

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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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,  $100 \text{ \AA}$ ,  $5 \mu\text{m}$ ,  $150 \times 4.6$  mm HPLC column. The elution  
profile used was as follows: solvent A =  $\text{H}_2\text{O}$  (0.1% AcOH (v/v)), Solvent B = MeCN  
(0.1% AcOH (v/v)), flow rate =  $1.5 \text{ mLmin}^{-1}$ ; initial = 80% A, 20 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  $100 \text{ \AA}$ ,  $150 \times 4.6$

5 mm HPLC column. The elution profile used was as follows: solvent A = H<sub>2</sub>O (0.1% TFA (v/v)), Solvent B = MeCN (0.1% TFA (v/v)), flow rate = 1 mLmin<sup>-1</sup>; 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 [<sup>124</sup>I]-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 H<sub>2</sub>O and then Na<sub>2</sub>CO<sub>3</sub> was added (245 µL, 2 M); the pH was checked by strip (pH = 5).

**Fig 6.** Analytical HPLC trace of purified [<sup>124</sup>I]-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 = H<sub>2</sub>O (0.1% TFA (v/v)), Solvent B = MeCN (0.1% TFA (v/v)), flow rate = 1 mLmin<sup>-1</sup>; 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<sup>-1</sup>) 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  $\mu$ m XB-C18 100 Å, 150  $\times$  4.6 mm HPLC column. The elution profile used was as follows: solvent A =  
10 H<sub>2</sub>O (0.1% TFA (v/v)), Solvent B = MeCN (0.1% TFA (v/v)), flow rate = 1 mLmin<sup>-1</sup>; 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 [ $^{124}\text{I}$ ]-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 3<sup>rd</sup> FN3  
20 domain of tenascin (TN3), or the 10<sup>th</sup> 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  $1 \times 10^{-6}$  M or less, for example about  $1 \times 10^{-7}$  M or less, about  $1 \times 10^{-8}$  M or less, about  $1 \times 10^{-9}$  M or less, about  $1 \times 10^{-10}$  M or less, about  $1 \times 10^{-11}$  M or less, about  $1 \times 10^{-12}$  M or less, or about  $1 \times 10^{-13}$  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  $1 \times 10^{-7}$  M, for example less than about  $1 \times 10^{-8}$  M, less than about  $1 \times 10^{-9}$  M, less than about  $1 \times 10^{-10}$  M, less than about  $1 \times 10^{-11}$  M, less than about  $1 \times 10^{-12}$  M, or less than about  $1 \times 10^{-13}$  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 CO<sub>2</sub> 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  $\mu$ 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<br>(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\\_sloning\\_com](http://www_sloning_com)). This technology uses a library of pre-made double stranded triplets that act as universal building blocks sufficient for thousands of gene synthesis processes. The triplet library represents all possible sequence combinations necessary to build any desired DNA molecule. The codon designations are according to the well known IUB code.

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 10<sup>th</sup> 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<sub>m</sub>") temperature, the temperature in ° Celsius (°C) at which half of the molecules become unfolded, using standard methods. Typically, the higher the T<sub>m</sub>, 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<sub>m</sub>.

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 (CH<sub>3</sub>COOH), 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)<sub>2</sub>, (SEQ ID

5 NO: 148), (GGGS)<sub>2</sub> (SEQ ID NO: 149), (GGGGS)<sub>5</sub> (SEQ ID NO: 150), (AP)<sub>2</sub> (SEQ ID  
NO: 151), (AP)<sub>5</sub> (SEQ ID NO: 152), (AP)<sub>10</sub> (SEQ ID NO: 153), (AP)<sub>20</sub> (SEQ ID NO:  
154) and A(EAAAK)<sub>5</sub>AAA (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  
10 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  
20 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  
25 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  
30 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, Ni<sup>2+</sup>, Flag tags, myc tags, heavy  
metals, enzymes, alkaline phosphatase, peroxidase, luciferase, electron  
donors/acceptors, acridinium esters, and colorimetric substrates. In a specific  
35 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 NH<sub>2</sub>-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  $\gamma$ -emitting radionuclide, Auger-emitting radionuclide,  
25    $\beta$ -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 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  $\text{Ba}^{2+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Cs}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cr}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Co}^{2+}$ ,  $\text{Co}^{3+}$ ,  $\text{Cu}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Cu}^{3+}$ ,  $\text{Ga}^{3+}$ ,  $\text{Gd}^{3+}$ ,  $\text{Au}^+$ ,  $\text{Au}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{F}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Mn}^{3+}$ ,  $\text{Mn}^{4+}$ ,  $\text{Mn}^{7+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Ni}^{3+}$ ,  $\text{Ag}^+$ ,  $\text{Sr}^{2+}$ ,  $\text{Sn}^{2+}$ ,  $\text{Sn}^{4+}$ , and  $\text{Zn}^{2+}$ . 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, 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, 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, 2<sup>nd</sup> 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<sup>+</sup> 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  
SYDLTGLKPGTEYTVSIYGVX<sub>7-12</sub>PLSAEFTT;  
wherein

- 5 X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>7</sub> is any amino acid; and  
X<sub>8</sub>, X<sub>9</sub>, X<sub>10</sub>, X<sub>11</sub> and X<sub>12</sub> 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)

- LPAPKNLVVSEVTEDSLRLSWX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>X<sub>8</sub>SFLIQYQESEKVGGEAINLTV  
PGSERSYDLTGLKPGTEYTVSIYGVX<sub>9</sub>X<sub>10</sub>X<sub>11</sub>X<sub>12</sub>X<sub>13</sub>SX<sub>14</sub>X<sub>15</sub>LSAEFTT; wherein  
30 X<sub>1</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;  
X<sub>2</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;  
X<sub>3</sub> Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or  
35 Val;  
X<sub>4</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;

- 5 X<sub>5</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;  
 X<sub>6</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;  
 X<sub>7</sub> is Phe, Ile, Leu, Val or Tyr;
- 10 X<sub>8</sub> is Asp, Glu or Thr;  
 X<sub>9</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;  
 X<sub>10</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
- 15 X<sub>11</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;  
 X<sub>12</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;  
 X<sub>13</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val;
- 20 X<sub>14</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val; and  
 X<sub>15</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or Val.

25

Table 1.

| Residue<br>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<sub>X1X2X3X4X5X6X7X8X9</sub>FD SFLIQYQESEKVG EAI  
25 VLT VPGSERSYDLTGLKPGTEYTVSIYGVX<sub>X10X11X12X13X14X15X16X17X18X19</sub>SNPL  
SAIFTT;  
wherein

- 5 X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>10</sub>, X<sub>11</sub>, X<sub>12</sub>, X<sub>13</sub>, X<sub>14</sub>, X<sub>15</sub> and X<sub>16</sub> is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y; and  
X<sub>7</sub>, X<sub>8</sub>, X<sub>9</sub>, X<sub>17</sub>, X<sub>18</sub> and X<sub>19</sub>, 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)**  
LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFLIQYQESEKVGAEIVLTPGSER  
SYDLTGLKPGTEYTVSIYGV X<sub>1</sub>X<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>X<sub>8</sub>X<sub>9</sub> X<sub>10</sub>X<sub>11</sub>X<sub>12</sub>SNPLSAIFTT;  
X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub> and X<sub>7</sub>, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y;  
and  
15 X<sub>8</sub>, X<sub>9</sub>, X<sub>10</sub>, X<sub>11</sub> and X<sub>12</sub> 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):**

LPAPKNLVVSRVTEDSARLSWTAPDAAFDSEFX<sub>1</sub>IX<sub>2</sub>YX<sub>3</sub>EX<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>GEAIVLTVP  
 GSERSYDLTGLKPGTEYX<sub>8</sub>VX<sub>9</sub>IX<sub>10</sub>GVKGGX<sub>11</sub>X<sub>12</sub>SX<sub>13</sub>PLSAIFTT;

Wherein

25 X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>10</sub>, X<sub>11</sub>, X<sub>12</sub> and X<sub>13</sub> 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 LPAPKNLVVSRVTEDSARLSWTAPDAAFDSFX<sub>1</sub>IX<sub>2</sub>YX<sub>3</sub>EX<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>GEAIX<sub>8</sub>LX<sub>9</sub>  
 VPGSERSYDLTGLKPGTEYX<sub>10</sub>VX<sub>11</sub>IX<sub>12</sub>GVKGGX<sub>13</sub>X<sub>14</sub>SX<sub>15</sub>PLX<sub>16</sub>AX<sub>17</sub>FTT;  
 wherein  
 X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>10</sub>, X<sub>11</sub>, X<sub>12</sub> and X<sub>13</sub> 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.

#### **20 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).

35

#### **Size Exclusion Chromatography Analysis**

- 5           Size exclusion chromatography was used to determine the aggregation state of anti-CD8 alpha FN3 domain molecules. Aliquots (10  $\mu$ 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 alpha FN3 domains were isolated that bound to recombinant human CD8 alpha greater
- 15          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  $\mu$ M and 0.2  $\mu$ 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<sup>-1</sup> 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  $\mu$ 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  $\mu$ 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  $\mu$ g/ml for 5 minutes at 30  $\mu$ l/minute flowrate. All FN3 domains were normalized to a 3  $\mu$ 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  $\mu$ 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  $\mu$ 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  
10 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  
20 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  
25 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><math>k_d</math> (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<sup>-1</sup>, 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<br>(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<br>(79)  | 3.48E+04                  | 6.60E-05    | 6.6        | 3.80E+05                   | 1.42E-05    | 0.04       |
| P282DR9P1359_F7<br>(81)  | 4.03E+04                  | 3.65E-04    | 12         | 4.04E+05                   | 7.99E-05    | 0.5        |
| P282DR9P1359_G7<br>(83)  | 6.84E+04                  | 5.51E-05    | 2.1        | 2.76E+05                   | 1.49E-05    | 0.05       |
| P282ER9P1360_C8<br>(89)  | 3.09E+04                  | 9.52E-05    | 4.1        | 2.18E+05                   | 4.71E-05    | 0.2        |
| P283AR9P1362_D6<br>(122) | 5.62E+04                  | 3.12E-05    | 0.98       | 1.55E+05                   | 1.00E-06    | 0.03       |
| P282DR9P1359_C5<br>(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  $\mu$ M anti-CD8A FN3 domain solution was combined with 10  $\mu$ L of 500 mM TCEP (Sigma, cat. # 646547), gently flushed with nitrogen, and incubated for 1 hour at room temperature.

10 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

15 centrifugation step, the resulting pellet was dissolved in 100 mM sodium phosphate 7.0, 1 mM EDTA and combined with 10  $\mu$ 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

20 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

25 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                  | $\leq 1 \text{ E-}05$ | $\leq 3.4$ | 3.73E+04                   | $\leq 1 \text{ E-}05$ | $\leq 0.3$ |
| CD8S370-DFO | 5.91E+03                  | 7.65E-05              | 13         | 4.64E+04                   | $\leq 2\text{E-}05$   | $\leq 0.3$ |

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  $\mu\text{M}$  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  $\text{EC}_{50}$  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 EC<sub>50</sub> values for top six anti-CD8A FN3 domain candidates.**

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

**EXAMPLE 8: ACTIVATION OF HUMAN T-CELLS**De 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  $\mu$ 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  $\mu$ g/mL and 0.01  $\mu$ 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  $\mu$ 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 $\mu$ M | Anti-CD3 $\mu$ g/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<br>(231) | 0.01 | 0 | 14973 | 776 | 353 | 435 | 331  |
| 022 | CD8S365<br>(232) | 1    | 0 | 13935 | 904 | 562 | 367 | 328  |
| 022 | CD8S365<br>(232) | 0.01 | 0 | 15156 | 697 | 243 | 504 | 323  |
| 022 | CD8S369<br>(233) | 1    | 0 | 13661 | 783 | 440 | 441 | 5122 |
| 022 | CD8S369<br>(233) | 0.01 | 0 | 16513 | 717 | 251 | 596 | 416  |
| 022 | TenCon           | 1    | 0 | 14920 | 702 | 284 | 447 | 334  |

5

**B**

| Donor | Sample<br>(SEQ ID<br>NO:) | Anti-<br>CD8A<br>FN3<br>conc $\mu$ M | Anti-CD3<br>$\mu$ g/mL | CD45RA<br>MFI | CD25<br>MFI | CD69<br>MFI | CD127<br>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<br>(234)          | 1                                    | 0                      | 8531          | 673         | 95          | 1368         | 589       |
| 146   | CD8S366<br>(234)          | 0.01                                 | 0                      | 9414          | 623         | 74          | 1615         | 559       |
| 146   | CD8S368<br>(229)          | 1                                    | 0                      | 8386          | 691         | 96          | 1301         | 561       |
| 146   | CD8S368<br>(229)          | 0.01                                 | 0                      | 9147          | 628         | 82          | 1424         | 586       |
| 146   | CD8S367<br>(230)          | 1                                    | 0                      | 8167          | 660         | 95          | 1322         | 581       |
| 146   | CD8S367<br>(230)          | 0.01                                 | 0                      | 8734          | 586         | 77          | 1479         | 571       |
| 146   | CD8S370<br>(231)          | 1                                    | 0                      | 8590          | 737         | 86          | 1362         | 583       |
| 146   | CD8S370<br>(231)          | 0.01                                 | 0                      | 7934          | 635         | 71          | 1526         | 559       |

|     |                  |      |   |      |     |    |      |      |
|-----|------------------|------|---|------|-----|----|------|------|
| 146 | CD8S365<br>(232) | 1    | 0 | 8344 | 813 | 85 | 1238 | 586  |
| 146 | CD8S365<br>(232) | 0.01 | 0 | 8460 | 628 | 80 | 1355 | 605  |
| 146 | CD8S369<br>(233) | 1    | 0 | 8778 | 681 | 92 | 1369 | 5690 |
| 146 | CD8S369<br>(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  $\mu\text{M}$  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<sup>+</sup> T cells for Donor 022 (A) and Donor 146 (B) in the presence of plate bound CD3.**

20 **A**

| Donor | Sample<br>(SEQ ID<br>NO:) | Anti-<br>CD8A<br>FN3<br>conc $\mu\text{M}$ | Anti-CD3<br>$\mu\text{g/mL}$ | CD45RA<br>MFI | CD25<br>MFI | CD69<br>MFI | CD127<br>MFI | CD137 MFI |
|-------|---------------------------|--------------------------------------------|------------------------------|---------------|-------------|-------------|--------------|-----------|
| 022   | PBS<br>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 $\mu$ M | Anti-CD3, $\mu$ 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

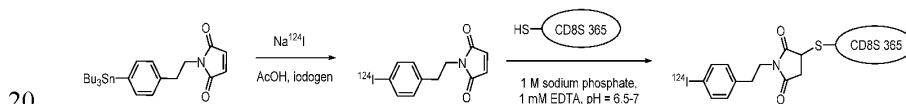
In order to determine if any of the changes observed in the activation markers resulting in changes in cytokine production, antigen-dependent T cell activation assays were also performed using two anti-CD8A FN3 domains. For one set of assays, either CMV reactive or M1 reactive human PBMCs were thawed 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 $\gamma$  Mabtech ELISpot plates in the presence or absence of 10  $\mu$ g/mL peptide. 1  $\mu$ 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 $\gamma$  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 $\gamma$  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 $\mu$ M in the presence or absence of 0.25  $\mu$ 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 $\gamma$  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 $\gamma$  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 [ $^{124}\text{I}$ ]-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 [ $^{124}\text{I}$ ]-IPEM CD8S 365**

To a 1.5 mL Eppendorf vial was added, in order, Na $^{124}\text{I}$  solution ( $\leq 13\ \mu\text{L}$ ,  $\leq 2.5\ \text{mCi}$ ), AcOH (5  $\mu\text{L}$  to acidify the solution), 1-(4-(tributylstannyl)phenethyl)-1H-pyrrole-2,5-dione (75  $\mu\text{L}$ , 1.00 mg/mL in MeCN) and iodogen (5  $\mu\text{L}$ , 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 [ $^{124}\text{I}$ ]-IPEM was collected in a 1 dram vial that had been pre-treated with Sigma-Cote<sup>TM</sup> (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  $\mu\text{L}$ .

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 [ $^{124}\text{I}$ ]-IPEM was then concentrated under vacuum at ambient temperature to a volume of <100  $\mu\text{L}$ .

5 Sodium phosphate buffer (1.0 M sodium phosphate, 1 mM EDTA, pH = 6.86) was added ( $\geq 25$   $\mu$ L) to bring the pH to  $\sim 6.5$ -7 (checked by strip). Lastly freshly reduced CD8S 365 (c  $\sim 4.57$  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  $\mu$ L of centyrin at c  $\sim 4.57$  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 ( $2 \times 1$  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  $< 500$   $\mu$ 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  $\mu$ L with PBS/10% EtOH if the volume was  $< 500$   $\mu$ L and then filtered through a Millex-GV 0.22  $\mu$ 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<sup>®</sup> 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 $\gamma$* <sup>null</sup> (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 10<sup>6</sup>  
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 mm<sup>3</sup> 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/cm<sup>2</sup>/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  $7.5 \times 10^6$  cells.

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## 5 Sequence listing

SEQ ID No. 1= Original Tencon Sequence

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSEFLIQYQESEKVGGEAINLTVPGSER  
SYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAEFTT

10

SEQ ID No. 2= TCL1 library

LPAPKNLVVSEVTEDSLRLSWTAPDAAFDSEFLIQYQESEKVGGEAINLTVPGSER  
SYDLTGLKPGTEYTVSIYGV(X)<sub>7-12</sub>PLSAEFTT;

wherein

15 X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>7</sub> is any amino acid; and

X<sub>8</sub>, X<sub>9</sub>, X<sub>10</sub>, X<sub>11</sub> and X<sub>12</sub> are any amino acid or deleted

SEQ ID No. 3=TCL2 library

LPAPKNLVVSEVTEDSLRLSWX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>X<sub>8</sub>SFLIQYQESEKVGGEAINLTV  
20 PGSERSYDLTGLKPGTEYTVSIYGVX<sub>9</sub>X<sub>10</sub>X<sub>11</sub>X<sub>12</sub>X<sub>13</sub>SX<sub>14</sub>X<sub>15</sub>LSAEFTT;

wherein

X<sub>1</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;

X<sub>2</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
25 or Val;

X<sub>3</sub> Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr or  
Val;

X<sub>4</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;

30 X<sub>5</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;

X<sub>6</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;

X<sub>7</sub> is Phe, Ile, Leu, Val or Tyr;

35 X<sub>8</sub> is Asp, Glu or Thr;

X<sub>9</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;

X<sub>10</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val;

40 X<sub>11</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp,  
Tyr or Val;

X<sub>12</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp,  
Tyr or Val;

X<sub>13</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp,  
45 Tyr or Val;

X<sub>14</sub> is Ala, Arg, Asn, Asp, Glu, Gln, Gly, His, Ile, Leu, Lys, Phe, Pro, Ser, Thr, Trp, Tyr  
or Val; and

X<sub>15</sub> 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)

LPAPKNLVVSRTEDSARLSWX<sub>1</sub>X<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>X<sub>8</sub>X<sub>9</sub>FDSFLIQYQESEKVGGEAI  
 VLTVPGSESYDLTGLKPGTEYTVSIYGVX<sub>10</sub>X<sub>11</sub>X<sub>12</sub>X<sub>13</sub>X<sub>14</sub>X<sub>15</sub>X<sub>16</sub>X<sub>17</sub>X<sub>18</sub>X<sub>19</sub>SNPL  
 SAIFTT;

10 wherein

X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>10</sub>, X<sub>11</sub>, X<sub>12</sub>, X<sub>13</sub>, X<sub>14</sub>, X<sub>15</sub> and X<sub>16</sub> are A, D, E, F, G, H, I, K, L,  
 N, P, Q, R, S, T, V, W or Y; and

X<sub>7</sub>, X<sub>8</sub>, X<sub>9</sub>, X<sub>17</sub>, X<sub>18</sub> and X<sub>19</sub>, 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 X<sub>1</sub>X<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>X<sub>8</sub>X<sub>9</sub> X<sub>10</sub>X<sub>11</sub>X<sub>12</sub>SNPLSAIFTT;

wherein

20 X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub> and X<sub>7</sub>, is A, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W or Y;  
and

X<sub>8</sub>, X<sub>9</sub>, X<sub>10</sub>, X<sub>11</sub> and X<sub>12</sub> 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 LPAPKNLVVSRTEDSARLSWTAPDAAFDSEFX<sub>1</sub>IX<sub>2</sub>YX<sub>3</sub>EX<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>GEAIVLTVP  
GSESYDLTGLKPGTEYX<sub>8</sub>VX<sub>9</sub>IX<sub>10</sub>GVKGGX<sub>11</sub>X<sub>12</sub>SX<sub>13</sub>PLSAIFTT;

wherein

X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>10</sub>, X<sub>11</sub>, X<sub>12</sub> and X<sub>13</sub> 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)

LPAPKNLVVSRTEDSARLSWTAPDAAFDSEFX<sub>1</sub>IX<sub>2</sub>YX<sub>3</sub>EX<sub>4</sub>X<sub>5</sub>X<sub>6</sub>X<sub>7</sub>GEAIX<sub>8</sub>LX<sub>9</sub>  
 VPGSESYDLTGLKPGTEYX<sub>10</sub>VX<sub>11</sub>IX<sub>12</sub>GVKGGX<sub>13</sub>X<sub>14</sub>SX<sub>15</sub>PLX<sub>16</sub>AX<sub>17</sub>FTT;

35 wherein

X<sub>1</sub>, X<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub>, X<sub>5</sub>, X<sub>6</sub>, X<sub>10</sub>, X<sub>11</sub>, X<sub>12</sub> and X<sub>13</sub> 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  
GATCTTCACCAACCGGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAG  
TCTAGCGGCCGCAACTGATCTTGGC
- 40 SEQ ID No. 27 = FG11  
GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC  
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA  
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC  
TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGACCGCGCC  
45 GGACGCGGCGTTCGACTCTTTCTGATCCAGTACCAGGAATCTGAAAAAGT  
TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG  
ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN  
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGAT  
CTTCACCAACCGGCGGTCACCATCACCATCACCATGGCAGCGGTTCTAGTCT  
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

SEQ ID No. 31 = FG7

GTGACACGGCGGTTAGAACGCGGCTACAATTAATACATAACCCCATCCCC  
TGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAGCGGATA  
ACAATTTACACAGGAAACAGGATCTACCATGCTGCCGGCGCCGAAAAACC  
40 TGGTTGTTTCTCGCGTTACCGAAGACTCTGCGCGTCTGTCTTGGACCGCGCC  
GGACGCGGCGTTCGACTCTTTCCTGATCCAGTACCAGGAATCTGAAAAAGT  
TGGTGAAGCGATCGTGCTGACCGTTCCGGGTTCTGAACGTTCTTACGACCTG  
ACCGGTCTGAAACCGGGTACCGAATACACCGTTTCTATCTACGGTGTTNNN  
45 NNNNNNNNNNNNNNNNNNNNNNNNTCTAACCCGCTGTCTGCGATCTTACCACCGGC  
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  
NKPAAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALNSIMYFSHFV  
PVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAGSGSGSDYKDDDDKDKT  
HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW  
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL  
PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES  
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNH  
YTQKSLSLSPGK

30

>SEQID No 36: CD8W13

SQFRVSPLDRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGAAASPTFLLYLSQNKP  
AAEGLDTQRFSGKRLGDTFVLTLSDFRRENEGYYFCSALNSIMYFSHFVPVFLPAKPT  
TTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDGGGGSDYKDDDDK  
GGGSHHHHHHDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH  
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES  
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKS  
LSLSPGK

40

>SEQID No. 37: mIgGK signal peptide  
Metdtlllwvlllwpvgstg

>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<br>TVPGSERSYDLTGKPGTEYTVSIYGVVDVDYNPTGRPVSSNPLSAIF<br>TT |

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

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

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

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

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

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|                  |     | SAIFTT                                                                                                         |
| P283BR9P1363_G7  | 145 | LPAPKNLVVSRVTEDSARLSWSAKKFP RHFD SFLIQYQESEKVG EAI<br>VLTVP GSERSYDLTGLKPGTEYTVSIYGV DVKWEGNRPVASNPLSA<br>IFTT |
| P283DR9P1364_A4  | 146 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFYIAYPEWVPVQGEAIV<br>LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGDWSEPLSAIFTT               |
| P283DR9P1364_A7  | 147 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFRIAYPEWVPVRGDAIV<br>LTVPGSERSYDLTGLKPGTEYWVIIQGVKGGTDSFPLSAIFTT                |
| P283DR9P1364_B1  | 148 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFYITYPEIPLGGEAIVLT<br>VPGSERSYDLTGLKPGTEYFVIIYG VKGGLLSSPLSAIFTT                |
| P283DR9P1364_B11 | 149 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFYISYPEWEQLGEAIV<br>LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGALSAPLSAIFTT                |
| P283DR9P1364_B4  | 150 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFAISYPEWPPPGEAIV<br>LTVPGSERSYDLTGLKPGTEYWVIILGVKGGDQSWPLSAIFTT                 |
| P283DR9P1364_C10 | 151 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFQIAYPEWPKDGEAI<br>VLTVP GSERSYDLTGLKPGTEYAVFIWGVKGGVYSNPLSAIFTT                |
| P283DR9P1364_D11 | 152 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFDIAYPEWPPKGEAIV<br>LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGIHSAPLSAIFTT                |
| P283DR9P1364_D8  | 153 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFQIAYPETPIQGEAIVL<br>TVPGSERSYDLTGLKPGTEYFVIIHGVKGGITSFPLSAIFTT                 |
| P283DR9P1364_D9  | 154 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFGISYPEWPP LGEAIV<br>LTVPGSERSYDLTGLKPGTEYWVIIFGVKGGERSWPLSAIFTT                |
| P283DR9P1364_E3  | 155 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFGIAYPELPIGGEAIVL<br>TVPGSERSYDLTGLKPGTEYFVIIRGVKGGT LSPPLSAIFTT                |
| P283DR9P1364_E5  | 156 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFWISYPEWVPVGEAI<br>VLTVP GSERSYDLTGLKPGTEYWVIIQGVKGGKLSWPLSAIFTT                |
| P283DR9P1364_E7  | 157 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFNIAYPEWVPVRGEAIV<br>LTVPGSERSYDLTGLKPGTEYWVIIYG VKGGDRSNPLSAIFTT               |
| P283DR9P1364_E8  | 158 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFSIAYPEWVPVHGEAIV<br>LTVPGSERSYDLTGLKPGTEYFVIIYG VKGGV LSEPLSAIFTT              |
| P283DR9P1364_E9  | 159 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFDIAYPEWPTKGEAIV<br>LTVPGSERSYDLTGLKPGTEYFVINGVKGGWRSFPLSAIFTT                  |
| P283DR9P1364_F2  | 160 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFWIAYPEWVPVGEAI<br>VLTVP GSERSYDLTGLKPGTEYFVIIQGVKGGFGSFPLSAIFTT                |
| P283DR9P1364_F6  | 161 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFTIAYPEREQDKWGE<br>AIVLTVPGSERSYDLTGLKPGTEYWVIIQGVKGGRPSTPLSAILTT               |
| P283DR9P1364_F8  | 162 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFAIAYPEWPPGEAIVL<br>TVPGSERSYDLTGLKPGTEYFVIIYG VKGGWTSPPLSAIFTT                 |
| P283DR9P1364_G10 | 163 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFSIAYPEWPGSGEAIV<br>LTVPGSERSYDLTGLKPGTEYFVIIFGVKGGSQSWPLSAIFTT                 |
| P283DR9P1364_G9  | 164 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFGIYWPEWVPVGGEAI<br>VLTVP GSERSYDLTGLKPGTEYWVNISGVKGG EYSFPLSAIFTT              |
| P283DR9P1364_H1  | 165 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFQISYPEWVPVHGEAIV<br>LTVPGSERSYDLTGLKPGTEYWVIIWGVKGG RQSWPLSAIFTT               |
| P283DR9P1364_H11 | 166 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFDIAYPELPLGGEAIVL<br>TVPGSERSYDLTGLKPGTEYFVIIWGVKGGDRSEPLSAIFTT                 |
| P283DR9P1364_H6  | 167 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFIIAYPETPVRGEAIVL<br>TVPGSERSYDLTGLKPGTEYFVIIIGVKGGQESFPLSAIFTT                 |
| P283DR9P1364_H9  | 168 | LPAPKNLVVSRVTEDSARLSWTAPDAAFD SFSISYIEYPEIPGGEAIV                                                              |

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

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

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

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

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

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

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SEQ ID. No. 270 Tencon25

LPAPKNLVVSEVTEDSARLSWTAPDAAFDSFLIQYQSEKVGAEIVLTVPGSER  
SYDLTGLKPGTEYTVSIYGVKGGHRSNPLSAIFTT

10 SEQ ID. NO: 271 Cyno CD8alpha

MRNQAPGRPKGATSPPLPTGSRAPPVAPELRAEPRPGERVMAPPVTALLPLV  
LLLHAARPNQFRVSPLGRTWNLGETVELKCQVLLSNPTSGCSWLFQPRGTAAR  
PTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLRDFRQENEGYYFCSALS  
NSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTTASQPLSLRPEACRPAAGGSVNT  
15 RGLDFACDIYWAPLAGACGVLLLSLVITLYCNHRNRRRVCKCPRPVKSGGK  
PSLSDRYV