

5 **CD8A-BINDING FIBRONECTIN TYPE III DOMAINS****Sequence Listing**

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety.

- 10 Said ASCII copy, created on December 5, 2017, is named JBI5112WOPCT_SL.txt and is 266,978 bytes in size.

Technical Field

- 15 The present invention relates to fibronectin type III (FN3) domains that specifically bind to cluster of differentiation 8a (CD8a). Such FN3 domains may be used for example, for medical imaging, diagnostics, and pharmaceutical therapy. Methods for the production of such molecules and diagnostic agents comprising them are also provided.

20 **Background**

- The rapidly evolving fields of cancer immunotherapy have recently led to the FDA approval of several new immunotherapies, with many more therapies presently in clinical trials for a variety of cancers. Furthermore, cellular, small molecule, antibody-based immunotherapies, and combinations thereof, are being rigorously tested
- 25 preclinically for clinical translation. The dynamic tumor microenvironment and tumor heterogeneity have become important topics in both preclinical and clinical studies (Hanahan D, Weinberg RA. Cell 2011;144:646–74; Mantovani A, Allavena P, Sica A, Balkwill F. Nature 2008; 454:436–44; Schreiber RD, Old LJ, Smyth MJ. Science 2011; 331:1565–70.), but the ability to
- 30 monitor changes in the immune status of primary lesions and metastatic cancers is limited. Current methods to monitor lymphocytes from whole blood or biopsies from heterogeneous tumors do not reflect the dynamic and spatial information likely required to monitor immune responses to therapeutic intervention, many of which elicit whole body changes in immune cell numbers and localization. Therefore, molecular imaging
- 35 methods that can noninvasively monitor both systemic and intratumoral alterations in immune cell numbers or localization during experimental therapies have the ability to increase the understanding of the dynamics of immunotherapeutic mechanism with the

- 5 potential to provide translatable methods for predicting and/or assessing clinical immunotherapeutic responses.

Analysis of tumor-infiltrating lymphocytes (TIL) has demonstrated the importance of tumor immune microenvironment and that the presence of cytotoxic CD8⁺ T cells can predict overall survival in breast, lung, ovarian, melanoma, and colorectal cancers (reviewed in refs. Pages F, et al., *Oncogene* 2010;29:1093–102. and Gooden MJ, et al. *Br J Cancer* 2011;105:93–103.). With the recent clinical successes of immunotherapies that alter the tumor immune microenvironment, including adoptive cell transfer (ACT) of T-cell receptor (TCR)- or chimeric antigen receptor-transduced cytotoxic T cells (Johnson LA, et al. *Blood* 2009;114:535–46; Rosenberg SA. *Sci Transl Med* 2012;4:127ps8.), agonistic antibodies targeting CD137 (4-1BB) and CD40 (Melero I, et al. *Clin Cancer Res* 2013;19:997–1008; Melero I, et al. *Nat Rev Cancer* 2007;7:95–106; Vinay DS, and Kwon BS. *Mol Cancer Ther* 2012;11:1062–70.), and antibody blockade of the checkpoint inhibitors CTLA-4, PD-1, and PD-L1 (Callahan MK, and Wolchok JD. *J Leukoc Biol* 2013;94:41–53; Shin DS, and Ribas A. *Curr Opin Immunol* 2015;33C:23–35; Topalian SL, et al. *Cancer Cell* 2015;27:450–61.), the ability to noninvasively monitor the tumor immune response to therapy has become of utmost importance.

SUMMARY

25 The present invention comprises CD8A-binding fibronectin type III (FN3) domains. Also described are related polynucleotides capable of encoding the provided FN3 domains, cells expressing the provided FN3 domains, as well as associated vectors. In addition, methods of using the provided FN3 domains are described. For example, the FN3 domains of the invention can be used to noninvasively and quantitatively monitor the presence and abundance of CD8⁺ T cells.

In some embodiments, the present invention comprises isolated FN3 domains, wherein the FN3 domains bind to a human CD8A comprising SEQ ID NO: 35. In other embodiments, the CD8A-specific FN3 domains bind to human CD8A and cynomolgus monkey CD8A. In yet other embodiments, the CD8A-specific FN3 domains are based on Tencon sequence of SEQ ID NO: 1. In further embodiments, the CD8A-specific FN3 domains are based on the Tencon27 sequence of SEQ ID NO: 4. In some embodiments, the albumin-specific FN3 domains are isolated from the library

5 comprising the sequence of SEQ ID NOs: 2, 3, 5, 6, 7 or 8. In some embodiments, the CD8A-specific FN3 domains do not activate CD8⁺ T-cells *in vitro* as measured by the enzyme-linked immunospot (ELISPOT) assay. In some embodiments, the CD8A-specific FN3 domains bind to human CD8A with an affinity (K_D) of between about 0.02 to about 6.6 nM as measured by surface plasmon resonance. In other
10 embodiments, the CD8A-specific FN3 domains have a cysteine substitution at residue position 54 of SEQ ID NOs 79, 81, 83, 89, 122 and 68. In other embodiments, the CD8A-specific FN3 domains comprise the amino acid sequence of SEQ ID NOs: 40-269. In other embodiments, the CD8A-specific FN3 domains are conjugated to a detectable label.

15 In addition to the described CD8A-specific FN3 domains, also provided are polynucleotide sequences capable of encoding the described FN3 domains. Vectors comprising the described polynucleotides are also provided, as are cells expressing the CD8A-specific FN3 domains herein. Also described are cells capable of expressing the disclosed vectors. These cells may be mammalian cells (such as 293F cells, CHO
20 cells), insect cells (such as Sf7 cells), yeast cells, plant cells, or bacteria cells (such as *E. coli*). A process for the production of the described FN3 domains is also provided.

The present invention also comprises methods of conjugating or otherwise associating the described CD8A-specific FN3 domains to various molecules for diagnostic purposes. For example, Zr-89 or I-124 are ideal fusion partners for creation
25 of diagnostic agents capable of detecting the presence of CD8⁺ T-cells. As such, the CD8A-specific FN3 domains have utility in cancer diagnostics using CD8A as a biomarker.

Another embodiment of the invention is a method of detecting CD8A-expressing cells in a biological sample comprising treating the biological sample with a
30 diagnostic agent comprising the described CD8A-specific FN3 domains. These methods are provided in the EXAMPLES.

Within the scope of the invention are kits including the disclosed CD8A-specific FN3 domains. The kits may be used to carry out the methods of using the CD8A-specific FN3 domains provided herein, or other methods known to those skilled
35 in the art. In some embodiments, the described kits may include the FN3 domains described herein and reagents for use in detecting the presence of human CD8A in a biological sample. The described kits may include one or more of the FN3 domains

5 described herein and a vessel for containing the FN3 domains when not in use,
instructions for use of the FN3 domains affixed to a solid support, and/or detectably
labeled forms of the FN3 domains, as described herein.

Brief Description of Drawings

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Fig. 1A-1D. The CD8S365-DFO conjugate does not activate T cells de novo and does not
modulate the antigen dependent activation of T cells in a 24 hour $\text{INF}\gamma$ EliSpot assay.

CMV reactive T cells were treated with 365-DFO in the absence (A) or presence (B) of
CMV peptides. A second M1 reactive donor was also tested in the absence (C) or

15 presence (D) of M1 peptides.

Fig. 2A and 2B. The CD8S365-DFO conjugate does not activate T cells de novo and
does not modulate the antigen dependent activation of T cells in a 6 day $\text{INF}\gamma$ MSD assay.

CMV reactive T cells were treated with 365-DFO in the absence (A) or presence (B) of

20 CMV peptides.

Fig. 3. Crude preparatory HPLC trace of $[^{124}\text{I}]$ -IPEM. Preparatory HPLC was
performed using a Waters 1525 Binary HPLC pump, a Waters 2489 dual wavelength
UV/Visible Detector ($\lambda = 214$ and 254 nm), a Bioscan Flow Count radiodetector (B-
25 FC-2000) and a Atlantis T3, $100 \text{ \AA}$, $5 \mu\text{m}$, 150×4.6 mm HPLC column. The elution
profile used was as follows: solvent A = H_2O (0.1% AcOH (v/v)), Solvent B = MeCN
(0.1% AcOH (v/v)), flow rate = 1.5 mLmin^{-1} ; initial = 80% A, $20 \text{ min} = 0\%$ A (linear
gradient). Multiple small molecule absorbance on UV-vis traces at 254 nm (top graph)
and 214 nm (middle graph) indicate presence of impurities and by-products in the crude
30 reaction mixture. Radiotrace (bottom graph) also shows expected baseline peaks due to
radiolabeled impurities.

Fig. 4. Analytical HPLC trace of $[^{124}\text{I}]$ -IPEM. Analytical HPLC was performed using a
Waters 1525 Binary HPLC pump, a Waters 2707 autosampler, a Waters 2489 dual
35 wavelength UV/Visible Detector ($\lambda = 214$ and 280 nm), a Bioscan Flow Count
radiodetector (B-FC-2000) and a Phenomenex Kinetex $5 \mu\text{m}$ XB-C18 $100 \text{ \AA}$, 150×4.6

5 mm HPLC column. The elution profile used was as follows: solvent A = H₂O (0.1% TFA (v/v)), Solvent B = MeCN (0.1% TFA (v/v)), flow rate = 1 mLmin⁻¹; initial = 90% A, 15 min = 0% A (linear gradient). Analytically pure [I-124] IPEM shows a single radiopeak (bottom graph) with a smooth baseline confirming successful purification. Please note that [I-124] IPEM is an organic small molecule; hence, lacking absorption at 280 nm (top graph) and 214 nm (middle graph).

Fig. 5. Radio TLC of purified [¹²⁴I]-IPEM CD8S365. The iTLC-SG plate (Agilent, cat #SGI0001) was read on a Bioscan AR-2000 radio-TLC imaging scanner. The radio TLC plate (Fig. 3) was co-spotted with 1 µL of NaI (0.1 M) and developed using citric acid (0.5 mM, pH = 5) as eluent. The origin = 20 mm and the solvent front = 100 mm. The radio TLC eluent was prepared by dissolving 96 mg of citric acid (Spectrum cat #CI131) in 25 mL of Trace Select H₂O and then Na₂CO₃ was added (245 µL, 2 M); the pH was checked by strip (pH = 5).

Fig 6. Analytical HPLC trace of purified [¹²⁴I]-IPEM CD8S 365. Analytical HPLC was performed using a Waters 1525 Binary HPLC pump, a Waters 2707 autosampler, a Waters 2489 dual wavelength UV/Visible Detector (λ = 214 and 280 nm), a Bioscan Flow Count radiodetector (B-FC-2000) and a Phenomenex Kinetex 5 µm XB-C18 100 Å, 150 × 4.6 mm HPLC column. The elution profile used was as follows: solvent A = H₂O (0.1% TFA (v/v)), Solvent B = MeCN (0.1% TFA (v/v)), flow rate = 1 mLmin⁻¹; initial = 90% A, 15 min = 0% A (linear gradient). Biomolecule (CD8S) absorbance at 280 nm (top graph) and small molecule (I124-IPEM) absorbance at 214 nm (middle graph) confirms successful conjugation reaction. UV and radio traces (bottom graph) indicate an analytically pure sample.

Fig. 7. MALDI-MS of IPEM CD8S365 (theoretical MW = 10786.12). The MALDI-MS analysis was performed at the Biointerfaces Institute using a Bruker UltrafleXtreme MALDI TOF/TOF in positive ion mode (linear detector). A saturated solution of sinapinic acid was prepared in TA30 solvent (30:70 (v/v) MeCN:0.1% TFA in water). The sample (c = 0.397 mgmL⁻¹) was mixed in a 1:1 ratio with the matrix solution and 1 µL was spotted on the plate. A protein solution was used as an external standard.

5 **Fig. 8.** Co-injection of [^{124}I]-IPEM CD8S365 with cold standard. Analytical HPLC was performed using a Waters 1525 Binary HPLC pump, a Waters 2707 autosampler, a Waters 2489 dual wavelength UV/Visible Detector ($\lambda = 214$ and 280 nm), a Bioscan Flow Count radiodetector (B-FC-2000) and a Phenomenex Kinetex 5 μm XB-C18 100 $\text{\AA}$, 150 $\times$ 4.6 mm HPLC column. The elution profile used was as follows: solvent A =
10 H_2O (0.1% TFA (v/v)), Solvent B = MeCN (0.1% TFA (v/v)), flow rate = 1 mLmin^{-1} ; initial = 90% A, 15 min = 0% A (linear gradient). Co-injection with cold sample leads to complete overlap of UV peaks (top and middle graphs), confirming the product's molecular identity (i.e. Cold and radiolabeled conjugates are identical except for the replacement of Iodine by Iodine-124)

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Fig. 9. Representative PET image showing CD8S365-IPEM5 radiolabeled with I-124, taken at 2 h post-injection. The image is a maximum intensity projection (anterior-posterior), with the spleen centered on the cross-hairs. The organs below the spleen are the kidneys, and the image is oriented to show the head at the top. The uptake in the
20 thyroid is evidence of some de-iodination of the protein.

Fig. 10. Time-activity curves for blood radioactivity in non-human primate for each anti-CD8A FN3 domain labeled with either Zr-89 or I-124.

25 **Fig. 11A and 11B.** Time-activity curves for organ radioactivity in NHP for each centyrin labeled with either Zr-89 or I-124. Fig. 11A includes kidneys, liver and spleen, while Fig. 11B is focused on the spleen. The 24 h time point for [^{124}I]-IPEM CD8S365 is missing due to a technical issue. The high uptake of Zr-89 in kidneys due to residualization of the isotope is largely absent from the I-124 data.

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Fig. 12A-12C. Confirmation of CD8 T cell depletion by Day 3 in blood taken from a non-human primate (12A). Also shown are changes in CD4 (12B) and CD3 T cells (12C).

35 **Fig. 13.** Representative PET image showing the 365 anti-CD8A FN3 domain radiolabeled with I-124, taken at 2 h post-injection in a CD8-depleted animal. The

5 image is a maximum intensity projection (anterior-posterior). This is to be compared against the non-depleted animal in Fig. 9, where the spleen is clearly visible above the kidney.

Fig. 14. Time-activity curves for blood radioactivity in cynomolgus monkeys for both
10 depleted and non-depleted animals after administration of [^{124}I]-IPEM CD8S365.

Fig. 15A and 15B. Time-activity curves for organ radioactivity in cynomolgus monkeys for both depleted and non-depleted animals. 15A includes kidneys, liver and spleen, while 15B is focused on the spleen.

15 **Fig. 16.** Representative PET image of a two identically treated mice showing the CD8S365-IPEM radiolabeled with I-124, taken at 3 h post-injection. The image is a 3D maximum intensity projection, overlaid on a CT scan. Tumor (formed from HEK-293-luc transfected to over-express huCD8+) and other organs are indicated by arrows. The
20 uptake in the thyroid is evidence of some de-iodination of the protein.

Fig. 17. Time-activity curve for blood radioactivity in mice bearing either HEK-293-luc CD8+ or HEK-293 parental tumors.

25 **Fig. 18.** Time-activity curve for tumor radioactivity in mice bearing either HEK-293-luc CD8+ or HEK-293 parental tumors.

Fig. 19. Uptake of the I-124 labeled CD8S365 in the HEK293 CD8 overexpressing cells, as a function of the number of implanted cells.

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DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Definitions

Various terms relating to aspects of the description are used throughout the
35 specification and claims. Such terms are to be given their ordinary meaning in the art unless otherwise indicated. Other specifically defined terms are to be construed in a manner consistent with the definitions provided herein.

5 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.

10 The term “about” as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of up to $\pm 10\%$ from the specified value, as such variations are appropriate to perform the disclosed methods. Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances
15 by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical
20 parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however,
25 inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

“Isolated” means a biological component (such as a nucleic acid, peptide or protein) has been substantially separated, produced apart from, or purified away from other biological components of the organism in which the component naturally occurs,
30 i.e., other chromosomal and extrachromosomal DNA and RNA, and proteins. Nucleic acids, peptides and proteins that have been “isolated” thus include nucleic acids and proteins purified by standard purification methods. “Isolated” nucleic acids, peptides and proteins can be part of a composition and still be isolated if such composition is not part of the native environment of the nucleic acid, peptide, or protein. The term also
35 embraces nucleic acids, peptides and proteins prepared by recombinant expression in a host cell as well as chemically synthesized nucleic acids. An “isolated” FN3 domain, as used herein, is intended to refer to an FN3 domain which is substantially free of

5 other FN3 domains having different antigenic specificities (for instance, an isolated FN3 domain that specifically binds to human serum albumin is substantially free of FN3 domains that specifically bind antigens other than human serum albumin). An isolated FN3 domain that specifically binds to an epitope, isoform or variant of human serum albumin may, however, have cross-reactivity to other related antigens, for
10 instance from other species (such as serum albumin species homologs).

The term “fibronectin type III (FN3) domain” (FN3 domain) as used herein 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
15 175:1910-1918, 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
20 domain of tenascin (TN3), or the 10th FN3 domain of fibronectin (FN10).

“Centyrin” as used herein 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. Examples of capture agents
25 include but are not limited to magnetic beads, ferrofluids, encapsulating reagents and the like.

The term “biological sample” refers to blood, tissue, marrow, sputum and the like.

The term “diagnostic reagent” refers to any substance that may be used to
30 analyze a biological sample, whether or not such substance is distributed as a single substance or in a combination with other substances in a diagnostic kit.

The term “substituting” or “substituted” or “mutating” or “mutated” as used herein refers to altering, deleting or inserting one or more amino acids or nucleotides in a polypeptide or polynucleotide sequence to generate a variant of that sequence.

35 The term “randomizing” or “randomized” or “diversified” or “diversifying” as used herein refers to making at least one substitution, insertion or deletion in a polynucleotide or polypeptide sequence.

5 “Variant” as used herein 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.

The term “specifically binds” or “specific binding” as used herein refers to the ability of the FN3 domain of the invention to bind to a predetermined antigen with a dissociation constant (K_D) of about 1×10^{-6} M or less, for example about 1×10^{-7} M or less, about 1×10^{-8} M or less, about 1×10^{-9} M or less, about 1×10^{-10} M or less, about 1×10^{-11} M or less, about 1×10^{-12} M or less, or about 1×10^{-13} M or less. Typically the FN3 domain of the invention binds to a predetermined antigen (i.e. human CD8A) with a K_D that is at least ten fold less than its K_D for a nonspecific antigen (for example BSA or casein) as measured by surface plasmon resonance using for example a Proteon Instrument (BioRad). The isolated FN3 domain of the invention that specifically binds to human CD8A may, however, have cross-reactivity to other related antigens, for example to the same predetermined antigen from other species (orthologs), such as *Macaca Fascicularis* (cynomolgous monkey, cyno) or *Pan troglodytes* (chimpanzee).

20 The term “library” refers to a collection of variants. The library may be composed of polypeptide or polynucleotide variants.

As used herein, the terms “CD8A” or “CD8” specifically include the human CD8 alpha protein, for example, as described in NCBI Reference Sequence: NP_001139345.1, NP_0011759.3, and NP_741969.1. CD8A is also known in the scientific literature as CD8a molecule, MAL, p32, Leu2, T-cell surface glycoprotein CD8 alpha chain, CD8 antigen, alpha polypeptide (p32), Leu2 T-lymphocyte antigen, OKT8 T-cell antigen, T-cell antigen Leu2, T-lymphocyte differentiation antigen T8/Leu-2, and T8 T-cell antigen.

30 “Tencon” as used herein 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. US2010/0216708.

The term “vector” means 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

5 biological components capable of duplicating a vector. The polynucleotide comprising a vector may be DNA or RNA molecules or a hybrid of these.

The term “expression vector” means 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.

10 The term “polynucleotide” means a molecule comprising a chain of nucleotides covalently linked by a sugar-phosphate backbone or other equivalent covalent chemistry. Double and single-stranded DNAs and RNAs are typical examples of polynucleotides.

The term “polypeptide” or “protein” means a molecule that comprises at least
15 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”.

The term “in combination with” as used herein means that two or more therapeutics can be administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

20

Compositions of matter

The present invention provides human CD8A binding FN3 domains and CD8A binding FN3 domains conjugated to detectable labels. The present invention provides polynucleotides encoding the FN3 domains of the invention or complementary nucleic
25 acids thereof, vectors, host cells, and methods of making and using them.

CD8A binding molecules

The present invention provides fibronectin type III (FN3) domains that bind specifically to CD8A, optionally conjugated to a detectable label. These molecules
30 may be widely used in preclinical applications and in cancer diagnostics using CD8A as a biomarker. 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 FN3 domains of the invention bind CD8A with high affinity and can
35 localize CD8-expressing cells, thereby providing an efficient way to deliver diagnostic reagents into tumor microenvironment.

5 One embodiment of the invention an isolated FN3 domain that specifically binds a human CD8A comprising the amino acid sequence of SEQ ID NO: 35.

 In some embodiment of the invention described herein, the FN3 domain of the invention cross-reacts with cynomolgus monkey CD8A having the amino acid sequence of SEQ ID NO: 271.

10 The FN3 domain of the invention may bind human, *Macaca Fascicularis* and/or *Pan troglodytes* CD8A with a dissociation constant (K_D) of less than about 1×10^{-7} M, for example less than about 1×10^{-8} M, less than about 1×10^{-9} M, less than about 1×10^{-10} M, less than about 1×10^{-11} M, less than about 1×10^{-12} M, or less than about 1×10^{-13} M as determined by surface plasmon resonance, as practiced by those of skill in the art. The
15 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.

20 In some embodiments, the CD8A binding FN3 domains comprise an initiator methionine (Met) linked to the N-terminus of the molecule.

 In some embodiments, the CD8A binding FN3 domains comprise a cysteine (Cys) linked to the FN3 domain.

 The addition of the N-terminal Met and/or the Cys may facilitate expression
25 and/or conjugation of second molecules.

 Another embodiment of the invention is an isolated FN3 domain that specifically binds human CD8A and wherein the CD8A-specific FN3 domain does not activate CD8+ T-cells *in vitro*. CD8+ T cell activation may be measured using standard methods. For example, the enzyme-linked immunospot (ELISPOT) assay
30 may be used. The ELISPOT assay employs the sandwich enzyme-linked immunosorbent assay (ELISA) technique. The interferon-gamma antibody is pre-coated onto a PVDF (polyvinylidene difluoride)-backed microplate. Appropriately stimulated cells (cells + peptides, FN3 domains, etc) are pipetted into the wells and the microplate is placed into a humidified 37 °C CO₂ incubator for a specified period of
35 time. During this incubation period, the immobilized interferon-gamma antibody, in the immediate vicinity of the secreting cells, binds the secreted interferon gamma. After washing away any cells and unbound substances, a second biotinylated interferon-

5 gamma antibody is added to the wells. Following a wash to remove any unbound
biotinylated antibody, alkaline-phosphatase conjugated to streptavidin is added.
Unbound enzyme is subsequently removed by washing and a substrate solution
(BCIP/NBT) is added. A blue-black colored precipitate forms and appears as spots at
the sites of interferon-gamma localization, with each individual spot representing an
10 individual interferon gamma-secreting cell. The spots can be counted with an
automated ELISpot reader system or manually, using a stereomicroscope. The isolated
CD8A binding FN3 domains of the invention do not activate CD8+ T-cells *in vitro*
when tested at 1 μ M concentrations as described in the EXAMPLES.

In some embodiments of the invention described herein, the isolated FN3
15 domain comprises the amino acid sequence of SEQ ID NOs: 40-269.

In some embodiments of the invention described herein, the CD8A-specific
FN3 domain has a cysteine substitution at residue position 54 of SEQ ID NOs 79, 81,
83, 89, 122 and 68.

Substitutions resulting in introduction of cysteine into a protein sequence may
20 be utilized to chemically conjugate small molecules such as cytotoxic agents, detectable
labels, half-life extension molecules, chelators, polyethylene glycol and/or nucleic acids
to the FN3 domain using standard chemistry.

In some embodiments, the FN3 domain specifically binding human CD8A
competes for binding to human CD8A with the FN3 domain of SEQ ID NOs: 229-234.
25 FN3 domains may be evaluated for their competition with a reference molecule for
binding human CD8A using well known *in vitro* methods. In an exemplary method,
HEK cells recombinantly expressing human CD8A may be incubated with unlabeled
reference molecule for 15 min at 4°C, followed by incubation with an excess of
fluorescently labeled test FN3 domain for 45 min at 4°C. After washing in PBS/BSA,
30 fluorescence may be measured by flow cytometry using standard methods. In another
exemplary method, extracellular portion of human CD8A may be coated on the surface
of an ELISA plate. Excess of unlabelled reference molecule may be added for about 15
minutes and subsequently biotinylated test FN3 domains may be added. After washes
in PBS/Tween, binding of the test biotinylated FN3 domain may be detected using
35 horseradish peroxidase (HRP)-conjugated streptavidine and the signal detected using
standard methods. It is readily apparent that in the competition assays, reference
molecule may be labelled and the test FN3 domain unlabeled. The test FN3 domain

5 may compete with the reference molecule when the reference molecule inhibits binding of the test FN3 domain, or the test FN3 domain inhibits binding of the reference molecule by 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% or 100%.

In some embodiments, the isolated FN3 domain that specifically binds human CD8A of the invention is conjugated to a chelator that can bind to a radioactive metal
10 and may be used as an imaging agent to evaluate tumor distribution, diagnosis for the presence of CD8-T cells inside tumors and /or efficacy of cancer treatment.

In some embodiments, the CD8A-specific FN3 domains are removed from the blood via renal and/or liver clearance.

15 **Isolation of CD8A 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
20 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
25 (FN3) domains that may be used to select novel molecules that bind CD8A. 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
30 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 the 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
35 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

5 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.

A library designed based on the Tencon sequence may also have randomized
 10 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. US2013/0226834. Library designed based on Tencon
 15 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 improved thermal stability. Exemplary Tencon variants are described in US Pat. Publ. No. 2011/0274623, and include Tencon27 (SEQ ID NO: 4) having substitutions E11R,
 20 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

5

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

10 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

15 (<http://www.sloming.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.

20 The FN3 domains specifically binding human CD8A 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.

25 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 CD8A 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

30 Wiley & Sons, Inc., New York). The identified FN3 domains specifically binding CD8A are further characterized for their inhibition of CD8A activity, internalization, stability, and other desired characteristics.

The FN3 domains specifically binding human CD8A of the invention may be generated using any FN3 domain as a template to generate a library and screening the

35 library for molecules specifically binding human CD8A using methods provided

5 within. Exemplar FN3 domains that may be used are the 3rd FN3 domain of tenascin C (TN3) (SEQ ID NO: 145), Fibcon (SEQ ID NO: 146), and the 10th FN3 domain of fibronectin (FN10) (SEQ ID NO: 147). 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
10 (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
15 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 of the invention described herein, the FN3 domain specifically binding human CD8A is based on Tencon sequence of SEQ ID NO: 1 or
20 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 specifically binding human CD8A 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
25 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).
30 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
35 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

5 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
10 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.

15 In one embodiment, the FN3 domains specifically binding human CD8A 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.

20 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.
25 hydrochloric acid (HCl), acetic acid (CH₃COOH), halogenated acetic acids), hydrophobic molecules (e.g. phospholipids), 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
30 formation of disulfide bonds.

The FN3 domains of the invention 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
35 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

5 NO: 148), (GGGS)₂ (SEQ ID NO: 149), (GGGGGS)₅ (SEQ ID NO: 150), (AP)₂ (SEQ ID NO: 151), (AP)₅ (SEQ ID NO: 152), (AP)₁₀ (SEQ ID NO: 153), (AP)₂₀ (SEQ ID NO: 154) and A(EAAAK)₅AAA (SEQ ID NO: 142). 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).

Diagnostic Agents

15 According to the invention, a CD8A-specific FN3 domain of the invention may comprise a detectable label. In an embodiment, the detectable label may be complexed with a chelating agent that is conjugated to the FN3 domain. In another embodiment, the detectable label may be complexed with a chelating agent that is conjugated to a linker that is conjugated to the FN3 domain. In still another embodiment, the detectable label may be coupled to a linker that is conjugated to the FN3 domain. In still yet another embodiment, a detectable label may be indirectly attached to a peptide of the invention by the ability of the label to be specifically bound by a second molecule. One example of this type of an indirectly attached label is a biotin label that can be specifically bound by the second molecule, streptavidin. Single, dual or multiple labeling may be advantageous. As used herein, a "detectable label" is any type of label which, when attached to an FN3 domain of the invention renders the FN3 domain detectable. A detectable label may also be toxic to cells or cytotoxic. In general, detectable labels may include luminescent molecules, chemiluminescent molecules, fluorochromes, fluorophores, fluorescent quenching agents, colored molecules, radioisotopes, radionuclides, cintillants, massive labels such as a metal atom (for detection via mass changes), biotin, avidin, streptavidin, protein A, protein G, antibodies or fragments thereof, Grb2, polyhistidine, Ni²⁺, Flag tags, myc tags, heavy metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron donors/acceptors, acridinium esters, and colorimetric substrates. In a specific embodiment, the detectable label is a radionuclide. The skilled artisan would readily recognize other useful labels that are not mentioned above, which may be employed in the operation of the present invention.

5 A detectable label emits a signal that can be detected by a signal transducing machine. In some cases, the detectable label can emit a signal spontaneously, such as when the detectable label is a radionuclide. In other cases, the detectable label emits a signal as a result of being stimulated by an external field such as when the detectable label is a relaxivity metal. Examples of signals include, without limitation, gamma
10 rays, X-rays, visible light, infrared energy, and radiowaves. Examples of signal transducing machines include, without limitation, gamma cameras including SPECT/CT devices, PET scanners, fluorimeters, and Magnetic Resonance Imaging (MRI) machines. As such, the detectable label comprises a label that can be detected using magnetic resonance imaging, scintigraphic imaging, ultrasound, or fluorescence.

15 Suitable fluorophores include, but are not limited to, fluorescein isothiocyanate (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. An FN3 domain of the invention can be labeled for fluorescence detection by labeling the agent
20 with a fluorophore using techniques well known in the art (see, e.g., Lohse et al., Bioconj Chem 8:503-509 (1997)). For example, many known dyes are capable of being coupled to NH₂-terminal amino acid residues. Alternatively, a fluorochrome such as fluorescein may be bound to a lysine residue of the peptide linker.

 A radionuclide may be a γ -emitting radionuclide, Auger-emitting radionuclide,
25 β -emitting radionuclide, an alpha-emitting radionuclide, or a positron-emitting radionuclide. A radionuclide may be a detectable label and/or a cytotoxic agent. Non-limiting examples of suitable radionuclides may include carbon-11, nitrogen-13, oxygen- 15, fluorine-18, fluorodeoxyglucose-18, phosphorous-32, scandium-47, copper-64, 65 and 67, gallium-67 and 68, bromine-75, 77 and 80m, rubidium-82,
30 strontium-89, zirconium-89, yttrium-86 and 90, ruthenium-95, 97, 103 and 105, rhenium-99m, 101, 105, 186 and 188, technetium-99m, rhodium-105, mercury-107, palladium-109, indium-111, silver-111, indium-113m, lanthanide-114m, tin-117m, tellurium-121 m, 122m and 125m, iodine-122, 123, 124, 125, 126, 131 and 133, praseodymium-142, promethium- 149, samarium-153, gadolinium-159, thulium-165,
35 167 and 168, dysprosium-165, holmium-166, lutetium-177, rhenium-186 and 188, iridium-192, platinum-193 and 195m, gold-199, thallium-201, titanium-201, astatine-211, bismuth-212 and 213, lead-212, radium-223, actinium-225, and nitride or oxide

5 forms derived there from. In a specific embodiment, a radionuclide is selected from the group consisting of copper-64, zirconium-89, yttrium-90, indium-111, and lutetium-177. In another specific embodiment, a radionuclide is selected from the group consisting of yttrium-90, indium-111, and lutetium-177. In an exemplary embodiment, a radionuclide is zirconium-89.

10 A variety of metal atoms may be used as a detectable label. The metal atom may generally be selected from the group of metal atoms comprised of metals with an atomic number of twenty or greater. For instance, the metal atoms may be calcium atoms, scandium atoms, titanium atoms, vanadium atoms, chromium atoms, manganese atoms, iron atoms, cobalt atoms, nickel atoms, copper atoms, zinc atoms, gallium
15 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,
20 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,
25 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 selected from the group comprising alkali metals
30 with an atomic number greater than twenty. In other embodiments, the metal atoms may be selected from the group comprising alkaline earth metals with an atomic number greater than twenty. In one embodiment, the metal atoms may be selected from the group of metals comprising the lanthanides. In another embodiment, the metal atoms may be selected from the group of metals comprising the actinides. In still
35 another embodiment, the metal atoms may be selected from the group of metals comprising the transition metals. In yet another embodiment, the metal atoms may be selected from the group of metals comprising the poor metals. In other embodiments,

5 the metal atoms may be selected from the group comprising gold atoms, bismuth atoms, tantalum atoms, and gadolinium atoms. In preferred embodiments, the metal atoms may be selected from the group comprising metals with an atomic number of 53 (i.e. iodine) to 83 (i.e. bismuth). In an alternative embodiment, the metal atoms may be atoms suitable for magnetic resonance imaging. In another alternative embodiment, the
 10 metal atoms may be selected from the group consisting of metals that have a K-edge in the x-ray energy band of CT. Preferred metal atoms include, but are not limited to, manganese, iron, gadolinium, gold, and iodine.

The metal atoms may be metal ions in the form of +1, +2, or +3 oxidation states. For instance, non-limiting examples include Ba^{2+} , Bi^{3+} , Cs^+ , Ca^{2+} , Cr^{2+} , Cr^{3+} ,
 15 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. For instance, non-limiting examples of metal oxides may include iron oxide, manganese oxide, or gadolinium oxide. Additional examples may include magnetite, maghemite, or a combination thereof.

20 According to the invention, an FN3 domain comprising a chelating agent may incorporate a radionuclide or metal atom. Incorporation of the radionuclide or metal atom with an FN3 domain-chelating agent complex may be achieved by various methods common in the art of coordination chemistry.

25 **Half-life extending moieties**

The FN3 domain specifically binding human CD8A of the invention may incorporate other subunits for example via covalent interaction. In one aspect of the invention, the FN3 domain of the invention further comprises a half-life extending moiety. Exemplary half-life extending moieties are albumin, albumin variants,
 30 albumin-binding proteins and/or domains, transferrin and fragments and analogues thereof, and Fc regions.

Additional moieties may be incorporated into the FN3 domain of the invention 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,
 35 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.

5 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 of the invention
10 by incorporating a cysteine residue to the C-terminus of the molecule, or engineering cysteines into residue positions that face away from the human CD8A binding face of the molecule, and attaching a pegyl group to the cysteine using well known methods. FN3 domain of the invention incorporating additional moieties may be compared for functionality by several well known assays. For example, altered properties due to
15 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.

20

Polynucleotides, vectors, host cells

The invention provides for nucleic acids encoding the FN3 domains specifically binding human CD8A of the invention as isolated polynucleotides or as portions of expression vectors or as portions of linear DNA sequences, including linear DNA
25 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
30 domains of the invention are also within the scope of the invention.

One embodiment of the invention is an isolated polynucleotide encoding the FN3 domain specifically binding human CD8A comprising the amino acid sequence of SEQ ID NOs: 40-269.

The polynucleotides of the invention may be produced by chemical synthesis
35 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

5 techniques such a 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
10 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.

Another embodiment of the invention is a vector comprising at least one
15 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
20 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.

25 Another embodiment of the invention is a host cell comprising the vector of the invention. The FN3 domain specifically binding human CD8A of the invention 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-
30 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).

35 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

5 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.

10 Another embodiment of the invention is a method of producing the isolated FN3 domain specifically binding human CD8A of the invention, comprising culturing the isolated host cell of the invention under conditions such that the isolated FN3 domain specifically binding human CD8A is expressed, and purifying the FN3 domain.

The FN3 domain specifically binding human CD8A may be purified from
15 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).

20

Kits for Detecting Human CD8A

Provided herein are kits for detecting CD8A in a biological sample. These kits include one or more of the CD8A-specific FN3 domains described herein and instructions for use of the kit.

25 The provided CD8A-specific FN3 domain may be in solution; lyophilized; affixed to a substrate, carrier, or plate; or detectably labeled.

The described kits may also include additional components useful for performing the methods described herein. By way of example, the kits may comprise means for obtaining a sample from a subject, a control or reference sample, e.g., a
30 sample from a subject having slowly progressing cancer and/or a subject not having cancer, one or more sample compartments, and/or instructional material which describes performance of a method of the invention and tissue specific controls or standards.

The means for determining the level of CD8A can further include, for example,
35 buffers or other reagents for use in an assay for determining the level of CD8A. The

- 5 instructions can be, for example, printed instructions for performing the assay and/or instructions for evaluating the level of CD8A.

The described kits may also include means for isolating a sample from a subject. These means can comprise one or more items of equipment or reagents that can be used to obtain a fluid or tissue from a subject. The means for obtaining a sample
 10 from a subject may also comprise means for isolating blood components, such as serum, from a blood sample. Preferably, the kit is designed for use with a human subject.

Uses of human CD8A binding FN3 domains of the invention

- 15 The FN3 domains specifically binding human CD8A of the invention may be used to diagnose human disease or specific pathologies in cells, tissues, organs, fluid, or, generally, a host, using CD8A as a biomarker. The methods of the invention may be used in an animal patient belonging to any classification. Examples of such animals include mammals such as humans, rodents, dogs, cats and farm animals.

20

EMBODIMENTS

The invention also provides the following non-limiting embodiments.

- 1) An isolated FN3 domain that specifically binds a human CD8A protein comprising the amino acid sequence of SEQ ID NO: 35.
- 25 2) The isolated FN3 domain of embodiment 1, wherein the FN3 domain cross-reacts with cynomolgus monkey CD8A protein comprising the amino acid sequence of of SEQ ID NO: 271.
- 3) The isolated FN3 domain of embodiment 2, wherein
 - a) the FN3 domain is based on a Tencon sequence of SEQ ID NO: 1;
 - 30 b) the FN3 domain is based on a Tencon27 sequence of SEQ ID NO: 4; and/or
 - c) the FN3 domain is isolated from the library comprising the sequence of SEQ ID NOs: 2, 3, 5, 6, 7 or 8.
- 4) The isolated FN3 domain of embodiment 3, wherein the FN3 domain is conjugated to a second molecule.
- 35 5) The isolated FN3 domain of embodiment 4, wherein the second molecule is a detectable label.

- 5 6) The isolated FN3 domain of embodiment 5, 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.
- 7) The isolated FN3 domain of embodiment 3, wherein the FN3 domain has a cysteine substitution at residue position 54 of SEQ ID NOs 79, 81, 83, 89, 122 and 68.
- 10 8) The isolated FN3 domain of embodiment 3, wherein the FN3 domain does not activate CD8⁺ T-cells *in vitro* as measured by the enzyme-linked immunospot (ELISPOT) assay.
- 9) The isolated FN3 domain of embodiment 3, wherein the FN3 domain competes for binding to human CD8A protein with the FN3 domain comprising the amino acid sequence of one of SEQ ID NOs: 229-234.
- 15 10) The isolated FN3 domain of embodiment 3, wherein the FN3 domain binds human CD8A protein with an affinity (K_D) of between about 0.02 to about 6.6 nM as measured by surface plasmon resonance performed according to the conditions described in Example 3.
- 20 11) The isolated FN3 domain of embodiment 3, wherein the FN3 domain comprises any one of the amino acid sequences of SEQ ID NOs: 40-269.
- 12) The isolated FN3 domain of embodiment 11, further comprising a methionine at the N-terminus of the FN3 domain.
- 25 13) The isolated FN3 domain of embodiment 3, wherein the FN3 domain is coupled to a half-life extending moiety.
- 14) The isolated FN3 domain of embodiment 13, wherein the half-life extending moiety is a CD8 binding molecule, a polyethylene glycol (PEG), CD8, CD8 variant, or at least a portion of an Fc region of an immunoglobulin.
- 30 15) An isolated polynucleotide encoding the FN3 domain of embodiment 3.
- 16) A vector comprising the polynucleotide of embodiment 15.
- 17) An isolated host cell comprising the vector of embodiment 16.
- 18) A method of producing an FN3 domain that specifically binds a human CD8A protein, comprising culturing the isolated host cell of embodiment 17 under conditions such that the FN3 domain is expressed, and purifying the FN3 domain.
- 35 19) A diagnostic kit comprising the FN3 domain of embodiment 3.
- 20) A diagnostic or capture agent comprising the FN3 domain of embodiment 3.

- 5 21) The diagnostic or capture agent of embodiment 20 wherein the FN3 domain has a cysteine substitution at residue position 54 of SEQ ID NOs 79, 81, 83, 89, 122 and/or 68.
- 22) The diagnostic agent of embodiment 21 wherein the substituted cysteine is conjugated to Zr-89 or I-124.
- 10 23) A method of detecting CD8-expressing cells in a biological sample comprising treating the biological sample with the diagnostic reagent of embodiment 20 and evaluating the binding of the biological sample to the FN3 domain of such diagnostic agent.
- 24) The method of embodiment 23 wherein the diagnostic agent has a cysteine
- 15 substitution at residue position 54 of SEQ ID NOs 79, 81, 83, 89, 122 and/or 68 and the substituted cysteine is conjugated to Zr-89 or I-124.

EXAMPLES

The following examples are provided to supplement the prior disclosure and to provide

20 a better understanding of the subject matter described herein. These examples should not be considered to limit the described subject matter. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be apparent to persons skilled in the art and are to be included within, and can be made without departing from, the true

25 scope of the invention.

EXAMPLE 1. CONSTRUCTION OF TENCON LIBRARIES WITH RANDOMIZED LOOPS

Tencon (SEQ ID NO: 1) is an immunoglobulin-like scaffold, fibronectin type

30 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

35 (FN3) domains that can be used to select novel molecules that bind to specific targets.

Tencon:

- 5 LPAPKNLVVSEVTEDSLRLSWTAPDAAFD SFLIQYQESEKVG EAINLTVPGSER
SYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT (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

- 10 TCL1 and TCL2 libraries are described in detail in Int. Pat. Publ. No.
WO2014081944A2.

Construction of TCL1 library

- A library designed to randomize only the FG loop of Tencon (SEQ ID NO: 1),
15 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 double-
stranded 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
20 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
25 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: 32). NNS codons were used in the construction of this
30 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.

- 35 TCL1 library (SEQ ID NO: 2)
LPAPKNLVVSEVTEDSLRLSWTAPDAAFD SFLIQYQESEKVG EAINLTVPGSER
SYDLTGLKPGTEYTVSIYGVX₇₋₁₂PLSAEFTT;
wherein

- 5 X₁, X₂, X₃, X₄, X₅, X₆, X₇ is any amino acid; and
X₈, X₉, X₁₀, X₁₁ and X₁₂ 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
10 were randomized and the distribution of amino acids at each position was strictly
controlled. Table 2 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
15 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
20 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 1 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%
25 valine. This distribution is devoid of methionine, cysteine, and STOP codons.

TCL2 library (SEQ ID NO: 3)

- LPAPKNLVVSEVTEDSLRLSWX₁X₂X₃X₄X₅X₆X₇X₈SFLIQYQESEKVGGEAINLTV
PGSERSYDLTGLKPGTEYTVSIYGVX₉X₁₀X₁₁X₁₂X₁₃SX₁₄X₁₅LSAEFTT; wherein
30 X₁ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;
X₂ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;
X₃ Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or
35 Val;
X₄ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;

- 5 X₅ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
 X₆ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
 X₇ is Phe, Ile, Leu, Val or Tyr;
- 10 X₈ is Asp, Glu or Thr;
 X₉ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
 X₁₀ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
- 15 X₁₁ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
 X₁₂ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
 X₁₃ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
- 20 X₁₄ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val; and
 X₁₅ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

25

Table 1.

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

5

*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)

20 LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFLIQYQESEKVG EAI VLT VPGSER
SYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

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

LPAPKNLVVSRVTEDSARLSW_{X1X2X3X4X5X6X7X8X9}FD SFLIQYQESEKVG EAI
25 VLT VPGSERSYDLTGLKPGTEYTVSIYGVX_{X10X11X12X13X14X15X16X17X18X19}SNPL
SAIFTT;
wherein

- 5 X₁, X₂, X₃, X₄, X₅, X₆, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₅ and X₁₆ is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and
X₇, X₈, X₉, X₁₇, X₁₈ and X₁₉, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.
- 10 **TCL9 (randomized FG loop) (SEQ ID NO: 6)**
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFLIQYQESEKVGAEIVLTVPGSER
SYDLTGLKPGTEYTVSIYGV X₁X₂X₃X₄X₅X₆X₇X₈X₉ X₁₀X₁₁X₁₂SNPLSAIFTT;
X₁, X₂, X₃, X₄, X₅, X₆ and X₇, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y;
and
15 X₈, X₉, X₁₀, X₁₁ and X₁₂ 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
20 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)

- 25 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
30 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,
35 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

5 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-
10 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
15 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 No. 13-16) 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.
20 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
25 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
30 templates 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.

35 Fragments encoding the FG loop were amplified by PCR using FG7 (SEQ ID No. 31), FG8 (SEQ ID No. 30), FG9 (SEQ ID No. 29), FG10 (SEQ ID No. 28), FG11 (SEQ ID No. 27), and FG12 (SEQ ID No. 26) as templates with oligonucleotides

5 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
10 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 NotI 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)
15 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
20 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
25 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
30 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
35 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

5 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-
 10 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
 15 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.

20

TCL14 library (SEQ ID NO: 7):

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFX₁IX₂YX₃EX₄X₅X₆X₇GEAIVLTVP
 GSERSYDLTGLKPGTEYX₈VX₉IX₁₀GVKGGX₁₁X₁₂SX₁₃PLSAIFTT;

Wherein

25 X₁, X₂, X₃, X₄, X₅, X₆, X₁₀, X₁₁, X₁₂ and X₁₃ are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y, 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;
 30 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.

35 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

5 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
10 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
15 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 2). TCL19 and TCL21 were
20 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 2. As with the TCL21 library, cysteine and methionine
25 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.
30 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 2.

35

TCL24 Library (SEQ ID NO: 8)

- 5 LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX₁IX₂YX₃EX₄X₅X₆X₇GEAIX₈LX₉
 VPGSERSYDLTGLKPGTEYX₁₀VX₁₁IX₁₂GVKGGX₁₃X₁₄SX₁₅PLX₁₆AX₁₇FTT;
 wherein
 X₁, X₂, X₃, X₄, X₅, X₆, X₁₀, X₁₁, X₁₂ and X₁₃ are A, D, E, F, G, H, I, K, L, N, P, Q, R, S,
 T, V or W.

10

**Table 2. Amino acid frequency (%) at each randomized position for TCL21,
 TCL23, and TCL24.**

<u>Amino Acid</u>	<u>TCL19</u>	<u>TCL21</u>	<u>TCL23</u>	<u>TCL24</u>
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

- 15 **Generation of TCL21, TCL23, and TCL24 libraries**

5 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
 10 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 CD8A

15

Design and Production of Human CD8 alpha antigens:

Two human CD8 alpha (Swiss Prot P01732) constructs were expressed and purified from HEK cells to produce recombinant protein for CIS-Display panning (Table 3).

20 **Table 3: CD8A constructs generated for use as antigens**

Construct	SEQID No.	Description
CD8W7	35	Human CD8 alpha residues 22-167 fused to Fc fragment of human IgG1
CD8W13	36	Human CD8 alpha residues 22-182 fused to Fc fragment of human IgG1

Each construct was designed to include a murine IgG Kappa secretion signal (SEQ ID No 3) and was fused to the Fc fragment of human IgG1 (SEQID No. 4). The CD8 alpha and Fc fragment sequences were connected by a linker containing a flag and
 25 polyhistidine tag sequence (SEQID No 5.)

Plasmids encoding these proteins were transfected into HEK 293-Expi cells by transient transfection and culture supernatants were harvested by centrifugation at 6000 x g and clarified with a 0.2 micron filter. Supernatants were loaded onto a HiTrap MabSure Select column (GE Healthcare) and CD8A proteins eluted in 0.1 M Na-Acetate pH 3.5 and neutralized by addition of 2M Tris pH 7. Each sample was then
 30 dialyzed into PBS pH 7.4 for biotinylation with a No Weigh EZ-Link-Sulfo-NHS-LC-Biotin biotinylation kit (Thermo Scientific).

5

Library screening

Cis-display was used to select human CD8 alpha-binding domains from the TCL18, TCL19, TCL21, TCL23, and TCL24 libraries. Biotinylated CD8W7 and CD8W13 were used for panning. For *in vitro* transcription and translation (ITT), 3 µg of library DNA were incubated with 0.1 mM complete amino acids, 1X S30 premix components, and 15 µL of S30 extract (Promega) in a total volume of 50 µL and incubated at 30°C. After 1 hour, 375 µL of blocking solution ((0.1% Casein (Thermo Fisher, Rockford, IL), 100 mg/ml Herring Sperm DNA (Promega, Madison, WI), 1 mg/mL heparin (Sigma-Aldrich, St. Louis, MO)) was added and the reaction was incubated on ice for 15 minutes. For selection, biotinylated antigen was added at concentrations of 400 nM (Round 1), 200 nM (Rounds 2 and 3) and 100 nM (Rounds 4 and 5). Bound library members were recovered using neutravidin magnetic beads (Thermo Fisher, Rockford, IL) (Rounds 1, 3, and 5) or streptavidin magnetic beads (Promega, Madison, WI) (Rounds 2 and 4) and unbound library members were removed by washing the beads 5-14 times with 500 µL PBST followed by 2 washes with 500 µL PBS. Additional selection rounds were performed in order to identify scaffold molecules with improved affinities. Briefly, outputs from round 5 were prepared as described above and subjected to additional iterative rounds of selection with the following changes: the biotinylated target concentration decreased to 25 nM (Rounds 6 and 7) or 2.5 nM (Rounds 8 and 9), and an additional 1 hour wash was performed in the presence of an excess of non-biotinylated target protein. The goal of these changes was to simultaneously select for binders with a potentially faster on-rate and a slower off-rate yielding a substantially lower K_D .

Following panning, selected FN3 domains were amplified by PCR using oligos Tcon6 (SEQID NO: 33) and Tcon5shortE86I (SEQID NO: 34), subcloned by annealing into a pET15-LIC and transformed into BL21-GOLD (DE3) cells (Agilent, Santa Clara, CA) for soluble expression in *E. coli* using standard molecular biology techniques. Single clones were picked and grown to saturation in 1 mL LB with ampicillin in 96 deepwell plates at 37°C. The following day, 25 µL was transferred to fresh 1 mL LB-Amp media in 96 deepwell plates and grown at 37 °C for 2 hours. IPTG was added at 1 mM final concentration and protein expression was induced at 30°C for 16 hours. The cells were harvested by centrifugation and subsequently lysed with

- 5 Bugbuster HT (EMD Chemicals, Gibbstown, NJ) supplemented with 0.2 mg/mL final Chicken Egg White Lysozyme (Sigma- Aldrich, St. Louis, MO). 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) with shaking at room temperature for 45 minutes.

10

Selection of FN3 domains that bind CD8A

- Neutravidin-coated plates were blocked for 1 hour in Starting Block T20 (Pierce) and then coated with biotinylated CD8W7 or CD8W13 (same antigen as in panning) or negative control (human Fc) for 1 hour. Plates were rinsed with TBST and
- 15 diluted lysate was applied to plates for 1 hour. Following additional rinses, wells were treated with HRP-conjugated anti-FN3 domain antibody (PAB25) for 1h and then assayed with POD (Roche). FN3 domain molecules with signals at least 10-fold above background were selected for further analysis.

Small Scale Expression and Purification of identified FN3 domains binding CD8A

- Isolated clones from unique hits identified by biochemical binding ELISA were combined into a single hit plate for growth in 96-well block plates; 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
- 25 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
- 30 lysed with BugBuster® HT lysis buffer (Novagen EMD Biosciences) and His-tagged Centyrins 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).

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Size Exclusion Chromatography Analysis

- 5 Size exclusion chromatography was used to determine the aggregation state of anti-CD8 alpha FN3 domain molecules. Aliquots (10 μ L) of each purified FN3 domain 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. Wild-type Tencon was included in each run as a control.
- 10 Agilent ChemStation software was used to analyse the elution profiles. Only those proteins with elution profiles similar to that of the tenascin consensus protein in the same run were considered for further characterization. After panning, ELISA screening and size exclusion chromatographic analysis, a total of 190 unique anti-human CD8
- 15 alpha FN3 domains were isolated that bound to recombinant human CD8 alpha greater than 10-fold over background and were free of aggregates by SEC (Table 4, SEQID no. 40-228, and 70).

Table 4. Summary of CD8A-binding FN3 domains identified from ELISA screens

Clone ID	SEQ ID NO:	Human T-cell	Human T-cell	kd (1/s)	Cyno T-cell	Cyno T-cell
		Binding 2 uM (MFI)	cell Binding 0.2 uM (MFI)		Binding 2 uM (MFI)	Binding 0.2 uM (MFI)
P282AR9P1356_A10	40	4258	2093	2.91E-04	10584	3122
P282AR9P1356_A4	41	16674	4380	8.61E-05	26447	8632
P282AR9P1356_A6	42	10835	3441	9.73E-05	31432	5783
P282AR9P1356_B9	43	17158	3670	2.95E-04	36397	5437
P282AR9P1356_D3	44	5963	2403	1.58E-04	13852	3365
P282AR9P1356_H1	45	14696	3234	1.14E-04	46317	5699
P282AR9P1356_H6	46	6646	2642	8.08E-05	14393	3205
P282BR9P1357_A9	47	3117	1074	5.90E-04	7281	1940
P282BR9P1357_B2	48	5931	2875	1.00E-04	17974	3841
P282BR9P1357_C10	49	9779	2901	4.58E-04	24476	5110
P282BR9P1357_C4	50	16809	4224	1.27E-04	41586	7064
P282BR9P1357_D12	51	15269	3899	8.76E-05	40450	7364
P282BR9P1357_D2	52	9606	1568	1.05E-03	25843	2525
P282BR9P1357_E5	53	6726	2587	2.10E-04	10563	4101
P282BR9P1357_G9	54	12733	2803	3.04E-04	41492	4635
P282BR9P1357_H3	55	11142	3033	2.85E-04	27090	5701
P282CR9P1358_C2	56	10086	1059	1.13E-03	55786	7047
P282CR9P1358_C5	57	2706	511	9.99E-04	25688	1831
P282CR9P1358_D10	58	28650	2764	3.11E-04	74051	4072

P282CR9P1358_F11	59	6420	749	1.35E-03	62412	6585
P282CR9P1358_F5	60	24427	3072	6.37E-04	85691	13667
P282DR9P1359_A12	61	32222	5952	8.12E-05	88032	15491
P282DR9P1359_A7	62	38382	8764	7.54E-04	83943	22803
P282DR9P1359_A8	63	21124	2113	6.38E-04	70263	7766
P282DR9P1359_B2	64	22228	2726	6.38E-04	60866	4472
P282DR9P1359_C10	65	27822	2879	9.91E-04	98481	15134
P282DR9P1359_C11	66	18176	1288	2.16E-03	19916	457
P282DR9P1359_C12	67	15106	944	9.78E-04	66538	3636
P282DR9P1359_C5	68	31017	5551	1.74E-04	95679	14183
P282DR9P1359_D12	69	4540	542	1.93E-03	37139	1746
P282DR9P1359_E11	70	40607	7578	2.65E-04	104291	33144
P282DR9P1359_E2	71	28491	4824	2.06E-03	77725	10939
P282DR9P1359_E3	72	4307	349	2.63E-03	52426	1625
P282DR9P1359_E5	73	24100	1954	1.01E-03	81183	13601
P282DR9P1359_E6	74	20507	1262	1.71E-03	61734	5065
P282DR9P1359_E8	75	26074	2919	1.19E-03	80973	16948
P282DR9P1359_F11	76	35639	6592	5.54E-04	86740	16146
P282DR9P1359_F2	77	18415	3047	7.22E-04	38228	4031
P282DR9P1359_F3	78	6343	646	1.06E-03	48861	3084
P282DR9P1359_F5	79	48931	8483	9.02E-05	113733	34709
P282DR9P1359_F6	80	19937	3782	3.89E-04	73219	10680
P282DR9P1359_F7	81	38323	6932	3.65E-04	96456	26331
P282DR9P1359_G4	82	26568	2670	5.17E-04	78619	6006
P282DR9P1359_G7	83	37626	6129	1.14E-04	69085	8769
P282DR9P1359_H5	84	919	278	4.49E-03	2252	500
P282ER9P1360_A9	85	23379	5344	1.33E-04	64694	8732
P282ER9P1360_C1	86	25874	6291	1.81E-04	64813	9679
P282ER9P1360_C4	87	19202	3459	1.07E-03	33427	3896
P282ER9P1360_C6	88	25942	5079	1.75E-04	52783	7579
P282ER9P1360_C8	89	30578	6013	1.56E-04	66829	10220
P282ER9P1360_D11	90	36755	3210	1.42E-04	76564	1937
P282ER9P1360_E4	91	26889	5030	1.91E-04	60757	6867
P282ER9P1360_F11	92	22442	3863	2.25E-04	48653	4407
P282ER9P1360_G10	93	26951	7046	2.07E-03	62701	22641
P282ER9P1360_G7	94	25438	5869	2.21E-04	69709	9921
P282ER9P1360_H10	95	2513	506	1.04E-03	27063	1887
P282ER9P1360_H2	96	15165	3479	2.69E-04	44563	4535
P282ER9P1360_H3	97	19992	4271	2.42E-04	65994	6441
P282FR9P1361_A3	98	7670	1661	7.57E-04	8476	740
P282FR9P1361_A5	99	32752	5213	1.92E-04	63541	8108

P282FR9P1361_C7	100	8538	1575	2.24E-03	11639	896
P282FR9P1361_D3	101	6881	1028	5.02E-03	14762	764
P282FR9P1361_E12	102	15794	1130	1.09E-03	63536	15052
P282FR9P1361_F1	103	5498	801	1.26E-03	9869	1392
P282FR9P1361_F11	104	2189	382	2.13E-03	2289	384
P282FR9P1361_F2	105	4610	498	4.96E-03	10883	462
P282FR9P1361_F3	106	5157	674	1.07E-02	9709	513
P282FR9P1361_F7	107	7001	1107	1.14E-03	1705	353
P282FR9P1361_G9	108	859	297	6.53E-03	3746	666
P282FR9P1361_H4	109	13056	3736	3.17E-04	26273	2504
P282FR9P1361_H5	110	5730	698	5.77E-03	11794	637
P283AR9P1362_A3	111	5535	1400	1.53E-03	17345	3533
P283AR9P1362_A4	112	6314	2539	3.02E-04	21218	4402
P283AR9P1362_B10	113	15380	3703	1.39E-04	35686	8380
P283AR9P1362_B2	114	13649	3505	1.60E-04	38828	6479
P283AR9P1362_B8	115	5737	1576	6.48E-04	12886	2271
P283AR9P1362_C12	116	7064	2616	9.94E-05	14808	3832
P283AR9P1362_C6	117	15955	4147	1.09E-03	17494	5690
P283AR9P1362_C7	118	10957	2792	1.86E-04	19690	5515
P283AR9P1362_D2	119	8650	2758	2.53E-04	17182	5333
P283AR9P1362_D3	120	9498	3484	1.25E-04	34619	6052
P283AR9P1362_D4	121	9832	2977	9.72E-05	25671	4101
P283AR9P1362_D6	122	13686	3664	2.64E-05	33547	7721
P283AR9P1362_D7	123	17327	3354	1.18E-04	27491	4849
P283AR9P1362_E9	124	6178	2010	3.27E-04	15869	2837
P283AR9P1362_F12	125	8970	2623	7.28E-05	26333	3794
P283AR9P1362_F2	126	9619	1366	2.11E-03	26443	5518
P283AR9P1362_F8	127	9195	3167	1.12E-04	23735	4571
P283AR9P1362_G11	128	12690	3531	1.02E-04	32484	6826
P283AR9P1362_G3	129	18512	4307	9.45E-05	35268	9198
P283AR9P1362_H11	130	5734	2268	1.80E-04	11588	3655
P283BR9P1363_A10	131	7886	2753	3.60E-04	27790	4105
P283BR9P1363_A8	132	11285	2536	3.53E-04	24234	3453
P283BR9P1363_B2	133	8358	2399	2.08E-04	14846	2819
P283BR9P1363_B6	134	14534	3453	2.69E-04	37691	6839
P283BR9P1363_C4	135	9073	2247	4.09E-04	23387	3266
P283BR9P1363_C8	136	16541	3739	3.35E-04	37175	9082
P283BR9P1363_D11	137	8692	2890	4.95E-04	20572	11630
P283BR9P1363_E4	138	10790	2498	3.29E-04	17702	2469
P283BR9P1363_E6	139	8239	2079	1.36E-03	16784	3715
P283BR9P1363_F2	140	14473	3274	2.88E-04	33286	5278

P283BR9P1363_F4	141	11933	2963	1.55E-04	20245	4479
P283BR9P1363_F6	142	10632	3229	8.21E-05	31568	4571
P283BR9P1363_G2	143	9640	3226	1.22E-04	15899	5383
P283BR9P1363_G5	144	14798	3307	1.40E-04	24945	4430
P283BR9P1363_G7	145	4639	2340	4.01E-05	7212	3022
P283DR9P1364_A4	146	9491	1024	1.09E-03	48337	6653
P283DR9P1364_A7	147	8985	435	1.97E-03	39870	2641
P283DR9P1364_B1	148	1477	666	1.56E-03	8617	746
P283DR9P1364_B11	149	4255	451	1.30E-03	22852	1590
P283DR9P1364_B4	150	45452	6062	1.09E-04	96492	20238
P283DR9P1364_C10	151	4936	649	1.29E-03	34234	2713
P283DR9P1364_D11	152	32293	4223	5.14E-04	70431	16240
P283DR9P1364_D8	153	656	244	6.61E-03	2484	365
P283DR9P1364_D9	154	42285	5245	4.30E-04	88300	19979
P283DR9P1364_E3	155	1285	317	2.53E-03	9128	887
P283DR9P1364_E5	156	17625	1269	8.25E-04	55654	5091
P283DR9P1364_E7	157	5394	442	2.43E-03	28732	2241
P283DR9P1364_E8	158	14321	1181	7.56E-04	59328	5510
P283DR9P1364_E9	159	4295	548	1.90E-03	19688	2096
P283DR9P1364_F2	160	39164	6252	1.61E-04	91474	16946
P283DR9P1364_F6	161	17215	1831	1.00E-03	33767	3161
P283DR9P1364_F8	162	6305	458	1.74E-03	36659	1302
P283DR9P1364_G10	163	6291	409	2.53E-03	10920	769
P283DR9P1364_G9	164	9892	401	7.79E-03	47097	2796
P283DR9P1364_H1	165	29248	3033	6.13E-04	54014	10610
P283DR9P1364_H11	166	11479	834	9.64E-04	60609	9459
P283DR9P1364_H6	167	2623	268	2.30E-03	6002	418
P283DR9P1364_H9	168	32763	4057	2.71E-04	54593	4556
P283ER9P1365_A1	169	25512	3862	4.67E-04	9676	1365
P283ER9P1365_A7	170	18513	1315	7.86E-04	36568	2960
P283ER9P1365_B6	171	22998	3397	2.88E-04	30081	2692
P283ER9P1365_C1	172	8004	644	1.15E-03	23975	1884
P283ER9P1365_E2	173	20011	2867	3.11E-04	17177	1905
P283ER9P1365_F4	174	24065	2596	2.16E-04	43243	2038
P283ER9P1365_G1	175	1280	318	3.67E-03	489	383
P283ER9P1365_G3	176	12481	2916	2.50E-03	3480	1470
P283ER9P1365_H3	177	17965	953	3.75E-04	19560	436
P283FR9P1366_A1	178	8782	516	2.26E-03	39384	1650
P283FR9P1366_A5	179	27649	3598	5.85E-04	67839	10945
P283FR9P1366_A9	180	1717	252	3.94E-03	8809	580
P283FR9P1366_B7	181	11365	899	1.15E-03	51186	4668

P283FR9P1366_C2	182	40957	4319	4.91E-04	89242	19288
P283FR9P1366_C3	183	1823	407	2.07E-03	4628	1044
P283FR9P1366_C4	184	33821	3754	5.36E-04	63373	10200
P283FR9P1366_C6	185	4541	483	1.43E-03	26242	1675
P283FR9P1366_D12	186	27793	1528	1.76E-03	87643	8143
P283FR9P1366_D6	187	32924	4554	5.09E-04	79621	10399
P283FR9P1366_D7	188	7517	566	3.54E-04	41434	2581
P283FR9P1366_D8	189	3394	413	1.34E-03	28181	2296
P283FR9P1366_E11	190	4594	567	1.41E-03	14194	1469
P283FR9P1366_F5	191	6880	720	1.04E-03	46414	4695
P283FR9P1366_F8	192	3970	369	4.03E-03	26970	2269
P283FR9P1366_F9	193	33559	6295	4.94E-04	84279	24622
P283FR9P1366_G1	194	3605	650	8.72E-04	39796	4981
P283FR9P1366_G5	195	8450	261	7.05E-04	36380	369
P283FR9P1366_G8	196	6857	574	1.08E-03	37144	3126
P283FR9P1366_H10	197	25020	2414	6.30E-04	75192	13854
P283FR9P1366_H11	198	18896	2331	1.39E-03	37386	3659
P283FR9P1366_H3	199	7671	632	1.21E-03	40770	3173
P283FR9P1366_H5	200	3137	252	3.18E-03	5091	477
P283FR9P1366_H6	201	43937	7129	2.05E-04	81542	18993
P283FR9P1366_H7	202	13778	567	1.77E-03	24435	1238
P283FR9P1366_H8	203	24942	4544	1.75E-04	61256	17144
P283FR9P1366_H9	204	8570	693	1.98E-03	36501	2877
P283GR7P1367_A11	205	11326	1029	6.35E-04	66691	5666
P283GR7P1367_B4	206	8302	446	5.18E-03	396	367
P283GR7P1367_B7	207	10865	739	1.27E-03	37518	3134
P283GR7P1367_B9	208	11242	1092	1.16E-03	2924	442
P283GR7P1367_C9	209	10989	896	2.21E-03	66977	5553
P283GR7P1367_E5	210	10014	1333	1.24E-03	3189	533
P283GR7P1367_F5	211	4565	601	1.08E-03	28950	2051
P283GR7P1367_G8	212	1463	450	3.85E-03	21031	1421
P283GR7P1367_H2	213	1621	390	2.35E-03	4207	864
P283GR7P1367_H8	214	5269	303	9.74E-03	20918	930
P283GR7P1367_H9	215	1714	434	1.47E-03	6121	918
P283HR7P1368_A10	216	13632	3233	5.13E-04	42326	4772
P283HR7P1368_B12	217	13399	1538	4.53E-05	18650	826
P283HR7P1368_C3	218	12727	2215	3.49E-04	13326	1306
P283HR7P1368_D1	219	14077	2312	1.66E-03	7850	1408
P283HR7P1368_D2	220	15246	1907	1.30E-03	11132	950
P283HR7P1368_D4	221	28979	6850	2.35E-04	52999	23549
P283HR7P1368_F10	222	18836	2661	1.65E-04	16121	1019

P283HR7P1368_F6	223	14325	3510	1.80E-04	20580	3541
P283HR7P1368_G1	224	31276	4940	2.15E-03	69817	11559
P283HR7P1368_G10	225	8122	753	1.45E-03	23790	2660
P283HR7P1368_G11	226	19305	2647	3.73E-04	14857	1343
P283HR7P1368_H1	227	15389	2460	5.52E-04	17285	1974
P283HR7P1368_H8	228	22758	1612	7.63E-04	35932	4888
Tencon25-His	270	341	219		337	336

5

Screen for Binding to T-cells from human and cynomolgus monkey donors

Binding of the 190 ELISA hits to human and cynomolgous monkey primary CD8 T cells was assessed by flow cytometry. The FN3 domain molecules were diluted to 2 μ M and 0.2 μ M in PBS and incubated with human or cynomolgous monkey CD8+ T cells in 96-well format. After 1 hour at 4°C, the cells were washed once with PBS and then resuspended with an anti-FN3 domain antibody (PAB25) solution. Following this incubation, the cells were washed twice with PBS and a PE conjugated secondary antibody and a viability dye were added. Finally, cells were washed and resuspended in PBS for flow cytometric analysis using a BD Canto Instrument. Cells were gating on live cells and median fluorescence intensity of the bound Centyrins (PE channel) was calculated using Cytobank software. Results are summarized in Table 4.

Off-rate analysis of anti-Human CD8 alpha Centyrins

Purified anti-CD8A FN3 domains were subjected to off-rate analysis using a Proteon surface plasmon resonance instrument in order to pick clones with the slowest off-rates for further characterization. Measured off-rates ranged from 2.64E-5 to 1.07E-2 sec⁻¹ as shown in Table 4.

Goat anti-human Fc IgG (Jackson immunoresearch, Cat# 109-005-098) was directly immobilized on a GLC sensor chip at 10 μ g/ml, pH5.0 via amine coupling (pH 5.0) on all 6 ligand channels in vertical orientation on the chip with a flow rate of 30 μ l/min in PBST (PBS, 0.005% Tween). The immobilized GAH-Fc IgG densities averaged about 6000 Response Units (Ru) with less than 1% variation among different channels. In house human CD8A-Fc was captured in vertical orientation at 3 different ligand densities, 10, 5, 2.5 μ g/ml for 5 minutes at 30 μ l/minute flowrate. All FN3 domains were normalized to a 3 μ M concentration, and tested for binding in horizontal orientation. All 6 analyte channels were used for FN3 domains to maximize the

5 screening throughput. The dissociation phase was monitored for 15 minutes at a flow rate of 100 μ L/min using PBST as running buffer. Regeneration of the surface was achieved by a short pulse of 0.85% phosphoric acid (18s contact time at 100 μ L/min). Data analyses were performed using Bio-Rad ProteOn Manager software (version 3.1.0.6). Raw data were double referenced by subtraction of the interspot (empty chip surface, no protein immobilized or captured) signals to correct the non-specific binding of the FN3 domain to the pre-coated GAH-Fc IgG surface, followed by a double correction using empty channel L6 where no hCD8A-Fc was captured. The processed binding data were locally fit to a 1:1 simple Langmuir binding model to extract the koff for each FN3 domain binding to captured hCD8A-Fc.

15

EXAMPLE 4: ENGINEERING OF ANTI-CD8A FN3 DOMAINS

A number of mutations were designed into top anti-CD8A candidates in order to eliminate post translational modification risks of oxidation (methionine, or tryptophan), deamidation (NS), isomerization (DG) and clipping (DP). Proline residues found in beta strands were also mutated as proline has a potential for destabilizing beta strands (Chiba T., et al. J Biol Chem. 2003;278:47016-24). Only residues derived from FN3 domain library-designed positions were considered for mutation. Variant sequences were chosen to either mimic similar chemical properties of the parent molecule (example tryptophan to tyrosine) or to replace the PTM risk amino acid with an amino acid found in other CD8A FN3 domains at that position. A full list of engineered sequences is found in Table 5. The dissociation rate between each mutant and recombinant CD8 alpha was measured by surface plasmon resonance to estimate relative binding strengths.

30 **Table 5. Dissociation rates of CD8A Centyrin mutants. Mutants are grouped according to the parent molecule.**

<u>Sample</u>	<u>k_d (1/s)</u>	<u>Mutations</u>	<u>SEQ ID NO:</u>
P282DR9P1359_C5	1.47E-04	Parent	68
CD8S402	4.84E-05	D40P	266
CD8S396	1.52E-04	W32Y	260

CD8S398	4.43E-04	W32S	262
CD8S397	6.60E-04	W32Q	261
CD8S399	1.34E-03	W38Y	263
CD8S401	1.27E-02	W38I	265
CD8S400	2.26E-02	W38L	264
CD8S404	3.09E-02	P36A	268
<hr/>			
P282DR9P1359_F5	5.78E-05	Parent	79
CD8S371	1.94E-04	W48Y	235
CD8S377	4.00E-04	W81E	241
CD8S374	4.03E-04	W81Y	238
CD8S372	5.71E-04	W48L	236
CD8S375	8.30E-04	W81L	239
CD8S376	8.46E-04	W81S	240
CD8S373	4.03E-03	W48I	237
<hr/>			
P282DR9P1359_G7	1.06E-05		83
CD8S379	4.97E-05	D43S	243
CD8S378	5.80E-05	D43E	242
CD8S388	7.54E-05	N81Q	252
CD8S387	1.25E-04	W83E	251
CD8S381	2.00E-04	W70F	245
CD8S383	7.47E-04	W74Y	247
CD8S380	1.21E-03	W70Y	244
CD8S382	2.47E-01	W70S	246
<hr/>			
P282ER9P1360_C8	1.79E-04	Parent	89
CD8S390	1.52E-04	W68Y	254
CD8S389	1.84E-04	W68F	253
CD8S391	3.20E-04	W68H	255
CD8S405	1.14E-03	P48T	269
<hr/>			
P282DR9P1359_F7	3.39E-04	Parent	81
CD8S403	1.33E-04	P36A	267
CD8S392	1.55E-03	W38Y	256
CD8S395	1.89E-03	W38H	259

CD8S393	2.55E-03	W38L	257
CD8S394	3.55E-03	W38I	258

5

From the data presented in Table 5, it is apparent that a number of mutations that reduce developability risks maintain dissociation rates similar to that of the parent molecule. Mutants CD8S402 (elimination of DP site), CD8S390 (elimination of Trp residue), and CD8S403 (removal of Pro from beta strand) resulted in slower dissociation rates than the parent appropriate molecule, indicative of tighter binding. A number of other mutations maintain binding similar to the parent molecule and thus might be preferred over the parent as these molecules pose less CMC related risks during development.

15 **EXAMPLE 5: AFFINITY MEASUREMENTS OF CD8A-BINDING FN3 DOMAINS.**

Nineteen anti-CD8A candidates were selected for full kinetic analysis of binding to recombinant human CD8 alpha. These candidates were selected from the above positive hits (Table 4) using the criteria of 1) strong relative binding to human T-cells, 2) strong relative binding to cyno T-cells, 3) minimal reduction in cell binding at 0.2 uM compared to 2 uM, 4) free of aggregates via SEC, 5) off-rates slower than 2.07E-3 sec⁻¹, 6) sequence diversity with respect to sequence families, and 7) relative propensity for sequences with potential developability challenges (oxidation, deamidation, clipping and hydrophobicity).

25 Affinities of the top 19 candidates, later a repeat of the top 6 candidates, binding to hCD8A-Fc were measured on a ProteOn XPR36 instrument (Bio-Rad) using GLC sensor chips under similar conditions to those for koff screening. Goat anti-human Fc antibody was directly immobilized on the chip by standard amine coupling at 10 µg/ml, pH 5.0 on all 6 ligand channels in vertical orientation on the chip with a flow rate of 30 µl/min in PBST (PBS, 0.005% Tween), achieving an average of 6200 Rus on each ligand channel. Human CD8A-Fc was then captured at five surface densities ranging from 200 to 1200 response units, leaving the 6th channel as empty channel control for GAH-Fc IgG surface. Binding was measured by flowing five different concentrations of anti-CD8A FN3 domains (1 µM diluted in a 3-fold dilution series) as analytes

5 simultaneously in the horizontal orientation over the captured hCD8A-Fc surfaces, with
a sixth analyte channel containing only running buffer PBST. All interactions were
measured at 100 uL/min flow rate with association and dissociation times being 4, 30
minutes respectively. Ligand surface regeneration was achieved by 1 short pulse of
0.85% phosphoric acid (18s contact time at 100 uL/min). Data analyses were
10 performed using Bio-Rad ProteOn Manager software (version 3.1.0.6). Raw data were
double referenced by subtraction of the interspot (empty chip surface, no protein
immobilized or captured) signals to correct the non-specific binding of the FN3 domain
to the pre-coated GAH-Fc IgG surface, followed by a double referencing using the
buffer blank response (to correct for any baseline drift resulting from ligand
15 dissociation over time). It has been consistently observed in multiple analyses that the
anti-CD8A FN3 domain binding data do not conform well to the 1:1 simple Langmuir
binding model, implying either the reagents issues and/or the intrinsically complicated
binding mechanisms that can't be accounted for using a simple 1:1 binding mode.
Given that the GAH-Fc capture of hCD8A-Fc format is the least disruptive relative to
20 other formats in introducing potential experimental artifacts (such as ligand activity
loss and/or artificial eptiopes/heterogeneous ligand population due to amine coupling),
it is considered that the results from the GAH-Fc capture experiments reported here
represent the most reliable ProteOn SPR data, despite the non-conforming 1:1
Langmuir fits observed in many instances. A heterogeneous ligand model was chosen
25 to fit the data assuming two different ligand species, either due to the heterogeneity in
the ligand protein population or due to potential different mechanisms for each FN3
domain binding to the 2 hCD8A monomers in the Fc fusion protein. In this case,
because each anti-CD8A FN3 domain would have separate affinities, the resultant
sensorgram reflects the sum of two independent reactions with two sets of rate
30 constants, which were reported for each FN3 domain binding.

35 **Table 6: Summary of kinetic affinities for top six anti-CD8A FN3 domain
candidates. Note: Affinity, K_D = k_d/k_a .**

Sample (SEQ ID NO:)	Lower Affinity Population			Higher Affinity Population		
	k_a (1/Ms)	k_d (1/s)	K_D (nM)	k_a (1/Ms)	k_d (1/s)	K_D (nM)
P282DR9P1359_F5 (79)	3.48E+04	6.60E-05	6.6	3.80E+05	1.42E-05	0.04
P282DR9P1359_F7 (81)	4.03E+04	3.65E-04	12	4.04E+05	7.99E-05	0.5
P282DR9P1359_G7 (83)	6.84E+04	5.51E-05	2.1	2.76E+05	1.49E-05	0.05
P282ER9P1360_C8 (89)	3.09E+04	9.52E-05	4.1	2.18E+05	4.71E-05	0.2
P283AR9P1362_D6 (122)	5.62E+04	3.12E-05	0.98	1.55E+05	1.00E-06	0.03
P282DR9P1359_C5 (68)	1.92E+04	1.27E-04	6.5	3.00E+05	5.79E-06	0.02

5

EXAMPLE 6: LABELING OF ANTI-CD8A FN3 DOMAINS WITH DFO AND 89ZR

Anti-CD8A FN3 domains were modified to include a single cysteine residue for conjugation of maleimide containing chelators or PET labels. Synthetic plasmid DNA encoding clones P282DR9P1359_F5, P282DR9P1359_F7, P282DR9P1359_G7, P282ER9P1360_C8, P283AR9P1362_D6, and P282DR9P1359_C5 with a mutation of residue E54 to cysteine were synthesized at DNA2.0 (Table 7). E54 was chosen as the position for mutation based on earlier studies that demonstrated maintenance of binding affinity, stability, and expression levels for other FN3 domains mutated at this residue (Goldberg S. et al. Protein Engineering Design and Selection 2016 Epub ahead of print).

Table 7: Modified anti-CD8A FN3 domain molecules

Original Clone	SEQID NO	Clone with E54C	SEQID No
P282DR9P1359_F5	79	CD8S368	229
P282DR9P1359_F7	81	CD8S367	230
P282DR9P1359_G7	83	CD8S370	231

P282ER9P1360_C8	89	CD8S365	232
P283AR9P1362_D6	122	CD8S369	233
P282DR9P1359_C5	68	CD8S366	234

5

Anti-CD8A FN3 domins modified with a free cysteine were conjugated to Deferoxamine (DFO) in order to chelate radiometals. 0.5 mL of a 100-500 μ M anti-CD8A FN3 domain solution was combined with 10 μ L of 500 mM TCEP (Sigma, cat. # 646547), gently flushed with nitrogen, and incubated for 1 hour at room temperature.

1.0 mL of saturated ammonium sulfate (4.02 M) was added to each tube to reach a final concentration of 3.2M before incubation on ice for 10 minutes and centrifugation at 16,000 x g or higher to pellet the protein. The resulting pellet was resuspended and washed in 1.0 mL of 3.2 M ammonium sulfate supplemented with 100 mM sodium phosphate pH 7.2 and 1 mM EDTA before centrifuging again. After the second

centrifugation step, the resulting pellet was dissolved in 100 mM sodium phosphate 7.0, 1 mM EDTA and combined with 10 μ L of 50 mM DFO solution to make a final molar ratio of 5:1 DFO to anti-CD8A. This reaction was allowed to proceed at room temperature for 30 minutes before quenching with 5.0 microliters of beta-mercaptoethanol. Excess DFO was finally removed by a variety of methods including

a second round of ammonium sulfate precipitation as described above, passing through a desalting column such as Zeba 7k column (Pierce Cat #89889), or by purification with nickle-NTA resin (Qiagen #30450). Anti-CD8A FN3domain-DFO conjugates were formulated in 1X PBS for further analysis.

Following conjugation to DFO, the binding of each anti-CD8A FN3 domain to recombinant human CD8 alpha was assessed by surface plasmon resonance as previously described. All samples retained tight binding to human CD8A following mutation of E54 to Cys and conjugation to DFO (Table 8).

30

Table 8. Binding affinity following DFO conjugation

Sample	Lower Affinity Population			Higher Affinity Population		
	k_a (1/Ms)	k_d (1/s)	K_D (nM)	k_a (1/Ms)	k_d (1/s)	K_D (nM)
CD8S365-DFO	4.41E+03	4.29E-05	9.73	6.80E+04	4.18E-05	0.6
CD8S366-DFO	5.85E+03	1.06E-04	18.2	7.95E+04	7.01E-05	0.9
CD8S367-DFO	1.09E+04	9.75E-04	89.1	8.45E+04	1.31E-04	1.55
CD8S368-DFO	7.32E+03	9.98E-05	13.6	1.08E+05	2.53E-05	0.23
CD8S369-DFO	2.87E+03	$\leq 1 \text{ E-}05$	≤ 3.4	3.73E+04	$\leq 1 \text{ E-}05$	≤ 0.3
CD8S370-DFO	5.91E+03	7.65E-05	13	4.64E+04	$\leq 2\text{E-}05$	≤ 0.3

5

EXAMPLE 7: BINDING OF ANTI-CD8A FN3 DOMAINS TO HUMAN AND CYNO T-CELLS.

A full dose response binding curve was generated for the nineteen selected anti-CD8A FN3 domains. Each candidate was diluted to 20 μM in PBS followed by a 1:3 dilution series to generate either an 11-point or an 18-point dose response curve. Human or cyno CD8+ T cells were incubated with the diluted FN3 domain for 1 hour at 4°C. Cells were washed once with PBS and incubated with an anti-centyrin antibody (PAB25) for 1 hour at 4°C. The cells were washed twice with PBS, followed by incubation with a PE-secondary, anti-CD3- PacB, anti-CD4-APC, and a viability dye. Finally, cells were washed and resuspended in PBS for flow cytometric analysis using a BD Canto Instrument. CD8 T cells were defined as live CD3+CD4- cells. Median fluorescence intensity of the bound Centyrins (PE channel) and % of cells showing positive staining calculated using Cytobank software. Results were graphed using Prism and EC_{50} values were calculated using the 4 parameter dose response variable slope equation.

A MesoScale Discovery-Cell Affinity Technology (MSD-CAT) based equilibrium cell-binding assay was performed to determine the affinity of the top six anti-CD8A candidates binding to primary human cytotoxic T cell surface CD8A receptors. Each anti-CD8A FN3 domain at a constant concentration of 50 pM was pre-incubated with 10 different concentrations of primary cytotoxic CD8 T cells (columns

2-11 in a row). Cell viability was checked prior to the binding measurements and a >85% viability was desired for valid analysis. Since these cells were from different donors, in case of donor-to-donor variations, only cells of the same donors were combined together. Each individual anti-CD8A FN3 domain binding was measured in replicates using cells from the same donors. Cells and FN3 domains were incubated overnight at 4°C on a rotator to reach equilibrium. Following the incubation the cells were spun down along with cell bound anti-CD8A FN3 domains and the unbound (free) anti-CD8A FN3 domains in the supernatants is quantified using MSD assays where biotinylated recombinant hCD8A-Fc protein was captured at 0.6 ug/mL in assay buffer to streptavidin MSD plates overnight ~16 hours at 4°C. After blocking the plate, supernatant with free anti-CD8A FN3 domains was added to the plate and incubated for 1 hr, then followed by SulfoTag pAb139 (In-house) detection at 1.6 ug/ml. A buffer control without any FN3 domain and hCD8A (plate background binding control) in column 1 and FN3 domain alone control without hCD8A (100% free/unbound) in column 12 were included. Mouse Anti-hCD8A mAb (mIgG1k, BD Biosciences, cat# 555364, clone RPA-T8) was included as a positive control. Tencon27 was included in the initial assay validation as a negative control and no significant binding was observed, and therefore, was not included in the later cell binding due to the cell availability. Plates were read immediately on the MSD Sector Imager 6000™ Reader for luminescence levels after adding MSD Read Buffer by diluting 1:4 of stock into H2O.

Raw MSD data were exported and analysed in Prism using a non-linear fit with variable slope function to derive the Bmax and Hillslope values. Only those with converged Bmax values and hillslope within the range of -1.5~-0.5 (ideal -1.0) will be considered for further analysis. Binding data were then normalized using the Bmax values to calculate the normalized % free FN3 domains. A surface CD8 density of 50,000 receptors per cell was used for the receptor concentration calculation. A saturation criterion of <20% free Centyrin at highest CD8 cell concentrations was required to determine the affinity using a “Solution Affinity Equation for normalized data” for a 1: 1 binding model.

Anti-CD8A FN3 domains bound to primary cells with affinities ranging from 0.167 to 2.81 nM (Table 9).

5 **Table 9: Summary of EC₅₀ values for top six anti-CD8A FN3 domain candidates.**

Clone ID (SEQ ID NO:)	EC50 Binding to Human T-cells by Flow Cytometry (nM)	EC50 Binding to cyno T-cells by Flow Cytometry (nM)	Affinity for Human T-cells by MSD-CAT (nM)
CD8S365 (232)	556.0	123.6	0.167
CD8S366 (234)	162.7	69.5	0.123
CD8S367 (230)	194.5	50.8	0.225
CD8S368 (229)	154.7	70.0	0.459
CD8S369 (233)	124.2	72.3	2.81
CD8S370 (231)	208.7	67.6	0.869

EXAMPLE 8: ACTIVATION OF HUMAN T-CELLSDe Novo Activation

10

In order to determine if the anti-CD8A FN3 domains activate T cells, a flow cytometry assay was performed to monitor changes in T cell activation markers. Six anti-CD8A FN3 domains were evaluated for T-cell activation. De novo activation was assessed by incubating the FN3 domains at either 1 μ M or 10 nM in duplicate with human pan-T

15 cells in media for 4 days. Two independent donors were tested. Plate bound anti-CD3 was used a positive control at 2 doses, 0.1 μ g/mL and 0.01 μ g/mL. PBS was used as a negative control. Cells were then stained with a viability dye and the following panel of antibodies: CD4-FITC, CD3-PerCP-Cy5.5, CD69-PacB, CD45RA-BV605, CD25-BV650, CD127-PE, and CD137-PE-Cy7. CD8+ cells were defined as live CD3+CD4-

20 cells and were profiled for each T-cell activation marker. Median fluorescence

- 5 intensity values were calculated using FlowJo software and replicate values were averaged. Results are summarized in Table 10A (donor 022) and 10B (donor 146). For 365, 366, 367, 368, and 370, small changes in the T cell activation markers were observed in only 1 out of the 2 donors tested at the highest dose level of 1 μ M. These changes were absent in both donors at the 10 nM dose, suggesting the molecules do not
- 10 activate T cells de novo at relevant concentrations. The 369 molecule does appear to significantly activate CD137 expression in both donors at the highest dose level.

Table 10: Median Fluorescence Intensity (MFI) values for various T cells activation markers on CD8+ T cells for Donor 022 (A) and Donor 146 (B)

15 **A**

Donor	Sample (SEQ ID NO:)	Anti-CD8A FN3 conc μ M	Anti-CD3 ug/mL	CD45RA MFI	CD25 MFI	CD69 MFI	CD127 MFI	CD137 MFI
022	PBS control	0	0	12856	571	223	651	296
022	PBS control	0	0.01	13133	707	403	517	343
022	PBS control	0	0.1	11394	1333	1694	158	529
022	CD8S366 (234)	1	0	15054	949	477	425	310
022	CD8S366 (234)	0.01	0	13336	814	230	586	301
022	CD8S368 (229)	1	0	12992	858	698	367	329
022	CD8S368 (229)	0.01	0	15262	677	276	489	306
022	CD8S367 (230)	1	0	15409	796	401	511	297
022	CD8S367 (230)	0.01	0	13666	723	261	502	312
022	CD8S370 (231)	1	0	12946	916	572	376	353

022	CD8S370 (231)	0.01	0	14973	776	353	435	331
022	CD8S365 (232)	1	0	13935	904	562	367	328
022	CD8S365 (232)	0.01	0	15156	697	243	504	323
022	CD8S369 (233)	1	0	13661	783	440	441	5122
022	CD8S369 (233)	0.01	0	16513	717	251	596	416
022	TenCon	1	0	14920	702	284	447	334

5

B

Donor	Sample (SEQ ID NO:)	Anti- CD8A FN3 conc μ M	Anti-CD3 μ g/mL	CD45RA MFI	CD25 MFI	CD69 MFI	CD127 MFI	CD137 MFI
146	PBS	0	0	7172	627	61	1313	500
146	PBS	0	0.01	8076	681	153	1296	617
146	PBS	0	0.1	5171	1462	1100	139	798
146	CD8S366 (234)	1	0	8531	673	95	1368	589
146	CD8S366 (234)	0.01	0	9414	623	74	1615	559
146	CD8S368 (229)	1	0	8386	691	96	1301	561
146	CD8S368 (229)	0.01	0	9147	628	82	1424	586
146	CD8S367 (230)	1	0	8167	660	95	1322	581
146	CD8S367 (230)	0.01	0	8734	586	77	1479	571
146	CD8S370 (231)	1	0	8590	737	86	1362	583
146	CD8S370 (231)	0.01	0	7934	635	71	1526	559

146	CD8S365 (232)	1	0	8344	813	85	1238	586
146	CD8S365 (232)	0.01	0	8460	628	80	1355	605
146	CD8S369 (233)	1	0	8778	681	92	1369	5690
146	CD8S369 (233)	0.01	0	7862	591	74	1498	784
146	TenCon	1	0	7325	609	78	1198	574
146	TenCon	0.01	0	7764	596	66	1281	530

5

Pan T-cell activation

In order to determine if the anti-CD8A FN3 domains can affect markers of T cell activation in pan-activated T cells, the anti-CD8A FN3 domains were also evaluated in combination with a low dose of plate bound CD3. In this assay, a sub-optimal concentration (0.01 $\mu\text{g/mL}$) of plate bound anti-CD3 was used to activate the T cells in the presence of either 1 μM or 10 nM anti-CD8A. After 4 days, the cells were assessed using the same panel and gating strategy as described above. Two independent donors were tested. Median fluorescence intensity values were calculated using FlowJo software and replicate values were averaged. Results are summarized in Tables 11A (donor 022) and 11B (donor 146).

Tables 11A and B: Median Fluorescence Intensity (MFI) values for various T cells activation markers on CD8⁺ T cells for Donor 022 (A) and Donor 146 (B) in the presence of plate bound CD3.

20 **A**

Donor	Sample (SEQ ID NO:)	Anti- CD8A FN3 conc μM	Anti-CD3 $\mu\text{g/mL}$	CD45RA MFI	CD25 MFI	CD69 MFI	CD127 MFI	CD137 MFI
022	PBS control	0	0	12856	571	223	651	296
022	PBS	0	0.01	13133	707	403	517	343

	control							
022	PBS control	0	0.1	11394	1333	1694	158	529
022	CD8S366 (234)	1	0.01	11918	892	1005	369	376
022	CD8S366 (234)	0.01	0.01	13417	1068	848	384	399
022	CD8S368 (229)	1	0.01	11311	1147	1279	260	428
022	CD8S368 (229)	0.01	0.01	13441	760	599	499	348
022	CD8S367 (230)	1	0.01	13271	1135	1127	367	385
022	CD8S367 (230)	0.01	0.01	14521	960	636	483	362
022	CD8S370 (231)	1	0.01	15138	1103	890	407	378
022	CD8S370 (231)	0.01	0.01	14230	875	612	431	355
022	CD8S365 (232)	1	0.01	14395	1112	907	380	407
022	CD8S365 (232)	0.01	0.01	14006	1175	1063	297	430
022	CD8S369 (233)	1	0.01	13735	877	759	464	5457
022	CD8S369 (233)	0.01	0.01	13864	842	617	450	498
022	TenCon	1	0.01	14687	791	553	408	358
022	TenCon	0.01	0.01	13090	759	630	464	368

5

B

Donor	Sample (SEQ ID NO:)	Anti-CD8A FN3 conc μ M	Anti-CD3, μ g/mL	CD45RA MFI	CD25 MFI	CD69 MFI	CD127 MFI	CD137 MFI
146	PBS	0	0	12856	571	223	651	296

	control							
146	PBS control	0	0.01	13133	707	403	517	343
146	PBS control	0	0.1	11394	1333	1694	158	529
146	CD8S366 (234)	1	0.01	6798	876	163	1095	632
146	CD8S366 (234)	0.01	0.01	8589	775	158	1077	637
146	CD8S368 (229)	1	0.01	6576	945	175	1105	662
146	CD8S368 (229)	0.01	0.01	7608	843	200	950	678
146	CD8S367 (230)	1	0.01	6447	897	173	1088	672
146	CD8S367 (230)	0.01	0.01	7899	801	175	1031	655
146	CD8S370 (231)	1	0.01	7327	992	169	1055	687
146	CD8S370 (231)	0.01	0.01	8676	790	183	946	675
146	CD8S365 (232)	1	0.01	6624	977	172	1059	670
146	CD8S365 (232)	0.01	0.01	7902	843	193	985	659
146	CD8S369 (233)	1	0.01	7660	933	165	1149	7114
146	CD8S369 (233)	0.01	0.01	7892	854	187	989	842
146	TenCon	1	0.01	8352	829	170	1026	658
146	TenCon	0.01	0.01	7627	761	185	1043	673

5

Cytokine Response

5

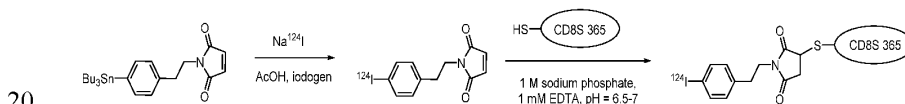
In order to determine if any of the changes observed in the activation markers resulting in changes in cytokine production, antigen-dependent T cell activation assays were also performed using two anti-CD8A FN3 domains. For one set of assays, either CMV reactive or M1 reactive human PBMCs were thawed and rested overnight at 37°C in 6 well plates. The following day, the PBMCs were harvested by pipetting, counted, and
10 plated onto IFN γ Mabtech ELISpot plates in the presence or absence of 10 μ g/mL peptide. 1 μ M anti-CD8A FN3-DFO conjugate was added to the wells and plates were allowed to incubate at 37°C for approximately 24 hours undisturbed. The cells were removed and the plates were washed 5 times with PBS. The supplied detection
15 antibody was added and plates were incubated for 2 hours. The plates were again washed and the kit substrate was added to each well. Plates were developed for approximately 5 minutes before the reaction was stopped by running the plate under water. Plates were dried upside down overnight in the dark. Plates were read on the AID EliSpot Reader and spot counts were generated using the AID EliSpot Software.
20 Results were graphed in Prism. Results are summarized in Fig. 1. In this assay, 365-DFO does not increase the number of IFN γ spots compared to media alone or non-CD8A binding TenCon control in the absence of peptide (Fig. 1A, 1C). Peptide and CD3 are included as positive controls. In the presence of peptide, the 365-DFO does not change the number of IFN γ spots compared to peptide alone or peptide with non-
25 CD8A binding tencon (Fig. 1B, 1D). Media is included as a negative control and CD3 is included as a positive control. These results suggest that the centyrin does not affect T cell activation.

To confirm these results in a longer-term assay, IFN-gamma levels were also measured in a 6-Day activation assay. For this study, CMV reactive PBMCs were
30 incubated in triplicate with anti-CD8A FN3 domains at 1 μ M in the presence or absence of 0.25 μ g/mL pp65 peptide. Cells were incubated for 6 days at 37°C. At each timepoint the cells were centrifuged and supernatant was harvested. Samples were stored at -80°C until analyzed. Thawed samples were analyzed for IFN-gamma using a single-plex Meso Scale Discovery (MSD) based ELISA. For this assay, a standard
35 curve was prepared as per manufacturer's instructions. Samples and standards were added to pre-coated 96 well MSD plates. After a 2 hour incubation, the kit detection antibody was added. After another 2 hour incubation, plates were washed three times

5 followed by the addition of the supplied read buffer. Plates were read on MSD Sector Imager 6000 plate reader. Raw MSD data files were analyzed against the standard curves generated using the MSD Discovery Workbench software. The analyzed data graphed using the Tibco Spotfire program. Results are summarized in Fig. 2. In this assay, 365-DFO does not increase the secretion of IFN γ into the media compared to
 10 media alone in the absence of peptide (Fig. 2A). CMV peptide is included as a positive control. In the presence of peptide, the 365-DFO also does not change the amount of IFN γ secretion compared to peptide alone (Fig. 2B). Media is included as a negative control. These results suggest that the centyrin does not affect T cell activation.

15 **EXAMPLE 9: LABELING OF ANTI-CD8A FN3 DOMAINS WITH I124/I125**

The current method to radiolabel CD8S365 with iodine-124 to produce [^{124}I]-IPEM CD8S 365 (Scheme 1) was adapted from literature procedures (*Bioconjugate Chem.* **1991**, 2, 435-440; *ChemistryOpen* **2015**, 4, 174-182).



Scheme 1: Synthesis of [^{124}I]-IPEM CD8S 365

To a 1.5 mL Eppendorf vial was added, in order, Na ^{124}I solution ($\leq 13\ \mu\text{L}$, $\leq 2.5\ \text{mCi}$), AcOH (5 μL to acidify the solution), 1-(4-(tributylstannyl)phenethyl)-1H-pyrrole-2,5-dione (75 μL , 1.00 mg/mL in MeCN) and iodogen (5 μL , 1.00 mg/mL in
 25 MeCN) solution. The reaction was left for 5 min at room temperature.

The crude reaction mixture was diluted with 0.5 mL of 20% EtOH/H $_2\text{O}$ and was purified directly on preparatory HPLC, the retention time = 14.4 min (Fig. 3). The [^{124}I]-IPEM was collected in a 1 dram vial that had been pre-treated with Sigma-CoteTM (then rinsed with 3 mL of 70% EtOH, followed by 3 mL of H $_2\text{O}$); total volume
 30 collected off preparatory HPLC <750 μL .

An aliquot ($\sim 5\text{--}25\ \mu\text{Ci}$) of the purified fraction was then injected on analytical HPLC (Fig. 4, retention time = 11.7 min).

The purified [^{124}I]-IPEM was then concentrated under vacuum at ambient temperature to a volume of <100 μL .

5 Sodium phosphate buffer (1.0 M sodium phosphate, 1 mM EDTA, pH = 6.86) was added (≥ 25 μ L) to bring the pH to ~ 6.5 -7 (checked by strip). Lastly freshly reduced CD8S 365 (c ~ 4.57 mg/mL in 100 mM sodium phosphate buffer, 1 mM EDTA, pH = 6.86), was added in appropriate amount to achieve targeted specific activity (ie. if targeting specific activity of 25 mCi/mg and 2.0 mCi of [^{124} I]-IPEM was collected add 17.5 μ L of centyrin at c ~ 4.57 mg/mL). The conjugation reaction was left
10 for 60 min at ambient temperature and the reaction progress was checked to verify that the conversion exceeded 90% by iTLC.

Purification consisted of diluting the reaction solution with PBS/10% EtOH (1 mL, pH = 7) transferring the reaction solution from the 1 dram vial into a Vivaspin 6
15 5kDa MWCO centrifugal filter (see appendix for the pre-conditioning). After the transfer, the reaction Eppendorf was rinsed with PBS/10% EtOH (2×1 mL, pH = 7) and the washings were added to the filter. The crude reaction mixture was centrifuged at 4000 rpm, at 20 $^{\circ}$ C for 30 min. Following centrifugation < 500 μ L of solution remained and was found to have a radiochemical purity (RCP) $> 95\%$ by radio TLC
20 (Fig. 5). The purified [^{124} I]-IPEM CD8S 365 was diluted to a volume of 500 μ L with PBS/10% EtOH if the volume was < 500 μ L and then filtered through a Millex-GV 0.22 μ m hydrophilic Durapore (PVDF) membrane.

The radiochemical yield from the protocol is $\sim 50\%$ with a radiochemical purity $\geq 95\%$ RCP by radio TLC). Analytical reverse phase HPLC was used to determine the
25 protein concentration and specific activity of the final product. The average integration of the peak at retention time = 7.3 min in the UV at $\lambda = 280$ nm was used to extrapolate the protein concentration from a calibration curve (Fig. 6 for a representative example). A co-injection with the non-radioactive cold standard IPEM CD8S 365 (MALDI analysis shown in Fig. 7) was also conducted (see Fig. 8). The bacterial endotoxin
30 concentration was measured using the Endosafe[®] portable test system using a 10X dilution with LAL reagent water.

35 **EXAMPLE 10: DETECTION OF CD8 EXPRESSION IN CYNOMOLGUS MONKEYS**

Two anti-CD8A FN3 molecules (CD8S365 and CD8S368) were selected for PET imaging in non-human primates (NHP). The anti-CD8A molecules were radiolabeled with either Zr-89 (Zevacor, Somerset, NJ) or I-124 (CPDC, Hamilton, Canada, and Zevacor, Somerset, NJ). Approximately 1-2 mCi of radiolabeled anti-CD8A molecules was(were) injected into the saphenous vein of a female NHP (cynomolgus macaque), while anesthetized with isoflurane in oxygen. Each animal was scanned in a large-bore microPET Focus 220 PET scanner (Siemens, Knoxville, TN), with the bed moved to accommodate the entire body of the animal (head to lower abdomen). Each scan lasted approximately 1 h, and scans were acquired at 15 min, 2 h, and 24 h after injection. PET images were reconstructed using a 2D maximum likelihood expectation maximization (ML-EM) algorithm, into 3D images of voxel size 1.898 x 1.898 x 0.796 mm, dimensions 128 x 128 x 475. Blood samples were obtained at multiple time points from the saphenous vein in the opposite leg to the injection, and the blood radioactivity counted in a well counter.

PET images were analyzed using PMOD v3.7 software (PMOD, Zurich, Switzerland). Regions-of-interest were drawn manually around organs such as spleen, kidneys and liver. Counts were converted to units of percent injected dose per gram of tissue (%ID/g), while blood radioactivity was presented as %ID. A representative PET image is shown in Fig. 9.

Blood kinetics for each NHP and each anti-CD8A FN3 domain molecule (labeled with either Zr-89 or I-124) are shown in Table 11, and summarized in Fig. 10. For the same animals and anti-CD8A molecules, the organ biodistributions are shown in Table 12 (units are %ID/g), and summarized in Fig. 11. The Zr-89 labeled molecules exhibited residualization of the radioisotope in the excretory organs, which caused a large background signal in the kidneys, potentially obscuring other nearby tissues. This was largely absent from the I-124 labeled molecules. The spleen uptake was very similar between the two different molecules and two different radioisotopes for all time points.

Table 11. Blood kinetics for each centyrin, radiolabeled with either Zr-89 or I-124 (entries are % ID).

Time (h)	365 Zr-89	Time (h)	368 Zr-89	Time (h)	365 I-124	Time (h)	368 I-124
0.38	32.49	0.25	75.64	0.40	73.53	0.33	50.56
0.62	17.62	0.50	44.01	0.65	44.59	0.57	37.07
1.18	9.03	0.75	32.06	0.92	29.79	0.87	19.33
2.00	6.12	1.00	24.20	1.13	24.51	1.17	16.01
3.70	3.77	1.50	18.82	2.00	17.14	1.37	12.84
24.00	1.36	2.00	14.22	3.33	10.92	2.07	12.18
		3.33	8.40			3.88	8.20
		24.00	1.94			23.03	1.24

5

For the same animals and anti-CD8A molecules, the organ biodistributions are shown in Table 12 (units are %ID/g), and summarized in Fig. 11.

Table 12. Organ uptake for the different centyrins, labeled with either Zr-89 or I-124

10 (entries are %ID/g).

365 Zr-89			
<u>Time (h)</u>	<u>Kidney</u>	<u>Spleen</u>	<u>Liver</u>
0.25 h	0.641	0.0386	0.0620
2 h	0.624	0.0218	0.0513
24 h	0.633	0.0136	0.0354

368 Zr-89			
<u>Time (h)</u>	<u>Kidney</u>	<u>Spleen</u>	<u>Liver</u>
0.25 h	0.575	0.0345	0.0740
2 h	0.664	0.0307	0.0688
24 h	0.931	0.0294	0.0508

365 I-124			
<u>Time (h)</u>	<u>Kidney</u>	<u>Spleen</u>	<u>Liver</u>
0.25 h	0.104	0.0324	0.0291
2 h	0.065	0.0222	0.0142
24 h	Not collected due to technical issue		

368 I-124			
<u>Time (h)</u>	<u>Kidney</u>	<u>Spleen</u>	<u>Liver</u>
0.25 h	0.292	0.0357	0.0439
2 h	0.140	0.0271	0.0241
24 h	0.0089	0.0022	0.0029

5

EXAMPLE 11: SPECIFICITY OF ANTI-CD8A FN3 DOMAINS IN CYNOMOLGUS MONKEYS

In order to test specificity of the anti-CD8A molecules, the same monkeys were treated with a chimeric CD8-depleting antibody (CM-T807 mouse V/human Fc anti-CD8 antibody) to reduce CD8+ T cells prior to imaging. Animals were administered s.c. with 10 mg/kg CD8 depleting antibody 3 days prior to imaging. CD8 depletion was confirmed by measuring the percentage of CD8 T cells in blood samples taken from each animal before and after depletion (Fig. 12).

Approximately 1-2 mCi of radiolabeled [I-124]CD8S365 anti-CD8 FN3 domain molecule was injected into the saphenous vein of a female NHP (cynomolgus macaque), while anesthetized with isoflurane in oxygen. Each animal was scanned in a large-bore microPET Focus 220 PET scanner (Siemens, Knoxville, TN), with the bed moved to accommodate the entire body of the animal (head to lower abdomen). Each scan lasted approximately 1 h, and scans were acquired at 15 min, 2 h, and 24 h after injection. PET images were reconstructed using a 2D maximum likelihood expectation maximization (ML-EM) algorithm, into 3D images of voxel size 1.898 x 1.898 x 0.796 mm, dimensions 128 x 128 x 475. Blood samples were obtained at multiple time points from the saphenous vein in the opposite leg to the injection, and the blood radioactivity counted in a well counter.

PET images were analyzed using PMOD v3.7 software (PMOD, Zurich, Switzerland). Regions-of-interest were drawn manually around organs such as spleen, kidneys and liver. Counts were converted to units of percent injected dose per gram of tissue (%ID/g), while blood radioactivity was presented as %ID. A representative PET image is shown in Fig. 13 for a depleted animal, showing a complete absence of the spleen signal seen in the non-depleted animal in Fig. 9.

Blood kinetics for each NHP, both depleted and non-depleted, are shown in Fig. 14, while the organ uptakes are shown in Fig. 15. There is little difference in blood kinetics between the depleted and non-depleted animals. Spleen uptake at the earliest time point are similar between depleted and non-depleted, since this is dominated by blood flow. However, at later time points (2 h) the spleen uptake in the depleted

- 5 animals is less than half that seen in the non-depleted animals, and is essentially at background levels, demonstrating CD8A specificity of the radiolabeled centyrin.

EXAMPLE 12: SENSITIVITY AND SPECIFICITY OF PET IMAGING IN CD8 OVER-EXPRESSING TUMORS

- 10 In order to determine the lowest number of cells that can be detected with the anti-CD8A FN3 domain molecules and PET, a study was performed in mice using different numbers of CD8 overexpressing cells. Forty 4-5 week old female NOD-*scid* *IL2 γ ^{null}* (NSG) mice (JAX Laboratory) were used, and acclimated for 7-10 days. Mice were group housed in IVC-cages under a 12-h light:dark cycle (lights on at 06:30 h) at
15 a temperature of 19 to 22 °C. Mice were fed a standard autoclaved laboratory chow and water *ad libitum*. Mice were ear-tagged and tails were tattooed 5-7 days prior to the start of the study to identify each animal.

- HEK-293 parental and HEK-293-luc CD8+ over-expressing cell lines were maintained as 2D-cultures. Mice were implanted subcutaneously with a total of 10⁶
20 tumor cells in a 1:1 medium to cultrex mix containing varying ratios of HEK-293-Luc CD8+ expressing cells and HEK-293 parental cells. Once tumors were palpable, approximately 10-14 days and 200-300 mm³ in size, the human CD8+ cells were visualized using [I-124]CD8-S365.

- Luciferase expression of HEK-293-Luc CD8+ cells was quantified *in vivo* using
25 bioluminescence imaging in an IVIS Spectrum optical imager (Perkin Elmer). Dynamic optical imaging was performed immediately after injection of 150 mg/kg D-luciferin to identify the peak light emission.

- Approximately 0.2-0.5 mCi of radiolabeled anti-CD8A FN3 domain molecules was injected into the tail vein while anesthetized with isoflurane in oxygen. Each
30 animal was scanned in an Inveon microPET-CT scanner (Siemens, Knoxville, TN) for 20 min static scan. Scans were acquired at 2-3 h post tracer injection. PET images were reconstructed using a 2D maximum likelihood expectation maximization (ML-EM) algorithm, into 3D images of voxel size 0.776 x 0.776 x 0.796 mm, dimensions 128 x 128 x 159.

- 35 PET images were analyzed using PMOD v3.7 software (PMOD, Zurich, Switzerland). Regions-of-interest were drawn manually around the tumor and other organs such as spleen, kidneys and liver. Counts were converted to units of percent

5 injected dose per gram of tissue (%ID/g). A representative PET image is shown in Fig. 16. Luciferase expression was quantified by drawing regions-of-interest in Living Image v4.4 software (Perkin Elmer). Light emission was measured in units of photons/sec/cm²/steradian.

Time-activity curves of radiolabeled anti-CD8A FN3 domain molecules in the
10 blood and tumor for both CD8+ HEK293 cells and parental cells are shown in Fig. 17 and Fig. 18. There is a significant increase in anti-CD8A FN3-binding in the CD8-expressing cells compared to the parentals, while the blood activity is the same for both. Uptake of the anti-CD8A FN3 by the CD8+ HEK293 cells is shown in Fig. 19, as a function of number of implanted cells. Based on these data, it is estimated that the
15 lowest level of detection is approximately 7.5×10^6 cells.

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25

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45

5 Sequence listing

SEQ ID No. 1= Original Tencon Sequence

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSEFLIQYQESEKVGGEAINLTVPGSER
SYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT

10

SEQ ID No. 2= TCL1 library

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSEFLIQYQESEKVGGEAINLTVPGSER
SYDLTGLKPGTEYTVSIYGV(X)₇₋₁₂PLSAEFTT;

wherein

15 X₁, X₂, X₃, X₄, X₅, X₆, X₇ is any amino acid; and

X₈, X₉, X₁₀, X₁₁ and X₁₂ are any amino acid or deleted

SEQ ID No. 3=TCL2 library

LPAPKNLVVSEVTEDSLRLSWX₁X₂X₃X₄X₅X₆X₇X₈SFLIQYQESEKVGGEAINLTV
20 PGSERSYDLTGLKPGTEYTVSIYGVX₉X₁₀X₁₁X₁₂X₁₃SX₁₄X₁₅LSAEFTT;

wherein

X₁ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;

X₂ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
25 or Val;

X₃ Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or
Val;

X₄ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;

30 X₅ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;

X₆ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;

X₇ is Phe, Ile, Leu, Val or Tyr;

35 X₈ is Asp, Glu or Thr;

X₉ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;

X₁₀ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val;

40 X₁₁ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp,
Tyr or Val;

X₁₂ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp,
Tyr or Val;

X₁₃ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp,
45 Tyr or Val;

X₁₄ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr
or Val; and

X₁₅ is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp,
Tyr or Val.

50

SEQ ID No. 4= Stabilized Tencon (Tencon 27)

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFLIQYQESEKVGGEAIVLTVPGSER
SYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

5

SEQ ID No. 5= TCL7 (FG and BC loops)

LPAPKNLVVSRTEDSARLSWX₁X₂X₃X₄X₅X₆X₇X₈X₉FDSFLIQYQESEKVGGEAI
 VLTVPGSESYDLTGLKPGTEYTVSIYGVX₁₀X₁₁X₁₂X₁₃X₁₄X₁₅X₁₆X₁₇X₁₈X₁₉SNPL
 SAIFTT;

10 wherein

X₁, X₂, X₃, X₄, X₅, X₆, X₁₀, X₁₁, X₁₂, X₁₃, X₁₄, X₁₅ and X₁₆ are A, D, E, F, G, H, I, K, L,
 N, P, Q, R, S, T, V, W or Y; and

X₇, X₈, X₉, X₁₇, X₁₈ and X₁₉, are A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or
 deleted

15

SEQ ID No. 6= TCL9 (FG loop)

LPAPKNLVVSRTEDSARLSWTAPDAAFDSEFLIQYQESEKVGGEAIVLTVPGSE
 SYDLTGLKPGTEYTVSIYGV X₁X₂X₃X₄X₅X₆X₇X₈X₉ X₁₀X₁₁X₁₂SNPLSAIFTT;

wherein

20 X₁, X₂, X₃, X₄, X₅, X₆ and X₇, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y;
and

X₈, X₉, X₁₀, X₁₁ and X₁₂ is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, Y or deleted.

SEQ ID No. 7= TCL14 library

25 LPAPKNLVVSRTEDSARLSWTAPDAAFDSEFX₁IX₂YX₃EX₄X₅X₆X₇GEAIVLTVP
GSESYDLTGLKPGTEYX₈VX₉IX₁₀GVKGGX₁₁X₁₂SX₁₃PLSAIFTT;

wherein

X₁, X₂, X₃, X₄, X₅, X₆, X₁₀, X₁₁, X₁₂ and X₁₃ are A, D, E, F, G, H, I, K, L, N, P, Q, R, S,
 T, V, W, Y, or M.

30

SEQ ID No. 8= TCL24 Library

TCL24 Library (SEQ ID NO: 8)

LPAPKNLVVSRTEDSARLSWTAPDAAFDSEFX₁IX₂YX₃EX₄X₅X₆X₇GEAIX₈LX₉
 VPGSESYDLTGLKPGTEYX₁₀VX₁₁IX₁₂GVKGGX₁₃X₁₄SX₁₅PLX₁₆AX₁₇FTT;

35 wherein

X₁, X₂, X₃, X₄, X₅, X₆, X₁₀, X₁₁, X₁₂ and X₁₃ are A, D, E, F, G, H, I, K, L, N, P, Q, R, S,
 T, V or W.

40 SEQ ID No. 9 = Sloning-FOR
GTGACACGGCGGTTAGAAC

SEQ ID No. 10 = Sloning-REV
 GCCTTTGGGAAGCTTCTAAG

45

SEQ ID No. 11 = POP2250
 CGGCGGTTAGAACGCGGCTACAATTAATAC

50 SEQ ID No. 12 = DigLigRev
CATGATTACGCCAAGCTCAGAA

SEQ ID No. 13 = BC9

5 GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNN
10 TGA AAAAGTTGGTGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCT
TTACGACCTGACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTA
CGGTGTTCTTAGAAGCTTCCCAAAGGC

SEQ ID No. 14 = BC8

15 GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNN
NNNNNNNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGA
20 AAAAGTTGGTGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTA
CGACCTGACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGG
TGTTCTTAGAAGCTTCCCAAAGGC

SEQ ID No. 15 = BC7

25 GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNN
NNNNNNNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAA
30 AGTTGGTGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGA
CCTGACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGT
CTTAGAAGCTTCCCAAAGGC

SEQ ID No. 16 = BC6

35 GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTGAAGTTACCGAAGACTCTCTGCGTCTGTCTTGGNNNNNNNN
NNNNNNNNNNNTTYGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT
40 TGGTGAAGCGATCAACCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCT
GACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTCTT
AGAAGCTTCCCAAAGGC

SEQ ID No. 17 = 130mer-L17A

45 CGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCCCTGTTGA
CAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATT
TCACACAGGAAACAGGATCTACCATGCTG

SEQ ID No. 18 = POP222ext

50 CGG CGG TTA GAA CGC GGC TAC AAT TAA TAC

SEQ ID No. 19 = LS1114

- 5 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
- 10 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
15 CATGCGGTCTCTCCGAAAAAGTTGGTGAAGCGATCGTCCTGACCGTTCCG
GGT
- SEQ ID No. 23 = SDG24
GGTGGTGAAGATCGCAGACAGCGGGTTAG
- 20 SEQ ID No. 24 = POP2222
CGGCGGTTAGAACGCGGCTAC
- SEQ ID No. 25 = SDG28
25 AAGATCAGTTGCGGCCGCTAGACTAGAACCGCTGCCACCGCCGGTGGTGAA
GATCGCAGAC
- SEQ ID No. 26 = FG12
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
30 TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGACCGCGCC
GGACGCGGCGTTCGACTCTTTCTGATCCAGTACCAGGAATCTGAAAAAGT
TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG
35 ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGC
GATCTTCACCACCGGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAG
TCTAGCGGCCGCAACTGATCTTGGC
- 40 SEQ ID No. 27 = FG11
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGACCGCGCC
45 GGACGCGGCGTTCGACTCTTTCTGATCCAGTACCAGGAATCTGAAAAAGT
TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG
ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGAT
CTTCACCACCGGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCT
50 AGCGGCCGCAACTGATCTTGGC
- SEQ ID No. 28 = FG10

5 GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCC
GGACGCGGCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT
10 TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG
ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTT
CACCACCGGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGC
GGCCGCAACTGATCTTGGC

15
SEQ ID No. 29 = FG9

GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
20 TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCC
GGACGCGGCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT
TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG
ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTAC
25 CACCGGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGC
CGCAACTGATCTTGGC

SEQ ID No. 30 = FG8

GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
30 TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCC
GGACGCGGCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT
TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG
ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN
35 NNNNNNNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTACCAC
CGGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGC
AACTGATCTTGGC

40
SEQ ID No. 31 = FG7

GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC
TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCC
45 GGACGCGGCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT
TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG
ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN
NNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTACCACCGGC
GGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCTAGCGGCCGCAACT
50 GATCTTGGC

5

SEQ ID NO: 32 FG loop of Tencon
KGGHRSN

10

SEQ ID No. 33 = Tcon 6
AAGAAGGAGAACCGGTATGCTGCCGGCGCCGAAAAAC

15

SEQ ID No. 34 = Tcon5E86Ishort
GAG CCG CCG CCA CCG GTT TAA TGG TGA TGG TGA
TGG TGA CCA CCG GTG GTG AAG ATC GCA GAC AG

20

> SEQID No 35: CD8W7

SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQ
NKPAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYFCSALNSIMYFSHFV
PVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAGSGSGSDYKDDDDKDKT
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNH
YTQKSLSLSPGK

30

>SEQID No 36: CD8W13

SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQNKPK
AAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYFCSALNSIMYFSHFVPVFLPAKPT
TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDGGGSDYKDDDDKKG
GGGSHHHHHHDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNNHYTQKS
LSLSPGK

40

>SEQID No. 37: mIgGK signal peptide
Metdtlllwvlllwpgstg

>SEQID No. 38: Human Fc

Dkthtccppcpapellggpsvflfppkpkdtlmisrtpevtcvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvsv
ltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytlppsrdeltnqvslclvkgfypsdiavewesngqpenny
kttpvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslspsgk

50

>SEQID No. 39: linker sequence
Ggggsdykdddkgggshhhhhh

Clone ID	SEQID No	Amino Acid Sequence
P282AR9P1356_A10	40	LPAPKNLVVSRVTEDSARLSWHTATNSFDSFLIQYQESEKVGIAIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVDYNPTGRPVSSNPLSAIF TT

P282AR9P1356_A4	41	LPAPKNLVVSRVTEDSARLSWVKRPNSFDSFLIQYQSEKVGGEAIVL TVPGSERSYDLTGLKPGTEYTVSIYGVDDVYEGRPWSNPLSAIFT T
P282AR9P1356_A6	42	LPAPKNLVVSRVTEDSARLSWSKTDSSFDSFLIQYQSEKVGGEAIVL TVPGSERSYDLTGLKPGTEYTVSIYGVDDVYIEGNPVFSNPLSAIFTT
P282AR9P1356_B9	43	LPAPKNLVVSRVTEDSARLSWPEGDRPFDSFLIQYQSEKVGGEAIV LTVPGSERSYDLTGLKPGTEYTVSIYGVDDVKWEGNRPVASNPLSAIF TT
P282AR9P1356_D3	44	LPAPKNLVVSRVTEDSARLSWTRHETSFDSFLIQYRESEKVGGEAIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVVEYDAAGNPKYSNPLSAIF TT
P282AR9P1356_H1	45	LPAPKNLVVSRVTEDSARLSWIPNPSSFDSFLIQYQSEKVGGEAIVLT VPGSERSYDLTGLKPGTEYTVSIYGVDDVFDVPGFSPHSNPLSAIFT T
P282AR9P1356_H6	46	LPAPKNLVVSRVTEDSARLSWRKRANSFDSFLIQYQSEKVGGEAIVL TVPGSERSYDLTGLKPGTEYTVSIYGVHVEYDQHGRPRWSNPLSAI FTT
P282BR9P1357_A9	47	LPAPKNLVVSRVTEDSARLSWKANRTTDLHFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDDVQYDGGQPLYSNPLS AIFTT
P282BR9P1357_B2	48	LPAPKNLVVSRVTEDSARLSWNPSEDPQRFDSFLIQYQSEKVGGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVDDVKWEGNRPVASNPLS AIFTT
P282BR9P1357_C10	49	LPAPKNLVVSRVTEDSARLSWWSNDNRPIFDSFLIQYQSEKVGGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVDDVKWEGNRPVASNPLS AIFTT
P282BR9P1357_C4	50	LPAPKNLVVSRVTEDSARLSWPFVSQNKPHFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDDVKWEGNRPVASNPL SAIFTT
P282BR9P1357_D12	51	LPAPKNLVVSRVTEDSARLSWGQYITAFSFDLSFLIQYQSEKVGGEA VLTVPGESESYDLTGLKPGTEYTVSIYGVDDVAWFQGGKPTWSNPLS AIFTT
P282BR9P1357_D2	52	LPAPKNLVVSRVTEDSARLSWIKDGHPRHFDLSFLIQYQSEKVGGEA VLTVPGESESYDLTGLKPGTEYTVSIYGVDDVYDRGQLISSNPLSAIF TT
P282BR9P1357_E5	53	LPAPKNLVVSRVTEDSARLSWWPRKYQRPFDLSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDDIEWIGNRPIASNPLSAI FTT
P282BR9P1357_G9	54	LPAPKNLVVSRVTEDSARLSWPIASQIHSPFDLSFLIQYQSEKVGGEA VLTVPGESESYDLTGLKPGTEYTVSIYGVDDVKYDIDSRPISSNPLSAIF TT
P282BR9P1357_H3	55	LPAPKNLVVSRVTEDSARLSWKKREYQDPGFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDDVKWEGNRPVASNPL SAIFTT
P282CR9P1358_C2	56	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFKIAYPEWPSNGEAI VLTVPGESESYDLTGLKPGTEYAVFIWGVKGGAFSNPLSAIFTT
P282CR9P1358_C5	57	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIAYPEWPDSEGEAI VLTVPGESESYDLTGLKPGTEYAVFIWGVKGGPLSHPLSAIFTT
P282CR9P1358_D10	58	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLISYPEYPPPGGEAIVL TVPGSERSYDLTGLKPGTEYFVIFGVKGGDTSWPLSAIFTT

P282CR9P1358_F11	59	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFLIAYPEWPIFEGEAI LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGEQSSPLSAIFTT
P282CR9P1358_F5	60	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWISYPEWPPDGEAI VLTVPGSERSYDLTGLKPGTEYFVIIWGVKGGETSAPLSAIFTT
P282DR9P1359_A12	61	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFQIAYPEWPPPREAIV LTVPGSERSYDLTGLKPGTEYFVVIQGVKGGESWPLSAIFTT
P282DR9P1359_A7	62	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFRIGYPELEKLGEGEAI VLTVPGSERSYDLTGLKPGTEYWVIIWGVKGGENSWPLSAIFTT
P282DR9P1359_A8	63	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFRIAYPEWVPVQGEAI LTVPGSERSYDLTGLKPGTEYFVIIYGVKGGELSPPLSAIFTT
P282DR9P1359_B2	64	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWIAYTEWPIPYEEAG QEGEAIVLTVPGSERSYDLTGLKPGTEYWVSIYGVKGGPNSQPLSAI FTT
P282DR9P1359_C10	65	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAIVYPEWPTDGEAI LTVPGSERSYDLTGLKPGTEYAVFIWGVKGGNQSWPLSAIFTT
P282DR9P1359_C11	66	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFKIAYPEFPPGGEAIV LTVPGSERSYDLTGLKPGTEYVIIIGVKGGTDSWPLSAIFTT
P282DR9P1359_C12	67	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYISYPEWVPVGEAIV LTVPGSERSYDLTGLKPGTEYWVIIYGVKGGALSVPLSAIFTT
P282DR9P1359_C5	68	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWITYPEWPDGGEAI IVLTVPGSERSYDLTGLKPGTEYFVVIYGVKGGEIYSPLSAIFTT
P282DR9P1359_D12	69	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFRIAYPETATWGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYGVKGGFESAPLSAIFTT
P282DR9P1359_E11	70	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYISYPEWPPVGEAIV LTVPGSERSYDLTGLKPGTEYWVIIYGVKGGAIPTLSAIFTT
P282DR9P1359_E2	71	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFNIFYPEIVTWGEAIV LTVPGSERSYDLTGLKPGTEYWVNIVGVKGGDNSWPLSAIFTT
P282DR9P1359_E3	72	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAIAYPELPLGGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYGVKGGVESFPLSAIFTT
P282DR9P1359_E5	73	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAISYPEWVPVGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYGVKGGGLYSAPLSAIFTT
P282DR9P1359_E6	74	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWIAYPEWVPVQGEAI VLTVPGSERSYDLTGLKPGTEYFVVIQGVKGGTPSWPLSAIFTT
P282DR9P1359_E8	75	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFQIAYPEWVPVGEAIV LTVPGSERSYDLTGLKPGTEYWVIIQGVKGGYTSWPLSAIFTT
P282DR9P1359_F11	76	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIFYPELPIHGEAIV LTVPGSERSYDLTGLKPGTEYWVNITGVKGGDFSWPLSAIFTT
P282DR9P1359_F2	77	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFNIAYPEALHPGYGEAI IVLTVPGSERSYDLTGLKPGTEYWVIIIGGVKGGQKSWPLSAIFTTGG HHHDHH
P282DR9P1359_F3	78	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYITYPEWVPVQGEAIV LTVPGSERSYDLTGLKPGTEYWVIIYGVKGGTESEPLSAIFTT
P282DR9P1359_F5	79	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFQIAYPEWPPPGGEAIV LTVPGSERSYDLTGLKPGTEYFVIIQGVKGGVESWPLSAIFTT
P282DR9P1359_F6	80	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPTTGGEAIV LTVPGSERSYDLTGLKPGTEYFVIIWGVKGGDHSAPLSAIFTT
P282DR9P1359_F7	81	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAIAYPEWPPQGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYGVKGGSYSAPLSAIFTT
P282DR9P1359_G4	82	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFIAYPEWPPPGGEAIV

		LTVPGSERSYDLTGLKPGPEYFVVIQGVKGGDPSFPLSAIFTTGGNH HHHH
P282DR9P1359_G7	83	LPAPKNLVVSRVTEDSARLSWTAPDAAFDFAITYIEKEHIEDGEAI VLTVPGSERSYDLTGLKPGTEYWVPIWGVKGGANSWPLSAIFTT
P282DR9P1359_H5	84	LPAPKNLVVSRVTEDSARLSWTAPDAAFDNFNIAYPEALHPGYGEA IVLTVPGSERSYDLTGLKPGTEYFVVIYGVKGGTNSEPLSAIFTT
P282ER9P1360_A9	85	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL PIPGSERSYDLTGLKPGTEYWVVITGVKGGAPSTPLGAIFTT
P282ER9P1360_C1	86	LPAPKNLVVSRVTEDSARLSWTTPDAAFDGILYEPVDSGEAITL PVPGSERSYDLTGLKPGTEYWVVITGVKGGAPSTPLGAIFTT
P282ER9P1360_C4	87	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGITYYEPNHGGEAIS LSVPGSERSYDPTGLKPGTEYWVVITGVKGGAPSTPLGAIFTT
P282ER9P1360_C6	88	LSAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL PIPGSERSYDLTGLKPGTEYWVVITGVKGGAPSTPLGAIFTT
P282ER9P1360_C8	89	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL PVPGSERSYDLTGLKPGTEYWVVITGVKGGAPSTPLGTIFTT
P282ER9P1360_D11	90	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL PVPGSERSYDLTGLKPGTEYFVIIVGVKGGYPSIPLGAFTT
P282ER9P1360_E4	91	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL PVLGSERSYDLTGLKPGTEYWVVITGVKGGAPSTPLGAIFTT
P282ER9P1360_F11	92	LPAPKNLVVSRVTEDSARLSWIAPDAAFDGILYEPVDSGEAITL VVPGSERSYDLTGLKPGTEYWVVILGVKGGNPSNPLGASFTT
P282ER9P1360_G10	93	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LFVPGSERSYDLTGLKPGTEYTVGIRGVKGGNFSTPLIAHFTT
P282ER9P1360_G7	94	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL PVPGSERSYDLTGLKPGTEYWVVITGVKGGAPSTPLGAILTT
P282ER9P1360_H10	95	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LPVPGSERSYDLTGLKPGTEYFVVIYGVKGGELSEPLTAQFTT
P282ER9P1360_H2	96	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL VVPGSERSYDLTGLKPGTEYWVVILGVKGGNPSNPLGASFTT
P282ER9P1360_H3	97	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL VVPGSERSYDLTGLKPGTEYWVVILGVKGGNPSNPLGASFTT
P282FR9P1361_A3	98	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LTVPGSERSYDLTGLKPGTEYDVAIVGVKGGNRSYPLSAIFTT
P282FR9P1361_A5	99	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL PVPGSERSYDLTGLKPGTEYWVVITGVKGGAPSTPLGAIFTT
P282FR9P1361_C7	100	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL VLTVPGSERSYDLTGLKPGTEYDVAIWGVKGGDVSYPLSAIFTT
P282FR9P1361_D3	101	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LTVPGSERSYDLTGLNPGTEYDVAISGVKGGPRSYPLSAIFTT
P282FR9P1361_E12	102	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LTVPGSERSYDLTGLKPGTEYGVFILGVKGGYPSAPLSAIFTT
P282FR9P1361_F1	103	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LTVPGSERSYDLTGLKPGTEYDVAIWGVKGGVRSYPLSAIFTT
P282FR9P1361_F11	104	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LTVPGSERSYDLTGLKPGTEYDVAIWGVKGGVRSYPLSAIFTT
P282FR9P1361_F2	105	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL LTVPGSERSYDLTGLKPGTEYDVAISGVKGGPRSYPLSAIFTT
P282FR9P1361_F3	106	LPAPKNLVVSRVTEDSARLSWTAPDAAFDGILYEPVDSGEAITL

		LTVPGSERSYDLTGLKPGTEYDVAISGAKGGPRSYPLSAIFTT
P282FR9P1361_F7	107	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSPFIWYREYATGEAIVL TVPGSERSYDLTGLKPGTEYDVVITGVKGGYPSYPLSAIFTT
P282FR9P1361_G9	108	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSPGITYWESPYAGEAIV LTVPGSERSYDLTGLKPGTEYGVFILGVKGGYPSAPLSAIFTT
P282FR9P1361_H4	109	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSPGIWYAEYGYSGEAIV LTVPGSERSYDLTGLKPGTEYDVAIYGVKGGSPSYPLSAIFTT
P282FR9P1361_H5	110	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSPDIWYAEYGPGEAIV LTVPGSERSYDLTGLKPGTEYDVAISGVKGGPRSYPLSAIFTT
P283AR9P1362_A3	111	LPAPKNLVVSRVTEDSARLSWKRIDSPFDSFLIQYQSEKVGAEIVL VPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_A4	112	LPAPKNLVVSRVTEDSARLSWIGHDSGFDSPFIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_B10	113	LPAPKNLVVSRVTEDSARLSWKRRWDSFDSFLIQYQSEKVGAEIV LTVPGSERSYDLTGLKPGTEYTVSIYGVVDVWFNGLPHHSNPLSAIF TT
P283AR9P1362_B2	114	LPAPKNLVVSRVTEDSARLSWAKHPNSFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVVNELLNPLFSNPLSAIFT T
P283AR9P1362_B8	115	LPAPKNLVVSRVTEDSARLSWWTSPLPFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_C12	116	LPAPKNLVVSRVTEDSARLSWAKNLHSFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_C6	117	LPAPKNLVVSRVTEDSARLSWYPSDPPFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVPNYHSRRSYYSNPLSAIFTT
P283AR9P1362_C7	118	LPAPKNLVVSRVTEDSARLSWVKRATSFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVRYNEGQPIWSNPLSAIFT T
P283AR9P1362_D2	119	LPAPKNLVVSRVTEDSARLSWQRPKSGFFDSFLIQYQSEKVGAEIV LTVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFT T
P283AR9P1362_D3	120	LPAPKNLVVSRVTEDSARLSWPVESNAFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVEYDQHGRPRWSNPLSAI FTT
P283AR9P1362_D4	121	LPAPKNLVVSRVTEDSARLSWVREHDSFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_D6	122	LPAPKNLVVSRVTEDSARLSWAKRPGAFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_D7	123	LPAPKNLVVSRVTEDSARLSWVKRATSFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_E9	124	LPAPKNLVVSRVTEDSARLSWVPSPWGFDSPFIQYQSEKVGAEIV LTVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFT T
P283AR9P1362_F12	125	LPAPKNLVVSRVTEDSARLSWARNITSFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_F2	126	LPAPKNLVVSRVTEDSARLSWRKKDHPFDSFLIQYQSEKVGAEIVL TVPGSERSYDLTGLKPGTEYTVSIYGVVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_F8	127	LPAPKNLVVSRVTEDSARLSWGYYHGHFDSFLIQYQSEKVGAEIV LTVPGSERSYDLTGLKPGTEYTVSIYGVVDVKWEGNRPVASNPLSAIF

		TT
P283AR9P1362_G11	128	LPAPKNLVVSRVTEDSARLSWRKEATSFDSFLIQYQSEKVGGEAIVL TVPGSERSYDLTGLKPGTEYTVSIYGVDVKYDIDSRPISSNPLSAIFTT
P283AR9P1362_G3	129	LPAPKNLVVSRVTEDSARLSWVKRATSFDSFLIQYQSEKVGGEAIVL TVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPLSAIFT T
P283AR9P1362_H11	130	LPAPKNLVVSRVTEDSARLSWPQIQGHFDSFLIQYQSEKVGGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKYDIDSRPISSNPLSAIF TT
P283BR9P1363_A10	131	LPAPKNLVVSRVTEDSARLSWQRADDILPYFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKYDIDSRPISSNPLSA IFTT
P283BR9P1363_A8	132	LPAPKNLVVSRVTEDSARLSWVRSDTARFFDSFLIQYQSEKVGGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKYDIDSRPISSNPLSAIF TT
P283BR9P1363_B2	133	LPAPKNLVVSRVTEDSARLSWASTVDPHPRFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKYDIDSRPISSNPLSA IFTT
P283BR9P1363_B6	134	LPAPKNLVVSRVTEDSARLSWQRHSDAHPFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPL SAIFTT
P283BR9P1363_C4	135	LPAPKNLVVSRVTEDSARLSWPVINTPLHFDSFLIQYQSEKVGGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVDVQYTATGQPERSNPLSA IFTT
P283BR9P1363_C8	136	LPAPKNLVVSRVTEDSARLSWAKTSDLHPLFDSFLIQYQSEKVGGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPLS AIFTT
P283BR9P1363_D11	137	LPAPKNLVVSRVTEDSARLSWNKKHDGQPTFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVVYEGSYPASSNPLSA IFTT
P283BR9P1363_E4	138	LPAPKNLVVSRVTEDSARLSWIKSETSQPAFDSFLIQYQSEKVGGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPLS AIFTT
P283BR9P1363_E6	139	LPAPKNLVVSRVTEDSARLSWYARKFISPFDSFLIQYQSEKVGGEAIV LTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPLSAIF TT
P283BR9P1363_F2	140	LPAPKNLVVSRVTEDSARLSWYRPDNRAGAFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKYDIDSRPISSNPLSA IFTT
P283BR9P1363_F4	141	LPAPKNLVVSRVTEDSARLSWERIVQTPHFDSFLIQYQSEKVGGEAI VLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPLSA IFTT
P283BR9P1363_F6	142	LPAPKNLVVSRVTEDSARLSWPPEAVTATSFDSFLIQYQSEKVGGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPLS AIFTT
P283BR9P1363_G2	143	LPAPKNLVVSRVTEDSARLSWPKNQTNRHFDSDFLIQYQSEKVGGEA IVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPLS AIFTT
P283BR9P1363_G5	144	LPAPKNLVVSRVTEDSARLSWYRATTPAPHFDSFLIQYQSEKVGGE AIVLTVPGSERSYDLTGLKPGTEYTVSIYGVDVKWEGNRPVASNPL

		SAIFTT
P283BR9P1363_G7	145	LPAPKNLVVSRVTEDSARLSWSAKKFP RHFD SFLIQYQESEKVG EAI VLTVP GSERSYDLTGLKPGTEYTVSIYGV DVKWEGNRPVASNPLSA IFTT
P283DR9P1364_A4	146	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFYIAYPEW PVQGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGDWSEPLSAIFTT
P283DR9P1364_A7	147	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFRIAYPEW PVRGDAIV LTVPGSERSYDLTGLKPGTEYWVIIQGV KGGTDSFPLSAIFTT
P283DR9P1364_B1	148	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFYITYPEIPLGGEAIVLT VPGSERSYDLTGLKPGTEYFVIIYG VKGGLLSSPLSAIFTT
P283DR9P1364_B11	149	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFYISYPEW EQLG EAI LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGALSAPLSAIFTT
P283DR9P1364_B4	150	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFAISYPEW PPPGEAIV LTVPGSERSYDLTGLKPGTEYWIIIGVK GGDQSWPLSAIFTT
P283DR9P1364_C10	151	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFQIAYPEW PKDGEAI VLTVP GSERSYDLTGLKPGTEYAVFIWGV KGGVYSNPLSAIFTT
P283DR9P1364_D11	152	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFDIAYPEW WPKGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGIHSAPLSAIFTT
P283DR9P1364_D8	153	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFQIAYPETPIQGEAIVL TVPGSERSYDLTGLKPGTEYFVIIHGV KGGITSFPLSAIFTT
P283DR9P1364_D9	154	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFGISYPEW WPLGEAIV LTVPGSERSYDLTGLKPGTEYWIIIFGV KGGERSWPLSAIFTT
P283DR9P1364_E3	155	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFGIAYPELPIGGEAIVL TVPGSERSYDLTGLKPGTEYFVIIRGV KGGTSLPPLSAIFTT
P283DR9P1364_E5	156	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFWISYPEW WVPGEAI VLTVP GSERSYDLTGLKPGTEYWVIIQGV KGGKLSWPLSAIFTT
P283DR9P1364_E7	157	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFNIAYPEW PVRGEAIV LTVPGSERSYDLTGLKPGTEYWVIIYG VKGGDRSNPLSAIFTT
P283DR9P1364_E8	158	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFSIAYPEW PVHGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGVLSEPLSAIFTT
P283DR9P1364_E9	159	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFDIAYPEW WTKGEAIV LTVPGSERSYDLTGLKPGTEYFVINGV KGGWRSFPLSAIFTT
P283DR9P1364_F2	160	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFWIAYPEW WVPGEAI VLTVP GSERSYDLTGLKPGTEYFVIIQGV KGGFGSFPLSAIFTT
P283DR9P1364_F6	161	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFTIAYPEREQDKWGE AIVLTVPGSERSYDLTGLKPGTEYWVIIQGV KGGRPSTPLSAILTT
P283DR9P1364_F8	162	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFAIAYPEW WPGGEAIVL TVPGSERSYDLTGLKPGTEYFVIIYG VKGGWTSPPLSAIFTT
P283DR9P1364_G10	163	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFSIAYPEW WPGSGEAIV LTVPGSERSYDLTGLKPGTEYFVIFGV KGGSQSWPLSAIFTT
P283DR9P1364_G9	164	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFGIYWPEW WVGGEAI VLTVP GSERSYDLTGLKPGTEYWVNISGV KGGEYSFPLSAIFTT
P283DR9P1364_H1	165	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFQISYPEW PVHGEAIV LTVPGSERSYDLTGLKPGTEYWVIIWGV KGGRQSWPLSAIFTT
P283DR9P1364_H11	166	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFDIAYPELPLGGEAIVL TVPGSERSYDLTGLKPGTEYFVIIWGV KGGDRSEPLSAIFTT
P283DR9P1364_H6	167	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFIIAYPETPVRGEAIVL TVPGSERSYDLTGLKPGTEYFVIIIGVK GGGQESFPLSAIFTT
P283DR9P1364_H9	168	LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFSISYIEYPEIPGGEAIV

		LTVPGSERSYDLTGLKPGTEYWVPIWGVKGGIQSWPLSAIFTT
P283ER9P1365_A1	169	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIAYVEWWHRGEAI SLPVPGSERSYDLTGLKPGTEYNVIITGVKGGIPSHPLGAIFTT
P283ER9P1365_A7	170	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIPYWESEVYGEAIA LPVPGSERSYDLTGLKPGTEYQVSIIGVKGGVYSQPLAAIFTT
P283ER9P1365_B6	171	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIGYAEPVVTGEAIS LSVPGSERSYDLTGLKPGTEYWVVIIGVKGGINSYPLGAIFTT
P283ER9P1365_C1	172	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIPYWESEVYGEAIA LPVTGSERSYDLTGLKPGTEYQVSIIGVKGGVYSQPLAAIFTT
P283ER9P1365_E2	173	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIPYRESEFRGEAIAL PVPGSERSYDLTGLKPGTKYRVIIIGVKGGFEFSQPLAAIFTT
P283ER9P1365_F4	174	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIPYRESEFRGEAIAL PVPGSERSYDLTGLKPGTKYSVIIIGVKGGFEFSQPLGAIFTT
P283ER9P1365_G1	175	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIPYRESEFRGEAIAL SVPGSERSYDLTGLKPGTKYRVIIIGVKGGFEFSQPLGAIFTT
P283ER9P1365_G3	176	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGISYEWAPNGEAI QLSVPGSERSYDLTGLKPGTEYHVVIIGVKGGEPHPLGAIFTT
P283ER9P1365_H3	177	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIPYRESEFRGEAIAL PVPGSERSYDLTGLKPGTKYRVIIIGVKGGFEFSQPLSAIFTT
P283FR9P1366_A1	178	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWITYPEWVPVGEAI VLTVPGSERSYDLTGLKPGTEYAVFIWGVKGGDASEPLSAIFTT
P283FR9P1366_A5	179	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWIAYPEWPTVGEAI VLTVPGSERSYDLTGLKPGTEYFVVIYGVKGGSPSPPLSAIFTT
P283FR9P1366_A9	180	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFNIAYPEWPGVGEAIV LTVPGSERSYDLTGLKPGTEYWVPIWGVKGGELSEPLSAIFTT
P283FR9P1366_B7	181	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWITYPEWPNVGEAI VLTVPGSERSYDLTGLKPGTEYWVVIWGVKGGVESPLSAIFTT
P283FR9P1366_C2	182	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFKISYPEWPPEGEAIV LTVPGSERSYDLTGLKPGTEYAVFIWCVKGGGEHSWPLSAIFTT
P283FR9P1366_C3	183	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFKIAYPEWPDGGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYGVKGGILSPPLSAIFTT
P283FR9P1366_C4	184	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFNIAYPEWPVRGEAIV LTVPGSERSYDLTGLKPGTEYWVVIIGVKGGEDSWPLSAIFTT
P283FR9P1366_C6	185	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFSIAYPEWVPVGEAIV LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGNYSDPLSAIFTT
P283FR9P1366_D12	186	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPLGGEAIV LTVPGSERSYDLTGLKPGTEYWVILGVKGGDQSWPLSAIFTT
P283FR9P1366_D6	187	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFNIFYPELVFPGEAIVL TVPGSERSYDLTGLKPGTEYWVNISGVKGGGEHSWPLSAIFTT
P283FR9P1366_D7	188	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFSIAYPELPVKGEAIVL TVPGSERSYDLTGLKPGTEYFVVIWGVKGGTYSGPLSAIFTT
P283FR9P1366_D8	189	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFIAYPEIPIAGEAIVLT VPGSERSYDLTGLKPGTEYFVIIYGVKGGDWSPLSAIFTT
P283FR9P1366_E11	190	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWVPVGEAIV LTVPGSERSYDLTGLKPGTEYWVIIGVKGGNISWPLSAIFTT
P283FR9P1366_F5	191	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIGYPEWPIKGEAIV LTVPGSERSYDLTGLKPGTEYWVIWGVKGGDRSEPLSAIFTT
P283FR9P1366_F8	192	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAIAYPEIAKWGEAIV LTVPGSERSYDLTGLKPGTEYFVIIYGVKGGVHSFPLSAIFTT

P283FR9P1366_F9	193	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEHIFYPELPIAGEAIVLT VPGSERSYDLTGLKPGTEYWVNISGVKGGYESWPLSAIFTT
P283FR9P1366_G1	194	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYISYPELPVEGEAIVL TVPGSERSYDLTGLKPGTEYWVIIWGVKGGATSEPLSAIFTT
P283FR9P1366_G5	195	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFQIAYPEYPALGEAIVL TVPGSERSYDLTGLKPGTEYFVIIIGVKGGDESFPLSAIFTT
P283FR9P1366_G8	196	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPELPIGGEAIVL TVPGSERSYDLTGLKPGTEYFVVIYGVKGGIHSAPLSAIFTT
P283FR9P1366_H10	197	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFNIAYPEWPPEGEAIV LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGHLSPLSAIFTT
P283FR9P1366_H11	198	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFQIQYLETAPDGEAIV LTVPGSERSYDLTGLKPGTEYVWIPGVKGGAFSPLSAIFTT
P283FR9P1366_H3	199	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAIAYPEWPIKGEAIVL TVPGSERSYDLTGLKPGTEYWVVIYGVKGGVFSEPLSAIFTT
P283FR9P1366_H5	200	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFIYIENKVNGEAIVLTV PGSERSYDLTGLKPGTEYHVTIGGVKGGTESNTLSAIFTT
P283FR9P1366_H6	201	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPVTGEAIV LTVPGSERSYDLTGLKPGTEYWVIIIGVKGGERSWPLSAIFTT
P283FR9P1366_H7	202	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFQIAYPEYPALGEAIVL TVPGSERSYDLTGLKPGTEYFVIIAGVKGGIQSWPLSAIFTT
P283FR9P1366_H8	203	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYISYPEWPGSGEAIV LTVPGSERSYDLTGLKPGTEYAVFIWCVKGGWLSPLSAIFTT
P283FR9P1366_H9	204	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFIAYPEWPVNGEAIV LTVPGSERSYDLTGLKPGTEYWVVIWGVKGGVNSYPLSAIFTT
P283GR7P1367_A11	205	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPTDGEAIV LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGSYSEPLSAIFTT
P283GR7P1367_B4	206	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFSILYYELPPSGEAIVLT VPGSERSYDLTGLKPGTEYTVSIFGVKGGDNSFPLSAIFTT
P283GR7P1367_B7	207	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPTDGEAIV LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGHWSYPLSAIFTT
P283GR7P1367_B9	208	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFIWYHEYHPRGEAIV LTVPSERSYDLTGLKPGTEYDVVISGVKGGHWSYPLSAIFTT
P283GR7P1367_C9	209	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFLIGYPEWPLGGEAIV LTVPGSERSYDLTGLKPGTEYWVIYGVKGGESDPLSAIFTT
P283GR7P1367_E5	210	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFIWYHEYHPRGEAIV LTVPGSERSYDLTGLKPGTEYDVVISGVKGGHWSYPLSAIFTT
P283GR7P1367_F5	211	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPTDGEAIV LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGALSRLSAIFTT
P283GR7P1367_G8	212	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIAYPEYVWGGEATS LGEAIVLTVPGSERSYDLTGLKPGTEYFVVITGVKGGGLGSYPLSAIFT T
P283GR7P1367_H2	213	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPTDGEAIV LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGGRSYPLSAIFTT
P283GR7P1367_H8	214	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFSINYWEEDPAGEAIV LTVPGSERSYDLTGLKPGTEYRVLIGGVKGGHGSPLSAIFTT
P283GR7P1367_H9	215	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFDIAYPEWPTDGEAIV LTVPGSERSYDLTGLKPGTEYFVVIYGVKGGGRSAPLSAIFTT
P283HR7P1368_A10	216	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWIFYLEPFRGEAIVL EVPGSERSYDLTGLKPGTEYSVDIRGVKGGDHSDPLWAYFTT

P283HR7P1368_B12		217	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIGYVEFTRAGEAISL SVPGSERSYDLTGLKPGTEYHVVIIGVKGGEP SHPLGAPFTT
P283HR7P1368_C3		218	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIGYAEP AVTGEAIS LSVPGSKRSYDLTGLKPGTEYVVVIIGVKGGINSYPLGASFTT
P283HR7P1368_D1		219	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGISYYEWAPNGEAI QLSVPGSERSYDLTGLKPGTEYHVVIIGVKGGEP SHPLGAPFTT
P283HR7P1368_D2		220	LPAPKNLVVSRVTEDSARLSWTAPDAAFNSEFGIGYAEP AVTGEAIS LSVPGSERSYDLTGLKPGTEYVVVIIGVKGGINSYPLGASFTT
P283HR7P1368_D4		221	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAIWCVEPIPEGEAIP FVPGSERSYDLTGLKPGTEYRVGIRGVKGGTFSSPLAAPFTT
P283HR7P1368_F10		222	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFYIPYRESEFRGEAIAL PVPGSERSYDLTGLKPGTKYRVIIIIGVKGGEFSQPLGAIFTT
P283HR7P1368_F6		223	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIGYIEWVHRGEAIS LHVPGSERSYDLTGLKPGTEYVVAIVGVKGGEPSTPLGAPFTT
P283HR7P1368_G1		224	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFLITYWIEIEPEGEAIFL GVPGSERSYDLTGLKPGTEYRVQINGVKGGTISYPLFAGFTT
P283HR7P1368_G10		225	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIAYVEWWHRGEAI SLPVPGSERSYDLTGLKPGTEYWVTILGVKGGIISTPLGASFTT
P283HR7P1368_G11		226	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIGYAEP AVTGEAIS LSVPGSERSYDLTGLKPGTEYVVVIIGVKGGINSYPLGASFTT
P283HR7P1368_H1		227	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGIAYIETARWGEAISL TVPGSERSYDLTGLKPGTEYNVVIIGVKGGTP SHPLGAPFTT
P283HR7P1368_H8		228	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGITYLDRNGEAI SL NVPGSERSYDLTGLKPGTEYVVVIIGVKGGINSYPLGASFTT
CD8S368		229	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFQIAYPEWPPP GEAIVLTVPGSCRSYDLTGLKPGTEYFVIIQGVKGGVESWPL LSAIFTT
CD8S367		230	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAIAYPEWPPQ GEAIVLTVPGSCRSYDLTGLKPGTEYFVVIYGVKGGSYSAP LSAIFTT
CD8S370		231	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFAITYIEKEHI EDGEAIVLTVPGSCRSYDLTGLKPGTEYWVPIWGVKGGANS WPLSAIFTT
CD8S365		232	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFGILYYEPVDS GEAITLPVPGSCRSYDLTGLKPGTEYWVITGVKGGAPSTP LGTIFTT
CD8S369		233	LPAPKNLVVSRVTEDSARLSWAKRPGAFDSFLIQYQSEKV GEAIVLTVPGSCRSYDLTGLKPGTEYTVSIYGVVDVKYDIDS RPISSNPLSAIFTT
CD8S366		234	LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFWITYPEWPD P GGEAIVLTVPGSCRSYDLTGLKPGTEYFVVIYGVKGGEIYS PLSAIFTT
Clone	SEQID No	Parent	Sequence
CD8S371	235	P282DR9P1359_F5	LPAPKNLVVSRVTEDSARLSWT

			APDAAFDSFQIAYPEYPPPGAI VLTVPGSERSYDLTGLKPGTEYF VIIQGVKGGVESWPLSAIFTT
CD8S372	236	P282DR9P1359_F5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFQIAYPELPPPGAI VLTVPGSERSYDLTGLKPGTEYFV IIQGVKGGVESWPLSAIFTT
CD8S373	237	P282DR9P1359_F5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFQIAYPEIPPPGAI VLTVPGSERSYDLTGLKPGTEYFV IIQGVKGGVESWPLSAIFTT
CD8S374	238	P282DR9P1359_F5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFQIAYPEWPPPGAI VLTVPGSERSYDLTGLKPGTEYF VIIQGVKGGVESYPLSAIFTT
CD8S375	239	P282DR9P1359_F5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFQIAYPEWPPPGAI VLTVPGSERSYDLTGLKPGTEYF VIIQGVKGGVESLPLSAIFTT
CD8S376	240	P282DR9P1359_F5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFQIAYPEWPPPGAI VLTVPGSERSYDLTGLKPGTEYF VIIQGVKGGVESPPLSAIFTT
CD8S377	241	P282DR9P1359_F5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFQIAYPEWPPPGAI VLTVPGSERSYDLTGLKPGTEYF VIIQGVKGGVESEPLSAIFTT
CD8S378	242	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEG IVLTVPGSERSYDLTGLKPGTEY WVPIWGVKGGANSWPLSAIFTT
CD8S379	243	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIESG IVLTVPGSERSYDLTGLKPGTEY WVPIWGVKGGANSWPLSAIFTT
CD8S380	244	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDG IVLTVPGSERSYDLTGLKPGTEY YVPIWGVKGGANSWPLSAIFTT
CD8S381	245	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDG IVLTVPGSERSYDLTGLKPGTEY FVPIWGVKGGANSWPLSAIFTT
CD8S382	246	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDG IVLTVPGSERSYDLTGLKPGTEY SVPIWGVKGGANSWPLSAIFTT
CD8S383	247	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDG IVLTVPGSERSYDLTGLKPGTEY WVPIYGVKGGANSWPLSAIFTT
CD8S384	248	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDG

			IVLTVPGSERSYDLTGLKPGTEY WVPIFGVKGGANSWPLSAIFTT
CD8S385	249	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDGEA IVLTVPGSERSYDLTGLKPGTEY WVPISGVKGGANSWPLSAIFTT
CD8S386	250	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDGEA IVLTVPGSERSYDLTGLKPGTEY WVPIWGVKGGANSYPLSAIFTT
CD8S387	251	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDGEA IVLTVPGSERSYDLTGLKPGTEY WVPIWGVKGGANSEPLSAIFTT
CD8S388	252	P282DR9P1359_G7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAITYIEKEHIEDGEA IVLTVPGSERSYDLTGLKPGTEY WVPIWGVKGGAQSWPLSAIFTT
CD8S389	253	P282ER9P1360_C8	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFGILYYEPVDSGEAI TLPVPGSERSYDLTGLKPGTEYF VVITGVKGGAPSTPLGTIFTT
CD8S390	254	P282ER9P1360_C8	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFGILYYEPVDSGEAI TLPVPGSERSYDLTGLKPGTEYY VVITGVKGGAPSTPLGTIFTT
CD8S391	255	P282ER9P1360_C8	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFGILYYEPVDSGEAI TLPVPGSERSYDLTGLKPGTEYH VVITGVKGGAPSTPLGTIFTT
CD8S392	256	P282DR9P1359_F7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAIAYPEYPPQGEAI VLTVPGSERSYDLTGLKPGTEYF VVIYGVKGGSYSAPLSAIFTT
CD8S393	257	P282DR9P1359_F7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAIAYPELPPQGEAI VLTVPGSERSYDLTGLKPGTEYF VVIYGVKGGSYSAPLSAIFTT
CD8S394	258	P282DR9P1359_F7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAIAYPEIPPQGEAIV LTVPGSERSYDLTGLKPGTEYFV VIYGVKGGSYSAPLSAIFTT
CD8S395	259	P282DR9P1359_F7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAIAYPEHPPQGEAI VLTVPGSERSYDLTGLKPGTEYF VVIYGVKGGSYSAPLSAIFTT
CD8S396	260	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFYITYPEWDPGGEA IVLTVPGSERSYDLTGLKPGTEY FVVIYGVKGGEIYSPLSAIFTT
CD8S397	261	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFQITYPEWDPGGEA IVLTVPGSERSYDLTGLKPGTEY

			FVVIYGVKGGGEIYSPLSAIFTT
CD8S398	262	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFSITYPEWDPGGEA IVLTVPGSERSYDLTGLKPGTEY FVVIYGVKGGGEIYSPLSAIFTT
CD8S399	263	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFWITYPEYPDPGGEA IVLTVPGSERSYDLTGLKPGTEY FVVIYGVKGGGEIYSPLSAIFTT
CD8S400	264	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFWITYPELPDPGGEA IVLTVPGSERSYDLTGLKPGTEY FVVIYGVKGGGEIYSPLSAIFTT
CD8S401	265	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFWITYPEIPDPGGEAI VLTVP GSERSYDLTGLKPGTEYF VVIYGVKGGGEIYSPLSAIFTT
CD8S402	266	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFWITYPEWPPPGGE AIVLTVPGSERSYDLTGLKPGTE YFVVIYGVKGGGEIYSPLSAIFTT
CD8S403	267	P282DR9P1359_F7	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFAIAYA EWPPQGEAI VLTVP GSERSYDLTGLKPGTEYF VVIYGVKGGSYSAPLSAIFTT
CD8S404	268	P282DR9P1359_C5	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFWITYAEWDPGGE AIVLTVPGSERSYDLTGLKPGTE YFVVIYGVKGGGEIYSPLSAIFTT
CD8S405	269	P282ER9P1360_C8	LPAPKNLVVSRVTEDSARLSWT APDAAFDSFGILYYEPVDSGEAI TLTVPGSERSYDLTGLKPGTEY WVVITGVKGGAPSTPLGTIFTT

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SEQ ID. No. 270 Tencon25

LPAPKNLVVSEVTEDSARLSWTAPDAAFDSFLIQYQSEKVG EAI VLTVP GSER
SYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

10 SEQ ID. NO: 271 Cyno CD8alpha

MRNQAPGRPKGATSPPLPTGSRAPPVAPELRAEPRPGERVMAPPVTALLPLV
LLLHAARPNQFRVSPLGRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGTAAR
PTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLRDFRQENEGYYFCSALS
NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTTASQPLSLRPEACRPAAGGSVNT
15 RGLDFACDIYIWAPLAGACGVLLLSLVITLYCNHRNRRRVCKCPRPVVKS GGK
PSLSDRYV

CLAIMS

1. An isolated fibronectin type III (FN3) domain that specifically binds to a human cluster of differentiation 8A (CD8A) protein comprising an amino acid sequence of any one of SEQ ID NO: 40-269, wherein the amino acid sequence can optionally have a residue substituted with a cysteine at a position that corresponds to residue 54 of a sequence set forth in any one of SEQ ID NOs: 79, 81, 83, 89, 122, or 68.
2. The isolated FN3 domain of claim 1, wherein the protein is conjugated to a second molecule.
3. The isolated FN3 domain of claim 2, wherein the second molecule is a detectable label.
4. The isolated 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 isolated FN3 domain of any one of claims 1 to 4, wherein the FN3 domain has a cysteine substitution at residue position 54 corresponding to an amino acid sequence set forth in any one of SEQ ID NOs: 79, 81, 83, 89, 122, or 68.
6. The isolated FN3 domain of any one of claims 1 to 5, further comprising a methionine at the N-terminus of the FN3 domain.
7. The isolated FN3 domain of any one of claims 1 to 6, wherein the FN3 domain is coupled to a half-life extending moiety.
8. The isolated FN3 domain of claim 7, wherein the half-life extending moiety is a CD8 binding molecule, albumin, an albumin variant, an albumin binding molecule, a polyethylene glycol (PEG), CD8, CD8 variant, or at least a portion of an Fc region of an immunoglobulin.

9. An isolated polynucleotide encoding the FN3 domain of any one of claims 1 to 8.
10. A vector comprising the polynucleotide of claim 9.
11. An isolated host cell comprising the vector of claim 10.
12. A method of producing the FN3 domain of any one of claims 1 to 8, comprising culturing an isolated host cell comprising a nucleic acid molecule encoding the FN3 domain under conditions such that the FN3 domain is expressed, and purifying the FN3 domain.
13. A diagnostic kit comprising the FN3 domain of any one of claims 1 to 8.
14. A capture agent comprising the FN3 domain of any one of claims 1 to 8.
15. The diagnostic kit of claim 13 or capture agent of claim 14, wherein the substituted cysteine is conjugated to Zr-89 or I-124.
16. A method of detecting CD8-expressing cells in a biological sample previously obtained from a subject, comprising:
 - contacting the biological sample with the capture agent of claim 15; and
 - evaluating binding of the biological sample to the FN3 domain of the capture agent.
17. The method of claim 17, wherein the capture agent has a cysteine substitution at residue position 54 corresponding to a sequence set forth in any one of SEQ ID NOs 79, 81, 83, 89, 122, or 68 and the substituted cysteine is conjugated to Zr-89 or I-124.
18. An isolated fibronectin type III (FN3) domain that specifically binds to a human cluster of differentiation 8A (CD8A) protein comprising an amino acid sequence of any one of SEQ ID NO: 229, SEQ ID NO: 230, SEQ ID NO: 231, SEQ ID NO: 232, or SEQ ID NO: 234.