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(54) Title: CD137 BINDING FIBRONECTIN TYPE III DOMAINS

(57) Abstract: FN3 domains that specifically bind to CD137, their conjugates, isolated nucleotides encoding the molecules, vectors, host cells, and methods of making and them are useful in therapeutic and diagnostic applications.

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CD137 BINDING FIBRONECTIN TYPE III DOMAINS

FIELD OF THE INVENTION

The present invention relates to fibronectin type III domains that specifically bind to cluster of differentiation 137 (CD137) and methods of making and using the molecules.

BACKGROUND OF THE INVENTION

Advances in understanding of the requirements for tumor antigen recognition and immune effector function indicate that a potential strategy to enhance an anti-tumor immune response is to provide co-stimulation through an auxiliary molecule. The current model for T-cell activation postulates that naive T-cells require two signals for full activation: (i) a signal provided through the binding of processed antigens presented to the T-cell receptor by major histocompatibility complex (MHC) class I molecules; and (ii) an additional signal provided by the interaction of co-stimulatory molecules on the surface of T-cells and their ligands on antigen presenting cells.

CD137 (4-1BB) is a member of the TNF receptor superfamily and is an activation-induced T-cell costimulatory molecule. The receptor was initially described in mice (B. Kwon et al., *P.N.A.S.* USA, 86:1963-7 (1989)), and later identified in humans (M. Alderson et al., *Eur. J. Immunol.,* 24: 2219-27 (1994); Z. Zhou et al., *Immunol. Lett.*, 45:67 (1995)). The interaction of CD137 and the CD137 ligand (4-1BBL) activates an important costimulatory pathway. Signaling via CD137 upregulates survival genes, enhances cell division, induces cytokine production, and prevents activation-induced cell death in T cells. The importance of the CD137 pathway has been underscored in a number of diseases, including cancer (see, e.g., U.S. Patent No.: 7,288,638).

Expression of CD137 has been shown to be predominantly on cells of lymphoid lineage such as activated T-cells, activated Natural Killer (NK) cells, NKT-cells, CD4CD25 regulatory T-cells, and also on activated thymocytes, and intraepithelial lymphocytes. In addition, CD137 has also been shown to be expressed on cells of myeloid origin like dendritic cells, monocytes, neutrophils,

and eosinophils. Even though CD137 expression is mainly restricted to immune/inflammatory cells, there have been reports describing its expression on endothelial cells associated with a small number of tissues from inflammatory sites and tumors.

The physiological events observed following CD137 stimulation on T-cells are mediated by NF-κB and PI3K/ERK1/2 signals with separate physiological functions. NF-κB signals trigger expression of Bcl-XL, an anti-apopotic molecule, thus resulting in increased survival, whereas PI3K and ERK1/2 signals are specifically responsible for CD137-mediated cell cycle progression (H. Lee et al., *J. Immunol.*, 169(9):4882-8 (2002)). The effect of CD137 activation on the inhibition of activation-induced cell death was shown *in vitro* by Hurtado et al. (J. Hurtado et al., *J. Immunol.,* 158(6):2600-9 (1997)), and in an *in vivo* system in which anti-CD137 monoclonal antibodies (mabs) were shown to produce long-term survival of superantigen-activated CD8+ T-cells by preventing clonal deletion (C. Takahashi et al., *J. Immunol.,* 162:5037 (1999)). Later, two reports demonstrated, under different experimental conditions, that the CD137 signal regulated both clonal expansion and survival of CD8+ T-cells (D. Cooper et al., *Eur. J. Immunol.,* 32(2):521-9 (2002); M. Maus et al., *Nat. Biotechnol.,* 20:143 (2002)).

Altogether, CD137 stimulation results in enhanced expansion, survival, and effector functions of newly primed CD8+ T-cells, acting, in part, directly on these cells. Both CD4+ and CD8+ T-cells have been shown to respond to CD137 stimulation, however, it appears that enhancement of T-cell function is greater in CD8+ cells ((W. Shuford et al., *J. Exp. Med.,* 186(1):47-55 (1997); I. Gramaglia et al., *Eur. J. Immunol.,* 30(2):392-402 (2000); C. Takahashi et al., *J. Immunol.,* 162:5037 (1999)). Based on the critical role of CD137 stimulation in CD8+ T-cell function and survival, agonism of the CD137/CD137L system provides a plausible approach for the treatment of tumors and viral pathogens.

Alternatively, while it has been shown that agonistic antibodies to CD137 and the ligand to CD137 enhance lymphocyte activation, the CD137 protein has the opposite effect. It inhibits proliferation of activated T lymphocytes and induces programmed cell death. These T cell-inhibitory activities of CD137 require

immobilisation of the protein, arguing for transmission of a signal through the ligand/coreceptor (Schwarz et al., *Blood* 87, 2839-2845 (1996); Michel et al., Immunology 98, 42-46 (1999)).

The known human CD137 ligand is expressed constitutively by monocytes and its expression is inducible in T lymphocytes (Alderson et al., *Eur. J. Immunol.* 24, 2219-2227 (1994)). Monocytes are activated by immobilised CD137 protein and their survival is profoundly prolonged by CD137. (Langstein et al., *J. Immunol.* 160, 2488-2494 (1998); Langstein et al., *J. Leuk. Biol.* 65, 829-833 (1999)). CD137 also induces proliferation in peripheral monocytes (Langstein et al., 1999b). Macrophage colony- stimulating factor (M-CSF) is essential for the proliferative and survival-enhancing activities of CD137 (Langstein et al., *J. Leuk. Biol.* 65, 829-833 (1999); Langstein et al., *Blood* 94, 3161-3168 (1999)).

Signalling through CD137 ligand has also been demonstrated in B cells where it enhances proliferation and immunoglobulin synthesis. This occurs at interactions of B cells with CD137-expressing T cells or follicular dendritic cells (Pauly et al., *J. Leuk. Biol.* 72, 35-42 (2002)). It was postulated that similarly to the CD40 receptor/ligand system, which mediates T cell help to B cells after first antigen encounter, the CD137 receptor/ligand system may mediate co-stimulation of B cells by FDC during affinity maturation (Pauly et al., *J. Leuk. Biol.* 72, 35-42 (2002)).

Furthermore, soluble forms of CD137 are generated by differential splicing and are selectively expressed by activated T cells (Michel et al., *Eur. J. Immunol.* 28, 290-295 (1998)). Soluble CD137 is antagonistic to membrane-bound or immobilised CD137, and levels of soluble CD137 correlate with activation induced cell death in T cells (DeBenedette et al., *J. Exp. Med.* 181, 985-992 (1995); Hurtado et al., *J. Immunol.* 155, 3360-3367 (1995); Michel et al., *Cytokine* 12, 742-746 (2000)).

Thus, considering the complicated picture for CD137 involvement in divergent mechanisms of action and different cell types, there exists a need for reagents to accurately detect CD137 in tumor tissues and other samples and for new therapeutics that modulate the interaction between CD137 and the 4-1BBL ligand or that modulate the interaction between CD137 and other cellular targets.

SUMMARY OF THE INVENTION

The invention provides an isolated FN3 domain that specifically binds to CD137 protein.

The invention also provides an isolated FN3 domain that specifically binds to CD137 protein comprising the amino acid sequence of SEQ ID NOs: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, or 224.

The invention also provides an isolated polynucleotide encoding the FN3 domain that specifically binds to CD137 protein.

The invention also provides a vector comprising the polynucleotide.

The invention also provides a host cell comprising the vector.

The invention also provides a method of producing the FN3 domain that specifically binds to CD137 protein, comprising culturing the isolated host cell under conditions that the FN3 domain that specifically binds to CD137 protein is expressed, and purifying the FN3 domain that specifically binds to CD137 protein.

The invention also provides a pharmaceutical composition comprising the FN3 domain that specifically binds to CD137 protein and a pharmaceutically acceptable carrier.

The invention also provides an anti-idiotypic antibody that specifically binds the FN3 domain that specifically binds to CD137 protein.

The invention also provides a kit comprising the FN3 domain.

The invention also provides a method of detecting CD137-expressing cancer cells in a tumor tissue, comprising

obtaining a sample of the tumor tissue from a subject; and

detecting whether CD137 protein is expressed in the tumor tissue by contacting the sample of the tumor tissue with the FN3 domain that specifically binds CD137 protein comprising the amino acid sequence of one of SEQ ID NOs: 45-224 and detecting the binding between CD137 protein and the FN3 domain.

The invention also provides a method of isolating CD137 expressing cells, comprising obtaining a sample from a subject;

contacting the sample with the FN3 domain that specifically binds to CD137 protein comprising the amino acid sequence of one of SEQ ID NOs: 45-224, and

isolating the cells bound to the FN3 domains.

The invention also provides a method of detecting CD137-expressing cancer cells in a tumor tissue, comprising

conjugating the FN3 domain that specifically binds to CD137 protein comprising the amino acid sequence of one of SEQ ID NOs: 45-224 to a detectable label to form a conjugate;

administering the conjugate to a subject; and

visualizing the CD137 expressing cancer cells to which the conjugate is bound.

DETAILED DESCRIPTION OF THE INVENTION

As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells, and the like.

"Fibronectin type III (FN3) domain" (FN3 domain) refers to a domain occurring frequently in proteins including fibronectins, tenascin, intracellular cytoskeletal proteins, cytokine receptors and prokaryotic enzymes (Bork and Doolittle, Proc Nat Acad Sci USA 89:8990-8994, 1992; Meinke *et al.*, J Bacteriol 175:1910-1918, 1993; Watanabe *et al.,* J Biol Chem 265:15659-15665, 1990). Exemplary FN3 domains are the 15 different FN3 domains present in human tenascin C, the 15 different FN3 domains present in human fibronectin (FN), and non-natural synthetic FN3 domains as described for example in U.S. Pat. No.

8,278,419. Individual FN3 domains are referred to by domain number and protein name, e.g., the 3rd FN3 domain of tenascin (TN3), or the 10th FN3 domain of fibronectin (FN10).

"Centyrin" refers to a FN3 domain that is based on the consensus sequence of the 15 different FN3 domains present in human tenascin C.

The term "capture agent" refers to substances that bind to a particular type of cells and enable the isolation of that cell from other cells. Exemplary capture agents are magnetic beads, ferrofluids, encapsulating reagents, molecules that bind the particular cell type and the like.

"Sample" refers to a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Exemplary samples are tissue biopsies, fine needle aspirations, surgically resected tissue, organ cultures, cell cultures and biological fluids such as blood, serum and serosal fluids, plasma, lymph, urine, saliva, cystic fluid, tear drops, feces, sputum, mucosal secretions of the secretory tissues and organs, vaginal secretions, ascites fluids, fluids of the pleural, pericardial, peritoneal, abdominal and other body cavities, fluids collected by bronchial lavage, synovial fluid, liquid solutions contacted with a subject or biological source, for example, cell and organ culture medium including cell or organ conditioned medium and lavage fluids and the like.

"Substituting" or "substituted" or 'mutating" or "mutated" refers to altering, deleting of inserting one or more amino acids or nucleotides in a polypeptide or polynucleotide sequence to generate a variant of that sequence.

"Variant" refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications for example, substitutions, insertions or deletions.

"Specifically binds" or "specific binding" refers to the ability of the FN3 domain of the invention to bind CD137 with a dissociation constant (K_D) of about $1x10^{-6}$ M or less, for example about $1x10^{-7}$ M or less, about $1x10^{-8}$ M or less, about $1x10^{-9}$ M or less, about $1x10^{-10}$ M or less, about $1x10^{-11}$ M or less, about $1x10^{-12}$ M or less, or about $1x10^{-13}$ M or less. Alternatively, "specific binding" refers to the ability of the FN3 domain of the invention to bind CD137 at least 5-fold above the negative control in standard ELISA assay. The isolated FN3 domain of the

invention that specifically binds CD137 may, however, have cross-reactivity to other related antigens, for example to the same predetermined antigen from other species (homologs), such as *Macaca Fascicularis* (cynomolgous monkey, cyno) *or Pan troglodytes* (chimpanzee).

"Library" refers to a collection of variants. The library may be composed of polypeptide or polynucleotide variants.

"Stability" refers to the ability of a molecule to maintain a folded state under physiological conditions such that it retains at least one of its normal functional activities, for example, binding to a predetermined antigen such as CD137.

"CD137" refers to human CD137 protein having the amino acid sequence of SEQ ID NO:44.

"Tencon" refers to the synthetic fibronectin type III (FN3) domain having the sequence shown in SEQ ID NO:1 and described in U.S. Pat. Publ. No. 2010/0216708.

A "cancer cell" or a "tumor cell" refers to a cancerous, pre-cancerous or transformed cell, either *in vivo*, *ex vivo*, and in tissue culture, that has spontaneous or induced phenotypic changes that do not necessarily involve the uptake of new genetic material. Although transformation can arise from infection with a transforming virus and incorporation of new genomic nucleic acid, or uptake of exogenous nucleic acid, it can also arise spontaneously or following exposure to a carcinogen, thereby mutating an endogenous gene. Transformation/cancer is exemplified by, e.g., morphological changes, immortalization of cells, aberrant growth control, foci formation, proliferation, malignancy, tumor specific markers levels, invasiveness, tumor growth or suppression in suitable animal hosts such as nude mice, and the like, *in vitro*, *in vivo*, and *ex vivo* (Freshney, Culture of Animal Cells: A Manual of Basic Technique (3rd ed. 1994)).

"Vector" refers to a polynucleotide capable of being duplicated within a biological system or that can be moved between such systems. Vector polynucleotides typically contain elements, such as origins of replication, polyadenylation signal or selection markers that function to facilitate the duplication or maintenance of these polynucleotides in a biological system.

Examples of such biological systems may include a cell, virus, animal, plant, and reconstituted biological systems utilizing biological components capable of duplicating a vector. The polynucleotide comprising a vector may be DNA or RNA molecules or a hybrid of these.

"Expression vector" refers to a vector that can be utilized in a biological system or in a reconstituted biological system to direct the translation of a polypeptide encoded by a polynucleotide sequence present in the expression vector.

"Polynucleotide" refers to a synthetic molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. cDNA is a typical example of a polynucleotide.

"Polypeptide" or "protein" refers to a molecule that comprises at least two amino acid residues linked by a peptide bond to form a polypeptide. Small polypeptides of less than about 50 amino acids may be referred to as "peptides".

"Valent" refers to the presence of a specified number of binding sites specific for an antigen in a molecule. As such, the terms "monovalent", "bivalent", "tetravalent", and "hexavalent" refer to the presence of one, two, four and six binding sites, respectively, specific for an antigen in a molecule.

"Subject" includes any human or nonhuman animal. "Nonhuman animal" includes all vertebrates, *e.g.,* mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows, chickens, amphibians, reptiles, etc. Except when noted, the terms "patient" or "subject" are used interchangeably.

"Isolated" refers to a homogenous population of molecules (such as synthetic polynucleotides or a polypeptide such as FN3 domains) which have been substantially separated and/or purified away from other components of the system the molecules are produced in, such as a recombinant cell, as well as a protein that has been subjected to at least one purification or isolation step. "Isolated FN3 domain" refers to an FN3 domain that is substantially free of other cellular material and/or chemicals and encompasses FN3 domains that are isolated to a higher purity, such as to 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% purity.

Compositions of matter

The present invention provides fibronectin type III (FN3) domains that specifically bind human CD137 protein (SEQ ID NO:44). These molecules can be used in therapeutic and diagnostic applications and in imaging. The present invention provides polynucleotides encoding the FN3 domains of the invention or complementary nucleic acids thereof, vectors, host cells, and methods of making and using them.

The invention provides an isolated FN3 domain that specifically binds CD137.

The FN3 domain of the invention may bind CD137 with a dissociation constant (K_D) of less than about $1x10^{-7}$ M, for example less than about $1x10^{-8}$ M, less than about $1x10^{-9}$ M, less than about $1x10^{-10}$ M, less than about $1x10^{-11}$ M, less than about $1x10^{-12}$ M, or less than about $1x10^{-13}$ M as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art. The measured affinity of a particular FN3 domain-antigen interaction can vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g., K_D, K_{on}, K_{off}) are made with standardized solutions of protein scaffold and antigen, and a standardized buffer, such as the buffer described herein.

The FN3 domain of the invention may bind CD137 at least 5-fold above the signal obtained for a negative control in standard ELISA assay.

In some embodiments, the FN3 domain that specifically binds CD137 comprises an initiator methionine (Met) linked to the N-terminus of the molecule.

In some embodiments, the FN3 domain that specifically binds CD137 comprises a cysteine (Cys) linked to a C-terminus of the FN3 domain.

The addition of the N-terminal Met and/or the C-terminal Cys may facilitate expression and/or conjugation of half-life extending molecules.

In some embodiments, the FN3 domain that specifically binds CD137 is internalized into a cell.

Internalization of the FN3 domain may facilitate delivery of a cytotoxic agent into tumor cells.

In some embodiments, the FN3 domain that specifically binds CD137 inhibits binding of the CD137 ligand (4-1BBL) to CD137.

Inhibition of binding of 4-1BBL to CD137 by the FN3 domains of the invention may be assessed using competition ELISA. In an exemplary assay, 1 μg/ml recombinant human CD137 is bound on wells of microtiter plates, the wells are washed and blocked, and 10 μg/ml of the test FN3 domain is added. Without washing, 7.5 μg/ml 4-1BBL is added into the wells and incubated for 30 min, after which 0.5 μg/ml anti-4-1BBL antibodies are added and incubated for 30 min. The plates are washed and 0.5 μg/mL neutravidin-HRP conjugate polyclonal antibody is added and incubated for 30 minutes. The plates are washed and POD Chemiluminescence substrate added immediately prior to reading the luminescence signal. The FN3 domains of the invention inhibit binding of 4-1BBL to CD137 when the binding of 4-1BBL is reduced by at least about 80%, 85%, 90%, 95% or 100%.

In some embodiments, the FN3 domain that specifically binds CD137 is a CD137 antagonist.

In some embodiments, the FN3 domain that specifically binds CD137 is a CD137 agonist.

"Antagonist" refers to a FN3 domain that specifically binds CD137 that suppresses at least one activity of CD137 function by inhibiting CD137 binding to to its natural ligand 4-1BBl or inhibiting CD137 binding to other molecules. A molecule is an antagonist when the at least one reaction or activity is suppressed by at least about 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% more than the at least one reaction or activity suppressed in the absence of the antagonist (*e.g.*, negative control), or when the suppression is statistically significant when compared to the suppression in the absence of the antagonist. A typical reaction or activity that is induced by 4-1BBL binding to CD137 is upregulation of survival genes, enhanced cell division, induced cytokine production, and prevention of activation-induced cell death in T cells.

The antagonistic FN3 domains that specifically bind CD137 may be used in the treatment of autoimmune or inflammatory diseases and in general diseases in which suppression of T cell responses is desirable.

"Agonist" refers to a FN3 domain that specifically binds CD137 that induces at least one reaction or activity that is induced by CD137. The FN3 domain is an agonist when the at least one reaction or activity is induced by at least about 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100% greater than the at least one reaction or activity induced in the absence of the agonist (*e.g.*, negative control), or when the induction is statistically significant when compared to the induction in the absence of the agonist. A typical reaction or activity that is induced by 4-1BBL binding to CD137 is upregulation of survival genes, enhanced cell division, induced cytokine production, and prevention of activation-induced cell death in T cells.

The agonistic FN3 domains that specifically bind CD137 may be used, for example, in the treatment of cancer or viral infections and in general in treatment of diseases in which activation of T cell responses is desirable.

In some embodiments, the FN3 domain that specifically binds CD137 does not inhibit 4-1BBL binding to CD137.

In some embodiments, the FN3 domain that specifically binds CD137 is based on Tencon sequence of SEQ ID NO:1 or Tencon 27 sequence of SEQ ID NO:4, optionally having substitutions at residues positions 11, 14, 17, 37, 46, 73, or 86 (residue numbering corresponding to SEQ ID NO:4).

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NOs: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, or 224.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:45.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:46.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:47.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:48.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:49.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:50.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:51.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:52.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:53.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:54.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:55.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:56.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:57.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:58.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:59.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:60.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:61.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:62.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:63.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:64.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:65.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:66.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:67.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:68.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:69.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:70.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:71.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:72.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:73.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:74.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:75.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:76.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:77.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:78.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:79.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:80.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:81.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:82.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:83.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:84.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:85.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:86.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:87.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:88.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:89.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:90.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:91.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:92.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:93.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:94.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:95.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:96.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:97.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:98.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:99.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:100.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:101.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:102.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:103.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:104.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:105.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:106.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:107.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:108.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:109.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:110.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:111.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:112.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:113.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:114.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:115.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:116.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:117.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:118.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:119.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:120.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:121.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:122.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:123.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:124.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:125.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:126.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:127.

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The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:130.

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The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:141.

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The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:181.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:182.

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The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:200.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:201.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:202.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:203.

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The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:209.

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The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:211.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:212.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:213.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:214.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:215.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:216.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:217.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:218.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:219.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:220.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:221.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:222.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:223.

The invention also provides an isolated FN3 domain that specifically binds CD137 comprising the amino acid sequence of SEQ ID NO:224.

In some embodiments, the isolated FN3 domain that specifically binds CD137 comprises an initiator methionine (Met) linked to the N-terminus of the molecule.

In some embodiments, the isolated FN3 domain that specifically binds CD137 comprises an amino acid sequence that is 62%, 63%, 64% , 65%, 66%,

67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to one of the amino acid sequences of SEQ ID NOs: 45-224.

Conjugates of the FN3 domains that specifically bind CD137 of the invention

The invention also provides an isolated FN3 domain that specifically binds CD137 conjugated to a heterologous molecule(s).

In some embodiments, the heterologous molecule is a detectable label or a cytotoxic agent.

The invention also provides an FN3 domain that specifically binds CD137 conjugated to a detectable label.

The invention also provides an FN3 domain that specifically binds CD137 conjugated to a cytotoxic agent.

In some embodiments, the detectable label is also a cytotoxic agent.

The FN3 domains that specifically bind CD137 of the invention conjugated to a detectable label can be used to evaluate expression of CD137 on samples such as tumor tissue *in vivo* or *in vitro*.

Detectable label includes compositions that when conjugated to the FN3 domains that specifically bind CD137 of the invention renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means.

Exemplary detectable labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes, electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, haptens, luminescent molecules, chemiluminescent molecules, fluorochromes, fluorophores, fluorescent quenching agents, colored molecules, radioactive isotopes, cintillants, avidin, streptavidin, protein A, protein G, antibodies or fragments thereof, polyhistidine, Ni^{2+}, Flag tags, myc tags, heavy metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron donors/acceptors, acridinium esters, and colorimetric substrates.

A detectable label may emit a signal spontaneously, such as when the detectable label is a radioactive isotope. In other cases the detectable label emits a signal as a result of being stimulated by an external field.

Exemplary radioactive isotopes may be γ-emitting, Auger-emitting, β-emitting, an alpha-emitting or positron-emitting radioactive isotope. Exemplary radioactive isotopes include ^{3}H, ^{11}C, ^{13}C, ^{15}N, ^{18}F, ^{19}F, ^{55}Co, ^{57}Co, ^{60}Co, ^{61}Cu, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{68}Ga, ^{72}As, ^{75}Br, ^{86}Y, ^{89}Zr, ^{90}Sr, ^{94m}Tc, ^{99m}Tc, ^{115}In, $^{123}1$, $^{124}1$, ^{125}I, $^{131}1$, ^{211}At, ^{212}Bi, ^{213}Bi, ^{223}Ra, ^{226}Ra, ^{225}Ac and ^{227}Ac.

Exemplary metal atoms are metals with an atomic number greater than 20, such as calcium atoms, scandium atoms, titanium atoms, vanadium atoms, chromium atoms, manganese atoms, iron atoms, cobalt atoms, nickel atoms, copper atoms, zinc atoms, gallium atoms, germanium atoms, arsenic atoms, selenium atoms, bromine atoms, krypton atoms, rubidium atoms, strontium atoms, yttrium atoms, zirconium atoms, niobium atoms, molybdenum atoms, technetium atoms, ruthenium atoms, rhodium atoms, palladium atoms, silver atoms, cadmium atoms, indium atoms, tin atoms, antimony atoms, tellurium atoms, iodine atoms, xenon atoms, cesium atoms, barium atoms, lanthanum atoms, hafnium atoms, tantalum atoms, tungsten atoms, rhenium atoms, osmium atoms, iridium atoms, platinum atoms, gold atoms, mercury atoms, thallium atoms, lead atoms, bismuth atoms, francium atoms, radium atoms, actinium atoms, cerium atoms, praseodymium atoms, neodymium atoms, promethium atoms, samarium atoms, europium atoms, gadolinium atoms, terbium atoms, dysprosium atoms, holmium atoms, erbium atoms, thulium atoms, ytterbium atoms, lutetium atoms, thorium atoms, protactinium atoms, uranium atoms, neptunium atoms, plutonium atoms, americium atoms, curium atoms, berkelium atoms, californium atoms, einsteinium atoms, fermium atoms, mendelevium atoms, nobelium atoms, or lawrencium atoms.

In some embodiments, the metal atoms may be alkaline earth metals with an atomic number greater than twenty.

In some embodiments, the metal atoms may be lanthanides.

In some embodiments, the metal atoms may be actinides.

In some embodiments, the metal atoms may be transition metals.

In some embodiments, the metal atoms may be poor metals.

In some embodiments, the metal atoms may be gold atoms, bismuth atoms, tantalum atoms, and gadolinium atoms.

In some embodiments, the metal atoms may be metals with an atomic number of 53 (i.e., iodine) to 83 (i.e., bismuth).

In some embodiments, the metal atoms may be atoms suitable for magnetic resonance imaging.

The metal atoms may be metal ions in the form of +1 , +2, or +3 oxidation states, such as Ba^{2+}, Bi^{3+}, Cs^{+}, Ca^{2+}, Cr^{2+}, Cr^{3+}, Cr^{6+}, Co^{2+}, Co^{3+}, Cu^{+}, Cu^{2+}, Cu^{3+}, Ga^{3+}, Gd^{3+}, Au^{+}, Au^{3+}, Fe^{2+}, Fe^{3+}, F^{3+}, Pb^{2+}, Mn^{2+}, Mn^{3+}, Mn^{4+}, Mn^{7+}, Hg^{2+}, Ni^{2+}, Ni^{3+}, Ag^{+}, Sr^{2+}, Sn^{2+}, Sn^{4+}, and Zn^{2+}. The metal atoms may comprise a metal oxide, such as iron oxide, manganese oxide, or gadolinium oxide.

Suitable dyes include any commercially available dyes such as, for example, 5(6)-carboxyfluorescein, IRDye 680RD maleimide or IRDye 800CW, ruthenium polypyridyl dyes, and the like.

Suitable fluorophores are fluorescein isothiocyante (FITC), fluorescein thiosemicarbazide, rhodamine, Texas Red, CyDyes (e.g., Cy3, Cy5, Cy5.5), Alexa Fluors (e.g., Alexa488, Alexa555, Alexa594; Alexa647), near infrared (NIR) (700-900 nm) fluorescent dyes, and carbocyanine and aminostyryl dyes.

The FN3 domains that specifically bind CD137 conjugated to a detectable label may be used as an imaging agent to evaluate tumor distribution, diagnosis for the presence of tumor cells and/or, recurrence of tumor.

In some embodiments, the FN3 domains that specifically bind CD137 of the invention are conjugated to a cytotoxic agent.

In some embodiments, the cytotoxic agent is a chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate).

The FN3 domains that specifically bind CD137 conjugated to a cytotoxic agent of the invention may be used in the targeted delivery of the cytotoxic agent to CD137 expressing tumor cell, and intracellular accumulation therein, wherein

systemic administration of these unconjugated cytotoxic agents may result in unacceptable levels of toxicity to normal cells.

In some embodiments, the cytotoxic agent is daunomycin, doxorubicin, methotrexate, vindesine, bacterial toxins such as diphtheria toxin, ricin, geldanamycin, maytansinoids or calicheamicin. The cytotoxic agent may elict their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

In some embodiments, the cytotoxic agent is an enzymatically active toxins such as diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, *sapaonaria officinalis* inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In some embodiments, the cytotoxic agent is a radionuclide, such as ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

In some embodiments, the cytotoxic agent is dolastatins or dolostatin peptidic analogs and derivatives, auristatin or monomethyl auristatin phenylalanine. Exemplary molecules are disclosed in U.S. Pat No. 5,635,483 and 5,780,588. Dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob Agents and Chemother. 45(12):3580-3584) and have anticancerand antifungal activity. The dolastatin or auristatin drug moiety may be attached to the FN3 domain of the invention through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172), or via any cysteine engineered into the FN3 domain.

The FN3 domains that specifically bind CD137 of the invention may be conjugated to a detectable label using known methods.

In some embodiments, the detectable label is complexed with a chelating agent.

In some embodiments, the detectable label is conjugated to the FN3 domain that specifically binds CD137 of the invention via a linker.

The detectable label or the cytotoxic moiety may be linked directly, or indirectly, to the FN3 domain that specifically binds CD137 of the invention using known methods. Suitable linkers are known in the art and include, for example, prosthetic groups, non-phenolic linkers (derivatives of N-succimidyl-benzoates; dodecaborate), chelating moieties of both macrocyclics and acyclic chelators, such as derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10,tetraacetic acid (DOTA), derivatives of diethylenetriaminepentaacetic avid (DTPA), derivatives of S-2-(4-Isothiocyanatobenzyl)-1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and derivatives of 1,4,8,11-tetraazacyclodocedan-1,4,8,11-tetraacetic acid (TETA), N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene) and other chelating moieties. Suitable peptide linkers are well known.

In some embodiment, the FN3 domain that specifically binds CD137 is removed from the blood via renal clearance.

Isolation of CD137 binding FN3 domains from a library based on Tencon sequence

Tencon (SEQ ID NO:1) is a non-naturally occurring fibronectin type III (FN3) domain designed from a consensus sequence of fifteen FN3 domains from human tenascin-C (Jacobs *et al.*, Protein Engineering, Design, and Selection, 25:107-117, 2012; U.S. Pat. Publ. No. 2010/0216708). The crystal structure of Tencon shows six surface-exposed loops that connect seven beta-strands as is characteristic to the FN3 domains, the beta-strands referred to as A, B, C, D, E, F, and G, and the loops referred to as AB, BC, CD, DE, EF, and FG loops (Bork and Doolittle, Proc Natl Acad Sci USA 89:8990-8992, 1992; U.S. Pat. No. 6,673,901). These loops, or selected residues within each loop, may be randomized in order to construct libraries of fibronectin type III (FN3) domains that may be used to select

novel molecules that bind CD137. **Table 1** shows positions and sequences of each loop and beta-strand in Tencon (**SEQ ID NO:1**).

Library designed based on Tencon sequence may thus have randomized FG loop, or randomized BC and FG loops, such as libraries TCL1 or TCL2 as described below. The Tencon BC loop is 7 amino acids long, thus 1, 2, 3, 4, 5, 6 or 7 amino acids may be randomized in the library diversified at the BC loop and designed based on Tencon sequence. The Tencon FG loop is 7 amino acids long, thus 1, 2, 3, 4, 5, 6 or 7 amino acids may be randomized in the library diversified at the FG loop and designed based on Tencon sequence. Further diversity at loops in the Tencon libraries may be achieved by insertion and/or deletions of residues at loops. For example, the FG and/or BC loops may be extended by 1-22 amino acids, or decreased by 1-3 amino acids. The FG loop in Tencon is 7 amino acids long, whereas the corresponding loop in antibody heavy chains ranges from 4-28 residues. To provide maximum diversity, the FG loop may be diversified in sequence as well as in length to correspond to the antibody CDR3 length range of 4-28 residues. For example, the FG loop can further be diversified in length by extending the loop by additional 1, 2, 3, 4 or 5 amino acids.

Library designed based on Tencon sequence may also have randomized alternative surfaces that form on a side of the FN3 domain and comprise two or more beta strands, and at least one loop. One such alternative surface is formed by amino acids in the C and the F beta-strands and the CD and the FG loops (a C-CD-F-FG surface). A library design based on Tencon alternative C-CD-F-FG surface is described in U.S. Pat. Publ. No. 2013/0226834. Library designed based on Tencon sequence also includes libraries designed based on Tencon variants, such as Tencon variants having substitutions at residues positions 11, 14, 17, 37, 46, 73, or 86 (residue numbering corresponding to SEQ ID NO:1), and which variants display improve thermal stability. Exemplary Tencon variants are described in US Pat. Publ. No. 2011/0274623, and include Tencon27 (SEQ ID NO:4) having substitutions E11R, L17A, N46V and E86I when compared to Tencon of SEQ ID NO:1.

Table 1. Tencon topology

FN3 domain	Tencon (SEQ ID NO:1)
A strand	1-12
AB loop	13-16
B strand	17-21
BC loop	22-28
C strand	29-37
CD loop	38-43
D strand	44-50
DE loop	51-54
E strand	55-59
EF loop	60-64
F strand	65-74
FG loop	75-81
G strand	82-89

Tencon and other FN3 sequence based libraries may be randomized at chosen residue positions using a random or defined set of amino acids. For example, variants in the library having random substitutions may be generated using NNK codons, which encode all 20 naturally occurring amino acids. In other diversification schemes, DVK codons may be used to encode amino acids Ala, Trp, Tyr, Lys, Thr, Asn, Lys, Ser, Arg, Asp, Glu, Gly, and Cys. Alternatively, NNS codons may be used to give rise to all 20 amino acid residues and simultaneously reducing the frequency of stop codons. Libraries of FN3 domains with biased amino acid distribution at positions to be diversified may be synthesized for example using Slonomics® technology (http:_//www_sloning_com). This technology uses a library of pre-made double stranded triplets that act as universal building blocks sufficient for thousands of gene synthesis processes. The triplet library represents all possible sequence combinations necessary to build any desired DNA molecule. The codon designations are according to the well-known IUB code.

The FN3 domains that specifically bind CD137 of the invention may be isolated by producing the FN3 library such as the Tencon library using *cis* display to ligate DNA fragments encoding the scaffold proteins to a DNA fragment encoding RepA to generate a pool of protein-DNA complexes formed after *in vitro* translation wherein each protein is stably associated with the DNA that encodes it (U.S. Pat. No. 7,842,476; Odegrip *et al.,* Proc Natl Acad Sci U S A 101, 2806-2810, 2004), and assaying the library for specific binding to PSMA by any method known in the art and described in the Example. Exemplary well known methods which can be used are ELISA, sandwich immunoassays, and competitive and non-competitive assays (see, e.g., Ausubel *et al.,* eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York). The identified FN3 domains that specifically bind CD137 are further characterized for their binding to CD137, modulation of CD137 activity, internalization, stability, and other desired characteristics.

The FN3 domains that specifically bind CD137 of the invention may be generated using any FN3 domain as a template to generate a library and screening the library for molecules specifically binding CD137 using methods provided within. Exemplar FN3 domains that may be used are the 3rd FN3 domain of tenascin C (TN3) (SEQ ID NO:32), Fibcon (SEQ ID NO:33), and the 10th FN3 domain of fibronectin (FN10) (SEQ ID NO:34). Standard cloning and expression techniques are used to clone the libraries into a vector or synthesize double stranded cDNA cassettes of the library, to express, or to translate the libraries *in vitro*. For example ribosome display (Hanes and Pluckthun, Proc Natl Acad Sci USA, 94, 4937-4942, 1997), mRNA display (Roberts and Szostak, Proc Natl Acad Sci USA, 94, 12297-12302, 1997), or other cell-free systems (U.S. Pat. No. 5,643,768) can be used. The libraries of the FN3 domain variants may be expressed as fusion proteins displayed on the surface for example of any suitable bacteriophage. Methods for displaying fusion polypeptides on the surface of a bacteriophage are well known (U.S. Pat. Publ. No. 2011/0118144; Int. Pat. Publ. No. WO2009/085462; U.S. Pat. No. 6,969,108; U.S. Pat. No. 6,172,197; U.S. Pat. No. 5,223,409; U.S. Pat. No. 6,582,915; U.S. Pat. No. 6,472,147).

In some embodiments. the FN3 domain that specifically binds CD137 is based on Tencon sequence of SEQ ID NO:1 or Tencon27 sequence of SEQ ID NO:4, the SEQ ID NO:1 or the SEQ ID NO:4, optionally having substitutions at residues positions 11, 14, 17, 37, 46, 73, and/or 86.

The FN3 domains that specifically bind CD137 of the invention may be modified to improve their properties such as improve thermal stability and reversibility of thermal folding and unfolding. Several methods have been applied to increase the apparent thermal stability of proteins and enzymes, including rational design based on comparison to highly similar thermostable sequences, design of stabilizing disulfide bridges, mutations to increase alpha-helix propensity, engineering of salt bridges, alteration of the surface charge of the protein, directed evolution, and composition of consensus sequences (Lehmann and Wyss, Curr Opin Biotechnol, 12, 371-375, 2001). High thermal stability may increase the yield of the expressed protein, improve solubility or activity, decrease immunogenicity, and minimize the need of a cold chain in manufacturing. Residues that may be substituted to improve thermal stability of Tencon (SEQ ID NO:1) are residue positions 11, 14, 17, 37, 46, 73, or 86, and are described in US Pat. Publ. No. 2011/0274623. Substitutions corresponding to these residues may be incorporated to the FN3 domain containing molecules of the invention.

Measurement of protein stability and protein lability can be viewed as the same or different aspects of protein integrity. Proteins are sensitive or “labile” to denaturation caused by heat, by ultraviolet or ionizing radiation, changes in the ambient osmolarity and pH if in liquid solution, mechanical shear force imposed by small pore-size filtration, ultraviolet radiation, ionizing radiation, such as by gamma irradiation, chemical or heat dehydration, or any other action or force that may cause protein structure disruption. The stability of the molecule can be determined using standard methods. For example, the stability of a molecule can be determined by measuring the thermal melting ("T_m") temperature, the temperature in ° Celsius (°C) at which half of the molecules become unfolded, using standard methods. Typically, the higher the T_m, the more stable the molecule. In addition to heat, the chemical environment also changes the ability of the protein to maintain a particular three dimensional structure.

In one embodiment, the FN3 domain that specifically binds CD137 of the invention may exhibit increased stability by at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% or more compared to the same domain prior to engineering measured by the increase in the T_m.

Chemical denaturation can likewise be measured by a variety of methods. Chemical denaturants include guanidinium hydrochloride, guanidinium thiocyanate, urea, acetone, organic solvents (DMF, benzene, acetonitrile), salts (ammonium sulfate, lithium bromide, lithium chloride, sodium bromide, calcium chloride, sodium chloride); reducing agents (e.g. dithiothreitol, beta-mercaptoethanol, dinitrothiobenzene, and hydrides, such as sodium borohydride), non-ionic and ionic detergents, acids (e.g. hydrochloric acid (HCl), acetic acid (CH_3COOH), halogenated acetic acids), hydrophobic molecules (e.g. phosopholipids), and targeted denaturants. Quantitation of the extent of denaturation can rely on loss of a functional property, such as ability to bind a target molecule, or by physiochemical properties, such as tendency to aggregation, exposure of formerly solvent inaccessible residues, or disruption or formation of disulfide bonds.

The FN3 domain that specifically binds CD137 may be generated as monomers, dimers, or multimers, for example, as a means to increase the valency and thus the avidity of target molecule binding, or to generate bi- or multispecific scaffolds simultaneously binding two or more different target molecules. The dimers and multimers may be generated by linking monospecific, bi- or multispecific protein scaffolds, for example, by the inclusion of an amino acid linker, for example a linker containing poly-glycine, glycine and serine, or alanine and proline. Exemplary linker include $(GS)_2$, (SEQ ID NO:35), $(GGGS)_2$ (SEQ ID NO:36), $(GGGGS)_5$ (SEQ ID NO:37), $(AP)_2$ (SEQ ID NO:38), $(AP)_5$ (SEQ ID NO:39), $(AP)_{10}$ (SEQ ID NO:40), $(AP)_{20}$ (SEQ ID NO:41) and $A(EAAAK)_5AAA$ (SEQ ID NO:42). The dimers and multimers may be linked to each other in a N-to C-direction. The use of naturally occurring as well as artificial peptide linkers to connect polypeptides into novel linked fusion polypeptides is well known in the literature (Hallewell *et al., J Biol Chem* 264, 5260-5268, 1989; Alfthan *et al.,*

Protein Eng. 8, 725-731, 1995; Robinson & Sauer, *Biochemistry* 35, 109-116, 1996; U.S. Pat. No. 5,856,456).

Half-life extending moieties

The FN3 domains that specifically bind CD137 may incorporate other subunits for example via covalent interaction. In one aspect of the invention, the FN3 domains that specifically bind CD137 further comprise a half-life extending moiety. Exemplary half-life extending moieties are albumin, albumin variants, albumin-binding proteins and/or domains, transferrin and fragments and analogues thereof, and Fc regions. An exemplary albumin variant is shown in SEQ ID NO:**43**. Amino acid sequences of the human Fc regions are well known, and include IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE Fc regions.

All or a portion of an antibody constant region may be attached to the FN3 domain that specifically binds CD137 to impart antibody-like properties, especially those properties associated with the Fc region, such as Fc effector functions such as C1q binding, complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, down regulation of cell surface receptors (e.g., B cell receptor; BCR), and may be further modified by modifying residues in the Fc responsible for these activities (for review; see Strohl, *Curr Opin Biotechnol.* 20, 685-691, 2009).

Additional moieties may be incorporated into the FN3 domains that specifically bind CD137 such as polyethylene glycol (PEG) molecules, such as PEG5000 or PEG20,000, fatty acids and fatty acid esters of different chain lengths, for example laurate, myristate, stearate, arachidate, behenate, oleate, arachidonate, octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like, polylysine, octane, carbohydrates (dextran, cellulose, oligo- or polysaccharides) for desired properties. These moieties may be direct fusions with the protein scaffold coding sequences and may be generated by standard cloning and expression techniques. Alternatively, well known chemical coupling methods may be used to attach the moieties to recombinantly produced molecules of the invention.

A pegyl moiety may for example be added to the FN3 domain that specifically binds CD137 by incorporating a cysteine residue to the C-terminus of the molecule, or engineering cysteines into residue positions that face away from the CD137 binding face of the molecule, and attaching a pegyl group to the cysteine using well known methods.

FN3 domains that specifically bind CD137 incorporating additional moieties may be compared for functionality by several well-known assays. For example, altered properties due to incorporation of Fc domains and/or Fc domain variants may be assayed in Fc receptor binding assays using soluble forms of the receptors, such as the FcγRI, FcγRII, FcγRIII or FcRn receptors, or using well known cell-based assays measuring for example ADCC or CDC, or evaluating pharmacokinetic properties of the molecules of the invention in *in vivo* models.

Polynucleotides, vectors, host cells

The invention also provides nucleic acids encoding the FN3 domains specifically binding CD137 as isolated polynucleotides or as portions of expression vectors or as portions of linear DNA sequences, including linear DNA sequences used for *in vitro* transcription/translation, vectors compatible with prokaryotic, eukaryotic or filamentous phage expression, secretion and/or display of the compositions or directed mutagens thereof. Certain exemplary polynucleotides are disclosed herein, however, other polynucleotides which, given the degeneracy of the genetic code or codon preferences in a given expression system, encode the FN3 domains of the invention are also within the scope of the invention.

The invention also provides an isolated polynucleotide encoding the FN3 domain specifically binding CD137 comprising the amino acid sequence of SEQ ID NOs: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170,

171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, or 224.

The polynucleotides of the invention may be produced by chemical synthesis such as solid phase polynucleotide synthesis on an automated polynucleotide synthesizer and assembled into complete single or double stranded molecules. Alternatively, the polynucleotides of the invention may be produced by other techniques such as PCR followed by routine cloning. Techniques for producing or obtaining polynucleotides of a given known sequence are well known in the art.

The polynucleotides of the invention may comprise at least one non-coding sequence, such as a promoter or enhancer sequence, intron, polyadenylation signal, a *cis* sequence facilitating RepA binding, and the like. The polynucleotide sequences may also comprise additional sequences encoding additional amino acids that encode for example a marker or a tag sequence such as a histidine tag or an HA tag to facilitate purification or detection of the protein, a signal sequence, a fusion protein partner such as RepA, Fc or bacteriophage coat protein such as pIX or pIII.

The invention also provides a vector comprising at least one polynucleotide of the invention. Such vectors may be plasmid vectors, viral vectors, vectors for baculovirus expression, transposon based vectors or any other vector suitable for introduction of the polynucleotides of the invention into a given organism or genetic background by any means. Such vectors may be expression vectors comprising nucleic acid sequence elements that can control, regulate, cause or permit expression of a polypeptide encoded by such a vector. Such elements may comprise transcriptional enhancer binding sites, RNA polymerase initiation sites, ribosome binding sites, and other sites that facilitate the expression of encoded polypeptides in a given expression system. Such expression systems may be cell-based, or cell-free systems well known in the art.

The invention also provides a host cell comprising the vector of the invention. The FN3 domain that specifically bind CD137 may be optionally

produced by a cell line, a mixed cell line, an immortalized cell or clonal population of immortalized cells, as well known in the art. See, e.g., Ausubel, *et al.,* ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, NY (1987-2001); Sambrook, *et al.,* Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, *et al.,* eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan *et al.,* Current Protocols in Protein Science, John Wiley & Sons, NY, NY, (1997-2001).

The host cell chosen for expression may be of mammalian origin or may be selected from COS-1, COS-7, HEK293, BHK21, CHO, BSC-1, He G2, SP2/0, HeLa, myeloma, lymphoma, yeast, insect or plant cells, or any derivative, immortalized or transformed cell thereof. Alternatively, the host cell may be selected from a species or organism incapable of glycosylating polypeptides, e.g. a prokaryotic cell or organism, such as BL21, BL21(DE3), BL21-GOLD(DE3), XL1-Blue, JM109, HMS174, HMS174(DE3), and any of the natural or engineered *E. coli spp, Klebsiella spp.,* or *Pseudomonas spp* strains.

The invention also provides a method of producing the isolated FN3 domain that specifically binds CD137, comprising culturing the isolated host cell of the invention under conditions such that the isolated FN3 domain that specifically binds CD137 is expressed, and purifying the FN3 domain.

The FN3 domains that specifically bind CD137 may be purified from recombinant cell cultures by well-known methods, for example by protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography, or high performance liquid chromatography (HPLC).

Anti-idiotypic antibodies

The present invention also provides an anti-idiotypic antibody binding to the FN3 domain.

The invention also provides an anti-idiotypic antibody that specifically binds the FN3 domain comprising the amino acid sequences of one of SEQ ID NOs: 45-224.

Kits

The invention also provides a kit comprising the FN3 domain that specifically binds CD137.

The kit may be used for therapeutic uses and as a diagnostic kit.

In some embodiments, the kit comprises the FN3 domain that specifically binds CD137 and reagents for detecting the FN3 domain. The kit can include one or more other elements including: instructions for use; other reagents, e.g., a label, an agent useful for chelating, or otherwise coupling, a radioprotective composition; devices or other materials for preparing the FN3 domain that specifically binds CD137 for administration for imaging, diagnostic or therapeutic purpose; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

In some embodiments, the kit comprises the FN3 domain that specifically binds CD137 comprising the amino acid sequences of one of SEQ ID NOs: 45-224.

Uses of CD137 binding FN3 domains of the invention

The FN3 domains that specifically bind CD137 may be used to diagnose, monitor, modulate, treat, alleviate, help prevent the incidence of, or reduce the symptoms of human disease or specific pathologies in cells, tissues, organs, fluid, or, generally, a host. The FN3 domains that specifically bind CD137 may also be used in imaging CD137 positive tumor tissue in a subject. The methods of the invention may be used with an animal patient belonging to any classification. Examples of such animals include mammals such as humans, rodents, dogs, cats and farm animals.

The invention provides a method of diagnosing a subject having, or who is likely to develop cancer of a tissue based on the expression of CD137 by cells of

the cancer tissue, methods of predicting success of immunotherapy, methods of prognosis, and methods of treatment.

The invention also provides a method of detecting CD137-expressing cancer cells in a tumor tissue, comprising

obtaining a sample of the tumor tissue from a subject;

detecting whether CD137 is expressed in the tumor tissue by contacting toe sample of the tumor tissues with the FN3 domain that specifically binds CD137 comprising the amino acid sequence of one of SEQ ID NOs: 45-224 and detecting the binding between CD137 and the FN3 domain.

The tissue can be tissue of any organ or anatomical system, for example lung, epithelial, connective, vascular, muscle, neural, skeletal, lymphatic, prostate, cervical, breast, spleen, gastric, intestinal, oral, esophageal, uterine, ovarian, renal or testicular tissue.

CD137 expression may be evaluated using known methods, such as immunohistochemistry or ELISA.

The invention also provides a method of isolating CD137 expressing cells, comprising

obtaining a sample from a subject;

contacting the sample with the FN3 domain that specifically binds CD137 comprising the amino acid sequence of one of SEQ ID NOs: 45-224, and

isolating the cells bound to the FN3 domains.

The invention also provides a method of detecting CD137-expressing cancer cells in a tumor tissue, comprising

conjugating the FN3 domain that specifically binds CD137 comprising the amino acid sequence of one of SEQ ID NOs: 45-224 to a detectable label to form a conjugate;

administering the conjugate to a subject; and

visualizing the CD137 expressing cancer cells to which the conjugate is bound.

The invention also provides a method of treating a subject having cancer, comprising administering to the subject a FN3 domain that specifically binds CD137 of the invention.

In some embodiments, the subject has a solid tumor.

In some embodiments, the subject has a hematological malignancy.

In some embodiments, the solid tumor is a melanoma.

In some embodiments, the solid tumor is a lung cancer.

In some embodiments, the solid tumor is a non-small cell lung cancer (NSCLC).

In some embodiments, the solid tumor is a squamous non-small cell lung cancer (NSCLC).

In some embodiments, the solid tumor is a non-squamous NSCLC.

In some embodiments, the solid tumor is a lung adenocarcinoma.

In some embodiments, the solid tumor is a renal cell carcinoma (RCC).

In some embodiments, the solid tumor is a mesothelioma.

In some embodiments, the solid tumor is a nasopharyngeal carcinoma (NPC).

In some embodiments, the solid tumor is a colorectal cancer.

In some embodiments, the solid tumor is a prostate cancer.

In some embodiments, the solid tumor is castration-resistant prostate cancer.

In some embodiments, the solid tumor is a stomach cancer.

In some embodiments, the solid tumor is an ovarian cancer.

In some embodiments, the solid tumor is a gastric cancer.

In some embodiments, the solid tumor is a liver cancer.

In some embodiments, the solid tumor is pancreatic cancer.

In some embodiments, the solid tumor is a thyroid cancer.

In some embodiments, the solid tumor is a squamous cell carcinoma of the head and neck.

In some embodiments, the solid tumor is a carcinomas of the esophagus or gastrointestinal tract.

In some embodiments, the solid tumor is a breast cancer.

In some embodiments, the solid tumor is a fallopian tube cancer.

In some embodiments, the solid tumor is a brain cancer.

In some embodiments, the solid tumor is an urethral cancer.

In some embodiments, the solid tumor is a genitourinary cancer.

In some embodiments, the solid tumor is an endometriosis.

In some embodiments, the solid tumor is a cervical cancer.

In some embodiments, the solid tumor is a metastatic lesion of the cancer.

In some embodiments, the hematological malignancy is a lymphoma, a myeloma or a leukemia.

In some embodiments, the hematological malignancy is a B cell lymphoma.

In some embodiments, the hematological malignancy is Burkitt's lymphoma.

In some embodiments, the hematological malignancy is Hodgkin's lymphoma.

In some embodiments, the hematological malignancy is a non-Hodgkin's lymphoma.

In some embodiments, the hematological malignancy is a myelodysplastic syndrome.

In some embodiments, the hematological malignancy is an acute myeloid leukemia (AML).

In some embodiments, the hematological malignancy is a chronic myeloid leukemia (CML).

In some embodiments, the hematological malignancy is a chronic myelomoncytic leukemia (CMML).

In some embodiments, the hematological malignancy is a multiple myeloma (MM).

In some embodiments, the hematological malignancy is a plasmacytoma. In some embodiments, the cancer is kidney cancer.

"Treat" or "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results

include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of the FN3 domains that specifically bind CD137 of the invention may vary according to factors such as the disease state, age, sex, and weight of the individual. Exemplary indicators of an effective FN3 domain that specifically binds CD137 is improved well-being of the patient, decrease or shrinkage of the size of a tumor, arrested or slowed growth of a tumor, and/or absence of metastasis of cancer cells to other locations in the body.

Administration/ Pharmaceutical Compositions

The invention provides for pharmaceutical compositions of the FN3 domains that specifically bind CD137, optionally conjugated to a detectable label or a cytotoxic drug of the invention and a pharmaceutically acceptable carrier. For therapeutic use, the FN3 domains that specifically bind CD137of the invention may be prepared as pharmaceutical compositions containing an effective amount of the domain or molecule as an active ingredient in a pharmaceutically acceptable carrier. "Carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the active compound is administered. Such vehicles can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine can be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate

physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the molecules of the invention in such pharmaceutical formulation can vary widely, *i.e.*, from less than about 0.5%, usually at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 21st Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, See especially pp. 958-989.

The mode of administration for therapeutic use of the FN3 domains of the invention may be any suitable route that delivers the agent to the host, such as parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, pulmonary; transmucosal (oral, intranasal, intravaginal, rectal), using a formulation in a tablet, capsule, solution, powder, gel, particle; and contained in a syringe, an implanted device, osmotic pump, cartridge, micropump; or other means appreciated by the skilled artisan, as well known in the art. Site specific administration may be achieved by for example intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intracardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravascular, intravesical, intralesional, vaginal, rectal, buccal, sublingual, intranasal, or transdermal delivery.

Pharmaceutical compositions can be supplied as a kit comprising a container that comprises the pharmaceutical composition as described herein. A pharmaceutical composition can be provided, for example, in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a

pharmaceutical composition. Such a kit can further comprise written information on indications and usage of the pharmaceutical composition.

While having described the invention in general terms, the embodiments of the invention will be further disclosed in the following examples that should not be construed as limiting the scope of the claims.

EXAMPLES

EXAMPLE 1. Construction of Tencon libraries with randomized loops

Tencon (SEQ ID NO:1) is an immunoglobulin-like scaffold, fibronectin type III (FN3) domain, designed from a consensus sequence of fifteen FN3 domains from human tenascin-C (Jacobs *et al.*, Protein Engineering, Design, and Selection, 25:107-117, 2012; U.S. Pat. No. 8,278,419). The crystal structure of Tencon shows six surface-exposed loops that connect seven beta-strands. These loops, or selected residues within each loop, can be randomized in order to construct libraries of fibronectin type III (FN3) domains that can be used to select novel molecules that bind to specific targets.

Tencon:

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGS ERSYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (SEQ ID NO 1):

Various libraries were generated using the tencon scaffold and various design strategies. In general, libraries TCL1 and TCL2 produced good binders. Generation of TCL1 and TCL2 libraries are described in detail in Int. Pat. Publ. No. WO/2014081944A2.

Construction of TCL1 library

A library designed to randomize only the FG loop of Tencon (**SEQ ID NO:1**), TCL1, was constructed for use with the *cis*-display system (Jacobs *et al.*, Protein Engineering, Design, and Selection, 25:107-117, 2012). In this system, a single-strand DNA incorporating sequences for a Tac promoter, Tencon library coding sequence, RepA coding sequence, cis-element, and ori element is produced.

Upon expression in an *in vitro* transcription/translation system, a complex is produced of the Tencon-RepA fusion protein bound in *cis* to the DNA from which it is encoded. Complexes that bind to a target molecule are then isolated and amplified by polymerase chain reaction (PCR), as described below.

Construction of the TCL1 library for use with *cis*-display was achieved by successive rounds of PCR to produce the final linear, double-stranded DNA molecules in two halves; the 5' fragment contains the promoter and Tencon sequences, while the 3' fragment contains the *repA* gene and the cis- and ori elements. These two halves are combined by restriction digest in order to produce the entire construct. The TCL1 library was designed to incorporate random amino acids only in the FG loop of Tencon, KGGHRSN (SEQ ID NO:55). NNS codons were used in the construction of this library, resulting in the possible incorporation of all 20 amino acids and one stop codon into the FG loop. The TCL1 library contains six separate sub-libraries, each having a different randomized FG loop length, from 7 to 12 residues, in order to further increase diversity.

TCL1 library (SEQ ID NO:2)

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGS ERSYDLTGLKPGTEYTVSIYGVX_{7-12}PLSAEFTT;

wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_7 is any amino acid; and
X_8, X_9, X_{10}, X_{11} and X_{12} are any amino acid or deleted

Construction of TCL2 Library

TCL2 library was constructed in which both the BC and the FG loops of Tencon were randomized and the distribution of amino acids at each position was strictly controlled. Table 3 shows the amino acid distribution at desired loop positions in the TCL2 library. The designed amino acid distribution had two aims. First, the library was biased toward residues that were predicted to be structurally important for Tencon folding and stability based on analysis of the Tencon crystal structure and/or from homology modeling. For example, position 29 was fixed to

be only a subset of hydrophobic amino acids, as this residue was buried in the hydrophobic core of the Tencon fold. A second layer of design included biasing the amino acid distribution toward that of residues preferentially found in the heavy chain HCDR3 of antibodies, to efficiently produce high-affinity binders (Birtalan *et al.*, J Mol Biol 377:1518-28, 2008; Olson *et al.*, Protein Sci 16:476-84, 2007). Towards this goal, the "designed distribution" in Table 2 refers to the distribution as follows: 6% alanine, 6% arginine, 3.9% asparagine, 7.5% aspartic acid, 2.5% glutamic acid, 1.5% glutamine, 15% glycine, 2.3% histidine, 2.5% isoleucine, 5% leucine, 1.5% lysine, 2.5% phenylalanine, 4% proline, 10% serine, 4.5% threonine, 4% tryptophan, 17.3% tyrosine, and 4% valine. This distribution is devoid of methionine, cysteine, and STOP codons.

TCL2 library (SEQ ID NO:3)

LPAPKNLVVSEVTEDSLRLSW$X_1X_2X_3X_4X_5X_6X_7X_8$SFLIQYQESEKVGEAINL TVPGSERSYDLTGLKPGTEYTVSIYGV$X_9X_{10}X_{11}X_{12}X_{13}SX_{14}X_{15}$LSAEFTT; wherein

X_1 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_2 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_3 Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_4 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_5 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_6 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_7 is Phe, Ile, Leu, Val or Tyr;

X_8 is Asp, Glu or Thr;

X_9 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{10} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{11} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{12} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{13} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;

X_{14} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val; and

X_{15} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

Table 2. Residue distribution in the TCL2 library

Residue Position*	WT residues	Distribution in the TCL2 library
22	T	designed distribution
23	A	designed distribution
24	P	50% P + designed distribution
25	D	designed distribution
26	A	20% A + 20% G + designed distribution
27	A	designed distribution
28	F	20% F, 20% I, 20% L, 20% V, 20% Y
29	D	33% D, 33% E, 33% T
75	K	designed distribution
76	G	designed distribution
77	G	designed distribution

78	H	designed distribution
79	R	designed distribution
80	S	100% S
81	N	designed distribution
82	P	50% P + designed distribution

*residue numbering is based on Tencon sequence of SEQ ID NO:1

Subsequently, these libraries were improved by various ways, including building of the libraries on a stabilized Tencon framework (U.S. Pat. No. 8,569,227) that incorporates substitutions E11R/L17A/N46V/E86I (Tencon27; SEQ ID NO:4) when compared to the wild type tencon as well as altering of the positions randomized in the BC and FG loops. Tencon27 is described in Int. Pat. Appl. No. WO2013049275. From this, new libraries designed to randomize only the FG loop of Tencon (library TCL9), or a combination of the BC and FG loops (library TCL7) were generated. These libraries were constructed for use with the cis-display system (Odegrip et al., Proc Natl Acad Sci U S A 101: 2806-2810, 2004). The details of this design are shown below:

Stabilized Tencon (Tencon27) (SEQ ID NO:4)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPG SERSYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

TCL7 (randomized FG and BC loops) (SEQ ID NO:5)

LPAPKNLVVSRVTEDSARLSW$X_1X_2X_3X_4X_5X_6X_7X_8X_9$FDSFLIQYQESEKVG EAIVLTVPGSERSYDLTGLKPGTEYTVSIYGV$X_{10}X_{11}X_{12}X_{13}X_{14}X_{15}X_{16}X_{17}X_{18}$ X_{19}SNPLSAIFTT;

wherein

X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15} and X_{16} is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and

X_7, X_8, X_9, X_{17}, X_{18} and X_{19}, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.

TCL9 (randomized FG loop) (SEQ ID NO:6)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPG SERSYDLTGLKPGTEYTVSIYGV $X_1X_2X_3X_4X_5X_6X_7X_8X_9$ $X_{10}X_{11}X_{12}$SNPLSAIFTT;

X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and

X_8, X_9, X_{10}, X_{11} and X_{12} is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.

For library construction, DNA fragments encoding randomized BC loops (lengths 6-9 positions) or FG loops (lengths 7-12 positions) were synthesized using Slonomics technology (Sloning Biotechnology GmbH) so as to control the amino acid distribution of the library and to eliminate stop codons. Two different sets of DNA molecules randomizing either the BC loop or the FG loops were synthesized independently and later combined using PCR to produce the full library product.

Construction of FG loop libraries (TCL9)

A set of synthetic DNA molecules consisting of a 5' Tac promoter followed by the complete gene sequence of Tencon with the exception of randomized codons in the FG loop was produced (SEQ ID NOs: 26-31). For FG loop randomization, all amino acids except cysteine and methionine were encoded at equal percentages. The lengths of the diversified portion are such that they encode for 7, 8, 9, 10, 11, or 12 amino acids in the FG loop. Sub-libraries of each length variation were synthesized individually at a scale of 2ug and then amplified by PCR using oligos Sloning-FOR (SEQ ID NO:9) and Sloning-Rev (SEQ ID NO:10).

The 3' fragment of the library is a constant DNA sequence containing elements for display, including a PspOMI restriction site, the coding region of the repA gene, and the cis- and ori elements. PCR reactions were performed to amplify this fragment using a plasmid (pCR4Blunt) (Invitrogen) as a template with M13 Forward and M13 Reverse primers. The resulting PCR products were digested by PspOMI overnight and gel-purified. To ligate the 5' portion of library DNA to the 3' DNA containing repA gene, 2 pmol (~540ng to 560ng) of 5' DNA was ligated to an equal molar (~1.25 µg) of 3' repA DNA in the presence of NotI and PspOMI enzyme and T4 ligase at 37°C overnight. The ligated library product was amplified by using 12 cycles of PCR with oligos POP2250 (SEQ ID NO:11) and DigLigRev (SEQ ID NO:12). For each sub-library, the resulting DNA from 12 PCR reactions were combined and purified by Qiagen spin column. The yield for each sub-library of TCL9 ranged from 32-34 µg.

Construction of FG/BC Loop libraries (TCL7)

The TCL7 library provides for a library with randomized Tencon BC and FG loops. In this library, BC loops of lengths 6-9 amino acids were mixed combinatorially with randomized FG loops of 7-12 amino acids in length. Synthetic Tencon fragments BC6, BC7, BC8, and BC9 (SEQ ID NOs: 13-16, respectively) were produced to include the Tencon gene encoding for the N-terminal portion of the protein up to and including residue VX such that the BC loop is replaced with either 6, 7, 8, or 9 randomized amino acids. These fragments were synthesized prior to the discovery of L17A, N46V and E83I mutations (CEN5243) but these mutations were introduced in the molecular biology steps described below. In order to combine this fragment with fragments encoding for randomized FG loops, the following steps were taken.

First, a DNA fragment encoding the Tac promoter and the 5' sequence of Tencon up to the nucleotide encoding for amino acid A17 (130mer-L17A, SEQ ID NO:17) was produced by PCR using oligos POP2222ext (SEQ ID NO:18) and LS1114 (SEQ ID NO:19). This was done to include the L17A mutation in the library (CEN5243). Next, DNA fragments encoding for Tencon residues R18-V75 including randomized BC loops were amplified by PCR using BC6, BC7,

BC8, or BC9 as a template and oligos LS1115 (SEQ ID NO:20) and LS1117 (SEQ ID NO:21). This PCR step introduced a BsaI site at the 3' end. These DNA fragments were subsequently joined by overlapping PCR using oligos POP2222ext and LS1117 as primers. The resulting PCR product of 240bp was pooled and purified by Qiagen PCR purification kit. The purified DNA was digested with BsaI-HF and gel purified.

Fragments encoding the FG loop were amplified by PCR using FG7, FG8, FG9, FG10, FG11, and FG12 as templates with oligonucleotides SDG10 (SEQ ID NO:22) and SDG24 (SEQ ID NO:23) to incorporate a BsaI restriction site and N46V and E86I variations (CEN5243).

The digested BC fragments and FG fragments were ligated together in a single step using a 3-way ligation. Four ligation reactions in the 16 possible combinations were set up, with each ligation reaction combining two BC loop lengths with 2 FG loop lengths. Each ligation contained ~300 ng of total BC fragment and 300 ng of the FG fragment. These 4 ligation pools were then amplified by PCR using oligos POP2222 (SEQ ID NO:24) and SDG28 SEQ ID NO:25). 7.5 µg of each reaction product were then digested with Not1 and cleaned up with a Qiagen PCR purification column. 5.2 µg of this DNA, was ligated to an equal molar amount of RepA DNA fragment (~14 µg) digested with PspOMI and the product amplified by PCR using oligos POP2222.

EXAMPLE 2: Generation of Tencon libraries having alternative binding surfaces

The choice of residues to be randomized in a particular library design governs the overall shape of the interaction surface created. X-ray crystallographic analysis of an FN3 domain containing scaffold protein selected to bind maltose binding protein (MBP) from a library in which the BC, DE, and FG loops were randomized was shown to have a largely curved interface that fits into the active site of MBP (Koide et al., Proc Natl Acad Sci U S A 104: 6632-6637, 2007). In contrast, an ankyrin repeat scaffold protein that was selected to bind to MBP was found to have a much more planar interaction surface and to bind to the outer surface of MBP distant from the active (Binz et al., Nat Biotechnol 22: 575-582,

2004). These results suggest that the shape of the binding surface of a scaffold molecule (curved vs. flat) may dictate what target proteins or specific epitopes on those target proteins are able to be bound effectively by the scaffold. Published efforts around engineering protein scaffolds containing FN3 domains for protein binding has relied on engineering adjacent loops for target binding, thus producing curved binding surfaces. This approach may limit the number of targets and epitopes accessible by such scaffolds.

Tencon and other FN3 domains contain two sets of CDR-like loops lying on the opposite faces of the molecule, the first set formed by the BC, DE, and FG loops, and the second set formed by the AB, CD, and EF loops. The two sets of loops are separated by the beta-strands that form the center of the FN3 structure. If the image of the Tencon is rotated by 90 degrees, an alternative surface can be visualized. This slightly concave surface is formed by the CD and FG loops and two antiparallel beta-strands, the C and the F beta-strands, and is herein called the C-CD-F-FG surface. The C-CD-F-FG surface can be used as a template to design libraries of protein scaffold interaction surfaces by randomizing a subset of residues that form the surface. Beta-strands have a repeating structure with the side chain of every other residue exposed to the surface of the protein. Thus, a library can be made by randomizing some or all surface exposed residues in the beta strands. By choosing the appropriate residues in the beta-strands, the inherent stability of the Tencon scaffold should be minimally compromised while providing a unique scaffold surface for interaction with other proteins.

Library TCL14 (SEQ ID NO:7), was designed into Tencon27 scaffold (SEQ ID NO:4).

A full description of the methods used to construct this library is described in US. Pat. Publ. No. 2013/0226834.

TCL14 library (SEQ ID NO:7):

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX_1IX_2YX_3E$X_4X_5X_6X_7$GEAIVL TVPGSERSYDLTGLKPGTEYX_8VX_9IX_{10}GVKGG$X_{11}X_{12}$SX_{13}PLSAIFTT;

wherein

X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12} and X_{13} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W,Y, C or M.

The two beta strands forming the C-CD-F-FG surface in Tencon27 have a total of 9 surface exposed residues that could be randomized; C-strand: S30, L32, Q34, Q36; F-strand: E66, T68, S70, Y72, and V74, while the CD loop has 6 potential residues: S38, E39, K40, V41, G42, and E43 and the FG loop has 7 potential residues: K75, G76, G77, H78, R79, S80, and N81. Select residues were chosen for inclusion in the TCL14 design due to the larger theoretical size of the library if all 22 residues were randomized.

Thirteen positions in Tencon were chosen for randomizing: L32, Q34 and Q36 in C-strand, S38, E39, K40 and V41 in CD-loop, T68, S70 and Y72 in F-strand, H78, R79, and N81 in FG-loop. In the C and F strands S30 and E66 were not randomized as they lie just beyond the CD and FG loops and do not appear to be as apparently a part of the C-CD-F-FG surface. For the CD loop, G42 and E43 were not randomized as glycine, providing flexibility, can be valuable in loop regions, and E43 lies at the junction of the surface. The FG loop had K75, G76, G77, and S80 excluded. The glycines were excluded for the reasons above while careful inspection of the crystal structures revealed S80 making key contacts with the core to help form the stable FG loop. K75 faces away from the surface of the C-CD-F-FG surface and was a less appealing candidate for randomization. Although the above mentioned residues were not randomized in the original TCL14 design, they could be included in subsequent library designs to provide additional diversity for de novo selection or for example for an affinity maturation library on a select TCL14 target specific hit.

Subsequent to the production of TCL14, 3 additional Tencon libraries of similar design were produced. These two libraries, TCL19, TCL21 and TCL23, are randomized at the same positions as TCL14 (see above) however the distribution of amino acids occurring at these positions is altered (Table 3). TCL19 and TCL21 were designed to include an equal distribution of 18 natural

amino acids at every position (5.55% of each), excluding only cysteine and methionine. TCL23 was designed such that each randomized position approximates the amino acid distribution found in the HCDR3 loops of functional antibodies (Birtalan et al., J Mol Biol 377: 1518-1528, 2008) as described in Table 3. As with the TCL21 library, cysteine and methionine were excluded.

A third additional library was built to expand potential target binding surface of the other libraries library. In this library, TCL24, 4 additional Tencon positions were randomized as compared to libraries TCL14, TCL19, TCL21, and TCL23. These positions include N46 and T48 from the D strand and S84 and I86 from the G strand. Positions 46, 48, 84, and 86 were chosen in particular as the side chains of these residues are surface exposed from beta-strands D and G and lie structurally adjacent to the randomized portions of the C and F strand, thus increasing the surface area accessible for binding to target proteins. The amino acid distribution used at each position for TCL24 is identical to that described for TCL19 and TCL21 in Table 3.

TCL24 Library (SEQ ID NO:8)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX_1IX_2YX_3E$X_4$$X_5$$X_6$$X_7$GEAI$X_8$ LX_9VPGSERSYDLTGLKPGTEYX_{10}VX_{11}IX_{12}GVKGG$X_{13}$$X_{14}SX_{15}PLX_{16}AX_{17}$FT T;

wherein

X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16} and X_{17} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, Y or W.

Table 3. Amino acid frequency (%) at each randomized position for TCL21, TCL23, and TCL24.

Amino Acid	TCL19	TCL21	TCL23	TCL24
Ala	5.6	5.6	6.0	5.6
Arg	5.6	5.6	6.0	5.6
Asn	5.6	5.6	3.9	5.6

Asp	5.6	5.6	7.5	5.6
Cys	0.0	0.0	0.0	0.0
Gln	5.6	5.6	1.5	5.6
Glu	5.6	5.6	2.5	5.6
Gly	5.6	5.6	15.0	5.6
His	5.6	5.6	2.3	5.6
Ile	5.6	5.6	2.5	5.6
Leu	5.6	5.6	5.0	5.6
Lys	5.6	5.6	1.5	5.6
Met	0.0	0.0	0.0	0.0
Phe	5.6	5.6	2.5	5.6
Pro	5.6	5.6	4.0	5.6
Ser	5.6	5.6	10.0	5.6
Thr	5.6	5.6	4.5	5.6
Trp	5.6	5.6	4.0	5.6
Tyr	5.6	5.6	17.3	5.6
Val	5.6	5.6	4.0	5.6

Generation of TCL21, TCL23, and TCL24 libraries

The TCL21 library was generated using Colibra library technology (Isogenica) in order to control amino acid distributions. TCL19, TCL23, and TCL24 gene fragments were generated using Slonomics technology (Morphosys) to control amino acid distributions. PCR was used to amplify each library following initial synthesis followed by ligation to the gene for RepA in order to be used in selections using the CIS-display system (Odegrip et al., Proc Natl Acad Sci U S A 101: 2806-2810, 2004) as described above for the loop libraries.

EXAMPLE 3: Selection of fibronectin type III (FN3) domains that bind CD137

Panning

FN3 domains specific for human CD137 were selected via CIS-Display (Odegrip *et al* 2004) using recombinant biotinylated CD137 protein (Fc-fusion protein, R&D Systems 838-4B). For *in vitro* transcription and translation (ITT), 3 µg of DNA from Centyrin libraries TCL18, TCL19, TCL21, TCL23, and TCL24 (See accompanying library description document) were incubated at 30°C with 0.1 mM complete amino acids, 1X S30 premix components, and 15 µL of S30 extract (Isogenica) in a total volume of 50 µL. After 1 hour, 375 µL of blocking solution (2% BSA in PBS, Invitrogen) was added and reactions were incubated on a cold block for 15 minutes. Unbound library members were removed by successive washes with TBST and TBS. After washing, DNA was eluted from the target protein by heating to 75°C for 10 minutes and amplified by PCR using KOD polymberase for further rounds of panning. High affinity binders were isolated by successively lowering the concentration of target CD137 during each round from 400 nM to 100 nM and increasing the washing stringency.

Outputs from the fifth round panning were subjected to four additional rounds of off-rate selection. Library transcription and translation was performed as described above after which, the ITT reactions were incubated with biotinylated recombinant CD137 proteins and captured on neutravidin or streptavidin coated magnetic beads, before being washed in TBST extensively then subsequently washed in 5 uM cold recombinant CD137 protein for 1 hour. The biotinylated target antigen concentration was reduced from 25 nM in rounds 6 and 7 to 2.5 nM in rounds 8 and 9.

Following panning, genes encoding the selected FN3 domains were amplified by PCR, subcloned into a pET vector modified to include a ligase independent cloning site, and transformed into BL21 (DE3) (Stratagene) cells for soluble expression in *E. coli* using standard molecular biology techniques. A gene sequence encoding a C-terminal poly-histidine tag was added to each Centyrin to enable purification and detection. Cultures were grown to an optical density of

0.6-0.8 in TB medium supplemented with 100 μg/mL carbenicillin in 1 mL 96-well blocks at 37°C before the addition of IPTG to 1 mM, at which point the temperature was reduced to 30°C. Cells were harvested approximately 16 hours later by centrifugation and frozen at -20°C. Cell lysis was achieved by incubating each pellet in 0.6 mL of BugBuster® HT lysis buffer (Novagen EMD Biosciences) supplemented with 0.2 mg/mL lysozyme with shaking at room temperature for 30 minutes.

Biochemical Screening for FN3 domains that Recombinant CD137

Streptavidin-coated Maxisorp plates (Nunc catalog 436110) were blocked for 1h in Starting Block T20 (Pierce) and then coated with biotinylated CD137 (using same antigen as in panning) or negative controls (an unrelated Fc-fused recombinant protein and human serum albumin) for 1h. Plates were rinsed with TBST and diluted lysate was applied to plates for 1h. Following additional rinses, wells were treated with HRP-conjugated anti-Centyrin antibody (PAB25) for 1h and then assayed with POD (Roche catalog 11582950001). The DNA from Centyrin lysates with signals at least 10-fold ELISA signal above that of Fc and HSA controls were sequenced resulting in 78 (Table 1) and 102 (Table 2) unique, readable Centyrin sequences isolated from Round 5 and Round 9 screening respectively.

High-throughput Expression of anti-CD137 FN3 domains

102 Isolated clones from unique hits identified by biochemical binding ELISA from Round 9 were combined for growth into 96-well block plate; clones grew in 1 mL cultures (LB media supplemented with kanamycin for selection) at 37°C overnight with shaking. For protein expression in 96-block plates, 1 mL TB media supplemented with kanamycin was inoculated with 50 uL of the overnight culture and grown at 37°C with continual shaking at 300rpm until OD_{600} = 0.6-1. Once the target OD was reached, protein expression was induced with addition of IPTG to 1 mM; plates were transferred to 30°C (300 rpm) for overnight growth. Overnight cultures were centrifuged to harvest the cells; bacterial pellets were stored at -80°C until ready for use. Pellets were lysed with BugBuster® HT lysis

buffer (Novagen EMD Biosciences) and His-tagged FN3 domains purified from the clarified lysates with His MultiTrap™ HP plates (GE Healthcare) and eluted in buffer containing 20 mM sodium phosphate, 500 mM sodium chloride, and 250 mM imidazole at pH 7.4. Purified samples were exchanged into PBS pH 7.4 for analysis using PD MultiTrap™ G-25 plates (GE Healthcare).

Size Exclusion Chromatography Analysis

Size exclusion chromatography was used to determine the aggregation state of anti-CD137 FN3 domains. Aliquots (10 μL) of each purified Centyrin were injected onto a Superdex 75 5/150 column (GE Healthcare) at a flow rate of 0.3 mL/min in a mobile phase of PBS pH 7.4. Elution from the column was monitored by absorbance at 280 nm. Tencon protein was included in each run as a control. Agilent ChemStation software was used to analyse the elution profiles. 46 anti-CD137 FN3 domains demonstrated a retention time between 5.2 and 6.4 minutes and only a single SEC peak indicative of monomeric protein.

Table 4. Summary of Round 5 Screening Hits

FN3 Domain	ELISA CD137-Fc (RLU)	ELISA Fc Control (RLU)	ELISA HSA (RLU)	SEQ ID No.
ISOP120AR5P1D2	907520	640	640	45
ISOP120AR5P1C3	927040	320	240	46
ISOP120AR5P1H3	769280	400	480	47
ISOP120AR5P1C4	708240	320	240	48
ISOP120AR5P1B5	500640	400	320	49
ISOP120AR5P1C5	425120	320	160	50
ISOP120AR5P1G7	568000	560	480	51
ISOP120AR5P1G8	541200	320	320	52
ISOP120AR5P1D9	636320	320	320	53
ISOP120AR5P1F11	714800	480	320	54
ISOP120AR5P1B12	864240	400	480	55
ISOP120BR5P1C1	437680	480	480	56
ISOP120BR5P1F1	541920	480	480	57
ISOP120BR5P1D2	360800	720	240	58
ISOP120BR5P1E2	882480	5680	4960	59
ISOP120BR5P1F2	298800	400	240	60
ISOP120BR5P1D3	1138560	240	400	61
ISOP120BR5P1H3	874560	2000	720	62

ISOP120BR5P1E4	942320	320	560	63
ISOP120BR5P1G4	580240	480	400	64
ISOP120BR5P1A5	503040	640	400	65
ISOP120BR5P1E6	779120	320	400	66
ISOP120BR5P1B7	564560	400	480	67
ISOP120BR5P1C7	306240	880	240	68
ISOP120BR5P1D8	941680	480	320	69
ISOP120BR5P1E8	906160	480	640	70
ISOP120BR5P1B9	358000	560	400	71
ISOP120BR5P1C9	1272800	320	560	72
ISOP120BR5P1D9	1224720	560	560	73
ISOP120BR5P1A10	573280	36160	26560	74
ISOP120BR5P1G11	485440	480	560	75
ISOP120GR5P1E1	1022960	320	320	76
ISOP120GR5P1G3	1335760	320	320	77
ISOP120GR5P1F5	1283680	400	400	78
ISOP120GR5P1H6	721440	400	400	79
ISOP120GR5P1E7	1130720	400	480	80
ISOP120GR5P1A10	626640	400	400	81
ISOP120GR5P1C10	501840	240	480	82
ISOP120GR5P1A11	1045760	480	320	83
ISOP120GR5P1B11	875360	320	160	84
ISOP120GR5P1H11	1310560	640	320	85
ISOP120HR5P1E2	1319040	720	2880	86
ISOP120HR5P1A3	1076480	560	240	87
ISOP120HR5P1B4	1185360	320	320	88
ISOP120HR5P1G4	346880	320	480	89
ISOP120HR5P1H4	630480	480	320	90
ISOP120HR5P1B5	519520	320	240	91
ISOP120HR5P1A6	1292720	640	400	92
ISOP120HR5P1G6	2035360	400	320	93
ISOP120HR5P1A7	986800	400	480	94
ISOP120HR5P1D7	1104240	320	320	95
ISOP120HR5P1E7	363120	480	480	96
ISOP120HR5P1H7	1527200	640	480	97
ISOP120HR5P1H8	2217040	320	400	98
ISOP120HR5P1D9	404720	480	400	99
ISOP120HR5P1F9	1177120	400	400	100
ISOP120ER5P1B4	499360	400	400	101
ISOP120ER5P1F4	536720	320	400	102
ISOP120ER5P1H4	1070240	480	560	103
ISOP120ER5P1E5	413120	240	320	104
ISOP120ER5P1B6	1351600	160	400	105

ISOP120ER5P1C6	495360	320	400	106
ISOP120ER5P1H6	588560	320	480	107
ISOP120ER5P1A7	1114080	400	400	108
ISOP120ER5P1A8	1897040	400	320	109
ISOP120ER5P1E10	810320	720	400	110
ISOP120ER5P1A11	1144160	320	320	111
ISOP120ER5P1B12	1441520	720	800	112
ISOP120FR5P1F1	1228320	480	640	113
ISOP120FR5P1C2	388960	240	400	114
ISOP120FR5P1H5	459680	400	560	115
ISOP120FR5P1A6	1404240	400	320	116
ISOP120FR5P1H6	356880	320	320	117
ISOP120FR5P1D7	1178800	400	480	118
ISOP120FR5P1F8	1197120	240	400	119
ISOP120FR5P1E9	1183360	320	400	120
ISOP120FR5P1E10	953040	240	320	121
ISOP120FR5P1A11	920080	480	480	122

Table 5. Summary of Round 9 Screening Hits

FN3 Domain Clone	ELISA CD137 (RLU)	ELISA Fc Control (RLU)	ELISA HSA (RLU)	SEC Retention Time (min)	SEC Peak Height (mAU)	Monomeric	SEQ ID No.
ISOP193AR9P1A11	8659280	1440	320	No peak		FALSE	123
ISOP193AR9P1A6	6739840	960	480	No peak		FALSE	124
ISOP193AR9P1B10	8120400	1520	480	5.355	84.76	TRUE	125
ISOP193AR9P1B12	2762240	2240	1440	5.712	39.72	TRUE	126
ISOP193AR9P1B4	5744400	960	480	5.495	94.79	TRUE	127
ISOP193AR9P1C10	7143200	3520	960	No peak		FALSE	128
ISOP193AR9P1E6	4179680	1200	720	No peak		FALSE	129
ISOP193AR9P1F4	3836000	1520	720	5.664	80.79	TRUE	130
ISOP193AR9P1F9	4710240	1600	560	5.317	142.48	TRUE	131
ISOP193AR9P1G11	5892800	2240	960	5.234	225.17		132
ISOP193AR9P1G5	4022880	1120	720	5.315	191.85	FALSE	133
ISOP193AR9P1G8	4255040	1440	720	No peak	1.40	FALSE	134
ISOP193AR9P1H8	3716320	3040	1040	5.982	36.60	TRUE	135
ISOP193BR9P1B10	9733920	2640	1200	5.884	66.69	TRUE	136
ISOP193BR9P1B12	6551440	14000	2880	No peak		FALSE	137
ISOP193BR9P1E6	4625840	2560	560	6.326	5.13	FALSE	138
ISOP193BR9P1G11	4988080	56880	9600	No peak		FALSE	139
ISOP193BR9P1G2	6145520	15920	2160	No peak		FALSE	140
ISOP193BR9P1G3	4710400	1360	640	7.974	7.68	FALSE	141

ISOP193BR9P1G6	8092720	2640	960	6.045	11.68	TRUE	142
ISOP193BR9P1G9	3725520	1200	720	6.028	4.71	FALSE	143
ISOP193BR9P1H2	3502960	11840	3280	6.055	55.87	FALSE	144
ISOP193BR9P1H3	5257440	2080	1360	No peak		FALSE	145
ISOP193BR9P1H6	8857840	8320	2880	5.787	64.85	FALSE	146
ISOP193ER9P1A10	14863840	1120	240	No peak		FALSE	147
ISOP193ER9P1A11	12781600	1200	640	6.015	35.38	TRUE	148
ISOP193ER9P1A3	14185440	3040	880	5.73	136.02	TRUE	149
ISOP193ER9P1A4	9806400	960	480	5.738	61.50	TRUE	150
ISOP193ER9P1A8	14274800	1440	400	5.892	14.52	TRUE	151
ISOP193ER9P1B4	16089360	1600	320	No peak		FALSE	152
ISOP193ER9P1B5	12675520	1200	400	6.033	8.43	TRUE	153
ISOP193ER9P1C10	8866800	2480	560	5.704	179.28	TRUE	154
ISOP193ER9P1C4	15455120	960	320	6.032	18.18	TRUE	155
ISOP193ER9P1C8	16680560	1040	400	5.862	19.39	TRUE	156
ISOP193ER9P1C9	14280160	880	560	5.668	20.02	TRUE	157
ISOP193ER9P1D4	16022720	1120	480	5.843	22.38	TRUE	158
ISOP193ER9P1D7	10954000	1680	400	5.92	80.79	TRUE	159
ISOP193ER9P1E1	14972480	1200	560	5.755	18.97	TRUE	160
ISOP193ER9P1E2	15691600	1040	560	6.296	20.36	TRUE	161
ISOP193ER9P1E4	12645760	1680	480	5.762	118.21	TRUE	162
ISOP193ER9P1E8	16401200	880	480	5.699	16.07	TRUE	163
ISOP193ER9P1F11	11182240	2240	400	No peak		FALSE	164
ISOP193ER9P1F7	15148960	1200	480	5.856	7.39	TRUE	165
ISOP193ER9P1F9	14980400	1840	400	7.819	4.92	FALSE	166
ISOP193ER9P1G11	14840160	1440	560	5.859	17.01	TRUE	167
ISOP193ER9P1G2	7192960	1680	720	5.677	24.15	TRUE	168
ISOP193ER9P1G4	13819760	1440	320	5.979	2.54	FALSE	169
ISOP193ER9P1G5	15073600	1440	400	No peak		FALSE	170
ISOP193ER9P1G9	12900320	1280	400	5.781	6.70	TRUE	171
ISOP193ER9P1H11	2080000	1360	640	5.991	39.63	TRUE	172
ISOP193ER9P1H2	5183360	1360	560	5.833	53.11	TRUE	173
ISOP193ER9P1H3	10515760	1520	400	6.073	7.45	FALSE	174
ISOP193FR9P1A11	5784000	3520	880	No peak		FALSE	175
ISOP193FR9P1A5	9072080	95120	26240	6.033	8.14	TRUE	176
ISOP193FR9P1C1	14116720	36800	8160	5.866	3.99	FALSE	177
ISOP193FR9P1C5	8660800	79280	19200	6.377	20.91	TRUE	178
ISOP193FR9P1C9	12306480	21040	4000	7.257	5.36	FALSE	179
ISOP193FR9P1D1	8132800	1680	640	No peak		FALSE	180
ISOP193FR9P1D5	6046880	51680	10800	5.948	4.84	TRUE	181
ISOP193FR9P1D7	2195360	15040	2640	6.077	1.98	FALSE	182
ISOP193FR9P1E1	11602480	2000	880	5.84	28.48	FALSE	183
ISOP193FR9P1E10	2051600	133120	31040	5.9	46.84	TRUE	184

ISOP193FR9P1F8	8573040	25680	5040	5.652	3.10	FALSE	185
ISOP193FR9P1G10	8908880	2480	880	6.864	2.25	FALSE	186
ISOP193FR9P1G11	10788560	60640	10960	5.945	5.46	TRUE	187
ISOP193FR9P1G2	7864240	2560	880	No peak		FALSE	188
ISOP193FR9P1G4	13950480	1840	640	5.834	5.47	TRUE	189
ISOP193FR9P1G7	5500720	42320	10960	5.897	9.89	TRUE	190
ISOP193FR9P1G8	14458880	25120	5040	5.874	5.87	FALSE	191
ISOP193FR9P1G9	12761120	33600	6800	6.413	6.02	FALSE	192
ISOP193FR9P1H6	11204000	88320	23040	5.712	7.91	TRUE	193
ISOP193FR9P1H9	2420400	2000	800	5.987	23.27	TRUE	194
ISOP193GR9P1A7	2153840	1040	480	5.634	10.89	TRUE	195
ISOP193GR9P1B3	3457040	880	320	5.768	3.78	FALSE	196
ISOP193GR9P1E10	10452960	1360	480	No peak		FALSE	197
ISOP193GR9P1F6	9846640	1360	400	5.656	4.72	FALSE	198
ISOP193GR9P1F7	3480640	880	400	5.712	2.95	FALSE	199
ISOP193GR9P1G9	3052480	960	480	5.645	7.32	TRUE	200
ISOP193GR9P1H2	5314000	1360	640	No peak		FALSE	201
ISOP193HR9P1A10	12663280	5680	1520	No peak		FALSE	202
ISOP193HR9P1A11	16644800	28320	4240	No peak		FALSE	203
ISOP193HR9P1A5	14895120	6080	2080	No peak		FALSE	204
ISOP193HR9P1A6	14635040	24960	5120	No peak		FALSE	205
ISOP193HR9P1A7	14786080	48880	12640	6.013	9.21	TRUE	206
ISOP193HR9P1B11	16579440	14960	4080	No peak		FALSE	207
ISOP193HR9P1B7	16384560	12960	2240	No peak		FALSE	208
ISOP193HR9P1C7	3436800	71360	10000	5.69	2.15	FALSE	209
ISOP193HR9P1C8	18185520	1360	560	6.475	4.54	TRUE	210
ISOP193HR9P1D11	14160720	48720	6240	5.936	5.80	TRUE	211
ISOP193HR9P1D8	6271280	10880	2640	5.79	4.93	TRUE	212
ISOP193HR9P1E2	9022400	13120	3840	5.801	4.22	FALSE	213
ISOP193HR9P1E3	17767600	1120	640	6.564	3.11	FALSE	214
ISOP193HR9P1E6	11258560	20080	3040	5.859	3.13	FALSE	215
ISOP193HR9P1E8	16318560	3120	1520	No peak		FALSE	216
ISOP193HR9P1F10	15810240	1280	960	No peak		FALSE	217
ISOP193HR9P1F8	16086000	31280	6080	No peak		FALSE	218
ISOP193HR9P1G10	15586960	1360	800	6.226	2.97	FALSE	219
ISOP193HR9P1G4	17180000	960	560	6.293	2.54	FALSE	220
ISOP193HR9P1G5	15137440	24160	3360	5.913	9.58	TRUE	221
ISOP193HR9P1G6	11499680	8160	1200	No peak		FALSE	222
ISOP193HR9P1H10	14818080	43920	10080	6.107	2.19	FALSE	223
ISOP193HR9P1H7	4604800	49840	13680	6.035	1.81	FALSE	224

EXAMPLE 4: Characterization of fibronectin type III (FN3) domains that bind CD137

Fluorescence-activated cell sorting (FACS)

Cell surface binding was analyzed via flow cytometry. Cenyrins were prepared at a maximal concentration of 500 nM in the presence of 125 nM anti His-mIgG1 antibody and then serially diluted in PBS/1% FCS buffer. Samples were then applied to approx 100 000 CHO-K1 cells expressing the extracellular domain of human CD137 on their surface. Unbound Centyrin/Antibody complexes were washed away and bound FN3 domains were detected by the addition of goat anti mouse-FITC labeled antibody. Samples were then aquired by flow cytometry. Subsequently, Mean flurescence intensity was plotted against the log Centyrin concentration and EC50 values were calculated by nonlinear regression using GraphPad Prism.

Binding analysis with Biacore

For selected FN3 domains, binding to recombinant CD137-Fc was evaluated using surface plasmon resonance (Biacore T100). For each cycle approximately 500 RU of recombinant human CD137-Fc-IgG1 was captured via an anti human IgG immobilized on the surface of a CM5 chip. Once CD137-Fc protein was captured, increasing concentrations of Centyrin candidates were injected for 120s and dissociation was then analyzed for 240s. A flow cell immobilized with just the anti human IgG1 antibody served as reference flow cell. Kd values were subsequently extrapolated using a 1:1 kinetic binding model (BiaEvaluation software).

Table 6. Centyrin binding analysis by FACS and Biacore

Centyrin Clone	EC50 FACS (nM)	Biacore (Kd)
ISOP193AR9P1B4	13.93	n.d.
ISOP193AR9P1F4	n.a.	n.d.
ISOP193AR9P1H8	n.a.	n.d.
ISOP193AR9P1F9	218	n.d.
ISOP193AR9P1B10	1.205	n.d.

ISOP193AR9P1G11	10.48	n.d.
ISOP193AR9P1B12	n.a.	n.d.
ISOP193BR9P1G6	n.a.	n.d.
ISOP193BR9P1B10	134	n.d.
ISOP193GR9P1A7	605.6	n.d.
ISOP193GR9P1G9	n.a.	n.d.
ISOP193GR9P1F11	508.9	n.d.
ISOP193HR9P1C8	3.268	n.d.
ISOP193ER9P1E1	12.55	1.059E-08
ISOP193ER9P1E2	15.31	1.094E-08
ISOP193ER9P1G2	22.94	6.563E-08
ISOP193ER9P1H2	27.46	n.d.
ISOP193ER9P1A3	10.22	8.3E-09
ISOP193ER9P1A4	27.1	1.172E-07
ISOP193ER9P1C4	18.91	1.31E-08
ISOP193ER9P1D4	12.86	9.552E-09
ISOP193ER9P1E4	278.7	n.d.
ISOP193ER9P1B5	7.342	n.d.
ISOP193ER9P1D5	29.61	n.d.
ISOP193ER9P1C7	19.07	n.d.
ISOP193ER9P1F7	24.99	n.d.
ISOP193ER9P1A8	16.96	n.d.
ISOP193ER9P1C8	12.43	n.d.
ISOP193ER9P1E8	17.67	n.d.
ISOP193ER9P1C9	14.1	2.51E-08
ISOP193ER9P1G9	15.26	5.986E-08
ISOP193ER9P1C10	31.01	1.077E-07
ISOP193ER9P1A11	10.56	n.d.
ISOP193ER9P1G11	18.69	n.d.
ISOP193ER9P1H11	184.5	n.d.
ISOP193ER9P1B12	19.87	n.d.
ISOP193FR9P1G4	26.93	n.d.
ISOP193FR9P1H9	72.75	n.d.

SEQUENCES

SEQ ID NO:1= Original Tencon Sequence
LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY
DLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT

SEQ ID NO:2= TCL1 library
LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSFLIQYQESEKVGEAINLTVPGSERSY
DLTGLKPGTEYTVSIYGV$(X)_{7-12}$PLSAEFTT;

wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_7 is any amino acid; and
X_8, X_9, X_{10}, X_{11} and X_{12} are any amino acid or deleted

SEQ ID NO:3=TCL2 library
LPAPKNLVVSEVTEDSLRLSWX$_1$X$_2$X$_3$X$_4$X$_5$X$_6$X$_7$X$_8$SFLIQYQESEKVGEAINLTVPGS
ERSYDLTGLKPGTEYTVSIYGVX$_9$X$_{10}$X$_{11}$X$_{12}$X$_{13}$SX$_{14}$X$_{15}$LSAEFTT;
wherein
X_1 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_2 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_3 Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_4 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_5 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_6 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_7 is Phe, Ile, Leu, Val or Tyr;
X_8 is Asp, Glu or Thr;
X_9 is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{10} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{11} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{12} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{13} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
X_{14} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val; and
X_{15} is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

SEQ ID NO:4= Stabilized Tencon
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

SEQ ID NO:5= TCL7 (FG and BC loops)
LPAPKNLVVSRVTEDSARLSWX$_1$X$_2$X$_3$X$_4$X$_5$X$_6$X$_7$X$_8$X$_9$FDSFLIQYQESEKVGEAIVLT
VPGSERSYDLTGLKPGTEYTVSIYGVX$_{10}$X$_{11}$X$_{12}$X$_{13}$X$_{14}$X$_{15}$X$_{16}$X$_{17}$X$_{18}$X$_{19}$SNPLSAIFTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15} and X_{16} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and
X_7, X_8, X_9, X_{17}, X_{18} and X_{19}, are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted

SEQ ID NO:6= TCL9 (FG loop)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGV $X_1X_2X_3X_4X_5X_6X_7X_8X_9$ $X_{10}X_{11}X_{12}$SNPLSAIFTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6 and X_7, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and
X_8, X_9, X_{10}, X_{11} and X_{12} is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.

TCL14 library (SEQ ID NO:7):
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX_1IX_2YX_3E$X_4X_5X_6X_7$GEAIVLTVPGS
ERSYDLTGLKPGTEYX$_8$VX$_9$IX$_{10}$GVKGGX$_{11}$X$_{12}$SX$_{13}$PLSAIFTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}, X_{12} and X_{13} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W,Y, C or M.

TCL24 Library (SEQ ID NO:8)
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX_1IX_2YX_3E$X_4X_5X_6X_7$GEAIX$_8$LX$_9$VPG
SERSYDLTGLKPGTEYX$_{10}$VX$_{11}$IX$_{12}$GVKGGX$_{13}$X$_{14}$SX$_{15}$PLX$_{16}$AX$_{17}$FTT;
wherein
X_1, X_2, X_3, X_4, X_5, X_6, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, X_{16} and X_{17} are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, Y or W.

SEQ ID NO:9 = Sloning-FOR
GTGACACGGCGGTTAGAAC

SEQ ID NO:10 = Sloning-REV
GCCTTTGGGAAGCTTCTAAG

SEQ ID NO:11 = POP2250
CGGCGGTTAGAACGCGGCTACAATTAATAC

SEQ ID NO:12 = DigLigRev
CATGATTACGCCAAGCTCAGAA

SEQ ID NO:13 = BC9
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNNNNNNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT
TGGTGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGA
CCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGA
AGCTTCCCAAAGGC

SEQ ID NO:14 = BC8
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNNNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGG
TGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCG
GTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGAAGC
TTCCCAAAGGC

SEQ ID NO:15 = BC7
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGA
AGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTC
TGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGAAGCTTCC
CAAAGGC

SEQ ID NO:16 = BC6
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNNNNNNNNN
NNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTCTTAGAAGCTTCCCA
AAGGC

SEQ ID NO:17 = 130mer-L17A
CGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTGTTGACA
ATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCAC
ACAGGAAACAGGATCTACCATGCTG

SEQ ID NO:18 = POP222ext
CGG CGG TTA GAA CGC GGC TAC AAT TAA TAC

SEQ ID NO:19 = LS1114
CCA AGA CAG ACG GGC AGA GTC TTC GGT AAC GCG AGA AAC AAC CAG
GTT TTT CGG CGC CGG CAG CAT GGT AGA TCC TGT TTC

SEQ ID NO:20 = LS1115
CCG AAG ACT CTG CCC GTC TGT CTT GG

SEQ ID NO:21 = LS1117
CAG TGG TCT CAC GGA TTC CTG GTA CTG GAT CAG GAA AGA GTC GAA

SEQ ID NO:22 = SDG10
CATGCGGTCTCTTCCGAAAAAGTTGGTGAAGCGATCGTCCTGACCGTTCCGGG
T

SEQ ID NO:23 = SDG24
GGTGGTGAAGATCGCAGACAGCGGGTTAG

SEQ ID NO:24 = POP2222
CGGCGGTTAGAACGCGGCTAC

SEQ ID NO:25 = SDG28
AAGATCAGTTGCGGCCGCTAGACTAGAACCGCTGCCACCGCCGGTGGTGAAG
ATCGCAGAC

SEQ ID NO:26 = FG12
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACC
GGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAAC
TGATCTTGGC

SEQ ID NO:27 = FG11
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGC
GGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGA
TCTTGGC

SEQ ID NO:28 = FG10
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTC
ACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTT
GGC

SEQ ID NO:29 = FG9
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTCACC
ATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTTGGC

SEQ ID NO:30 = FG8
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG

TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTCACCATC
ACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTTGGC

SEQ ID NO:31 = FG7
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTG
TTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAA
TTTCACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACCTGGTTG
TTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCCGGACGCG
GCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGTTGGTGAAGC
GATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTGACCGGTCTGA
AACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNNNNNNNNNNNNN
NNNNNNNTCTAACCCGCTGTCTGCGATCTTCACCACCGGCGGTCACCATCACC
ATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACTGATCTTGGC

Table 7. FN3 Domains, Linkers, and Albumin variant

Clone	SEQ ID NO:	AA Sequence
3rd FN3 domain of tenascin C (TN3	32	DAPSQIEVKDVTDTTALITWFKPLAEIDGIELTYGIKDVP GDRTTIDLTEDENQYSIGNLKPDTEYEVSLISRR GDMSSNPAKETFTT
Fibcon	33	LDAPTDLQVTNVTDTSITVSWTPPSATITGYRITYTPSNG PGEPKELTVPPSSTSVTITGLTPGVEYVVSLYAL KDNQESPPLVGTQTT
10th FN3 domain of fibronectin	34	VSDVPRDLEVVAATPTSLLISWDAPAVTVRYYRITYGET GGNSPVQEFTVPGSKSTATISGLKPGVDYTITVY AVTGRGDSPASSKPISINYRT
Linker	35	GSGS
Linker	36	GGGSGGGS
Linker	37	GGGGSGGGGSGGGGSGGGGSGGGGS
Linker	38	APAP
Linker	39	APAPAPAPAP
Linker	40	APAPAPAPAPAPAPAPAPAP

Linker	41	APAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAPAP
Linker	42	EAAAKEAAAKEAAAKEAAAKEAAAKAAA
Albumin variant	43	DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQSPFED HVKLVNEVTEFAKTCVADESAENCDKSLHTLFG DKLCTVATLRETYGEMADCCAKQEPERNECFL QHKDDNPNLPRLVRPEVDVMCTAFHDNEETFL KKYLYEIARRHPYFYAPELLFFAKRYKAAFTEC CQAADKAACLLPKLDELRDEGKASSAKQRLKC ASLQKFGERAFKAWAVARLSQRFPKAEFAEVSK LVTDLTKVHTECCHGDLLECADDRADLAKYICE NQDSISSKLKECCEKPLLEKSHCIAEVENDEMPA DLPSLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVVLLLRLAKTYETTLEKCCAAAD PHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLG EYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGK VGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLH EKTPVSDRVTKCCTESLVNRRPCFSALEVDETY VPKEFNAETFTFHADICTLSEKERQIKKQTALVE LVKHKPKATKEQLKAVMDDFAAFVEKCCKAD DKETCFAEEGKKLVAASQAALGL

SEQ ID NO:44 = human CD137

MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSA
GGQRTCDICRQCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQGQ
ELTKKGCKDCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCGPSPAD
LSPGASSVTPPAPAREPGHSPQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYI
FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

CD137 binding fibronectin type III domains

SEQ ID NO:45 ISOP120AR5P1D2
LPAPKNLVVSRVTEDSARLSWDFAYFKFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVGRFYTVYYSNPLSAIFTT

SEQ ID NO:46 ISOP120AR5P1C3
LPAPKNLVVSRVTEDSARLSWSPVDADFTFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:47 ISOP120AR5P1H3
LPAPKNLVVSRVTEDSARLSWKWISHEPLEFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:48 ISOP120AR5P1C4
LPAPKNLVVSRVTEDSARLSWAFQWHIFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVGQPYTVYDSNPLSAIFTT

SEQ ID NO:49 ISOP120AR5P1B5
LPAPKNLVVSRVTEDYARLSWKYGEHIIWFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVKGQHDHDSNPLSAIFTT

SEQ ID NO:50 ISOP120AR5P1C5
LPAPKNLVVSRVTEDSARLSWTLPNIHFDSFLIQYQESEKVGEAIVLTVPGSERSYD
LTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:51 ISOP120AR5P1G7
LPAPKNLVVSRVTEDSARLSWSQHYLSPIPFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:52 ISOP120AR5P1G8
LPAPKNLVVSRVTEDSARLSWHATFGDPFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:53 ISOP120AR5P1D9
LPAPKNLVVSRVTEDSARLSWNTDWVHTFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:54 ISOP120AR5P1F11
LPAPKNLVVSRVTEDSARLSWTNEQITKYGFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPATEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:55 ISOP120AR5P1B12
LPAPKNLVVSRVTEDSARLSWDGDKWANFKFDSFLIQYQESEKVGEAIVLTVPGS
ERSYDLTGLKPGTEYTVSIYGVGLHYIVYDSNPLSAIFTT

SEQ ID NO:56 ISOP120BR5P1C1
LPAPKNLVVSRVTEDSARLSWVREDAYAFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVSSLHWVVHDSNPLSAIFTT

SEQ ID NO:57 ISOP120BR5P1F1
LPAPKNLVVSRVTEDSARLSWTFHPTFEGFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVKWTVLRPWLSNPLSAIFTT

SEQ ID NO:58 ISOP120BR5P1D2
LPAPKNLVVSRVTEDSARLSWIRKHNHVKWFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVGFLIDTDDSNPLSAIFTT

SEQ ID NO:59 ISOP120BR5P1E2
LPAPKNLVVSRVTEDSARLSWAQELDHFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVYWTWWVRWNSNPLSAIFTT

SEQ ID NO:60 ISOP120BR5P1F2
LPAPKNLVVSRVTEDSARLSWTFHPTFEGFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVKWYAGIGYPVSNPLSAIFTT

SEQ ID NO:61 ISOP120BR5P1D3
LPAPKNLVVSRVTEDSARLSWSEHPTPFATFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVWWVENHFPVSNPLSAIFTT

SEQ ID NO:62 ISOP120BR5P1H3
LPAPKNLVVSRVTEDSARLSWEESRQFFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVVHRAWLRWNGSNPLSAIFTT

SEQ ID NO:63 ISOP120BR5P1E4
LPAPKNLVVSRVTEDSARLSWDDQFEDWFDSFLIQYQESEQVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVHTRDWTAWNASNPLSAIFTT

SEQ ID NO:64 ISOP120BR5P1G4
LPAPKNLVVSRVTEDSARLSWAGHYRKIRNFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVKFPYYYATADSNPLSAIFTT

SEQ ID NO:65 ISOP120BR5P1A5
LPAPKNLVVSRVTEDSARLSWAGHYRKIRNFDSFLIQYQESEKVGEAIVLTVPGSK
RSYDLTGLKPGTEYTVSIYGVKFPYYYATADSNPLSAIFTT

SEQ ID NO:66 ISOP120BR5P1E6
LPAPKNLVVSRVTEDSARLSWLEGANAEFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVHWVGPWYPVSNPLSAIFTT

SEQ ID NO:67 ISOP120BR5P1B7
LPAPKNLVVSRVTEDSARLSWGAKTRQFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVWWVENHFPVSNPLSAIFTT

SEQ ID NO:68 ISOP120BR5P1C7
LPAPKNLVVSRVTEDSARLSWNVTQKEFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVGNRYYTVYDSNPLSAIFTT

SEQ ID NO:69 ISOP120BR5P1D8
LPAPKNLVVSRVTEDSARLSWKNHTQEWEFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVPIAWLAWTSTSNPLSAIFTT

SEQ ID NO:70 ISOP120BR5P1E8
LPAPKNLVVSRVTEDSARLSWNGGEYWVPRFDSFLIQYQESEKVGEAIVLTVPGS
ERSYDLTGLKPGTEYTVSIYGVVWLQWISWTDSNPLSAIFTT

SEQ ID NO:71 ISOP120BR5P1B9
LPAPKNLVVSRVTEDSARLSWAVEFNPTKFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVWWFEQWYPVSNPLSAIFTT

SEQ ID NO:72 ISOP120BR5P1C9
LPAPKNLVVSRVTEDSARLSWAWNRHDFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVHWTVLRPFIDSNPLSAIFTT

SEQ ID NO:73 ISOP120BR5P1D9
LPAPKNLVVSRVTEDSARLSWTINSHIFDSFLIQYQESEKVGEAIVLTVPGSERSYD
LTGLKPGTEYTVSIYGVWGTKYWQAQSNPLSAIFTT

SEQ ID NO:74 ISOP120BR5P1A10
LPAPKNLVVSRVTEDSARLSWTEEDITHLRFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVYWTWWVRWNSNPLSAIFTT

SEQ ID NO:75 ISOP120BR5P1G11
LPAPKNLVVSRVTEDSARLSWTKRHFYTFDSFLIQYQESEKVGEAIVLTVPGSERS
YYLTGLKPGTENTVSIYGVHGNHPYTDAPANPLSAIFTT

SEQ ID NO:76 ISOP120GR5P1E1
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFIIQYAEDSSWGEAINLHVPGSERSY
DLTGLKPGTEYHVHIYGVKGGEASNPLWAWFTT

SEQ ID NO:77 ISOP120GR5P1G3
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFYIRYWEFCHSGEAIELSVPGSERSY
DLTGLKPGTEYFVRIVGVKGGRVSLPLGAKFTT

SEQ ID NO:78 ISOP120GR5P1F5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIDYWEVESEGEAIVLFVPGSERSY
DLTGLKPGTEYHVHIVGVKGGTPSYPLWADFTT

SEQ ID NO:79 ISOP120GR5P1H6
LPAPKNLVVSRVTEDSARLSWTNEQITKYGFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:80 ISOP120GR5P1E7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIDYWEVESEGEAIILFVPGSERSY
DLTGLKPGTEYHVHIVGVKGGTPSYPLWADFTT

SEQ ID NO:81 ISOP120GR5P1A10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIPYIEVETIGEAIWLHVPGSERSYD
LTGLKPGTEYSVGINGVKGGHTSNPLSARFTT

SEQ ID NO:82 ISOP120GR5P1C10
LPAPKNLVVSRVTEDSARLSWTAPDGAFDSFEIPYIEVETIGEAIWLHVPGSERSYD
LTGLKPGTEYSVGINGVKGGHTSNPLSARFTT

SEQ ID NO:83 ISOP120GR5P1A11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFGIPYWEWTTEGEAIQLIVPGSERSY
DLTGLKPATEYHVHIVGVKGGSFSEPLPADFTT

SEQ ID NO:84 ISOP120GR5P1B11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFNIKYWEANLYGEAIVLTVPGSERS
YGLTGLKPGTEYRVHIRGVKGGINSFPLVAVFTT

SEQ ID NO:85 ISOP120GR5P1H11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFYIAYWEYWGNGEAIGLIVPGSERS
YDLTGLKPGTEYHVHIVGVKGGAGSVPLWANFTT

SEQ ID NO:86 ISOP120HR5P1E2
LPAPKNLVVSHVTEDSARLSWTAPDAAFDSFEIYYLEGGRGEAIVLTVPGSERSYD
LTVLKPGTEYLGTIYGVKCGWASNPLSAIFTT

SEQ ID NO:87 ISOP120HR5P1A3
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYAEFGYYGEAIVLTVPGSERSY
DLTGLKPGTEYTVTIYGVKGGWYSTPLSAIFTT

SEQ ID NO:88 ISOP120HR5P1B4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSIYYGEYYNLGEAIVLTVPGSERSY
DLTGLKPGTEYVVTIYGVKGGGYSNPLSAIFTT

SEQ ID NO:89 ISOP120HR5P1G4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYREYWYSGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGWYSDPLSAIFTT

SEQ ID NO:90 ISOP120HR5P1H4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDILYLEPYQEGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYYSLPLSAIFTT

SEQ ID NO:91 ISOP120HR5P1B5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFIIRYIEEGYYGEAIVLTVPGSERSYD
LTGLKPGTEYHVGIEGVKGGYYSYPLSAIFTT

SEQ ID NO:92 ISOP120HR5P1A6
LPAPKNLVVSRVTEDSARLSWTAPDGAFDSFEIYYLEGGRGEAIVLTVPGSERSYD
LTGLKPGTEYLVTIYGIKCGWASNPLSAIFTT

SEQ ID NO:93 ISOP120HR5P1G6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGLDSQPLSAIFTT

SEQ ID NO:94 ISOP120HR5P1A7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYAEPRYYGEAIVLTVPGSERSY
DLTGLKPGTEYTVTIYGVKGGYYSSPLSAIFTT

SEQ ID NO:95 ISOP120HR5P1D7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYLESWTRGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGSYSRPLSAIFTT

SEQ ID NO:96 ISOP120HR5P1E7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFQIYYLEQLGYGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKVCEQSYPLSAIFTT

SEQ ID NO:97 ISOP120HR5P1H7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYGEPGNLGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGDYSSPLSAIFTT

SEQ ID NO:98 ISOP120HR5P1H8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYYSGPLSAIFTT

SEQ ID NO:99 ISOP120HR5P1D9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYRELDFQGEAIVLTVPGSERSY
DLTGLKPGTEYLVIIYGVKGGSYSYTLSAIFTT

SEQ ID NO:100 ISOP120HR5P1F9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFYIYYREHWTIGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGAYSNPLSAIFTT

SEQ ID NO:101 ISOP120ER5P1B4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSILYGEPPALGEAIVLTVPGSERSY
DLTGLKPGTEYWVTIYGVKGGVFSHPLSAIFTT

SEQ ID NO:102 ISOP120ER5P1F4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFVIRYIEDTVMGEAIVLTVPGSERSY
DLTGLKPGTEYHVSIEGVKGGPSSLPLSAIFTT

SEQ ID NO:103 ISOP120ER5P1H4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFNIMYLEDVQCGEAIVLTVPGSERS
YDLTGLKPGTEYHVGINGVKGGLRSFPLSAIFTT

SEQ ID NO:104 ISOP120ER5P1E5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFRISYLEDVYYGEAIVLTVPGSERSY
DLTGLKPGTEYHVGIHGVKGGIDSFPLSAIFTT

SEQ ID NO:105 ISOP120ER5P1B6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYGEHWKLGEAIVLTVQGSERS
YDLTGLKPGTEYLVTIYGVKGGQWSFPLSAIFTT

SEQ ID NO:106 ISOP120ER5P1C6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSIYYGEWHALGEAIVLTVPGSERSY
DLTGLKPGTEYVVTIYGVKGGTYSLPLSAISTT

SEQ ID NO:107 ISOP120ER5P1H6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFSIYYGEWHALGEAIVLTVPGSERSY
DLTGLKPGTEYVVTIYGVKGGTYSLPLSAIFTT

SEQ ID NO:108 ISOP120ER5P1A7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFNIGYYERIIPGEAIVLTVPGSERSYD
LTGLKPGTEYSVLICGVKGGKGSIPLSAIFTT

SEQ ID NO:109 ISOP120ER5P1A8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSMPLSAIFTT

SEQ ID NO:110 ISOP120ER5P1E10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYMEDFHSGEAIVLTVPGSERSY
DLTGLKPGTEYWVTIYGVEGGTGSLPLSAIFTT

SEQ ID NO:111 ISOP120ER5P1A11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYKELRAEGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGSVSIPLSAIFTT

SEQ ID NO:112 ISOP120ER5P1B12
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIYYIEWTAYGEAIVLTVPGSERSY
DLTGLKPGTEYVVRISGVKCGIVSFPLSAIFTT

SEQ ID NO:113 ISOP120FR5P1F1
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFTIYYFENENGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:114 ISOP120FR5P1C2
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDINYFEQPKGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGPYSPPLSAIFTT

SEQ ID NO:115 ISOP120FR5P1H5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSLQIYYFEWVVGGEAIVLTVPGSERS
YDLTGLKLGTEYLVTIYGVKGGNFSDPLSAIFTT

SEQ ID NO:116 ISOP120FR5P1A6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFIIRYLEDISYGEAIVLTVPGSERSYD
LTGLKPGTEYHVGIEGVKGGNVSFPLSAIFTT

SEQ ID NO:117 ISOP120FR5P1H6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFGIPYLEDIEVGEAIVLTVPGSERSYD
LAGLKPGTEYHVGIYGVKGGEQSFPLSAIFTT

SEQ ID NO:118 ISOP120FR5P1D7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFIIRYLEDISYGEAIVLTVPGSERSYD
LTGLKPGTEYHVGIEGVKGGNVSWPLSAIFTT

SEQ ID NO:119 ISOP120FR5P1F8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFIIRYLEDISYGEAIVLTVPGSERSYD
LTGLKPGTEYHVGIEGVKGGNVSWPLSAIFTT

SEQ ID NO:120 ISOP120FR5P1E9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFIIYYPEYISNGEAIVLTVPGSERSYD
LTGLKPGTEYHVTIGVKGGHSWPLSAIFTT

SEQ ID NO:121 ISOP120FR5P1E10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIHYTEQPSKGEAIVLTVPGSERSY
DLTGLKPGTEYQVPIGVKGGTQSCPLSAIFTT

SEQ ID NO:122 ISOP120FR5P1A11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFTIYYFENENGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGHWSRPLSAIFTT

SEQ ID NO:123 ISOP193AR9P1A11
LPAPKNLVVSRVTEDSARLSWALSSVHAYFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVQYVDGFFKSNPLSAIFTT

SEQ ID NO:124 ISOP193AR9P1A6
LPAPKNLVVSRVTEDSARLSWKFGEVAFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:125 ISOP193AR9P1B10
LPAPKNLVVSRVTEDSARLSWAFQWHIFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:126 ISOP193AR9P1B12
LPAPKNLVVSRVTEDSARLSWTNEQITKYGFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVGAPYTVYDSNPLSAIFTT

SEQ ID NO:127 ISOP193AR9P1B4
LPAPKNLVVSRVTEDSARLSWRDLQYHTFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:128 ISOP193AR9P1C10
LPAPKNLVVSRVTEDSARLSWPNHISIFDSFLIQYQESEKVGEAIVLTVPGSERSYD
LTGLKPGTEYTVSIYGVGRFYTVFDSNPLSAIFTT

SEQ ID NO:129 ISOP193AR9P1E6
LPAPKNLVVSRVTEDSARLSWKFHSPTFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIVTT

SEQ ID NO:130 ISOP193AR9P1F4
LPAPKNLVVSRVTEDSARLSWLEQEQFVNHFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVQYVDGFFKSNPLSAIFTT

SEQ ID NO:131 ISOP193AR9P1F9
LPAPKNLVVSRVTEDSARLSWPLFASDLNIFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:132 ISOP193AR9P1G11
LPAPKNLVVSRVTEDSARLSWTNEQITKYGFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:133 ISOP193AR9P1G5
LPAPKNLVVSRVTEDSARLSWRISDRLPLFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:134 ISOP193AR9P1G8
LPAPKNLVVSRVTEDSARLSWHATFGDPFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVGRHYTVYDSNPLSAIFTT

SEQ ID NO:135 ISOP193AR9P1H8
LPAPKNLVVSRVTEDSARLSWTNEQITKYGFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVGRFYTVFDSNPLSAIFTT

SEQ ID NO:136 ISOP193BR9P1B10
LPAPKNLVVSRVTEDSARLSWAWNRHDFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVHWTVLRPFIDSNPLSAIFTT

SEQ ID NO:137 ISOP193BR9P1B12
LPAPKNLVVSRVTEDSARLSWPDESRPVRFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVLRPWIYATNDSNPLSAIFTT

SEQ ID NO:138 ISOP193BR9P1E6
LPAPKNLVVSRVTEDSARLSWGAITALFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVKFPYYYYATADSNPLSAIFTT

SEQ ID NO:139 ISOP193BR9P1G11
LPAPKNLVVSRVTEDSARLSWAGHYRKIRNFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVKFPYYYATADSNPLSAIFTT

SEQ ID NO:140 ISOP193BR9P1G2
LPAPKNLVVSRVTEDSARLSWAGHYRKIRNFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVAEHWYYATQDSNPLSAIFTT

SEQ ID NO:141 ISOP193BR9P1G3
LPAPKNLVVSRVTEDSARLSWAQSNQQFDSFLIQYQESEKVGEAIVLTVPGSERSY
DLTGLKPGTEYTVSIYGVVWQNWVAYNSNPLSAIFTT

SEQ ID NO:142 ISOP193BR9P1G6
LPAPKNLVVSRVTEDSARLSWDDQFEDWFDSFLIQYQESEQVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVHTRDWTAWNASNPLSAIFTT

SEQ ID NO:143 ISOP193BR9P1G9
LPAPKNLVVSRVTEDSARLSWKQVTVAPEFDSFLIQYQESEKVGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVKFPYYYATADSNPLSAIFTT

SEQ ID NO:144 ISOP193BR9P1H2
LPAPKNLVVSRVTEDSARLSWPDESRPVRFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVHTRDWTAWNASNPLSAIFTT

SEQ ID NO:145 ISOP193BR9P1H3
LPAPKNLVVSRVTEDSARLSWNRLDSEWVAFDSFLIQYQESEKVGEAIVLTVPGSE
RSYDLTGLKPGTEYTVSIYGVVFRPWLAYNSNPLSAIFTT

SEQ ID NO:146 ISOP193BR9P1H6
LPAPKNLVVSRVTEDSARLSWPDESRPVRFDSFLIQYQESEKVGEAIVLTVPGSERS
YDLTGLKPGTEYTVSIYGVVGQWKYATADSNPLSAIFTT

SEQ ID NO:147 ISOP193ER9P1A10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGHFSGPLSAIFTT

SEQ ID NO:148 ISOP193ER9P1A11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIQYQEYVAHGEAIVLTVPGSERS
YDLTGLKPGTEYHVRISGVKGGGVSWPLSAIFTT

SEQ ID NO:149 ISOP193ER9P1A3
LPAPKNLVVSRVTEDSARLSWTTPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSKPLSAIFTT

SEQ ID NO:150 ISOP193ER9P1A4
LPAPKNLIVSRVTEDSARLSWTAPDAAFDSFEIYYKELRAEGEAIVLTVPGSERSYD
LTGLKPGTEYLVTIYGVKGGSVSIPLSAIFTT

SEQ ID NO:151 ISOP193ER9P1A8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSLDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSMPLSAIFTT

SEQ ID NO:152 ISOP193ER9P1B4
LPAPKNLVVSHVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSMPLSAIFTT

SEQ ID NO:153 ISOP193ER9P1B5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIQYQEYVAHGEAIVLTVPGSERS
YDLTGLKPGTEYHVRISGVKGGGVSWPLSAIVTT

SEQ ID NO:154 ISOP193ER9P1C10
LLAPKNLVVSRVTEDSARLSWIAPDAAFDSFEIYYKELRAEGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGSVSIPLSAIFTT

SEQ ID NO:155 ISOP193ER9P1C4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGIWSVPLSAIFTT

SEQ ID NO:156 ISOP193ER9P1C8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGTYSLPLSAIFTT

SEQ ID NO:157 ISOP193ER9P1C9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGELSKPLSAISTT

SEQ ID NO:158 ISOP193ER9P1D4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGTYSPPLSAIFTT

SEQ ID NO:159 ISOP193ER9P1D7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYGEHWKLGEAIVLTVPGSERS
YDLTGLKPGTEYLVTIYGVKGGMSSNPLSAIFTT

SEQ ID NO:160 ISOP193ER9P1E1
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGSVSIPLSAIFTT

SEQ ID NO:161 ISOP193ER9P1E2
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGFWSQPLSAIFTT

SEQ ID NO:162 ISOP193ER9P1E4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIIYQEYVKSGEAIVLTVPGSERSY
DLTGLKPGTEYHVRIGGVKGGLLSLPLSAIFTT

SEQ ID NO:163 ISOP193ER9P1E8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGELSKPLSAIFTT

SEQ ID NO:164 ISOP193ER9P1F11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIQYQEYVAHGEAIVLTVPGSERS
YDLTGLKPGTEYHVRISGVKGGGVSWPLSAISTT

SEQ ID NO:165 ISOP193ER9P1F7
LPAPKNLVVSRVTEDSAHLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSMPLSAIFTT

SEQ ID NO:166 ISOP193ER9P1F9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFHIYYGEHYNLGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGFWSTPLSAIFTT

SEQ ID NO:167 ISOP193ER9P1G11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSMPLSAIFTT

SEQ ID NO:168 ISOP193ER9P1G2
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEQPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSMPLSAIFTT

SEQ ID NO:169 ISOP193ER9P1G4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGNFSFPLSAIFTT

SEQ ID NO:170 ISOP193ER9P1G5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYLSMPLSAIFTT

SEQ ID NO:171 ISOP193ER9P1G9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGNGSSPLSAIFTT

SEQ ID NO:172 ISOP193ER9P1H11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYFEHPVGGEAIVLTVPGSERSY
DLTGLKPGTEYLVAIYGVKGGVFSHPLSAIFTT

SEQ ID NO:173 ISOP193ER9P1H2
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFRISYLEDVYYGEAIVLTVPGSERSY
DLTGLKPGTEYHVGIHGVKGGIDSFPLSAIFTT

SEQ ID NO:174 ISOP193ER9P1H3
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYLEVRNRGEAIVLTVPGSERSY
DLTGLKPGTEYHVGIAGVKGGFHSFPLSAIFTT

SEQ ID NO:175 ISOP193FR9P1A11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIQYWEGWEWGEAIVLTVPGSERS
YDLTGLKPGTEYLVTIYGVKGGHWSRPLSAIFTT

SEQ ID NO:176 ISOP193FR9P1A5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYIEPIAPGEAIVLTVPGSERSYD
LTGLKPGTEYWVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:177 ISOP193FR9P1C1
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFENENGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:178 ISOP193FR9P1C5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFHIYYLEQYSRGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:179 ISOP193FR9P1C9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFQIYYFEWVVGGEAIVLTVPGSERS
YDLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:180 ISOP193FR9P1D1
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFFIQYLEDVTNGEAIVLTVPGSERSY
DLTGLKPGTEYRVPIAGVKGGRDSQPLSAIFTT

SEQ ID NO:181 ISOP193FR9P1D5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFYIRYIEDVDFGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:182 ISOP193FR9P1D7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYAEYFKNGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAISTT

SEQ ID NO:183 ISOP193FR9P1E1
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFIIRYLEDISYGEAIVLTVPGSERSYD
LTGLKPGTEYHVGIEGVKGGNVSWPLSAIFTT

SEQ ID NO:184 ISOP193FR9P1E10
LPAPKNLVVSRVTEDSARLSWTTPDAAFDSFHIHYLEGEWGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:185 ISOP193FR9P1F8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDINYFENELGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:186 ISOP193FR9P1G10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFTIYYFENENGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGHWSRPLSAIFTT

SEQ ID NO:187 ISOP193FR9P1G11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFTIYYFENENGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:188 ISOP193FR9P1G2
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFTIYYFENENGGEAIVLTVPGSERSY
DLTGLKPDTEYLVTIYGVKGGHWSRPLSAIFTT

SEQ ID NO:189 ISOP193FR9P1G4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFENELGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGDWSDPLSAIFTT

SEQ ID NO:190 ISOP193FR9P1G7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFHIHYLEGEWGGEAIVLTVPGSERS
YDLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:191 ISOP193FR9P1G8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFENELGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:192 ISOP193FR9P1G9
LPAPKNLFVSRVTEDSARLSWTAPDAAFDSFQIYYREQWWDGEAIVLTVPGSERS
YDLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:193 ISOP193FR9P1H6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDINYFEQPKGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCDWSDPLSAIFTT

SEQ ID NO:194 ISOP193FR9P1H9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYDELRNPGEAIVLTVPGSERSY
DLTGLKPGTEYAVTIYGVKGGRYSPPLSAIFTT

SEQ ID NO:195 ISOP193GR9P1A7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIFYHEFANPGEAIDLPVPGSERSY
DLTGLKPGTEYDVRIYGVKGGTASIPLDAEFTT

SEQ ID NO:196 ISOP193GR9P1B3
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIGYVEWTANGEAIVLIVPGSERSY
DLTGLKPGTEYVVRIRGGVKGGDSSFPLRADFTT

SEQ ID NO:197 ISOP193GR9P1E10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAISYTESIRQGEAIWLWVPGSERSY
DLTGLKPGTEYEVTIGGVKGGIRSYPLWAWFTT

SEQ ID NO:198 ISOP193GR9P1F6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIDYWEVESEGEAIVLFVPGSERSY
DLTGLKPGTEYHVHIVGVKGGTPSYPLWADFTT

SEQ ID NO:199 ISOP193GR9P1F7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIPYVEYYPSGEAIVLNVPGSERSY
DLTGLKPGTEYGVTIWGIKGGNESVPLTARFTT

SEQ ID NO:200 ISOP193GR9P1G9
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFAIFYHEFANSGEAIDLPVPGSERSY
DLTGLKPGTEYDVRIYGVKGGTASIPLDAEFTT

SEQ ID NO:201 ISOP193GR9P1H2
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIPYIEVETIGEAIWLHVPGSERSYD
LTGLKPGTEYSVGINGVKGGHTSNPLSARFTT

SEQ ID NO:202 ISOP193HR9P1A10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYLEPWGGGEAIVLTVPGSERSY
DLTGLKPGTEYWVTIYGVKVCLGSNPLSAIFTT

SEQ ID NO:203 ISOP193HR9P1A11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYLEGGRGEAIVLTVPGSERSYD
LTGLKPGTEYLVTIYGVKCGWASNPLSAIFTT

SEQ ID NO:204 ISOP193HR9P1A5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCGYSAPLSAIVTT

SEQ ID NO:205 ISOP193HR9P1A6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVSIYGVKGCGYSDPLSAIFTT

SEQ ID NO:206 ISOP193HR9P1A7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKVCNASTPLSAIFTT

SEQ ID NO:207 ISOP193HR9P1B11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCGYSDPLSAIFTT

SEQ ID NO:208 ISOP193HR9P1B7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVSIYGVKGCGYSDPLSAIFTT

SEQ ID NO:209 ISOP193HR9P1C7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYLELDSDGEAIVLTVPGSERSY
DLTGLKPGTEYIVTIYGVKVCTGSRPLSAIFTT

SEQ ID NO:210 ISOP193HR9P1C8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGGYSTPLSAIFTT

SEQ ID NO:211 ISOP193HR9P1D11
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKVCEQSYPLSAIFTT

SEQ ID NO:212 ISOP193HR9P1D8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYLESGRDGEAIVLTVPGSERSY
DLTGLKPGTEYLVSIYGVKGCGYSDPLSAIFTT

SEQ ID NO:213 ISOP193HR9P1E2
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYLEWCSGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCAASDPLSAIFTT

SEQ ID NO:214 ISOP193HR9P1E3
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGAYSNPLSAIFTT

SEQ ID NO:215 ISOP193HR9P1E6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYAEFGYYGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCAASDPLSAIFTT

SEQ ID NO:216 ISOP193HR9P1E8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGDYSPPLSAIFTT

SEQ ID NO:217 ISOP193HR9P1F10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGYYSGPLSAIFTT

SEQ ID NO:218 ISOP193HR9P1F8
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKVCYYSTPLSAIFTT

SEQ ID NO:219 ISOP193HR9P1G10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGDYSPPLSAISTT

SEQ ID NO:220 ISOP193HR9P1G4
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYFELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGGDYSPPLSAIFTT

SEQ ID NO:221 ISOP193HR9P1G5
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKGCAASDPLSAIFTT

SEQ ID NO:222 ISOP193HR9P1G6
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKVCEQSYPLSAISTT

SEQ ID NO:223 ISOP193HR9P1H10
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFDIYYYELRLGGEAIVLTVPGSERSY
DLTGLKPGTEYLVTIYGVKVCLGSNPLSAIFTT

SEQ ID NO:224 ISOP193HR9P1H7
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFEIYYREPHYGGEAIVLTVPGSERSY
DLTGLKPGTEYWVTIYGVKVCLGSNPLSAIFTT

WHAT IS CLAIMED

1) An isolated FN3 domain that specifically binds to CD137.

2) The FN3 domain of claim 1, wherein the FN3 domain is based on Tencon 27 (SEQ ID NO:4) or Tencon 1 (SEQ ID NO:1), or on the amino acid sequence of SEQ ID NO:4, optionally having substitutions at residue positions 11, 14, 17, 37, 46, 73 and/or 86 corresponding to SEQ ID NO:4.

3) The FN3 domain of claim 1, wherein the FN3 domain is conjugated to a detectable label, a cytotoxic agent, or both.

4) The FN3 domain of claim 3, wherein the detectable label is a radioactive isotope, magnetic beads, metallic beads, colloidal particles, a fluorescent dye, an electron-dense reagent, an enzyme, biotin, digoxigenin, or hapten.

5) The FN3 domain of claim 4, wherein the detectable label is auristatin, monomethyl auristatin phenylalanine, dolostatin, chemotherapeutic agent, a drug, a growth inhibitory agent, a toxin, or a radioactive isotope.

6) The FN3 domain of claim 1, comprising the amino acid sequence of SEQ ID NOs: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, or 224.

7) The FN3 domain of claim 6, further comprising a methionine at the N-terminus of the FN3 domain.

8) The FN3 domain of claim 7, wherein the FN3 domain is coupled to a half-life extending moiety.

9) The FN3 domain of claim 8, wherein the half-life extending moiety is an albumin binding molecule, a polyethylene glygol (PEG), albumin, albumin variant, or at least a portion of an Fc region of an immunoglobulin.

10) An isolated polynucleotide encoding the FN3 domain of claim 6.

11) A vector comprising the polynucleotide of claim 10.

12) A host cell comprising the vector of claim 11.

13) A method of producing an FN3 domain that specifically binds CD137, comprising culturing the isolated host cell of claim 12 under conditions that the FN3 domain is expressed, and purifying the FN3 domain.

14) A pharmaceutical composition comprising the FN3 domain of any of claim 6 and a pharmaceutically acceptable carrier.

15) An anti-idiotypic antibody that specifically binds the FN3 domain of claim 6.

16) A kit comprising the FN3 domain of claim 6.

17) A method of detecting CD137-expressing cancer cells in a tumor tissue, comprising

a) obtaining a sample of the tumor tissue from a subject; and

b) detecting whether CD137 is expressed in the tumor tissue by contacting the sample of the tumor tissue with the FN3 domain that specifically binds CD137 comprising the amino acid sequence of one of SEQ ID NOs: 45-224 and detecting the binding between CD137 and the FN3 domain.

18) A method of isolating CD137 expressing cells, comprising

a) obtaining a sample from a subject;

b) contacting the sample with the FN3 domain that specifically binds CD137 comprising the amino acid sequence of one of SEQ ID NOs: 45-224, and

c) isolating the cells bound to the FN3 domain.

19) A method of detecting CD137-expressing cancer cells in a tumor tissue, comprising

a) conjugating the FN3 domain that specifically binds CD137 comprising the amino acid sequence of one of SEQ ID NOs: 45-224 to a detectable label to form a conjugate;

b) administering the conjugate to a subject; and

c) visualizing the CD137 expressing cancer cells to which the conjugate is bound.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/65983

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 51/08, A61K 49/00, G01N 33/53 (2018.01)
CPC - A61K 51/088, A61K 49/0004, G01N 33/53, G01N 33/534

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	WO 2016/086036 A2 (BRISTOL-MYERS SQUIBB COMPANY) 2 June 2016 (2.06.2016) para [00058]; para [000122]; para [000135]; para [000137-000138]	1, 3-5 --- 2 --- 6-9, 14, 16
Y	WO 2010/051274 A2 (CENTOCOR ORTHO BIOTECH INC et al.) 6 May 2010 (6.05.2010) Claim 2	2
A	US 2015/0210756 A1 (NEW YORK UNIVERSITY et al.) 30 July 2015 (30.07.2015) para [0017]; claim 28	6-9, 14, 16

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 2018	26APR 2018

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/65983

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

----Please see continuation in first extra sheet ----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-9, 14, 16 limited to SEQ ID NO: 45

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/65983

Continuation of Box No. III. Observations where unity of invention is lacking.

Group I+: Claims 1-9, 14, 16, drawn to an isolated FN3 domain that specifically binds to CD137, pharmaceutical composition comprising said FN3 domain, antibody that binds said FN3 domain, and kit comprising said FN3 domain. The FN3 domain compositions will be searched to the extent that the FN3 domain amino acid sequence encompasses SEQ ID NO: 45. It is believed that claims 1-9, 14, 16, limited to SEQ ID NO: 45 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass compositions comprising a FN3 domain having the amino acid sequence of SEQ ID NO: 45. Additional FN3 domain amino acid sequences will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected FN3 domain amino acid sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a compositions comprising a FN3 domain having the amino acid sequence of SEQ ID NO: 46, i.e. claims 1-9, 14, 16, limited to SEQ ID NO: 46.

Group II: Claims 10-13, drawn to a polynucleotide encoding a FN3 domain, vector comprising said polynucleotide, host cell comprising said vector, and method of using said host cell.

Group III: Claim 15, drawn to an anti-idiotypic antibody that specifically binds the FN3 domain.

Group IV+: Claims 17-19, drawn to methods of detecting CD137-expressing cells. Group III+ will be searched upon payment of additional fees. The detection methods may be searched for an additional fee and election as such, for example, to the extent that the FN3 domain amino acid sequence encompasses SEQ ID NO: 45, i.e. claims 17-19, limited to SEQ ID NO: 45. Additional FN3 domain amino acid sequences will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected FN3 domain amino acid sequence(s). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be detection methods comprising a FN3 domain having the amino acid sequence of SEQ ID NO: 46, i.e. claims 17-19, limited to SEQ ID NO: 46.

The inventions listed as Groups I+, II, III, IV+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I+ requires compositions of matter comprising FN3 domain protein, not required by Groups II and III.

Group II requires compositions of matter comprising polynucleotide encoding a FN3 domain, vector comprising said polynucleotide, host cell comprising said vector, and method of using said host cell, not required by Groups I+ and III.

Group III requires an anti-idiotypic antibody that specifically binds the FN3 domain

Group IV+ requires method steps for detecting CD137-expressing cells, not required by Groups I+ and II.

No technical features are shared between the FN3 domain amino acid sequences of the inventions listed as Groups I+ and the inventions listed as Groups III+ and, accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Groups I+ and the inventions listed as Groups III+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Common Technical Features

The feature shared by the inventions listed as Groups I+, II, III, IV+ is a FN3 domain that specifically binds CD 137.

The feature shared by the inventions listed as Groups I+, II, and III is the FN3 domain of claim 1

Further features shared by the inventions listed as Groups I+ are the pharmaceutical composition of claim 14 and the kit of claim 16.

The feature shared by the inventions listed as Groups III+ are the detection methods of claims 17, 18, and 19.

----Please see continuation in next extra sheet ----------

Continuation of Box No. III. Observations where unity of invention is lacking.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by WO 2016/086036 A2 to Bristol-Myers Squibb Co. (hereinafter 'Bristol-Myers Squibb').

Bristol-Myers Squibb discloses [claim 1] an isolated FN3 domain (para [000135] - "Conventional separation and purification techniques known in the art can be used to purify PEGylated FBS proteins"; para [000122] - "Exemplary FBS moieties may be derived from tenascin, a protein that is composed of 15 Fn3 domains"). Bristol-Myers Squibb does not expressly teach that the FN3 domain specifically binds to CD137. However, Bristol-Myers Squibb does teach that the FN3 domain serves as the binding moeity of a 18F labeled imaging agent that can specifically bind to several targets, including CD137 (para [000137] - "Provided herein are 18F labeled imaging agents . . . In certain embodiments, the moiety is . . . an alternative scaffold, such as an Fn3 (e.g., a human Fn3) domain, such as an FBS"; para [000138] - "In certain embodiments, an 18F labeled imaging agent comprises a moiety that binds specifically to an immuno-oncology target (receptor or ligand), such as . . . CD137"). Given that Bristol-Myers Squibb does teach that a FN3 domain can serve as a binding moeity that targets and binds to targets including CD137, one of ordinary skill in the art would have found it obvious to obtain a FN3 domain that specifically binds to CD137.

Bristol-Myers Squibb discloses [claim 14] a pharmaceutical composition comprising the FN3 domain and a pharmaceutically acceptable carrier (para [000147] - "Further provided are compositions, e.g., a pharmaceutical compositions, containing one or a combination of 18F-labeled targeting agents, described herein, formulated together with a pharmaceutically acceptable carrier"; para [000137] - "Provided herein are 18F labeled imaging agents . . . In certain embodiments, the moiety is . . . an alternative scaffold, such as an Fn3 (e.g., a human Fn3) domain").

Bristol-Myers Squibb discloses [claim 16] a kit comprising the FN3 domain (para [000169] - "Also provided are kits for producing the 18F -radiolabeled targeting compositions described herein and instructions for use. Kits typically include a packaged combination of reagents in predetermined amounts with instructions and a label indicating the intended use of the contents of the kit"; para [000137] - "Provided herein are 18F labeled imaging agents . . . In certain embodiments, the moiety is . . . an alternative scaffold, such as an Fn3 (e.g., a human Fn3) domain").

Bristol-Myers Squibb discloses [claim 17] a method of detecting cancer cells in a tumor tissue (para [000179] - "In certain aspects, provided is a method of diagnosing the presence of a disease in a subject"; para [000180] - "In some embodiments, the disease is a solid cancer, hematopoietic cancer, hematological cancer"), comprising
a) obtaining a sample of the tumor tissue from a subject (para [00075] - "The term "sample" can refer to a tissue sample, cell sample, a fluid sample, and the like. The sample may be taken from a subject"); and
b) detecting whether a target is expressed in the tumor tissue by contacting the sample of the tumor tissue with the FN3 domain and detecting the binding of the FN3 domain (para [000179] - "the method comprising administering to a subject in need thereof an 18F-radiolabeled protein-based probe which binds to a target molecule associated with the presence of the disease, and obtaining a radio-image of at least a portion of the subject to detect the presence or absence of the 18F-radiolabeled protein-based probe"; para [000157] - "the 18F-labeled targeting agent can be used to image target-positive cells or tissues, e.g., target expressing tumors").
Bristol-Myers Squibb does not expressly teach that target expressed in the cancer cell is CD 137, and that the FN3 domain specifically binds to CD137. However, Bristol-Myers Squibb does teach that the FN3 domain serves as the binding moeity of a 18F labeled imaging agent that can specifically bind to several targets on tumor cells (para [000107] - "the 18F-labeled peptides or protein may be selected to bind directly to a targeted cell, tissue, pathogenic organism or other target for imaging and/or detection"; para [000136] - "In certain embodiments, the moiety binds to a cell surface molecule, e.g., a cell surface molecule on a tumor cell or a cell in the tumor"; para [000137] - "Provided herein are 18F labeled imaging agents . . . In certain embodiments, the moiety is . . . an alternative scaffold, such as an Fn3 (e.g., a human Fn3) domain, such as an FBS"), where the target can include CD137 (para [000138] - "In certain embodiments, an 18F labeled imaging agent comprises a moiety that binds specifically to an immuno-oncology target (receptor or ligand), such as . . . CD137"). Given that Bristol-Myers Squibb does teach that a FN3 domain can serve as a binding moeity that targets and binds to tumor cell targets including CD137, one of ordinary skill in the art would have found it obvious to obtain a FN3 domain that specifically binds to CD137 for targeting CD137 expressing cancer cells.

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Continuation of Box No. III. Observations where unity of invention is lacking.

Bristol-Myers Squibb discloses [claim 18] a method of isolating CD137 expressing cells (para [000179] - "In certain aspects, provided is a method of diagnosing the presence of a disease in a subject"; para [000180] - "In some embodiments, the disease is a solid cancer, hematopoietic cancer, hematological cancer"), comprising
a) obtaining a sample from a subject (para [00075] - "The term "sample" can refer to a tissue sample, cell sample, a fluid sample, and the like. The sample may be taken from a subject"); and
b) contacting the sample with the FN3 domain (para [000179] - "the method comprising administering to a subject in need thereof an 18F-radiolabeled protein-based probe which binds to a target molecule associated with the presence of the disease, and obtaining a radio-image of at least a portion of the subject to detect the presence or absence of the 18F-radiolabeled protein-based probe").
Bristol-Myers Squibb does not expressly teach that the cancer cell expresses CD 137, and that the FN3 domain specifically binds to CD137. However, Bristol-Myers Squibb does teach that the FN3 domain serves as the binding moeity of a 18F labeled imaging agent that can specifically bind to several targets on tumor cells (para [000107] - "the 18F-labeled peptides or protein may be selected to bind directly to a targeted cell, tissue, pathogenic organism or other target for imaging and/or detection"; para [000136] - "In certain embodiments, the moiety binds to a cell surface molecule, e.g., a cell surface molecule on a tumor cell or a cell in the tumor"; para [000137] - "Provided herein are 18F labeled imaging agents . . . In certain embodiments, the moiety is . . . an alternative scaffold, such as an Fn3 (e.g., a human Fn3) domain, such as an FBS"), where the target can include CD137 (para [000138] - "In certain embodiments, an 18F labeled imaging agent comprises a moiety that binds specifically to an immuno-oncology target (receptor or ligand), such as . . . CD137"). Given that Bristol-Myers Squibb does teach that a FN3 domain can serve as a binding moeity that targets and binds to tumor cell targets including CD137, one of ordinary skill in the art would have found it obvious to obtain a FN3 domain that specifically binds to CD137 for targeting CD137 expressing cancer cells.
Bristol-Myers Squibb also does not teach that the method further comprises c) isolating the cells bound to the FN3 domain. However, Bristol-Myers Squibb does teach that the FN3 domain is used to visualize the cancer cells to which the conjugate is bound (para [000179] - "obtaining a radio-image of at least a portion of the subject to detect the presence or absence of the 18F-radiolabeled protein-based probe"; para [000157] - "the 18F-labeled targeting agent can be used to image target-positive cells or tissues, e.g., target expressing tumors"). Given that cells bound to the FN2 domain are easily visualized and distinguished from cells that are not bound by the FN2 domain, one of ordinary skill in the art would have found it obvious to further isolate the cells bound to the FN3 domain from cells that are not bound by the FN2 domain.

Bristol-Myers Squibb discloses [claim 19] a method of detecting CD 137-expressing cancer cells in a tumor tissue (para [000179] - "In certain aspects, provided is a method of diagnosing the presence of a disease in a subject"; para [000180] - "In some embodiments, the disease is a solid cancer, hematopoietic cancer, hematological cancer"), comprising
a) conjugating the FN3 domain to a detectable label to form a conjugate (para [000137] - "Provided herein are 18F labeled imaging agents . . . In certain embodiments, the moiety is . . . an alternative scaffold, such as an Fn3 (e.g., a human Fn3) domain, such as an FBS");
b) administering the conjugate to a subject (para [000179] - "the method comprising administering to a subject in need thereof an 18F-radiolabeled protein-based probe which binds to a target molecule associated with the presence of the disease"); and
c) visualizing the cancer cells to which the conjugate is bound (para [000179] - "obtaining a radio-image of at least a portion of the subject to detect the presence or absence of the 18F-radiolabeled protein-based probe"; para [000157] - "the 18F-labeled targeting agent can be used to image target-positive cells or tissues, e.g., target expressing tumors").
Bristol-Myers Squibb does not expressly teach that the cancer cell expresses CD 137, and that the FN3 domain specifically binds to CD137. However, Bristol-Myers Squibb does teach that the FN3 domain serves as the binding moeity of a 18F labeled imaging agent that can specifically bind to several targets on tumor cells (para [000107] - "the 18F-labeled peptides or protein may be selected to bind directly to a targeted cell, tissue, pathogenic organism or other target for imaging and/or detection"; para [000136] - "In certain embodiments, the moiety binds to a cell surface molecule, e.g., a cell surface molecule on a tumor cell or a cell in the tumor"; para [000137] - "Provided herein are 18F labeled imaging agents . . . In certain embodiments, the moiety is . . . an alternative scaffold, such as an Fn3 (e.g., a human Fn3) domain, such as an FBS"), where the target can include CD137 (para [000138] - "In certain embodiments, an 18F labeled imaging agent comprises a moiety that binds specifically to an immuno-oncology target (receptor or ligand), such as . . . CD137"). Given that Bristol-Myers Squibb does teach that a FN3 domain can serve as a binding moeity that targets and binds to tumor cell targets including CD137, one of ordinary skill in the art would have found it obvious to obtain a FN3 domain that specifically binds to CD137 for targeting CD137 expressing cancer cells.

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Groups I+, II, III, IV+ therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.