

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 (DARPin)s 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 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 examples. For example, in several 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 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 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 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 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] In one aspect, there is provided a polypeptide for binding a target of interest, the polypeptide comprising an amino acid sequence comprising: MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKKGKNPEVEX₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (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 specific binding of the polypeptide to the target of interest is greater than the binding of a polypeptide according to SEQ ID NO:1 to the target of interest, and optionally wherein the polypeptide does not contain SEQ ID NO:50 and wherein the modifications to SEQ ID NO:1 comprise conservative or non-conservative substitutions and do not include a substitution with a cysteine or a proline. Several examples of the disclosure 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 examples, there is provided a binding agent that binds with a high degree of specificity to a target of interest. In several examples the binding agent is a non-antibody agent. In several examples, the binding agent is a polypeptide. In several 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 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 examples, there is provided a polypeptide for binding a target of interest, the polypeptide comprising an amino acid sequence comprising MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKKGKNPEVEX₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (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 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 examples, the polypeptide does not contain the sequence of SEQ ID NO:50.

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

used. In some examples, the substitutions do not include substitution with a cysteine (e.g., no cysteines are added to the sequence). In some examples, wherein the substitutions do not include substitution with a proline (e.g., no prolines are added to the sequence). In some 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 examples, the target of interest specifically bound by the polypeptide is a cancer antigen. In some examples, the cancer antigen specifically bound by polypeptide is PD-L1. In several such 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 examples, the cancer antigen specifically bound by polypeptide is CD137. In some such 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 examples, the cancer antigen specifically bound by polypeptide is CD123. In some such examples, the polypeptide comprises or consists essentially of an amino acid sequence selected from SEQ ID NOS: 92-127. In some examples, a combination of cancer antigens is targeted, for example by coupling or otherwise combining various target-binding polypeptides. In some examples, two, three, four or more different cancer antigens are targeted. In some 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 examples is a method for transforming a reference polypeptide into a polypeptide having specific binding for a target of interest. In one aspect, there is provided 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, and wherein the modification comprises one or more conservative or non-conservative amino acid substitutions and does not include a substitution with a cysteine or a proline; 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; and optionally further comprising identifying potentially immunogenic amino acid residues in a candidate binding polypeptide and modifying at least one of the potentially immunogenic amino acid residues in the candidate polypeptide, wherein the modification comprises an amino acid substitution. In some examples, 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 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 examples the amino acid residues to be modified are solvent accessible or solvent inaccessible

amino acids. In several examples, a greater degree of solvent accessible amino acids are modified, while in some examples a greater degree of solvent inaccessible amino acids are modified. In some 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 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 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 examples, the modification to reduce immunogenicity comprises an amino acid substitution (e.g., conservative and/or non-conservative substitutions).

[0013] In one aspect, there is provided a de novo binding domain polypeptide (DBDpp), wherein the DBDpp comprises three anti-parallel alpha helices and is a variant of a synthetic polypeptide, wherein the DBDpp immunospecifically 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 has a dissociation constant (KD) between about 10^{-4} M and about 10^{-12} M, optionally wherein, the amino acid sequence comprises MGSWX5EFX8X9RLX12AIX15X16RLX19ALGGSEAEELAAFEKEIAAFESLQAYKGKGNPEVEX55LRX58X59AAX62IR X65X66LQAYRHN (SEQ ID NO:4), and wherein Xn is a natural or non-natural amino acid, and optionally, wherein Xn is not cysteine or proline, and optionally 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, and optionally wherein the DBDpp binds to a tumor. In several 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 examples, the DBDpp has a dissociation constant (KD) between about 10^{-4} M and about 10^{-12} M. In some 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 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (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 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 examples, the target-binding agent (e.g., a polypeptide with specificity for a target of interest) is labeled. Depending on the 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 examples, the label is a biotin moiety. In several examples, a streptavidin moiety can be used. In some examples, a His-tag, FLAG-tag or other tag is used. In some examples, the label is luciferase, green fluorescent protein, red fluorescent protein, or other similar agent.

[0015] In one aspect, there is provided a fusion protein comprising the de novo binding domain polypeptide (DBDpp) as disclosed herein and further comprising one or more additional DBDpp exhibiting binding specificity for a tumor target. In several 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 example, the target-binding agent may optionally comprise a pharmaceutically acceptable carrier.

[0016] In several 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.). In one aspect, there is provided a kit comprising the de novo binding domain polypeptide (DBDpp) as disclosed herein.

[0017] In one aspect, there is provided an isolated nucleic acid molecule encoding the de novo binding domain polypeptide (DBDpp) as disclosed herein. In another aspect, there is provided a vector comprising the isolated nucleic acid molecule as disclosed herein, and optionally further comprising a nucleotide sequence which regulates the expression of the de novo binding domain polypeptide (DBDpp) encoded by the nucleic acid molecule. In a further aspect, there is provided a host cell comprising the nucleic acid molecule as disclosed herein or the vector as disclosed herein. In a further aspect, there is provided a cell line engineered to express the de novo binding domain polypeptide (DBDpp) as disclosed herein. Several examples also provide for isolated nucleic acid molecules encoding the any of the target-binding polypeptides disclosed herein. Still additional examples provide for a vector (e.g., a plasmid, viral vector, or non-viral vector) containing the isolated nucleic acid molecule. Several such 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 examples, the vector further comprises an additional nucleotide sequence which regulates the expression of the polypeptide encoded by the nucleic acid molecule. In several examples, the additional nucleic acid sequence is an inducible promoter.

[0018] Further provided for in several examples are host cells that comprise the nucleic acid molecules encoding the any of the target-binding polypeptides disclosed herein. In several examples such examples, the host cell (e.g., a cell line) is engineered to express the target-binding polypeptides disclosed herein. In some examples, the expression of the target-binding polypeptides by the host cells allows production and isolation of the target-binding polypeptides. In some 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 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 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 example, may be a suspension tumor or a solid tumor.

[0021] In one aspect, there is provided a chimeric antigen receptor (CAR), wherein the CAR comprises a targeting domain, a transmembrane domain, and an intracellular signaling domain, wherein the targeting domain comprises a de novo binding domain polypeptide (DBDpp) as disclosed herein, optionally wherein 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. In another aspect, there is provided a chimeric antigen receptor (CAR), wherein the CAR comprises the fusion protein as disclosed herein. Several 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 examples, the targeting domain is made up of, at least in part, a target-binding polypeptide as disclosed herein. In several 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 example, the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, 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 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. In one aspect, there is provided an isolated nucleic acid sequence comprising a sequence encoding a chimeric antigen

receptor (CAR), wherein the CAR comprises a de novo binding domain polypeptide (DBDpp) as disclosed herein, a transmembrane domain, and an intracellular signaling domain.

[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 one aspect, there is provided a cell comprising a nucleic acid sequence comprising a sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain derived from a de novo binding domain polypeptide (DBDpp), a transmembrane domain, and a signaling domain, optionally wherein the de novo binding domain polypeptide (DBDpp) binds to a tumor antigen associated with a hematologic malignancy or a solid tumor, and optionally 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/IL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof. In several examples, the polypeptide binds specifically to a tumor antigen (and thus functions to deliver the cell expressing the CAR to the tumor. In several examples, the tumor antigen is associated with a hematologic malignancy. In additional examples, tumor antigen is associated with a solid tumor. Both solid and hematologic tumors can be simultaneously targeted in some examples. In several 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/IL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof. Depending on the example, the cell expressing the CAR can be a T cell or a natural killer (NK) cell. In several 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 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 one aspect is 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). In another aspect, there is provided virus-like particles (VLPs) produced by the mammalian cell as disclosed herein, 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). Depending on the examples, the VLPs produced by the mammalian cells are suitable for use as immunogens for antibody generation. In some such 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). In one aspect, there is provided use of a T cell comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment of cancer, wherein the CAR comprises: a target binding domain comprising 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 the amino acid sequence contains no cysteine or proline residues, wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and wherein 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, 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. There are also provided, in several 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: MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKGKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (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 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 example, the immune cell can be a T cell. In some 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 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 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 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 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 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 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/IL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof.

[0030] In several 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 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 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] In one aspect, there is provided use of an immune cell comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament 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, and optionally wherein the immune cells is a T cell or a natural killer (NK) cell. There are also provided, in several 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 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 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 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 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 examples, the cytotoxic effects result from degranulation of the T cells. Advantageously, in several 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 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 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 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 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 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 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESELQAYKKGKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (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 examples the method further comprises dissociating the composition from the target of interest and recovering the target of interest. In several examples, the target of interest can be eluted from the composition, thereby purifying (wholly or partially) the target of interest.

[0036] Depending on the 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 examples. In several examples, the polypeptide agent is coupled to the solid support through non-covalent association, while in other examples, the polypeptide agent is coupled to the solid support through covalent bonding. Depending on the example, the supports, and the target of interest, combinations of covalent and non-covalent association can also be used.

[0037] In several 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 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 example.

[0038] In some 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 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 examples, allow for production of the polypeptide in larger scale for use in protein purification.

[0040] Also provided for in several 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 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 examples, the solid support comprises one or more of a bead, a glass slide, a chip, a gelatin, or an agarose. In several 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 examples.

[0042] In some examples, the portion of the sample that is not bound to the composition is discarded. In some 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 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 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 examples, the polypeptide of the composition further comprises a tag, such as a peptide tag. In several examples, the peptide tag comprises a hexahistidine moiety or a FLAG tag. Depending on the 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 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 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: MGSW₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVE

ALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESSELQAYKKGKNPEVEX₅
₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKKGK
 GNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG
 KGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPE
 VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),
 MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂
 X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),
 MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESSELQAYZ₂NPEVEX₅₀LRX₅₃
 X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEV
 EX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) and MGSWX₅EFX₈
 X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPEVEALX₅₂X₅₃E
 AX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (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 examples, Z₁ and/or Z₂ can comprise between about 2 to about 30 natural or non-natural amino acids.

[0047] In several 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 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 examples. Additionally, in several examples, the amino acid substitution at one or more residues comprises a substitution at a solvent accessible residue. In some examples, the amino acid substitution at one or more residues comprises a substitution at a solvent inaccessible residue. In some examples, substitutions (whether

conservative or non-conservative) can optionally be made at both solvent accessible and solvent inaccessible residues. In several 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 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: MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAE₃₀LAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), MGSWAEFKQRLAAIKTRLEALGGSEAE₃₂LAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVE ALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3), MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAE₃₀LAAFEKEIAAFESELQAYKGKGNPEVEX₅ LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4), MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAE₃₂LAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGK GNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5), MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAE₃₀LAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG KGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6), MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPE VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7), MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂ X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8), MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESELQAYZ₂NPEVEX₅₀LRX₅₃ X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9), MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEV EX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) and MGSWX₅EFX₈ X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPEVEALX₅₂X₅₃E AX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), and combinations thereof, wherein the amino acid sequence is not SEQ ID NO:1. In several 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 Z₁ and/or Z₂ is 2 to 30 natural or non-natural amino acids. In several examples, the polypeptide agent is attached to the solid support by covalent bonding,

by non-covalent association, or combinations thereof. In several 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 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

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG
KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),

MGSWAEFKQRLAAIKTRLEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVE
ALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),

MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIAAFESSELQAYKGKGNPEVEX₅
₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGK
GNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),

MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG
KGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPE
VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),

MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂
X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),

MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESSELQAYZ₂NPEVEX₅₀LRX₅₃
X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEV
EX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) and MGSWX₅EFX₈
X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPEVEALX₅₂X₅₃E
AX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), and combinations thereof, wherein the amino acid
sequence is not SEQ ID NO:1. In several 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 Z₁ and/or Z₂ is 2
to 30 natural or non-natural amino acids. In several 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 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 examples. In several examples, the amino acid substitution at one or more residues comprises a substitution at a solvent accessible residue. In several examples, the amino acid substitution at one or more residues comprises a substitution at a solvent inaccessible residue. Some examples employ substitutions at both solvent accessible and inaccessible residues. In several 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 examples, the solid support may comprise one or more of a bead, glass slide, chip, gelatin, or agarose.

[0051] In several 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 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:

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAE₃₀LAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG
KGNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2),

MGSWAEFKQRLAAIKTRLEALGGSEAE₃₂LA₃₂AFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVE
ALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3),

MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAE₃₀LA₃₀FEKEIAAFES₃₀ELQAYKGKGNPEVEX₅
₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4),

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAE₃₂LA₃₂AFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGK
GNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5),

MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAE₃₀LAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKG
KGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6),

MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPE
VEALRKEAAAIRDELQAYRHN (SEQ ID NO:7),

MGSWAEFKQRLAAIKTRLEALZ₁EAELAA₃₀AFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂
X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8),

MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFES₃₀ELQAYZ₂NPEVEX₅₀LRX₅₃

X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9),
 MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEV
 EX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10) and MGSWX₅EFX₈
 X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPEVEALX₅₂X₅₃E
 AX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), and combinations thereof, wherein the amino acid
 sequence is not SEQ ID NO:1. In several 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 Z₁ and/or
 Z₂ is 2 to 30 natural or non-natural amino acids.

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

[0053] In several examples of the composition, the polypeptide agent is conjugated to a chromatography bead, resin, glass slide, chip, gelatin, or agarose. In several examples, the tag comprises polyhistidyl tag, a myc tag, or a FLAG tag. Combinations of tags may also be used in several examples. In several examples, the polypeptide agent is conjugated to a detectable agent or tag by covalent binding. In several examples of the composition, the polypeptide agent is a fusion protein. In several 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 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 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 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 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 examples). In several 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 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 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 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 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 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 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 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 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 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 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 example, L21 and Y45 are also included in the group of substituted, solvent inaccessible residues. In an additional 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 example, the DBDpp is attached to a solid support. In a further 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 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 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 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 examples, the host cell displays the DBDpp on its surface. In additional examples, the host cell is a prokaryote or a eukaryote that display the DBDpp on its surface. In a further example, the host cell is a phage that displays the DBDpp on its surface. In a further 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 example, a DBDpp comprises an amino acid sequence selected from the group consisting of: (a) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIA AFESSELQAYKKGKNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₅₅, X₅₈, X₅₉, X₆₂, X₆₅, and/or X₆₆, is a natural and/or non-natural amino acid residue; (b) MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKKGKNPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:2), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇, X₃₀, X₃₃, X₃₄, X₃₇, X₄₀, X₄₁, and/or X₄₄, is a natural and/or non-natural amino acid residue; (c) MGSWAEFKQRLAAIKTRLEALGGSEAELAA FX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKKGKNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:3), wherein X₃₂, X₃₃, X₃₆, X₃₉, X₄₀, X₄₃, X₅₇, X₅₈, X₆₁, X₆₄, X₆₅, and/or X₆₈, is a natural and/or non-natural amino acid residue, and; (d) MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELAAFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKKGKNPEVEX₅₅LRX₅₈X₅₉AA X₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇, X₃₂, X₃₃, X₃₆, X₃₉, X₄₀, X₄₃, X₅₅, X₅₈, X₅₉, X₆₂, X₆₅, and/or X₆₆, is a natural and/or non-natural amino acid residue; and (e) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKKGKNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₃₀, X₃₃, X₃₄, X₃₇, X₄₀, X₄₁, X₄₄, X₅₇, X₅₈, X₆₁, X₆₄, X₆₅, and/or X₆₈, is a natural and/or non-natural amino acid residue; and wherein the DBDpp specifically binds a target of interest. In several 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 example, X_n is a natural amino acid residue. In a further example, X_n is a natural amino acid residue other than cysteine or proline. In still additional examples, X_n is a deletion of an amino acid (e.g., optionally a null position in the sequence). In an additional example, the DBDpp is a fusion protein. In another 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 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 example, the DBDpp specifically binds a target disclosed herein. In an additional 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 examples, the host cell is a prokaryote or a eukaryote that display the DBDpp on its surface. In some examples, the host cell displays the DBDpp on its surface. In a further example, the host cell is a phage that displays the DBDpp on its surface. In a further 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 example, the DBDpp is attached to a solid support. In a further 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) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAF EKEIAAFESSELQAYZ₂NPEVEX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₅₀, X₅₃, X₅₄, X₅₇, X₆₀, and/or X₆₁, is a natural and/or non-natural amino acid residue, and Z₁ and/or Z₂ is 2 to 30 natural and/or non-natural amino acid residues; (b) MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂LAX₃₅FEX₃₈ X₃₉LQX₄₂YZ₂NPEVEALRKEAAAIRDELQAYRHN (SEQ ID NO:7), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇, X₂₈, X₃₁, X₃₂, X₃₅, X₃₈, X₃₉, and/or X₄₂, is a natural and/or non-natural amino acid residue, and Z₁ and/or Z₂ is 2 to 30 natural and/or non-natural amino acid residues; (c) MGSWAEFKQRLAAIKTRLEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂ X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8), wherein X₃₀, X₃₁, X₃₄, X₃₇, X₃₈, X₄₁, X₅₂, X₅₃, X₅₆, X₅₉, X₆₀, and/or X₆₃, is a natural and/or non-natural amino acid residue, and Z₁ and/or Z₂ is 2 to 30 natural and/or non-natural amino acid residues; (d) MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇ LEALZ₁EAELAAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁ LQAYRHN (SEQ ID NO:10), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇, X₃₀, X₃₁, X₃₄, X₃₇, X₃₈, X₄₁, X₅₀, X₅₃, X₅₄, X₅₇, X₆₀, and/or X₆₁, is a natural and/or non-natural amino acid residue, and Z₁ and/or

Z_2 is 2 to 30 natural and/or non-natural amino acid residues; and (e) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:11), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₂₈, X₃₁, X₃₂, X₃₅, X₃₈, X₃₉, X₄₂, X₅₂, X₅₃, X₅₆, X₅₉, X₆₀, and/or X₆₃, is a natural and/or non-natural amino acid residue, and Z₁ and/or Z₂ is 2 to 30 natural and/or non-natural amino acid residues; and wherein the DBDpp specifically binds a target of interest. In several 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 example, X_n is a natural amino acid residue. In a further example, X_n is a natural amino acid residue other than cysteine or proline. In still additional examples, X_n is a deletion of an amino acid (e.g., optionally a null position in the sequence). In still additional examples, Z₁ and/or Z₂ are deletions of amino acids (e.g., optionally null positions in the sequence). In an additional example, the DBDpp is a fusion protein. In another 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 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 example, the DBDpp specifically binds a target disclosed herein. In an additional 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 examples, the host cell displays the DBDpp on its surface. In a further example, the host cell is a phage that displays the DBDpp on its surface. In additional examples, the host cell is a prokaryote or a eukaryote that display the DBDpp on its surface. In a further example, the host cell is a human immune cell that expresses a DBDpp fusion protein on its surface. In one example, the DBDpp is attached to a solid support. In a further 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 examples, the host cell is a viral particle, or a bacterial, yeast, fungal, or plant cell. In a particular example, the host cell is a mammalian cell. In another example, the mammalian cell is an immune cell. In a further example, the host cell is a human immune cell. In some examples, the host cell displays the DBDpp as a fusion protein on the cell surface. In a further 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 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 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 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 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 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 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 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 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 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 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 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 example, the library is a vector library or a host cell library. In an additional example, the vector library is a library of host cells. In another example, the host cell library comprises a plurality of host cells that display the DBDpp on their surface. In a further example, the host cells are phage that display the DBDpp on their surface. In some 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 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 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 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 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 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 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 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 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 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 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 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 example, the vector library is contained in host cells (e.g., viral particles). In another example, the library comprises a plurality of host cells that display the DBDpp on their surface. In a further example, the host cells are phage that display the DBDpp on their surface. In some 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 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) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELA AFEKEIAAFESSELQAYKGKGNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:4), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₅₅, X₅₈, X₅₉, X₆₂, X₆₅, and/or X₆₆, is a natural and/or non-natural amino acid residue; (b) MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALGG SEAELAX₃₀FEX₃₃X₃₄IAX₃₇FEX₄₀X₄₁LQX₄₄YKGKGNPEVEALRKEAAAIRDELQAYR HN (SEQ ID NO:2), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇, X₃₀, X₃₃, X₃₄, X₃₇, X₄₀, X₄₁, and/or X₄₄, is a natural and/or non-natural amino acid residue; (c) MGSWAEFKQRLAAIKTRLEA LGGSEAELA AFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ ELX₆₈AYRHN (SEQ ID NO:3), wherein X₃₂, X₃₃, X₃₆, X₃₉, X₄₀, X₄₃, X₅₇, X₅₈, X₆₁, X₆₄, X₆₅, and/or X₆₈, is a natural and/or non-natural amino acid residue, and; (d) MGSWX₅X₆FKX₉ X₁₀LAX₁₃IKX₁₆X₁₇LEALGGSEAELA AFX₃₂X₃₃EIX₃₆AFX₃₉X₄₀ELX₄₃AYKGKGNPEVE X₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (SEQ ID NO:5), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇,

X₃₂, X₃₃, X₃₆, X₃₉, X₄₀, X₄₃, X₅₅, X₅₈, X₅₉, X₆₂, X₆₅, and/or X₆₆, is a natural and/or non-natural amino acid residue; and (e) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAX₃₀FEX₃₃X₃₄IX₃₇FEX₄₀X₄₁LQX₄₄YKGKGNPEVEALX₅₇X₅₈EAX₆₁AIX₆₄X₆₅ELX₆₈AYRHN (SEQ ID NO:6), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₃₀, X₃₃, X₃₄, X₃₇, X₄₀, X₄₁, X₄₄, X₅₇, X₅₈, X₆₁, X₆₄, X₆₅, and/or X₆₈, is a natural and/or non-natural amino acid residue; and wherein the DBDpp specifically binds a target of interest. In an additional example, X_n is a natural amino acid residue. In a further example, X_n is a natural amino acid residue other than cysteine or proline. In still additional examples, X_n is a deletion of an amino acid (e.g., optionally a null position in the sequence). In an additional example, a plurality of the vectors in the library encode a DBDpp fusion protein. In another 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 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 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 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 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 example, the host cells display DBDpp on their surface. In a further example, the host cells are phage that display DBDpp on their surface. In some 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)

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

[0063] In one example, a vector library comprises a plurality of nucleic acids encoding DBDpp comprising an amino acid sequence selected from the group consisting of: (a) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALZ₁EAELAAFEKEIAAFESEQLQAYZ₂NPEVE X₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:9), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₅₀, X₅₃, X₅₄, X₅₇, X₆₀, and/or X₆₁, is a natural and/or non-natural amino acid residue, and Z₁ and Z₂ is 2 to 30 natural and/or non-natural amino acid residues; (b) MGSWX₅X₆FKX₉X₁₀LA X₁₃IKX₁₆X₁₇LEALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPEVEALRKEAAA IRDELQAYRHN (SEQ ID NO:7), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇, X₂₈, X₃₁, X₃₂, X₃₅, X₃₈, X₃₉, and/or X₄₂, is a natural and/or non-natural amino acid residue, and Z₁ and Z₂ is 2 to 30 natural and/or non-natural amino acid residues; (c) MGSWAEFKQRLAAIKTRLEALZ₁EAEL AAFX₃₀X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ELX₆₃AYRHN (SEQ ID NO:8), wherein X₃₀, X₃₁, X₃₄, X₃₇, X₃₈, X₄₁, X₅₂, X₅₃, X₅₆, X₅₉, X₆₀, and/or X₆₃, is a natural and/or non-natural amino acid residue, and Z₁ and Z₂ is 2 to 30 natural and/or non-natural amino acid residues; (d) MGSWX₅X₆FKX₉X₁₀LAX₁₃IKX₁₆X₁₇LEALZ₁EAELAAAFX₃₀ X₃₁EIX₃₄AFX₃₇X₃₈ELX₄₁AYZ₂NPEVEX₅₀LRX₅₃X₅₄AAX₅₇IRX₆₀X₆₁LQAYRHN (SEQ ID NO:10), wherein X₅, X₆, X₉, X₁₀, X₁₃, X₁₆, X₁₇, X₃₀, X₃₁, X₃₄, X₃₇, X₃₈, X₄₁, X₅₀, X₅₃, X₅₄, X₅₇, X₆₀, and/or X₆₁, is a natural and/or non-natural amino acid residue, and Z₁ and Z₂ is 2 to 30 natural and/or non-natural amino acid residues; and (e) MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RL X₁₉ALZ₁EAELAX₂₈FEX₃₁X₃₂IAX₃₅FEX₃₈X₃₉LQX₄₂YZ₂NPEVEALX₅₂X₅₃EAX₅₆AIX₅₉X₆₀ ELX₆₃AYRHN (SEQ ID NO:11), wherein X₅, X₈, X₉, X₁₂, X₁₅, X₁₆, X₁₉, X₂₈, X₃₁, X₃₂, X₃₅, X₃₈, X₃₉, X₄₂, X₅₂, X₅₃, X₅₆, X₅₉, X₆₀, and/or X₆₃, is a natural and/or non-natural amino acid residue, and Z₁ and Z₂ 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 example, X_n is a natural amino acid residue. In a further example, X_n is a natural amino acid residue other than cysteine or proline. In still additional examples, X_n is a deletion of an amino acid (e.g., optionally a null position in the sequence). In an additional example, a plurality of the vectors in the library encode a DBDpp fusion protein. In another 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 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 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 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 example, a plurality of the vectors of the vector library are contained in host cells. [In another example, the host cells (e.g., viral particles) display DBDpp on their surface. In a further example, the host cells are phage that display DBDpp on their surface. In some examples, the host cells are mammalian cells. In some 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 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 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 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 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 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 examples, the target is recovered by elution. In one example, the DBDpp is immobilized on a solid support. In a further example, the elution of the bound target is monitored by ultra violet light absorption, or other visualization or chemical-based detection technique. In some 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 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 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 examples. One 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 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.”

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:

MGSWX₅EFX₈X₉RLX₁₂AIX₁₅X₁₆RLX₁₉ALGGSEAELAAFEKEIA

AFESELQAYKKGKNPEVEX₅₅LRX₅₈X₅₉AAX₆₂IRX₆₅X₆₆LQAYRHN (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 specific binding of the polypeptide to the target of interest is greater than the binding of a polypeptide according to SEQ ID NO:1 to the target of interest, and optionally wherein the polypeptide does not contain SEQ ID NO:50 and wherein the modifications to SEQ ID NO:1 comprise conservative or non-conservative substitutions and do not include a substitution with a cysteine or a proline.

2. The polypeptide of Claim 1, wherein the target of interest specifically bound by the polypeptide is a cancer antigen, optionally wherein the cancer antigen is PD-L1, CD137, or CD123.

3. The polypeptide of Claim 1,

- (a) wherein the polypeptide specifically binds PD-L1 and 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, and SEQ ID NO:44;
- (b) wherein the polypeptide specifically binds 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;
- (c) wherein the polypeptide specifically binds CD123 and wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 92-126 and SEQ ID NO:127; or
- (d) wherein the polypeptide competes with the polypeptide of (c) for binding to CD123.

4. 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, and wherein the modification comprises one or more conservative or non-conservative amino acid substitutions and does not include a substitution with a cysteine or a proline;

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;

and optionally further comprising identifying potentially immunogenic amino acid residues in a candidate binding polypeptide and modifying at least one of the potentially immunogenic amino acid residues in the candidate polypeptide, wherein the modification comprises an amino acid substitution.

5. A de novo binding domain polypeptide (DBDpp), wherein the DBDpp comprises three anti-parallel alpha helices and is a variant of a synthetic polypeptide, wherein the DBDpp immunospecifically 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 has a dissociation constant (KD) between about 10^{-4} M and about 10^{-12} M,

optionally wherein, the amino acid sequence comprises MGSWX5EFX8X9RLX12AIX15X16RLX19ALGGSEAELAAFEKEIAAFESLQAYKGGKGNPEVEX55LRX58X59AAX62IR X65X66LQAYRHN (SEQ ID NO:4), and wherein Xn is a natural or non-natural amino acid, and optionally, wherein Xn is not cysteine or proline, and optionally 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, and

optionally wherein the DBDpp binds to a tumor.

6. A fusion protein comprising the de novo binding domain polypeptide (DBDpp) of Claim 5 and further comprising one or more additional DBDpp exhibiting binding specificity for a tumor target.

7. A de novo binding domain polypeptide (DBDpp) according to Claim 5 or 6, wherein the DBDpp is labeled and optionally, 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.

8. A de novo binding domain polypeptide (DBDpp) according to any one of Claims 5-7 wherein the DBDpp is conjugated to a therapeutic or cytotoxic agent.

9. A de novo binding domain polypeptide (DBDpp) according to any one of Claims 5-8, further comprising a pharmaceutically acceptable carrier.

10. A kit comprising the de novo binding domain polypeptide (DBDpp) according to any one of Claims 5-9.

11. An isolated nucleic acid molecule encoding the de novo binding domain polypeptide (DBDpp) according to Claim 5 or Claim 6.

12. A vector comprising the isolated nucleic acid molecule of Claim 11, and optionally further comprising a nucleotide sequence which regulates the expression of the de novo binding domain polypeptide (DBDpp) encoded by the nucleic acid molecule.

13. A host cell comprising the nucleic acid molecule of Claim 11 or the vector of Claim 12.

14. A cell line engineered to express the de novo binding domain polypeptide (DBDpp) of Claim 5.

15. A chimeric antigen receptor (CAR), wherein the CAR comprises a targeting domain, a transmembrane domain, and an intracellular signaling domain, wherein the targeting domain comprises a de novo binding domain polypeptide (DBDpp) according to Claim 5 or Claim 6, optionally wherein 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.

16. A chimeric antigen receptor (CAR) according to Claim 15, wherein the intracellular signaling domain comprises a costimulatory signaling region, optionally wherein the costimulatory signaling region comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 4-1BB, 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.

17. A chimeric antigen receptor (CAR), wherein the CAR comprises the fusion protein of claim 6.

18. An isolated nucleic acid sequence comprising a sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises a de novo binding domain polypeptide (DBDpp) according to Claim 5, a transmembrane domain, and an intracellular signaling domain.

19. A cell comprising a nucleic acid sequence comprising a sequence encoding a chimeric antigen receptor (CAR), wherein the CAR comprises an antigen binding domain derived from a de novo binding domain polypeptide (DBDpp), a transmembrane domain, and a signaling domain, optionally wherein the de novo binding domain polypeptide (DBDpp) binds to a tumor antigen associated with a hematologic malignancy or a solid tumor, and optionally

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/IL3Ra, cMet, PSMA, Glycolipid F77, EGFRvIII, GD2, NY-ESO- 1, MAGE A3, and combinations thereof.

20. A cell according to Claim 19, 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 de novo binding domain polypeptide (DBDpp) binds to its corresponding tumor antigen.

21. 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.

22. Virus-like particles (VLPs) produced by the mammalian cell of Claim 21, 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).

23. Use of a T cell comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament for the treatment of cancer, wherein the CAR comprises:

a target binding domain comprising 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 the amino acid sequence contains no cysteine or proline residues, wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, and wherein 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,

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.

24. Use of an immune cell comprising a chimeric antigen receptor (CAR) for the manufacture of a medicament 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,

and optionally wherein the immune cells is a T cell or a natural killer (NK) cell.