cysteines, either intra-domain or inter-domain, that can lead to lower expression, product yield and product quality.

[0005] Structure found in protein subdomains has provided another source of structural constraint. Structures such as fibronectin type III repeats (adnectins), z-proteins (affibodies), knottins, lipocalins (anticalins) and ankyrin repeats (DARPins) have been developed with antibody-like affinities against a variety of different targets (Hey et al., Trends in Biotech. 23(10):514-422, 2005). These domains typically contain two features that are analogous to the frameworks and complementarity determining regions (CDRs) found in antibody variable domains: a structural scaffold that imparts high thermodynamic stability and residues or loops that form the basis of the display library's variability.

SUMMARY

[0006] In general, there remains a substantial unmet need for new target-binding agents and compositions, and particularly for such agents containing alternative binding scaffolds (e.g., non-antibody scaffolds). In several ~~embodiment~~examples, agents of particular interest may be characterized by, for example, substantially reduced production costs and/or comparable or superior reagent, diagnostic and/or therapeutic properties as compared to antibodies. The present disclosure provides such desirable agents in several ~~embodiment~~examples. For example, in several ~~embodiment~~examples, the present disclosure provides certain polypeptide agents that are characterized by high target binding affinity and by a non-antibody structural scaffold. Alternatively or additionally, in several ~~embodiment~~examples, target-binding agents, such as the polypeptides disclosed herein, for example resulting from the production methods disclosed herein have advantages including, for example, highly target-specific binding. In some ~~embodiment~~examples, this can advantageously be used to target therapeutics (e.g., immune cells) to particular cells (e.g., diseased cells), thereby reducing or eliminating off-target effects. In some ~~embodiment~~examples, the agents provided herein, such as the target-specific polypeptides, can be used as protein therapeutics to bind cells or soluble factors involved in disease. In some ~~embodiment~~examples, the provided agents can be used to purify targets (e.g., proteins or other targets) with a high degree of specificity, which may, for example, result in higher purity and/or reduced downstream processing to purify a target.

[0007] Several ~~embodiment~~examples of the ~~invention~~disclosures~~s~~ disclosed herein relate to agents that specifically bind targets of interest, such as the de novo binding domain (DBD) containing polypeptides (DBDpp) disclosed herein. Nucleic acids encoding the DBDpp and vectors and host cells containing the nucleic acids are also provided, as are DBDpp libraries and methods for producing and screening such libraries and the DBDpp identified from such libraries and/or screens. DBDpp including DBDpp fusion proteins are also provided, as are methods of making and using the DBDpp. Non-limiting examples of such uses include, but are not limited to, affinity purification, target analysis, diagnostic and/or therapeutic applications.

[0008] In several ~~embodiment~~examples, there is provided a binding agent that binds with a high degree of specificity to a target of interest. In several ~~embodiment~~examples the binding agent is a non-antibody agent. In several ~~embodiment~~examples, the binding agent is a polypeptide. In several ~~embodiment~~examples, there are provided polypeptides for binding a target of interest that have a sequence that differs, at least at one position, from the sequence of SEQ ID NO:1. In several such ~~embodiment~~examples, the agent (e.g., a polypeptide) exhibits specific binding to the target of interest, that binding being greater than the binding of a polypeptide according to SEQ ID NO:1 to the target of interest. In several ~~embodiment~~examples, there is provided a polypeptide for binding a target of interest, the polypeptide comprising an amino acid sequence comprising $MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX_5$ $_5LRX_{58}X_{59}AAX_{62}IRX_{65}X_{66}LQAYRHN$ (SEQ ID NO:4), wherein the sequence differs in sequence from the sequence of SEQ ID NO:1 (e.g., by modifications to the amino acid sequence of SEQ ID NO:1). In several ~~embodiment~~examples, the polypeptide specifically binds a target of interest (such as a cancer marker or other distinctive marker related to a target of interest), and the specific binding of the polypeptide to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest. In several ~~embodiment~~examples, the polypeptide does not contain the sequence of SEQ ID NO:50.

[0009] In several ~~embodiment~~examples, the polypeptide has a sequence that differs from SEQ ID NO:1 because certain selected amino acid positions have been modified. In some ~~embodiment~~examples, the modifications comprise substitutions. In several ~~embodiment~~examples, the substitutions are conservative substitutions, while in some ~~embodiment~~examples, the substitutions are non-conservative substitutions. In still additional ~~embodiment~~examples, combinations of conservative and non-conservative substitutions are

used. In some ~~embodiment~~examples, the substitutions do not include substitution with a cysteine (e.g., no cysteines are added to the sequence). In some ~~embodiment~~examples, wherein the substitutions do not include substitution with a proline (e.g., no prolines are added to the sequence). In some ~~embodiment~~examples, neither cysteine nor proline is substituted into the sequence of the polypeptide.

[0010] Various targets of interest can be bound by the agents disclosed herein. For example, in several ~~embodiment~~examples, the target of interest specifically bound by the polypeptide is a cancer antigen. In some ~~embodiment~~examples, the cancer antigen specifically bound by polypeptide is PD-L1. In several such ~~embodiment~~examples, target-binding polypeptide comprises or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO: 43, SEQ ID and NO:44. In some ~~embodiment~~examples, the cancer antigen specifically bound by polypeptide is CD137. In some such ~~embodiment~~example, the polypeptide comprises or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO: 17, SEQ ID NO:18, and SEQ ID NO:19. In some ~~embodiment~~examples, the cancer antigen specifically bound by polypeptide is CD123. In some such ~~embodiment~~examples, the polypeptide comprises or consists essentially of an amino acid sequence selected from SEQ ID NOS: 92-127. In some ~~embodiment~~examples, a combination of cancer antigens is targeted, for example by coupling or otherwise combining various target-binding polypeptides. In some ~~embodiment~~examples, two, three, four or more different cancer antigens are targeted. In some ~~embodiment~~examples, multiple target-binding polypeptides are used to enhance the ability and/or capacity to bind a single target (e.g., dimers, trimers, etc.)

[0011] Additionally provided for in several ~~embodiment~~examples is a method for transforming a reference polypeptide into a polypeptide having specific binding for a target of interest, the method comprising modifying a plurality of amino acid residues from a reference polypeptide to generate a plurality of candidate binding polypeptides, packaging the plurality of candidate binding polypeptides in a plurality of vectors to generate a candidate library, and screening the candidate library for candidate binding polypeptides that exhibit specific binding to the target of interest. In several ~~embodiment~~examples, the reference polypeptide comprises a variant of a non-naturally occurring polypeptide and comprises three anti-parallel alpha helices joined by linker peptides. In several ~~embodiment~~examples the amino acid residues to be modified are solvent accessible or solvent inaccessible

amino acids. In several ~~embodiment~~examples, a greater degree of solvent accessible amino acids are modified, while in some ~~embodiment~~examples a greater degree of solvent inaccessible amino acids are modified. In some ~~embodiment~~examples, the modification comprises amino acid substitutions. As discussed above, the substitutions can comprise conservative amino acid substitutions, non-conservative amino acid substitutions, and/or combinations thereof. Optionally, in several ~~embodiment~~examples, the substitution does not comprise substitution in of a cysteine, does not comprise substitution in of a proline, and in some cases does not comprise substitution in of a cysteine or a proline.

[0012] In several ~~embodiment~~examples, the method further comprises identifying potentially immunogenic amino acid residues in the candidate binding polypeptides and modifying at least one of the potentially immunogenic amino acid residues (e.g., to reduce the potential immunogenicity of the resultant polypeptides that bind a target of interest). In several ~~embodiment~~examples, the modification to reduce immunogenicity comprises an amino acid substitution (e.g., conservative and/or non-conservative substitutions).

[0013] In several ~~embodiment~~examples, there is provided a de novo binding domain polypeptide (DBDpp) that comprises or consists essentially of three anti-parallel alpha helices, the DBDpp being a variant of a synthetic polypeptide, wherein the DBDpp immunospecifically binds to a protein that is at least 95% identical to CD123. In several ~~embodiment~~examples, the DBDpp has a dissociation constant (KD) between about 10^{-4}M and about 10^{-12}M. In some ~~embodiment~~examples, the target to which the DBDpp immunospecifically binds comprises amino acids 19-305 of CD123 (SEQ ID NO: 187). There is also provided herein a DBDpp having an amino acid sequence $MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX_5$ $_5LRX_{58}X_{59}AAX_{62}IRX_{65}X_{66}LQAYRHN$ (SEQ ID NO:4), and wherein X_n is a natural or non-natural amino acid. Moreover, there is also provided for a DBDpp having an amino acid sequence at least 85% identical to the amino acid sequence of any one of SEQ ID NO:60 – SEQ ID NO: 136. Still further ~~embodiment~~examples provide for a fusion protein that binds to CD123 (or other target of interest disclosed herein) and further comprises one or more additional DBDpp exhibiting binding specificity for a tumor target.

[0014] In several ~~embodiment~~examples, the target-binding agent (e.g., a polypeptide with specificity for a target of interest) is labeled. Depending on the ~~embodiment~~example, various labels can be used, including but not limited to an enzymatic label, a fluorescent label, a luminescent label, and a

bioluminescent label. In some ~~embodiment~~examples, the label is a biotin moiety. In several ~~embodiment~~examples, a streptavidin moiety can be used. In some ~~embodiment~~examples, a His-tag, FLAG-tag or other tag is used. In some ~~embodiment~~examples, the label is luciferase, green fluorescent protein, red fluorescent protein, or other similar agent.

[0015] In several ~~embodiment~~examples, the target-binding agent (e.g., a polypeptide) is conjugated to a therapeutic or cytotoxic agent (e.g., chemotherapeutic agent, radiotherapeutic agent, etc.). Depending on the ~~embodiment~~example, the target-binding agent may optionally comprise a pharmaceutically acceptable carrier.

[0016] In several ~~embodiment~~examples, there are provided kits comprising any of the target-binding agents disclosed herein (e.g., a therapeutic kit, a diagnostic kit, a kit for research use, etc.).

[0017] Several ~~embodiment~~examples also provide for isolated nucleic acid molecules encoding the any of the target-binding polypeptides disclosed herein. Still additional ~~embodiment~~examples provide for a vector (e.g., a plasmid, viral vector, or non-viral vector) containing the isolated nucleic acid molecule. Several such ~~embodiment~~examples may also include standard components for expression of protein encoded by the nucleic acid (e.g., promoters, packaging components, etc.). For example, in several ~~embodiment~~examples, the vector further comprises an additional nucleotide sequence which regulates the expression of the polypeptide encoded by the nucleic acid molecule. In several ~~embodiment~~examples, the additional nucleic acid sequence is an inducible promoter.

[0018] Further provided for in several ~~embodiment~~examples are host cells that comprise the nucleic acid molecules encoding the any of the target-binding polypeptides disclosed herein. In several ~~embodiment~~examples such ~~embodiment~~examples, the host cell (e.g., a cell line) is engineered to express the target-binding polypeptides disclosed herein. In some ~~embodiment~~examples, the expression of the target-binding polypeptides by the host cells allows production and isolation of the target-binding polypeptides. In some ~~embodiment~~examples, the expression results in the target-binding polypeptides expressed on the surface and/or integral to the membrane of the cells.

[0019] Also provided for herein are de novo binding domain polypeptides (DBDpp) that compete with the polypeptides disclosed herein for binding to CD123 (or other targets of interest). In several ~~embodiment~~examples, there are also provided polypeptides that compete with those disclosed herein for binding to other targets of interest, including CD123, PD-L1, CD19, CD22, and the like (or other targets disclosed herein). Competitors that are provided for include full or partial agonists,

full or partial antagonists, and the like. Those agents that compete for binding to a target of interest (either to the same epitope, an overlapping epitope, or a non-overlapping epitope that leads to steric or other hindrances to the agent binding a target of interest) can be identified by competitive binding assays.

[0020] Also provided for herein are polypeptides (either alone or expressed by a cell) that bind to a tumor. In several ~~embodiment~~examples, the binding is based on the polypeptide having been generated and identified as having specific binding for one or more markers expressed by the tumor. The tumor, depending on the ~~embodiment~~example, may be a suspension tumor or a solid tumor.

[0021] Several ~~embodiment~~examples, also provide for a chimeric antigen receptor (CAR), wherein the CAR includes a targeting domain, a transmembrane domain, and an intracellular signaling domain. In several ~~embodiment~~examples, the targeting domain is made up of, at least in part, a target-binding polypeptide as disclosed herein. In several ~~embodiment~~examples, the intracellular signaling domain is selected from the group consisting of a human CD3 zeta domain, 41BB domain, a CD28 domain and any combination thereof. Depending on the ~~embodiment~~example, the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-lBB, OX40, CD30, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof. In several ~~embodiment~~examples, the CAR comprises a fusion protein that includes an additional target-binding polypeptide. Also provided for are isolated nucleic acid sequences encoding CARs that include the target-binding polypeptides as part (or all) of the targeting region.

[0022] Further provided for herein are cells comprising a nucleic acid sequence encoding a CAR, wherein the CAR comprises an antigen binding domain made up of, at least in part, a polypeptide that binds a target of interest, a transmembrane domain, and a signaling domain. In several ~~embodiment~~examples, the polypeptide binds specifically to a tumor antigen (and thus functions to deliver the cell expressing the CAR to the tumor. In several ~~embodiment~~examples, the tumor antigen is associated with a hematologic malignancy. In additional ~~embodiment~~examples, tumor antigen is associated with a solid tumor. Both solid and hematologic tumors can be simultaneously targeted in some ~~embodiment~~examples. In several ~~embodiment~~examples, the tumor antigen is selected from the group consisting of CD137, PD-L1, CD123, CTLA4, CD47, KIR, DR5, TIM3, PD1, EGFR, TCR, CD19, CD20, CD22,

ROR 1, mesothelin, CD33/lL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof. Depending on the ~~embodiment~~example, the cell expressing the CAR can be a T cell or a natural killer (NK) cell. In several ~~embodiment~~examples, the cell (whether T cell, NK cell or other cell type) exhibits an anti-tumor immunity when the polypeptide binds to its corresponding tumor antigen.

[0023] Still additional ~~embodiment~~examples provide for amino acids having the sequence of SEQ ID 4, wherein X_n is not cysteine or proline.

[0024] Also provided for in several ~~embodiment~~examples are mammalian cells that generate membrane-bound virus-like particles (VLPs), wherein the mammalian cell is engineered to express a fusion protein comprising a de novo binding domain polypeptide (DBDpp) fused to a chimeric antigen receptor (CAR), the fusion protein being expressed on the generated VLPs (e.g., as transmembrane proteins). Depending on the ~~embodiment~~examples, the VLPs produced by the mammalian cells are suitable for use as immunogens for antibody generation. In some such ~~embodiment~~examples, the antibodies are directed against the de novo binding domain polypeptide (DBDpp) (e.g., the antibodies bind to the DBDpp and can be used to detect the DBDpp, isolate the DBDpp, etc.

[0025] The target-binding polypeptides disclosed herein are also useful in a therapeutic context, e.g., for treatment and/or diagnosis of a disease, such as a cancer (e.g., a solid or hematologic malignancy). Thus, there are provided, in several ~~embodiment~~examples methods of treating a subject having cancer, comprising administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises a target binding domain, wherein the target binding domain comprises a polypeptide having an amino acid sequence comprising: $MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX_5$ $_5LRX_{58}X_{59}AAX_{62}IRX_{65}X_{66}LQAYRHN$ (SEQ ID NO:4), a transmembrane domain, and an intracellular domain (comprising a signaling domain). Upon administration to a subject having cancer, the target binding domain specifically binds to a target of interest expressed by a cancer cell, and the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell, thereby treating the cancer. In several ~~embodiment~~examples, the polypeptide has a sequence that differs from SEQ ID NO:1 (e.g., the polypeptide is generated by modifying the amino acid sequence of SEQ ID NO:1). As a result of the differing sequence, the

polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest.

[0026] Depending on the ~~embodiment~~example, the immune cell can be a T cell. In some ~~embodiment~~examples, the immune cell is a NK cell. Other immune cells, and/or combinations of different immune cell types can optionally be used. In some ~~embodiment~~examples, combinations of cell types (e.g., NK cells and T cells) are advantageous because they act synergistically to treat a cancer. When combinations are used, the various cell types can target the same or different (or overlapping) tumor antigens.

[0027] In several ~~embodiment~~examples wherein T cells are used, the binding of the target of interest stimulates the T cell to initiate intracellular signaling, produce cytokines, and degranulate, leading to the cytotoxic effects on the cancer cell. Additionally, in several ~~embodiment~~examples, the T cell proliferates in response to binding the target of interest. Advantageously, however, the activity of the T cell does not result in the T cells exhibiting a phenotype associated with T cell exhaustion. In several ~~embodiment~~examples where T cells are used, the transmembrane domain of the CAR comprises 41BB or CD28, and the cytoplasmic domain comprises an alpha, beta, or zeta chain of the T cell receptor.

[0028] In several ~~embodiment~~examples where NK cells are used, the transmembrane domain comprises CD28, and the cytoplasmic domain comprises a zeta chain of the T cell receptor.

[0029] In several ~~embodiment~~examples, the CAR-containing immune cells are designed to bind to a target of interest expressed by the cancer cell, such as a tumor antigen selected from the group consisting of CD137, PD-L1, CD123, CTLA4, CD47, KIR, DR5, TIM3, PD1, EGFR, TCR, CD19, CD20, CD22, ROR 1, mesothelin, CD33/lL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof.

[0030] In several ~~embodiment~~examples, the CAR further comprises a second polypeptide having an amino acid of SEQ ID NO:4, the polypeptide being able to specifically bind a second target of interest expressed by a cancer cell, and wherein the second polypeptide's specific binding the second target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the second target of interest. In several ~~embodiment~~examples, the generation of the polypeptide that makes up at least a portion of the targeting domain of the CAR does not include substituting a cysteine or a proline into SEQ ID NO: 1.

[0031] In several ~~embodiment~~examples, the administration of the immune cells with a CAR is intravenous, though other routes, such as intra-arterial, intramuscular, local, or other acceptable route can be used for a given treatment scenario.

[0032] There are also provided, in several ~~embodiment~~examples, methods of treating a subject having cancer, comprising, administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises a target binding domain, wherein the target binding domain comprises a polypeptide having an amino acid sequence selected from of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest, a transmembrane domain, and an intracellular domain, wherein the intracellular domain comprises a signaling domain, wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and wherein the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell, thereby treating the cancer. As discussed above, depending on the ~~embodiment~~example, the immune cell can be a T cell, a NK cell, or other type of immune cell (or combinations of various types). In one ~~embodiment~~example, the transmembrane domain comprises 41BB or CD28, wherein the cytoplasmic domain comprises an alpha, beta, or zeta chain of the T cell receptor, and wherein the immune cell is a T cell. In some such ~~embodiment~~examples, upon binding the target of interest, the T cell is stimulated to initiate intracellular signaling, produce cytokines, proliferates and degranulates, leading to the cytotoxic effects on the cancer cell, without the T cells exhibiting a phenotype associated with T cell exhaustion.

[0033] Further ~~embodiment~~examples provide for a method of treating a subject having cancer, the method comprising intravenously administering to the subject an immune cell comprising a chimeric antigen receptor (CAR) expressed on a T cell, wherein the CAR comprises a target binding domain comprising a polypeptide having an amino acid sequence comprising, the polypeptide having an amino acid sequence of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, however, no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID

NO:3, SEQ ID NO:4, SEQ ID NO:5, or SEQ ID NO:6, the polypeptide capable of specifically binding a target of interest expressed by a cancer cell with a binding to the target of interest that is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest, a transmembrane domain selected from 41BB and CD28, and an intracellular domain, wherein the intracellular domain comprises a signaling domain selected from an alpha, beta, or zeta chain of the T cell receptor, wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and wherein the binding of the target of interest induces the T cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell. In several ~~embodiment~~examples, the cytotoxic effects result from degranulation of the T cells. Advantageously, in several ~~embodiment~~examples, the activation and cytotoxic activity of the T cells is not associated with the T cells exhibiting a phenotype associated with T cell exhaustion. In several ~~embodiment~~examples, the CAR optionally further comprises a second target binding domain comprising a second polypeptide having a different target than the target binding domain. In still further ~~embodiment~~examples, additional targeting domains can optionally be included to enhance binding capacity to a marker, or impart binding specificity to other markers.

[0034] Additionally provided for in several ~~embodiment~~examples, is the use of an immune cell comprising a chimeric antigen receptor (CAR) for the treatment of cancer, wherein the CAR comprises a target binding domain comprising a polypeptide having an amino acid sequence comprising, the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest, a transmembrane domain selected from 41BB and CD28, and an intracellular domain, wherein the intracellular domain comprises a signaling domain selected from an alpha, beta, or zeta chain of the T cell receptor, wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and wherein the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytotoxic effects

on the cancer cell. Depending on the ~~embodiment~~example the immune cells can be a T cell or a natural killer (NK) cell.

[0035] In addition to binding domain compositions, methods for generating, screening and using same, there are also provided methods for purifying targets of interest. Thus, provided for herein, in several ~~embodiment~~examples, is a method for purifying a target of interest comprising contacting a sample comprising a target of interest with a composition comprising a polypeptide agent attached to a solid support, wherein the polypeptide agent has an amino acid sequence comprising MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX$_5$ $_5$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:4), wherein the polypeptide has an amino acid sequence that differs from SEQ ID NO:1, wherein the polypeptide specifically binds the target of interest, wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest, the contacting performed under conditions that permit binding of the composition to the target of interest, and removing a portion of the sample that is not bound to the composition. In several ~~embodiment~~examples the method further comprises dissociating the composition from the target of interest and recovering the target of interest. In several ~~embodiment~~examples, the target of interest can be eluted from the composition, thereby purifying (wholly or partially) the target of interest.

[0036] Depending on the ~~embodiment~~example, the solid support may be a bead, a glass slide, a chip, a gelatin, or an agarose. Combinations of supports may be used in certain ~~embodiment~~examples. In several ~~embodiment~~examples, the polypeptide agent is coupled to the solid support through non-covalent association, while in other ~~embodiment~~examples, the polypeptide agent is coupled to the solid support through covalent bonding. Depending on the ~~embodiment~~example, the supports, and the target of interest, combinations of covalent and non-covalent association can also be used.

[0037] In several ~~embodiment~~examples, the polypeptide agent of the composition further comprises a peptide tag, wherein the peptide tag comprises a hexahistidine moiety or a FLAG tag. In some ~~embodiment~~examples, the polypeptide agent of the composition further comprises a streptavidin moiety. Other types of tags, e.g., enzymes, colorimetric, bioluminescent and/or fluorescent tags can be used, depending on the ~~embodiment~~example.

[0038] In some ~~embodiment~~examples, the solid support comprises a bead, and the composition is suitable for use in affinity chromatography to purify the target of interest.

[0039] In several ~~embodiment~~examples, a nucleic acid molecule encoding the polypeptide is packaged in an expression vector that is used to transduce a cell line to cause the cell line to express the polypeptide. Such ~~embodiment~~examples, allow for production of the polypeptide in larger scale for use in protein purification.

[0040] Also provided for in several ~~embodiment~~examples is a method for purifying a target of interest comprising contacting a sample comprising a target of interest with a composition comprising a virus-like particle coupled to a solid support, wherein the virus-like particle expresses a polypeptide as a membrane protein, the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein the polypeptide has an amino acid sequence that differs from SEQ ID NO:1, wherein the polypeptide specifically binds the target of interest, wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest; and the contacting performed under conditions that permit binding of the composition to the target of interest; and removing a portion of the sample that is not bound to the composition. In several ~~embodiment~~examples, wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6 when generating the polypeptide.

[0041] In several ~~embodiment~~examples, the solid support comprises one or more of a bead, a glass slide, a chip, a gelatin, or an agarose. In several ~~embodiment~~examples, the polypeptide of the composition further comprises a peptide tag, wherein the peptide tag comprises a hexahistidine moiety or a FLAG tag. As discussed herein, other types of tags may be used in additional ~~embodiment~~examples.

[0042] In some ~~embodiment~~examples, the portion of the sample that is not bound to the composition is discarded. In some ~~embodiment~~examples, the portion of the sample that is not bound to the composition is contacted with the composition a second time to capture additional target of interest, thereby improving the overall yield of the purification.

[0043] In several ~~embodiment~~examples, the method further comprises contacting the portion of the sample that is not bound to the composition with an antibody directed against the polypeptide of the composition, the antibody being generated from membrane bound virus-like particles (VLP) expressing the polypeptide released from a mammalian cell is engineered to express a fusion protein

comprising the polypeptide fused to a chimeric antigen receptor (CAR), the fusion protein being expressed on the generated VLPs, wherein the antibodies are suitable for use in an assay to detect residual polypeptides detached from the solid support.

[0044] Not only are there provided methods for purifying a target (e.g., removing the target from a larger sample), but several ~~embodiment~~examples provide for a method for removing one or more contaminants from a sample comprising a target of interest, the method comprising contacting a sample comprising a target of interest with a composition comprising a virus-like particle coupled to a solid support, wherein the virus-like particle expresses a polypeptide as a membrane protein, the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, wherein the polypeptide has an amino acid sequence that differs from SEQ ID NO:1, wherein the polypeptide specifically binds one or more contaminants to be removed from a sample comprising the target of interest, wherein the polypeptide's specific binding to one or more contaminants is greater than binding of a polypeptide according to SEQ ID NO:1 to the one or more contaminants; the contacting performed under conditions that permit binding of the composition to the one or more contaminants; and collecting a portion of the sample that is not bound to the composition. As discussed above, in several ~~embodiment~~examples, the polypeptide of the composition further comprises a tag, such as a peptide tag. In several ~~embodiment~~examples, the peptide tag comprises a hexahistindine moiety or a FLAG tag. Depending on the ~~embodiment~~examples, the solid support may comprise a bead, a glass slide, a chip, a gelatin, or an agarose and the virus-like particles are coupled to the solid support through non-covalent association. In some ~~embodiment~~examples, the portion of the sample that collected is contacted with the composition a second time to remove additional contaminants from the sample.

[0045] Also provided for herein are compositions for use in protein purification. In several ~~embodiment~~examples, there is provided an affinity resin comprising a polypeptide agent having an amino acid sequence comprising a sequence selected from the group consisting of: MGSWX_5X_6FKX_9X_{10}LAX_{13}IK$X_{16}X_{17}$LEALGGSEAELAX_{30}FE$X_{33}X_{34}$IAX_{37}FE$X_{40}X_{41}$LQX_{44}YKG KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), MGSWAEFKQRLAAIKTRLEALGGSEAELAAF$X_{32}X_{33}$EIX_{36}AF$X_{39}X_{40}$ELX_{43}AYKGKGNPEVE

$ALX_{57}X_{58}EAX_{61}AIX_{64}X_{65}ELX_{68}AYRHN$ (SEQ ID NO:3),
$MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX_5$
$_5LRX_{58}X_{59}AAX_{62}IRX_{65}X_{66}LQAYRHN$ (SEQ ID NO:4),
$MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALGGSEAELAAFX_{32}X_{33}EIX_{36}AFX_{39}X_{40}ELX_{43}AYKGK$
$GNPEVEX_{55}LRX_{58}X_{59}AAX_{62}IRX_{65}X_{66}LQAYRHN$ (SEQ ID NO:5),
$MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALGGSEAELAX_{30}FEX_{33}X_{34}IAX_{37}FEX_{40}X_{41}LQX_{44}YKG$
$KGNPEVEALX_{57}X_{58}EAX_{61}AIX_{64}X_{65}ELX_{68}AYRHN$ (SEQ ID NO:6),
$MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALZ_1EAELAX_{28}FEX_{31}X_{32}IAX_{35}FEX_{38}X_{39}LQX_{42}YZ_2NPE$
$VEALRKEAAAIRDELQAYRHN$ (SEQ ID NO:7),
$MGSWAEFKQRLAAIKTRLEALZ_1EAELAAFX_{30}X_{31}EIX_{34}AFX_{37}X_{38}ELX_{41}AYZ_2NPEVEALX_{52}$
$X_{53}EAX_{56}AIX_{59}X_{60}ELX_{63}AYRHN$ (SEQ ID NO:8),
$MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALZ_1EAELAAFEKEIAAFESELQAYZ_2NPEVEX_{50}LRX_{53}$
$X_{54}AAX_{57}IRX_{60}X_{61}LQAYRHN$ (SEQ ID NO:9),
$MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALZ_1EAELAAFX_{30}X_{31}EIX_{34}AFX_{37}X_{38}ELX_{41}AYZ_2NPEV$
$EX_{50}LRX_{53}X_{54}AAX_{57}IRX_{60}X_{61}LQAYRHN$ (SEQ ID NO:10) and $MGSWX_5EFX_8$
$X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALZ_1EAELAX_{28}FEX_{31}X_{32}IAX_{35}FEX_{38}X_{39}LQX_{42}YZ_2NPEVEALX_{52}X_{53}E$
$AX_{56}AIX_{59}X_{60}ELX_{63}AYRHN$ (SEQ ID NO:11), as well as combinations thereof, and wherein the amino acid sequence is not SEQ ID NO:1.

[0046] In any of the sequences listed above, any of the X positions (e.g., "X_n") can be a natural or non-natural amino acid; wherein each X_n is the same or different natural or non-natural amino acid. Additionally, in several ~~embodiment~~examples, Z_1 and/or Z_2 can comprise between about 2 to about 30 natural or non-natural amino acids.

[0047] In several ~~embodiment~~examples, the polypeptide agent has an amino acid sequence that differs from SEQ ID NO:1 by an amino acid substitution at one or more residues. Depending on the ~~embodiment~~examples the amino acid substitution at one or more residues can comprise a conservative substitution, or a non-conservative substitution. Combinations of conservative and non-conservative substitutions may also be use, in several ~~embodiment~~examples. Additionally, in several ~~embodiment~~examples, the amino acid substitution at one or more residues comprises a substitution at a solvent accessible residue. In some ~~embodiment~~examples, the amino acid substitution at one or more residues comprises a substitution at a solvent inaccessible residue. In some ~~embodiment~~examples, substitutions (whether

conservative or non-conservative) can optionally be made at both solvent accessible and solvent inaccessible residues. In several ~~embodiment~~examples, the polypeptide agent has an amino acid sequence that differs from SEQ ID NO:1 by an amino acid deletion at one or more residues.

[0048] In several ~~embodiment~~examples, there is provided a method of making an affinity resin comprising attaching to a solid support a polypeptide agent having an amino acid sequence comprising a sequence selected from the group consisting of: $MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALGGSEAELAX_{30}FEX_{33}X_{34}IAX_{37}FEX_{40}X_{41}LQX_{44}YKG$ KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), $MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX_{32}X_{33}EIX_{36}AFX_{39}X_{40}ELX_{43}AYKGKGNPEVE$ $ALX_{57}X_{58}EAX_{61}AIX_{64}X_{65}ELX_{68}AYRHN$ (SEQ ID NO:3), $MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX_5$ $_5LRX_{58}X_{59}AAX_{62}IRX_{65}X_{66}LQAYRHN$ (SEQ ID NO:4), $MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALGGSEAELAAFX_{32}X_{33}EIX_{36}AFX_{39}X_{40}ELX_{43}AYKGK$ $GNPEVEX_{55}LRX_{58}X_{59}AAX_{62}IRX_{65}X_{66}LQAYRHN$ (SEQ ID NO:5), $MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALGGSEAELAX_{30}FEX_{33}X_{34}IAX_{37}FEX_{40}X_{41}LQX_{44}YKG$ $KGNPEVEALX_{57}X_{58}EAX_{61}AIX_{64}X_{65}ELX_{68}AYRHN$ (SEQ ID NO:6), $MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALZ_1EAELAX_{28}FEX_{31}X_{32}IAX_{35}FEX_{38}X_{39}LQX_{42}YZ_2NPE$ VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7), $MGSWAEFKQRLAAIKTRLEALZ_1EAELAAFX_{30}X_{31}EIX_{34}AFX_{37}X_{38}ELX_{41}AYZ_2NPEVEALX_{52}$ $X_{53}EAX_{56}AIX_{59}X_{60}ELX_{63}AYRHN$ (SEQ ID NO:8), $MGSWX_5EFX_8X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALZ_1EAELAAFEKEIAAFESELQAYZ_2NPEVEX_{50}LRX_{53}$ $X_{54}AAX_{57}IRX_{60}X_{61}LQAYRHN$ (SEQ ID NO:9), $MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALZ_1EAELAAFX_{30}X_{31}EIX_{34}AFX_{37}X_{38}ELX_{41}AYZ_2NPEV$ $EX_{50}LRX_{53}X_{54}AAX_{57}IRX_{60}X_{61}LQAYRHN$ (SEQ ID NO:10) and $MGSWX_5EFX_8$ $X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALZ_1EAELAX_{28}FEX_{31}X_{32}IAX_{35}FEX_{38}X_{39}LQX_{42}YZ_2NPEVEALX_{52}X_{53}E$ $AX_{56}AIX_{59}X_{60}ELX_{63}AYRHN$ (SEQ ID NO:11), and combinations thereof, wherein the amino acid sequence is not SEQ ID NO:1. In several ~~embodiment~~examples, the X positions of the sequences (e.g., "X_n") can comprise a natural or non-natural amino acid; wherein each X_n is the same or different natural or non-natural amino acid; and/or wherein Z_1 and/or Z_2 is 2 to 30 natural or non-natural amino acids. In several ~~embodiment~~examples, the polypeptide agent is attached to the solid support by covalent bonding,

by non-covalent association, or combinations thereof. In several ~~embodiment~~examples, the solid support comprises one or more of a bead, glass slide, chip, gelatin, or agarose.

[0049] Further provided for protein purification, in several ~~embodiment~~examples, is a composition comprising a solid support coupled to a polypeptide agent having an amino acid sequence comprising a sequence selected from the group consisting of MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALGGSEAELAX$_{30}$FEX$_{33}$X$_{34}$IAX$_{37}$FEX$_{40}$X$_{41}$LQX$_{44}$YKG KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGKGNPEVE ALX$_{57}$X$_{58}$EAX$_{61}$AIX$_{64}$X$_{65}$ELX$_{68}$AYRHN (SEQ ID NO:3), MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX$_5$ $_5$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:4), MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALGGSEAELAAFX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGK GNPEVEX$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:5), MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAX$_{30}$FEX$_{33}$X$_{34}$IAX$_{37}$FEX$_{40}$X$_{41}$LQX$_{44}$YKG KGNPEVEALX$_{57}$X$_{58}$EAX$_{61}$AIX$_{64}$X$_{65}$ELX$_{68}$AYRHN (SEQ ID NO:6), MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALZ$_1$EAELAX$_{28}$FEX$_{31}$X$_{32}$IAX$_{35}$FEX$_{38}$X$_{39}$LQX$_{42}$YZ$_2$NPE VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7), MGSWAEFKQRLAAIKTRLEALZ$_1$EAELAAFX$_{30}$X$_{31}$EIX$_{34}$AFX$_{37}$X$_{38}$ELX$_{41}$AYZ$_2$NPEVEALX$_{52}$ X$_{53}$EAX$_{56}$AIX$_{59}$X$_{60}$ELX$_{63}$AYRHN (SEQ ID NO:8), MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALZ$_1$EAELAAFEKEIAAFESELQAYZ$_2$NPEVEX$_{50}$LRX$_{53}$ X$_{54}$AAX$_{57}$IRX$_{60}$X$_{61}$LQAYRHN (SEQ ID NO:9), MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALZ$_1$EAELAAFX$_{30}$X$_{31}$EIX$_{34}$AFX$_{37}$X$_{38}$ELX$_{41}$AYZ$_2$NPEV EX$_{50}$LRX$_{53}$X$_{54}$AAX$_{57}$IRX$_{60}$X$_{61}$LQAYRHN (SEQ ID NO:10) and MGSWX$_5$EFX$_8$ X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALZ$_1$EAELAX$_{28}$FEX$_{31}$X$_{32}$IAX$_{35}$FEX$_{38}$X$_{39}$LQX$_{42}$YZ$_2$NPEVEALX$_{52}$X$_{53}$E AX$_{56}$AIX$_{59}$X$_{60}$ELX$_{63}$AYRHN (SEQ ID NO:11), and combinations thereof, wherein the amino acid sequence is not SEQ ID NO:1. In several ~~embodiment~~examples, X_n is a natural or non-natural amino acid; wherein each X_n is the same or different natural or non-natural amino acid; and/or Z_1 and/or Z_2 is 2 to 30 natural or non-natural amino acids. In several ~~embodiment~~examples, the polypeptide agent has an amino acid sequence that differs from SEQ ID NO:1 by an amino acid substitution at one or more residues.

[0050] Depending on the ~~embodiment~~example, the amino acid substitution at one or more residues may comprise a conservative substitution or may comprise a non-conservative substitution. Combinations of conservative and non-conservative substitutions may also be used, in certain ~~embodiment~~examples. In several ~~embodiment~~examples, the amino acid substitution at one or more residues comprises a substitution at a solvent accessible residue. In several ~~embodiment~~examples, the amino acid substitution at one or more residues comprises a substitution at a solvent inaccessible residue. Some ~~embodiment~~examples employ substitutions at both solvent accessible and inaccessible residues. In several ~~embodiment~~examples, the polypeptide agent has an amino acid sequence that differs from SEQ ID NO:1 by an amino acid deletion at one or more residues. Depending on the ~~embodiment~~examples, the solid support may comprise one or more of a bead, glass slide, chip, gelatin, or agarose.

[0051] In several ~~embodiment~~examples, the polypeptides disclosed herein can be used in protein analytics, such as function as detectable agents or tags. As such, there is provided herein, in several ~~embodiment~~examples, a composition comprising a polypeptide agent conjugated to a detectable agent and/or tag, wherein the polypeptide agent has an amino acid sequence comprising a sequence selected from the group consisting of: MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALGGSEAELAX$_{30}$FEX$_{33}$X$_{34}$IAX$_{37}$FEX$_{40}$X$_{41}$LQX$_{44}$YKG KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGKGNPEVE ALX$_{57}$X$_{58}$EAX$_{61}$AIX$_{64}$X$_{65}$ELX$_{68}$AYRHN (SEQ ID NO:3), MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX$_5$ $_5$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:4), MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALGGSEAELAAFX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGK GNPEVEX$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:5), MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAX$_{30}$FEX$_{33}$X$_{34}$IAX$_{37}$FEX$_{40}$X$_{41}$LQX$_{44}$YKG KGNPEVEALX$_{57}$X$_{58}$EAX$_{61}$AIX$_{64}$X$_{65}$ELX$_{68}$AYRHN (SEQ ID NO:6), MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALZ$_1$EAELAX$_{28}$FEX$_{31}$X$_{32}$IAX$_{35}$FEX$_{38}$X$_{39}$LQX$_{42}$YZ$_2$NPE VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7), MGSWAEFKQRLAAIKTRLEALZ$_1$EAELAAFX$_{30}$X$_{31}$EIX$_{34}$AFX$_{37}$X$_{38}$ELX$_{41}$AYZ$_2$NPEVEALX$_{52}$ X$_{53}$EAX$_{56}$AIX$_{59}$X$_{60}$ELX$_{63}$AYRHN (SEQ ID NO:8), MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALZ$_1$EAELAAFEKEIAAFESELQAYZ$_2$NPEVEX$_{50}$LRX$_{53}$

$X_{54}AAX_{57}IRX_{60}X_{61}LQAYRHN$ (SEQ ID NO:9), $MGSWX_5X_6FKX_9X_{10}LAX_{13}IKX_{16}X_{17}LEALZ_1EAELAAFX_{30}X_{31}EIX_{34}AFX_{37}X_{38}ELX_{41}AYZ_2NPEV$ $EX_{50}LRX_{53}X_{54}AAX_{57}IRX_{60}X_{61}LQAYRHN$ (SEQ ID NO:10) and $MGSWX_5EFX_8$ $X_9RLX_{12}AIX_{15}X_{16}RLX_{19}ALZ_1EAELAX_{28}FEX_{31}X_{32}IAX_{35}FEX_{38}X_{39}LQX_{42}YZ_2NPEVEALX_{52}X_{53}E$ $AX_{56}AIX_{59}X_{60}ELX_{63}AYRHN$ (SEQ ID NO:11), and combinations thereof, wherein the amino acid sequence is not SEQ ID NO:1. In several ~~embodiment~~examples, X_n is a natural or non-natural amino acid; wherein each X_n is the same or different natural or non-natural amino acid; and/or wherein Z_1 and/or Z_2 is 2 to 30 natural or non-natural amino acids.

[0052] In several ~~embodiment~~examples, the detectable agent comprises a chromogen. In several ~~embodiment~~examples, the detectable agent comprises a fluorescent dye. In several ~~embodiment~~examples, the detectable agent comprises a radionuclide. In such ~~embodiment~~examples, the detectable agent is quantifiable.

[0053] In several ~~embodiment~~examples of the composition, the polypeptide agent is conjugated to a chromatography bead, resin, glass slide, chip, gelatin, or agarose. In several ~~embodiment~~examples, the tag comprises polyhistidyl tag, a myc tag, or a FLAG tag. Combinations of tags may also be used in several ~~embodiment~~examples. In several ~~embodiment~~examples, the polypeptide agent is conjugated to a detectable agent or tag by covalent binding. In several ~~embodiment~~examples of the composition, the polypeptide agent is a fusion protein. In several ~~embodiment~~examples, the polypeptide agent is multimeric.

[0054] De novo binding domain (DBD) containing polypeptides (DBDpp) that specifically bind targets of interest are provided, as are nucleic acids encoding the provided DBDpp, vectors containing the nucleic acids and host cells containing the nucleic acids and vectors. DBDpp libraries, methods for producing and screening such libraries and the DBDpp identified from such libraries and screens are also provided. DBDpp such as DBDpp fusion proteins, are also provided as are methods of making and using the DBDpp. Such uses include, but are not limited to, affinity purification, and diagnostic and therapeutic applications.

[0055] In one ~~embodiment~~example, a DBDpp is provided whose amino acid sequence differs (e.g., due to amino acid modifications) from that of a reference scaffold having the sequence of SEQ ID NO:1. The reference scaffold is a variant of a non-naturally occurring and targetless (e.g., to Applicant's knowledge, no target is presently known) antiparallel three helical bundle reference polypeptide originally engineered as an exercise in protein folding (see, Walsh et al., PNAS

96:5486–5491 (1999) incorporated by reference herein in its entirety). It has been discovered, and is disclosed herein in several ~~embodiment~~examples, that polypeptides containing modifications of the targetless reference scaffold having the amino acid sequence of SEQ ID NO:1 are able to specifically bind targets of interest. While not wishing to be bound by theory, it is believed that in designing the DBD, the structural constraints of surface-exposed residues (that can be modified) confer the ability of the surface exposed residues to specifically bind a target of interest.

[0056] In one ~~embodiment~~example, a DBDpp agent comprises a polypeptide whose amino acid sequence shows homology with SEQ ID NO:1 but differs from SEQ ID NO:1 by modification of one or more amino acids. According to several ~~embodiment~~examples, the target-binding agents (e.g., the DBDpp) provided herein specifically bind to a target of interest (such as a marker associated with cancer or a tumor, such as CD123, CD137, PD-L1, CD19, CD22, NY-ESO, MAGE A3, as non-limiting ~~embodiment~~examples). In several ~~embodiment~~examples, a provided target-binding agent (e.g., a DBDpp) comprises a total of 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 amino acid residues that have been modified as compared to SEQ ID NO:1; and wherein the agent specifically binds a target of interest. In another ~~embodiment~~example, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 of the modified amino acid residues are substitutions. In another ~~embodiment~~example, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, or 5 to 50 of the modified amino acid residues are conservative substitutions. In another ~~embodiment~~example, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, or 5 to 50 of the modified amino acid residues are non-conservative substitutions. In a further ~~embodiment~~example, 5 to 15, 5 to 20, 5 to 25, 5 to 30, 5 to 35, 5 to 40, or 5 to 45 of the amino acid residue modifications are conservative substitutions and 5 to 15, 5 to 20, 5 to 25, 5 to 30, 5 to 35, 5 to 40, or 5 to 45 of the amino acid residue modifications are non-conservative substitutions. In additional ~~embodiment~~examples, 1 to 25, 1 to 30, 1 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 of the substitutions are at amino acid residues of SEQ ID NO:1 selected from the group consisting of: M1, G2, S3, W4, A5, E6, K8, Q9, R10, A12, A13, K15, T16, R17, E19, A20, L21, G22, G23, S24, E25, A26, E27, A29, A30, E32, K33, E34, A36, A37, E39, S40, E41, Q43, A44, Y45, K46, G47, K48, G49, N50, P51, E52, E54, A55, R57, K58, E59, A61, A62, R64, D65, E66, Q68, A69, Y70, R71, H72, and N73. In a further ~~embodiment~~example, 1 to 20, 1 to 30, or 1 to 40 of the substitutions are at amino acid residues of SEQ ID NO:1 selected from the group consisting of: G2, S3, W4, A5, E6, K8, Q9, R10, A12, A13, K15, T16, R17, E19, A20, A29, A30, E32, K33, E34, A36, A37, E39, S40, E41, Q43, A44, E52, E54,

A55, R57, K58, E59, A61, A62, R64, D65, E66, Q68, A69, and Y70. In an optional further ~~embodiment~~example, the DBDpp optionally further comprises an amino acid sequence wherein 1 to 5, 1 to 10, 1 to 15, 5 to 10 or 5 to 15 of the residues corresponding to the solvent inaccessible residues of the amino acid sequence of SEQ ID NO:1 are substituted and wherein the DBDpp specifically binds a target of interest. In several ~~embodiment~~examples, the DBDpp comprise an amino acid sequence wherein about 1 to about 5, about 1 to about 10, about 1 to about 15, about 5 to about 10, about 5 to about 15 (or more) of the residues that correspond to the solvent accessible or the solvent inaccessible residues of the amino acid sequence of SEQ ID NO:1 are substituted. In several ~~embodiment~~examples, the substitution of both accessible and inaccessible residues confers a greater degree of target specificity as compared to substitution of only accessible or only inaccessible residues. In a further optional ~~embodiment~~example, the substituted residues corresponding to a solvent inaccessible residue of SEQ ID NO:1 are selected from the group consisting of: F7, L11, I14, L18, L28, F31, I35, F38, L42, V53, L56, A60, I63, and L67, and Y70. In an additional ~~embodiment~~example, L21 and Y45 are also included in the group of substituted, solvent inaccessible residues. In an additional ~~embodiment~~example, the DBDpp is a fusion protein (e.g., the DBDpp is fused, conjugated, or otherwise associated with another molecule, directly or indirectly, such as a therapeutic or diagnostic agent). In one ~~embodiment~~example, the DBDpp is attached to a solid support. In a further ~~embodiment~~example, the solid support is selected from the group consisting of: a bead, a glass slide, a chip, a gelatin, and an agarose. In an additional ~~embodiment~~example, the DBDpp specifically binds a target of interest selected from the group consisting of: a nucleic acid, an oligosaccharide, a peptide, a protein, a cell surface antigen, and a small organic molecule. In a further ~~embodiment~~example, the DBDpp specifically binds a protein selected from the group consisting of: an immunoglobulin, an enzyme, a hormone, a serum protein, a cell surface protein, a therapeutic protein, a tumor-specific antigen (TSA), a cancer-specific antigen (CSA), and a protein containing a peptide tag. In another ~~embodiment~~example, the DBDpp specifically binds a target disclosed herein. Nucleic acids encoding the DBDpp and vectors containing the nucleic acids are also provided. Host cells (including viral particles) containing the nucleic acids and vectors are also provided. In some ~~embodiment~~examples, the host cell displays the DBDpp on its surface. In additional ~~embodiment~~examples, the host cell is a prokaryote or a eukaryote that display the DBDpp on its surface. In a further ~~embodiment~~example, the host cell is a phage that displays the DBDpp on its surface. In a further ~~embodiment~~example, the host cell

is a human immune cell that expresses a DBDpp fusion protein on its surface. Libraries comprising a plurality of DBDpp are also provided.

[0057] In one ~~embodiment~~example, a DBDpp comprises an amino acid sequence selected from the group consisting of: (a) MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAAFEKEIA AFESELQAYKGKGNPEVEX$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:4), wherein X$_5$, X$_8$, X$_9$, X$_{12}$, X$_{15}$, X$_{16}$, X$_{19}$, X$_{55}$, X$_{58}$, X$_{59}$, X$_{62}$, X$_{65}$, and/or X$_{66}$, is a natural and/or non-natural amino acid residue; (b) MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALGGSEAELAX$_{30}$ FEX$_{33}$X$_{34}$IAX$_{37}$FEX$_{40}$X$_{41}$LQX$_{44}$YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$, X$_{30}$, X$_{33}$, X$_{34}$, X$_{37}$, X$_{40}$, X$_{41}$, and/or X$_{44}$, is a natural and/or non-natural amino acid residue; (c) MGSWAEFKQRLAAIKTRLEALGGSEAELAA FX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGKGNPEVEALX$_{57}$X$_{58}$EAX$_{61}$AIX$_{64}$X$_{65}$ELX$_{68}$AYRHN (SEQ ID NO:3), wherein X$_{32}$, X$_{33}$, X$_{36}$, X$_{39}$, X$_{40}$, X$_{43}$, X$_{57}$, X$_{58}$, X$_{61}$, X$_{64}$, X$_{65}$, and/or X$_{68}$, is a natural and/or non-natural amino acid residue, and; (d) MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$ X$_{17}$LEALGGSEAELAAFX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGKGNPEVEX$_{55}$LRX$_{58}$X$_{59}$AA X$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:5), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$, X$_{32}$, X$_{33}$, X$_{36}$, X$_{39}$, X$_{40}$, X$_{43}$, X$_{55}$, X$_{58}$, X$_{59}$, X$_{62}$, X$_{65}$, and/or X$_{66}$, is a natural and/or non-natural amino acid residue; and (e) MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAX$_{30}$FEX$_{33}$X$_{34}$IAX$_{37}$ FEX$_{40}$X$_{41}$LQX$_{44}$YKGKGNPEVEALX$_{57}$X$_{58}$EAX$_{61}$AIX$_{64}$X$_{65}$ELX$_{68}$AYRHN (SEQ ID NO:6), wherein X$_5$, X$_8$, X$_9$, X$_{12}$, X$_{15}$, X$_{16}$, X$_{19}$, X$_{30}$, X$_{33}$, X$_{34}$, X$_{37}$, X$_{40}$, X$_{41}$, X$_{44}$, X$_{57}$, X$_{58}$, X$_{61}$, X$_{64}$, X$_{65}$, and/or X$_{68}$, is a natural and/or non-natural amino acid residue; and wherein the DBDpp specifically binds a target of interest. In several ~~embodiment~~examples, a DBDpp comprises, consists of, or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5. In an additional ~~embodiment~~example, X$_n$ is a natural amino acid residue. In a further ~~embodiment~~example, X$_n$ is a natural amino acid residue other than cysteine or proline. In still additional ~~embodiment~~examples, X$_n$ is a deletion of an amino acid (e.g., optionally a null position in the sequence). In an additional ~~embodiment~~example, the DBDpp is a fusion protein. In another ~~embodiment~~example, the DBDpp specifically binds a target of interest selected from the group consisting of: a nucleic acid, an oligosaccharide, a peptide, a protein, a cell surface antigen, and a small organic molecule. In a further ~~embodiment~~example, the DBDpp specifically binds a protein selected from the group consisting of: an immunoglobulin, an enzyme, a hormone, a serum protein, a cell surface protein, a therapeutic

protein, a TSA, a CSA, and a protein containing a peptide tag. In a further ~~embodiment~~example, the DBDpp specifically binds a target disclosed herein. In an additional ~~embodiment~~example, a library containing a plurality of DBDpp is provided. Nucleic acids encoding the DBDpp and vectors containing the nucleic acids are also provided. Host cells (including viral particles) containing the nucleic acids and vectors are also provided. In some ~~embodiment~~examples, the host cell is a prokaryote or a eukaryote that display the DBDpp on its surface. In some ~~embodiment~~examples, the host cell displays the DBDpp on its surface. In a further ~~embodiment~~example, the host cell is a phage that displays the DBDpp on its surface. In a further ~~embodiment~~example, the host cell is a human immune cell (e.g., B-cell, T-cell, killer T-cell, helper T-cell, regulatory T-cell, antigen presenting cell, natural killer cell, and the like) that expresses one or more DBDpp fusion proteins on its surface. In one ~~embodiment~~example, the DBDpp is attached to a solid support. In a further ~~embodiment~~example, the solid support is selected from the group consisting of: a bead, a glass slide, other glass or plastic-based materials (e.g., a filter or filter device), a filtration material (e.g., glass fiber, steel wool, polyethersulfone, etc.), a chip, a gelatin, and an agarose, and combinations thereof.

[0058] Also provided is an isolated DBDpp that comprises an amino acid sequence selected from the group consisting of: (a) MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALZ$_1$EAELAAF EKEIAAFESELQAYZ$_2$NPEVEX$_{50}$LRX$_{53}$X$_{54}$AAX$_{57}$IRX$_{60}$X$_{61}$LQAYRHN (SEQ ID NO:9), wherein X$_5$, X$_8$, X$_9$, X$_{12}$, X$_{15}$, X$_{16}$, X$_{19}$, X$_{50}$, X$_{53}$, X$_{54}$, X$_{57}$, X$_{60}$, and/or X$_{61}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and/or Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; (b) MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALZ$_1$EAELAX$_{28}$FEX$_{31}$X$_{32}$IAX$_{35}$FEX$_{38}$ X$_{39}$LQX$_{42}$YZ$_2$NPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:7), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$, X$_{28}$, X$_{31}$, X$_{32}$, X$_{35}$, X$_{38}$, X$_{39}$, and/or X$_{42}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and/or Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; (c) MGSWAEFKQRLAAIKTRLEALZ$_1$EAELAAFX$_{30}$X$_{31}$EIX$_{34}$AFX$_{37}$X$_{38}$ELX$_{41}$AYZ$_2$NPEVEALX$_{52}$ X$_{53}$EAX$_{56}$AIX$_{59}$X$_{60}$ELX$_{63}$AYRHN (SEQ ID NO:8), wherein X$_{30}$, X$_{31}$, X$_{34}$, X$_{37}$, X$_{38}$, X$_{41}$, X$_{52}$, X$_{53}$, X$_{56}$, X$_{59}$, X$_{60}$, and/or X$_{63}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and/or Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; (d) MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$ LEALZ$_1$EAELAAFX$_{30}$X$_{31}$EIX$_{34}$AFX$_{37}$X$_{38}$ELX$_{41}$AYZ$_2$NPEVEX$_{50}$LRX$_{53}$X$_{54}$AAX$_{57}$IRX$_{60}$X$_{61}$ LQAYRHN (SEQ ID NO:10), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$, X$_{30}$, X$_{31}$, X$_{34}$, X$_{37}$, X$_{38}$, X$_{41}$, X$_{50}$, X$_{53}$, X$_{54}$, X$_{57}$, X$_{60}$, and/or X$_{61}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and/or

Z_2 is 2 to 30 natural and/or non-natural amino acid residues; and (e) MGSWX_5EFX_8 X_9RLX_{12}AI$X_{15}X_{16}$RLX_{19}ALZ_1EAELAX_{28}FE$X_{31}X_{32}$IAX_{35}FE$X_{38}X_{39}$LQX_{42}YZ_2NPEVEAL $X_{52}X_{53}$EAX_{56}AI$X_{59}X_{60}$ELX_{63}AYRHN (SEQ ID NO:11), wherein X_5, X_8, X_9, X_{12}, X_{15}, X_{16}, X_{19}, X_{28}, X_{31}, X_{32}, X_{35}, X_{38}, X_{39}, X_{42}, X_{52}, X_{53}, X_{56}, X_{59}, X_{60}, and/or X_{63}, is a natural and/or non-natural amino acid residue, and Z_1 and/or Z_2 is 2 to 30 natural and/or non-natural amino acid residues; and wherein the DBDpp specifically binds a target of interest. In several ~~embodiment~~examples, a DBDpp comprises, consists of, or consists essentially of an amino acid sequence selected from the group consisting of SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, and SEQ ID NO:10, and SEQ ID NO:11. In an additional ~~embodiment~~example, X_n is a natural amino acid residue. In a further ~~embodiment~~example, X_n is a natural amino acid residue other than cysteine or proline. In still additional ~~embodiment~~examples, X_n is a deletion of an amino acid (e.g., optionally a null position in the sequence). In still additional ~~embodiment~~examples, Z_1 and/or Z_2 are deletions of amino acids (e.g., optionally null positions in the sequence). In an additional ~~embodiment~~example, the DBDpp is a fusion protein. In another ~~embodiment~~example, the DBDpp specifically binds a target of interest selected from the group consisting of: a nucleic acid, an oligosaccharide, a peptide, a protein, a cell surface antigen, and a small organic molecule. In a further ~~embodiment~~example, the DBDpp specifically binds a protein selected from the group consisting of: an immunoglobulin, an enzyme, a hormone, a serum protein, a cell surface protein, a therapeutic protein, a TSA, a CSA, and a protein containing a peptide tag. In a further ~~embodiment~~example, the DBDpp specifically binds a target disclosed herein. In an additional ~~embodiment~~example, a library containing a plurality of DBDpp is provided. Nucleic acids encoding the DBDpp and vectors containing the nucleic acids are also provided. Host cells, including viral particles, containing the nucleic acids are also provided. In some ~~embodiment~~examples, the host cell displays the DBDpp on its surface. In a further ~~embodiment~~example, the host cell is a phage that displays the DBDpp on its surface. In additional ~~embodiment~~examples, the host cell is a prokaryote or a eukaryote that display the DBDpp on its surface. In a further ~~embodiment~~example, the host cell is a human immune cell that expresses a DBDpp fusion protein on its surface. In one ~~embodiment~~example, the DBDpp is attached to a solid support. In a further ~~embodiment~~example, the solid support is selected from the group consisting of: a bead, a glass slide, a chip, a gelatin, and an agarose.

[0059] Nucleic acids encoding a DBDpp such as a DBDpp fusion protein are also provided. Additionally provided are vectors containing nucleic acids encoding DBDpp (e.g., DBDpp

fusion proteins) and host cells containing the nucleic acids and vectors. In some ~~embodiment~~examples, the host cell is a viral particle, or a bacterial, yeast, fungal, or plant cell. In a particular ~~embodiment~~example, the host cell is a mammalian cell. In another ~~embodiment~~example, the mammalian cell is an immune cell. In a further ~~embodiment~~example, the host cell is a human immune cell. In some ~~embodiment~~examples, the host cell displays the DBDpp as a fusion protein on the cell surface. In a further ~~embodiment~~example, the host cell is a human immune cell that displays a DBDpp on the cell surface. Additionally provided herein are vector libraries comprising nucleic acids encoding a plurality of DBDpp.

[0060] Also provided is a library containing a plurality of DBDpp. In one ~~embodiment~~example, the DBDpp library comprises a plurality of DBDpp containing a different amino acid sequences and that comprise the amino acid sequence of SEQ ID NO:1 wherein a total of 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 amino acid residues (including any number between those listed) have been modified; and wherein the DBDpp specifically binds a target of interest. In another ~~embodiment~~example, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 (including any number between those listed) of the modified amino acid residues are substitutions. In another ~~embodiment~~example, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, or 5 to 50 (including any number between those listed) of the modified amino acid residues are conservative substitutions. In another ~~embodiment~~example, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, or 5 to 50 (including any number between those listed) of the modified amino acid residues are non-conservative substitutions. In a further ~~embodiment~~example, 5 to 15, 5 to 20, 5 to 25, 5 to 30, 5 to 35, 5 to 40, or 5 to 45 (including any number between those listed) of the amino acid residue modifications are conservative substitutions and 5 to 15, 5 to 20, 5 to 25, 5 to 30, 5 to 35, 5 to 40, or 5 to 45(including any number between those listed) of the amino acid residue modifications are non-conservative substitutions. In additional ~~embodiment~~examples, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 (including any number between those listed) of the substitutions are at one or more amino acid residues of SEQ ID NO:1 selected from the group consisting of: M1, G2, S3, W4, A5, E6, K8, Q9, R10, A12, A13, K15, T16, R17, E19, A20, L21, G22, G23, S24, E25, A26, E27, A29, A30, E32, K33, E34, A36, A37, E39, S40, E41, Q43, A44, Y45, K46, G47, K48, G49, N50, P51, E52, E54, A55, R57, K58, E59, A61, A62, R64, D65, E66, Q68, A69, Y70, R71, H72, and N73. In a further ~~embodiment~~example, 1 to 20, 1 to 30, or 1 to 40 (including any number between those listed) of the substitutions are at one or more of amino acid residues of SEQ ID NO:1 selected from the group consisting of: G2, S3, W4, A5, E6,

K8, Q9, R10, A12, A13, K15, T16, R17, E19, A20, A29, A30, E32, K33, E34, A36, A37, E39, S40, E41, Q43, A44, E52, E54, A55, R57, K58, E59, A61, A62, R64, D65, E66, Q68, A69, and Y70. In another ~~embodiment~~example, the library comprises at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 (including any range between those numbers listed, such as 2-10, 5-25, 50-100, 250-1000, etc.) different DBDpp that specifically binding different targets (or DBDpp that have differential specificity for a given target). In a further ~~embodiment~~example, the different targets bound by DBDpp in the library are selected from the group consisting of: a nucleic acid, an oligosaccharide, a peptide, a protein, a cell surface antigen, and a small organic molecule. In a further ~~embodiment~~example, the library comprises at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 (including any range between those numbers listed, such as 2-10, 5-25, 50-100, 250-1000, etc.) different DBDpp that specifically bind a protein target selected from the group consisting of: an immunoglobulin, an enzyme, a hormone, a serum protein, a cell surface protein, a therapeutic protein, a TSA, a CSA, and a protein containing a peptide tag. In a further ~~embodiment~~example, the library comprises at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 (including any range between those numbers listed, such as 2-10, 5-25, 50-100, 250-1000, etc.) different DBDpp that specifically bind a target disclosed herein. In an additional ~~embodiment~~example, the library is a vector library or a host cell library. In an additional ~~embodiment~~example, the vector library is a library of host cells. In another ~~embodiment~~example, the host cell library comprises a plurality of host cells that display the DBDpp on their surface. In a further ~~embodiment~~example, the host cells are phage that display the DBDpp on their surface. In some ~~embodiment~~examples, the vector library comprises: (a) nucleic acids encoding 3 DBDpp that specifically bind to different targets; (b) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same target; (c) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same epitope of a target; (d) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to different epitopes of a target; (e) nucleic acids encoding 3 DBDpp having different sequences that compete for binding to the same target; or (f) 3 different nucleic acid sequences encoding the same DBDpp sequence. Host cells containing the vectors are also provided.

[0061] Also provided is a vector library comprising a plurality of different nucleic acid sequences encoding DBDpp, that comprise the amino acid sequence of SEQ ID NO:1 wherein a total of 1 to 5, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 amino acid residues have been modified (or any number in between those listed); and wherein the DBDpp specifically

binds a target of interest. In another ~~embodiment~~example, 1 to 5, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 of the modified amino acid residues (or any number in between those listed) encoded by the nucleic acids sequences are substitutions. In another ~~embodiment~~example, 1 to 5, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, or 5 to 50 of the modified amino acid residues (or any number in between those listed) are conservative substitutions. In another ~~embodiment~~example, 1 to 5, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, or 5 to 50 of the encoded modified amino acid residues (or any number in between those listed) are non-conservative substitutions. In a further ~~embodiment~~example, 1 to 5, 5 to 15, 5 to 20, 5 to 25, 5 to 30, 5 to 35, 5 to 40, or 5 to 45 of the encoded amino acid residue modifications(or any number in between those listed) are conservative substitutions and 1 to 5, 5 to 15, 5 to 20, 5 to 25, 5 to 30, 5 to 35, 5 to 40, or 5 to 45 of the encoded amino acid residue modifications (or any number in between those listed) are non-conservative substitutions. In additional ~~embodiment~~examples, 1 to 5, 5 to 25, 5 to 30, 5 to 35, 5 to 40, 5 to 45, 5 to 50, 5 to 55, or 5 to 60 of the encoded substitutions (or any number in between those listed) are at amino acid residues of SEQ ID NO:1 selected from the group consisting of one or more of: M1, G2, S3, W4, A5, E6, K8, Q9, R10, A12, A13, K15, T16, R17, E19, A20, L21, G22, G23, S24, E25, A26, E27, A29, A30, E32, K33, E34, A36, A37, E39, S40, E41, Q43, A44, Y45, K46, G47, K48, G49, N50, P51, E52, E54, A55, R57, K58, E59, A61, A62, R64, D65, E66, Q68, A69, Y70, R71, H72, and N73. In a further ~~embodiment~~example, 1 to 20, 1 to 30, or 1 to 40 of the encoded substitutions (or any number in between those listed) are at amino acid residues of SEQ ID NO:1 selected from the group consisting of one or more of: G2, S3, W4, A5, E6, K8, Q9, R10, A12, A13, K15, T16, R17, E19, A20, A29, A30, E32, K33, E34, A36, A37, E39, S40, E41, Q43, A44, E52, E54, A55, R57, K58, E59, A61, A62, R64, D65, E66, Q68, A69, and Y70. In a further ~~embodiment~~example, the nucleic acids optionally encode a DBDpp that further comprises an amino acid sequence wherein 1 to 5, 5 to 15, 5 to 20, 5 to 25, 5 to 30, 5 to 35, 5 to 40, or 5 to 45 (or any number in between those listed) of the residues corresponding to the solvent inaccessible residues of the amino acid sequence of SEQ ID NO:1 are substituted and wherein the DBDpp specifically binds a target of interest. In another ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind different targets (or have varied affinity for the same target). In a further ~~embodiment~~example, the different targets bound by DBDpp in the library are selected from the group consisting of: a nucleic acid, an oligosaccharide, a peptide, a protein, a cell surface antigen, and a small organic molecule. In a further ~~embodiment~~example, the

library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind a protein target selected from the group consisting of: an immunoglobulin, an enzyme, a hormone, a serum protein, a cell surface protein, a therapeutic protein, a TSA, a CSA, and a protein containing a peptide tag. In a further ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind a target disclosed herein. In an additional ~~embodiment~~example, the vector library is contained in host cells (e.g., viral particles). In another ~~embodiment~~example, the library comprises a plurality of host cells that display the DBDpp on their surface. In a further ~~embodiment~~example, the host cells are phage that display the DBDpp on their surface. In some ~~embodiment~~examples, the vector library comprises: (a) nucleic acids encoding 3 DBDpp that specifically bind to different targets; (b) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same target; (c) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same epitope of a target; (d) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to different epitopes of a target; (e) nucleic acids encoding 3 DBDpp having different sequences that compete for binding to the same target; or (f) 3 different nucleic acid sequences encoding the same DBDpp sequence. Host cells containing the vectors are also provided.

[0062] In one ~~embodiment~~example, a vector library comprises a plurality of different nucleic acids encoding DBDpp, wherein the encoded DBDpp comprises an amino acid sequence selected from the group consisting of: (a) MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELA AFEKEIAAFESELQAYKGKGNPEVEX$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:4), wherein X$_5$, X$_8$, X$_9$, X$_{12}$, X$_{15}$, X$_{16}$, X$_{19}$, X$_{55}$, X$_{5}$8, X$_{59}$, X$_{62}$, X$_{65}$, and/or X$_{66}$, is a natural and/or non-natural amino acid residue; (b) MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALGG SEAELAX$_{30}$FEX$_{33}$X$_{34}$IAX$_{37}$FEX$_{40}$X$_{41}$LQX$_{44}$YKGKGNPEVEALRKEAAAIRDELQAYR HN (SEQ ID NO:2), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$, X$_{30}$, X$_{33}$, X$_{34}$, X$_{37}$, X$_{40}$, X$_{41}$, and/or X$_{44}$, is a natural and/or non-natural amino acid residue; (c) MGSWAEFKQRLAAIKTRLEA LGGSEAELAAFX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGKGNPEVEALX$_{57}$X$_{58}$EAX$_{61}$AIX$_{64}$X$_{65}$ ELX$_{68}$AYRHN (SEQ ID NO:3), wherein X$_{32}$, X$_{33}$, X$_{36}$, X$_{39}$, X$_{40}$, X$_{43}$, X$_{57}$, X$_{58}$, X$_{61}$, X$_{64}$, X$_{65}$, and/or X$_{68}$, is a natural and/or non-natural amino acid residue, and; (d) MGSWX$_5$X$_6$FKX$_9$ X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALGGSEAELAAFX$_{32}$X$_{33}$EIX$_{36}$AFX$_{39}$X$_{40}$ELX$_{43}$AYKGKGNPEVE X$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:5), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$,

X_{32}, X_{33}, X_{36}, X_{39}, X_{40}, X_{43}, X_{55}, X_{58}, X_{59}, X_{62}, X_{65}, and/or X_{66}, is a natural and/or non-natural amino acid residue; and (e) MGSWX_5EFX_8X_9RLX_{12}AI$X_{15}X_{16}$RLX_{19}ALGGSEAELAX_{30} FE$X_{33}X_{34}$IAX_{37}FE$X_{40}X_{41}$LQX_{44}YKGKGNPEVEALX$_{57}X_{58}$EAX_{61}AI$X_{64}X_{65}$ELX_{68}AYRHN (SEQ ID NO:6), wherein X_5, X_8, X_9, X_{12}, X_{15}, X_{16}, X_{19}, X_{30}, X_{33}, X_{34}, X_{37}, X_{40}, X_{41}, X_{44}, X_{57}, X_{58}, X_{61}, X_{64}, X_{65}, and/or X_{68}, is a natural and/or non-natural amino acid residue; and wherein the DBDpp specifically binds a target of interest. In an additional ~~embodiment~~example, X_n is a natural amino acid residue. In a further ~~embodiment~~example, X_n is a natural amino acid residue other than cysteine or proline. In still additional ~~embodiment~~examples, X_n is a deletion of an amino acid (e.g., optionally a null position in the sequence). In an additional ~~embodiment~~example, a plurality of the vectors in the library encode a DBDpp fusion protein. In another ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind different targets. In a further ~~embodiment~~example, the different targets bound by DBDpp encoded by the nucleic acids in the library are selected from the group consisting of: a nucleic acid, an oligosaccharide, a peptide, a protein, a cell surface antigen, and a small organic molecule. In a further ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind a protein target selected from the group consisting of: an immunoglobulin, an enzyme, a hormone, a serum protein, a cell surface protein, a therapeutic protein, a TSA, a CSA, and a protein containing a peptide tag. In a further ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind a target disclosed herein. In an additional ~~embodiment~~example, a plurality of the vectors of the vector library are contained in host cells (e.g., viral particles such as phage), E. coli, yeast, and mammalian cells. In another ~~embodiment~~example, the host cells display DBDpp on their surface. In a further ~~embodiment~~example, the host cells are phage that display DBDpp on their surface. In some ~~embodiment~~examples, the vector library comprises: (a) nucleic acids encoding 3 DBDpp that specifically bind to different targets; (b) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same target; (c) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same epitope of a target; (d) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to different epitopes of a target; (e) nucleic acids encoding 3 DBDpp having different sequences that compete for binding to the same target; or (f) 3

different nucleic acid sequences encoding the same DBDpp sequence. Host cells containing the vectors are also provided.

[0063] In one ~~embodiment~~example, a vector library comprises a plurality of nucleic acids encoding DBDpp comprising an amino acid sequence selected from the group consisting of: (a) MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALZ$_1$EAELAAFEKEIAAFESELQAYZ$_2$NPEVE X$_{50}$LRX$_{53}$X$_{54}$AAX$_{57}$IRX$_{60}$X$_{61}$LQAYRHN (SEQ ID NO:9), wherein X$_5$, X$_8$, X$_9$, X$_{12}$, X$_{15}$, X$_{16}$, X$_{19}$, X$_{50}$, X$_{53}$, X$_{54}$, X$_{57}$, X$_{60}$, and/or X$_{61}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; (b) MGSWX$_5$X$_6$FKX$_9$X$_{10}$LA X$_{13}$IKX$_{16}$X$_{17}$LEALZ$_1$EAELAX$_{28}$FEX$_{31}$X$_{32}$IAX$_{35}$FEX$_{38}$X$_{39}$LQX$_{42}$YZ$_2$NPEVEALRKEAAA IRDELQAYRHN (SEQ ID NO:7), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$, X$_{28}$, X$_{31}$, X$_{32}$, X$_{35}$, X$_{38}$, X$_{39}$, and/or X$_{42}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; (c) MGSWAEFKQRLAAIKTRLEALZ$_1$EAEL AAFX$_{30}$X$_{31}$EIX$_{34}$AFX$_{37}$X$_{38}$ELX$_{41}$AYZ$_2$NPEVEALX$_{52}$X$_{53}$EAX$_{56}$AIX$_{59}$X$_{60}$ELX$_{63}$AYRHN (SEQ ID NO:8), wherein X$_{30}$, X$_{31}$, X$_{34}$, X$_{37}$, X$_{38}$, X$_{41}$, X$_{52}$, X$_{53}$, X$_{56}$, X$_{59}$, X$_{60}$, and/or X$_{63}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; (d) MGSWX$_5$X$_6$FKX$_9$X$_{10}$LAX$_{13}$IKX$_{16}$X$_{17}$LEALZ$_1$EAELAAFX$_{30}$ X$_{31}$EIX$_{34}$AFX$_{37}$X$_{38}$ELX$_{41}$AYZ$_2$NPEVEX$_{50}$LRX$_{53}$X$_{54}$AAX$_{57}$IRX$_{60}$X$_{61}$LQAYRHN (SEQ ID NO:10), wherein X$_5$, X$_6$, X$_9$, X$_{10}$, X$_{13}$, X$_{16}$, X$_{17}$, X$_{30}$, X$_{31}$, X$_{34}$, X$_{37}$, X$_{38}$, X$_{41}$, X$_{50}$, X$_{53}$, X$_{54}$, X$_{57}$, X$_{60}$, and/or X$_{61}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; and (e) MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RL X$_{19}$ALZ$_1$EAELAX$_{28}$FEX$_{31}$X$_{32}$IAX$_{35}$FEX$_{38}$X$_{39}$LQX$_{42}$YZ$_2$NPEVEALX$_{52}$X$_{53}$EAX$_{56}$AIX$_{59}$X$_{60}$ ELX$_{63}$AYRHN (SEQ ID NO:11), wherein X$_5$, X$_8$, X$_9$, X$_{12}$, X$_{15}$, X$_{16}$, X$_{19}$, X$_{28}$, X$_{31}$, X$_{32}$, X$_{35}$, X$_{38}$, X$_{39}$, X$_{42}$, X$_{52}$, X$_{53}$, X$_{56}$, X$_{59}$, X$_{60}$, and/or X$_{63}$, is a natural and/or non-natural amino acid residue, and Z$_1$ and Z$_2$ is 2 to 30 natural and/or non-natural amino acid residues; and wherein the DBDpp specifically binds a target of interest. In an additional ~~embodiment~~example, X$_n$ is a natural amino acid residue. In a further ~~embodiment~~example, X$_n$ is a natural amino acid residue other than cysteine or proline. In still additional ~~embodiment~~examples, X$_n$ is a deletion of an amino acid (e.g., optionally a null position in the sequence). In an additional ~~embodiment~~example, a plurality of the vectors in the library encode a DBDpp fusion protein. In another ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind different targets. In a

further ~~embodiment~~example, the different targets bound by DBDpp encoded by the nucleic acids in the library are selected from the group consisting of: a nucleic acid, an oligosaccharide, a peptide, a protein, a cell surface antigen, and a small organic molecule. In a further ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind a protein target selected from the group consisting of: an immunoglobulin, an enzyme, a hormone, a serum protein, a cell surface protein, a therapeutic protein, a TSA, a CSA, and a protein containing a peptide tag. In a further ~~embodiment~~example, the library comprises nucleic acids encoding at least 2, 3, 4, 5, 10, 25, 50, 75, 100, 250, 500, or 1000 different DBDpp that specifically bind a target disclosed herein. In an additional ~~embodiment~~example, a plurality of the vectors of the vector library are contained in host cells. [In another ~~embodiment~~example, the host cells (e.g., viral particles) display DBDpp on their surface. In a further ~~embodiment~~example, the host cells are phage that display DBDpp on their surface. In some ~~embodiment~~examples, the host cells are mammalian cells. In some ~~embodiment~~examples, the vector library comprises: (a) nucleic acids encoding 3 DBDpp that specifically bind to different targets; (b) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same target; (c) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to the same epitope of a target; (d) nucleic acids encoding 3 DBDpp having different sequences that specifically bind to different epitopes of a target; (e) nucleic acids encoding 3 DBDpp having different sequences that compete for binding to the same target; or (f) 3 different nucleic acid sequences encoding the same DBDpp sequence. Host cells containing the vectors are also provided.

[0064] The DBDpp according to several ~~embodiment~~examples provided herein possess activities that include but are not limited to target binding, the ability to bind, link, and/or otherwise associate with a target of interest (e.g., a purification target, a therapeutic target, a diagnostic target, a peptide tag, and a serum protein such as human serum albumin (HSA) or an immunoglobulin) in vitro or in vivo and the ability to serve as a reactive site for linking or associating proteins such as DBDpp fusion proteins with additional moieties (e.g., a solid support), and/or other modifications. The DBDpp provided herein can also possess additional desirable properties and/or functionalities useful in manufacturing, purification, formulation and biological, diagnostic, and therapeutic applications.

[0065] In some ~~embodiment~~examples, a DBDpp is used to bind, detect, quantitate, remove, and/or purify a target of interest in a sample containing the target.

[0066] One non-limiting ~~embodiment~~example provides a method for detecting a target of interest in a sample, comprising: (a) contacting the sample with a DBDpp that specifically binds the target, under conditions suitable for specific binding of the DBDpp to the target, to form a target/DBDpp complex, and (b) detecting the presence of the complex and/or captured target. In one ~~embodiment~~example, the DBDpp is immobilized on a solid support.

[0067] Also provided is a method for quantifying a target of interest in a sample containing the target, comprising: (a) contacting the sample with a DBDpp that specifically binds the target and that is immobilized on a solid support, under conditions suitable for specific binding of the DBDpp to the target, to form a target/DBDpp complex and (b) detecting the presence of the target/DBDpp complex and/or captured target, wherein quantitative detection of the product indicates, or is otherwise able to be correlated with, the quantity of the target in the sample.

[0068] Some ~~embodiment~~examples provide methods for purifying a target of interest from a sample containing the target that comprises: (a) contacting a sample containing a target of interest with a DBDpp that specifically binds the target, under conditions suitable for specific binding of the DBDpp to the target, and (b) recovering the bound target. In some ~~embodiment~~examples, the target is recovered by elution. In one ~~embodiment~~example, the DBDpp is immobilized on a solid support. In a further ~~embodiment~~example, the elution of the bound target is monitored by ultra violet light absorption, or other visualization or chemical-based detection technique. In some ~~embodiment~~examples, methods are provided to remove an undesired target of interest from a sample and wherein the bound undesired target is discarded directly or is eluted (or otherwise collected or separated) and then discarded.

[0069] An additional ~~embodiment~~example provides a method of screening a library of DBDpp for a DBDpp that specifically binds a target of interest, that comprises: (a) obtaining a plurality of host cells (e.g., viral particles, phage, bacteria, and/or mammalian cells) displaying a library of DBDpp on their surface; (b) contacting the plurality of host cells with a target of interest under conditions suitable for specific binding of the target to a DBDpp; and (c) determining the binding of the target to the DBDpp. In one ~~embodiment~~example, the host cells are phage that display the DBDpp on their surface.

[0070] Methods of using DBDpp in diagnostic and therapeutic applications are also provided, in several ~~embodiment~~examples. One ~~embodiment~~example provides a method of treating a disease or disorder comprising administering a therapeutically effective amount of a DBDpp (e.g., a DBDpp fusion protein) that specifically binds a therapeutic target of interest to a subject in need thereof. In

some ~~embodiment~~examples, the disease or disorder is cancer, a disease or disorder of the immune system, or an infection. Methods of treating a disease or disorder that comprises co-administering an additional therapeutic agent along with a DBDpp are also provided.

[0071] Additionally provided are methods for treating or preventing cancer comprising administering a DBDpp-CAR T lymphocyte to a patient (e.g., predisposed to or having a cancer) that expresses a tumor antigen on the surface of target cells, and wherein the DBDpp specifically binds the antigen.

[0072] Certain methods summarized above and set forth in further detail below describe certain actions taken by a practitioner; however, it should be understood that they can also include the instruction of those actions by another party. Thus, actions such as "administering a T cell comprising a target specific binding polypeptide-CAR" include "instructing the administration of a T cell comprising a target specific binding polypeptide-CAR."

[0072a] In one aspect, the present disclosure refers to a de novo binding domain polypeptide (DBDpp), comprising three anti-parallel alpha helices joined by linker peptides, wherein (a) the DBDpp is a synthetic peptide derived from modifications to the amino acid sequence of SEQ ID NO:1, wherein the modifications consist of 1 to 30 conservative or non-conservative amino acid substitutions selected from positions 1-6, 8-10, 12, 13, 15-17, 19, 20-27, 29, 30, 32-34, 36, 37, 39-41, 43-52, 54, 55, 57-59, 61, 62, 64-66, and 68-73 of SEQ ID NO: 1; (b) the DBDpp specifically binds a target of interest, wherein the specific binding of the DBDpp to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and (c) the DBDpp does not comprise the amino acid sequence of SEQ ID NO:50.

[0072b] In another aspect, the present disclosure refers to a fusion protein comprising a first and second DBDpp as disclosed herein wherein the first and second DBDpp exhibits binding specificity for a tumor target, optionally wherein the first and second DBDpp exhibits binding specificity for different tumor targets.

[0072c] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the DBDpp as disclosed herein, or the fusion protein as disclosed herein, and further comprising a pharmaceutically acceptable carrier.

[0072d] In another aspect, the present disclosure refers to a kit comprising the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0072e] In another aspect, the present disclosure refers to an isolated nucleic acid molecule encoding the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0072f] In another aspect, the present disclosure refers to a vector comprising the isolated nucleic acid molecule as disclosed herein and optionally further comprising a nucleotide sequence which regulates the expression of the DBDpp, or fusion protein encoded by the nucleic acid molecule.

[0072g] In another aspect, the present disclosure refers to a host cell comprising the nucleic acid molecule as disclosed herein.

[0072h] In another aspect, the present disclosure refers to a cell line comprising the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0072i] In another aspect, the present disclosure refers to a chimeric antigen receptor (CAR), wherein the CAR comprises (a) a targeting domain, (b) a transmembrane domain, and (c) an intracellular signaling domain, wherein the targeting domain comprises the DBDpp as disclosed herein, or the fusion protein as disclosed herein.

[0072j] In another aspect, the present disclosure refers to an isolated nucleic acid molecule comprising a sequence encoding the CAR as disclosed herein.

[0072k] In another aspect, the present disclosure refers to a cell comprising a nucleic acid molecule comprising a sequence encoding the CAR as disclosed herein, optionally wherein the cell is a T cell or a natural killer (NK) cell, and optionally wherein the cell exhibits an anti-tumor immunity when the DBDpp, or fusion protein binds to its corresponding tumor antigen.

[0072l] In another aspect, the present disclosure refers to use of an immune cell comprising the CAR as disclosed herein in the manufacture of a medicament for treating cancer

[0072m] In another aspect, the present disclosure refers to use of an immune cell comprising a chimeric antigen receptor (CAR) in the manufacture of a medicament for treating cancer, wherein the CAR comprises: (a) target binding domain comprising a de novo binding domain polypeptide (DBDpp) comprising three anti-parallel alpha helices joined by linker peptides, wherein, (i) the DBDpp is a synthetic peptide derived from modifications to the amino acid sequence of SEQ ID NO:1, wherein the modifications consist of 1 to 30 conservative or non-conservative amino acid substitutions selected from positions 1-6, 8-10, 12, 13, 15-17, 19, 20-27, 29, 30, 32-34, 36, 37, 39-41, 43-52, 54, 55, 57-59, 61, 62, 64-66, and 68-73 of SEQ ID NO: 1; (ii) the DBDpp

specifically binds a target of interest, wherein the specific binding of the DBDpp to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and (iii) the DBDpp does not comprise the amino acid sequence of SEQ ID NO:50, (b) a transmembrane domain selected from 41BB and CD28, and (c) an intracellular domain, wherein the intracellular domain comprises a signaling domain selected from an alpha, beta, or zeta chain of the T cell receptor.

[0072n] In another aspect, the present disclosure refers to virus-like particles (VLPs) comprising a fusion polypeptide comprising the DBDpp as disclosed herein wherein the VLPs are suitable for use as immunogens for antibody generation, wherein said antibodies are directed against the DBDpp.

BRIEF DESCRIPTION OF THE DRAWINGS

[0073] FIGS. 1A-B. Schematic depicting SEQ ID NO:1 derived homology model of DBDpp. Transverse view illustrating the helices and three faces of domain (FIG. 1A). Longitudinal view illustrating position of residue E19, N-terminus (NT) and C-terminus (CT) (FIG. 1B).

[0074] FIGS. 2A-J. Schematic representation of different homology models of DBDpp based off the reference scaffold of SEQ ID NO:1. The residues targeted for modification in the Face libraries (F1, F2, and F3) and Combined libraries (C1 and C2) of DBDpp are darkly shaded. Longitudinal and transverse perspective views of the F1 library are shown in FIG.2A and FIG. 2B respectively. Longitudinal and transverse perspective views of the F2 library are shown in FIG.2C and FIG. 2D respectively. Longitudinal and transverse perspective views of the F3 library are shown in FIG.2E and FIG. 2F respectively. Longitudinal and transverse perspective views of the C1 library are shown in FIG.2G and FIG.2H respectively. Longitudinal and transverse perspective views of the C2 library are shown in FIG.2I and FIG. 2J respectively. N-terminus (NT) and C-terminus (CT) for each model are indicated.

[0075] FIGS. 3A-D. FIG. 3A. Schematic depiction of phage display construct for use in accordance with several embodiments disclosed herein. FIG. 3B. Depicts a linear vector map for the

WHAT IS CLAIMED IS:

~~1. A polypeptide for binding a target of interest, the polypeptide comprising:~~

~~an amino acid sequence comprising:~~

~~MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAAFEKEIAAFESELQAYK GKGNPEVEX$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:4),~~

~~wherein the polypeptide is derived from modifications to the amino acid sequence of SEQ ID NO:1,~~

~~wherein the polypeptide specifically binds a target of interest, and~~

~~wherein the polypeptide the specific binding the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest.~~

~~2. The polypeptide of Claim 1, wherein the polypeptide does not contain SEQ ID NO:50.~~

~~3. The polypeptide of Claim 1, wherein the modifications to SEQ ID NO:1 comprise substitutions.~~

~~4. The polypeptide of Claim 3, wherein the substitutions are conservative substitutions.~~

~~5. The polypeptide of Claim 3, wherein the substitutions are non-conservative substitutions.~~

~~6. The polypeptide of Claim 3, wherein the substitutions do not include substitution of an amino acid of SEQ ID NO:1 with a cysteine.~~

~~7. The polypeptide of Claim 3, wherein the substitutions do not include substitution of an amino acid of SEQ ID NO:1 with a proline.~~

8. The polypeptide of Claim 1, wherein the target of interest specifically bound by the polypeptide is a cancer antigen.

9. The polypeptide of Claim 8, wherein the cancer antigen specifically bound by polypeptide is PD-L1.

10. The polypeptide of Claim 9, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO: 43, SEQ ID and NO:44.

11. The polypeptide of Claim 8, wherein the cancer antigen specifically bound by polypeptide is CD137 and wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO: 17, SEQ ID NO:18, and SEQ ID NO:19.

12. The polypeptide of Claim 8, wherein the cancer antigen specifically bound by polypeptide is CD123.

13. The polypeptide of Claim 12, wherein the polypeptide comprises an amino acid sequence selected from SEQ ID NOS: 92-127.

14. A method for transforming a reference polypeptide into a polypeptide having specific binding for a target of interest, the method comprising:

modifying a plurality of amino acid residues from a reference polypeptide to generate a plurality of candidate binding polypeptides;

wherein the reference polypeptide comprises a variant of a non-naturally occurring polypeptide and comprises three anti-parallel alpha helices joined by linker peptides,

wherein the amino acid residues to be modified are solvent accessible or solvent inaccessible,

wherein the modification comprises amino acid substitutions;

~~packaging the plurality of candidate binding polypeptides in a plurality of vectors to generate a candidate library; and~~

~~screening the candidate library for candidate binding polypeptides that exhibit specific binding to the target of interest.~~

~~15. The method of Claim 14, wherein the substitutions are conservative amino acid substitutions.~~

~~16. The method of Claim 14, wherein the substitutions are non-conservative amino acid substitutions.~~

~~17. A method according to any one of Claims 14-16, wherein the substitution does not comprise substitution in of a cysteine.~~

~~18. A method according to any one of Claims 14-17, wherein the substitution does not comprise substitution in of a proline.~~

~~19. A method according to any one of Claims 14-18, further comprising identifying potentially immunogenic amino acid residues in the candidate binding polypeptides and modifying at least one of the potentially immunogenic amino acid residues, wherein the modification comprises an amino acid substitution.~~

~~20.~~1. A de novo binding domain polypeptide (DBDpp), ~~wherein the DBDpp comprises~~ comprising three anti-parallel alpha helices ~~and is a variant of a synthetic polypeptide, wherein the DBDpp immunospecifically binds to a protein that is at least 95% identical to CD123~~ joined by linker peptides, wherein~~.~~

(a) the the DBDpp is a synthetic peptide derived from modifications to the amino acid sequence of SEQ ID NO:1, wherein the modifications consist of 1 to 30 conservative or non-conservative amino acid substitutions selected from positions 1-6, 8-10, 12, 13, 15-17, 19, 20-27, 29, 30, 32-34, 36, 37, 39-41, 43-52, 54, 55, 57-59, 61, 62, 64-66, and 68-73 of SEQ ID NO: 1;

(b) the DBDpp specifically binds a target of interest, wherein the specific binding of the DBDpp to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and

(c) the DBDpp does not comprise the amino acid sequence of SEQ ID NO:50.

~~21. The de novo binding domain polypeptide (DBDpp) of Claim 20, wherein the DBDpp has a dissociation constant (KD) between about 10^{-4}M and about 10^{-12}M.~~

~~22. A de novo binding domain polypeptide (DBDpp) according to Claim 20 or 21, wherein the protein to which the DBDpp immunospecifically binds comprises amino acids 19-305 of CD123 (SEQ ID NO: 187).~~

~~23. A de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-22, wherein the amino acid sequence comprises: MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAAFEKEIAAFESELQAYKGK GNPEVEX$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN **(SEQ ID NO:4)**, and wherein X$_n$ is a natural or non-natural amino acid.~~

~~24. A de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-22, wherein the DBDpp comprises an amino acid sequence at least 85% identical to the amino acid sequence of any one of SEQ ID NO:60 – SEQ ID NO: 136.~~

2. The de novo binding domain polypeptide (DBDpp) of claim 1, wherein the modifications consist of 1 to 30 conservative or non-conservative amino acid substitutions selected from positions 2-6, 8-10, 12, 13, 15-17, 19, 20, 29, 30, 32-34, 36, 37, 39-41, 43, 44, 52, 54, 55, 57-59, 61, 62, 64-66, 68, 69, and 70 of SEQ ID NO: 1.

3. The DBDpp of claim 1 or claim 2 comprising an amino acid sequence of MGSWX5X6FKX9X10LAX13IKX16X17LEALGGSEAELAX30FEX33X34IAX37FEX40 X41LQX44YKGKGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX32X33EIX36AFX39X40ELX43AYKG

KGNPEVEALX57X58EAX61AIX64X65ELX68AYRHN (SEQ ID NO:3), MGSWX5EFX8X9RLX12AIX15X16RLX19ALGGSEAELAAFEKEIAAFESELQAYKGK GNPEVEX55LRX58X59AAX62IRX65X66LQAYRHN (SEQ ID NO:4), MGSWX5X6FKX9X10LAX13IKX16X17LEALGGSEAELAAFX32X33EIX36AFX39X40 ELX43AYKGKGNPEVEX55LRX58X59AAX62IRX65X66LQAYRHN (SEQ ID NO:5), MGSWX5EFX8X9RLX12AIX15X16RLX19ALGGSEAELAX30FEX33X34IAX37FEX40 X41LQX44YKGKGNPEVEALX57X58EAX61AIX64X65ELX68AYRHN (SEQ ID NO:6), wherein Xn is a natural or non-natural amino acid.

4. The DBDpp of any one of claims 1 to 3 that specifically binds to a protein comprising amino acids 19-305 of CD123 (SEQ ID NO: 187) or a protein that is at least 95% identical to CD123, optionally wherein the DBDpp is characterized by one or more of

(a) the DBDpp binds to the protein comprising amino acids 19-305 of CD123 (SEQ ID NO: 187) or the protein that is at least 95% identical to CD123 with a dissociation constant (KD) between 10-4M and 10-12M,

(b) the DBDpp comprises an amino acid sequence of MGSWX5EFX8X9RLX12AIX15X16RLX19ALGGSEAELAAFEKEIAAFESEL QAYKGKGNPEVEX55LRX58X59AAX62IRX65X66LQAYRHN (SEQ ID NO:4), wherein Xn is a natural or non-natural amino acid,

(c) the DBDpp comprises an amino acid sequence of MGSWX5EFX8X9RLX12AIX15X16RLX19ALGGSEAELAAFEKEIAAFESEL QAYKGKGNPEVEX55LRX58X59AAX62IRX65X66LQAYRHN (SEQ ID NO:4), wherein Xn is a natural or non-natural amino acid, and wherein Xn is not cysteine or proline, and

(d) the DBDpp comprises an amino acid sequence at least 85% identical to the amino acid sequence of any one of SEQ ID NO:60 – SEQ ID NO: 136, and

(e) the DBDpp is capable of binding to a tumor.

~~25.~~5. A fusion protein comprising ~~the~~ a first and second ~~de novo binding domain polypeptide (~~DBDpp~~) of~~ according to any one of ~~Claims~~ claims ~~20-24~~1 to 4, wherein the first and second DBDpp exhibits ~~and further comprising one or more additional DBDpp~~

~~exhibiting~~ binding specificity for a tumor target, optionally wherein the first and second DBDpp exhibits binding specificity for different tumor targets.

~~26.~~6. ~~A de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-25,~~ The DBDpp of any one of claims 1 to 4, or the fusion protein of claim 5, wherein the DBDpp, or fusion protein is labeled, and optionally~~.~~

~~27. The de novo binding domain polypeptide (DBDpp) of Claim 26,~~ wherein the label is a biotin moiety or is selected from the group consisting of an enzymatic label, a fluorescent label, a luminescent label, and a bioluminescent label.

~~28. The de novo binding domain polypeptide (DBDpp) of Claim 26, wherein the label is a biotin moiety.~~

~~29.~~7. ~~A de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-28~~The DBDpp of any one of claims 1 to 4 and 6, or the fusion protein of claims 5 or claim 6, wherein target-binding polypeptide, the DBDpp or fusion protein is conjugated to a therapeutic or cytotoxic agent.

~~30.~~8. ~~A de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-29,~~A pharmaceutical composition comprising the DBDpp of any one of claims 1 to 4, 6 and 7, or the fusion protein of any one of claims 5 to 7, and further comprising a pharmaceutically acceptable carrier.

~~31.~~9. A kit comprising the DBDpp of any one of claims 1 to 4, 6 and 7, or the fusion protein of any one of claims 5 to 7~~the de novo binding domain polypeptide (DBDpp) of any one of Claims 20-30~~.

~~32.~~10. An isolated nucleic acid molecule encoding the DBDpp of any one of claims 1 to 4, or the fusion protein of claim 5~~the de novo binding domain polypeptide (DBDpp) of any one of Claims 20-30~~.

~~33.~~11. A vector comprising the isolated nucleic acid molecule of ~~Claim 32~~claim 10~~.~~

~~34. The vector of Claim 33~~, and optionally further comprising a nucleotide sequence which regulates the expression of the ~~de novo binding domain polypeptide (DBDpp)~~ DBDpp, or fusion protein encoded by the nucleic acid molecule.

~~35.~~12. A host cell comprising the nucleic acid molecule of ~~Claim 33~~claim 10 or the vector of claim 11.

~~36.~~13. A cell line comprising the DBDpp of any one of claims 1 to 4, or the fusion protein of claim 5 ~~engineered to express the de novo binding domain polypeptide (DBDpp) of any one of Claims 20-30~~.

~~37. A de novo binding domain polypeptide (DBDpp) that competes with the DBDpp according to any one of Claims 20-30 for binding to CD123~~.

~~38. A de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-30 that binds to a tumor.~~

~~39.~~14. A chimeric antigen receptor (CAR), wherein the CAR comprises (a) a targeting domain, (b) a transmembrane domain, and (c) an intracellular signaling domain, wherein the targeting domain comprises the DBDpp of any one of claims 1 to 4, or the fusion protein of claim 5 ~~a de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-30~~.

~~40.~~15. The ~~chimeric antigen receptor (CAR) of Claim 39~~CAR of claim 14, which is characterized by one or more of ~~wherein~~

(a) the intracellular signaling domain is selected from the group consisting of a human CD3 zeta domain, 41BB domain, a CD28 domain and any combination thereof,

(b) the DBDpp, or fusion protein binds to a tumor antigen associated with a hematologic malignancy or a solid tumor

(c) the DBDpp, or fusion protein binds to a tumor antigen selected from the group consisting of CD137, PD-L1, CD123, CTLA4, CD47, KIR, DR5, TIM3, PD1, EGFR, TCR, CD19, CD20, CD22, ROR 1, mesothelin, CD33/lL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof

(d) the intracellular signaling domain comprises a costimulatory signaling region, and.

41. A chimeric antigen receptor (CAR) according to any one of Claims 39 or 40, wherein (e) the intracellular signaling domain comprises a costimulatory signaling region comprises comprising the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-lBB, OX40, CD30, CD40, PD-1, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.

42. A chimeric antigen receptor (CAR), wherein the CAR comprises the fusion protein of claim 25.

43.16. An isolated nucleic acid molecule sequence comprising a sequence encoding a chimeric antigen receptor (CAR), wherein the CAR of claim 14 or claim 15comprises a de novo binding domain polypeptide (DBDpp) according to any one of Claims 20-30, a transmembrane domain, and an intracellular signaling domain.

44.17. A cell comprising a nucleic acid sequence molecule comprising a sequence encoding a chimeric antigen receptor (CAR)the CAR of claim 14 or 15, optionally wherein the cell is a T cell or a natural killer (NK) cell, and optionally wherein the cell exhibits an

anti-tumor immunity when the DBDpp, or fusion protein binds to its corresponding tumor antigen ~~wherein the CAR comprises an antigen binding domain derived from a de novo binding domain polypeptide (DBDpp), a transmembrane domain, and a signaling domain~~.

~~45. The cell of claim 44, wherein the de novo binding domain polypeptide (DBDpp) binds to a tumor antigen.~~

~~46. The cell of claim 45, wherein the tumor antigen is associated with a hematologic malignancy.~~

~~47. The cell of claim 45, wherein the tumor antigen is associated with a solid tumor.~~

~~48. The cell of claim 45, wherein the tumor antigen is selected from the group consisting of CD137, PD-L1, CD123, CTLA4, CD47, KIR, DR5, TIM3, PD1, EGFR, TCR, CD19, CD20, CD22, ROR-1, mesothelin, CD33/lL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO-1, MAGE-A3, and combinations thereof.~~

~~49. A cell according to any one of Claims 44-48, wherein the cell is a T cell or a natural killer (NK) cell~~

~~50. The cell of Claim 49, wherein the cell exhibits an anti-tumor immunity when the de novo binding domain polypeptide (DBDpp) binds to its corresponding tumor antigen.~~

~~51. An amino acid sequence comprising SEQ ID 4, wherein X_n is not cysteine or proline.~~

~~52. A mammalian cell that generates membrane-bound virus-like particles (VLPs), wherein the mammalian cell is engineered to express a fusion protein comprising a de novo binding domain polypeptide (DBDpp) fused to a chimeric antigen receptor (CAR), the fusion protein being expressed on the generated VLPs.~~

~~53. Virus-like particles (VLPs) produced by the mammalian cell of Claim 52, wherein the VLPs are suitable for use as immunogens for antibody generation, wherein said antibodies are directed against the de novo binding domain polypeptide (DBDpp).~~

~~54. A polypeptide that competes with the DBDpp according to any one of Claims 20-30 for binding to CD123.~~

~~55. A method of treating a subject having cancer, the method comprising:~~

~~administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises:~~

~~a target binding domain, wherein the target binding domain comprises a polypeptide having an amino acid sequence comprising:~~

~~MGSWX$_5$EFX$_8$X$_9$RLX$_{12}$AIX$_{15}$X$_{16}$RLX$_{19}$ALGGSEAELAAFEKEIAAFESEL QAYKGKGNPEVEX$_{55}$LRX$_{58}$X$_{59}$AAX$_{62}$IRX$_{65}$X$_{66}$LQAYRHN (SEQ ID NO:4),~~

~~wherein the polypeptide is derived from modifications to the amino acid sequence of SEQ ID NO:1,~~

~~wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and~~

~~wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest,~~

~~a transmembrane domain, and~~

~~an intracellular domain, wherein the intracellular domain comprises a signaling domain,~~

~~wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and~~

~~wherein the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytoxic effects on the cancer cell, thereby treating the cancer.~~

~~56. The method of Claim 55, wherein the immune cell is a T cell.~~

~~57. The method of Claim 55, wherein the immune cell is a NK cell.~~

~~58. The method of Claim 55, wherein the administration is intravenous.~~

~~59. The method of Claim 55, wherein transmembrane domain comprises 41BB or CD28, wherein the cytoplasmic domain comprises an alpha, beta, or zeta chain of the T cell receptor, and wherein the immune cell is a T cell.~~

~~60. The method of Claim 59, wherein, upon binding the target of interest, the T cell is stimulated to initiate intracellular signaling, produce cytokines, and degranulate, leading to the cytotoxic effects on the cancer cell.~~

~~61. The method of Claim 60, wherein the T cell proliferates in response to binding the target of interest and does not exhibit a phenotype associated with T cell exhaustion.~~

~~62. The method of Claim 55, wherein transmembrane domain comprises CD28, wherein the cytoplasmic domain comprises a zeta chain of the T cell receptor, and wherein the immune cell is a NK cell.~~

~~63. The method of Claim 55, wherein the target of interest expressed by the cancer cell is one or more of tumor antigen is selected from the group consisting of CD137, PD-L1, CD123, CTLA4, CD47, KIR, DR5, TIM3, PD1, EGFR, TCR, CD19, CD20, CD22, ROR-1, mesothelin, CD33/IL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO-1, MAGE-A3, and combinations thereof.~~

~~64. The method of Claim 55, wherein the chimeric antigen receptor (CAR) further comprises a second polypeptide having an amino acid of SEQ ID NO:4, the polypeptide able to specifically bind a second target of interest expressed by a cancer cell, and wherein the second polypeptide's specific binding the second target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the second target of interest.~~

65. The method of Claim 55, wherein the modifications do not include substituting a cysteine or a proline into SEQ ID NO: 1.

66. A method of treating a subject having cancer, the method comprising,

administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises:

a target binding domain, wherein the target binding domain comprises a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6,

wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6,

wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and

wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest,

a transmembrane domain, and

an intracellular domain, wherein the intracellular domain comprises a signaling domain,

wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and

wherein the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell, thereby treating the cancer.

67. The method of Claim 66, wherein the immune cell is a T cell.

68. The method of Claim 66, wherein the immune cell is a NK cell.

69. The method of Claim 66, wherein transmembrane domain comprises 41BB or CD28, wherein the cytoplasmic domain comprises an alpha, beta, or zeta chain of the T cell receptor, and wherein the immune cell is a T cell.

70. The method of Claim 69, wherein, upon binding the target of interest, the T cell is stimulated to initiate intracellular signaling, produce cytokines, proliferates and degranulates, leading to the cytotoxic effects on the cancer cell, and wherein the T cells do not exhibit a phenotype associated with T cell exhaustion.

71. A method of treating a subject having cancer, the method comprising,

intravenously administering to the subject an immune cell comprising a chimeric antigen receptor (CAR) expressed on a T cell, wherein the CAR comprises:

a target binding domain comprising a polypeptide having an amino acid sequence comprising, the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6,

wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6,

wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and

wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest,

a transmembrane domain selected from 41BB and CD28, and

an intracellular domain, wherein the intracellular domain comprises a signaling domain selected from an alpha, beta, or zeta chain of the T cell receptor,

wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and

wherein the binding of the target of interest induces the T cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell.

~~72. The method of Claim 71, wherein, cytotoxic effects result from degranulation of the T cells.~~

~~73. The method of Claim 71, wherein after activation, the T cells do not exhibit a phenotype associated with T cell exhaustion.~~

~~74. The method of Claim 71, wherein the chimeric antigen receptor (CAR) further comprises a second target binding domain comprising a second polypeptide having a different target than the target binding domain.~~

~~75.~~18. Use of an ~~T~~ immune cell comprising ~~a chimeric antigen receptor (CAR)~~ the CAR of claim 14 or claim 15 in the manufacture of a medicament for ~~the treatment of~~treating cancer~~, wherein the CAR comprises:~~

~~a target binding domain comprising a polypeptide having an amino acid sequence comprising, the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6,~~

~~wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6,~~

~~wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and~~

~~wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest,~~

~~a transmembrane domain selected from 41BB and CD28, and~~

~~an intracellular domain, wherein the intracellular domain comprises a signaling domain selected from an alpha, beta, or zeta chain of the T cell receptor,~~

~~wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and~~

~~wherein the binding of the target of interest induces the T cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell~~.

~~76.~~19. Use of an immune cell comprising a chimeric antigen receptor (CAR) in the manufacture of a medicament for ~~the treatment of~~ treating cancer, wherein the CAR comprises:

(a) a target binding domain comprising a de novo binding domain polypeptide (DBDpp) comprising three anti-parallel alpha helices joined by linker peptides, wherein ~~a polypeptide having an amino acid sequence comprising, the polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6~~, (i) the DBDpp is a synthetic peptide derived from modifications to the amino acid sequence of SEQ ID NO:1, wherein the modifications consist of 1 to 30 conservative or non-conservative amino acid substitutions selected from positions 1-6, 8-10, 12, 13, 15-17, 19, 20-27, 29, 30, 32-34, 36, 37, 39-41, 43-52, 54, 55, 57-59, 61, 62, 64-66, and 68-73 of SEQ ID NO: 1; (ii) the DBDpp specifically binds a target of interest, wherein the specific binding of the DBDpp to the target of interest is greater than the binding of a reference polypeptide comprising the amino acid sequence of SEQ ID NO:1 to the target of interest; and (iii) the DBDpp does not comprise the amino acid sequence of SEQ ID NO:50,

~~wherein no cysteine or proline residues are substituted into any of SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6,~~

~~wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and~~

~~wherein the polypeptide's specific binding to the target of interest is greater than binding of a polypeptide according to SEQ ID NO:1 to the target of interest,~~

(b) a transmembrane domain selected from 41BB and CD28, and

(c) an intracellular domain, wherein the intracellular domain comprises a signaling domain selected from an alpha, beta, or zeta chain of the T cell receptor~~,~~

~~wherein, upon administration to a subject having cancer, the target binding domain specifically binds to the target of interest expressed by a cancer cell, and~~

~~wherein the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytotoxic effects on the cancer cell~~.

~~77.~~20. The use according to ~~Claim 76~~claim 19, wherein the immune cell~~s~~ is a T cell or a natural killer (NK) cell.

21. Virus-like particles (VLPs) comprising a fusion polypeptide comprising the DBDpp of any one of claims 1 to 4, wherein the VLPs are suitable for use as immunogens for antibody generation, wherein said antibodies are directed against the DBDpp.