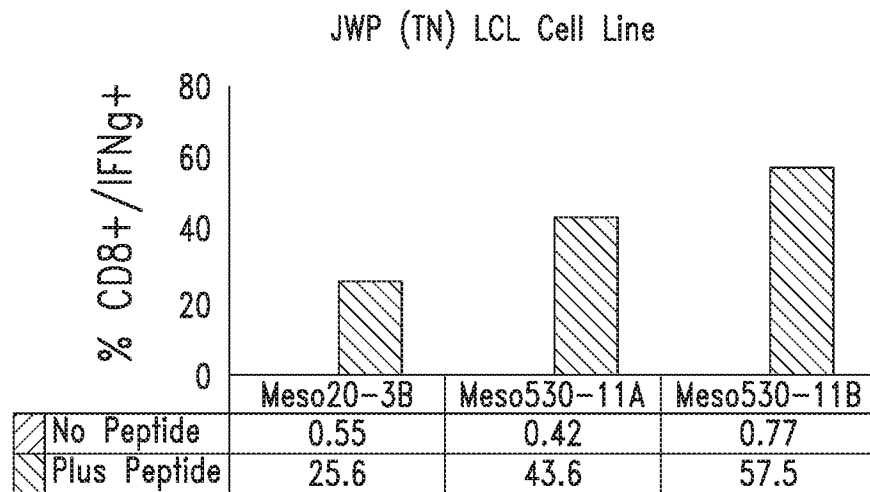
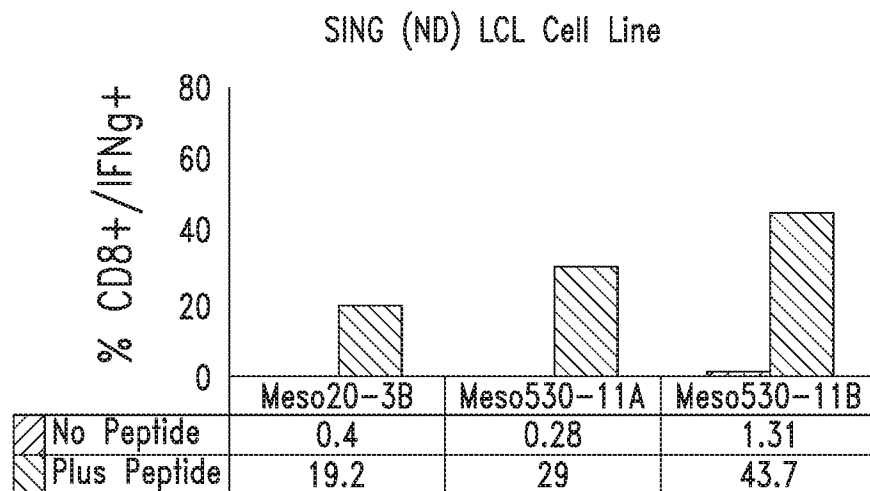


FIG. 11B

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Initials (Prot.)	HLA-A	HLA-B	HLA-Cw
DAH #2 (956)	A1,A2	B7,B15	C1,C7
DMJ (1334)	A2,A2	B44,B44	C5,C7
FGA (1334)	A2,A30	B7,B44	C7,C7
GWB (956)	A2,A11	B15,B44	C3,C5
JAS (956)	A1,A68	B44,B55	C3,C5
JRL #2 (956)	A1,A2	B8,B55	C3,C7
JWP (TN)	A2,A33	B7,B15	C3,C7
SING (ND)	A24,A32	B35,B40	C2,C4

FIG. 12A*FIG. 12B**FIG. 12C*

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DAH#2 (956) LCL Cell Line

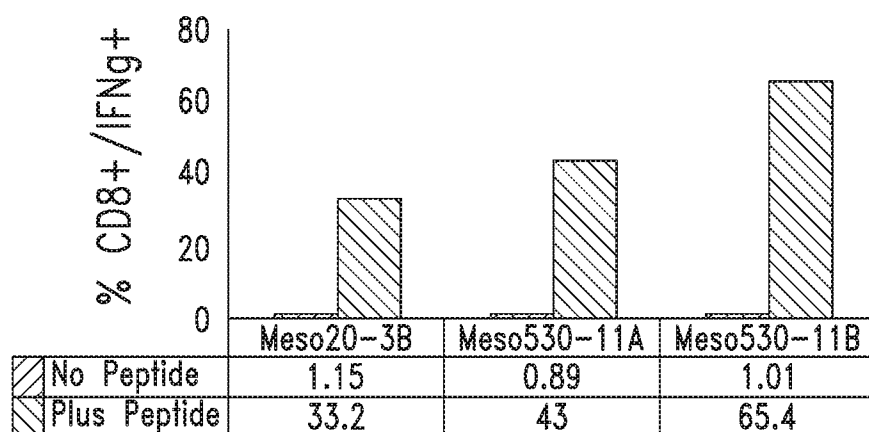


FIG. 12D

DMJ (1334) LCL Cell Line

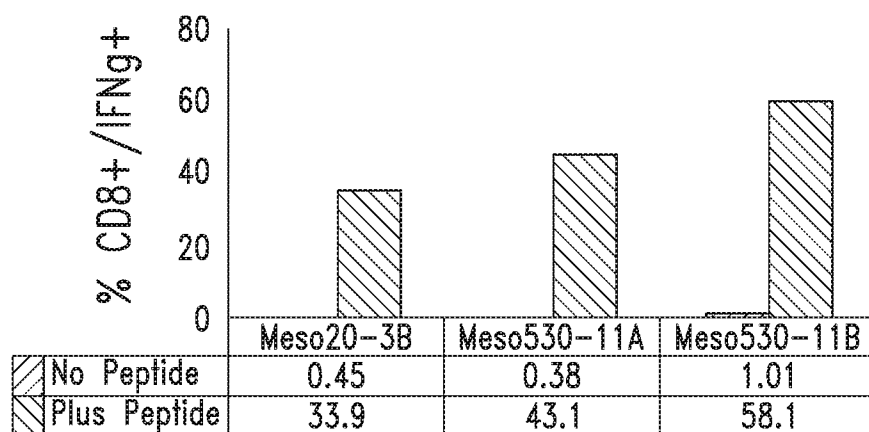


FIG. 12E

FGA (1334) LCL Cell Line

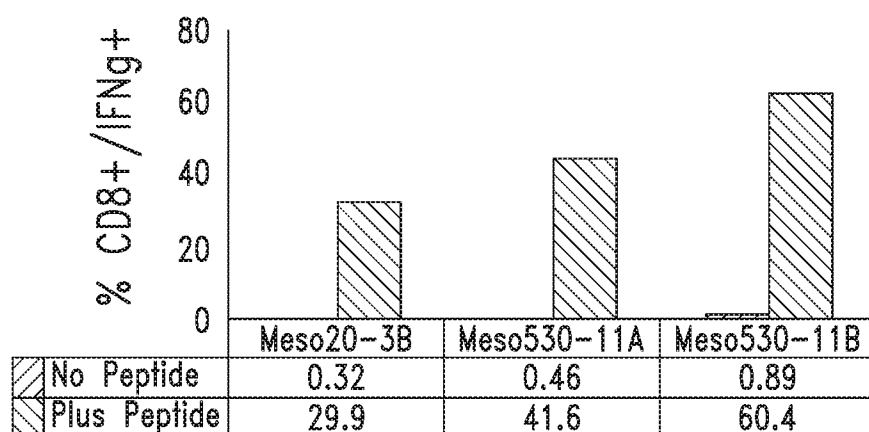


FIG. 12F

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GWB (956) LCL Cell Line

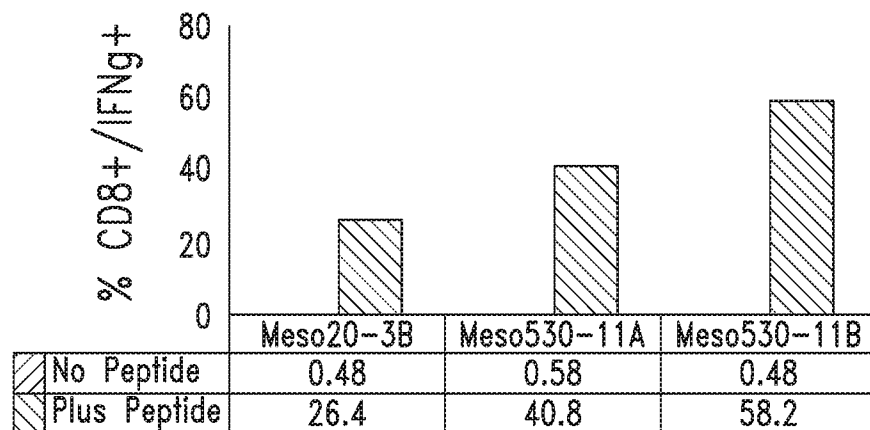


FIG. 12G

JAS (956) LCL Cell Line

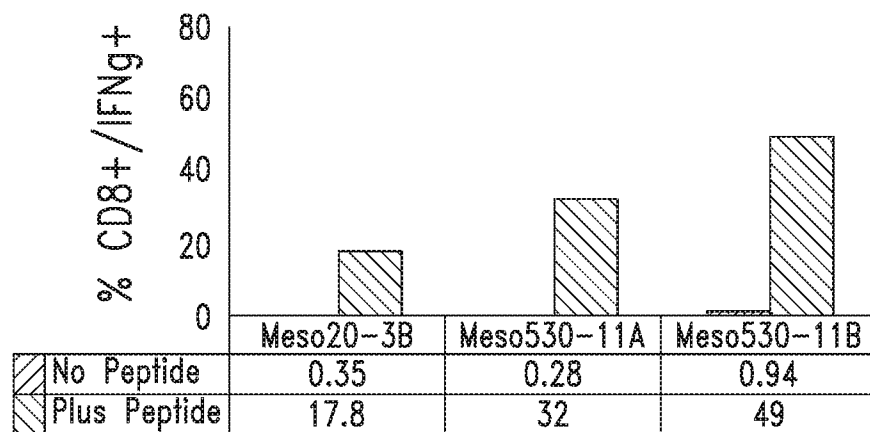


FIG. 12H

JRL#2 (956) LCL Cell Line

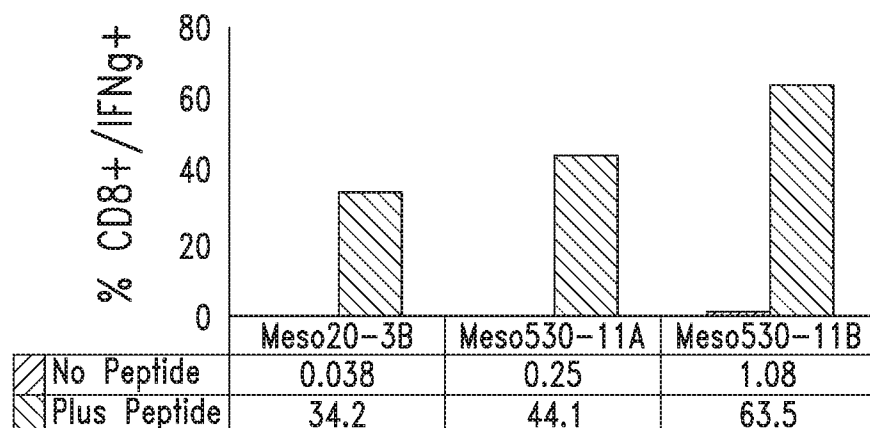
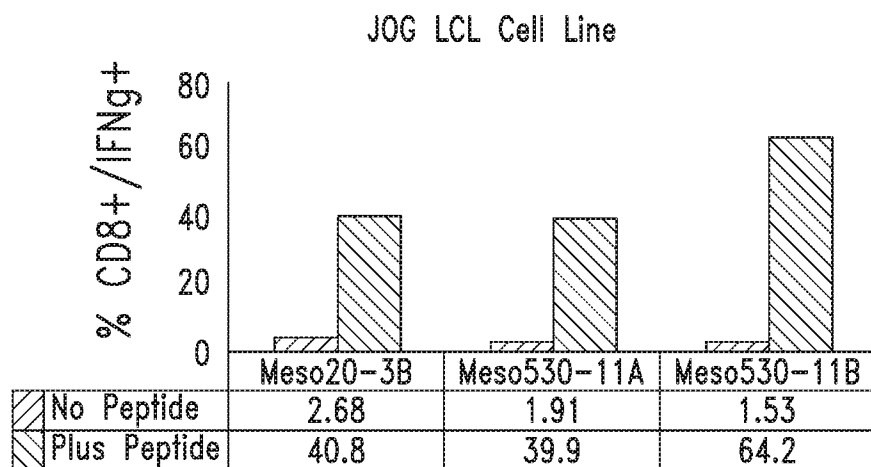
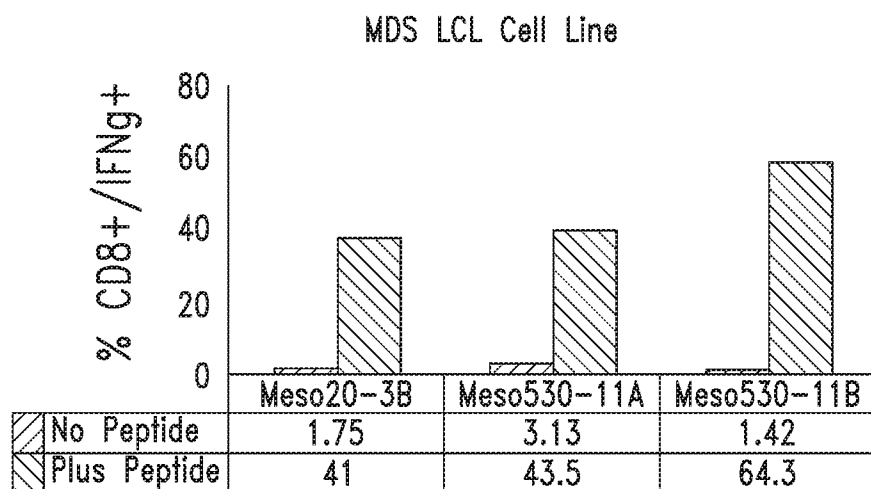


FIG. 12I

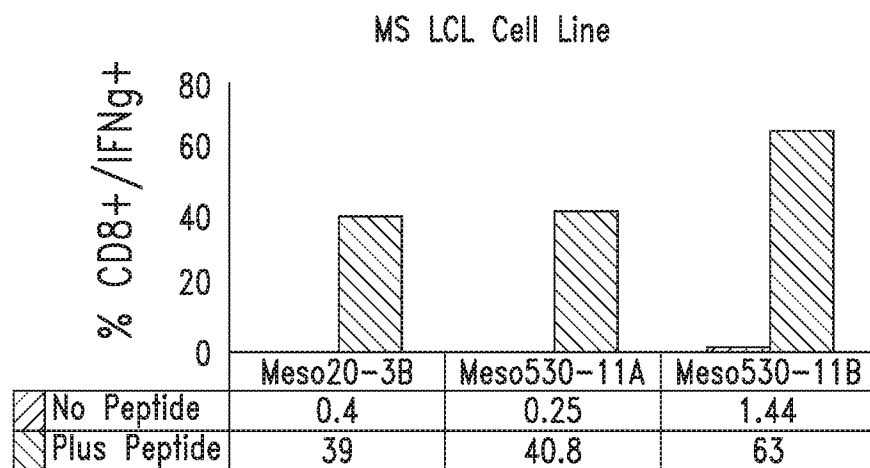
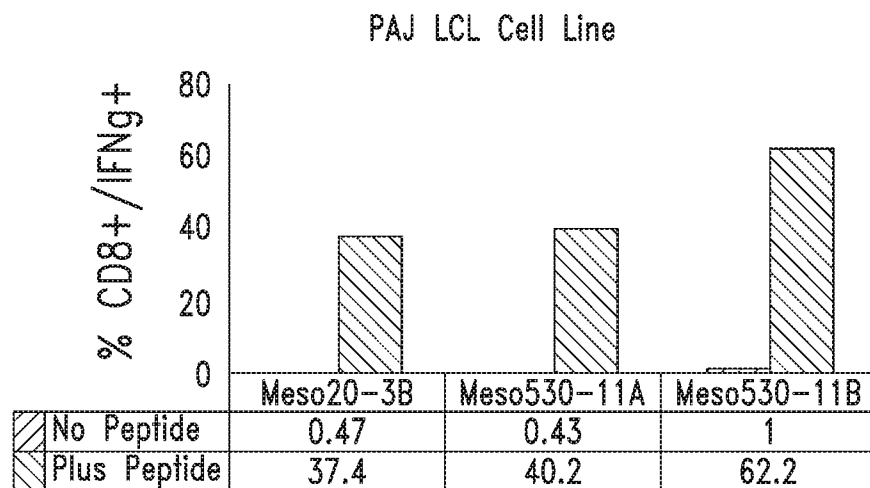
Initials (Prot.)	HLA-A	HLA-B	HLA-Cw
FAH	A3, A68	B13:02:01, B40	C2, C6:02:01
CLP	A2, A3	B40, B51	C3, C15
JOG	A3, A29	B40, B44	C3, C16
MS	A11, A11	B13:01:01, B15	C4, C12
GIM	A2, A30	B7, B13:02:01	C6:02:01, C7
MDS	A1, A24	B7, B57	C6:02:01, C7
PAJ	A1, A3	B8, B49	C7, C7

FIG. 13A

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*FIG. 13B**FIG. 13C*

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*FIG. 13D**FIG. 13E*

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CLP LCL Cell Line

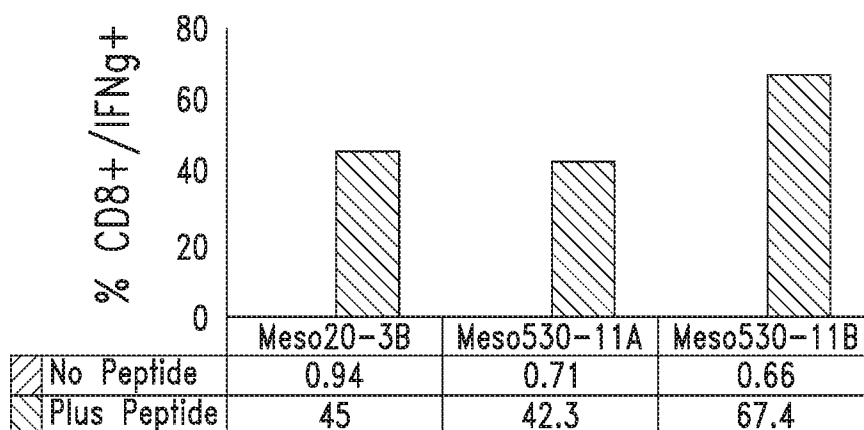


FIG. 13F

GIM LCL Cell Line

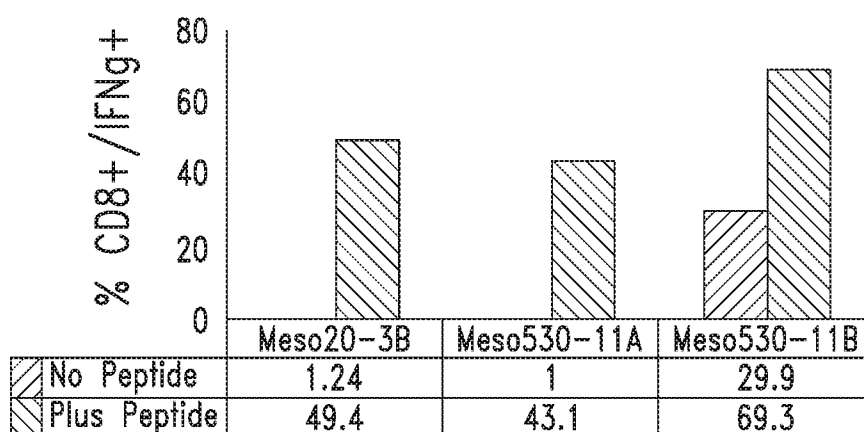


FIG. 13G

FAH (1334) LCL Cell Line

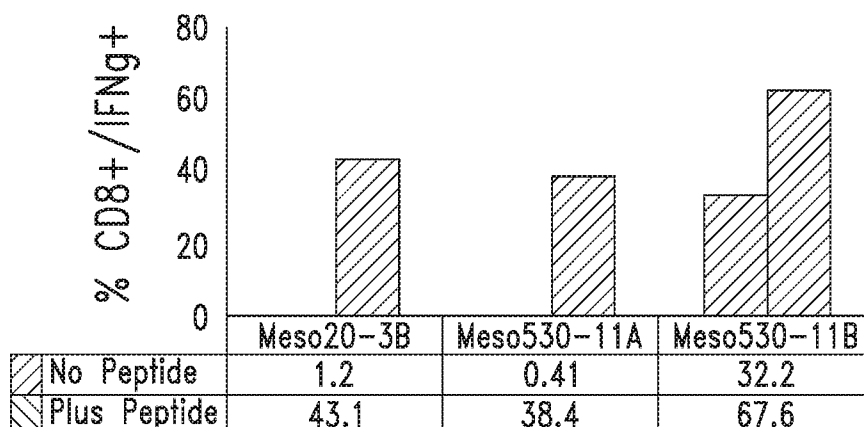


FIG. 13H

SEQUENCE LISTING

<110> Fred Hutchinson Cancer Research Center
Juno Therapeutics, Inc.
Schmitt, Thomas M.
Chapuis, Aude G.
Greenberg, Philip D.

<120> IMMUNOTHERAPY TARGETING MESOTHELIN

<130> 360056.475W0

<140> PCT

<141> 2019-11-08

<150> US 62/758,397

<151> 2018-11-09

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aagagcagcc cgccctgaac gacagccggt actgtctgag cagcagactg agagtgtccg
660

ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc tacggcctga
720

gcgagaacga cgagtggacc caggaccggg ccaagcccgt gaccagatc gtgtctgctg
780

aggcctgggg cagagccgat tgcggcttca ccagcgagag ctaccagcag ggcgtgctga
840

gcgccaccat cctgtacgag atcctgctgg gcaaggccac cctgtacgcc gtgctggtgt
900

ccgccctggg gctgatggcc atggtcaagc ggaaggacag ccggggcggt tccggagcca
960

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cgaacttctc tctgttaaag caagcaggag acgtggaaga aaaccccggg cccatgtggg
1020
gcgcttttct tctgtatgtg agcatgaaga tgggcggaac agctggacag tctctggaac
1080
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1140
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1200
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1260
gatctgactc ttacggatat ctgctgctgc aggagctgca gatgaaggat agcgccagct
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1500
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1560
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1620
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1680
agctggtgga aaagagcttc gagacagaca ccaacctgaa cttccagaac ctcagcgtga
1740
tcggcttccg gatcctgctg ctgaagggtg ccggcttcaa cctgctgatg accctgcggc
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t g t g g t c c a g                               c t g a g t c g a c
1820

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<210> 6

<211> 312

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-3B Protein TCRbeta -
with signal peptide

<400> 6

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Met Gly Ser Trp Thr Leu Cys Cys Val Ser Leu Cys Ile Leu Val Ala
 1           5           10           15
Lys His Thr Asp Ala Gly Val Ile Gln Ser Pro Arg His Glu Val Thr
          20           25           30

```

Glu	Met	Gly	Gln	Glu	Val	Thr	Leu	Arg	Cys	Lys	Pro	Ile	Ser	Gly	His	
		35					40					45				
Asp	Tyr	Leu	Phe	Trp	Tyr	Arg	Gln	Thr	Met	Met	Arg	Gly	Leu	Glu	Leu	
	50					55					60					
Leu	Ile	Tyr	Phe	Asn	Asn	Asn	Val	Pro	Ile	Asp	Asp	Ser	Gly	Met	Pro	
65					70					75					80	
Glu	Asp	Arg	Phe	Ser	Ala	Lys	Met	Pro	Asn	Ala	Ser	Phe	Ser	Thr	Leu	
				85					90					95		
Lys	Ile	Gln	Pro	Ser	Glu	Pro	Arg	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	
			100					105					110			
Ser	Ser	Phe	Thr	Ser	Gly	Ser	Tyr	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	
		115					120					125				
Arg	Leu	Thr	Val	Thr	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	
	130					135					140					
Ala	Val	Phe	Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	
145					150					155					160	
Thr	Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	
				165				170						175		
Ser	Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	
			180					185					190			
Pro	Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	
		195					200					205				
Leu	Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	
	210					215					220					
Asn	His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	
225					230					235					240	
Glu	Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	
				245					250					255		
Glu	Ala	Trp	Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln	
			260					265					270			
Gln	Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	
		275					280					285				
Ala	Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	
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Val	Lys	Arg	Lys	Asp	Ser	Arg	Gly									
305					310											

<210> 7

<211> 266

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-3B Protein TCRalpha -
with signal peptide

<400> 7

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Ala	Gly	Gln	Ser	Leu	Glu	Gln	Pro	Ser	Glu	Val	Thr	Ala	Val	Glu	Gly
			20					25					30		
Ala	Ile	Val	Gln	Ile	Asn	Cys	Thr	Tyr	Gln	Thr	Ser	Gly	Phe	Tyr	Gly
		35					40					45			
Leu	Ser	Trp	Tyr	Gln	Gln	His	Asp	Gly	Gly	Ala	Pro	Thr	Phe	Leu	Ser
	50					55					60				
Tyr	Asn	Ala	Leu	Asp	Gly	Leu	Glu	Glu	Thr	Gly	Arg	Phe	Ser	Ser	Phe
65					70					75					80
Leu	Ser	Arg	Ser	Asp	Ser	Tyr	Gly	Tyr	Leu	Leu	Leu	Gln	Glu	Leu	Gln
				85					90					95	
Met	Lys	Asp	Ser	Ala	Ser	Tyr	Phe	Cys	Ala	Val	Asn	Asp	Ala	Ser	Lys
			100					105					110		
Ile	Ile	Phe	Gly	Ser	Gly	Thr	Arg	Leu	Ser	Ile	Arg	Pro	Asn	Ile	Gln
		115					120					125			
Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser	Lys	Ser	Ser	Asp
	130					135					140				
Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln	Thr	Asn	Val	Ser
145					150					155					160
Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp	Lys	Cys	Val	Leu	Asp
				165					170					175	
Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala	Val	Ala	Trp	Ser	Asn
			180					185					190		
Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn	Asn	Ser	Ile	Ile	Pro
		195					200					205			
Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser	Cys	Asp	Val	Lys	Leu
	210					215					220				
Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu	Asn	Phe	Gln	Asn	Leu
225					230					235					240
Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys	Val	Ala	Gly	Phe	Asn
				245					250					255	
Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser						
			260					265							

<210> 8

<211> 600

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-3B Protein CO-TCR
beta-P2A-TCR alpha

<400> 8

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Lys	His	Thr	Asp	Ala	Gly	Val	Ile	Gln	Ser	Pro	Arg	His	Glu	Val	Thr	
			20					25					30			
Glu	Met	Gly	Gln	Glu	Val	Thr	Leu	Arg	Cys	Lys	Pro	Ile	Ser	Gly	His	
		35					40					45				
Asp	Tyr	Leu	Phe	Trp	Tyr	Arg	Gln	Thr	Met	Met	Arg	Gly	Leu	Glu	Leu	
	50					55					60					
Leu	Ile	Tyr	Phe	Asn	Asn	Asn	Val	Pro	Ile	Asp	Asp	Ser	Gly	Met	Pro	
65				70						75					80	
Glu	Asp	Arg	Phe	Ser	Ala	Lys	Met	Pro	Asn	Ala	Ser	Phe	Ser	Thr	Leu	
				85					90					95		
Lys	Ile	Gln	Pro	Ser	Glu	Pro	Arg	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	
			100					105					110			
Ser	Ser	Phe	Thr	Ser	Gly	Ser	Tyr	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	
		115					120					125				
Arg	Leu	Thr	Val	Thr	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	
	130					135					140					
Ala	Val	Phe	Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	
145					150					155					160	
Thr	Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	
				165					170					175		
Ser	Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	
			180					185					190			
Pro	Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	
		195					200					205				
Leu	Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	
	210					215					220					
Asn	His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	
225					230					235					240	
Glu	Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	
				245					250					255		
Glu	Ala	Trp	Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln	
			260					265					270			
Gln	Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	
		275					280					285				
Ala	Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	
	290					295					300					
Val	Lys	Arg	Lys	Asp	Ser	Arg	Gly	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser	
305					310					315					320	
Leu	Leu	Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Trp	
				325					330					335		
Gly	Ala	Phe	Leu	Leu	Tyr	Val	Ser	Met	Lys	Met	Gly	Gly	Thr	Ala	Gly	

			340					345					350				
Gln	Ser	Leu	Glu	Gln	Pro	Ser	Glu	Val	Thr	Ala	Val	Glu	Gly	Ala	Ile		
		355					360					365					
Val	Gln	Ile	Asn	Cys	Thr	Tyr	Gln	Thr	Ser	Gly	Phe	Tyr	Gly	Leu	Ser		
	370					375					380						
Trp	Tyr	Gln	Gln	His	Asp	Gly	Gly	Ala	Pro	Thr	Phe	Leu	Ser	Tyr	Asn		
385					390					395					400		
Ala	Leu	Asp	Gly	Leu	Glu	Glu	Thr	Gly	Arg	Phe	Ser	Ser	Phe	Leu	Ser		
			405					410						415			
Arg	Ser	Asp	Ser	Tyr	Gly	Tyr	Leu	Leu	Leu	Gln	Glu	Leu	Gln	Met	Lys		
		420					425						430				
Asp	Ser	Ala	Ser	Tyr	Phe	Cys	Ala	Val	Asn	Asp	Ala	Ser	Lys	Ile	Ile		
	435					440					445						
Phe	Gly	Ser	Gly	Thr	Arg	Leu	Ser	Ile	Arg	Pro	Asn	Ile	Gln	Asn	Pro		
	450				455						460						
Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser	Lys	Ser	Ser	Asp	Lys	Ser		
465					470					475					480		
Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln	Thr	Asn	Val	Ser	Gln	Ser		
			485				490							495			
Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp	Lys	Cys	Val	Leu	Asp	Met	Arg		
		500					505						510				
Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala	Val	Ala	Trp	Ser	Asn	Lys	Ser		
	515					520					525						
Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn	Asn	Ser	Ile	Ile	Pro	Glu	Asp		
	530				535					540							
Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser	Cys	Asp	Val	Lys	Leu	Val	Glu		
545					550					555					560		
Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu	Asn	Phe	Gln	Asn	Leu	Ser	Val		
			565				570							575			
Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys	Val	Ala	Gly	Phe	Asn	Leu	Leu		
		580					585						590				
Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser										
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<210> 9

<211> 939

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B - WT-TCR beta

<400> 9

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60

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gctggagtta tccagtcacc ccgccatgag gtgacagaga tgggacaaga agtgactctg
120
agatgtaaac caatttcagg ccacaactcc cttttctggg acagacagac catgatgcgg
180
ggactggagt tgctcattta ctttaacaac aacgttccga tagatgattc agggatgccc
240
gaggatcgat tctcagctaa gatgcctaata gcatcattct ccactctgaa gatccagccc
300
tcagaaccca gggactcagc tgtgtacttc tgtgccagca gttcgcctga caggctgggc
360
gagcagtact tcggggccggg caccaggctc acggtcacag aggacctgaa aaacgtgttc
420
ccacccgagg tcgctgtgtt tgagccatca gaagcagaga tctccacac ccaaaaggcc
480
acactgggtg gcctggccac aggtttctac cccgaccacg tggagctgag ctggtgggtg
540
aatgggaagg aggtgcacag tggggtcagc acagaccgc agccctcaa ggagcagccc
600
gccctcaatg actccagata ctgcctgagc agccgcctga gggctctcggc caccttctgg
660
cagaaccccc gcaaccactt ccgctgtcaa gtccagttct acgggctctc ggagaatgac
720
gagtggaccc aggatagggc caaacctgtc acccagatcg tcagcgccga ggcctggggt
780
agagcagact gtggcttcac ctccgagtct taccagcaag gggtcctgtc tgccaccatc
840
ctctatgaga tcttgctagg gaaggccacc ttgtatgccg tgctgggtcag tgccctcgtg
900
ctgatggcca      tgggtcaagag      aaaggattcc      agaggctag
939

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<210> 10

<211> 939

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B - CO-TCRbeta

<400> 10

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60
gctggagtga ttcagagccc tagacatgag gtgaccgaaa tgggacagga ggtgacactg
120
agatgtaagc ccatttctgg ccacaacagc ctgttctggg acagacagac catgatgagg
180

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ggactggaac  tgctgatcta  cttcaacaac  aacgtgccca  tcgacgatag  cggcatgcct
240
gaggacagat  ttagcgccaa  gatgcctaata  gccagctttt  ctaccctgaa  gatccagccc
300
tctgagccca  gagattctgc  cgtgtacttt  tgtgccagct  cttctcctga  tagactggga
360
gagcagtact  ttggccctgg  cacaagactg  acagtgacag  aggacctgaa  gaacgtgttc
420
ccccagagg  tggccgtggt  cgagcctagc  gaggccgaga  tcagccacac  ccagaaagcc
480
accctcgtgt  gcctggccac  cggctttttac  cccgaccacg  tggaactgtc  ttggtgggtc
540
aacggcaaag  aggtgcacag  cggcgtctgc  accgaccccc  agcccctgaa  agagcagccc
600
gccctgaacg  acagccggta  ctgtctgagc  agcagactga  gagtgtccgc  caccttctgg
660
cagaaccccc  ggaaccactt  cagatgccag  gtgcagttct  acggcctgag  cgagaacgac
720
gagtggaccc  aggaccgggc  caagcccgtg  acccagatcg  tgtctgctga  ggccctggggc
780
agagccgatt  gcggcttcac  cagcgagagc  taccagcagg  gcgtgctgag  cgccaccatc
840
ctgtacgaga  tcctgctggg  caaggccacc  ctgtacgccg  tgctggtgtc  cgccctggtg
900
ctgatggcca      tgggtcaagcg      gaaggacagc      cggggctga
939

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<210> 11

<211> 819

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B - WT-TCR alpha

<400> 11

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agccaacaga  aggaggtgga  gcaggatcct  ggaccactca  gtgttccaga  gggagccatt
120
gtttctctca  actgcactta  cagcaacagt  gcttttcaat  acttcatgtg  gtacagacag
180
tattccagaa  aaggccctga  gttgctgatg  tacacatact  ccagtggtaa  caaagaagat
240
ggaaggttta  cagcacaggt  cgataaatcc  agcaagtata  tctccttggt  catcagagac
300

```

```

tcacagccca  gtgattcagc  cacctacctc  tgtgcaggag  ggggaaacac  acctcttgtc
360
tttggaagagg  gcacaagact  ttctgtgatt  gcaaataatcc  agaaccctga  ccctgccgtg
420
taccagctga  gagactctaa  atccagtgac  aagtctgtct  gcctattcac  cgattttgat
480
tctcaaacaa  atgtgtcaca  aagtaaggat  tctgatgtgt  atatcacaga  caaaactgtg
540
ctagacatga  ggtctatgga  cttcaagagc  aacagtgtctg  tggcctggag  caacaaatct
600
gactttgcat  gtgcaaacgc  cttcaacaac  agcattattc  cagaagacac  cttcttcccc
660
agcccagaaa  gttcctgtga  tgtcaagctg  gtcgagaaaa  gctttgaaac  agatacgaac
720
ctaaactttc  aaaacctgtc  agtgattggg  ttccgaatcc  tcctcctgaa  agtggccggg
780
t t t a a t c t g c      t c a t g a c g c t      g c g g c t g t g g      t c c a g c t g a
819

```

<210> 12

<211> 819

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B - CO-TCR alpha

<400> 12

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tctcagcaga  aagaagtgga  gcaggatcct  ggacctctgt  ctgtgcctga  aggagctatt
120
gtgagcctga  attgcaccta  cagcaatagc  gccttccagt  acttcatgtg  gtaccggcag
180
tacagcagaa  agggccctga  actgctgatg  tacacctact  ctagcggcaa  taaggaagat
240
ggccgggttta  cagctcaggt  ggacaagagc  agcaagtaca  tcagcctgtt  catcagggat
300
tctcagccta  gcgattctgc  cacctacctg  tgtgctggag  gcggaaatac  acctctgggtt
360
tttggaagagg  gcaccagact  gtctgtgatc  gccaacatcc  agaaccccga  ccctgctgtg
420
taccagctgc  gggacagcaa  gagcagcgac  aagagcgtgt  gcctgttcac  cgacttcgac
480
agccagacca  acgtgtccca  gagcaaggac  agcgacgtgt  acatcaccga  taagtgcgtg
540

```

```

ctggacatgc ggagcatgga cttcaagagc aacagcgccg tggcctggtc caacaagagc
600
gacttcgcct gcgccaacgc cttcaacaac agcattatcc ccgaggacac attcttccca
660
agccccgaga gcagctgcga cgtgaagctg gtggaaaaga gcttcgagac agacaccaac
720
ctgaacttcc agaacctcag cgtgatcggc ttccggatcc tgctgctgaa ggtggccggc
780
ttcaacctgc      tgatgacct      gcggctgtgg      tccagctga
819

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<210> 13

<211> 1838

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B - CO-TCR beta-P2A-
TCR alpha

<400> 13

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aacacacaga tgctggagtg attcagagcc ctagacatga ggtgaccgaa atgggacagg
120
aggtgacact gagatgtaag cccatttctg gccacaacag cctgttctgg tacagacaga
180
ccatgatgag gggactggaa ctgctgatct acttcaacaa caacgtgccc atcgacgata
240
gcggcatgcc tgaggacaga tttagcgcca agatgcctaa tgccagcttt tctaccctga
300
agatccagcc ctctgagccc agagattctg ccgtgtactt ttgtgccagc tcttctcctg
360
atagactggg agagcagtac tttggccctg gcacaagact gacagtgaca gaggacctga
420
agaacgtgtt cccccagag gtggccgtgt tcgagcctag cgaggccgag atcagccaca
480
cccagaaagc caccctcgtg tgcctggcca ccggctttta ccccgaccac gtggaactgt
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600
aagagcagcc cgccctgaac gacagccggt actgtctgag cagcagactg agagtgtccg
660
ccaccttctg gcagaacccc cggaaccact tcagatgcca ggtgcagttc tacggcctga
720

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gcgagaacga	cgagtggacc	caggaccggg	ccaagcccgt	gacccagatc	gtgtctgctg
780					
aggcctgggg	cagagccgat	tgcggcttca	ccagcgagag	ctaccagcag	ggcgtgctga
840					
gcgccaccat	cctgtacgag	atcctgctgg	gcaaggccac	cctgtacgcc	gtgctggtgt
900					
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960					
cgaacttctc	tctgttaaag	caagcaggag	acgtggaaga	aaaccccgggt	cccatgatga
1020					
agagcctgcg	agtcctcctg	gtgattctgt	ggttacagct	gtcttgggtg	tggtctcagc
1080					
agaaagaagt	ggagcaggat	cctggacctc	tgtctgtgcc	tgaaggagct	attgtgagcc
1140					
tgaattgcac	ctacagcaat	agcgccttcc	agtacttcat	gtggtaccgg	cagtacagca
1200					
gaaagggccc	tgaactgctg	atgtacacct	actctagcgg	caataaggaa	gatggccggt
1260					
ttacagctca	ggtggacaag	agcagcaagt	acatcagcct	gttcatcagg	gattctcagc
1320					
ctagcgattc	tgccacctac	ctgtgtgctg	gaggcgga	tacacctctg	gtttttggaa
1380					
aaggcaccag	actgtctgtg	atcgccaaca	tccagaaccc	cgaccctgct	gtgtaccagc
1440					
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1500					
ccaacgtgtc	ccagagcaag	gacagcgacg	tgtacatcac	cgataagtgc	gtgctggaca
1560					
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1620					
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1680					
agagcagctg	cgacgtgaag	ctggtggaaa	agagcttcga	gacagacacc	aacctgaact
1740					
tccagaacct	cagcgtgatc	ggcttccgga	tcctgctgct	gaaggtggcc	ggcttcaacc
1800					
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1838					

<210> 14

<211> 312

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B - Protein TCR beta -
with signal peptide

<400> 14

Met 1	Asp	Ser	Trp	Thr 5	Phe	Cys	Cys	Val	Ser 10	Leu	Cys	Ile	Leu	Val 15	Ala
Lys	His	Thr	Asp 20	Ala	Gly	Val	Ile	Gln 25	Ser	Pro	Arg	His	Glu 30	Val	Thr
Glu	Met	Gly 35	Gln	Glu	Val	Thr	Leu 40	Arg	Cys	Lys	Pro	Ile 45	Ser	Gly	His
Asn 50	Ser	Leu	Phe	Trp	Tyr	Arg 55	Gln	Thr	Met	Met	Arg 60	Gly	Leu	Glu	Leu
Leu 65	Ile	Tyr	Phe	Asn 70	Asn	Asn	Val	Pro	Ile	Asp 75	Asp	Ser	Gly	Met	Pro 80
Glu	Asp	Arg	Phe 85	Ser	Ala	Lys	Met	Pro	Asn 90	Ala	Ser	Phe	Ser	Thr 95	Leu
Lys	Ile	Gln 100	Pro	Ser	Glu	Pro	Arg 105	Asp	Ser	Ala	Val	Tyr 110	Phe	Cys	Ala
Ser	Ser 115	Ser	Pro	Asp	Arg	Leu	Gly 120	Glu	Gln	Tyr	Phe	Gly 125	Pro	Gly	Thr
Arg 130	Leu	Thr	Val	Thr	Glu	Asp 135	Leu	Lys	Asn	Val	Phe 140	Pro	Pro	Glu	Val
Ala 145	Val	Phe	Glu	Pro 150	Ser	Glu	Ala	Glu	Ile	Ser 155	His	Thr	Gln	Lys	Ala 160
Thr	Leu	Val	Cys 165	Leu	Ala	Thr	Gly	Phe	Tyr 170	Pro	Asp	His	Val	Glu 175	Leu
Ser	Trp	Trp 180	Val	Asn	Gly	Lys	Glu 185	Val	His	Ser	Gly	Val 190	Cys	Thr	Asp
Pro	Gln 195	Pro	Leu	Lys	Glu	Gln 200	Pro	Ala	Leu	Asn	Asp 205	Ser	Arg	Tyr	Cys
Leu 210	Ser	Ser	Arg	Leu	Arg 215	Val	Ser	Ala	Thr	Phe	Trp 220	Gln	Asn	Pro	Arg
Asn 225	His	Phe	Arg	Cys 230	Gln	Val	Gln	Phe	Tyr	Gly 235	Leu	Ser	Glu	Asn	Asp 240
Glu	Trp	Thr	Gln 245	Asp	Arg	Ala	Lys	Pro	Val 250	Thr	Gln	Ile	Val	Ser 255	Ala
Glu	Ala	Trp 260	Gly	Arg	Ala	Asp	Cys 265	Gly	Phe	Thr	Ser	Glu 270	Ser	Tyr	Gln
Gln	Gly 275	Val	Leu	Ser	Ala	Thr 280	Ile	Leu	Tyr	Glu	Ile 285	Leu	Leu	Gly	Lys
Ala 290	Thr	Leu	Tyr	Ala	Val 295	Leu	Val	Ser	Ala	Leu	Val 300	Leu	Met	Ala	Met
Val 305	Lys	Arg	Lys	Asp 310	Ser	Arg	Gly								

<210> 15
 <211> 272
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B - Protein TCR alpha
 - with signal peptide

<400> 15

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Ser	Trp	Val	Trp	Ser	Gln	Gln	Lys	Glu	Val	Glu	Gln	Asp	Pro	Gly	Pro
		20						25					30		
Leu	Ser	Val	Pro	Glu	Gly	Ala	Ile	Val	Ser	Leu	Asn	Cys	Thr	Tyr	Ser
		35					40					45			
Asn	Ser	Ala	Phe	Gln	Tyr	Phe	Met	Trp	Tyr	Arg	Gln	Tyr	Ser	Arg	Lys
	50					55					60				
Gly	Pro	Glu	Leu	Leu	Met	Tyr	Thr	Tyr	Ser	Ser	Gly	Asn	Lys	Glu	Asp
65					70				75					80	
Gly	Arg	Phe	Thr	Ala	Gln	Val	Asp	Lys	Ser	Ser	Lys	Tyr	Ile	Ser	Leu
				85				90						95	
Phe	Ile	Arg	Asp	Ser	Gln	Pro	Ser	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala
			100					105						110	
Gly	Gly	Gly	Asn	Thr	Pro	Leu	Val	Phe	Gly	Lys	Gly	Thr	Arg	Leu	Ser
		115					120					125			
Val	Ile	Ala	Asn	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg
	130					135					140				
Asp	Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp
145					150				155					160	
Ser	Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr
				165					170					175	
Asp	Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser
			180					185					190		
Ala	Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe
		195					200					205			
Asn	Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser
	210					215					220				
Ser	Cys	Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn
225					230					235				240	
Leu	Asn	Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu
				245					250					255	
Lys	Val	Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser
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<210> 16
<211> 606
<212> PRT
<213> Artificial Sequence
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<220>

**<223> Synthetic sequence Meso20-16B - Protein TCR
beta-P2A-TCR alpha**

<400> 16

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Lys	His	Thr	Asp 20	Ala	Gly	Val	Ile	Gln 25	Ser	Pro	Arg	His	Glu 30	Val	Thr
Glu	Met	Gly 35	Gln	Glu	Val	Thr	Leu 40	Arg	Cys	Lys	Pro	Ile 45	Ser	Gly	His
Asn 50	Ser	Leu	Phe	Trp	Tyr	Arg 55	Gln	Thr	Met	Met	Arg 60	Gly	Leu	Glu	Leu
Leu 65	Ile	Tyr	Phe	Asn 70	Asn	Asn	Val	Pro	Ile	Asp 75	Asp	Ser	Gly	Met	Pro 80
Glu	Asp	Arg	Phe 85	Ser	Ala	Lys	Met	Pro	Asn 90	Ala	Ser	Phe	Ser	Thr 95	Leu
Lys	Ile	Gln	Pro 100	Ser	Glu	Pro	Arg	Asp 105	Ser	Ala	Val	Tyr	Phe 110	Cys	Ala
Ser	Ser	Ser 115	Pro	Asp	Arg	Leu 120	Gly	Glu	Gln	Tyr	Phe 125	Gly	Pro	Gly	Thr
Arg 130	Leu	Thr	Val	Thr	Glu	Asp 135	Leu	Lys	Asn	Val	Phe 140	Pro	Pro	Glu	Val
Ala 145	Val	Phe	Glu	Pro	Ser 150	Glu	Ala	Glu	Ile	Ser 155	His	Thr	Gln	Lys	Ala 160
Thr	Leu	Val	Cys 165	Leu	Ala	Thr	Gly	Phe	Tyr 170	Pro	Asp	His	Val	Glu 175	Leu
Ser	Trp	Trp	Val 180	Asn	Gly	Lys	Glu	Val 185	His	Ser	Gly	Val	Cys 190	Thr	Asp
Pro	Gln	Pro 195	Leu	Lys	Glu	Gln 200	Pro	Ala	Leu	Asn	Asp 205	Ser	Arg	Tyr	Cys
Leu	Ser 210	Ser	Arg	Leu	Arg 215	Val	Ser	Ala	Thr	Phe	Trp 220	Gln	Asn	Pro	Arg
Asn 225	His	Phe	Arg	Cys 230	Gln	Val	Gln	Phe	Tyr	Gly 235	Leu	Ser	Glu	Asn	Asp 240
Glu	Trp	Thr	Gln 245	Asp	Arg	Ala	Lys	Pro	Val 250	Thr	Gln	Ile	Val	Ser 255	Ala
Glu	Ala	Trp	Gly 260	Arg	Ala	Asp 265	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln
Gln	Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys

		275					280				285				
Ala	Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met
	290					295					300				
Val	Lys	Arg	Lys	Asp	Ser	Arg	Gly	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser
305					310					315					320
Leu	Leu	Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Met
			325						330					335	
Lys	Ser	Leu	Arg	Val	Leu	Leu	Val	Ile	Leu	Trp	Leu	Gln	Leu	Ser	Trp
			340					345					350		
Val	Trp	Ser	Gln	Gln	Lys	Glu	Val	Glu	Gln	Asp	Pro	Gly	Pro	Leu	Ser
		355					360					365			
Val	Pro	Glu	Gly	Ala	Ile	Val	Ser	Leu	Asn	Cys	Thr	Tyr	Ser	Asn	Ser
	370					375					380				
Ala	Phe	Gln	Tyr	Phe	Met	Trp	Tyr	Arg	Gln	Tyr	Ser	Arg	Lys	Gly	Pro
385					390					395					400
Glu	Leu	Leu	Met	Tyr	Thr	Tyr	Ser	Ser	Gly	Asn	Lys	Glu	Asp	Gly	Arg
			405						410					415	
Phe	Thr	Ala	Gln	Val	Asp	Lys	Ser	Ser	Lys	Tyr	Ile	Ser	Leu	Phe	Ile
			420					425					430		
Arg	Asp	Ser	Gln	Pro	Ser	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Gly	Gly
		435					440					445			
Gly	Asn	Thr	Pro	Leu	Val	Phe	Gly	Lys	Gly	Thr	Arg	Leu	Ser	Val	Ile
	450					455					460				
Ala	Asn	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser
465					470					475					480
Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln
			485						490					495	
Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp	Lys
			500					505					510		
Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala	Val
		515					520					525			
Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn	Asn
	530					535					540				
Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser	Cys
545					550					555					560
Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu	Asn
			565						570					575	
Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys	Val
			580					585					590		
Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser		
		595					600					605			

<210> 17
 <211> 930
 <212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A WT-TCR beta

<400> 17

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actgaagtta cccagacacc aaaacacctg gtcattggaa tgacaaataa gaagtctttg
120
aaatgtgaac aacatatggg gcacagggct atgtattggg acaagcagaa agctaagaag
180
ccaccggagc tcatgtttgt ctacagctat gagaaactct ctataaatga aagtgtgcca
240
agtcgcttct cacctgaatg cccaacagc tctctcttaa accttcacct acacgccctg
300
cagccagaag actcagccct gtatctctgc gccagcagcc acgggtccct gaacactgaa
360
gctttctttg gacaaggcac cagactcaca gttgtagagg acctgaacaa ggtgttccca
420
cccgaggctg ctgtgtttga gccatcagaa gcagagatct cccacacca aaaggccaca
480
ctgggtgtgc tggccacagg cttcttcccc gaccacgtgg agctgagctg gtgggtgaat
540
gggaaggagg tgcacagtgg ggtcagcacg gaccgcagc ccctcaagga gcagcccgcc
600
ctcaatgact ccagatactg cctgagcagc cgcctgaggg tctcggccac cttctggcag
660
aacccccgca accacttccg ctgtcaagtc cagttctacg ggctctcgga gaatgacgag
720
tggaaccagg atagggccaa acccgtcacc cagatcgtca gcgccgaggc ctggggtaga
780
gcagactgtg gctttacctc ggtgtcctac cagcaagggg tcctgtctgc caccatcctc
840
tatgagatcc tgctagggaa ggccaccctg tatgctgtgc tggtcagcgc cttgtgtttg
900
a t g g c c a t g g           t c a a g a g a a a           g g a t t t c t g a
930
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<210> 18

<211> 930

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A CO-TCR beta

<400> 18

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acagaggtga cccagacacc taagcatctg gtgatgggca tgaccaacaa gaagagcctg
120
aagtgtgaac agcacatggg ccatagggcc atgtactggg acaagcagaa ggccaagaaa
180
cctcctgagc tgatgttcgt gtacagctac gagaagctga gcatcaacga gagcgtgccc
240
agcagatfff ctcctgagtg ccctaatagc tctctgctga atctgcacct gcatgctctg
300
cagcctgagg attctgctct gtacctgtgt gcttcttctc acggatctct gaatacagag
360
gccttcttcg gccagggcac aagactgaca gtggttgagg atctgaacaa ggtgttcccc
420
ccagaggtgg ccgtgttcga gccttctgag gccgagatct cccacaccca gaaagccacc
480
ctcgtgtgcc tggccaccgg ctttttcccc gaccacgtgg aactgtcttg gtgggtcaac
540
ggcaaagagg tgcactccgg cgtgtgcacc gatccccagc ctctgaaaga acagcccgcc
600
ctgaacgaca gccggtactg cctgagcagc agactgagag tgtccgccac cttctggcag
660
aacccccgga accacttcag atgccagggt cagttctacg gcctgagcga gaacgacgag
720
tggaaccagg acagagccaa gcccgtgaca cagatcgtgt ctgccgaagc ctggggcaga
780
gccgattgcg gctttacctc cgtgtcctat cagcagggcg tgctgagcgc cacaatcctg
840
tacgagatcc tgctgggcaa ggccaccctg tacgccgtgc tgggtgtctgc cctgggtgctg
900
a t g g c c a t g g           t c a a g c g g a a           g g a c t t c t g a
930
```

<210> 19

<211> 831

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A WT-TCR alpha

<400> 19

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120
ataaactgca cttacacaga cagctcctcc acctacttat actggtataa gcaagaacct
180
ggagcaggtc tccagttgct gacgtatatatt ttttcaaata tggacatgaa acaagaccaa
240
agactcactg ttctattgaa taaaaaggat aaacatctgt ctctgcgcat tgcagacacc
300
cagactgggg actcagctat ctacttctgt gcagagaccc cgggggtatgg tggtgctaca
360
aacaagctca tctttggaac tggcactctg cttgctgtcc agccaaatat ccagaacctt
420
gacctgccc tgtaccagct gagagactct aaatccagtg acaagtctgt ctgcctattc
480
accgattttg attctcaaac aaatgtgtca caaagtaagg attctgatgt gtatatcaca
540
gacaaaactg tgctagacat gaggtctatg gacttcaaga gcaacagtgc tgtggcctgg
600
agcaacaaat ctgactttgc atgtgcaaac gccttcaaca acagcattat tccagaagac
660
accttcttcc ccagcccaga aagttcctgt gatgtcaagc tggtcgagaa aagctttgaa
720
acagatacga acctaaactt tcaaaacctg tcagtgattg ggttccgaat cctcctcctg
780
aaagtggccg ggtttaatct gctcatgacg ctgcggctgt ggtccagctg a
831

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<210> 20

<211> 831

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A C0-TCR alpha

<400> 20

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120
atcaattgca cctacaccga tagcagcagc acctacctgt actggtacaa gcaggagcct
180
ggagctggat tacagctgct gacatacatc ttcagcaaca tggacatgaa gcaggaccag
240

```

```

aggctgaccg  tgctgctgaa  caagaaggac  aagcacctgt  ctctgagaat  tgccgataca
300
cagacaggcg  atagcgccat  ctacttctgt  gccgagacac  ctggctatgg  aggagctacc
360
aataagctga  ttttcggcac  aggcacactg  ttagctgtgc  agcccaacat  ccagaatccc
420
gatcctgctg  tgtaccagct  gcgggacagc  aagagcagcg  acaagagcgt  gtgcctgttc
480
accgacttcg  acagccagac  caacgtgtcc  cagagcaagg  acagcgacgt  gtacatcacc
540
gataagtgcg  tgctggacat  gcggagcatg  gacttcaaga  gcaacagcgc  cgtggcctgg
600
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780
aaggtggccg  gcttcaacct  gctgatgacc  ctgcggtgt  ggtccagctg  a
831

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<210> 21

<211> 1841

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A C0-TCR beta-P2A-TCR
alpha

<400> 21

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tgcctatcga  tacagagggtg  acccagacac  ctaagcatct  ggtgatgggc  atgaccaaca
120
agaagagcct  gaagtgtgaa  cagcacatgg  gccatagggc  catgtactgg  tacaagcaga
180
aggccaagaa  acctcctgag  ctgatgttcg  tgtacagcta  cgagaagctg  agcatcaacg
240
agagcgtgcc  cagcagattt  tctcctgagt  gccctaatag  ctctctgctg  aatctgcacc
300
tgcattgctt  gcagcctgag  gattctgctc  tgtacctgtg  tgcttcttct  cacggatctc
360
tgaatacaga  ggccttcttc  ggccagggca  caagactgac  agtgggttgag  gatctgaaca
420

```

aggtgttccc 480	cccagaggtg	gccgtgttcg	agccttctga	ggccgagatc	tcccacaccc
agaaagccac 540	cctcgtgtgc	ctggccaccg	gctttttccc	cgaccacgtg	gaactgtctt
ggtgggtcaa 600	cggcaaagag	gtgcactccg	gcgtgtgcac	cgatccccag	cctctgaaag
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ccacaatcct 900	gtacgagatc	ctgctgggca	aggccaccct	gtacgccgtg	ctggtgtctg
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gcttcagctt 1080	cctgttttctg	tggctgcagc	tggattgtat	gagcagaggc	gaagatgtgg
aacagagcct 1140	gttcctgagc	gttagagagg	gcgatagctc	tgtgatcaat	tgcacctaca
ccgatagcag 1200	cagcacctac	ctgtactggc	acaagcagga	gcctggagct	ggattacagc
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ccatctactt 1380	ctgtgccgag	acacctggct	atggaggagc	taccaataag	ctgattttcg
gcacaggcac 1440	actgttagct	gtgcagccca	acatccagaa	tcccgatcct	gctgtgtacc
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acatgcggag 1620	catggacttc	aagagcaaca	gcgccgtggc	ctgggtccaac	aagagcgact
tcgcctgcgc 1680	caacgccttc	aacaacagca	ttatccccga	ggacacattc	ttcccaagcc
ccgagagcag 1740	ctgcgacgtg	aagctggtgg	aaaagagctt	cgagacagac	accaacctga
acttccagaa 1800	cctcagcgtg	atcggcttcc	ggatcctgct	gctgaagggtg	gccggcttca

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1841

<210> 22

<211> 309

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A Protein C0-TCR beta
- with signal peptide

<400> 22

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Val	Pro	Ile	Asp 20	Thr	Glu	Val	Thr	Gln 25	Thr	Pro	Lys	His	Leu 30	Val	Met
Gly	Met	Thr 35	Asn	Lys	Lys	Ser	Leu 40	Lys	Cys	Glu	Gln	His 45	Met	Gly	His
Arg	Ala 50	Met	Tyr	Trp	Tyr	Lys 55	Gln	Lys	Ala	Lys	Lys 60	Pro	Pro	Glu	Leu
Met 65	Phe	Val	Tyr	Ser	Tyr 70	Glu	Lys	Leu	Ser	Ile 75	Asn	Glu	Ser	Val	Pro 80
Ser	Arg	Phe	Ser 85	Pro	Glu	Cys	Pro	Asn 90	Ser	Ser	Leu	Leu	Asn 95	Leu	His
Leu	His	Ala	Leu 100	Gln	Pro	Glu	Asp 105	Ser	Ala	Leu	Tyr	Leu 110	Cys	Ala	Ser
Ser	His	Gly 115	Ser	Leu	Asn	Thr	Glu 120	Ala	Phe	Phe	Gly	Gln 125	Gly	Thr	Arg
Leu	Thr 130	Val	Val	Glu	Asp 135	Leu	Asn	Lys	Val	Phe	Pro 140	Pro	Glu	Val	Ala
Val 145	Phe	Glu	Pro	Ser	Glu 150	Ala	Glu	Ile	Ser	His 155	Thr	Gln	Lys	Ala	Thr 160
Leu	Val	Cys	Leu 165	Ala	Thr	Gly	Phe	Phe	Pro 170	Asp	His	Val	Glu	Leu 175	Ser
Trp	Trp	Val	Asn 180	Gly	Lys	Glu	Val	His 185	Ser	Gly	Val	Cys	Thr 190	Asp	Pro
Gln	Pro	Leu 195	Lys	Glu	Gln	Pro	Ala 200	Leu	Asn	Asp	Ser	Arg 205	Tyr	Cys	Leu
Ser	Ser 210	Arg	Leu	Arg	Val 215	Ser	Ala	Thr	Phe	Trp	Gln 220	Asn	Pro	Arg	Asn
His 225	Phe	Arg	Cys	Gln	Val 230	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu 240
Trp	Thr	Gln	Asp 245	Arg	Ala	Lys	Pro	Val	Thr 250	Gln	Ile	Val	Ser	Ala	Glu 255

Ala	Trp	Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Val	Ser	Tyr	Gln	Gln
			260					265					270		
Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	Ala
		275					280					285			
Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	Val
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Lys	Arg	Lys	Asp	Phe											
305															

<210> 23

<211> 276

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A Protein C0-TCR
alpha - with signal peptide

<400> 23

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1				5					10					15	
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			20				25						30		
Val	Arg	Glu	Gly	Asp	Ser	Ser	Val	Ile	Asn	Cys	Thr	Tyr	Thr	Asp	Ser
		35				40					45				
Ser	Ser	Thr	Tyr	Leu	Tyr	Trp	Tyr	Lys	Gln	Glu	Pro	Gly	Ala	Gly	Leu
	50				55						60				
Gln	Leu	Leu	Thr	Tyr	Ile	Phe	Ser	Asn	Met	Asp	Met	Lys	Gln	Asp	Gln
65					70					75				80	
Arg	Leu	Thr	Val	Leu	Leu	Asn	Lys	Lys	Asp	Lys	His	Leu	Ser	Leu	Arg
				85					90					95	
Ile	Ala	Asp	Thr	Gln	Thr	Gly	Asp	Ser	Ala	Ile	Tyr	Phe	Cys	Ala	Glu
			100				105					110			
Thr	Pro	Gly	Tyr	Gly	Gly	Ala	Thr	Asn	Lys	Leu	Ile	Phe	Gly	Thr	Gly
		115					120					125			
Thr	Leu	Leu	Ala	Val	Gln	Pro	Asn	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val
	130					135					140				
Tyr	Gln	Leu	Arg	Asp	Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe
145					150				155					160	
Thr	Asp	Phe	Asp	Ser	Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp
				165					170					175	
Val	Tyr	Ile	Thr	Asp	Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe
			180					185					190		
Lys	Ser	Asn	Ser	Ala	Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys
		195					200					205			

Ala	Asn	Ala	Phe	Asn	Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro
	210					215					220				
Ser	Pro	Glu	Ser	Ser	Cys	Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu
225					230					235					240
Thr	Asp	Thr	Asn	Leu	Asn	Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg
				245					250					255	
Ile	Leu	Leu	Leu	Lys	Val	Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg
			260					265					270		
Leu	Trp	Ser	Ser												
			275												

<210> 24

<211> 607

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A Protein
Vbeta-P2A-Valpha

<400> 24

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			20					25					30		
Gly	Met	Thr	Asn	Lys	Lys	Ser	Leu	Lys	Cys	Glu	Gln	His	Met	Gly	His
		35					40					45			
Arg	Ala	Met	Tyr	Trp	Tyr	Lys	Gln	Lys	Ala	Lys	Lys	Pro	Pro	Glu	Leu
	50					55					60				
Met	Phe	Val	Tyr	Ser	Tyr	Glu	Lys	Leu	Ser	Ile	Asn	Glu	Ser	Val	Pro
65					70					75					80
Ser	Arg	Phe	Ser	Pro	Glu	Cys	Pro	Asn	Ser	Ser	Leu	Leu	Asn	Leu	His
				85					90					95	
Leu	His	Ala	Leu	Gln	Pro	Glu	Asp	Ser	Ala	Leu	Tyr	Leu	Cys	Ala	Ser
			100					105					110		
Ser	His	Gly	Ser	Leu	Asn	Thr	Glu	Ala	Phe	Phe	Gly	Gln	Gly	Thr	Arg
		115					120					125			
Leu	Thr	Val	Val	Glu	Asp	Leu	Asn	Lys	Val	Phe	Pro	Pro	Glu	Val	Ala
	130					135					140				
Val	Phe	Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr
145					150					155					160
Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Phe	Pro	Asp	His	Val	Glu	Leu	Ser
				165					170					175	
Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	Pro
			180					185					190		

Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	Leu
		195					200					205			
Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	Asn
	210					215					220				
His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu
225					230					235					240
Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu
			245					250						255	
Ala	Trp	Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Val	Ser	Tyr	Gln	Gln
			260					265					270		
Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	Ala
		275					280					285			
Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	Val
	290					295					300				
Lys	Arg	Lys	Asp	Phe	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser	Leu	Leu	Lys
305					310					315					320
Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Lys	Thr	Phe	Ala
			325						330					335	
Gly	Phe	Ser	Phe	Leu	Phe	Leu	Trp	Leu	Gln	Leu	Asp	Cys	Met	Ser	Arg
			340					345					350		
Gly	Glu	Asp	Val	Glu	Gln	Ser	Leu	Phe	Leu	Ser	Val	Arg	Glu	Gly	Asp
		355					360					365			
Ser	Ser	Val	Ile	Asn	Cys	Thr	Tyr	Thr	Asp	Ser	Ser	Ser	Thr	Tyr	Leu
	370					375					380				
Tyr	Trp	Tyr	Lys	Gln	Glu	Pro	Gly	Ala	Gly	Leu	Gln	Leu	Leu	Thr	Tyr
385					390					395					400
Ile	Phe	Ser	Asn	Met	Asp	Met	Lys	Gln	Asp	Gln	Arg	Leu	Thr	Val	Leu
			405						410					415	
Leu	Asn	Lys	Lys	Asp	Lys	His	Leu	Ser	Leu	Arg	Ile	Ala	Asp	Thr	Gln
			420					425					430		
Thr	Gly	Asp	Ser	Ala	Ile	Tyr	Phe	Cys	Ala	Glu	Thr	Pro	Gly	Tyr	Gly
		435					440					445			
Gly	Ala	Thr	Asn	Lys	Leu	Ile	Phe	Gly	Thr	Gly	Thr	Leu	Leu	Ala	Val
	450					455					460				
Gln	Pro	Asn	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp
465					470					475					480
Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser
			485					490						495	
Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp
			500					505					510		
Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala
		515					520					525			
Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn
	530					535					540				
Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser

545					550					555					560
Cys	Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu
				565					570					575	
Asn	Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys
			580					585					590		
Val	Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser	
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<210> 25

<211> 939

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B CO-TCR beta

<400> 25

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120					
agatgttcta	gccagtctgg	ccacaataca	gtgagctggt	atcagcaggc	tttaggacag
180					
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240					
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300					
gaactggacg	attctgctct	gtacctgtgt	gccagctctt	ttgctggcgg	aagaagcgat
360					
accagttatt	ttggacctgg	aaccagactg	acagtgctgg	aggacctgaa	gaacgtgttc
420					
ccccagagg	tggccgtggt	cgagcctagc	gaggccgaga	tcagccacac	ccagaaagcc
480					
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540					
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600					
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660					
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720					
gagtggaccc	aggaccgggc	caagcccgtg	accagatcg	tgtctgctga	ggcctggggc
780					
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840					

ctgtacgaga tcctgctggg caaggccacc ctgtacgccg tgctggtgtc cgccctggtg
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939

<210> 26

<211> 831

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B C0-TCR alpha

<400> 26

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120
aactgcagct tcacagatag cgccatctac aacctgcagt ggttcagaca ggatcctgga
180
aaaggcctga caagcctgct gctgattcag agctctcaga gagagcagac atctggaaga
240
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300
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360
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420
gatcctgccg tgtaccagct gcgggacagc aagagcagcg acaagagcgt gtgcctgttc
480
accgacttcg acagccagac caacgtgtcc cagagcaagg acagcgacgt gtacatcacc
540
gataagtgcg tgctggacat gcggagcatg gacttcaaga gcaacagcgc cgtggcctgg
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660
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720
acagacacca acctgaactt ccagaacctc agcgtgatcg gcttccggat cctgctgctg
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831

<210> 27

<211> 1843

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B -TCR beta-P2A-TCR
alpha

<400> 27

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120					
cctgagatgt	tctagccagt	ctggccacaa	tacagtgagc	tggtatcagc	aggctttagg
180					
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240					
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300					
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360					
cgatacccag	tattttggac	ctggaaccag	actgacagtg	ctggaggacc	tgaagaacgt
420					
gttcccccca	gaggtggccg	tggtcgagcc	tagcgaggcc	gagatcagcc	acaccagaa
480					
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540					
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600					
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660					
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720					
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780					
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840					
catcctgtac	gagatcctgc	tgggcaaggc	caccctgtac	gccgtgctgg	tgtccgccct
900					
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960					
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1020					
gggactactg	attctgtggc	tgcaactgca	atgggtgagc	agcaaacagg	aggttaccca
1080					
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1140					

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agatagcgcc atctacaacc tgcagtgggtt cagacaggat cctggaaaag gcctgacaag
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1320
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1380
tggaaggggc acaaagctga ccgtgaatcc ctacatccag aaccctgata ctgccgtgta
1440
ccagctgcgg gacagcaaga gcagcgacaa gagcgtgtgc ctgttcaccg acttcgacag
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1620
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1843

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<210> 28

<211> 312

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B Protein C0-TCR beta
- with signal peptide

<400> 28

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Gly Ser Val Glu Thr Gly Val Thr Gln Ser Pro Thr His Leu Ile Lys
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Thr Arg Gly Gln Gln Val Thr Leu Arg Cys Ser Ser Gln Ser Gly His
          35          40          45
Asn Thr Val Ser Trp Tyr Gln Gln Ala Leu Gly Gln Gly Pro Gln Phe
 50          55          60
Ile Phe Gln Tyr Tyr Arg Glu Glu Glu Asn Gly Arg Gly Asn Phe Pro
65          70          75          80

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Val	Asn	Ala	Leu	Glu	Leu	Asp	Asp	Ser	Ala	Leu	Tyr	Leu	Cys	Ala	Ser	
			100					105					110			
Ser	Phe	Ala	Gly	Gly	Arg	Ser	Asp	Thr	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	
		115					120					125				
Arg	Leu	Thr	Val	Leu	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	
	130					135					140					
Ala	Val	Phe	Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	
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Thr	Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	
			165						170					175		
Ser	Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	
		180						185					190			
Pro	Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	
	195						200					205				
Leu	Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	
	210					215					220					
Asn	His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	
225				230					235						240	
Glu	Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	
			245						250					255		
Glu	Ala	Trp	Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln	
		260						265					270			
Gln	Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	
	275						280					285				
Ala	Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	
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Val	Lys	Arg	Lys	Asp	Ser	Arg	Gly									
305					310											

<210> 29

<211> 276

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B Protein CO-TCR
alpha - with signal peptide

<400> 29

Met	Glu	Thr	Leu	Leu	Gly	Leu	Leu	Ile	Leu	Trp	Leu	Gln	Leu	Gln	Trp	
1				5				10					15			
Val	Ser	Ser	Lys	Gln	Glu	Val	Thr	Gln	Ile	Pro	Ala	Ala	Leu	Ser	Val	
			20					25					30			

Pro	Glu	Gly	Glu	Asn	Leu	Val	Leu	Asn	Cys	Ser	Phe	Thr	Asp	Ser	Ala
		35					40					45			
Ile	Tyr	Asn	Leu	Gln	Trp	Phe	Arg	Gln	Asp	Pro	Gly	Lys	Gly	Leu	Thr
	50					55					60				
Ser	Leu	Leu	Leu	Ile	Gln	Ser	Ser	Gln	Arg	Glu	Gln	Thr	Ser	Gly	Arg
65					70					75					80
Leu	Asn	Ala	Ser	Leu	Asp	Lys	Ser	Ser	Gly	Arg	Ser	Thr	Leu	Tyr	Ile
				85					90					95	
Ala	Ala	Ser	Gln	Pro	Gly	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Val	Arg
			100					105					110		
Leu	Leu	Phe	Ala	Gln	Gly	Gly	Ser	Glu	Lys	Leu	Val	Phe	Gly	Lys	Gly
		115					120					125			
Thr	Lys	Leu	Thr	Val	Asn	Pro	Tyr	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val
	130					135					140				
Tyr	Gln	Leu	Arg	Asp	Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe
145					150					155					160
Thr	Asp	Phe	Asp	Ser	Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp
				165					170					175	
Val	Tyr	Ile	Thr	Asp	Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe
			180					185					190		
Lys	Ser	Asn	Ser	Ala	Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys
		195					200					205			
Ala	Asn	Ala	Phe	Asn	Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro
	210					215					220				
Ser	Pro	Glu	Ser	Ser	Cys	Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu
225					230					235					240
Thr	Asp	Thr	Asn	Leu	Asn	Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg
				245					250					255	
Ile	Leu	Leu	Leu	Lys	Val	Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg
			260					265					270		
Leu	Trp	Ser	Ser												
		275													

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<211> 610

<212> PRT

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<223> Synthetic sequence Meso530-11B Protein CO-TCR
beta-P2A-TCR alpha

<400> 30

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			20					25					30		
Thr	Arg	Gly	Gln	Gln	Val	Thr	Leu	Arg	Cys	Ser	Ser	Gln	Ser	Gly	His
		35					40					45			
Asn	Thr	Val	Ser	Trp	Tyr	Gln	Gln	Ala	Leu	Gly	Gln	Gly	Pro	Gln	Phe
	50					55					60				
Ile	Phe	Gln	Tyr	Tyr	Arg	Glu	Glu	Glu	Asn	Gly	Arg	Gly	Asn	Phe	Pro
65					70					75					80
Pro	Arg	Phe	Ser	Gly	Leu	Gln	Phe	Pro	Asn	Tyr	Ser	Ser	Glu	Leu	Asn
				85					90					95	
Val	Asn	Ala	Leu	Glu	Leu	Asp	Asp	Ser	Ala	Leu	Tyr	Leu	Cys	Ala	Ser
			100					105					110		
Ser	Phe	Ala	Gly	Gly	Arg	Ser	Asp	Thr	Gln	Tyr	Phe	Gly	Pro	Gly	Thr
		115					120					125			
Arg	Leu	Thr	Val	Leu	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val
	130					135					140				
Ala	Val	Phe	Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala
145					150					155					160
Thr	Leu	Val	Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu
			165					170						175	
Ser	Trp	Trp	Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp
			180					185					190		
Pro	Gln	Pro	Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys
		195					200					205			
Leu	Ser	Ser	Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg
	210					215					220				
Asn	His	Phe	Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp
225					230					235					240
Glu	Trp	Thr	Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala
				245					250					255	
Glu	Ala	Trp	Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln
			260					265					270		
Gln	Gly	Val	Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys
		275					280					285			
Ala	Thr	Leu	Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met
	290					295					300				
Val	Lys	Arg	Lys	Asp	Ser	Arg	Gly	Gly	Ser	Gly	Ala	Thr	Asn	Phe	Ser
305					310					315					320
Leu	Leu	Lys	Gln	Ala	Gly	Asp	Val	Glu	Glu	Asn	Pro	Gly	Pro	Met	Glu
				325				330						335	
Thr	Leu	Leu	Gly	Leu	Leu	Ile	Leu	Trp	Leu	Gln	Leu	Gln	Trp	Val	Ser
			340					345					350		
Ser	Lys	Gln	Glu	Val	Thr	Gln	Ile	Pro	Ala	Ala	Leu	Ser	Val	Pro	Glu
		355					360					365			
Gly	Glu	Asn	Leu	Val	Leu	Asn	Cys	Ser	Phe	Thr	Asp	Ser	Ala	Ile	Tyr

370						375						380				
Asn	Leu	Gln	Trp	Phe	Arg	Gln	Asp	Pro	Gly	Lys	Gly	Leu	Thr	Ser	Leu	
385					390					395					400	
Leu	Leu	Ile	Gln	Ser	Ser	Gln	Arg	Glu	Gln	Thr	Ser	Gly	Arg	Leu	Asn	
				405					410					415		
Ala	Ser	Leu	Asp	Lys	Ser	Ser	Gly	Arg	Ser	Thr	Leu	Tyr	Ile	Ala	Ala	
			420					425					430			
Ser	Gln	Pro	Gly	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Val	Arg	Leu	Leu	
		435					440					445				
Phe	Ala	Gln	Gly	Gly	Ser	Glu	Lys	Leu	Val	Phe	Gly	Lys	Gly	Thr	Lys	
	450					455					460					
Leu	Thr	Val	Asn	Pro	Tyr	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	
465					470					475					480	
Leu	Arg	Asp	Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	
				485				490						495		
Phe	Asp	Ser	Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	
			500					505					510			
Ile	Thr	Asp	Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	
		515					520					525				
Asn	Ser	Ala	Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	
	530					535					540					
Ala	Phe	Asn	Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	
545					550				555						560	
Glu	Ser	Ser	Cys	Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	
			565					570						575		
Thr	Asn	Leu	Asn	Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	
			580				585						590			
Leu	Leu	Lys	Val	Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	
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<210> 31

<211> 9

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<223> Synthetic sequence Msln20-28

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Ser Leu Leu Phe Leu Leu Phe Ser Leu

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5

<210> 32

<211> 9
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<400> 32
Val Leu Pro Leu Thr Val Ala Glu Val
1 5

<210> 33
<211> 11
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<220>
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<400> 33
Cys Ala Val Asn Asp Ala Ser Lys Ile Ile Phe
1 5 10

<210> 34
<211> 14
<212> PRT
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<220>
<223> Synthetic sequence Meso20-3B CDR3 beta

<400> 34
Cys Ala Ser Ser Phe Thr Ser Gly Ser Tyr Glu Gln Tyr Phe
1 5 10

<210> 35
<211> 11
<212> PRT
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<400> 35
Cys Ala Gly Gly Gly Asn Thr Pro Leu Val Phe

1 5 10

<210> 36
<211> 14
<212> PRT
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<400> 36
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1 5 10

<210> 37
<211> 16
<212> PRT
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<220>
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<400> 37
Cys Ala Glu Thr Pro Gly Tyr Gly Gly Ala Thr Asn Lys Leu Ile Phe
1 5 10 15

<210> 38
<211> 14
<212> PRT
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Cys Ala Ser Ser His Gly Ser Leu Asn Thr Glu Ala Phe Phe
1 5 10

<210> 39
<211> 17
<212> PRT
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<220>
<223> Synthetic sequence Meso530-11B CDR3 alpha

<400> 39

Cys Ala Val Arg Leu Leu Phe Ala Gln Gly Gly Ser Glu Lys Leu Val
1 5 10 15
Phe

<210> 40

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B CDR3 beta

<400> 40

Cys Ala Ser Ser Phe Ala Gly Gly Arg Ser Asp Thr Gln Tyr Phe
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<210> 41

<211> 66

<212> DNA

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<220>

<223> Synthetic sequence Porcine teschovirus
self-cleaving peptide P2A-1

<400> 41

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g g t c c a
66

<210> 42

<211> 63

<212> DNA

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self-cleaving peptide P2A-2

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60

c		c		t
63				

<210> 43
 <211> 65
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 self-cleaving peptide P2A-3

<400> 43
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 g c c c t
 65

<210> 44
 <211> 66
 <212> DNA
 <213> Artificial Sequence

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 self-cleaving peptide P2A-4

<400> 44
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<210> 45
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 self-cleaving peptide P2A-5

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g          g          a          c          c          t
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<210> 46
<211> 66
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<220>
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g          g          a          c          c          t
66

<210> 47
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<223> Synthetic sequence Thoseaassigna virus 2A T2A

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c          c          t
63

<210> 48
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<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic sequence Equine rhinitis A virus E2A

<400> 48
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c          c          t          g          g          a          c          c          t
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<210> 49
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<220>
<223> Synthetic sequence Foot-and-Mouth disease virus 2
F2A

<400> 49
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75

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<211> 630
<212> PRT
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<223> Synthetic sequence human MSLN, isoform 1

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20 25 30
Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
35 40 45
Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
50 55 60
Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65 70 75 80
Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
85 90 95
Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
100 105 110
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
115 120 125
Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
130 135 140
Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145 150 155 160

Arg	Leu	Leu	Pro	Ala	Ala	Leu	Ala	Cys	Trp	Gly	Val	Arg	Gly	Ser	Leu	165	170	175
Leu	Ser	Glu	Ala	Asp	Val	Arg	Ala	Leu	Gly	Gly	Leu	Ala	Cys	Asp	Leu	180	185	190
Pro	Gly	Arg	Phe	Val	Ala	Glu	Ser	Ala	Glu	Val	Leu	Leu	Pro	Arg	Leu	195	200	205
Val	Ser	Cys	Pro	Gly	Pro	Leu	Asp	Gln	Asp	Gln	Gln	Glu	Ala	Ala	Arg	210	215	220
Ala	Ala	Leu	Gln	Gly	Gly	Gly	Pro	Pro	Tyr	Gly	Pro	Pro	Ser	Thr	Trp	225	230	235
Ser	Val	Ser	Thr	Met	Asp	Ala	Leu	Arg	Gly	Leu	Leu	Pro	Val	Leu	Gly	245	250	255
Gln	Pro	Ile	Ile	Arg	Ser	Ile	Pro	Gln	Gly	Ile	Val	Ala	Ala	Trp	Arg	260	265	270
Gln	Arg	Ser	Ser	Arg	Asp	Pro	Ser	Trp	Arg	Gln	Pro	Glu	Arg	Thr	Ile	275	280	285
Leu	Arg	Pro	Arg	Phe	Arg	Arg	Glu	Val	Glu	Lys	Thr	Ala	Cys	Pro	Ser	290	295	300
Gly	Lys	Lys	Ala	Arg	Glu	Ile	Asp	Glu	Ser	Leu	Ile	Phe	Tyr	Lys	Lys	305	310	315
Trp	Glu	Leu	Glu	Ala	Cys	Val	Asp	Ala	Ala	Leu	Leu	Ala	Thr	Gln	Met	325	330	335
Asp	Arg	Val	Asn	Ala	Ile	Pro	Phe	Thr	Tyr	Glu	Gln	Leu	Asp	Val	Leu	340	345	350
Lys	His	Lys	Leu	Asp	Glu	Leu	Tyr	Pro	Gln	Gly	Tyr	Pro	Glu	Ser	Val	355	360	365
Ile	Gln	His	Leu	Gly	Tyr	Leu	Phe	Leu	Lys	Met	Ser	Pro	Glu	Asp	Ile	370	375	380
Arg	Lys	Trp	Asn	Val	Thr	Ser	Leu	Glu	Thr	Leu	Lys	Ala	Leu	Leu	Glu	385	390	395
Val	Asn	Lys	Gly	His	Glu	Met	Ser	Pro	Gln	Ala	Pro	Arg	Arg	Pro	Leu	405	410	415
Pro	Gln	Val	Ala	Thr	Leu	Ile	Asp	Arg	Phe	Val	Lys	Gly	Arg	Gly	Gln	420	425	430
Leu	Asp	Lys	Asp	Thr	Leu	Asp	Thr	Leu	Thr	Ala	Phe	Tyr	Pro	Gly	Tyr	435	440	445
Leu	Cys	Ser	Leu	Ser	Pro	Glu	Glu	Leu	Ser	Ser	Val	Pro	Pro	Ser	Ser	450	455	460
Ile	Trp	Ala	Val	Arg	Pro	Gln	Asp	Leu	Asp	Thr	Cys	Asp	Pro	Arg	Gln	465	470	475
Leu	Asp	Val	Leu	Tyr	Pro	Lys	Ala	Arg	Leu	Ala	Phe	Gln	Asn	Met	Asn	485	490	495
Gly	Ser	Glu	Tyr	Phe	Val	Lys	Ile	Gln	Ser	Phe	Leu	Gly	Gly	Ala	Pro	500	505	510
Thr	Glu	Asp	Leu	Lys	Ala	Leu	Ser	Gln	Gln	Asn	Val	Ser	Met	Asp	Leu			

		515					520				525				
Ala	Thr	Phe	Met	Lys	Leu	Arg	Thr	Asp	Ala	Val	Leu	Pro	Leu	Thr	Val
	530					535					540				
Ala	Glu	Val	Gln	Lys	Leu	Leu	Gly	Pro	His	Val	Glu	Gly	Leu	Lys	Ala
545					550					555					560
Glu	Glu	Arg	His	Arg	Pro	Val	Arg	Asp	Trp	Ile	Leu	Arg	Gln	Arg	Gln
				565					570					575	
Asp	Asp	Leu	Asp	Thr	Leu	Gly	Leu	Gly	Leu	Gln	Gly	Gly	Ile	Pro	Asn
			580					585					590		
Gly	Tyr	Leu	Val	Leu	Asp	Leu	Ser	Met	Gln	Glu	Ala	Leu	Ser	Gly	Thr
	595					600					605				
Pro	Cys	Leu	Leu	Gly	Pro	Gly	Pro	Val	Leu	Thr	Val	Leu	Ala	Leu	Leu
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Leu	Ala	Ser	Thr	Leu	Ala										
625					630										

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<210> 52
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 <212> PRT
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<400> 52
 Ser Ala Leu Phe Leu Leu Phe Ser Leu
 1 5

<210> 53
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 <212> PRT
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<223> Synthetic sequence Msln20-28 Ala variant 3

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Ser Leu Ala Phe Leu Leu Phe Ser Leu
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<210> 54

<211> 9

<212> PRT

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<223> Synthetic sequence Msln20-28 Ala variant 4

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Ser Leu Leu Ala Leu Leu Phe Ser Leu
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<210> 55

<211> 9

<212> PRT

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<223> Synthetic sequence Msln20-28 Ala variant 5

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Ser Leu Leu Phe Ala Leu Phe Ser Leu
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<210> 56

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Synthetic sequence Msln20-28 Ala variant 6

<400> 56

Ser Leu Leu Phe Leu Ala Phe Ser Leu
1 5

<210> 57

<211> 9

<212> PRT
<213> Artificial Sequence

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<223> Synthetic sequence Msln20-28 Ala variant 7

<400> 57

Ser Leu Leu Phe Leu Leu Ala Ser Leu
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<210> 58

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Synthetic sequence Msln20-28 Ala variant 8

<400> 58

Ser Leu Leu Phe Leu Leu Phe Ala Leu
1 5

<210> 59

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Msln20-28 Ala variant 9

<400> 59

Ser Leu Leu Phe Leu Leu Phe Ser Ala
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<210> 60

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Msln20-28 TCR epitope consensus
sequence

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<221> VARIANT

<222> 1,2,7,8,9

<223> Xaa = any amino acid, optionally alanine

<400> 60

Xaa Xaa Leu Phe Leu Leu Xaa Xaa Xaa
1 5

<210> 61

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Synthetic sequence Msln530-538 TCR epitope
consensus sequence 1

<220>

<221> VARIANT

<222> 3,5,6,9

<223> Xaa = any amino acid, optionally alanine

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Val Leu Xaa Leu Xaa Xaa Ala Glu Xaa
1 5

<210> 62

<211> 9

<212> PRT

<213> Artificial Sequence

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<223> Synthetic sequence Msln530-538 TCR epitope
consensus sequence 2

<220>

<221> VARIANT

<222> 1,5,9

<223> Xaa = any amino acid, optionally alanine

<400> 62

Xaa Leu Pro Leu Xaa Val Ala Glu Xaa
1 5

<210> 63
<211> 9
<212> PRT
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<220>
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<400> 63
Val Leu Leu Leu Ser Leu Ala Glu Ile
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<210> 64
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<210> 65
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<212> PRT
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1 5

<210> 66
<211> 9
<212> PRT
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<220>
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<400> 66

Val Leu Pro Leu Leu Val Ala Glu Ala

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5

<210> 67

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence ULK1 peptide

<400> 67

Val Leu Tyr Leu Lys Val Ala Glu Leu

1

5

<210> 68

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence OR2J2 peptide

<400> 68

Val Leu Ala Leu Gly Ile Ala Glu Cys

1

5

<210> 69

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence DNHD1 peptide

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<210> 70

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence AL1L1 peptide

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<210> 71

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence GALP peptide

<400> 71

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<210> 72

<211> 9

<212> PRT

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<223> Synthetic sequence OR2J1 peptide

<400> 72

Val Leu Ala Leu Gly Thr Ala Glu Cys
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<210> 73

<211> 9

<212> PRT

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<220>

<223> Synthetic sequence ADPRH peptide

<400> 73

Val Met His Leu Ala Thr Ala Glu Ala
1 5

<210> 74

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence CHPF2 peptide

<400> 74

Val Leu Pro Leu Ile Val Ala Glu Ala
1 5

<210> 75

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence LRCH3 peptide

<400> 75

Asp Leu Pro Leu Arg Val Ala Glu Ile
1 5

<210> 76

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence IN80B peptide

<400> 76

Met Leu Pro Leu Pro Val Ala Glu Gly
1 5

<210> 77

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence CEAM3 peptide

<400> 77

Ser Met Pro Leu Ser Val Ala Glu Gly
1 5

<210> 78
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-3B CDR1 beta

<400> 78
Ser Gly His Asp Tyr
1 5

<210> 79
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-3B, Meso20-16B CDR2 beta

<400> 79
Phe Asn Asn Asn Val Pro
1 5

<210> 80
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-3B CDR1 alpha

<400> 80
Thr Ser Gly Phe Tyr Gly
1 5

<210> 81
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-3B CDR2 alpha v1

<400> 81

Asn Ala Leu Asp Gly
1 5

<210> 82
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-16B CDR1 beta

<400> 82
Ser Gly His Asn Ser
1 5

<210> 83
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-16B / Meso20-3B /
Meso530 -11A / Meso530 -11B CDR1 beta consensus

<220>
<221> VARIANT
<222> 1
<223> Xaa = M, S, or T

<220>
<221> VARIANT
<222> 4
<223> Xaa = D, E, N, Q, R or K

<220>
<221> VARIANT
<222> 5
<223> Xaa = Y, S, A, V, I, L, or T

<400> 83
Xaa Gly His Xaa Xaa
1 5

<210> 84

<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-16B / Meso20-3B /
Meso530 CDR2 beta consensus

<220>
<221> VARIANT
<222> 4
<223> Xaa = D or N

<220>
<221> VARIANT
<222> 5
<223> Xaa = Y, S, or T

<400> 84
Ser Gly His Xaa Xaa
1 5

<210> 85
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-16B CDR1 alpha

<400> 85
Asn Ser Ala Phe Gln Tyr
1 5

<210> 86
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-16B CDR2 alpha v1

<400> 86
Thr Tyr Ser Ser Gly
1 5

<210> 87
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso530-11A CDR1 beta

<400> 87
Met Gly His Arg Ala
1 5

<210> 88
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso530-11A CDR2 beta

<400> 88
Tyr Ser Tyr Glu Lys Leu
1 5

<210> 89
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso530-11A CDR1 alpha

<400> 89
Ser Ser Ser Thr Tyr Leu
1 5

<210> 90
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso530-11A CDR2 alpha

<400> 90

Ile Phe Ser Asn Met Asp Met

1

5

<210> 91

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B CDR1 beta

<400> 91

Ser Gly His Asn Thr

1

5

<210> 92

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B CDR2 beta

<400> 92

Tyr Tyr Arg Glu Glu Glu

1

5

<210> 93

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B CDR1 alpha

<400> 93

Asp Ser Ala Ile Tyr Asn

1

5

<210> 94

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B CDR2 alpha

<400> 94

Ile Gln Ser Ser Gln Arg Glu
1 5

<210> 95

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-3B V beta (mature)

<400> 95

Asp Ala Gly Val Ile Gln Ser Pro Arg His Glu Val Thr Glu Met Gly
1 5 10 15
Gln Glu Val Thr Leu Arg Cys Lys Pro Ile Ser Gly His Asp Tyr Leu
20 25 30
Phe Trp Tyr Arg Gln Thr Met Met Arg Gly Leu Glu Leu Ile Tyr
35 40 45
Phe Asn Asn Asn Val Pro Ile Asp Asp Ser Gly Met Pro Glu Asp Arg
50 55 60
Phe Ser Ala Lys Met Pro Asn Ala Ser Phe Ser Thr Leu Lys Ile Gln
65 70 75 80
Pro Ser Glu Pro Arg Asp Ser Ala Val Tyr Phe Cys Ala Ser Ser Phe
85 90 95
Thr Ser Gly Ser Tyr Glu Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr
100 105 110
Val Thr

<210> 96

<211> 108

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-3B V alpha (mature)

<400> 96

Gly Gln Ser Leu Glu Gln Pro Ser Glu Val Thr Ala Val Glu Gly Ala
1 5 10 15
Ile Val Gln Ile Asn Cys Thr Tyr Gln Thr Ser Gly Phe Tyr Gly Leu

			20					25				30			
Ser	Trp	Tyr	Gln	Gln	His	Asp	Gly	Gly	Ala	Pro	Thr	Phe	Leu	Ser	Tyr
		35					40					45			
Asn	Ala	Leu	Asp	Gly	Leu	Glu	Glu	Thr	Gly	Arg	Phe	Ser	Ser	Phe	Leu
	50					55					60				
Ser	Arg	Ser	Asp	Ser	Tyr	Gly	Tyr	Leu	Leu	Leu	Gln	Glu	Leu	Gln	Met
65					70					75					80
Lys	Asp	Ser	Ala	Ser	Tyr	Phe	Cys	Ala	Val	Asn	Asp	Ala	Ser	Lys	Ile
			85					90						95	
Ile	Phe	Gly	Ser	Gly	Thr	Arg	Leu	Ser	Ile	Arg	Pro				
			100					105							

<210> 97

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B V beta (mature)

<400> 97

Asp	Ala	Gly	Val	Ile	Gln	Ser	Pro	Arg	His	Glu	Val	Thr	Glu	Met	Gly
1				5				10					15		
Gln	Glu	Val	Thr	Leu	Arg	Cys	Lys	Pro	Ile	Ser	Gly	His	Asn	Ser	Leu
		20					25					30			
Phe	Trp	Tyr	Arg	Gln	Thr	Met	Met	Arg	Gly	Leu	Glu	Leu	Leu	Ile	Tyr
	35					40					45				
Phe	Asn	Asn	Asn	Val	Pro	Ile	Asp	Asp	Ser	Gly	Met	Pro	Glu	Asp	Arg
	50				55				60						
Phe	Ser	Ala	Lys	Met	Pro	Asn	Ala	Ser	Phe	Ser	Thr	Leu	Lys	Ile	Gln
65				70				75							80
Pro	Ser	Glu	Pro	Arg	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Ser	Ser	Ser
			85				90						95		
Pro	Asp	Arg	Leu	Gly	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg	Leu	Thr
			100				105						110		
Val	Thr														

<210> 98

<211> 109

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B V alpha (mature)

<400> 98

Gln	Lys	Glu	Val	Glu	Gln	Asp	Pro	Gly	Pro	Leu	Ser	Val	Pro	Glu	Gly
1				5				10						15	
Ala	Ile	Val	Ser	Leu	Asn	Cys	Thr	Tyr	Ser	Asn	Ser	Ala	Phe	Gln	Tyr
			20					25					30		
Phe	Met	Trp	Tyr	Arg	Gln	Tyr	Ser	Arg	Lys	Gly	Pro	Glu	Leu	Leu	Met
		35					40					45			
Tyr	Thr	Tyr	Ser	Ser	Gly	Asn	Lys	Glu	Asp	Gly	Arg	Phe	Thr	Ala	Gln
	50					55					60				
Val	Asp	Lys	Ser	Ser	Lys	Tyr	Ile	Ser	Leu	Phe	Ile	Arg	Asp	Ser	Gln
65					70					75					80
Pro	Ser	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Gly	Gly	Gly	Asn	Thr	Pro
				85					90					95	
Leu	Val	Phe	Gly	Lys	Gly	Thr	Arg	Leu	Ser	Val	Ile	Ala			
			100					105							

<210> 99

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A V beta (mature)

<400> 99

Asp	Thr	Glu	Val	Thr	Gln	Thr	Pro	Lys	His	Leu	Val	Met	Gly	Met	Thr
1				5				10						15	
Asn	Lys	Lys	Ser	Leu	Lys	Cys	Glu	Gln	His	Met	Gly	His	Arg	Ala	Met
			20					25					30		
Tyr	Trp	Tyr	Lys	Gln	Lys	Ala	Lys	Lys	Pro	Pro	Glu	Leu	Met	Phe	Val
		35					40					45			
Tyr	Ser	Tyr	Glu	Lys	Leu	Ser	Ile	Asn	Glu	Ser	Val	Pro	Ser	Arg	Phe
	50					55					60				
Ser	Pro	Glu	Cys	Pro	Asn	Ser	Ser	Leu	Leu	Asn	Leu	His	Leu	His	Ala
65					70					75					80
Leu	Gln	Pro	Glu	Asp	Ser	Ala	Leu	Tyr	Leu	Cys	Ala	Ser	Ser	His	Gly
				85					90					95	
Ser	Leu	Asn	Thr	Glu	Ala	Phe	Phe	Gly	Gln	Gly	Thr	Arg	Leu	Thr	Val
			100					105					110		

Val

<210> 100

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A V alpha (mature)

<400> 100

Gly	Glu	Asp	Val	Glu	Gln	Ser	Leu	Phe	Leu	Ser	Val	Arg	Glu	Gly	Asp	
1				5				10						15		
Ser	Ser	Val	Ile	Asn	Cys	Thr	Tyr	Thr	Asp	Ser	Ser	Ser	Thr	Tyr	Leu	
			20					25						30		
Tyr	Trp	Tyr	Lys	Gln	Glu	Pro	Gly	Ala	Gly	Leu	Gln	Leu	Leu	Thr	Tyr	
		35					40					45				
Ile	Phe	Ser	Asn	Met	Asp	Met	Lys	Gln	Asp	Gln	Arg	Leu	Thr	Val	Leu	
	50					55					60					
Leu	Asn	Lys	Lys	Asp	Lys	His	Leu	Ser	Leu	Arg	Ile	Ala	Asp	Thr	Gln	
65					70					75					80	
Thr	Gly	Asp	Ser	Ala	Ile	Tyr	Phe	Cys	Ala	Glu	Thr	Pro	Gly	Tyr	Gly	
				85					90					95		
Gly	Ala	Thr	Asn	Lys	Leu	Ile	Phe	Gly	Thr	Gly	Thr	Leu	Leu	Ala	Val	
			100					105						110		
Gln	Pro															

<210> 101

<211> 114

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B V beta (mature)

<400> 101

Glu	Thr	Gly	Val	Thr	Gln	Ser	Pro	Thr	His	Leu	Ile	Lys	Thr	Arg	Gly	
1				5				10						15		
Gln	Gln	Val	Thr	Leu	Arg	Cys	Ser	Ser	Gln	Ser	Gly	His	Asn	Thr	Val	
			20					25						30		
Ser	Trp	Tyr	Gln	Gln	Ala	Leu	Gly	Gln	Gly	Pro	Gln	Phe	Ile	Phe	Gln	
		35					40					45				
Tyr	Tyr	Arg	Glu	Glu	Glu	Asn	Gly	Arg	Gly	Asn	Phe	Pro	Pro	Arg	Phe	
	50					55					60					
Ser	Gly	Leu	Gln	Phe	Pro	Asn	Tyr	Ser	Ser	Glu	Leu	Asn	Val	Asn	Ala	
65					70					75					80	
Leu	Glu	Leu	Asp	Asp	Ser	Ala	Leu	Tyr	Leu	Cys	Ala	Ser	Ser	Phe	Ala	
			85					90						95		

Gly Gly Arg Ser Asp Thr Gln Tyr Phe Gly Pro Gly Thr Arg Leu Thr
100 105 110
Val Leu

<210> 102
<211> 116
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso530-11B V alpha (mature)

<400> 102
Lys Gln Glu Val Thr Gln Ile Pro Ala Ala Leu Ser Val Pro Glu Gly
1 5 10 15
Glu Asn Leu Val Leu Asn Cys Ser Phe Thr Asp Ser Ala Ile Tyr Asn
20 25 30
Leu Gln Trp Phe Arg Gln Asp Pro Gly Lys Gly Leu Thr Ser Leu Leu
35 40 45
Leu Ile Gln Ser Ser Gln Arg Glu Gln Thr Ser Gly Arg Leu Asn Ala
50 55 60
Ser Leu Asp Lys Ser Ser Gly Arg Ser Thr Leu Tyr Ile Ala Ala Ser
65 70 75 80
Gln Pro Gly Asp Ser Ala Thr Tyr Leu Cys Ala Val Arg Leu Leu Phe
85 90 95
Ala Gln Gly Gly Ser Glu Lys Leu Val Phe Gly Lys Gly Thr Lys Leu
100 105 110
Thr Val Asn Pro
115

<210> 103
<211> 293
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence Meso20-3B TCR beta (mature)

<400> 103
Asp Ala Gly Val Ile Gln Ser Pro Arg His Glu Val Thr Glu Met Gly
1 5 10 15
Gln Glu Val Thr Leu Arg Cys Lys Pro Ile Ser Gly His Asp Tyr Leu
20 25 30
Phe Trp Tyr Arg Gln Thr Met Met Arg Gly Leu Glu Leu Leu Ile Tyr

		35				40				45					
Phe	Asn	Asn	Val	Pro	Ile	Asp	Asp	Ser	Gly	Met	Pro	Glu	Asp	Arg	
50					55					60					
Phe	Ser	Ala	Lys	Met	Pro	Asn	Ala	Ser	Phe	Ser	Thr	Leu	Lys	Ile	Gln
65					70					75					80
Pro	Ser	Glu	Pro	Arg	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Ser	Ser	Phe
				85					90					95	
Thr	Ser	Gly	Ser	Tyr	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg	Leu	Thr
			100					105					110		
Val	Thr	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	Ala	Val	Phe
		115					120					125			
Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr	Leu	Val
130						135					140				
Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	Ser	Trp	Trp
145					150					155					160
Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	Pro	Gln	Pro
				165				170						175	
Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	Leu	Ser	Ser
			180					185					190		
Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	Asn	His	Phe
		195					200					205			
Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu	Trp	Thr
210					215						220				
Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu	Ala	Trp
225					230					235					240
Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln	Gln	Gly	Val
				245					250					255	
Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	Ala	Thr	Leu
			260					265					270		
Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	Val	Lys	Arg
		275					280					285			
Lys	Asp	Ser	Arg	Gly											
290															

<210> 104

<211> 249

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-3B TCR alpha (mature) -
Cys mod

<400> 104

Gly Gln Ser Leu Glu Gln Pro Ser Glu Val Thr Ala Val Glu Gly Ala

1				5					10					15		
Ile	Val	Gln	Ile	Asn	Cys	Thr	Tyr	Gln	Thr	Ser	Gly	Phe	Tyr	Gly	Leu	
			20					25					30			
Ser	Trp	Tyr	Gln	Gln	His	Asp	Gly	Gly	Ala	Pro	Thr	Phe	Leu	Ser	Tyr	
		35					40					45				
Asn	Ala	Leu	Asp	Gly	Leu	Glu	Glu	Thr	Gly	Arg	Phe	Ser	Ser	Phe	Leu	
	50					55					60					
Ser	Arg	Ser	Asp	Ser	Tyr	Gly	Tyr	Leu	Leu	Leu	Gln	Glu	Leu	Gln	Met	
65					70					75					80	
Lys	Asp	Ser	Ala	Ser	Tyr	Phe	Cys	Ala	Val	Asn	Asp	Ala	Ser	Lys	Ile	
				85					90					95		
Ile	Phe	Gly	Ser	Gly	Thr	Arg	Leu	Ser	Ile	Arg	Pro	Asn	Ile	Gln	Asn	
			100					105					110			
Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser	Lys	Ser	Ser	Asp	Lys	
		115					120					125				
Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln	Thr	Asn	Val	Ser	Gln	
	130					135					140					
Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp	Lys	Cys	Val	Leu	Asp	Met	
145					150					155					160	
Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala	Val	Ala	Trp	Ser	Asn	Lys	
				165					170					175		
Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn	Asn	Ser	Ile	Ile	Pro	Glu	
			180					185					190			
Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser	Cys	Asp	Val	Lys	Leu	Val	
		195					200					205				
Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu	Asn	Phe	Gln	Asn	Leu	Ser	
	210					215					220					
Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys	Val	Ala	Gly	Phe	Asn	Leu	
225					230					235					240	
Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser								
				245												

<210> 105

<211> 293

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B TCR beta (mature) -
Cys mod

<400> 105

Asp	Ala	Gly	Val	Ile	Gln	Ser	Pro	Arg	His	Glu	Val	Thr	Glu	Met	Gly	
1				5				10					15			
Gln	Glu	Val	Thr	Leu	Arg	Cys	Lys	Pro	Ile	Ser	Gly	His	Asn	Ser	Leu	

			20					25				30					
Phe	Trp	Tyr	Arg	Gln	Thr	Met	Met	Arg	Gly	Leu	Glu	Leu	Leu	Ile	Tyr		
		35					40					45					
Phe	Asn	Asn	Asn	Val	Pro	Ile	Asp	Asp	Ser	Gly	Met	Pro	Glu	Asp	Arg		
	50					55					60						
Phe	Ser	Ala	Lys	Met	Pro	Asn	Ala	Ser	Phe	Ser	Thr	Leu	Lys	Ile	Gln		
65					70					75					80		
Pro	Ser	Glu	Pro	Arg	Asp	Ser	Ala	Val	Tyr	Phe	Cys	Ala	Ser	Ser	Ser		
				85					90					95			
Pro	Asp	Arg	Leu	Gly	Glu	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg	Leu	Thr		
			100					105					110				
Val	Thr	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	Ala	Val	Phe		
		115					120					125					
Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr	Leu	Val		
	130					135					140						
Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	Ser	Trp	Trp		
145					150					155					160		
Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	Pro	Gln	Pro		
				165				170						175			
Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	Leu	Ser	Ser		
			180					185					190				
Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	Asn	His	Phe		
		195					200					205					
Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu	Trp	Thr		
	210					215					220						
Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu	Ala	Trp		
225					230					235					240		
Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln	Gln	Gly	Val		
				245				250						255			
Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	Ala	Thr	Leu		
			260					265					270				
Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	Val	Lys	Arg		
		275					280					285					
Lys	Asp	Ser	Arg	Gly													
		290															

<210> 106

<211> 250

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso20-16B TCR alpha (mature) -
Cys mod

<400> 106

Gln	Lys	Glu	Val	Glu	Gln	Asp	Pro	Gly	Pro	Leu	Ser	Val	Pro	Glu	Gly
1				5				10						15	
Ala	Ile	Val	Ser	Leu	Asn	Cys	Thr	Tyr	Ser	Asn	Ser	Ala	Phe	Gln	Tyr
			20					25					30		
Phe	Met	Trp	Tyr	Arg	Gln	Tyr	Ser	Arg	Lys	Gly	Pro	Glu	Leu	Leu	Met
		35					40					45			
Tyr	Thr	Tyr	Ser	Ser	Gly	Asn	Lys	Glu	Asp	Gly	Arg	Phe	Thr	Ala	Gln
	50					55					60				
Val	Asp	Lys	Ser	Ser	Lys	Tyr	Ile	Ser	Leu	Phe	Ile	Arg	Asp	Ser	Gln
65					70					75					80
Pro	Ser	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Gly	Gly	Gly	Asn	Thr	Pro
			85					90					95		
Leu	Val	Phe	Gly	Lys	Gly	Thr	Arg	Leu	Ser	Val	Ile	Ala	Asn	Ile	Gln
			100					105					110		
Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp	Ser	Lys	Ser	Ser	Asp
		115					120					125			
Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser	Gln	Thr	Asn	Val	Ser
	130					135					140				
Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp	Lys	Cys	Val	Leu	Asp
145					150					155					160
Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala	Val	Ala	Trp	Ser	Asn
			165					170					175		
Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn	Asn	Ser	Ile	Ile	Pro
		180						185					190		
Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser	Cys	Asp	Val	Lys	Leu
	195						200					205			
Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu	Asn	Phe	Gln	Asn	Leu
	210					215					220				
Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys	Val	Ala	Gly	Phe	Asn
225					230					235					240
Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser						
			245					250							

<210> 107

<211> 290

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A TCR beta (mature) -
Cys mod

<400> 107

Asp Thr Glu Val Thr Gln Thr Pro Lys His Leu Val Met Gly Met Thr

1				5				10					15			
Asn	Lys	Lys	Ser	Leu	Lys	Cys	Glu	Gln	His	Met	Gly	His	Arg	Ala	Met	
			20					25					30			
Tyr	Trp	Tyr	Lys	Gln	Lys	Ala	Lys	Lys	Pro	Pro	Glu	Leu	Met	Phe	Val	
		35					40					45				
Tyr	Ser	Tyr	Glu	Lys	Leu	Ser	Ile	Asn	Glu	Ser	Val	Pro	Ser	Arg	Phe	
	50					55					60					
Ser	Pro	Glu	Cys	Pro	Asn	Ser	Ser	Leu	Leu	Asn	Leu	His	Leu	His	Ala	
65					70					75					80	
Leu	Gln	Pro	Glu	Asp	Ser	Ala	Leu	Tyr	Leu	Cys	Ala	Ser	Ser	His	Gly	
				85					90					95		
Ser	Leu	Asn	Thr	Glu	Ala	Phe	Phe	Gly	Gln	Gly	Thr	Arg	Leu	Thr	Val	
			100					105					110			
Val	Glu	Asp	Leu	Asn	Lys	Val	Phe	Pro	Pro	Glu	Val	Ala	Val	Phe	Glu	
		115					120					125				
Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr	Leu	Val	Cys	
		130				135					140					
Leu	Ala	Thr	Gly	Phe	Phe	Pro	Asp	His	Val	Glu	Leu	Ser	Trp	Trp	Val	
145					150					155					160	
Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	Pro	Gln	Pro	Leu	
				165					170					175		
Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	Leu	Ser	Ser	Arg	
			180					185					190			
Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	Asn	His	Phe	Arg	
		195					200					205				
Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu	Trp	Thr	Gln	
		210				215					220					
Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu	Ala	Trp	Gly	
225					230					235					240	
Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Val	Ser	Tyr	Gln	Gln	Gly	Val	Leu	
				245					250					255		
Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	Ala	Thr	Leu	Tyr	
			260					265					270			
Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	Val	Lys	Arg	Lys	
		275					280					285				
Asp	Phe															
	290															

<210> 108

<211> 255

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11A TCR alpha (mature)

- Cys mod

<400> 108

Gly	Glu	Asp	Val	Glu	Gln	Ser	Leu	Phe	Leu	Ser	Val	Arg	Glu	Gly	Asp
1				5					10					15	
Ser	Ser	Val	Ile	Asn	Cys	Thr	Tyr	Thr	Asp	Ser	Ser	Ser	Thr	Tyr	Leu
			20					25					30		
Tyr	Trp	Tyr	Lys	Gln	Glu	Pro	Gly	Ala	Gly	Leu	Gln	Leu	Leu	Thr	Tyr
		35					40					45			
Ile	Phe	Ser	Asn	Met	Asp	Met	Lys	Gln	Asp	Gln	Arg	Leu	Thr	Val	Leu
	50					55					60				
Leu	Asn	Lys	Lys	Asp	Lys	His	Leu	Ser	Leu	Arg	Ile	Ala	Asp	Thr	Gln
65				70					75						80
Thr	Gly	Asp	Ser	Ala	Ile	Tyr	Phe	Cys	Ala	Glu	Thr	Pro	Gly	Tyr	Gly
				85					90					95	
Gly	Ala	Thr	Asn	Lys	Leu	Ile	Phe	Gly	Thr	Gly	Thr	Leu	Leu	Ala	Val
			100					105					110		
Gln	Pro	Asn	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu	Arg	Asp
		115					120					125			
Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe	Asp	Ser
	130					135					140				
Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile	Thr	Asp
145				150					155						160
Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn	Ser	Ala
				165					170					175	
Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala	Phe	Asn
		180					185						190		
Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu	Ser	Ser
		195					200					205			
Cys	Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr	Asn	Leu
	210					215					220				
Asn	Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu	Leu	Lys
225				230						235					240
Val	Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser	Ser	
				245					250					255	

<210> 109

<211> 293

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B TCR beta (mature) -
Cys mod

<400> 109

Glu	Thr	Gly	Val	Thr	Gln	Ser	Pro	Thr	His	Leu	Ile	Lys	Thr	Arg	Gly
1				5					10					15	
Gln	Gln	Val	Thr	Leu	Arg	Cys	Ser	Ser	Gln	Ser	Gly	His	Asn	Thr	Val
			20					25					30		
Ser	Trp	Tyr	Gln	Gln	Ala	Leu	Gly	Gln	Gly	Pro	Gln	Phe	Ile	Phe	Gln
		35					40					45			
Tyr	Tyr	Arg	Glu	Glu	Glu	Asn	Gly	Arg	Gly	Asn	Phe	Pro	Pro	Arg	Phe
	50					55					60				
Ser	Gly	Leu	Gln	Phe	Pro	Asn	Tyr	Ser	Ser	Glu	Leu	Asn	Val	Asn	Ala
65					70					75					80
Leu	Glu	Leu	Asp	Asp	Ser	Ala	Leu	Tyr	Leu	Cys	Ala	Ser	Ser	Phe	Ala
			85						90					95	
Gly	Gly	Arg	Ser	Asp	Thr	Gln	Tyr	Phe	Gly	Pro	Gly	Thr	Arg	Leu	Thr
			100					105					110		
Val	Leu	Glu	Asp	Leu	Lys	Asn	Val	Phe	Pro	Pro	Glu	Val	Ala	Val	Phe
		115					120					125			
Glu	Pro	Ser	Glu	Ala	Glu	Ile	Ser	His	Thr	Gln	Lys	Ala	Thr	Leu	Val
	130					135					140				
Cys	Leu	Ala	Thr	Gly	Phe	Tyr	Pro	Asp	His	Val	Glu	Leu	Ser	Trp	Trp
145					150					155					160
Val	Asn	Gly	Lys	Glu	Val	His	Ser	Gly	Val	Cys	Thr	Asp	Pro	Gln	Pro
			165					170						175	
Leu	Lys	Glu	Gln	Pro	Ala	Leu	Asn	Asp	Ser	Arg	Tyr	Cys	Leu	Ser	Ser
			180					185					190		
Arg	Leu	Arg	Val	Ser	Ala	Thr	Phe	Trp	Gln	Asn	Pro	Arg	Asn	His	Phe
		195					200					205			
Arg	Cys	Gln	Val	Gln	Phe	Tyr	Gly	Leu	Ser	Glu	Asn	Asp	Glu	Trp	Thr
	210					215					220				
Gln	Asp	Arg	Ala	Lys	Pro	Val	Thr	Gln	Ile	Val	Ser	Ala	Glu	Ala	Trp
225					230					235					240
Gly	Arg	Ala	Asp	Cys	Gly	Phe	Thr	Ser	Glu	Ser	Tyr	Gln	Gln	Gly	Val
			245						250					255	
Leu	Ser	Ala	Thr	Ile	Leu	Tyr	Glu	Ile	Leu	Leu	Gly	Lys	Ala	Thr	Leu
			260					265					270		
Tyr	Ala	Val	Leu	Val	Ser	Ala	Leu	Val	Leu	Met	Ala	Met	Val	Lys	Arg
		275					280					285			
Lys	Asp	Ser	Arg	Gly											
	290														

<210> 110

<211> 257

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B TCR alpha (mature)
- Cys mod

<400> 110

Lys	Gln	Glu	Val	Thr	Gln	Ile	Pro	Ala	Ala	Leu	Ser	Val	Pro	Glu	Gly
1				5					10					15	
Glu	Asn	Leu	Val	Leu	Asn	Cys	Ser	Phe	Thr	Asp	Ser	Ala	Ile	Tyr	Asn
			20					25					30		
Leu	Gln	Trp	Phe	Arg	Gln	Asp	Pro	Gly	Lys	Gly	Leu	Thr	Ser	Leu	Leu
		35					40					45			
Leu	Ile	Gln	Ser	Ser	Gln	Arg	Glu	Gln	Thr	Ser	Gly	Arg	Leu	Asn	Ala
	50					55					60				
Ser	Leu	Asp	Lys	Ser	Ser	Gly	Arg	Ser	Thr	Leu	Tyr	Ile	Ala	Ala	Ser
65					70					75					80
Gln	Pro	Gly	Asp	Ser	Ala	Thr	Tyr	Leu	Cys	Ala	Val	Arg	Leu	Leu	Phe
				85					90					95	
Ala	Gln	Gly	Gly	Ser	Glu	Lys	Leu	Val	Phe	Gly	Lys	Gly	Thr	Lys	Leu
			100					105					110		
Thr	Val	Asn	Pro	Tyr	Ile	Gln	Asn	Pro	Asp	Pro	Ala	Val	Tyr	Gln	Leu
		115					120					125			
Arg	Asp	Ser	Lys	Ser	Ser	Asp	Lys	Ser	Val	Cys	Leu	Phe	Thr	Asp	Phe
	130					135					140				
Asp	Ser	Gln	Thr	Asn	Val	Ser	Gln	Ser	Lys	Asp	Ser	Asp	Val	Tyr	Ile
145					150					155					160
Thr	Asp	Lys	Cys	Val	Leu	Asp	Met	Arg	Ser	Met	Asp	Phe	Lys	Ser	Asn
				165					170					175	
Ser	Ala	Val	Ala	Trp	Ser	Asn	Lys	Ser	Asp	Phe	Ala	Cys	Ala	Asn	Ala
			180					185					190		
Phe	Asn	Asn	Ser	Ile	Ile	Pro	Glu	Asp	Thr	Phe	Phe	Pro	Ser	Pro	Glu
		195					200					205			
Ser	Ser	Cys	Asp	Val	Lys	Leu	Val	Glu	Lys	Ser	Phe	Glu	Thr	Asp	Thr
	210					215					220				
Asn	Leu	Asn	Phe	Gln	Asn	Leu	Ser	Val	Ile	Gly	Phe	Arg	Ile	Leu	Leu
225					230					235					240
Leu	Lys	Val	Ala	Gly	Phe	Asn	Leu	Leu	Met	Thr	Leu	Arg	Leu	Trp	Ser
				245					250					255	
Ser															

<210> 111

<211> 831

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B WT TCR alpha

<400> 111

```
atggagaccc tcttgggcct gcttatcctt tggctgcagc tgcaatgggt gagcagcaaa
60
caggaggtga cgcagattcc tgcagctctg agtgtcccag aaggagaaaa cttggttctc
120
aactgcagtt tcaactgatag cgctattttac aacctccagt ggtttaggca ggaccctggg
180
aaaggtctca catctctgtt gcttattcag tcaagtcaga gagagcaaac aagtggaaga
240
cttaatgcct cgctggataa atcatcagga cgtagtactt tatacattgc agcttctcag
300
cctggtgact cagccaccta cctctgtgct gtgaggctat tattcgctca gggcggatct
360
gaaaagctgg tcttttgaaa gggaacgaaa ctgacagtaa acccatatat ccagaaccct
420
gaccctgccg tgtaccagct gagagactct aaatccagtg acaagtctgt ctgcctattc
480
accgattttg attctcaaac aaatgtgtca caaagtaagg attctgatgt gtatatcaca
540
gacaaaactg tgctagacat gaggtctatg gacttcaaga gcaacagtg c tgtggcctgg
600
agcaacaaat ctgactttgc atgtgcaaac gccttcaaca acagcattat tccagaagac
660
accttcttcc ccagcccaga aagttcctgt gatgtcaagc tggtcgagaa aagctttgaa
720
acagatacga acctaaactt tcaaaacctg tcagtgattg gggtccgaat cctcctcctg
780
aaagtggccg ggtttaatct gctcatgacg ctgcggctgt ggtccagctg a
831
```

<210> 112

<211> 939

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence Meso530-11B WT TCR beta

<400> 112

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atgggccctg ggctcctctg ctgggtgctg ctttgtctcc tgggagcagg ctcagtggag
60
actggagtca ccaaagtcc cacacacctg atcaaaacga gaggacagca agtgactctg
120
```

```

agatgctctt ctcagtctgg gcacaacact gtgtcctggg accaacaggc cctgggtcag
180
gggccccagt ttatctttca gtattatagg gaggaagaga atggcagagg aaacttcctt
240
cctagattct caggtctcca gttccctaata tatagctctg agctgaatgt gaacgccttg
300
gagctggacg actcggccct gtatctctgt gccagcagct tcgcgggggg gcgatcagat
360
acgcagtatt ttggcccagg caccgggctg acagtgctcg aggacctgaa aaacgtgttc
420
ccacccgagg tcgctgtgtt tgagccatca gaagcagaga tctcccacac ccaaaaggcc
480
acactgggtg gcctggccac aggcttctac cccgaccacg tggagctgag ctgggtgggtg
540
aatgggaagg aggtgcacag tggggtcagc acagaccgc agccccctcaa ggagcagccc
600
gccctcaatg actccagata ctgcctgagc agccgcctga gggctctcggc caccttcttg
660
cagaaccccc gcaaccactt ccgctgtcaa gtccagttct acgggctctc ggagaatgac
720
gagtggaccc aggatagggc caaacctgtc acccagatcg tcagcgccga ggcctggggg
780
agagcagact gtggcttcac ctccgagtct taccagcaag gggtcctgtc tgccaccatc
840
ctctatgaga tcttgctagg gaaggccacc ttgtatgccg tgctggtcag tgccctcgtg
900
ctgatggcca tgggtcaagag aaaggattcc agaggctag
939

```

<210> 113

<211> 22

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Porcine teschovirus
self-cleaving peptide P2A-1

<400> 113

```

Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln Ala Gly Asp Val
 1             5             10             15
Glu Glu Asn Pro Gly Pro
                20

```

<210> 114

<211> 21
<212> PRT
<213> Artificial Sequence

<220>

<223> Synthetic sequence Porcine teschovirus
self-cleaving peptide P2A-2

<400> 114

Ser	Gly	Ala	Thr	Asn	Phe	Ser	Leu	Leu	Lys	Gln	Ala	Gly	Asp	Val	Glu
1				5					10					15	
Glu	Asn	Pro	Gly	Pro											
			20												

<210> 115

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Thoseaasigna virus 2A T2A

<400> 115

Gly	Ser	Gly	Glu	Gly	Arg	Gly	Ser	Leu	Leu	Thr	Cys	Gly	Asp	Val	Glu
1				5				10						15	
Glu	Asn	Pro	Gly	Pro											
			20												

<210> 116

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Equine rhinitis A virus E2A

<400> 116

Gly	Ser	Gly	Gln	Cys	Thr	Asn	Tyr	Ala	Leu	Leu	Lys	Leu	Ala	Gly	Asp
1				5				10						15	
Val	Glu	Ser	Asn	Pro	Gly	Pro									
			20												

<210> 117

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence Foot-and-Mouth disease virus 2
F2A

<400> 117

Gly	Ser	Gly	Val	Lys	Gln	Thr	Leu	Asn	Phe	Asp	Leu	Leu	Lys	Leu	Ala
1				5					10					15	
Gly	Asp	Val	Glu	Ser	Asn	Pro	Gly	Pro							
			20					25							

<210> 118

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence - Meso20-3B CDR2 alpha v2

<400> 118

Asn	Ala	Leu	Asp	Gly	Leu
1				5	

<210> 119

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence - Meso20-16B CDR2 alpha v2

<400> 119

Thr	Tyr	Ser	Ser	Gly	Asn
1				5	

<210> 120

<211> 1833

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence - Meso530-11B
C0-Beta-P2A-Alpha: (alternate optimization)

<400> 120

atgggacctg	gattgctttg	ttgggtgctg	ctgtgtctgc	tcggagccgg	atctgtggaa
60					
acaggcgtga	cacagagccc	cacacacctg	atcaagacca	gaggccagca	agtgaccctg
120					
agatgcagct	ctcagagcgg	ccacaatacc	gtgtcctggg	atcagcaggc	cctcggacag
180					
ggccctcagt	tcattcttcca	gtactacaga	gaggaagaga	acggcagagg	caacttccca
240					
cctagattca	gcggcctgca	gttccccaac	tacagcagcg	agctgaacgt	gaacgccctg
300					
gaactggatg	acagcgccct	gtacctgtgc	gccagttctt	ttgccggcgg	aagaagcgac
360					
accagtgact	ttggacctgg	caccagactg	accgtgctgg	aagatctgaa	gaacgtgttc
420					
ccacctgagg	tggccgtggt	cgagccttct	gaggccgaga	tcagccacac	acagaaagcc
480					
acactcgtgt	gtctggccac	cggcttctat	cccgatcacg	tggaactgtc	ttgggtgggtc
540					
aacggcaaag	aggtgcacag	cggcgtctgt	accgatcctc	agcctctgaa	agagcagccc
600					
gctctgaacg	acagcagata	ctgcctgagc	agcagactga	gagtgtccgc	caccttctgg
660					
cagaacccca	gaaaccactt	cagatgccag	gtgcagttct	acggcctgag	cgagaacgat
720					
gagtggaccc	aggatagagc	caagcctgtg	actcagatcg	tgtctgccga	agcctggggc
780					
agagccgatt	gtggctttac	cagcgagtct	taccagcagg	gcgtgctgtc	tgccaccatc
840					
ctgtatgaga	tcctgctggg	caaagccact	ctgtacgccg	tgctgggttc	tgccctgggtg
900					
ctgatggcca	tgggtcaagcg	gaaggatagc	agaggcgga	gcggcgccac	aaacttctca
960					
ctgctgaaac	aggccggcga	cgtggaagag	aatcccggac	ctatggaaac	actgctggga
1020					
ctgctgatcc	tgtggctgca	gcttcagtgg	gtgtccagca	agcaagaagt	gaccagatt
1080					
cctgccgcac	tgtctgtgcc	tgagggcgag	aatctgggtc	tgaactgctc	cttcaccgac
1140					
agcgccatct	acaacctgca	gtggttcaga	caggaccccg	gcaagggact	gacaagcctg
1200					
ctgctgattc	agagcagcca	gagagagcag	accagcggca	gactgaatgc	cagcctggat
1260					
aagtcctccg	gcagaagcac	cctgtatatc	gccgcttctc	agcctggcga	tagcgccaca
1320					

tatctgtgtg ccggtgcggct gctgtttgcc caaggcggat ctgagaagct ggtgttcggc
1380
aagggcacca agctgacagt gaacccttac attcagaacc ccgatacctgc cgtgtaccag
1440
ctgagagaca gcaagagcag cgacaagagc gtgtgcctgt tcaccgactt cgacagccag
1500
accaacgtgt cccagagcaa ggacagcgac gtgtacatca ccgataagtg cgtgctggac
1560
atgcggagca tggacttcaa gagcaacagc gccgtggcct ggtccaacaa gagcgatttc
1620
gcctgcgcca acgccttcaa caacagcatt atccccgagg acacattctt cccaagtcct
1680
gagtccagct gcgacgtgaa actgggtggaa aagagcttcg agacagacac caacctgaac
1740
ttccagaacc tgagcgtgat cggttccgg atcctgctcc tgaaagtggc cggttcaac
1800
c t g c t g a t g a c c c t g c g a c t g t g g t c c a g c t a a
1833

<210> 121

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence - Meganuclease sequence

<400> 121

Leu Ala Gly Leu Ile Asp Ala Asp Gly

1

5

<210> 122

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic sequence - Meganuclease sequence

<400> 122

Gly Ile Tyr Tyr Ile Gly

1

5

<210> 123

<211> 5

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic sequence - Meganuclease sequence

<220>
<221> VARIANT
<222> 3
<223> Xaa = D or E

<220>
<221> VARIANT
<222> 4
<223> Xaa = any amino acid

<400> 123
Pro Asp Xaa Xaa Lys
1 5