

SINGLE-VARIABLE DOMAIN T-CELL RECEPTORS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 63/606,453, filed December 5, 2023, the disclosure of which is incorporated herein by reference in its entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] The present application contains a Sequence Listing in electronic format, which is hereby incorporated by reference in its entirety. The accompanying Sequence Listing file, named 065115-501001WO-US.xml, was created November 26, 2024, and is 70 KB in size.

BACKGROUND

[0003] T-cell receptors (TCRs) are expressed on the surface of T-cells and are responsible for recognizing peptide antigens bound to major histocompatibility complexes (MHCs) on the surface of antigen-presenting cells. When a T-cell receptor engages its cognate peptide-major histocompatibility complex (pMHC), the TCR elicits signals leading to activation of the T-cell. In some instances, T cell therapies have produced clinical responses, including killing of cancer cells. However, barriers to effective T-cell therapy include toxicity, modest anti-cancer activity, and limited tumor infiltration. Further, transduction of exogenous TCR genes into patients has resulted in mispairing of exogenous and endogenous TCR chains. Thus, approaches for improving the efficacy and safety of TCR-based therapy are needed. This disclosure provides solutions to at least some of these problems.

SUMMARY

[0004] Provided herein is a therapeutic, comprising: a single-variable domain (svd)-T cell receptor (TCR) that can bind to a first epitope of a first peptide-MHC complex (pMHC); and an antigen binding domain that can bind to a second epitope; wherein the therapeutic can be soluble. In some embodiments, the antigen binding domain comprises multiple separate polypeptides. In some embodiments, the antigen binding domain comprises a second svd-TCR. In some embodiments, the antigen binding domain comprises a second svd-TCR, an antibody, an antibody fragment, an antibody binding domain or a TCR binding domain. In some embodiments, the antigen binding domain can be a single polypeptide. In some embodiments, the svd-TCR and the antigen binding domain together are a single polypeptide chain. In some embodiments, the svd-TCR and the antigen binding domain can be non-covalently linked together, or wherein the svd-TCR can be non-covalently linked with the antigen binding domain. In some embodiments, the svd-TCR can be covalently linked with the antigen binding domain. In some embodiments, the svd-TCR or the antigen

binding domain can be linked to a scaffold. In some embodiments, the scaffold comprises an antibody fragment crystallizable (Fc) region, an antibody constant domain, a TCR constant domain, albumin, a nanocage, ferritin, or lumazine synthase, or a fragment thereof.

[0005] Provided herein is a therapeutic, comprising: a polypeptide, comprising a first single-variable domain TCR (svd-TCR) that can bind to a first epitope of a first peptide-MHC complex (pMHC), and a second svd-TCR that can bind to a second epitope, wherein the polypeptide can be soluble.

[0006] In some embodiments, the first epitope or the second epitope can be on a cell. In some embodiments, the cell can be a diseased cell. In some embodiments, the disease can be cancer, an autoimmune disease, an infectious disease, or a rare disease. In some embodiments, the first epitope or second epitope can be a cancer antigen, a neoantigen, a viral antigen, or a bacterial antigen. In some embodiments, the second epitope can be the same as the first epitope. In some embodiments, the second epitope can be different from the first epitope. In some embodiments, the second epitope comprises a surface antigen. In some embodiments, the second epitope comprises a non-pMHC surface protein. In some embodiments, the second epitope can be of a second pMHC. In some embodiments, an MHC of the pMHC can be a class I MHC or a class II MHC.

[0007] In some embodiments, any of the therapeutics of the present disclosure can further comprise a T-cell engager or NK-cell engager. In some embodiments, the T-cell engager can be a CD3 engager. In some embodiments, the first or second epitope may not be a superantigen. In some embodiments, the svd-TCR or the antigen binding domain comprises a TCR variable domain. In some embodiments, the TCR variable domain comprises a $V\alpha$, $V\beta$, $V\gamma$, or $V\delta$ variable domain, or a binding fragment thereof. In some embodiments, the TCR variable domain comprises a $V\beta$ variable domain, or a binding fragment thereof. In some embodiments, the TCR variable domain comprises a mammalian variable domain or a binding fragment thereof, or is at least 90% identical to a mammalian variable domain sequence. In some embodiments, the TCR variable domain comprises a non-mammalian variable domain or a binding fragment thereof, or is at least 90% identical to a non-mammalian variable domain sequence.

[0008] In some embodiments, the TCR variable domain of the svd-TCR or the antigen binding domain can be derived from a TRAV, TRBV, TRGV, or TRDV gene. In some embodiments, the TCR variable domain of the svd-TCR or the antigen binding domain can be derived from a TRBV1, TRBV2, TRBV3-1, TRBV3-2, TRBV4-1, TRBV4-2, TRBV4-3, TRBV5-1, TRBV5-2, TRBV5-3, TRBV5-4, TRBV5-5, TRBV5-6, TRBV5-7, TRBV5-8, TRBV6-1, TRBV6-2, TRBV6-3, TRBV6-4, TRBV6-5, TRBV6-6, TRBV6-7, TRBV6-8, TRBV6-9, TRBV7-1, TRBV7-2, TRBV7-3, TRBV7-4, TRBV7-5, TRBV7-6, TRBV7-7, TRBV7-8, TRBV7-9, TRBV8-1, TRBV8-2, TRBV9, TRBV10-1, TRBV10-2, TRBV10-3, TRBV11-1, TRBV11-2, TRBV11-3, TRBV12-1, TRBV12-2, TRBV12-3, TRBV12-4, TRBV12-5, TRBV13, TRBV14, TRBV15, TRBV16, TRBV17, TRBV18, TRBV19, TRBV20-1, TRBV21-1, TRBV22-1, TRBV23-1, TRBV24-1, TRBV25-1, TRBV26, TRBV27, TRBV28, TRBV29-1, or TRBV30 gene or a fragment thereof. In some embodiments,

the svd-TCR includes a TRBJ1-1, TRBJ1-2, TRBJ1-3, TRBJ1-4, TRBJ1-5, TRBJ1-6, TRBJ2-1, TRBJ2-2, TRBJ2-2P, TRBJ2-3, TRBJ2-4, TRBJ2-5, TRBJ2-6, or TRBJ2-7 gene or a fragment or derivative thereof. In some embodiments, the TCR variable domain does not comprise a TCR transmembrane domain or a portion thereof. In some embodiments, the TCR variable domain comprises a portion of constant domain. In some embodiments, the TCR variable domain does not comprise a TCR constant domain or a portion thereof. In some embodiments, the TCR variable domain comprises a CDR of a human, mouse, or macaque or a derivative thereof. In some embodiments, the TCR variable domain comprises a CDR3 having a length of 10-30 amino acids.

[0009] In some embodiments, the svd-TCR or the antigen binding domain comprises a glycosylation site. In some embodiments, the glycosylation site comprises a NX[S/T] amino acid motif, wherein X is any residue except proline. In some embodiments, the glycosylation site comprises an amino acid motif selected from the group consisting of: NYS, NVT, NLT, NLS, NVS, NET, NES, NMS, NFT, or NGT.

[0010] In some embodiments, the TCR variable domain of the svd-TCR or the antigen binding domain comprises a hydrophobic residue mutated to a non-hydrophobic residue relative to a wild type or non-mutated TCR variable domain. In some embodiments, the hydrophobic residue can be from the BetaCON1 region, Alpha1 region, or Alpha2 region shown in any one of **FIG. 7A-7C** or CDR1, CDR2, or CDR3 shown in **FIG. 7D**. In some embodiments, the hydrophobic residue comprises a residue from the BetaCON1 region, Alpha1 region, or Alpha2 region shown in any one of **FIG. 7A-7C** or CDR1, CDR2, or CDR3 shown in **FIG. 7D**. In some embodiments, the hydrophobic residue comprises a residue in a BetaCON1 region, Alpha1 region, or Alpha2 region of **FIG. 7C**.

[0011] In some embodiments, the hydrophobic residue can be an exposed hydrophobic residue. In some embodiments, the exposed hydrophobic residue can come into contact with another TCR variable domain when not in a svd-TCR format. In some embodiments, the exposed hydrophobic residue can be within a V α or a V β of the TCR variable domain. In some embodiments, the exposed hydrophobic residue can be within a β constant domain of the TCR variable domain.

[0012] In some embodiments, the mutation to the non-hydrophobic residue can result in an increase in the hydrophilicity of the therapeutic or a polypeptide of the therapeutic by about 4 to about 8 units, with respect to the Kyte and Doolittle scale, relative to a wild type or non-mutated TCR variable domain. In some embodiments, inclusion of the non-hydrophobic residue can result in an increase in the charge density of the therapeutic or a polypeptide of the therapeutic by about +1 or about -1, relative to a wild type or non-mutated TCR variable domain.

[0013] In some embodiments, the TCR variable domain of the svd-TCR or the antigen binding domain comprises a mutated residue located at a buried surface or core of the svd-TCR or the antigen binding domain, relative to a wild type or non-mutated TCR variable domain. In some embodiments, inclusion of the

mutated residue can result in an increase in production, stability, a specific binding activity, or a functional activity of the svd-TCR or the antigen binding domain.

[0014] In some embodiments, any of the therapeutics in the present disclosure can have a mass of at least 10 kDa, at least 15 kDa, at least 20 kDa, at least 25 kDa, at least 30 kDa, at least 35 kDa, at least 40 kDa, at least 50 kDa, at least 60 kDa, at least 70 kDa, at least 80 kDa, at least 90 kDa, at least 100 kDa, at least 125 kDa, at least 150 kDa, at least 175 kDa, at least 200 kDa, at least 250 kDa, at least 300 kDa, at least 400 kDa, at least 500 kDa, at least 600 kDa, at least 700 kDa, at least 800 kDa, at least 900 kDa, or at least 1000 kDa. In some embodiments, any of the therapeutics in the present disclosure can have a mass of less than 10 kDa, less than 15 kDa, less than 20 kDa, less than 25 kDa, less than 30 kDa, less than 35 kDa, less than 40 kDa, less than 50 kDa, less than 60 kDa, less than 70 kDa, less than 80 kDa, less than 90 kDa, less than 100 kDa, less than 125 kDa, less than 150 kDa, less than 175 kDa, less than 200 kDa, less than 250 kDa, less than 300 kDa, less than 400 kDa, less than 500 kDa, less than 600 kDa, less than 700 kDa, less than 800 kDa, less than 900 kDa, or less than 1000 kDa.

[0015] In some embodiments, the mass can be a molecular mass estimated based on a predicted chemical structure or sequence. In some embodiments, the therapeutic or a polypeptide of the therapeutic can have a molecular mass no greater than 30 kDa. In some embodiments, the svd-TCR or the antigen binding domain can have a molecular mass of about 10-13 kDa.

[0016] In some embodiments, any of the therapeutics of the present disclosure further comprise an additional binding protein. In some embodiments, the additional binding protein can be coupled with the svd-TCR or the antigen binding domain. In some embodiments, the additional binding protein comprises an antibody or binding fragment thereof or a single-chain variable fragment (scFv).

[0017] In some embodiments, any of the therapeutics of the present disclosure can further comprise a third svd-TCR. In some embodiments, any of the therapeutics of the present disclosure can further comprise a fourth svd-TCR. In some embodiments, any of the therapeutics of the present disclosure can further comprise a fifth svd-TCR. In some embodiments, any of the therapeutics of the present disclosure can further comprise a sixth svd-TCR.

[0018] In some embodiments, any of the therapeutics of the present disclosure can further comprise a biologically active group. In some embodiments, the biologically active group can be another peptide or a small molecule conjugate. In some embodiments, the biologically active group can be coupled to a polypeptide of the therapeutic. In some embodiments, the therapeutic can be coupled with a binding protein, an antibody, an antibody binding fragment, a scFv, a therapeutic moiety, a detectable moiety, an immune cell modulator or engager, a checkpoint inhibitor, a biologically active group, an anti-cancer agent, an anti-infection agent, an immune checkpoint inhibitor, a CD3 engager, or a radionuclide.

[0019] In some embodiments, any of the therapeutics of the present disclosure can further comprise a linker connecting the svd-TCR and the antigen binding domain to each other. In some embodiments, any of

the therapeutics of the present disclosure can further comprise a linker connecting the svd-TCR or the antigen binding domain with the biologically active group or binding protein. In some embodiments, any of the therapeutics of the present disclosure can further comprise a linker connecting the svd-TCR or the antigen binding domain with a scaffold.

[0020] In some embodiments, the linker can have a length of 2-30 amino acids or 9-25 amino acids. In some embodiments, the linker comprises a hinge region sequence of an immunoglobulin. In some embodiments, the linker can be a GS linker. In some embodiments, the GS linker comprises GS_n, GGS_n, GGGS_n, or GGGGS_n.

[0021] Provided herein is a pharmaceutical composition comprising: any of the therapeutics of the present disclosure and a pharmaceutically acceptable carrier.

[0022] Further provided herein is a nucleic acid encoding any of the therapeutics of the present disclosure.

[0023] Also provided herein is methods comprising administering any of the therapeutics of the present disclosure to a subject.

[0024] Also provided herein is a kit comprising: any of the therapeutics of the present disclosure; and an instruction.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0026] **FIG. 1A** is schematics illustrating a TCR giving access to fragments of intracellular proteins through pHLA presentation and a larger target space compared to antibodies.

[0027] **FIG. 1B** is schematics illustrating relative size and structure of an example svd-TCR compared to some other targeting polypeptides.

[0028] **FIG. 1C** is schematics illustrating that antigens associated or specifically expressed in cancer are often MHC presented antigens, and increased specificity may sometimes be needed to target cancer associated antigens to limit on-target off-cancer toxicity.

[0029] **FIG. 1D** is schematics illustrating a full size TCR interacting with an MHC peptide (left) and svd-TCR interacting with an MHC peptide (right).

[0030] **FIG. 1E** is schematics illustrating exemplary polypeptides including svd-TCR moieties, including multi-specific and multivalent formats described herein to increase specificity to target tissue without restricting tissue access due to prohibitive size. Options for increasing specificity of targeting include targeting the same pMHC twice and tuning avidity, using different modalities (*e.g.*, polypeptide attached to an antibody or binding fragment thereof), and targeting two different pMHC.

[0031] FIG. 2A is schematics illustrating exemplary TCR structures formed by $\alpha\beta$ or $\gamma\delta$ heterodimers and recognition of TCRs by MHC class I and MHC class II molecules.

[0032] FIG. 2B is schematics illustrating generation of diverse variable domains by recombination. The V region may include three hypervariable complementary-determining regions (CDRs) where binding occurs; CDR1 and CDR2 may come with the V gene and play a role in both interaction and stabilization of the TCR-MHC complex; CDR3 is usually the most variable, may be encoded by VDJ or VJ segments and determine most of the binding specificity of the TCRs to the antigen-MHC complex. The β and δ chain may undergo V(D)J recombination, while the α and γ chain may undergo VJ recombination

[0033] FIG. 3 is schematics illustrating use of multivalent polypeptides to achieve high avidity to reduce on-target off-tissue toxicity by increasing selectivity for high antigen expressing cells. The schematics show that multivalency may allow for combination of multiple low affinity receptors to achieve selectivity for high antigen expressing cells. This can include combining 2 svd-TCRs, 3 svd-TCRs, 4 svd-TCRs, or more svd-TCRs. Multivalency can also be achieved in a therapeutic using 2 or 3 targeting moieties that are not in a single polypeptide.

[0034] FIG. 4A is schematics illustrating targeting two different pMHCs to the same cell that increase specificity of targeting paired with a CD3 engager.

[0035] FIG. 4B is schematics illustrating pairing of a svd-TCR with another modality (*e.g.*, antibody or fragment of antibody) that increase specificity of targeting paired with a CD3 engager.

[0036] FIG. 4C is schematics illustrating targeting the same epitope multiple times that increase specificity of targeting paired with a CD3 engager.

[0037] FIG. 5 is a plot illustrating comparison of CDR3 lengths among human TRBV genes using information from IMGT.com.

[0038] FIG. 6A is plots illustrating CDR3 lengths of various human TRBV genes in combination with some TRBJ genes, any of which may be included in a svd-TCR or other composition herein.

[0039] FIG. 6B is plots illustrating CDR3 lengths of various human TRBV genes in combination with some TRBJ genes, any of which may be included in a svd-TCR or other composition herein.

[0040] FIG. 7A is a hydrophobicity plot of human TRBV genes. Beta constant (BetaCon1) and alpha variable (Alpha1, Alpha2) exposed interfaces which might be useful to mutate to increase stability or solubility of a svd-TCR are highlighted.

[0041] FIG. 7B is a charge plot of human TRBV genes. Beta constant (BetaCon1) and alpha variable (Alpha1, Alpha 2) exposed interfaces which might be useful to mutate to increase stability or solubility of a svd-TCR are highlighted.

[0042] FIG. 7C is sequence alignments illustrating specific amino acid sequences of TRBV regions including exposed beta constant interface and alpha variable interfaces.

[0043] FIG. 7D illustrates all beta variable domain sequences observed in the Protein Database (PDB) with >75% sequence identity to TRBV5-8. These V genes share a high sequence identity with the V gene reported to act as svd, at the exposed interface, any aspect of which may be used in a svd-TCR or other composition herein. Highlighted amino acids may be mutated to, for example, increase stability of a svd-TCR.

[0044] FIG. 8 is schematics illustrating that increased specificity created by multivalent and multi-specific svd-TCR constructs binding to pMHC targets can be paired with a CD3 engager as effector function. Some embodiments include 2, 3, 4, 5, or more svd-TCRs, each of which may target a different MHC peptide complex.

[0045] FIG. 9 is schematics illustrating exemplary polypeptides, including combinations of effectors functions that can be linked to single variable domain (svd) containing polypeptides. Those can include antibodies and fragment thereof, drug conjugate, chemotherapeutic agent, radioisotopes and cytokines. Effector functions may be joined to a scaffold.

[0046] FIG. 10 is schematics illustrating exemplary polypeptide constructs including a plurality of svd-TCR connected by peptide linkers (two middle panels) and a svd-TCR connected to an additional protein binding moiety by a linker (right panel). Examples of approximate sizes of the polypeptide constructs are shown.

[0047] FIG. 11A is schematics illustrating exemplary therapeutics including polypeptides comprising svd-TCRs that may be connected to all or part of a constant domain. Some embodiments include an Fc domain. An effector domain may be included with the therapeutic or as part of the polypeptide.

[0048] FIG. 11B is schematics illustrating exemplary therapeutics including polypeptides comprising svd-TCRs that may be connected to all or part of a constant domain. Some embodiments include an Fc domain. An effector domain may be included with the therapeutic or as part of the polypeptide.

[0049] FIG. 12 is schematics illustrating exemplary therapeutics that include svd-TCRs on separate chains, scaffolds, and an effector protein.

[0050] FIG. 13A illustrates exemplary TRBV genes, some including sites where N-glycosylation is predicted, and some including sites where N-glycosylation is not expected. Predicted N-glycosylation sites are underlined, and sites where N-glycosylation is not expected are shown in italics. Predictions are based on a NGlycNet score.

[0051] FIG. 13B illustrates exemplary TRBJ genes, some including sites where N-glycosylation is predicted, and some including sites where N-glycosylation is not expected. Predicted N-glycosylation sites are underlined, and sites where N-glycosylation is not expected are shown in italics. Predictions are based on a NGlycNet score.

[0052] FIG. 14A is schematics illustrating two svd-TCR-Fc constructs encoding NY-ESO-1 and MAGE-A3-5 generated by fusing an svd-TCR with a human IgG1 Fc segment.

[0053] FIG. 14B is SDS-PAGE illustrating the purity of two svd-TCR-Fc construct encoding NY-ESO-1 and MAGE-A3-5.

[0054] FIG. 15 is graphs illustrating melting temperatures of six structurally distinct control TCR-Fc fusion proteins analyzed by DSF.

[0055] FIG. 16 is a graph illustrating the presence of aggregation based on the data from the SEC-MALS instrument.

[0056] FIG. 17A is schematic illustrating NY-ESO-1 svd-TCR-Fc fusion protein binding to NY-ESO-1 peptide loaded MHC.

[0057] FIG. 17B is a graph illustrating the binding signal of NY-ESO-directed svd-TCR-Fc fusion protein.

[0058] FIG. 18A is schematic illustrating NY-ESO-1 svd-TCR-Fc fusion protein not recognizing MAGE-A3 peptide loaded MHC.

[0059] FIG. 18B is a graph illustrating absence of SPR activity of the NY-ESO-1 svd-TCR-FC fusion protein to MAGE-A3 peptide loaded MHC.

[0060] Certain figures included herein were created using BioRender.com.

DETAILED DESCRIPTION

[0061] The present disclosure describes polypeptides and therapeutics that can include a single-variable domain TCR (svd-TCR) capable of binding to an epitope, such as an epitope on a peptide-MHC complex (pMHC). The polypeptide or therapeutic may also incorporate an additional svd-TCR or a binding protein other than an svd-TCR. In some embodiments, the polypeptide or therapeutic can be soluble. The polypeptide or therapeutic may include any of the structures or sequences shown in FIG. 1A-18B.

[0062] The following descriptions and examples illustrate embodiments of the present disclosure in detail. Although the present disclosure has been described in some details by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications can be practiced within the scope of the appended claims.

[0063] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0064] Although various features of the disclosure can be described in the context of a single embodiment, the features can also be provided separately or in any suitable combination. Conversely, although the present disclosure can be described herein in the context of separate embodiments for clarity, the present disclosure can also be implemented in a single embodiment. It is to be understood that the present disclosure is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are variations and modifications of the present disclosure, which are encompassed within its scope.

I. COMPOSITIONS OF THE DISCLOSURE

[0065] T cell receptor (TCR) is a heterodimeric cell surface protein of the immunoglobulin superfamily that participate in the activation of T cells in response to the binding of an antigen. The TCR complex can consist of TCR α / β chains and CD3 γ / δ / ϵ / ζ subunits, which can associate through hydrophobic interactions (see **FIGS. 1D and 2A** for a full TCR structure). Somatic VDJ recombination enables the generation of distinct TCR α and TCR β chains, and TCR α β heterodimers are generally responsible for antigen recognition by binding to peptide-MHC complexes. CD3 can transmit the TCR-triggered signal through immunoreceptor tyrosine-based activation motifs (ITAMs) in its cytoplasmic tail, but it is generally not directly involved in antigen recognition.

[0066] TCRs are capable of eliciting an immunogenic response from antigens that are indicative of a disease and a critical component of T cell function, enabling T cells to recognize and respond to specific antigens presented by major histocompatibility complex (MHC) molecules on the surface of antigen-presenting cells.

[0067] Single variable domain T cell receptor (svd-TCR) of the present disclosure is a variable domain of a T cell receptor that can specifically bind to an epitope in the absence of a second TCR variable domain. For example, an svd-TCR comprising a β chain variable domain ($V\beta$) can bind to an epitope independent of and/or in the absence of an α chain variable domain ($V\alpha$), and an svd-TCR comprising a $V\alpha$ variable domain can bind to an epitope independent of and/or in the absence of a $V\beta$ variable domain.

[0068] Accordingly, provided herein is, *inter alia*, a composition comprising one or more svd-TCRs configured to bind one or more epitopes. The composition can comprise a therapeutic or polypeptide, which are described below in detail. The composition can also comprise one or more additional components, such as a binding protein (*e.g.*, an antibody or a binding fragment thereof, a single-chain variable fragment (scFv), a checkpoint inhibitor), a detectable moiety, a biologically active group (*e.g.*, another peptide or a small molecule conjugate), or an antibody-drug conjugate.

[0069] Additionally, the present disclosure describes svd-TCRs engineered to function in a conditional manner, activating in response to the presence of any one of multiple distinct target antigens. This design of the svd-TCRs allows for the mitigation of off-tissue toxicity by requiring recognition of at least one of the specified antigens to elicit T-cell activation, thereby enhancing specificity and adaptability in environments where target expression varies. This multi-targeting approach of the svd-TCRs enables targeting of diseased tissues while reducing unintended effects on healthy tissues.

A. Therapeutics and Polypeptides

[0070] Provided herein, *inter alia*, are therapeutics comprising a single-variable domain T cell receptor (svd-TCR) (**FIG. 1D**). In some embodiments, the therapeutic can include a recombinant protein such as a

polypeptide comprising a first svd-TCR and a second svd-TCR. In some embodiments, the therapeutic can be or include a polypeptide. Where the polypeptide is described herein, a broader therapeutic application is also contemplated. The therapeutic or polypeptide can be encoded by a nucleic acid construct. In some embodiments, the therapeutic can include a pharmaceutical composition or a kit. In some embodiments, the therapeutic can be administered to a subject in need thereof.

[0071] Also provided herein, *inter alia*, are polypeptides comprising svd-TCRs capable of binding a target protein (*e.g.*, an epitope (*e.g.*, a cancer antigen, a peptide-MHC complex, etc.) (**FIG. 1C**)). The polypeptides provided herein can selectively binding at least one target epitope. For example, the polypeptide provided herein may be specific for an epitope associated with a disease (*e.g.*, cancer, an autoimmune disease, an infectious disease, or a rare disease) or a cell (*e.g.*, a cancer cell, an infected cell, or an otherwise diseased cell).

[0072] In some embodiments, the therapeutic is the polypeptide. In some embodiments, the therapeutic can include the polypeptide. In some embodiments, the therapeutic can include multiple polypeptides. In some embodiments, the therapeutic and/or polypeptide of the present disclosure can be multi-specific, comprising one or more svd-TCRs. In some embodiments, the therapeutic and/or polypeptide of the present disclosure can be bispecific, comprising two svd-TCRs. In some embodiments, the therapeutic and/or polypeptide of the present disclosure can be tri-specific, comprising two svd-TCRs and a T-cell engager or NK-cell engager. In some embodiments, the T-cell engager can be a CD3 engager.

[0073] Production of the polypeptide provided herein can circumvent difficulties associated with generating T cells expressing endogenous or engineered TCRs specific for an epitope. For example, the polypeptide can be produced using expression systems, such as mammalian or bacterial expression systems, that are commonly employed by those skilled in the art, thereby eliminating the need for isolation and/or expansion of T cells derived from a subject or a donor. In some embodiments, a mammalian expression system can include, but not limited to, HEK293T cells. *In vitro* expanded T cells can exhibit terminal differentiation or reduced stability of the T cell, and co-receptors expressed on the surface of the T cell derived from a subject or a donor may contribute to off-target or non-specific interactions. Thus, in some embodiments, production of the polypeptide (*e.g.*, in a bacterial or mammalian expression system) does not involve the expansion of T cells derived from a donor or subject. In some embodiments, production of the polypeptide does not involve the modification of T cells derived from a donor or subject.

[0074] In some embodiments, use of the polypeptide described herein (*e.g.*, for treatment of a disease such as cancer, an autoimmune disease, an infectious disease, or a rare disease) can offer advantages over the use of T cells expressing endogenous or engineered TCRs. For example, it is contemplated that administration of the polypeptide to a subject can effectively treat the disease (*e.g.*, cancer, an autoimmune disease, an infectious disease, or a rare disease) while minimizing the risk of non-specific activation of proinflammatory

cytokines (*e.g.*, cytokine release syndrome, cytokine storm, cytokine-associated toxicity) that are commonly associated with cell therapies, such as CAR T-cell therapy.

[0075] In some embodiments, the therapeutic comprises a single-variable domain T-cell receptor (svd-TCR) that binds to a first epitope of a first peptide-MHC complex (pMHC) and an antigen binding domain that binds to a second epitope (*e.g.*, see **FIG. 1E**). Peptide-major histocompatibility complex (peptide-MHC complex or pMHC) refer to a complex comprising an epitope (peptide) bound to a multimeric cell surface protein (*e.g.*, MHC) that presents the epitope to immune cells (*e.g.*, T cells). Major histocompatibility complex (MHC) refers to a multimeric cell surface protein complex that binds to an epitope for presentation to immune cells. MHC molecules can include MHC class I (MHC-I) or MHC class II (MHC-II). See **FIG. 2A** for illustration of MHC structures.

[0076] In some embodiments, the therapeutic can be soluble. In some embodiments, the antigen binding domain comprises an antibody, an antibody fragment, an antibody binding domain or a TCR binding domain. In some embodiments, the antigen binding domain can comprise multiple separate polypeptides. In some embodiments, the antigen binding domain can be a single polypeptide.

[0077] In some embodiments, the svd-TCR and the antigen binding domain can be non-covalently linked. In some embodiments, the svd-TCR can be covalently linked to the antigen binding domain. In some embodiments, the svd-TCR and the antigen binding domain together form a single polypeptide chain. In some embodiments, the antigen binding domain can comprise a second svd-TCR.

[0078] In some embodiments, a svd-TCR or antigen binding domain can include or be derived at least in part from a TCR domain. In some embodiments, the antigen binding domain can include or be derived at least in part from an antibody domain. In some embodiments, the therapeutic or polypeptide can comprise a first svd-TCR that binds to a first epitope, can be soluble, and can comprise at least two targeting antigen binding domain.

[0079] In some embodiments, the polypeptide, which can be soluble, can comprise a first single-variable domain TCR (svd-TCR) that binds to a first epitope of a first peptide-MHC complex (pMHC) and a second svd-TCR that binds to a second epitope.

[0080] In some embodiments, the svd-TCR does not comprise multiple polypeptide chains. In some embodiments, the svd-TCR can be a single polypeptide chain. In some embodiments, the svd-TCR comprises a single polypeptide chain. In some embodiments, the svd-TCR binds to an epitope independent of any other variable domain or protein. Illustrative epitopes recognized by svd-TCRs can include peptide:MHC complexes (pMHC complexes).

[0081] In some embodiments, the svd-TCR can engage antigens through complementarity-determining regions (CDRs). For example, when the svd-TCR includes a V α variable domain, it can comprise CDR1 α , CDR2 α , and CDR3 α . Similarly, when the svd-TCR includes a V β variable domain, it can comprise CDR1 β ,

CDR2 β , and CDR3 β . In some embodiments, the V α or V β variable domains of the svd-TCR can be derived from either natural or recombinant sources.

[0082] In some embodiments, in native TCRs, the complementary-determining regions (CDRs) of the TCR α and TCR β chains can be either germline-encoded (in the case of CDR1 and CDR2) or the result of somatic rearrangement (in the case of CDR3). The TCR α gene locus comprises variable (V) and joining (J) gene segments (V α and J α), while the TCR β locus can include variable (V), diversity (D), and joining (J) gene segments (V β , D β , and J β). Accordingly, the TCR α chain can be generated through VJ recombination, whereas the TCR β chain can be generated through VDJ recombination. See **FIG. 2B** for illustration of generating diverse variable domains by recombination.

[0083] In some embodiments, the svd-TCR can further include a constant region. In some embodiments, the constant region can include one or more mutations that stabilizes the svd-TCR.

B. Epitopes and Multivalency

[0084] The polypeptide provided herein can be specific for an epitope associated with a pathogen or a cell such as a specific type of cell. For example, the epitope can be associated with a disease cell. An example of a diseased cell can include a cancer cell or a cell of a subject with an autoimmune disease, an infectious disease, or a rare disease. In some embodiments, the epitope can be associated with an antigen expressed on the surface of a cancer cell. In some embodiments, the epitope can be associated with an antigen expressed by a cancer cell or an antigen expressed at higher levels by a cancer cell compared to a healthy cell. The epitope can be expressed from a gene that is incorrectly expressed (*e.g.*, at higher or lower levels) compared to a healthy control or wild-type cell, or from a gene that is mutated. In some embodiments, the epitope can be associated with a germline cancer gene. For example, the epitope can be associated with a germline cancer antigen. A germline cancer gene can include a genetic mutation inherited from a parent (*e.g.*, a genetic mutation present in the sperm or egg). For example, in a germline cancer gene, the genetic mutation derived from the initial sperm or egg cell can be passed to the embryo. Thus, a germline cancer antigen can include a gene whose expression occurs in germ cells and can therefore be passed to offspring. Mutations occurring in such genes can result in pathogenesis. In some embodiments, the germline cancer antigen can include a germline gene that is expressed or overexpressed in cancer and may or may not be mutated.

[0085] In some embodiments, the epitope can be associated with a tumor-associated antigen, such as one produced from a tumor-differentiated gene). A tumor-differentiated gene can be one that is expressed at higher levels in well-differentiated cancer cells. For example, such genes can be overexpressed in cancer cells that are well-differentiated and retain properties similar to the healthy cells of the tissue from which the cancer cells originated. In some embodiments, the epitope can be associated with a tumor-associated gene.

[0086] In some embodiments, the second epitope of the polypeptide described herein can be the same as the first epitope. For example, both the first and second epitopes can be the same epitope associated with a

cancer antigen. Accordingly, in some embodiments, the polypeptide can be a multivalent polypeptide (FIG. 3). Multivalent polypeptide refers to a polypeptide comprising at least two binding domains (*e.g.*, svd-TCR), where each binding domain binds to an epitope (*e.g.*, a cancer antigen, a pMHC, etc.). The binding domains of a multivalent polypeptide can bind the same epitope. The binding domains can be covalently linked to each other through linkers, such as peptide linkers or chemical linkers.

[0087] In some embodiments, the second epitope can be different from the first epitope. For example, the first epitope is associated with a first cancer antigen, while the second epitope is associated with a second cancer antigen (FIG. 1E). Accordingly, in some embodiments, the polypeptide described herein can be a multi-specific polypeptide. Multi-specific polypeptide refers to a polypeptide comprising at least two binding domains (*e.g.*, svd-TCR), where each of the binding domain simultaneously binds to a distinct epitope (*e.g.*, a cancer antigen, a pMHC, etc.).

[0088] In some embodiments, the second epitope is associated with a second pMHC. In some embodiments, the MHC of the pMHC is a class I MHC or a class II MHC. The MHC of the pMHC can be a class I MHC or a class II MHC. In some embodiments, the MHC of the pMHC can be a class I MHC. In some embodiments, the MHC of the pMHC can be a class II MHC. In some embodiments, the MHC of the first pMHC can be a class I MHC or class II MHC, and the MHC of the second pMHC can also be either a class I MHC or a class II MHC.

[0089] In some embodiments, the first svd-TCR can bind the first epitope with a binding affinity that is at least 0.05-fold, 0.1-fold, 0.2-fold, 0.3-fold, 0.4-fold, 0.5-fold, 0.6-fold, 0.7-fold, 0.8-fold, 0.9-fold, 1-fold, 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, or 2-fold that of a naturally occurring TCR comprising the svd-TCR and a second TCR variable domain. In some embodiments, the second svd-TCR binds the second epitope with a binding affinity that is at least 0.05-fold, 0.1-fold, 0.2-fold, 0.3-fold, 0.4-fold, 0.5-fold, 0.6-fold, 0.7-fold, 0.8-fold, 0.9-fold, 1-fold, 1.1-fold, 1.2-fold, 1.3-fold, 1.4-fold, 1.5-fold, 1.6-fold, 1.7-fold, 1.8-fold, 1.9-fold, or 2-fold that of a naturally occurring TCR comprising the second svd-TCR and a second TCR variable domain.

[0090] In some embodiments, the polypeptide can comprise a plurality of svd-TCRs, wherein the plurality of svd-TCRs binds to the same epitope. Multivalent binding of the plurality of svd-TCRs to the epitope can enhance binding avidity, thereby reducing off-target or non-specific interactions. For example, polypeptides with the plurality of svd-TCRs that binds the same epitope can selectively target cancer cells expressing higher levels of the epitope compared to healthy, non-cancerous cells.

[0091] In some embodiments, the polypeptide can comprise a plurality of svd-TCRs, wherein each svd-TCR of the plurality of svd-TCRs independently binds to a distinct epitope. This configuration allows the polypeptide to target tumors with cancer cells expressing diverse expression patterns of cancer antigens. In

some embodiments, the polypeptide is designed to target tumors containing cancer cells that independently express different antigens. Additionally, the polypeptide can be effective in targeting cancer cells with epitopes that change during disease progression. For example, in some embodiments, the polypeptide can comprise a plurality of svd-TCRs, with each svd-TCR of the plurality of svd-TCRs independently binding to an epitope expressed at different stages of cancer progression.

[0092] An svd-TCR can be configured to bind a specific antigen of interest. Non-limiting exemplary antigens include cancer antigens, neoantigens, viral antigens, and bacterial antigens. In some embodiments, the svd-TCR binds to a cancer antigen. In some embodiments, the svd-TCR binds to a neoantigen. In some embodiments, the svd-TCR binds to a viral antigen. In some embodiments, the svd-TCR binds to a bacterial antigen.

[0093] A superantigen is a protein that can trigger an intense immune response by directly binding to TCRs on T cells and MHC molecules on antigen-presenting cells (APCs). Unlike conventional antigens, which activate specific subsets of T cells, superantigens non-specifically activate a large proportion of T cells, potentially leading to an overwhelming immune reaction. In some embodiments, the first or second epitope is not a superantigen. In some embodiments, an svd-TCR does not bind to a superantigen. In some embodiments, an svd-TCR (*e.g.*, a first or a second svd-TCR) is specific for an epitope excludes superantigens. In some embodiments, an svd-TCR binds to a superantigen. In some embodiments, an antigen binding domain does not bind to a superantigen. In some embodiments, an antigen binding domain is specific for an epitope that excludes superantigens. In some embodiments, an antigen binding domain binds a superantigen.

[0094] In some embodiments, an svd-TCR binds to a protein on the surface of a cancer cell. In some embodiments, an svd-TCR binds to a pMHC. In some embodiments, multiple svd-TCRs bind the same pMHC. In some embodiments, multiple svd-TCRs of a therapeutic bind different or distinct pMHCs. In some embodiments, an antigen binding domain of a therapeutic binds to a surface antigen. In some embodiments, an antigen binding domain of a therapeutic binds a pMHC, which may be the same or different from the pMHC targeted by an svd-TCR. In some embodiments, the antigen binding domain is or comprises an svd-TCR, such as a second svd-TCR.

C. TCR Domains

[0095] Disclosed herein are, *inter alia*, TCR domains, including TCR variable domains, which may be incorporated into the therapeutic. In some embodiments, a TCR variable domain can be part of an svd-TCR or an antigen binding domain. In some embodiments, a TCR domain can comprise multiple TCR variable domains or multiple TCR domains. In some embodiments, a first or second svd-TCR can include a TCR variable domain. In some embodiments, the TCR variable domain can include a TCR α chain variable domain ($V\alpha$) or a binding fragment thereof, a TCR β chain variable domain ($V\beta$) or a binding fragment

thereof, a TCR γ chain variable domain ($V\gamma$) or a binding fragment thereof, or a TCR δ variable domain ($V\delta$) or a binding fragment thereof. See **FIG. 2A** for TCR structures.

[0096] In some embodiments, the TCR variable domain can be a $V\alpha$ or a binding fragment thereof. In some embodiments, the TCR variable domain can include a $V\beta$ or a binding fragment thereof. In some embodiments, the TCR variable domain includes a $V\gamma$ or a binding fragment thereof. In some embodiments, the TCR variable domain can include a $V\delta$ or a binding fragment thereof. In some embodiments, the first TCR variable domain can include a $V\alpha$ or a binding fragment thereof. In some embodiments, the first TCR variable domain can include a $V\beta$ or a binding fragment thereof. In some embodiments, the first TCR variable domain can include a $V\gamma$ or a binding fragment thereof. In some embodiments, the first TCR variable domain can include a $V\delta$ or a binding fragment thereof. In some embodiments, the second TCR variable domain can include a $V\alpha$ or a binding fragment thereof. In some embodiments, the second TCR variable domain can include a $V\beta$ or a binding fragment thereof. In some embodiments, the second TCR variable domain can include a $V\gamma$ or a binding fragment thereof. In some embodiments, the second TCR variable domain can include a $V\delta$ or a binding fragment thereof.

[0097] In some embodiments, the TCR variable domain can include a mammalian variable domain (*e.g.*, human, marsupial such as opossum, or monotreme such as platypus) or a binding fragment thereof. In some embodiments, the sequence of the TCR domain can have high sequence identity to the mammalian variable domain. For example, the TCR variable domain sequence can have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 90% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 91% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 92% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 93% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 94% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 95% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 96% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 97% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 98% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 99% sequence identity to the mammalian variable domain sequence. In some embodiments, the TCR variable domain is a mammalian variable domain.

[0098] In some embodiments, the TCR variable domain can include a variable domain or a binding fragment thereof from human. In some embodiments, the sequence of the TCR domain can have high sequence identity to the human variable domain. For example, the TCR variable domain sequence can have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 90% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 91% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 92% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 93% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 94% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 95% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 96% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 97% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 98% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 99% sequence identity to the human variable domain sequence. In some embodiments, the TCR variable domain is a human variable domain.

[0099] In some embodiments, the TCR variable domain can include a variable domain or a binding fragment thereof from an opossum. In some embodiments, the sequence of the TCR domain can have high sequence identity to the opossum variable domain. For example, the TCR variable domain sequence can have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 90% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 91% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 92% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 93% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 94% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 95% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 96% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 97% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable

domain sequence can have at least 98% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 99% sequence identity to the opossum variable domain sequence. In some embodiments, the TCR variable domain is an opossum variable domain.

[0100] In some embodiments, the TCR variable domain can include a variable domain or a binding fragment thereof from a marsupial. In some embodiments, the sequence of the TCR domain can have high sequence identity to the marsupial variable domain. For example, the TCR variable domain sequence can have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 90% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 91% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 92% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 93% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 94% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 95% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 96% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 97% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 98% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 99% sequence identity to the marsupial variable domain sequence. In some embodiments, the TCR variable domain is a marsupial variable domain.

[0101] In some embodiments, the TCR variable domain can include a non-mammalian variable domain or a binding fragment thereof. In some embodiments, the sequence of the TCR domain can have high sequence identity to the non-mammalian variable domain. For example, the TCR variable domain sequence can have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 90% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 91% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 92% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 93% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 94% sequence identity to the non-mammalian variable domain sequence. In some

embodiments, the TCR variable domain sequence can have at least 95% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 96% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 97% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 98% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain sequence can have at least 99% sequence identity to the non-mammalian variable domain sequence. In some embodiments, the TCR variable domain is a non-mammalian variable domain.

[0102] In some embodiments, the TCR variable domain sequence can have at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 90% sequence identity any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 91% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 92% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 93% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 94% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 95% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 96% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 97% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 98% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain sequence can have at least 99% sequence identity to any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74. In some embodiments, the TCR variable domain is any one of the sequences set forth in SEQ ID NOs: 1-61 and 64-74.

[0103] In some embodiments, the TCR variable domain can exclude a TCR transmembrane domain or any portion thereof. In some embodiments, the TCR variable domain can lack the entire TCR transmembrane domain. In some embodiments, the TCR variable domain can lack a portion of a TCR transmembrane domain.

[0104] In some embodiments, the TCR variable domain can include a portion of a TCR constant domain. Alternatively, in some embodiments, the TCR variable domain can exclude a TCR constant domain or any

portion thereof. For example, the TCR variable domain can lack the entire constant domain, or it may specifically omit a portion of the constant domain.

[0105] In some embodiments, the TCR variable domain can include or be derived from a complementarity-determining region (CDR), such as CDR3, originating from *e.g.*, a human, mouse, or macaque. For example, the TCR variable domain can include or be derived from a CDR (*e.g.*, CDR3) of a human, a CDR (*e.g.*, CDR3) of a mouse, a CDR (*e.g.*, CDR3) of a macaque.

[0106] In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 4 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 6 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 8 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 10 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 12 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 14 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 16 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 18 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 20 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 22 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 24 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 26 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 28 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 30 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 32 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 34 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 36 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 with a length ranging from about 38 to about 40 amino acids.

[0107] In some embodiments, the TCR variable domain can include a CDR3 having a length of about 4 to about 38 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 4 to about 36 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 4 to about 34 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 4 to about 32 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 4 to about 30 amino acids. In some embodiments, the

CDR3 having a length of about 32 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 33 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 34 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 35 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 36 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 37 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 38 to about 40 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 39 to about 40 amino acids.

[0113] In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 39 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 38 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 37 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 36 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 35 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 34 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 32 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30 to about 31 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, or about 40 amino acids.

[0114] In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 20 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 22 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 24 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 26 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 28 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 30 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 32 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 34 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 36 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 38 amino acids. In some embodiments, the TCR variable domain can include a CDR3 having a length of at least 40 amino acids.

1. TCR Genes

[0115] In some embodiments, the TCR variable domain of an antigen binding domain or a svd-TCR (*e.g.*, the first or second svd-TCR) can be derived from a T cell receptor α variable gene (TRAV), T cell receptor β variable gene (TRBV), T cell receptor γ variable gene (TRGV), or T cell receptor δ variable gene (TRDV). In some embodiments, an antigen binding domain can be derived from a TRAV gene, TRBV gene, TRGV gene, or TRDV gene. In some embodiments, the TCR variable domain (*e.g.*, of the first or second svd-TCR) can be derived from any combination of TRAV, TRBV, TRGV or TRDV genes, including sequences or embodiments described herein. TRAV, TRBV, TRGV or TRDV genes can be identified by IMGT database accession numbers as available at the time of filing, with non-limiting examples shown in **FIGS. 5, 6A-6B, and 7D**. In some embodiments, the TCR variable domain of a first or second svd-TCR, or of an antigen binding domain, can be derived from a TRAV gene. In some embodiments, the TCR variable domain of a first or second svd-TCR, or of an antigen binding domain, can be derived from a TRBV gene. In some embodiments, the TCR variable domain of a first or second svd-TCR, or of an antigen binding domain, can be derived from a TRGV gene. In some embodiments, the TCR variable domain of a first or second svd-TCR, or of an antigen binding domain, can be derived from a TRDV gene. In some embodiments, the TCR variable domain can be derived from a $V\mu$ gene, such as when the variable domain originates from an opossum gene. Where a TCR variable domain is described as being derived from a gene, it can either be encoded directly by the gene or by another gene derived from or similar to the referenced gene.

[0116] In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-8 gene. In some embodiments, the TRBV5-8 gene can be or include the TRBV5-8*01 allele.

[0117] In some embodiments, the polypeptides described herein can be derived from T cell receptor β diversity (TRBD) genes. The TRBD genes can be identified using IMGT data base accession numbers available as of the effective filing date. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBD gene. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBD1 gene. In some embodiments, the TRBD1 gene can be TRBD1*01 allele. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBD2 gene. In some embodiments, the TRBD2 gene can be TRBD2*01 allele. In some embodiments, the TRBD2 gene can be allele TRBD2*02.

[0118] In some embodiments, the polypeptides described herein can be derived from T cell receptor β joining (TRBJ) genes. The TRBJ genes can be identified using IMGT data base accession numbers available as of filing date. An svd-TCR can be derived from a TRBJ gene or a TRAJ gene. In some embodiments, when the svd-TCR is derived at least in part from a TRBJ gene or a TRAJ gene, the svd-TCR can include a region, or fragment thereof, or a segment derived from such a region or fragment. Thus, in some

embodiments, the TCR variable domain of an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ gene. In some embodiments, the TCR variable domain of an svd-TCR can be derived from a TRBJ2-1 gene. In some embodiments, the TRBJ2-1 gene can be TRBJ2-1*01 allele. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-3 gene. In some embodiments, the TRBJ2-3 gene can be TRBJ2-3*01 allele. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-7 gene. In some embodiments, the TRBJ2-7 gene can be TRBJ2-7*01 allele. Non-limiting examples of TRAJ and TRBJ genes are shown in **Table 2** and **Table 4**.

[0119] In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a combination of specific TCR genes. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBV5-8 gene, a TRBD2 gene, and a TRBJ2-7 gene. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBV5-8 gene, a TRBD2 gene, and a TRBJ2-1 gene. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBV5-8 gene, a TRBD2 gene, and a TRBJ2-3 gene. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBV5-8 gene, a TRBD1 gene, and a TRBJ2-7 gene. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR is derived from a TRBV5-8 gene, a TRBD1 gene, and a TRBJ2-1 gene. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBV5-8 gene, a TRBD1 gene, and a TRBJ2-3 gene. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a gene depicted in **FIGS. 5, 6A-6B, 7C-7D, and 13A-13B**.

[0120] In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can be derived from a TRBV1 gene, such as, but not limited to, TRBV1*01 allele identified by the IMGT accession number L36092.

[0121] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV2 gene, such as, but not limited to, TRBV2*01 allele identified by the IMGT accession number L36092, TRBV2*02 allele identified by the IMGT accession number M62379, or TRBV2*03 allele identified by the IMGT accession number M64351. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV2-1 gene, such as, but not limited to, TRBV2-1*01 allele.

[0122] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV3 gene, such as, but not limited to, TRBV3*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV3-1 gene, such as, but not limited to, TRBV3-1*01

allele identified by the IMGT accession number U07977 or TRBV3-1*02 allele identified by the IMGT accession number L06889. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV3-2 gene, such as, but not limited to, TRBV3-2*01 allele identified by the IMGT accession number L36092, TRBV3-2*02 allele identified by the IMGT accession number U07978, or TRBV3-2*03 allele identified by the IMGT accession number M33240.

[0123] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV4 gene, such as, but not limited to, TRBV4*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV4-1 gene, such as, but not limited to, TRBV4-1*01 allele identified by the IMGT accession number U07977 or TRBV4-1*02 allele identified by the IMGT accession number M13855. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV4-2 gene, such as, but not limited to, TRBV4-2*01 allele identified by the IMGT accession number U07975 or TRBV4-2*02 allele identified by the IMGT accession number X58811. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV4-3 gene, such as, but not limited to, TRBV4-3*01 allele identified by the IMGT accession number U07978, TRBV4-3*02 allele identified by the IMGT accession number X58812, TRBV4-3*03 allele identified by the IMGT accession number L06888, or TRBV4-3*04 allele identified by the IMGT accession number X57616.

[0124] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5 gene, such as, but not limited to, TRBV5*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-1 gene, such as, but not limited to, TRBV5-1*01 allele identified by the IMGT accession number L36092 or TRBV5-1*02 allele identified by the IMGT accession number M14271. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-2 gene, such as, but not limited to, TRBV5-2*01 allele identified by the IMGT accession number L36092. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-3 gene, such as, but not limited to, TRBV5-3*01 allele identified by the IMGT accession number X61439 or TRBV5-3*02 allele identified by the IMGT accession number AF009660. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-4 gene, such as, but not limited to, TRBV5-4*01 allele identified by the IMGT accession number L36092, TRBV5-4*02 allele identified by the IMGT accession number X57615, TRBV5-4*03 allele identified by the IMGT accession number S50547, or TRBV5-4*04 allele identified by the IMGT accession number X58804. In some embodiments, the TCR variable domain of

an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-5 gene, such as, but not limited to, TRBV5-5*01 allele identified by the IMGT accession number L36092, TRBV5-5*02 allele identified by the IMGT accession number X57611, or TRBV5-5*03 allele identified by the IMGT accession number X58801. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-6 gene, such as, but not limited to, TRBV5-6*01 allele identified by IMGT accession number L36092. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-7 gene, such as, but not limited to, TRBV5-7*01 allele identified by IMGT accession number L36092. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV5-8 gene, such as, but not limited to, TRBV5-8*01 allele identified by IMGT accession number L36092 or TRBV5-8*02 allele identified by IMGT accession number X58803.

[0125] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-1 gene, such as, but not limited to, TRBV6-1*01 allele identified by the IMGT accession number X61446. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-2 gene, such as, but not limited to, TRBV6-2*01 allele identified by the IMGT accession number X61445. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-3 gene, such as, but not limited to, TRBV6-3*01 allele identified by the IMGT accession number U07978. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-4 gene, such as, but not limited to, TRBV6-4*01 allele identified by the IMGT accession number X61653 or TRBV6-4*02 allele identified by the IMGT accession number AF009660. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-5 gene, such as, but not limited to, TRBV6-5*01 allele identified by the IMGT accession number L36092. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-6 gene, such as, but not limited to, TRBV6-6*01 allele identified by the IMGT accession number L36092, TRBV6-6*02 allele identified by the IMGT accession number AF009662, TRBV6-6*03 allele identified by the IMGT accession number X58815, TRBV6-6*04 allele identified by the IMGT accession number X74848, or TRBV6-6*05 allele identified by the IMGT accession number L06892. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-7 gene, such as, but not limited to, TRBV6-7*01 allele identified by the IMGT accession number L36092. In some embodiments, the TCR

variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-8 gene, such as, but not limited to, TRBV6-8*01 allele identified by the IMGT accession number L36092. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV6-9 gene, such as, but not limited to, TRBV6-9*01 allele identified by the IMGT accession number X61447.

[0126] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-1 gene, such as, but not limited to, TRBV7-1*01 allele identified by the IMGT accession number X61444. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-2 gene, such as, but not limited to, TRBV7-2*01 allele identified by the IMGT accession number X61442, TRBV7-2*02 allele identified by the IMGT accession number L36190, TRBV7-2*03 allele identified by the IMGT accession number U07975, or TRBV7-2*04 allele identified by the IMGT accession number M27387. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-3 gene, such as, but not limited to, TRBV7-3*01 allele identified by the IMGT accession number X61440, TRBV7-3*02 allele identified by the IMGT accession number M97943, TRBV7-3*03 allele identified by the IMGT accession number AF009660, TRBV7-3*04 allele identified by the IMGT accession number X74843, or TRBV7-3*05 allele identified by the IMGT accession number M13550. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-4 gene, such as, but not limited to, TRBV7-4*01 allele identified by the IMGT accession number L36092 or TRBV7-4*02 allele identified by the IMGT accession number L13762. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-5 gene, such as, but not limited to, TRBV7-5*01 allele identified by the IMGT accession number L36092 or TRBV7-5*02 allele identified by the IMGT accession number AF009663. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-6 gene, such as, but not limited to, TRBV7-6*01 allele identified by the IMGT accession number L36092 or TRBV7-6*02 allele identified by the IMGT accession number X58806. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-7 gene, such as, but not limited to, TRBV7-7*01 allele identified by the IMGT accession number L36092 or TRBV7-7*02 allele identified by the IMGT accession number X57607. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-8 gene, such as, but not limited to, TRBV7-8*01 allele identified by the IMGT accession number M11953, TRBV7-8*02 allele identified by the IMGT accession number X61441, or TRBV7-8*03

allele identified by the IMGT accession number M27384. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV7-9 gene, such as, but not limited to, TRBV7-9*01 allele identified by the IMGT accession number L36092, TRBV7-9*02 allele identified by the IMGT accession number M15564, TRBV7-9*03 allele identified by the IMGT accession number AF009663, TRBV7-9*04 allele identified by the IMGT accession number M14261, TRBV7-9*05 allele identified by the IMGT accession number M27385, TRBV7-9*06 allele identified by the IMGT accession number X74844, or TRBV7-9*07 allele identified by the IMGT accession number L14854.

[0127] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV8 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV8-1 gene, such as, but not limited to, TRBV8-1*01 allele identified by the IMGT accession number L36092 or TRBV8-1*02 identified by the IMGT accession number IMGT000021. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV8-2 gene, such as, but not limited to, TRBV8-2*01 allele identified by the IMGT accession number L36092 or TRBV8-2*02 allele identified by the IMGT accession number IMGT000021.

[0128] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV9 gene, such as, but not limited to, TRBV9*01 allele identified by the IMGT accession number L36092, TRBV9*02 allele identified by the IMGT accession number AF009660, or TRBV9*03 allele identified by the IMGT accession number M27380.

[0129] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV10 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV10-1 gene, such as, but not limited to, TRBV10-1*01 allele identified by the IMGT accession number L36092, TRBV10-1*02 allele identified by the IMGT accession number AF009660, or TRBV10-1*03 allele identified by the IMGT accession number U17051. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV10-2 gene, such as, but not limited to, TRBV10-2*01 allele identified by the IMGT accession number L36092 or TRBV10-2*02 allele identified by the IMGT accession number IMGT000021. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV10-3 gene, such as, but not limited to, TRBV10-3*01 allele identified by the IMGT accession number U03115, TRBV10-3*02 allele identified by the IMGT accession number U17047, TRBV10-3*03 allele identified by the IMGT accession number L33101, or TRBV10-3*04 allele identified by the IMGT accession number L33102.

[0130] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV11 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV11-1 gene, such as, but not limited to, TRBV11-1*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV11-2 gene, such as, but not limited to, TRBV11-2*01 allele, TRBV11-2*02 allele, or TRBV11-2*03 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV11-3 gene, such as, but not limited to, TRBV11-3*01 allele, TRBV11-3*02 allele, TRBV11-3*03 allele, or TRBV11-3*04 allele.

[0131] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV12 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV12-1 gene, such as, but not limited to, TRBV12-1*01 allele identified by the IMGT accession number X07224. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV12-2 gene, such as, but not limited to, TRBV12-2*01 allele identified by the IMGT accession number X06936. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV12-3 gene, such as, but not limited to, TRBV12-3*01 allele identified by the IMGT accession number X07192. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV12-4 gene, such as, but not limited to, TRBV12-4*01 allele identified by the IMGT accession number K02546 or TRBV12-4*02 allele identified by the IMGT accession number M14264. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV12-5 gene, such as, but not limited to, TRBV12-5*01 allele identified by the IMGT accession number X07223.

[0132] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV13 gene, such as, but not limited to, TRBV13*01 allele identified by the IMGT accession number U03115 or TRBV13*02 allele identified by the IMGT accession number M62378. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV13-1 gene, such as, but not limited to, TRBV13-1*01 allele or TRBV13-1*02 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV13-2 gene, such as, but not limited to, TRBV13-2*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV13-3 gene, such as, but not limited to, TRBV13-3*01 allele.

[0133] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV14 gene, such as, but not limited to, TRBV14*01 allele identified by the IMGT accession number X06154 or TRBV14*02 allele identified by the IMGT accession number X57722.

[0134] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV15 gene, such as, but not limited to, TRBV15*01 allele identified by the IMGT accession number U03115, TRBV15*02 allele identified by the IMGT accession number IMGT000021, or TRBV15*03 allele identified by the IMGT accession number M62376.

[0135] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV16 gene, such as, but not limited to, TRBV16*01 allele identified by the IMGT accession number L26231, TRBV16*02 allele identified by the IMGT accession number U03115, or TRBV16*03 allele identified by the IMGT accession number L26054.

[0136] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV17 gene, such as, but not limited to, TRBV17*01 allele identified by the IMGT accession number U03115 or TRBV17*02 allele identified by the IMGT accession number IMGT000021.

[0137] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV18 gene, such as, but not limited to, TRBV18*01 allele identified by the IMGT accession number L36092.

[0138] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV19 gene, such as, but not limited to, TRBV19*01 allele identified by the IMGT accession number L36092, TRBV19*02 allele identified by the IMGT accession number U48259, or TRBV19*03 allele identified by the IMGT accession number M97725.

[0139] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV20 gene, such as, but not limited to, TRBV20*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV20-1 gene, such as, but not limited to, TRBV20-1*01 allele identified by the IMGT accession number M11955, TRBV20-1*02 allele identified by the IMGT accession number X72719, TRBV20-1*03 allele identified by the IMGT accession number M11954, TRBV20-1*04 allele identified by the IMGT accession number M14263, TRBV20-1*05 allele identified by the IMGT accession number X57604, TRBV20-1*06 allele identified by the IMGT accession number D13088, or TRBV20-1*07 allele identified by the IMGT accession number X74852.

[0140] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV21 gene. In some embodiments, the TCR

variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV21-1 gene, such as, but not limited to, TRBV21-1*01 allele identified by the IMGT accession number L36092 or TRBV21-1*02 allele identified by the IMGT accession number IMGT000021.

[0141] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV22 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV22-1 gene, such as, but not limited to, TRBV22-1*01 allele identified by the IMGT accession number L36092.

[0142] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV23 gene. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV23-1 gene, such as, but not limited to, TRBV23-1*01 allele identified by the IMGT accession number L36092.

[0143] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV24 gene, such as, but not limited to, TRBV24*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV24-1 gene, such as, but not limited to, TRBV24-1*01 allele identified by the IMGT accession number M11951 or TRBV24-1*02 allele identified by the IMGT accession number IMGT000021.

[0144] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV25-1 gene, such as, but not limited to, TRBV25-1*01 allele identified by the IMGT accession number L36092.

[0145] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV26 gene, such as, but not limited to, TRBV26*01 allele identified by the IMGT accession number L36092.

[0146] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV27 gene, such as, but not limited to, TRBV27*01 allele identified by the IMGT accession number L36092.

[0147] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV28 gene, such as, but not limited to, TRBV28*01 allele identified by the IMGT accession number U08314.

[0148] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV29 gene, such as, but not limited to, TRBV29*01 allele. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV29-1 gene, such as, but not limited to,

TRBV29-1*01 allele identified by the IMGT accession number L36092, TRBV29-1*02 allele identified by the IMGT accession number M13847, or TRBV29-1*03 allele identified by the IMGT accession number X04926.

[0149] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV30 gene, such as, but not limited to, TRBV30*01 allele identified by the IMGT accession number L36092, TRBV30*02 allele identified by the IMGT accession number Z13967, TRBV30*03 allele identified by the IMGT accession number IMGT000027, TRBV30*04 allele identified by the IMGT accession number M13554, or TRBV30*05 allele identified by the IMGT accession number L06893.

[0150] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBV31 gene, such as, but not limited to, TRBV31*01 allele.

[0151] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBVA gene, such as, but not limited to, TRBVA*01 allele identified by the IMGT accession number L36092 or TRBVA*02 allele identified by the IMGT accession number IMGT000021.

[0152] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBVB gene, such as, but not limited to, TRBVB*01 allele identified by the IMGT accession number L36092 or TRBVB*02 allele.

[0153] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBVC gene, such as, but not limited to, TRBVC*01 allele identified by the IMGT accession number L36092 or TRBVC*02 allele.

[0154] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ1-1 gene, such as, but not limited to, TRBJ1-1*01 allele identified by the IMGT accession number K02545. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ1-2 gene, such as, but not limited to, TRBJ1-2*01 allele identified by the IMGT accession number K02545. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ1-3 gene, such as, but not limited to, TRBJ1-3*01 allele identified by the IMGT accession number M14158. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ1-4 gene, such as, but not limited to, TRBJ1-4*01 allele identified by the IMGT accession number M14158. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ1-5 gene, such as, but not limited to, TRBJ1-5*01 allele identified by the IMGT accession number M14158. In some embodiments, the TCR variable domain of an

antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ1-6 gene, such as, but not limited to, TRBJ1-6*01 allele identified by the IMGT accession number M14158 or TRBJ1-6*02 allele identified by the IMGT accession number L36092 or U66061.

[0155] In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-1 gene, such as, but not limited to, TRBJ2-1*01 allele identified by the IMGT accession number X02987. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-2 gene, such as, but not limited to, TRBJ2-2*01 allele identified by the IMGT accession number X02987. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-2P gene, such as, but not limited to, TRBJ2-2P*01 allele identified by the IMGT accession number X02987. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-3 gene, such as, but not limited to, TRBJ2-3*01 allele identified by the IMGT accession number X02987. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-4 gene, such as, but not limited to, TRBJ2-4*01 allele identified by the IMGT accession number X02987. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-5 gene, such as, but not limited to, TRBJ2-5*01 allele identified by the IMGT accession number X02987. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-6 gene, such as, but not limited to, TRBJ2-6*01 allele identified by the IMGT accession number X02987. In some embodiments, the TCR variable domain of an antigen-binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can be derived from a TRBJ2-7 gene, such as, but not limited to, TRBJ2-7*01 allele identified by the IMGT accession number X02987 or TRBJ2-7*02 allele identified by the IMGT accession number X02987.

[0156] In some embodiments, any of the aforementioned genes can be excluded. For example, the TRBV gene may not include or be not derived from TRBV5-8, TRBJ2-1, TRBJ2-3, or TRBJ2-7. In some embodiments, the TCR variable domain of the first svd-TCR excludes a TRBV5-8 gene. In some embodiments, the TCR variable domain of the first svd-TCR excludes a TRBJ2-1 gene. In some embodiments, the TCR variable domain of the first svd-TCR excludes a TRBJ2-3 gene. In some embodiments, the TCR variable domain of the first svd-TCR excludes a TRBJ2-7 gene. In some embodiments, the TCR variable domain of the second svd-TCR excludes a TRBV5-8 gene. In some embodiments, the TCR variable domain of the second svd-TCR excludes a TRBJ2-1 gene. In some embodiments, the TCR variable domain of the second svd-TCR excludes a TRBJ2-3 gene. In some embodiments, the TCR variable domain of the second svd-TCR excludes a TRBJ2-7 gene.

[0157] Various embodiments of the TCR domains as described herein are found in **Tables 1-4**. In some embodiments, the TCR domain can be a subgroup, gene, allele, sequence, or allelic variation of the TCR domains in **Table 1**. In some embodiments, the TCR domain can be a subgroup, gene, allele, sequence, or allelic variation of the TCR domains in **Table 2**. In some embodiments, the TCR domain can be a subgroup, gene, allele, or allelic variation of the TCR domains in **Table 3**. In some embodiments, the TCR domain can be a subgroup, gene, allele, or allelic variation of the TCR domains in **Table 4**. In some embodiments, the TCR domain can be derived from any of the TCR domains described in **Tables 1-4**.

[0158] In some embodiments, the TCR domain can include or be derived from any of the sequences (*e.g.*, a TRBV or TRBJ sequence) in **Table 1** or **Table 2**. In some embodiments, the TCR domain can exhibit a degree of sequence identity to any of the sequences in **Table 1** or **Table 2**. For example, the sequence can be at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of the sequences in **Table 1** or **Table 2**.

Table 1. Non-limiting exemplary T cell receptor β variable (TRBV) domains and sequences

IMGT subgroup	IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:	Allelic variation
TRBV2	TRBV2	TRBV2*01	EPEVTQTPSHQVTQMGEVILRCVPISNHL YFY WYRQILGQKVEFLVSFYNN EISEKSEIFDDQFS VERPDG SNFTLKIRSTKLEDSAMYFCASSE	1	
		TRBV2*02			R22H
		TRBV2*03			n/a
TRBV3	TRBV3-1	TRBV3-1*01	DTAVSQTPKYLVTQMGN DKSIKCEQNLGHDT MYWYKQDSKKFLKIMFSYNNKELIINETV PNR FSPKSPDKAHLNLHINSLELGDSAVYFCASSQ	2	
		TRBV3-1*02			H86K
TRBV4	TRBV4-1	TRBV4-1*01	DTEVTQTPKHLVMGMTNKKSLKCEQHMGHR AMYWYKQKAKKPELMFVYSYEKLSINESVPS RFSPEC PNSSLNLHLHALQPEDSALYLCASSQ	3	
		TRBV4-1*02			n/a
	TRBV4-2	TRBV4-2*01	ETGVTQTPRHLVMGMTNKKSLKCEQHLGHNA MYWYKQSAKKPELMFVYNFKEQTENNSVPS RFSPEC PNSSHLFLHLHTLQPEDSALYLCASSQ	4	
		TRBV4-2*02			F88C
	TRBV4-3	TRBV4-3*01	ETGVTQTPRHLVMGMTNKKSLKCEQHLGHNA MYWYKQSAKKPELMFVYSLEERVENNSVPS RFSPEC PNSSHLFLHLHTLQPEDSALYLCASSQ	5	
		TRBV4-3*02			F88S
		TRBV4-3*03			n/a
		TRBV4-3*04			n/a

IMGT subgroup	IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:	Allelic variation
TRBV5	TRBV5-1	TRBV5-1*01	KAGVTQTPRYLIKTRGQQVTLSCSPISGHRVS WYQQTPGQGLQFLFEYFSETQRNKGNFPRFS GRQFSNSRSEMNVSTLELGDSALYLCASSL	6	
		TRBV5-1*02			K1R, Y10H, S22G, P46L, P72L
	TRBV5-4	TRBV5-4*01	ETGVTQSPTHLIKTRGQQVTLRCSSQSGHNTVS WYQQALGQGPQFIFQYYREEENGRGNFPPRFS GLQFPNYSELNVNALELDDSAALYLCASSL	7	
		TRBV5-4*02			S86N
		TRBV5-4*03			n/a
		TRBV5-4*04			F71S
	TRBV5-5	TRBV5-5*01	DAGVTQSPTHLIKTRGQQVTLRCSPISGHKSVS WYQQVLGQGPQFIFQYYEKEERGRGNFPDRFS ARQFPNYSELNVNALLLGDSALYLCASSL	8	
		TRBV5-5*02			Q18H
		TRBV5-5*03			G28E
	TRBV5-6	TRBV5-6*01	DAGVTQSPTHLIKTRGQQVTLRCSPKSGHDTV SWYQQALGQGPQFIFQYYEEEEERQRGNFPDRF SGHQFPNYSELNVNALLLGDSALYLCASSL	9	
	TRBV5-8	TRBV5-8*01	EAGVTQSPTHLIKTRGQQATLRCSPISGHTSVY WYQQALGLGLQFLWYDEGEERNRGNFPDRF SGRQFPNYSELNVNALELEDSALYLCASSL	10	
		TRBV5-8*02			F52L
TRBV6	TRBV6-1	TRBV6-1*01	NAGVTQTPKFQVLKTGQSMTLQCAQDMNHNS MYWYRQDPGMGLRLIYYSASEGTTDKGEVPN GYNVSRNLNKRFLSLRLESAAPSQTSVYFCASSE	11	
	TRBV6-2	TRBV6-2*01	NAGVTQTPKFRVLKTGQSMTLLCAQDMNHEY MYWYRQDPGMGLRLIHYSVGEGTTAKGEVPD GYNVSRLLKKQNFLGLLESAAPSQTSVYFCASS Y	12	n/a
	TRBV6-3	TRBV6-3*01	NAGVTQTPKFRVLKTGQSMTLLCAQDMNHEY MYWYRQDPGMGLRLIHYSVGEGTTAKGEVPD GYNVSRLLKKQNFLGLLESAAPSQTSVYFCASS Y	13	n/a
	TRBV6-4	TRBV6-4*01	IAGITQAPTSQILAAGRRMTLRCTQDMRHNAM YWYRQDLGLGLRLIHYSNTAGTTGKGEVPDG YSVSRANTDDFPLTLASAVPSQTSVYFCASSD	14	
		TRBV6-4*02			R18S
	TRBV6-5	TRBV6-5*01	NAGVTQTPKFQVLKTGQSMTLQCAQDMNHEY MSWYRQDPGMGLRLIHYSVGAGITDQGEVPN GYNVSRSTTEDFPLRLLSAAPSQTSVYFCASSY	15	
	TRBV6-6	TRBV6-6*01	NAGVTQTPKFRILKIGQSMTLQCTQDMNHNH MYWYRQDPGMGLKLIYYSVGAGITDKGEVPN GYNVSRSTTEDFPLRLELAAPSQTSVYFCASSY	16	

IMGT subgroup	IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:	Allelic variation
		TRBV6-6*02			T24A
		TRBV6-6*03			T24A
		TRBV6-6*04			N30E
		TRBV6-6*05			T24A, P96A
	TRBV6-8	TRBV6-8*01	NAGVTQTPKFHILKTGQSMTLQCAQDMNHGY MSWYRQDPGMGLRLIYYSAAGTTDKKEVPNG YNVSRLNTEDFPLRLVSAAPSQTSVYLCASSY	17	
	TRBV6-9	TRBV6-9*01	NAGVTQTPKFHILKTGQSMTLQCAQDMNHGY LSWYRQDPGMGLRRIHYSVAAGITDKGEVPD GYNVSRNTEDEFLRLVSAAPSQTSVYFCASSY	18	
TRBV7	TRBV7-2	TRBV7-2*01	GAGVSQSPSNKVTEKGKDVELRCDPISGHTAL YWYRQSLGQGLEFLIYFQGNAPDKSGLPSDR FSAERTGGSVSTLTIQRTQQEDSAVYLCASSL	19	
		TRBV7-2*02			S45R, G84E
		TRBV7-2*03			S45R, G84E, A105T
		TRBV7-2*04			n/a
	TRBV7-3	TRBV7-3*01	GAGVSQTPSNKVTEKGKYVELRCDPISGHTAL YWYRQSLGQGPFLIYFQGTGAADDGSLPNDR FFAVRPEGSVSTLKIQRTERGDSAVYLCASSL	20	
		TRBV7-3*04			n/a
		TRBV7-3*05			F52L
	TRBV7-4	TRBV7-4*01	GAGVSQSPRYKVAKRGRDVALRCDPISGHVTL YWYRQTLGQGSEVLTYQSDAQRDKSGRPSG RFSAPERPSVSTLKIQRTEQGDSAVYLCASSL	21	
	TRBV7-6	TRBV7-6*01	GAGVSQSPRYKVTKRGQDVALRCDPISGHVSL YWYRQALGQGPEFLTYFNIEAQQDKSGLPND RFSAPERPEGSISTLTIQRTEQRDSAMYRCASSL	22	
		TRBV7-6*02			n/a
	TRBV7-7	TRBV7-7*01	GAGVSQSPRYKVTKRGQDVTLRCDPISSHATL YWYQALGQGPEFLTYFNIEAQPDKSGLPSD RFSAPERPEGSISTLTIQRTEQRDSAMYRCASSL	23	
		TRBV7-7*02			A30V
	TRBV7-8	TRBV7-8*01	GAGVSQSPRYKVAKRQDVALRCDPISGHVSL FWYQALGQGPEFLTYFQNEAQLDKSGLPSDR FFAERPEGSVSTLKIQRTEQGDSAVYLCASSL	24	
		TRBV7-8*02			Q96K
		TRBV7-8*03			n/a
	TRBV7-9	TRBV7-9*01	DTGVSQNPRHKITKRQNVTFRCDPISSEHNRLY WYRQTLGQGPEFLTYFQNEAQLDKSGLPSDR SAERPKEGSFSTLEIQRTEQGDSAMYLCASSL	25	
		TRBV7-9*02			K11N
		TRBV7-9*03			N7D

IMGT subgroup	IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:	Allelic variation
		TRBV7-9*04			D1I, T2S, Q>H, T45N, L46P, R70G, F76>I
		TRBV7-9*05			F86L
		TRBV7-9*06			F86L
		TRBV7-9*07			Q96E
TRBV9	TRBV9	TRBV9*01	DSGVTQTPKHLITATGQRVTLRCSRSGDLSVY WYQQSLDQGLQFLIQYYNGEERAKGNILERFS AQQFPDLHSELNLSSLELGDSALYFCASSV	26	
		TRBV9*02			Q55H
		TRBV9*03			n/a
TRBV10	TRBV10-1	TRBV10-1*01	DAEITQSPRHKITETGRQVTLACHQTWNHNNM FWYRQDLGHGLRLIHYSYGVQDTNKGEVSDG YSVSRNTEDLPLTLESAASSQTSVYFCASSE	27	
		TRBV10-1*02			Q60H
	TRBV10-2	TRBV10-2*01	DAGITQSPRYKITETGRQVTLMCHQTWSHSYM FWYRQDLGHGLRLIYYSAADITDKGEVPDGY VVSRSKTENFPLTLESATRSQTSVYFCASSE	28	
		TRBV10-2*02			n/a
	TRBV10-3	TRBV10-3*01	DAGITQSPRHKVTETGTPVTLRCHQTENHRYM YWYRQDPGHGLRLIHYSYGVKDTDKGEVSDG YSVSRSKTEDFLLTLESATSSQTSVYFCAISE	29	
		TRBV10-3*02			n/a
		TRBV10-3*03			n/a
		TRBV10-3*04			n/a
TRBV11	TRBV11-1	TRBV11-1*01	EAEVAQSPRYKITEKSQAVAFWCDPISGHATL YWYRQILGQGPELLVQFQDESVDSDSQLPKDR FSAERLKGVDSTLKIQPAELGDSAMYLCASSL	30	
	TRBV11-2	TRBV11-2*01	EAGVAQSPRYKIIEKRQSVAFWCNPISGHATLY WYQQILGQGPKLLIQFNNGVVDSDSQLPKDRF SAERLKGVDSTLKIQPAKLEDSAVYLCASSL	31	
		TRBV11-2*02			D98N
		TRBV11-2*03			na
	TRBV11-3	TRBV11-3*01	EAGVVQSPRYKIIEKKQPVAFWCNPISGHNTLY WYLQNLGQGPELLIRYENEEAVDDSQLPKDRF SAERLKGVDSTLKIQPAELGDSAVYLCASSL	32	
		TRBV11-3*02			L43R
		TRBV11-3*03			V101M
		TRBV11-3*04			L43R
TRBV12	TRBV12-3	TRBV12-3*01	DAGVIQSPRHEVTEMGQEVTLRCKPISGHNSLF WYRQTMMRGLELLIYFNNNVPIDDSGMPEDRF SAKMPNASFSTLKIQPSEPRDSAVYFCASSL	33	

IMGT subgroup	IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:	Allelic variation
	TRBV12-4	TRBV12-4*01	DAGVIQSPRHEVTEMGQEVTLRCKPISGHDYL FWYRQTMMRGLELLIYFNNNVPIDDSGMPEDR FSAKMPNASFSTLKIQPSEPRDSAVYFCASSL	34	
		TRBV12-4*02			K90R
	TRBV12-5	TRBV12-5*01	DARVTQTTPRHKVTEMGQEVTMRCQPILGHNT VFWYRQTMMQGLELLAYFRNRAPLDDSGMP KDRFSAEMPDATLATLKIQPSEPRDSAVYFCAS GL	35	
TRBV13	TRBV13	TRBV13*01	AAGVIQSPRHLIKEKRETATLKCYPPIRHDTVY WYQQGPGQDPQFLISFYEKMQSDKGSIPDRFS AQQFSDYHSELNMSLELGDSALYFCASSL	36	
		TRBV13*02			K13R, L53F
TRBV14	TRBV14	TRBV14*01	EAGVTQFPSSHVIEKGQTVTLRCDPISGHDNLY WYRRVMGKEIKFLLHFVKESKQDESGMPNNR FLAERTGGTYSTLKVQPAELED SGVYFCASSQ	37	
		TRBV14*02			na
TRBV15	TRBV15	TRBV15*01	DAMVIQNPRYQVTQFGKPVTLSCSQTLNHNV MYWYQQKSSQAPKLLFHYDKDFNNEADTPD NFQSRRPNTSFCFLDIRSPGLGDTAMYLCA TSR	38	
		TRBV15*02			T99A
		TRBV15*03			Q11R, D58N, T99A, L103Q
TRBV16	TRBV16	TRBV16*01	GEEVAQTPKHLVRGEGQKAKLYCAPIKGHSY VFWYQQVLKNEFKFLISFQENNVFDETGMPE RFS AKCLPNSPCSLEIQATKLEDSAVYFCASSQ	39	
		TRBV16*03			I54V
TRBV18	TRBV18	TRBV18*01	NAGVMQNPRHLVRRRGQEARLRCSPMKGHSH VYWYRQLPEEGLKFMVYLQKENIIDESGMPKE RFSAEFPKEGPSILRIQQVVRGDSAA YFCASSP	40	
TRBV19	TRBV19	TRBV19*01	DGGITQSPKYLFRKEGQNVTLSC EQNLNHDAM YWYRQDPGQGLRLIYYSQIVNDFQKG DIAEGY SVSREKKESFPLTVTSAQKNPTAFYLCASSI	41	
		TRBV19*02			D45V, Q57H
		TRBV19*03			Q57H
TRBV20	TRBV20-1	TRBV20-1*01	GAVVSQHPSWVICKSGT SVKIECRSLDFQATT MFWYRQFPKQSLMLMATSNEGSKATYEQGVE KDKFLINHASLTLSTLTVTSAHPEDSSFYICSAR	42	
		TRBV20-1*02			W10R
		TRBV20-1*03			S60C
		TRBV20-1*04			W10R, Q48K
		TRBV20-1*05			W10R, Q48K

IMGT subgroup	IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:	Allelic variation
		TRBV20-1*06			W10R, Q48K
		TRBV20-1*07			W10R, Q48K, L52Q, M53I
TRBV24	TRBV24-1	TRBV24-1*01	DADVTQTPRNRITKTGKRIMLECSQTKGHDRM YWYRQDPGLGLRLIYYSFDVKDINKGEISDGY SVSRQAQAKFSLSLESAIPNQTALYFCATSDL	43	
		TRBV24-1*02			R44Q
TRBV25	TRBV25-1	TRBV25-1*01	EADIYQTPRYLVIGTGKKITLECSQTMGHDKM YWYQQDPGMELHLIHYSYGVNSTEKGDLSSES TVSRIRTEHFPLTLESARPSHTSQYLCASSE	44	
TRBV27	TRBV27	TRBV27*01	EAQVTQNPRYLITVTGKKLTVTCSQNMNHEY MSWYRQDPGLGLRQIYYSMNVEVTDKGDVPE GYKVSRRKEKRNFLILESPSPNQTSLYFCASSL	45	
TRBV28	TRBV28	TRBV28*01	DVKVTQSSRYLVKRTGEKVFLECVQDMDHEN MFWYRQDPGLGLRLIYFSYDVKMKEKGDIEPG YSVSREKKERFSLILESASTNQTSMYLCASSL	46	
TRBV29	TRBV29-1	TRBV29-1*01	SAVISQKPSRDICQRGTSLTIQCQVDSQVTMMF WYRQQPGQSLTLIATANQGSEATYESGFVIDK FPISRPNLTFSTLTVSNMSPEDSSIYLCSSVE	47	
		TRBV29-1*02			T88S
		TRBV29-1*03			M39I
TRBV30	TRBV30	TRBV30*01	SQTIHQWPATLVQPVGSPLSLECTVEGTSNPNL YWYRQAAGRGLQLLFYSVGIGQISSEVPQNLS ASRPQDRQFILSSKKLLLSDSGFYLCAWS	48	
		TRBV30*02			n/a
		TRBV30*04			V57I, G65D
		TRBV30*05			n/a

Table 2. Non-limiting exemplary T cell receptor β joining (TRBJ) genes and sequences

IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:
TRBJ1-1	TRBJ1-1*01	NTEAFFGQGTRLTVV	49
TRBJ1-2	TRBJ1-2*01	NYGYTFGSGTRLTVV	50
TRBJ1-3	TRBJ1-3*01	SGNTIYFGECSWLTVV	51
TRBJ1-4	TRBJ1-4*01	TNEKLFFGSGTQLSVL	52
TRBJ1-5	TRBJ1-5*01	SNQPQHFGDGTRLSIL	53
TRBJ1-6	TRBJ1-6*01	SYNSPLHFGNGTRLTVT	54
	TRBJ1-6*02		
TRBJ2-1	TRBJ2-1*01	SYNEQFFGPGTRLTVL	55
TRBJ2-2	TRBJ2-2*01	NTGELFFGEGSRLTVL	56
TRBJ2-3	TRBJ2-3*01	STDTQYFGPGTRLTVL	57

IMGT gene name	IMGT allele name	Sequence	SEQ ID NO:
TRBJ2-4	TRBJ2-4*01	AKNIQYFGAGTRLSVL	58
TRBJ2-5	TRBJ2-5*01	QETQYFGPGTRLLVL	59
TRBJ2-6	TRBJ2-6*01	SGANVLTFGAGSRLTVL	60
TRBJ2-7	TRBJ2-7*01	SYEQYFGPGTRLTVT	61

Table 3. Non-limiting exemplary T cell receptor α variable (TRAV) genes and alleles

IMGT subgroup	IMGT gene name	IMGT allele name
TRAV1	TRAV1-1	TRAV1-1*01
		TRAV1-1*02
	TRAV1-2	TRAV1-2*01
		TRAV1-2*02
		TRAV1-2*03
TRAV2	TRAV2	TRAV2*01
		TRAV2*02
TRAV3	TRAV3	TRAV3*01
TRAV4	TRAV4	TRAV4*01
TRAV5	TRAV5	TRAV5*01
TRAV6	TRAV6	TRAV6*01
		TRAV6*02
		TRAV6*03
		TRAV6*04
		TRAV6*05
		TRAV6*06
		TRAV6*07
TRAV7	TRAV7	TRAV7*01
TRAV8	TRAV8-1	TRAV8-1*01
		TRAV8-1*02
	TRAV8-2	TRAV8-2*01
		TRAV8-2*02
		TRAV8-2*03
	TRAV8-3	TRAV8-3*01
		TRAV8-3*02
		TRAV8-3*03
	TRAV8-4	TRAV8-4*01
		TRAV8-4*02
		TRAV8-4*03
		TRAV8-4*04
		TRAV8-4*05
		TRAV8-4*06
		TRAV8-4*07
	TRAV8-6	TRAV8-6*01

IMGT subgroup	IMGT gene name	IMGT allele name
		TRAV8-6*02
TRAV9	TRAV9-1	TRAV9-1*01
	TRAV9-2	TRAV9-2*01
		TRAV9-2*02
		TRAV9-2*03
		TRAV9-2*04
TRAV10	TRAV10	TRAV10*01
		TRAV10*02
TRAV12	TRAV12-1	TRAV12-1*01
		TRAV12-1*02
	TRAV12-2	TRAV12-2*01
		TRAV12-2*02
		TRAV12-2*03
	TRAV12-3	TRAV12-3*01
		TRAV12-3*02
TRAV13	TRAV13-1	TRAV13-1*01
		TRAV13-1*02
		TRAV13-1*03
	TRAV13-2	TRAV13-2*01
		TRAV13-2*02
TRAV14	TRAV14/DV4	TRAV14/DV4*01
		TRAV14/DV4*02
		TRAV14/DV4*03
		TRAV14/DV4*04
TRAV16	TRAV16	TRAV16*01
TRAV17	TRAV17	TRAV17*01
TRAV18	TRAV18	TRAV18*01
TRAV19	TRAV19	TRAV19*01
TRAV20	TRAV20	TRAV20*01
		TRAV20*02
		TRAV20*03
		TRAV20*04
TRAV21	TRAV21	TRAV21*01
		TRAV21*02
TRAV22	TRAV22	TRAV22*01
TRAV23	TRAV23/DV6	TRAV23/DV6*01
		TRAV23/DV6*02
		TRAV23/DV6*03
		TRAV23/DV6*04
		TRAV23/DV6*05
TRAV24	TRAV24	TRAV24*01

IMGT subgroup	IMGT gene name	IMGT allele name
		TRAV24*02
TRAV25	TRAV25	TRAV25*01
TRAV26	TRAV26-1	TRAV26-1*01
		TRAV26-1*02
		TRAV26-1*03
	TRAV26-2	TRAV26-2*01
		TRAV26-2*02
TRAV27	TRAV27	TRAV27*01
		TRAV27*02
		TRAV27*03
TRAV29	TRAV29/DV5	TRAV29/DV5*01
		TRAV29/DV5*02
		TRAV29/DV5*04
TRAV30	TRAV30	TRAV30*01
		TRAV30*02
		TRAV30*03
		TRAV30*04
		TRAV30*05
TRAV34	TRAV34	TRAV34*01
TRAV35	TRAV35	TRAV35*01
		TRAV35*02
TRAV36	TRAV36/DV7	TRAV36/DV7*01
		TRAV36/DV7*02
		TRAV36/DV7*03
		TRAV36/DV7*04
		TRAV36/DV7*05
TRAV38	TRAV38-1	TRAV38-1*01
		TRAV38-1*02
		TRAV38-1*03
		TRAV38-1*04
	TRAV38-2/DV8	TRAV38-2/DV8*01
TRAV39	TRAV39	TRAV39*01
TRAV40	TRAV40	TRAV40*01
TRAV41	TRAV41	TRAV41*01

Table 4. Non-limiting exemplary T cell receptor α joining (TRAJ) genes and alleles

IMGT gene name	IMGT allele name
TRAJ3	TRAJ3*01
TRAJ4	TRAJ4*01
TRAJ5	TRAJ5*01
TRAJ6	TRAJ6*01
TRAJ7	TRAJ7*01
TRAJ8	TRAJ8*01
TRAJ9	TRAJ9*01
TRAJ10	TRAJ10*01
TRAJ11	TRAJ11*01
TRAJ12	TRAJ12*01
TRAJ13	TRAJ13*01
	TRAJ13*02
TRAJ14	TRAJ14*01
TRAJ15	TRAJ15*01
	TRAJ15*02
TRAJ16	TRAJ16*01
	TRAJ16*02
TRAJ17	TRAJ17*01
TRAJ18	TRAJ18*01
TRAJ20	TRAJ20*01
TRAJ21	TRAJ21*01
TRAJ22	TRAJ22*01
TRAJ23	TRAJ23*01
	TRAJ23*02
TRAJ24	TRAJ24*01
	TRAJ24*02
	TRAJ24*03
TRAJ26	TRAJ26*01
TRAJ27	TRAJ27*01
TRAJ28	TRAJ28*01

IMGT gene name	IMGT allele name
TRAJ29	TRAJ29*01
TRAJ30	TRAJ30*01
TRAJ31	TRAJ31*01
TRAJ32	TRAJ32*01
	TRAJ32*02
TRAJ33	TRAJ33*01
TRAJ34	TRAJ34*01
TRAJ35	TRAJ35*01
TRAJ36	TRAJ36*01
TRAJ37	TRAJ37*01
	TRAJ37*02
TRAJ38	TRAJ38*01
TRAJ39	TRAJ39*01
TRAJ40	TRAJ40*01
TRAJ41	TRAJ41*01
TRAJ42	TRAJ42*01
TRAJ43	TRAJ43*01
TRAJ44	TRAJ44*01
TRAJ45	TRAJ45*01
TRAJ46	TRAJ46*01
TRAJ47	TRAJ47*01
	TRAJ47*02
TRAJ48	TRAJ48*01
TRAJ49	TRAJ49*01
TRAJ50	TRAJ50*01
TRAJ52	TRAJ52*01
TRAJ53	TRAJ53*01
TRAJ54	TRAJ54*01
TRAJ56	TRAJ56*01
TRAJ57	TRAJ57*01

[0159] In some embodiments, the therapeutic can include a TCR domain comprising an N-linked glycan or an N-linked glycosylation site. In some embodiments, the N-linked glycosylation can be recombinantly introduced to the therapeutic. For example, an svd-TCR or an antigen binding domain can be engineered to incorporate the N-linked glycosylation site. In some embodiments, the N-linked glycosylation can be present in the therapeutic when a TCR domain (*e.g.*, a TCR variable domain) is included in the therapeutic, wherein the TCR domain can be derived from a TCR domain gene that naturally includes an N-linked glycosylation site. N-linked glycans or N-linked glycosylation sites can contribute to increasing the thermal stability or

solubility of the polypeptides or the therapeutics described herein. In some embodiments, an svd-TCR can be glycosylated or comprise a glycosylation site. In some embodiments, an antigen binding domain can be glycosylated or comprise or a glycosylation site.

[0160] N-linked glycosylation can occur on an asparagine residue. The asparagine residue can be part of a glycosylation motif such as a NX[S/T] amino acid motif. In some embodiments, X can be any residue except proline. In some embodiments, the glycosylation motif can be NYS amino acid sequence. In some embodiments, the glycosylation motif can be NYS, NVT, NLT, NLS, NVS, NET, NES, NMS, NFT, or NGT amino acid sequences. In some embodiments, the polypeptides or the therapeutics described herein can be modified to replace a non-N-glycosylation motif to a N-glycosylation motif. For example, an amino acid motif such as NYS, NPT, NQT, NNS, NSS, NTS, or NAS can be modified or replaced with a different motif. Non-limiting exemplary TRBV and TRBJ genes comprising glycosylation motifs or non-glycosylation motifs are shown in **FIG. 13A-13B**. Any of the TRBV genes can be used or modified in the creation of a therapeutic described herein.

2. *Mutated Residues*

[0161] In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR) can include one or more modifications. A modification can increase the stability the polypeptide. A modification can increase the solubility of the polypeptide. A modification can decrease the aggregation propensity of the polypeptide. In some embodiments, the TCR variable domain of an antigen binding domain or an svd-TCR can include one or more modifications that increase the binding affinity of an antigen binding domain or an svd-TCR to a target (*e.g.*, a first epitope and/or a second epitope).

[0162] In some embodiments, the modification can be a hydrophobic residue. In some embodiments, the modification can be a non-hydrophobic residue. In some embodiments, the TCR variable domain (*e.g.*, of a first or second svd-TCR, or of an antigen binding domain) can include a hydrophobic residue mutated to a non-hydrophobic residue. In some embodiments, the hydrophobic residue can be an exposed hydrophobic residue. The exposed hydrophobic residue can be at a constant domain-contact site. In some embodiments, the incorporation of the non-hydrophobic residue can alter the hydrophobicity or hydrophilicity of the polypeptide.

[0163] In some embodiments, the modified hydrophobic residue, when not in an svd-TCR format, would otherwise interact with another TCR variable domain. For example, in a TCR molecule comprising a first TCR variable domain and a second TCR variable domain, the exposed hydrophobic residue of the first TCR variable domain can interact with one or more residues of the second TCR variable domain. In some embodiments, the exposed hydrophobic residue of the V α chain can interact with one or more residues of the V β chain in a TCR molecule comprising an V α chain and a V β chain.

[0164] In some embodiments, the exposed hydrophobic residue can be within a $V\alpha$ variable domain of the TCR variable domain. In some embodiments, the exposed hydrophobic residue can be within a β constant domain of the TCR variable domain.

[0165] In some embodiments, the modification can be a charged residue. In some embodiments, the modification can be an uncharged residue. In some embodiments, the charged residues can include positively charged residues or negatively charged residues. In some embodiments, the charged residue can be part of an otherwise basic or acidic amino acid. In some embodiments, inclusion of a charged or uncharged residue can change the charge density of the polypeptide. The change in charge density can be positive or negative depending on amino acid used. In some embodiments, the charge density can be changed by about +1 or -1. In some embodiments, inclusion of a charged or uncharged residue can increase the charge density of the polypeptide by about +1 or more. In some embodiments, inclusion of a charged or uncharged residue can decrease the charge density of the polypeptide by about -1 or more.

[0166] In some embodiments, modifying an exposed hydrophobic residue to a non-hydrophobic residue within an α variable ($V\alpha$) variable domain or a β constant domain can increase the expression yield of the polypeptide described herein. In some embodiments, modifying an exposed hydrophobic residue to a non-hydrophobic residue within an α variable ($V\alpha$) variable domain or a β constant domain can increase the thermostability (T_m) of the polypeptide described herein. In some embodiments, modifying an exposed hydrophobic residue to a non-hydrophobic residue within an α variable ($V\alpha$) variable domain or a β constant domain can decrease the aggregation propensity (T_{agg}) of the polypeptide described herein.

[0167] In some embodiments, the inclusion of a mutated residue (*e.g.*, a non-hydrophobic residue, a hydrophilic residue, etc.) can increase production, stability, a specific binding activity, or a functional activity of an svd-TCR (*e.g.*, the first or second svd-TCR) or an antigen binding domain. For example, as described above, the mutated residue can improve stability of an antigen binding domain or an svd-TCR (*e.g.*, a first or second svd-TCR). In another example, the mutated residue can increase the expression levels of the polypeptide, such as enhancing its production in an expression system.

[0168] In some embodiments, the mutated residue can enhance binding affinity to a target (*e.g.*, a first epitope or a second epitope). In some embodiments, the mutated residue can improve the binding specificity of the first svd-TCR or the second svd-TCR to a first and/or second epitope. To influence affinity, the mutations can be within the CDR3 loop that interacts with the peptide, antigen, or MHC.

[0169] In some embodiments, the modification can be in an amino acid that interacts with an antigen or an MHC, potentially altering the binding affinity of the polypeptide. In some embodiments, the modification impacts the binding affinity of the polypeptide or of an svd-TCR of the polypeptide. In some embodiments, the modification can be within a CDR loop of the polypeptide. For example, a mutation in the CDR or CDR loop can affect the binding affinity of the polypeptide (*e.g.*, a binding affinity of a svd-TCR of a polypeptide). In some embodiments, the mutation can be in a CDR of the polypeptide.

[0170] In some embodiments, the TCR variable domain of the svd-TCR, or the antigen binding domain can comprise a hydrophobic residue mutated to a non-hydrophobic residue, compared to a wild type or non-mutated TCR variable domain. In some embodiments, the hydrophobic residue can originate from regions illustrated in any of **FIG. 7A-7D**. In some embodiments, the hydrophobic residue can comprise a residue of **FIG. 7A-7D**. In some embodiments, the hydrophobic residue can be located in a BetaCON1 region, Alpha1 region, or Alpha2 region. In some embodiments, the hydrophobic residue can be located in a BetaCON1 region. In some embodiments, the hydrophobic residue can be located in an Alpha1 region. In some embodiments, the hydrophobic residue can be located in an Alpha2 region. In some embodiments, the hydrophobic residue can be located in a BetaCON1 region, Alpha1 region, or Alpha2 region of **FIG. 7C**. In some embodiments, replacing the hydrophobic residue with a non-hydrophobic residue can increase the hydrophilicity of the therapeutic or a polypeptide component of the therapeutic by about 4 to about 8 units on the Kyte and Doolittle scale hydrophobicity scale, compared to wild type or non-mutated TCR variable domain. In some embodiments, replacing the hydrophobic residue with a non-hydrophobic residue can alter the charge density of the therapeutic or a polypeptide component of the therapeutic by about +1 or about -1, relative to the wild type or non-mutated TCR variable domain. In some embodiments, incorporating the mutated residue can enhance production, stability, specific binding activity, or functional activity of the svd-TCR or the antigen binding domain, as compared to a wild type or non-mutated TCR variable domain.

D. Sizes of Therapeutics and Polypeptides

[0171] Described herein are the sizes of the polypeptides or therapeutics. Sizes specified for therapeutics are also contemplated to apply to polypeptides, and vice versa. Furthermore, any aspects described herein in relation to a polypeptide are also contemplated to apply to a therapeutic, where applicable. Similarly, aspects described herein in relation to a therapeutic are contemplated to apply to a polypeptide, where applicable.

[0172] The size of the polypeptides or therapeutics described herein can vary. The size of the polypeptide or therapeutic can range from about 13 kilodaltons (kDa) to about 165 kDa or from about 10 kDa to about 200 kDa. In some embodiments, specific molecular sizes of the polypeptide or therapeutic can include about 13 kDa, about 26 kDa, about 27 kDa, about 28 kDa, about 39 kDa, about 40 kDa, about 41 kDa, about 42 kDa, about 163 kDa, about 164 kDa, about 165 kDa, or any ranges in between. Non-limiting exemplary sizes are illustrated in **FIG. 10**. The size can refer to or include the molecular mass of the polypeptide or therapeutic. Such size variations can depend on the specific design, structural components, or functional domains included in the polypeptide or therapeutic.

[0173] In some embodiments, the therapeutic has a mass of at least 10 kDa, at least 15 kDa, at least 20 kDa, at least 25 kDa, at least 30 kDa, at least 35 kDa, at least 40 kDa, at least 50 kDa, at least 60 kDa, at least 70 kDa, at least 80 kDa, at least 90 kDa, at least 100 kDa, at least 125 kDa, at least 150 kDa, at least 175 kDa, at least 200 kDa, at least 250 kDa, at least 300 kDa, at least 400 kDa, at least 500 kDa, at least 600 kDa, at

least 700 kDa, at least 800 kDa, at least 900 kDa, or at least 1000 kDa. In some embodiments, the therapeutic has a mass of less than 10 kDa, less than 15 kDa, less than 20 kDa, less than 25 kDa, less than 30 kDa, less than 35 kDa, less than 40 kDa, less than 50 kDa, less than 60 kDa, less than 70 kDa, less than 80 kDa, less than 90 kDa, less than 100 kDa, less than 125 kDa, less than 150 kDa, less than 175 kDa, less than 200 kDa, less than 250 kDa, less than 300 kDa, less than 400 kDa, less than 500 kDa, less than 600 kDa, less than 700 kDa, less than 800 kDa, less than 900 kDa, or less than 1000 kDa. A mass can be of a molecular mass. A molecular mass can be estimated based on a chemical structure. A molecular mass can be estimated based on a predicted sequence. A molecular mass can be estimated using known molecular masses of constituents of the therapeutic.

[0174] In some embodiments, a polypeptide described herein can have a molecular mass below 200 kDa. In some embodiments, the polypeptide's molecular mass can be less than 175 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 150 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 125 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 100 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 75 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 70 kDa. In some embodiments, the polypeptide's molecular mass can be less than 65 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 60 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 55 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 50 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 45 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 40 kDa. In some embodiments, the molecular mass of the polypeptide can be less than 35 kDa.

[0175] For the polypeptide described herein, in some embodiments, the polypeptide can have a molecular mass no greater than 30 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 29 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 28 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 27 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 26 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 25 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 24 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 23 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 22 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 21 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 20 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 19 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 18 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 17 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 16 kDa. In some embodiments, the polypeptide can have a molecular mass no greater

than 15 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 14 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 13 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 12 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 11 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 10 kDa.

[0176] In some embodiments, the molecular mass of the polypeptide can be at least or greater than any of the aforementioned molecular masses. The polypeptide can include 1, 2, 3, 4, 5, or more svd-TCRs. In some embodiments, an svd-TCR can have a molecular mass of about 10-13 kDa. In some embodiments, an svd-TCR can have a molecular mass of about 11-14 kDa. In some embodiments, an svd-TCR can have a molecular mass of about 13-15 kDa. In some embodiments, an svd-TCR can have a molecular mass of about 10 kDa. In some embodiments, an svd-TCR can have a molecular mass of about 11 kDa. In some embodiments, an svd-TCR can have a molecular mass of about 12 kDa. In some embodiments, an svd-TCR can have a molecular mass of about 13 kDa.

[0177] In some embodiments, the polypeptide can include at least two svd-TCR. In some embodiments, the first svd-TCR and the second svd-TCR can be attached by a linker, such as a polypeptide linker. For example, the C-terminus of the first svd-TCR can be attached to the N-terminus of the second svd-TCR by a linker. In some embodiments, the polypeptide can have a molecular mass of about 26 to about 28 kDa. In some embodiments, the polypeptide can have a molecular mass of about 26 kDa. In some embodiments, the polypeptide can have a molecular mass of about 27 kDa. In some embodiments, the polypeptide can have a molecular mass of about 28 kDa.

[0178] In some embodiments, the polypeptide can comprise three svd-TCRs or four svd-TCRs. In some embodiments, the polypeptide can comprise three svd-TCRs (*e.g.*, a first svd-TCR, a second svd-TCR, and a third svd-TCR). In some embodiments, the polypeptide can comprise four svd-TCRs (*e.g.*, a first svd-TCR, a second svd-TCR, a third svd-TCR, and a fourth svd-TCR). In some embodiments, three svd-TCRs can be attached by linkers (*e.g.*, a polypeptide linkers). For example, the C-terminus of the first svd-TCR can be connected to the N-terminus of the second svd-TCR by a first linker, and the C-terminus of the second svd-TCR can be connected to the N-terminus of the third svd-TCR by a second linker. In some embodiments, four svd-TCRs can be attached by linkers (*e.g.*, a polypeptide linker). For example, the C-terminus of the first svd-TCR can be connected to the N-terminus of the second svd-TCR by a first linker, the C-terminus of the second svd-TCR can be connected to the N-terminus of the third svd-TCR by a second linker, and the C-terminus of the third svd-TCR can be connected to the N-terminus of the fourth svd-TCR by a third linker. In some embodiments, the polypeptide can have a molecular mass of about 39 to about 42 kDa. In some embodiments, the polypeptide can have a molecular mass of about 39 kDa. In some embodiments, the polypeptide can have a molecular mass of about 40 kDa. In some embodiments, the polypeptide can have a

molecular mass of about 41 kDa. In some embodiments, the polypeptide can have a molecular mass of about 42 kDa.

[0179] In some embodiments, wherein the polypeptide or therapeutic includes at least two svd-TCRs, the most stable-svd-TCR can be attached to the next stable svd-TCR in a direction from the N-terminus to the C-terminus. In some embodiments, the stability of the svd-TCR can be measured by its melting temperature. In some embodiments, the therapeutic can include an svd-TCR attached to a scaffold, such as an Fc scaffold, and can have more than one svd-TCR.

[0180] In some embodiments, the polypeptide can be attached to an additional binding protein, as further described below. In some embodiments, the polypeptide can be attached to an antibody or a fragment thereof. In some embodiments, the binding protein can be attached to the C-terminus of the first svd-TCR or the second- svd-TCR of the polypeptide. In some embodiments, the therapeutic can have a molecular mass ranging from about 163 to about 165 kDa. In some embodiments, the therapeutic can have a molecular mass of about 163 kDa. In some embodiments, the therapeutic can have a molecular mass of about 164 kDa. In some embodiments, the polypeptide can have a molecular mass of about 165 kDa.

[0181] In some embodiments, the polypeptide can have a molecular mass no greater than 100 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 95 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 90 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 85 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 80 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 75 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 70 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 65 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 60 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 55 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 50 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 45 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 40 kDa. In some embodiments, the polypeptide can have a molecular mass no greater than 35 kDa.

E. Additional Components of the Therapeutics or Polypeptides

[0182] The therapeutic or polypeptide provided herein may be attached to an additional component, such as, but not limited to, an additional binding protein, therapeutic moiety, detectable moiety, enzyme, or toxin. In some embodiments, the therapeutic or polypeptide can be attached to an additional binding protein. In some embodiments, the therapeutic or polypeptide can be attached to an additional therapeutic moiety. In some embodiments, the therapeutic moiety can be an anti-cancer agent. An anti-cancer agent or anticancer agent refers to a composition (*e.g.*, compound, drug, antagonist, inhibitor, modulator) with antineoplastic

properties or the ability to inhibit the growth or proliferation of cells. In some embodiments, the anti-cancer agent can be a chemotherapeutic. In some embodiments, the anti-cancer agent can be an agent identified herein as having utility in methods of treating cancer. In some embodiments, the anti-cancer agent can be an agent approved by the FDA or similar regulatory agency outside of the USA for the treatment of cancer.

[0183] In some embodiments, the polypeptide or therapeutic can be attached to a detectable moiety. A detectable agent or detectable moiety refers to a composition, substance, element, or compound that can be detected by suitable means such as spectroscopic, photochemical, biochemical, immunochemical, chemical, magnetic resonance imaging, or other physical techniques. In some embodiments, the detectable moiety can include a detectable protein.

[0184] In some embodiments, the polypeptide or therapeutic can be linked to a binding protein targets an additional antigen or a molecule expressed on a target cell (*e.g.*, cancer cell) or an effector cell (*e.g.*, an immune cell). In some embodiments, the binding protein can interact with a ligand or receptor involved in activating one or more components of the immune system (*e.g.*, T cells), thereby facilitating activation or proliferation of immune cells. In some embodiments, the binding protein can function as or include an immune cell (*e.g.*, T cell or NK cell) engager. In some embodiments, the binding protein can serve as a T-cell engager. T-cell engager refers to a molecule capable of binding an immune cell and redirecting it toward a target (*e.g.*, a cancer cell, a tumor, etc.). For example, a T-cell engager can bind a protein expressed on the surface of an immune cell. In some embodiments, the polypeptide or the therapeutic disclosed herein can target a cancer antigen expressed on the surface of a cancer cell while also incorporating a T-cell engager, thus recruiting T cells to engage with and attack the cancer cell.

[0185] In some embodiments, the binding protein can function as or include an NK-cell engager, such as, but not limited to, CD16 (FcγRIII) or NKG2D. An NK-cell engager is a protein that binds to an NK cell. For example, the NK-cell engager can interact with a protein expressed on the surface of an NK cell. Accordingly, in some embodiments, the polypeptide or therapeutic can form a bridge between an NK cell and a cancer cell, activating the NK cell and inducing apoptosis in the cancer cell. In some embodiments, the binding protein can function as an M1-cell engager, such as CD80 or CD86. An M1 macrophage engager or M1-cell engager is a protein that binds to an M1 macrophage. In some embodiments, the M1-cell engager can bind to a protein expressed on the surface of an M1 macrophage. The binding of an M1-cell engager to a macrophage can increase the expression of pro-inflammatory cytokines, thereby eliciting an immune response.

[0186] In some embodiments, the binding protein can be a checkpoint inhibitor, which can bind to an immune checkpoint protein. An immune checkpoint, immune checkpoint protein, or checkpoint protein are used interchangeably herein and refer to a molecule capable of modulating the duration and amplitude of physiological immune responses. An immune checkpoint molecule can stimulate (increase) an immune response. In some embodiments, the checkpoint protein can be a cellular receptor. Similarly, an immune

checkpoint inhibitor or checkpoint inhibitor refers to a substance (*e.g.*, an antibody or fragment thereof, a small molecule) that is capable of inhibiting or negatively affecting (*e.g.*, decreasing) the activity or function of a checkpoint protein. This inhibition can occur through decreasing expression or reducing the activity of the checkpoint protein relative to its activity or function in the absence of the inhibitor. The checkpoint inhibitor can, at least in part, partially or completely block stimulation, decrease, prevent, or delay activation, or inactivate, desensitize, or down-regulate signal transduction, enzymatic activity, or the amount of a checkpoint protein. A checkpoint inhibitor can inhibit a checkpoint protein by binding to it and partially or totally blocking its activity, decreasing its functionality, preventing or delaying its activation, inactivating it, desensitizing it, or down-regulating its activity. This action can involve reducing the activity or expression of the checkpoint protein and its associated signaling pathways. In some embodiments, the checkpoint inhibitor can be an antibody. In some embodiments, the checkpoint inhibitor can be an antibody fragment. In some embodiments, the checkpoint inhibitor can be an antibody variant. In some embodiments, the checkpoint inhibitor can be a single-chain variable fragment (scFv).

[0187] In some embodiments, the binding protein can bind to a cytokine. In some embodiments, the interaction between the binding protein and the cytokine can increase or upregulate the activation or proliferation of an immune cell.

[0188] In some embodiments, the binding protein can include an antibody, a binding fragment thereof, or a single-chain variable fragment (scFv). In some embodiments, the binding protein can comprise an antibody or a binding fragment thereof. In some embodiments, the binding protein can be an antibody. In some embodiments, the antibody or binding fragment thereof bind to a protein expressed on the surface of an immune cell. In some embodiments, the interaction between the antibody or binding fragment thereof and the immune cell protein can upregulate or enhance the activation of the immune cell.

[0189] In some embodiments, the conditions within the tumor microenvironment (TME) can suppress or inhibit the ability of immune cells (*e.g.*, T cells) to kill cancer cells. For example, chemokines present in the TME can prevent apoptosis of cancer cells or promote the proliferation of cancer cells. The polypeptides described herein, including various embodiments, are designed to effectively recruit immune cells to the TME, thereby enhancing the killing of cancer cells compared to conditions where the svd-TCR is absent. In some embodiments, the polypeptide can include a plurality of svd-TCRs (*e.g.*, at least two, three, or four svd-TCRs) and can also include a binding protein. The multivalent binding capacity of the polypeptide or therapeutic to epitopes expressed on cancer cells, combined with the simultaneous binding of the appended binding protein to an immune cell, can enhance the recruitment of immune cells to the TME. In some embodiments, the binding of multiple svd-TCRs (*e.g.*, at least two, three, or four svd-TCR) to epitopes expressed on a cancer cell, coupled with the binding of a binding protein to an immune cell, can increase apoptosis of cancer cells.

[0190] In some embodiments, the polypeptide or therapeutic can include a single svd-TCR or multiple svd-TCRs. In some embodiments, the polypeptide or the therapeutic can include at least one svd-TCR, at least two svd-TCRs, at least three svd-TCRs, at least four svd-TCRs, at least five svd-TCRs, or more svd-TCRs. In some embodiments, the polypeptide or therapeutic can include two svd-TCRs. In some embodiments, the polypeptide or therapeutic can include a third, fourth, or fifth svd-TCR.

[0191] In some embodiments, the polypeptide or therapeutic can include or be coupled with a biologically active group, such as another peptide or a small molecule conjugate. The biologically active group, referred to as an effector, can enhance the functionality of the polypeptide or therapeutic. In some embodiments, the polypeptide or therapeutic can be attached (*e.g.*, linked) to a small molecule or an anti-cancer agent, which can include a chemotherapeutic agent. In some embodiments, the polypeptide or therapeutic can be attached to a detectable moiety, such as a radionucleotide or a detectable protein, for enabling visualization or tracking. In some embodiments, the polypeptide or therapeutic can be attached to an immune checkpoint inhibitor to modulate immune responses. In some embodiments, the polypeptide or therapeutic can be attached to a protein, such as a cytokine or an antibody, for additional biological activity. In some embodiments, the biologically active group can comprise a toxin for targeted cytotoxicity or an enzyme for specific biochemical activities.

[0192] In some embodiments, the polypeptide or therapeutic can be attached to an antibody-drug conjugate. In some embodiments, the polypeptide or therapeutic can be coupled with a cluster of differentiation 3 (CD3) engager to facilitate immune cell targeting (see **FIGS. 4A-4C**). In some embodiments, the polypeptide or therapeutic can be linked to an immune cell modulator to regulate immune cell activity. In some embodiments, the polypeptide or therapeutic can comprise a radioactive isotope. In some embodiments, the polypeptide or therapeutic can be attached to a radionuclide, providing capabilities for imaging or targeted radiotherapy. These attachments exemplify various types of effectors that can be integrated with the polypeptide or therapeutic to enhance or diversify its functionality.

[0193] In some embodiments, an svd-TCR or the antigen binding domain of the therapeutic or polypeptide can be linked to a scaffold. In some embodiments, an antigen binding domain can be linked to a scaffold. The scaffold can include an antibody fragment crystallizable (Fc) region, an antibody constant domain, a TCR constant domain, albumin, a nanocage, ferritin, or lumazine synthase, or a fragment thereof. In some embodiments, the scaffold can include an antibody fragment crystallizable (Fc) region, an antibody constant domain, or a TCR constant domain. In some embodiments, the scaffold can include an Fc region, or a fragment thereof. In some embodiments, the scaffold can include an Fc region. In some embodiments, the scaffold can include a fragment of an Fc region. In some embodiments, the Fc scaffold (or fragment thereof) can include or be derived from an antibody allotype such as IgG, IgA, IgM, IgD, or IgE. In some embodiments, the scaffold can include albumin or an albumin fragment. In some embodiments, the scaffold can include a nanocage. Non-limiting exemplary nanocages can include E2p or I53-50. In some

embodiments, the scaffold can include a nanocage (*e.g.*, a ferritin nanocage), lumazine synthase, and E2p or I53-50.

F. Linkers

[0194] In some embodiments, the polypeptide or therapeutic can comprise a linker. In an embodiment, a linker can serve to connect the first and second svd-TCRs to one another. In another embodiment, a linker can join an svd-TCR with an antigen binding domain. In some embodiments, a linker can join an svd-TCR with an effector or a scaffold. In some embodiments, the linker can be a peptide linker or a chemical linker. In some embodiments, the peptide linker can connect the C-terminus of the first svd-TCR to the N-terminus of the second-svd-TCR. In some embodiments, the peptide linker can connect the C-terminus of the second svd-TCR to the N-terminus of the first-svd-TCR. For example, when the polypeptide or therapeutic comprises three svd-TCRs, the first svd-TCR, the second svd-TCR, and the third svd-TCR can be joined by linkers (*e.g.*, polypeptide linkers or chemical linkers). For example, the first linker can connect the C-terminus of the first svd-TCR to the N-terminus of the second svd-TCR, and the second linker can connect the C-terminus of the second svd-TCR to the N-terminus of the third svd-TCR. In some embodiments, the polypeptide or therapeutic can comprise an additional binding protein, which can be attached to the first svd-TCR or the second svd-TCR using a linker (*e.g.*, a polypeptide linker or a chemical linker). In some embodiments, the additional binding protein can be attached to the N-terminus or the C-terminus of an antigen binding domain or an svd-TCR (*e.g.*, a first or a second svd-TCR) via a linker.

[0195] In some embodiments, the polypeptide or therapeutic can comprise a linker that can connect an svd-TCR or an antigen binding domain to a biologically active group or binding protein. In some embodiments, the polypeptide or therapeutic comprises a linker that can connect the first or second svd-TCR to a biologically active group or binding protein. In some embodiments, the linker can be a peptide linker or a chemical linker. In some embodiments, the linker can be a peptide linker.

[0196] In some embodiments, the peptide linker can have a length of about 2 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 4 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 6 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 8 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 10 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 12 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 14 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 16 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 18 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 20 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 22 to about 30 amino acids. In some embodiments, the peptide linker can have a length of about 24 to about 30 amino acids. In some

linker can have a length of about 3 to about 15 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 14 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 13 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 12 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 11 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 10 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 9 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 8 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 7 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 6 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 5 amino acids. In some embodiments, the peptide linker can have a length of about 3 to about 4 amino acids. In some embodiments, the peptide linker can have a length of about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20 amino acids.

[0200] In some embodiments, the linker can allow for conformational flexibility and/or low aggregation propensity. In some embodiments, the linker can exclude a protease-cleaving sequence. In some embodiments, the linker can have low or no immunogenicity, reducing the likelihood of triggering an unwanted immune response upon administration. In some embodiments, the linker can include a hinge region sequence derived from an immunoglobulin. In some embodiments, the linker can comprise glycine and serine residues, such as a glycine-serine linker (a GS linker). In some embodiments, the GS linker can comprise a GS_n , GGS_n , $GGGS_n$ or $GGGS_n$ sequence, where n represents a number of glycine-serine repeats. In some embodiments, the linker can comprise a GS_n sequence. In some embodiments, the linker can comprise a GGS_n sequence. In some embodiments, the linker can comprise a $GGGS_n$ sequence. In some embodiments, the linker can comprise a $GGGS_n$ sequence.

[0201] In some embodiments, the linker can be a 2A peptide linker, such as, but not limited to, T2A, P2A, E2A, F2A, IRES, an internal ribosome entry site (IRES) element, a flexible and cleavable linker, such as, but not limited to, a GS linker, thrombin cleavage site, TEV protease cleavage site, or other cleavable linkers, such as, but not limited to, 3C protease cleavage site or sortase A recognition site.

G. Engineered Cells

[0202] The compositions of the present disclosure can be introduced and expressed in cells, such as T cells. The methods of introducing and expressing the compositions of the present disclosure are well known in the art. In some embodiments, the compositions of the present disclosure can be introduced and expressed in a cell by introducing nucleic acids (*i.e.*, DNA or RNA) encoding the compositions. The nucleic acids can include an expression construct and can be utilized to produce the polypeptide or therapeutic in a cell. In the context of an expression vector, the vector can be readily introduced into a host cell by any method in the art.

For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means. In some embodiments, the expression vector can be a viral vector. In some embodiments, the viral vector can be a lentiviral vector, an adeno virus vector, an adeno-associated virus vector, or a retroviral vector. In some embodiments, the viral vector can be a lentiviral vector.

[0203] In some embodiments, the polypeptide or the therapeutic can be attached to a cell (see **FIGS. 4A-4C**). In some embodiments, the polypeptide or therapeutic can be part of a cell. In some embodiments, the polypeptide or therapeutic can be inside a cell. In some embodiments, the polypeptide or therapeutic can bind or be configured to bind a cell. In some embodiments, the polypeptide or therapeutic can bind or be bound to a cellular protein. In some embodiments, the polypeptide or therapeutic can be encoded or synthesized by a cell. The cell can originate from a subject, be cultured externally, or exist in or on a subject. In some embodiments, the cell can be engineered or transfected with nucleic acids (*e.g.*, RNA or DNA) encoding the therapeutic or polypeptide and subsequently cultured or expanded. These cells or their progeny can then be administered or introduced into a subject, which can be the same subject from which the cells originated or a different subject.

[0204] In some embodiments, the therapeutic or polypeptide incorporate a receptor protein in addition to an svd-TCR. For example, a cell can contain the polypeptide or therapeutic that includes both an svd-TCR and the receptor protein. In some embodiments, the receptor protein can include a cell surface receptor, such as a chimeric antigen receptor (CAR). In some embodiments, the therapeutic or polypeptide can include a CAR in addition to an svd-TCR. For example, a cell can include the polypeptide or therapeutic with an svd-TCR and a CAR. In some embodiments, the cell can be a CAR-T cell or a CAR-NK cell. These cells, comprising the therapeutic or polypeptide along with the receptor protein, can be administered to a subject. The administered cells can be either autologous (from the same subject) or allogeneic (from a different donor) relative to the subject.

H. Pharmaceutical Compositions

[0205] Provided herein are, *inter alia*, pharmaceutical compositions comprising the therapeutic or polypeptide of the present disclosure. The therapeutic or polypeptide can comprise one or more svd-TCRs configured to bind one or more epitopes. The therapeutic or polypeptide can also comprise one or more additional components, such as a binding protein (*e.g.*, an antibody or a binding fragment thereof, a single-chain variable fragment (scFv), a checkpoint inhibitor), a detectable moiety, a biologically active group (*e.g.*, another peptide or a small molecule conjugate), or an antibody-drug conjugate.

[0206] According to embodiments of the present disclosure, a therapeutically effective amount of the pharmaceutical compositions can stimulate an immune response in a subject in need thereof, preferably results in treatment of a disease, disorder, or condition; prevents or slows the progression of the disease, disorder, or condition; or reduces or completely alleviates symptoms associated with the immune disease,

disorder, or condition. In some embodiments, the disease, disorder, or condition can be a cancer, an infection, an autoimmune disease, or a rare disease.

[0207] In some embodiments, the pharmaceutical compositions of the present disclosure can comprise the compositions of the disclosure (*i.e.*, the therapeutic and/or polypeptide) in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents, or excipients. In some embodiments, the pharmaceutical composition can comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextran, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (*e.g.*, aluminum hydroxide); and preservatives. In some embodiments, the pharmaceutical composition can comprise excipients, chemical additives, co-solutes, or co-solvents that can stabilize the polypeptide while in solution form (also in dried or frozen forms). Non-limiting examples of the excipients, chemical additives, co-solutes, or co-solvents include but are not limited to sugars/polyols such as sucrose, lactose, glycerol, xylitol, sorbitol, mannitol, maltose, inositol, trehalose, glucose; polymers such as serum albumin (bovine serum albumin (BSA), human SA or recombinant HA), dextran, PVA, hydroxypropyl methylcellulose (HPMC), polyethyleneimine, gelatin, polyvinylpyrrolidone (PVP), hydroxyethylcellulose (HEC); non-aqueous solvents such as: polyhydric alcohols, (*e.g.*, PEG, ethylene glycol and glycerol) dimethylsulfoxide (DMSO) and dimethylformamide (DMF); amino acids such as proline, L-serine, sodium glutamic acid, alanine, glycine, lysine hydrochloride, sarcosine and gamma-aminobutyric acid; surfactants such as Tween-80, Tween-20, SDS, polysorbate, polyoxyethylene copolymer; and miscellaneous excipients such as potassium phosphate, sodium acetate, ammonium sulfate, magnesium sulfate, sodium sulfate, trimethylamine N-oxide, betaine, metal ions (*e.g.*, zinc, copper, calcium, manganese, and magnesium), CHAPS, monolaurate, 2-O-beta-mannoglycerate or any combination of the above.

[0208] In some embodiments, the pharmaceutical compositions can be administered in any suitable manner, including but not limited to aerosol inhalation, injection, ingestion, transfusion, implantation, or transplantation. The pharmaceutical compositions described herein may be administered to a patient through various routes, such as subcutaneous, intradermal, intratumoral, intranasal, intranodal, intramedullary, intramuscular, intravenous (*i.v.*), intraperitoneal, transdermal, oral, or intra-cranial administration.

[0209] In some embodiments, the pharmaceutical compositions can be formulated for intravenous administration. The pharmaceutical compositions can be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration can be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages can be determined by clinical trials. For intravenous administration, suitable carriers include, but are not limited to, physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.), or phosphate buffered saline (PBS). In all cases, the pharmaceutical compositions should be sterile and should be fluid to the extent that easy syringability exist. It can be stable under the conditions of manufacture and

storage, and can be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants, *e.g.*, sodium dodecyl sulfate. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In some embodiments, isotonic agents, *e.g.*, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride, can be included in the pharmaceutical compositions. Prolonged absorption of the injectable pharmaceutical compositions can be brought about by including in the pharmaceutical composition an agent which delays absorption, for example, aluminum monostearate and gelatin. Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions can be prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above.

[0210] In some embodiment, the pharmaceutical composition can be administered to a patient by intradermal or subcutaneous injection. In another embodiment, the pharmaceutical compositions can be preferably administered by intravenous injection. In some embodiments, the pharmaceutical compositions can be directly injected into specific sites such as a tumor, lymph node, or site of infection.

[0211] In some embodiments, T cells comprising the therapeutic and/or polypeptide can be activated and expanded using methods known in the art for expanding T cells to therapeutic levels and can be administered to a patient in conjunction with other relevant treatment modalities. These modalities can include, but are not limited to, treatment with agents such as antiviral therapy, cidofovir, interleukin-2, Cytarabine (also known as ARA-C), natalizumab for patients with multiple sclerosis (MS), efalizumab for psoriasis patients, or other relevant treatments for progressive multifocal leukoencephalopathy (PML) patients. The timing of administration can vary, with the T cell compositions administered prior to, simultaneously with, or following the other therapeutic modalities.

[0212] When an immunologically effective amount, an anti-tumor effective amount, a tumor-inhibiting effective amount, or therapeutic amount is indicated, the precise amount of the pharmaceutical composition of the present disclosure to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, or condition of the patient (subject).

[0213] In some embodiments, the pharmaceutical compositions can be utilized in combination with chemotherapy, radiation, immunosuppressive agents, or other therapeutic approaches. Examples of such immunosuppressive agents include, but are not limited to, cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, as well as antibodies or other immune-ablative agents such as CAMPATH, anti-

CD3 antibodies or other antibody therapies, cytoxin, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, and FR901228. These agents exert their effects through various mechanisms, such as the inhibition of the calcium-dependent phosphatase calcineurin (*e.g.*, cyclosporin and FK506) or the inhibition of the p70S6 kinase, which is critical for growth factor-induced signaling (*e.g.*, rapamycin) as described in prior studies (Liu et al., *Cell* **66**:807-815 (1991); Henderson et al., *Immun.* **73**:316-321 (1991); Bierer et al., *Curr. Opin. Immun.* **5**:763-773 (1993)).

I. Kits

[0214] Also provided herein are various kits for the practice of the methods described herein. In particular, some embodiments of the disclosure provide kits for use in methods of modulating T-cell activation in a subject. Some other embodiments relate to kits for use in methods of preventing a health condition in a subject in need thereof. Some other embodiments relate to kits for use in methods of treating a health condition in a subject in need thereof. For example, provided herein, in some embodiments, are kits that include one or more of the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, recombinant cells comprising the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, cell cultures, and/or the pharmaceutical compositions as provided and described herein, as well as written instructions for using the same.

[0215] In some embodiments, provided herein are kits that include one or more of the therapeutic and/or polypeptides as described herein, as well as written instructions for using the same in practicing a method described herein. In some embodiments, provided herein are kits that include nucleic acids encoding the therapeutic and/or polypeptide as described herein, as well as written instructions for using the same in practicing a method described herein. In some embodiments, provided herein are kits that include one or more of recombinant cells comprising the therapeutic and/or polypeptides as provided and described herein, as well as written instructions for using the same in practicing a method described herein. In some embodiments, provided herein are kits that include one or more of the pharmaceutical compositions as described herein, as well as written instructions for using the same in practicing a method described herein.

[0216] In some embodiments, the kits of the disclosure further include one or more means useful for the administration of any one of the provided therapeutic and/or polypeptide, nucleic acids encoding the therapeutic and/or polypeptide, recombinant cells comprising the therapeutic and/or polypeptide, cell cultures, and pharmaceutical compositions to a subject. For example, in some embodiments, the kits of the disclosure further include one or more syringes (including pre-filled syringes) and/or catheters (including pre-filled syringes) used to administer any one of the provided therapeutic and/or polypeptide, nucleic acids, recombinant cells, cell cultures, or pharmaceutical compositions to a subject. In some embodiments, a kit can have one or more additional therapeutic agents that can be administered simultaneously or sequentially with

the other kit components for a desired purpose, *e.g.*, for diagnosing, preventing, or treating a condition in a subject in need thereof.

[0217] Any of the above-described kits can further include one or more additional reagents, where such additional reagents can be selected from: dilution buffers; reconstitution solutions, wash buffers, control reagents, control expression vectors, negative controls, positive controls, reagents suitable for *in vitro* production and/or preparation of the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, recombinant cells comprising the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, cell cultures, and/or the pharmaceutical compositions of the disclosure.

[0218] In some embodiments, the components of a kit can be in separate containers. In some other embodiments, the components of a kit can be combined in a single container. Accordingly, in some embodiments of the disclosure, the kit includes one or more of the compositions described herein, *e.g.*, the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, recombinant cells comprising the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, cell cultures, and/or the pharmaceutical compositions of the disclosure in one container (*e.g.*, in a sterile glass or plastic vial) and a further therapeutic agent in another container (*e.g.*, in a sterile glass or plastic vial).

[0219] In some embodiments, the kit can include a combination of the compositions described herein, including the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, recombinant cells comprising the therapeutic and/or polypeptides, nucleic acids encoding the therapeutic and/or polypeptide, cell cultures, and/or the pharmaceutical compositions of the disclosure, in combination with one or more further therapeutic agents formulated together, optionally, in a pharmaceutical composition, in a single, common container.

[0220] In instances where the kit includes a pharmaceutical composition for parenteral administration to a subject, the kit can include a device (*e.g.*, an injection device or catheter) for performing such administration. For example, the kit can include one or more needles (*e.g.*, hypodermic needles) or other injection devices as discussed above containing one or more of the compositions described herein, *e.g.*, chimeric polypeptides, CARs, nucleic acids, recombinant cells, and pharmaceutical compositions of the disclosure.

[0221] In some embodiments, a kit can further include instructions for using the components of the kit to practice the methods disclosed herein. For example, the kit can include a package insert including information concerning the pharmaceutical compositions and dosage forms in the kit. Generally, such information aids patients and physicians in using the enclosed pharmaceutical compositions and dosage forms effectively and safely. For example, the following information regarding a combination of the disclosure may be supplied in the insert: pharmacokinetics, pharmacodynamics, clinical studies, efficacy parameters, indications and usage, contraindications, warnings, precautions, adverse reactions, overdose, proper dosage and administration, how supplied, proper storage conditions, references, manufacturer/distributor information and intellectual property information.

[0222] The instructions for practicing the methods are generally recorded on a suitable recording medium. For example, the instructions can be printed on a substrate, such as paper or plastic, etc. The instructions can be present in the kit as a package insert, in the labeling of the container of the kit or components thereof (*e.g.*, associated with the packaging or subpackaging), etc. The instructions can be present as an electronic storage data file present on a suitable computer readable storage medium, *e.g.*, CD-ROM, diskette, flash drive, etc. In some instances, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source (*e.g.*, via the internet), can be provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions can be recorded on a suitable substrate.

II. METHODS OF THE DISCLOSURE

[0223] Provided herein are, *inter alia*, methods of producing the therapeutics or polypeptides of the disclosure and methods of treatment with the therapeutics or polypeptides of the disclosure.

A. Production Methods

[0224] The svd-TCRs of the present disclosure and their derivatives can be identified, expressed, and characterized for various therapeutic applications.

[0225] The process of identifying an svd-TCR can involve using *e.g.*, yeast surface display libraries, where diverse svd-TCR candidates can be generated through mutagenesis and data mining of TCR beta domain sequences. Subsequently, the svd-TCR candidates can be screened using various techniques such as, but not limited to, fluorescence-activated cell sorting (FACS) and magnetic bead sorting to select svd-TCR variants with enhanced binding affinity, stability, and specificity for target peptide-MHC complexes (pMHCs). Iterative rounds of selection and mutagenesis can be conducted to further improve the desired properties of the svd-TCRs.

[0226] The svd-TCRs can be produced in a mammalian or bacterial expression systems. In mammalian systems, svd-TCRs can be expressed in *e.g.*, Exp CHO cells using codon-optimized plasmids, and the expressed svd-TCRs can be purified through *e.g.*, affinity and size-exclusion chromatography. In bacterial systems, svd-TCRs can be expressed in *e.g.*, *E. coli* using a periplasmic expression approach, and the expressed svd-TCRs can be purified using *e.g.*, nickel affinity chromatography. Analytical techniques such as SDS-PAGE, ELISA, and Western blotting can confirm the expression, purity, and molecular weight of the svd-TCRs.

[0227] In some embodiments, an svd-TCR can be linked to an Fc region of an antibody. For svd-TCR-Fc fusion proteins, the methods can involve linking svd-TCR domains to *e.g.*, human IgG1 Fc regions to create bivalent molecules with enhanced binding potential. The svd-TCR-Fc fusion proteins can be expressed in

mammalian systems and purified using *e.g.*, Protein A/G affinity chromatography followed by *e.g.*, size-exclusion chromatography for improved purity. Various well-known analytical techniques can validate the structure and molecular weight of the fusion proteins.

[0228] Various well-known methodologies can be utilized for the biochemical characterization of svd-TCRs and their derivatives (*e.g.*, svd-TCR-Fc fusion proteins). For example, thermostability of svd-TCRs can be evaluated through thermal shift assays using *e.g.*, differential scanning fluorimetry (DSF) or differential scanning calorimetry (DSC), which can measure melting temperatures (T_m) to assess the stability of the proteins. Mutations that can improve the thermostability can be identified by comparing the T_m values of modified proteins to parental variants, wherein the thermostable variants can be selected for further development.

[0229] The size and molecular weight of svd-TCRs and their derivatives (*e.g.*, svd-TCR-Fc fusion proteins) can be determined using *e.g.*, size-exclusion chromatography with multi-angle light scattering (SEC-MALS). This approach can provide insights into the molecular size, monodispersity, and aggregation state of the proteins. Hydrodynamic and gyration radii of the svd-TCRs and their derivatives (*e.g.*, svd-TCR-Fc fusion proteins) can be calculated using light scattering and small-angle X-ray scattering (SAXS) techniques.

[0230] Solubility and aggregation propensity of svd-TCRs and their derivatives (*e.g.*, svd-TCR-Fc fusion proteins) can be assessed using *e.g.*, dynamic light scattering (DLS) and SEC-MALS, which can help evaluate protein stability and detect higher-order aggregates in solution. The svd-TCR proteins exhibiting monodispersity and solubility at specified concentrations can be identified, ensuring their suitability for therapeutic use.

[0231] Hydrophobicity and hydrophilicity of svd-TCRs and their derivatives (*e.g.*, svd-TCR-Fc fusion proteins) can be analyzed to address issues of aggregation and instability associated with exposed hydrophobic patches. Well-known computational tools and hydrophobicity scales can be used to identify hydrophobic residues, which can then be replaced with hydrophilic ones derived from analogous positions in structurally validated proteins. Such modifications can enhance protein solubility and stability while retaining functional integrity.

[0232] Evaluating binding affinity, specificity, and poly-reactivity of the svd-TCRs and their derivatives (*e.g.*, svd-TCR-Fc fusion proteins) can provide insights into their specificity for targeting peptide-MHC (pMHC) complexes.

[0233] Binding affinity can be assessed by measuring the interaction between svd-TCR molecules, either in monomeric or multimeric formats, and their target pMHC complexes. For example, Surface Plasmon Resonance (SPR) can be utilized to quantify binding dynamics, including dissociation constants (K_D) and apparent affinities. In some embodiments, biotinylated pMHC complexes can be immobilized on sensor chips, and svd-TCR proteins can be introduced at varying concentrations. Data from such experiments can be

analyzed to determine the strength and specificity of the interaction between svd-TCRs and their cognate antigens.

[0234] The ability of svd-TCRs binding target pMHC complexes containing relevant peptides can be tested *e.g.*, using SPR. The binding profiles of svd-TCRs can be compared across different peptide-MHC complexes to confirm selective recognition of the target antigens. Non-specific binding can be minimized through the use of appropriate buffers and assay conditions.

[0235] In some embodiments, poly-reactivity testing can evaluate whether svd-TCRs exhibit undesired interactions with unrelated molecular structures on cell surfaces. For example, immunofluorescence microscopy can be used to observe the binding of svd-TCRs to target-expressing cells, which can confirm the absence of binding to cells lacking the specific pMHC complex. Secondary antibodies conjugated to fluorescent dyes or direct labeling methods can also be employed to visualize svd-TCR binding to target antigens.

[0236] The biological activity of svd-TCRs and their derivatives (*e.g.*, svd-TCR-Fc fusion proteins) can be determined by analyzing their binding to specific pMHC proteins on *e.g.*, human cell surfaces, their role in T cell activation, and their ability to redirect natural killer (NK) cells toward target cells expressing a pMHC of interest.

[0237] In some embodiments, cell surface binding assays can be conducted using immunofluorescence labeling and flow cytometry or fluorescence microscopy to measure the interaction of svd-TCR molecules, including Fc fusion proteins, with pMHC proteins on the surface of human cells. For example, the pMHC-expressing cells can be incubated with svd-TCR proteins, and binding can be detected through *e.g.*, fluorescence-conjugated antibodies. Fluorescence intensity and dissociation constants (K_D) can provide insights into the affinity and specificity of svd-TCR-pMHC interactions.

[0238] T cell activation assays can evaluate the ability of svd-TCR-anti-CD3 fusion proteins to redirect CD3⁺ T cells toward target cells displaying a pMHC antigen. This can be assessed using *e.g.*, ELISPOT assays to measure interferon- γ secretion as an indicator of T cell activation. Additionally, real-time microscopy with apoptosis markers such as Caspase-3/7 can be used to monitor the cytotoxic effects of T cell-mediated killing of antigen-positive tumor cells. In some embodiments, various svd-TCR concentrations can be tested to establish dose-dependent efficacy.

[0239] NK cell redirection can be assessed with svd-TCR-Fc fusion proteins modified to enhance binding to Fc γ RIII receptors on NK cells. For example, NK cells can be co-cultured with peptide-pulsed target cells expressing specific pMHC complexes, and NK cell-mediated cytotoxicity can be evaluated by measuring lactate dehydrogenase release and degranulation markers like perforin and CD107a.

B. Methods of Treatment

[0240] The therapeutics or polypeptides of the present disclosure or cells comprising the therapeutic or polypeptide of the present disclosure can be used as a medicament in the treatment of a disease, disorder, or condition in a subject. In some embodiments, the disease, disorder, or condition can be a cancer, an infection, an autoimmune disease, or a rare disease.

[0241] Accordingly, provided herein are, *inter alia*, methods of treatment with the therapeutics or polypeptides of the disclosure or with the cells comprising the therapeutics or polypeptides of the disclosure. The therapeutics or polypeptides comprises svd-TCRs or their derivatives (*e.g.*, svd-TCR-Fc fusion proteins).

[0242] In some embodiments, an administration of the therapeutics or polypeptides of the disclosure can be parenteral administration, *i.e.*, subcutaneous, intramuscular, intravenous, intraperitoneal, intracerebrospinal, intra-articular, intrasynovial, and/or intrathecal administrations. Parenteral administration can be by bolus injection or continuous infusion. The pharmaceutical composition comprising the therapeutics or polypeptides for injection can be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. In some embodiments, the pharmaceutical composition comprising the therapeutics or polypeptides can be delivered using Inject-ease™, Genject™, injector pens such as GenPen™, and needleless devices such as MediJector™ and BioJector™.

[0243] The therapeutics or polypeptides can also be formulated as a depot preparation. Such long-acting formulations can be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Accordingly, in some embodiments, the formulations can be modified with suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil), ion exchange resins, or as sparingly soluble derivatives, for example, in the form of a sparingly soluble salt.

[0244] The cells engineered to comprise the therapeutics or polypeptides (*e.g.*, T cells) of the present disclosure can be allogeneic or autologous to a subject who receives the treatment. In instances wherein the cells are allogeneic, preferably the cells are MHC or HLA histocompatible relative to the subject to be treated and/or are modified to impair or eliminate expression or functionality of the cells' endogenous TCRs and/or MHCs. In some instances, allogeneic T cells can be preferred, especially if the T cells of the subject to be treated are diseased and/or possess some property that renders them less than ideal for therapeutic use. In some instances, allogeneic T cells can be preferred, especially if the T cells are obtained from healthy donors as they may better migrate or traffic to desired sites.

[0245] In some embodiments, the cell therapy, *e.g.*, adoptive cell therapy, *e.g.*, adoptive T cell therapy, can be carried out by autologous transfer, in which the cells can be isolated and/or otherwise prepared from the subject who is to receive the cell therapy, or from a sample derived from such a subject. Thus, in some embodiments, the cells can be derived from a subject, *e.g.*, patient, in need of a treatment and the cells, following isolation and processing can be administered to the same subject.

[0246] In some embodiments, the cell therapy, *e.g.*, adoptive cell therapy, *e.g.*, adoptive T cell therapy, can be carried out by allogeneic transfer, in which the cells can be isolated and/or otherwise prepared from a subject other than a subject who is to receive or who ultimately receives the cell therapy, *e.g.*, a first subject. In such embodiments, the cells then can be administered to a different subject, *e.g.*, a second subject, of the same species. In some embodiments, the first and second subjects can be genetically identical. In some embodiments, the first and second subjects can be genetically similar. In some embodiments, the second subject can express the same HLA class or supertype as the first subject.

[0247] The subject referred to herein can be any living subject. In a preferred embodiment, the subject can be a mammal. The mammal referred to herein can be any mammal. In some embodiments, the mammal can be a human.

[0248] In some embodiments, the subject, to whom the cells, cell populations, or compositions can be administered is a primate, preferably a human. In some embodiments, the primate can be a monkey or an ape. The subject can be male or female and can be any suitable age, including infant, juvenile, adolescent, adult, and geriatric subjects. In some examples, the patient or subject can be a validated animal model for disease, adoptive cell therapy, and/or for assessing toxic outcomes, such as cytokine release syndrome (CRS). In some embodiments, the subject has a cancer, an infection, an autoimmune disease, or a rare disease

[0249] In some embodiments, the cells, populations, and compositions can be administered to a subject having the particular disease or condition to be treated, *e.g.*, via adoptive cell therapy, such as adoptive T cell therapy. In some embodiments, the cells or compositions can be administered to the subject, such as a subject having or at risk for the disease or condition. In some embodiments, the methods thereby treat, *e.g.*, ameliorate one or more symptom of the disease or condition.

[0250] In some embodiments, the T cells comprising the therapeutics or polypeptides of the present disclosure can undergo *in vivo* expansion and can persist for an extended amount of time.

[0251] Once the T cells comprising the therapeutics or polypeptides of the present disclosure are administered to a subject (*e.g.*, a human), the biological activity of the T cells populations can be measured by any of a number of known methods. Parameters to assess include specific binding of an engineered or natural T cell or other immune cell to antigen, *in vivo*, *e.g.*, by imaging, or *ex vivo*, *e.g.*, by ELISA or flow cytometry. In some embodiments, the biological activity can be measured by assessing clinical outcome, such as the reduction in disease symptoms.

[0252] The T cells comprising the therapeutics or polypeptides of the present disclosure can be administered in a number of ways depending upon whether local or systemic treatment is desired. In the case of adoptive cell therapy, methods for administration of cells for adoptive cell therapy are known and can be used in connection with the provided compositions.

[0253] Formulations comprising populations of the T cells comprising the therapeutics or polypeptides of the present disclosure can include pharmaceutically acceptable excipient(s). Excipients included in the

formulations can have different purposes depending, for example, on the T cells, the subpopulation of T cells used, and the mode of administration. Examples of generally used excipients include, without limitation: saline, buffered saline, dextrose, water-for-injection, glycerol, ethanol, and combinations thereof, stabilizing agents, solubilizing agents and surfactants, buffers and preservatives, tonicity agents, bulking agents, and lubricating agents. The formulations comprising populations of T cells comprising the therapeutics or polypeptides of the present disclosure can typically have been prepared and cultured in the absence of any non-human components, such as animal serum (*e.g.*, bovine serum albumin).

[0254] The cells or population of cells can be administered in one or more doses. In some embodiments, an effective amount of cells can be administered as a single dose. In some embodiments, an effective amount of cells can be administered as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The dosage administered can be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired. In some embodiments, administration can be an intravenous administration.

[0255] In some embodiments, the methods of the disclosure can involve administering an effective amount or number of the T cells comprising the therapeutics or polypeptides of the present disclosure to a subject in need thereof. This administering step can be accomplished using any method of implantation delivery in the art. For example, the recombinant cells can be infused directly in the subject's bloodstream or otherwise administered to the subject.

III. DEFINITIONS

[0256] While various embodiments and aspects of the present disclosure are shown and described herein, it will be apparent to those skilled in the art that such embodiments and aspects are provided by way of example only. Numerous variations, changes, and substitutions can be made by those skilled in the art without departing from scope of the present disclosure. It should be understood that alternative approaches, methods, or configurations to those described herein may also be utilized in practicing the present disclosure.

[0257] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in the application including, without limitation, patents, patent applications, articles, books, manuals, and treatises are hereby expressly incorporated by reference in their entirety for any purpose.

[0258] The abbreviations used herein have their conventional meaning within the chemical and biological arts. The chemical structures and formulae set forth herein are constructed according to the standard rules of chemical valency known in the chemical arts.

[0259] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art. See, *e.g.*, Singleton et al., *DICTIONARY OF*

MICROBIOLOGY AND MOLECULAR BIOLOGY^{GY} 2nd ed., J. Wiley & Sons (New York, NY 1994); Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL, Cold Springs Harbor Press (Cold Springs Harbor, NY 1989). Any methods, devices and materials similar or equivalent to those described herein can be used in the practice of this invention. The following definitions are provided to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[0260] Throughout this application, various embodiments may be presented in a range format. It should be understood that the use of a range format is intended for convenience and brevity and should not be construed as a rigid limitation on the scope of the disclosure. The description of a range should be considered to explicitly disclose all the possible subranges as well as each individual numerical value within that range. For example, the description of a range such as from 1 to 6 should be understood to specifically disclose subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6, and so on, as well as individual numbers within that range, including 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0261] As used in the specification and claims, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a sample” includes a plurality of samples, including mixtures thereof.

[0262] The terms “determining,” “measuring,” “evaluating,” “assessing,” “assaying,” and “analyzing” are used interchangeably herein to refer to various forms of measurement. These terms encompass determining whether an element is present or not, which includes, but is not limited to, detection. The scope of these terms extends to quantitative determinations, qualitative determinations, or a combination of both quantitative and qualitative determinations. Assessing can be relative or absolute, depending on the context in which it is performed. Additionally, the term “detecting the presence of” can include determining the quantity of amount of a substance present, as well as determining whether it is present or absent.

[0263] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *i.e.*, the limitations of the measurement system. Where particular values are described in the application and claims, unless otherwise stated the term “about” meaning within an acceptable error range for the particular value should be assumed.

[0264] Amino acids may be referred to herein using their commonly known three-letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Similarly, nucleotides may be referred to using their widely accepted single-letter codes.

[0265] An amino acid or nucleotide “base position” can be denoted by a number that sequentially identifies each amino acid residue or nucleotide base in the reference sequence, with numbering based on its position relative to the N-terminus for amino acids or 5'-end for nucleotides. It should be noted, however,

that due to occurrences such as deletions, insertions, truncations, fusions, the positional numbering in a test sequence determined by counting sequentially from the N-terminus or 5'-end will not necessarily correspond directly to the same numbered position in the reference sequence. For example, if a variant sequence includes a deletion relative to the aligned reference sequence, there will be no amino acid or nucleotide in the variant sequence corresponding to the position of the deletion in the reference sequence. Conversely, when an insertion occurs in the aligned reference sequence, the inserted elements will not correspond to any numbered position in the variant reference sequence. Similarly, truncations or fusions can result in stretches of amino acids or nucleotides in either the reference or the aligned sequence that do not correspond to any part of the other sequence.

[0266] The terms “numbered with reference to” or “corresponding to,” as used in the context of the numbering of amino acid or polynucleotide sequences, refer to the numbering of residues within a specified reference sequence when a given amino acid or polynucleotide sequence is compared to the reference sequence. In this context, an amino acid residue in a protein is considered to “correspond” to a given residue in the reference sequence if it occupies the same essential structural position within the protein as the given residue. A person skilled in the art will readily recognize the identity and location of residues corresponding to a specific position in a protein when examining other proteins with different numbering systems. For example, by performing a sequence alignment between a protein and a reference protein sequence, the identity and location of residues corresponding to specific positions in the reference protein can be identified in other protein sequences that align with the reference sequence. If a selected residue in a particular protein occupies the same essential spatial or structural position as a glutamic acid at position 138 in the reference sequence, then the selected residue corresponds to the glutamic acid at position 138. In this instance, a simple primary sequence alignment can be used to establish homology and determine which position in the selected protein aligns with glutamic acid 138 in the reference sequence. In some cases, a three-dimensional structural alignment can be employed instead of or in addition to primary sequence alignment. When using structural alignment, the structure of the selected protein is aligned to maximize correspondence with the reference structure, such that an amino acid in the selected protein occupying the same essential spatial or structural position as glutamic acid at position 138 in the reference sequence corresponds to glutamic acid 138. This approach considers spatial and structural relationships rather than solely relying on sequence homology. Whether using primary sequence alignment or structural alignment, the correspondence between residues is determined based on their functional or structural equivalence within the protein context.

[0267] With regard to amino acid sequences, a skilled artisan will understand that individual substitutions, deletions, or additions to a nucleic acid, peptide, polypeptide, or protein sequence that alter, add, or delete a single amino acid or a small percentage of amino acids in the encoded sequence are referred to as “conservatively modified variants,” provided the alteration results in the substitution of an amino acid with a chemically similar amino acid. Such substitutions are based on the chemical and functional similarity of

amino acids, and conservative substitution tables that list functionally similar amino acids are well established and widely recognized in the art. Conservatively modified variants retain the fundamental properties of the original sequence due to the chemical similarity of the substituted amino acids. These modifications are distinct from, but inclusive of, naturally occurring polymorphic variants, interspecies homologs, and alleles, which are also encompassed within the scope of the disclosure. Thus, conservatively modified variants represent a subset of sequence alterations that maintain the functional integrity of the original sequence while accommodating slight chemical changes through substitutions, additions, or deletions of amino acids.

[0268] The term “conservatively modified variants” may apply to both amino acid and nucleic acid sequences. With respect to nucleic acid sequences, “conservatively modified variants” may refer to those sequences that encode identical or essentially identical amino acid sequences. Due to the degeneracy of the genetic code, multiple nucleic acid sequences can encode the same protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at any position in a nucleic acid sequence where alanine is specified by a codon, the codon can be altered to any of the synonymous codons (*e.g.*, from GCA to GCC, GCG, or GCU) without changing the amino acid sequence of the resulting polypeptide. Such variations in nucleic acid sequences, commonly referred to as “silent variations,” represent one category of conservatively modified variants. These silent variations do not alter the encoded polypeptide sequence but result in different nucleotide sequences that are functionally equivalent. It is understood that every nucleic acid sequence disclosed herein, which encodes a polypeptide, inherently encompasses all possible silent variations of the nucleic acid sequence. A skilled artisan will recognize that each codon in a nucleic acid sequence, except for the codons AUG (ordinarily the only codon for methionine) and TGG (ordinarily the only codon for tryptophan), can be substituted with one of its synonymous codons without altering the function or identity of the encoded polypeptide. As such, all silent variations of a nucleic acid sequence that encode a polypeptide are inherently described and included within each nucleic acid sequence disclosed herein.

[0269] The terms “identical” or percent “identity,” when used in reference to two or more nucleic acids or polypeptide sequences, refer to sequences or subsequences that are either entirely the same or have a specified percentage of amino acid residues or nucleotides that are identical. The percentage identity may range from about 60% identity, with higher thresholds including 65%, 70%, 75%, 75%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region. This identity can be determined by comparing the sequences and aligning them for maximum correspondence over a defined comparison window or designated region. Such comparisons can be carried out using sequence comparison algorithms such as a BLAST or BLAST 2.0 with default parameters as described herein, or alternatively by manual alignment and visual inspection (*e.g.*, see NCBI web site www.ncbi.nlm.nih.gov/BLAST/ or similar resources). Sequences demonstrating such level of identity can be described as being “substantially

identical.” This definition further encompasses sequences that represent the complement of a test sequence and extends to sequences that include deletions, additions, and/or substitutions. The assessment of identity may account for these alterations, such as insertions or gaps, using preferred algorithms designed for sequence comparison, which allow for the presence of such variations while still enabling an accurate determination of correspondence. Preferably, the region of comparison for identity is at least about 25 amino acids or nucleotides in length, and more preferably extends over a region ranging from 50 to 100 amino acids or nucleotides or longer.

[0270] The term “percentage of sequence identity” refers to a measure obtained by comparing two optimally aligned sequences over a defined comparison window. The comparison window represents a region where the polynucleotide or polypeptide sequences are aligned to achieve maximum correspondence. This alignment may involve introducing additions or deletions (*i.e.*, gaps) into one of the sequences to ensure optimal alignment with the reference sequence, which itself does not contain such additions or deletions. To determine the percentage of sequence identity, the number of positions where an identical nucleic acid base or amino acid residue is present in both sequences within the comparison window can be identified. This count of identical positions is referred to as the number of matched positions. The number of matched positions is then divided by the total number of positions within the comparison window, including any positions accounted for by gaps introduced during alignment. The resulting value is then multiplied by 100 to yield the percentage of sequence identity.

[0271] An amino acid or nucleotide base “position” can be identified by a number that sequentially designates each amino acid (or nucleotide base) in the reference sequence, starting from the N-terminus (or 5'-end). However, due to factors such as deletions, insertions, truncations, or fusions that may occur in a test sequence, determining an optimal alignment can result in discrepancies between the numbering of amino acid residues in the test sequence and their corresponding positions in the reference sequence. For example, when a variant includes a deletion relative to the aligned reference sequence, there will be no amino acid in the variant corresponding to the position in the reference sequence at the site of the deletion. Conversely, if an insertion is present in the aligned reference sequence, the inserted region will not correspond to a numbered amino acid position in the reference sequence. Similarly, in cases of truncations or fusions, stretches of amino acids may exist in either the reference sequence or the aligned test sequence that lack correspondence to any amino acid in the other sequence.

[0272] A “comparison window,” as used herein, refers to a segment consisting of any number of contiguous positions, such as a full-length sequence or a range from 20 to 600 positions, or about 50 to about 200, or about 100 to about 150 amino acids or nucleotides. Within this segment, a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences have been optimally aligned. Methods for aligning sequences for comparison are well established in the field. Optimal alignment can be performed using techniques such as the local homology algorithm of Smith and

Waterman, *Adv. Appl. Math.* **2**:482 (1970); the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* **48**:443 (1970); the search for similarity method of Pearson and Lipman, *Proc. Nat'l. Acad. Sci.* **85**:2444 (1988); computerized implementations of these algorithms (*e.g.*, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI); or by manual alignment and visual inspection, as detailed in sources such as Ausubel et al., *Current Protocols in Molecular Biology* (1995 supplement).

[0273] An example of an algorithm suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, including its updated version BLAST 2.0, as described in Altschul et al. *Nucleic Acids Research* **25**:3389-3402 (1997) and Altschul et al. *Journal of Molecular Biology* **215**:403-410 (1990). Software implementing these algorithms is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). The algorithm functions by first identifying high scoring sequence pairs (HSPs), achieved by locating short words of a specified length, denoted as W, within the query sequence. These words must either match exactly or meet a predetermined positive threshold score T when aligned with a word of the same length in a database sequence. The threshold score T is referred to as the neighborhood word score threshold, as described in Altschul et al., *supra*. The identified neighborhood word hits serve as seeds to initiate searches for longer HSPs that incorporate them. The algorithm extends these word hits bidirectionally along the sequences as long as the cumulative alignment score increases. Cumulative scores are calculated using specific parameters: for nucleotide sequences, M represents the reward score for a pair of matching residues (always greater than 0), and N represents the penalty score for mismatching residues (always less than 0). For amino acid sequences, a scoring matrix is applied to calculate the cumulative score. The extension of word hits in either direction stops under the following conditions: (1) the cumulative alignment score drops by a quantity X from its maximum value achieved, (2) the cumulative score falls to zero or below because of the accumulation of negative-scoring residue alignments, or (3) the end of either sequence is reached. The parameters W (word length), T (threshold score), and X (drop-off value) govern the sensitivity and speed of the alignment in the BLAST algorithm. For nucleotide sequences, the BLASTN program uses default values, which include a word length (W) of 11, an expectation value (E) of 10, a match reward score (M) of 5, a mismatch penalty score (N) of -4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses default values that include a word length of 3, an expectation value of 10, and the BLOSUM62 scoring matrix (as described in Henikoff and Henikoff, *Proc. Nat'l. Acad. Sci.* **89**:10915 (1989)), along with alignment parameters of B=50 and other default values of M = 5 and N = -4. Both BLASTN and BLASTP perform comparisons of both strands in their default configurations.

[0274] The BLAST algorithm can also perform a statistical analysis to evaluate the similarity between two sequences, as described in Karlin and Altschul, *Proc. Nat'l. Acad. Sci.* **90**:5873-5787 (1993). Among the measures of similarity that the BLAST algorithm provides is the smallest sum probability (P(N)), which

serves as an indicator of the likelihood that a match between two nucleotide or amino acid sequences occurs purely by chance. For example, a nucleic acid sequence is deemed similar to a reference sequence if the smallest sum probability in the comparison of the test nucleic acid to the reference nucleic acid is less than approximately 0.2, with increasing stringency and preference for probabilities of less than approximately 0.01, and most preferably, less than approximately 0.001.

[0275] For specific proteins described herein, the named protein can encompass any of the protein's naturally occurring forms, variants, or homologs that retain the activity of the protein (*e.g.*, within at least 50%, 75%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% activity compared to the native protein). In some embodiments, variants or homologs exhibit at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% amino acid sequence identity across the entire sequence or a portion of the sequence (*e.g.*, a 50, 100, 150, or 200 continuous amino acid portion) compared to a naturally occurring form. In other embodiments, the protein is the protein as identified by its NCBI sequence reference. In further embodiments, the protein is the protein as identified by its NCBI sequence reference, homolog, or functional fragment thereof.

[0276] The term "CD8," as referred to herein, is a transmembrane glycoprotein that functions as a co-receptor for the T cell receptor (TCR). Similar to the TCR, CD8 interacts with a major histocompatibility complex (MHC) molecule, but it is specific for the class I MHC protein; see ENTREZ No. 925 and UNIPROT No. P01732, both of which are incorporated by reference herein.

[0277] A "gene" can encompass a segment of a nucleic acid involved in producing a protein; a gene may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). The leader, the trailer, as well as the introns, may contain regulatory elements essential for the transcription and translation of a gene. In some embodiments, a gene may comprise a nucleic acid, such as a DNA sequence, involved in producing a protein.

[0278] The term "allele" refers to varying forms of the genomic DNA located at a given site. For example, "allele" refers to a genetic variant at a specific genomic location (locus). Alleles include silent nucleotide substitutions as well as nucleotide substitutions that alter the amino acid sequence of the encoded protein. In some instances, alleles may result in alternative RNA splicing.

[0279] The terms "plasmid," "vector," or "expression vector" can refer to a nucleic acid molecule that encodes genes and/or regulatory elements necessary for the expression of genes. Expression of a gene from a plasmid can occur in *cis* or in *trans*. If a gene is expressed in *cis*, the gene and the regulatory elements are encoded by the same plasmid. Expression in *trans* may refer to instances where the gene and the regulatory elements are encoded by separate plasmids.

[0280] As used herein, the term "construct" can refer to any recombinant nucleic acid molecule. In some embodiments, a construct includes an expression cassette, plasmid, cosmid, virus, autonomously replicating polynucleotide molecule, phage, or linear or circular, single-stranded or double-stranded DNA or RNA polynucleotide molecule. A construct may be derived from any source and may be capable of genomic

integration or autonomous replication, including a nucleic acid molecule where one or more nucleic acid sequences have been linked in a functionally operative manner, *e.g.*, operably linked.

[0281] The term “expression” can encompass any step involved in the production of a polypeptide, including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion. Expression can be detected using conventional techniques for detecting proteins (*e.g.*, ELISA, Western blotting, flow cytometry, immunofluorescence, immunohistochemistry, etc.).

[0282] “Control experiment” is used in accordance with its plain ordinary meaning and refers to an experiment in which the subjects or reagents of the experiment are treated as in a parallel experiment, except for the omission of a procedure, reagent, or variable of the experiment

[0283] A “control” or “standard control” can refer to a sample, measurement, or value that serves as a reference, usually a known reference, for comparison to a test sample, measurement, or value. For example, a test sample can be taken from a patient suspected of having a given disease (*e.g.*, cancer, an infection, an autoimmune disease, or a rare disease) and compared to a known normal (non-diseased) individual (*e.g.*, a standard control subject). A standard control can also represent an average measurement or value gathered from a population of similar individuals (*e.g.*, standard control subjects) that do not have a given disease (*i.e.*, standard control population), *e.g.*, healthy individuals with a similar medical background, same age, weight, etc. A standard control value can also be obtained from the same individual, *e.g.*, from an earlier-obtained sample from the patient prior to disease onset. For example, a control can be devised to compare therapeutic benefit based on pharmacological data (*e.g.*, half-life) or therapeutic measures (*e.g.*, comparison of side effects). Controls are also valuable for determining the significance of data. For example, if values for a given parameter are widely variant in controls, variation in test samples will not be considered significant. One of skill in the art will recognize that standard controls can be designed for assessment of any number of parameters (*e.g.*, RNA levels, protein levels, specific cell types, specific bodily fluids, specific tissues, etc.). One of skill in the art will understand which standard controls are most appropriate in a given situation and be able to analyze data based on comparisons to standard control values. Standard controls are also valuable for determining the significance (*e.g.*, statistical significance) of data. For example, if values for a given parameter are widely variant in standard controls, variation in test samples will not be considered significant.

[0284] “Patient,” “subject,” or “subject in need thereof” can refer to a living organism suffering from or prone to a disease or condition that can be treated by the administration of a pharmaceutical composition as provided herein. Non-limiting examples include humans, other mammals, bovines, rats, mice, dogs, monkeys, goats, sheep, cows, deer, and other non-mammalian animals. In some embodiments, a patient is human.

[0285] The terms “disease” or “condition” can refer to a state of being or health status of a patient or subject that is capable of being treated with the compounds or methods provided herein. The disease can include or be cancer, an infection, an autoimmune disease, or a rare disease.

[0286] The terms “treating” or “treatment” refers to achieving beneficial or desired results in addressing an injury, disease, pathology or condition. The beneficial or desired results can include any objective or subjective parameter, such as alleviation or amelioration of symptoms; abatement or diminishment of the extent of a disease; stabilizing (*i.e.*, not worsening) the state of disease; partial or total remission of the disease or diminishing the reoccurrence of the disease; making the injury, pathology, or condition more tolerable to the patient; slowing the rate of degeneration, decline, or disease progression; preventing the transmission of a disease; making the final point of degeneration less debilitating; shortening the duration of a disease; improving a patient’s physical or mental well-being; or any combination of these outcomes. The treatment or amelioration of symptoms can be based on objective parameters, such as physical examinations, neuropsychiatric exams, or other diagnostic assessments, or subjective evaluations of a patient’s condition. Treating or treatment can also include the prevention of an injury, pathology, condition, or disease. Accordingly, treating or treatment can refer to achieving a measurable reduction in the severity of an established disease, condition, or symptom, with reductions ranging from 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 90%, to 100%. For example, a method for treating a disease can be considered a treatment when it results in at least 10% reduction in one or more symptoms of the disease in a subject as compared to a control. The reduction can occur at any percentage between 10% and 100%, as compared to baseline or control levels. It is understood that treatment does not necessarily imply a cure or complete ablation of the disease, condition, or its symptoms. Additionally, references to terms such as decreasing, reducing, or inhibiting are used to describe changes of 10% or greater relative to control levels. These terms can encompass a spectrum of effects, including partial improvements, but do not necessarily require complete resolution or elimination of the condition or its symptoms. In some embodiments, treatment can also include prophylactic approaches, in which an active agent can be administered to a subject in a therapeutically effective amount to prevent the onset or progression of a condition. Prophylactic treatment can consist of a single administration or multiple administrations over a variable duration. The treatment period can depend on factors such as the severity of the condition, the age of the subject, the concentration of the active agent, the activity of the composition, or a combination of these factors. Effective dosages of the agent can increase or decrease during the treatment or prophylactic regimen based on diagnostic assessments. Effective dosages and changes in dosage can be determined by a skilled person in the art. In some embodiments, chronic administration can be required. In some embodiments, treatment can exclude prophylactic approaches.

[0287] The term “prevent” can refer to a decrease in the occurrence of disease symptoms in a patient. The prevention can be complete (no detectable symptoms) or partial, such that fewer symptoms are observed than would likely occur in the absence of treatment.

[0288] A “therapeutic agent,” as used herein, can refer to an agent (*e.g.*, compound or composition described herein) that, when administered to a subject, will have the intended prophylactic effect, *e.g.*, preventing or delaying the onset (or recurrence) of an injury, disease, pathology, or condition, or reducing the

likelihood of the onset (or recurrence) of an injury, disease, pathology, or condition, or their symptoms, or the intended therapeutic effect, *e.g.*, treatment or amelioration of an injury, disease, pathology, or condition, or their symptoms, including any objective or subjective parameter of treatment such as abatement; remission; diminishing of symptoms; making the injury, pathology, or condition more tolerable to the patient; slowing the rate of degeneration or decline; making the final point of degeneration less debilitating; or improving a patient's physical or mental well-being.

[0289] The term "soluble" refers to a polypeptide that may not be readily sedimented under low G-force centrifugation (*e.g.*, less than about 30,000 revolutions per minute in a standard centrifuge) from an aqueous buffer. For example, the polypeptide may not be readily sedimented in cell media. In some embodiments, the polypeptide can remain in aqueous solution at a temperature greater than about 5-37 °C at or near neutral pH, wherein the aqueous solution includes a low concentration of an anionic or non-ionic detergent, or wherein there is an absence of an anionic or non-ionic detergent.

IV. EMBODIMENTS

[0290] Embodiment 1. A therapeutic, comprising: a single-variable domain (svd)-T cell receptor (TCR) that binds to a first epitope of a first peptide-MHC complex (pMHC); and an antigen binding domain that binds to a second epitope; wherein the therapeutic is soluble.

[0291] Embodiment 2. The therapeutic of embodiment 1, wherein the antigen binding domain comprises multiple separate polypeptides.

[0292] Embodiment 3. The therapeutic of embodiment 2, wherein the antigen binding domain comprises a second svd-TCR.

[0293] Embodiment 4. The therapeutic of embodiment 3, wherein the antigen binding domain comprises a second svd-TCR, an antibody, an antibody fragment, an antibody binding domain or a TCR binding domain.

[0294] Embodiment 5. The therapeutic of embodiment 1, wherein the antigen binding domain is a single polypeptide.

[0295] Embodiment 6. The therapeutic of embodiment 1, wherein the svd-TCR and the antigen binding domain together are a single polypeptide chain.

[0296] Embodiment 7. The therapeutic of any one of embodiments 1-6, wherein the svd-TCR and the antigen binding domain are non-covalently linked together, or wherein the svd-TCR is non-covalently linked with the antigen binding domain.

[0297] Embodiment 8. The therapeutic of any one of embodiments 1-6, wherein the svd-TCR is covalently linked with the antigen binding domain.

[0298] Embodiment 9. The therapeutic of any one of embodiments 1-8, wherein the svd-TCR or the antigen binding domain is linked to a scaffold.

- [0299] Embodiment 10. The therapeutic of embodiment 9, wherein the scaffold comprises an antibody fragment crystallizable (Fc) region, an antibody constant domain, a TCR constant domain, albumin, a nanocage, ferritin, or lumazine synthase, or a fragment thereof.
- [0300] Embodiment 11. A therapeutic, comprising: a polypeptide, comprising a first single-variable domain TCR (svd-TCR) that binds to a first epitope of a first peptide-MHC complex (pMHC), and a second svd-TCR that binds to a second epitope.
- [0301] Embodiment 12. The therapeutic of embodiment 11, wherein the polypeptide is soluble.
- [0302] Embodiment 13. The therapeutic of any one of embodiments 1-12, wherein the first epitope or the second epitope is on a cell.
- [0303] Embodiment 14. The therapeutic of embodiment 13, wherein the cell is a diseased cell.
- [0304] Embodiment 15. The therapeutic of embodiment 14, wherein the disease is cancer, an autoimmune disease, an infectious disease, or a rare disease.
- [0305] Embodiment 16. The therapeutic of embodiment 15, wherein the first epitope or second epitope is a cancer antigen, a neoantigen, a viral antigen, or a bacterial antigen.
- [0306] Embodiment 17. The therapeutic of any one of embodiments 1-16, wherein the second epitope is the same as the first epitope.
- [0307] Embodiment 18. The therapeutic of any one of embodiments 1-16, wherein the second epitope is different from the first epitope.
- [0308] Embodiment 19. The therapeutic of any one of embodiments 1-18, wherein the second epitope comprises a surface antigen.
- [0309] Embodiment 20. The therapeutic of embodiment 19, wherein the second epitope comprises a non-pMHC surface protein.
- [0310] Embodiment 21. The therapeutic of embodiment 19, wherein the second epitope is of a second pMHC.
- [0311] Embodiment 22. The therapeutic of embodiment 21, wherein an MHC of the pMHC is a class I MHC or a class II MHC.
- [0312] Embodiment 23. The therapeutic of any one of embodiments 1-22, further comprising a T-cell engager or NK-cell engager.
- [0313] Embodiment 24. The therapeutic of claim 23, wherein the T-cell engager is a CD3 engager.
- [0314] Embodiment 25. The therapeutic of any one of embodiments 1-24, wherein the first or second epitope is not a superantigen.
- [0315] Embodiment 26. The therapeutic of any one of embodiments 1-25, wherein the svd-TCR or the antigen binding domain comprises a TCR variable domain.
- [0316] Embodiment 27. The therapeutic of embodiments 26, wherein the TCR variable domain comprises a V α , V β , V γ , or V δ variable domain, or a binding fragment thereof.

- [0317] Embodiment 28. The therapeutic of embodiments 27, wherein the TCR variable domain comprises a V β variable domain, or a binding fragment thereof.
- [0318] Embodiment 29. The therapeutic of any one of embodiments 26-28, wherein the TCR variable domain comprises a mammalian variable domain or a binding fragment thereof, or is at least 90% identical to a mammalian variable domain sequence.
- [0319] Embodiment 30. The therapeutic of any one of embodiments 26-28, wherein the TCR variable domain comprises a non-mammalian variable domain or a binding fragment thereof, or is at least 90% identical to a non-mammalian variable domain sequence.
- [0320] Embodiment 31. The therapeutic of any one of embodiments 1-30, wherein the TCR variable domain of the svd-TCR or the antigen binding domain is derived from a TRAV, TRBV, TRGV, or TRDV gene.
- [0321] Embodiment 32. The therapeutic of embodiment 31, wherein the TCR variable domain of the svd-TCR or the antigen binding domain is derived from a TRBV1, TRBV2, TRBV3-1, TRBV3-2, TRBV4-1, TRBV4-2, TRBV4-3, TRBV5-1, TRBV5-2, TRBV5-3, TRBV5-4, TRBV5-5, TRBV5-6, TRBV5-7, TRBV5-8, TRBV6-1, TRBV6-2, TRBV6-3, TRBV6-4, TRBV6-5, TRBV6-6, TRBV6-7, TRBV6-8, TRBV6-9, TRBV7-1, TRBV7-2, TRBV7-3, TRBV7-4, TRBV7-5, TRBV7-6, TRBV7-7, TRBV7-8, TRBV7-9, TRBV8-1, TRBV8-2, TRBV9, TRBV10-1, TRBV10-2, TRBV10-3, TRBV11-1, TRBV11-2, TRBV11-3, TRBV12-1, TRBV12-2, TRBV12-3, TRBV12-4, TRBV12-5, TRBV13, TRBV14, TRBV15, TRBV16, TRBV17, TRBV18, TRBV19, TRBV20-1, TRBV21-1, TRBV22-1, TRBV23-1, TRBV24-1, TRBV25-1, TRBV26, TRBV27, TRBV28, TRBV29-1, or TRBV30 gene or a fragment thereof.
- [0322] Embodiment 33. The therapeutic of embodiment 32, wherein the svd-TCR includes a TRBJ1-1, TRBJ1-2, TRBJ1-3, TRBJ1-4, TRBJ1-5, TRBJ1-6, TRBJ2-1, TRBJ2-2, TRBJ2-2P, TRBJ2-3, TRBJ2-4, TRBJ2-5, TRBJ2-6, or TRBJ2-7 gene or a fragment or derivative thereof.
- [0323] Embodiment 34. The therapeutic of any one of embodiments 26-33, wherein the TCR variable domain does not comprise a TCR transmembrane domain or a portion thereof.
- [0324] Embodiment 35. The therapeutic of any one of embodiments 26-34, wherein the TCR variable domain comprises a portion of constant domain.
- [0325] Embodiment 36. The therapeutic of any one of embodiments 26-34, wherein the TCR variable domain does not comprise a TCR constant domain or a portion thereof.
- [0326] Embodiment 37. The therapeutic of any one of embodiments 26-36, wherein the TCR variable domain comprises a CDR of a human, mouse, or macaque or a derivative thereof.
- [0327] Embodiment 38. The therapeutic of embodiments 37, wherein the TCR variable domain comprises a CDR3 having a length of 10-30 amino acids.
- [0328] Embodiment 39. The therapeutic of any one of embodiments 1-38, wherein the svd-TCR or the antigen binding domain comprises a glycosylation site.

- [0329] Embodiment 40. The therapeutic of embodiments 39, wherein the glycosylation site comprises a NX[S/T] amino acid motif, wherein X is any residue except proline.
- [0330] Embodiment 41. The therapeutic of embodiment 40, wherein the glycosylation site comprises an amino acid motif selected from the group consisting of: NYS, NVT, NLT, NLS, NVS, NET, NES, NMS, NFT, or NGT.
- [0331] Embodiment 42. The therapeutic of any one of embodiments 1-41, wherein the TCR variable domain of the svd-TCR or the antigen binding domain comprises a hydrophobic residue mutated to a non-hydrophobic residue relative to a wild type or non-mutated TCR variable domain.
- [0332] Embodiment 43. The therapeutic of embodiment 42, wherein the hydrophobic residue is from a region shown in any of FIG. 7A-7D.
- [0333] Embodiment 44. The therapeutic of any one of embodiments 42-43, wherein the hydrophobic residue comprises a residue in any of FIG. 7A-7D.
- [0334] Embodiment 45. The therapeutic of any one of embodiments 42-44, wherein the hydrophobic residue comprises a residue in a BetaCON1 region, Alpha1 region, or Alpha2 region of FIG. 7C.
- [0335] Embodiment 46. The therapeutic of any one of embodiments 42-45, wherein the hydrophobic residue is an exposed hydrophobic residue.
- [0336] Embodiment 47. The therapeutic of embodiment 46, wherein the exposed hydrophobic residue comes into contact with another TCR variable domain when not in a svd-TCR format.
- [0337] Embodiment 48. The therapeutic of any one of embodiments 46-47, wherein the exposed hydrophobic residue is within a V α or a V β of the TCR variable domain.
- [0338] Embodiment 49. The therapeutic of any one of embodiments 46-47, wherein the exposed hydrophobic residue is within a β constant domain of the TCR variable domain.
- [0339] Embodiment 50. The therapeutic of embodiment 42, wherein the mutation to the non-hydrophobic residue results in an increase in the hydrophilicity of the therapeutic or a polypeptide of the therapeutic by about 4 to about 8 units, with respect to the Kyte and Doolittle scale, relative to a wild type or non-mutated TCR variable domain.
- [0340] Embodiment 51. The therapeutic of embodiment 50, wherein inclusion of the non-hydrophobic residue results in an increase in the charge density of the therapeutic or a polypeptide of the therapeutic by about +1 or about -1, relative to a wild type or non-mutated TCR variable domain.
- [0341] Embodiment 52. The therapeutic of any one of embodiments 1-52, wherein the TCR variable domain of the svd-TCR or the antigen binding domain comprises a mutated residue located at a buried surface or core of the svd-TCR or the antigen binding domain, relative to a wild type or non-mutated TCR variable domain.

[0342] Embodiment 53. The therapeutic of embodiment 52, wherein inclusion of the mutated residue results in an increase in production, stability, a specific binding activity, or a functional activity of the svd-TCR or the antigen binding domain.

[0343] Embodiment 54. The therapeutic of any one of embodiments 1-53, wherein the therapeutic has a mass of at least 10 kDa, at least 15 kDa, at least 20 kDa, at least 25 kDa, at least 30 kDa, at least 35 kDa, at least 40 kDa, at least 50 kDa, at least 60 kDa, at least 70 kDa, at least 80 kDa, at least 90 kDa, at least 100 kDa, at least 125 kDa, at least 150 kDa, at least 175 kDa, at least 200 kDa, at least 250 kDa, at least 300 kDa, at least 400 kDa, at least 500 kDa, at least 600 kDa, at least 700 kDa, at least 800 kDa, at least 900 kDa, or at least 1000 kDa.

[0344] Embodiment 55. The therapeutic of any one of embodiments 1-53, wherein the therapeutic has a mass of less than 10 kDa, less than 15 kDa, less than 20 kDa, less than 25 kDa, less than 30 kDa, less than 35 kDa, less than 40 kDa, less than 50 kDa, less than 60 kDa, less than 70 kDa, less than 80 kDa, less than 90 kDa, less than 100 kDa, less than 125 kDa, less than 150 kDa, less than 175 kDa, less than 200 kDa, less than 250 kDa, less than 300 kDa, less than 400 kDa, less than 500 kDa, less than 600 kDa, less than 700 kDa, less than 800 kDa, less than 900 kDa, or less than 1000 kDa.

[0345] Embodiment 56. The therapeutic of any one of embodiments 54-55, wherein the mass is a molecular mass estimated based on a predicted chemical structure or sequence.

[0346] Embodiment 57. The therapeutic of any one of embodiments 54-56, wherein the therapeutic or a polypeptide of the therapeutic has a molecular mass no greater than 30 kDa.

[0347] Embodiment 58. The therapeutic of embodiment 57, wherein the svd-TCR or the antigen binding domain has a molecular mass of about 10-13 kDa.

[0348] Embodiment 59. The therapeutic of any one of embodiments 1-58, further comprising an additional binding protein.

[0349] Embodiment 60. The therapeutic of embodiment 59, wherein the additional binding protein is coupled with the svd-TCR or the antigen binding domain.

[0350] Embodiment 61. The therapeutic of embodiment 59, wherein the additional binding protein comprises an antibody or binding fragment thereof or a single-chain variable fragment (scFv).

[0351] Embodiment 62. The therapeutic of any one of embodiments 1-61, further comprising a third svd-TCR.

[0352] Embodiment 63. The therapeutic of embodiment 62, further comprising a fourth svd-TCR.

[0353] Embodiment 64. The therapeutic of embodiment 63, further comprising a fifth svd-TCR.

[0354] Embodiment 65. The therapeutic of embodiment 64, further comprising a sixth svd-TCR.

[0355] Embodiment 66. The therapeutic of any one of embodiments 1-65, further comprising a biologically active group.

- [0356] Embodiment 67. The therapeutic of embodiment 66, wherein the biologically active group is another peptide or a small molecule conjugate.
- [0357] Embodiment 68. The therapeutic of any one of embodiments 66-67, wherein the biologically active group is coupled to a polypeptide of the therapeutic.
- [0358] Embodiment 69. The therapeutic of any one of embodiments 1-68, wherein the therapeutic is coupled with a binding protein, an antibody, an antibody binding fragment, a scFv, a therapeutic moiety, a detectable moiety, an immune cell modulator or engager, a checkpoint inhibitor, a biologically active group, an anti-cancer agent, an anti-infection agent, an immune checkpoint inhibitor, a CD3 engager, or a radionuclide.
- [0359] Embodiment 70. The therapeutic of any one of embodiments 1-69, further comprising a linker connecting the svd-TCR and the antigen binding domain to each other.
- [0360] Embodiment 71. The therapeutic of any one of embodiments 59-68, further comprising a linker connecting the svd-TCR or the antigen binding domain with the biologically active group or binding protein.
- [0361] Embodiment 72. The therapeutic of any one of embodiments 1-71, further comprising a linker connecting the svd-TCR or the antigen binding domain with a scaffold.
- [0362] Embodiment 73. The therapeutic of any one of embodiments 70-72, wherein the linker has a length of 2-30 amino acids or 9-25 amino acids.
- [0363] Embodiment 74. The therapeutic of any one of embodiments 70-73, wherein the linker comprises a hinge region sequence of an immunoglobulin.
- [0364] Embodiment 75. The therapeutic of any one of embodiments 70-73, wherein the linker is a GS linker.
- [0365] Embodiment 76. The therapeutic of embodiment 75, wherein the GS linker comprises GS_n , GGS_n , $GGGS_n$, or $GGGGS_n$.
- [0366] Embodiment 77. A pharmaceutical composition comprising: the therapeutic of any one of embodiments 1-76; and a pharmaceutically acceptable carrier.
- [0367] Embodiment 78. A nucleic acid encoding the therapeutic of any one of embodiments 1-76.
- [0368] Embodiment 79. A method comprising administering the therapeutic of any one of embodiments 1-76 to a subject.
- [0369] Embodiment 80. A kit comprising: the therapeutic of any one of embodiments 1-76; and an instruction.

* * * * *

[0370] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All

publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

EXAMPLES

[0371] The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1.1: Methods for identifying an svd-TCR candidate

[0372] This Example describes two methods for producing an svd-TCR that contains only the TCR beta chain variable region specifically binding to a pMHC antigen.

[0373] The svd-TCR is developed through *in vitro* selection from a yeast surface display library of mutant svd-TCR sequences. The svd-TCR constructs are isolated from svd-TCR display libraries, generated by data mining the observed TCR beta domain sequence space available in public databases and incorporating mutations aimed at increasing expression, stability, and/or affinity for a target pMHC antigen. TCR beta domain sequences are selected from databases such as, but not limited to, VDJdb (<https://vdjdb.cdr3.net/>) and STCRDab (<https://opig.stats.ox.ac.uk/webapps/stcrdab-stcrpred/>). These sequences may be explicitly defined in terms of all amino acids present the TCR beta domain or provided as individual TRBV, TRBD, TRBJ, and CDR3 sequences, allowing the complete amino acid sequence to be reconstructed using *in silico* methods such as, but not limited to, Stitchr (<https://github.com/JamieHeather/stitchr>).

[0374] The yeast surface display library comprises a plurality of *Saccharomyces cerevisiae* yeast host cells, each of which expresses an svd-TCR candidate protein fused at the C terminus to the N terminus of the Aga2p subunit protein for display on the extracellular surface of the yeast host cell. Each yeast host cell displays a distinct svd-TCR, resulting in a diverse library of svd-TCRs across the yeast population.

[0375] The plurality of yeast cells expressing diversity of svd-TCR molecules on their surface is screened by flow cytometry, FACS, magnetic bead sorting, or any methodological variant thereof, with pMHC tetramers labeled with a fluorescent tag or affinity tag. This process enables the identification svd-TCR variants that are specific for the cognate pMHC or a variant thereof.

[0376] Selection or modification of svd-TCR for higher affinity to a target pMHC may include any or all of the following six steps.

[0377] (1) The library encoding a diverse set of mutant svd-TCR fusion protein, genetically linked to the N terminus of the Aga2p subunit protein, is cloned into an appropriate expression vector system, which may include, but is not limited to, the pJYDC3, pJYDC2, and/or pJYDC1 yeast surface display expression plasmid vectors for expression on the yeast cell surface. Preferably, the fusion protein is expressed under the transcriptional control of the GAL1 promoter and is secreted extracellularly via the appS4 leader sequence.

[0378] (2) FACS or electromagnetic bead selection for the development of mutagenic svd-TCR libraries and stabilized variants directed to a specific pMHC. Mutagenic libraries are employed to enable stable surface expression on yeast due to potential instability of svd-TCR proteins arising from the absence of stabilized constant regions or a paired alpha chain. Alternative display formats may be used for mutation selection, including, though not limited to, phages and mammalian cell displays. These can involve affinity-based binding to immobilized pMHC ligand (in phage display), magnetic particle selection via antigen binding (in yeast display), or fluorescently activated cell sorting using labeled peptide-MHC antigen (in yeast display). Various methods may be used to select variants, including the use of antibodies targeting TCR V β to recognize folded epitopes, fluorescently activated cell sorting (FACS), or electromagnetic bead selection. These techniques are applied in the Examples to isolate variants with improved antibody binding.

[0379] (3) The svd-TCR clones isolated from the mutagenesis library selection are evaluated for thermal stability, and the stabilized variants are selected and sequenced to serve as templates for affinity maturation. Typically, single site mutations that contribute to increased surface expression levels on yeast and greater stability in solution are identified.

[0380] (4) Stabilized svd-TCR sequences are typically used as templates for the second-generation development of svd-TCR libraries. In the present disclosure, yeast-displayed variants with specific binding to an indicated pMHC target antigen are selected from either the first svd-TCR library to identify svd-TCR constructs or from the second-generation library to improve binding to pMHC, using electromagnetic bead selection and/or fluorescence-activated cell sorting (FACS). Additional selection methods, including but not limited to panning by phage display, magnetic selection, or FACS by mammalian display, may also be employed.

[0381] (5) The svd-TCR clones isolated from the selection of first- or second-generation svd-TCR libraries are evaluated for specific binding to their cognate pMHC. The plasmid is extracted from the yeast clone and sequenced.

[0382] (6) If further improvement in affinity is needed, the svd-TCR clones selected in step 5 can be used as templates for the development of third-generation libraries, allowing for additional rounds of selection.

Example 1.2 Methods for protein expression of an svd-TCR in a mammalian system

[0383] This Example describes experiments for the protein expression of an svd-TCR in a mammalian system.

[0384] Svd-TCR expression plasmids containing a single svd-TCR identified from library screening and possessing either an N- or C-terminal affinity tag, such as, but not limited to, a 6 $\times$ His tag, Strep II tag, FLAG tag, or any other affinity tag, are transfected into ExpiCHO cells at scales of at 2.5 ml, 50 ml, 500 ml, or 1000 ml.

[0385] The specific method for expression of an svd-TCR in a mammalian system comprised the following steps: construction of the svd-TCR genetically linked to an affinity is encoded within an ExpiCHO expression plasmid by molecular cloning. ExpiCHO cells are passaged one day before transfection, and the constructed plasmid is mixed with a transfection reagent and added dropwise to the ExpiCHO cell culture. The culture is thoroughly mixed, and after 18-22 hours of expression at 37°C, a feed medium is added according to the manufacturer's protocol. After feeding, the cells are incubated at 32°C for 5 days post-transfection. A second feed is added, and after 10-12 days, the resulting supernatant is clarified by centrifugation and is filtered through a 0.22 µm filter. The svd-TCR protein in the filtered culture supernatants is purified by affinity chromatography using the appropriate affinity resin or IMAC resin if a 6× His Tag is used. For additional purification to enhance the purity and/or mono-dispersity of the final protein product, the material eluted from the affinity purification is applied to a size exclusion chromatography column, either a Superdex 75 Increase or Superdex 200 Increase, and purified by isostatic elution in phosphor-buffer saline. The resulting svd-TCR is analyzed for purity using SDS-PAGE.

Example 1.3 Methods for protein expression of an svd-TCR in a bacterial system

[0386] This Example describes experiments performed for the protein expression of an svd-TCR in a bacterial system.

[0387] The specific method for expression of an svd-TCR in a bacterial system comprised the following steps. The svd-TCRs were cloned into an *E. coli* expression vector containing two C-terminal affinity tags: a hexahistidine (6× His) tag and a human influenza hemagglutinin (HA) tag. The constructs were electroporated into SS320 *E. coli* cells, and svd-TCRs were expressed in the periplasmic space using the PelB signal sequence. A 100 mL culture of SS320 *E. coli* was grown at 37 °C until an optical density at 600 nm (OD₆₀₀) of 0.6 was achieved. Svd-TCR expression was induced with IPTG at a final concentration of 1.5 mM, followed by overnight incubation at 30 °C.

[0388] After overnight incubation, the culture was centrifuged, and the cell pellet was resuspended in 40 mL of 1× Na₂HPO₄ (25 mM Na₂HPO₄, pH 8.0, 150 mM NaCl) and an equivalent volume of 2× Na₂HPO₄ shock buffer (50 mM Na₂HPO₄, pH 8.0, 300 mM NaCl, 1 M sucrose, 2 mM EDTA) containing a protease inhibitor cocktail tablet. The mixture was incubated at room temperature for 15 minutes with periodic shaking, followed by centrifugation to remove the supernatant. The resulting pellet was resuspended in 30 mL of cold deionized water supplemented with 5 mM MgSO₄ and 0.5 mg/mL lysozyme. This suspension was incubated on ice for 15 minutes and then centrifuged to collect the supernatant for further purification on a nickel affinity column.

[0389] A sample of the extract was tested for the presence of svd-TCR using an enzyme-linked immunosorbent assay (ELISA). Extracted proteins were coated onto an ELISA plate and subsequently washed with PBST. Following the wash, 0.05 mL of anti-HA-HRP antibody, diluted 1:5000 in PBS, was

added. The presence of svd-TCR in the bound protein extract was detected by adding the HRP substrate, TMB. Absorbance was measured at 450 nm to quantify the presence of svd-TCR.

[0390] In parallel, 30 ml of 2× Na₂HPO₄ pre-binding buffer (100 mM Na₂HPO₄, pH 8.0, 1 M NaCl, 20 mM imidazole, 0.02% NaN₃) was added to the remainder of the supernatant, and the mixture was loaded onto the affinity column. After loading, the column was washed twice with 30 ml of wash buffer (50 mM Na₂HPO₄, pH 8.0, 500 mM NaCl, 10 mM imidazole). The captured protein was eluted with 5 mL of elution buffer (20 mM phosphate, 500 mM NaCl, 500 mM imidazole).

[0391] Purified svd-TCR was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein samples were combined with SDS sample buffer containing sodium dodecyl sulfate (SDS) and a reducing agent. The mixture was heated at 95 °C for 5 minutes to ensure complete denaturation of the proteins. Denatured samples were then loaded into the wells of the polyacrylamide gel, which was run in Tris-Glycine-SDS buffer at a constant voltage of 150 V. Following electrophoresis, the gel was stained with Coomassie Brilliant Blue to visualize the separated proteins.

[0392] The svd-TCR monomers derived from the TRBV5-8 gene were expressed and purified from bacteria. The svd-TCR monomers were individually produced in 100 mL cultures of SS320 *E. coli*, and their expression and periplasmic localization were detected by ELISA. The svd-TCRs were purified from the bacterial periplasm using nickel affinity columns, detected on an SDS-PAGE gel, and confirmed by Western blotting, with major bands observed at the expected molecular weights, taking into account the added His and HA tags, and appearing between the 15 and 20 kDa markers of the molecular weight ladder.

Example 1.4 Methods for protein expression of an svd-TCR-Fc fusion

[0393] This Example describes experiments performed for the protein expression of an svd-TCR in a bacterial system.

[0394] Svd-TCR-Fc proteins comprise an svd-TCR domain genetically linked to the CH2 and CH3 domains of an IgG1 Fc region. This configuration enables natural pairing between one CH2-CH3 set and a second CH2- CH3 set, resulting in a soluble homodimeric protein in which the svd-TCR-Fc fusion protein possesses two svd-TCR domains (**FIG. 14A**). This design allows one svd-TCR domain to bind to a first epitope on a peptide-MHC complex, while the second svd-TCR domain binds to a second epitope. Svd-TCR-Fc fusion proteins incorporating the human IgG1 Fc domain are thus bivalent, with two svd-TCR domains.

[0395] Svd-TCR-Fc constructs encoding NY-ESO-1 and MAGE-A3-5 (**FIG. 14A**) were generated by fusing an svd-TCR with a human IgG1 Fc segment. This was done by constructing a fusion expression vector that encodes for a protein in which the C-terminal end of the svd-TCR amino acid sequence was linked with the N-terminal end of the human IgG1 Fc segment amino acid sequence. The fusion expression vector plasmid was then transformed into ExpiCHO cells, and induced expression was carried out to obtain the svd-TCR-Fc chimeric protein fused with the Fc segment. An svd-TCR may be genetically conjugated to the

human Fc directly or via an intermediary linker comprising Glycine and Serine residues, ranging from 2 to 20 amino acids in length. Svd-TCR-Fc expression constructs were transfected into ExpiCHO cells at scales of 2.5 ml, 50 ml, 500 ml, or 1000 ml. In alternative embodiments, svd-TCR-Fc proteins can be conjugated in different formats.

[0396] The specific method for expressing and purifying an svd-TCR-Fc comprises the following steps. Construction of the svd-TCR genetically conjugated to a human IgG1 Fc segment was encoded within a mammalian expression plasmid via molecular cloning. The primary expression system for svd-TCRs and svd-TCR-Fc fusion proteins was ExpiCHO, but Expi293 cells or other eukaryotic expression system for biopharmaceutical manufacturing can also be used. The svd-TCR and/or svd-TCR-Fc encoding nucleotide sequence was codon-optimized for expression in cells specific to the chosen eukaryotic expression system.

[0397] In this example, ExpiCHO cells were passaged one day before transfection, and the constructed plasmid was mixed with a transfection reagent, then added dropwise to the ExpiCHO cell culture and thoroughly mixed. After 18-22 hours of expression at 37°C, a feed medium was added according to the manufacturer's protocol. Following the feed, the cells were incubated at 32°C for 5 days post-transfection. A second feed was added, and after 10-12 days, the resulting supernatant was clarified by centrifugation and filtered through a 0.22 µm filter.

[0398] The svd-TCR protein in the filtered culture supernatants was purified using Protein A/G affinity purification, with the target svd-TCR-Fc protein eluted using 100 mM glycine (pH 3.0). When additional purification steps were required to enhance the purity and/or mono-dispersity of the final protein product, the material eluted from the affinity purification step was applied to a size exclusion chromatography column, either Superdex 75 Increase or Superdex 200 Increase, and purified by isostatic elution into phosphor-buffer saline. The resulting svd-TCR-Fc was analyzed to determine purity using SDS-PAGE (**FIG. 14B**) and analytical size-exclusion chromatography (aSEC).

[0399] Svd-TCR-Fc proteins derived from the TRBV5-8 gene were individually expressed from either 350 mL or 600 mL ExpiCHO cell cultures, with protein yields ranging from 4.5 to 41.7 mg/L. These yields were calculated by measuring the absorbance at 280 nm (Abs₂₈₀) and dividing by the extinction coefficients specific to each svd-TCR-Fc amino acid sequence. All svd-TCR-Fc fusion proteins were visible in the peaks of aSEC profiles and appeared on SDS-PAGE under reducing conditions, with the major bands at the expected molecular weights near the 75 kDa marker on the molecular weight ladder (**FIG. 14B**).

Example 2.1 Methods for biochemical svd-TCR characterization – Thermostability

[0400] This Example describes the experiments performed to evaluate thermostability of svd-TCR and svd-TCR-Fc fusion proteins by measuring their melting temperatures (T_m), molecular weights, and size distribution profiles using DSF, DSC, and SEC-MALS techniques.

[0401] The melting temperatures (T_m) of svd-TCR-Fc molecules were measured to assess the protein's thermostability. The T_m value was determined using a thermal shift assay performed on purified protein samples. The methodology for the thermal shift assay can be selected from either differential scanning fluorimetry (DSF) or differential scanning calorimetry (DSC). An svd-TCR variant was considered stabilized if it possesses mutations that increase its thermostability by at least 2 °C compared to the parent svd-TCR.

[0402] The specific method for DSF comprised the following steps. Purified svd-TCR protein at a concentration within the range of 0.1 mg/ml to 2 mg/ml in 1× PBS buffer was mixed with SYPRO Orange fluorescent dye at a final dye concentration of 5-10× the manufacturer's recommended concentration. The mixture was assessed in triplicate and read from a 96-well PCR plate sealed with optical-grade PCR plate seals. A real-time PCR machine or thermal performed a temperature ramp from 25 °C to 95 °C at a rate of 1 °C per minute, while fluorescence was monitored at the emission wavelength appropriate for the dye (approximately 600 nm). A buffer-only control and a protein-without-dye control were used to determine baseline fluorescence. The T_m value was determined by plotting the fluorescence signal values against temperature for the protein samples, calibration standards, and controls to generate a thermal denaturation curve. The T_m value for the svd-TCR protein samples was calculated by fitting the fluorescence signal values against temperature data points and identifying the inflection point of the denaturation curve.

[0403] Thermal shift assays using the DSF methodology were conducted to assess the thermostability of svd-TCR-Fc fusion proteins by measuring their melting temperatures (T_m). Svd-TCR-Fc fusion proteins were classified as thermostable if their calculated T_m value was within 5 °C of a control TCR-Fc fusion protein with binding specificity to a pMHC protein complex. Six structurally distinct control TCR-Fc fusion proteins were analyzed by DSF, yielding melting curves with T_m values ranging from 62.1 °C to 66.7 °C (FIG. 15). In comparison, all svd-TCR-Fc proteins exhibited T_m values between 65.8 °C and 66.0 °C, confirming that all seven svd-TCR-Fc proteins were thermostable. The results are shown in Table 5.

Table 5. Calculated values from DSF assays and SEC-MALS

ID	Format	pHLA-A*02:01	T_m (°C)	Mono-dispersity (%)	Calculated Size (kDa)
Oh-VB-MAGE-A3-1	svd-TCR-Fc	MAGE-A3	65.9	84.1	77.5
Oh-VB-MAGE-A3-2	svd-TCR-Fc	MAGE-A3	65.9	82.2	77.1
Oh-VB-MAGE-A3-4	svd-TCR-Fc	MAGE-A3	66	69.4	76.3
Oh-VB-MAGE-A3-5	svd-TCR-Fc	MAGE-A3	65.8	95.2	77
Oh-VB-NY-ESO-1	svd-TCR-Fc	NY-ESO-1	65.8	100	76
Oh-VB-NY-ESO-2	svd-TCR-Fc	NY-ESO-1	65.7	91	77.3
Oh-VB-NY-ESO-3	svd-TCR-Fc	NY-ESO-1	66	85.7	76.2
1G4	TCR-Fc	NY-ESO-1	64.4	100	150
1G4c113	TCR-Fc	NY-ESO-1	65	95.4	152
c5c1	TCR-Fc	NY-ESO-1	66	100	151
ImmTAC_a24+b52	TCR-Fc	NY-ESO-1	66.7	100	152

ImmTAC_a82+b65	TCR-Fc	NY-ESO-1	66.5	100	153
NYE_S3	TCR-Fc	NY-ESO-1	62.1	100	152
3M4E5	TCRm (Fab)	NY-ESO-1	64	100	56

[0404] The specific method for DSC comprises the following steps. Purified svd-TCR protein at a concentration within the range of 0.5 mg/ml to 5 mg/ml is dialyzed into 1× PBS buffer overnight at 4 °C. The svd-TCR protein solution and the remaining dialyzation buffer are collected for analyte and baseline measurements, respectively. The TA Instruments Nano DSC calorimeter sample cell and reference cell are purged with inert nitrogen gas to eliminate any environmental oxygen. The calorimetric baseline DSC signal is determined by filling the sample and reference cells with svd-TCR protein solution and dialyzation buffer, respectively. A temperature ramp from 25 °C to 110 °C at a scanning rate of 1 °C per minute and at a pressure of 2 or 3 atm is applied to collect the calorimetric data. The final calorimetric data are corrected by subtracting the calorimetric baseline between the initial and the final state by using the sigmoidal baseline function in NanoAnalyze software (TA Instruments). The T_m value is then calculated following the manufacturer's instructions.

Example 2.2 Methods for biochemical svd-TCR characterization – Size

[0405] This Example describes the experiments performed to evaluate the size, molecular weight, and monodispersity of purified svd-TCR and svd-TCR-Fc fusion proteins expressed in mammalian cells.

[0406] The svd-TCR protein purified after transgenic expression in mammalian cells was assessed for size (molecular weight) and monodispersity using size-exclusion chromatography with multi-angle light scattering (SEC-MALS). SEC is a technique to separate molecules according to their size and/or hydrodynamic radius as they pass through a porous stationary phase, with larger molecules eluting more quickly than smaller ones. MALS is a label-free, non-destructive method that measures the angle-dependent scattering of laser light by macromolecules at multiple angles, providing information about the molecular weight and size of particles in solution. Other methods for quantifying the size include integrating a Quasi-Elastic Light Scattering (QELS) module integrated into the MALS detector. This technique calculates the hydrodynamic radius (R_h), defined as the radius of an equivalent solid sphere diffusing at the same rate as a molecule. Additionally, Small-angle X-ray scattering (SAXS) can be employed to determine the radius of gyration (R_g), which provides a measure of the spatial distribution of atoms within a molecular structure.

[0407] The specific method for SEC-MALS comprised the following steps. The purified svd-TCR protein sample was injected onto a SEC column, either a Superdex 75 Increase or Superdex 200 Increase. The eluting protein was detected by absorption at 280 nm, and the concentration of the eluting protein was measured by monitoring change in refractive index and/or absorption at 280 nm. After elution, the protein entered the MALS detector, which measured the scattered light at multiple angles and provided data on the angular

dependence of light scattering. From this data, the molecular weight and mono- or poly-dispersity of the protein were determined.

[0408] The molecular weight of an svd-TCR can also be estimated using specialized software or databases that sum the molecular weights of each amino acid in the sequence, with adjustments for post-translational modifications.

[0409] To determine the size of monodisperse, purified svd-TCR-Fc fusion proteins, SEC-MALS was performed using a Mini Dawn TREOS instrument with a Superdex 200 Increase 10-300GL column. A UV spectrometer measured absorption at 280 nm, while an Optilab rEX measured the sample's refractive index. For each svd-TCR-Fc protein, 70 μ L of protein in 1 $\times$ PBS buffer at a concentration of 1 mg/mL was loaded and run at a flow rate of 0.75 mL/min. A peak corresponding to the expected molecular weight of each svd-TCR-Fc protein, plus the additional mass of the N-linked glycans, was detected for each of the three NY-ESO-1-directed entities as well as for the four MAGE-A3-directed entities (see **Table 5**). Using the aforementioned methods, svd-TCR proteins demonstrated a range of sizes from 12 kDa to 77.5 kDa.

Example 2.3 Methods for biochemical svd-TCR characterization – Solubility/aggregation

[0410] This Example describes the experiments performed to assess the solubility, size distribution, and aggregation propensity of svd-TCR and svd-TCR-Fc fusion protein.

[0411] Solubility and aggregation of svd-TCR protein and svd-TCR-Fc fusion protein were measured using dynamic light scattering (DLS) techniques. DLS is a technique used to determine the size distribution profile of a protein in solution by analyzing the intensity of scattered light. Soluble protein exists in a monodispersed state with a uniform size distribution; however, when a protein becomes insoluble or aggregates, DLS detects an increase in particle size and polydispersity in the solution.

[0412] Protein solubility is an important factor in the manufacturing, formulation, and administration of protein-based biotherapeutic material. Protein insolubility can lead to issues such as protein aggregation. In the context of biomolecular therapeutics, aggregation referred to various types of undesired interactions or characteristics that could result in yield loss during manufacturing, reduced stability during formulation, and/or unintended immunogenic responses or other adverse effects upon administration.

[0413] The specific method for DLS comprised the following steps. A Wyatt Technology DynaPro 99 instrument and thermostat (20 °C) were warmed for 15 minutes before the experiment, and the Dynamics software was connected to the instrument. The acquisition time for multiple acquisitions was set to 10 seconds, and the laser power was set to 100%. The quartz cuvette was thoroughly washed with deionized water and methanol before the protein sample (at a concentration of approximately 1 μ g/mL to 10 μ g/mL in PBS, 15 μ L) was added. Air bubbles were removed by tapping the cuvette before inserting it into the holder, with the frosted side facing left. If the intensity was too high (indicated by a warning message on the screen), the sample was further diluted with PBS until the intensity fell within the normal range. The data collected

included hydrodynamic radius, polydispersity, predicted average molecular weight, percentage strength, and percentage mass.

[0414] Another technique that can be used to determine the size distribution profile of a protein in solution, assessing its solubility and propensity for aggregation, is SEC-MALS. As described in **Example 2.2**, SEC-MALS provides information on the molecular weight and mono- or poly-dispersity of a protein in solution. By concentrating a protein sample to a specific concentration, a known mass of protein was injected into the SEC-MALS instrument. The protein was quantified by its 280 nm absorption in the SEC-MALS instrument, allowing calculation of a mass fraction to confirm whether all injected protein remains in solution, indicating solubility. Additionally, any protein signal from the SEC-MALS instrument that elutes with a calculated mass larger than the expected molecular weight of a monodisperse sample suggests the presence of aggregation (**FIG. 16**), and the mass fraction of both monodisperse and polydisperse materials was determined.

[0415] To confirm that the svd-TCR-Fc fusion proteins were soluble at a concentration of 1 mg/mL and to detect any higher-order aggregates of svd-TCR-Fc fusion dimers, trimers, or tetramers, SEC-MALS was performed on a Mini Dawn TREOS instrument with a Superdex 200 Increase 10-300GL column. A volume of 70 μ L of svd-TCR-Fc protein suspended in 1 $\times$ PBS buffer at a concentration of 1 mg/mL was obtained for each svd-TCR-Fc construct, indicating that the proteins were soluble at this concentration. Protein solutions were loaded onto the SEC-MALS instrument and run at a flow rate of 0.75 mL/min. Mass fractions were calculated for each peak in the size-exclusion profile as well as for any higher-order assemblies. Therefore, one svd-TCR was characterized as having the expected molecular weight without observed polydispersity.

Example 2.4 Methods for biochemical svd-TCR characterization – Hydrophobicity/Hydrophilicity

[0416] This Example describes the experiments performed to identify and replace exposed hydrophobic residues on svd-TCR proteins with hydrophilic residues.

[0417] Exposed hydrophobic patches on protein surfaces are a significant factor contributing to protein aggregation and instability. Structurally ordered protein domains have both hydrophilic and hydrophobic regions on their surface. These hydrophobic regions can form continuous or discontinuous clusters of aliphatic amino acids with non-polar side chains. Typically, hydrophobic residues are buried within the protein core, shielded from the surrounding aqueous environment to minimize exposure to water. Hydrophobic patches are also commonly found at the interfaces between protein chains in stable heterodimeric proteins, such as a TCRs or BCRs. When these heterodimeric proteins exist as single-chain monodimeric proteins, exposure of hydrophobic patches can lead to aggregation and instability. Identifying hydrophobic residues within TCR proteins supports the design, selection, and optimization of svd-TCR molecules.

[0418] The specific method for identifying hydrophobic residues and replacing them with residues of greater hydrophilicity comprised the following steps. A protein sequence of an svd-TCR molecule is converted to FASTA format and quantified for each residue using an amino acid hydrophobicity scale (*e.g.*, Kyte-Doolittle) with the Alakazam R programming package from the Immcantation analysis framework for Adaptive Immune Receptor Repertoire sequencing (AIRR-seq). Residues with greater hydrophilicity are selected from positionally equivalent residues naturally occurring in human and/or non-human TR gene sequences. Residues with greater hydrophilicity can be selected from positionally equivalent residues naturally occurring in human and/or non-human TR gene sequences or from positionally analogous residues in VHH domains or Nanobodies, which are structurally validated and annotated in the Protein Data Bank (PDB). Assignment of positionally equivalent or analogous residues in TRBV, TRBJ, and Nanobody sequences is performed by amino acid sequence alignment using the ClustalOmegaCommandline function from the Bio.Align.Applications Python package. To retain corresponding residue positions throughout the alignment protocol, amino acid profiles for TR genes and VH genes – relating to svd-TCRs and VHH/Nanobodies, respectively – are independently generated using IMGT numbering schemes and then aligned. Exposed hydrophobic positions are compared to hydrophilic, positionally equivalent or analogous residues, and mutations are manually selected to engineer svd-TCR constructs with enhanced hydrophilicity.

Example 3.1: Methods for characterizing svd-TCR binding – Affinity and avidity

[0419] This Example describes the experiments performed to evaluate the binding affinity and specificity of svd-TCR molecules to pMHC ligands.

[0420] For generated svd-TCR molecules, functionality can be assessed by their ability to bind the relevant svd-TCR ligand, which is a pMHC. Binding can be determined by measuring the interaction between the svd-TCR – tested as either a monomeric or multimeric entity – and its target pMHC complex.

[0421] Binding of a monomeric svd-TCR to a monomeric pMHC allows for the determination of binding affinity (K_D), while binding of a multimeric svd-TCR to a monomeric pMHC provides the apparent binding affinity (apparent- K_D). This interaction can be described as high affinity if a monomeric or multimeric svd-TCR binds to a monomeric pMHC with a K_D or apparent- K_D of $\leq 1 \mu\text{M}$ and/or a k_{off} of $1 \times 10^{-3} \text{ s}^{-1}$ or slower. Surface Plasmon Resonance (SPR) is the preferred method, using a BIAcore SPR instrument to measure interactions between svd-TCR molecules and pMHC complexes. Binding measurements can be conducted using a Biacore T200™, Biacore 3000™, Biacore 2000™, or any equivalent system. The specific method for determining binding affinity involves generating pMHC protein complexes with site-specific biotinylation, which are then immobilized on a streptavidin-coated binding surface of a Biacore SPR biosensor, enabling efficient testing of svd-TCR protein binding to specific pMHC protein complexes.

[0422] The following is an exemplary protocol for generating pMHC complex protein material for SPR binding analysis (O'Callaghan et al., *Anal. Biochem.* **266**:9-15 (1999)). The identity of the MHC and peptide

binder was provided as an example but is generally interchangeable with any other MHC and peptide tested for binding affinity to a generated svd-TCR molecule. An HLA-A*0201 heavy chain (truncated at Pro-276) with a C-terminal AviTag and $\beta 2m$ was separately expressed in *E. coli* as inclusion bodies. These inclusion bodies were isolated by sonication, followed by successive wash and centrifugation steps using 0.5% Triton X-100. The inclusion bodies were dissolved in 6 M guanidine, 10 mM dithiothreitol (DTT), 10 mM EDTA, 50 mM Tris pH 8.1, and 100 mM NaCl. Soluble TCR was refolded by rapid dilution of a mixture of the dissolved α - and β -chain inclusion bodies into 5 M urea, 0.4 M L-arginine, 100 mM Tris pH 8.1, 3.7 mM cystamine, and 6.6 mM β ME at 4 °C to a final concentration of 60 mg/L. The refold mixture was dialyzed for 24 hours against 10 volumes of 10 mM Tris pH 8.1, followed by an additional dialysis against 10 volumes of 10 mM Tris pH 8.1 at 4 °C. The refolded MHC protein material was purified using ion exchange chromatography and eluted with a linear 0-500 mM NaCl gradient, followed by size exclusion chromatography (Superdex 75). Peptide-MHC complexes were generated by refolding in the presence of NY-ESO-1/MAGE-A3 peptides or other target peptides specified for the generated svd-TCR molecule.

[0423] Site-specific biotinylation of the AviTag was carried out using biotin ligase (BirA) prior to size exclusion chromatography. Biotinylation of pMHC molecules was performed by buffer exchanging the purified protein material into 10 mM Tris pH 8.1, 5 mM NaCl using a Pharmacia fast desalting column equilibrated with the same buffer. Immediately upon elution, the protein-containing fractions were chilled on ice in the presence of a protease inhibitor cocktail (Calbiochem). Biotinylation reagents (1 mM biotin, 5 mM ATP buffered to pH 8, 7.5 mM $MgCl_2$, and 5 μ g/mL BirA enzyme, purified according to O'Callaghan et al., *Anal. Biochem.* **266**:9-15 (1999)) were then added, and the mixture was incubated at room temperature for 12 to 16 hours to produce biotinylated pMHC protein material. The biotinylated pMHC protein material was stored at -80 °C with a protease inhibitor cocktail (Roche).

[0424] The following is an exemplary protocol for the SPR assay. Activated CM-5 BIAcore™ chips were coated with streptavidin using amine coupling according to the manufacturer's instructions. Biotinylated pMHC protein complexes were then injected onto the chip flow cells at various concentrations, optimized for each sample to achieve an appropriate response signal. To assess non-specific interactions during the SPR assay, probe flow cells were prepared with pMHC bound on the surface of different flow cells. The unreacted activated surface was blocked with ethanolamine hydrochloric acid solution to complete the coupling process. The assay was performed by passing specific, serially diluted concentrations of monomeric or multimeric svd-TCR over the flow cells and measuring the SPR response, with dose-response (5-9 concentrations) in duplicate. The binding experiments produced sensorgrams obtained from the SPR instrument, and K_D and/or apparent- K_D values were calculated from binding curves fitted using a 1:1 interaction model (Price & Dwek, *Principles and Problems in Physical Chemistry for Biochemists*, 2nd Edition, 1979, Clarendon Press, Oxford).

[0425] SPR assays were performed using a Biacore T200 instrument with monomeric pMHC complexes composed of biotinylated HLA-A*02-01: β 2m loaded with either the NY-ESO-1 peptide (amino acid sequence: SLLMWITQV) (SEQ ID NO: 62) (**FIG. 17A**) or the MAGE-A3 peptide (FLWGPRALV) (SEQ ID NO: 63), each independently, immobilized onto streptavidin sensor chips in a running buffer containing 20 mM phosphate (pH 7.4), 137 mM NaCl, 2.7 mM KCl, and 0.05% surfactant P20, at a flow rate of 30 μ L/min. Using the same running buffer and flow rate, multimeric svd-TCR-Fc fusion protein at five concentrations (35, 12, 4, 1.3, and 0.4 μ M) was applied to the pMHC-loaded sensor chips. A binding signal was measured for one NY-ESO-1-directed svd-TCR-Fc fusion protein, with an apparent- K_D determined to be 14 μ M (**FIG. 17B**).

Example 3.2: Methods for characterizing svd-TCR binding – Peptide specificity for pMHC

[0426] This Example describes the experiments performed to assess the binding specificity of svd-TCR-Fc, confirming specific binding to the target pMHC complex with the relevant peptide and showing no detectable binding to an irrelevant pMHC complex under identical SPR assay conditions.

[0427] The generated svd-TCR molecules are considered functional if their binding specificity demonstrates recognition of the target MHC protein complex only when the relevant target peptide antigen is bound within the pMHC complex. Cross-reactivity refers to the recognition of pMHC antigens that share structural resemblance to the primary pMHC antigen targeted by an svd-TCR. Specificity for the peptide within the pMHC complex is defined as minimal recognition of the target MHC protein complex when it either lacks any bound peptide or is bound by an irrelevant peptide with less than 50% sequence identity to the target peptide, for which the svd-TCR has high affinity in the pMHC complex.

[0428] To test specificity, the generated svd-TCR molecules were assessed in monomeric form and/or fused to an Fc scaffold as a multimer. Binding specificity was determined by measuring the interaction between the svd-TCR – tested as a monomeric or multimeric entity – and its target pMHC complex using SPR. The affinity/apparent-affinity was compared to that of the svd-TCR for an immobilized, biotinylated specific MHC complex, purified as previously described but without any bound peptide or with an irrelevant peptide. An svd-TCR was identified as specific for a pMHC complex if the SPR binding signal for the unbound MHC or irrelevant pMHC was at or below the limit of detection, equivalent to the baseline measurement. To reduce the likelihood of non-specific interactions in SPR assays where high protein concentration was required, buffering additives such as 0.02% Tween20 and/or 0.1% BSA were used.

[0429] To characterize the binding specificity of the NY-ESO-1-directed svd-TCR-Fc, which exhibited a measurable apparent K_D of 14 μ M (as described in **Example 3.1**), an SPR assay was conducted under identical parameters using the irrelevant MAGE-A3 peptide (FLWGPRALV) (SEQ ID NO: 63) loaded onto HLA-A*02-01: β 2m as the target pMHC complex (**FIG. 18A**). No detectable binding signal was observed at the assessed concentrations of svd-TCR-Fc protein (35, 12, 4, 1.3, and 0.4 μ M) (**FIG. 18B**).

Example 3.3: Methods for characterizing svd-TCR binding – Lack of poly-reactivity

[0430] This Example describes the experiments for evaluating the specificity and poly-reactivity of svd-TCR proteins by assessing their binding to target pMHC complexes on cell surfaces.

[0431] For generated svd-TCR proteins, poly-reactivity refers to the undesired recognition of diverse molecular structures of unrelated pMHC antigens and/or other biomolecules on or around the extracellular surface without strict specificity, potentially misdirecting the svd-TCR towards unintended biological targets.

[0432] The reactivity of an svd-TCR for its target pMHC antigen is determined by immunofluorescence microscopy. Observing punctate cell surface staining by immunofluorescence microscopy when cells expressing the specific pMHC antigen on their surface are labeled with an svd-TCR-Fc fusion protein provides confirmatory evidence of svd-TCR reactivity for a specific pMHC protein complex. To identify poly-reactivity, svd-TCR-Fc fusion proteins with known specificity for a pMHC expressed on the surface of a human cell are used as primary antibodies for labeling human cells by immunofluorescence microscopy. Observing punctate cell surface staining when cells lacking the specific pMHC antigen are labeled by an svd-TCR-Fc fusion protein confirms poly-reactivity for the svd-TCR under evaluation.

[0433] The specific method for immunofluorescence microscopy with an svd-TCR fusion protein comprises the following steps. First, an svd-TCR-Fc fusion protein is expressed from ExpiCHO cells, as described in **Example 1.2**, and purified using methods previously outlined to achieve a final protein purity of >90%, confirmed by SDS-PAGE analysis. Next, antigen-positive cells expressing a specific MHC protein complex on their surface are obtained by peptide pulsing at a suitable peptide concentration to reach an antigen presentation level comparable to that of cancer cells (for example, 10 nM peptide, as described in Bossi et al., *Oncoimmunol.* **2**(11):e26840 (2013)) or by using cells that naturally present the peptide. When a specific MHC protein complex cannot be readily identified in available human cell lines, the human erythroleukemic cell line K562 is used as the antigen-presenting cell for svd-TCR labeling. The K562 cancer cell line lacks natural surface expression of MHC class I and II alleles but does express β -2 microglobulin, as described in Lozzio and Lozzio, *Blood* **45**:321–334 (1975). Thus, transgenic expression of any MHC allele, such as the human HLA-A*02:01 alpha chain, results in surface expression of MHC complexes capable of forming cell surface pMHCs via peptide pulsing at an appropriate peptide concentration. Evidence of poly-reactivity for a specific svd-TCR is generated using cells that do not express the cognate MHC complex and have not been peptide pulsed with the target. Additionally, peptide specificity for pMHC by an svd-TCR is evaluated by labeling cells expressing the cognate MHC complex that have not been pulsed with the target peptide.

[0434] Labeling of pMHC protein complexes on the surface of cells is determined based on the assumption that the fluorescence signal corresponds to an svd-TCR bound to its cognate pMHC ligand on the target cell surface, as detected by single-molecule fluorescence. This is facilitated by using svd-TCR-Fc

fusion proteins in which the Fc region is derived from the human IgG1 amino acid sequence, allowing binding by a secondary antibody labeled with a fluorophore visible under fluorescence microscopy. The secondary antibody can be a goat anti-human IgG antibody covalently conjugated to fluorescein or any other fluorophore-conjugated anti-human IgG secondary antibody. In cases where high background signal is detected with an anti-human IgG secondary, a direct immunofluorescence assay is performed as an alternative, using biotinylated svd-TCR material to label pMHC antigen-expressing cells, followed by labeling with streptavidin-R phycoerythrin (PE) conjugates. Individual PE molecules are then imaged using three-dimensional fluorescence microscopy.

[0435] For staining adherent cells, a cancer cell line, such as K562 cells transiently expressing the MHC of interest, is plated onto chamber well slides and pulsed with a target peptide to generate surface-localized pMHC protein complexes. Cells are incubated in a solution containing the svd-TCR-Fc fusion protein of interest at a concentration of 5 µg/mL in PBS with 0.5% BSA for 30 minutes at 4 °C. The svd-TCR solution is then removed, and cells are washed three times with PBS. When a secondary anti-human IgG antibody conjugated to a fluorophore is used, a PBS solution with a secondary antibody concentration between 50 and 500 ng/mL is applied to label the cells. If a biotinylated svd-TCR-Fc fusion protein is used, cells are incubated in streptavidin-PE solution (5 µg/mL streptavidin-PE in PBS containing 0.5% BSA) at room temperature in the dark for 20 minutes. The fluorophore-containing solution is then removed, and cells are washed five times with PBS.

[0436] Fluorescence microscopy is carried out using an Axiovert 200M (Zeiss) microscope with a 63× oil objective (Zeiss). Illumination is provided by a Lambda LS light source containing a 300W Xenon Arc lamp (Sutter), with light intensity adjusted to optimal levels by placing 0.3 and 0.6 neutral density filters into the light path. Excitation and emission spectra are separated using a TRITC/DiI filter set (Chroma). Cells are imaged in three dimensions through z-stack acquisition (21 planes, 1 µm apart). Image acquisition and analysis are performed using Metamorph software (Universal Imaging) as described in Irvine et al., *Nature* **419**: 845-849 (2002) and Purbhoo et al., *Nature Immunology* **5**:524-530 (2004).

Example 4.1: Methods to characterize biological activity of svd-TCR – Cell surface binding

[0437] This Example describes the experiments for evaluating the biological activity of svd-TCR molecules by measuring their binding to cognate pMHC proteins on human cell surfaces.

[0438] The biological activity of an svd-TCR molecule, in the context of monomers and/or oligomers (e.g., Fc fusion proteins), initially depends on its ability to bind to its cognate pMHC protein when the pMHC is localized on the surface of human cells. Immunofluorescence labeling followed by flow cytometric analysis or fluorescence microscopy is the established method for making such determinations. Protocols for generating human cancer cell lines expressing specific pMHC proteins on the cell surface, as well as

protocols for fluorescence microscopy, are provided previously. See Lorenz et al. *Hum Gene Ther.*

28(12):1158-1168 (2017).

[0439] The specific method for flow cytometry with an svd-TCR fusion protein comprises the following steps. Cells expressing surface-localized pMHC are incubated with 100 μ L of serial dilutions of svd-TCR-Fc fusion proteins, starting at 100 μ g/mL in FACS buffer (Ca/Mg²⁺-free PBS, 0.5-1% BSA) for 45 minutes on ice. After washing three times with FACS buffer, the cells are incubated with 0.1 mL of 15 μ g/mL FITC-conjugated goat anti-mouse IgG antibody under the same conditions. For negative controls, cells are incubated with mouse anti-His IgG (13/45/31) and then with goat anti-mouse IgG FITC-conjugated antibodies without svd-TCR-Fc fusion protein. The cells are then washed again and resuspended in 0.2 mL FACS buffer containing 2 μ g/mL propidium iodide to exclude dead cells. Fluorescence of 10,000 living cells is measured using a Beckman-Coulter FC500 MPL flow cytometer with the MXR program (Beckman-Coulter, Krefeld, Germany) or a Millipore Guava EasyCyte flow cytometer with the Incyte program (Merck Millipore, Schwalbach, Germany). Average fluorescence intensities are calculated using the CXP program (Beckman-Coulter, Krefeld, Germany) or Incyte (Merck Millipore, Schwalbach, Germany). For analysis, 0.5 million cells are used for staining with the Beckman-Coulter FC500 MPL, and 0.25 million cells per staining are used with the Millipore Guava EasyCyte. After subtracting the fluorescence intensity of cells stained with secondary and tertiary reagents only, KD values are calculated using a one-site binding model (hyperbola) in GraphPad Prism software (GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla, California, USA).

Example 4.2: Methods to characterize biological activity of svd-TCR – T cell activation

[0440] This Example describes the experiments for assessing the ability of svd-TCR-anti-CD3 fusion proteins to specifically redirect CD3⁺ T cells towards target cells expressing a pMHC of interest.

[0441] An exemplary intended biological function of an svd-TCR is an embodiment in which the svd-TCR is fused to an anti-CD3 interacting protein – such as IgG, IgA, IgM, IgE, IgD, Fab, Nanobody, scFv, Affibody, DARPin, or another CD3-binding domain – to enable the svd-TCR to mediate potent and specific redirection of CD3⁺ T cells. This redirection is assessed by ELISPOT assay, using interferon- γ (IFN- γ) secretion as a readout for T cell activation towards target cells expressing a pMHC of interest. The specific method for redirecting CD3⁺ T cells with an svd-TCR fusion protein comprises the following steps.

[0442] Assays are performed using a human IFN- γ ELISPOT kit (BD Biosciences). Target cells are prepared at a density of 1×10^6 /mL in assay medium (RPMI 1640 containing 10% heat-inactivated FBS and 1% penicillin-streptomycin-L-glutamine) and plated at 50,000 cells per well in a volume of 50 μ L. Peripheral blood mononuclear cells (PBMCs), isolated from fresh donor blood, are used as effector cells and plated at 10,000–50,000 cells per well in a volume of 50 μ L (the exact number of cells used for each experiment depends on the donor and may be adjusted to produce a response within a suitable range for the assay).

Varying concentrations of svd-TCR-anti-CD3 fusion proteins are used, spanning the anticipated clinically relevant range, and added to the well in a volume of 50 μ L.

[0443] Plates are prepared according to the manufacturer's instructions. Target cells, effector cells, and svd-TCR-anti-CD3 fusion protein molecules are added to the relevant wells, with the volume adjusted to 200 μ L with assay medium. All reactions are performed in triplicate. Control wells are prepared by omitting svd-TCR-anti-CD3 fusion proteins, effector cells, or target cells. Plates are incubated overnight at 37°C with 5% CO₂. The following day, plates are washed three times with wash buffer (1 $\times$ PBS containing 0.05% P20, prepared in deionized water). The primary detection antibody is then added to each well in a 50 μ L volume, and plates are incubated at room temperature for 2 hours before being washed again three times. Secondary detection is performed by adding 50 μ L of diluted streptavidin-HRP to each well, incubating at room temperature for 1 hour, followed by another wash step. Just before use, one drop (20 μ L) of AEC chromogen is added to each 1 mL of AEC substrate, mixed, and 50 μ L is added to each well. Spot development is monitored, and plates are washed with tap water to stop the reaction. Plates are dried at room temperature for at least 2 hours before counting spots with a CTL analyzer and Immunospot software (Cellular Technology Limited).

[0444] The ability of svd-TCR-anti-CD3 fusion proteins to mediate potent redirected T cell killing of antigen-positive tumor cells is investigated using the IncuCyte platform (Essen BioScience). This assay provides real-time detection by microscopy of Caspase-3/7 release, a marker of apoptosis.

[0445] Assays are performed using the CellPlayer 96-well Caspase-3/7 apoptosis assay kit (Essen BioScience, Cat. No. 4440) following the manufacturer's protocol. Briefly, target cells (NCI-H1703 – antigen +ve HLA-A*02+ve and NCI-H441 – antigen –ve HLA-A*02+ve) are plated at 5,000 cells per well and incubated overnight for adhesion. svd-TCR-anti-CD3 fusion protein solutions are prepared at concentrations between 0.5 nM and 0.01 nM, with 25 μ L of each concentration added to the relevant wells. Effector cells are used at an effector-to-target cell ratio of 10:1 (50,000 cells per well). A control sample without svd-TCR-anti-CD3 fusion protein is also included. NucView assay reagent is prepared at 30 μ M, and 25 μ L is added to each well, adjusting the final volume to 150 μ L (resulting in a 5 μ M final concentration). The plate is placed in the IncuCyte instrument, and images are taken every 2 hours (1 image per well) over 3 days. The number of apoptotic cells in each image is recorded as apoptotic cells per mm², with assays performed in triplicate.

Example 4.3: Methods to characterize biological activity of svd-TCR – NK cell redirection

[0446] This Example describes the experiments for assessing the ability of svd-TCR-Fc fusion proteins to specifically redirect NK cells toward target cells expressing a pMHC of interest.

[0447] An exemplary intended biological function of an svd-TCR-Fc fusion protein is to specifically redirect NK cells toward target cells that express a pMHC of interest. An *in vitro* method for assessing the capacity of an svd-TCR-Fc fusion protein to redirect NK cells toward cells expressing a target pMHC

involves the following steps. First, svd-TCR-Fc fusion proteins with enhanced NK cell binding capacity are generated by introducing mutations within the CD2 domain (S239D, A330L, I332E) in the Fc portion to increase FcγRIII receptor binding affinity (referred to as svd-TCR-Fc-DLE). The specific svd-TCR-Fc-DLE protein is expressed and purified from ExpiCHO cells. To analyze NK cell redirection by the svd-TCR-Fc-DLE format, an *in vitro* cellular co-culture system is established using a peptide-pulsed human cancer cell line expressing an MHC of interest loaded with a specific target peptide, serving as the artificial target cell population. The co-culture also includes primary human NK cells isolated from donor PBMCs and purified by flow cytometry to a final purity of >80% CD56⁺ cells, as previously described (Reusch et al., *MAbs*. 6:728–39 (2014)). NK cell-mediated cytotoxicity is analyzed after a 4-hour co-culture, as a function of svd-TCR-Fc-DLE concentration, by measuring the release of lactate dehydrogenase (LDH) upon cell lysis compared to that induced by the addition of lysis buffer, which is defined as 100% cytotoxicity. A molecular indicator of NK cell activation, known as degranulation, is assessed by the release of lytic granule contents (perforin and granzymes) onto the surface of the target cell. Cytotoxic NK cell degranulation is further evaluated by immunofluorescence staining of the degranulation marker CD107a using flow cytometry.

CLAIMS

1. A therapeutic, comprising:
a single-variable domain (svd)-T cell receptor (TCR) that binds to a first epitope of a first peptide-MHC complex (pMHC); and
an antigen binding domain that binds to a second epitope;
wherein the therapeutic is soluble.
2. The therapeutic of claim 1, wherein the antigen binding domain comprises multiple separate polypeptides.
3. The therapeutic of claim 2, wherein the antigen binding domain comprises a second svd-TCR.
4. The therapeutic of claim 3, wherein the antigen binding domain comprises a second svd-TCR, an antibody, an antibody fragment, an antibody binding domain or a TCR binding domain.
5. The therapeutic of claim 1, wherein the antigen binding domain is a single polypeptide.
6. The therapeutic of claim 1, wherein the svd-TCR and the antigen binding domain together are a single polypeptide chain.
7. The therapeutic of claim 1, wherein the svd-TCR and the antigen binding domain are non-covalently linked together, or wherein the svd-TCR is non-covalently linked with the antigen binding domain.
8. The therapeutic of claim 1, wherein the svd-TCR is covalently linked with the antigen binding domain.
9. The therapeutic of claim 1, wherein the svd-TCR or the antigen binding domain is linked to a scaffold.
10. The therapeutic of claim 9, wherein the scaffold comprises an antibody fragment crystallizable (Fc) region, an antibody constant domain, a TCR constant domain, albumin, a nanocage, ferritin, or lumazine synthase, or a fragment thereof.
11. A therapeutic, comprising:
a polypeptide, comprising
a first single-variable domain TCR (svd-TCR) that binds to a first epitope of a first peptide-MHC complex (pMHC), and
a second svd-TCR that binds to a second epitope;
wherein the polypeptide is soluble.
12. The therapeutic of claims 1 or 11, wherein the first epitope or the second epitope is on a cell.
13. The therapeutic of claim 12, wherein the cell is a diseased cell.
14. The therapeutic of claim 13, wherein the disease is cancer, an autoimmune disease, an infectious disease, or a rare disease.

15. The therapeutic of claim 14, wherein the first epitope or second epitope is a cancer antigen, a neoantigen, a viral antigen, or a bacterial antigen.
16. The therapeutic of claims 1 or 11, wherein the second epitope is the same as the first epitope.
17. The therapeutic of claims 1 or 11, wherein the second epitope is different from the first epitope.
18. The therapeutic of claims 1 or 11, wherein the second epitope comprises a surface antigen.
19. The therapeutic of claim 18, wherein the second epitope comprises a non-pMHC surface protein.
20. The therapeutic of claim 18, wherein the second epitope is of a second pMHC.
21. The therapeutic of claim 20, wherein an MHC of the pMHC is a class I MHC or a class II MHC.
22. The therapeutic of claims 1 or 11, further comprising a T-cell engager or NK-cell engager.
23. The therapeutic of claim 22, wherein the T-cell engager is a CD3 engager.
24. The therapeutic of claims 1 or 11, wherein the first or second epitope is not a superantigen.
25. The therapeutic of claims 1 or 11, wherein the svd-TCR or the antigen binding domain comprises a TCR variable domain.
26. The therapeutic of claim 25, wherein the TCR variable domain comprises a V α , V β , V γ , or V δ variable domain, or a binding fragment thereof.
27. The therapeutic of claim 26, wherein the TCR variable domain comprises a V β variable domain, or a binding fragment thereof.
28. The therapeutic of claim 25, wherein the TCR variable domain comprises a mammalian variable domain or a binding fragment thereof, or is at least 90% identical to a mammalian variable domain sequence.
29. The therapeutic of claim 25, wherein the TCR variable domain comprises a non-mammalian variable domain or a binding fragment thereof, or is at least 90% identical to a non-mammalian variable domain sequence.
30. The therapeutic of claims 1 or 11, wherein the TCR variable domain of the svd-TCR or the antigen binding domain is derived from a TRAV, TRBV, TRGV, or TRDV gene.
31. The therapeutic of claim 30, wherein the TCR variable domain of the svd-TCR or the antigen binding domain is derived from a TRBV1, TRBV2, TRBV3-1, TRBV3-2, TRBV4-1, TRBV4-2, TRBV4-3, TRBV5-1, TRBV5-2, TRBV5-3, TRBV5-4, TRBV5-5, TRBV5-6, TRBV5-7, TRBV5-8, TRBV6-1, TRBV6-2, TRBV6-3, TRBV6-4, TRBV6-5, TRBV6-6, TRBV6-7, TRBV6-8, TRBV6-9, TRBV7-1, TRBV7-2, TRBV7-3, TRBV7-4, TRBV7-5, TRBV7-6, TRBV7-7, TRBV7-8, TRBV7-9, TRBV8-1,

- TRBV8-2, TRBV9, TRBV10-1, TRBV10-2, TRBV10-3, TRBV11-1, TRBV11-2, TRBV11-3, TRBV12-1, TRBV12-2, TRBV12-3, TRBV12-4, TRBV12-5, TRBV13, TRBV14, TRBV15, TRBV16, TRBV17, TRBV18, TRBV19, TRBV20-1, TRBV21-1, TRBV22-1, TRBV23-1, TRBV24-1, TRBV25-1, TRBV26, TRBV27, TRBV28, TRBV29-1, or TRBV30 gene or a fragment thereof.
32. The therapeutic of claim 31, wherein the svd-TCR comprises a TRBJ1-1, TRBJ1-2, TRBJ1-3, TRBJ1-4, TRBJ1-5, TRBJ1-6, TRBJ2-1, TRBJ2-2, TRBJ2-2P, TRBJ2-3, TRBJ2-4, TRBJ2-5, TRBJ2-6, or TRBJ2-7 gene or a fragment or derivative thereof.
33. The therapeutic of claim 25, wherein the TCR variable domain does not comprise a TCR transmembrane domain or a portion thereof.
34. The therapeutic of claim 25, wherein the TCR variable domain comprises a portion of constant domain.
35. The therapeutic of claim 25, wherein the TCR variable domain does not comprise a TCR constant domain or a portion thereof.
36. The therapeutic of claim 25, wherein the TCR variable domain comprises a CDR of a human, mouse, or macaque or a derivative thereof.
37. The therapeutic of claim 36, wherein the TCR variable domain comprises a CDR3 having a length of 10-30 amino acids.
38. The therapeutic of claims 1 or 11, wherein the svd-TCR or the antigen binding domain comprises a glycosylation site.
39. The therapeutic of claim 38, wherein the glycosylation site comprises a NX[S/T] amino acid motif, wherein X is any residue except proline.
40. The therapeutic of claim 39, wherein the glycosylation site comprises an amino acid motif selected from the group consisting of: NYS, NVT, NLT, NLS, NVS, NET, NES, NMS, NFT, or NGT.
41. The therapeutic of claims 1 or 11, wherein the TCR variable domain of the svd-TCR or the antigen binding domain comprises a hydrophobic residue mutated to a non-hydrophobic residue relative to a wild type or non-mutated TCR variable domain.
42. The therapeutic of claim 41, wherein the hydrophobic residue is from the BetaCON1 region, Alpha1 region, or Alpha2 region shown in any one of **FIG. 7A-7C** or CDR1, CDR2, or CDR3 shown in **FIG. 7D**.
43. The therapeutic of claim 41, wherein the hydrophobic residue comprises a residue from the BetaCON1 region, Alpha1 region, or Alpha2 region shown in any one of **FIG. 7A-7C** or CDR1, CDR2, or CDR3 shown in **FIG. 7D**.

44. The therapeutic of claim 41, wherein the hydrophobic residue comprises a residue in a BetaCON1 region, Alpha1 region, or Alpha2 region of **FIG. 7C**.
45. The therapeutic of claim 41, wherein the hydrophobic residue is an exposed hydrophobic residue.
46. The therapeutic of claim 45, wherein the exposed hydrophobic residue comes into contact with another TCR variable domain when not in a svd-TCR format.
47. The therapeutic of claim 45, wherein the exposed hydrophobic residue is within a V α or a V β of the TCR variable domain.
48. The therapeutic of claim 45, wherein the exposed hydrophobic residue is within a β constant domain of the TCR variable domain.
49. The therapeutic of claim 41, wherein the mutation to the non-hydrophobic residue results in an increase in the hydrophilicity of the therapeutic or a polypeptide of the therapeutic by about 4 to about 8 units, with respect to the Kyte and Doolittle scale, relative to a wild type or non-mutated TCR variable domain.
50. The therapeutic of claim 49, wherein inclusion of the non-hydrophobic residue results in an increase in the charge density of the therapeutic or a polypeptide of the therapeutic by about +1 or about -1, relative to a wild type or non-mutated TCR variable domain.
51. The therapeutic of claims 1 or 11, wherein the TCR variable domain of the svd-TCR or the antigen binding domain comprises a mutated residue located at a buried surface or core of the svd-TCR or the antigen binding domain, relative to a wild type or non-mutated TCR variable domain.
52. The therapeutic of claim 51, wherein inclusion of the mutated residue results in an increase in production, stability, a specific binding activity, or a functional activity of the svd-TCR or the antigen binding domain.
53. The therapeutic of claims 1 or 11, wherein the therapeutic has a mass of at least 10 kDa, at least 15 kDa, at least 20 kDa, at least 25 kDa, at least 30 kDa, at least 35 kDa, at least 40 kDa, at least 50 kDa, at least 60 kDa, at least 70 kDa, at least 80 kDa, at least 90 kDa, at least 100 kDa, at least 125 kDa, at least 150 kDa, at least 175 kDa, at least 200 kDa, at least 250 kDa, at least 300 kDa, at least 400 kDa, at least 500 kDa, at least 600 kDa, at least 700 kDa, at least 800 kDa, at least 900 kDa, or at least 1000 kDa.
54. The therapeutic of claims 1 or 11, wherein the therapeutic has a mass of less than 10 kDa, less than 15 kDa, less than 20 kDa, less than 25 kDa, less than 30 kDa, less than 35 kDa, less than 40 kDa, less than 50 kDa, less than 60 kDa, less than 70 kDa, less than 80 kDa, less than 90 kDa, less than 100 kDa, less than 125 kDa, less than 150 kDa, less than 175 kDa, less than 200 kDa, less than 250 kDa, less than

300 kDa, less than 400 kDa, less than 500 kDa, less than 600 kDa, less than 700 kDa, less than 800 kDa, less than 900 kDa, or less than 1000 kDa.

55. The therapeutic of claims 1 or 11, wherein the mass is a molecular mass estimated based on a predicted chemical structure or sequence.
56. The therapeutic of claims 1 or 11, wherein the therapeutic or a polypeptide of the therapeutic has a molecular mass no greater than 30 kDa.
57. The therapeutic of claim 56, wherein the svd-TCR or the antigen binding domain has a molecular mass of about 10-13 kDa.
58. The therapeutic of claims 1 or 11, further comprising an additional binding protein.
59. The therapeutic of claim 58, wherein the additional binding protein is coupled with the svd-TCR or the antigen binding domain.
60. The therapeutic of claim 58, wherein the additional binding protein comprises an antibody or binding fragment thereof or a single-chain variable fragment (scFv).
61. The therapeutic of claims 1 or 11, further comprising a third svd-TCR.
62. The therapeutic of claim 61, further comprising a fourth svd-TCR.
63. The therapeutic of claim 62, further comprising a fifth svd-TCR.
64. The therapeutic of claim 63, further comprising a sixth svd-TCR.
65. The therapeutic of claims 1 or 11, further comprising a biologically active group.
66. The therapeutic of claim 65, wherein the biologically active group is another peptide or a small molecule conjugate.
67. The therapeutic of claim 65, wherein the biologically active group is coupled to a polypeptide of the therapeutic.
68. The therapeutic of claims 1 or 11, wherein the therapeutic is coupled with a binding protein, an antibody, an antibody binding fragment, a scFv, a therapeutic moiety, a detectable moiety, an immune cell modulator or engager, a checkpoint inhibitor, a biologically active group, an anti-cancer agent, an anti-infection agent, an immune checkpoint inhibitor, a CD3 engager, or a radionuclide.
69. The therapeutic of claims 1 or 11, further comprising a linker connecting the svd-TCR and the antigen binding domain to each other.
70. The therapeutic of claims 1 or 11, further comprising a linker connecting the svd-TCR or the antigen binding domain with the biologically active group or binding protein.

71. The therapeutic of claims 1 or 11, further comprising a linker connecting the svd-TCR or the antigen binding domain with a scaffold.
72. The therapeutic of claims 1 or 11, wherein the linker has a length of 2-30 amino acids or 9-25 amino acids.
73. The therapeutic of claims 1 or 11, wherein the linker comprises a hinge region sequence of an immunoglobulin.
74. The therapeutic of claims 1 or 11, wherein the linker is a GS linker.
75. The therapeutic of claim 74, wherein the GS linker comprises GS_n, GGS_n, GGGS_n, or GGGGS_n.
76. A pharmaceutical composition comprising:
the therapeutic of claims 1 or 11; and
a pharmaceutically acceptable carrier.
77. A nucleic acid encoding the therapeutic of claims 1 or 11.
78. A method comprising administering the therapeutic of claims 1 or 11 to a subject.
79. A kit comprising:
the therapeutic of claims 1 or 11; and
an instruction.