

D-DOMAIN CONTAINING POLYPEPTIDES AND USES THEREOF

BACKGROUND

[0001] Antibody-based reagents have accelerated the pace of biological research and development. Antibody compositions represent one of the most important and successful classes of therapeutic and diagnostic agents utilized in the pharmaceutical industry. However, cost, time and efficacy have motivated the development of alternative affinity reagents.

[0002] A variety of non-antibody binding formats have emerged for applications historically served by antibodies. While many successes have been reported for unstructured, linear peptides, more robust results have been achieved by imposing a structural constraint on the peptide sequence – typically through the introduction of a disulfide bond. This constraint affords higher affinity and greater specificity through the more favorable thermodynamics of fixed-shape complementarity and surface presentations of residues (*e.g.*, hydrophobic amino acids) that might otherwise be buried and therefore not target-facing (Ladner, Trends in Biotech. 13(10): 426-430 (1995)). Conversely, formats that contain disulfide bonds are typically prone to improper pairing of cysteines, either intra-domain or inter-domain, that can lead to lower expression, product yield and product quality.

[0003] 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

[0003a] In one aspect, the present disclosure refers to a protein comprising a D Domain (DD) target binding domain wherein the DD is a member selected from the group consisting of: (a) a DD that specifically binds BCMA and comprises the amino acid sequence of SEQ ID NO: 11-305, or 306; (b) a DD that specifically binds CD123 and comprises the amino acid sequence of SEQ ID NO: 307-739, or 740; (c) a DD that specifically binds AFP and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895; (d) a DD that specifically binds AFP p26 and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895; (e) a DD that specifically binds CS1 and comprises

the amino acid sequence of SEQ ID NO: 896-909, or 910; and (f) a DD that specifically binds HER2 and comprises the amino acid sequence of SEQ ID NO: 911-949, or 950.

[0003b] In another aspect, the present disclosure refers to a chimeric antigen receptor (CAR) which comprises a target binding domain comprising the protein disclosed herein.

[0003c] In yet another aspect, the present disclosure refers to an isolated nucleic acid encoding the protein disclosed herein or the CAR disclosed herein.

[0003d] In yet another aspect, the present disclosure refers to a vector comprising the nucleic acid disclosed herein, wherein optionally the nucleic acid is operably linked with a nucleotide sequence which regulates the expression of the protein encoded by the nucleic acid, wherein optionally the vector is a lentiviral vector.

[0003e] In yet another aspect, the present disclosure refers to a host cell comprising the nucleic acid disclosed herein or the vector disclosed herein, wherein optionally the cell is a T cell or a natural killer (NK) cell.

[0003f] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the protein disclosed herein, the CAR disclosed herein, the nucleic acid disclosed herein, the vector disclosed herein, or the cell disclosed herein.

[0003g] In yet another aspect, the present disclosure refers to a kit comprising the protein disclosed herein or the CAR disclosed herein.

[0003h] In yet another aspect, the present disclosure refers to use of a protein disclosed herein, the CAR disclosed herein, the nucleic acid disclosed herein, the vector disclosed herein, the cell disclosed herein, or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating cancer.

[0003i] In yet another aspect, the present disclosure refers to use of a protein disclosed herein, the CAR disclosed herein, the nucleic acid disclosed herein, the vector disclosed herein, the cell disclosed herein, or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a B cell associated disorder (*e.g.*, monoclonal gammopathy of determined significance), (MGUS)).

[0003j] In yet another aspect, the present disclosure refers to use of a protein disclosed herein, the CAR disclosed herein, the nucleic acid disclosed herein, the vector disclosed herein, the cell disclosed

herein, or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating a disorder of the immune system.

[0003k] In yet another aspect, the present disclosure refers to use of an immune cell comprising a chimeric antigen receptor (CAR) in the manufacture of a medicament for treating cancer in a subject, wherein the CAR comprises: (a) a target binding domain comprising a DD having an amino acid sequence selected from the group consisting of SEQ ID NO: 11-949, and 950, optionally wherein the DD specifically binds a target of interest expressed by a cancer cell; (b) a transmembrane domain and an intracellular domain; wherein the intracellular domain comprises a signaling domain, and wherein, upon administration to a subject, 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.

[0004] There remains a substantial unmet need for new target-binding compositions, and particularly for such agents containing alternative binding scaffolds (*e.g.*, non-antibody scaffolds). 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 novel target-binding D domain (DD) polypeptides that are based on a non-antibody structural scaffold. In some examples, the D domain polypeptides (DDpps) are characterized by high target binding affinity and by a non-antibody structural scaffold. In some

examples, the DDpps are target-specific binding polypeptides that 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 provided DDpps are used as therapeutics to bind cells or soluble factors involved in disease.

[0005] In some examples, the disclosure provides a protein comprising a D Domain (DD) target binding domain (DDpp) wherein the DD specifically binds a target of interest selected from the group consisting of BCMA (SEQ ID NO: 7), CD123 (SEQ ID NO: 8), CS1 (SEQ ID NO: 925), HER2 (SEQ ID NO: 927), AFP (SEQ ID NO: 9), AFP p26 (SEQ ID NO: 10), or a fragment thereof. In some examples, the DDpp are monovalent or multivalent. In some examples, the DDpp are monospecific or multispecific. In further examples, the monospecific and multivalent. In other examples, the DDpp are multispecific and multivalent. Fusion proteins comprising one or more DD are also provided, as are methods of making and using the fusion proteins. Nucleic acids encoding the DDpps and vectors and host cells containing the nucleic acids are also provided. Non-limiting examples of such uses include, but are not limited to target analysis, and diagnostic and therapeutic applications.

[0006] In additional examples, the disclosure provides a protein comprising a D Domain (DD) target binding domain (DDpp) wherein the DD is a member selected from the group consisting of: (a) a DD that specifically binds BCMA (SEQ ID NO: 7) and comprises the amino acid sequence of SEQ ID NO: 11-305, or 306; (b) a DD that specifically binds CD123 (SEQ ID NO: 8) and comprises the amino acid sequence of SEQ ID NO: 307-739, or 740; (c) a DD that specifically binds AFP (SEQ ID NO: 9) or a fragment thereof, and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895; (d) a DD that specifically binds AFP p26 (SEQ ID NO: 10) and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895, (e) a DD that specifically binds CS1 (SEQ ID NO: 925) or a fragment thereof, and comprises the amino acid sequence of SEQ ID NO: 896-909, or 910, and (f) a DD that specifically binds HER2 or a fragment thereof, and comprises the amino acid sequence of SEQ ID NO: 911-949, or 950. Proteins comprising variants of (a)-(f) that retain the ability to specifically bind their respective targets are also provided. In some examples, the DDpp is fused to a heterologous polypeptide. In some examples, the heterologous polypeptide comprises a full-length antibody or an antibody fragment. In some examples, the DD is fused to: the amino terminus of a full-length antibody heavy chain; the amino terminus of a full-length antibody light chain; the

carboxyl terminus of a full-length antibody heavy chain; or the carboxyl terminus of a full-length antibody light chain. In other examples, the DD is fused to an antibody fragment which is an Fc. In additional examples, the heterologous polypeptide comprises a member selected from the group consisting of: (i) a transmembrane domain; (ii) a membrane associating domain; (iii) human serum albumin or a fragment thereof; (iv) AFP or a fragment thereof; (v) AFP p26 or a fragment thereof; (vi) the extracellular domain of a receptor or a fragment thereof; and (vii) the extracellular domain of an intracellular receptor (*e.g.*, a nuclear protein) or a fragment thereof. In some examples, the protein contains a heterologous polypeptide that comprises the extracellular domain, or a fragment of an extracellular domain of BCMA (SEQ ID NO: 7) or CD123 (SEQ ID NO: 8) or CD19 (SEQ ID NO: 3) or CS1 (SEQ ID NO: 925). In some examples, the protein contains a heterologous polypeptide that comprises the extracellular domain, or a fragment of an extracellular domain, of a receptor selected from the group consisting of: CD19, CD20, CD22, HVEM, BTLA, DR3, CD37; TSLPR, IL7R, and gp96. In some examples, the protein contains a heterologous polypeptide that comprises an antigenic portion of a serum protein (*e.g.*, AFP, and AFP p26). In some examples, the protein contains a heterologous polypeptide that comprises an antigenic portion of an intracellular protein (*e.g.*, a nuclear protein). In some examples, the protein is labeled. In further examples, the label is selected from the group consisting of an enzymatic label, a fluorescent label, a luminescent label, a bioluminescent label, and a biotin moiety. In additional examples, the protein is conjugated to a therapeutic or cytotoxic agent. In some examples, the protein contains a heterologous polypeptide that binds to one or more with major histocompatibility complex (MHC) class I or class II complexes.

[0007] In some examples, a DD of the DDpp specifically binds BCMA. In some examples, the DD specifically binds a BCMA protein having an amino acid sequence consisting of SEQ ID NO: 7. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In other examples, the BCMA-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In some examples, the DDpp comprises multiple target-binding domains (*e.g.*, dimers, trimers, *etc.*). In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD that bind BCMA. In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD that have the same sequence. In some examples, the DDpp comprises 2, 3, 4, 5 or more than 5, DD that bind to

different epitopes of BCMA. In some examples, the DDpp comprises a DD that specifically binds BCMA and further comprises 2, 3, 4, 5 or more than 5, additional different DDs or target-binding binding domains (*e.g.*, scFvs) that bind to BCMA or a different target antigen. In some examples, the DDpp comprises a DD that specifically binds BCMA and further comprises one or more additional DDs or other target-binding binding domains that bind one or more antigens expressed on the surface of a B cell. In some examples, the DDpp comprises a DD that specifically binds BCMA and further comprises one or more additional DDs or other target-binding binding domains that bind one or more cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different targets. In further examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens expressed on the surface of a cancer cell. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, cancer antigens expressed on the surface of different cancer cells.

[0008] In some examples, the DDpp is a fusion protein comprising a DD that specifically binds BCMA. In some examples, the DDpp fusion protein comprises a DD that specifically binds a BCMA protein having an amino acid sequence consisting of SEQ ID NO: 7. In some examples, the DDpp is a fusion protein comprising a DD that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In other examples, the BCMA-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In some examples, the DDpp fusion protein comprises a full-length antibody or a portion (fragment) of an antibody. In some example, the DDpp fusion protein comprises a full length IgG antibody (*e.g.*, IgG1, IgG2, IgG2, or IgG4). In further examples, the DDpp comprises a commercially approved therapeutic antibody (*e.g.*, rituximab, ofatumumab, ocrelizumab, veltuzumab, MEDI-551, epratuzumab, belimumab, tabalumab, AMG-557, MEDI-570, and NN882). In other examples, the BCMA-binding DDpp is an Fc fusion protein.

[0009] In some examples, the DDpp fusion protein comprises a BCMA-binding DD operably linked to a serum protein. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In other examples, the BCMA-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In further examples, the DDpp fusion protein comprises human serum albumin or a fragment thereof. In some examples, the DDpp

fusion protein comprises AFP or AFP p26, or a fragment thereof. In some examples, the DDpp fusion protein comprises AFP (*e.g.*, SEQ ID NO: 9), or a fragment thereof. In other examples, the DDpp fusion protein comprises AFP p26 (SEQ ID NO: 10), or a fragment thereof.

[0010] In some examples, the DDpp fusion protein is a soluble protein comprising one or more target-binding DDpp and a p29 protein (*e.g.*, having the sequence of SEQ ID NO: 10, 928, 929, 930, 931, 932, 933, or 934). Such fusion proteins containing p29 sequences have been discovered herein to have surprisingly long serum half-life. In some examples, the soluble DDpp fusion protein has a plasma half-life *in vivo* of at least 1 hour, at least 2 hours, at least 4 hours, at least 8 hours, at least 16 hours, at least 32 hours, at least 64 hours, or more. In some examples, the soluble fusion protein has an *in vivo* plasma half-life of at least 1 hour, at least 2 hours, at least 4 hours, at least 8 hours, at least 16 hours, at least 32 hours, at least 64 hours, or more hours 65 hours, or 1-10 hours, 2-10 hours, 4-10 hours, 6-10 hours, or 6-9 hours in a mouse. In some examples, the soluble DDpp fusion protein has an *in vivo* plasma half-life of at least 1 hour, at least 2 hours, at least 4 hours, at least 8 hours, at least 16 hours, at least 32 hours, at least 64 hours, or more hours 65 hours, or 1-10 hours, 2-10 hours, 4-10 hours, 6-10 hours, or 6-9 hours, in a human.

[0011] In some examples, the BCMA-binding DDpp fusion protein comprises the extracellular domain of a receptor or a fragment thereof. In some examples, the DDpp fusion protein comprises a DD that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In other examples, the BCMA-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In further examples, the CD123-binding DDpp fusion protein comprises the extracellular domain of CD123 (SEQ ID NO: 8), or a fragment thereof. In some examples, the BCMA-binding DDpp fusion protein comprises the extracellular domain of a receptor selected from the group consisting of: CD19, CD20, CD22, HVEM, BTLA, DR3, CD37, CS1, TSLPR, IL7R, and gp96, or a fragment thereof.

[0012] In additional examples, the BCMA-binding DDpp fusion protein comprises an intracellular protein (*e.g.*, a nuclear protein) or a fragment thereof. In some examples, the DDpp fusion protein comprises a DD that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In other examples, the BCMA-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In some examples, the BCMA-binding DDpp fusion protein comprises a

fragment of a serum protein (*e.g.*, HSA, AFP, and AFP p26), an extracellular domain of a receptor (*e.g.*, BCMA, CD123, CS1, and CD19), or an intracellular protein (*e.g.*, a nuclear protein), consisting of 5-500, 5-400, 5-300, 5-200, 5-100, 5-50, 10-500, 10-400, 10-300, 10-200, 10-100, or 10-50 amino acid residues.

[0013] In some examples, a DD of the DDpp specifically binds CD123. In some examples, the DDpp specifically binds a CD123 protein having an amino acid sequence consisting of SEQ ID NO: 8. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In other examples, the CD123-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In some examples, the DDpp comprises multiple target-binding domains (*e.g.*, dimers, trimers, *etc.*). In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that bind CD123. In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that have the same sequence. In some examples, the DDpp comprises 2, 3, 4, 5 or more than 5, DD that bind to different epitopes of CD123. In some examples, the DDpp comprises a DD that specifically binds CD123 and further comprises 2, 3, 4, 5 or more than 5, additional different DDs or target-binding binding domains (*e.g.*, scFvs) that bind to BCMA or a different target antigen. In some examples, the DDpp comprises a DD that specifically binds CD123 and further comprises one or more additional DDs or other target-binding binding domains that bind one or more antigens expressed on the surface of a B cell. In some examples, the DDpp comprises a DD that specifically binds CD123 and further comprises one or more additional DDs or other target-binding binding domains that bind one or more cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different targets. In further examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens expressed on the surface of a cancer cell. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, cancer antigens expressed on the surface of different cancer cells.

[0014] In some examples, the DDpp is a fusion protein comprising a DD that specifically binds CD123. In some examples, the DD specifically binds a CD123 protein having an amino acid sequence consisting of SEQ ID NO: 8. In some examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In other examples, the CD123-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from

the group consisting of SEQ ID NO: 307-739, and 740. In some examples, the DDpp fusion protein comprises a full-length antibody or a portion (fragment) of an antibody. In some examples, the DDpp fusion protein comprises a full length IgG antibody (*e.g.*, IgG1, IgG2, IgG2, or IgG4). In further examples, the DDpp comprises a commercially approved therapeutic antibody (*e.g.*, rituximab, ofatumumab, ocrelizumab, veltuzumab, MEDI-551, epratuzumab, belimumab, tabalumab, AMG-557, MEDI-570, and NN8828). In other examples, the CD123-binding DDpp is an Fc fusion protein.

[0015] In some examples, the DDpp fusion protein comprises a CD123-binding DD operably linked to a serum protein. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In other examples, the CD123-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In further examples, the DDpp fusion protein comprises human serum albumin or a fragment thereof. In some examples, the DDpp fusion protein comprises AFP or AFP p26, or a fragment thereof. In some examples, the DDpp fusion protein comprises AFP (*e.g.*, SEQ ID NO: 9), or a fragment thereof. In other examples, the DDpp fusion protein comprises AFP p26 (SEQ ID NO: 10), or a fragment thereof.

[0016] In some examples, the CD123-binding DDpp fusion protein comprises the extracellular domain of a receptor or a fragment thereof. In some examples, the CD123-binding DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In other examples, the CD123-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In further examples, the CD123-binding DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7) or CD123 (SEQ ID NO: 8), or a fragment thereof. In further examples, the CD123-binding DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7), or CD123 (SEQ ID NO: 8), or CS1 (SEQ ID NO: 925), or a fragment thereof. In some examples, the CD123-binding DDpp fusion protein comprises the extracellular domain of a receptor selected from the group consisting of: CD19, CD20, CD22, HVEM, BTLA, DR3, CD37, CS1, TSLPR, IL7R, and gp96, or a fragment thereof.

[0017] In additional examples, the CD123-binding DDpp fusion protein comprises an intracellular protein (*e.g.*, a nuclear protein) or a fragment thereof. In some examples, the CD123-binding DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID

NO: 307-739, and 740. In other examples, the CD123-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740.

[0018] In some examples, the CD123-binding DDpp fusion protein comprises a fragment of a serum protein (*e.g.*, HSA, AFP, and AFP 26), an extracellular domain of a receptor (*e.g.*, BCMA, CS1, CD123, and CD19), or an intracellular protein (*e.g.*, a nuclear protein), consisting of 5-500, 5-400, 5-300, 5-200, 5-100, 5-50, 10-500, 10-400, 10-300, 10-200, 10-100, or 10-50 amino acid residues.

[0019] In some examples, a DD of the DDpp specifically binds CS1. In some examples, the DDpp specifically binds a CS1 protein having an amino acid sequence consisting of SEQ ID NO: 925. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In other examples, the CS1-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In some examples, the DDpp comprises multiple target-binding domains (*e.g.*, dimers, trimers, *etc.*). In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that bind CS1. In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that have the same sequence. In some examples, the DDpp comprises 2, 3, 4, 5 or more than 5, DD that bind to different epitopes of CS1. In some examples, the DDpp comprises a DD that specifically binds CS1 and further comprises 2, 3, 4, 5 or more than 5, additional different DDs or target-binding binding domains (*e.g.*, scFvs) that bind to BCMA or a different target antigen. In some examples, the DDpp comprises a DD that specifically binds CS1 and further comprises one or more additional DDs or other target-binding binding domains that bind one or more antigens expressed on the surface of a B cell. In some examples, the DDpp comprises a DD that specifically binds CS1 and further comprises one or more additional DDs or other target-binding binding domains that bind one or more cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different targets. In further examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens expressed on the surface of a cancer cell. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, cancer antigens expressed on the surface of different cancer cells.

[0020] In some examples, the DDpp is a fusion protein comprising a DD that specifically binds CS1. In some examples, the DD specifically binds a CS1 protein having an amino acid sequence

consisting of SEQ ID NO: 925. In some examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In other examples, the CS1-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In some examples, the DDpp fusion protein comprises a full-length antibody or a portion (fragment) of an antibody. In some examples, the DDpp fusion protein comprises a full length IgG antibody (*e.g.*, IgG1, IgG2, IgG2, or IgG4). In further examples, the DDpp comprises a commercially approved therapeutic antibody (*e.g.*, rituximab, ofatumumab, ocrelizumab, veltuzumab, MEDI-551, epratuzumab, belimumab, tabalumab, AMG-557, MEDI-570, and NN8828). In other examples, the CS1-binding DDpp is an Fc fusion protein.

[0021] In some examples, the DDpp fusion protein comprises a CS1-binding DD operably linked to a serum protein. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In other examples, the CS1-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In further examples, the DDpp fusion protein comprises human serum albumin or a fragment thereof. In some examples, the DDpp fusion protein comprises AFP or AFP p26, or a fragment thereof. In some examples, the DDpp fusion protein comprises AFP (*e.g.*, SEQ ID NO: 9), or a fragment thereof. In other examples, the DDpp fusion protein comprises AFP p26 (SEQ ID NO: 10), or a fragment thereof.

[0022] In some examples, the CS1-binding DDpp fusion protein comprises the extracellular domain of a receptor or a fragment thereof. In some examples, the CS1-binding DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In other examples, the CS1-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In further examples, the CS1-binding DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7), CD123 (SEQ ID NO: 8), or CS1 (SEQ ID NO: 925), or a fragment thereof. In some examples, the CS1-binding DDpp fusion protein comprises the extracellular domain of a receptor selected from the group consisting of: CD19, CD20, CD22, HVEM, BTLA, DR3, CD37, CS-1, TSLPR, IL7R, and gp96, or a fragment thereof.

[0023] In additional examples, the CS1-binding DDpp fusion protein comprises an intracellular protein (*e.g.*, a nuclear protein) or a fragment thereof. In some examples, the CS1-binding DDpp

fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In other examples, the CS1-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910.

[0024] In some examples, the CS1-binding DDpp fusion protein comprises a fragment of a serum protein (*e.g.*, HSA, AFP, and AFP 26), an extracellular domain of a receptor (*e.g.*, BCMA, CS1, CD123, and CD19), or an intracellular protein (*e.g.*, a nuclear protein), consisting of 5-500, 5-400, 5-300, 5-200, 5-100, 5-50, 10-500, 10-400, 10-300, 10-200, 10-100, or 10-50 amino acid residues.

[0025] In some examples, a DD of the DDpp specifically binds AFP or a fragment thereof. In some examples, the DDpp specifically binds an AFP protein having an amino acid sequence consisting of SEQ ID NO: 9 or a fragment thereof. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the DDpp comprises multiple target-binding domains (*e.g.*, dimers, trimers, *etc.*). In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that bind AFP or a fragment thereof. In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that have the same sequence. In some examples, the DDpp comprises 2, 3, 4, 5 or more than 5, DD that bind to different epitopes of AFP or a fragment thereof. In some examples, the DDpp comprises a DD that specifically binds AFP or a fragment thereof and further comprises 2, 3, 4, 5 or more than 5, additional different DDs or target-binding binding domains (*e.g.*, scFvs) that bind to AFP, an AFP fragment, or a different target antigen. In some examples, the DDpp comprises a DD that specifically binds AFP or a fragment thereof and further comprises one or more additional DDs or other target-binding binding domains that bind one or more antigens expressed on the surface of a B cell. In some examples, the DDpp comprises a DD that specifically binds AFP and further comprises one or more additional DDs or other target-binding binding domains that bind one or more cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different targets. In further examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens expressed on the surface of a cancer cell. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, cancer antigens expressed on the surface of different cancer cells.

[0026] In some examples, the DDpp is a fusion protein comprising a DD that specifically binds AFP, or a fragment thereof. In some examples, the DD specifically binds an AFP protein having an amino acid sequence consisting of SEQ ID NO: 9, or a fragment thereof. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the fusion protein comprises an AFP-binding DD operably linked to a full-length antibody or a portion (fragment) of an antibody. In some examples, the DDpp fusion protein comprises a full length IgG antibody (*e.g.*, IgG1, IgG2, IgG2, or IgG4). In further examples, the DDpp comprises a commercially approved therapeutic antibody (*e.g.*, rituximab, ofatumumab, ocrelizumab, veltuzumab, MEDI-551, epratuzumab, belimumab, tabalumab, AMG-557, MEDI-570, and NN882). In some examples, the AFP-binding DDpp is an Fc fusion protein.

[0027] In some examples, the DDpp fusion protein comprises a DD that specifically binds AFP or an AFP fragment, operably linked to a serum protein. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In further examples, the DDpp fusion protein comprises human serum albumin or a fragment thereof.

[0028] In some examples, the DDpp fusion protein comprises a DD that specifically binds AFP or an AFP fragment and further comprises the extracellular domain of a receptor or a fragment thereof. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7) or CD123 (SEQ ID NO: 8), or a fragment thereof. In some examples, the DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7), or CD123 (SEQ ID NO: 8), or CS1 (SEQ ID NO: 925), or a fragment thereof. In some examples, the DDpp fusion protein comprises the extracellular domain of a receptor selected from the group consisting of: CD19, CD20, CD22, HVEM, BTLA, DR3, CD37; TSLPR, IL7R, and gp96, or a fragment thereof.

[0029] In additional examples, the DDpp fusion protein comprises a DD that specifically binds AFP or an AFP fragment and further comprises an intracellular protein (*e.g.*, a nuclear protein) or a fragment thereof. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895.

[0030] In some examples, the AFP-binding DDpp fusion protein comprises a fragment of a serum protein (*e.g.*, HSA), an extracellular domain of a receptor (*e.g.*, BCMA, CS1, CD123, and CD19), or an intracellular protein (*e.g.*, a nuclear protein), consisting of 5-500, 5-400, 5-300, 5-200, 5-100, 5-50, 10-500, 10-400, 10-300, 10-200, 10-100, or 10-50 amino acid residues.

[0031] In some examples, a DD of the DDpp specifically binds AFP p26. In some examples, the DDpp specifically binds AFP p26 having an amino acid sequence consisting of SEQ ID NO: 10, or a fragment thereof. In some examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In further examples, the DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In further examples, the DD specifically binds AFP p26 having an amino acid sequence consisting of SEQ ID NO: 10, but does not specifically bind AFP having an amino acid sequence consisting of SEQ ID NO: 9. In some examples, the DDpp comprises multiple target-binding domains (*e.g.*, dimers, trimers, *etc.*). In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that bind AFP p26. In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that have the same sequence. In some examples, the DDpp comprises 2, 3, 4, 5 or more than 5, DD that bind to different epitopes of AFP p26. In some examples, the DDpp comprises a DD that specifically binds AFP p26 and further comprises 2, 3, 4, 5 or more than 5, additional different DDs or target-binding binding domains (*e.g.*, scFvs) that bind to AFP p26 or a different target antigen. In some examples, the DDpp comprises a DD that specifically binds AFP p26 and further comprises one or more additional DDs or other target-binding binding domains that bind one or more antigens expressed on the surface of a B cell. In some examples, the DDpp comprises a DD that specifically binds AFP p26 and further comprises one or more additional DDs or other target-binding binding domains that bind one or more cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different targets. In further examples, the

DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens expressed on the surface of a cancer cell. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, cancer antigens expressed on the surface of different cancer cells.

[0032] In some examples, the DDpp is a fusion protein comprising a DD that specifically binds AFP p26. In some examples, the DD specifically binds AFP p26 having an amino acid sequence consisting of SEQ ID NO: 10. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP p26-binding DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, a DD of the DDpp fusion protein specifically binds AFP p26 but does not specifically bind AFP having an amino acid sequence consisting of SEQ ID NO: 9. In further examples, the DDpp is a fusion protein comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP p26-binding DDpp is an Fc fusion protein.

[0033] In some examples, the DDpp fusion protein comprises a DD that specifically binds AFP p26. In other examples, the DDpp fusion protein comprises an AFP p26-binding DD operably linked to a serum protein. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP p26-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In further examples, the DDpp fusion protein comprises human serum albumin or a fragment thereof.

[0034] In some examples, the DDpp fusion protein comprises an AFP p26-binding DD and further comprises the extracellular domain of a receptor or a fragment thereof. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP p26-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7) or CD123 (SEQ ID NO: 8), or a fragment thereof. In some

examples, the DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7), CD123 (SEQ ID NO: 8), or CS1 (SEQ ID NO: 925), or a fragment thereof. In some examples, the DDpp fusion protein comprises the extracellular domain of a receptor selected from the group consisting of: CD19, CD20, CD22, HVEM, BTLA, DR3, CD37; TSLPR, IL7R, and gp96, or a fragment thereof.

[0035] In additional examples, the DDpp fusion protein comprises an AFP p26-binding DD and further comprises an intracellular protein (*e.g.*, a nuclear protein) or a fragment thereof. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In other examples, the AFP p26-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the AFP p26-binding DDpp fusion protein comprises a fragment of a serum protein (*e.g.*, HSA), an extracellular domain of a receptor (*e.g.*, BCMA, CS1, CD123, and CD19), or an intracellular protein (*e.g.*, a nuclear protein), consisting of 5-500, 5-400, 5-300, 5-200, 5-100, 5-50, 10-500, 10-400, 10-300, 10-200, 10-100, or 10-50 amino acid residues.

[0036] In some examples, a DD of the DDpp specifically binds HER2. In some examples, the DDpp specifically binds a HER2 protein having an amino acid sequence consisting of SEQ ID NO: 927. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In other examples, the HER2-binding DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In some examples, the DDpp comprises multiple target-binding domains (*e.g.*, dimers, trimers, *etc.*). In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that bind HER2. In some examples, the DDpp comprises 2, 3, 4, 5, or more than 5, DD, that have the same sequence. In some examples, the DDpp comprises 2, 3, 4, 5 or more than 5, DD that bind to different epitopes of HER2. In some examples, the DDpp comprises a DD that specifically binds HER2 and further comprises 2, 3, 4, 5 or more than 5, additional different DDs or target-binding binding domains (*e.g.*, scFvs) that bind to BCMA or a different target antigen. In some examples, the DDpp comprises a DD that specifically binds HER2 and further comprises one or more additional DDs or other target-binding binding domains that bind one or more antigens expressed on the surface of a cancer cell. In some examples, the DDpp comprises a DD that specifically binds HER2 and

further comprises one or more additional DDs or other target-binding binding domains that bind one or more cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different targets. In further examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, different cancer antigens expressed on the surface of a cancer cell. In some examples, the DDpp specifically binds 2, 3, 4, 5, or more than 5, cancer antigens expressed on the surface of different cancer cells.

[0037] In some examples, the DDpp is a fusion protein comprising a DD that specifically binds HER2. In some examples, the DD specifically binds a HER2 protein having an amino acid sequence consisting of SEQ ID NO: 927. In some examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In other examples, the HER2-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In some examples, the DDpp fusion protein comprises a full-length antibody or a portion (fragment) of an antibody. In some examples, the DDpp fusion protein comprises a full length IgG antibody (*e.g.*, IgG1, IgG2, IgG2, or IgG4). In further examples, the DDpp comprises a commercially approved therapeutic antibody. In other examples, the HER2-binding DDpp is an Fc fusion protein.

[0038] In some examples, the DDpp fusion protein comprises a HER2-binding DD operably linked to a serum protein. In some examples, the DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In other examples, the HER2-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In further examples, the DDpp fusion protein comprises human serum albumin or a fragment thereof. In some examples, the DDpp fusion protein comprises AFP or AFP p26, or a fragment thereof. In some examples, the DDpp fusion protein comprises AFP (*e.g.*, SEQ ID NO: 9), or a fragment thereof. In other examples, the DDpp fusion protein comprises AFP p26 (SEQ ID NO: 10), or a fragment thereof.

[0039] In some examples, the HER2-binding DDpp fusion protein comprises the extracellular domain of a receptor or a fragment thereof. In some examples, the HER2-binding DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In other examples, the HER2-binding DDpp fusion protein comprises a variant of

an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In further examples, the HER2-binding DDpp fusion protein comprises the extracellular domain of BCMA (SEQ ID NO: 7), CD123 (SEQ ID NO: 8), or CS1 (SEQ ID NO: 925), or a fragment thereof. In some examples, the HER2-binding DDpp fusion protein comprises the extracellular domain of a receptor selected from the group consisting of: CD19, CD20, CD22, HVEM, BTLA, DR3, CD37, CS-1, TSLPR, IL7R, and gp96, or a fragment thereof.

[0040] In additional examples, the HER2-binding DDpp fusion protein comprises an intracellular protein (*e.g.*, a nuclear protein) or a fragment thereof. In some examples, the HER2-binding DDpp fusion protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In other examples, the HER2-binding DDpp fusion protein comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950.

[0041] In some examples, the HER2-binding DDpp fusion protein comprises a fragment of a serum protein (*e.g.*, HSA, AFP, and AFP 26), an extracellular domain of a receptor (*e.g.*, BCMA, CS1, CD123, and CD19), or an intracellular protein (*e.g.*, a nuclear protein), consisting of 5-500, 5-400, 5-300, 5-200, 5-100, 5-50, 10-500, 10-400, 10-300, 10-200, 10-100, or 10-50 amino acid residues.

[0042] In some examples, the DDpp fusion protein comprises a full length antibody. In further examples, the DDpp is a fusion protein comprising a full length antibody that specifically binds a cancer antigen. In further examples, the DDpp is a fusion protein comprising a full length antibody, wherein the antibody specifically binds a cancer antigen believed to be expressed by the cancer of the subject to which the DDpp fusion protein is administered.

[0043] In some examples, the disclosed DDpp (*e.g.*, a DDpp fusion protein) is labeled. Labels that can be used to label the DDpp include but are 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 some examples, the label is a streptavidin moiety. In some examples, the label is a His-tag or a FLAG tag. In some examples, the label is luciferase, green fluorescent protein, red fluorescent protein, or other similar agent.

[0044] In other examples, the DDpp fusion protein is attached to a solid support. In some examples, the solid support is selected from the group consisting of: a bead, a glass slide, a chip, a gelatin, and an agarose.

[0045] In some examples, the DDpp (*e.g.*, a DDpp fusion protein) is associated with a liposome. In some examples, the DDpp is associated with the liposome through covalent binding. In some examples, DDpp is a fusion protein. In further examples, the DDpp is a CAR. In additional examples, the DDpp is associated with the liposome through ionic binding but not covalent binding.

[0046] In some examples, the target-binding DDpp is conjugated to a therapeutic or cytotoxic agent (*e.g.*, a chemotherapeutic agent or a radiotherapeutic agent).

[0047] In additional examples, the disclosure provides a chimeric antigen receptor (CAR) which comprises a target binding domain comprising a DD disclosed herein (*e.g.*, a DD comprising the amino acid sequence of SEQ ID NO: 11-949, or 950). In some examples, the DD binds BCMA and comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In some examples, the DD binds CD123 and comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In some examples, the DD binds AFP and comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the DD binds AFP p26 and comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the CAR comprises, a target binding domain, a transmembrane domain, and an intracellular signaling domain. In some examples, the CAR transmembrane domain comprises a 41BB or CD28 transmembrane domain. In some examples the CAR comprises an intracellular signaling domain selected from the group consisting of a domain of a human T cell receptor alpha, beta, or zeta chain; a human 41BB domain; a human CD28 domain; and any combination thereof. In some examples, the CAR intracellular signaling domain comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 41BB, OX40, CD30, CD40, PD1, 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 some examples the CAR further comprises a second target binding domain having the same or a different target than the DD target binding domain. In some examples, the CAR is expressed in an immune cell. In some examples, the immune cell is a T cell (CAR-T cell) or a natural killer (NK) cell (CAR-NK cell). In some examples, the CAR is associated with a liposome.

[0048] In some examples, the CAR comprises 2, 3, 4, 5, or more than 5, DD and/or other binding domains (*e.g.*, scFv) that specifically bind a target of interest (*e.g.*, BCMA or CD123) expressed on the surface of the cancer cell. In additional examples, the CAR comprises 2, 3, 4, 5, or more than 5, DD or other binding domains (*e.g.*, scFv) that specifically bind a second, different target of interest, expressed on the surface of the cancer cell. In additional examples, the administered CAR further comprises 2, 3, 4, 5, or more than 5, DD or other binding domains (*e.g.*, scFv) that specifically binds a second, different target of interest, expressed by a second, different cancer cell or a vascular endothelial cell.

[0049] Nucleic acids encoding the disclosed DDpp (*e.g.*, DDpp fusion proteins) are also provided. Additionally provided are vectors (*e.g.*, plasmids, viral vectors, and non-viral vectors) containing nucleic acids encoding the DDpp (*e.g.*, DDpp fusion proteins) and host cells containing the nucleic acids and vectors. In some examples, the vector comprises a nucleotide sequence which regulates the expression of the polypeptide encoded by the nucleic acid molecule. In further examples, the vector comprises an inducible promoter sequence. In additional examples, the vector includes one or more additional standard components for expression of a protein encoded a nucleic acid (*e.g.*, promoters, packaging components, *etc.*). In some examples, the vector is a lentiviral vector.

[0050] The disclosure also provides host cells that comprise the nucleic acid molecules encoding a target-binding DDpp disclosed herein. In some examples, the host cells (*e.g.*, cells of a cell line) are engineered to express a protein containing a DD disclosed herein (*e.g.*, a DD having the amino acid sequence of SEQ ID NO: 11-949, or 950). In some examples, the expression of the DDpp by the host cells allows production and isolation of the DDpp. In some examples, the expression results in the DDpp being expressed on the surface and/or integral to the membrane of the host cells. In some examples, the host cell is a viral particle, or a bacterial, yeast, fungal, or plant cell. In other examples, the host cell is a mammalian cell. In a further example, the mammalian cell is an immune cell. In one example, the host cell is a human immune cell. In some examples, the human immune cell is a T cell. In other examples, the human immune cell is a natural killer (NK) cell. In some examples, the human immune cell displays the DDpp on its cell surface.

[0051] The disclosure further provides a host cell expressing a protein comprising a DD disclosed herein. In some examples, the host cell expresses a chimeric antigen receptor (CAR) comprising a DD disclosed herein. In some examples, the CAR comprises a target binding domain that

comprises a DD that comprising an amino acid sequence selected from SEQ ID NO: 11-305, and 306; SEQ ID NO: 307-739, and 740; SEQ ID NO: 741-874 and 886-895; SEQ ID NO: 896-909 and 910; or SEQ ID NO: 911-949, and 950 and a transmembrane domain. In some examples, the CAR further comprise an intracellular domain (comprising a signaling domain). In some examples, the CAR immune cell is a T cell. In some examples, the CAR immune cell is a NK cell. In some examples, the CAR immune cell is not a T cell or an NK cell. In some examples, the host cell is an immune cell that further comprises a second CAR polypeptide having a DD or other binding domain (*e.g.*, scFv) that specifically binds the same or a different target (*e.g.*, a different epitope of the same target, or a second target of interest) expressed by the cancer cell) as the first CAR expressed by the host immune cell.

[0052] Also provided are mammalian cells that generate membrane-bound virus-like particles (VLPs), wherein the mammalian cell is engineered to express a fusion protein comprising D domain polypeptide (DDpp) fused to a chimeric antigen receptor (CAR), the fusion protein being expressed on the generated VLPs (*e.g.*, as transmembrane proteins). Depending on the examples, the VLPs produced by the mammalian cells are suitable for use as immunogens for antibody generation.

[0053] Pharmaceutical compositions containing a protein comprising a DD disclosed herein, nucleic acids encoding the proteins, vectors containing the nucleic acids, and host cells containing the nucleic acids and or vectors are also provided. As are kits containing one or more of the disclosed target-binding DDpps (*e.g.*, DDpp fusion proteins such as DD-Fc and DD-CAR), nucleic acid molecules, vectors, and host cells (*e.g.*, a therapeutic kit, a diagnostic kit, a kit for research use, *etc.*).

[0054] DDpp provided herein possess activities that include but are not limited to the ability to specifically bind a target of interest (*e.g.*, a therapeutic target and/or diagnostic target such as BCMA, CD123, CS1, HER2, AFP, and AFP p26, a peptide tag, and a serum protein such as alpha-fetoprotein, 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 a protein such as a DDpp fusion protein with one or more additional moieties (*e.g.*, a solid support), and/or other modifications. The DDpp provided herein can also possess additional desirable properties and/or functionalities useful in manufacturing, formulation and biological, diagnostic, and therapeutic applications.

[0055] In some examples, the DDpp is used to bind, detect, and/or quantitate, a target of interest (*e.g.*, BCMA, CD123, CS1, HER2, AFP, or AFP p26) in a sample containing the target. In one

example, the disclosure provides a method for detecting a target of interest (*e.g.*, BCMA, CD123, CS1, HER2, AFP, or AFP p26) in a sample, comprising: (a) contacting the sample with a DDpp containing a DD that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-949, and 950, that specifically binds the target, under conditions suitable for specific binding of the DDpp to the target, to form a target/DDpp complex, and (b) detecting the presence of the complex and/or captured target. In some examples, the DDpp is immobilized on a solid support. In some examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305 or 306, and the DDpp is used to bind, detect, and/or quantitate, BCMA or a fusion protein comprising BCMA in a sample. In some examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739 or 740, and the DDpp is used to bind, detect, and/or quantitate, CD123 or a fusion protein comprising CD123 in a sample. In some examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874 or 886-895, and the DDpp is used to bind, detect, and/or quantitate, AFP p26, or a fusion protein comprising AFP p26 in a sample. In some examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909 or 910, and the DDpp is used to bind, detect, and/or quantitate, CS1, or a fusion protein comprising CS1 in a sample. In some examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949 or 950, and the DDpp is used to bind, detect, and/or quantitate, HER2, or a fusion protein comprising HER2 in a sample.

[0056] Also provided is a method for quantifying a target of interest (*e.g.*, BCMA, CD123, CS1, HER2, AFP, or AFP p26) in a sample containing the target, comprising: (a) contacting the sample with a DDpp containing a DD that comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-949, and 950, that specifically binds the target and that is immobilized on a solid support, under conditions suitable for specific binding of the DDpp to the target, to form a target/DDpp complex and (b) detecting the presence of the target/DDpp 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 or a fusion protein containing the target in the sample.

[0057] In additional examples, the provided DDpps are used in protein analytics. In some examples, the DDpps are conjugated to a detectable agent and/or tag. In some examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305,

and 306; SEQ ID NO: 307-739, and 740; SEQ ID NO: 741-874 and 886-895; SEQ ID NO: 896-909 and 910; or SEQ ID NO: 911-949, and 950. In other examples, the DDpp comprises a variant of an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306; SEQ ID NO: 307-739, and 740; or SEQ ID NO: 741-874 and 886-895; SEQ ID NO: 896-909 and 910; or SEQ ID NO: 911-949, and 950. In some examples, the DDpp is conjugated to a detectable agent. In one example, the detectable agent comprises a chromogen. In another example, the detectable agent comprises a fluorescent dye. In an additional example, the detectable agent comprises a radionuclide. In some examples, the DDpp is conjugated to the detectable agent by covalent binding. In some examples, the DDpp is a fusion protein. In additional examples, the DDpp is multimeric. In additional examples, the DDpp is conjugated to a tag. In some examples, the tag is a member selected from the group consisting of: a polyhistidyl tag, a myc tag, and a FLAG tag. In further examples, the DDpp is conjugated to a combination of tags (*e.g.*, a polyhistidyl tag and a FLAG tag). In some examples, the DDpp is conjugated to the tag(s) by covalent binding. In some examples, the DDpp is a fusion protein. In some examples, the DDpp is multimeric.

[0058] In additional examples, the DDpp is conjugated to a solid support or tag. In some examples, the solid support is a chromatography bead, resin, glass slide, chip, gelatin, or agarose.

[0059] Methods of using DDpp in diagnostic and therapeutic applications are also provided. In one example, the disclosure provides a method of treating a disease or disorder comprising administering a therapeutically effective amount of a DDpp (*e.g.*, a DDpp fusion protein) that specifically binds a therapeutic target of interest (*e.g.*, BCMA, CD123, CS1, HER2, AFP, or AFP p26) to a subject in need thereof. In some examples, the disease or disorder is cancer, a B cell malignancy, 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 disclosed DDpp are also provided. In some examples, the disease or disorder is multiple myeloma. In some examples, the disease or disorder is breast cancer or ovarian cancer.

[0060] The target binding DDpps disclosed herein have uses that include diagnostic and therapeutic applications. In some examples, the DDpps are 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).

[0061] In some examples, the disclosure provides a method of treating a subject having cancer, the method comprising: administering to the subject a therapeutically effective amount of: a protein

(i.e., DDpp) containing a DD disclosed herein (e.g., disclosed in Table 1, or having the amino acid sequence of SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector. In some examples, the DDpp comprises a DD amino acid sequence that specifically binds BCMA. In some examples, a DD of the DDpp specifically binds BCMA having an amino acid sequence consisting of SEQ ID NO: 7. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In some examples, the DDpp comprises a DD amino acid sequence that specifically binds CD123. In some examples, a DD of the DDpp specifically binds CD123 having an amino acid sequence consisting of SEQ ID NO: 8. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In some examples, the DDpp comprises a DD amino acid sequence that specifically binds CS1. In some examples, a DD of the DDpp specifically binds CS1 having an amino acid sequence consisting of SEQ ID NO: 925. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In some examples, the DDpp comprises a DD amino acid sequence that specifically binds HER2. In some examples, a DD of the DDpp specifically binds HER2 having an amino acid sequence consisting of SEQ ID NO: 927. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In some examples, the DDpp comprises a DD amino acid sequence that specifically binds AFP. In some examples, a DD of the DDpp specifically binds AFP having an amino acid sequence consisting of SEQ ID NO: 9. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874 and 886-895. In some examples, the DDpp specifically binds AFP p26 having an amino acid sequence consisting of SEQ ID NO: 10. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874 and 886-895. In some examples, a DD of the DDpp specifically binds AFP p26 but does not specifically bind AFP having an amino acid sequence consisting of SEQ ID NO: 9. In further examples, the DDpp comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874 and 886-895.

[0062] In some examples, the disclosure provides a method of treating a subject having a B cell malignancy, said method comprising: administering to the subject an effective amount of: a protein containing a DD disclosed herein (e.g., disclosed in Table 1, or having the amino acid sequence of

SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector. In some examples, the B cell malignancy is selected from the group consisting of: a lymphoma (*e.g.*, a Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL), a leukemia, and a myeloma. In some examples, the B cell malignancy is selected from the group consisting of: acute lymphocytic leukemia, chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, and diffuse large B-cell lymphoma, plasmacytoma, and multiple myeloma.

[0063] In some examples, the disclosure provides a method of treating a subject having cancer, the method comprising: administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), wherein the CAR comprises: a target binding domain comprising a DD having an amino acid sequence selected from the group consisting of SEQ ID NO: 11-949, and 950, wherein the polypeptide specifically binds a target of interest and a transmembrane domain. In further examples, the target of interest is expressed by a cancer cell. In some examples, the DD specifically binds BCMA having an amino acid sequence consisting of SEQ ID NO: 7. In further examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In some examples, the DD specifically binds CD123 having an amino acid sequence consisting of SEQ ID NO: 8. In further examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In some examples, the DD specifically binds CS1 having an amino acid sequence consisting of SEQ ID NO: 925. In further examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In some examples, the DD specifically binds HER2 having an amino acid sequence consisting of SEQ ID NO: 927. In further examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 911-949, and 950. In some examples, the DD specifically binds AFP p26 having an amino acid sequence consisting of SEQ ID NO: 10. In further examples, the DD comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 741-874, and 886-895. In some examples, the CAR comprises an intracellular domain. In further examples, 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 (*e.g.*, BCMA and CD123), 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. In some examples, the immune cell is a T cell. In other examples, the immune cell is a NK cell. In some examples, the administered CAR immune cell is not a T cell or an NK cell. In further examples, a combination of different CAR immune cell types (*e.g.*, NK cells and T cells) is administered to the subject. In some examples, the immune cell administration is intravenous. In some examples, a combination of different CAR immune cell types is administered intravenously to the subject. In some examples, the cancer is multiple myeloma. In some examples, the cancer is breast cancer or ovarian cancer.

[0064] In some examples, the disclosure provides a method of treating a subject having a B cell malignancy, said method comprising: administering to the subject an immune cell comprising a chimeric antigen receptor (CAR), 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: 11-949, and 950, wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, a transmembrane domain and an intracellular domain. In some examples, the target binding domain specifically binds BCMA having an amino acid sequence consisting of SEQ ID NO: 7. In further examples, the target binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-305, and 306. In some examples, the target binding domain specifically binds CD123 having an amino acid sequence consisting of SEQ ID NO: 8. In further examples, the target binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 307-739, and 740. In some examples, the target binding domain specifically binds CS1 having an amino acid sequence consisting of SEQ ID NO: 925. In further examples, the target binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 896-909, and 910. In some examples, the CAR comprises an intracellular domain. In further examples, 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 malignant B cell (*e.g.*, BCMA, CS1, and CD123), and wherein the binding of the target of interest induces the immune cell to generate cytotoxic signals that result in cytotoxic effects on the malignant B cancer cell. In some examples, the B cell malignancy is selected from the group consisting of: a lymphoma (*e.g.*, a Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL)), a leukemia, and a myeloma. In some examples, the B cell malignancy is selected from the group

consisting of: acute lymphocytic leukemia, chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, plasmacytoma and multiple myeloma. In some examples, the B cell malignancy is multiple myeloma. In some examples, the immune cell is a T cell. In other examples, the immune cell is a NK cell. In some examples, the administered CAR immune cell is not a T cell or an NK cell. In further examples, a combination of different CAR immune cell types (*e.g.*, NK cells and T cells) is administered to the subject. In some examples, the immune cell administration is intravenous. In some examples, a combination of different CAR immune cell types is administered intravenously to the subject.

[0065] In some examples, the disclosure provides a method of treating a subject having a B cell associated disorder, said method comprising: administering to the subject an effective amount of: a protein containing a DD disclosed herein (*e.g.*, a DD having an amino acid sequence disclosed in Table 1, or having the amino acid sequence of SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector.

[0066] In some examples, the disclosure provides a method of treating a subject having a disorder of the immune system, said method comprising: administering to the subject an effective amount of: a protein comprising a DD disclosed herein (*e.g.*, a DD having an amino acid sequence disclosed in Table 1, or having the amino acid sequence of SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector. In some examples, the disorder of the immune system is an autoimmune disease such as rheumatoid arthritis.

[0067] In some examples, the disclosure provides for the use of a composition for treating cancer, wherein the composition comprises: a protein containing a DD disclosed herein (*e.g.*, a DD having an amino acid sequence disclosed in Table 1, or having the amino acid sequence of SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector. In some examples, the cancer is a B cell malignancy. In some examples, the B cell malignancy is selected from the group consisting of: a lymphoma (*e.g.*, a Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL), a leukemia, and a myeloma. In some examples, the B cell malignancy is selected from the group consisting of: acute lymphocytic leukemia, chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, diffuse large

B-cell lymphoma, plasmacytoma, and multiple myeloma. In some examples, the cancer is multiple myeloma. In some examples, the cancer is breast cancer or ovarian cancer.

[0068] In some examples, the disclosure provides for the use of a composition for treating a B cell malignancy, wherein the composition comprises: a protein containing a DD disclosed herein (*e.g.*, a DD having an amino acid sequence disclosed in Table 1, or having the amino acid sequence of SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector. In some examples, the B cell malignancy is selected from the group consisting of: a lymphoma (*e.g.*, a Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL), a leukemia, and a myeloma. In some examples, the B cell malignancy is selected from the group consisting of: acute lymphocytic leukemia, chronic lymphocytic leukemia, follicular lymphoma, mantle cell lymphoma, diffuse large B-cell lymphoma, plasmacytoma and multiple myeloma. In some examples, the B cell malignancy is multiple myeloma.

[0069] In some examples, the disclosure provides for the use of a composition for the treatment of a B cell associated disorder, wherein the composition comprises: a protein containing a DD disclosed herein (*e.g.*, a DD having an amino acid sequence disclosed in Table 1, or having the amino acid sequence of SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector.

[0070] In some examples, the disclosure provides for the use of a composition for the treatment of a disorder of the immune system, wherein the composition comprises: a protein containing a DD disclosed herein (*e.g.*, a DD having an amino acid sequence disclosed in Table 1, or having the amino acid sequence of SEQ ID NO: 11-949, or 950); a nucleic acid encoding the protein; a vector containing the nucleic acid; or a host cell containing the nucleic acid or vector. In some examples, disorder of the immune system is an autoimmune disease such as rheumatoid arthritis.

[0071] In additional examples, the disclosure provides for 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: 11-949, and 950, wherein the polypeptide specifically binds a target of interest expressed by a cancer cell, 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. In some examples, the immune cell is a T cell or a natural killer (NK) cell. In some examples, the immune cell is a T cell. In some examples, the immune cell is a natural killer (NK) cell. In some examples, the immune cell is not a T cell or an NK cell. In further examples, a combination of different CAR immune cell types (*e.g.*, T cells and NK cells) is used. In some examples, the cancer is multiple myeloma. In some examples, the cancer is breast cancer or ovarian cancer.

[0072] In additional examples, the disclosure provides for use of an immune cell comprising a chimeric antigen receptor (CAR) for the treatment of a B cell malignancy, 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: 11-949, or 950, wherein the polypeptide specifically binds a target of interest (*e.g.*, a target expressed by a malignant B cell, such as BCMA, CS1, or CD123, wherein the target binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11-306, 307-740, and 896-910), 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 malignant B 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 malignant B cell. In some examples, the immune cell is a T cell or a natural killer (NK) cell. In some examples, the immune cell is a T cell. In some examples, the immune cell is a natural killer (NK) cell. In some examples, the immune cell is not a T cell or an NK cell. In further examples, a combination of different CAR immune cell types (*e.g.*, T cells and NK cells) is used. In some examples, the B cell malignancy is multiple myeloma.

[0073] In additional examples, the disclosure provides for use of an immune cell comprising a chimeric antigen receptor (CAR) for the treatment of a disorder of the immune system, 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: 11-949, and 950, wherein the polypeptide specifically binds a target of interest expressed by the immune cell to be targeted, a transmembrane domain and an intracellular domain, wherein the

intracellular domain comprises a signaling domain, wherein, upon administration to a subject having the disorder of the immune system, the target binding domain specifically binds to the target of interest expressed by the targeted immune cell, and wherein the binding of the target of interest induces the recombinant immune cell to generate cytotoxic signals that result in cytotoxic effects on the targeted immune cell. In some examples, the immune cell is a T cell or a natural killer (NK) cell. In some examples, the immune cell is not a T cell or an NK cell. In some examples, the immune cell is a natural killer (NK) cell. In some examples, the immune cell is not a T cell or an NK cell. In further examples, a combination of different CAR immune cell types (*e.g.*, T cells and NK cells) is used.

[0074] The methods summarized above and/or set forth herein 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."

[0075] In some examples, the disclosure provides:

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- [1.] A protein comprising a D Domain (DD) target binding domain wherein the DD is a member selected from the group consisting of:
 - (a) a DD that specifically binds BCMA and comprises the amino acid sequence of SEQ ID NO: 11-305, or 306;
 - (b) a DD that specifically binds CD123 and comprises the amino acid sequence of SEQ ID NO: 307-739, or 740;
 - (c) a DD that specifically binds AFP and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895;
 - (d) a DD that specifically binds AFP p26 and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895;
 - (e) a DD that specifically binds CS1 and comprises the amino acid sequence of SEQ ID NO: 896-909, or 910; and
 - (f) a DD that specifically binds HER2 and comprises the amino acid sequence of SEQ ID NO: 911-949, or 950;
- [2.] the protein of [1], wherein the DD is fused to a heterologous polypeptide;

WHAT IS CLAIMED IS:

1. A protein comprising a D Domain (DD) target binding domain wherein the DD is a member selected from the group consisting of:
 - (a) a DD that specifically binds BCMA and comprises the amino acid sequence of SEQ ID NO: 11-305, or 306;
 - (b) a DD that specifically binds CD123 and comprises the amino acid sequence of SEQ ID NO: 307-739, or 740;
 - (c) a DD that specifically binds AFP and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895;
 - (d) a DD that specifically binds AFP p26 and comprises the amino acid sequence of SEQ ID NO: 741-874, or 886-895;
 - (e) a DD that specifically binds CS1 and comprises the amino acid sequence of SEQ ID NO: 896-909, or 910; and
 - (f) a DD that specifically binds HER2 and comprises the amino acid sequence of SEQ ID NO: 911-949, or 950.
2. The protein of claim 1 wherein the DD is fused to a heterologous polypeptide.
3. The protein of claim 2, wherein the heterologous polypeptide comprises
 - (a) a full-length antibody or an antibody fragment;
 - (b) an Fc region;
 - (c) the extracellular domain, or a fragment of an extracellular domain, of a receptor selected from the group consisting of: BCMA, CD123, CS1, and CD19;
 - (d) a transmembrane domain;
 - (e) a membrane associating domain;
 - (f) human serum albumin or a fragment thereof;
 - (g) AFP or a fragment thereof;
 - (h) AFP p26 or a fragment thereof; and/or
 - (i) the extracellular domain of a receptor or a fragment thereof.
4. The protein according to any one of claims 1-3, which is labeled and/or conjugated to a therapeutic or cytotoxic agent, wherein optionally the label is selected from the group consisting of an enzymatic label, a fluorescent label, a luminescent label, a bioluminescent label and a biotin moiety.

5. A chimeric antigen receptor (CAR) which comprises a target binding domain comprising the protein according to any one of claims 1-3
6. The CAR of claim 5, which comprises, a target binding domain, a transmembrane domain, and an intracellular signaling domain, and optionally wherein
 - (a) the transmembrane domain comprises a 41BB or CD28 transmembrane domain;
 - (b) the intracellular signaling domain is selected from the group consisting of a domain of a human T cell receptor alpha, beta, or zeta chain; a human 41BB domain; a human CD28 domain; and any combination thereof; and/or
 - (c) the intracellular signaling domain comprises the intracellular domain of a costimulatory molecule selected from the group consisting of CD27, CD28, 41BB, OX40, CD30, CD40, PD1, lymphocyte function-associated antigen- 1 (LFA-1), CD2, CD7, LIGHT, NKG2C, NKG2D, B7-H3, a ligand that specifically binds with CD83, and any combination thereof.
7. The protein according to any one of claims 1-4 or the CAR according to claim 5 or claim 6, which further comprises a second target binding domain having the same or a different target than the DD target binding domain.
8. An isolated nucleic acid encoding the protein according to any one of claims 1-4 and 7 or the CAR according to any one of claims 5-7.
9. A vector comprising the nucleic acid of claim 8, wherein optionally the nucleic acid is operably linked with a nucleotide sequence which regulates the expression of the protein encoded by the nucleic acid, wherein optionally the vector is a lentiviral vector.
10. A host cell comprising the nucleic acid according to claim 8 or the vector according to claim 9, wherein optionally the cell is a T cell or a natural killer (NK) cell.
11. A pharmaceutical composition comprising the protein according to any one of claims 1-4 and 7, the CAR according to any one of claims 5-7, the nucleic acid of claim 8, the vector of claim 9, or the cell according to claim 10.
12. A kit comprising the protein according to any one of claims 1-4 and 7 or the CAR according to any one of claims 5-7.
13. Use of a protein according to any one of claims 1-4 and 7, the CAR according to any one of claims 5-7, the nucleic acid of claim 8, the vector of claim 9, the cell according to

- claim 10, or the pharmaceutical composition according to claim 11 in the manufacture of a medicament for treating cancer.
14. The use of claim 13, wherein
- (a) the cancer is a B cell malignancy selected from the group consisting of: a lymphoma (*e.g.*, a Hodgkin's lymphoma and non-Hodgkin's lymphoma (NHL), a leukemia, a plasmacytoma and a myeloma;
 - (b) the B cell malignancy is selected from the group consisting of: chronic lymphocytic leukaemia, follicular lymphoma, mantle cell lymphoma, and diffuse large B-cell lymphoma, plasmacytoma and multiple myeloma; and/or
 - (c) the cancer is a myeloid malignancy selected from the group consisting of: chronic myeloid leukemia, acute myeloid leukemia, leukemia, plasmacytoma and myeloma.
15. Use of a protein according to any one of claims 1-4 and 7, the CAR according to any one of claims 5-7, the nucleic acid of claim 8, the vector of claim 9, the cell according to claim 10, or the pharmaceutical composition according to claim 11 in the manufacture of a medicament for treating a B cell associated disorder (*e.g.*, monoclonal gammopathy of determined significance), (MGUS)).
16. Use of a protein according to any one of claims 1-4 and 7, the CAR according to any one of claims 5-7, the nucleic acid of claim 8, the vector of claim 9, the cell according to claim 10, or the pharmaceutical composition according to claim 11 in the manufacture of a medicament for treating a disorder of the immune system.
17. The use of claim 16, wherein the disorder of the immune system is an autoimmune disease, wherein the autoimmune disease is optionally rheumatoid arthritis.
18. Use of an immune cell comprising a chimeric antigen receptor (CAR) in the manufacture of a medicament for treating cancer in a subject, wherein the CAR comprises:
- (a) a target binding domain comprising a DD having an amino acid sequence selected from the group consisting of SEQ ID NO: 11-949, and 950, optionally wherein the DD specifically binds a target of interest expressed by a cancer cell;
 - (b) a transmembrane domain and an intracellular domain; wherein the intracellular domain comprises a signaling domain, and

wherein, upon administration to a subject, 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.

19. The use of claim 18, wherein the immune cell is a T cell or an NK cell.