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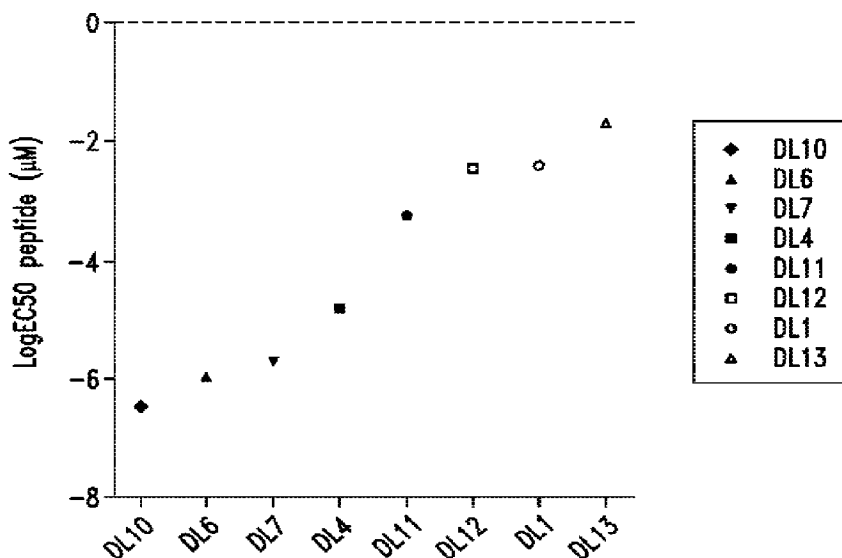


FIG. 4C

(57) Abrégé/Abstract:

The present disclosure provides binding proteins specific for human Wilms tumor protein 1 (WT-1) epitopes, as well as host cells that express the binding proteins. Also provided are polynucleotides that encode a binding protein and vectors that comprise a polynucleotide. Related methods and uses of the presently disclosed compositions are provided for treating diseases or disorders associated with WT-1 expression, such as various cancers.



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Abstract:

The present disclosure provides binding proteins specific for human Wilms tumor protein 1 (WT-1) epitopes, as well as host cells that express the binding proteins. Also provided are polynucleotides that encode a binding protein and vectors that comprise a polynucleotide. Related methods and uses of the presently disclosed compositions are provided for treating diseases or disorders associated with WT-1 expression, such as various cancers.

T-CELL IMMUNOTHERAPY SPECIFIC FOR WT-1

STATEMENT REGARDING SEQUENCE LISTING

The Sequence Listing associated with this application is provided in text
5 format in lieu of a paper copy, and is hereby incorporated by reference into the
specification. The name of the text file containing the Sequence Listing is
360056_473WO_SEQUENCE_LISTING.txt. The text file is 109 KB, was created on
August 17, 2020, and is being submitted electronically via EFS-Web.

BACKGROUND

10 T cell receptor (TCR) gene therapy is an emerging treatment approach designed
to overcome obstacles associated with conventional T cell adoptive immunotherapy,
such as the extensive time and labor required to isolate, characterize, and expand tumor
antigen-specific T cells (Schmitt *et al.*, *Hum. Gene Ther.* 20:1240, 2009). Another
hurdle is that most identified tumor antigens that can be targeted by T cell
15 immunotherapy are over-expressed self-proteins, so high affinity T cells specific for
these antigens are generally eliminated during thymic selection, and are rare or non-
existent in the peripheral repertoire.

Strategies have been considered for enhancing the affinity of TCRs intended for
use in TCR gene therapy (*e.g.*, Udyavar *et al.*, *J. Immunol.* 182:4439, 2009; Zhao *et al.*,
20 *J. Immunol.* 179:5845, 2007; Richman and Kranz, *Biomol. Eng.* 24:361, 2007).

A precondition for targeted T-cell therapy achieving a maximal clinical effect
that would be accompanied by minimal immunological toxicity involves identifying
disease-associated antigens with high expression in and presentation by, for example, a
malignant cell compartment, but without significant expression in normal tissue. For
25 example, several acute myeloid leukemia (AML) associated antigens have been
described, and Wilms tumor protein 1 (WT-1) has been shown to be expressed in the
leukemia stem cell (LSC) compartment of the majority of AML patients at levels
significantly higher than in physiological hematopoietic stem cells (HSCs). WT-1 has
been targeted in clinical trials both with adoptive T-cell transfer and peptide vaccination

(see, e.g., U.S. Pat. Nos. 7,342,092; 7,608,685; 7,622,119). In addition, WT-1 expression has been reported to be a marker of minimal residual disease because increased transcript levels in patients with AML in morphologic remission were predictive of overt clinical relapse (Inoue *et al.*, *Blood* 84:3071, 1994; Ogawa *et al.*,
5 *Blood* 101:1698, 2003).

Since WT-1 is an intracellular (usually nuclear) protein, immunotherapies targeting WT-1 have used cellular approaches aimed at generating WT-1-specific CD8⁺ cytotoxic T lymphocyte (CTL) responses that recognize peptides presented on the cell surface by MHC class I molecules. For induction of a CTL response, intracellular
10 proteins are usually degraded by the proteasome or endo/lysosomes, with the resulting peptide fragments binding to MHC class I or class II molecules. These peptide-MHC complexes are displayed on the cell surface where they are bound by T cells via the peptide-MHC-TCR interaction. Peptides derived from the WT-1 protein can theoretically be used in a vaccine in humans to induce human leukocyte antigen (HLA)-
15 restricted cytotoxic CD8⁺ T cells that are capable of killing tumor cells. However, because WT-1 is a self-protein, immunization may only elicit responses by T cells with low affinity TCRs. In addition, antibodies against WT-1 are detectable in patients with hematopoietic malignancies and solid tumors, which show that WT-1 can be a highly immunogenic antigen (Gaiger *et al.*, *Clin. Cancer Res.* 7 (Suppl. 3):761, 2001).

20 Clearly there is a need for alternative gene therapies for use as highly specific, WT-1 targeted immunotherapies directed against various cancers, such as leukemia and tumors. The presently disclosed embodiments address this need and provide other related advantages.

BRIEF SUMMARY

25 The present disclosure provides, according to certain embodiments, binding proteins that are capable of binding to a RMFPNAPYL (SEQ ID NO:94):human leukocyte antigen (HLA) complex, wherein the binding proteins comprise (a) a T cell receptor (TCR) α chain variable (V α) domain comprising the CDR3 amino acid sequence (CDR3 α) according to any one of SEQ ID NOs.: 19, 22, 25, or 28, or a variant
30 thereof, and a TCR β chain variable (V β) domain, wherein the V β optionally comprises

or consists of an amino acid sequence having at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to the amino acid sequence set forth in any one of SEQ ID NOs.:5-8 or 13-16; (b) a TCR V β domain comprising the CDR3 amino acid sequence (CDR3 β) according to any one of SEQ ID NOs.:31, 34, 37, 5 or 40, or a variant thereof, and a TCR V α domain, wherein the V α optionally comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.: 1-4 or 9-12; or (c) a TCR V α domain of (a), and a TCR V β domain of (b).

In certain embodiments, a binding protein is capable of binding to a
 10 RMFPNAPYL (SEQ ID NO.:94):human leukocyte antigen (HLA) complex with an IFN γ production pEC₅₀ of 4.0, 4.5, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, or higher (*e.g.*, an immune cell (such as, for example, a T cell, a NK-T cell, or a NK cell) comprising the binding protein has a SEQ ID NO.:94:HLA IFN γ production pEC₅₀ of 4.0, 4.5, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2,
 15 6.3, 6.4, 6.5, or higher).

In certain embodiments, the HLA comprises HLA-A*0201.

In certain embodiments, the binding protein is human, humanized, or chimeric.

In any of the herein disclosed embodiments, a binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):human leukocyte antigen (HLA)
 20 complex, wherein the binding protein comprises a TCR V β domain and a TCR V α domain, wherein: (i) the V β domain comprises or consists of an amino acid sequence having at least 90% identity (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) to the amino acid sequence of any one of SEQ ID NOs.: 5-8 or 13-16; and/or (ii) the V α domain comprises or consists of an amino acid sequence having at
 25 least 90% identity to the amino acid sequence of any one of SEQ ID NOs.:1- 4 or 9-12.

In certain embodiments, the encoded binding protein is a TCR, a CAR, or a scTCR.

Also provided herein modified immune cells that comprise a heterologous polynucleotide that encodes a binding protein as disclosed herein. In certain
 30 embodiments, the immune cell comprises a T cell, a NK cell, a NK-T cell, or any combination thereof. In certain embodiments, an immune cell encoding a binding

protein as disclosed herein is activated in the presence of a peptide according to SEQ ID NO.:94 (*e.g.*, comprised in a peptide:HLA complex). In certain embodiments, an immune cell encoding a binding protein as disclosed herein is capable of killing a target cell expressing a SEQ ID NO: 94:HLA complex.

5 Also provided are isolated polynucleotides that encode a binding protein according to the present disclosure. In certain embodiments, the encoded binding protein comprises: (i) the amino acid sequence set forth in any one of SEQ ID NOs.: 19, 22, 25, 28, 31, 34, 37, or 40; (ii) the amino acid sequence set forth in any one of SEQ ID NOs.: 17, 20, 23, 26, 29, 32, 35, or 38; (iii) the amino acid sequence set forth in
10 any one of SEQ ID NOs.: 18, 21, 24, 27, 30, 33, 36, or 39; (iv) an amino acid sequence comprising, consisting of, or having at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the amino acid sequence set forth in any one of SEQ ID NOs: 1-4 or 9-12; (v) an amino acid sequence comprising, consisting of, or having at least 90% identity to the amino acid sequence set forth in
15 any one of SEQ ID NOs: 5-8 or 13-16; (vi) an amino acid sequence comprising, consisting of, or having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.: 46-53; (vii) an amino acid sequence comprising, consisting of, or having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.: 54-61; or (viii) any combination of (i)-(vii).

20 In certain embodiments, the polynucleotide comprises or consists of a polynucleotide having at least 75% (*e.g.*, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) identity to the nucleotide sequence set forth in any one of SEQ ID NOs.: 62-81. In certain embodiments, the polynucleotide is codon-optimized for expression in a host cell, such an immune cell (*e.g.*, human T cell).

25 Vectors that contain a polynucleotide as described herein are also provided, as well as compositions that comprise a presently disclosed modified cell, binding protein, polynucleotide, and/or vector.

 Also provided are methods for treating a subject having a disease or disorder associated with WT-1 expression or activity, wherein the methods comprise
30 administering to the subject an effective amount of a modified immune cell, binding protein, polynucleotide, or vector as disclosed herein.

In certain embodiments, the disease or disorder is a hematological malignancy or a solid cancer. For example, the hematological malignancy to be treated may be acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloid leukemia (AML, including refractory and relapsed AML, and including acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelocytic leukemia, acute myelomonocytic leukemia (*e.g.*, with or without eosinophilia), acute monocytic leukemia, acute erythroid leukemia, and acute megakaryoblastic leukemia), chronic myelogenous leukemia (CML), chronic myelocytic leukemia, chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM, including refractory and relapsed MM). Exemplary solid cancer to be treated may be biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, breast carcinoma, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, glioblastoma, melanoma, diffuse peritoneal mesothelioma, malignant pleural mesothelioma, glioma, astrocytoma, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, hepatocellular carcinoma, lung cancer, non small-cell lung cancer, malignant melanoma, osteosarcoma, ovarian cancer (*e.g.*, epithelial ovarian cancer, ovarian carcinoma), fallopian tube cancer, endometrial carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, osteogenic sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, uterine carcinosarcoma, or uterine cancer.

These and other aspects and embodiments of the present disclosure will be further understood upon reference to the following detailed description and attached drawings. 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 are incorporated herein by reference in their entirety, as if each was incorporated individually. Aspects and embodiments of the disclosure can be modified, if necessary,

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows identification of WT-1-specific T cell lines. Briefly, WT-1-specific polyclonal T cell lines (CD8+) obtained from 4 healthy donors were cultured with peptide-pulsed autologous dendritic cells, stained with CD8-independent (CD8i) WT-1 peptide/HLA-A tetramer, and sorted for high tetramer staining cells. Twenty-four (24) WT-1-specific clonotypes were identified. TCR repertoire analysis was performed by sequencing. The fold-enrichment of each clonotype within the sorted versus unsorted populations (y-axis) and the CD3 surface expression (x-axis) of each clonotype are shown.

Figures 2A-2C show that exemplary WT-1-specific binding proteins are expressed in transduced host cells. Ten (10) WT-1 specific TCRs from the 24 clonotypes shown in Figure 1 were used to transduce TCR α /TCR β -deficient J76 Jurkat T cells. WT-1:HLA-A tetramer binding and CD3 expression of the transduced cells (shown in light grey) was compared to cells transduced with a reference WT-1-specific TCR (shown in dark grey) having an α chain according to SEQ ID NO.:82 and a β chain according to SEQ ID NO.:83 and described by Schmitt *et al.* (*Nat. Biotechnol.* 35:1188, 2017).

Figures 3A and 3B show that binding proteins of the present disclosure are functional in transduced host cells. (A) Jurkat cells expressing *Nur77*-dtTomato reporter (reporting antigen-specific signaling in human T cells; *see* Ahsouri and Weiss, *J Immunol* 198(2):657-668 (2017)) were transduced with WT-1-specific TCR and incubated for 24h with APCs loaded with WT-1 peptide at the indicated concentrations. (B) *Nur77* activation peptide EC₅₀ values of the indicated TCRs.

Figures 4A-4C show further functional characterization of exemplary binding proteins of the present disclosure. (A) Peptide/MHC tetramer binding by CD8+ T cells that were transduced to express a WT-1-specific TCR as indicated. (B) IFN γ production (as determined by flow cytometry) by expanded antigen-specific CD8+ T cells in a 4 hour co-culture with T2 target cells pulsed with titrated concentrations of

peptide. Shown is the percentage of IFN- γ + T cells. (C) IFN γ peptide EC₅₀ for each TCR was determined by fitting the percentage of IFN γ -producing cells to dose-response curves by linear regression.

Figures 5A and 5B show lysis of tumor cells by T cells expressing an exemplary binding protein of the present disclosure (TCR DL6 or DL10), as compared to lysis by T cells expressing a reference TCR (α chain according to SEQ ID NO.:82; β chain according to SEQ ID NO.:83). (A) Lysis of HLA-A2-transduced tumor cell line MDA-MB-468 cells. (B) Lysis of naturally HLA-A2+ tumor line Panc-1 cells. Both (A) and (B) show results from 4h assays in which target cells were loaded with ⁵¹Cr and TCR-transduced T cell-mediated killing was calculated by measuring Cr-release in response to decreasing doses of effector T cells relative to tumor cell targets (E:T; x-axis).

Figure 6 shows IFN- γ production by exemplary TCR DL10 of the present disclosure, as compared to the reference TCR (α chain according to SEQ ID NO.:82; β chain according to SEQ ID NO.:83), in the presence of T2 target cells pulsed with peptide.

Figure 7 shows reduced growth of Panc-1 tumor cells in the presence of T cells expressing exemplary TCR DL10 of the present disclosure, as compared to tumor cells alone or in the presence of T cells expressing the reference TCR (α chain according to SEQ ID NO.:82; β chain according to SEQ ID NO.:83). Cell proliferation/survival was monitored over 7 days in an IncuCyte assay. Error bars indicate standard error of the mean.

DETAILED DESCRIPTION

In certain aspects, the present disclosure provides binding protein (*e.g.*, TCRs, CARs, scTCRs) having specificity for WT-1 peptide antigen associated with a major histocompatibility complex (MHC) (*e.g.*, human leukocyte antigen (HLA)), polynucleotides that encode a binding protein, vectors, modified immune cells that encode and/or express the binding proteins, and related compositions. The presently disclosed WT-1 specific compositions are useful in, for example, adoptive immunotherapy to treat cancer. By way of background, most tumor targets for

T cell-based immunotherapies are self-antigens since tumors arise from previously normal tissue. For example, such tumor-associated antigens (TAAs) may be expressed at high levels in a cancer cell, but may not be expressed or may be minimally expressed in other cells. During T cell development in the thymus, T cells that bind weakly to self-antigens are allowed to survive in the thymus, which can undergo further development to increase specificity against foreign invaders, while T cells that bind strongly to self-antigens are eliminated by the immune system since such cells would mount an undesirable autoimmune response. Hence, T cells are sorted by their relative ability to bind to antigens to prepare the immune system to respond against a foreign invader (*i.e.*, recognition of non-self-antigen) while at the same time preventing an autoimmune response (*i.e.*, recognition of self-antigen). This tolerance mechanism limits naturally occurring T cells that can recognize tumor (self) antigens with high affinity and, therefore, eliminates the T cells that would effectively eliminate tumor cells. Consequently, isolating T cells having high affinity TCRs specific for tumor antigens is difficult because such cells are essentially eliminated by the immune system.

An advantage of the instant disclosure is to provide a binding protein specific for a WT-1 peptide, wherein the binding protein is capable of binding to a WT-1 (SEQ ID NO.:94):HLA (*e.g.*, HLA-A*0201) complex. In certain embodiments, the binding protein is capable of binding to the WT-1 independent or in the absence of CD8, is capable of more efficiently associating with a CD3 protein as compared to an endogenous TCR, is capable of initiating a signal to a host cell expressing the binding protein to produce a cytokine, or any combination thereof. In certain embodiments, a binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO: 94):HLA complex with an IFN γ production pEC₅₀ of 4.0, 4.5, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, or higher; and/or wherein the binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.: 94):HLA complex with a higher IFN γ production pEC₅₀ as compared to a TCR comprising an α -chain amino acid sequence of SEQ ID NO: 82 and a β -chain amino acid sequence of SEQ ID NO: 83.

The compositions and methods described herein will in certain embodiments have therapeutic utility for the treatment of diseases and conditions associated with WT-1 expression (*e.g.*, detectable WT-1 expression at a level that is greater in

magnitude, in a statistically significant manner, than the level of WT-1 expression that is detectable in a normal or disease-free cell). Such diseases include various diseases and disorders, such as hematological malignancies and solid cancers. Non-limiting examples of these and related uses are described herein and include *in vitro*, *ex vivo* and
5 *in vivo* stimulation of WT-1 antigen-specific T-cell responses, such as by the use of recombinant immune cells, such as T cells, encoding and/or expressing a heterologous binding protein specific for a WT-1 peptide (*e.g.*, RMFPNAPYL, SEQ ID NO.: 94).

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.

10 Additional definitions are set forth throughout this disclosure.

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 one hundredth of an integer), unless otherwise indicated. Also, any number range recited
15 herein relating to any physical feature, such as polymer subunits, size or thickness, are to be understood to include any integer within the recited range, unless otherwise indicated. As used herein, the term "about" means $\pm 20\%$ of the indicated range, value, or structure, unless otherwise indicated. 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
20 the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include," "have" and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

In addition, it should be understood that the individual compounds, or groups of
25 compounds, derived from the various combinations of the structures and substituents described herein, are disclosed by the present application to the same extent as if each compound or group of compounds was set forth individually. Thus, selection of particular structures or particular substituents is within the scope of the present disclosure.

30 The term "consisting essentially of" is not equivalent to "comprising", and refers to the specified materials or steps, or to those that do not materially affect the basic

characteristics of a claimed invention. For example, a protein domain, region, or module (*e.g.*, a binding domain, hinge region, linker module) 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).

As used herein, the term "host" refers to a cell (*e.g.*, T cell) or microorganism targeted for genetic modification with a heterologous or exogenous nucleic acid molecule to produce a polypeptide of interest (*e.g.*, enhanced affinity anti-WT-1 TCR). In certain embodiments, a host cell may optionally already possess or be modified to include other genetic modifications that confer desired properties related or unrelated to biosynthesis of the heterologous or exogenous protein (*e.g.*, inclusion of a detectable marker; deleted, altered or truncated endogenous TCR; increased co-stimulatory factor expression). In certain embodiments, a host cell is a human hematopoietic progenitor cell transduced with heterologous or exogenous nucleic acid molecule encoding a TCR α chain specific for a WT-1 antigen peptide.

As used herein, an "immune system cell" means any cell of the immune system that originate from a hematopoietic stem cell in the bone marrow, which gives rise to two major lineages, a myeloid progenitor cell (which give rise to myeloid cells such as monocytes, macrophages, dendritic cells, megakaryocytes and granulocytes) and a lymphoid progenitor cell (which give 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 natural killer 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 interacts

with a TCR on the surface of a T cell. In certain embodiments, an immune system cell is a human immune system cell.

As used herein, a "hematopoietic progenitor cell" is a cell that can be derived from hematopoietic stem cells or fetal tissue and is capable of further differentiation
5 into mature cells types (*e.g.*, immune system cells). Exemplary hematopoietic progenitor cells include those with a CD24^{Lo} Lin⁻ CD117⁺ phenotype or those found in the thymus (referred to as progenitor thymocytes).

"Major histocompatibility complex" (MHC) refers to glycoproteins that deliver peptide antigens to a cell surface. MHC class I molecules are heterodimers having a
10 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
15 molecules deliver peptides originating in the vesicular system to the cell surface, where they are recognized by CD4⁺ T cells. Human MHC are referred to as human leukocyte antigen (HLA).

A "T cell" is an immune system cell that matures in the thymus and produces T cell receptors (TCRs). T cells can be naïve (not exposed to antigen; increased
20 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
25 expression of CD54RA as compared to naïve T cells), stem cell memory 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}). Effector T cells (T_E) refers to a antigen-experienced CD8⁺ cytotoxic T lymphocytes that has decreased expression of CD62L, CCR7, CD28, and are positive for granzyme and
30 perforin as compared to T_{CM}.

"T cell receptor" (TCR) refers to an immunoglobulin superfamily member (having a variable binding domain, a constant domain, a 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 other immunoglobulins (*e.g., antibodies*), the extracellular portion of TCR chains (*e.g., α -chain, 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 other 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.* 87:9138, 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, rabbit or other mammal.

The term "variable region" or "variable domain" refers to the domain of an immunoglobulin superfamily binding protein (*e.g., a TCR α -chain or β -chain (or γ chain and δ chain for $\gamma\delta$ TCRs)*) that is involved in binding of the immunoglobulin superfamily binding protein (*e.g., TCR*) to antigen. In the case of a TCR, the variable domains of the α chain and β chain (V α and V β , respectively) 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 separate DNA segments, the variable gene segment, the diversity gene segment, and the joining gene segment (V-D-J). A single V α or V β domain, or a functional fragment or portion thereof, may be sufficient to confer antigen-binding specificity. Furthermore, 5 TCRs that bind a particular antigen may be isolated using a V α or V β domain from a TCR that binds the antigen to screen a library of complementary V α or V β domains, respectively.

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 10 amino acids within immunoglobulin (e.g., TCR) variable regions, which confer antigen specificity and/or binding affinity and are separated from one another by framework regions. In general, there are three CDRs in each TCR α -chain variable region (α CDR1, α CDR2, α CDR3) and three CDRs in each TCR β -chain variable region (β CDR1, β CDR2, β CDR3). In TCRs, CDR3 is thought to be the main CDR 15 responsible for recognizing processed antigen. Typically, CDR1 and CDR2 mainly interact with the MHC.

CDR1 and CDR2 are encoded within the variable gene segment of a TCR variable region-coding sequence, whereas CDR3 is encoded by the region spanning the variable and joining segments for V α , or the region spanning variable, diversity, and 20 joining segments for V β . Thus, if the identity of the variable gene segment of a V α or V β is known, the sequences of their corresponding CDR1 and CDR2 can be deduced. Compared with CDR1 and CDR2, CDR3 is typically significantly more diverse because of the addition and loss of nucleotides during the recombination process.

TCR variable domain sequences can be aligned to a numbering scheme (e.g., 25 IMGT, Kabat, Chothia, Enhanced Chothia, Contact, and Aho), allowing equivalent residue positions to be annotated and for different molecules to be compared using, for example, ANARCI software tool (2016, Bioinformatics 15:298-300). A numbering scheme provides a standardized delineation of framework regions and CDRs in the TCR variable domains. Accordingly, it will be understood that a CDR from a TCR V α 30 or V β region or domain may have a particular sequence according to a particular numbering scheme, and may have a slightly shorter, longer, or shifted (e.g., partially

overlapping) sequence according to a different numbering scheme. In certain presently disclosed embodiments, CDRs are determined using the IMGT numbering scheme; for example, using IMGT V-Quest (imgt.org/IMGTindex/V-QUEST.php).

"CD3" is known in the art as a multi-protein complex of six chains (*see*, Abbas and Lichtman, 2003; Janeway *et al.*, p172 and 178, 1999). In mammals, the complex comprises 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 highly 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 T cell receptor 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 wishing to be bound by theory, it is believed that 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.

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.

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).

As used herein, the term "CD8 co-receptor" or "CD8" means the cell surface glycoprotein CD8, which can be expressed by T cells as a homodimer comprising two CD8 α chains, or as a heterodimer comprising an α chain and a β chain. The CD8 co-

receptor is believed to assist in the function of cytotoxic T cells (CD8⁺) and functions through signaling via its cytoplasmic tyrosine phosphorylation pathway (Gao and Jakobsen, *Immunol. Today* 21:630-636, 2000; Cole and Gao, *Cell. Mol. Immunol.* 1:81-88, 2004). In particular, and without wishing to be bound by theory, it is believed that the CD8 co-receptor binds to an MHC-I protein complex expressed on the surface of an antigen-expressing cell, and that this binding in the context of TCR:antigen-MHC binding initiates or assists in a T cell signaling pathway that produces an immune response (e.g., transcription and expression of cytokines, calcium secretion, cytolytic activity, or the like) against the antigen-expressing cell. In humans, eight (8) different CD8 beta chain isoforms are known ("M1"- "M8"; see UniProtKB identifiers P10966-1, 2, 3, 4, 6, 7, 8, and 9); of these, isoforms 1, 2, 4, and 5 are thought to associate with the cell membrane in nature, while isoforms 3, 6, 7, and 8 are believed to associate with extracellular regions or be secreted. Also in humans, three CD8 alpha chain isoforms are known (see UniProtKB identifiers P01732-1, 2, and 3).

"CD4" refers to an immunoglobulin co-receptor glycoprotein that assists the TCR in communicating with antigen-presenting cells (see, Campbell & Reece, *Biology* 909 (Benjamin Cummings, Sixth Ed., 2002); UniProtKB P01730). CD4 is found on the surface of immune cells such as T helper cells, monocytes, macrophages, and dendritic cells, and typically includes four immunoglobulin domains (D1 (comprising an Ig-like V-type domain), D2, D3, and D4 (respectively comprising Ig-like C2-type domains 1, 2, and 3)) that are expressed at the cell surface. During antigen presentation, CD4 is recruited, along with the TCR complex, to bind to different regions of the MHCII molecule (CD4 binds MHCII β 2, while the TCR complex binds MHCII α 1/ β 1). Without wishing to be bound by theory, it is believed that close proximity to the TCR complex allows CD4-associated kinase molecules to phosphorylate the immunoreceptor tyrosine activation motifs (ITAMs) present on the cytoplasmic domains of CD3. This activity is thought to amplify the signal generated by the activated TCR in order to produce various types of T helper cells.

A "binding domain" (also referred to as a "binding region" or "binding moiety"), as used herein, refers to a peptide, oligopeptide, polypeptide, or protein that possesses the ability to specifically and non-covalently associate, unite, or combine with a target

(*e.g.*, WT-1 or WT-1 peptide:MHC complex). A binding domain includes any naturally occurring, synthetic, semi-synthetic, or recombinantly produced binding partner for a biological molecule, a molecular complex (*i.e.*, complex comprising two or more biological molecules), or other target of interest. Exemplary binding domains include
 5 single chain immunoglobulin variable regions (*e.g.*, scTCR, scFv), receptor ectodomains, ligands (*e.g.*, cytokines, chemokines), or synthetic polypeptides selected for the specific ability to bind to a biological molecule, a molecular complex or other target of interest.

As used herein, "specifically binds" or "specific for" refers to an association or
 10 union of a binding protein (*e.g.*, TCR receptor) or a binding domain (or fusion protein thereof) 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 $10^5 M^{-1}$ (which equals the ratio of the on-rate [k_{on}] to the off-rate [k_{off}] for this association reaction), while not significantly associating or uniting with any other
 15 molecules or components in a sample. Binding proteins or binding domains (or fusion proteins thereof) may be classified as "high affinity" binding proteins or binding domains (or fusion proteins thereof) or as "low affinity" binding proteins or binding domains (or fusion proteins thereof). "High affinity" binding proteins or binding domains refer to those binding proteins or binding domains having a K_a of at least 10^7
 20 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 proteins or binding domains refer to those binding proteins or binding domains having a K_a of up to $10^7 M^{-1}$, up to $10^6 M^{-1}$, 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 $10^{-13} M$).

25 In certain embodiments, a receptor or binding domain may have "enhanced affinity," which refers to a selected or engineered receptors or binding domain with stronger binding to a target antigen than a wild type (or parent) binding domain. 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, due to a K_d
 30 (dissociation constant) for the target antigen that is less than that of the wild type binding domain, due to an off-rate (k_{off}) for the target antigen that is less than that of the

wild type binding domain, or a combination thereof. In certain embodiments, enhanced affinity TCRs may be codon optimized to enhance expression in a particular host cell, such as T cells (Scholten *et al.*, *Clin. Immunol.* 119:135, 2006).

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, activation, and proliferation. For example, T cells that biologically (immunologically) respond *in vitro* to a low antigen dose by producing cytokines, being cytotoxic, expressing an activation marker, 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.

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 can 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.

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 – $\log_{10}(\text{EC}_{50})$ (see, e.g., Figure 4(C)). For example, if the EC_{50} equals 1 μM (10^{-6} M), the $\log_{10}(\text{EC}_{50})$ value is –6. Another value used is pEC_{50} , which is defined as the negative logarithm of the EC_{50} ($-\log_{10}(\text{EC}_{50})$). In the above example, the EC_{50} equaling 1 μM has a pEC_{50} value of 6. In certain embodiments, the functional

5 avidity of a binding protein of this disclosure will be a measure of its ability to promote $\text{IFN}\gamma$ production by immune cells (e.g., T cells, NK-T cells, NK cells), which can be measured using assays known in the art and/or described herein. "High functional avidity" TCRs or binding domains thereof refer to those TCRs or binding domains thereof having a EC_{50} of at least 10^{-4} M, at least about 10^{-5} M, or at least about 10^{-6} M.

10 In some embodiments, a pEC_{50} is used to describe the amount of peptide antigen required for 50% of immune cells to express the activation marker *Nur77*.

A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, as well as determining binding domain or fusion protein affinities, such as Western blot, ELISA, analytical

15 ultracentrifugation, spectroscopy and surface plasmon resonance (Biacore®) analysis (see, e.g., Scatchard *et al.*, *Ann. N.Y. Acad. Sci.* 51:660, 1949; Wilson, *Science* 295:2103, 2002; Wolff *et al.*, *Cancer Res.* 53:2560, 1993; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent), and peptide:MHC tetramer staining.

The term "WT-1-specific immunoglobulin superfamily binding protein" or

20 "WT-1-specific binding protein" refers in certain embodiments to a protein or polypeptide of the present disclosure that specifically binds to WT-1 or a peptide thereof, optionally in complex with a HLA molecule. The term "WT-1 binding domain" or "WT-1 binding fragment" refer to a domain or portion of a WT-1-specific binding protein responsible for the specific WT-1 binding. A WT-1-specific binding

25 domain alone (*i.e.*, without any other portion of a WT-1-specific binding protein) can be soluble. Exemplary WT-1-specific binding domains include those from a WT-1-specific TCR, such as can be found in a TCR or a scTCR (e.g., single chain $\alpha\beta$ TCR proteins such as $V\alpha$ -L- $V\beta$, $V\beta$ -L- $V\alpha$, $V\alpha$ -C α -L- $V\alpha$, or $V\alpha$ -L- $V\beta$ -C β , wherein $V\alpha$ and $V\beta$ are TCR α and β variable domains respectively, C α and C β are TCR α and β constant

30 domains, respectively, and L is a linker) and scFv fragments as described herein, which can be derived from an anti-WT-1 TCR or antibody.

"WT-1 antigen" or "WT-1 peptide antigen" refer to a naturally or synthetically produced portion of a WT-1 protein ranging in length from about 7 amino acids to about 15 amino acids, which can form a complex with a MHC (*e.g.*, HLA) molecule and such a complex can bind with a TCR specific for a WT-1 peptide:MHC (*e.g.*, HLA) complex. Principles of antigen processing by antigen presenting cells (APC) (such as dendritic cells, macrophages, lymphocytes or other cell types), and of antigen presentation by APC to T cells, including major histocompatibility complex (MHC)-restricted presentation between immunocompatible (*e.g.*, sharing at least one allelic form of an MHC gene that is relevant for antigen presentation) APC and T cells, are well established (*see, e.g.*, Murphy, Janeway's Immunobiology (8th Ed.) 2011 Garland Science, NY; chapters 6, 9 and 16). For example, processed antigen peptides originating in the cytosol (*e.g.*, tumor antigen, intracellular pathogen) are generally from about 7 amino acids to about 11 amino acids in length and will associate with class I MHC molecules, whereas peptides processed in the vesicular system (*e.g.*, bacterial, viral) will vary in length from about 10 amino acids to about 25 amino acids and associate with class II MHC molecules. Since WT-1 is an internal host protein, WT-1 antigen peptides will be presented in the context of class I MHC. In particular embodiments, a WT-1 peptide is RMFPNAPYL (SEQ ID NO.:94), which is known to associate with human class I HLA (and, more specifically, associates with allele HLA-A*201).

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 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, for instance, or about four to about 20 amino acids or about eight to about 15 amino acids or about 15 to about 25 amino acids.

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 in a protein. A polynucleotide encoding a fusion protein may be constructed using PCR, recombinantly engineered, or the like, or such fusion proteins

can be synthesized. 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.*, an immune cell, such as, for example, a T cell) locates to the cell surface, where the fusion protein is anchored to the cell membrane (*e.g.*, via a transmembrane component or domain) and comprises an extracellular component (*e.g.*, capable of associating with a MHC molecule) and an intracellular component (*e.g.*, containing a signaling domain, effector domain, co-stimulatory domain or portions or combinations thereof).

"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, for example, 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).

An "altered domain" or "altered protein" refers to a motif, region, domain, peptide, polypeptide, or protein with a 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, TCR β constant domain) of at least 85% (*e.g.*, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.5%).

As used herein, "nucleic acid" or "nucleic acid molecule" or "polynucleotide" refers to any of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), oligonucleotides, fragments generated, for example, by the polymerase chain reaction (PCR) or by *in vitro* translation, and fragments generated by any of ligation, scission, endonuclease action, or exonuclease action. In certain embodiments, the polynucleotides of the present disclosure are produced by PCR. Polynucleotides may be composed of monomers that are naturally occurring nucleotides (such as deoxyribonucleotides and ribonucleotides), analogs of naturally occurring nucleotides (*e.g.*, α -enantiomeric forms of naturally-occurring nucleotides), or a combination of both. Modified nucleotides can have modifications in or replacement of sugar moieties, or pyrimidine or purine base moieties. Nucleic acid monomers can be linked by

phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. Polynucleotides can be either single stranded or double stranded.

5 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 materials in the natural system, is isolated. Such nucleic acid could be part of a vector
10 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 following the coding region "leader and trailer" as well as intervening sequences
15 (introns) between individual coding segments (exons).

Any host cell, binding protein, polynucleotide, or vector of the present disclosure can be "isolated".

As used herein, the terms "recombinant", "engineered", and "modified" refer to a cell, microorganism, nucleic acid molecule, or vector that has been modified by
20 introduction of an exogenous nucleic acid molecule, or refers to a cell or microorganism that has been altered 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
25 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.

30 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.

A "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well known in the art (*see, e.g.*, WO 97/09433 at page 10; Lehninger, Biochemistry, 2nd Edition; Worth Publishers, Inc. NY, NY, pp.71-77, 1975; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA, p. 8, 1990). A variety of criteria known to persons skilled in the art indicate whether an amino acid that is substituted at a particular position in a peptide or polypeptide is conservative (or similar). For example, a similar amino acid or a conservative amino acid substitution is one in which an amino acid residue is replaced with an amino acid residue having a similar side chain. Similar amino acids may be included in the following categories: amino acids with basic side chains (*e.g.*, lysine, arginine, histidine); amino acids with acidic side chains (*e.g.*, aspartic acid, glutamic acid); amino acids with uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine, histidine); amino acids with nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan); amino acids with beta-branched side chains (*e.g.*, threonine, valine, isoleucine), and amino acids with aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan). Proline, which is considered more difficult to classify, shares properties with amino acids that have aliphatic side chains (*e.g.*, leucine, valine, isoleucine, and alanine). In certain circumstances, substitution of glutamine for glutamic acid or asparagine for aspartic acid may be considered a similar substitution in that glutamine and asparagine are amide derivatives of glutamic acid and aspartic acid, respectively. 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 GENWORKS, Align, the BLAST algorithm, or other algorithms described herein and practiced in the art).

In certain embodiments, proteins (*e.g.*, binding proteins) according to the present disclosure comprise a variant sequence as compared to a reference sequence (*e.g.*, a variant TCR CDR as compared to a reference TCR CDR disclosed herein). 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. Variants of polynucleotides and polypeptides of this disclosure are also contemplated. Variant nucleic acid molecules or polypeptides are at least 70%, 75%, 80%, 85%, 90%, and are preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 99.9% identical to a defined or reference polynucleotide or polypeptide (respectively) as described herein, or that, for a polynucleotide, hybridize to a reference 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 binding protein or a binding domain thereof having a functionality described herein, such as specifically binding a target molecule. 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.

Variants can also refer to fragments (*e.g.*, a portion resulting from truncation, cleavage, or the like) of a defined or reference sequence, and fragments can be of any length shorter than the length of the defined or reference sequence. As used herein, a "functional portion" or "functional fragment" refers to a polypeptide or polynucleotide that comprises only a domain, 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). 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
5 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
10 domain, costimulatory molecule, or costimulatory domain.

In certain embodiments, a variant binding protein or a portion (e.g., binding domain) or fragment thereof can comprise one or more amino acid substitutions relative to a parent or reference binding protein or domain, wherein the one or more amino acid substitutions remove, change, or attenuate, a potential undesired feature or
15 characteristic, if present in the parent or reference binding domain or protein; e.g., an amino acid sequence that is potentially immunogenic, or an amino acid sequence that may provide an undesired glycosylation site, an undesired deamidation site, an undesired oxidation site, an undesired isomerization site, or a reduction in thermodynamic stability, or that may result in mis-pairing or mis-folding in a binding
20 protein (e.g., unpaired cysteine residues in close proximity). Amino acid sequences, patterns, and motifs that may provide for an undesired feature or characteristic are known (see, e.g., Seeliger *et al.*, *mAbs* 7(3): 505–515 (2015)).

In certain embodiments, an amino acid substitution comprises a substitution to remove a somatic mutation, such as, for example, a reversion to a germline-encoded
25 amino acid. For example, in certain embodiments, a variant of a reference CDR amino acid sequence, or of a TCR variable domain sequence or TCR constant region sequence, comprises a substitution to remove or attenuate a potential undesired feature or characteristic. It will be understood that such variants are selected so as not to compromise, or substantially compromise, a desired function (e.g., binding specificity
30 and/or affinity for a WT-1 antigen:HLA complex).

The term "construct" refers to any polynucleotide that contains a recombinant nucleic acid. 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. Vectors may be, for example, plasmids, 5 cosmids, viruses, a RNA vector or a linear or circular DNA or RNA molecule that may include chromosomal, non-chromosomal, semi-synthetic or synthetic nucleic acids. Exemplary vectors are those capable of autonomous replication (episomal vector) or expression of nucleic acids to which they are linked (expression vectors).

Viral vectors include retrovirus, adenovirus, parvovirus (*e.g.*, adeno-associated 10 viruses), coronavirus, negative strand RNA viruses such as ortho-myxovirus (*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, herpesvirus (*e.g.*, Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus 15 (*e.g.*, vaccinia, fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus, reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include avian leukosis-sarcoma, mammalian C-type, B-type viruses, D type viruses, HTLV-BLV group, lentivirus, spumavirus (Coffin, J. M., *Retroviridae: The viruses and their replication*, In *Fundamental Virology*, Third 20 Edition, B. N. Fields *et al.*, Eds., Lippincott-Raven Publishers, Philadelphia, 1996).

"Lentiviral vector," as used herein, means 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 (packaging, envelope and 25 transfer) or more plasmids 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 30 integration into the DNA of infected cells.

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 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.

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 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.

The term "expression", as used herein, refers to the process by which a polypeptide is produced based on the nucleic acid sequence of a gene. The process includes both transcription and translation.

The term "introduced" in the context of inserting a nucleic acid sequence into a cell, means "transfection", or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid sequence 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, "heterologous" or "exogenous" nucleic acid molecule, construct or sequence refers to a nucleic acid molecule or portion of a nucleic acid molecule that is not native to a host cell, but may be homologous to a nucleic acid molecule or portion of a nucleic acid molecule from the host cell. The source of the heterologous or

exogenous nucleic acid molecule, construct or sequence may be from a different genus or species. In certain embodiments, a heterologous or exogenous nucleic acid molecule is added (*i.e.*, not endogenous or native) to a host cell or host genome by, for example, conjugation, transformation, transfection, electroporation, or the like, wherein the added
5 molecule may integrate into the host genome or exist as extra-chromosomal genetic material (*e.g.*, as a plasmid or other form of self-replicating vector), and may be present in multiple copies. In addition, "heterologous" refers to a non-native enzyme, protein or other activity encoded by an exogenous nucleic acid molecule introduced into the host cell, even if the host cell encodes a homologous protein or activity. Moreover, a cell
10 comprising a "modification" or a "heterologous" polynucleotide or binding protein includes progeny of that cell, regardless of whether the progeny were themselves transduced, transfected, or otherwise manipulated or changed.

As described herein, more than one heterologous or exogenous nucleic acid molecule can be introduced into a host cell as separate nucleic acid molecules, as a
15 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. For example, as disclosed herein, a host cell can be modified to express two or more heterologous or exogenous nucleic acid molecules encoding desired TCR specific for a WT-1 antigen peptide (*e.g.*, TCR α and TCR β). When two or more exogenous nucleic
20 acid molecules are introduced into a host cell, it is understood that the two more exogenous nucleic acid molecules can be introduced as a single nucleic acid molecule (*e.g.*, on a single vector), on separate vectors, integrated into the host chromosome at a single site or multiple sites. The number of referenced heterologous nucleic acid molecules or protein activities refers to the number of encoding nucleic acid molecules
25 or the number of protein activities, not the number of separate nucleic acid molecules introduced into a host cell. Exogenous nucleic acid molecules (*e.g.*, encoding a binding protein or a CD8 co-receptor of the present disclosure) can also be introduced into the genome of a host cell by gene-editing techniques, *e.g.*, using a CRISPR-Cas system, a meganuclease, or the like.

30 As used herein, the term "endogenous" or "native" refers to a gene, protein, or activity that is normally present in a host cell. Moreover, a gene, protein or activity that

is mutated, overexpressed, shuffled, duplicated or otherwise altered as compared to a parent gene, protein or activity is still considered to be endogenous or native to that particular host cell. For example, an endogenous control sequence from a first gene (*e.g.*, promoter, translational attenuation sequences) may be used to alter or regulate expression of a second native gene or nucleic acid molecule, wherein the expression or regulation of the second native gene or nucleic acid molecule differs from normal expression or regulation in a parent cell.

The term "homologous" or "homolog" refers to a molecule or activity found in or derived from a host cell, species or strain. For example, a heterologous or exogenous nucleic acid molecule may be homologous to a native host cell gene, and may optionally have an altered expression level, a different sequence, an altered activity, or any combination thereof.

"Sequence identity," as used herein, refers to the percentage of amino acid residues in one sequence that are identical with the amino acid residues in another reference polypeptide sequence after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. The percentage sequence identity values can be generated using the NCBI BLAST2.0 software as defined by Altschul *et al.* (1997) "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402, with the parameters set to default values.

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).

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, pre-malignant cells, as well as non-neoplastic or non-malignant hyperproliferative disorders (*e.g.*, adenoma, fibroma, lipoma, leiomyoma, hemangioma, 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.

Binding Proteins Specific for WT-1 Antigen Peptides

In certain embodiments, the instant disclosure provides a binding protein, comprising (a) a T cell receptor (TCR) α chain variable ($V\alpha$) domain comprising a CDR3 amino acid sequence (CDR3 α) according to any one of SEQ ID NOs: 19, 22, 25, or 28, or a variant thereof, and a TCR $V\beta$ domain; (b) a TCR $V\beta$ domain comprising a CDR3 amino acid sequence (CDR3 β) according to any one of SEQ ID NOs: 31, 34, 37, or 40, or a variant thereof, and a TCR $V\alpha$ domain; or (c) a TCR $V\alpha$ domain of (a), and a TCR $V\beta$ domain of (b), wherein the binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.: 94):human leukocyte antigen (HLA) complex wherein, optionally, the HLA comprises HLA-A*0201.

In certain embodiments, the binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.: 94):HLA complex with an IFN γ production pEC₅₀ of 4.0, 4.5, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, or higher.

In certain embodiments, the $V\beta$ domain of (a), (b), or (c) comprises or consists of an amino acid sequence having at least 90% (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%) identity to the amino acid sequence set forth in any one of SEQ ID NOs: 5-8 or 13-16, and/or the $V\alpha$ domain of (a), (b), or (c) comprises or consists of any amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs: 1-4 or 9-12.

In certain embodiments, the CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 31 and the CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 19. In certain embodiments, the CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34, and the CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 22. In certain embodiments, the CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 37, and the CDR3 α comprises or consists of the amino acid

sequence set forth in SEQ ID NO: 25. In certain embodiments, the CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 40, and the CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 28.

In certain embodiments, the binding protein comprises a V α domain comprising
5 a CDR1 α , a CDR2 α , and a CDR3 α , and a V β domain comprising a CDR1 β , a CDR2 β , and a CDR3 β .

In certain embodiments, the V β domain comprises the CDR1 β amino acid sequence of any one of SEQ ID NOs: 29, 32, 35, or 38, or a variant thereof, and/or the CDR2 β amino acid sequence of any one of SEQ ID NOs: 30, 33, 36, or 39, or a variant
10 thereof. In certain embodiments, the V α domain comprises the CDR1 α amino acid sequence of any one of SEQ ID NOs: 17, 20, 23, or 26, or a variant thereof, and/or the CDR2 α amino acid sequence of any one of SEQ ID NOs: 18, 21, 24, or 27, or a variant thereof.

In particular embodiments, the TCR V α and V β domains comprise, respectively,
15 CDR1 α , CDR2 α , CDR3 α , and CDR1 β , CDR2 β , CDR3 β and amino acid sequences of: (i) SEQ ID NOs: 26-28 and 38-40, respectively; (ii) SEQ ID NOs: 23, 27, 28, and 38-40, respectively; (iii) SEQ ID NOs: 17-19 and 29-31, respectively; (iv) SEQ ID NOs: 20-22 and 32-34, respectively; or (v) SEQ ID NOs: 23-25 and 35-37, respectively.

In any of the presently disclosed embodiments, the binding protein binds to a
20 WT-1 peptide:HLA-A*201 complex. In any of the presently disclosed embodiments, the binding protein is capable of binding to a WT-1 peptide:HLA complex on a cell surface independent of CD8 or in the absence of CD8.

In certain embodiments, a binding protein as described herein includes variant polypeptide species that have one or more amino acid substitutions, insertions, or
25 deletions in the amino acid sequence relative to the sequences of SEQ ID NOS: 1-61 as presented herein. In certain embodiments, a substitution is or comprises a conservative substitution. Conservative substitutions of amino acids are well known and may occur naturally or may be introduced when the binding protein is recombinantly produced. Amino acid substitutions, deletions, and additions may be introduced into a protein
30 using mutagenesis methods known in the art (*see, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual*, 3d ed., Cold Spring Harbor Laboratory Press, NY,

2001). Oligonucleotide-directed site-specific (or segment specific) mutagenesis procedures may be employed to provide an altered polynucleotide that has particular codons altered according to the substitution, deletion, or insertion desired.

Alternatively, random or saturation mutagenesis techniques, such as alanine scanning
5 mutagenesis, error prone polymerase chain reaction mutagenesis, and oligonucleotide-directed mutagenesis may be used to prepare immunogen polypeptide variants (*see, e.g., Sambrook et al., supra*).

In certain embodiments, species (or variants) of a particular binding protein specific for WT-1 may include a protein that has at least 85%, 86%, 87%, 88%, 89%,
10 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity to any of the exemplary amino acid sequences disclosed herein (*e.g., SEQ ID NOs.:1-61*).

In certain embodiments, a binding protein is provided that comprises a V β domain having at least 90% (*e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,*
15 *99%, or 100%*) sequence identity to an amino acid sequence as set forth in any one of SEQ ID NOs.: 1-4 or 9-12, and/or a V α domain having at least 90% sequence identity to an amino acid sequence as set forth in any one of SEQ ID NOs.: 5-8 or 13-16, wherein the binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO:94):HLA complex. In further embodiments, any one or more of the β or α CDR
20 amino acid sequences as provided herein can be present in the V β domain and/or the V α domain, respectively.

In certain embodiments, the binding protein comprises a V β domain comprising or consisting of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs.: 5-8 or 13-16, and/or a V α domain comprising or consisting of an amino acid
25 sequence having at least 90% identity to any one of SEQ ID NOs.: 1-4 or 9-12, provided that(a) at least three or four of the CDRs have no mutations, (b) the CDRs that do have mutations have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof, and (c) the binding protein retains its ability to bind to a RMFPNAPYL (SEQ ID NO:94):HLA complex, optionally with an
30 IFN γ production pEC₅₀ of 4.0, 4.5, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, or higher.

In certain embodiments, a binding protein comprises a TCR variable domain comprising an amino acid sequence encoded by a human TCR V, D, and/or J allele. By way of background, during lymphocyte development, V α exons are assembled from different variable and joining gene segments (V-J), and V β exons are assembled from different variable, diversity, and joining gene segments (V-D-J). The TCR α chromosomal locus has 70-80 variable gene segments and 61 joining gene segments. The TCR β chromosomal locus has 52 variable gene segments, and two separate clusters of each containing a single diversity gene segment, together with six or seven joining gene segments. Functional V α and V β gene exons are generated by the recombination of a variable gene segment with a joining gene segment for V α , and a variable gene segment with a diversity gene segment and a joining gene segment for V β . Nucleotide and amino acid sequences according to TCR gene segments of various alleles are known in the art and can be found on the ImMunoGeneTics website; for example, at imgt.org/IMGTrepertoire/LocusGenes/listIG_TR/TR/human/Hu_TRgroup.html.

It will be understood that while a polynucleotide encoding a binding protein can comprise a same nucleotide sequence according to a TCR gene segment as disclosed herein, any nucleotide sequence that encodes the amino acid sequence of the gene segment may be used within the context of the present disclosure.

In certain embodiments, the binding protein comprises a V β domain comprising (i) an amino acid sequence according to a TRBJ02-03 gene segment; and/or (ii) an amino acid sequence according to a TRBV06-05 gene segment, an amino acid sequence according to a TRBV07-09 gene segment, or an amino acid sequence according to a TRBV20-01 gene segment. In certain embodiments, the binding protein comprises a V α domain comprising (i) an amino acid sequence according to a TRAJ43 gene segment; and/or (ii) an amino acid sequence according to a TRAV20-02 gene segment; an amino acid sequence encoded by a TRAV38DV08 gene segment; or an amino acid sequence according to a TRAV38-01 gene segment. In related embodiments, the V β domain comprises an amino acid sequence according to a TRBJ02-03 gene segment and the V α domain comprises an amino acid sequence according to a TRAJ43 gene segment; the V β domain comprises an amino acid sequence according to TRBV06-05 gene segment and the V α domain comprises an amino acid sequence according to a

TRAV20-02 gene segment; the V β domain comprises an amino acid sequence according to TRBV07-09 gene segment and the V α domain comprises an amino acid sequence according to a TRAV38DV08 gene segment; or the V β domain comprises an amino acid sequence according to a TRBV20-01 gene segment and the V α domain
 5 comprises an amino acid sequence according to a TRAV38-01 gene segment.

In the aforementioned embodiments, the amino acid sequence according to a TRA or TRB gene segment is 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 amino acids in length, and including any length of amino acids between these exemplary
 10 lengths (*e.g.*, 11, 12, 13, 14 amino acids, or the like).

In certain embodiments, a binding protein is provided that is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):HLA complex, wherein the binding protein comprises a TCR V α domain and a TCR V β domain. In certain embodiments, the V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.: 1 or
 15 9 and the V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.: 5 or 13. In certain embodiments, the V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.: 2 or 10 and the V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.: 6 or 14. In certain embodiments, the V α domain comprises or consists of the amino acid sequence set
 20 forth in SEQ ID NO.: 3 or 11 and the V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.: 7 or 15. In certain embodiments, the V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.: 4 or 12 and the V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.: 8 or 16.

In any of the presently disclosed embodiments, a binding protein can comprise an α -chain constant domain (C α) or a fragment thereof and/or a β -chain constant domain (C β) or a fragment or portion thereof. In certain embodiments, the C α comprises or consists of an amino acid sequence having at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) sequence identity to any one of
 30 SEQ ID NOs.:41-44. In certain embodiments, the C β comprises or consists of an amino acid sequence having at least 90% sequence identity to SEQ ID NO:45.

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)*).

5 In certain embodiments, the binding protein is a T cell receptor (TCR), a chimeric antigen receptor or an antigen-binding fragment of a TCR, any of which can be chimeric, humanized or human. In further embodiments, an antigen-binding fragment of the TCR comprises a single chain TCR (scTCR) or a chimeric antigen receptor (CAR).

10 "Chimeric antigen receptor" (CAR) refers to a fusion protein that is engineered to contain two or more naturally occurring amino 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 can include an extracellular portion comprising an antigen-binding domain (e.g., obtained
15 or derived from an immunoglobulin or immunoglobulin-like molecule, such as a TCR binding domain derived or obtained from a TCR specific for a cancer antigen, a scFv derived or obtained from an antibody, or an antigen-binding domain derived or obtained from a killer immunoreceptor from an NK cell) linked to a transmembrane domain and one or more intracellular signaling domains (optionally containing co-stimulatory
20 domain(s)) (*see, e.g., Sadelain et al., Cancer Discov., 3(4):388 (2013); see also Harris and Kranz, Trends Pharmacol. Sci., 37(3):220 (2016), Stone et al., Cancer Immunol. Immunother., 63(11):1163 (2014), and Walseng et al., Scientific Reports 7:10713 (2017), which CAR constructs and methods of making the same are incorporated by reference herein*). CARs of the present disclosure that specifically bind to a WT-1
25 antigen (e.g., in the context of a peptide:HLA complex) comprise a TCR V α domain and a V β domain, or a functional fragment or portion thereof.

In certain embodiments, a WT-1 specific binding protein is a TCR. In related embodiments, the binding protein (a) comprises a TCR α -chain having at least 90% (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) identity to,
30 comprising, or consisting of the amino acid sequence as set forth in SEQ ID NO: 50 or 46, and a TCR β -chain having at least 90% identity to, comprising, or consisting of the

amino acid sequence set forth in SEQ ID NO: 58 or 54; (b) comprises a TCR α -chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO: 51 or 47, and a TCR β -chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO: 59 or 55;

5 (c) comprises a TCR α -chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO: 52 or 48, and a TCR β -chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO: 60 or 56; or (d) comprises a TCR α -chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID

10 NO:53 or 49, and a TCR β -chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO: 61 or 57.

In certain embodiments, the binding protein comprises a fusion protein comprising (i) an extracellular component comprising a binding domain that comprises a T cell receptor (TCR) α chain variable ($V\alpha$) domain and a T cell receptor (TCR) β chain variable ($V\beta$) domain, wherein the $V\alpha$ domain comprises the CDR3 amino acid sequence (CDR3 α) according to any one of SEQ ID NOs: 19, 22, 25, or 28, or a variant thereof, and the TCR $V\beta$ domain comprises the CDR3 amino acid sequence (CDR3 β) according to any one of SEQ ID NOs: 31, 34, 37, or 40, or a variant thereof; (ii) an intracellular component; and (iii) a transmembrane component disposed between the

15 extracellular component and the intracellular component. In certain embodiments, the fusion protein is a CAR.

20

In certain embodiments, the binding protein comprises a fusion protein comprising (i) an extracellular component comprising a binding domain that specifically binds to a RMFPNAPYL (SEQ ID NO:94) peptide:HLA complex, wherein

25 the binding domain comprises a T cell receptor (TCR) α chain variable ($V\alpha$) domain and a T cell receptor (TCR) β chain variable ($V\beta$) domain, wherein the $V\alpha$ domain comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs: 1, 2, 3, 4, 9, 10, 11, or 12, or a variant thereof, and the $V\beta$ domain comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs: 5, 6, 7, 8, 13, 14, 15, or 16, or a variant thereof, optionally provided

30 that: (a) at least three or four of the CDRs have no mutations, (b) the CDRs that do have

mutations have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof; and (c) the fusion protein retains its ability to bind to a RMFPNAPYL (SEQ ID NO:94):HLA complex; (ii) an intracellular component; and (iii) a transmembrane component disposed between the extracellular
5 component and the intracellular component.

It will be understood that any of the presently disclosed binding domains, including the exemplary CDR and variable domain sequences and variants thereof, can be comprised in a fusion protein according to the present disclosure.

In any of the embodiments described herein, an encoded polypeptide of this
10 disclosure can comprise a "signal peptide" (also known as a leader sequence, leader peptide, or transit peptide). Signal peptides target newly synthesized polypeptides to their appropriate location inside or outside the cell. Amino acid sequences of exemplary proteins or polypeptides of the present disclosure that comprise a signal peptide are provided in, for example, SEQ ID NOs.:1-8, 46-49, and 54-57. A signal
15 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. In any of the herein disclosed embodiments, a binding protein or fusion protein comprises, or is, a mature protein, or is or comprises a
20 pre-protein. Amino acid sequences of exemplary mature proteins or polypeptides of the present disclosure are provided in SEQ ID NOs.:9-16, 50-53, and 58-61.

In certain embodiments, a binding protein of the present disclosure comprises one or more junction amino acids.

In certain embodiments, a binding protein comprises a linker. In certain
25 embodiments, a linker is comprised of about two to about 35 amino acids, for instance, or about four to about 20 amino acids or about eight to about 15 amino acids or about 15 to about 25 amino acids. Exemplary linkers include glycine-serine linkers (*e.g.*, SEQ ID NOs.:95 and 96).

In certain embodiments, there is provided a composition comprising a
30 WT-specific binding protein according to any one of the aforementioned embodiments and a pharmaceutically acceptable carrier, diluent, or excipient.

In certain embodiments, a WT-1-specific binding protein is provided in soluble form (see, e.g., Walseng et al., PLoS One doi:10.1371/journal.pone.0119559 (2015)), and can optionally be conjugated to a cytotoxic agent and/or a detectable agent. 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.

In certain embodiments, nucleic acid molecules encoding a binding protein specific for WT-1 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; Warren et al., 2011 *Genome Res.* 21:790) and may be employed in the course of practicing the embodiments according to the present disclosure. 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.* 17:742, 2009; Till et al., *Blood* 112:2261, 2008; Wang et al., *Hum. Gene Ther.* 18:712, 2007; Kuball et al., *Blood* 109:2331, 2007; US 2011/0243972; US2011/0189141; 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 enhanced affinity TCRs specific for WT-1 peptide antigen RMFPNAPYL (SEQ ID NO.:94) complexed with an HLA.

In certain embodiments, WT-1-specific binding proteins or domains as
5 described herein, may 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
10 embodiments, the binding protein is capable of promoting an antigen-specific T cell response against human WT-1 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
15 response is directed against a WT-1-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.
20 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
25 Francisco, CA (1979); Green and Reed, *Science* 281:1309 (1998) and references cited therein).

"MHC-peptide tetramer staining" refers to an assay used to detect antigen-specific T cells, which features a tetramer of MHC molecules, each comprising an identical peptide having an amino acid sequence that is cognate (*e.g.*, identical or
30 related to) at least one antigen (*e.g.*, WT-1), wherein the complex is capable of binding T cell receptors specific for the cognate antigen. Each of the MHC molecules may be

tagged with a biotin molecule. Biotinylated MHC/peptides are 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.

Polynucleotides and Vectors

Also provided are polynucleotides (*e.g.*, isolated polynucleotides) that encode a binding protein or a fragment or portion thereof according to the present disclosure. Isolated or recombinant polynucleotides encoding binding proteins specific for WT-1 as described herein may be produced and prepared according to various methods and techniques of the molecular biology or polypeptide purification arts.

In certain embodiments, a polynucleotide is codon optimized for efficient expression in a target host cell. Codon optimization can be performed using known techniques and tools, *e.g.*, using the GenScript® OptimumGene™ tool; GeneArt® GeneOptimizer™ (Sigma), or the like. Codon-optimized sequences include sequences that are partially codon-optimized (*i.e.*, at least one codon is optimized for expression in the host cell) and those that are fully codon-optimized. Codon optimization for expression in

certain immune host cells is disclosed in, for example, Scholten *et al.*, *Clin. Immunol.* 119:135, 2006.

As one of skill in the art will recognize, a polynucleotide may refer to a single- or a double-stranded DNA, cDNA or RNA in any form, and may include a positive and
5 a negative strand of the nucleic acid which complement each other, including anti-sense DNA, cDNA and RNA. Also included are siRNA, microRNA, RNA—DNA hybrids, ribozymes, and other various naturally occurring or synthetic forms of DNA or RNA.

In some embodiments, a polynucleotide encoding a binding protein of the present disclosure comprises a polynucleotide having at least 75% identity (*e.g.*, 75%,
10 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) to the polynucleotide sequence set forth in any one of SEQ ID NOs:62-81.

In certain embodiments, the polynucleotide encoding a binding protein comprises (i) a TCR β chain-encoding polynucleotide having at least 75% identity to the
15 polynucleotide sequence set forth in SEQ ID NO: 74, and a TCR α chain-encoding polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO: 66; (ii) TCR β chain-encoding a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO: 75, and a TCR α chain-encoding polynucleotide having at least 75% identity to the polynucleotide sequence
20 set forth in SEQ ID NO: 67; (iii) a TCR β chain-encoding polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO: 76, and a TCR α chain-encoding polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO: 68; or (iv) a TCR β chain-encoding polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO: 77,
25 and a TCR α chain-encoding polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO: 69.

In some embodiments, a single polynucleotide encodes a binding protein as described herein, or, alternatively, the binding protein may be encoded by more than one polynucleotide. In other words, components or portions of a binding protein may
30 be encoded by two or more polynucleotides, which may be contained on a single nucleic acid molecule or may be contained on two or more nucleic acid molecules.

In certain embodiments, a polynucleotide encoding two or more components or portions of a binding protein of the present disclosure comprises 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 disclosure, such as a TCR (*e.g.*, alpha- and beta-chains) or CAR, 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 polypeptide with the parts separated by a cleavable or removable segment. For instance, self-cleaving peptides useful for expression of separable polypeptides encoded by a single polynucleotide or vector are known in the art and include, for example, a Porcine teschovirus-1 2A (P2A-1) peptide, a Porcine teschovirus-2 2A (P2A-2) peptide, a Thossea asigna virus 2A (T2A) peptide, an Equine rhinitis A virus (ERAV) 2A (E2A) peptide, and a Foot-and-Mouth disease virus 2A (F2A) peptide. Exemplary nucleotide and amino acid sequences of self-cleaving peptides are provided in SEQ ID NOs: 84-93. In certain embodiments, the polynucleotide encoding a self-cleaving peptide comprises or consists of a polynucleotide sequence having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs: 89-93. In certain embodiments, the encoded self-cleaving peptide comprises or consists of an amino acid sequence having at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the amino acid sequence set forth in any one of SEQ ID NOs: 84-88.

It will be understood that various arrangements of polynucleotides encoding two polypeptides of interest separated by a self-cleaving peptide (*e.g.*, β -chain-encoding polynucleotide-self-cleaving peptide- α -chain-encoding polynucleotide; α -chain-encoding polynucleotide-self-cleaving peptide- β -chain-encoding polynucleotide) are contemplated.

Accordingly, in some embodiments, the polynucleotide encoding a binding protein has a structure comprising or having 5'-end to 3'-end of (TCR β domain (*e.g.*,

chain)-encoding polynucleotide)-(self-cleaving peptide-encoding polynucleotide)-(TCR α domain (*e.g.*, chain)-encoding polynucleotide).

In certain embodiments, the polynucleotide encoding a binding protein comprises or consists of a polynucleotide sequence having at least 75% (*e.g.*, 75%,
 5 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 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: 78-81. In certain embodiments, the polynucleotide comprises a polynucleotide sequence comprising or consisting of the polynucleotide sequence set forth in any one of SEQ ID NOs: 78-81.

10 In further aspects, there is provided an isolated polynucleotide encoding a binding protein wherein the encoded binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):HLA complex and comprises a TCR V α domain and a TCR V β domain that comprise: (i) the amino acid sequence set forth in any one of SEQ ID NOs: 19, 22, 25, 28, 31, 34, 37, or 40; (ii) the amino acid sequence set forth in
 15 any one of SEQ ID NOs: 17, 20, 23, 26, 29, 32, 35, or 38; (iii) the amino acid sequence set forth in any one of SEQ ID NOs: 18, 21, 24, 27, 30, 33, 36, or 39; (iv) an amino acid sequence comprising, consisting of, or having at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to the amino acid sequence set forth in any one of SEQ ID NOs: 1-4 or 9-12; (v) an amino acid sequence comprising,
 20 consisting of, or having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs: 5-8 or 13-16; (vi) an amino acid sequence comprising, consisting of, or having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs: 46-53; (vii) an amino acid sequence comprising, consisting of, or having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID
 25 NOs: 54-61; or (viii) any combination of (i)-(vii).

In further embodiments, an encoded binding protein is expressed as part of a transgene construct that encodes, and/or a modified immune cell can 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
 30 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 PCT publication no. WO 2018/058002, the polynucleotides, transgene constructs, and accessory components, including the nucleotide and amino acid sequences 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.

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 (Erbix) 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 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).

Other accessory components useful for modified immune 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 immune cells having 25 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 expanded for inclusion in a product of desired purity.

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
30 detection and positive selection of immune cells transduced with a polynucleotide comprising a selection marker. RQR is a selection marker that 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
5 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
10 clinically accepted pharmaceutical antibody, rituximab, that allows for the selective deletion of a transgene expressing engineered T cell (Philip *et al.*, 2014).

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
20 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
25 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
30 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.

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

By way of background, inclusion of CD4⁺ T cells in an immunotherapy cell product can provide antigen-induced IL-2 secretion and augment persistence and function of introduced cytotoxic CD8⁺ T cells (*see, e.g., Kennedy et al., Immunol. Rev.* 222:129 (2008); Nakanishi *et al., Nature* 462(7272):510 (2009)). In certain
10 circumstances, a class I restricted TCR in CD4⁺ T cells may require the transfer of a CD8 co-receptor to enhance sensitivity of the TCR to class I HLA peptide complexes. CD4 co-receptors differ in structure to CD8 and have been shown to be ineffective substitutes for CD8 co-receptors (*see, e.g., Stone & Kranz, Front. Immunol.* 4:244 (2013); *see also Cole et al., Immunology* 137(2):139 (2012). Thus, another accessory
15 protein for use in the compositions and methods of this disclosure comprises a CD8 co-receptor or component thereof.

Engineered immune cells comprising a heterologous polynucleotide encoding a binding protein of the present disclosure may, in certain embodiments, further comprise a heterologous polynucleotide encoding a CD8 co-receptor protein, or a beta-chain or
20 alpha-chain component thereof.

An encoded CD8 co-receptor includes, in some embodiments, an α -chain or a fragment or variant thereof. An amino acid sequence of the human CD8 co-receptor α -chain precursor is known and is provided at, for example, UniProtKB –P30433 (*see also* UniProtKB – P31783; -P10732; and -P10731).

25 An encoded CD8 co-receptor includes, in some embodiments, a β -chain or a fragment or variant thereof. An amino acid sequence of the human CD8 co-receptor β -chain precursor is known and is provided at, for example, UniProtKB –P10966 (*see also* UniProtKB – Q9UQ56; -E9PD41; Q8TD28; and -P30434; and -P05541).

Without wishing to be bound by theory, it is believed that distance from the host cell
30 surface is important for RQR polypeptides to function as selection markers/safety switches (Philip *et al.*, 2010 (*supra*)). In some embodiments, the encoded RQR

polypeptide is contained in a β -chain, an α -chain, or both, or a fragment or variant of either or both, of the encoded CD8 co-receptor. In specific embodiments, a modified immune cell comprises a heterologous polynucleotide encoding iCasp9 and a heterologous polynucleotide encoding a recombinant CD8 co-receptor protein that
5 comprises a β -chain containing a RQR polypeptide and further comprises a CD8 α -chain.

An isolated polynucleotide of this disclosure may further comprise a polynucleotide encoding a safety switch protein, a selection marker, a CD8 co-receptor beta chain, or a CD8 co-receptor alpha chain as disclosed herein, or may comprise a
10 polynucleotide encoding any combination thereof.

In certain embodiments, a modified immune cell of the present disclosure further comprises: (i) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain; (ii) a
15 heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; or (iii) the polynucleotide of (i) and the polynucleotide of (ii), wherein, optionally, the host cell comprises a CD4+ T cell, a CD8+ T cell, or both.

In further embodiments, the modified immune cell comprises: (a) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain; (b) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain, wherein,
25 optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; and (c) a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of (a) and the polynucleotide of (b).

In some embodiments, a polynucleotide encoding a binding protein further comprises a polynucleotide that encodes a selection marker.

30 Standard techniques may be used for recombinant DNA, peptide and oligonucleotide synthesis, immunoassays and tissue culture and transformation (*e.g.*,

electroporation, 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);
- 5 *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*
- 10 *Cell Protocols* (Methods in Molecular Medicine) (Christopher A. Klug, and Craig T. Jordan Eds., 2001); *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).

Certain embodiments include a polynucleotide contained in a vector. One of

15 skill in the art can readily ascertain suitable vectors for use with certain embodiments disclosed herein. A typical vector may comprise a polynucleotide capable of transporting another nucleic acid to which it has been linked, or which is capable of replication in a host organism. Some examples of vectors include plasmids, viral vectors, cosmids, and others. Some vectors may be capable of autonomous replication

20 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. According to

25 related embodiments, it is further understood that, if one or more agents (*e.g.*, polynucleotides encoding binding proteins specific for WT-1, or variants 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 the same agent) may be introduced to a cell or cell population or administered to a subject.

30 In certain embodiments, polynucleotides of the present disclosure may be operatively linked to 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. Expression control sequences may include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation
5 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 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
10 interest.

In certain embodiments, the vector comprises a plasmid vector or a viral vector (*e.g.*, a vector selected from lentiviral vector or a γ -retroviral vector). Viral vectors include retrovirus, adenovirus, parvovirus (*e.g.*, adeno-associated viruses), coronavirus, negative strand RNA viruses such as ortho-myxovirus (*e.g.*, influenza virus),
15 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, herpesvirus (*e.g.*, Herpes Simplex virus types 1 and 2, Epstein-Barr virus, cytomegalovirus), and poxvirus (*e.g.*, vaccinia, fowlpox and canarypox). Other viruses include Norwalk virus, togavirus, flavivirus,
20 reoviruses, papovavirus, hepadnavirus, and hepatitis virus, for example. Examples of retroviruses include avian leukosis-sarcoma, mammalian C-type, B-type viruses, D type viruses, HTLV-BLV 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).

"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
25 reticuloendotheliosis viruses. "Lentiviral vector," as used herein, means HIV-based lentiviral vectors for gene delivery, which can be integrative or non-integrative, have
30

relatively large packaging capacity, and can transduce a range of different cell types. Lentiviral vectors are usually generated following transient transfection of three (packaging, envelope and transfer) or more plasmids into producer cells. Like HIV, lentiviral vectors enter the target cell through the interaction of viral surface

5 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.

In certain embodiments, the viral vector can be a gammaretrovirus, e.g.,
10 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
15 vectors and packaging cells for transducing mammalian host cells with viral particles 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 Verhoeven et al., Methods Mol.
20 Biol. 506:97, 2009. Retroviral and lentiviral vector constructs and expression systems 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
25 HSV (Krisky et al., Gene Ther. 5:1517, 1998).

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
30 Harbor Lab), or plasmid vectors (such as Sleeping Beauty or other transposon vectors).

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
5 internal ribosome entry sites (IRES), furin cleavage sites, viral 2A peptide, or any combination thereof.

In certain embodiments, a vector is capable of delivering the polynucleotide or transgene 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
10 polynucleotide or transgene 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 stem cell memory T cell, a $\gamma\delta$ T cell, a natural killer cell, a dendritic cell, or any combination thereof. In further embodiments, a vector is capable of delivering a transgene construct to a naïve T cell, a central memory T cell, an effector memory T cell, or any
15 combination thereof. In some embodiments, a vector that encodes a polynucleotide or transgene construct of the present disclosure may further comprise a polynucleotide that encodes a nuclease that can be used to perform a chromosomal 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 polynucleotide or transgene or portion thereof to
20 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 a polynucleotide or transgene construct of this disclosure.

Host Cells

25 Also provided herein are host cells that comprise a heterologous polynucleotide of the present disclosure (e.g., encoding any presently disclosed binding protein or fusion protein and optionally encoding one or more additional proteins, such as a selection marker a self-cleaving peptide, a CD8 co-receptor polypeptide, or any combination thereof) and/or express any binding protein as disclosed herein. Such a
30 host cell may be generated by, for example, transfection or transduction with a vector of the present disclosure, and/or by gene-editing.

In some embodiments, a host cell comprises an immune system cell, which may be any human immune system cell, such as those exemplary immune system cells described herein. In certain embodiments, the host cell comprises a T cell, a NK cell, a NK-T cell, or any combination thereof. In further embodiments, the T cell comprises a
5 CD8⁺ T cell, a CD4⁺ T cell, or both.

Accordingly, in particular embodiments, a modified immune cell comprises a heterologous polynucleotide that encodes a binding protein according to the present disclosure, wherein the encoded binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):human leukocyte antigen (HLA) complex.

10 In certain embodiments, the encoded binding protein is capable of binding to the SEQ ID NO.:94:HLA complex with an IFN γ production pEC₅₀ of 4.0, 4.5, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, or higher.

In certain embodiments, the peptide:HLA complex comprises a peptide:HLA-A*201 complex.

15 In any of the presently disclosed embodiments, 50% or more of the modified immune cells in a population produce interferon-gamma (IFN- γ) when the population of the modified immune cells is co-cultured for 4 hours with antigen-presenting cells (*e.g.*, T2 cells, Jurkat cells, dendritic cells, expressing an HLA such as HLA-A*0201) pulsed with a peptide comprising or consisting of the amino acid sequence
20 RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻⁴ μ M, or 10⁻⁵ μ M, or 10⁻⁶ μ M in the co-culture. In certain embodiments, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, or more of the modified immune cells in a population are Nur77⁺ (*i.e.*, express Nur77) when the population of the modified immune cells is co-cultured with antigen-presenting cells (also called "target cells" in some contexts) pulsed with a
25 peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of about 10⁻² μ M in the co-culture, wherein the antigen-presenting cells express optionally comprise T2 cells, Jurkat cells, or both, and are optionally HLA-A*0201+.

In some embodiments, 10%, 15%, 20%, or more of the modified immune cells
30 in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of

the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10^{-3} μ M in the co-culture.

In some embodiments, a binding protein has a Nur77 expression pEC50 (SEQ ID NO.:94:HLA, such as HLA-A*0201) of 1.0, 1.1, 1.2, 1.3, 1.4, 1.4, 1.5, 1.6, 1.7, 1.8,
5 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, or more.

In any of the presently disclosed embodiments, a modified immune cell is capable of killing at least 11%, 12%, 13%, 14%, 15%, 20%, 25%, or more of the HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1. In certain
10 embodiments, the modified immune cell is capable of killing 10% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 10:1. In certain embodiments, the modified immune cell is capable of killing about 5% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the
15 MDA-MB-468 cells are present in the sample at a ratio of 1:1.

In any of the presently disclosed embodiments, killing can be measured using a 4hr co-culture assay, optionally using labeled chromium (*e.g.*, ⁵¹Cr), as described herein.

In certain embodiments, a modified immune cell is capable of killing 21%, 22%,
20 23%, 24%, 25%, or more of the Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1. In further embodiments, a modified immune cell is capable of killing 10%, 15%, or more of the Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 10:1. In further embodiments, a modified immune cell is capable of killing 10%, 15% or more
25 of the Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 9:1, 8:1, 7:1, 6:1, or 5:1.

In certain embodiments, a modified immune cell: has increased killing activity against HLA-A*0201⁺ MDA-MB-468 cells and/or against Panc1 cells in a 4-hour co-culture, and/or has improved IFN- γ production in a 4-hour co-culture with peptide-
30 pulsed target cells, and/or further reduces proliferation of Panc-1 cancer cells (*e.g.*, over 60, 75, 100, 125, 150, or more hours, as measured using an IncuCyte assay, at an 8:1

effector:target cell ratio), as compared to a reference immune cell comprising a polynucleotide that encodes a T cell receptor comprising a TCR α chain having the amino acid sequence set forth in SEQ ID NO: 82 and a TCR β chain having the amino acid sequence set forth in SEQ ID NO: 83.

- 5 In some embodiments, a modified immune cell of the present disclosure prevents or substantially prevents growth and/or proliferation of Panc-1 cancer cells for over 50, 100, 150 hours, or more, wherein, optionally, the modified immune cell and the Panc-1 cancer cells are present at a 8:1 effector:target cell ratio.

 In any of the presently disclosed embodiments, killing can be measured using a
10 4hr co-culture assay, optionally using labeled chromium (*e.g.*, ^{51}Cr), as described herein.

 It will be understood that a "reference" immune cell is of a same immune cell type and is phenotypically identical or substantially identical to the subject modified immune cell, with the exception of the encoded binding protein. For example, for a
15 modified CD8 $^{+}$ human T cell, a reference immune cell is a CD8 $^{+}$ human T cell, and can be, for example, from the same source or donor as the modified immune cell.

 In any of the aforementioned embodiments, an encoded binding protein can be a TCR, a CAR, or a scTCR. In any of the aforementioned embodiments, an encoded binding protein is capable of binding to a WT-1 peptide (SEQ ID NO.:94):HLA
20 complex on a cell surface independent of CD8 or in the absence of CD8.

 In any of the presently disclosed embodiments, a binding protein expressed by a modified immune cell can comprise a TCR, a single-chain TCR (scTCR), a chimeric antigen receptor (CAR), or any combination thereof.

 Any suitable immune cell may be modified to include a heterologous
25 polynucleotide encoding a binding protein of this disclosure, including, for example, a T cell, a NK cell, a NK-T cell, a macrophage, a monocyte, or a dendritic cell. In some embodiments, a modified immune cell comprises a CD4 $^{+}$ T cell, a CD8 $^{+}$ T cell, or both. In some embodiments, a modified immune cell comprises a CD4-CD8- double negative T cell or a $\gamma\delta$ T cell. In some embodiments, the T cell is a naïve T cell, a central
30 memory T cell, an effector memory T cell, or any combination thereof. Methods for transfecting/transducing T cells with desired nucleic acids have been described (*e.g.*,

U.S. Patent Application Pub. No. US 2004/0087025) as have adoptive transfer procedures using T cells of desired target-specificity (*e.g.*, Schmitt *et al.*, *Hum. Gen.* 20:1240, 2009; Dossett *et al.*, *Mol. Ther.* 17:742, 2009; Till *et al.*, *Blood* 112:2261, 2008; Wang *et al.*, *Hum. Gene Ther.* 18:712, 2007; Kuball *et al.*, *Blood* 109:2331, 2007; 5 US 2011/0243972; US 2011/0189141; 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.

Any appropriate method can be used to transfect or transduce the cells, for example, the T cells, or to administer the polynucleotides or compositions of the present 10 methods. Known methods for delivering polynucleotides to host cells include, for example, use of cationic polymers, lipid-like molecules, and certain commercial products such as, for example, IN-VIVO-JET PEI. Other methods include *ex vivo* transduction, injection, electroporation, DEAE-dextran, sonication loading, liposome-mediated transfection, receptor-mediated transduction, microprojectile bombardment, 15 transposon-mediated transfer, and the like. Still further methods of transfecting or transducing host cells employ vectors, described in further detail herein. In certain embodiments, a host cell is gene-edited to comprise in its genome a polynucleotide that encodes a binding protein (or other protein or polypeptide) as provided herein.

In any of the foregoing embodiments, a modified immune cell may modified to 20 reduce or eliminate expression (*e.g.*, by a chromosomal gene knockout as described herein) of one or more endogenous genes that encode a polypeptide involved in immune signaling or other related activities, and/or to comprise a heterologous polynucleotide as provided herein. Exemplary gene knockouts include those that encode PD-1, LAG-3, CTLA4, TIM3, an HLA molecule, a TCR molecule, or the like. 25 Without wishing to be bound by theory, certain endogenously expressed immune cell proteins may be recognized as foreign by an allogeneic host receiving the modified immune cells, which may result in elimination of the modified immune cells (*e.g.*, an HLA allele), or may downregulate the immune activity of the modified immune cells (*e.g.*, PD-1, LAG-3, CTLA4), or may interfere with the binding activity of a 30 heterologously expressed binding protein of the present disclosure (*e.g.*, an endogenous TCR of a modified T cell that binds a non-WT-1 antigen and may interferes with the

modified immune cell binding a cell that expresses WT-1 antigen), or may compete for expression with a heterologous binding protein.

Accordingly, decreasing or eliminating expression or activity of such endogenous genes or proteins can improve the activity, tolerance, expression of a binding protein, or persistence of the modified immune cells in an autologous or
5 allogeneic host setting, and may allow for universal administration of the cells (*e.g.*, to any recipient regardless of HLA type). In certain embodiments, a modified immune cell is a donor cell (*e.g.*, allogeneic) or an autologous cell. In certain embodiments, a modified immune cell of this disclosure comprises a chromosomal gene knockout of
10 one or more of a gene that encodes PD-1, LAG-3, CTLA4, TIM3, TIGIT, an HLA component (*e.g.*, a gene that encodes an α 1 macroglobulin, an α 2 macroglobulin, an α 3 macroglobulin, a β 1 microglobulin, or a β 2 microglobulin), or a TCR component (*e.g.*, a gene that encodes a TCR variable region or a TCR constant region) (*see, e.g.*, Torikai *et al.*, *Nature Sci. Rep.* 6:21757 (2016); Torikai *et al.*, *Blood* 119(24):5697 (2012); and
15 Torikai *et al.*, *Blood* 122(8):1341 (2013), the gene-editing techniques, compositions, and adoptive cell therapies of which are herein incorporated by reference in their entirety).

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

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 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.
30 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 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 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.

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 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 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 ZFN molecule.

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

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
5 (canonical) code for DNA recognition of these TALEs has been determined such that 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
10 are also known (*see, e.g.*, U.S. Patent Publication No. US 2011/0301073, which atypical 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
15 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.

As used herein, a "clustered regularly interspaced short palindromic
20 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
25 (*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
30 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
5 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 programed to target a desired sequence (*Xie et al., PLOS One 9:e100448, 2014; U.S. Pat. Appl.*
10 *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.

15 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.

20 Alternative Cas nucleases may be used, including but not limited to, Cas 12, Cas 13, and Cas 14 nucleases, and variants thereof. For example, Cas nucleases disclosed in WO 2019/178427, which is hereby incorporated by reference in its entirety (including the Cas nucleases, CRISPR-Cas systems, and related methods disclosed therein), may be utilized.

25 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: 97), GIY-YIG (SEQ ID NO:98), HNH, His-Cys box and PD-(D/E)XK (SEQ ID
30 NO:99). 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).

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. 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.

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 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.

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

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.

Any of the foregoing gene-editing techniques can be used to introduce a
5 polynucleotide of the present disclosure (*e.g.*, encoding a binding protein and/or a CD8 co-receptor polypeptide) into a host cell genome. In some embodiments, a heterologous polynucleotide is introduced into a locus encoding an endogenous TCR component, HLA component, PD-1, LAG-3, CTLA4, TIM3, or TIGIT, or a safe harbor locus such as *Rosa26*, *AAVS1*, *CCR5*, or the like. In certain embodiments, a heterologous
10 polynucleotide encoding a binding protein and/or encoding a CD8 co-receptor polypeptide is introduced into a host cell *TRAC* locus. In further embodiments, a chromosomal knockout of a host cell *TRBC* locus is introduced.

Accordingly, in certain embodiments, a host cell (*e.g.*, modified immune cell) is provided that comprises, in an endogenous *TRAC* locus, a heterologous polynucleotide
15 encoding a binding protein of the present disclosure, a CD8 co-receptor of the present disclosure, or both. In further embodiments, the host cell comprises a chromosomal knockout of an endogenous *TRBC* locus.

In another aspect, compositions are provided herein that comprise a modified immune cell of the present disclosure (and/or a polynucleotide, vector, or binding
20 protein) and a pharmaceutically acceptable carrier, diluent, or excipient.

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
25 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
30 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

then 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).

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
5 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 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
10 substantially no naïve T cells. In still further embodiments, a unit dose comprises (i) a composition comprising at least about 70% modified CD4+ T cells, combined with (ii) a composition comprising at least about 70% 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 comprises (i) a composition comprising at least about
15 80% modified CD4+ T cells, combined with (ii) a composition comprising at least about 80% 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 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
20 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 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.
25 It will be appreciated that a unit dose of the present disclosure may comprise a modified immune cell as described herein (i.e., expressing a binding protein specific for a WT-1 antigen according to SEQ ID NO:94) and a modified immune cell expressing a binding protein specific for a different antigen (e.g., a different WT-1 antigen (*e.g.*, comprising SEQ ID NO.:100), or an antigen from a different protein or target, such as,
30 for example, BCMA, 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), mesothelin, 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, KRAS peptide bound to HLA, LTβR, LIFRβ, LRP5, MUC1, OSMRβ, TCRα, TCRβ, CD19, CD20, CD22, CD25, CD28, CD30, CD33, CD52, CD56, CD79a, CD79b, CD80, CD81, CD86, CD123, CD171, CD276, B7H4, TLR7, TLR9, PTCH1, HA1-H, Robo1, α-fetoprotein (AFP), Frizzled, OX40, PRAME, BRAF, Core Binding Factor, MR-1 (Crowther *et al.*, *Nature Immunol.* 21:175-185 (2020)), and SSX-2, or the like). For example, a unit dose can comprise modified CD8⁺ T cells expressing a binding protein that specifically binds to a WT-1:HLA complex and modified CD4⁺ T cells (and/or modified CD8⁺ T cells) expressing a binding protein (e.g., a CAR) that specifically binds to a CD19 antigen.

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.

In some embodiments, a composition is provided that comprises (i) a modified immune cell as disclosed herein and further comprises (ii) an immune cell comprising a polynucleotide that encodes a binding protein that comprises a TCR Vα and a TCR Vβ and is capable of specifically binding to a VLDFAPPGA (SEQ ID NO.:100):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201, and wherein, optionally, the modified immune cell of (i) and the immune cell of (ii) are each independently selected from a T cell, a NK cell, and NK-T cell.

In certain further embodiments, the binding protein of the immune cell in (ii) comprises: (a) CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β amino acid sequences of SEQ ID NOs.:101-103 and 105-107, respectively, and wherein, optionally, (1) the Vα has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:104 and/or (2) the Vβ has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:108; (b) CDR1α, CDR2α, CDR3α, CDR1β, CDR2β, and CDR3β amino acid sequences of SEQ

ID NOs.:109-110 and 113-115, respectively, and wherein, optionally, (1) the V α has at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:112 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:116; or (c) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:117-119 and 121-123, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:120 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:124.

Uses

In certain aspects, the instant disclosure is directed to methods for treating a disease or disorder associated with WT-1 expression by administering to human subject in need thereof an effective amount of a binding protein, polynucleotide, vector, cell, or composition according to any the presently disclosed embodiments.

Treat" or "treatment" or "ameliorate" refers to medical management of a disease, disorder, or condition of a subject (*e.g.*, a human or a non-human mammal, such as a primate, horse, cat, dog, goat, mouse, or rat). 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.

A "therapeutically effective amount" or "effective amount", as used herein, refers to an amount of modified immune 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.

As used herein, "statistically significant" refers to a p value of 0.050 or less when calculated using the Students t-test and indicates that it is unlikely that a particular event or result being measured has arisen by chance.

Subjects that can be treated according to methods of the present disclosure are, in general, human and other primate subjects, such as monkeys and apes for veterinary medicine and/or research purposes. Mice or rats can also be used for research purposes. In any of the aforementioned embodiments, the subject may be a human subject. The subject 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 modified immune cell or unit dose 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 target cells (*e.g.*, leukemia cells). 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 particular form of the active ingredient; and the method of administration.

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.

As used herein, the term "adoptive immune therapy" or "adoptive immunotherapy" refers to administration of naturally occurring or genetically engineered, disease- or 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).

A condition associated with WT-1 expression includes any disorder or condition in which underactivity, overactivity or improper activity of a WT-1 cellular or
5 molecular event is present, and typically results from unusually high (with statistical significance) levels of WT-1 expression in afflicted cells (*e.g.*, leukemic cells), relative to normal cells. A subject having such a disorder or condition would benefit from treatment with a composition or method of the presently described embodiments. Some conditions associated with WT-1 overexpression thus may include acute as well as
10 chronic disorders and diseases, such as those pathological conditions that predispose the subject to a particular disorder.

Diseases and disorders associated with WT-1 expression include the presence of dysplastic, cancerous and/or transformed cells in the subject, including, for example neoplastic, tumor, non-contact inhibited or oncogenically transformed cells, or the like
15 (*e.g.*, solid cancers; hematologic cancers including lymphomas and leukemias, such as acute myeloid leukemia, chronic myeloid leukemia, etc.), which are known in the art and for which criteria for diagnosis and classification are established (*e.g.*, Hanahan and Weinberg, 2011 *Cell* 144:646; Hanahan and Weinberg 2000 *Cell* 100:57; Cavallo et al., 2011 *Canc. Immunol. Immunother.* 60:319; Kyrigideis et al., 2010 *J. Carcinog.* 9:3). In
20 certain embodiments, such cancer cells may be cells of acute myeloid leukemia, B-cell lymphoblastic leukemia, T-cell lymphoblastic leukemia, or myeloma, including cancer stem cells that are capable of initiating and serially transplanting any of these types of cancer (see, *e.g.*, see Park et al. 2009 *Molec. Therap.* 17:219). According to certain embodiments, virtually any type of cancer that is characterized by WT-1 overexpression
25 may be treated through the use of compositions and methods disclosed herein, including hematological cancers (*e.g.*, leukemia including acute myeloid leukemia (AML), T or B cell lymphomas, myeloma, and others). 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
30 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. Also contemplated within the presently disclosed embodiments are specific embodiments wherein only one of the above types of disease is included, or where specific conditions may be excluded regardless of whether or not they are characterized by WT-1 overexpression.

- 5 Some examples of conditions associated with WT-1 overexpression include hyperproliferative disorders and proliferative disorders in a subject including tumors, neoplasms, cancer, malignancy, etc. In addition to activated or proliferating cells, a hyperproliferative or proliferative disorder may also include an aberration or dysregulation of cell death processes, whether by necrosis or apoptosis. Such
- 10 aberration of cell death processes may be associated with a variety of conditions, including cancer (including primary, secondary malignancies as well as metastasis), or other conditions.

 In certain embodiments, presently methods are useful for treating a hematological malignancy or a solid cancer. Exemplary hematological malignancies

15 include acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloid leukemia (AML, including refractory and relapsed AML, and including acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelocytic leukemia, acute myelomonocytic leukemia (*e.g.*, with or without eosinophilia), acute monocytic leukemia, acute erythroid leukemia, and acute

20 megakaryoblastic leukemia), chronic myelogenous leukemia (CML), chronic myelocytic leukemia, chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM, including refractory and relapsed MM).

 In certain embodiments, presently disclosed methods are useful for treating a

25 solid cancer, such as biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, breast carcinoma, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, glioblastoma, melanoma, diffuse peritoneal mesothelioma, malignant

30 pleural mesothelioma, glioma, astrocytoma, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, hepatocellular carcinoma, lung cancer, non

small-cell lung cancer, malignant melanoma, osteosarcoma, ovarian cancer (*e.g.*, epithelial ovarian cancer, ovarian carcinoma), fallopian tube cancer, endometrial carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, 5 rhabdomyosarcoma, skin cancer, soft tissue sarcoma, osteogenic sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, uterine carcinosarcoma, or uterine cancer.

Also provided are pharmaceutical compositions that comprise a binding protein, polynucleotide, vector, host cell, and/or modified immune cell (optionally in 10 combination with an immune cell encoding a binding protein that is capable of binding to a SEQ ID NO.:100:HLA complex) for use according to any of the methods described herein. 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 15 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 20 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).

An effective amount of a pharmaceutical composition refers to an amount sufficient, at dosages and for periods of time needed, to achieve the desired clinical 25 results or beneficial treatment, as described herein. An effective amount may be delivered in one or more administrations. If the administration is to a subject already known or confirmed to have a disease or disease-state, the term "therapeutic amount" may be used in reference to treatment, whereas "prophylactically effective amount" may be used to describe administering an effective amount to a subject that is 30 susceptible or at risk of developing a disease or disease-state (*e.g.*, recurrence) as a preventative course.

In certain embodiments, a method comprises administering a modified immune cell as provided herein to a subject who has received, is receiving, or will receive an immune cell comprising a polynucleotide that encodes a binding protein that comprises a TCR V α and a TCR V β and is capable of specifically binding to a VLDFAPPGA (SEQ ID NO.:100):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201, and wherein, optionally, the modified immune cell and the immune cell are each independently selected from a T cell, a NK cell, and NK-T cell. In certain embodiments, the the binding protein that is capable of specifically binding to the VLDFAPPGA (SEQ ID NO.:100):HLA complex comprises: (a) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:101-103 and 105-107, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:104 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:108;

(b) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:109-110 and 113-115, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:112 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:116; or (c) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:117-119 and 121-123, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:120 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:124.

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 in the art and may be performed using samples obtained from a subject before and after treatment.

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 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.

Certain methods of treatment or prevention contemplated herein include administering a host cell (which may be autologous, allogeneic or syngeneic) comprising a desired nucleic acid molecule as described herein that is stably integrated into the chromosome of the cell, or is present as an extrachromosomal nucleic acid molecule. For example, such a cellular composition may be generated *ex vivo* using autologous, allogeneic or syngeneic immune system cells (*e.g.*, T cells, antigen-presenting cells, natural killer cells) in order to administer a desired, WT-1-targeted T-cell composition to a subject as an adoptive immunotherapy. In certain embodiments, the immune 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 dendritic cell, or any combination thereof. In further embodiments, the immune cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

The amount of cells in a composition or unit dose is at least one cell (for example, one modified CD8⁺ T cell subpopulation (*e.g.*, optionally comprising memory and/or naïve CD8⁺ T cells); one modified 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, up to 10¹⁰, or up to 10¹¹ cells. In certain embodiments, the cells are administered in a range from about 10⁴ to about 10¹¹ 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 10⁵ cells/kg. In some embodiments, an administered dose comprises up to about 1 x 10⁶ cells/kg. In some embodiments, an administered dose comprises up to about 3.3 x 10⁶

cells/kg. In some embodiments, an administered dose comprises up to about 1×10^7 cells/kg. In certain embodiments, a modified immune cell is administered to a subject at 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 modified immune cell is
5 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 cells included therein. For example, cells modified to contain a binding protein will comprise a cell population containing at least 30%, 35%, 40%, 45%, 50%, 55%, 60%,
10 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 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
15 a range of time. A clinically relevant number of immune 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 modified immune 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
20 modified immune cells comprised in the unit dose is autologous to the subject.

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 use. In certain embodiments, a unit dose comprises a recombinant host cell as described herein at a dose of about 10^4
25 cells/ m^2 to about 10^{11} cells/ m^2 . The development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, parenteral or intravenous administration or formulation.

In certain embodiments, a pharmaceutical composition that comprises a modified immune cell as disclosed herein further comprises a pharmaceutically
30 acceptable carrier, diluent, 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.

5 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
10 can be utilized. An infusion medium can be supplemented with human serum albumin or other human serum components.

 If the subject composition is administered parenterally, the composition may also include sterile aqueous or oleaginous solution or suspension. Suitable non-toxic parenterally acceptable diluents or solvents include water, Ringer's solution, isotonic
15 salt solution, 1,3-butanediol, ethanol, propylene glycol or polythethylene glycols in mixtures with water. Aqueous solutions or suspensions may further comprise 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
20 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.

25 As used herein, administration of a composition or therapy refers to delivering the same to a subject, regardless of the route or mode of delivery. 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
30 condition, disease or disease state. Co-administration with an adjunctive therapy may include simultaneous and/or sequential delivery of multiple agents in any order and on

any dosing schedule (*e.g.*, modified immune 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).

In certain embodiments, a plurality of doses of a modified immune cell or
5 binding protein as described herein is administered to the subject, which may be administered at intervals between administrations of about two to about four weeks. In further embodiments, a cytokine is administered sequentially, provided that the subject was administered the modified immune cell at least three or four times before cytokine administration. In certain embodiments, the cytokine is administered subcutaneously
10 (*e.g.*, IL-2, IL-15, IL-21). In still further embodiments, the subject being treated is further receiving immunosuppressive therapy, such as calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof.

Treatment or prevention methods of this disclosure may be administered to a
15 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 modified immune cell is receiving or had previously received a hematopoietic cell transplant (HCT; including myeloablative and non-myeloablative HCT). In some
20 embodiments, the modified immune cell may be administered at least two to at least three months after the non-myeloablative hematopoietic cell transplant. 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
25 cell, or a cell from amniotic fluid. Accordingly, in certain embodiments, a modified immune 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
30 TCR component, or both.

In some embodiments, the subject receiving the modified immune cell or binding protein has previously received lymphodepleting chemotherapy. In further embodiments, the lymphodepleting chemotherapy comprises cyclophosphamide, fludarabine, anti-thymocyte globulin, or a combination thereof.

5 In certain embodiments, the subject has previously received therapy for AML, or is at-risk for developing or progressing AML.

 Methods according to this disclosure may further include administering one or more additional agents to treat the disease or disorder in a combination therapy. In some embodiments, the method further comprises administering a modified immune
10 cell or binding protein with (concurrently, simultaneously, or sequentially) an immune checkpoint inhibitor. In some embodiments, the method comprises administering a modified immune cell with an agonist of a stimulatory immune checkpoint agent. In further embodiments, a combination therapy comprises administering a modified immune cell with a secondary therapy, such as a chemotherapeutic agent, a radiation
15 therapy, a surgery, an antibody, or any combination thereof.

 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 those molecules that partially or
20 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, A2aR, immunosuppressive
25 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.

 An immune suppression agent inhibitor (also referred to as an immune checkpoint inhibitor) may be a compound, an antibody, an antibody fragment or fusion polypeptide (e.g., Fc fusion, such as CTLA4-Fc or LAG3-Fc), an antisense molecule, a
30 ribozyme or RNAi molecule, or a low molecular weight organic molecule. In any of the embodiments disclosed herein, a method may comprise a modified immune cell or

binding protein with one or more inhibitor of any one of the following immune suppression components, singly or in any combination.

In certain embodiments, a modified immune cell or binding protein is used in combination with a PD-1 inhibitor, for example a PD-1-specific antibody or binding
5 fragment thereof, such as pidilizumab, nivolumab, pembrolizumab, MEDI0680 (formerly AMP-514), AMP-224, BMS-936558 or any combination thereof. In further embodiments, a modified immune cell or binding protein of the present disclosure is used in combination with a PD-L1 specific antibody or binding fragment thereof, such as BMS-936559, durvalumab (MEDI4736), atezolizumab (RG7446), avelumab
10 (MSB0010718C), MPDL3280A, or any combination thereof.

In certain embodiments, a modified immune cell or binding protein of the present disclosure is used in combination with a LAG3 inhibitor, such as LAG525, IMP321, IMP701, 9H12, BMS-986016, or any combination thereof.

In certain embodiments, a modified immune cell or binding protein is used in
15 combination with an inhibitor of CTLA4. In particular embodiments, a modified immune cell or binding protein is used in combination with a CTLA4 specific antibody or binding fragment thereof, such as ipilimumab, tremelimumab, CTLA4-Ig fusion proteins (e.g., abatacept, belatacept), or any combination thereof.

In certain embodiments, a modified immune cell or binding protein is used in
20 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 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.

25 In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of CD244.

In certain embodiments, a modified immune cell or binding protein is used 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
30 2010/084158.

In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of TIM3.

In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of Gal9.

5 In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of adenosine signaling, such as a decoy adenosine receptor.

In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of A2aR.

10 In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of KIR, such as lirilumab (BMS-986015).

In certain embodiments, a modified immune cell is used in combination with an inhibitor of an inhibitory cytokine (typically, a cytokine other than TGF β) or Treg development or activity.

15 In certain embodiments, a modified immune cell or binding protein 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. 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
20 (1-MT)-tira-pazamine, or any combination thereof.

In certain embodiments, a modified immune cell or binding protein is used in combination with an arginase inhibitor, such as N(omega)-Nitro-L-arginine methyl 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
25 combination thereof.

In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of VISTA, such as CA-170 (Curis, Lexington, Mass.).

In certain embodiments, a modified immune cell is used in combination with an inhibitor of TIGIT such as, for example, COM902 (Compugen, Toronto, Ontario
30 Canada), an inhibitor of CD155, such as, for example, COM701 (Compugen), or both.

In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of PVRIG, PVRL2, or both. Anti-PVRIG antibodies 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.

- 5 In certain embodiments, an modified immune cell or binding protein is used in combination with a LAIR1 inhibitor.

In certain embodiments, a modified immune cell or binding protein is used in combination with an inhibitor of CEACAM-1, CEACAM-3, CEACAM-5, or any combination thereof.

- 10 In certain embodiments, a modified immune cell or binding protein is used in combination with an agent that increases the activity (i.e., is an agonist) of a stimulatory immune checkpoint molecule. For example an modified immune cell or binding protein can be used in combination with a CD137 (4-1BB) agonist (such as, for example, urelumab), a CD134 (OX-40) agonist (such as, for example, MEDI6469,
15 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 example, IL-2) an agonist of GITR (such as, for example, humanized monoclonal antibodies described in PCT Patent Publication No. WO
20 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 modified immune cell or binding protein with one or more agonist of a stimulatory immune checkpoint molecule, including any of the foregoing, singly or in any combination.

- 25 In certain embodiments, a combination therapy comprises a modified immune cell or binding protein and a secondary therapy comprising one or more of: an antibody or antigen binding-fragment thereof that is specific for a cancer antigen expressed by the non-inflamed solid tumor, a radiation treatment, a surgery, a chemotherapeutic agent, a cytokine, RNAi, or any combination thereof.

- 30 In certain embodiments, a combination therapy method comprises administering a modified immune cell or binding protein 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.

- 5 In certain embodiments, a combination therapy method comprises administering a modified immune cell or binding protein 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
- 10 analog, purine analog, and sugar-modified analog), 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
- 15 antagonists and related inhibitors (mercaptopurine, 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),
- 20 DNA damaging agents (actinomycin, amsacrine, anthracyclines, bleomycin, busulfan, camptothecin, carboplatin, chlorambucil, cisplatin, cyclophosphamide, Cytosan, dactinomycin, daunorubicin, doxorubicin, epirubicin, hexamethylmelamineoxaliplatin, iphosphamide, melphalan, merchlorheptamine, mitomycin, mitoxantrone, nitrosourea, plicamycin, procarbazine, taxol, taxotere, temozolamide, teniposide,
- 25 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;
- 30 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.

Cytokines may be used to manipulate host immune response towards anticancer activity. See, e.g., Floros & Tarhini, *Semin. Oncol.* 42(4):539-548, 2015. 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 modified immune cell or binding protein of this disclosure.

In still further aspects, methods are provided for manufacturing a composition of the present disclosure. In certain embodiments, the methods comprise combining (i) an aliquot of a host cell transduced with a vector of the present disclosure with (ii) a pharmaceutically acceptable carrier. In certain embodiments, vectors of the present
5 disclosure are used to transfect/transduce a host cell (e.g., a T cell) for use in adoptive transfer therapy (e.g., targeting a cancer antigen).

In some embodiments, the methods further comprise, prior to the aliquotting, culturing the transduced host cell and selecting the transduced cell as having incorporated (i.e., expressing) the vector. In further embodiments, the methods
10 comprise, following the culturing and selection and prior to the aliquotting, expanding the transduced host cell. In any of the embodiments of the instant methods, the manufactured composition or unit dose may be frozen for later use. Any appropriate host cell can be used for manufacturing a composition or unit dose according to the instant methods, including, for example, a hematopoietic stem cell, a T cell, a primary T
15 cell, a T cell line, a NK cell, or a NK-T cell. In specific embodiments, the methods comprise a host cell which is a CD8⁺ T cell, a CD4⁺ T cell, or both.

The present disclosure also includes the following exemplary embodiments.

Embodiment 1. A modified immune cell, comprising a heterologous polynucleotide that encodes a binding protein, wherein the encoded binding protein
20 comprises: (a) a T cell receptor (TCR) α chain variable ($V\alpha$) domain comprising the CDR3 amino acid sequence (CDR3 α) according to any one of SEQ ID NOs.:28, 19, 22, or 25, or a variant thereof, and a TCR β -chain variable ($V\beta$) domain; or (b) a TCR $V\beta$ domain comprising the CDR3 amino acid sequence (CDR3 β) according to any one of SEQ ID NOs.:40, 31, 34, or 37, or a variant thereof, and a TCR $V\alpha$ domain; or (c) a
25 TCR $V\alpha$ domain of (a), and a TCR $V\beta$ domain of (b), wherein the encoded binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):human leukocyte antigen (HLA), wherein, optionally, the HLA comprises HLA-A*0201.

Embodiment 2. The modified immune cell of embodiment 1, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID
30 NO.:94):HLA complex with an IFN γ production pEC₅₀ of 4.0 or higher, or 4.5 or higher.

Embodiment 3. The modified immune cell of embodiment 1 or 2, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94): HLA complex with an IFN γ production pEC₅₀ of 5.0 or higher.

Embodiment 4. The modified immune cell of any one of embodiments 1-3, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94): HLA complex with an IFN γ production pEC₅₀ of 5.5 or higher.

Embodiment 5. The modified immune cell of any one of embodiments 1-4, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94): HLA complex with an IFN γ production pEC₅₀ of 6.0 or higher.

Embodiment 6. The modified immune cell of any one of embodiments 1-5, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94): HLA complex with an IFN γ production pEC₅₀ of 6.5 or higher.

Embodiment 7. The modified immune cell of any one of embodiments 1-6, wherein the HLA comprises HLA-A*201.

Embodiment 8. The modified immune cell of any one of embodiments 1-7, wherein 50% or more of the modified immune cells in a population produce IFN- γ when the population of the modified immune cells is co-cultured for 4 hours with antigen-presenting cells (*e.g.*, HLA-A*0201+) pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻⁴ μ M, 10⁻⁵ μ M, or 10⁻⁶ μ M in the co-culture, wherein the antigen-presenting cells optionally comprise T2 cells.

Embodiment 9. The modified immune cell of any one of embodiments 1-8, wherein 10% or more of the modified immune cells in a population are Nur77⁺ when the population of the modified immune cells is co-cultured (*e.g.*, for about 24 hours) with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻² μ M in the co-culture, wherein the antigen-presenting cells optionally comprise T2 cells, Jurkat cells, or both.

Embodiment 10. The modified immune cell of embodiment 9, wherein 15% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured (*e.g.*, for about 24 hours) with

antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10^{-2} μ M in the co-culture.

Embodiment 11. The modified immune cell of embodiment 9 or 10,
5 wherein 20% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured (*e.g.*, for about 24 hours) with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10^{-2} μ M in the co-culture.

10 Embodiment 12. The modified immune cell of any one of embodiments 9-11, wherein 40% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured (*e.g.*, for about 24 hours) with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of
15 10^{-2} μ M in the co-culture.

Embodiment 13. The modified immune cell of any one of embodiments 9-12, wherein 50% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured (*e.g.*, for about 24 hours) with antigen-presenting cells pulsed with a peptide comprising or consisting of
20 the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10^{-2} μ M in the co-culture.

Embodiment 14. The modified immune cell of any one of embodiments 1-13, wherein 10% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured (*e.g.*, for about 24
25 hours) with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10^{-3} μ M in the co-culture.

Embodiment 15. The modified immune cell of embodiment 14, wherein
15% or more of the modified immune cells in the population are Nur77⁺ when the
30 population of the modified immune cells is co-cultured (*e.g.*, for about 24 hours) with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino

acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10^{-3} μ M in the co-culture.

Embodiment 16. The modified immune cell of embodiment 14 or 15, wherein 20% or more of the modified immune cells in the population are Nur77⁺ when
5 the population of the modified immune cells is co-cultured (*e.g.*, for about 24 hours) with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO: 94) at a peptide concentration of about 10^{-3} μ M in the co-culture.

Embodiment 17. The modified immune cell of any one of embodiments 1-
10 16, wherein the modified immune cell is capable of killing 11% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

Embodiment 18. The modified immune cell of embodiment 17, wherein the modified immune cell is capable of killing 12% or more of HLA-A*0201⁺ MDA-
15 MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

Embodiment 19. The modified immune cell of embodiment 17 or 18, wherein the modified immune cell is capable of killing 14% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-
20 468 cells are present in the sample at a ratio of 30:1.

Embodiment 20. The modified immune cell of any one of embodiments 17-19, wherein the modified immune cell is capable of killing 15% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

Embodiment 21. The modified immune cell of any one of embodiments 17-20, wherein the modified immune cell is capable of killing 20% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

Embodiment 22. The modified immune cell of any one of embodiments
30 17-21, wherein the modified immune cell is capable of killing 25% or more of HLA-

A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

Embodiment 23. The modified immune cell of any one of embodiments 1-22, wherein the modified immune cell is capable of killing 10% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 10:1.

Embodiment 24. The modified immune cell of any one of embodiments 1-23, wherein the modified immune cell is capable of killing about 5% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 1:1.

Embodiment 25. The modified immune cell of any one of embodiments 1-24, wherein the modified immune cell is capable of killing 21% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

Embodiment 26. The modified immune cell of embodiment 25, wherein the modified immune cell is capable of killing 22% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

Embodiment 27. The modified immune cell of embodiment 25 or 26, wherein the modified immune cell is capable of killing 23% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

Embodiment 28. The modified immune cell of any one of embodiments 25-27, wherein the modified immune cell is capable of killing 24% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

Embodiment 29. The modified immune cell of any one of embodiments 25-28, wherein the modified immune cell is capable of killing 25% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

Embodiment 30. The modified immune cell of any one of embodiments 1-29, wherein the modified immune cell has increased killing activity against HLA-

A*0201⁺ MDA-MB-468 cells and/or against Panc1 cells in a 4-hour co-culture as compared to a reference immune cell comprising a polynucleotide that encodes a T cell receptor comprising a TCR α chain having the amino acid sequence set forth in SEQ ID NO.:82 and a TCR β chain having the amino acid sequence set forth in SEQ ID NO.:83.

5 Embodiment 31. The modified immune cell of any one of embodiments 1-30, wherein the encoded binding protein is capable of binding to a WT-1 peptide:HLA complex on a cell surface independent of CD8 or in the absence of CD8.

 Embodiment 32. The modified immune cell of any one of embodiments 1-31, wherein: (i) the encoded V β domain comprises or consists of an amino acid
10 sequence having at least 90% identity to the amino acid sequence of any one of SEQ ID NOs.:16, 5, 6, 7, 8, 13, 14, or 15; and/or (ii) the encoded V α domain comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence of any one of SEQ ID NOs.:12, 1, 2, 3, 4, 9, 10, or 11.

 Embodiment 33. The modified immune cell of any one of embodiments 1-15 32, wherein the encoded V β domain comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs.: 16, 5, 6, 7, 8, 13, 14, or 15, and/or the encoded V α domain comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs.:12, 1, 2, 3, 4, 9, 10, or 11, provided that: (i)at least three or four of the CDRs have no mutations; (ii) the CDRs that do have
20 mutations have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof; and (iii) the encoded binding protein retains its ability to bind to a RMFPNAPYL (SEQ ID NO.:94):HLA complex, optionally with an interferon-gamma (IFN γ) production pEC50 of 4.0, 4.5, 5.0, 5.1, 5.2., 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, or higher.

25 Embodiment 34. The modified immune cell of any one of embodiments 1-33, wherein: (i) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO.:40, and the encoded CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO.:28; (ii) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 31, and the encoded
30 CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 19; (iii) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in

SEQ ID NO: 34, and the encoded CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 22; or (iv) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 37, and the encoded CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 25.

5 Embodiment 35. The modified immune cell of any one of embodiments 1-34, wherein: (i) the encoded V β domain comprises the CDR1 β amino acid sequence of any one of SEQ ID NOs.:38, 29, 32, or 35 and/or the CDR2 β amino acid sequence of any one of SEQ ID NOs.: 39, 30, 33, or 36; and/or (ii) the encoded V α domain comprises the CDR1 α amino acid sequence of any one of SEQ ID NOs.:26, 17, 20, or
10 23, and/or the CDR2 α amino acid sequence of any one of SEQ ID NOs.:27, 18, 21, or 24.

 Embodiment 36. The modified immune cell of any one of embodiments 1-35, wherein the encoded TCR V α and V β domains comprise CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of: (i) SEQ ID NOs.:26-28 and
15 38-40, respectively; (ii) SEQ ID NOs.:23, 27, 28, and 38-40, respectively; (iii) SEQ ID NOs.: 17-19 and 29-31, respectively; (iv) SEQ ID NOs.: 20-22 and 32-34, respectively; or (v) SEQ ID NOs.: 23-25 and 35-37, respectively.

 Embodiment 37. The modified immune cell of any one of embodiments 1-36, wherein the encoded TCR V β domain comprises: (i) an amino acid sequence
20 according to a TRBJ02-03 gene segment; and/or (ii) an amino acid sequence according to a TRBV06-05 gene segment, an amino acid sequence according to a TRBV07-09 gene segment, or an amino acid sequence according to a TRBV20-01 gene segment.

 Embodiment 38. The modified immune cell of any one of embodiments 1-37, wherein the encoded TCR V α domain comprises: (i) an amino acid sequence
25 according to a TRAJ43 gene segment; and/or (ii) an amino acid sequence according to a TRAV20-02 gene segment; an amino acid sequence according to a TRAV38DV08 gene segment; or an amino acid sequence according to a TRAV38-01 gene segment.

 Embodiment 39. The modified immune cell of embodiment 38, wherein:
30 (i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBJ02-03 gene segment; and (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAJ43 gene segment.

Embodiment 40. The modified immune cell of embodiment 38, wherein:
(i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBV06-05 gene segment; and (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAV20 gene segment.

5 Embodiment 41. The modified immune cell of embodiment 38, wherein:
(i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBV07-09 gene segment; and (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAV38DV08 gene segment.

Embodiment 42. The modified immune cell of embodiment 38, wherein:
10 (i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBV20-01 gene segment; and (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAV38-01 gene segment.

Embodiment 43. The modified immune cell of any one of embodiments 1-42, wherein: (i) the encoded V β domain comprises or consists of the amino acid
15 sequence set forth in SEQ ID NO.:16 or 8, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:12 or 4; (ii) the encoded V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:13 or 5, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:9 or 1; (iii) the encoded V β domain comprises or
20 consists of the amino acid sequence set forth in SEQ ID NO.:14 or 6, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:10 or 2; or (iv) the encoded V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:15 or 7, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:11 or 3.

25 Embodiment 44. The modified immune cell of any one of embodiments 1-43, wherein the encoded binding protein further comprises: (i) a TCR α chain constant domain (C α), or a fragment thereof; and/or (ii) a TCR β chain constant domain (C β), or a fragment thereof.

Embodiment 45. The modified immune cell of embodiment 44, wherein
30 the encoded C α comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs.:41-44.

Embodiment 46. The modified immune cell of embodiment 44 or 45, wherein the encoded C β comprises or consists of an amino acid sequence having at least 90% identity to SEQ ID NO.:45.

Embodiment 47. The modified immune cell of any one of embodiments 5 44–46, wherein the encoded binding protein comprises: (i) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:61 or 57, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:53 or 49; (ii) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid 10 sequence set forth in SEQ ID NO.:58 or 54, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:50 or 46; (iii) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:59 or 55, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid 15 sequence set forth in SEQ ID NO.:51 or 52; or (iv) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:60 or 56, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:52 or 48.

Embodiment 48. The modified immune cell of any one of embodiments 1- 20 47, wherein the encoded binding protein is a TCR, a chimeric antigen receptor (CAR), or a single-chain TCR (scTCR).

Embodiment 49. The modified immune cell of any one of embodiments 1- 48, wherein the immune cell is a human immune system cell.

Embodiment 50. The modified immune cell of any one of embodiments 1- 25 49, wherein the immune cell is a T cell, optionally a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁺ CD8⁺ double negative T cell, or a $\gamma\delta$ T cell, a natural killer cell, a NK-T cell, a dendritic cell, or any combination thereof.

Embodiment 51. The modified immune cell of embodiment 50, wherein the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, a stem 30 cell memory T cell, or any combination thereof.

Embodiment 52. The modified immune cell of any one of embodiments 1-51, wherein the polynucleotide encoding the binding protein is codon-optimized for expression in the immune cell.

Embodiment 53. The modified immune cell of any one of embodiments 1-52, wherein the polynucleotide encoding the binding protein comprises a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs.:62-77.

Embodiment 54. The modified immune cell of embodiment 53, wherein the polynucleotide encoding the binding protein comprises: (i) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:77, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:69; (ii) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:74, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:66; (iii) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:75, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:67; or (iv) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:76, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:68.

Embodiment 55. The modified immune cell of any one of embodiments 1-54, wherein the polynucleotide encoding a binding protein further comprises a polynucleotide encoding a self-cleaving peptide disposed between the V β -encoding polynucleotide and the V α -encoding polynucleotide.

Embodiment 56. The modified immune cell of embodiment 55, wherein the encoded self-cleaving peptide comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:84-88.

Embodiment 57. The modified immune cell of embodiment 55 or 56, wherein the polynucleotide encoding a self-cleaving peptide comprises or consists of a polynucleotide sequence having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs.:89-93.

Embodiment 58. The modified immune cell of any one of embodiments 55-57, wherein the polynucleotide encoding a binding protein has a structure from 5'-end to 3'-end of (TCR β chain-encoding polynucleotide)-(self-cleaving peptide-encoding polynucleotide)-(TCR α chain-encoding polynucleotide).

5 Embodiment 59. The modified immune cell any one of embodiments 55-58, wherein the polynucleotide encoding a binding protein comprises a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs.:78-81.

10 Embodiment 60. The modified immune cell of embodiment 53, wherein the polynucleotide encoding a binding protein comprises a polynucleotide comprising or consisting of the polynucleotide sequence set forth in any one of SEQ ID NOs.:78-81.

15 Embodiment 61. The modified immune cell of any one of embodiments 1-60, further comprising: (i) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain; (ii) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; or (iii) the polynucleotide of (i) and the polynucleotide of (ii),
20 wherein, optionally, the host cell comprises a CD4⁺ T cell.

Embodiment 62. The host cell of embodiment 61, comprising:
(a) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain; (b) the heterologous polynucleotide encoding a
25 polypeptide comprising an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; and
(c) a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of (a) and the polynucleotide of (b).

Embodiment 63. The modified immune cell of any one of embodiments 1-
30 62, wherein the immune cell comprises a chromosomal gene knockout of a PD-1 gene;

a LAG3 gene; a TIM3 gene; a CTLA4 gene; an HLA component gene; a TCR component gene; or any combination thereof.

Embodiment 64. The modified immune cell of embodiment 63, wherein the chromosomal gene knockout comprises a knockout of an HLA component gene
5 selected from an $\alpha 1$ macroglobulin gene; an $\alpha 2$ macroglobulin gene; an $\alpha 3$ macroglobulin gene; a $\beta 1$ microglobulin gene; or a $\beta 2$ microglobulin gene; or any combination thereof.

Embodiment 65. The modified immune cell of embodiment 63 or 64, wherein the chromosomal gene knockout comprises a knockout of a TCR component
10 gene selected from a TCR α variable region gene; a TCR β variable region gene; a TCR constant region gene; or any combination thereof.

Embodiment 66. An isolated polynucleotide encoding a binding protein, wherein the encoded binding protein comprises a TCR $V\alpha$ domain and a TCR $V\beta$ domain and is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):HLA complex,
15 wherein: (i) the encoded $V\alpha$ domain comprises the CDR3 amino acid sequence set forth in any one of SEQ ID NOs.:28, 19, 22, or 25; (ii) the encoded $V\beta$ domain comprises the CDR3 amino acid sequence set forth in any one of SEQ ID NOs.:40, 31, 34, or 37; (iii) the encoded $V\alpha$ domain comprises the CDR1 amino acid sequence set forth in any one of SEQ ID NOs.:26, 17, 20, or 23; (iv) the encoded $V\beta$ domain comprises the CDR1
20 amino acid sequence set forth in any one of SEQ ID NOs.:38, 32, or 35; (v) the encoded $V\alpha$ domain comprises the CDR2 amino acid sequence set forth in any one of SEQ ID NOs.:27, 18, 21, or 24; (vi) the encoded $V\beta$ domain comprises the CDR2 amino acid sequence set forth in any one of SEQ ID NOs.:39, 30, 33, or 36; (vii) the encoded $V\alpha$ domain comprises or consists of an amino acid sequence having at least 90% identity to
25 the amino acid sequence set forth in any one of SEQ ID NOs.:4, 1-3, 12, or 9-11; (viii) the encoded $V\beta$ domain comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:8, 5-7, 16, or 13-15; (ix) the encoded TCR $V\alpha$ is comprised in a TCR α chain, wherein the TCR α chain comprises or consists of an amino acid sequence having at least 90%
30 identity to the amino acid sequence set forth in any one of SEQ ID NOs.:53 and 46-52; (x) the encoded TCR $V\beta$ is comprised in a TCR β chain, wherein the TCR β chain an

amino acid sequence comprising, consisting of, or having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:61 and 54-60; or (xi) any combination of (i)-(x).

Embodiment 67. The isolated polynucleotide of embodiment 66, wherein
5 the polynucleotide is codon-optimized for expression in a host cell, wherein, optionally, the host cell is a human T cell, NK cell, or NK-T cell.

Embodiment 68. The isolated polynucleotide of embodiment 66 or 67, wherein the polynucleotide comprises a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs: 62-81.

10 Embodiment 69. A vector, comprising the polynucleotide of any one of embodiments 66-68.

Embodiment 70. The vector of embodiment 69, wherein the vector is a lentiviral vector or a retroviral vector.

Embodiment 71. A host cell, comprising the polynucleotide of any one of
15 embodiments 66-68 and capable of expressing the encoded binding protein.

Embodiment 72. A composition, comprising: (i) the modified immune cell of any one of embodiments 1-65; (ii) the polynucleotide of any one of embodiments 66-68; (iii) the vector of embodiment 69 or 70; and/or (iv) the host cell of embodiment 71; and a pharmaceutically acceptable carrier, excipient, or diluent.

20 Embodiment 73. The composition of embodiment 72, comprising:
(i) the modified immune cell of any one of embodiments 1-65; and (ii) an immune cell comprising a polynucleotide that encodes a binding protein that comprises a TCR V α and a TCR V β and is capable of specifically binding to a VLDFAPPGA (SEQ ID NO.:100):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201, and
25 wherein, optionally, the modified immune cell of (i) and the immune cell of (ii) are each independently selected from a T cell, a NK cell, and NK-T cell.

Embodiment 74. The composition of embodiment 73, wherein the binding protein of the immune cell in (ii) comprises: (a) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:101-103 and 105-107,
30 respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:104 and/or (2) the V β

has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:108; (b) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:109-110 and 113-115, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:112 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:116; or (c) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:117-119 and 121-123, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:120 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:124.

Embodiment 75. A method for treating a subject having a disease or disorder associated with WT-1 expression, comprising administering to the subject an effective amount of: (i) the modified immune cell of any one of embodiments 1-65; (ii) the polynucleotide of any one of embodiments 66-68; (iii) the vector of embodiment 69 or 70; (iv) the host cell of embodiment 71; and/or (v) the composition of any one of embodiments 72-74.

Embodiment 76. The method of embodiment 75, comprising administering the modified immune cell of any one of embodiments 1-65 to a subject who has received, is receiving, or will receive an immune cell comprising a polynucleotide that encodes a binding protein that comprises a TCR V α and a TCR V β and is capable of specifically binding to a VLDFAPPGA (SEQ ID NO.:100):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201, and wherein, optionally, the modified immune cell and the immune cell are each independently selected from a T cell, a NK cell, and NK-T cell.

Embodiment 77. The method of embodiment 76, wherein the binding protein that is capable of specifically binding to the VLDFAPPGA (SEQ ID NO.:100):HLA complex comprises: (a) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:101-103 and 105-107, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:104 and/or (2) the V β has at least 90%

identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:108; (b) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:109-110 and 113-115, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:112 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:116; or (c) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:117-119 and 121-123, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:120 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:124.

Embodiment 78. The method of any one of embodiments 75-77, wherein the disease or disorder is a hematological malignancy or a solid cancer.

Embodiment 79. The method of embodiment 78, wherein the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloid leukemia (AML), including refractory and relapsed AML, and including acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelocytic leukemia, acute myelomonocytic leukemia (*e.g.*, with or without eosinophilia), acute monocytic leukemia, acute erythroid leukemia, and acute megakaryoblastic leukemia), chronic myelogenous leukemia (CML), chronic myelocytic leukemia, chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM, including refractory and relapsed MM).

Embodiment 80. The method of embodiment 78, wherein the solid cancer is selected from biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, breast carcinoma, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, glioblastoma, melanoma, diffuse peritoneal mesothelioma, malignant pleural mesothelioma, glioma, astrocytoma, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, hepatocellular carcinoma, lung cancer, non

small-cell lung cancer, malignant melanoma, osteosarcoma, ovarian cancer (e.g., epithelial ovarian cancer, ovarian carcinoma), fallopian tube cancer, endometrial carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, 5 rhabdomyosarcoma, skin cancer, soft tissue sarcoma, osteogenic sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, uterine carcinosarcoma, or uterine cancer.

Embodiment 81. The method according to any one of embodiments 75-80, wherein the modified immune cell is modified *ex vivo* to comprise the heterologous 10 polynucleotide.

Embodiment 82. The method according to any one of embodiments 75-81, wherein the modified immune cell is allogeneic, syngeneic, or autologous to the subject.

Embodiment 83. The method according to any one of embodiments 75-82, 15 wherein the modified immune cell is a hematopoietic progenitor cell or a human immune system cell.

Embodiment 84. The method according to embodiment 83, 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 dendritic cell, or any combination thereof.

Embodiment 85. The method according to embodiment 84, wherein the T 20 cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

Embodiment 86. The method according to any one of embodiments 75-85, wherein the method comprises administering a plurality of doses of the modified 25 immune cell to the subject.

Embodiment 87. The method according to embodiment 86, wherein the plurality of doses are administered at intervals between administrations of about two to about four weeks.

Embodiment 88. The method according to any one of embodiments 75-87, 30 wherein the modified immune cell is administered to the subject at a dose of about 10⁴ cells/kg to about 10¹¹ cells/kg.

Embodiment 89. The method according to any one of embodiments 75-88, wherein the method further comprises administering a cytokine to the subject.

Embodiment 90. The method according to embodiment 89, wherein the cytokine is IL-2, IL-15, IL-21 or any combination thereof.

5 Embodiment 91. The method according to embodiment 90, wherein the cytokine is IL-2 and is administered concurrently or sequentially with the modified immune cell.

Embodiment 92. The method according to embodiment 91, wherein the cytokine is administered sequentially, provided that the subject was administered the
10 modified immune cell at least three or four times before cytokine administration.

Embodiment 93. The method according to any one of embodiments 89-92, wherein the cytokine is IL-2 and is administered subcutaneously.

Embodiment 94. The method according to any one of embodiments 75-93, wherein the subject is further receiving immunosuppressive therapy.

15 Embodiment 95. The method according to embodiment 94, wherein the immunosuppressive therapy is selected from calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof.

Embodiment 96. The method according to any one of embodiments 75-95,
20 wherein the subject has received a non-myeloablative or a myeloablative hematopoietic cell transplant.

Embodiment 97. The method according to embodiment 96, wherein the subject is administered the modified immune cell at least three months after the non-myeloablative hematopoietic cell transplant.

25 Embodiment 98. The method according to embodiment 97, wherein the subject is administered the modified immune cell at least two months after the myeloablative hematopoietic cell transplant.

Embodiment 99. The method of any one of embodiments 75-98, wherein the subject has previously received therapy for AML.

30 Embodiment 100. A composition, comprising: (i) the modified immune cell of any one of embodiments 1-65; (ii) the polynucleotide of any one of embodiments 66-

68; (iii) the vector of embodiment 69 or 70; and/or (iv) the host cell of embodiment 71, for use in the treatment of a disease or disorder associated with WT-1 expression.

Embodiment 101. A composition, comprising: (i) the modified immune cell of any one of embodiments 1-65; (ii) the polynucleotide of any one of embodiments 66-
 5 68; (iii) the vector of embodiment 69 or 70; and/or (iv) the host cell of embodiment 71, for use in the manufacture of a medicament for the treatment of a disease or disorder associated with WT-1 expression.

Embodiment 102. The composition for use according to embodiment 100 or 101, wherein the disease or disorder is a hematological malignancy or a solid cancer.

10 Embodiment 103. The composition for use according to embodiment 102, wherein the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloid leukemia (AML, including refractory and relapsed AML, and including acute
 myeloblastic leukemia, acute promyelocytic leukemia, acute myelocytic leukemia, acute
 15 myelomonocytic leukemia (*e.g.*, with or without eosinophilia), acute monocytic leukemia, acute erythroid leukemia, and acute megakaryoblastic leukemia), chronic myelogenous leukemia (CML), chronic myelocytic leukemia, chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM, including refractory and relapsed MM).

20 Embodiment 104. The composition for use according to embodiment 102, wherein the solid cancer is selected from biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, breast carcinoma, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma,
 25 glioblastoma multiforme, glioblastoma, melanoma, diffuse peritoneal mesothelioma, malignant pleural mesothelioma, glioma, astrocytoma, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, hepatocellular carcinoma, lung cancer, non small-cell lung cancer, malignant melanoma, osteosarcoma, ovarian cancer (*e.g.*, epithelial ovarian cancer, ovarian carcinoma), fallopian tube cancer, endometrial
 30 carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma,

rhabdomyosarcoma, skin cancer, soft tissue sarcoma, osteogenic sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, uterine carcinosarcoma, or uterine cancer.

Embodiment 105. A binding protein comprising a T cell receptor (TCR) α chain variable ($V\alpha$) domain and a TCR β chain variable ($V\beta$) domain, wherein: the $V\alpha$ domain comprises: (i) the CDR3 amino acid sequence ($CDR3\alpha$) according to any one of SEQ ID NOs.:28, 19, 22, or 25, or 28, or a variant thereof; (ii) the CDR1 amino acid sequence ($CDR1\alpha$) of any one of SEQ ID NOs.:26, 17, 20, or 23; (iii) the CDR2 amino acid sequence ($CDR2\alpha$) of any one of SEQ ID NOs.: 27, 18, 21, or 24; (iv) an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:1-4 or 9-12; or (v) any combination of (i)-(iv); and the $V\beta$ domain comprises: (vi) the CDR3 amino acid sequence ($CDR3\beta$) according to any one of SEQ ID NOs.: 40, 31, 34, or 37, or a variant thereof; (vii) the CDR1 amino acid sequence ($CDR1\beta$) of any one of SEQ ID NOs.:38, 29, 32, or 35; (viii) the CDR2 amino acid sequence ($CDR2\beta$) of any one of SEQ ID NOs.:39, 30, 33, or 36; (ix) an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:5-8 or 13-16; or (x) any combination of (vi)-(x), and wherein the binding protein of embodiment 105, which is capable of binding to a RMFPNAPYL (SEQ ID NO:94):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201.

Embodiment 106. The binding protein of embodiment 105, further comprising: (1) a TCR α chain constant domain ($C\alpha$), or a fragment thereof; and/or (2) a TCR β chain constant domain ($C\beta$), or a fragment thereof.

Embodiment 107. The binding protein of embodiment 106, wherein the $C\alpha$ comprises or consists of the amino acid sequence of any one of SEQ ID NOs.:41-44.

Embodiment 108. The binding protein of embodiment 106 or 107, wherein the $C\beta$ comprises or consists of the amino acid sequence of SEQ ID NO.:45.

Embodiment 109. An isolated polynucleotide encoding the binding protein of any one of embodiments 105-108.

Embodiment 110. The polynucleotide of embodiment 109, which is codon-optimized for expression in a host cell.

Embodiment 111. A modified immune cell, comprising the polynucleotide of any one of embodiments 109-110, wherein the polynucleotide is heterologous to the immune cell.

5

SEQUENCES

SEQ ID NO:1 (#4 α chain variable domain with leader sequence)

MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSDRGSQS
FFWYRQYSGKSPELIMFIYSNGDKEDGRFTAQLNKASQYVSLLRDSQPSDSAT
10 YLCAVNIGNHDMRFGAGTRLTVKPN

SEQ ID NO:2 (#6 α chain variable domain with leader sequence)

MEKMLECAFIVLWLQLGWLSGEDQVTQSPEALRLQEGESSLNCSYTVSGLRG
LFWYRQDPGKGPEFLFTLYSAGEEKEKERLKATLTKKESFLHITAPKPEDSATY
15 LCAVQTMMDGNQFYFGTGTSLTVIPN

SEQ ID NO:3 (#7 α chain variable domain with leader sequence)

MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTLSCITYDTSESDYY
LFWYKQPPSRQMILVIRQEAYKQQNATENRFSVNFQKAASFSLKISDSQLGDA
20 AMYFCASSPGTYKYIFGTGTRLKVLAN

SEQ ID NO:4 (#10 α chain variable domain with leader sequence)

MTRVSLWAVVSTCLESGMAQTVTQSQPEMSVQEAETVTLSCITYDTSESNY
YLFWYKQPPSRQMILVIRQEAYKQQNATENRFSVNFQKAASFSLKISDSQLGD
25 TAMYFCAFNPWENYQNFVFGPGTRLSVLPY

SEQ ID NO:5 (#4 β chain variable domain with leader sequence)

MGCRLCCAVLCLLGAVPIDTEVTQTPKHLVMGMTNKKSLKCEQHMGHRAM
YWKQKAKKPELMFVYSYEKLSINESVPSRFSPECNSSLNLHLHALQPEDS
30 ALYLCASSQGTSGADTQYFGPGTRLTVLE

SEQ ID NO:6 (#6 β chain variable domain with leader sequence)

MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYM
SWYRQDPGMGLRLIHYSVGAGITDQGEVPNGYNVSRSTEDFPLRLLSAAPSQT
35 SVYFCASSYSLWDLQETQYFGPGTRLLVLE

SEQ ID NO:7 (#7 β chain variable domain with leader sequence)

MGTSLLCWMALCLLGADHADTGVSQDPRHKITKRGQNVTFRCDPISSEHNRLY
WYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERPKGFSFSTLEIQRTEQGDS
40 AMYLCASSFSDGGATDTQYFGPGTRLTVLE

SEQ ID NO:8 (#10 β chain variable domain with leader sequence)

MLLLLLLLGPAGSGLGAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQ
FPKQSLMLMATSNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYI
45 CSARPHSLTDTQYFGPGTRLTVLE

SEQ ID NO:9 (#4 α chain variable domain, leader sequence removed)

QKEVEQNSGPLSVPEGAIASLNCTYSDRGSQSFFWYRQYSGKSPELIMFIYSNG
DKEDGRFTAQLNKASQYVSLLRDSQPSDSATYLCAVNIGNHDMRFGAGTRLT
5 VKPN

SEQ ID NO:10 (#6 α chain variable domain, leader sequence removed)

EDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTLYSAG
EEKEKERLKATLTKKESFLHITAPKPEDSATYLCAVQTM DGNQFYFGTGTS LTV
10 IPN

SEQ ID NO:11 (#7 α chain variable domain, leader sequence removed)

AQTVTQSQPEMSVQEAETVTL SCTYDTSES DY LFWYKQPPSRQMILVIRQEA
YKQQNATENRFSVNFQKAAKSFSLKISDSQLGDAAMYFCASSPGTYKYIFGTGT
15 RLKVLAN

SEQ ID NO:12 (#10 α chain variable domain, leader sequence removed)

AQTVTQSQPEMSVQEAETVTL SCTYDTSES NY LFWYKQPPSRQMILVIRQEA
YKQQNATENRFSVNFQKAAKSFSLKISDSQLGDTAMYFCAFNPWENYGQNFVF
20 GPGTRLSVLPY

SEQ ID NO:13 (#4 β chain variable domain, leader sequence removed)

DTEVTQTPKHLVMGMTNKKSLKCEQHMGHRAMYWYKQKAKKPPELMFVYS
YEKLSINESVPSRFSPEC PNSSLNLHLHALQPEDSALYLCASSQGTSGADTQYF
25 GPGTRLTVLE

SEQ ID NO:14 (#6 β chain variable domain, leader sequence removed)

NAGVTQTPKFQVLKTGQSM TLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVG
AGITDQGEVPNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYSLWDLQETQY
30 FPGPTRLLVLE

SEQ ID NO:15 (#7 β chain variable domain, leader sequence removed)

DTGVSQDPRHKITKRGNVTFRCDPISEHNRLYWYRQTLGQGPEFLTYFQNEA
QLEKSRLLSDRFSAERP KGSFSTLEIQRTEQGDSAMYL CASSFS DGGATDTQYF
35 GPGTRLTVLE

SEQ ID NO:16 (#10 β chain variable domain, leader sequence removed)

GAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQFPKQSLMLMATSNEG
SKATYEQGVEKDKFLINHASLTLSTLTV TSAHPEDSSFYIC SARPHSLTDTQYFG
40 PGTRLTVLE

SEQ ID NO:17 (#4 CDR1 α)

DRGSQS

SEQ ID NO:18 (#4 CDR2 α)

IYSNGD

SEQ ID NO:19 (#4 CDR3 α)

CAVNIGNHDMRF

SEQ ID NO:20 (#6 CDR1 α)
VSGLRG

5 SEQ ID NO:21 (#6 CDR2 α)
LYSAGEE

SEQ ID NO:22 (#6 CDR3 α)
CAVQTM DGNQFYF

10 SEQ ID NO:23 (#7 CDR1 α)
TSESDYY

SEQ ID NO:24 (#7 CDR2 α)
15 QEAYKQQN

SEQ ID NO:25 (#7 CDR3 α)
CASSPGTYKYIF

20 SEQ ID NO:26 (#10 CDR1 α)
TSESNYY

SEQ ID NO:27 (#10 CDR2 α)
QEAYKQQN

25 SEQ ID NO:28 (#10 CDR3 α)
CAFNPWENYGQNFVF

SEQ ID NO:29 (#4 CDR1 β)
30 MGHRA

SEQ ID NO:30 (#4 CDR2 β)
YSYEKL

35 SEQ ID NO:31 (#4 CDR3 β)
CASSQGTSGADTQYF

SEQ ID NO:32 (#6 CDR1 β)
MNHEY

40 SEQ ID NO:33 (#6 CDR2 β)
SVGAGI

SEQ ID NO:34 (#6 CDR3 β)
45 CASSYSLWDLQETQYF

SEQ ID NO:35 (#7 CDR1 β)
SEHNR

SEQ ID NO:36 (#7 CDR2 β)

FQNEAQ

SEQ ID NO:37 (#7 CDR3 β)

5 CASSFSDDGGATDTQYF

SEQ ID NO:38 (#10 CDR1 β)

DFQATT

10 **SEQ ID NO:39 (#10 CDR2 β)**

SNEGSKA

SEQ ID NO:40 (#10 CDR3 β)

CSARPHSLDTQYF

15

SEQ ID NO:41 (#4 TCR α constant domain)IQNPDPAPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRS
MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTN
LNFQNLVIGFRILLKLVAGFNLLMTLRLWSS

20

SEQ ID NO:42 (#6 TCR α constant domain)IQNPDPAPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRS
MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTN
LNFQNLVIGFRILLKLVAGFNLLMTLRLWSS

25

SEQ ID NO:43 (#7 TCR α constant domain)IQNPDPAPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRS
MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTN
LNFQNLVIGFRILLKLVAGFNLLMTLRLWSS

30

SEQ ID NO:44 (#10 TCR α constant domain)IQNPDPAPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDMRS
MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTN
LNFQNLVIGFRILLKLVAGFNLLMTLRLWSS

35

SEQ ID NO:45 (TCR β constant domain)DLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSWVNGKEVHS
GVCTDPQPLKEQPALNDSRYCLSSRLRVSAFTWQNPRNHFRCQVQFYGLSEND
EWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYA
40 VLVSALVLMAMVKRKDSRG**SEQ ID NO:46 (#4 α chain with leader sequence)**MKSLRVLLVILWLQLSWVWSQQKEVEQNSGPLSVPEGAIASLNCTYSRGRGSQS
FFWYRQYSGKSPELIMFIYSNGDKEDGRFTAQLNKASQYVSLIRDSQPSDSAT
45 YLCAVNIGNHDMRFGAGTRLTVKPNIQNPDPAPVYQLRDSKSSDKSVCLFTDFD
SQTNVVSQSKDSDVYITDKCVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSII
PEDTFFPSPESSCDVKLVEKSFETDTNLFQNLVIGFRILLKLVAGFNLLMTLRL
WSS

SEQ ID NO:47 (#6 α chain with leader sequence)

MEKMLECAFIVLWLQLGWLSGEDQVTQSPEALRLQEGESSLNCSYTVSGLRG
 LFWYRQDPGKGPEFLFTLYSAGEEKEKERLKATLTKKESFLHITAPKPEDSATY
 LCAVQTM DGNQFYFGTGTSLTVIPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDS
 5 QTNVSQSKDSDVYITDKCVLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIP
 EDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLK VAGFNLLMTLRL
 WSS

SEQ ID NO:48 (#7 α chain with leader sequence)

10 MACPGFLWALVISTCLEFSMAQTVTQSQPEMSVQEAETVTL SCTYDTSESDYY
 LFWYKQPPSRQMILVIRQEAYKQQNATENRFSVNFQKA AKSFS LKISDSQLGDA
 AMYFCASSPGTYKYIFGTGTRLKVLANIQNPDPAVYQLRDSKSSDKSVCLFTDF
 DSQTNVSQSKDSDVYITDKCVLDMRSMDFKSN SAVAWSNKSDFACANAFNNS
 IIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLK VAGFNLLMTL
 15 RLWSS

SEQ ID NO:49 (#10 α chain with leader sequence)

MTRVSLWAVVVSTCLESGMAQTVTQSQPEMSVQEAETVTL SCTYDTSESNY
 YLFWYKQPPSRQMILVIRQEAYKQQNATENRFSVNFQKA AKSFS LKISDSQLGD
 20 TAMYFCAFNPWENYGQNFVFGPGTRLSVLPYIQNPDP AVYQLRDSKSSDKSVC
 LFTDFDSQTNVSQSKDSDVYITDKCVLDMRSMDFKSN SAVAWSNKSDFACAN
 AFNNSIIPEDTFFPSPESSCDVKLVEKSFETDTNLFQNL SVIGFRILLK VAGFN
 LMTLRLWSS

SEQ ID NO:50 (#4 α chain, leader sequence removed)

25 QKEVEQNSGPLSVPEGAIASLNCTYS DRGSQSFFWYRQYSGKSP ELIMFIYSNG
 DKEDGRFTAQLNKASQYVSLLRDSQPSDSATYLC AVNIGNHDMRFGAGTRLT
 VKPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLD
 MRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFE
 30 TDTNLFQNL SVIGFRILLK VAGFNLLMTLRLWSS

SEQ ID NO:51 (#6 α chain, leader sequence removed)

EDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTLYSAG
 EEKEKERLKATLTKKESFLHITAPKPEDSATYLC AVQTM DGNQFYFGTGTSLTV
 35 IPNIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKCVLDM
 RSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETD
 TNLFQNL SVIGFRILLK VAGFNLLMTLRLWSS

SEQ ID NO:52 (#7 α chain, leader sequence removed)

40 AQTVTQSQPEMSVQEAETVTL SCTYDTSESDYYLFWYKQPPSRQMILVIRQEA
 YKQQNATENRFSVNFQKA AKSFS LKISDSQLGDAAMYFCASSPGTYKYIFGTGT
 RLKVLANIQNPDPAVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYITDKC
 VLDMRSMDFKSN SAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEK
 SFETDTNLFQNL SVIGFRILLK VAGFNLLMTLRLWSS

45

SEQ ID NO:53 (#10 α chain, leader sequence removed)

AQTVTQSQPEMSVQEAETVTL SCTYDTSESNYLFWYKQPPSRQMILVIRQEA
 YKQQNATENRFSVNFQKA AKSFS LKISDSQLGDTAMYFCAFNPWENYGQNFV
 GPGTRLSVLPYIQNPDP AVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKDSDVYI

TDKCVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVK
LVEKSFETDTNLFQNL SVIGFRILLK VAGFNLLMTLRLWSS

SEQ ID NO:54 (#4 β chain with leader sequence)

5 MGCRLCCAVLCLLGAVPIDTEVTQTPKHLVMGMTNKKSLKCEQHMGHRAM
YWKQKAKKPPPELMFVYSYEKLSINESVPSRFSPECNSSLNLHLHALQPEDS
ALYLCASSQGTSGADTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQK
ATLVCLATGFYDPDHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSS
RLRVSAATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRAD
10 CGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG

SEQ ID NO:55 (#6 β chain with leader sequence)

MSIGLLCCAALSLLWAGPVNAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYM
SWYRQDPGMGLRLIHYSVGAGITDQGEVNGYNVSRSTTEDFPLRLLSAAPSQT
15 SVYFCASSYSLWDLQETQYFGPGTRLVLEDLKNVFPPEVAVFEPSEAEISHTQ
KATLVCLATGFYDPDHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLS
SRLRVSAATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA
DCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG

SEQ ID NO:56 (#7 β chain with leader sequence)

20 MGTSLLCWMALCLLGADHADTGVSDPRHKITKRGQNVTFRCDPISEHNRLY
WYRQTLGQGPEFLTYFQNEAQLEKSRLLSDRFSAERPKGFSFSTLEIQRTEQGDS
AMLYCASSFSDGGATDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQ
KATLVCLATGFYDPDHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLS
25 SRLRVSAATFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRA
DCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG

SEQ ID NO:57 (#10 β chain with leader sequence)

MLLLLLLLGPAGSGLGAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFYWRQ
30 FPKQSLMLMATSNEGSKATYEQGVKDKFLINHASLTLSTLTVTSAHPEDSSFYI
CSARPHSLTDTQYFGPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCL
ATGFYDPDHVELSWWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSA
TFWQNPRNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSE
SYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG
35

SEQ ID NO:58 (#4 β chain, leader sequence removed)

DTEVTQTPKHLVMGMTNKKSLKCEQHMGHRAMYWKQKAKKPPPELMFVYS
YEKLSINESVPSRFSPECNSSLNLHLHALQPEDSALYLCASSQGTSGADTQYF
GPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSW
40 WWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSAATFWQNPRNHFRCQ
VQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYE
ILLGKATLYAVLVSALVLMAMVKRKDSRG

SEQ ID NO:59 (#6 β chain, leader sequence removed)

45 NAGVTQTPKFQVLKTGQSMTLQCAQDMNHEYMSWYRQDPGMGLRLIHYSVG
AGITDQGEVNGYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSYSLWDLQETQY
FGPGTRLVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELS
WWVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSAATFWQNPRNHFRC

QVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILY
EILLGKATLYAVLVSALVLMAMVKRKDSRG

SEQ ID NO:60 (#7 β chain, leader sequence removed)

5 DTGVSQDPRIHKITKRGNVTFRCDPISSEHNRLYWYRQTLGQGPEFLTYFQNEA
QLEKSRLLSDRFSAERPCKGSFSTLEIQRTEQGDSAMYLCASSFSDGGATDTQYF
GPGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSW
WVNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSAATFWQNPРНHFRСQ
VQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYE
10 ILLGKATLYAVLVSALVLMAMVKRKDSRG

SEQ ID NO:61 (#10 β chain, leader sequence removed)

GAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFYRQFPKQSLMLMATSNEG
SKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYICСARPHSLTDTQYFG
15 PGTRLTVLEDLKNVFPPEVAVFEPSEAEISHTQKATLVCLATGFYDPDHVELSWW
VNGKEVHSGVCTDPQPLKEQPALNDSRYCLSSRLRVSAATFWQNPРНHFRСQVQ
FYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILL
GKATLYAVLVSALVLMAMVKRKDSRG

20 SEQ ID NO:62 (#4 α gene fragment)

ATGAAATCCTTGAGAGTTTTACTAGTGATCCTGTGGCTTCAGTTGAGCTGGG
TTTGGAGCCAACAGAAGGAGGTGGAGCAGAATTCTGGACCCCTCAGTGTTT
CAGAGGGAGCCATTGCCTCTCTCAACTGCACTTACAGTGACCGAGGTTCCC
AGTCCTTCTTCTGGTACAGACAATATTCTGGGAAAAGCCCTGAGTTGATAAT
25 GTTCATATACTCCAATGGTGACAAAGAAGATGGAAGGTTTACAGCACAGCT
CAATAAAGCCAGCCAGTATGTTTCTCTGCTCATCAGAGACTCCCAGCCCAGT
GATTCAGCCACCTACCTCTGTGCCGTGAACATAGGAAACCATGACATGCGC
TTTGGAGCAGGGACCAGACTGACAGTAAAACCAAATATCCAGAACCCTGAC
CCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCC
30 TATTCACCGATTTTGATTCTCAAACAAATGTGTCAAAAGTAAGGATTCTGA
TGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTTCAA
GAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAA
CGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCAGAA
AGTTCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAAC
35 CTAAACTTTCAAAACCTGTCAAGTATTGGGTTCCGAATCCTCCTCCTGAAAG
TGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTGA

SEQ ID NO:63 (#6 α gene fragment)

ATGGAGAAAATGTTGGAGTGTGCATTCATAGTCTTGTGGCTTCAGCTTGGCT
40 GGTTGAGTGGAGAAGACCAGGTGACGCAGAGTCCCGAGGCCCTGAGACTCC
AGGAGGGAGAGAGTAGCAGTCTCAACTGCAGTTACACAGTCAGCGGTTTAA
GAGGGCTGTTCTGGTATAGGCAAGATCCTGGGAAAGGCCCTGAATTCCTCT
TCACCCTGTATTAGCTGGGGAAGAAAAGGAGAAAGAAAGGCTAAAAGCC
ACATTAACAAAGAAGGAAAGCTTTCTGCACATCACAGCCCCTAAACCTGAA
45 GACTCAGCCACTTATCTCTGTGCTGTGCAGACCATGGACGGTAACCAAGTTCT
ATTTTGGGACAGGGACAAGTTTGACGGTCATTCCAAATATCCAGAACCCTG
ACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCT
GCCTATTACCGATTTTGATTCTCAAACAAATGTGTCAAAAGTAAGGATTC
TGATGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATGGACTT

CAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGC
 AAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCAGCCCA
 GAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACG
 AACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCGAATCCTCCTCCTGA
 5 AAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGCTGA

SEQ ID NO:64 (#7 α gene fragment)

ATGGCATGCCCTGGCTTCCTGTGGGCACTTGTGATCTCCACCTGTCTTGAAT
 TTAGCATGGCTCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGG
 10 AGGCAGAGACCGTGACCCTGAGCTGCACATATGACACCAGTGAGAGTGATT
 ATTATTTATTCTGGTACAAGCAGCCTCCCAGCAGGCAGATGATTCTCGTTAT
 TCGCCAAGAAGCTTATAAGCAACAGAATGCAACAGAGAATCGTTTCTCTGT
 GAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTCAGACTCACA
 GCTGGGGGATGCCGCGATGTATTTCTGTGCTTCCAGTCCAGGAACCTACAA
 15 ATACATCTTTGGAACAGGCACCAGGCTGAAGGTTTTAGCAAATATCCAGAA
 CCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTC
 TGTCTGCCTATTCACCGATTTTGATTCTCAAAACAAATGTGTACAAAGTAAG
 GATTCTGATGTGTATATCACAGACAAAACCTGTGCTAGACATGAGGTCTATG
 GACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCA
 20 TGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTCTTCCCCA
 GCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAG
 ATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCGAATCCTCCT
 CCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGTGGTCCAGC
 TGA
 25

SEQ ID NO:65 (#10 α gene fragment)

ATGACACGAGTTAGCTTGCTGTGGGCAGTCGTGGTCTCCACCTGTCTTGAAT
 CCGGCATGGCCAGACAGTCACTCAGTCTCAACCAGAGATGTCTGTGCAGG
 AGGCAGAGACTGTGACCCTGAGTTGCACATATGACACCAGTGAGAGTAATT
 30 ATTATTTGTTCTGGTACAAACAGCCTCCCAGCAGGCAGATGATTCTCGTTAT
 TCGCCAAGAAGCTTATAAGCAACAGAATGCAACGGGAGAATCGTTTCTCTGT
 GAACTTCCAGAAAGCAGCCAAATCCTTCAGTCTCAAGATCTCAGACTCACA
 GCTGGGGGACACTGCGATGTATTTCTGTGCTTTCAACCCTTGGGAGAACTAT
 GGTCAGAAATTTGTCTTTGGTCCCGGAACCAGATTGTCCGTGCTGCCCTATA
 35 TCCAGAACCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTG
 ACAAGTCTGTCTGCCTATTCACCGATTTTGATTCTCAAAACAAATGTGTACAA
 AAGTAAGGATTCTGATGTGTATATCACAGACAAAACCTGTGCTAGACATGAG
 GTCTATGGACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGA
 CTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTCCAGAAGACACCTTC
 40 TTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTT
 GAAACAGATACGAACCTAAACTTTCAAAACCTGTCAGTGATTGGGTTCGA
 ATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGGCTGT
 GGTCCAGCTGA

45 SEQ ID NO:66 (#4 α gene fragment, codon-optimized)

ATGAAGAGCCTGAGAGTCCTGCTGGTGATTTTGTGGCTGCAGCTGTCTTGGG
 TTTGGTCTCAGCAGAAAGAAGTGGAGCAGAATAGCGGCCCTCTGTCTGTTC
 CTGAAGGCGCTATTGCTAGCCTGAATTGCACATACAGCGATAGAGGATCTC
 AGAGCTTCTTCTGGTACCGGCAGTACAGCGGCAAGAGCCCAGAACTGATCA

TGTTTCATCTACAGCAATGGCGACAAGGAGGATGGCAGGTTTACAGCCCAGC
 TGAACAAGGCCAGCCAGTATGTTTCTCTGCTGATCAGAGATAGCCAGCCTA
 GCGATTCTGCCACCTACCTGTGTGCCGTGAACATCGGAAATCACGACATGA
 GATTTGGAGCCGGCACAAGACTGACCGTGAAGCCCAATATCCAGAACCCTG
 5 ATCCTGCTGTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTGT
 GCCTGTTACCCGACTTCGACAGCCAGACCAACGTGTCCCAGAGCAAGGACA
 GCGACGTGTACATCACCGATAAGTGCGTGCTGGACATGCGGAGCATGGACT
 TCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCG
 CCAACGCCTTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAAGCCC
 10 CGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACA
 CCAACCTGAACTTCCAGAACCCTCAGCGTGATCGGCTTCCGGATCCTGCTGCT
 GAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTG
 A

15 SEQ ID NO:67 (#6 α gene fragment, codon-optimized)

ATGGAGAAGATGCTGGAGTGTGCGTTCATCGTTCTGTGGCTGCAACTTGGAT
 GGCTGTCTGGAGAGGATCAGGTTACACAGTCTCCTGAAGCCCTGAGACTGC
 AAGAAGGAGAAAGCTCTAGCCTGAACTGCAGCTACACAGTGTCTGGACTGA
 GAGGCCTGTTCTGGTACAGACAGGATCCTGGAAAAGGCCAGAGTTCCTGT
 20 TTACCCTGTATTCTGCCGGCGAGGAGAAGGAGAAAGAGAGACTGAAAGCTA
 CCCTGACCAAGAAGGAGAGCTTCCTGCACATTACCGCCCCCAAACCTGAGG
 ATTCTGCCACATATCTGTGTGCTGTGCAGACCATGGATGGCAACCAGTTCTA
 CTTCCGGCACAGGCACATCTCTGACCGTTATCCCCAATATCCAGAACCCTGAT
 CCTGCCGTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTGTGC
 25 CTGTTACCCGACTTCGACAGCCAGACCAACGTGTCCCAGAGCAAGGACAGC
 GACGTGTACATCACCGATAAGTGCGTGCTGGACATGCGGAGCATGGACTTC
 AAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCC
 AACGCCTTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAAGCCCC
 GAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACAC
 30 CAACCTGAACTTCCAGAACCCTCAGCGTGATCGGCTTCCGGATCCTGCTGCTG
 AAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA

SEQ ID NO:68 (#7 α gene fragment, codon-optimized)

ATGGCTTGTCTGGATTCTTATGGGCTCTGGTGATCAGCACCTGTCTGGAGT
 35 TCTCTATGGCCCAGACAGTGACACAGTCTCAGCCTGAAATGTCTGTGCAGG
 AAGCCGAAACCGTGACACTGTCTTGACCTACGATACAAGCGAGAGCGACT
 ACTACCTGTTCTGGTACAAGCAGCCTCCCTCTAGGCAGATGATCCTGGTGAT
 TAGACAGGAGGCCACAAACAGCAGAATGCCACCGAGAACCGGTTTAGCGT
 GAACTTCCAGAAAGCCGCCAAGAGCTTCAGCCTGAAAATCTCTGACAGCCA
 40 GCTGGGAGATGCTGCCATGTACTTTTGTGCCAGCTCTCCAGGCACCTACAAG
 TACATTTTTTGGCACCGGCACCAGACTGAAGGTGCTGGCCAATATCCAGAAT
 CCCGATCCTGCCGTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGC
 GTGTGCCTGTTACCCGACTTCGACAGCCAGACCAACGTGTCCCAGAGCAAG
 GACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGACATGCGGAGCATG
 45 GACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCC
 TGCGCCAACGCCTTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAA
 GCCCCGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACA
 GACACCAACCTGAACTTCCAGAACCCTCAGCGTGATCGGCTTCCGGATCCTG

CTGCTGAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCA
GCTGA

SEQ ID NO:69 (#10 α gene fragment, codon-optimized)

5 ATGACCAGAGTTAGCCTGTTATGGGCTGTGGTGGTGAGCACATGTCTGGAA
TCTGGAATGGCCCAGACAGTGACACAGTCTCAGCCTGAAATGTCTGTGCAG
GAAGCCGAAACCGTTACACTGAGCTGCACCTACGATACAAGCGAGAGCAAC
TACTACCTGTTCTGGTACAAGCAGCCCCCTTCTAGGCAGATGATCCTGGTGA
TCAGACAGGAGGCCTATAAACAGCAGAATGCCACCGAGAACCGGTTTAGCG
10 TGAAGTTCCAGAAAGCCGCCAAGAGCTTCAGCCTGAAAATCTCTGACAGCC
AGCTGGGCGATACAGCCATGTACTTTTGTGCCTTCAACCCCTGGGAGAACTA
TGGCCAGAATTTCTGTGTTTCGGCCCTGGCACCAGACTGTCTGTTCTGCCTTAT
ATCCAGAACCCCGATCCTGCTGTGTACCAGCTGCGGGACAGCAAGAGCAGC
GACAAGAGCGTGTGCCTGTTACCCGACTTCGACAGCCAGACCAACGTGTCC
15 CAGAGCAAGGACAGCGACGTGTACATCACCGATAAGTGCGTGCTGGACATG
CGGAGCATGGACTTCAAGAGCAACAGCGCCGTGGCCTGGTCCAACAAGAGC
GACTTCGCCTGCGCCAACGCCTTCAACAACAGCATTATCCCCGAGGACACA
TTCTTCCCAAGCCCCGAGAGCAGCTGCGACGTGAAGCTGGTGGAAAAGAGC
TTCGAGACAGACACCAACCTGAACTTCCAGAACCTCAGCGTGATCGGCTTC
20 CGGATCCTGCTGCTGAAGGTGGCCGGCTTCAACCTGCTGATGACCCTGCGG
CTGTGGTCCAGCTGA

SEQ ID NO:70 (#4 β gene fragment)

ATGGGCTGCAGGCTGCTCTGCTGTGCGGTTCTCTGTCTCCTGGGAGCAGTTC
25 CCATAGACACTGAAGTTACCCAGACACCAAAACACCTGGTCATGGGAATGA
CAAATAAGAAGTCTTTGAAATGTGAACAACATATGGGGCACAGGGCTATGT
ATTGGTACAAGCAGAAAGCTAAGAAGCCACCGAGCTCATGTTTGTCTACA
GCTATGAGAACTCTCTATAAATGAAAGTGTGCCAAGTCGTTCTCACCTGA
ATGCCCCAACAGCTCTCTCTTAAACCTTCACCTACACGCCCTGCAGCCAGAA
30 GACTCAGCCCTGTATCTCTGCGCCAGCAGCCAAGGGACTAGCGGGGCAGAT
ACGCAGTATTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAGGACCTGAAA
AACGTGTTCCACCCGAGGTCGCTGTGTTTGAAGCATCAGAAGCAGAGATC
TCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCC
GACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGG
35 GGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTC
CAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCAGAA
CCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAAT
GACGAGTGGACCCAGGATAGGGCCAAACCTGTCAACCAGATCGTCAGCGCC
GAGGCTTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACCAGCAA
40 GGGGTCTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCT
TGTATGCCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAGAA
AGGATTCCAGAGGCTAG

SEQ ID NO:71 (#6 β gene fragment)

45 ATGAGCATCGGCCTCCTGTGCTGTGCAGCCTTGTCTCTCCTGTGGGCAGGTC
CAGTGAATGCTGGTGTCACTCAGACCCCAAAATTCCAGGTCTGAAGACAG
GACAGAGCATGACACTGCAGTGTGCCAGGATATGAACCATGAATACATGT
CCTGGTATCGACAAGACCCAGGCATGGGGCTGAGGCTGATTCACTACTCAG
TTGGTGCTGGTATCACTGACCAAGGAGAAGTCCCCAATGGCTACAATGTCT

CCAGATCAACCACAGAGGATTTCCCGCTCAGGCTGCTGTCGGCTGCTCCCTC
 CCAGACATCTGTGTACTTCTGTGCCAGCAGTTACTCTCTTTGGGACCTTCAA
 GAGACCCAGTACTTCGGGGCCAGGCACGCGGCTCCTGGTGCTCGAGGACCTG
 AAAAACGTGTTCCCAACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAG
 5 ATCTCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTAC
 CCCGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAG
 TGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGA
 CTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCA
 GAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAG
 10 AATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTCAGC
 GCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACCAG
 CAAGGGGTCTGTCTGCCACCATCTCTATGAGATCTTGCTAGGGAAGGCC
 ACCTTGTATGCCGTGCTGGTCAGTGGCCTCGTGCTGATGGCCATGGTCAAGA
 GAAAGGATTCCAGAGGCTAG

15

SEQ ID NO:72 (#7 β gene fragment)

ATGGGCACCAGCCTCCTCTGCTGGATGGCCCTGTGTCTCCTGGGGGCAGATC
 ACGCAGATACTGGAGTCTCCAGGACCCAGACACAAGATCACAAAGAGG
 GGACAGAATGTAACCTTCAGGTGTGATCCAATTTCTGAACACAACCGCCTTT
 20 ATTGGTACCGACAGACCCTGGGGCAGGGCCAGAGTTTCTGACTTACTTCC
 AGAATGAAGCTCAACTAGAAAAATCAAGGCTGCTCAGTGATCGGTTCTCTG
 CAGAGAGGCCTAAGGGATCTTTCTCCACCTTGGAGATCCAGCGCACAGAGC
 AGGGGGACTCGGCCATGTATCTCTGTGCCAGCAGCTTTTCAGACGGGGGGG
 CTACAGATACGCAGTATTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAGG
 25 ACCTGAAAAACGTGTTCCCAACCCGAGGTCGCTGTGTTTGAGCCATCAGAAG
 CAGAGATCTCCACACCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCT
 TCTACCCCGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGC
 ACAGTGGGGTCAGCACAGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCA
 ATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTG
 30 GCAGAACCCCCGCAACCACTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCG
 GAGAATGACGAGTGGACCCAGGATAGGGCCAAACCTGTCACCCAGATCGTC
 AGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTAC
 CAGCAAGGGGTCTGTCTGCCACCATCTCTATGAGATCTTGCTAGGGAAG
 GCCACCTTGTATGCCGTGCTGGTCAGTGGCCTCGTGCTGATGGCCATGGTCA
 35 AGAGAAAGGATTCCAGAGGCTAG

SEQ ID NO:73 (#10 β gene fragment)

ATGCTGCTGCTTCTGCTGCTTCTGGGGCCAGCAGGCTCCGGGCTTGGTGCTG
 TCGTCTCTCAACATCCGAGCTGGGTTATCTGTAAGAGTGGAACTCTGTGAA
 40 GATCGAGTGCCGTTCCCTGGACTTTCAGGCCACAACCTATGTTTTGGTATCGT
 CAGTTCGCCGAAACAGAGTCTCATGCTGATGGCAACTTCCAATGAGGGCTCC
 AAGGCCACATACGAGCAAGGCGTCGAGAAGGACAAGTTTCTCATCAACCAT
 GCAAGCCTGACCTTGTCCACTCTGACAGTGACCAGTGCCCATCTGAAGAC
 AGCAGCTTCTACATCTGCAGTGCTAGACCCCATCTCTCACAGATACGCAGT
 45 ATTTTGGCCCAGGCACCCGGCTGACAGTGCTCGAGGACCTGAAAAACGTGT
 TCCCAACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCACA
 CCCAAAAGGCCACACTGGTGTGCCTGGCCACAGGCTTCTACCCCGACCACG
 TGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGGGGTCAGC
 ACAGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATAC

TGCCTGAGCAGCCGCCTGAGGGTCTCGGCCACCTTCTGGCAGAACCCCCGC
 AACCACCTTCCGCTGTCAAGTCCAGTTCTACGGGCTCTCGGAGAATGACGAG
 TGGACCCAGGATAGGGCCAAACCTGTACCCAGATCGTCAGCGCCGAGGCC
 TGGGGTAGAGCAGACTGTGGCTTCACCTCCGAGTCTTACCAGCAAGGGGTC
 5 CTGTCTGCCACCATCCTCTATGAGATCTTGCTAGGGAAGGCCACCTTGTATG
 CCGTGCTGGTCAGTGCCCTCGTGCTGATGGCCATGGTCAAGAGAAAGGATT
 CCAGAGGCTAG

SEQ ID NO:74 (#4 β gene fragment, codon-optimized)

10 ATGGGCTGTAGACTGTTGTGTTGTGCTGTGCTGTGTTGGGAGCTGTGC
 CTATCGATACAGAGGTGACCCAGACCCCTAAACATCTGGTTATGGGCATGA
 CCAACAAGAAGAGCCTGAAGTGCGAGCAGCACATGGGCCATAGGGCCATG
 TATTGGTATAAGCAGAAGGCCAAGAAACCTCCTGAGCTGATGTTTCGTGTAC
 AGCTACGAGAAGCTGAGCATCAACGAGAGCGTGCCCAGCAGATTTTCTCCT
 15 GAGTGCCCTAATTCTAGCCTGCTGAATCTGCACCTGCATGCTCTGCAGCCTG
 AGGATTCTGCTCTGTACCTGTGTGCTTCTTCTCAGGGCACATCTGGAGCTGA
 TACACAGTACTTCGGACCTGGCACAAAGACTGACAGTGCTGGAAGACCTGAA
 GAACGTGTTCCCCCAGAGGTGGCCGTGTTTCGAGCCTAGCGAGGCCGAGAT
 CAGCCACACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACCCC
 20 GACCACGTGGAAGTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGC
 GTCTGCACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTGAACGACAGC
 CGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAAC
 CCCCGBAACCCTTCAGATGCCAGGTGCAGTTCTACGGCCTGAGCGAGAAC
 GACGAGTGGACCCAGGACCGGGCCAAGCCCGTGACCCAGATCGTGTCTGCT
 25 GAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAGCTACCAGCAG
 GCGGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCACC
 CTGTACGCCGTGCTGGTGTCCGCCCTGGTGCTGATGGCCATGGTCAAGCGG
 AAGGACAGCCGGGGC

30 SEQ ID NO:75 (#6 β gene fragment, codon-optimized)

ATGTCTATCGGTCTGCTGTGCTGTGCTGCTCTTTCTGCTTTGGGCTGGACC
 TGTGAATGCTGGAGTTACACAAACCCCCAAGTTCCAAGTGCTGAAGACAGG
 ACAGAGCATGACCCTGCAGTGTGCTCAGGACATGAATCACGAGTACATGAG
 CTGGTACAGACAGGATCCTGGAATGGGCCTGAGGCTGATCCACTACTCTGT
 35 TGGAGCCGGAATTACAGATCAGGGAGAAAGTGCCAAATGGCTACAACGTGA
 GCAGGAGCACAAACCGAGGACTTCCCCCTTAAGACTGTTGTCTGCTGCTCCATC
 TCAGACAAGCGTGTACTTTTGCGCCAGCTCCTACTCTCTGTGGGATCTGCAG
 GAAACCCAGTACTTTGGACCAGGCACAAGACTGTTAGTGCTGGAGGACCTG
 AAGAACGTGTTCCCCCAGAGGTGGCCGTGTTTCGAGCCTAGCGAGGCCGAG
 40 ATCAGCCACACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACC
 CCGACCACGTGGAAGTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCG
 GCGTCTGCACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTGAACGACA
 GCCGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGA
 ACCCCCGGAACCCTTCAGATGCCAGGTGCAGTTCTACGGCCTGAGCGAGA
 45 ACGACGAGTGGACCCAGGACCGGGCCAAGCCCGTGACCCAGATCGTGTCTG
 CTGAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAGCTACCAGC
 AGGGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCA
 CCCTGTACGCCGTGCTGGTGTCCGCCCTGGTGCTGATGGCCATGGTCAAGCG
 GAAGGACAGCCGGGGC

SEQ ID NO:76 (#7 β gene fragment, codon-optimized)

ATGGGCACATCTCTTCTCTGCTGGATGGCTCTTTGTCTGCTTGGAGCCGATC
 ATGCCGATACAGGAGTTAGCCAGGATCCTAGACACAAGATCACCAAGAGA
 5 GGCCAGAATGTGACCTTCCGGTGCATCCTATCTCTGAGCACAACAGGCTG
 TACTGGTACAGACAAACACTGGGACAAGGACCTGAGTTCCTGACCTACTTC
 CAGAACGAAGCCCAGCTGGAGAAGTCTAGACTTCTGAGCGACAGATTTAGC
 GCCGAGAGACCTAAAGGCAGCTTTAGCACCTGGAGATCCAGAGAACAGA
 ACAGGGCGATTCTGCCATGTACCTGTGTGCTAGCAGCTTTTCTGATGGAGGC
 10 GCCACCGATACACAGTATTTCCGACCTGGCACAAGACTGACAGTGCTGGAG
 GACCTGAAGAACGTGTTCCCCCAGAGGTGGCCGTGTTTCGAGCCTAGCGAG
 GCCGAGATCAGCCACACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGC
 TTTTACCCCGACCACGTGGAAGTGTCTTGGTGGGTCAACGGCAAAGAGGTG
 CACAGCGGCGTCTGCACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTG
 15 AACGACAGCCGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTC
 TGGCAGAACCCCCGGAACCACTTCAGATGCCAGGTGCAGTTCTACGGCCTG
 AGCGAGAACGACGAGTGGACCCAGGACCGGGCCAAGCCCGTGACCCAGAT
 CGTGTCTGCTGAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAG
 CTACCAGCAGGGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGG
 20 CAAGGCCACCCTGTACGCCGTGCTGGTGTCCGCCCTGGTGCTGATGGCCATG
 GTCAAGCGGAAGGACAGCCGGGGC

SEQ ID NO:77 (#10 β gene fragment, codon-optimized)

ATGCTGCTTCTTCTCCTCCTTCTCGGACCTGCTGGATCTGGATTAGGAGCTGT
 25 TGTGTCTCAGCACCTTCTTGGGTGATCTGTAAAAGCGGCACAAGCGTGAA
 GATCGAGTGCAGAAGCCTGGACTTTCAGGCCACAACCATGTTCTGGTATAG
 GCAGTTCCCCAAGCAGTCTCTGATGCTGATGGCCACCTCTAATGAGGGCTCT
 AAGGCCACATATGAACAGGGAGTGGAGAAGGACAAGTTCCTGATCAACCA
 CGCCTCTCTGACCCTGTCTACCCTGACAGTTACATCTGCCCACCCTGAGGAT
 30 AGCAGCTTTTACATCTGTAGCGCCAGACCTCACAGCCTGACCGATACACAG
 TACTTTGGCCCTGGCACAAGACTGACAGTGTTAGAAGACCTGAAGAACGTG
 TTCCCCCAGAGGTGGCCGTGTTTCGAGCCTAGCGAGGCCGAGATCAGCCAC
 ACCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACCCCGACCAC
 GTGGAAGTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCTGC
 35 ACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTGAACGACAGCCGGTAC
 TGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGG
 AACCCTTCAGATGCCAGGTGCAGTTCTACGGCCTGAGCGAGAACGACGAG
 TGGACCCAGGACCGGGCCAAGCCCGTGACCCAGATCGTGTCTGCTGAGGCC
 TGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAGCTACCAGCAGGGCGTG
 40 CTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCACCCTGTAC
 GCCGTGCTGGTGTCCGCCCTGGTGCTGATGGCCATGGTCAAGCGGAAGGAC
 AGCCGGGGC

SEQ ID NO:78 (#4 β -P2A-#4 α , codon-optimized)

45 ATGGGCTGTAGACTGTTGTGTTGTGCTGTGCTGTGTCTGTTGGGAGCTGTGC
 CTATCGATACAGAGGTGACCCAGACCCCTAAACATCTGGTTATGGGCATGA
 CCAACAAGAAGAGCCTGAAGTGCAGCAGCACATGGGCCATAGGGCCATG
 TATTGGTATAAGCAGAAGGCCAAGAAACCTCCTGAGCTGATGTTTCGTGTAC
 AGCTACGAGAAGCTGAGCATCAACGAGAGCGTGCCAGCAGATTTTCTCCT

GAGTGCCCTAATTCTAGCCTGCTGAATCTGCACCTGCATGCTCTGCAGCCTG
 AGGATTCTGCTCTGTACCTGTGTGCTTCTTCTCAGGGCACATCTGGAGCTGA
 TACACAGTACTTCGGACCTGGCACAAGACTGACAGTGCTGGAAGACCTGAA
 GAACGTGTTCCCCCAGAGGTGGCCGTGTTTCGAGCCTAGCGAGGCCGAGAT
 5 CAGCCACACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACCCC
 GACCACGTGGAAGTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGC
 GTCTGCACCGACCCCCAGCCCCTGAAAGAGCAGCCCGCCCTGAACGACAGC
 CGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAAC
 CCCCGBAACCCTTCAGATGCCAGGTGCAGTTCTACGGCCTGAGCGAGAAC
 10 GACGAGTGGACCCAGGACCGGGCCAAGCCCGTGACCCAGATCGTGTCTGCT
 GAGGCCTGGGGCAGAGCCGATTGCGGCTTCACCAGCGAGAGCTACCAGCAG
 GGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCACC
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 15 GCAGGAGACGTGGAAGAAAACCCCGGTCCCATGAAGAGCCTGAGAGTCCT
 GCTGGTGAATTTGTGGCTGCAGCTGTCTTGGGTTTGGTCTCAGCAGAAAGAA
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 TGAATTGCACATACAGCGATAGAGGATCTCAGAGCTTCTTCTGGTACCGGC
 AGTACAGCGGCAAGAGCCCAGAACTGATCATGTTTCATCTACAGCAATGGCG
 20 ACAAGGAGGATGGCAGGTTTACAGCCCAGCTGAACAAGGCCAGCCAGTAT
 GTTTCTCTGCTGATCAGAGATAGCCAGCCTAGCGATTCTGCCACCTACCTGT
 GTGCCGTGAACATCGGAAATCACGACATGAGATTTGGAGCCGGGCACAAGAC
 TGACCGTGAAGCCCAATATCCAGAACCCTGATCCTGCTGTGTACCAGCTGC
 GGGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGACTTCGACA
 25 GCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATACCGATA
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 TTATCCCCGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGCGACGTGA
 AGCTGGTGGAAAAGAGCTTCGAGACAGACACCAACCTGAACTTCCAGAACC
 30 TCAGCGTGATCGGCTTCCGGATCCTGCTGCTGAAGGTGGCCGGCTTCAACCT
 GCTGATGACCCTGCGGCTGTGGTCCAGCTGA

SEQ ID NO:79 (#6 β -P2A-#6 α , codon-optimized)

ATGTCTATCGGTCTGCTGTGCTGTGCTGCTCTTTCTCTGCTTTGGGCTGGACC
 35 TGTGAATGCTGGAGTTACACAAACCCCCAAGTTCCAAGTGCTGAAGACAGG
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 CTGGTACAGACAGGATCCTGGAATGGGCTGAGGCTGATCCACTACTCTGT
 TGGAGCCGGAATTACAGATCAGGGAGAAAGTGCCAAATGGCTACAACGTGA
 GCAGGAGCACAAACCGAGGACTTCCCCCTTAAGACTGTTGTCTGCTGCTCCATC
 40 TCAGACAAGCGTGTACTTTTGGCCAGCTCCTACTCTCTGTGGGATCTGCAG
 GAAACCCAGTACTTTGGACCAGGCACAAGACTGTTAGTGCTGGAGGACCTG
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 ATCAGCCACACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACC
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 ACGACGAGTGGACCCAGGACCGGGCCAAGCCCGTGACCCAGATCGTGTCTG
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AGGGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGGCAAGGCCA
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 5 GTGCGTTCATCGTTCTGTGGCTGCAACTTGGATGGCTGTCTGGAGAGGATCA
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 CCTGAACTGCAGCTACACAGTGTCTGGACTGAGAGGCCTGTTCTGGTACAG
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 GAGGAGAAGGAGAAAGAGAGACTGAAAGCTACCCTGACCAAGAAGGAGA
 10 GCTTCCTGCACATTACCGCCCCAAACCTGAGGATTCTGCCACATATCTGTG
 TGCTGTGCAGACCATGGATGGCAACCAGTTCTACTTCGGCACAGGCACATC
 TCTGACCGTTATCCCCAATATCCAGAACCCTGATCCTGCCGTGTACCAGCTG
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 AGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATCACCGAT
 15 AAGTGCGTGCTGGACATGCGGAGCATGGACTTCAAGAGCAACAGCGCCGTG
 GCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAACAACAGC
 ATTATCCCCGAGGACACATTCTTCCAAGCCCCGAGAGCAGCTGCGACGTG
 AAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAACCTGAACTTCCAGAAC
 CTCAGCGTGATCGGCTTCCGGATCCTGCTGCTGAAGGTGGCCGGCTTCAACC
 20 TGCTGATGACCCTGCGGCTGTGGTCCAGCTGA

SEQ ID NO:80 (#7 β -P2A-#7 α , codon-optimized)

ATGGGCACATCTCTTCTCTGCTGGATGGCTCTTTGTCTGCTTGGAGCCGATC
 ATGCCGATACAGGAGTTAGCCAGGATCCTAGACACAAGATCACCAAGAGA
 25 GGCCAGAATGTGACCTTCCGGTGGGATCCTATCTCTGAGCACAACAGGCTG
 TACTGGTACAGACAAACACTGGGACAAGGACCTGAGTTCTGACCTACTTC
 CAGAACGAAGCCCAGCTGGAGAAGTCTAGACTTCTGAGCGACAGATTTAGC
 GCCGAGAGACCTAAAGGCAGCTTTAGCACCTGGAGATCCAGAGAACAGA
 ACAGGGCGATTCTGCCATGTACCTGTGTGCTAGCAGCTTTTCTGATGGAGGC
 30 GCCACCGATACACAGTATTTCCGACCTGGCACAAGACTGACAGTGCTGGAG
 GACCTGAAGAACGTGTTCCCCCAGAGGTGGCCGTGTTTCGAGCCTAGCGAG
 GCCGAGATCAGCCACACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGC
 TTTTACCCCGACCACGTGGAAGTGTCTTGGTGGGTCAACGGCAAAGAGGTG
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 35 AACGACAGCCGGTACTGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTC
 TGGCAGAACCCCGGAACCACTTCAGATGCCAGGTGCAGTTCTACGGCCTG
 AGCGAGAACGACGAGTGGACCCAGGACCGGGCCAAGCCCGTGACCCAGAT
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 CTACCAGCAGGGCGTGCTGAGCGCCACCATCCTGTACGAGATCCTGCTGGG
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 45 TGACACTGTCTTGACCTACGATACAAGCGAGAGCGACTACTACCTGTTCTG
 GTACAAGCAGCCTCCCTCTAGGCAGATGATCCTGGTGATTAGACAGGAGGC
 CTACAAACAGCAGAATGCCACCGAGAACCGGTTTAGCGTGAACCTTCCAGAA
 AGCCGCCAAGAGCTTCAGCCTGAAAATCTCTGACAGCCAGCTGGGAGATGC
 TGCCATGTACTTTTGTGCCAGCTCTCCAGGCACCTACAAGTACATTTTGGC

ACCGGCACCAGACTGAAGGTGCTGGCCAATATCCAGAATCCCGATCCTGCC
 GTGTACCAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTC
 ACCGACTTCGACAGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTG
 TACATCACCGATAAGTGCCTGCTGGACATGCGGAGCATGGACTTCAAGAGC
 5 AACAGCGCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCC
 TTCAACAACAGCATTATCCCCGAGGACACATTCTTCCCAAGCCCCGAGAGC
 AGCTGCGACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAACCT
 GAACTTCCAGAACCTCAGCGTGATCGGCTTCCGGATCCTGCTGCTGAAGGT
 GGCCGGCTTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA
 10
SEQ ID NO:81 (#10 β -P2A-#10 α , codon-optimized)
 ATGCTGCTTCTTCTCCTCCTTCTCGGACCTGCTGGATCTGGATTAGGAGCTGT
 TGTGTCTCAGCACCCCTTCTTGGGTGATCTGTAAAAGCGGCACAAGCGTGAA
 GATCGAGTGCAGAAGCCTGGACTTTCAGGCCACAACCATGTTCTGGTATAG
 15 GCAGTTCCCCAAGCAGTCTCTGATGCTGATGGCCACCTCTAATGAGGGCTCT
 AAGGCCACATATGAACAGGGAGTGGAGAAGGACAAGTTCCTGATCAACCA
 CGCCTCTCTGACCCTGTCTACCCTGACAGTTACATCTGCCCACCCTGAGGAT
 AGCAGCTTTTACATCTGTAGCGCCAGACCTCACAGCCTGACCGATACACAG
 TACTTTGGCCCTGGCACAAGACTGACAGTGTTAGAAGACCTGAAGAACGTG
 20 TTCCCCCAGAGGTGGCCGTGTTTCGAGCCTAGCGAGGCCGAGATCAGCCAC
 ACCCAGAAAGCCACCCTCGTGTGCCTGGCCACCGGCTTTTACCCCGACCAC
 GTGGAAGTGTCTTGGTGGGTCAACGGCAAAGAGGTGCACAGCGGCGTCTGC
 ACCGACCCCCAGCCCCTGAAAGAGCAGCCCCGCCCTGAACGACAGCCGGTAC
 TGTCTGAGCAGCAGACTGAGAGTGTCCGCCACCTTCTGGCAGAACCCCCGG
 25 AACCACCTCAGATGCCAGGTGCAGTTCTACGGCCTGAGCGAGAACGACGAG
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 GCCGTGCTGGTGTCCGCCCTGGTGCTGATGGCCATGGTCAAGCGGAAGGAC
 30 AGCCGGGGCGGTTCCGGAGCCACGAACCTTCTCTCTGTAAAGCAAGCAGGA
 GACGTGGAAGAAAACCCCGGTCCCATGACCAGAGTTAGCCTGTTATGGGCT
 GTGGTGGTGAGCACATGTCTGGAATCTGGAATGGCCCAGACAGTGACACAG
 TCTCAGCCTGAAATGTCTGTGCAGGAAGCCGAAACCGTTACACTGAGCTGC
 ACCTACGATACAAGCGAGAGCAACTACTACCTGTTCTGGTACAAGCAGCCC
 35 CCTTCTAGGCAGATGATCCTGGTGATCAGACAGGAGGCCTATAAACAGCAG
 AATGCCACCGAGAACCGGTTTAGCGTGAACCTTCCAGAAAGCCGCCAAGAGC
 TTCAGCCTGAAAATCTCTGACAGCCAGCTGGGCGATACAGCCATGTACTTTT
 GTGCCTTCAACCCCTGGGAGAACTATGGCCAGAATTTTCGTGTTCCGGCCCTGG
 CACCAGACTGTCTGTCTGCTTATATCCAGAACCCCGATCCTGCTGTGTAC
 40 CAGCTGCGGGACAGCAAGAGCAGCGACAAGAGCGTGTGCCTGTTACCGAC
 TTCGACAGCCAGACCAACGTGTCCCAGAGCAAGGACAGCGACGTGTACATC
 ACCGATAAGTGCCTGCTGGACATGCGGAGCATGGACTTCAAGAGCAACAGC
 GCCGTGGCCTGGTCCAACAAGAGCGACTTCGCCTGCGCCAACGCCTTCAAC
 AACAGCATTATCCCCGAGGACACATTCTTCCCAAGCCCCGAGAGCAGCTGC
 45 GACGTGAAGCTGGTGGAAAAGAGCTTCGAGACAGACACCAACCTGAACTTC
 CAGAACCTCAGCGTGATCGGCTTCCGGATCCTGCTGCTGAAGGTGGCCGGC
 TTCAACCTGCTGATGACCCTGCGGCTGTGGTCCAGCTGA

SEQ ID NO:82 (Reference TCR α chain)

MTSIRAVFIFLWLQLDLVNGENVEQHPSTLSVQEGDSAVIKCTYSDSASNYFPW
 YKQELGKRPLIIDIRSNVGEKKDQRIAVTLNKTAKHFSLHITETQPEDSAVYFC
 AATEDYQLIWGAGTKLIKPDIONPDPAVYQLRDSKSSDKSVCLFTDFDSQTNV
 SQSKSDSVYITDKTVLDMRSMDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFF
 5 PSESSCDVKLVEKSFETDTNLFQNL SVIGFRILLKLVAGFNLLMTLRLWSS

SEQ ID NO:83 (Reference TCR β chain)

MSNQVLCCVVLGANTVDGGITQSPKYLFRKEGQNVTLSCQNLNHDAMY
 WYRQDPGQGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTA
 FYLCASSPGALYEQYFGPGTRLTVTEDLKNVFPPEVAVFEPSEAEISHTQKATLV
 10 CLATGFYDPDHVELSWWVNGKEVHSGVSTDPQPLKEQPALNDSRYCLSSRLRVS
 ATFWQNPRNHFRQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTS
 ESYQQGVLSATILYEILLGKATLYAVLVSAVLMLMAMVKRKDSRG

SEQ ID NO:84 (Porcine teschovirus-1 2A (P2A-1) peptide)

GSGATNFSLLKQAGDVEENPGP

15 SEQ ID NO:85 (Porcine teschovirus-2 2A (P2A-2) peptide)

SGATNFSLLKQAGDVEENPGP

SEQ ID NO:86 (Thosea asigna virus 2A (T2A) peptide)

GSGEGRGSLLTCGDVEENPGP

SEQ ID NO:87 (Equine rhinitis A virus (ERAV) 2A (E2A) peptide)

20 GSGQCTNYALLKLAGDVESNPGP

SEQ ID NO:88 (Foot-and-mouth disease virus 2A (F2A) peptide)

GSGVKQTLNFDLLKLAGDVESNPGP

SEQ ID NO:89 (Porcine teschovirus-1 2A (P2A-1) peptide – nt sequence)

GGAAGTGGAGCTACGAATTTTCTTTATTAAAAACAAGCAGGAGATGTTGAG
 25 GAGAATCCCGGTCCA

SEQ ID NO:90 (Porcine teschovirus-2 2A (P2A-2) peptide – nt sequence)

AGCGGCGCCACCAACTTCAGCCTGCTGAAACAGGCCGCGACGTGGAAGA
 GAACCCTGGCCCT

SEQ ID NO:91 (Thosea asigna virus 2A (T2A) peptide – nt sequence)

30 GGAAGCGGAGAGGGCAGAGGAAGTCTGCTAACATGCGGTGACGTCGAGGA
 GAATCCTGGACCT

SEQ ID NO:92 (Equine rhinitis A virus (ERAV) 2A (E2A) peptide – nt sequence)

GGAAGCGGACAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTT
 GAGAGCAACCCTGGACCT

35 SEQ ID NO:93 (Foot-and-mouth disease virus 2A (F2A) peptide – nt sequence)

GGAAGCGGAGTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGA
 GACGTGGAGTCCAACCCTGGACCT

SEQ ID NO:94 (WT-1 peptide antigen)

RMFPNAPYL

SEQ ID NO:95 (G-S linker)

GGGGS

SEQ ID NO:96

5 GlyxSery, wherein X and Y are each independently a non-zero integer

SEQ ID NO:97 – meganuclease recognition site

LAGLIDADG

SEQ ID NO:98 – meganuclease recognition site

GIY-YIG

10 **SEQ ID NO:99 – meganuclease recognition site**

PD-(D/E)XK

SEQ ID NO.:100 (WT-1 aa 37-45)

VLDFAPPGA

15

SEQ ID NO.:101 (TCR 10.1 CDR1 α)

DSAIYN

SEQ ID NO.:102 (TCR 10.1 CDR2 α)

20 IQSSQRE

SEQ ID NO.:103 (TCR 10.1 CDR3 α)

CAVKETSGSRLTF

25 **SEQ ID NO.:104 (TCR 10.1 V α)**

KQEVTTQIPAALSVPEGENVLNCSTFDSAIFYNLQWFRQDPGKGLTSLLLIQSSQR
EQTSGRNLNASLDKSSGRSTLYIAASQPGDSATYLCVKETSGSRLTFGEGTQLT
VNP

30 **SEQ ID NO.:105 (TCR 10.1 CDR1 β)**

SGHVS

SEQ ID NO.:106 (TCR 10.1 CDR2 β)

FNIEAQ

35

SEQ ID NO.:107 (TCR 10.1 CDR3 β)

CASSLTGSIEQYF

SEQ ID NO.:108 (TCR 10.1 V β)

40 GAGVSQSPRYKVTKRGQDVALRCDPISGHVSLYWYRQALGQGPEFLTYFNIE
AQQDKSGLPNDRFSAERPEGSISTLTIQRTQQRDSAMYRCASSLTGSIEQYFGP
GTRLTVTE

SEQ ID NO.:109 (TCR 13.1 CDR1 α)

NSMFDY

SEQ ID NO.:110 (TCR 13.1 CDR2 α)
ISSIKDK

5

SEQ ID NO.:111 (TCR 13.1 CDR3 α)
CAASGIGDYKLSF

SEQ ID NO.:112 (TCR 13.1 V α)
10 DQQVKQNSPSLSVQEGRISILNCDYTNSMFDYFLWYKKYPAEGPTFLISSIKD
KNEDGRFTVFLNKS AKHL SLHIVSPQPGDSAVYFCAASGIGDYKLSFGAGTTVT
VRAN

SEQ ID NO.:113 (TCR 13.1 CDR1 β)
15 PRHDT

SEQ ID NO.:114 (TCR 13.1 CDR2 β)
FYEKMQ

20 **SEQ ID NO.:115 (TCR 13.1 CDR3 β)**
CASSLRLGRETQYF

SEQ ID NO.:116 (TCR 13.1 V β)
AAGVIQSPRHLIKEKRETATLKCYPPIRHDTVYWYQQGPGQDPQFLISFYEKMQ
25 SDKGSIPDRFSAQQFS DYHSELNMSSLELGDSALYFCASSLRLGRETQYFGPGTR
LLVLE

SEQ ID NO.:117 (TCR 16.1 CDR1 α)
30 VSGLRG

SEQ ID NO.:118 (TCR 16.1 CDR2 α)
LYSAGEE

SEQ ID NO.:119 (TCR 16.1 CDR3 α)
35 CAVITGFQKLVF

SEQ ID NO.:120 (TCR 16.1 V α)
EDQVTQSPEALRLQEGESSLNCSYTVSGLRGLFWYRQDPGKGPEFLFTLYSAG
EEKEKERL KATLTKKESFLHITAPKPEDSATYLCAVITGFQKL VFGTGTRLLVSP
40 N

SEQ ID NO.:121 (TCR 16.1 CDR1 β)
MNHEY

45 **SEQ ID NO.:122 (TCR 16.1 CDR2 β)**
SMNVEV

SEQ ID NO.:123 (TCR 16.1 CDR3 β)

CASSFSGGTYEQYF

SEQ ID NO.:124 (TCR 16.1 V β)

EAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNV
 5 EVTDKGDVPEGYKVSRRKEKRNFLILESPSPNQTSLYFCASSFSGGTYEQYFGPG
 TRLTVTE

EXAMPLES

EXAMPLE 1

10 IDENTIFICATION AND CHARACTERIZATION OF TCRs SPECIFIC FOR A WT-1 PEPTIDE

CD8⁺ T cells from four donors were cultured with autologous dendritic cells (DCs) pulsed with the WT-1 peptide RMFPNAPYL (SEQ ID NO:94) (Ho, Greenberg *et al.*, 2006). The T cell lines were combined, stained with CD8-independent (CD8i) WT-1 peptide:HLA tetramer and sorted for high tetramer staining cells. A total of 40
 15 polyclonal T cell lines with high tetramer staining were identified. The sorted T cell samples and a fraction of the total unsorted sample were both analyzed by TCR repertoire analysis (Adaptive Biotechnologies) to quantitate each TCR clonotype. A fraction of high tetramer-staining cells was also analyzed by single-cell RNA sequencing and TCR pairing (10X genomics). 24 TCR clonotypes were identified as
 20 WT-1-specific by comparison of the fold-enrichment of each clonotype within the sorted versus unsorted population and the CD3 surface expression of each clonotype (Figure 1). Of these, 10 were selected for synthesis. TCR α /TCR β -deficient J76 Jurkat T cells were transduced to express the candidate TCRs. The affinity of each candidate for WT-1 was assessed by measuring the relative WT-1 peptide:HLA tetramer staining
 25 as compared to CD3 expression, which is a marker of total transgenic TCR surface expression. The results were compared to those of cells expressing the WT-1 peptide:HLA-specific TCR having an α chain according to SEQ ID NO:82 and a β chain according to SEQ ID NO:83. Data are shown in Figures 2A-2C.

TCR-transduced Jurkat cells expressing the *Nur77*-tomato activation marker
 30 were assessed for activation in the presence of peptide. Data are shown in Figure 3A.

CD8⁺ T cells isolated from donor PMBCs were transduced to express one of eight TCRs selected for further study. After one week, the transduced cells were sorted for WT-1 peptide:HLA tetramer staining and CD8 expression (Figure 4A). Tetramer⁺ CD8⁺ T cells were expanded and assayed for IFN γ production. The expanded antigen-specific cells were cultured for four hours with T2 target cells pulsed with titrated concentrations of WT-1 peptide, and IFN γ production was determined by flow cytometry (Figure 4B). The percentage of IFN γ -producing cells was fit to dose-response curves by non-linear regression to calculate the peptide EC₅₀ for each TCR (Figure 4C).

T cells expressing the two TCRs with the lowest EC₅₀ values were compared to T cells expressing the reference WT-1 specific TCR for the ability to induce tumor cell lysis. Tumor cell line MDA-MB-468, which was transduced to express HLA-A2, and the naturally HLA_A2⁺ tumor cell line Panc-1 were loaded with ⁵¹Cr. TCR-transduced T cell-mediated killing was calculated by measuring Cr release in response to decreasing doses of effector T cells relative to tumor cell targets (Figures 5A and 5B).

T cells expressing exemplary TCR DL10 were further investigated for IFN- γ production (Figure 6) and the ability to reduce growth of tumor cell line cells (Figure 7). For IFN- γ production, primary CD8⁺ T cells were transduced with a reference TCR or with TCRDL10 and cultured with the peptide-pulsed T2 target cells as indicated in Figure 6. After 4 hours, IFN γ production was assessed by intracellular flow cytometry and percent IFN γ positivity was fit to a dose response curve by nonlinear regression in Graphpad Prism. To assess the ability to control tumor growth, primary CD8⁺ T cells were transduced with a reference TCR (comprising a TCR α chain having the amino acid sequence set forth in SEQ ID NO: 82 and a TCR β chain having the amino acid sequence set forth in SEQ ID NO: 83) or with TCRDL10 and cultured in triplicate with the HLA-A2⁺ WT1⁺ pancreatic tumor cell line Panc-1 (transduced to express a red fluorescence protein) at a ratio of 8:1 in an IncuCyte assay, and cell proliferation/survival was monitored over 7 days. Error bars indicate standard error of the mean.

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. Patent Application No. 62/889,519, filed August 20, 2019, are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if

- 5 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 not be construed to limit the claims to the specific embodiments disclosed in the specification
- 10 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

What is claimed is:

1. A modified immune cell, comprising a heterologous polynucleotide that encodes a binding protein, wherein the encoded binding protein comprises:
 - (a) a T cell receptor (TCR) α chain variable ($V\alpha$) domain comprising the CDR3 amino acid sequence (CDR3 α) according to any one of SEQ ID NOs.:28, 19, 22, or 25, or a variant thereof, and a TCR β -chain variable ($V\beta$) domain; or
 - (b) a TCR $V\beta$ domain comprising the CDR3 amino acid sequence (CDR3 β) according to any one of SEQ ID NOs.:40, 31, 34, or 37, or a variant thereof, and a TCR $V\alpha$ domain; or
 - (c) a TCR $V\alpha$ domain of (a), and a TCR $V\beta$ domain of (b),wherein the encoded binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):human leukocyte antigen (HLA) complex, wherein, optionally, the HLA comprises HLA-A*0201.
2. The modified immune cell of claim 1, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94):HLA complex with an IFN γ production pEC₅₀ of 4.0 or higher, or 4.5 or higher.
3. The modified immune cell of claim 1 or 2, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94):HLA complex with an IFN γ production pEC₅₀ of 5.0 or higher.
4. The modified immune cell of any one of claims 1-3, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94):HLA complex with an IFN γ production pEC₅₀ of 5.5 or higher.

5. The modified immune cell of any one of claims 1-4, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94):HLA complex with an IFN γ production pEC₅₀ of 6.0 or higher.

6. The modified immune cell of any one of claims 1-5, wherein the encoded binding protein is capable of binding to the RMFPNAPYL (SEQ ID NO.:94):HLA complex with an IFN γ production pEC₅₀ of 6.5 or higher.

7. The modified immune cell of any one of claims 1-6, wherein the HLA comprises HLA-A*201.

8. The modified immune cell of any one of claims 1-7, wherein 50% or more of the modified immune cells in a population produce IFN- γ when the population of the modified immune cells is co-cultured for 4 hours with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻⁴ μ M, 10⁻⁵ μ M, or 10⁻⁶ μ M in the co-culture, wherein the antigen-presenting cells optionally comprise T2 cells.

9. The modified immune cell of any one of claims 1-8, wherein 10% or more of the modified immune cells in a population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻² μ M in the co-culture, wherein the antigen-presenting cells optionally comprise T2 cells, Jurkat cells, or both.

10. The modified immune cell of claim 9, wherein 15% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻² μ M in the co-culture.

11. The modified immune cell of claim 9 or 10, wherein 20% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻² μM in the co-culture.

12. The modified immune cell of any one of claims 9-11, wherein 40% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻² μM in the co-culture.

13. The modified immune cell of any one of claims 9-12, wherein 50% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻² μM in the co-culture.

14. The modified immune cell of any one of claims 1-13, wherein 10% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻³ μM in the co-culture.

15. The modified immune cell of claim 14, wherein 15% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO.:94) at a peptide concentration of 10⁻³ μM in the co-culture.

16. The modified immune cell of claim 14 or 15, wherein 20% or more of the modified immune cells in the population are Nur77⁺ when the population of the modified immune cells is co-cultured with antigen-presenting cells pulsed with a peptide comprising or consisting of the amino acid sequence RMFPNAPYL (SEQ ID NO: 94) at a peptide concentration of about 10⁻³ μM in the co-culture.

17. The modified immune cell of any one of claims 1-16, wherein the modified immune cell is capable of killing 11% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

18. The modified immune cell of claim 17, wherein the modified immune cell is capable of killing 12% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

19. The modified immune cell of claim 17 or 18, wherein the modified immune cell is capable of killing 14% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

20. The modified immune cell of any one of claims 17-19, wherein the modified immune cell is capable of killing 15% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

21. The modified immune cell of any one of claims 17-20, wherein the modified immune cell is capable of killing 20% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

22. The modified immune cell of any one of claims 17-21, wherein the modified immune cell is capable of killing 25% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 30:1.

23. The modified immune cell of any one of claims 1-22, wherein the modified immune cell is capable of killing 10% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 10:1.

24. The modified immune cell of any one of claims 1-23, wherein the modified immune cell is capable of killing about 5% or more of HLA-A*0201⁺ MDA-MB-468 cells in a sample when the modified immune cell and the MDA-MB-468 cells are present in the sample at a ratio of 1:1.

25. The modified immune cell of any one of claims 1-24, wherein the modified immune cell is capable of killing 21% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

26. The modified immune cell of claim 25, wherein the modified immune cell is capable of killing 22% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

27. The modified immune cell of claim 25 or 26, wherein the modified immune cell is capable of killing 23% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

28. The modified immune cell of any one of claims 25-27, wherein the modified immune cell is capable of killing 24% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

29. The modified immune cell of any one of claims 25-28, wherein the modified immune cell is capable of killing 25% or more of Panc1 cells in a sample when the modified immune cell and the Panc1 cells are present at a ratio of 30:1.

30. The modified immune cell of any one of claims 1-29, wherein the modified immune cell has increased killing activity against HLA-A*0201⁺ MDA-MB-468 cells and/or against Panc1 cells in a 4-hour co-culture as compared to a reference immune cell comprising a polynucleotide that encodes a T cell receptor comprising a TCR α chain having the amino acid sequence set forth in SEQ ID NO.:82 and a TCR β chain having the amino acid sequence set forth in SEQ ID NO.:83.

31. The modified immune cell of any one of claims 1-30, wherein the encoded binding protein is capable of binding to a WT-1 peptide:HLA complex on a cell surface independent of CD8 or in the absence of CD8.

32. The modified immune cell of any one of claims 1-31, wherein:

(i) the encoded V β domain comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence of any one of SEQ ID NOs.:16, 5, 6, 7, 8, 13, 14, or 15; and/or

(ii) the encoded V α domain comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence of any one of SEQ ID NOs.:12, 1, 2, 3, 4, 9, 10, or 11.

33. The modified immune cell of any one of claims 1-32, wherein the encoded V β domain comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs.: 16, 5, 6, 7, 8, 13, 14, or 15, and/or the encoded V α domain comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs.:12, 1, 2, 3, 4, 9, 10, or 11, provided that:

(i) at least three or four of the CDRs have no mutations;

(ii) the CDRs that do have mutations have only up to two amino acid substitutions, up to a contiguous five amino acid deletion, or a combination thereof; and

(iii) the encoded binding protein retains its ability to bind to a RMFPNAPYL (SEQ ID NO.:94):HLA complex, optionally with an interferon-gamma (IFN γ) production pEC50 of 4.0 or higher.

34. The modified immune cell of any one of claims 1-33, wherein:

(i) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO.:40, and the encoded CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO.:28;

(ii) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 31, and the encoded CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 19;

(iii) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 34, and the encoded CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 22; or

(iv) the encoded CDR3 β comprises or consists of the amino acid sequence set forth in SEQ ID NO: 37, and the encoded CDR3 α comprises or consists of the amino acid sequence set forth in SEQ ID NO: 25.

35. The modified immune cell of any one of claims 1-34, wherein:

(i) the encoded V β domain comprises the CDR1 β amino acid sequence of any one of SEQ ID NOs.:38, 29, 32, or 35 and/or the CDR2 β amino acid sequence of any one of SEQ ID NOs.: 39, 30, 33, or 36; and/or

(ii) the encoded V α domain comprises the CDR1 α amino acid sequence of any one of SEQ ID NOs.:26, 17, 20, or 23, and/or the CDR2 α amino acid sequence of any one of SEQ ID NOs.:27, 18, 21, or 24.

36. The modified immune cell of any one of claims 1-35, wherein the encoded TCR V α and V β domains comprise CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of:

(i) SEQ ID NOs.:26-28 and 38-40, respectively;

(ii) SEQ ID NOs.:23, 27, 28, and 38-40, respectively;

- (iii) SEQ ID NOs.: 17-19 and 29-31, respectively;
- (iv) SEQ ID NOs.: 20-22 and 32-34, respectively; or
- (v) SEQ ID NOs.: 23-25 and 35-37, respectively.

37. The modified immune cell of any one of claims 1-36, wherein the encoded TCR V β domain comprises:

- (i) an amino acid sequence according to a TRBJ02-03 gene segment; and/or
- (ii) an amino acid sequence according to a TRBV06-05 gene segment, an amino acid sequence according to a TRBV07-09 gene segment, or an amino acid sequence according to a TRBV20-01 gene segment.

38. The modified immune cell of any one of claims 1-37, wherein the encoded TCR V α domain comprises:

- (i) an amino acid sequence according to a TRAJ43 gene segment; and/or
- (ii) an amino acid sequence according to a TRAV20-02 gene segment; an amino acid sequence according to a TRAV38DV08 gene segment; or an amino acid sequence according to a TRAV38-01 gene segment.

39. The modified immune cell of claim 38, wherein:

- (i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBJ02-03 gene segment; and
- (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAJ43 gene segment.

40. The modified immune cell of claim 38, wherein:

- (i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBV06-05 gene segment; and
- (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAV20 gene segment.

41. The modified immune cell of claim 38, wherein:

- (i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBV07-09 gene segment; and
- (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAV38DV08 gene segment.

42. The modified immune cell of claim 38, wherein:

- (i) the encoded TCR V β domain comprises an amino acid sequence according to a TRBV20-01 gene segment; and
- (ii) the encoded TCR V α domain comprises an amino acid sequence according to a TRAV38-01 gene segment.

43. The modified immune cell of any one of claims 1-42, wherein:

- (i) the encoded V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:16 or 8, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:12 or 4;
- (ii) the encoded V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:13 or 5, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:9 or 1;
- (iii) the encoded V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:14 or 6, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:10 or 2; or
- (iv) the encoded V β domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:15 or 7, and the encoded V α domain comprises or consists of the amino acid sequence set forth in SEQ ID NO.:11 or 3.

44. The modified immune cell of any one of claims 1-43, wherein the encoded binding protein further comprises:

- (i) a TCR α chain constant domain (C α), or a fragment thereof; and/or
- (ii) a TCR β chain constant domain (C β), or a fragment thereof.

45. The modified immune cell of claim 44, wherein the encoded C α comprises or consists of an amino acid sequence having at least 90% identity to any one of SEQ ID NOs.:41-44.

46. The modified immune cell of claim 44 or 45, wherein the encoded C β comprises or consists of an amino acid sequence having at least 90% identity to SEQ ID NO.:45.

47. The modified immune cell of any one of claims 44-46, wherein the encoded binding protein comprises:

(i) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:61 or 57, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:53 or 49;

(ii) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:58 or 54, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:50 or 46;

(iii) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:59 or 55, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:51 or 47; or

(iv) a TCR β chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:60 or 56, and a TCR α chain having at least 90% identity to, comprising, or consisting of the amino acid sequence set forth in SEQ ID NO.:52 or 48.

48. The modified immune cell of any one of claims 1-47, wherein the encoded binding protein is a TCR, a chimeric antigen receptor (CAR), or a single-chain TCR (scTCR).

49. The modified immune cell of any one of claims 1-48, wherein the immune cell is a human immune system cell.

50. The modified immune cell of any one of claims 1-49, wherein the immune cell is a T cell, optionally a CD4⁺ T cell, a CD8⁺ T cell, a CD4⁺CD8⁺ double negative T cell, or a $\gamma\delta$ T cell, a natural killer cell, a NK-T cell, a dendritic cell, or any combination thereof.

51. The modified immune cell of claim 50, wherein the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, a stem cell memory T cell, or any combination thereof.

52. The modified immune cell of any one of claims 1-51, wherein the polynucleotide encoding the binding protein is codon-optimized for expression in the immune cell.

53. The modified immune cell of any one of claims 1-52, wherein the polynucleotide encoding the binding protein comprises a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs.:62-77.

54. The modified immune cell of claim 53, wherein the polynucleotide encoding the binding protein comprises:

(i) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:77, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:69;

(ii) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:74, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:66;

(iii) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:75, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:67; or

(iv) a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:76, and a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in SEQ ID NO.:68.

55. The modified immune cell of any one of claims 1-54, wherein the polynucleotide encoding a binding protein further comprises a polynucleotide encoding a self-cleaving peptide disposed between the V β -encoding polynucleotide and the V α -encoding polynucleotide.

56. The modified immune cell of claim 55, wherein the encoded self-cleaving peptide comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:84-88.

57. The modified immune cell of claim 55 or 56, wherein the polynucleotide encoding a self-cleaving peptide comprises or consists of a polynucleotide sequence having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs.:89-93.

58. The modified immune cell of any one of claims 55-57, wherein the polynucleotide encoding a binding protein has a structure from 5'-end to 3'-end of (TCR β chain-encoding polynucleotide)-(self-cleaving peptide-encoding polynucleotide)-(TCR α chain-encoding polynucleotide).

59. The modified immune cell any one of claims 55-58, wherein the polynucleotide encoding a binding protein comprises a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs.:81 or 78-80.

60. The modified immune cell of claim 53, wherein the polynucleotide encoding a binding protein comprises a polynucleotide comprising or consisting of the polynucleotide sequence set forth in any one of SEQ ID NOs.:81 or 78-80.

61. The modified immune cell of any one of claims 1-60, further comprising:

(i) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor α chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain;

(ii) a heterologous polynucleotide encoding a polypeptide that comprises an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; or

(iii) the polynucleotide of (i) and the polynucleotide of (ii), wherein, optionally, the host cell comprises a CD4⁺ T cell.

62. The host cell of claim 61, comprising:

(a) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor α chain wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor α chain;

(b) the heterologous polynucleotide encoding a polypeptide comprising an extracellular portion of a CD8 co-receptor β chain, wherein, optionally, the encoded polypeptide is or comprises a CD8 co-receptor β chain; and

(c) a polynucleotide encoding a self-cleaving peptide disposed between the polynucleotide of (a) and the polynucleotide of (b).

63. The modified immune cell of any one of claims 1-62, wherein the immune cell comprises a chromosomal gene knockout of a PD-1 gene; a LAG3 gene; a TIM3 gene; a CTLA4 gene; an HLA component gene; a TCR component gene; or any combination thereof.

64. The modified immune cell of claim 63, wherein the chromosomal gene knockout comprises a knockout of an HLA component gene selected from an $\alpha 1$ macroglobulin gene; an $\alpha 2$ macroglobulin gene; an $\alpha 3$ macroglobulin gene; a $\beta 1$ microglobulin gene; or a $\beta 2$ microglobulin gene; or any combination thereof.

65. The modified immune cell of claim 63 or 64, wherein the chromosomal gene knockout comprises a knockout of a TCR component gene selected from a TCR α variable region gene; a TCR β variable region gene; a TCR constant region gene; or any combination thereof.

66. An isolated polynucleotide encoding a binding protein, wherein the encoded binding protein comprises a TCR V α domain and a TCR V β domain and is capable of binding to a RMFPNAPYL (SEQ ID NO.:94):HLA complex, wherein the HLA optionally comprises HLA-A*0201, and wherein:

(i) the encoded V α domain comprises the CDR3 amino acid sequence set forth in any one of SEQ ID NOs.:28, 19, 22, or 25;

(ii) the encoded V β domain comprises the CDR3 amino acid sequence set forth in any one of SEQ ID NOs.:40, 31, 34, or 37;

(iii) the encoded V α domain comprises the CDR1 amino acid sequence set forth in any one of SEQ ID NOs.:26, 17, 20, or 23;

(iv) the encoded V β domain comprises the CDR1 amino acid sequence set forth in any one of SEQ ID NOs.:38, 32, or 35;

(v) the encoded V α domain comprises the CDR2 amino acid sequence set forth in any one of SEQ ID NOs.:27, 18, 21, or 24;

(vi) the encoded V β domain comprises the CDR2 amino acid sequence set forth in any one of SEQ ID NO.:39, 30, 33, or 36;

(vii) the encoded V α domain comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:4, 1-3, 12, or 9-11;

(viii) the encoded V β domain comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:8, 5-7, 16, or 13-15;

(ix) the encoded TCR V α is comprised in a TCR α chain, wherein the TCR α chain comprises or consists of an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:53 and 46-52;

(x) the encoded TCR V β is comprised in a TCR β chain, wherein the TCR β chain an amino acid sequence comprising, consisting of, or having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:61 and 54-60; or

(xi) any combination of (i)-(x).

67. The isolated polynucleotide of claim 66, wherein the polynucleotide is codon-optimized for expression in a host cell, wherein, optionally, the host cell is a human T cell, NK cell, or NK-T cell.

68. The isolated polynucleotide of claim 66 or 67, wherein the polynucleotide comprises a polynucleotide having at least 75% identity to the polynucleotide sequence set forth in any one of SEQ ID NOs: 62-81.

69. A vector, comprising the polynucleotide of any one of claims 66-68.

70. The vector of claim 69, wherein the vector is a lentiviral vector or a retroviral vector.

71. A host cell, comprising the polynucleotide of any one of claims 66-68 and capable of expressing the encoded binding protein.

72. A composition, comprising:

- (i) the modified immune cell of any one of claims 1-65;
- (ii) the polynucleotide of any one of claims 66-68;
- (iii) the vector of claim 69 or 70; and/or

- (iv) the host cell of claim 71;
and a pharmaceutically acceptable carrier, excipient, or diluent.

73. The composition of claim 72, comprising:

- (i) the modified immune cell of any one of claims 1-65; and
- (ii) an immune cell comprising a polynucleotide that encodes a binding protein that comprises a TCR V α and a TCR V β and is capable of specifically binding to a VLDFAPPGA (SEQ ID NO.:100):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201, and wherein, optionally, the modified immune cell of (i) and the immune cell of (ii) are each independently selected from a T cell, a NK cell, and NK-T cell.

74. The composition of claim 73, wherein the binding protein of the immune cell in (ii) comprises:

- (a) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:101-103 and 105-107, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:104 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:108;
- (b) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:109-110 and 113-115, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:112 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:116; or
- (c) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:117-119 and 121-123, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:120 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:124.

75. A method for treating a subject having a disease or disorder associated with WT-1 expression, comprising administering to the subject an effective amount of: (i) the modified immune cell of any one of claims 1-65; (ii) the polynucleotide of any one of claims 66-68; (iii) the vector of claim 69 or 70; (iv) the host cell of claim 71; and/or (v) the composition of any one of claims 72-74.

76. The method of claim 75, comprising administering the modified immune cell of any one of claims 1-65 to a subject who has received, is receiving, or will receive an immune cell comprising a polynucleotide that encodes a binding protein that comprises a TCR V α and a TCR V β and is capable of specifically binding to a VLDFAPPGA (SEQ ID NO.:100):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201, and wherein, optionally, the modified immune cell and the immune cell are each independently selected from a T cell, a NK cell, and NK-T cell.

77. The method of claim 76, wherein the binding protein that is capable of specifically binding to the VLDFAPPGA (SEQ ID NO.:100):HLA complex comprises:

(a) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:101-103 and 105-107, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:104 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:108;

(b) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:109-110 and 113-115, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:112 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:116; or

(c) CDR1 α , CDR2 α , CDR3 α , CDR1 β , CDR2 β , and CDR3 β amino acid sequences of SEQ ID NOs.:117-119 and 121-123, respectively, and wherein, optionally, (1) the V α has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:120 and/or (2) the V β has at least 90% identity to, comprises, or consists of the amino acid sequence set forth in SEQ ID NO.:124.

78. The method of any one of claims 75-77, wherein the disease or disorder is a hematological malignancy or a solid cancer.

79. The method of claim 78, wherein the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloid leukemia (AML, including refractory and relapsed AML, and including acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelocytic leukemia, acute myelomonocytic leukemia (*e.g.*, with or without eosinophilia), acute monocytic leukemia, acute erythroid leukemia, and acute megakaryoblastic leukemia), chronic myelogenous leukemia (CML), chronic myelocytic leukemia, chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM, including refractory and relapsed MM).

80. The method of claim 78, wherein the solid cancer is selected from biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, breast carcinoma, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma multiforme, glioblastoma, melanoma, diffuse peritoneal mesothelioma, malignant pleural mesothelioma, glioma, astrocytoma, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, hepatocellular carcinoma, lung cancer, non small-cell lung cancer, malignant melanoma, osteosarcoma, ovarian cancer (*e.g.*, epithelial ovarian cancer, ovarian carcinoma), fallopian tube cancer, endometrial carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, osteogenic sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, uterine carcinosarcoma, or uterine cancer.

81. The method according to any one of claims 75-80, wherein the modified immune cell is modified *ex vivo* to comprise the heterologous polynucleotide.

82. The method according to any one of claims 75-81, wherein the modified immune cell is allogeneic, syngeneic, or autologous to the subject.

83. The method according to any one of claims 75-82, wherein the modified immune cell is a hematopoietic progenitor cell or a human immune system cell.

84. The method according to claim 83, 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 dendritic cell, or any combination thereof.

85. The method according to claim 84, wherein the T cell is a naïve T cell, a central memory T cell, an effector memory T cell, or any combination thereof.

86. The method according to any one of claims 75-85, wherein the method comprises administering a plurality of doses of the modified immune cell to the subject.

87. The method according to claim 86, wherein the plurality of doses are administered at intervals between administrations of about two to about four weeks.

88. The method according to any one of claims 75-87, wherein the modified immune cell is administered to the subject at a dose of about 10⁴ cells/kg to about 10¹¹ cells/kg.

89. The method according to any one of claims 75-88, wherein the method further comprises administering a cytokine to the subject.

90. The method according to claim 89, wherein the cytokine is IL-2, IL-15, IL-21, or any combination thereof.

91. The method according to claim 90, wherein the cytokine is IL-2 and is administered concurrently or sequentially with the modified immune cell.

92. The method according to claim 91, wherein the cytokine is administered sequentially, provided that the subject was administered the modified immune cell at least three or four times before cytokine administration.

93. The method according to any one of claims 89-92, wherein the cytokine is IL-2 and is administered subcutaneously.

94. The method according to any one of claims 75-93, wherein the subject is further receiving immunosuppressive therapy.

95. The method according to claim 94, wherein the immunosuppressive therapy is selected from calcineurin inhibitors, corticosteroids, microtubule inhibitors, low dose of a mycophenolic acid prodrug, or any combination thereof.

96. The method according to any one of claims 75-95, wherein the subject has received a non-myeloablative or a myeloablative hematopoietic cell transplant.

97. The method according to claim 96, wherein the subject is administered the modified immune cell at least three months after the non-myeloablative hematopoietic cell transplant.

98. The method according to claim 97, wherein the subject is administered the modified immune cell at least two months after the myeloablative hematopoietic cell transplant.

99. The method of any one of claims 75-98, wherein the subject has previously received therapy for AML.

100. A composition, comprising:

- (i) the modified immune cell of any one of claims 1-65;
- (ii) the polynucleotide of any one of claims 66-68;

- (iii) the vector of claim 69 or 70; and/or
- (iv) the host cell of claim 71,

for use in the treatment of a disease or disorder associated with WT-1 expression.

101. A composition, comprising:

- (i) the modified immune cell of any one of claims 1-65;
- (ii) the polynucleotide of any one of claims 66-68;
- (iii) the vector of claim 69 or 70; and/or
- (iv) the host cell of claim 71,

for use in the manufacture of a medicament for the treatment of a disease or disorder associated with WT-1 expression.

102. The composition for use according to claim 100 or 101, wherein the disease or disorder is a hematological malignancy or a solid cancer.

103. The composition for use according to claim 102, wherein the hematological malignancy is selected from acute lymphoblastic leukemia (ALL), acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloid leukemia (AML, including refractory and relapsed AML, and including acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelocytic leukemia, acute myelomonocytic leukemia (*e.g.*, with or without eosinophilia), acute monocytic leukemia, acute erythroid leukemia, and acute megakaryoblastic leukemia), chronic myelogenous leukemia (CML), chronic myelocytic leukemia, chronic eosinophilic leukemia (CEL), myelodysplastic syndrome (MDS), non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM, including refractory and relapsed MM).

104. The composition for use according to claim 102, wherein the solid cancer is selected from biliary cancer, bladder cancer, bone and soft tissue carcinoma, brain tumor, breast cancer, breast carcinoma, cervical cancer, colon cancer, colorectal adenocarcinoma, colorectal cancer, desmoid tumor, embryonal cancer, endometrial cancer, esophageal cancer, gastric cancer, gastric adenocarcinoma, glioblastoma

multiforme, glioblastoma, melanoma, diffuse peritoneal mesothelioma, malignant pleural mesothelioma, glioma, astrocytoma, gynecological tumor, head and neck squamous cell carcinoma, hepatic cancer, hepatocellular carcinoma, lung cancer, non small-cell lung cancer, malignant melanoma, osteosarcoma, ovarian cancer (*e.g.*, epithelial ovarian cancer, ovarian carcinoma), fallopian tube cancer, endometrial carcinoma, pancreatic cancer, pancreatic ductal adenocarcinoma, primary astrocytic tumor, primary thyroid cancer, prostate cancer, renal cancer, renal cell carcinoma, rhabdomyosarcoma, skin cancer, soft tissue sarcoma, osteogenic sarcoma, testicular germ-cell tumor, urothelial cancer, uterine sarcoma, uterine carcinosarcoma, or uterine cancer.

105. A binding protein comprising a T cell receptor (TCR) α chain variable ($V\alpha$) domain and a TCR β chain variable ($V\beta$) domain, wherein:

the $V\alpha$ domain comprises:

(i) the CDR3 amino acid sequence ($CDR3\alpha$) according to any one of SEQ ID NOs.:28, 19, 22, or 25, or a variant thereof;

(ii) the CDR1 amino acid sequence ($CDR1\alpha$) of any one of SEQ ID NOs.:26, 17, 20, or 23;

(iii) the CDR2 amino acid sequence ($CDR2\alpha$) of any one of SEQ ID NOs.: 27, 18, 21, or 24;

(iv) an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs.:1-4 or 9-12; or

(v) any combination of (i)-(iv); and

the $V\beta$ domain comprises:

(vi) the CDR3 amino acid sequence ($CDR3\beta$) according to any one of SEQ ID NOs.: 40, 31, 34, or 37, or a variant thereof;

(vii) the CDR1 amino acid sequence ($CDR1\beta$) of any one of SEQ ID NOs.:38, 29, 32, or 35;

(viii) the CDR2 amino acid sequence ($CDR2\beta$) of any one of SEQ ID NOs.:39, 30, 33, or 36;

(ix) an amino acid sequence having at least 90% identity to the amino acid sequence set forth in any one of SEQ ID NOs:5-8 or 13-16; or

(x) any combination of (vi)-(x),

wherein the binding protein is capable of binding to a RMFPNAPYL (SEQ ID NO:94):HLA complex, wherein, optionally, the HLA comprises HLA-A*0201.

106. The binding protein of claim 105, further comprising:

(1) a TCR α chain constant domain (C α), or a fragment thereof; and/or

(2) a TCR β chain constant domain (C β), or a fragment thereof.

107. The binding protein of claim 106, wherein the C α comprises or consists of the amino acid sequence of any one of SEQ ID NOs.:41-44.

108. The binding protein of claim 106 or 107, wherein the C β comprises or consists of the amino acid sequence of SEQ ID NO.:45.

109. An isolated polynucleotide encoding the binding protein of any one of claims 105-108.

110. The polynucleotide of claim 109, which is codon-optimized for expression in a host cell.

111. A modified immune cell, comprising the polynucleotide of any one of claims 109-110, wherein the polynucleotide is heterologous to the immune cell.

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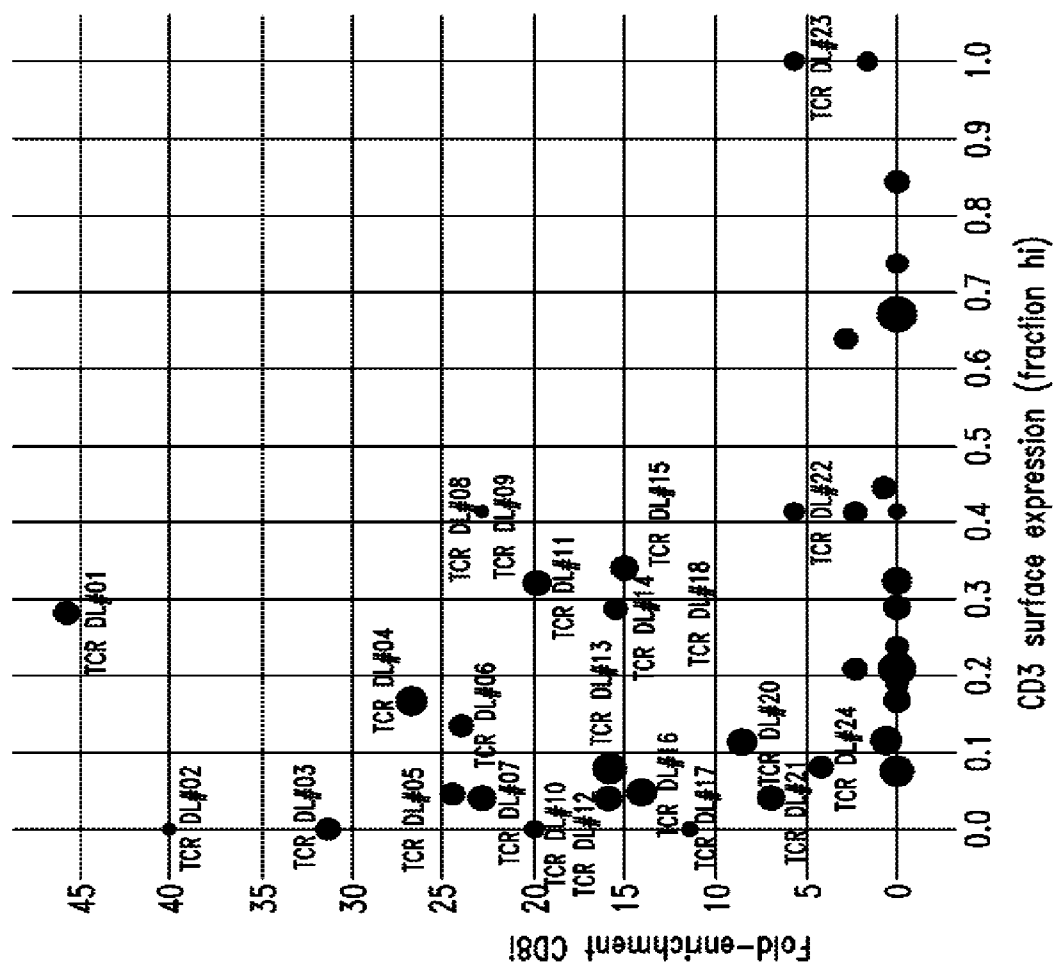


FIG. 1

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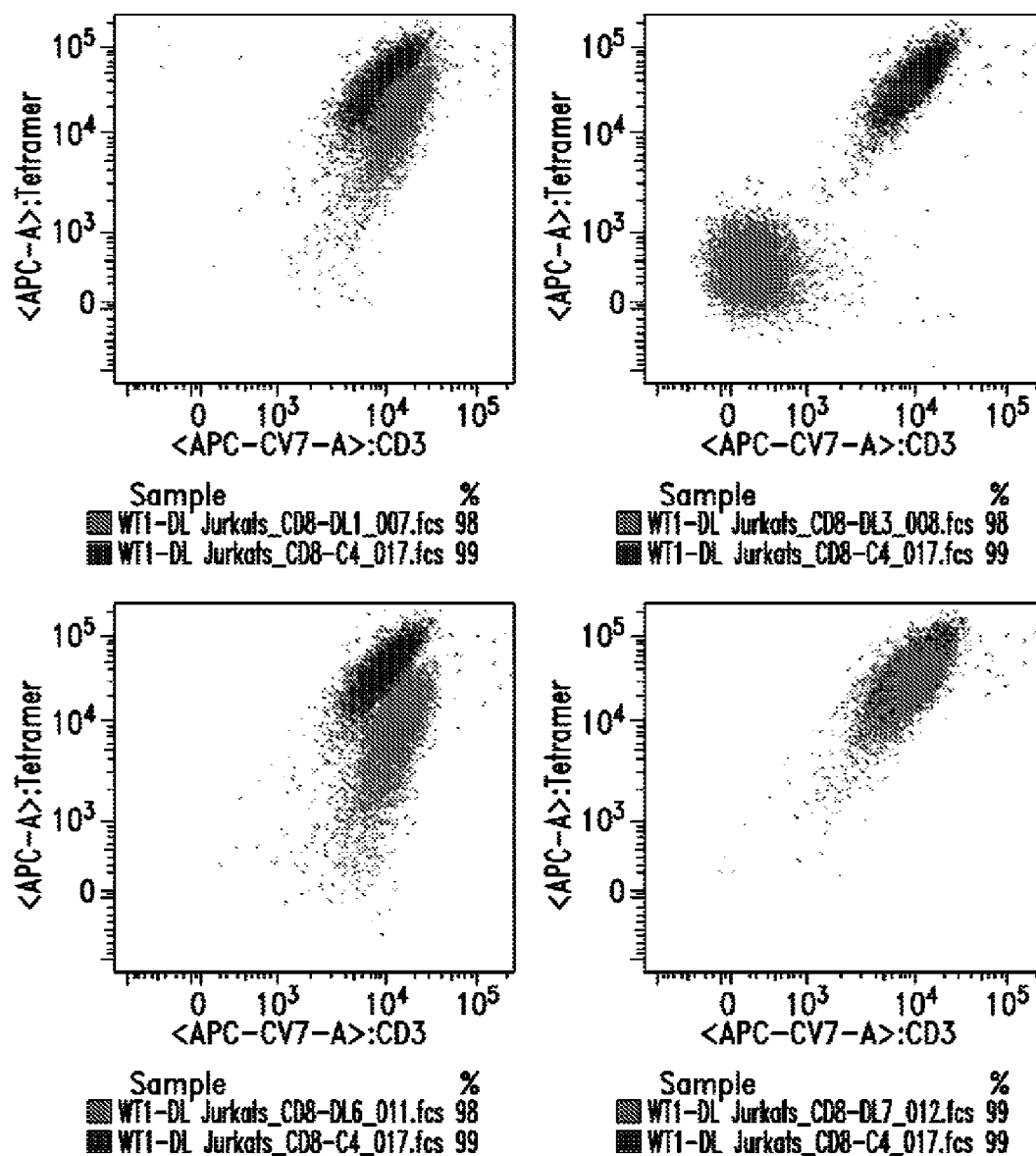


FIG. 2A

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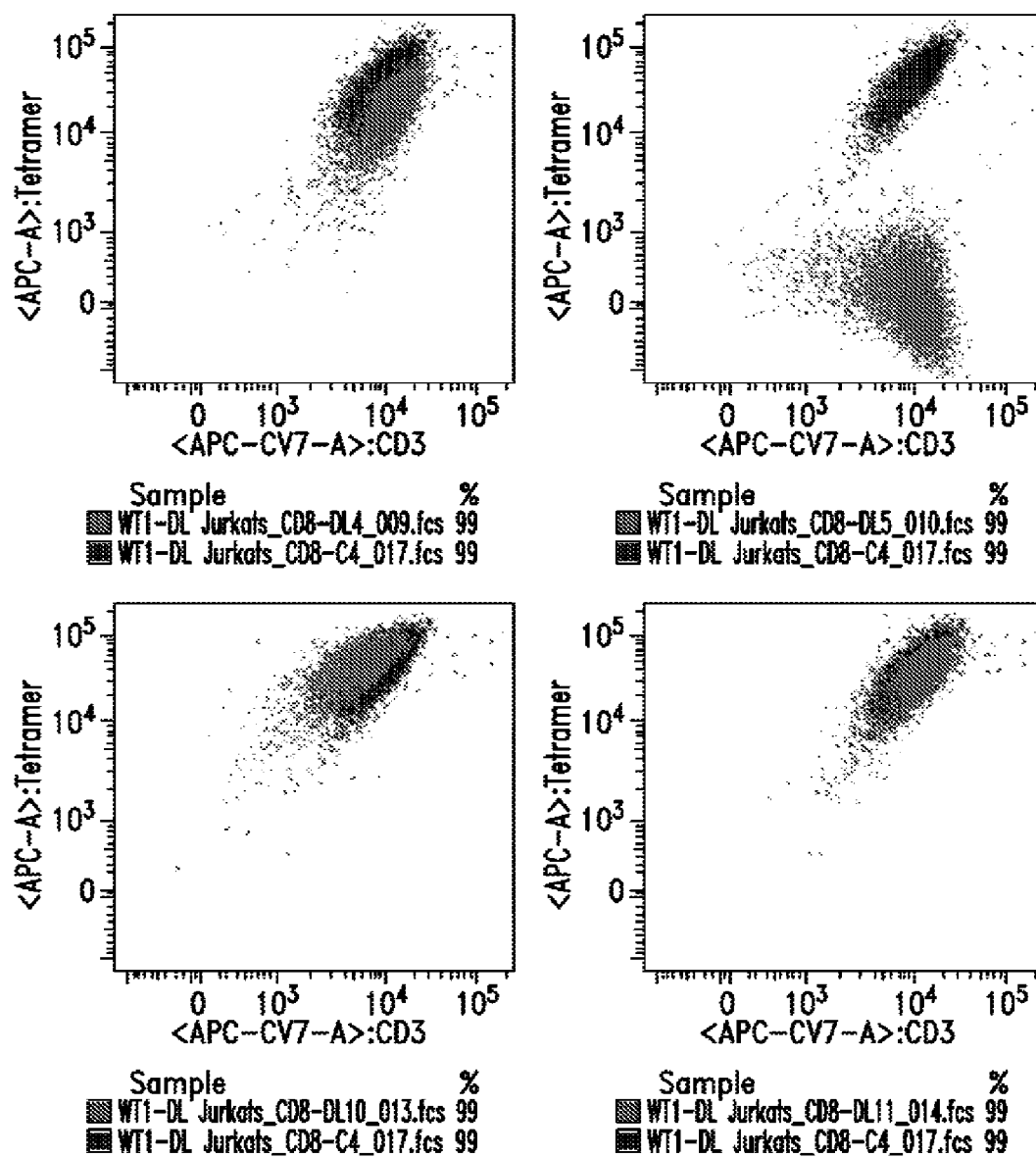


FIG. 2B

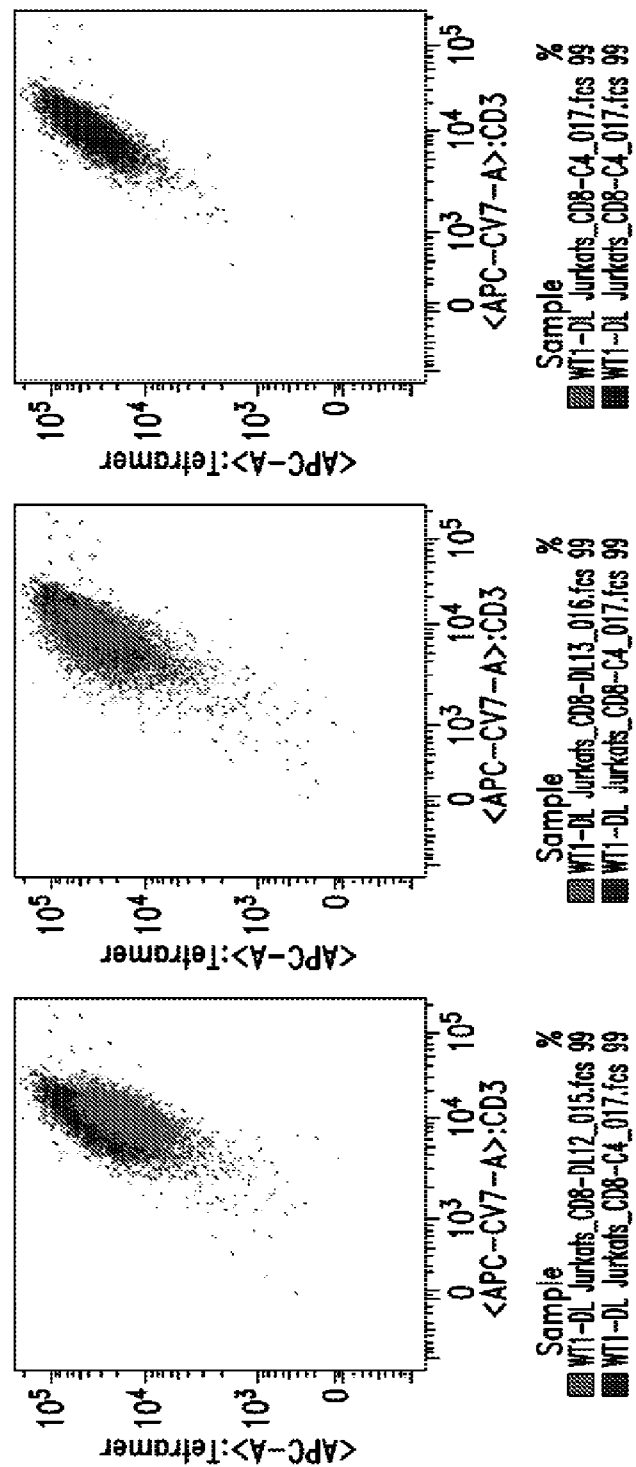


FIG. 2C

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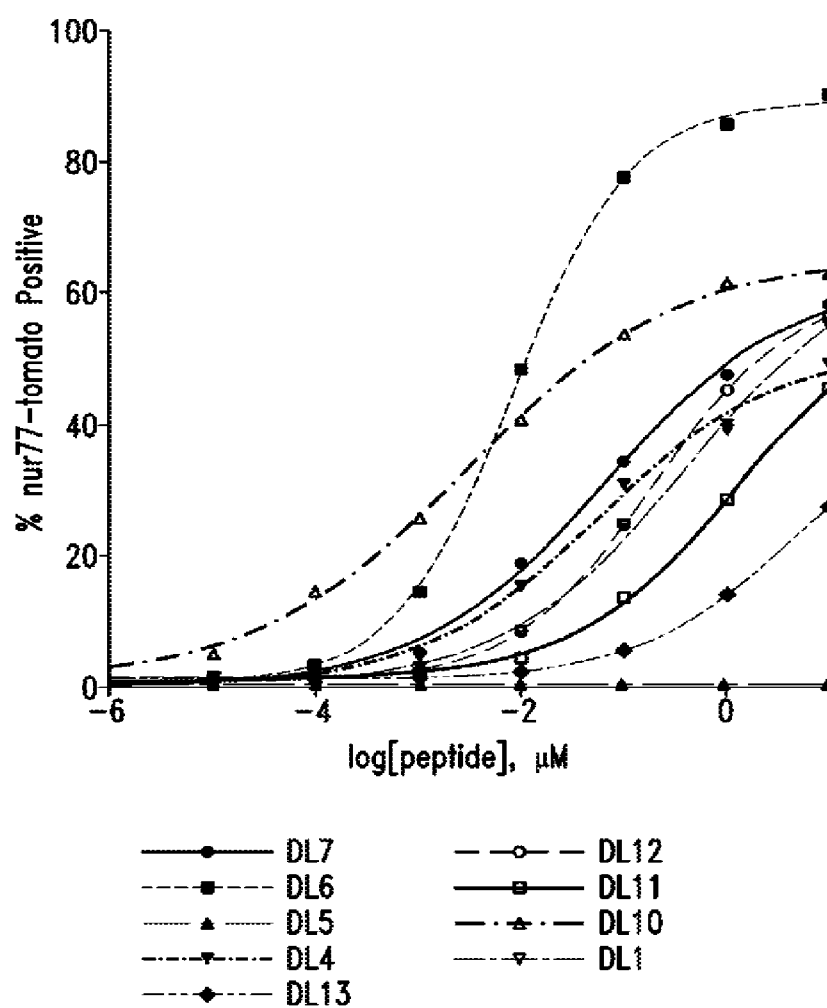
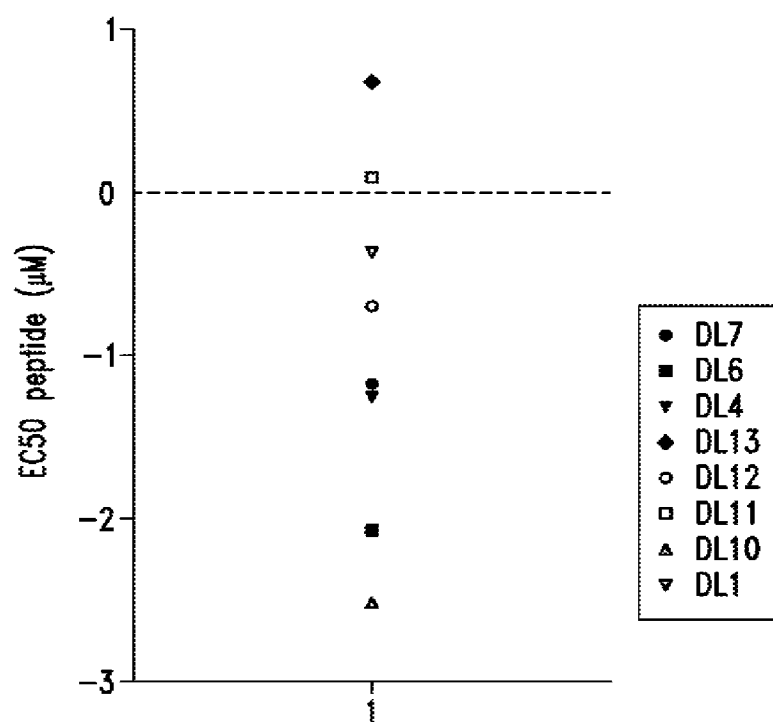


FIG. 3A

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*FIG. 3B*

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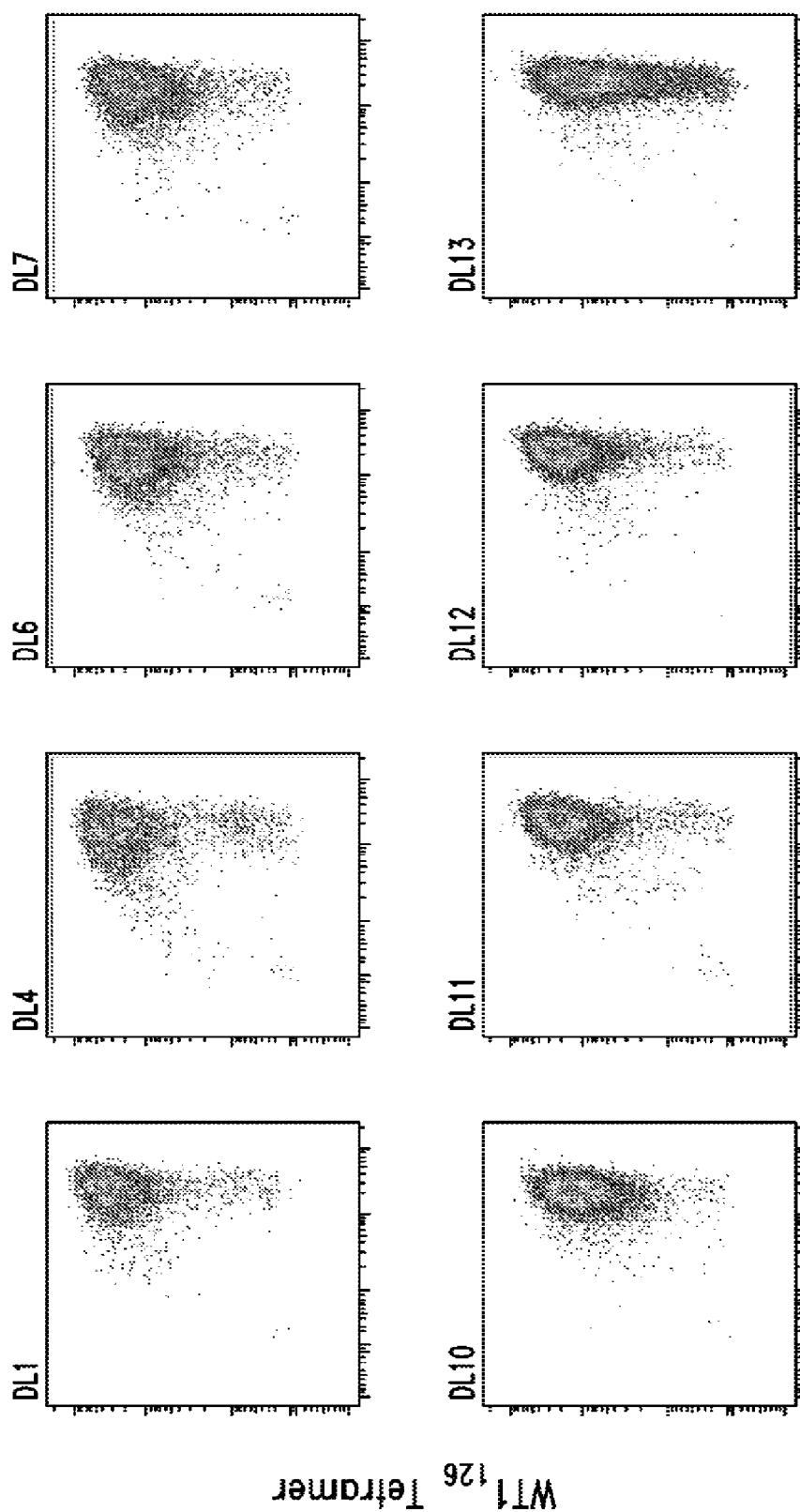
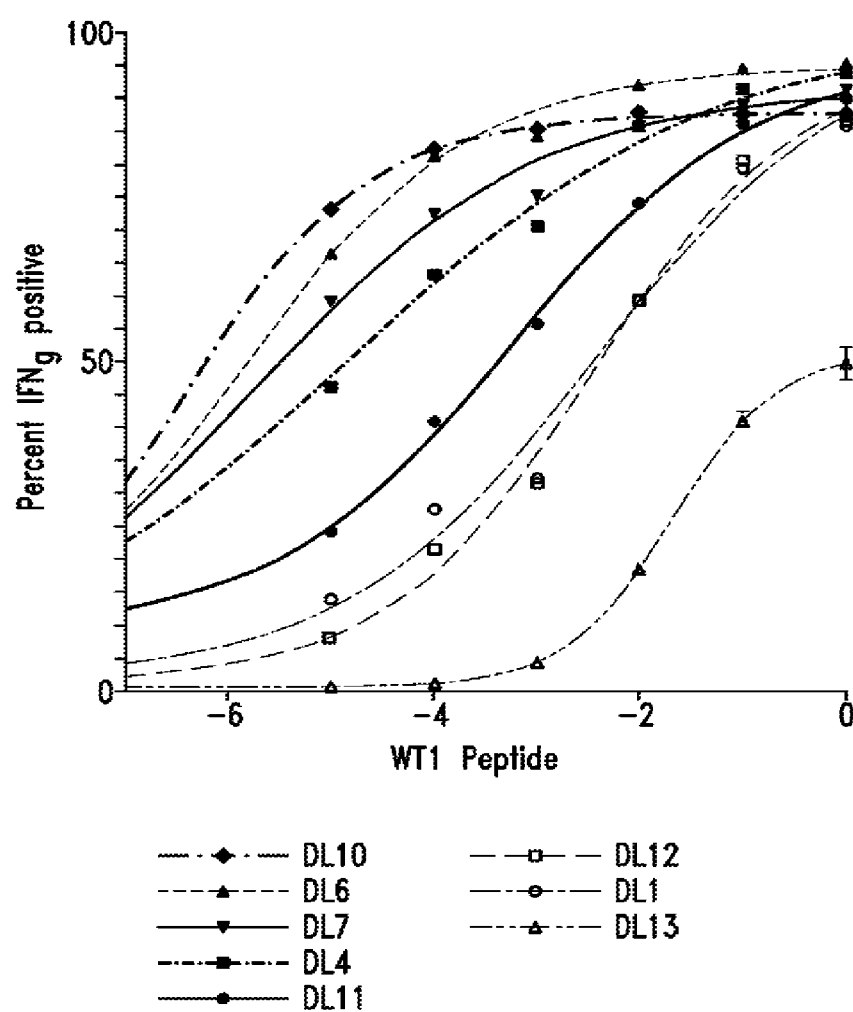
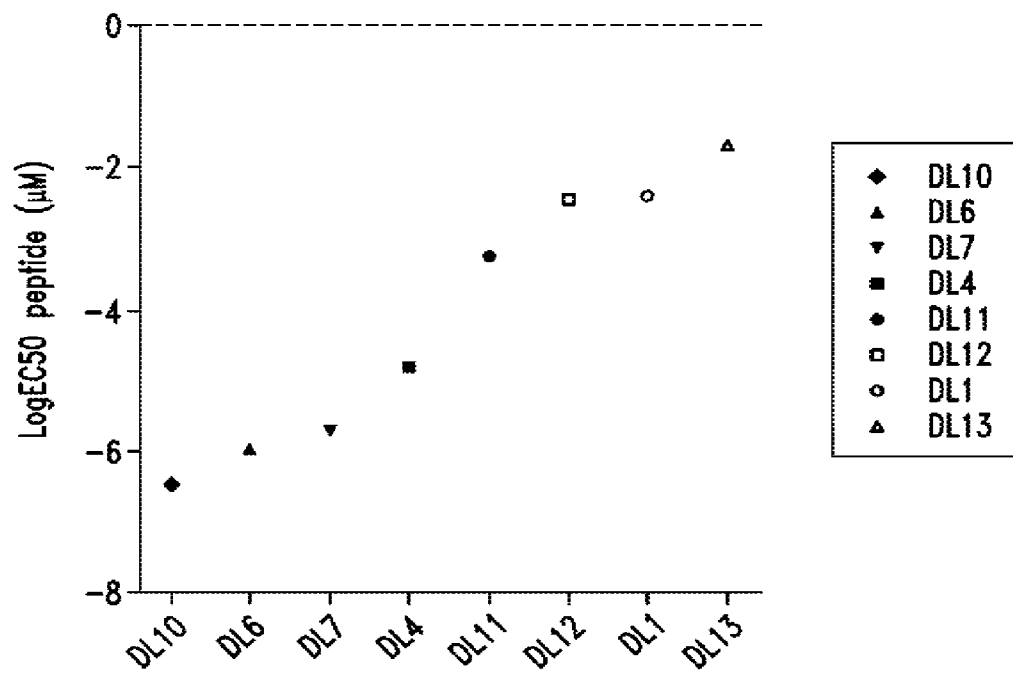


FIG. 4A

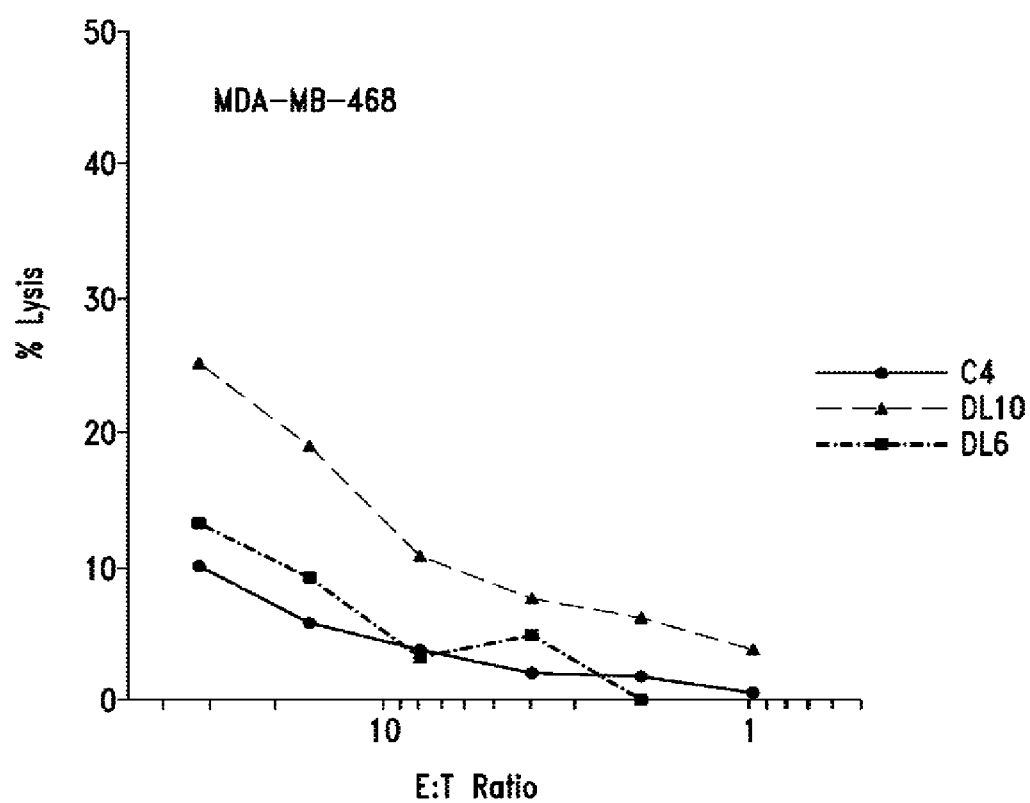
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*FIG. 4B*

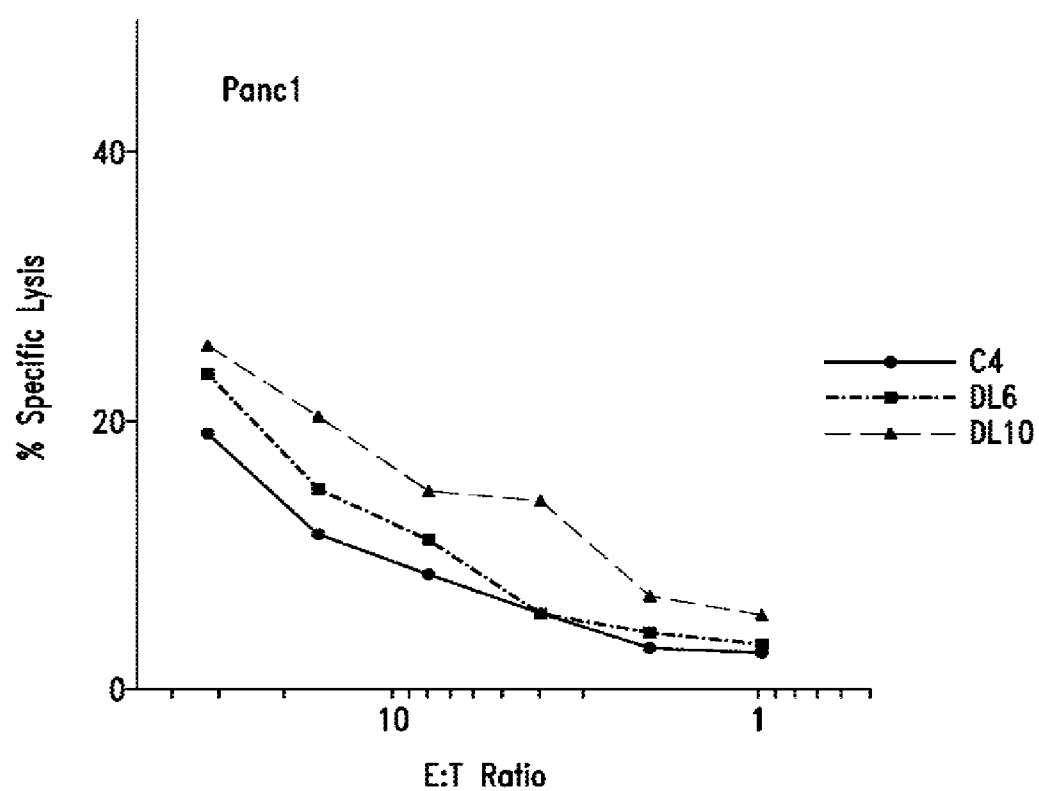
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*FIG. 4C*

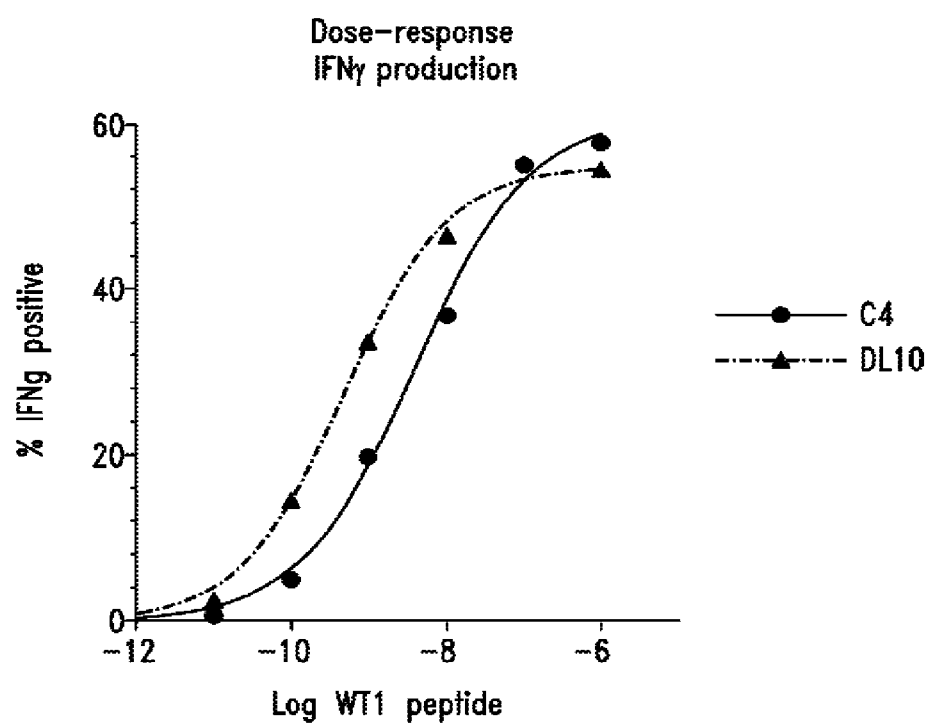
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*FIG. 5A*

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*FIG. 5B*

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*FIG. 6*

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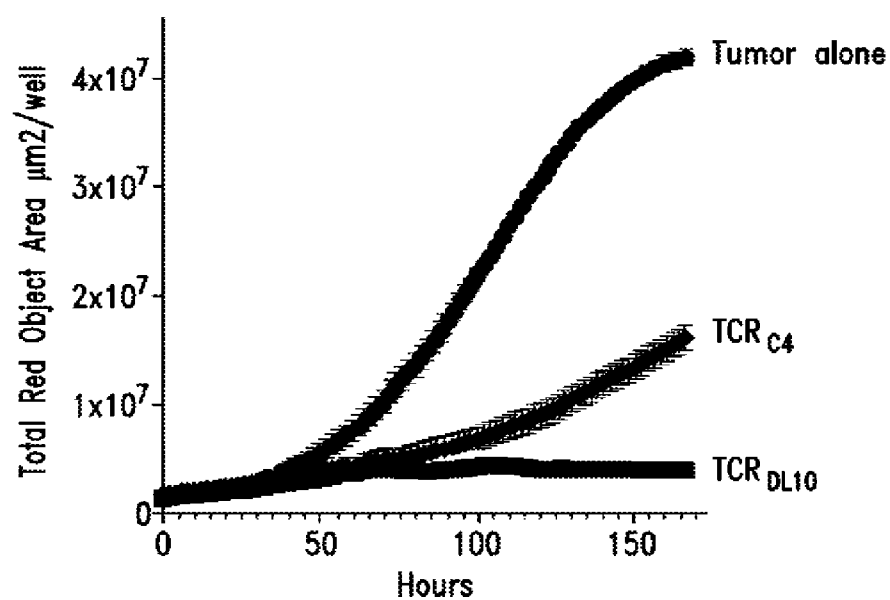


FIG. 7

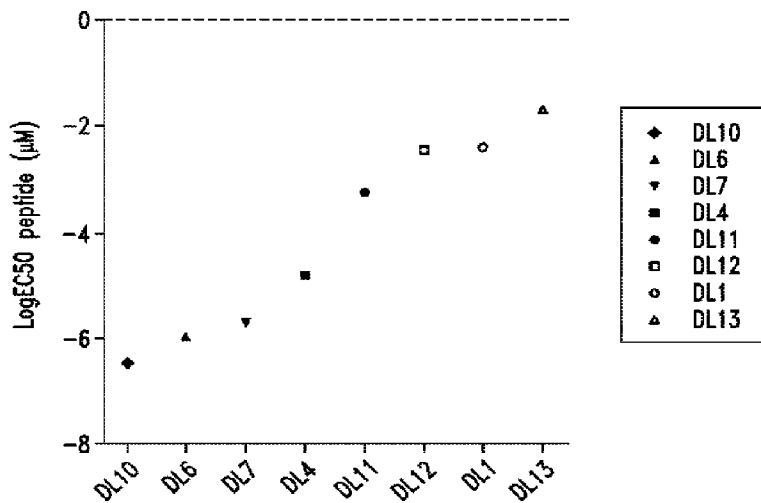


FIG. 4C