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CD3 BINDING POLYPEPTIDES

RELATED INVENTIONS

[001] This invention is related to PCT application PCT/US12/034575 filed April 20, 2012 titled "Prostate-Specific Membrane Antigen Binding Proteins And Related Compositions And Methods" which claims priority to US provisional patent application 61/478,449 filed April 22, 2011, and is also related to U.S. Application No. 61/636,557, all three of which are incorporated by reference in their entireties.

FIELD OF INVENTION

[002] The present invention relates to mono-specific and multi-specific protein therapeutics that bind or interact with CD3. This includes antibodies, fragments thereof, scFvs, Fabs, di-scFvs single domain antibodies and polypeptides containing an antibodies or antibody fragments.

ACCOMPANYING SEQUENCE LISTING

[003] The contents of the text file (Name: "2479_113PC03_SequenceListing_ascii.txt", Size: 459,722 bytes; Date of Creation: April 16, 2013) submitted electronically herewith are incorporated herein by reference in their entirety.

BACKGROUND

[004] Targeting the TCR complex on human T-cells with anti-CD3 monoclonal antibodies has been used or suggested for treatment of autoimmune disease and related disorders such as in the treatment of organ allograft rejection. Mouse monoclonal antibodies specific for human CD3, such as OKT3 (Kung *et al.* (1979) Science 206: 347-9), were the first generation of such treatments. Although OKT3 has strong immunosuppressive potency, its clinical use was hampered by serious side effects linked to its immunogenic and mitogenic potentials (Chatenoud (2003) Nature Reviews 3:123-132). It induced an anti-globulin response, promoting its own rapid clearance and neutralization (Chatenoud *et al.* (1982) Eur. J. Immunol. 137:830-8). In addition, OKT3 induced T-cell proliferation and cytokine production *in vitro* and led to a large scale release of cytokine *in vivo* (Hirsch *et al.* (1989) J. Immunol 142: 737-43, 1989). The cytokine release (also referred to as "cytokine storm") in turn led to a "flu-like" syndrome, characterized by fever, chills, headaches, nausea, vomiting, diarrhea, respiratory distress, septic meningitis and hypotension (Chatenoud, 2003). Such serious side effects limited the more widespread use of OKT3 in transplantation as well as the extension of its use to other clinical fields such as autoimmunity (*Id.*).

[005] To reduce the side effects of the first generation of anti-CD3 monoclonal antibodies, a second generation of genetically engineered anti-CD3 monoclonal antibodies had been developed not only by grafting complementarity-determining regions (CDRs) of murine anti-CD3 monoclonal antibodies into human IgG sequences, but also by introducing non-FcR-binding mutations into the Fc to reduce occurrence of cytokine storm (Cole *et al.* (1999) Transplantation 68: 563; Cole *et al.* (1997) J. Immunol. 159: 3613). See also PCT Publication No. WO2010/042904 which is herein incorporated by reference in its entirety.

[006] In addition to monospecific therapeutics that target CD3, multispecific polypeptides that bind selectively to T-cells and tumor cells could offer a mechanism to redirect T-cell cytotoxicity towards the tumor cells and treatment of cancer. One problem, however, to designing a bispecific or multispecific T-cell-recruiting antibody has been to maintain specificity while simultaneously overriding the regulation of T-cell activation by multiple regulatory pathways.

[007] A need remains for improved anti-CD3 monospecific and multispecific molecules. Although previous improvements have been made in the Fc portion of CD3 targeting molecules to reduce the likelihood of cytokine storm, there is still a need for anti-CD3 therapeutics that have an increased half-life over prior art molecules, can be effectively manufactured, exhibit improved T-cell binding and/or improved redirected T-cell cytotoxicity (in the case of multispecific molecules designed for treatment of cancer).

SUMMARY OF THE INVENTION

[008] In one aspect, the present invention includes a CD3 binding polypeptide comprising a CD3 binding domain with a reduced isoelectric point as compared to a binding domain with the amino acid sequence of SEQ ID NO:41. In certain embodiments, the CD3 binding domain comprises a VH and VL region, each VH and VL region containing framework regions. To reduce the isoelectric point (pI) of the binding domain and/or polypeptide, two or more amino acids can be modified in the framework regions by substituting positively charged amino acids with neutral amino acids and/or substituting neutral amino acids with negatively charged amino acids. For instance, R and T can be substituted with S, Q can be substituted with E, Y can be substituted with F, and T can be substituted with V. In one embodiment, *e.g.*, to reduce the risk of immunogenicity, the amino acids that are substituted into the sequence are prevalent in the human germline IgG sequence or contained in the human germline sequence at the same or proximate position, *e.g.*, in a corresponding human germline IgG sequence. In some embodiments, the human germline IgG sequence comprises SEQ ID NOs:43 or 44.

[009] In another embodiment of the invention, the CD3 binding polypeptide comprises modifications in the prehinge region that result in lowering the pI of the prehinge

region or entire polypeptide. The prehinge region is in the junction between the binding domain and hinge region. For instance, a three amino acid prehinge region with the sequence RRT can be replaced with the sequence SSS to reduce the isoelectric point. In some embodiments, a prehinge region has a reduced isoelectric point as compared to a CD3 binding polypeptide with an RRT prehinge region.

[0010] CD3 binding polypeptides can be designed to lower the isoelectric point, e.g., by mutating the framework region(s) of the VH and/or VL regions and/or the prehinge region. The reduction in isoelectric point can be by 0.5 to 2.5 or more units.

[0011] In one embodiment of the invention, the CD3 binding polypeptide is an antibody, e.g., humanized. In another embodiment it is a small modular immunopharmaceutical protein (SMIP). In some embodiments, a CD3 binding polypeptide comprises a single-chain variable fragment (scFv).

[0012] In yet another embodiment, it is a multispecific or bispecific polypeptide. For instance, the CD3 binding polypeptide can comprise a CD3 binding domain and a tumor antigen binding domain. In one embodiment, the bispecific or multispecific molecule redirects T-cell cytotoxicity to a tumor cell.

[0013] In another embodiment of the invention, the CD3 polypeptide exists as a homodimer or a heterodimer.

DESCRIPTION OF THE FIGURES

[0014] Figure 1 is a graph depicting the theoretical pI of humanized Cris-7 SMIP polypeptides previously generated compared to the theoretical pI of about 200 other SMIP polypeptides.

[0015] Figure 2A and 2B illustrate the clipping of CD3 binding SMIP polypeptides that contain a higher pI than the CD3 polypeptides of the invention. Figure 2A is a Capillary Electrophoresis in SDS that quantifies the low molecular weight clip content of purified proteins. Figure 2B contains illustrations of clipped CD3 SMIP molecules. The sequences shown in Figure 2B correspond to amino acids 252-260 of SEQ ID NO:4, 243-252 of SEQ ID NO:4 and 251-260 of SEQ ID NO:240.

[0016] Figure 3 is an amino acid sequence alignment of Cris-7 mouse VH and derived humanized VH sequences. Cris7 VH mouse, H1, H2, H3, H4, H5, H6 and the consensus sequence correspond to SEQ ID NOs: 45, 22, 24, 26, 28, 30, 32 and 229, respectively.

[0017] Figure 4 is an amino acid sequence alignment of Cris-7 mouse VL and derived humanized VL sequences. Cris7 VL mouse, L1, L2, L3, L4 and the consensus sequence correspond to SEQ ID NOs: 46, 34, 36, 38, 40 and 230, respectively.

[0018] Figure 5 is an amino acid sequence alignment of the H3 and germline sequences IGHV1-69*01 and IGHV3-30*01. The H3, IGHV1-69*01, IGHV3-30*01 and consensus sequences correspond to SEQ ID NOs: 26, 43, 44 and 331, respectively.

[0019] Figure 6 is an amino acid sequence alignment of the J kappa region of the VL region of the germline sequence IGKV1-5*01 and L1. The J kappa region of the VL region (IGKV1-5*01), L1 chain and consensus sequences correspond to SEQ ID NOs: 232, 34 and 233, respectively. The listed IGKJ1*01, IGKJ2*01, IGKJ3*01, IGKJ4*01 and IGKJ5*01 sequences correspond to SEQ ID NOs: 234-238, respectively.

[0020] Figures 7A and 7B depicts an amino acid sequence alignment of various CD3 binding polypeptide SMIP molecules with reduced theoretical pI as compared to DRA209. The sequences listed for DRA209, DRA219, DRA222, DRA221, DRA223, DRA224, DRA225 and the consensus sequence correspond to amino acids 1-423 of SEQ ID NO:4, amino acids 1-423 of SEQ ID NO:6, amino acids 1-423 of SEQ ID NO:8, amino acids 1-423 of SEQ ID NO:10, amino acids 1-423 of SEQ ID NO:12, amino acids 1-426 of SEQ ID NO:14, amino acids 1-426 of SEQ ID NO:16 and SEQ ID NO:239, respectively.

[0021] Figure 8 is an amino acid sequence alignment of pI variants DRA233 and DRA234 compared to DRA161. The sequences listed for DRA161, DRA233, DRA234 and the consensus sequence correspond to SEQ ID NOs: 240, 18, 20 and 241, respectively.

[0022] Figure 9 is an illustration of how empirical pI values were determined.

[0023] Figure 10 is a graph showing the empirical pI of DRA209 SMIP and scFv.

[0024] Figure 11 is a graph showing the empirical pI of DRA161 SMIP compared to pI variants DRA233 and DRA234.

[0025] Figure 12 is a graph showing that the SMIP pI variants bound to T-cells the same as DRA209 and DRA161 with the exception of DRA227. DRA227 SMIP is used as a control and it does not contain a CD3 or T-cell binding domain. The letter "a" following each molecule designation indicates that the particular molecule was produced in CHO cells and the four digit number is the lot number.

[0026] Figure 13 depicts images of non-reduced SDS-PAGE showing that the CD3 binding SMIP constructs DRA233 and DRA234 are less prone to fragmentation than SMIP DRA161 (which shares the same VH and VL as DRA209).

[0027] Figure 14 depicts images of reduced SDS-PAGE showing that the CD3 binding SMIP constructs DRA233 and DRA234 are less prone to fragmentation than SMIP DRA161 (which shares the same VH and VL as DRA209).

[0028] Figure 15 are graphs and a table showing that pI variants DRA233 and DRA234 are predominately expressed intact (e.g., no clipping).

[0029] Figure 16 are images showing non-reduced CE-SDS of DRA161 (purified).

[0030] Figure 17 shows data of a PK study of DRA234 and DRA233.

[0031] Figure 18 provides data from a WinNonLin Analysis for DRA233 and DRA234 as described in Example 14.

[0032] Figure 19 is a table of PK estimates for DRA233 and DRA234.

[0033] Figure 20 is a table of PK parameters for DRA161 compared to DRA233 (with and without the 504 hour time point) and DRA234.

[0034] Figure 21 depicts results from target-dependent T-cell proliferation assays using different homodimer bispecific polypeptide molecules that target CD19. Figures 21A and 21B show results for CD4+ T-cell proliferation and CD8+ T-cell proliferation, respectively, as described in Example 10.

[0035] Figure 22 depicts results from a target-dependent T-cell proliferation assay using different heterodimer bispecific polypeptide molecules that target CD19. Figures 22A and 22B show results for CD8+ T-cell proliferation and CD4+ T-cell proliferation, respectively, as described in Example 10.

[0036] Figure 23 depicts results from a redirected T-cell cytotoxicity assay using different polypeptide heterodimers and homodimers that target CD19. Figure 23A shows results for the homodimeric bispecific polypeptides TSC129a, TSC233 and TSC234. Figure 23B shows results for the heterodimeric bispecific polypeptides TSC127, TSC227 and TSC228.

[0037] Figure 24 depicts results from a redirected T-cell cytotoxicity assay using TSC275, TSC277, TSC278 and TSC279 which are different bispecific polypeptide homodimers that target RON.

[0038] Figure 25 depicts results for T-cell binding of bispecific polypeptide heterodimers and homodimers. Figures 25A and 25B show dose-dependent binding of bispecific molecules TSC228 (heterodimer) and TSC249 (homodimer) to Jurkat cells.

[0039] Figure 26 depicts results of target-dependent T-cell proliferation by polypeptide homodimers targeting PSMA. Figure 26A shows results for C4-2B cells expressing the target PSMA antigen upon treatment with TSC249. Figure 26B shows results for DU-145 cells that did not express the target PSMA antigen upon treatment with TSC249. Figure 26C shows the results when isolated T-cells were treated with TSC249.

[0040] Figure 27 depicts results from a redirected T-cell cytotoxicity assay using the homodimeric bispecific polypeptides TSC194 and TSC249 that target both PSMA.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The invention provides CD3 binding polypeptides with improved characteristics compared to prior art CD3 binding polypeptides. The molecules of the invention have been designed to exhibit a reduced isoelectric point by modifying amino acids in the heavy and/or light chain framework regions and/or a prehinge region to reduce the

isoelectric point of the molecule. In one embodiment, the modifications are made in the J kappa region of the VL region. In some embodiments, modifications are made by substituting amino acids with a positive charge with amino acids with a neutral charge and/or substituting amino acids with a neutral charge with a negative charge.

[0042] In some embodiments, a CD3 binding polypeptide of the invention has a theoretical pI of about 0.5 to 2.5 units less than the anti-CD3 DRA209 SMIP (SEQ ID NO:4). For instance, DRA209 has a theoretical pI of 9, whereas pI variants DRA219 (SEQ ID NO:6) has a theoretical pI of 8.4, DRA221 (SEQ ID NO:10) has a theoretical pI of 8.2, DRA222 (SEQ ID NO:8) has a theoretical pI of 7.5, DRA223 (SEQ ID NO:12) has a theoretical pI of 7.2 and DRA224 (SEQ ID NO:14) has a theoretical pI of 6.8. In some embodiments, a CD3 binding polypeptide has an empirical isoelectric point of at least 1 pI unit less than a polypeptide of SEQ ID NO:4.

[0043] Surprisingly, pI variants exhibit an improved half-life and express better in CHO cells than DRA209, *e.g.*, see Example 4 below which describes a method for measuring half-life. Moreover, multispecific constructs comprising the DRA222 scFv exhibited improved properties as compared to similar constructs with other anti-CD3 binding domains such as the DRA209 binding domain (see related PCT Publication No. WO2012/145714).

[0044] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited herein, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. In the event that one or more of the incorporated documents or portions of documents define a term that contradicts that term's definition in the application, the definition that appears in this application controls.

[0045] In the present description, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated. As used herein, "about" means $\pm 20\%$ of the indicated range, value, or structure, unless otherwise indicated. It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components unless otherwise indicated. The use of the alternative (*e.g.*, "or") should be understood to mean either one, both, or any combination thereof of the alternatives. As used herein, the terms "include" and "comprise" are used synonymously. In addition, it should be understood that the polypeptides comprising the various combinations of the components (*e.g.*, domains or regions) and substituents described herein, are disclosed by the present application to the

same extent as if each polypeptide was set forth individually. Thus, selection of particular components of individual polypeptides is within the scope of the present disclosure.

[0046] As used herein, the term “binding domain” or “binding region” refers to the domain, region, portion, or site of a protein, polypeptide, oligopeptide, or peptide that possesses the ability to specifically recognize and bind to a target molecule, such as an antigen, ligand, receptor, substrate, or inhibitor (e.g., CD3, or a tumor associated antigen such as RON, CD19, CD37 or PSMA). Exemplary binding domains include single-chain antibody variable regions (e.g., domain antibodies, sFv, scFv, and scFab), receptor ectodomains, and ligands (e.g., cytokines, chemokines). In certain embodiments, the binding domain comprises or consists of an antigen binding site (e.g., comprising a variable heavy chain sequence and variable light chain sequence or three light chain complementary determining regions (CDRs) and three heavy chain CDRs from an antibody placed into alternative framework regions (FRs) (e.g., human FRs optionally comprising one or more amino acid substitutions). A variety of assays are known for identifying binding domains of the present disclosure that specifically bind a particular target, including Western blot, ELISA, phage display library screening, and BIACORE® interaction analysis. As used herein, a CD3 binding polypeptide can have a “CD3 binding domain” and, optionally, a “second binding domain.” The CD3 binding domain may be located at either the amino- or carboxyl-terminus. In certain embodiments, the CD3 binding domain comprises a humanized scFv derived from a mouse monoclonal antibody (e.g., Cris-7 or HuM291). For instance, the CD3 binding domain may be comprised of a VH region and VL region derived from a mouse monoclonal antibody wherein the VH and VL regions are joined with a linker such as a (Gly₄Ser)₃ (SEQ ID NO:76) linker. In other embodiments, the CD3 binding domain consists essentially of or consists of a humanized scFv derived from a mouse monoclonal antibody. In some embodiments, a CD3 binding domain of the invention comprises heavy chain CDR1, CDR2 and CDR3 and light chain CDR1, CDR2 and CDR3 of murine Cris-7 antibody or HuM291.

[0047] A binding domain “specifically binds” a target if it binds the target with an affinity or K_a (i.e., an equilibrium association constant of a particular binding interaction with units of 1/M) equal to or greater than 10^5 M^{-1} , while not significantly binding other components present in a test sample. Binding domains may be classified as “high affinity” binding domains and “low affinity” binding domains. “High affinity” binding domains refer to those binding domains with a K_a of at least 10^7 M^{-1} , at least 10^8 M^{-1} , at least 10^9 M^{-1} , at least 10^{10} M^{-1} , at least 10^{11} M^{-1} , at least 10^{12} M^{-1} , or at least 10^{13} M^{-1} . “Low affinity” binding domains refer to those binding domains with a K_a of up to 10^7 M^{-1} , up to 10^6 M^{-1} , up to 10^5 M^{-1} . Alternatively, affinity may be defined as an equilibrium dissociation constant (K_d) of a particular binding interaction with units of M (e.g., 10^{-5} M to 10^{-13} M). Affinities of binding

domain polypeptides and single chain polypeptides according to the present disclosure can be readily determined using conventional techniques (see, e.g., Scatchard et al. (1949) Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

[0048] "CD3" is known in the art as a multi-protein complex of six chains (see, e.g., Abbas and Lichtman, 2003; Janeway *et al.*, p. 172 and 178, 1999), which are subunits of the T-cell receptor complex. In mammals, the CD3 subunits of the T-cell receptor complex are a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, and a homodimer of CD3 ζ chains. The CD3 γ , CD3 δ , and CD3 ϵ chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3 γ , CD3 δ , and CD3 ϵ chains are negatively charged, which is a characteristic that allows these chains to associate with the positively charged T-cell receptor chains. The intracellular tails of the CD3 γ , CD3 δ , and CD3 ϵ chains each contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM, whereas each CD3 ζ chain has three. It is believed the ITAMs are important for the signaling capacity of a TCR complex. CD3 as used in the present disclosure may be from various animal species, including human, monkey, mouse, rat, or other mammals.

[0049] As used herein, a "conservative substitution" is recognized in the art as a substitution of one amino acid for another amino acid that has similar properties. Exemplary conservative substitutions are well-known in the art (see, e.g., WO97/09433, page 10, published March 13, 1997; Lehninger, Biochemistry, Second Edition; Worth Publishers, Inc. NY:NY (1975), pp.71-77; Lewin, Genes IV, Oxford University Press, NY and Cell Press, Cambridge, MA (1990), p. 8). In certain embodiments, a conservative substitution includes a leucine to serine substitution.

[0050] As used herein, the term "derivative" refers to a modification of one or more amino acid residues of a peptide by chemical or biological means, either with or without an enzyme, e.g., by glycosylation, alkylation, acylation, ester formation, or amide formation.

[0051] As used herein, a polypeptide or amino acid sequence "derived from" a designated polypeptide or protein refers to the origin of the polypeptide. In one embodiment, the polypeptide or amino acid sequence which is derived from a particular sequence has an amino acid sequence that is essentially identical to that sequence or a portion thereof, wherein the portion consists of at least 10-20 amino acids, at least 20-30 amino acids, or at least 30-50 amino acids, or at least 50-150 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the sequence. In one embodiment, a humanized or chimeric binding domain is derived from the VH and/or VL of an antibody generated by another animal. For instance, a humanized binding domain or chimeric binding domain can be derived from the VH and VL regions of a murine antibody. For

example, humanization of murine antibodies can be performed by modifying framework regions by methods known in the art.

[0052] Polypeptides derived from another polypeptide may have one or more mutations relative to the starting polypeptide, e.g., one or more amino acid residues which have been substituted with another amino acid residue or which has one or more amino acid residue insertions or deletions. The polypeptide may comprise an amino acid sequence which is not naturally occurring. Such variations necessarily have less than 100% sequence identity or similarity with the starting polypeptide. In one embodiment, the variant will have an amino acid sequence from about 60% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the starting polypeptide. In another embodiment, the variant will have an amino acid sequence from about 75% to less than 100%, from about 80% to less than 100%, from about 85% to less than 100%, from about 90% to less than 100%, from about 95% to less than 100% amino acid sequence identity or similarity with the amino acid sequence of the starting polypeptide.

[0053] As used herein, unless otherwise provided, a position of an amino acid residue in a variable region of an immunoglobulin molecule is numbered according to the Kabat numbering convention (Kabat, *Sequences of Proteins of Immunological Interest*, 5th ed. Bethesda, MD: Public Health Service, National Institutes of Health (1991)), and a position of an amino acid residue in a constant region of an immunoglobulin molecule is numbered according to EU nomenclature (Ward *et al.*, 1995 *Therap. Immunol.* 2:77-94).

[0054] As used herein, the term "dimer" refers to a biological entity that consists of two subunits associated with each other via one or more forms of intramolecular forces, including covalent bonds (e.g., disulfide bonds) and other interactions (e.g., electrostatic interactions, salt bridges, hydrogen bonding, and hydrophobic interactions), and is stable under appropriate conditions (e.g., under physiological conditions, in an aqueous solution suitable for expressing, purifying, and/or storing recombinant proteins, or under conditions for non-denaturing and/or non-reducing electrophoresis). A "heterodimer" or "heterodimeric protein," as used herein, refers to a dimer formed from two different polypeptides. A heterodimer does not include an antibody formed from four polypeptides (*i.e.*, two light chains and two heavy chains). In one embodiment of the invention, a heterodimer is created using Emergent's Interceptor platform which comprises a heterodimerization domain. A "homodimer" or "homodimeric protein," as used herein, refers to a dimer formed from two identical polypeptides. In certain embodiments of the invention, the homodimer is created using Emergent's SMIP, PIMS or Scorpion platform.

[0055] As used herein, a "hinge region" or a "hinge" refers to a polypeptide derived from (a) an interdomain region of a transmembrane protein (e.g., a type I transmembrane protein); or (b) a stalk region of a type II C-lectin. For example, a hinge region may be

derived from an interdomain region of an immunoglobulin superfamily member; suitable hinge regions within this particular class include (i) immunoglobulin hinge regions (made up of, for example, upper and/or core region(s)) or functional variants thereof, including wild-type and altered immunoglobulin hinges, and (ii) regions (or functional variants thereof) that connect immunoglobulin V-like or immunoglobulin C-like domains.

[0056] A "wild-type immunoglobulin hinge region" refers to a naturally occurring upper and middle hinge amino acid sequences interposed between and connecting the CH1 and CH2 domains (for IgG, IgA, and IgD) or interposed between and connecting the CH1 and CH3 domains (for IgE and IgM) found in the heavy chain of an antibody. In certain embodiments, a wild type immunoglobulin hinge region sequence is human, and may comprise a human IgG hinge region.

[0057] An "altered wild-type immunoglobulin hinge region" or "altered immunoglobulin hinge region" refers to (a) a wild type immunoglobulin hinge region with up to 30% amino acid changes (e.g., up to 25%, 20%, 15%, 10%, or 5% amino acid substitutions or deletions), or (b) a portion of a wild type immunoglobulin hinge region that has a length of about 5 amino acids (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids) up to about 120 amino acids (for instance, having a length of about 10 to about 40 amino acids or about 15 to about 30 amino acids or about 15 to about 20 amino acids or about 20 to about 25 amino acids), has up to about 30% amino acid changes (e.g., up to about 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% amino acid substitutions or deletions or a combination thereof), and has an IgG core hinge region as disclosed in PCT Publications WO2011/090762 and WO2011/090754.

[0058] As used herein, the term "humanized" refers to a process of making an antibody or immunoglobulin binding proteins and polypeptides derived from a non-human species (e.g., mouse or rat) less immunogenic to humans, while still retaining antigen-binding properties of the original antibody, using genetic engineering techniques. In some embodiments, the binding domain(s) of an antibody or immunoglobulin binding proteins and polypeptides (e.g., light and heavy chain variable regions, Fab, scFv) are humanized. Non-human binding domains may be humanized using techniques known as CDR grafting (Jones *et al.*, *Nature* 321:522 (1986)) and variants thereof, including "reshaping" (Verhoeyen, *et al.*, 1988 *Science* 239:1534-1536; Riechmann, *et al.*, 1988 *Nature* 332:323-337; Tempest, *et al.*, *Bio/Technol* 1991 9:266-271), "hyperchimerization" (Queen, *et al.*, 1989 *Proc Natl Acad Sci USA* 86:10029-10033; Co, *et al.*, 1991 *Proc Natl Acad Sci USA* 88:2869-2873; Co, *et al.*, 1992 *J Immunol* 148:1149-1154), and "veneering" (Mark, *et al.*, "Derivation of therapeutically active humanized and veneered anti-CD18 antibodies. In: Metcalf BW, Dalton BJ, eds. Cellular adhesion: molecular definition to therapeutic potential. New York: Plenum Press, 1994: 291-312). If derived from a non-human source, other regions of the antibody or

immunoglobulin binding proteins and polypeptides, such as the hinge region and constant region domains, may also be humanized.

[0059] An "immunoglobulin constant region" or "constant region" is a term defined herein to refer to a peptide or polypeptide sequence that corresponds to or is derived from part or all of one or more constant region domains. In one embodiment, the constant region does contain all the constant region domains of a source antibody. In one embodiment, the constant region comprises IgG CH2 and CH3 domains, for instance, IgG1 CH2 and CH3 domains. In some embodiments, a constant region does not comprise a CH1 domain. In certain embodiments, the constant region domains making up the constant sub-region are human. In some embodiments, the constant region domains of a fusion protein of this disclosure lack or have minimal effector functions of antibody-dependent cell-mediated cytotoxicity (ADCC) and complement activation and complement-dependent cytotoxicity (CDC), while retaining the ability to bind some F_C receptors (such as F_CRn, the neonatal Fc receptor) and retaining a relatively long half life *in vivo*.

[0060] In other variations, a fusion protein of this disclosure includes constant domains that retain such effector function of one or both of ADCC and CDC. In certain embodiments, a binding domain of this disclosure is fused to a human IgG constant region (for instance, IgG1), wherein the constant region has one or more of the following amino acids mutated: leucine at position 234 (L234), leucine at position 235 (L235), glycine at position 237 (G237), glutamate at position 318 (E318), lysine at position 320 (K320), lysine at position 322 (K322), or any combination thereof (numbering according to EU). For example, any one or more of these amino acids can be changed to alanine. In a further embodiment, an IgG Fc domain such as IgG1 has each of L234, L235, G237, E318, K320, and K322 (according to EU numbering) mutated, *e.g.*, to an alanine (*i.e.*, L234A, L235A, G237A, E318A, K320A, and K322A, respectively), and optionally an N297A mutation as well (*i.e.*, essentially eliminating glycosylation of the CH2 domain).

[0061] As used here the term "small modular immunopharmaceutical proteins" or "SMIP" is used to refer to protein scaffold as generally disclosed in, for example, in US Patent Application Publication Nos. 2003/0133939, 2003/0118592, and 2005/0136049, which are incorporated herein by reference in their entirety. "SMIP molecules" described in the Examples and throughout the disclosure herein should be understood to be binding proteins comprising SMIP scaffolding, *e.g.*, in order from amino to carboxyl-terminus, a first binding domain, a hinge region, and an immunoglobulin constant region. "CD3-specific SMIP molecules" should be understood to be CD3-binding proteins comprising SMIP scaffolding.

[0062] As used here the term "PIMS" is used to refer to protein scaffold as generally disclosed in, for example, in US Patent Application Publication No. 2009/0148447, which is

incorporated herein in its entirety by reference. "PIMS molecules" described in the Examples and throughout the disclosure herein should be understood to be binding proteins comprising PIMS scaffolding, e.g., in order from amino to carboxyl-terminus, an immunoglobulin constant region, a hinge region and a first binding domain. "CD3-specific PIMS molecules" should be understood to be CD3-binding proteins comprising PIMS scaffolding.

[0063] "Fc region" or "Fc domain" refers to a polypeptide sequence corresponding to or derived from the portion of a source antibody that is responsible for binding to antibody receptors on cells and the C1q component of complement. Fc stands for "fragment crystalline," the fragment of an antibody that will readily form a protein crystal. Distinct protein fragments, which were originally described by proteolytic digestion, can define the overall general structure of an immunoglobulin protein. As originally defined in the literature, the Fc fragment consists of the disulfide-linked heavy chain hinge regions, CH2, and CH3 domains. However, more recently the term has been applied to a single chain consisting of CH3, CH2, and at least a portion of the hinge sufficient to form a disulfide-linked dimer with a second such chain. For a review of immunoglobulin structure and function, see Putnam, *The Plasma Proteins*, Vol. V (Academic Press, Inc., 1987), pp. 49-140; and Padlan, *Mol. Immunol.* 31:169-217, 1994. As used herein, the term Fc includes variants of naturally occurring sequences.

[0064] As used herein, "isoelectric point" or pI is the pH at which net charge is zero.

[0065] As used herein, the term "Interceptor" is used to refer to a monospecific or multispecific heterodimeric protein scaffold as generally disclosed in PCT Publications WO2011/090762 and WO2011/090754. The Interceptor molecules described herein should be understood to be CD3 binding proteins comprising two non-identical polypeptide chains, each polypeptide chain comprising an immunoglobulin heterodimerization domain. The interfacing immunoglobulin heterodimerization domains are different. In one embodiment, the immunoglobulin heterodimerization domain comprises a CH1 domain or a derivative thereof. In another embodiment, the immunoglobulin heterodimerization domain comprises a CL domain or a derivative thereof. In one embodiment, the CL domain is a C κ or C λ isotype or a derivative thereof.

[0066] As used herein, the "stalk region" of a type II C-lectin refers to the portion of the extracellular domain of the type II C-lectin that is located between the C-type lectin-like domain (CTLN; e.g., similar to CTLN of natural killer cell receptors) and the transmembrane domain. For example, in the human CD94 molecule (GenBank Accession No. AAC50291.1, PRI November 30, 1995), the extracellular domain corresponds to amino acid residues 34-179, whereas the CTLN corresponds to amino acid residues 61-176. Accordingly, the stalk region of the human CD94 molecule includes amino acid residues 34-60, which is found

between the membrane and the CTLD (see Boyington *et al.*, *Immunity* 10:75, 1999; for descriptions of other stalk regions, see also Beavil *et al.*, *Proc. Nat'l. Acad. Sci. USA* 89:753, 1992; and Figdor *et al.*, *Nature Rev. Immunol.* 2:77, 2002). These type II C-lectins may also have from six to 10 junction amino acids between the stalk region and the transmembrane region or the CTLD. In another example, the 233 amino acid human NKG2A protein (GenBank Accession No. P26715.1, PRI June 15, 2010) has a transmembrane domain ranging from amino acids 71-93 and an extracellular domain ranging from amino acids 94-233. The CTLD is comprised of amino acids 119-231, and the stalk region comprises amino acids 99-116, which is flanked by junctions of five and two amino acids. Other type II C-lectins, as well as their extracellular ligand-bind domains, interdomain or stalk regions, and CTLDs are known in the art (see, e.g., GenBank Accession Nos. NP_001993.2; AAH07037.1, PRI July 15, 2006; NP_001773.1, PRI June 20, 1010; AAL65234.1, PRI January 17, 2002; and CAA04925.1, PRI November 14, 2006, for the sequences of human CD23, CD69, CD72, NKG2A and NKG2D and their descriptions, respectively).

[0067] As used herein, the "interdomain region" of a transmembrane protein (e.g., a type I transmembrane protein) refers to a portion of the extracellular domain of the transmembrane protein that is located between two adjacent domains. Examples of interdomain regions include regions linking adjacent Ig domains of immunoglobulin superfamily members (e.g., an immunoglobulin hinge region from IgG, IgA, IgD, or IgE; the region linking the IgV and IgC2 domains of CD2; or the region linking the IgV and IgC domains of CD80 or CD86). Another example of an interdomain region is the region linking the non-Ig and IgC2 domain of CD22, a type I sialic acid-binding Ig-like lectin.

[0068] A polypeptide region "derived from" a stalk region of a type II C-lectin, or "derived from" a transmembrane protein interdomain region (e.g., an immunoglobulin hinge region), refers to an about five to about 150 amino acid sequence all or at least a portion of which includes (i) a wild-type stalk region or interdomain region sequence; (ii) a fragment of the wild-type stalk region or interdomain region sequence; (iii) a polypeptide having at least 80%, 85%, 90%, or 95% amino acid sequence identity with either (i) or (ii); or (iv) either (i) or (ii) in which one, two, three, four, or five amino acids have a deletion, insertion, substitution, or any combination thereof, for instance, the one or more changes are substitutions or the one or more mutations include only one deletion. In some embodiments, a derivative of a stalk region is more resistant to proteolytic cleavage as compared to the wild-type stalk region sequence, such as those derived from about eight to about 20, about 10 to about 25 or about 15 to about 25 amino acids of NKG2A, NKG2D, CD23, CD64, CD72, or CD94.

[0069] As used herein, the term "junction amino acids" or "junction amino acid residues" refers to one or more (e.g., about 2-10) amino acid residues between two adjacent regions or domains of a polypeptide, such as between a hinge and an adjacent

immunoglobulin constant sub-region or between a hinge and an adjacent binding domain or between a peptide linker that links two immunoglobulin variable domains and an adjacent immunoglobulin variable domain. Junction amino acids may result from the construct design of a polypeptide (e.g., amino acid residues resulting from the use of a restriction enzyme site during the construction of a nucleic acid molecule encoding a polypeptide). Junction amino acids between a binding domain and hinge region are referred to herein as the prehinge region, e.g., can result from adding a restriction site into the encoding nucleotide sequence. In one embodiment, the prehinge region consists of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 15 amino acids. The prehinge region sequence of DRA209 and DRA161 is not part of the murine Cris-7 or human immunoglobulin germline sequence. The prehinge region of DRA209 and DRA161 is the amino acid sequence RRT, while it is SSS for DRA219, DRA221, DRA222, DRA223, DRA224, DRA225, DRA228, DRA229, DRA233, DRA234, TSC249, TSC250, TSC251, TSC252, TSC295, TSC296, TSC301 and TSC302. Binding molecules of the invention may or may not include a prehinge region.

[0070] As used herein, the phrase a "linker between CH3 and CH1 or CL" refers to one or more (e.g., about 2-12) amino acid residues between the C-terminus of a CH3 domain (e.g., a wild type CH3 or a mutated CH3) and the N-terminus of a CH1 domain or CL domain (e.g., Ck).

[0071] As used herein, the term "patient in need" refers to a patient at risk of, or suffering from, a disease, disorder or condition that is amenable to treatment or amelioration with a CD3 binding protein or polypeptide or a composition thereof provided herein.

[0072] As used herein, the term "peptide linker" refers to an amino acid sequence that connects a heavy chain variable region to a light chain variable region and provides a spacer function compatible with interaction of the two sub-binding domains so that the resulting polypeptide retains a specific binding affinity to the same target molecule as an antibody that comprises the same light and heavy chain variable regions. In certain embodiments, a linker is comprised of five to about 35 amino acids, for instance, about 15 to about 25 amino acids.

[0073] As used herein, the term "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce allergic or other serious adverse reactions in a majority of subjects when administered using routes well known in the art. Molecular entities and compositions approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans are considered to be "pharmaceutically acceptable."

[0074] As used herein, the term "promoter" refers to a region of DNA involved in binding RNA polymerase to initiate transcription.

[0075] As used herein, the terms "nucleic acid," "nucleic acid molecule," or "polynucleotide" refer to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the terms encompass nucleic acids containing analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (e.g., degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.* (1991) *Nucleic Acid Res.* 19:5081; Ohtsuka *et al.* (1985) *J. Biol. Chem.* 260:2605-2608; Cassol *et al.* (1992); Rossolini *et al.* (1994) *Mol. Cell. Probes* 8:91-98). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene. As used herein, the terms "nucleic acid," "nucleic acid molecule," or "polynucleotide" are intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogs of the DNA or RNA generated using nucleotide analogs, and derivatives, fragments and homologs thereof.

[0076] The term "expression" refers to the biosynthesis of a product encoded by a nucleic acid. For example, in the case of nucleic acid segment encoding a polypeptide of interest, expression involves transcription of the nucleic acid segment into mRNA and the translation of mRNA into one or more polypeptides.

[0077] The terms "expression unit" and "expression cassette" are used interchangeably herein and denote a nucleic acid segment encoding a polypeptide of interest and capable of providing expression of the nucleic acid segment in a host cell. An expression unit typically comprises a transcription promoter, an open reading frame encoding the polypeptide of interest, and a transcription terminator, all in operable configuration. In addition to a transcriptional promoter and terminator, an expression unit may further include other nucleic acid segments such as, e.g., an enhancer or a polyadenylation signal.

[0078] The term "expression vector," as used herein, refers to a nucleic acid molecule, linear or circular, comprising one or more expression units. In addition to one or more expression units, an expression vector may also include additional nucleic acid segments such as, for example, one or more origins of replication or one or more selectable markers. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

[0079] As used herein, Scorpion is a term used to refer to a multi-specific binding protein scaffold. Multi-specific binding proteins and polypeptides are disclosed, for instance,

in PCT Application Publication No. WO 2007/146968, U.S. Patent Application Publication No. 2006/0051844, PCT Application Publication No. WO 2010/040105, PCT Application Publication No. WO2010/003108, and U.S. Patent No. 7,166,707. A Scorpion polypeptide comprises two binding domains (the domains can be designed to specifically bind the same or different targets), two linkers, and an immunoglobulin constant sub-region. Linkers for Scorpion molecules are described in PCT Application Publication No. WO2010/003108. In some embodiments, a linker sequence is between about 2-45 amino acids, or 2-38 amino acids, or 5-45 amino acids. In some embodiments, a linker is an antibody hinge region (e.g., from IgG) or a C-lectin stalk region. Scorpion proteins are homodimeric proteins comprising two identical, disulfide-bonded Scorpion polypeptides.

[0080] As used herein, the term "sequence identity" refers to a relationship between two or more polynucleotide sequences or between two or more polypeptide sequences. When a position in one sequence is occupied by the same nucleic acid base or amino acid residue in the corresponding position of the comparator sequence, the sequences are said to be "identical" at that position. The percentage "sequence identity" is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of "identical" positions. The number of "identical" positions is then divided by the total number of positions in the comparison window and multiplied by 100 to yield the percentage of "sequence identity." Percentage of "sequence identity" is determined by comparing two optimally aligned sequences over a comparison window. The comparison window for nucleic acid sequences may be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900 or 1000 or more nucleic acids in length. The comparison window for polypeptide sequences may be, for instance, at least 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300 or more amino acids in length. In order to optimally align sequences for comparison, the portion of a polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions termed gaps while the reference sequence is kept constant. An optimal alignment is that alignment which, even with gaps, produces the greatest possible number of "identical" positions between the reference and comparator sequences. Percentage "sequence identity" between two sequences can be determined using the version of the program "BLAST 2 Sequences" which was available from the National Center for Biotechnology Information as of September 1, 2004, which program incorporates the programs BLASTN (for nucleotide sequence comparison) and BLASTP (for polypeptide sequence comparison), which programs are based on the algorithm of Karlin and Altschul (Proc. Natl. Acad. Sci. USA 90(12):5873-5877, 1993). When utilizing "BLAST 2 Sequences," parameters that were default parameters as of September 1, 2004, can be used for word

size (3), open gap penalty (11), extension gap penalty (1), gap dropoff (50), expect value (10) and any other required parameter including but not limited to matrix option. Two nucleotide or amino acid sequences are considered to have “substantially similar sequence identity” or “substantial sequence identity” if the two sequences have at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity relative to each other.

[0081] As used herein, a “polypeptide” or “polypeptide chain” is a single, linear and contiguous arrangement of covalently linked amino acids. It does not include two polypeptide chains that link together in a non-linear fashion, such as via an interchain disulfide bond (e.g., a half immunoglobulin molecule in which a light chain links with a heavy chain via a disulfide bond). Polypeptides may have or form one or more intrachain disulfide bonds. With regard to polypeptides as described herein, reference to amino acid residues corresponding to those specified by a SEQ ID NO includes post-translational modifications of such residues.

[0082] A “protein” is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

[0083] The terms “amino-terminal” (N-terminal) and “carboxyl-terminal” (C-terminal) are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl-terminus of the reference sequence, but is not necessarily at the carboxyl-terminus of the complete polypeptide.

[0084] “T-cell receptor” (TCR) is a molecule found on the surface of T-cells that, along with CD3, is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. It consists of a disulfide-linked heterodimer of the highly variable α and β chains in most T-cells. In other T-cells, an alternative receptor made up of variable γ and δ chains is expressed. Each chain of the TCR is a member of the immunoglobulin superfamily and possesses one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end (see Abbas and Lichtman, *Cellular and Molecular Immunology* (5th Ed.), Editor: Saunders, Philadelphia, 2003; Janeway *et al.*, *Immunobiology: The Immune System in Health and Disease*, 4th Ed., Current Biology Publications, p148,

149, and 172, 1999). TCR as used in the present disclosure may be from various animal species, including human, mouse, rat, or other mammals.

[0085] "TCR complex," as used herein, refers to a complex formed by the association of CD3 chains with other TCR chains. For example, a TCR complex can be composed of a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, a homodimer of CD3 ζ chains, a TCR α chain, and a TCR β chain. Alternatively, a TCR complex can be composed of a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, a homodimer of CD3 ζ chains, a TCR γ chain, and a TCR δ chain.

[0086] "A component of a TCR complex," as used herein, refers to a TCR chain (e.g., TCR α , TCR β , TCR γ or TCR δ), a CD3 chain (e.g., CD3 γ , CD3 δ , CD3 ϵ or CD3 ζ), or a complex formed by two or more TCR chains or CD3 chains (e.g., a complex of TCR α and TCR β , a complex of TCR γ and TCR δ , a complex of CD3 ϵ and CD3 δ , a complex of CD3 γ and CD3 ϵ , or a sub-TCR complex of TCR α , TCR β , CD3 γ , CD3 δ , and two CD3 ϵ chains).

[0087] "Antibody-dependent cell-mediated cytotoxicity" and "ADCC," as used herein, refer to a cell-mediated process in which nonspecific cytotoxic cells that express Fc γ Rs (e.g., monocytic cells such as Natural Killer (NK) cells and macrophages) recognize bound antibody (or other protein capable of binding Fc γ Rs) on a target cell and subsequently cause lysis of the target cell. In principle, any effector cell with an activating Fc γ R can be triggered to mediate ADCC. The primary cells for mediating ADCC are NK cells, which express only Fc γ RIII, whereas monocytes, depending on their state of activation, localization, or differentiation, can express Fc γ RI, Fc γ RII, and Fc γ RIII. For a review of Fc γ R expression on hematopoietic cells, see, e.g., Ravetch *et al.*, 1991, *Annu. Rev. Immunol.*, 9:457-92.

[0088] The term "having ADCC activity," as used herein in reference to a polypeptide or protein, means that the polypeptide or protein (for example, one comprising an immunoglobulin hinge region and an immunoglobulin constant sub-region having CH2 and CH3 domains, such as derived from IgG (e.g., IgG1)), is capable of mediating antibody-dependent cell-mediated cytotoxicity (ADCC) through binding of a cytolytic Fc receptor (e.g., Fc γ RIII) on a cytolytic immune effector cell expressing the Fc receptor (e.g., an NK cell).

[0089] "Complement-dependent cytotoxicity" and "CDC," as used herein, refer to a process in which components in normal serum ("complement"), together with an antibody or other C1q-complement-binding protein bound to a target antigen, exhibit lysis of a target cell expressing the target antigen. Complement consists of a group of serum proteins that act in concert and in an orderly sequence to exert their effect.

[0090] The terms "classical complement pathway" and "classical complement system," as used herein, are synonymous and refer to a particular pathway for the activation of complement. The classical pathway requires antigen-antibody complexes for initiation

and involves the activation, in an orderly fashion, of nine major protein components designated C1 through C9. For several steps in the activation process, the product is an enzyme that catalyzes the subsequent step. This cascade provides amplification and activation of large amounts of complement by a relatively small initial signal.

[0091] The term "having CDC activity," as used herein in reference to a polypeptide or protein, means that the polypeptide or protein (for example, one comprising an immunoglobulin hinge region and an immunoglobulin constant sub-region having CH2 and CH3 domains, such as derived from IgG (e.g., IgG1)) is capable of mediating complement-dependent cytotoxicity (CDC) through binding of C1q complement protein and activation of the classical complement system.

[0092] "Redirected T-cell cytotoxicity" and "RTCC," as used herein, refer to a T-cell-mediated process in which a cytotoxic T-cell is recruited to a target cell using a multi-specific protein that is capable of specifically binding both the cytotoxic T-cell and the target cell, and whereby a target-dependent cytotoxic T-cell response is elicited against the target cell.

[0093] As used herein, the term "treatment," "treating," or "ameliorating" refers to either a therapeutic treatment or prophylactic/preventative treatment. A treatment is therapeutic if at least one symptom of disease in an individual receiving treatment improves or a treatment may delay worsening of a progressive disease in an individual, or prevent onset of additional associated diseases.

[0094] As used herein, the term "transformation," "transfection," and "transduction" refer to the transfer of nucleic acid (e.g., a nucleotide polymer) into a cell. As used herein, the term "genetic transformation" refers to the transfer and incorporation of DNA, especially recombinant DNA, into a cell. The transferred nucleic acid may be introduced into a cell via an expression vector.

[0095] As used herein, the term "variant" or "variants" refers to a nucleic acid or polypeptide differing from a reference nucleic acid or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the reference nucleic acid or polypeptide. For instance, a variant may exhibit at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98% or at least about 99% sequence identity compared to the active portion or full length reference nucleic acid or polypeptide.

[0096] The terms "light chain variable region" (also referred to as "light chain variable domain" or "VL") and "heavy chain variable region" (also referred to as "heavy chain variable domain" or "VH") refer to the variable binding region from an antibody light and heavy chain, respectively. The variable binding regions are made up of discrete, well-defined sub-regions known as "complementarity determining regions" (CDRs) and "framework regions" (FRs). In one embodiment, the FRs are humanized. The term "CL" refers to an "immunoglobulin light

chain constant region" or a "light chain constant region," *i.e.*, a constant region from an antibody light chain. The term "CH" refers to an "immunoglobulin heavy chain constant region" or a "heavy chain constant region," which is further divisible, depending on the antibody isotype into CH1, CH2, and CH3 (IgA, IgD, IgG), or CH1, CH2, CH3, and CH4 domains (IgE, IgM). A "Fab" (fragment antigen binding) is the part of an antibody that binds to antigens and includes the variable region and CH1 domain of the heavy chain linked to the light chain via an inter-chain disulfide bond.

[0097] The present disclosure provides, *inter alia*, polypeptides and proteins comprising binding domains, in particular, a first binding domain that specifically binds CD3. The polypeptides and proteins comprising binding domains of this disclosure may comprise one or more of the following: an immunoglobulin constant sub-region(s), a linker peptide(s), a hinge region(s), an immunoglobulin heterodimerization domain(s), an immunoglobulin dimerization domain(s), one or more junctional amino acids, a tag(s), *etc.* These components of the disclosed polypeptides and proteins are described in further detail herein.

[0098] Additionally, the CD3 binding polypeptides and proteins disclosed herein may be in the form of an antibody or a fusion protein of any of a variety of different formats (*e.g.*, the fusion protein may be in the form of a SMIP protein, a PIMS protein, a Scorpion protein or an Interceptor protein).

[0099] In some embodiments, a CD3 binding polypeptide comprises a second binding domain. In some embodiments, a CD3 binding polypeptide comprises, from amino to carboxyl terminus, a CD3 binding domain, a hinge region, a constant region, and a second binding domain. In other embodiments, a CD3 binding polypeptide comprises, from amino to carboxyl terminus, a second binding domain, a hinge region, a constant region, and a CD3 binding domain.

[00100] A CD3 binding protein in accordance with the present invention generally includes (a) at least one CD3 binding polypeptide chain comprising a CD3 binding domain as set forth herein. In certain variations, the CD3 binding polypeptide further includes (b) a hinge region carboxyl-terminal to the CD3 binding domain, and (c) an immunoglobulin constant region (*e.g.*, a SMIP polypeptide). In further variations, the CD3 binding polypeptide further includes (d) a second hinge region carboxyl-terminal to the immunoglobulin constant sub-region, and (e) a second binding domain carboxyl-terminal to the second hinge region (*e.g.*, a Scorpion polypeptide). In some embodiments, a CD3 binding polypeptide comprises a CD3 binding domain with framework regions, a prehinge region and a hinge region.

[00101] In some embodiments, a CD3 binding polypeptide comprises three or more modified amino acids as compared to SEQ ID NO:42, for example, the modifications being substitutions of positively charged amino acids with neutral amino acids and/or substitutions

of neutral amino acids with negatively charged amino acids, *e.g.*, in the framework and/or pre-hinge regions. In some embodiments, at least two, at least three, at least four, 3-5 or 3-10 amino acids are modified in the framework regions of VH and/or VL by substituting positively charged amino acids with neutral amino acids and/or substituting neutral amino acids with negatively charged amino acids.

[00102] In some embodiment of the invention, a CD3 binding polypeptide comprises modifications in the pre-hinge region, *e.g.*, that result in lowering the pI of the pre-hinge region or entire polypeptide. The pre-hinge region is in the junction between the binding domain and hinge region. For instance, a three amino acid pre-hinge region with the sequence RRT can be replaced with the sequence SSS or SST to reduce the isoelectric point. In some embodiments, a pre-hinge region has a reduced isoelectric point as compared to a CD3 binding polypeptide with an RRT pre-hinge region.

[00103] In one embodiment, to reduce the risk of immunogenicity, amino acids are substituted into the sequence that are prevalent in the human germline IgG sequence or contained in the human germline sequence at the same or proximate position, *e.g.*, in a corresponding human germline IgG sequence. In some embodiments, the human germline IgG sequence comprises SEQ ID NOs:43 or 44.

[00104] In some embodiments, at least one amino acid modification is within the J kappa (Jk) region of the VL region as compared to the Cris-7 VL Jk region mouse sequence LQIT (SEQ ID NO:252). In some embodiments, a Jk region comprises the amino acid sequence VEIK (SEQ ID NO:253), *e.g.*, in place of LQIT (SEQ ID NO:252).

[00105] In some embodiments, a CD3 binding polypeptide comprises a hinge region and a constant region, for example, a CD3 binding polypeptide may comprise (a) a hinge region amino-terminal to the CD3 binding domain, and (b) an immunoglobulin constant region amino-terminal to the hinge region (*e.g.*, a PIMS polypeptide). In some embodiments, a CD3 binding polypeptide comprises, from amino to carboxyl terminus, a CD3 binding domain, a hinge region and a constant region. In some embodiments, a CD3 binding polypeptide comprises, from amino to carboxyl terminus, a constant region, a hinge region and a CD3 binding domain.

[00106] Typically, CD3 binding polypeptides of the above formats (SMIP, Scorpion, or PIMS) are capable of homodimerization, typically through disulfide bonding, via the immunoglobulin constant region and/or hinge region (*e.g.*, via an immunoglobulin constant region comprising IgG CH2 and CH3 domains and an IgG hinge region). Thus, in certain aspects of the present invention, two identical CD3 binding polypeptides homodimerize to form a dimeric CD3 binding protein.

[00107] In other embodiments, a CD3 binding polypeptide further includes a heterodimerization domain that is capable of heterodimerization with a different

heterodimerization domain in a second, non-identical polypeptide chain. In certain variations, the second polypeptide chain for heterodimerization includes a second binding domain. Accordingly, in certain aspects of the present invention, two non-identical polypeptide chains, one comprising the CD3 binding domain and the second optionally comprising a second binding domain, dimerize to form a heterodimeric protein with one to four binding domains. In some embodiments, a CD3 binding polypeptide comprises, from amino to carboxyl terminus, a CD3 binding domain, a hinge region, a constant region, a heterodimerization domain and optionally a second binding domain.

[00108] In some embodiments, a second binding domain binds or interacts with a target molecule and the CD3 binding polypeptide induces T-cell cytotoxicity. This could be in a non-dimer, heterodimer or homodimer format. In some embodiments, a second binding domain binds or interacts with a tumor associated antigen. In some embodiments, a CD3 binding polypeptide induces T-cells to lyse tumor cells and/or induces polyclonal T- cell activation and expansion in the vicinity of a tumor.

[00109] Polypeptides comprising an anti-CD3 binding domain of the invention can be or comprise antibodies or antibody derivatives including functional antibody fragments or derivatives of fragments which retain binding specificity. In some embodiments, the invention includes fusion proteins and other polypeptides that contain variable heavy and/or light chain domains.

[00110] In some embodiments of the invention, a CD3 binding polypeptide comprises a CD3 binding domain that comprises a VH region sequence or VL region sequence selected from the group consisting of SEQ ID NOs:28, 30, 32, 38 and 40 . In some embodiments, a CD3 binding domain comprises a VH region selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30 and 32 and a VL region selected from the group consisting of SEQ ID NOs:38 and 40. In some embodiments, a CD3 binding domain comprises a VH region selected from the group consisting of SEQ ID NO:28, 30 and 32 and a VL region selected from the group consisting of SEQ ID NO:34, 36, 38 and 40. In some embodiments, the VH region comprises SEQ ID NO:28 and the VL region comprises SEQ ID NO:34; the VH region comprises SEQ ID NO:28 and the VL region comprises SEQ ID NO:38; the VH region comprises SEQ ID NO:26 and the VL region comprises SEQ ID NO:38; or the VH region comprises SEQ ID NO:26 and the VL region comprises SEQ ID NO:34. In some embodiments, a CD3 binding polypeptide comprises a CD3 binding domain that comprises a heavy chain CDR1, CDR2 and CDR3 and a light chain CDR1, CDR2 and CDR3 and wherein the heavy chain CDR3 comprises SEQ ID NO:51. In some embodiments, a CD3 binding polypeptide includes a heavy chain CDR1 comprising SEQ ID NO:49, a CDR2 comprising SEQ ID NO:50 and a CDR3 comprising SEQ ID NO:51 and a

light chain CDR1 comprising SEQ ID NO:52, a CDR2 comprising SEQ ID NO:53, and a CDR3 comprising SEQ ID NO:54.

[00111] In some embodiments, a CD3 binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18 and 20.

[00112] The invention includes a multispecific binding protein comprising dimerized single chain polypeptides, each single chain polypeptide comprising, from amino to carboxyl terminus, a first binding domain, an N-terminus linker, an immunoglobulin constant region, a C-terminus linker and a CD3 binding domain of the invention. Likewise, the invention includes a multispecific binding protein comprising dimerized single chain polypeptides, each single chain polypeptide comprising, from amino to carboxyl terminus, a CD3 binding domain of the invention, an N-terminus linker, an immunoglobulin constant region, a C-terminus linker and a first binding domain. In some embodiments, the N-terminus linker may comprise or may consist essentially of an immunoglobulin hinge region.

[00113] In another aspect of the invention, the multispecific binding protein comprises a single chain polypeptide comprising, from amino to carboxyl terminus, a first binding domain, an N-terminus linker, an immunoglobulin constant region, a C-terminus linker and a CD3 binding domain. Likewise, the invention includes a multispecific binding protein comprising, from amino to carboxyl terminus, a CD3 binding domain, an N-terminus linker, an immunoglobulin constant region, a C-terminus linker and a first binding domain.

[00114] The invention includes a multispecific binding protein comprising a first binding domain linked via a linker domain to a second binding domain (e.g., a scFv linked via a linker to another scFv). For instance, the invention includes a multispecific binding protein comprising a first binding domain (in V_H -linker- V_L or V_L -linker- V_H orientation) linked via a peptide linker domain to a CD3 binding domain (in V_H -linker- V_L or V_L -linker- V_H orientation). In some embodiments, a bispecific protein in the scFv-linker-scFv format may comprise variable heavy and variable light domains derived from an antibody to a T-cell antigen (such as CD3) including, but not limited to, the variable domains disclosed herein. A linker separating the scFv domains may comprise $((Gly_4)Ser)_n$ wherein $n = 1-5$ (SEQ ID NOs:74-78). In one embodiment, the linker is $((Gly_4)Ser)_3$ (SEQ ID NO:76). The linker may also comprise about 8-12 amino acids. In some embodiments, a protein of the invention in this format ("bispecific single chain antibody"), does not comprise an Fc region and as a result, has no Fc-related effector function.

[00115] The invention includes essentially any type of polypeptide containing a binding domain or domains as described herein. This includes antibodies, fragments thereof, scFvs, Fabs, di-scFvs single domain antibodies, diabodies, dual variable domain binding proteins and polypeptides containing an antibody or antibody fragments. Further types of polypeptides that are included in the invention are described herein.

[00116] In some embodiments, V_H and V_L domains are used as scFvs and attached/fused to other polypeptides or amino acid sequences in a manner that allows the scFvs and V_H/V_L to bind the target. In some embodiments, a V_H and V_L domain as described herein is used in an antibody or fragment thereof. For example the V_H and V_L domains are incorporated into the natural position for V_H and V_L domains in an antibody. In some embodiments, a CD3 binding polypeptide of the invention is an antibody, e.g., with a V_L chain and V_H chain as described herein.

[00117] In one embodiment of the invention, a multispecific protein is a scFv dimer or diabody rather than a whole antibody. Diabodies and scFv dimers can be constructed without an Fc region, using only variable domains. Diabodies are bivalent, bispecific antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a peptide linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., Antibody Engineering (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

[00118] In one embodiment of the invention, a multispecific protein is a disulfide-stabilized diabody. For instance, a multispecific antibody may comprise two distinct polypeptides that are co-expressed to generate a covalently linked heterodimeric complex with one binding site for each of 2 specificities. In this embodiment, each Fv is formed by the association of a V_L partner on one chain with a V_H partner on the second chain in a V_{LA} - V_{HB} (first chain) and V_{LB} - V_{HA} (second chain) configuration. This can be stabilized by either of two alternative carboxy terminal heterodimerization domains: a pairing of VEPKSC (SEQ ID NO:254) on one chain and FNRGEC (SEQ ID NO:255) on the other or a pairing of oppositely charged coiled-coil domains. See, for instance, Moore *et al.*, 2011, *Blood*. 117:4542-4551. In this embodiment, a multispecific binding protein may comprise a first chain with a CD3 binding domain V_H linked to first binding domain V_L and the second chain comprises a CD3 binding domain V_L linked to a first binding domain V_H , and the two chains are linked via a disulfide bond at the C-termini. A disulfide-stabilized diabody may be designed using variable heavy and light chains derived from known antibodies including, for instance, the variable heavy and light chains disclosed herein.

[00119] In another embodiment, a multispecific binding protein is a dual variable domain binding protein capable of binding a first binding site (e.g., a tumor antigen) and a TCR complex with specificity. In this embodiment, a recombinant protein comprises a polypeptide chain, wherein said polypeptide chain comprises $VD1-(X1)_n-VD2-C--(X2)_n$, wherein $VD1$ is a first variable domain, $VD2$ is a second variable domain, C is a constant

domain, X1 is a linker (e.g., a polypeptide linker of about 10 to 20 amino acids in length), X2 represents an Fc region and n is 0 or 1. See, for instance, U.S. Patent No. 8,258,268.

[00120] In one embodiment of the invention, a multispecific binding protein comprises one, two, three or more polypeptide chains. For instance, the invention includes a multispecific protein with a first chain comprising V_{H1} - V_{L2} , a second chain comprising $CH2$ - $CH3$ - V_{L1} - V_{H2} and a third chain comprising $CH2$ - $CH3$. In this embodiment, the V_{H1} and V_{L1} may correspond to a first binding domain's variable domains, and V_{H2} and V_{L2} may correspond to anti-CD3 variable domains. Alternatively, the V_{H1} and V_{L1} may correspond to anti-CD3 variable domains, and the V_{H2} and V_{L2} may correspond to a first binding domain's variable domains.

[00121] In other embodiments, a multispecific binding protein is composed of dimerized single chain polypeptide chains comprising different single chain polypeptides (a heterodimer). In this format ("multispecific heterodimer"), each polypeptide chain comprising a binding domain, N-terminus linker, constant region, C-terminus linker and optionally another binding domain and does further include a heterodimerization domain. In certain variations, the second polypeptide chain for heterodimerization includes additional binding domains. Accordingly, in certain embodiments of the present invention, a heterodimeric recombinant protein may contain two, three or four binding different binding domains. See PCT publication No. WO2011/090762 for some examples and related methods.

[00122] As indicated above, a CD3 binding polypeptide of the present disclosure comprises a binding domain that specifically binds CD3. In some variations, the CD3 binding domain is capable of competing for binding to CD3 with a single-chain Fv (scFv) having an amino acid sequence as shown in SEQ ID NO:41. In particular embodiments, the CD3 binding domain comprises (i) an immunoglobulin heavy chain variable region (V_H) comprising CDRs HCDR1, HCDR2, and HCDR3, and (ii) an immunoglobulin light chain variable region (V_L) comprising CDRs LCDR1, LCDR2, and LCDR3. Suitable CD3 binding domains include those having V_L and V_H regions derived from murine monoclonal antibody Cris-7 (Reinherz, E. L. et al. (eds.), Leukocyte typing II., Springer Verlag, New York, (1986). In some such embodiments, LCDR3 has the amino acid sequence set forth in SEQ ID NO:54 and/or HCDR3 has the amino acid sequence set forth in SEQ ID NO:51; and LCDR1 and LCDR2 optionally have the amino acid sequences as set forth in SEQ ID NO:52 and SEQ ID NO:53, respectively, and HCDR1 and HCDR2 optionally have the amino acid sequences as set forth in SEQ ID NO:49 and SEQ ID NO:50, respectively. In some embodiments, for example, LCDR1, LCDR2, and LCDR3 have the amino acid sequences respectively shown in SEQ ID NOs:52, 53, and 54; and/or HCDR1, HCDR2, and HCDR3 have the amino acid sequences as respectively shown in SEQ ID NOs:49, 50, and 51.

[00123] Exemplary anti-CD3 antibodies from which the binding domain of this disclosure may be derived include Cris-7 monoclonal antibody (Reinherz, E. L. *et al.* (eds.), *Leukocyte typing II.*, Springer Verlag, New York, (1986)

(V_L = QVLTQSPAIMSAFPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDSSKLASG VPARFSGSGSGTSSYSLTISSMETEDAATYYCQQWSRNPPTFGGGTKLQITR (SEQ ID NO:46) and

V_H = QVQLQQSGAELARPGASVKMSCKASGYTFTRSTMHWVKQRPGQGLEWIGYINPSSA YTNYNQKFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCASPQVHYDYNQFPYWGGQTL VTVSA (SEQ ID NO:45)); HuM291 (Chau *et al.* (2001) *Transplantation* 71:941-950

(V_L = DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKVEIK (SEQ ID NO:72) and

V_H = QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSG YTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYDYDGFAYWGQGTLLV TVSS (SEQ ID NO:73); BC3 monoclonal antibody (Anasetti *et al.* (1990) *J. Exp. Med.*

172:1691); OKT3 monoclonal antibody (Ortho multicenter Transplant Study Group (1985) *N. Engl. J. Med.* 313:337) and derivatives thereof such as OKT3 ala-ala (also referred to as

OKT3 AA-FL or OKT3 FL), a humanized, Fc variant with alanine substitutions at positions 234 and 235 (Herold *et al.* (2003) *J. Clin. Invest.* 11:409); visilizumab (Carpenter *et al.*

(2002) *Blood* 99:2712), G19-4 monoclonal antibody (Ledbetter *et al.*, 1986, *J. Immunol.*

136:3945) and 145-2C11 monoclonal antibody (Hirsch *et al.* (1988) *J. Immunol.* 140: 3766).

An exemplary anti-TCR antibody is the BMA031 monoclonal antibody (Borst *et al.* (1990) *Human Immunology* 29:175-188).

[00124] In some embodiments, a binding domain is a single-chain Fv fragment (scFv) that comprises V_H and V_L regions specific for a target of interest. In certain embodiments, the V_H and V_L regions are human.

[00125] In one embodiment of the invention, CD3 binding polypeptides have an empirical and/or theoretical pI that is at least 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5 or more units less than that of the DRA209 SMIP. In some embodiments, a CD3 binding polypeptide of the invention has an empirical and/or theoretical pI that is from about 0.25 to about 2.5, 0.5 to about 2.5, about 0.5 to about 2.0, about 0.5 to about 1.5, about 0.5 to about 1.25, about 0.5 to about 1.0, about 0.5 to about 0.75, about 0.75 to about 1.0, about 0.75 to about 1.25, about 0.75 to about 1.5, about 0.75 to about 2.0, about 1.0 to about 1.5, about 1.0 to about 2.0, or about 1.0 to about 2.5 units less than that of the DRA209 SMIP. In some embodiments, the CD3 polypeptides of the invention have an empirical or theoretical pI that is less than about 8.9, 8.8, 8.7, 8.6, 8.5, 8.4, 8.3, 8.2, 8.1, 8, 7.5, 7.25, 7.0 or 6.75. In some embodiments, the CD3 polypeptides of the invention have an empirical or theoretical pI that

is about 8.9, 8.8, 8.7, 8.6, 8.5, 8.4, 8.3, 8.2, 8.1, 8, 7.5, 7.25, 7.2, 7.0, 6.8 or 6.75. In some embodiments, the CD3 polypeptides of the invention have an empirical or theoretical pI that is from about 7.9 to about 8.7, 7.9 to about 8.6, 8.0 to about 8.6, 8.0 to about 8.5, 8.0 to about 8.4, 8.0 to about 8.3, 8.0 to about 8.2, 8.0 to about 8.1, about 8.1 to about 8.6, about 8.2 to about 8.6, about 8.3 to about 8.6, about 8.4 to about 8.6, about 8.5 to about 8.6, about 8.5 to about 8.3, about 8.4 to about 8.2, or about 8.3 to about 8.1.

[00126] In certain embodiments, a CD3 binding domain comprises or is a scFv with a reduced pI (empirical and/or theoretical) by at least about 0.25, 0.5, 1, 1.25, 1.5, 2, 2.5 or more units as compared to the scFv of SEQ ID NO:41. In some embodiments, a CD3 binding domain comprises or is a scFv with a reduced pI (empirical and/or theoretical) that is from about 0.25 to about 2.5, 0.5 to about 2.5, about 0.5 to about 2.0, about 0.5 to about 1.5, about 0.5 to about 1.25, about 0.5 to about 1.0, about 0.5 to about 0.75, about 0.75 to about 1.0, about 0.75 to about 1.25, about 0.75 to about 1.5, about 0.75 to about 2.0, about 1.0 to about 1.5, about 1.0 to about 2.0, or about 1.0 to about 2.5 units less than that of the scFv of SEQ ID NO:41. Such scFvs include the scFvs contained within SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20 or an scFv that is at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.5%, or 100% identical to an amino acid sequence of scFvs contained within SEQ ID NOs: 6, 8, 10, 12, 14, 16, 18, and 20.

[00127] In some embodiments, a CD3 binding domain of the invention is about 80% to about 99%, about 82% to about 99%, about 84% to about 99%, about 86% to about 99%, about 88% to about 99%, about 90% to about 99%, about 92% to about 99%, about 94% to about 99%, about 96% to about 99%, about 97% to about 99%, about 98% to about 99%, about 80% to about 85%, about 85% to about 90%, about 85% to about 95%, about 90% to about 97%, about 90% to about 95%, about 90% to about 93%, about 90% to about 91%, about 95% to about 96%, about 96% to about 97%, about 97% to about 98%, about 96% to about 97% or about 96% to about 98% identical to SEQ ID NO:41.

[00128] In some embodiments, a CD3 binding domain of the invention is a variant of SEQ ID NO:41, wherein one or more amino acids in the amino acid sequence have been mutated to decrease the pI of the binding domain. Mutations include substitution of an amino acid(s) with another amino acid(s) to lower the pI of the binding domain, insertion of an amino acid(s) to lower the pI of the binding molecule and/or deletion of an amino acid(s) to lower the pI of the binding domain. In one embodiment, the mutations are substitutions. In some embodiments, the mutation(s) are in the framework regions of the variable chain(s). In some embodiments, a variant has a mutation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids

[00129] In one embodiment, the CD3 binding domain comprises or is a scFv comprising a heavy chain or a light chain selected from the group consisting of SEQ ID NOs: 28, 30, 32, 38 and 40. In another embodiment, the CD3 binding domain comprises a VH region selected from the group consisting of SEQ ID NOs: 22, 24, 26, 28, 30 and 32, and a VL region selected from the group consisting of SEQ ID NOs: 38 and 40. In another embodiment, the CD3 binding domain comprises a VH region selected from the group consisting of SEQ ID NOs: 28, 30 and 32 and a VL region selected from the group consisting of SEQ ID NOs: 34, 36, 38 and 40. In another embodiment, the CD3 binding domain comprises SEQ ID NOs: 28 and 34. In another embodiment, the CD3 binding domain comprises SEQ ID NOs: 28 and 38. In another embodiment, the CD3 binding domain comprises SEQ ID NOs: 26 and 38. In another embodiment, the CD3 binding domain comprises SEQ ID NOs: 26 and 34. In some embodiments, a CD3 binding domain comprises a VL region selected from the group consisting of SEQ ID NOs: 38 and 40 and a VH region selected from the group consisting of SEQ ID NOs: 22, 24, 26, 28, 30 and 32. In further embodiments, each CDR comprises no more than one, two, or three substitutions, insertions or deletions, as compared to that from a monoclonal antibody or fragment or derivative thereof that specifically binds to a target of interest (e.g., CD3).

[00130] In some embodiments, a CD3 binding domain binds or interacts with the CD3 ϵ subunit of the T-cell receptor complex on T-cells. In some embodiments, the CD3 binding domain competes for binding to CD3 ϵ with the Cris-7 or HuM291 monoclonal antibody.

[00131] In some embodiments, a CD3 binding polypeptide induces internalization of a T-cell receptor complex.

[00132] In some variations, the binding domain is a single-chain Fv (scFv) comprising immunoglobulin V_L and V_H regions joined by a peptide linker. The use of peptide linkers for joining V_L and V_H regions is well-known in the art, and a large number of publications exist within this particular field. A widely used peptide linker is a 15mer consisting of three repeats of a Gly-Gly-Gly-Gly-Ser (SEQ ID NO:74) amino acid sequence (Gly₄Ser)₃ (SEQ ID NO:76). In some embodiments, a peptide linker comprises or consists of any one of SEQ ID NOs:74-78. Other linkers have been used, and phage display technology, as well as selective infective phage technology, has been used to diversify and select appropriate linker sequences (Tang *et al.*, *J. Biol. Chem.* 271, 15682-15686, 1996; Hennecke *et al.*, *Protein Eng.* 11, 405-410, 1998). In certain embodiments, the V_H and V_L regions are joined by a peptide linker having an amino acid sequence comprising the formula (Gly₄Ser)_n, wherein n=1-5 (SEQ ID NOs:74-78, respectively). Other suitable linkers may be obtained by optimizing a simple linker (e.g., (Gly₄Ser)_n) through random mutagenesis.

[00133] In particular embodiments, a binding domain comprises humanized immunoglobulin V_L and/or V_H regions. Techniques for humanizing immunoglobulin V_L and V_H regions are known in the art and are discussed, for example, in United States Patent Application Publication No. 2006/0153837.

[00134] "Humanization" is expected to result in an antibody that is less immunogenic, with complete retention of the antigen-binding properties of the original molecule. In order to retain all of the antigen-binding properties of the original antibody, the structure of its antigen binding site should be reproduced in the "humanized" version. This can be achieved by grafting only the nonhuman CDRs onto human variable framework domains and constant regions, with or without retention of critical framework residues (Jones *et al.*, *Nature* 321:522 (1986); Verhoeven *et al.*, *Science* 239:1539 (1988)) or by recombining the entire nonhuman variable domains (to preserve ligand-binding properties), but "cloaking" them with a human-like surface through judicious replacement of exposed residues (to reduce antigenicity) (Padlan, *Molec. Immunol.* 28:489 (1991)).

[00135] Essentially, humanization by CDR grafting involves recombining only the CDRs of a non-human antibody onto a human variable region framework and a human constant region. Theoretically, this should substantially reduce or eliminate immunogenicity (except if allotypic or idiotypic differences exist). However, it has been reported that some framework residues of the original antibody also may need to be preserved (Reichmann *et al.*, *Nature*, 332:323 (1988); Queen *et al.*, *Proc. Natl. Acad. Sci. USA*, 86:10,029 (1989)).

[00136] The framework residues that need to be preserved are amenable to identification through computer modeling. Alternatively, critical framework residues may potentially be identified by comparing known antigen-binding site structures (Padlan, *Molec. Immunol.*, 31(3):169-217 (1994), incorporated herein by reference).

[00137] The residues that potentially affect antigen binding fall into several groups. The first group comprises residues that are contiguous with the antigen site surface, which could therefore make direct contact with antigens. These residues include the amino-terminal residues and those adjacent to the CDRs. The second group includes residues that could alter the structure or relative alignment of the CDRs, either by contacting the CDRs or another peptide chain in the antibody. The third group comprises amino acids with buried side chains that could influence the structural integrity of the variable domains. The residues in these groups are usually found in the same positions (Padlan, 1994, *supra*) although their positions as identified may differ depending on the numbering system (see Kabat *et al.*, "Sequences of proteins of immunological interest, 5th ed., Pub. No. 91-3242, U.S. Dept. Health & Human Services, NIH, Bethesda, Md., 1991).

[00138] Although some examples described herein involve the humanization of scFv, SMIP, Scorpion, and Interceptor molecules, and not antibodies, knowledge about humanized antibodies in the art is applicable to the polypeptides according to the invention.

[00139] In certain embodiments, a hinge is a wild-type human immunoglobulin hinge region. In certain other embodiments, one or more amino acid residues may be added at the amino- or carboxyl-terminus of a wild type immunoglobulin hinge region as part of a fusion protein construct design. For example, additional junction amino acid residues at the hinge amino-terminus can be "RT," "RSS," "TG," or "T," or at the hinge carboxyl-terminus can be "SG", or a hinge deletion can be combined with an addition, such as Δ P with "SG" added at the carboxyl-terminus.

[00140] In certain embodiments, a hinge is an altered immunoglobulin hinge in which one or more cysteine residues in a wild type immunoglobulin hinge region is substituted with one or more other amino acid residues (*e.g.*, serine or alanine).

[00141] Exemplary altered immunoglobulin hinges include, but are not limited to, an immunoglobulin human IgG1 hinge region having one, two or three cysteine residues found in a wild type human IgG1 hinge substituted by one, two or three different amino acid residues (*e.g.*, serine or alanine). An altered immunoglobulin hinge may additionally have a proline substituted with another amino acid (*e.g.*, serine or alanine). For example, the above-described altered human IgG1 hinge may additionally have a proline located carboxyl-terminal to the three cysteines of wild type human IgG1 hinge region substituted by another amino acid residue (*e.g.*, serine, alanine). In one embodiment, the prolines of the core hinge region are not substituted.

[00142] In certain embodiments, a hinge polypeptide comprises or is a sequence that is at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to a wild type immunoglobulin hinge region, such as a wild type human IgG1 hinge, a wild type human IgG2 hinge, or a wild type human IgG4 hinge.

[00143] In further embodiments, a hinge present in a CD3 binding polypeptide may be a hinge that is not based on or derived from an immunoglobulin hinge (*i.e.*, not a wild-type immunoglobulin hinge or an altered immunoglobulin hinge). Examples for such hinges include peptides of about five to about 150 amino acids derived from an interdomain region of a transmembrane protein or stalk region of a type II C-lectin, for instance, peptides of about eight to 25 amino acids and peptides of about seven to 18 amino acids.

[00144] In certain embodiments, interdomain or stalk region hinges have seven to 18 amino acids and can form an α -helical coiled coil structure. In certain embodiments, interdomain or stalk region hinges contain 0, 1, 2, 3, or 4 cysteines. Exemplary interdomain

or stalk region hinges are peptide fragments of the interdomain or stalk regions, such as ten to 150 amino acid fragments from the stalk regions of CD69, CD72, CD94, NKG2A and NKG2D.

[00145] In certain embodiments, hinge sequences have about 5 to 150 amino acids, 5 to 10 amino acids, 10 to 20 amino acids, 20 to 30 amino acids, 30 to 40 amino acids, 40 to 50 amino acids, 50 to 60 amino acids, 5 to 60 amino acids, 5 to 40 amino acids, 8 to 20 amino acids, or 10 to 15 amino acids. The hinge may be primarily flexible, but may also provide more rigid characteristics or may contain primarily α -helical structure with minimal β -sheet structure. The lengths or the sequences of the hinges may affect the binding affinities of the binding domains to which the hinges are directly or indirectly (via another region or domain, such as an heterodimerization domain) connected as well as one or more activities of the Fc region portions to which the hinges are directly or indirectly connected.

[00146] In certain embodiments, hinge sequences are stable in plasma and serum and are resistant to proteolytic cleavage. The first lysine in the IgG1 upper hinge region may be mutated to minimize proteolytic cleavage, for instance, the lysine may be substituted with methionine, threonine, alanine or glycine, or is deleted.

[00147] In some embodiments of the invention, the CD3 binding polypeptide is capable of forming a heterodimer with a second polypeptide chain and comprises a hinge region: (a) immediately amino-terminal to an immunoglobulin constant region (e.g., amino-terminal to a CH2 domain wherein the immunoglobulin constant region includes CH2 and CH3 domains, or amino-terminal to a CH3 domain wherein the immunoglobulin regions includes CH3 and CH4 domains), (b) interposed between and connecting a binding domain (e.g., scFv) and a immunoglobulin heterodimerization domain, (c) interposed between and connecting an immunoglobulin heterodimerization domain and an immunoglobulin constant region (e.g., wherein the immunoglobulin constant region includes CH2 and CH3 domains or CH3 and CH4 domains), (d) interposed between and connecting an immunoglobulin constant region and a binding domain, (e) at the amino-terminus of a polypeptide chain, or (f) at the carboxyl-terminus of a polypeptide chain. A polypeptide chain comprising a hinge region as described herein can be capable of associating with a different polypeptide chain to form a homodimeric or heterodimeric protein provided herein, and the dimer formed will contain a binding domain that retains its target specificity and/or its specific target binding affinity.

[00148] In certain embodiments, a hinge present in a polypeptide that forms a heterodimer with another polypeptide chain may be an immunoglobulin hinge, such as a wild-type immunoglobulin hinge region or an altered immunoglobulin hinge region thereof. In certain embodiments, a hinge of one polypeptide chain of a heterodimeric protein is identical to a corresponding hinge of the other polypeptide chain of the heterodimer. In certain other

embodiments, a hinge of one chain is different from that of the other chain (e.g., in their length and/or sequence). The different hinges in the different chains allow different manipulation of the binding affinities of the binding domains to which the hinges are connected, so that the heterodimer is able to bind to the target of one binding domain over the target of the other binding domain. For example, in certain embodiments, a heterodimeric protein has a CD3- or TCR-binding domain in one chain and a second binding domain (such as a tumor antigen binding domain) in another chain. Having two different hinges in the two chains may allow the heterodimer to bind to the tumor antigen first, and then to a CD3 or other TCR component second. Thus, the heterodimer may recruit CD3⁺ T-cells to tumor antigen-expressing cells (e.g., PSMA-expressing tumor cells), which in turn may damage or destroy the tumor antigen-expressing cells.

[00149] Exemplary hinge regions suitable for use in accordance with the present invention are shown in the Tables 1 and 2 below.

Table 1: Exemplary hinge regions

Hinge Region	Amino Acid Sequence
sss(s)-hlgG1 hinge	EPKSSDKTHTSPPSS (SEQ ID NO:112)
csc(s)-hlgG1 hinge	EPKSCDKTHTSPPCS (SEQ ID NO:113)
ssc(s)-hlgG1 hinge	EPKSSDKTHTSPPCS (SEQ ID NO:114)
scc(s)-hlgG1 hinge	EPKSSDKTHTCPPCS (SEQ ID NO:115)
css(s)-hlgG1 hinge	EPKSCDKTHTSPPSS (SEQ ID NO:116)
scs(s)-hlgG1 hinge	EPKSSDKTHTCPPSS (SEQ ID NO:117)
ccc(s)-hlgG1 hinge	EPKSCDKTHTSPPCS (SEQ ID NO:118)
ccc(p)-hlgG1 hinge	EPKSCDKTHTSPPCP (SEQ ID NO:119)
sss(p)-hlgG1 hinge	EPKSSDKTHTSPPSP (SEQ ID NO:120)
csc(p)-hlgG1 hinge	EPKSCDKTHTSPPCP (SEQ ID NO:121)
ssc(p)-hlgG1 hinge	EPKSSDKTHTSPPCP (SEQ ID NO:122)
scc(p)-hlgG1 hinge	EPKSSDKTHTCPPCP (SEQ ID NO:123)
css(p)-hlgG1 hinge	EPKSCDKTHTSPPSP (SEQ ID NO:124)
scs(p)-hlgG1 hinge	EPKSSDKTHTCPPSP (SEQ ID NO:125)
Scppcp	SCPPCP (SEQ ID NO:126)
STD1	NYGGGGSGGGGSGGGGSGNS (SEQ ID NO:127)
STD2	NYGGGGSGGGGSGGGGSGNYGGGGSGGGGSGGGGSGNS (SEQ ID NO:128)
H1	NS (SEQ ID NO:129)
H2	GGGGSGNS (SEQ ID NO:130)
H3	NYGGGGSGNS (SEQ ID NO:131)
H4	GGGGSGGGGSGNS (SEQ ID NO:132)
H5	NYGGGGSGGGGSGNS (SEQ ID NO:133)
H6	GGGGSGGGGSGGGGSGNS (SEQ ID NO:134)
H7	GCPPCPNS (SEQ ID NO:135)

H9	GSPSPNS (SEQ ID NO:136)
(G4S)3	GGGGSGGGSGGGGS (SEQ ID NO:137)
H105	SGGGSGGGSGGGGS (SEQ ID NO:138)
(G4S)4	GGGGSGGGSGGGSGGGGS (SEQ ID NO:139)
H75 (NKG2A quadruple mutant)	QRHNNSSLNTGTQMAGHSPNS (SEQ ID NO:140)
H83 (NKG2A derived)	SSLNTGTQMAGHSPNS (SEQ ID NO:141)
H106 (NKG2A derived)	QRHNNSSLNTGTQMAGHS (SEQ ID NO:142)
H81 (NKG2D derived)	EVQIPLTESYSPNS (SEQ ID NO:143)
H91 (NKG2D derived)	NSLANQEVQIPLTESYSPNS (SEQ ID NO:144)
H94	SGGGSGGGSGGGGSPNS (SEQ ID NO:145)

Table 2: Exemplary hinge regions (derived from H7 hinge, stalk region of a type II C-lectin, or interdomain region of a type I transmembrane protein)

<u>Hinge Region</u>	<u>Amino Acid Sequence</u>	<u>Molecule and/or hinge from which derived</u>
H16	LSVKADFLTPSIGN (SEQ ID NO:146)	CD80
H17	LSVKADFLTPSISCPNPCNS (SEQ ID NO:147)	CD80 + H7
H18	LSVLANSQPEIGN (SEQ ID NO:148)	CD86
H19	LSVLANSQPEISCPNPCNS (SEQ ID NO:149)	CD86 + H7
H20	LKIQERVSKPKISNS (SEQ ID NO:150)	CD2
H21	LKIQERVSKPKISCPNPCNS (SEQ ID NO:151)	CD2 + H7
H22	LNVSERPFPPHIQNS (SEQ ID NO:152)	CD22
H23	LDVSERPFPPHIQSCPPNPCNS (SEQ ID NO:153)	CD22 + H7
H24	REQLAEVTLCLKANS (SEQ ID NO:154)	CD80
H25	REQLAEVTLCLKACPPNPCNS (SEQ ID NO:155)	CD80 + H7
H26	RIHQMNSELSVLANS (SEQ ID NO:156)	CD86
H27	RIHQMNSELSVLACPPNPCNS (SEQ ID NO:157)	CD86 + H7
H28	DTKGKNVLEKIFSNS (SEQ ID NO:158)	CD2
H30	LPPETQESQEVTLNS (SEQ ID NO:159)	CD22
H32	RIHLNVSERPFPPNS (SEQ ID NO:160)	CD22
H33	RIHLNVSERPFPPCPNPCNS (SEQ ID NO:161)	CD22 + H7
H36	GCPPCPGGGGSNS (SEQ ID NO:162)	H7
H40	GCPPCPANS (SEQ ID NO:163)	H7
H41	GCPPCPANS (SEQ ID NO:164)	H7
H42	GCPPCPNS (SEQ ID NO:165)	H7
H44	GGGASCPPCPGNS (SEQ ID NO:166)	H7
H45	GGGASCPPCAGNS (SEQ ID NO:167)	H7
H46	GGGASCPPCANS (SEQ ID NO:168)	H7
H47	LSVKADFLTPSIGN (SEQ ID NO:169)	CD80
H48	ADFLTPSIGN (SEQ ID NO:170)	CD80

H50	LSVLNFSQPEIGNS (SEQ ID NO:171)	CD86
H51	LSVLNFSQPEIGNS (SEQ ID NO:172)	CD86
H52	SQPEIVPISNS (SEQ ID NO:173)	CD86
H53	SQPEIVPISCPPCPNS (SEQ ID NO:174)	CD86 + H7
H54	SVLANFSQPEISCPPCPNS (SEQ ID NO:175)	CD86 + H7
H55	RIHQMNSELSVLANS (SEQ ID NO:176)	CD86
H56	QMNSELSVLANS (SEQ ID NO:177)	CD86
H57	VSERPFPNS (SEQ ID NO:178)	CD22
H58	KPFFTCGSADTCPNS (SEQ ID NO:179)	CD72
H59	KPFFTCGSADTCPNS (SEQ ID NO:180)	CD72
H60	QYNCPGQYTFSPNS (SEQ ID NO:181)	CD69
H61	EPAFTPGPNIELQKSDCPNS (SEQ ID NO:182)	CD94
H62	QRHNSSLNTRTQKARHCPNS (SEQ ID NO:183)	NKG2A
H63	NSLFNQEVQIPLTESYCPNS (SEQ ID NO:184)	NKG2D

[00150] In certain embodiments, a CD3 binding polypeptide or protein of the invention may comprise an “immunoglobulin dimerization domain” or “immunoglobulin heterodimerization domain.”

[00151] An “immunoglobulin dimerization domain” or “immunoglobulin heterodimerization domain,” as used herein, refers to an immunoglobulin domain of a polypeptide chain that interacts or associates with a second immunoglobulin domain of a second polypeptide chain, wherein the interaction of the first and second immunoglobulin heterodimerization domains substantially contributes to or efficiently promotes heterodimerization of the first and second polypeptide chains (*i.e.*, the formation of a dimer between two different polypeptide chains, which is also referred to as a “heterodimer” or “heterodimeric protein”). The interactions between immunoglobulin heterodimerization domains “substantially contributes to or efficiently promotes” the heterodimerization of first and second polypeptide chains if there is a statistically significant reduction in the dimerization between the first and second polypeptide chains in the absence of the immunoglobulin heterodimerization domain of the first polypeptide chain and/or the immunoglobulin heterodimerization domain of the second polypeptide chain. In certain embodiments, when the first and second polypeptide chains are co-expressed, at least 60%, at least about 60% to about 70%, at least about 70% to about 80%, at least 80% to about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the first and second polypeptide chains form heterodimers with each other. Representative immunoglobulin heterodimerization domains include an immunoglobulin CH1 domain, an immunoglobulin CL domain (*e.g.*, Cκ or Cλ isotypes), or derivatives thereof, including wild-type immunoglobulin

CH1 and CL domains and altered (or mutated) immunoglobulin CH1 and CL domains, such as provided herein.

[00152] Dimerization/heterodimerization domains may be used where it is desired to form heterodimers from two non-identical polypeptide chains, where one or both polypeptide chains comprise a binding domain. In certain embodiments, one polypeptide chain member of certain heterodimers described herein does not contain a binding domain. As indicated above, a heterodimeric protein of the present disclosure comprises an immunoglobulin heterodimerization domain in each polypeptide chain. The immunoglobulin heterodimerization domains in the polypeptide chains of a heterodimer are different from each other and thus may be differentially modified to facilitate heterodimerization of both chains and to minimize homodimerization of either chain. As shown in PCT Publication No. WO2011/090762, immunoglobulin heterodimerization domains provided herein allow for efficient heterodimerization between different polypeptides and facilitate purification of the resulting heterodimeric protein.

[00153] As provided herein, immunoglobulin heterodimerization domains useful for promoting heterodimerization of two different single chain polypeptides (e.g., one short and one long) according to the present disclosure include immunoglobulin CH1 and CL domains, for instance, human CH1 and CL domains. In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type CH1 domain, such as a wild type IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain. In further embodiments, an immunoglobulin heterodimerization domain is a wild-type human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain as set forth in SEQ ID NOS:114, 186-192 and 194, respectively, of PCT Publication No. WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type human IgG1 CH1 domain as set forth in SEQ ID NO:114 of WO2011/090762 (said sequence incorporated by reference herein), which is the same as SEQ ID NO:80 of the present application.

[00154] In further embodiments, an immunoglobulin heterodimerization domain is an altered immunoglobulin CH1 domain, such as an altered IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain. In certain embodiments, an immunoglobulin heterodimerization domain is an altered human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM CH1 domain. In still further embodiments, a cysteine residue of a wild-type CH1 domain (e.g., a human CH1) involved in forming a disulfide bond with a wild type immunoglobulin CL domain (e.g., a human CL) is deleted or substituted in the altered immunoglobulin CH1 domain such that a disulfide bond is not formed between the altered CH1 domain and the wild-type CL domain.

[00155] In certain embodiments, an immunoglobulin heterodimerization domain is a wild-type CL domain, such as a wild type C κ domain or a wild type C λ domain. In particular embodiments, an immunoglobulin heterodimerization domain is a wild type human C κ or human C λ domain as set forth in SEQ ID NOS:112 and 113, respectively, of WO2011/090762 (said sequences incorporated by reference herein), which are the same as SEQ ID NOs:81 & 82, respectively, of the present application. In further embodiments, an immunoglobulin heterodimerization domain is an altered immunoglobulin CL domain, such as an altered C κ or C λ domain, for instance, an altered human C κ or human C λ domain.

[00156] In certain embodiments, a cysteine residue of a wild-type CL domain (e.g., a human CL) involved in forming a disulfide bond with a wild type immunoglobulin CH1 domain (e.g., a human CH1) is deleted or substituted in the altered immunoglobulin CL domain. Such altered CL domains may further comprise an amino acid deletion at their amino-termini. An exemplary C κ domain is set forth in SEQ ID NO:141 of WO2011/090762 (said sequence incorporated by reference herein, which is SEQ ID NO:83 of the present application), in which the first arginine and the last cysteine of the wild type human C κ domain are both deleted. In certain embodiments, only the last cysteine of the wild type human C κ domain is deleted in the altered C κ domain because the first arginine deleted from the wild type human C κ domain may be provided by a linker that has an arginine at its carboxyl-terminus and links the amino-terminus of the altered C κ domain with another domain (e.g., an immunoglobulin sub-region, such as a sub-region comprising immunoglobulin CH2 and CH3 domains). An exemplary C λ domain is set forth in SEQ ID NO:140 of WO2011/090762 (said sequence incorporated by reference herein, which is SEQ ID NO:84 of the present application), in which the first arginine of a wild type human C λ domain is deleted and the cysteine involved in forming a disulfide bond with a cysteine in a CH1 domain is substituted by a serine.

[00157] In further embodiments, an immunoglobulin heterodimerization domain is an altered C κ domain that contains one or more amino acid substitutions, as compared to a wild type C κ domain, at positions that may be involved in forming the interchain-hydrogen bond network at a C κ -C κ interface. For example, in certain embodiments, an immunoglobulin heterodimerization domain is an altered human C κ domain having one or more amino acids at positions N29, N30, Q52, V55, T56, S68 or T70 that are substituted with a different amino acid. The numbering of the amino acids is based on their positions in the altered human C κ sequence as set forth in SEQ ID NO:141 of WO2011/090762 (said sequence incorporated by reference herein, which is SEQ ID NO:83 of the present application). In certain embodiments, an immunoglobulin heterodimerization domain is an altered human C κ domain having one, two, three or four amino acid substitutions at positions N29, N30, V55, or T70. The amino acid used as a substitute at the above-noted positions

may be an alanine, or an amino acid residue with a bulk side chain moiety such as arginine, tryptophan, tyrosine, glutamate, glutamine, or lysine. Additional amino acid residues that may be used to substitute amino acid residues of the wild type human Ck sequence at the above noted positions (e.g., N30) include aspartate, methionine, serine and phenylalanine. Exemplary altered human Ck domains are set forth in SEQ ID NOS:142-178 of WO2011/090762 (said sequences incorporated by reference herein). Altered human Ck domains are those that facilitate heterodimerization with a CH1 domain, but minimize homodimerization with another Ck domain. Representative altered human Ck domains are set forth in SEQ ID NOS:160 (N29W V55A T70A), 161 (N29Y V55A T70A), 202 (T70E N29A N30A V55A), 167 (N30R V55A T70A), 168 (N30K V55A T70A), 170 (N30E V55A T70A), 172 (V55R N29A N30A), 175 (N29W N30Y V55A T70E), 176 (N29Y N30Y V55A T70E), 177 (N30E V55A T70E), 178 (N30Y V55A T70E), 838 (N30D V55A T70E), 839 (N30M V55A T70E), 840 (N30S V55A T70E), and 841 (N30F V55A T70E) of WO2011/090762 (said sequences incorporated by reference herein).

[00158] In certain embodiments, in addition to or alternative to the mutations in Ck domains described herein, both the immunoglobulin heterodimerization domains (e.g., immunoglobulin CH1 and CL domains) of a polypeptide heterodimer have mutations so that the resulting immunoglobulin heterodimerization domains form salt bridges (*i.e.*, ionic interactions) between the amino acid residues at the mutated sites. For example, the immunoglobulin heterodimerization domains of a polypeptide heterodimer may be a mutated CH1 domain in combination with a mutated Ck domain. In the mutated CH1 domain, valine at position 68 (V68) of the wild type human CH1 domain is substituted by an amino acid residue having a negative charge (e.g., aspartate or glutamate), whereas leucine at position 29 (L29) of a mutated human Ck domain in which the first arginine and the last cysteine have been deleted is substituted by an amino acid residue having a positive charge (e.g., lysine, arginine or histidine). The charge-charge interaction between the amino acid residue having a negative charge of the resulting mutated CH1 domain and the amino acid residue having a positive charge of the resulting mutated Ck domain forms a salt bridge, which stabilizes the heterodimeric interface between the mutated CH1 and Ck domains. Alternatively, V68 of the wild type CH1 may be substituted by an amino acid residue having a positive charge, whereas L29 of a mutated human Ck domain in which the first arginine and the last cysteine have been deleted may be substituted by an amino acid residue having a negative charge. Exemplary mutated CH1 sequences in which V68 is substituted by an amino acid with either a negative or positive charge are set forth in SEQ ID NOS:844 and 845 of WO2011/090762 (said sequences incorporated by reference herein). Exemplary mutated Ck sequences in which L29 is substituted by an amino acid with either a negative or

positive charge are set forth in SEQ ID NOS:842 and 843 of WO2011/090762 (said sequences incorporated by reference herein).

[00159] Positions other than V68 of human CH1 domain and L29 of human Ck domain may be substituted with amino acids having opposite charges to produce ionic interactions between the amino acids in addition or alternative to the mutations in V68 of CH1 domain and L29 of Ck domain. Such positions can be identified by any suitable method, including random mutagenesis, analysis of the crystal structure of the CH1-Ck pair to identify amino acid residues at the CH1-Ck interface, and further identifying suitable positions among the amino acid residues at the CH1-Ck interface using a set of criteria (*e.g.*, propensity to engage in ionic interactions, proximity to a potential partner residue, etc.).

[00160] In certain embodiments, polypeptide heterodimers of the present disclosure contain only one pair of immunoglobulin heterodimerization domains. For example, a first chain of a polypeptide heterodimer may comprise a CH1 domain as an immunoglobulin heterodimerization domain, while a second chain may comprise a CL domain (*e.g.*, a Ck or CA) as an immunoglobulin heterodimerization domain. Alternatively, a first chain may comprise a CL domain (*e.g.*, a Ck or CA) as an immunoglobulin heterodimerization domain, while a second chain may comprise a CH1 domain as an immunoglobulin heterodimerization domain. As set forth herein, the immunoglobulin heterodimerization domains of the first and second chains are capable of associating to form a heterodimeric protein of this disclosure.

[00161] In certain other embodiments, heterodimeric proteins of the present disclosure may have two pairs of immunoglobulin heterodimerization domains. For example, a first chain of a heterodimer may comprise two CH1 domains, while a second chain may have two CL domains that associate with the two CH1 domains in the first chain. Alternatively, a first chain may comprise two CL domains, while a second chain may have two CH1 domains that associate with the two CL domains in the first chain. In certain embodiments, a first polypeptide chain comprises a CH1 domain and a CL domain, while a second polypeptide chain comprises a CL domain and a CH1 domain that associate with the CH1 domain and the CL domain, respectively, of the first polypeptide chain.

[00162] In the embodiments where a heterodimeric protein comprises only one heterodimerization pair (*e.g.*, one immunoglobulin heterodimerization domain in each chain), the immunoglobulin heterodimerization domain of each chain may be located amino-terminal to the immunoglobulin constant sub-region of that chain. Alternatively, the immunoglobulin heterodimerization domain in each chain may be located carboxyl-terminal to the immunoglobulin constant sub-region of that chain.

[00163] In the embodiments where a heterodimeric protein comprises two heterodimerization pairs (*e.g.*, two immunoglobulin heterodimerization domains in each chain), both immunoglobulin heterodimerization domains in each chain may be located

amino-terminal to the immunoglobulin constant sub-region of that chain. Alternatively, both immunoglobulin heterodimerization domains in each chain may be located carboxyl-terminal to the immunoglobulin constant sub-region of that chain. In further embodiments, one immunoglobulin heterodimerization domain in each chain may be located amino-terminal to the immunoglobulin constant sub-region of that chain, while the other immunoglobulin heterodimerization domain of each chain may be located carboxyl-terminal to the immunoglobulin constant sub-region of that chain. In other words, in those embodiments, the immunoglobulin constant sub-region is interposed between the two immunoglobulin heterodimerization domains of each chain.

[00164] As indicated herein, in certain embodiments, CD3 binding polypeptides of the present disclosure (e.g., small modular immunopharmaceutical proteins (SMIPs), homodimer bispecifics (e.g., Scorpion), heterodimer mono- and multi-specific therapeutics (e.g., Interceptor) comprise an immunoglobulin constant region in each polypeptide chain. The term "constant region" is used interchangeably with constant sub-region and immunoglobulin constant region herein. The inclusion of an immunoglobulin constant region can slow clearance of the homodimeric and heterodimeric proteins formed from two CD3 binding polypeptide chains from circulation after administration to a subject.

[00165] By mutations or other alterations, the immunoglobulin constant region further enables relatively easy modulation of dimeric polypeptide effector functions (e.g., ADCC, ADCP, CDC, complement fixation, and binding to Fc receptors), which can either be increased or decreased depending on the disease being treated, as known in the art and described herein.

[00166] In certain embodiments, an immunoglobulin constant region of one or both of the polypeptide chains of the polypeptide homodimers and heterodimers of the present disclosure will be capable of mediating one or more of these effector functions.

[00167] In other embodiments, one or more of these effector functions are reduced or absent in an immunoglobulin constant region of one or both of the polypeptide chains of the polypeptide homodimers and heterodimers of the present disclosure, as compared to a corresponding wild-type immunoglobulin constant region. For instance, it is particularly useful for monospecific CD3 binding polypeptides as well as CD3 binding polypeptides comprising a second binding domain, e.g., designed to elicit redirected T-cell toxicity (RTCC), to have an immunoglobulin constant region with reduced or no effector function relative to a corresponding wild-type immunoglobulin constant region. In one embodiment, the constant region is modified to not fix complement. The constant region can also be modified to not bind to one or more Fc γ receptors such as CD16, CD32 and CD64.

[00168] An immunoglobulin constant sub-region present in CD3 binding polypeptides of the present disclosure may comprise of or is derived from part or all of: a

CH2 domain, a CH3 domain, a CH4 domain, or any combination thereof. For example, an immunoglobulin constant sub-region may comprise a CH2 domain, a CH3 domain, both CH2 and CH3 domains, both CH3 and CH4 domains, two CH3 domains, a CH4 domain, two CH4 domains, and a CH2 domain and part of a CH3 domain. In some embodiments, a constant region comprises CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD or any combination thereof; an immunoglobulin CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM or any combination thereof; or immunoglobulin CH3 and CH4 domains of IgE, IgM or a combination thereof. In some embodiments, a constant region consists essentially of a CH2 domain and CH3 domain.

[00169] A CH2 domain that may form an immunoglobulin constant region of a CD3 binding polypeptide of the present disclosure may be a wild type immunoglobulin CH2 domain or an altered immunoglobulin CH2 domain thereof from certain immunoglobulin classes or subclasses (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD) and from various species (including human, mouse, rat, and other mammals). In some embodiments, a constant region is modified to reduce or eliminate effector function, reduce or not fix complement and/or reduce binding to or not bind to Fc γ receptors. In some embodiments, an Fc γ receptor is selected from the group consisting of CD16, CD32 and CD64.

[00170] In certain embodiments, a CH2 domain is a wild type human immunoglobulin CH2 domain, such as wild type CH2 domains of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, or IgD, as set forth in SEQ ID NOS:115, 199-201 and 195-197, respectively, of PCT Publication WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, the CH2 domain is a wild type human IgG1 CH2 domain as set forth in SEQ ID NO:115 of WO2011/090762 (said sequence incorporated by reference herein, which is SEQ ID NO:85 of the present application). In some embodiments, a constant region comprises or consists of the amino acid sequence of SEQ ID NO:55

[00171] In certain embodiments, a CH2 domain is an altered immunoglobulin CH2 region (e.g., an altered human IgG1 CH2 domain) that comprises an amino acid substitution at the asparagine of position 297 (e.g., asparagine to alanine). Such an amino acid substitution reduces or eliminates glycosylation at this site and abrogates efficient Fc binding to Fc γ R and C1q. The sequence of an altered human IgG1 CH2 domain with an Asn to Ala substitution at position 297 is set forth in SEQ ID NO:324 of WO2011/090762 said (sequence incorporated by reference herein, which is SEQ ID NO:86 of the present application).

[00172] In certain embodiments, a CH2 domain is an altered immunoglobulin CH2 region (e.g., an altered human IgG1 CH2 domain) that comprises at least one substitution or deletion at positions 234 to 238. For example, an immunoglobulin CH2 region can comprise

a substitution at position 234, 235, 236, 237 or 238, positions 234 and 235, positions 234 and 236, positions 234 and 237, positions 234 and 238, positions 234-236, positions 234, 235 and 237, positions 234, 236 and 238, positions 234, 235, 237, and 238, positions 236-238, or any other combination of two, three, four, or five amino acids at positions 234-238. In addition or alternatively, an altered CH2 region may comprise one or more (e.g., two, three, four or five) amino acid deletions at positions 234-238, for instance, at one of position 236 or position 237 while the other position is substituted. The above-noted mutation(s) decrease or eliminate the antibody-dependent cell-mediated cytotoxicity (ADCC) activity or Fc receptor-binding capability of a polypeptide heterodimer that comprises the altered CH2 domain. In certain embodiments, the amino acid residues at one or more of positions 234-238 has been replaced with one or more alanine residues. In further embodiments, only one of the amino acid residues at positions 234-238 have been deleted while one or more of the remaining amino acids at positions 234-238 can be substituted with another amino acid (e.g., alanine or serine).

[00173] In certain other embodiments, a CH2 domain is an altered immunoglobulin CH2 region (e.g., an altered human IgG1 CH2 domain) that comprises one or more amino acid substitutions at positions 253, 310, 318, 320, 322, and 331. For example, an immunoglobulin CH2 region can comprise a substitution at position 253, 310, 318, 320, 322, or 331, positions 318 and 320, positions 318 and 322, positions 318, 320 and 322, or any other combination of two, three, four, five or six amino acids at positions 253, 310, 318, 320, 322, and 331. The above-noted mutation(s) decrease or eliminate the complement-dependent cytotoxicity (CDC) of a polypeptide heterodimer that comprises the altered CH2 domain.

[00174] In certain other embodiments, in addition to the amino acid substitution at position 297, an altered CH2 region (e.g., an altered human IgG1 CH2 domain) can further comprise one or more (e.g., two, three, four, or five) additional substitutions at positions 234-238. For example, an immunoglobulin CH2 region can comprise a substitution at positions 234 and 297, positions 234, 235, and 297, positions 234, 236 and 297, positions 234-236 and 297, positions 234, 235, 237 and 297, positions 234, 236, 238 and 297, positions 234, 235, 237, 238 and 297, positions 236-238 and 297, or any combination of two, three, four, or five amino acids at positions 234-238 in addition to position 297. In addition or alternatively, an altered CH2 region may comprise one or more (e.g., two, three, four or five) amino acid deletions at positions 234-238, such as at position 236 or position 237. The additional mutation(s) decreases or eliminates the antibody-dependent cell-mediated cytotoxicity (ADCC) activity or Fc receptor-binding capability of a polypeptide heterodimer that comprises the altered CH2 domain. In certain embodiments, the amino acid residues at one or more of positions 234-238 have been replaced with one or more alanine residues. In

further embodiments, only one of the amino acid residues at positions 234-238 has been deleted while one or more of the remaining amino acids at positions 234-238 can be substituted with another amino acid (e.g., alanine or serine).

[00175] In certain embodiments, in addition to one or more (e.g., 2, 3, 4, or 5) amino acid substitutions at positions 234-238, a mutated CH2 region (e.g., an altered human IgG1 CH2 domain) in a fusion protein of the present disclosure may contain one or more (e.g., 2, 3, 4, 5, or 6) additional amino acid substitutions (e.g., substituted with alanine) at one or more positions involved in complement fixation (e.g., at positions I253, H310, E318, K320, K322, or P331). Examples of mutated immunoglobulin CH2 regions include human IgG1, IgG2, IgG4 and mouse IgG2a CH2 regions with alanine substitutions at positions 234, 235, 237 (if present), 318, 320 and 322. An exemplary mutated immunoglobulin CH2 region is mouse IGHG2c CH2 region with alanine substitutions at L234, L235, G237, E318, K320, and K322.

[00176] In still further embodiments, in addition to the amino acid substitution at position 297 and the additional deletion(s) or substitution(s) at positions 234-238, an altered CH2 region (e.g., an altered human IgG1 CH2 domain) can further comprise one or more (e.g., two, three, four, five, or six) additional substitutions at positions 253, 310, 318, 320, 322, and 331. For example, an immunoglobulin CH2 region can comprise a (1) substitution at position 297, (2) one or more substitutions or deletions or a combination thereof at positions 234-238, and one or more (e.g., 2, 3, 4, 5, or 6) amino acid substitutions at positions I253, H310, E318, K320, K322, and P331, such as one, two, three substitutions at positions E318, K320 and K322. The amino acids at the above-noted positions may be substituted by alanine or serine.

[00177] In certain embodiments, an immunoglobulin CH2 region polypeptide comprises: (i) an amino acid substitution at the asparagines of position 297 and one amino acid substitution at position 234, 235, 236 or 237; (ii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at two of positions 234-237; (iii) an amino acid substitution at the asparagine of position 297 and amino acid substitutions at three of positions 234-237; (iv) an amino acid substitution at the asparagine of position 297, amino acid substitutions at positions 234, 235 and 237, and an amino acid deletion at position 236; (v) amino acid substitutions at three of positions 234-237 and amino acid substitutions at positions 318, 320 and 322; or (vi) amino acid substitutions at three of positions 234-237, an amino acid deletion at position 236, and amino acid substitutions at positions 318, 320 and 322.

[00178] Exemplary altered immunoglobulin CH2 regions with amino acid substitutions at the asparagine of position 297 include: human IgG1 CH2 region with alanine substitutions at L234, L235, G237 and N297 and a deletion at G236 (SEQ ID NO:325 of

WO2011/090762, said sequence incorporated by reference herein), human IgG2 CH2 region with alanine substitutions at V234, G236, and N297 (SEQ ID NO:326 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at F234, L235, G237 and N297 and a deletion of G236 (SEQ ID NO:322 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at F234 and N297 (SEQ ID NO:343 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at L235 and N297 (SEQ ID NO:344 of WO2011/090762, said sequence incorporated by reference herein), human IgG4 CH2 region with alanine substitutions at G236 and N297 (SEQ ID NO:345 of WO2011/090762, said sequence incorporated by reference herein), and human IgG4 CH2 region with alanine substitutions at G237 and N297 (SEQ ID NO:346 of WO2011/090762, said sequence incorporated by reference herein).

[00179] In certain embodiments, in addition to the amino acid substitutions described above, an altered CH2 region (e.g., an altered human IgG1 CH2 domain) may contain one or more additional amino acid substitutions at one or more positions other than the above-noted positions. Such amino acid substitutions may be conservative or non-conservative amino acid substitutions. For example, in certain embodiments, P233 may be changed to E233 in an altered IgG2 CH2 region (see, e.g., SEQ ID NO:326 of WO2011/090762, said sequence incorporated by reference herein). In addition or alternatively, in certain embodiments, the altered CH2 region may contain one or more amino acid insertions, deletions, or both. The insertion(s), deletion(s) or substitution(s) may anywhere in an immunoglobulin CH2 region, such as at the N- or C-terminus of a wild type immunoglobulin CH2 region resulting from linking the CH2 region with another region (e.g., a binding domain or an immunoglobulin heterodimerization domain) via a hinge.

[00180] In certain embodiments, an altered CH2 region in a polypeptide of the present disclosure comprises or is a sequence that is at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to a wild type immunoglobulin CH2 region, such as the CH2 region of wild type human IgG1, IgG2, or IgG4, or mouse IgG2a (e.g., IGHG2c).

[00181] An altered immunoglobulin CH2 region in a CD3 binding polypeptide of the present disclosure may be derived from a CH2 region of various immunoglobulin isotypes, such as IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, and IgD, from various species (including human, mouse, rat, and other mammals). In certain embodiments, an altered immunoglobulin CH2 region in a fusion protein of the present disclosure may be derived from a CH2 region of human IgG1, IgG2 or IgG4, or mouse IgG2a (e.g., IGHG2c), whose sequences are set forth in SEQ ID NOS:115, 199, 201, and 320 of WO2011/090762 (said sequences incorporated by reference herein).

[00182] In certain embodiments, an altered CH2 domain is a human IgG1 CH2 domain with alanine substitutions at positions 235, 318, 320, and 322 (*i.e.*, a human IgG1 CH2 domain with L235A, E318A, K320A and K322A substitutions) (SEQ ID NO:595 of WO2011/090762, said sequence incorporated by reference herein), and optionally an N297 mutation (*e.g.*, to alanine). In certain other embodiments, an altered CH2 domain is a human IgG1 CH2 domain with alanine substitutions at positions 234, 235, 237, 318, 320 and 322 (*i.e.*, a human IgG1 CH2 domain with L234A, L235A, G237A, E318A, K320A and K322A substitutions) (SEQ ID NO:596 of WO2011/090762, said sequence incorporated by reference herein), and optionally an N297 mutation (*e.g.*, to alanine).

[00183] In certain embodiments, an altered CH2 domain is an altered human IgG1 CH2 domain with mutations known in the art that enhance immunological activities such as ADCC, ADCP, CDC, complement fixation, Fc receptor binding, or any combination thereof.

[00184] The CH3 domain that may form an immunoglobulin constant region of a CD3 binding polypeptide of the present disclosure may be a wild type immunoglobulin CH3 domain or an altered immunoglobulin CH3 domain thereof from certain immunoglobulin classes or subclasses (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM) of various species (including human, mouse, rat, and other mammals). In certain embodiments, a CH3 domain is a wild type human immunoglobulin CH3 domain, such as wild type CH3 domains of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM as set forth in SEQ ID NOS:116, 208-210, 204-207, and 212, respectively of WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, the CH3 domain is a wild type human IgG1 CH3 domain as set forth in SEQ ID NO:116 of WO2011/090762 (said sequence incorporated by reference herein). In certain embodiments, a CH3 domain is an altered human immunoglobulin CH3 domain, such as an altered CH3 domain based on or derived from a wild-type CH3 domain of human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM antibodies. For example, an altered CH3 domain may be a human IgG1 CH3 domain with one or two mutations at positions H433 and N434 (positions are numbered according to EU numbering). The mutations in such positions may be involved in complement fixation. In certain other embodiments, an altered CH3 domain may be a human IgG1 CH3 domain but with one or two amino acid substitutions at position F405 or Y407. The amino acids at such positions are involved in interacting with another CH3 domain. In certain embodiments, an altered CH3 domain may be an altered human IgG1 CH3 domain with its last lysine deleted. The sequence of this altered CH3 domain is set forth in SEQ ID NO:761 of WO2011/090762 (said sequence incorporated by reference herein).

[00185] In certain embodiments, CD3 binding polypeptides forming a polypeptide heterodimer comprise a CH3 pair that comprises so called "knobs-into-holes" mutations (see, Marvin and Zhu, *Acta Pharmacologica Sinica* 26:649-58, 2005; Ridgway *et al.*, *Protein*

Engineering 9:617-21, 1966). More specifically, mutations may be introduced into each of the two CH3 domains of each polypeptide chain so that the steric complementarity required for CH3/CH3 association obligates these two CH3 domains to pair with each other. For example, a CH3 domain in one single chain polypeptide of a polypeptide heterodimer may contain a T366W mutation (a "knob" mutation, which substitutes a small amino acid with a larger one), and a CH3 domain in the other single chain polypeptide of the polypeptide heterodimer may contain a Y407A mutation (a "hole" mutation, which substitutes a large amino acid with a smaller one). Other exemplary knobs-into-holes mutations include (1) a T366Y mutation in one CH3 domain and a Y407T in the other CH3 domain, and (2) a T366W mutation in one CH3 domain and T366S, L368A and Y407V mutations in the other CH3 domain.

[00186] The CH4 domain that may form an immunoglobulin constant region of CD3 binding polypeptides of the present disclosure may be a wild type immunoglobulin CH4 domain or an altered immunoglobulin CH4 domain thereof from IgE or IgM molecules. In certain embodiments, the CH4 domain is a wild type human immunoglobulin CH4 domain, such as wild type CH4 domains of human IgE and IgM molecules as set forth in SEQ ID NOS:213 and 214, respectively, of WO2011/090762 (said sequences incorporated by reference herein). In certain embodiments, a CH4 domain is an altered human immunoglobulin CH4 domain, such as an altered CH4 domain based on or derived from a CH4 domain of human IgE or IgM molecules, which have mutations that increase or decrease an immunological activity known to be associated with an IgE or IgM Fc region.

[00187] In certain embodiments, an immunoglobulin constant region of CD3 binding polypeptides of the present disclosure comprises a combination of CH2, CH3 or CH4 domains (*i.e.*, more than one constant region domain selected from CH2, CH3 and CH4). For example, the immunoglobulin constant sub-region may comprise CH2 and CH3 domains or CH3 and CH4 domains. In certain other embodiments, the immunoglobulin constant region may comprise two CH3 domains and no CH2 or CH4 domains (*i.e.*, only two or more CH3). The multiple constant region domains that form an immunoglobulin constant sub-region may be based on or derived from the same immunoglobulin molecule, or the same class or subclass immunoglobulin molecules. In certain embodiments, the immunoglobulin constant sub-region is an IgG CH2CH3 (*e.g.*, IgG1 CH2CH3, IgG2 CH2CH3, and IgG4 CH2CH3) and may be a human (*e.g.*, human IgG1, IgG2, and IgG4) CH2CH3. For example, in certain embodiments, the immunoglobulin constant sub-region comprises (1) wild type human IgG1 CH2 and CH3 domains, (2) human IgG1 CH2 with N297A substitution (*i.e.*, CH2(N297A)) and wild type human IgG1 CH3, or (3) human IgG1 CH2(N297A) and an altered human IgG1 CH3 with the last lysine deleted.

[00188] Alternatively, the multiple constant region domains may be based on or derived from different immunoglobulin molecules, or different classes or subclasses immunoglobulin molecules. For example, in certain embodiments, an immunoglobulin constant sub-region comprises both human IgM CH3 domain and human IgG1 CH3 domain. The multiple constant region domains that form an immunoglobulin constant sub-region may be directly linked together or may be linked to each other via one or more (e.g., about 2-10) amino acids.

[00189] Exemplary immunoglobulin constant sub-regions are set forth in SEQ ID NOS:305-309, 321, 323, 341, 342, and 762 of WO2011/090762 (said sequences incorporated by reference herein).

[00190] In certain embodiments, the immunoglobulin constant regions of both CD3 binding polypeptide chains of a homodimer or heterodimer are identical to each other. In certain other embodiments, the immunoglobulin constant sub-region of one polypeptide chain of a heterodimeric protein is different from the immunoglobulin constant sub-region of the other polypeptide chain of the heterodimer. For example, one immunoglobulin constant sub-region of a heterodimeric protein may contain a CH3 domain with a "knob" mutation, whereas the other immunoglobulin constant sub-region of the heterodimeric protein may contain a CH3 domain with a "hole" mutation.

[00191] Some embodiments of the invention involve using bispecific or multispecific binding molecules that (i) target a TCR complex (e.g., CD3) on human T-cells and (ii) a cell surface protein, e.g., to redirect T-cell cytotoxicity towards the cell with the cell surface protein, e.g., towards a tumor cell expressing a tumor antigen for the treatment of cancer.

[00192] In certain embodiments, a CD3 binding protein may comprise one or more additional binding domains (e.g., a second binding domain) that bind a target other than CD3. These other target molecules may comprise, for example, a tumor associated antigen. In one embodiment of the invention, the one or more additional binding domains bind or interact with one or more of the following tumor antigens: RON, c-Met, CEACAM-6, PSMA, EpCAM, CEA, PCTA-1, STEAP-1, STEAP-2, PSCA, PSA, PAP, ALCAM (CD166), PECAM-1, EphA2, CD151, CA-125/MUC16, MUC-1, MAGE-1, TROP2, IGF1R, TGFR2, GHRHR, GHR, IL-6R, gp130, TNFR2, OSMR β , Patched-1, Frizzled, Robo1, LT β R, CD19, CD25, CD26, CD27, CD30, CD33, CD44, CD44v6, CD63, CD80, CD81, CD86, CD100, CD151, CXCR4, CCR5, HER-2/ErbB1, HER-3/ErbB3, HER-4/ErbB4, EGFR/ErbB1, EGFRvIII isoform, MUC2, MUC3, MUC4, MUC5_{AC}, MUC5_b, MUC7, β hCG, Lewis-Y, ganglioside GD3, 9-O-Acetyl-GD3, GM2, Globo H, fucosyl GM1, Poly SA, GD2, Carbonic anhydrase IX (MN/CA IX), Sonic Hedgehog (Shh), Wue-1, Plasma Cell Antigen, (membrane-bound) IgE, Melanoma Chondroitin Sulfate Proteoglycan (MCSP), CCR8, TNF-alpha precursor, mesothelin, A33 Antigen, Ly-6; desmoglein 4, E-cadherin neoepitope, Fetal Acetylcholine

Receptor, CA19-9 marker, Muellierian Inhibitory Substance (MIS) Receptor type II, sTn (sialylated Tn antigen; TAG-72), FAP (fibroblast activation antigen), endosialin, LG, SAS, BCMA, TWEAKR/Fn14, FGFR4, VEGFR1, VEGFR2, SSX1, and SSX2.

[00193] In certain embodiments, the CD3 binding domain binds the TCR binding domain for recruitment of T-cells to target cells expressing a tumor antigen. In certain embodiments, a CD3 binding polypeptide may comprise a CD3 binding domain that specifically binds a TCR complex or a component thereof (e.g., TCR α , TCR β , CD3 γ , CD3 δ , and CD3 ϵ) and another binding domain that specifically binds to a tumor associated antigen.

[00194] PSMA is a highly restricted prostate-cancer-related cell membrane antigen. In prostate cancer cells, PSMA is expressed 1000-fold higher than on normal prostate epithelium (Su *et al.*, *Cancer Res.* 1995 44:1441-1443). Expression of PSMA increases with prostate cancer progression and is typically highest in metastatic disease, hormone refractory cases, and higher-grade lesions (Israeli *et al.*, *Cancer Res.* 1994, 54:1807-1811; Wright *et al.*, *Urologic Oncology: Seminars and Original Investigations* 1995 1:18-28; Wright *et al.*, *Urology* 1996 48:326-332; Sweat *et al.*, *Urology* 1998 52:637-640). Additionally, PSMA is abundantly expressed on the neovasculature of a variety of other solid tumors, including bladder, pancreas, melanoma, lung and kidney cancers, but not on normal neovasculature (Chang *et al.*, *Urology* 2001 57:801-805; Divgi *et al.*, *Clin. Cancer Res.* 1998 4:2729-3279). Binding domains for targeting PSMA include, but are not limited to, those described in PCT Publication No. WO2012/145714, which is herein incorporated by reference in its entirety.

[00195] In some embodiments, a PSMA binding domain comprises an amino acid sequence that is at least 90%, at least 95% or 100% identical to an amino acid sequence selected from the group consisting of (i) amino acids 1-107 and 124-243 of SEQ ID NO:212; (ii) amino acids 1-107 and 124-243 of SEQ ID NO:226; or (iii) amino acids 1-107 and 124-243 of SEQ ID NO:216. In some embodiments, a protein or molecule of the invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO:212, 214, 216 and 226. PSMA binding domains that can be used in accordance with the invention are also described in PCT Publication No. WO2012/145714 including PSMA binding domains comprising amino acid sequences SEQ ID NOs:19, 21, 30, 31, 34, or 35 of WO2012/145714 or comprises a VL chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5 and 23 and a VH chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2, 25 and 27 of WO2012/145714.

[00196] RON (recepteur d'origine Nantaise, also known as MST1R) is a receptor-type protein tyrosine kinase that is essential to embryonic development and also plays an important role in inflammatory responses (Camp *et al.* *Ann. Surg. Oncol.* 12:273-281

(2005)). RON is mostly expressed in epithelial-derived cell types, and it has been suggested that RON, like a number of other receptor-type tyrosine kinases, may play a role in the progression of malignant epithelial cancers (Wang et al. Carcinogenesis 23:1291-1297 (2003)). Activation of RON initiates downstream signaling pathways that have been implicated in tumorigenic activities such as cell proliferation, inhibition of apoptosis, and cell motility. RON represents a therapeutic target for epithelial cancers, *inter alia*, because of its signaling properties and/or the overexpression of RON in colorectal, breast, ovarian, and pancreatic carcinomas.

[00197] Binding domains for targeting RON include, but are not limited to, those described in the Examples section and those described in PCT Publication WO2011/090761 which is herein incorporated by reference in its entirety. In some embodiments, an anti-RON binding domain comprises (a) a VL domain comprising i. a CDR1 amino acid sequence of SEQ ID NO:87, a CDR2 amino acid sequence of SEQ ID NO:88, and a CDR3 amino acid sequence of SEQ ID NO:89; or ii. a CDR1 amino acid sequence of SEQ ID NO:90, a CDR2 amino acid sequence of SEQ ID NO:91, and a CDR3 amino acid sequence of SEQ ID NO:92; or (b) a VH domain comprising i. a CDR1 amino acid sequence of SEQ ID NO:93, a CDR2 amino acid sequence of SEQ ID NO:94, and a CDR3 amino acid sequence of SEQ ID NO:95; or ii. a CDR1 amino acid sequence of SEQ ID NO:96, a CDR2 amino acid sequence of SEQ ID NO:97, and a CDR3 amino acid sequence of SEQ ID NO:98; or (c) a VL of (a) and a VH of (b). In one embodiment, the VL domain comprises an amino acid sequence of any one of SEQ ID NOS:99 or 100, and the VH domain comprises an amino acid sequence of any one of SEQ ID NOS:101, 102 and 103. In another embodiment, the VL and VH domains are humanized. In certain embodiments, the humanized VL comprises an amino acid sequence of any one of SEQ ID NOS:104, 105 and 106, and the humanized VH domain comprises an amino acid sequence of any one of SEQ ID NOS:107-111. In some embodiments, an anti-RON binding domain comprises a VL domain comprising an amino acid sequence of any one of SEQ ID NO:99 and 100, and a VH domain comprising an amino acid sequence of any one of SEQ ID NO:101, 102 and 103. In some embodiments, an anti-RON binding domain comprises a VL domain comprising an amino acid sequence of any one of SEQ ID NOS:104, 105, and 106, and a VH domain comprising an amino acid sequence of any one of SEQ ID NOS:107-111.

[00198] In some embodiments, a RON binding domain comprises an amino acid sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:187 and/or comprises an amino acid sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:188. In some embodiments, a RON binding domain comprises (i) SEQ ID NO:187, wherein amino acid 43 (alanine) is substituted with another amino acid, e.g., with lysine (A43K) or threonine (A43T), (ii) SEQ ID NO:188,

wherein amino acid 38 (glutamine) and/or 113 (glutamine) is substituted with another amino acid, e.g., with glutamic acid and/or arginine such as Q38R and/or Q113E, or (iii) any combination thereof. In some embodiments, an anti-RON/anti-CD3 bispecific molecule comprises or consists of an amino acid sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOs:186, 190, 192 or 194. In some embodiments, the invention provides a nucleic acid that encodes an anti-RON/anti-CD3 bispecific molecule, wherein the nucleic acid comprises a nucleotide sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOs:185, 189, 191 or 193.

[00199] CD19 is found on the surface of B-cells including most malignant B cells. Targeting CD19 can be used to inhibit and treat B-cell related diseases, leukemias and lymphomas.

[00200] In some embodiments, a CD19 binding domain comprises an amino acid sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to amino acids 1-111 of SEQ ID NO:196 and/or comprises an amino acid sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to amino acids 128-251 of SEQ ID NO:196. In some embodiments, an anti-CD19/anti-CD3 bispecific molecule comprises or consists of an amino acid sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOs:196, 198, 200, 202, 204 or 206. In some embodiments, the invention provides a nucleic acid that encodes an anti-CD19/anti-CD3 bispecific molecule, wherein the nucleic acid comprises a nucleotide sequence that is at least 90%, 92%, 94%, 96%, 97%, 98%, 99% or 100% identical to any one of SEQ ID NOs:195, 197, 199, 201, 203 or 206.

[00201] HER2 is also known as human ErbB2. Overexpression of HER2 (frequently but not uniformly due to gene amplification) has also been observed in breast cancers, ovarian cancers and other carcinomas including carcinomas of the stomach, endometrium, salivary gland, lung, kidney, colon, thyroid, pancreas and bladder. Binding domains for targeting HER2 include, but are not limited to, those described in PCT Publication WO2009/055074 which is herein incorporated by reference in its entirety.

[00202] In some embodiments, a CD3 binding polypeptide comprises an amino acid sequence that is at least 90%, at least 95% or 100% identical to SEQ ID NO: 57, 59, 61, 63, 65, 67, 69, 71, 186, 190, 192, 194, 196, 198, 200, 202, 204 or 206.

[00203] In some embodiments, a CD3 binding polypeptide is part of a heterodimer. In some embodiments, a heterodimer comprises a pair of single chain polypeptides wherein the single chain pair comprises amino acid sequences that are at least 90%, at least 95% or 100% identical to the pairs selected from SEQ ID NO:210 & 247, SEQ ID NO:210 & 218, SEQ ID NO:210 & 220, SEQ ID NO:208 & 249, SEQ ID NO:208 & 222, or SEQ ID NO:208 &

224, SEQ ID NO:212 & 218, SEQ ID NO:216 & 222, SEQ ID NO:228 & 226, or SEQ ID NO:214 & 218.

[00204] The invention also includes nucleic acids (e.g., DNA or RNA) encoding a CD3 binding polypeptide as described herein, or one or more polypeptide chains of a dimeric or heterodimeric CD3 binding protein as described herein. Nucleic acids of the invention include nucleic acids having a region that is substantially identical to a polynucleotide as provided in SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, 19, 27, 29, 31, 37 and 39 or nucleic acids comprising a coding region that codes for the same or a similar polypeptide. In certain embodiments, a nucleic acid in accordance with the present invention has at least 80%, typically at least about 90%, and more typically at least about 95% or at least about 98% identity to a polypeptide-encoding polynucleotide as provided in SEQ ID NOs: 5, 7, 9, 11, 13, 15, 17, 19, 27, 29, 31, 37 and 39. Nucleic acids of the invention also include complementary nucleic acids. In some instances, the sequences will be fully complementary (no mismatches) when aligned. In other instances, there may be up to about a 20% mismatch in the sequences. In some embodiments of the invention are provided nucleic acids encoding both first and second polypeptide chains of a heterodimeric CD3 binding protein of the invention. The nucleic acid sequences provided herein can be exploited using codon optimization, degenerate sequence, silent mutations, and other DNA techniques to optimize expression in a particular host, and the present invention encompasses such sequence modifications.

[00205] Polynucleotide molecules comprising a desired polynucleotide sequence are propagated by placing the molecule in a vector. The invention also includes expression vectors containing nucleic acids of the invention and/or that express polypeptides of the invention. Viral and non-viral vectors are used, including plasmids. The choice of plasmid will depend on the type of cell in which propagation is desired and the purpose of propagation. Certain vectors are useful for amplifying and making large amounts of the desired DNA sequence. Other vectors are suitable for expression in cells in culture. Still other vectors are suitable for transfer and expression in cells in a whole animal or person. The choice of appropriate vector is well within the skill of the art. Many such vectors are available commercially. The partial or full-length polynucleotide is inserted into a vector typically by means of DNA ligase attachment to a cleaved restriction enzyme site in the vector. Alternatively, the desired nucleotide sequence can be inserted by homologous recombination *in vivo*. Typically this is accomplished by attaching regions of homology to the vector on the flanks of the desired nucleotide sequence. Regions of homology are added by ligation of oligonucleotides, or by polymerase chain reaction using primers comprising both the region of homology and a portion of the desired nucleotide sequence, for example.

[00206] For expression, an expression cassette or system may be employed. To express a nucleic acid encoding a polypeptide disclosed herein, a nucleic acid molecule encoding the polypeptide, operably linked to regulatory sequences that control transcriptional expression in an expression vector, is introduced into a host cell. The invention includes host cells comprising a nucleic acid and/or expression vector of the invention, e.g., those that code for a polypeptide of the invention. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene which is suitable for selection of cells that carry the expression vector. The gene product encoded by a polynucleotide of the invention is expressed in any convenient expression system, including, for example, bacterial, yeast, insect, amphibian and mammalian systems. In the expression vector, the polypeptide-encoding polynucleotide is linked to a regulatory sequence as appropriate to obtain the desired expression properties. These can include promoters, enhancers, terminators, operators, repressors, and inducers. The promoters can be regulated (e.g., the promoter from the steroid inducible pIND vector (Invitrogen)) or constitutive (e.g., promoters from CMV, SV40, Elongation Factor, or LTR sequences). These are linked to the desired nucleotide sequence using the techniques described above for linkage to vectors. Any techniques known in the art can be used. Accordingly, the expression vector will generally provide a transcriptional and translational initiation region, which may be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination region.

[00207] An expression cassette ("expression unit") may be introduced into a variety of vectors, e.g., plasmid, BAC, YAC, bacteriophage such as lambda, P1, M13, etc., plant or animal viral vectors (e.g., retroviral-based vectors, adenovirus vectors), and the like, where the vectors are normally characterized by the ability to provide selection of cells comprising the expression vectors. The vectors may provide for extrachromosomal maintenance, particularly as plasmids or viruses, or for integration into the host chromosome. Where extrachromosomal maintenance is desired, an origin sequence is provided for the replication of the plasmid, which may be low- or high copy-number. A wide variety of markers are available for selection, particularly those which protect against toxins, more particularly against antibiotics. The particular marker that is chosen is selected in accordance with the nature of the host, where in some cases, complementation may be employed with auxotrophic hosts. Introduction of the DNA construct may use any convenient method, including, e.g., conjugation, bacterial transformation, calcium-precipitated DNA, electroporation, fusion, transfection, infection with viral vectors, biolistics, and the like.

[00208] Accordingly, proteins for use within the present invention can be produced in genetically engineered host cells according to conventional techniques. Suitable host

cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells (including cultured cells of multicellular organisms), particularly cultured mammalian cells. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001), and Ausubel *et al.*, *Short Protocols in Molecular Biology* (4th ed., John Wiley & Sons, 1999).

[00209] For example, for recombinant expression of a homodimeric CD3 binding protein comprising two identical CD3 binding polypeptide chains as described herein, an expression vector will generally include a nucleic acid segment encoding the CD3 binding polypeptide, operably linked to a promoter. For recombinant expression of a heterodimeric CD3 binding protein, comprising different first and second polypeptide chains, the first and second polypeptide chains may be co-expressed from separate vectors in the host cell for expression of the entire heterodimeric protein. Alternatively, for the expression of heterodimeric CD3 binding proteins, the first and second polypeptide chains are co-expressed from separate expression units in the same vector in the host cell for expression of the entire heterodimeric protein. The expression vector(s) are transferred to a host cell by conventional techniques, and the transfected cells are then cultured by conventional techniques to produce the encoded polypeptide(s) to produce the corresponding CD3 binding protein.

[00210] To direct a recombinant protein into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence) is provided in the expression vector. The secretory signal sequence may be that of the native form of the recombinant protein, or may be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is operably linked to the polypeptide-encoding DNA sequence, *i.e.*, the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, *e.g.*, Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830). In particular variations, a secretory signal sequence for use in accordance with the present invention has the amino acid sequence MEAPAQLLFLLLLWLPTTG (SEQ ID NO:79).

[00211] Cultured mammalian cells are suitable hosts for production of recombinant proteins for use within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, *Cell*

14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981; Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann *et al.*, *EMBO J.* 1:841-845, 1982), DEAE-dextran mediated transfection (Ausubel *et al.*, *supra*), and liposome-mediated transfection (Hawley-Nelson *et al.*, *Focus* 15:73, 1993; Ciccarone *et al.*, *Focus* 15:80, 1993). The production of recombinant polypeptides in cultured mammalian cells is disclosed by, for example, Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Examples of suitable mammalian host cells include African green monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44; CHO DXB11 (Hyclone, Logan, UT); see also, e.g., Chasin *et al.*, *Som. Cell. Molec. Genet.* 12:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658). Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, Virginia. Strong transcription promoters can be used, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patents Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

[00212] Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants." Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." Exemplary selectable markers include a gene encoding resistance to the antibiotic neomycin, which allows selection to be carried out in the presence of a neomycin-type drug, such as G-418 or the like; the gpt gene for xanthine-guanine phosphoribosyl transferase, which permits host cell growth in the presence of mycophenolic acid/xanthine; and markers that provide resistance to zeocin, bleomycin, blastocidin, and hygromycin (see, e.g., Gatignol *et al.*, *Mol. Gen. Genet.* 207:342, 1987; Drocourt *et al.*, *Nucl. Acids Res.* 18:4009, 1990). Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. An exemplary amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g., hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

[00213] Other higher eukaryotic cells can also be used as hosts, including insect cells, plant cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar *et al.*, *J. Biosci. (Bangalore)* 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino *et al.*, U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463.

[00214] Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV). See King and Possee, *The Baculovirus Expression System: A Laboratory Guide* (Chapman & Hall, London); O'Reilly *et al.*, *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press., New York 1994); and *Baculovirus Expression Protocols. Methods in Molecular Biology* (Richardson ed., Humana Press, Totowa, NJ, 1995). Recombinant baculovirus can also be produced through the use of a transposon-based system described by Luckow *et al.* (*J. Virol.* 67:4566-4579, 1993). This system, which utilizes transfer vectors, is commercially available in kit form (BAC-TO-BAC kit; Life Technologies, Gaithersburg, MD). The transfer vector (*e.g.*, PFASTBAC1; Life Technologies) contains a Tn7 transposon to move the DNA encoding the protein of interest into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See Hill-Perkins and Possee, *J. Gen. Virol.* 71:971-976, 1990; Bonning *et al.*, *J. Gen. Virol.* 75:1551-1556, 1994; and Chazenbalk and Rapoport, *J. Biol. Chem.* 270:1543-1549, 1995. In addition, transfer vectors can include an in-frame fusion with DNA encoding a polypeptide extension or affinity tag as disclosed above. Using techniques known in the art, a transfer vector containing a protein-encoding DNA sequence is transformed into *E. coli* host cells, and the cells are screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, such as Sf9 cells. Recombinant virus that expresses the protein of interest is subsequently produced. Recombinant viral stocks are made by methods commonly used in the art.

[00215] For protein production, the recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda* (*e.g.*, Sf9 or Sf21 cells) or *Trichoplusia ni* (*e.g.*, HIGH FIVE cells; Invitrogen, Carlsbad, CA). See generally Glick and Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA* (ASM Press, Washington, D.C., 1994). See also U.S. Patent No. 5,300,435. Serum-free media are used to grow and maintain the cells. Suitable media formulations are known in the art and can be obtained from commercial suppliers. The cells are grown up from an inoculation density of approximately $2-5 \times 10^5$ cells to a density of $1-2 \times 10^6$ cells, at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more

typically near 3. Procedures used are generally described in available laboratory manuals (see, e.g., King and Possee, *supra*; O'Reilly *et al.*, *supra*; Richardson, *supra*).

[00216] Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). An exemplary vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936; and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii*, and *Candida maltosa* are known in the art. See, e.g., Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459-3465, 1986; Cregg, U.S. Patent No. 4,882,279; and Raymond *et al.*, *Yeast* 14:11-23, 1998. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533. Production of recombinant proteins in *Pichia methanolica* is disclosed in U.S. Patents Nos. 5,716,808; 5,736,383; 5,854,039; and 5,888,768.

[00217] Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus*, and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well-known in the art (see, e.g., Sambrook and Russell, *supra*). When expressing a recombinant protein in bacteria such as *E. coli*, the protein may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured protein can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione,

followed by dialysis against a buffered saline solution. In the alternative, the protein may be recovered from the cytoplasm in soluble form and isolated without the use of denaturants. The protein is recovered from the cell as an aqueous extract in, for example, phosphate buffered saline. To capture the protein of interest, the extract is applied directly to a chromatographic medium, such as an immobilized antibody or heparin-Sepharose column. Secreted proteins can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding. Antibodies, including single-chain antibodies, can be produced in bacterial host cells according to known methods. See, e.g., Bird *et al.*, *Science* 242:423-426, 1988; Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988; and Pantoliano *et al.*, *Biochem.* 30:10117-10125, 1991.

[00218] Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell.

[00219] CD3 binding proteins can be purified by conventional protein purification methods, typically by a combination of chromatographic techniques. See generally *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988); Scopes, *Protein Purification: Principles and Practice* (Springer-Verlag, New York 1994). Proteins comprising an immunoglobulin Fc region can be purified by affinity chromatography on immobilized protein A or protein G. Additional purification steps, such as gel filtration, can be used to obtain the desired level of purity or to provide for desalting, buffer exchange, and the like.

[00220] The invention also includes compositions comprising a CD3 binding polypeptide of the invention and a pharmaceutically acceptable carrier, diluent and/or excipient.

[00221] In one embodiment of the invention, a monospecific CD3 binding polypeptide is administering to a patient suffering from an autoimmune disease such as rheumatoid arthritis. In another embodiment of the invention, a monospecific CD3 binding polypeptide of the invention is administered to a subject about to undergo an organ transplant.

[00222] In another aspect, the present invention provides a method for treating a disorder characterized by overexpression of a tumor antigen, such as cancer. Generally, such methods include administering to a subject in need of such treatment a therapeutically effective amount of a CD3 binding protein comprising a second binding domain that binds the tumor antigen as described herein. In some embodiments, the CD3 binding protein induces redirected T-cell cytotoxicity (RTCC) against tumor antigen-expressing cells in the subject.

[00223] In certain variations of the method, the disorder is a cancer. In other variations, the disorder is an autoimmune disease. The invention also provides methods for treating cancer or an autoimmune disorder comprising administering a therapeutically effective amount of the compositions or CD3 binding polypeptides described herein to a patient in need thereof.

[00224] In each of the embodiments of the treatment methods described herein, the CD3 binding protein is delivered in a manner consistent with conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, an effective amount of the CD3 binding protein is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder.

[00225] For administration, the CD3 binding protein is formulated as a pharmaceutical composition. A pharmaceutical composition comprising a CD3 binding protein can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic molecule is combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to those in the art. (See, e.g., Gennaro (ed.), *Remington's Pharmaceutical Sciences* (Mack Publishing Company, 19th ed. 1995).) Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc.

[00226] A pharmaceutical composition comprising a CD3 binding protein is administered to a subject in a therapeutically effective amount. According to the methods of the present invention, a CD3 binding protein may be administered to subjects by a variety of administration modes, including, for example, by intramuscular, subcutaneous, intravenous, intra-atrial, intra-articular, parenteral, intranasal, intrapulmonary, transdermal, intrapleural, intrathecal, and oral routes of administration. For prevention and treatment purposes, administration to a subject may be, e.g., in a single bolus delivery, via continuous delivery

(e.g., continuous transdermal delivery) over an extended time period, or in a repeated administration protocol (e.g., on an hourly, daily, or weekly basis).

[00227] A “therapeutically effective amount (or dose)” or “effective amount (or dose)” of a molecule or composition is that amount that produces a statistically significant effect in amelioration of one or more symptoms of the disorder, such as a statistically significant reduction in disease progression or a statistically significant improvement in organ function. The exact dose will be determined by the clinician according to accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, *etc.* Determination of dose is within the level of ordinary skill in the art. When referring to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered serially or simultaneously (e.g., in the same formulation or concurrently in separate formulations).

[00228] Determination of effective dosages in this context is typically based on animal model studies followed up by human clinical trials and is guided by determining effective dosages and administration protocols that significantly reduce the occurrence or severity of the subject disorder in model subjects. Effective doses of the compositions of the present invention vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, whether treatment is prophylactic or therapeutic, as well as the specific activity of the composition itself and its ability to elicit the desired response in the individual. Usually, the patient is a human, but in some diseases, the patient can be a nonhuman mammal. Typically, dosage regimens are adjusted to provide an optimum therapeutic response, e.g., to optimize safety and efficacy.

EXAMPLES

EXAMPLE 1: DESIGN AND CONSTRUCTION OF ANTI-CD3 SMIP MOLECULES WITH REDUCED THEORETICAL pI

[00229] L1, L2, H1, H2 and H3 (see Figures 3 & 4) are heavy and light chains of humanized Cris-7 that were previously constructed and analyzed for binding and activity. H3L1 was used to make SMIP DRA209 (SEQ ID NOs: 3 and 4; IgG4 AA ADCC-CDC null Fc) and SMIP DRA161 (IgG4 AA N297A Fc). Although these constructs exhibit improved characteristics over OKT3 and other anti-CD3 therapeutics in the prior art, the constructs exhibit significantly shorter half-life, higher theoretical pI (see Figure 1) and “clipping” at the binding domain or prehinge region when expressed in CHO cells (see Figures 2A and 2B) as compared to other SMIP molecules.

[00230] Anti-CD3 pI variants were designed to reduce the pI of the binding domain and/or prehinge region. To make the anti-CD3 pI variants, new heavy and light chains (L3,

L4, H4, H5, and H6) were ordered and synthesized by Blue Heron. See Table 3 and Figures 3 and 4.

Table 3: CD3 Variable Heavy and Light Chains

Name	SEQ ID NOs:
H1	SEQ ID NO:21 nucleic acid (na) SEQ ID NO:22 amino acid (aa)
H2	SEQ ID NO:23 (na) SEQ ID NO:24 (aa)
H3	SEQ ID NO:25 (na) SEQ ID NO:26 (aa)
H4	SEQ ID NO:27 (na) SEQ ID NO:28 (aa)
H5	SEQ ID NO:29 (na) SEQ ID NO:30 (aa)
H6	SEQ ID NO:31 (na) SEQ ID NO:32 (aa)
L1	SEQ ID NO:33 (na) SEQ ID NO:34 (aa)
L2	SEQ ID NO:35 (na) SEQ ID NO:36 (aa)
L3	SEQ ID NO:37 (na) SEQ ID NO:38 (aa)
L4	SEQ ID NO:39 (na) SEQ ID NO:40 (aa)

[00231] H4 has 2 point mutations compared to H3. One mutation replaced a positive charge residue (K to Q) while the other mutation introduced a negative charge (Q to E). Both changes were designed to reduce the pI of the protein (either by removing a positive charge or introducing a negative charge). The mutations were performed on the framework sequence to avoid loss of affinity and new residues introduced were verified to be present in the germline sequence at that position to avoid the risk of immunogenicity. By using the germline sequence as a guide to reduce the pI of an already humanized binding domain, amino acid substitutions can be made to add amino acids that are prevalent in the germline sequence. These amino acids may be at the same location in the germline sequence or a proximate position. See Figure 5.

[00232] H5 was designed by "re-humanizing" the heavy chain sequence (as compared to DRA209 and DRA161) in such a way to create a lower pI as compared to DRA209 and DRA161. This was done by designing the heavy chain as compared to the VH1 framework of IGHV1-3*01 heavy chain germline sequence.

[00233] H6 was designed by "re-humanizing" the heavy chain sequence (as compared to DRA209 and DRA161) in such a way to create a lower pI as compared to DRA209 and DRA161. In other words, the starting sequence used to design H6 was the germline sequence rather than a previously humanized sequence. Specifically, this was

done by designing the heavy chain using the VH3 framework of IGHV3-21*01 heavy chain sequence.

[00234] L3 has 6 points mutation compared to L1 where RW were mutated (*i.e.*, replacing the positive charge residue, R to L) to LL (LL was used because LL is more common in the germline sequence than LW) and part of the J kappa (Jk) region (LQIT; SEQ ID NO:252) was replaced with Jk4 sequence (VEIK; SEQ ID NO:253). Jk4 is a better match Jk region based on the Cris-7 H_CDR3 sequence. See Figure 6. Again, mutations were only performed in the framework region and new residues introduced were verified to be present at that position in the germline sequence to avoid the risk of immunogenicity.

[00235] L4 was designed by "re-humanizing" the light chain sequence (as compared to DRA209 and DRA161) in such a way to create a lower pI as compared to DRA209 and DRA161. L4 was re-humanized using the framework of Vk3 of IGKV3-11*01 light chain sequence as a guide.

[00236] All the new light and heavy chains are expected to have lower pI based on theoretical calculations. Vector NTI (Invitrogen) was used to help put together the sequences (the sequences are attached). The germline sequences were obtained from NCBI website (<http://www.ncbi.nlm.nih.gov/igblast/>).

[00237] Variants were constructed that comprised substituted amino acids in the pre-hinge region of SMIP molecules that are otherwise identical to DRA209 and DRA161. The pre-hinge region is a short amino acid sequence that connects the binding domain to the hinge region, *e.g.*, can result from adding a restriction site into the encoding nucleotide sequence.

[00238] One variant made involved replacing the RRT sequence in the pre-hinge region of DRA209 with SSS by PCR mutagenesis. This was done intentionally to remove two positively charged residues (arginine). We have experimentally shown that this change translated to reduction in pI of the protein. This construct still had the H3L1 combination and was given the new name, DRA219. Aside from removing positive charged residues (two arginine), this SSS mutation also introduced an XhoI restriction site that allowed for easy assembly of the newer pI variant constructs.

[00239] Using the same Fc tail as DRA209, the following combinations of H and L were used to construct SMIP constructs using standard molecular biology techniques: a) H4 L1 (with IgG4 AA ADCC-CDC null Fc; also referred to herein as DRA222; SEQ ID NOs: 7 and 8), b) H4L3 (with IgG4 AA ADCC-CDC null Fc; also referred to herein as DRA221; SEQ ID NOs:9 and 10), c) H3L3 (with IgG4 AA ADCC-CDC null Fc; also referred to herein as DRA223; SEQ ID NOs: 11 and 12), d) H5L4 (with IgG4 AA ADCC-CDC null Fc; also referred to herein as DRA224; SEQ ID NOs: 13 and 14), and e) H6L4 (with IgG4 AA ADCC-CDC null Fc; also referred to herein as DRA225; SEQ ID NOs: 15 and 16). See Figures 7A and 7B.

[00240] All these constructs were transfected into HEK cells to generate protein for in vitro binding studies. All of the constructs expressed the desired protein except the DRA225 construct, which had no apparent expression. All of the other constructs were found (i) to bind CD3 with varying degrees (DRA222 having some of the best binding) and (ii) to have a lower pI than the parent molecule, DRA209. The measured pIs are found in Table 4.

Table 4: Measured pIs

Construct	Measured pI
DRA209	9.0
DRA219	8.4
DRA221	8.2
DRA222	7.5
DRA223	7.2
DRA224	6.8

[00241] DRA219, DRA221, DRA222, DRA223 and DRA224 all inhibited human mixed lymphocyte reactions (MLR; data not shown) and the amount of inhibition correlated with their measured ability to bind CD3.

[00242] DRA219 (H3 L1 IgG4 AA ADCC-CDC null FC; SEQ ID NOs: 5 and 6) and DRA222 (SEQ ID NOs: 7 and 8) both retained binding to CD3 and had a lower measured pI and were further cloned into the pEE12.4 vector which codes for a different Fc tail (IgG4 AA N297A) to generate two additional constructs – DRA233 (H3L1; SEQ ID NOs: 17 and 18) and DRA234 (H4L1; SEQ ID NOs: 19 and 20) respectively. Furthermore, DRA219 and DRA222 were cloned into pEE12.4 vector for expression in CHO and named as DRA228 and DRA229 respectively. Essentially DRA219 is equivalent to DRA228 and DRA222 is equivalent to DRA229. All the constructs (DRA228, DRA229, DRA233 and DRA234) were subsequently transfected into CHO cells to generate proteins for binding studies, protein characterization and PK studies. See Figure 8.

[00243] Proteins were purified from cell supernatant by Protein A affinity chromatography using an AKTA Purifier HPLC system. Pre-packed POROS A columns (Life Technologies) were used for in vitro preparations and pre-packed MabSuRe Protein A columns (GE Healthcare) were used for in vivo material. Samples were loaded in the presence of PBS and eluted with citrate buffer. Eluents were neutralized with TRIS buffer.

[00244] Capillary Isoelectric Focusing was performed on a Beckman PA 800 instrument and used to determine the isoelectric point (pI) of purified proteins. Analysis was performed according to standard Beckman cIEF protocol. Samples were prepared in a solution of 3M urea in cIEF gel (Beckman) supplemented with Pharmalyte 3-10 ampholytes (GE Healthcare), cathodic and anodic stabilizers, and synthetic peptide pI markers (Beckman). Samples were introduced into a neutral capillary (Beckman) and then focused by submerging the capillary inlet in anolyte solution, the outlet in catholyte solution, and applying a 25 kV electrical potential across the capillary for 15 minutes. After focusing, the

sample was mobilized by chemical means to pass all separated components across a fixed wavelength detector measuring absorbance at 280 nm. The isoelectric point (pI) was calculated for all samples from a standard curve constructed of results for the synthetic peptide pI markers. See Figures 9-11. The constructs had the following measured pIs: DRA228=8.4; DRA229=7.5; DRA233=8.6; DRA234=8.0, whereas the DRA161=9.2 and DRA209=8.8. Therefore, all of the pI variants that were expressed had a lower pI than their respective parental constructs, DRA161 or DRA209.

EXAMPLE 2: ANTI-CD3 SMIP MOLECULES BIND T-CELLS

[00245] Human peripheral blood mononuclear cells (PBMC) were isolated from fresh whole blood using standard density gradient centrifugation and washed three times in PBS with 0.2% BSA. Cells were plated in 96-well plates U-bottom plates at 200,000 PBMCs/well and labeled with 50ul of antibodies at the nM concentrations as indicated in Figure 12 for 30 min on ice. Plates were washed three times and labeled for 30 min with 50ul of an antibody cocktail containing optimal concentrations of anti-CD5 –APC plus F(ab')₂ goat anti-hu IgG Fc-PE. Plates were washed three times and fixed in 120ul of 1% paraformaldehyde in PBS, at 4C overnight. All antibody incubations and washes were performed on ice, with cold PBS, 0.2%BSA, 2nM EDTA. Samples were analyzed by flow cytometry using a BD LSRII analyzer. Sixty microliters were collected from each well. Collected samples were analyzed using Flowjo software by gating on T-cells (FCS vs SSC, CD5+ cells). The mean fluorescence intensity (MFI) on the PE channel is shown in the graph. The DRA227 SMIP was used only as a control and does not contain a CD3 or T-cell binding domain and was not modified to reduce pI. See Figure 12. All the tested pI variants bound to CD3 and DRA228 and DRA229 retained binding activity very similar to their parental constructs.

EXAMPLE 3: ANTI-CD3 SMIP MOLECULES WITH REDUCED pI EXPRESS BETTER IN CHO CELLS

[00246] Size exclusion chromatography: Size exclusion chromatography was used to assess the high-molecular weight aggregate content of purified proteins. The HPLC system was an Agilent 1200 series equipped with a quaternary pump, solvent degasser, DAD, and temperature-controlled autosampler. A Tosoh TSKgel G3000SWxl column equilibrated in a running buffer of 200 mM potassium phosphate and 250 mM potassium chloride at pH 7.2 was used for chromatographic separation. Solvent flow rate was 1 mL/min and sample injection mass was 50 µg. Column effluent was monitored with the Agilent DAD detector at a wavelength of 280 nm.

[00247] SDS-PAGE: Denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to assess the integrity of purified proteins. Samples were prepared with Novex sample buffer (Invitrogen) and heated at 85°C for 3 minutes to ensure full denaturation prior to loading into 4-20% Novex Tris-Glycine gels (Invitrogen). Samples were optionally reduced by adding Novex reducing agent prior to the heating step. Proteins were mobilized and separated by applying 125V across the gels for 110 minutes in an electrophoresis chamber filled with Tris-Glycine buffer (Invitrogen, from 10X stock). Protein bands were visualized by staining with SimplyBlue Safe Stain (Invitrogen). See Figures 13-14. The tested pI variants demonstrated reduced clipping as compared to their parental molecule, DRA161.

[00248] CE-SDS: Capillary Electrophoresis in SDS was performed on a Beckman PA 800 instrument and used to quantify the low molecular weight clip content of purified proteins. Samples were prepared at a 1 mg/mL concentration in 50% SDS sample buffer (Beckman) and heated at 85°C for 3 minutes to ensure full denaturation prior to analysis. Samples were electrokinetically injected (10 kV for 20 s) into a bare-fused silica capillary filled with SDS gel buffer (Beckman) and separated by applying a voltage of 15 kV across the capillary (reverse polarity) for 30 minutes. Absorbance at 220 nm and 280 nm was measured at the detector window. See Figures 15-16. This showed that the quality of protein of the pI variants was better than the parental molecule, e.g., because of lower apparent proteolysis, sometimes referred to herein as "clipping".

EXAMPLE 4: ANTI-CD3 SMIP pI VARIANT MOLECULES EXHIBIT INCREASED HALF-LIFE

[00249] Female BALB/c mice were injected intravenously (IV) at time 0 with 200 μ L of PBS containing 200 μ g of DRA234 or DRA233. Three mice per group were injected for each time point. Anesthetized mice were exsanguinated via cardiac puncture at the indicated time points after injection, and serum was collected as described below. Serum concentrations of DRA234 and DRA233 were determined using enzyme linked immunosorbent assays (ELISA). Pharmacokinetic disposition parameters for each protein were estimated by non-compartmental analysis using WinNonlin™ Professional software (v5.3) and applying the precompiled model 201 for IV bolus administration and sparse sampling.

[00250] At time 0, mice were injected IV with 200 μ L of PBS containing 200 μ g (~10 mg/kg) of DRA233 or DRA234. Three mice per group were injected for each time point. Anesthetized mice were exsanguinated via cardiac puncture and serum was collected at t = 15 minutes, and 2, 6, 24, 48, 72, 96, 168, 336 and 504 hours after injection. Serum concentrations for each protein were determined using a specific sandwich ELISA. Results

are expressed as mean serum concentration (in $\mu\text{g/mL}$) $\pm$ SD over time. Data are shown in linear (A) and log (B) format. See Figure 17.

[00251] Anti-drug antibodies (ADA) were detected using a sandwich ELISA, with DRA161 (original form of DRA233 and DRA234) coated on plates to capture ADA, and anti-mouse IgG (H&L) HRP to detect bound ADA. Bound complexes were measured using a peroxidase substrate, with results being read on a fluorescent plate reader. Results were expressed as mean fluorescent units (FU) vs. serum dilution. Levels of ADA were clearly seen at 504 hours for DRA233, with possible low levels starting at 336 hours; however there did not appear to be ADA in any of the DRA234 samples. (Data not shown).

[00252] The mean serum concentration vs time profile is plotted for all DRA233 PK time points in and without data from 504 hours. The serum concentration vs time profile for DRA234 is found in Figure 18C. Results are expressed as the observed data set and the predicted values calculated by WinNonLin™ software. The Rsq value and Rsq adjusted values are the goodness of fit statistics for the terminal elimination phase, before and after adjusting for the number of points used in the estimation of HL_Lambda z. The approximate half-life for DRA233 was 95 hours using only time points without detectable levels of anti-drug antibodies, and 84 hours for DRA234. See Figure 18.

[00253] PK estimates from WinNonLin program in tabular format: The pharmacokinetic analysis was carried out using WinNonlin™ Professional (v5.3) software, applying non-compartmental analysis using sparse sampling and the precompiled model 201 for IV bolus administration with uniform weighting. The area under the serum concentration-time curve (AUC) from time 0 to the last measurable concentration (Tlast) was estimated using the linear trapezoidal with linear/log interpolation calculation method. The following pharmacokinetic disposition parameters were estimated: maximum observed serum concentration (Cmax), concentration at the last measurable concentration (Clast), time to reach the maximum observed serum concentration (Tmax), apparent terminal elimination half-life (HL lambda z), AUC from time of dosing to the last measurable concentration (AUCall), AUC from dosing with time extrapolated to infinity (AUCINF_obs), serum clearance (Cl_obs), volume of distribution based on the terminal phase (Vz_obs), and mean residence time (MRT). See Figure 19 and 20. This Example shows that the tested pl variants had longer *in vivo* serum half-life and slower clearance in mice as compared to the parent molecule, DRA161.

EXAMPLE 5: BISPECIFIC HOMODIMER MOLECULES - ANTI-CD3 AND ANTI-PSMA

[00254] Bispecific CD3 and PSMA binding homodimers were constructed using standard techniques. These constructs, TSC249, TSC250, TSC251, TSC252, TSC295, TSC296, TSC301 and TSC302 are described in Table 5. Insertion of the N-terminal scFv

binding domain was accomplished through digestion of the parental template and scFv insert with either the restriction enzymes HindIII and XhoI or AgeI and XhoI, desired fragments were identified and isolated by agarose gel purification, and ligation. Insertion of the C-terminal scFv binding domain was accomplished through digestion of the parental template and scFv insert with the restriction enzymes EcoRI and NotI, desired fragments were identified and isolated by agarose gel purification, and ligation.

Table 5: Binding Polypeptide Sequences and Components

Name	SEQ ID NOs
humanized TSC249 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv)	SEQ ID NO:56 nucleic acid (na) SEQ ID NO:57 amino acid (aa)
humanized TSC250 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H81 linker)	SEQ ID NO:58 (na) SEQ ID NO:59 (aa)
humanized TSC251 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H83 linker)	SEQ ID NO:60 (na) SEQ ID NO:61 (aa)
humanized TSC252 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H91 linker)	SEQ ID NO:62 (na) SEQ ID NO:63 (aa)
Humanized TSC295 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H9 linker)	SEQ ID NO:64 (na) SEQ ID NO:65 (aa)
Humanized TSC296 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H94 linker)	SEQ ID NO:66 (na) SEQ ID NO:67 (aa)
Humanized TSC301 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H105 linker)	SEQ ID NO:68 (na) SEQ ID NO:69 (aa)
Humanized TSC302 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, with H106 linker)	SEQ ID NO:70 (na) SEQ ID NO:71 (aa)

EXAMPLE 6: BISPECIFIC HOMODIMER MOLECULES - ANTI-CD3 AND ANTI-RON

[00255] Bispecific molecules with both CD3 and RON binding homodimers were constructed using standard techniques. These constructs, TSC275, TSC277, TSC278 and TSC279, are described in Table 6. TSC275 contains the VL and VH chains of hu4C04, SEQ ID NOs:187 and 188, respectively, which are part of the RON binding domain. TSC277, TSC278 and TSC279 are the same as TSC275 except for the mutations/substitutions as indicated in Table 6.

Table 6: Binding Polypeptide Sequences and Components

Name	Brief Description	Nucleic Acid Sequence (SEQ ID NO)	Amino Acid Sequence (SEQ ID NO)
TSC275	hu4C04 x DRA222 null2	185	186
TSC277	hu4C04 (A43K Q240E) x DRA222 null2	189	190
TSC278	hu4C04 (A43T) x DRA222 null2	191	192
TSC279	hu4C04 (Q165R) x DRA222 null2	193	194

EXAMPLE 7: BISPECIFIC HOMODIMER MOLECULES - ANTI-CD3 AND ANTI-CD19

[00256] Bispecific molecules with both CD3 and CD19 binding homodimers were constructed using standard techniques, e.g., see PCT Publication No. WO2007/146968. These constructs, TSC233, TSC234, TSC235, TSC240, TSC241 and TSC242, are described in Table 7. TSC233 contains the VL (amino acids 1-111 of SEQ ID NO:196) and VH (amino acids 128-251 of SEQ ID NO:196) chains of HD37 which are part of the CD19 binding domain.

Table 7: Binding Polypeptide Sequences and Components

Name	Brief Description	Nucleic Acid Sequence (SEQ ID NO)	Amino Acid Sequence (SEQ ID NO)
TSC129a	HD37 x Cris7 scorpion, H75 linker	244	245
TSC233	HD37 x DRA221 scorpion, H75 linker	195	196
TSC234	HD37 x DRA222 scorpion, H75 linker	197	198
TSC235	HD37 x DRA224 scorpion, H75 linker	199	200
TSC240	HD37 x DRA222 scorpion, H81 linker	201	202
TSC241	HD37 x DRA222 scorpion, H83 linker	203	204
TSC242	HD37 x DRA222 scorpion, H91 linker	205	206

EXAMPLE 8: BISPECIFIC HETERODIMER MOLECULES - ANTI-CD3 AND ANTI-CD19

[00257] Different heterodimeric bispecific molecules comprising a CD19 binding domain and a CD3 binding domain were constructed similar to as described in PCT Publication WO2011/090762 by performing co-transfections of nucleic acids encoding the amino acid sequences as indicated in Table 8. Heterodimers were made by co-expressing two different polypeptides chains, one polypeptide chain comprising an immunoglobulin CH1 heterodimerization domain and the other polypeptide chain comprising an immunoglobulin CL heterodimerization domain.

Table 8: Cotransfection for ANTI-CD3 AND ANTI-CD19 Heterodimer Constructions

Heterodimer Designation	Binding Domain in Each Heterodimer	Constructs Used in Co-transfection (na & aa sequences)	
TSC127	Cris 7 scFv x HD37	TSC096 SEQ ID NOs:209 & 210	TSC125 SEQ ID NOs 242 & 243
TSC224	DRA221 scFv* x HD37	TSC096 SEQ ID NOs:209 & 210	TSC218 SEQ ID NOs:246 & 247
TSC225	DRA222 scFv** x HD37	TSC096 SEQ ID NOs:209 & 210	TSC219 SEQ ID NOs:217 & 218
TSC226	DRA224 scFv*** x HD37	TSC096 SEQ ID NOs:209 & 210	TSC220 SEQ ID NOs:219 & 220
TSC227	HD37 X DRA221 scFv*	TSC049	TSC221

Heterodimer Designation	Binding Domain in Each Heterodimer	Constructs Used in Co-transfection (na & aa sequences)	
		SEQ ID NOs:207 & 208	SEQ ID NOs:248 & 249
TSC228	HD37 X DRA222 scFv**	TSC049 SEQ ID NOs:207 & 208	TSC222 SEQ ID NOs:221 & 222
TSC229	HD37 X DRA224 scFv***	TSC049 SEQ ID NOs:207 & 208	TSC223 SEQ ID NOs:223 & 224

* amino acid 1-244 of SEQ ID NO:10

** amino acid 1-244 of SEQ ID NO:8

*** amino acid 1-247 of SEQ ID NO:14

[00258] TSC049 comprises from its amino- to carboxyl-terminus: HD37 (anti-CD19) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. TSC096 comprises from its amino- to carboxyl-terminus: HD37 (anti-CD19) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Ck(YAE). TSC125 comprises from its amino- to carboxyl-terminus: Cris7 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. TSC218 comprises from its amino- to carboxyl-terminus: DRA221 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. TSC219 comprises from its amino- to carboxyl-terminus: DRA222 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. TSC220 comprises from its amino- to carboxyl-terminus: DRA224 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. TSC221 comprises from its amino- to carboxyl-terminus: DRA221 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Ck(YAE). TSC222 comprises from its amino- to carboxyl-terminus: DRA222 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Ck(YAE). TSC223 comprises from its amino- to carboxyl-terminus: DRA224 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Ck(YAE).

EXAMPLE 9: BISPECIFIC HETERODIMER MOLECULES - ANTI-CD3 AND ANTI-PSMA

[00259] Different heterodimeric bispecific molecules comprising a PSMA binding domain and a CD3 binding domain were constructed similar to as described in PCT Publication WO2011/090762 by performing co-transfections of nucleic acids encoding the amino acid sequences as indicated in Table 9. Heterodimers were made by co-expressing two different polypeptide chains, one polypeptide chain comprising an immunoglobulin CH1 heterodimerization domain and the other polypeptide chain comprising an immunoglobulin CL heterodimerization domain. Some PSMA binding domains are described in PCT Publication No. WO2011/090761.

Table 9: Cotransfection for ANTI-CD3 AND ANTI-PSMA Heterodimer Constructions

Heterodimer Designation	Binding Domain in Each Heterodimer	Constructs Used in Co-transfection (na & aa sequences)	
TSC236	DRA222 scFv* x humanized 107-1A4 VL-VH#2 scFv**	TSC192 SEQ ID NOs:211 & 212	TSC219 SEQ ID NOs:217 & 218
TSC237	humanized 107-1A4 scFv** x DRA222 scFv*	TSC195 SEQ ID NOs:215 & 216	TSC222 SEQ ID NOs:221 & 222
TSC264	humanized 107-1A4 VL-VH#1 scFv*** x DRA222 scFv*	TSC258 SEQ ID NOs:227 & 228	TSC254 SEQ ID NOs:225 & 226
TSC265	DRA222 scFv* x humanized 107-1A4 VL-VH#1 scFv**	TSC193 SEQ ID NOs:213 & 214	TSC219 SEQ ID NOs:217 & 218

* amino acid 1-244 of SEQ ID NO:8

** amino acid 1-243 of SEQ ID NO:216

*** amino acid 1-243 of SEQ ID NO:226

[00260] "Humanized 107-1A4 VL-VH#2 scFv" and "humanized 107-1A4 VL-VH#1 scFv" are humanized scFvs based on the anti-human-PSMA 107-1A4 monoclonal antibody and are also described in PCT Publication No. WO2011/090761. The "humanized 107-1A4 VL-VH#2 scFv" and "humanized 107-1A4 VL-VH#1 scFv" correspond to amino acids amino acid 1-243 of SEQ ID NO:216 & 226, respectively.

[00261] TSC192 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#2 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Ck(YAE). TSC195 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#2 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. TSC193 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#1 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Ck(YAE).

[00262] TSC219 comprises from its amino- to carboxyl-terminus: DRA222 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human CH1. TSC222 comprises from its amino- to carboxyl-terminus: DRA222 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, and human Ck(YAE). TSC254 comprises from its amino- to carboxyl-terminus: humanized 107-1A4 (anti-PSMA) VL-VH#1 scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, linker, and human CH1. TSC258 comprises from its amino- to carboxyl-terminus: DRA222 (anti-CD3) scFv, human IgG1 SCC-P hinge, human IgG1 CH2, human IgG1 CH3, linker, and human Ck(YAE).

EXAMPLE 10: TARGET-DEPENDENT T-CELL PROLIFERATION BY POLYPEPTIDE
HETERODIMERS AND HOMODIMERS TARGETING CD19

[00263] To compare the effectiveness of different bispecific polypeptide heterodimer molecules at inducing target-dependent T-cell activation and proliferation, three different homodimeric bispecific molecules (TSC129a, TSC233, and TSC234 (SEQ ID NOs: 245, 196 and 198, respectively)) with a common anti-CD19 binding domain (HD37) and three different anti-CD3 binding domains (Cris7 for TSC129a, the scFv binding domain from DRA221 (amino acid 1-244 of SEQ ID NO:10) for TSC233, the scFv binding domain from DRA222 (amino acid 1-244 of SEQ ID NO:8) for TSC234) were compared. Additionally, three different heterodimeric bispecific molecules (TSC127, TSC227, TSC228) with three different anti-CD3 binding domains (Cris7 for TSC127, scFv binding domain from DRA221 (amino acid 1-244 of SEQ ID NO:10) for TSC227, and scFv binding domain from DRA222 (amino acid 1-244 of SEQ ID NO:8) for TSC228) were also compared.

[00264] Daudi Burkitt's lymphoma cells (CD19+) were obtained from ATCC (Manassas, VA) and cultured according to the provided protocol. Peripheral blood mononuclear cells (PBMC) were isolated from human blood using standard ficoll gradients. The isolated cells were washed in saline buffer. T-cells were additionally isolated from these PBMC using a Pan T-cell Isolation Kit II from Miltenyi Biotec (Bergisch Gladbach, Germany) using the manufacturer's protocol.

[00265] Proliferation was assessed by labeling isolated T-cell populations with carboxyfluorescein diacetate succinimidyl ester (CFSE). CFSE-labeled T-cells were plated in U-bottom 96-well plates at 100,000 T-cells/well, with 33,000 tumor cells/well, to achieve T-cell to tumor cell ratios of approximately 3:1. Concentrations of test molecules ranging from 1 nM to 5 fM were added to the cell mixtures in a total of 200 μ L/well in RPMI 1640 media supplemented with 10% human or bovine serum, sodium pyruvate and non-essential amino acids. Plates were incubated at 37°C, 5% CO₂ in humidified incubators. After 3 days, cells were labeled with antibodies for flow cytometric analysis. Cells were labeled and washed in their original plates to minimize cell losses during transfers, and all labeling was done in saline buffer with 0.2% bovine serum albumin. First, cells were pre-incubated with 100 μ g/ml human IgG at room temperature for 15 min. Subsequently, cells were incubated with a mixture (total volume 50 μ L) of the following dye-labeled antibodies: CD5-PE, CD4-APC, CD8-Pacific Blue, CD25-PE-Cy7, as well as 7-Amino Actinomycin D (7AAD hereafter) for 40 min. Plates were washed twice, resuspended in 80 to 120 μ L volumes and ran immediately in a BD LSRII flow cytometer to acquire 80% of the contents of each well. The sample files were analyzed using FlowJo software to calculate the percentages and numbers of cells that had undergone at least one cell division, according to their CFSE profile, by gating

sequentially on activated, live CD4+ or CD8+ T-cells (7AAD-, CD5+ CD25+ CD4+ or 7AAD- CD5+ CD25+ CD8+, respectively). Mean values and standard deviations were calculated using Microsoft Excel software. Graphs were plotted using Microsoft Excel or Graphpad Prism.

[00266] Analysis of live CD4+ and CD8+ populations from Daudi cells treated with whole T-cells revealed a significant increase in both the total number of cells and percent proliferating cells in the presence of Daudi cells displaying the target CD19 antigen (Figures 21 & 22).

[00267] For the homodimeric bispecific polypeptides (TSC129a, TSC233, TSC234), proliferation was comparable between CD4+ and CD8+ cells (Figure 21). Proliferation induced by TSC129a and TSC234 was comparable between CD4+ and CD8+ cells (Figure 21), suggesting that pl variant anti-CD3 scFv binding domain from DRA222 had essentially equivalent activity to the parental Cris7 binding domain. The profile of T-cell proliferation induced by TSC233 differed slightly from TSC129a and TSC234, with a decreased induction of CD4+ proliferation (Figure 21A) and a slightly increased induction of CD8+ proliferation (Figure 21B). This suggested that the pl variant anti-CD3 scFv binding domain from DRA221 was not completely equivalent in activity to the parental Cris7 binding domain.

[00268] For the heterodimeric bispecific polypeptides (TSC127, TSC227, TSC228), proliferation was higher for CD8+ T-cells than CD4+ T-cells in the presence of Daudi cells (Figure 22). Proliferation induced by TSC127 and TSC228 was comparable between CD4+ and CD8+ cells (Figure 22), suggesting that pl variant anti-CD3 scFv binding domain from DRA222 had essentially equivalent activity to the parental Cris7 binding domain. The profile of T-cell proliferation induced by TSC227 differed slightly from TSC127 and TSC228, with a decreased induction of CD4+ proliferation, and comparable induction of CD8+ proliferation. Again, this suggested that the pl variant anti-CD3 scFv binding domain from DRA221 was not completely equivalent in activity to the parental Cris7 binding domain.

EXAMPLE 11: REDIRECTED T-CELL CYTOTOXICITY BY POLYPEPTIDE HETERODIMERS AND HOMODIMERS TARGETING CD19

[00269] To compare the effectiveness of different bispecific polypeptide heterodimer molecules at inducing target-dependent T-cell cytotoxicity, three different homodimeric bispecific molecules (TSC129a, TSC233, and TSC234 (SEQ ID NOs: 245, 196 and 198, respectively)) with a common anti-CD19 binding domain (HD37) and three different anti-CD3 binding domains (Cris7 for TSC129a, the scFv binding domain from DRA221 (amino acid 1-244 of SEQ ID NO:10) for TSC233, the scFv binding domain from DRA222 (amino acid 1-244 of SEQ ID NO:8) for TSC234)) were compared. Additionally, three different

heterodimeric bispecific molecules (TSC127, TSC227, TSC228) with three different anti-CD3 binding domains (Cris7 for TSC127, scFv binding domain from DRA221 (amino acid 1-244 of SEQ ID NO:10) for TSC227, and scFv binding domain from DRA222 (amino acid 1-244 of SEQ ID NO:8) for TSC228) were also compared.

[00270] Daudi Burkitt's lymphoma cells (CD19+) were obtained from ATCC (Manassas, VA) and cultured according to the provided protocol. Peripheral blood mononuclear cells (PBMC) were isolated from human blood using standard ficoll gradients and washed in saline buffer. T cells were additionally isolated from PBMC using a Pan T-cell Isolation Kit II from Miltenyi Biotec (Bergisch Gladbach, Germany) using the manufacturer's protocol.

[00271] Cytotoxicity was assessed by a ^{51}Cr release assay. Approximately 5×10^6 Daudi cells were treated with 0.3 mCi of ^{51}Cr and incubated for 75 minutes at 37°C . After 75 minutes, cells were washed 3 times with media (RPMI + 10% FBS) and resuspended in 11.5 mL of media. From this suspension, 50 μL was dispensed per well into 96 well U-bottom plates (approximately 20,000 cells/well). Concentrations of bispecific molecules ranging from 500 pM to 0.1 pM were added to the Daudi cells, bringing the total volume to 100 μL /well. Target cells were incubated at room temperature for 15 minutes. Then 100 μL of isolated T-cells (approximately 200,000) were added to bring the T-cell to target cell ratio to 10:1. 50 μL of 0.8% NP-40 was added to a control well containing target cells, left for 15 minutes, then 100 μL of media was added to provide a total lysis control.

[00272] Plates were incubated for 4 hours, then spun at 1500 rpm for 3 minutes and 25 μL of supernatant was transferred from each well to the corresponding well of a 96-well Luma sample plate. Sample plates were allowed to air dry in a chemical safety hood for 18 hours, and then radioactivity was read on a Topcount scintillation counter using a standard protocol.

[00273] Analysis of cytotoxicity data showed T-cell directed cytotoxicity with the Daudi cells in the presence of T-cells and anti-CD19 directed bispecific molecules (Figure 23).

[00274] For the homodimeric bispecific polypeptides (TSC129a, TSC233, TSC234), observed cytotoxicity was comparable between TSC129a and TSC234 (Figure 23A), suggesting that the DRA222 pl variant had equivalent activity to the parental Cris7 binding domain. A slight decrease in cytotoxicity was observed with TSC233, suggesting that the DRA221 pl variant had less activity than the parental Cris7 binding domain.

[00275] For the heterodimeric bispecific polypeptides (TSC127, TSC227, TSC228), observed cytotoxicity was comparable between TSC127 and TSC228 (Figure 23B), suggesting that the DRA222 scFv binding domain pl variant had equivalent activity to the parental Cris7 binding domain. A slight decrease in cytotoxicity was observed with TSC227,

suggesting that the DRA221 scFv binding domain pl variant had less activity than the parental Cris7 binding domain.

EXAMPLE 12: REDIRECTED T-CELL CYTOTOXICITY BY POLYPEPTIDE HOMODIMERS TARGETING RON

[00276] To compare the effectiveness of different bispecific polypeptide heterodimer molecules at inducing target-dependent T-cell cytotoxicity, four different homodimeric bispecific molecules (TSC275, TSC277, TSC278, and TSC279) with a common anti-CD3 binding domain (scFv binding domain from DRA222 (amino acid 1-244 of SEQ ID NO:8)) and four variants of the same anti-RON binding domain (hu4C04 scFv for TSC275, hu4C04 scFv (A43K/Q240E) for TSC277, hu4C04 scFv (A43T) for TSC278, and hu4C04 scFv (Q165R) for TSC279) were compared.

[00277] MDA-MB-453 breast cancer cells (RON+) were obtained from ATCC (Manassas, VA) and cultured according to the protocol provided by ATCC. Peripheral blood mononuclear cells (PBMC) were isolated from human blood using standard ficoll gradients and washed in saline buffer. T-cells were additionally isolated from PBMC using a Pan T-cell Isolation Kit II from Miltenyi Biotec (Bergisch Gladbach, Germany) using the manufacturer's protocol.

[00278] Cytotoxicity was assessed by a ^{51}Cr release assay. Approximately 5×10^6 Daudi cells were treated with 0.3 mCi of ^{51}Cr and incubated for 75 minutes at 37°C . After 75 minutes, cells were washed 3 times with media (RPMI + 10% FBS) and resuspended in 11.5 mL of media. From this suspension, 50 μL was dispensed per well into 96 well U-bottom plates (approximately 20,000 cells/well). Concentrations of bispecific molecules ranging from 10 nM to 0.005 fM were added to the Daudi cells, bringing the total volume to 100 μL /well. Target cells were incubated at room temperature for 15 minutes. Then 100 μL of isolated T-cells (approximately 100,000) were added to bring the T-cell to target cell ratio to 5:1. 50 μL of 0.8% NP-40 was added to a control well containing target cells, left for 15 minutes, then 100 μL of media was added to provide a total lysis control.

[00279] Plates were incubated for 24 hours, spun at 1500 rpm for 3 minutes, and 25 μL of supernatant was transferred from each well to the corresponding well of a 96-well Luma sample plate. Sample plates were allowed to air dry in a chemical safety hood for 18 hours, and then radioactivity was read on a Topcount scintillation counter using a standard protocol.

[00280] Analysis of cytotoxicity data showed T-cell directed cytotoxicity with the MDA-MB-453 cells in the presence of T-cells and anti-RON directed bispecific molecules (Figure 24).

[00281] For the homodimeric bispecific polypeptides (TSC275, TSC277, TSC278 and TSC279), observed cytotoxicity was generally comparable (Figure 24), suggesting that mutations within the ORN196 binding domain did not significantly affect overall activity. However, slightly lower maximum lysis was observed for TSC278 compared to the other three variants (Figure 24). Maximum lysis was observed between concentrations of 10 pM and 10 nM.

EXAMPLE 13: T-CELL BINDING OF BISPECIFIC POLYPEPTIDE HETERODIMERS AND HOMODIMERS

[00282] To compare the effectiveness of bispecific polypeptide molecules featuring a pl variant CD3 binding domain (the scFv found in DRA222 (amino acid 1-244 of SEQ ID NO:8)) at binding to T-cells, the on-cell binding characteristics of an anti-CD19 x anti-CD3 bispecific polypeptide heterodimer, TSC228 (as described in Example 8, Table 8), were compared to that of an anti-PSMA x anti-CD3 bispecific polypeptide homodimer TSC249 (as described in Example 5, Table 5), were compared in two independent experiments.

[00283] Jurkat (CD3⁺) T cell leukemia cells were obtained from ATCC (Manassas, VA), and cultured according to the protocol provided by ATCC. Binding was assessed by incubating 5×10^5 Jurkat cells for 30 minutes at 4°C with serially diluted bispecific molecules TSC228 or TSC249, in concentrations from 250 nM to 0.125 nM (TSC249) or 125 nM to 1 pM (TSC228). The cells were washed three times and then incubated with goat anti-human IgG-FITC (1:200 dilution) for another 30 minutes at 4°C. The cells were then washed again three times, fixed in 1% paraformaldehyde and read on a FACS-Calibur instrument.

[00284] Analysis of the FSC high, SSC high subset in FlowJo v7.5 (Tree Star, Inc, Ashland, OR) showed dose-dependent binding of bispecific molecules TSC228 and TSC249 to Jurkat cells (Figures 25A & B, respectively). The TSC228 bispecific polypeptide heterodimer, which possessed only one anti-CD3 binding domain, bound with a lower apparent affinity ($EC_{50} = 24.5$ nM) than the TSC249 bispecific polypeptide homodimer ($EC_{50} = 1.9$ nM), which possessed two anti-CD3 binding domains. This suggests that the TSC249 bispecific polypeptide heterodimer bound with some avidity.

EXAMPLE 14: TARGET-DEPENDENT T-CELL PROLIFERATION BY BISPECIFIC POLYPEPTIDE HOMODIMERS TARGETING PSMA

[00285] To determine the effectiveness of different bispecific polypeptide heterodimer molecules at inducing target-dependent T-cell activation and proliferation, a homodimeric bispecific molecule (TSC249, see Example 5, Table 5) with an anti-PSMA binding domain (hu107-1A4) and an anti-CD3 binding domain (the scFv from DRA222 (amino acid 1-244 of SEQ ID NO:8)) was tested on prostate cancer cell lines expressing PSMA, prostate cancer cell lines not expressing PSMA, and T-cells without target cells present.

[00286] C4-2B prostate carcinoma cells (PSMA+) were obtained from MD Anderson Cancer Center (Houston, TX) and cultured according to the provided protocol. DU-145 prostate carcinoma cells (PSMA-) were obtained from ATCC (Manassas, VA) and cultured according to the provided protocol. Peripheral blood mononuclear cells (PBMC) were isolated from human blood using standard ficoll gradients, and further were washed in saline buffer. T-cells were then isolated from these PBMC using a Pan T-cell Isolation Kit II from Miltenyi Biotec (Bergisch Gladbach, Germany) using the manufacturer's protocol.

[00287] Proliferation was assessed by labeling isolated T cell populations with carboxyfluorescein diacetate succinimidyl ester (CFSE). Three individual experiments were set up simultaneously. CFSE-labeled T cells were mixed with the TSC249 molecule alone; CFSE-labeled T cells were mixed with DU-145 cells and the TSC249 molecule; and CFSE-labeled T cells were mixed with C4-2B cells and the TSC249 molecule. CFSE-labeled T-cells were plated in U-bottom 96-well plates at 100,000 cells/well, with 33,000 tumor cells/well, to achieve approximate T-cell to tumor cell ratios of 3:1. Concentrations of test molecules ranging from 20 nM to 5 fM were added to the cell mixtures in a total of 200 μ L/well in RPMI 1640 media supplemented with 10% human or bovine serum, sodium pyruvate and non-essential amino acids. Plates were incubated at 37°C, 5% CO₂ in humidified incubators. After 3 days, cells were labeled with antibodies for flow cytometric analysis. Cells were labeled and washed in their original plates to minimize cell losses during transfers, and all labeling was done in saline buffer with 0.2% bovine serum albumin. First, cells were pre-incubated with 100 μ g/ml human IgG at room temperature for 15 min. Subsequently, cells were incubated with a mixture (total volume 50 μ l) of the following dye-labeled antibodies: CD5-PE, CD4-APC, CD8-Pacific Blue, CD25-PE-Cy7, as well as 7-Amino Actinomycin D (7AAD hereafter) for 40 min. Plates were washed twice, resuspended in 80 to 120 μ l volumes and ran immediately in a BD LSRII flow cytometer to acquire 80% of the contents of each well. The sample files were analyzed using FlowJo software to calculate the percentages and numbers of cells that had undergone at least one cell division,

according to their CFSE profile, by gating sequentially on activated, live CD4+ or CD8+ T cells (7AAD-, CD5+ CD25+ CD4+ or 7AAD- CD5+ CD25+ CD8+, respectively). Mean values and standard deviations were calculated using Microsoft Excel software. Graphs were plotted using Microsoft Excel or Graphpad Prism.

[00288] Analysis of live CD4+ and CD8+ populations from C4-2B cells treated with whole T cells revealed a significant increase in both the total number of cells in the presence of C4-2B cells expressing the target PSMA antigen upon treatment with TSC249 (Figure 26A). This increase was not seen in the presence of DU-145 cells that did not express the target PSMA antigen after treatment with TSC249 (Figure 26B). Finally, this increase was also not seen when isolated T cells were treated with TSC249 (Figure 26C). This confirmed the target-dependent nature of the T cell proliferation induced by TSC249 in the presence of tumor cells expressing the target antigen.

EXAMPLE 15: IN VIVO TESTING OF BISPECIFIC TARGETING OF CD3 AND PSMA

[00289] To confirm effectiveness of an anti-CD3 and anti-PSMA bispecific molecule at inhibiting tumor growth *in vivo*, a PSMA-directed molecule can be evaluated using any one or more of the following.

[00290] Prophylactic treatment, or prevention of tumor engraftment of subcutaneous tumors: Cultured, PSMA-expressing tumor cell lines (such as LNCaP, LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1, LuCaP 58, LuCaP 70, LuCaP 77) are mixed with human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) and injected subcutaneously into immunodeficient mice (such as SCID, NOD/SCID, etc.). An anti-PSMA bispecific molecule is injected intravenously on the day of injection and on several subsequent days. Dose-dependent inhibition of tumor outgrowth, as assessed by tumor volume, indicates that the respective molecule has efficacy against PSMA-expressing tumors *in vivo*.

[00291] Therapeutic treatment or regression of previously established subcutaneous tumors: Cultured, PSMA-expressing tumor cell lines (such as LNCaP, LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1AI, LuCaP 58, LuCaP 70, and LuCaP 77) are injected subcutaneously into immunodeficient mice (such as SCID, NOD/SCID, etc.). Tumor growth is monitored, and the study is initiated when tumors show signs of established growth (typically a volume of ~200 mm³). Human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) are injected intravenously along with an anti-PSMA bispecific molecule on the day of injection. The anti-PSMA bispecific molecule is injected several subsequent days. Dose-dependent inhibition of tumor growth, as assessed by tumor volume, indicates that the respective molecule has efficacy against PSMA-expressing tumors *in vivo*.

[00292] Prophylactic treatment, or prevention of tumor engraftment of intra-tibial tumors: Cultured, PSMA-expressing tumor cell lines (such as LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1, LuCaP 58, LuCaP 70, LuCaP 77) are mixed with human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) and injected intra-tibially into immunodeficient mice (such as SCID, NOD/SCID, etc.). An anti-PSMA bispecific molecule is injected intravenously on the day of injection and on several subsequent days. Dose-dependent inhibition of tumor growth, as assessed by serum biomarkers, radiography, fluorescent imaging, weight loss, and other proxy measurements of tumor volume, indicates that the respective molecule has efficacy against PSMA-expressing tumors *in vivo*.

[00293] Therapeutic treatment or regression of previously established intra-tibial tumors: Cultured, PSMA-expressing tumor cell lines (such as LNCaP C4-2, LNCaP C4-2B, VCaP, CWR22Rv1, LAPC4, MDA-PCa-2b, LuCaP 23.1AI, LuCaP 58, LuCaP 70, and LuCaP 77) are injected intra-tibially into immunodeficient mice (such as SCID, NOD/SCID, etc.). Tumor growth is monitored, and the study is initiated when tumors show signs of established growth (typically a volume of ~200 mm³). Human lymphocytes (either human peripheral blood mononuclear cells or purified T-cells) are injected intravenously along with an anti-PSMA bispecific molecule on the day of injection. The anti-PSMA bispecific molecule is injected several subsequent days. Dose-dependent inhibition of tumor growth, as assessed by serum biomarkers, radiography, fluorescent imaging, weight loss, and other proxy measurements of tumor volume, indicates that the respective molecule has efficacy against PSMA expressing tumors *in vivo*.

EXAMPLE 16: REDIRECTED T-CELL CYTOTOXICITY BY POLYPEPTIDE HETERODIMERS AND HOMODIMERS TARGETING PSMA

[00294] To compare the effectiveness of different bispecific polypeptide heterodimer molecules at inducing target-dependent T-cell cytotoxicity, two different homodimeric bispecific molecules (TSC194, TSC249) with a common anti-PSMA binding domain (hu107-1A4) and two different anti-CD3 binding domains (a Cris7 scFv for TSC194, the scFv from DRA222 for TSC249) were compared. TSC194 is a homodimeric bispecific ((huVL-VH#2 107-1A4 scFv-Fc-Cris7 scFv) (SEQ ID NO:250 & 251)

[00295] C4-2B cells (PSMA+) were obtained from MD Anderson and cultured according to published culture conditions in RPMI-1640 media (Life Technologies, Carlsbad, CA) plus 10% FBS. Peripheral blood mononuclear cells (PBMCs) were isolated from human blood using standard Ficoll gradients and washed in saline buffer. T-cells were additionally isolated from PBMC using a Pan T-cell Isolation Kit II from Miltenyi Biotec (Bergisch Gladbach, Germany) using the manufacturer's protocol.

[00296] Cytotoxicity was assessed by a ^{51}Cr release assay. Approximately 5×10^6 C4-2 cells were treated with 0.3 mCi of ^{51}Cr and incubated for 75 minutes at 37°C . After 75 minutes, cells were washed 3 times with media (RPMI + 10% FBS) and resuspended in 11.5 mL of media. From this suspension, 50 μL was dispensed per well into 96 well U-bottom plates (approximately 20,000 cells/well). Concentrations of bispecific molecules ranging from 500 pM to 0.2 pM were added to the C4-2 cells, bringing the total volume to 100 μL /well. Target cells were incubated at room temperature for 15 minutes. Then 100 μL of isolated T-cells (approximately 200,000) were added to bring the T-cell to target cell ratio to 10:1. 50 μL of 0.8% NP-40 was added to a control well containing target cells, left for 15 minutes, then 100 μL of media was added to provide a total lysis control.

[00297] Plates were incubated for 4 hours, spun at 1500 rpm for 3 minutes, and 25 μL of supernatant was transferred from each well to the corresponding well of a 96-well Luma sample plate. Sample plates were allowed to air dry in a chemical safety hood for 18 hours, and then radioactivity was read on a Topcount scintillation counter using a standard protocol.

[00298] Analysis of cytotoxicity data showed T-cell directed cytotoxicity with the C4-2 cells in the presence of T-cells and anti-PSMA directed bispecific molecules (Figure 27). For the homodimeric bispecific polypeptides (TSC194, TSC249), observed cytotoxicity was comparable between TSC194 and TSC249 (Table 10), suggesting that the DRA222 anti-CD3 scFv binding domain pl variant had equivalent activity to the parental Cris7 binding domain.

Table 10: Observed Cytotoxicity

	EC ₅₀ for RTCC
TSC194	2.8 $\pm$ 0.6 pM
TSC249	2.9 $\pm$ 1.0 pM

CLAIMS

The invention claimed is:

1. A CD3 binding polypeptide comprising a CD3 binding domain with a reduced isoelectric point as compared to a binding domain with the amino acid sequence of SEQ ID NO:41.
2. The CD3 binding polypeptide of claim 1, wherein said binding domain comprises a VH region and a VL region and wherein the VH region and the VL region comprise framework regions.
3. The CD3 binding polypeptide of claim 2, wherein two or more amino acids are modified in the framework regions of VH and/or VL by substituting positively charged amino acids with neutral amino acids and/or substituting neutral amino acids with negatively charged amino acids.
4. The CD3 binding polypeptide of claim 3, wherein the modified amino acids of the framework regions are substituted with amino acids contained within a corresponding human germline IgG sequence.
5. The CD3 binding polypeptide of claim 4, wherein the human germline IgG sequence comprises SEQ ID NO:43
6. The CD3 binding polypeptide of claim 4, wherein the human germline IgG sequence comprises SEQ ID NO:44
7. The CD3 binding polypeptide of claims 1-3 further comprising a prehinge region.
8. The CD3 binding polypeptide of claim 7, wherein said prehinge region has a reduced isoelectric point as compared to a CD3 binding polypeptide with an RRT prehinge region.
9. A CD3 binding polypeptide comprising a CD3 binding domain with framework regions, a prehinge region and a hinge region,

wherein the CD3 binding polypeptide has three or more modified amino acids as compared to SEQ ID NO:42, said modifications being substitutions of positively charged amino acids with neutral amino acids and/or substitutions of neutral amino acids with negatively charged amino acids in the framework and/or prehinge regions.

10. The CD3 binding polypeptide of claim 9, wherein at least two or more amino acids are modified in the framework regions of VH and/or VL by substituting positively charged amino acids with neutral amino acids and/or substituting neutral amino acids with negatively charged amino acids.

11. The CD3 binding polypeptide of claims 3 and 9, wherein at least four or more amino acids are modified in the framework regions of VH and/or VL by substituting positively charged amino acids with neutral amino acids and/or substituting neutral amino acids with negatively charged amino acids.

12. The CD3 binding polypeptide of claims 3 and 9, wherein three to five amino acids are modified in the framework regions of VH and/or VL by substituting positively charged amino acids with neutral amino acids and/or substituting neutral amino acids with negatively charged amino acids.

13. The CD3 binding polypeptide of claims 3 and 9, wherein three to ten amino acids are modified in the framework regions of VH and/or VL by substituting positively charged amino acids with neutral amino acids and/or substituting neutral amino acids with negatively charged amino acids.

14. The CD3 binding polypeptide of claims 8-9, wherein the CD3 binding polypeptide pre-hinge region comprises the amino acid sequence SSS or SST.

15. The CD3 binding polypeptide of claims 1-8, wherein the CD3 binding polypeptide further comprises a hinge region and a constant region.

16. The CD3 binding polypeptide of claims 1-15, wherein the CD3 binding polypeptide has an empirical isoelectric point of at least 1 pI unit less than a polypeptide of SEQ ID NO:4.

17. The CD3 binding polypeptide of claim 1, wherein the CD3 binding polypeptide is a humanized antibody, a single chain Fv (scFv) or a SMIP.

18. The CD3 binding polypeptide of claim 15, wherein the CD3 binding polypeptide comprises, from amino to carboxyl terminus, the CD3 binding domain, the hinge region and the constant region.

19. The CD3 binding polypeptide of claim 15, wherein the binding polypeptide comprises, from amino to carboxyl terminus, the constant region, the hinge region and the CD3 binding domain.

20. The CD3 binding polypeptide of claim 15, wherein the binding polypeptide further comprises a second binding domain.

21. The CD3 binding polypeptide of claim 20, wherein the binding polypeptide comprises, from amino to carboxyl terminus, the CD3 binding domain, the hinge region, the constant region, and the second binding domain.

22. The CD3 binding polypeptide of claim 20, wherein the CD3 binding polypeptide comprises, from amino to carboxyl terminus, the second binding domain, the hinge region, the constant region, and the CD3 binding domain.

23. The CD3 binding polypeptide of claim 15, wherein the CD3 binding polypeptide further comprises a heterodimerization domain.

24. The CD3 binding polypeptide of claim 23, wherein the CD3 binding polypeptide comprises, from amino to carboxyl terminus, the CD3 binding domain, the hinge region, the constant region and the heterodimerization domain.

25. The CD3 binding polypeptide of claims 1-24, wherein the CD3 binding polypeptide further comprises a second binding domain.

26. The CD3 binding polypeptide of claim 25 wherein the second binding domain binds or interacts with a target molecule and the CD3 binding polypeptide induces T-cell cytotoxicity.

27. The CD3 binding polypeptide of claim 26, wherein the second binding domain binds or interacts with a tumor associated antigen.

28. The CD3 binding polypeptide of claim 27, wherein the CD3 binding polypeptide induces T-cells to lyse tumor cells.

29. The CD3 binding polypeptide of claims 27, wherein the CD3 binding polypeptide induces polyclonal T- cell activation and expansion in the vicinity of a tumor.

30. The CD3 binding polypeptide of claims 27-29, wherein the tumor associated antigen is selected from the group consisting of RON, c-Met, CEACAM-6, PSMA, EpCAM, CEA, PCTA-1, STEAP-1, STEAP-2, PSCA, PSA, PAP, ALCAM (CD166), PECAM-1, EphA2, CD151, CA-125/MUC16, MUC-1, MAGE-1, TROP2, IGF1R, TGFBR2, GHRHR, GHR, IL-6R, gp130, TNFR2, OSMR β , Patched-1, Frizzled, Robo1, LT β R, CD25, CD26, CD27, CD30, CD33, CD44, CD44v6, CD63,

CD80, CD81, CD86, CD100, CD151, CXCR4, CCR5, HER-2/ErbB1, HER-3/ErbB3, HER-4/ErbB4, EGFR/ErbB1, EGFRvIII isoform, MUC2, MUC3, MUC4, MUC5_{AC}, MUC5_b, MUC7, β hCG, Lewis-Y, ganglioside GD3, 9-O-Acetyl-GD3, GM2, Globo H, fucosyl GM1, Poly SA, GD2, Carbonic anhydrase IX (MN/CA IX), Sonic Hedgehog (Shh), Wue-1, Plasma Cell Antigen, (membrane-bound) IgE, Melanoma Chondroitin Sulfate Proteoglycan (MCSP), CCR8, TNF-alpha precursor, mesothelin, A33 Antigen, Ly-6; desmoglein 4, E-cadherin neoepitope, Fetal Acetylcholine Receptor, CA19-9 marker, Muellierian Inhibitory Substance (MIS) Receptor type II, sTn (sialylated Tn antigen; TAG-72), FAP (fibroblast activation antigen), endosialin, LG, SAS, BCMA, TWEAKR/Fn14, FGFR4, VEGFR1, VEGFR2, SSX1, and SSX2.

31. The CD3 binding polypeptide of claims 1-30, wherein the CD3 binding domain binds or interacts with the CD3 ϵ subunit of the T-cell receptor complex on T-cells.

32. The CD3 binding polypeptide of claims 1-31, wherein the CD3 binding polypeptide induces internalization of T-cell receptor complex.

33. The CD3 binding polypeptide of claims 1-32, wherein the CD3 binding domain is a humanized CD3 binding domain derived from murine Cris-7 monoclonal antibody or HuM291.

34. The CD3 binding polypeptide of claim 33, wherein the CD3 binding domain comprises heavy chain CDR1, CDR2 and CDR3 and light chain CDR1, CDR2 and CDR3 of murine Cris-7 antibody or HuM291.

35. The CD3 binding polypeptide of claim 1, wherein the CD3 binding domain comprises a VH region sequence or VL region sequence selected from the group consisting of SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:38 and SEQ ID NO:40 .

36. The CD3 binding polypeptide of claim 1, wherein the CD3 binding domain comprises a VH region selected from the group consisting of SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30 and SEQ ID NO:32 and a VL region selected from the group consisting of SEQ ID NO:38 and SEQ ID NO:40.

37. The CD3 binding polypeptide of claim 1, wherein the CD3 binding domain comprises a VH region selected from the group consisting of SEQ ID NO:28, SEQ ID

NO:30 and SEQ ID NO:32 and a VL region selected from the group consisting of SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38 and SEQ ID NO:40.

38. The CD3 binding polypeptide of claim 2, wherein the VH region comprises SEQ ID NO:28 and the VL region comprises SEQ ID NO:34.

39. The CD3 binding polypeptide of claim 2, wherein the VH region comprises SEQ ID NO:28 and the VL region comprises SEQ ID NO:38.

40. The CD3 binding polypeptide of claim 2, wherein the VH region comprises SEQ ID NO:26 and the VL region comprises SEQ ID NO:38.

41. The CD3 binding polypeptide of claims 9 and 14, wherein the VH region comprises SEQ ID NO:26 and the VL region comprises SEQ ID NO:34.

42. The CD3 binding polypeptide of claim 1, wherein said CD3 binding polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20.

43. The CD3 binding polypeptide of claim 1, wherein the CD3 binding domain comprises a heavy chain CDR1, CDR2 and CDR3 and a light chain CDR1, CDR2 and CDR3 and wherein the heavy chain CDR3 comprises SEQ ID NO:51.

44. The CD3 binding polypeptide of claim 43, wherein the heavy chain CDR1 comprises SEQ ID NO:49, CDR2 comprises SEQ ID NO:50 and CDR3 comprises SEQ ID NO:51 and light chain CDR1 comprises SEQ ID NO:52, CDR2 comprises SEQ ID NO:53, and CDR3 comprises SEQ ID NO:54.

45. The CD3 binding polypeptide of claim 15, wherein the constant region comprises CH2 and CH3 domains of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD or any combination thereof; an immunoglobulin CH3 domain of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, IgM or any combination thereof; or immunoglobulin CH3 and CH4 domains of IgE, IgM or a combination thereof.

46. The CD3 binding polypeptide of claim 15, wherein the constant region comprises CH2 and CH3.

47. The CD3 binding polypeptide of claim 15, wherein the constant region consists essentially of a CH2 domain and CH3 domain.

48. The CD3 binding polypeptide of claims 45-47, wherein the constant region is modified to reduce or eliminate effector function.

49. The CD3 binding polypeptide of claims 45-47, wherein the constant region is modified to not fix complement.

50. The CD3 binding polypeptide of claims 45-47, wherein the constant region is modified to not bind to Fc γ receptors.

51. The CD3 binding polypeptide of claim 50, wherein the Fc γ receptors are selected from the group consisting of CD16, CD32 and CD64.

52. The CD3 binding polypeptide of claim 15, wherein the constant region comprises the amino acid sequence of SEQ ID NO:55.

53. A nucleic acid encoding any one of the polypeptides of claims 1-52.

54. An isolated nucleic acid comprising the nucleic acid selected from the group consisting of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:27 and SEQ ID NO:37.

55. An expression vector comprising the nucleic acid of claim 53 or 54.

56. A recombinant host cell comprising the expression vector of claim 32.

57. A composition comprising the CD3 binding polypeptide of any of claims 1-52 and a pharmaceutically acceptable carrier, diluent or excipient.

58. A method for treating cancer or an autoimmune disorder comprising administering a therapeutically effective amount of the composition of claim 57 or a CD3 binding polypeptide of claims 1-52 to a patient in need thereof.

59. The CD3 binding polypeptide of claims 1-52, wherein said CD3 binding polypeptide forms a dimer.

60. The CD3 binding polypeptide of claim 59, wherein the dimer is a homodimer or heterodimer.

61. The CD3 binding polypeptide of claim 3, wherein at least one amino acid modification is within the J kappa (Jk) region of the VL region as compared to the Cris-7 VL Jk region mouse sequence LQIT (SEQ ID NO:252).

62. The CD3 binding polypeptide of claim 61, wherein the Jk region comprises the amino acid sequence VEIK (SEQ ID NO:253).

63. The CD3 binding polypeptide of claim 25, wherein the second binding domain comprises an amino acid sequence that is at least 90%, at least 95% or 100% identical to an amino acid sequence selected from the group consisting of amino acids 1-107 and 124-243 of SEQ ID NO:212; amino acids 1-107 and 124-243 of SEQ ID NO:226; amino acids 1-107 and 124-243 of SEQ ID NO:216; and 1-111 and 128-251 of SEQ ID NO:196;

64. The CD3 binding polypeptide of claim 1, wherein the binding polypeptide comprises an amino acid sequence that is at least 90%, at least 95% or 100% identical to SEQ ID NO: 57, 59, 61, 63, 65, 67, 69, 71, 186, 190, 192, 194, 196, 198, 200, 202, 204 or 206.

65. The CD3 binding polypeptide of claim 1, wherein the polypeptide is part of a heterodimer.

66. The CD3 binding polypeptide of claim 65, wherein the heterodimer comprises a pair of single chain polypeptides wherein the single chain pair comprises amino acid sequences that are at least 90%, at least 95% or 100% identical to the pairs selected from SEQ ID NO:210 & 247, SEQ ID NO:210 & 218, SEQ ID NO:210 & 220, SEQ ID NO:208 & 249, SEQ ID NO:208 & 222, or SEQ ID NO:208 & 224, SEQ ID NO:212 & 218, SEQ ID NO:216 & 222, SEQ ID NO:228 & 226, or SEQ ID NO:214 & 218.

67. The CD3 binding polypeptide of claim 1, wherein the CD3 binding domain is about 90% to about 99% identical to SEQ ID NO:41.

Theoretical pI of previously humanized anti-CD3 SMIP molecules
as compared to other SMIP molecules

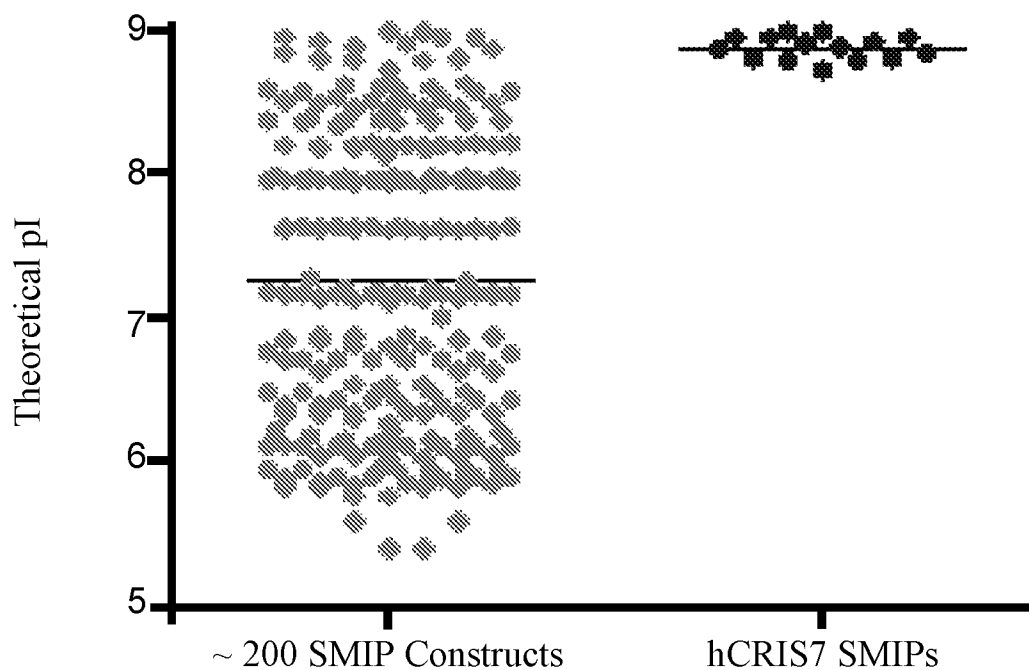
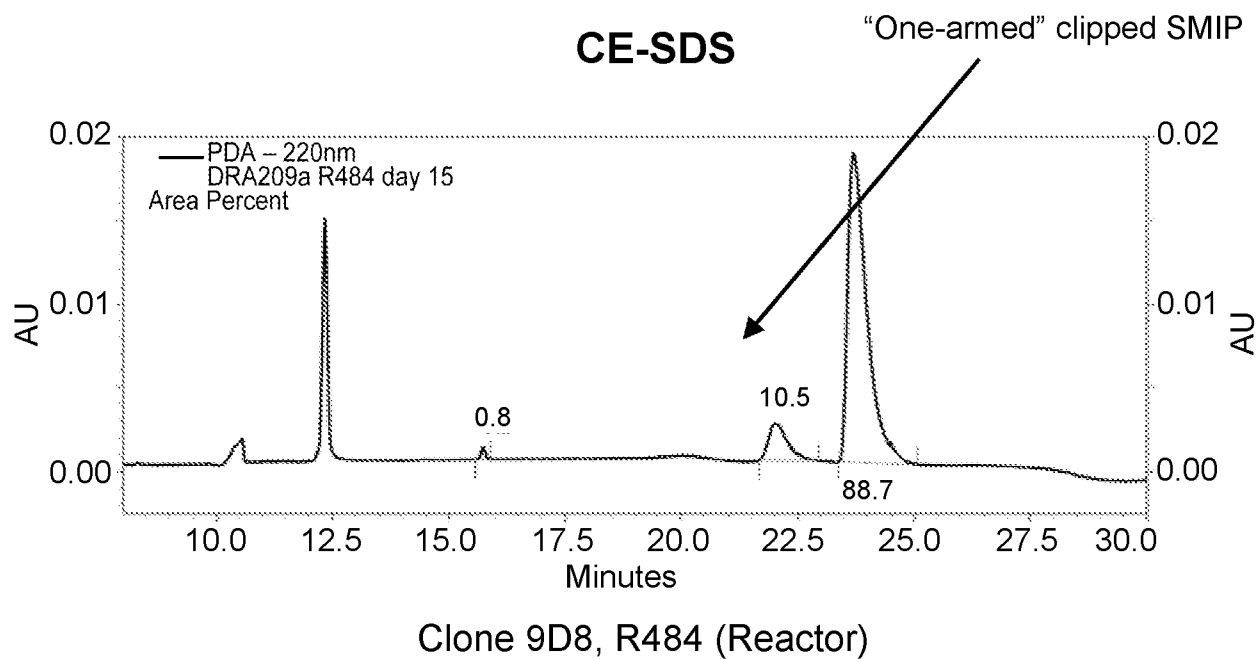
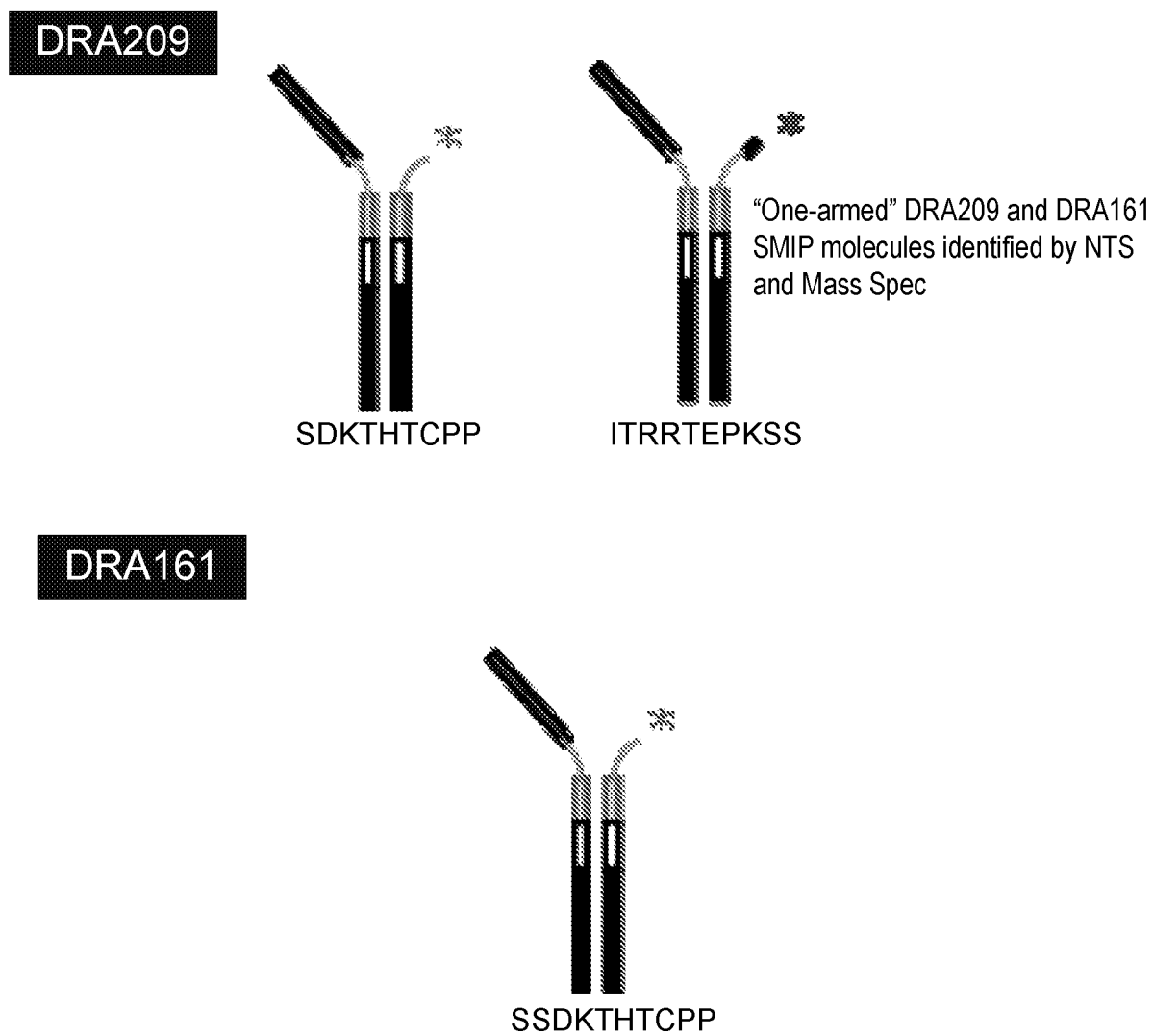


FIG. 1

**FIG. 2A**

**FIG. 2B**

	(1)	1	10	20	30	40	51
Cris7 VH mouse	(1)	QVQLQQSGAE	EPGASVKN	SCKASGYTFTTR	STMHWVRQ	APGQGLEW	IGYI
H1 chain	(1)	QVQLVQSGAE	EVKRPASV	KNVSCKASGYTFTTR	STMHWVRQ	APGQGLEW	IGYI
H2 chain	(1)	QVQLVQSGGG	GVVQFGR	SLRLSCKASGYTFTTR	STMHWVRQ	APGQGLEW	IGYI
H3 chain	(1)	QVQLVQSGGG	GVVQFGR	SLRLSCKASGYTFTTR	STMHWVRQ	APGQGLEW	IGYI
H4 chain	(1)	QVQLVQSGGG	GVVQFGR	SLRLSCKASGYTFTTR	STMHWVRQ	APGQGLEW	IGYI
H6 chain	(1)	EVQLVQSGGG	IVRPGG	SLRLSCAASGFTTR	STMHWVRQ	APGQGLEW	IVYI
H5 chain	(1)	QVQLVQSGAE	EVKRPASV	KNVSCKASGYTFTTR	STMHWVRQ	APGQRL	EWNGYI
Consensus	(1)	QVQLVQSGGG	VVRPGASL	RLSCKASGYTFTTR	STMHWVRQ	APGQGLEW	IGYI
Section 2							
	(52)	52	60	70	80	90	102
Cris7 VH mouse	(52)	NPSSAYTNYN	QKFKL	ATLTADKSS	STAYMQLSSL	TSEDSAVYYC	ASPVQH
H1 chain	(52)	NPSSAYTNYN	QKFKL	ATLTADKSS	STAYMQLSSL	TSEDSAVYYC	ASPVQH
H2 chain	(52)	NPSSAYTNYN	QKFPEDR	ATLTADKSS	STAYMQLSSL	TSEDSAVYYC	ASPVQH
H3 chain	(52)	NPSSAYTNYN	QKFPDR	FTTISADKSK	STAPLQMD	SLRPELDTG	VYFCARPVH
H4 chain	(52)	NPSSAYTNYN	QKFKDR	FTTISADKSK	STAPLQMD	SLRPELDTG	VYFCARPVH
H6 chain	(52)	NPSSAYTNYN	QKFKDR	FTTISRDN	AKMSLYLQ	MSLRPELDT	AVYYCARPVH
H5 chain	(52)	NPSSAYTNYN	QKFPEDR	VTTITRDT	SASTAYMQLSSL	TSEDSAVYYC	ASPVQH
Consensus	(52)	NPSSAYTNYN	QKFKDR	TITADKSK	STAYMQLSSL	TSEDSAVYYC	ASPVQH
Section 3							
	(103)	103	110	121			
Cris7 VH mouse	(103)	YDNGFPYWGQ	STLVT	VSA			
H1 chain	(103)	YDNGFPYWGQ	STLVT	VSS			
H2 chain	(103)	YDNGFPYWGQ	STLVT	VSS			
H3 chain	(103)	YDNGFPYWGQ	STPVT	VSS			
H4 chain	(103)	YDNGFPYWGQ	STPVT	VSS			
H6 chain	(103)	YDNGFPYWGQ	STLVT	VSS			
H5 chain	(103)	YDNGFPYWGQ	STLVT	VSS			
Consensus	(103)	YDNGFPYWGQ	STLVT	VSS			

FIG. 3

		Section 1						
		(1)	1	10	20	30	40	50
Cris7 VL mouse	(1)	AQCIVLTQSPAIMSAPPGERVTMTCSA3SSSVSYMNNWYQQKSGTSPKRWIYDS						
L4 chain	(1)	--EIVLTQSPATLSLSPGERATLSCSA3SSSVSYMNNWYQQKPGCAPRLLIYDS						
L1 chain	(1)	AQDIQMTQSPSSLSASVGDRVTMTCSA3SSSVSYMNNWYQQKPGKAPKRWIYDS						
L2 chain	(1)	AQDIQMTQSPSSLSASVGDRVTITCSA3SSSVSYMNNWYQQTEGKAPKRWIYDS						
L3 chain	(1)	AQDIQMTQSPSSLSASVGDRVTMTCSA3SSSVSYMNNWYQQKPGKAPRLLIYDS						
Consensus	(1)	AQDIQMTQSPSSLSASVGDRVTMTCSA3SSSVSYMNNWYQQKPGKAPKRWIYDS						
		Section 2						
		(53)	53	60	70	80	90	104
Cris7 VL mouse	(53)	SKLASGVPARFSGSGSGTSYSLTISSMETEDAAITYYCCQWSRNPPTFGGGTF						
L4 chain	(51)	SKLASGVPARFSGSGSGTDFTLTISSGLEEDFAVYYCCQWSRNPPTFGGGTF						
L1 chain	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCCQWSRNPPTFGGGTF						
L2 chain	(53)	SKLASGVPARFSGSGSGTDFTLTISSSLQPEDFATYYCCQWSRNPPTFGGGTF						
L3 chain	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCCQWSRNPPTFGGGTF						
Consensus	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCCQWSRNPPTFGGGTF						
		Section 3						
		(105)	105	108				
Cris7 VL mouse	(105)	LQIT						
L4 chain	(103)	WEIN						
L1 chain	(105)	LQIT						
L2 chain	(105)	LQIT						
L3 chain	(105)	WEIN						
Consensus	(105)	LQIT						

FIG. 4

								Section 1
	(1)	1	10	20	30	40	53	
H3 chain	(1)	QVQLVQSGGGVVPGRSLRLSCKASGFTTFRSTMHWVRQAPGKGLEWIGYINP						
IGHV1-69*01	(1)	QVQLVQSGAEVKRPGSSVVFVSCKASGGTFSSYATISWVRQAPGQGLEWNGGIIP						
IGHV3-30*01	(1)	QVQLVESGGGVVPGRSLRLSCAASGFTTFSSYAMHWVRQAPGKGLEWYAVISY						
Consensus	(1)	QVQLVQSGGGVVPGRSLRLSCKASGFTTFSSYAMHWVRQAPGKGLEWIG I P						
								Section 2
	(54)	54	60	70	80	90	106	
H3 chain	(54)	SSAYTNYNQEFKDRFTISADKSKSTAFLOMDSLRFEDTGVTFCARPQVHYDYN						
IGHV1-69*01	(54)	IFGTANYAQKFCGRVTITADESTSTAYNELSSLRSEDYAVYYCAR-----						
IGHV3-30*01	(54)	DGSNRYTADSVKGRFTISRNSNTLYLQMNSLRAEDYAVYYCAR-----						
Consensus	(54)	A NYAQKFKGRFTISAD SKSTAYLQM SLRAEDYAVYYCAR						
								Section 3
	(107)	107	121					
H3 chain	(107)	GFPYWGQGTPVTVSS						
IGHV1-69*01	(99)	-----						
IGHV3-30*01	(99)	-----						
Consensus	(107)							

FIG. 5

Jk region		Section 1										Section 2		Section 3	
		1	10	20	30	40	50	60	70	80	90	100	110	120	130
IGKV1-5*01	(1)	--DIQMTQSP	STLSASVGD	RVTTTCRA	SQSISSTIA	WYQQK	QKAEK	LI	IYDAS						
L1 chain	(1)	AQDIQMTQSP	STLSASVGD	RVTTTCRA	SQSISSTIA	WYQQK	QKAEK	LI	IYDAS						
Consensus	(1)	DIQMTQSP	STLSASVGD	RVTTTCRA	SQSISSTIA	WYQQK	QKAEK	LI	IYDAS						
		(55)	55	60	70	80	90	100	110	120	130	140	150	160	170
IGKV1-5*01	(53)	SLESGV	PARFSGSG	STFTLT	ISSIQP	DDFAT	YCCQ	NSYS	---						
L1 chain	(54)	KLASGV	PARFSGSG	STFTLT	ISSIQP	DDFAT	YCCQ	NSYS	---						
Consensus	(55)	L	SGV	PARFSGSG	STFTLT	ISSIQP	DDFAT	YCCQ	NSYS	---					
		(109)	109	110	120	130	140	150	160	170	180	190	200	210	220
IGKV1-5*01	(96)	---													
L1 chain	(108)	T													
Consensus	(109)	---													
Species	Gene	Allele	AccNum	Domain label	Functionality	Sequence									
Homsap	IGKJ1	IGKJ1*01	J00242		F	WTGQGTKEIK									
Homsap	IGKJ2	IGKJ2*01	J00242		F	YTFGQGTKEIK									
Homsap	IGKJ3	IGKJ3*01	J00242		F	FTGPGTKVDIK									
Homsap	IGKJ4	IGKJ4*01	J00242		F	LTGSGGTKEIK									
Homsap	IGKJ5	IGKJ5*01	J00242		F	ITGQGTKEIK									

JK4 is a better fit because of sequence similarity to the end of the CDR3 sequence

FIG. 6

[illegible]

FIG. 7A

	214	220	230	240	250	260	270	Section 4
Translation of DRA209 (214)	T	I	S	S	L	Q	P	284
Translation of DRA209 (211)	T	I	S	S	L	Q	P	
Translation of DRA219 (211)	T	I	S	S	L	Q	P	
Translation of DRA222 (211)	T	I	S	S	L	Q	P	
Translation of DRA221 (211)	T	I	S	S	L	Q	P	
Translation of DRA223 (211)	T	I	S	S	L	Q	P	
Translation of DRA224 (214)	T	I	S	S	L	Q	P	
Translation of DRA225 (214)	T	I	S	S	L	Q	P	
Consensus (214)	T	I	S	S	L	Q	P	
Translation of DRA209 (285)	L	M	I	S	R	T	P	355
Translation of DRA219 (282)	L	M	I	S	R	T	P	
Translation of DRA222 (282)	L	M	I	S	R	T	P	
Translation of DRA221 (282)	L	M	I	S	R	T	P	
Translation of DRA223 (282)	L	M	I	S	R	T	P	
Translation of DRA224 (285)	L	M	I	S	R	T	P	
Translation of DRA225 (285)	L	M	I	S	R	T	P	
Consensus (285)	L	M	I	S	R	T	P	
Translation of DRA209 (358)	A	V	S	N	K	G	L	426
Translation of DRA219 (353)	A	V	S	N	K	G	L	
Translation of DRA222 (353)	A	V	S	N	K	G	L	
Translation of DRA221 (353)	A	V	S	N	K	G	L	
Translation of DRA223 (353)	A	V	S	N	K	G	L	
Translation of DRA224 (358)	A	V	S	N	K	G	L	
Translation of DRA225 (358)	A	V	S	N	K	G	L	
Consensus (358)	A	V	S	N	K	G	L	

FIG. 7B

FIG. 8

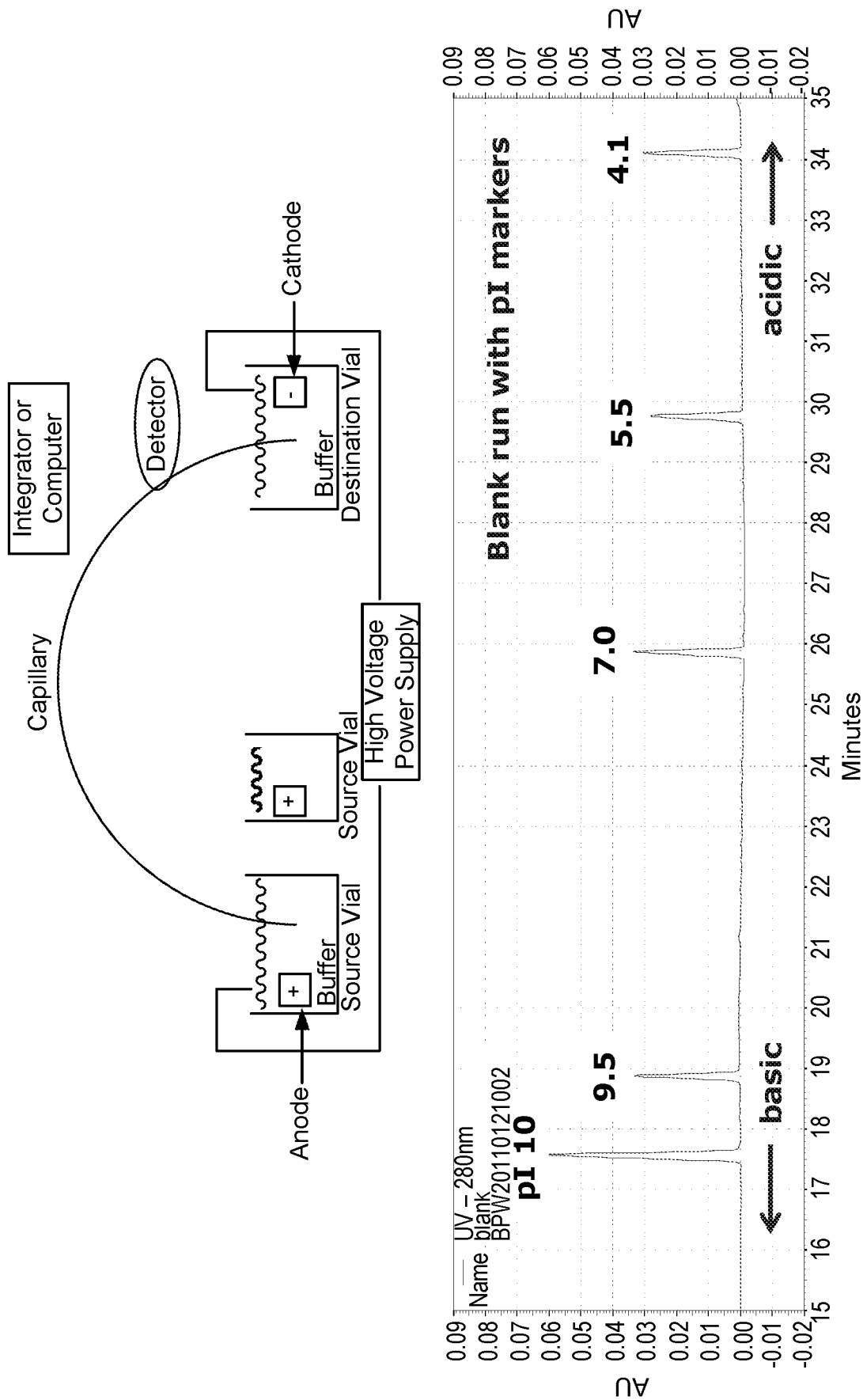
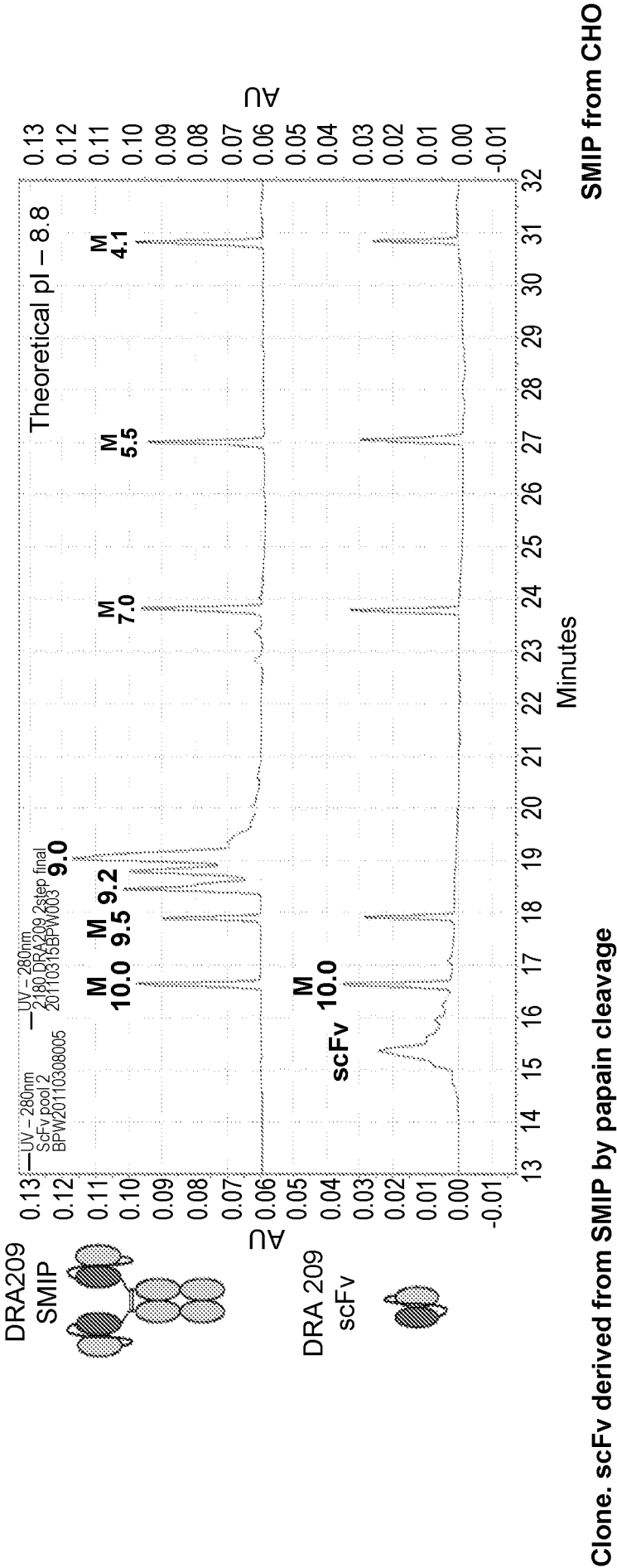
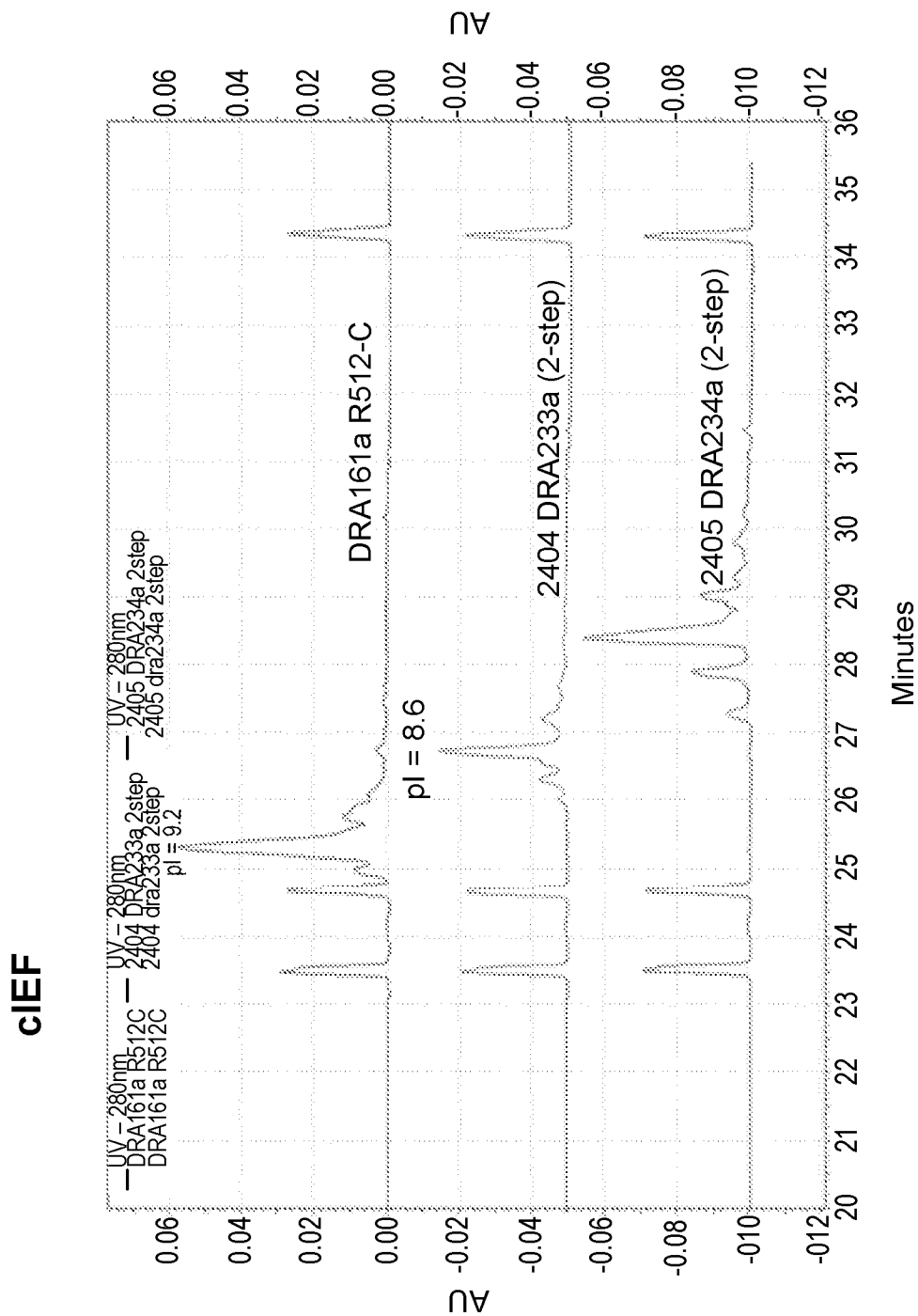


FIG.9

**cIEF: Empirical pI of SMIP and scFv Are
Even Higher Than Theoretical pI**





DRA233a and DRA234a Expressed in CHO cells

FIG. 11

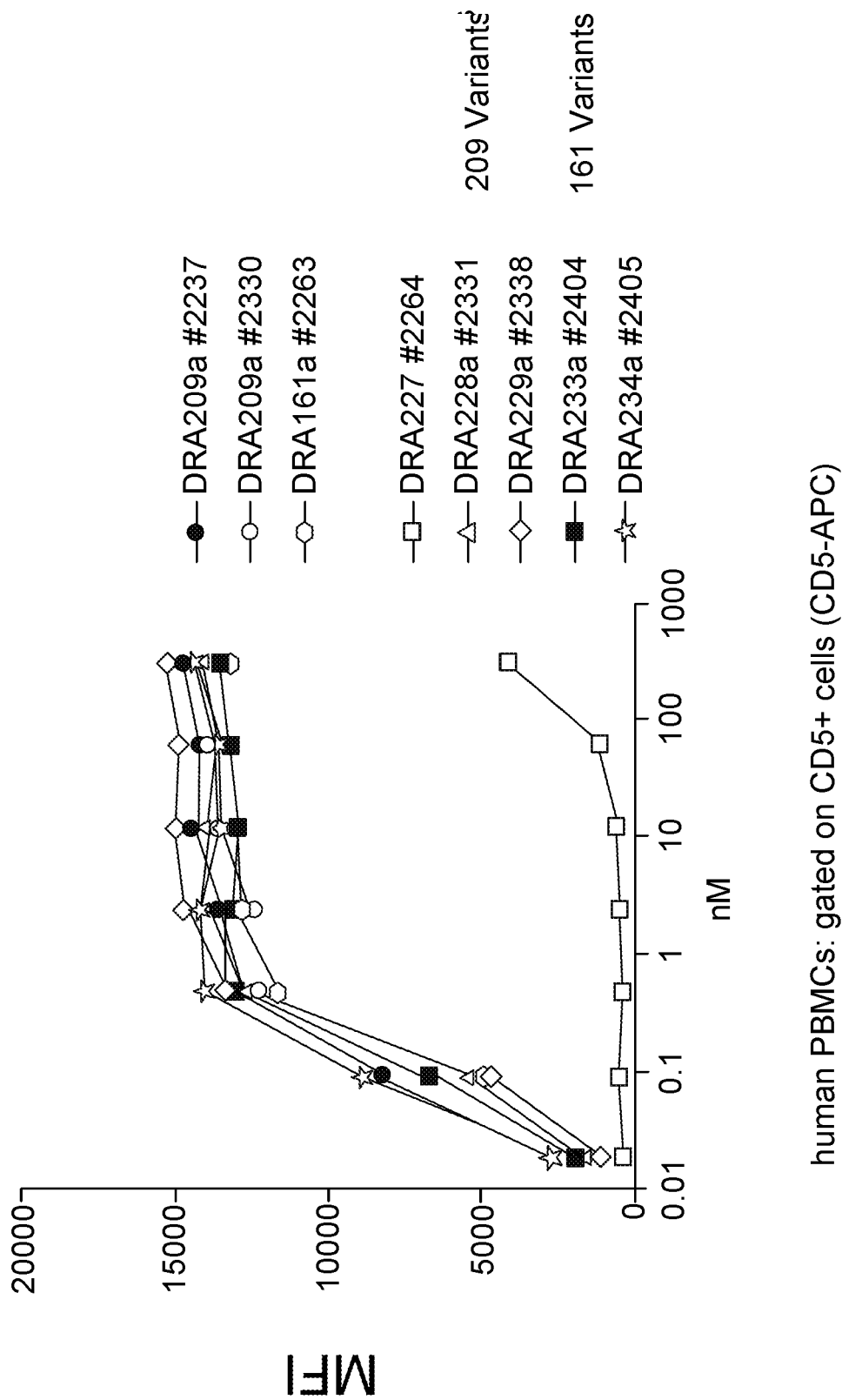
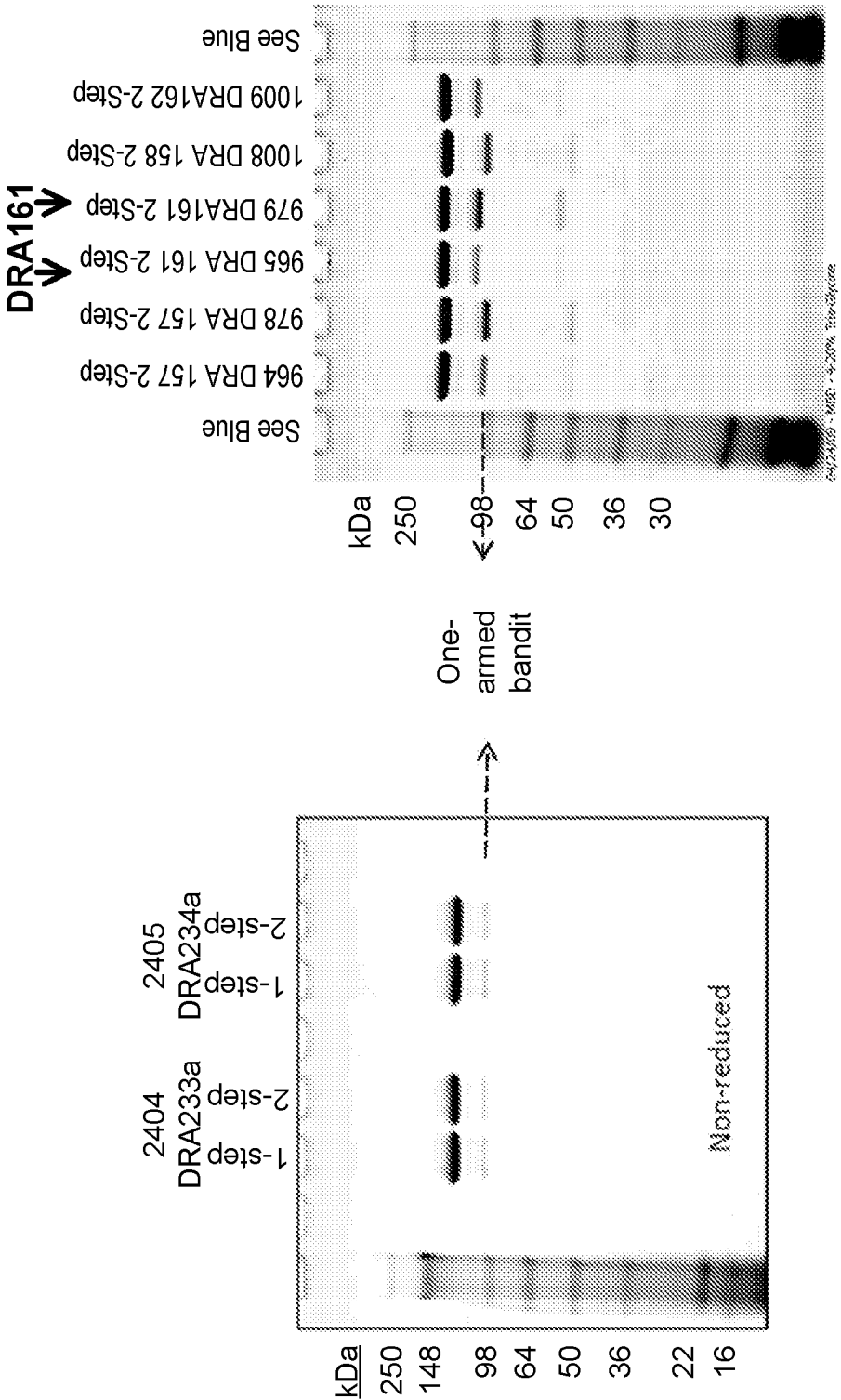


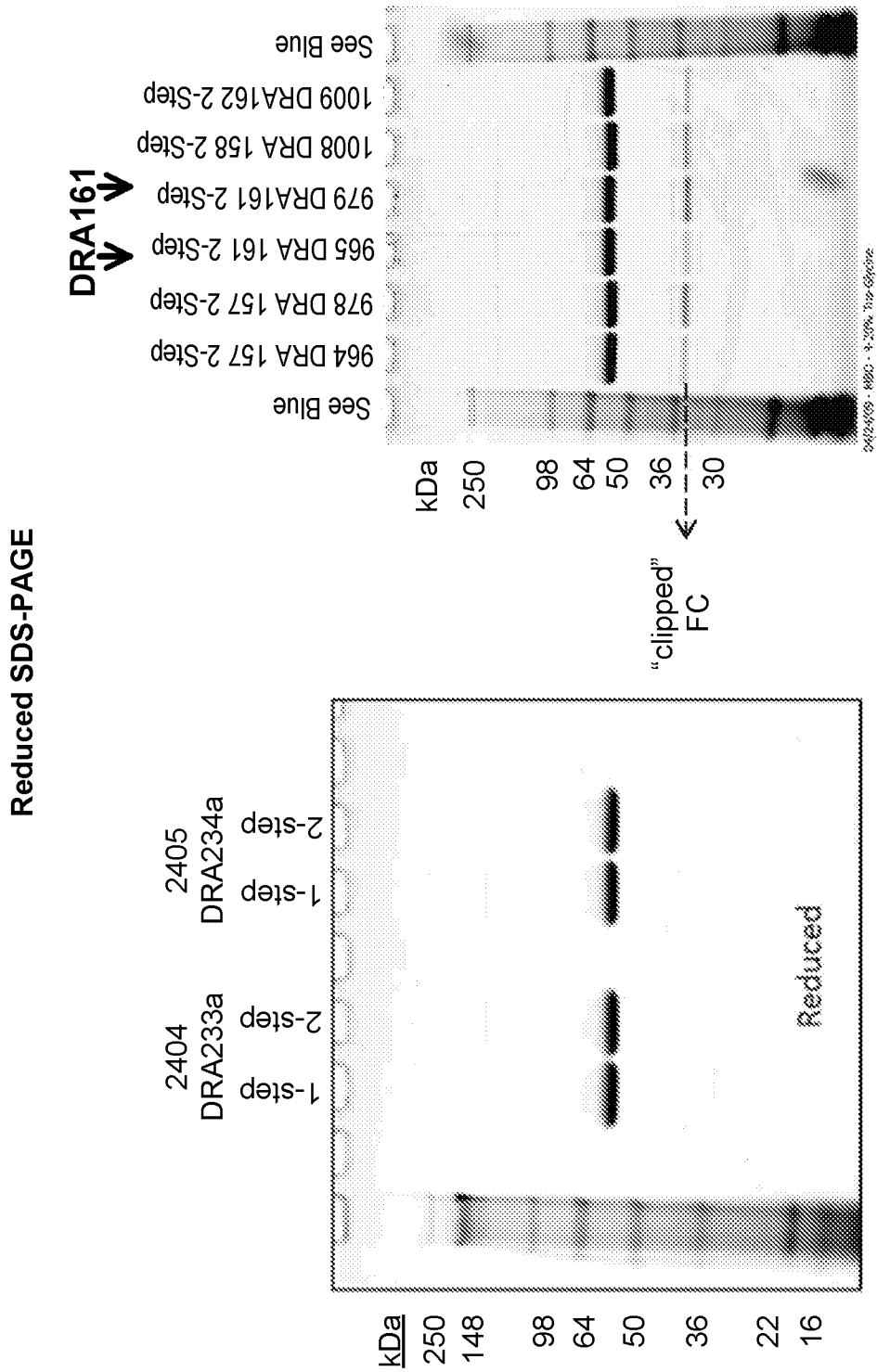
FIG. 12

Non-reduced SDS-PAGE: Data shows pl Variant SMIPs are less prone to fragmentation



DRA161a, DRA233a and DRA234a Expressed in CHO cells (Bulk)

FIG. 13



DRA161a, DRA233a and DRA234a Expressed in CHO cells (Bulk)

FIG. 14

Non-reduced CE-SDS of pI Variant SMIPs

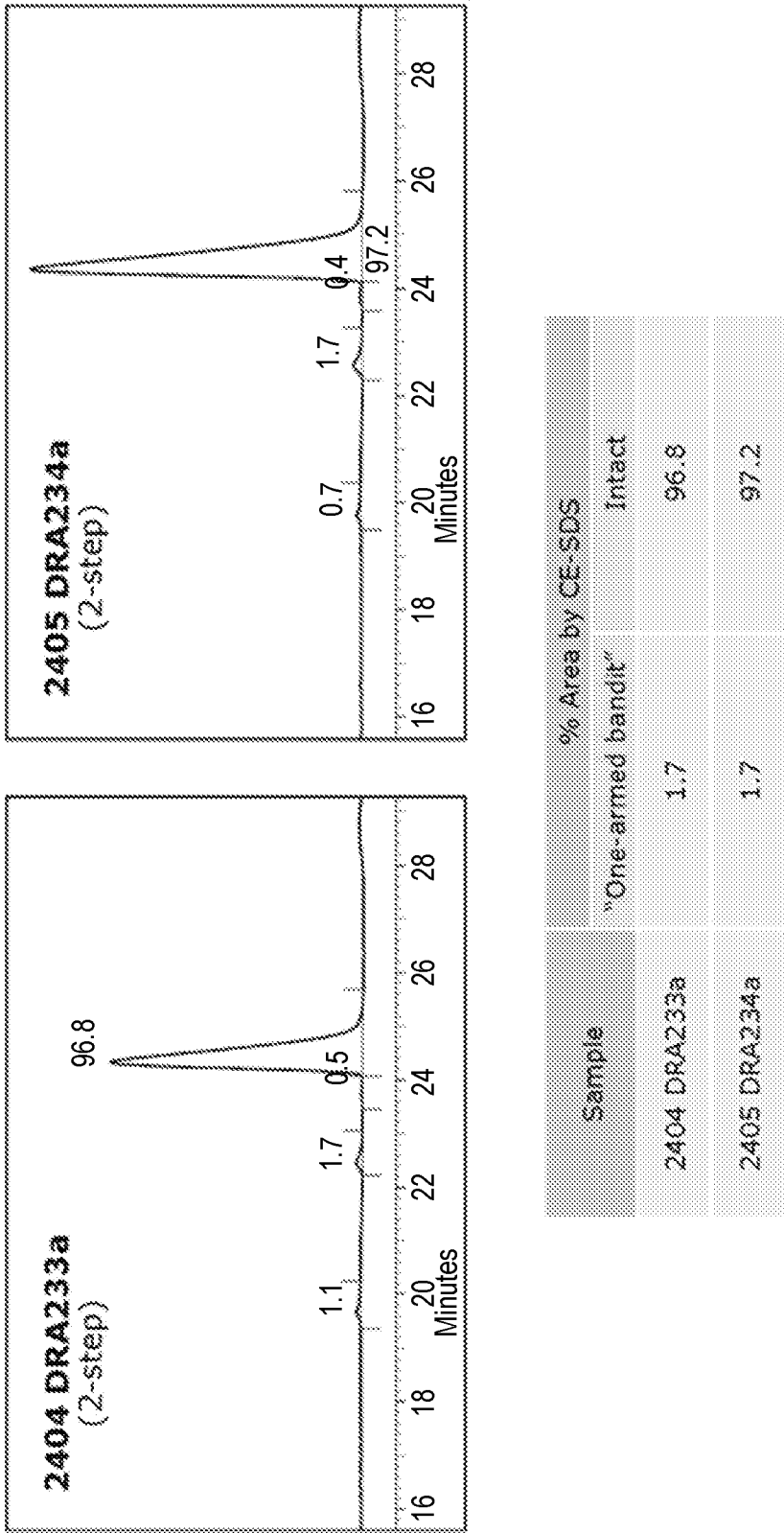


FIG. 15

Non-reduced CE-SDS of DRA 161 (bulk)
purified at large scale by PAD

pI Variants of
DRA161/209

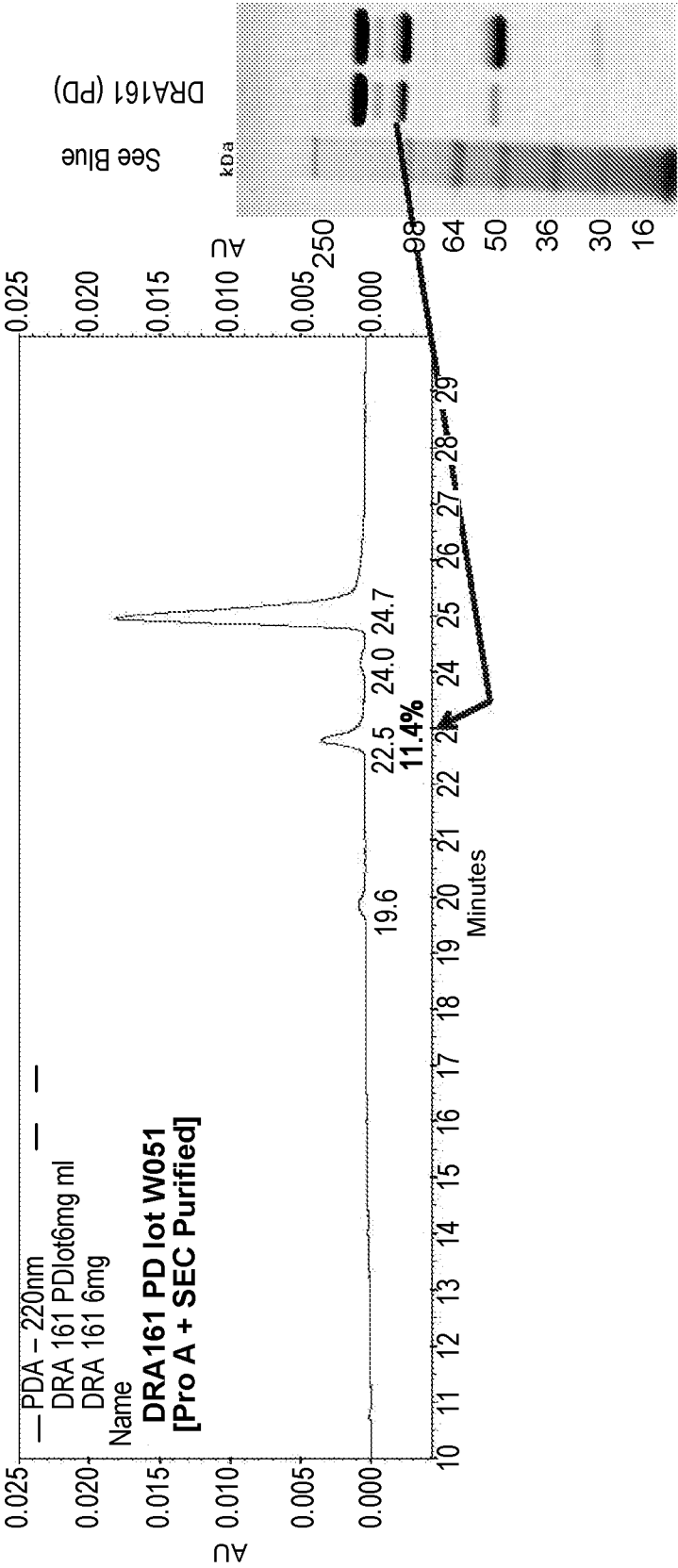


FIG. 16

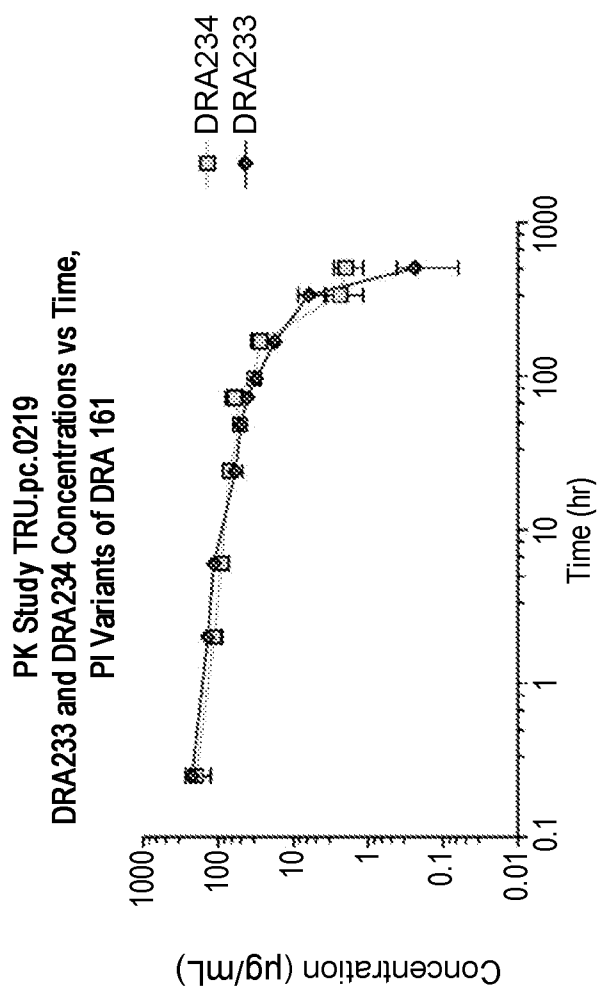
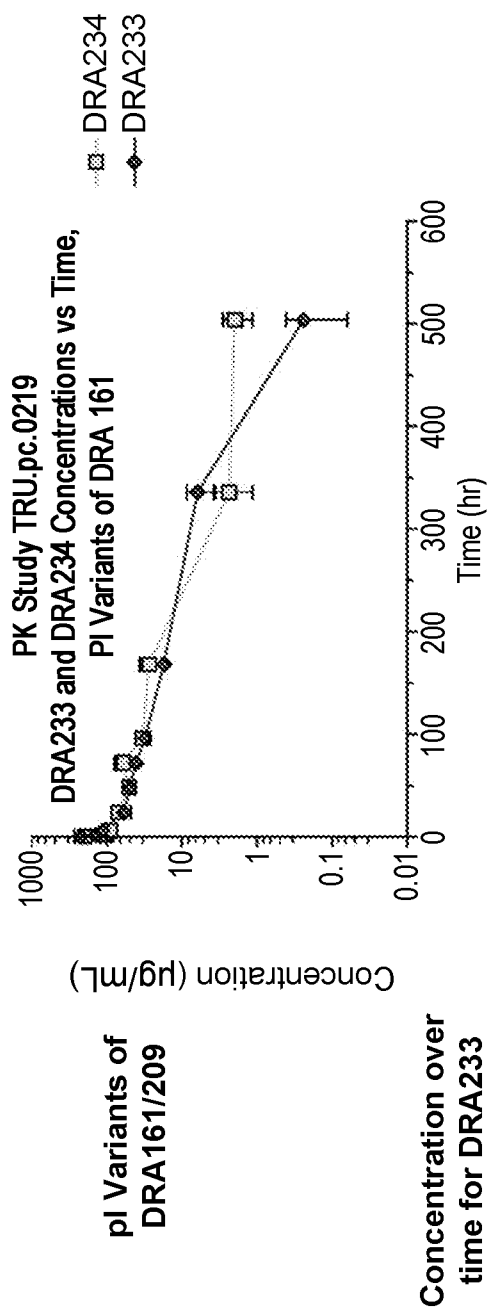


FIG. 17

WinNonLin Analysis

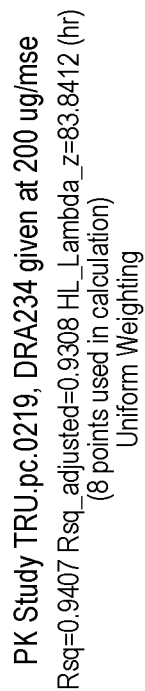
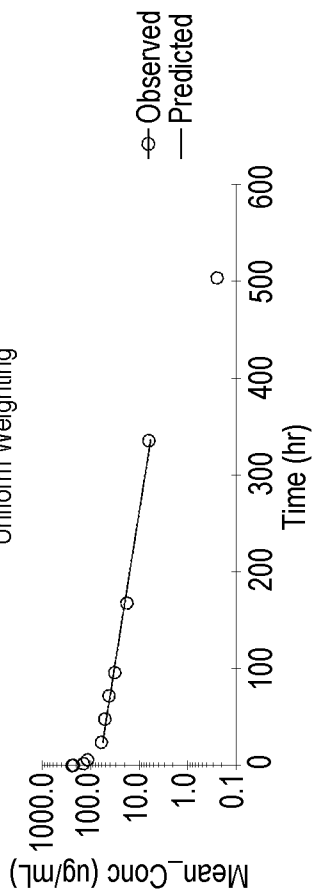
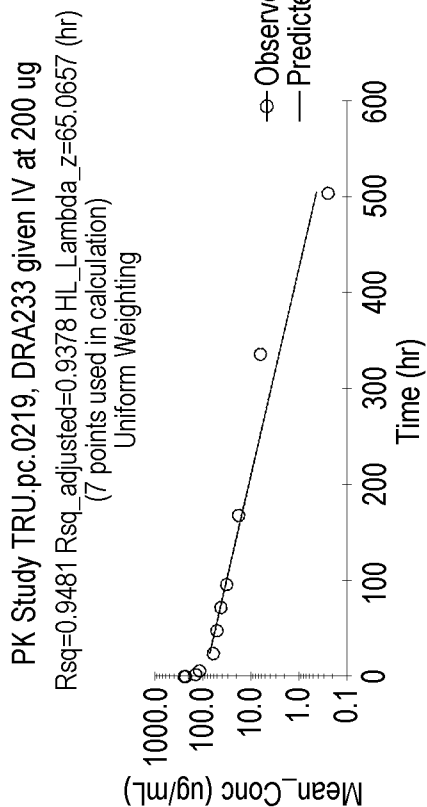


FIG. 18

	Estimate	Units	DRA233 w/o 504 hr	DRA233	DRA234
pl Variants of DRA 161/209	Rsqr		0.9918	0.9481	0.9407
	Rsqr_adjusted		0.9898	0.9378	0.9308
	Corr_XY		-0.9959	-0.9737	-0.9699
	No_points_lambda_z		6	7	8
	Lambda_z	1/hr	0.0073	0.0107	0.0083
	Lambda_z_lower	hr	24	24	6
	Lambda_z_upper	hr	336	504	504
	HL_Lambda_z	hr	95.12	65.07	83.84
	Tmax	hr	0.25	0.25	0.25
	Cmax	ug/mL	223.67	223.67	198.91
PK Estimates for DRA233 and DRA234	SE_Cmax	ug/mL	14.44	14.44	42.40
	Cmax_D	kg*ug/mL/mg	20.98	20.98	18.84
	C0	ug/mL	239.3	239.3	215.5
	Tlast	hr	504	504	504
	Clast	ug/mL	0.2412	0.2412	2.0063
	AUClast	hr*ug/mL	10029	10029	10991
	SE_AUClast	hr*ug/mL	363.4	363.4	278.1
	AUCall	hr*ug/mL	10029	10029	10991
	SE_AUCall	hr*ug/mL	363.4	363.4	278.1
	AUCINF_obs	hr*ug/mL	10062	10051	11234
	AUCINF_D_obs	hr*kg*ug/mL/mg	943.9	942.9	1063.8
	AUC_%Extrap_obs	%	0.329	0.225	2.160
	AUC_%Back_Ext_obs	%	0.575	0.576	0.4612
	Vz_obs	mL/kg	145.39	99.55	113.7
	Cl_obs	mL/hr/kg	1.059	1.061	0.94
	AUMClast	hr*hr*ug/mL	1027660	1027660	1122005
	AUMCINF_obs	hr*hr*ug/mL	1048881	1041195	1273670
	AUMC_%Extrap_obs	%	2.023	1.300	11.91
	MRTlast	hr	102.47	102.47	102.08
	MRTINF_obs	hr	104.24	103.59	113.38
	Vss_obs	mL/kg	110.44	109.86	106.58
	mean dose	mg/kg	10.66	10.66	10.56

FIG. 19

pl Variants of
DRA161/209
**Humanized CRIS7 Constructs: Comparison
of PK parameters**

Parameter	Estimates			
	DRA161	DRA233 w/o 504 hr*	DRA233	DRA234
Empirical pl	9.2	8.6	8.6	8.0
(Half-life, hr)	59.3	95.1	65.1	83.8
(Clearance, mL/hr/kg)	2.15	1.1	1.1	0.9

* Excluding
the last
time point

DRA 234 shows significantly higher half-life than DRA 161

FIG. 20

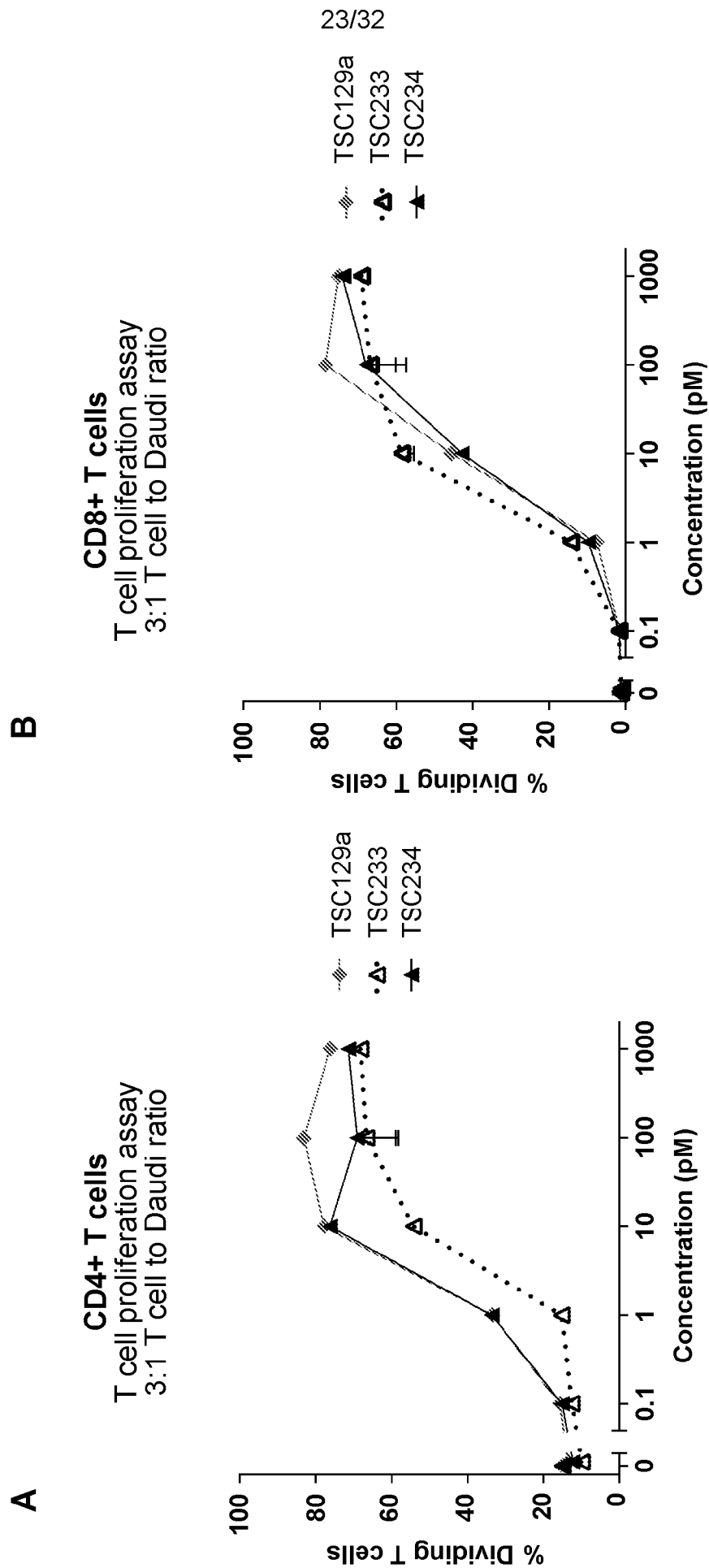


FIG. 21

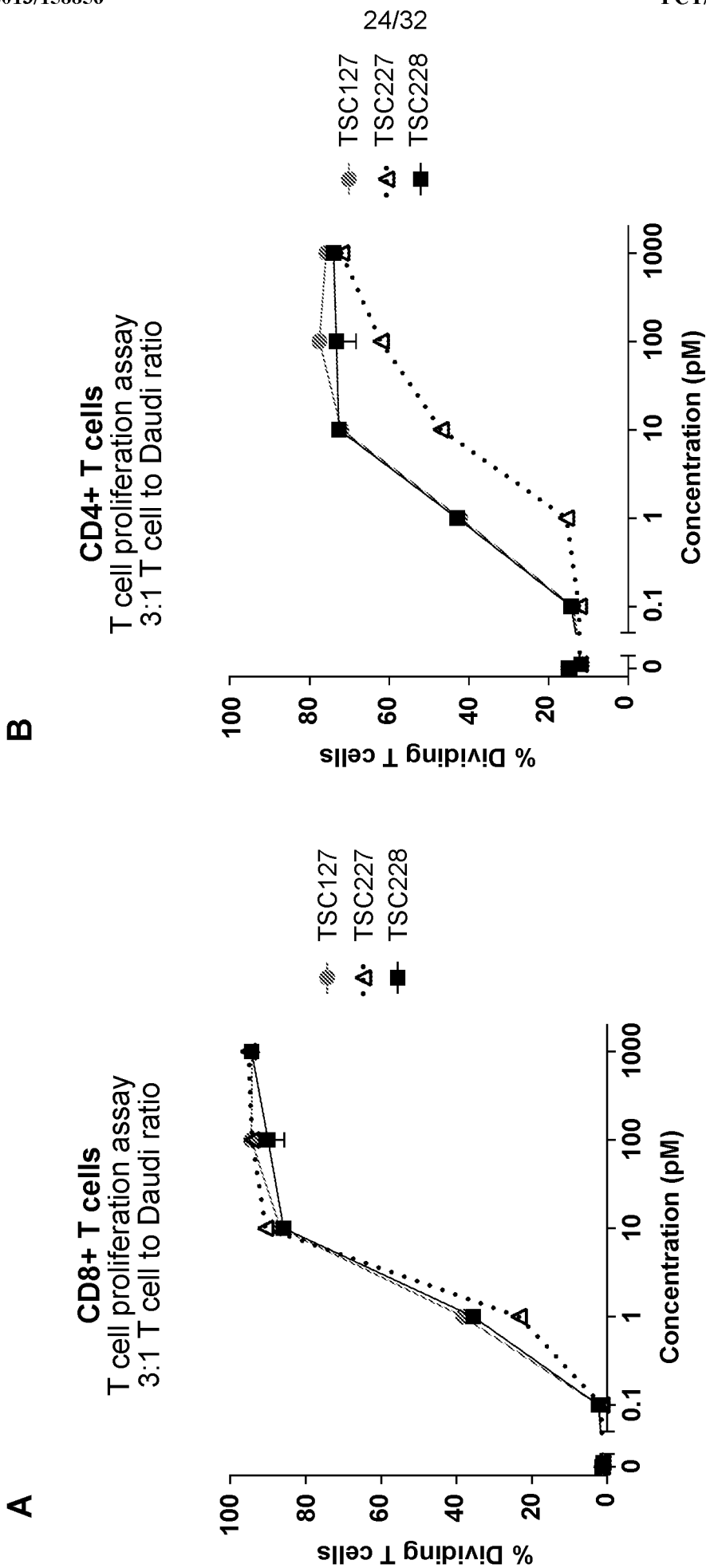


FIG. 22

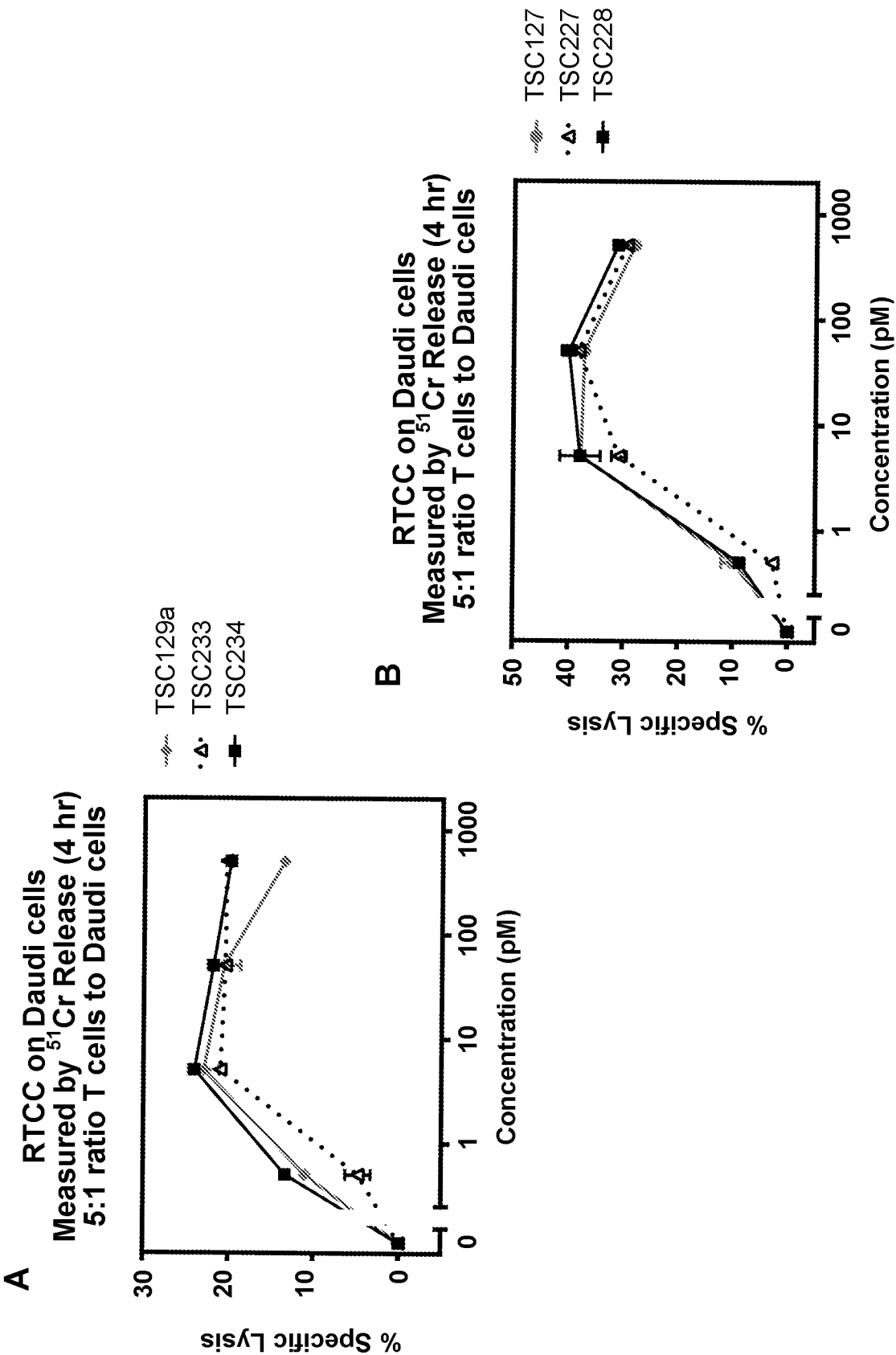


FIG. 23

RTCC on MDA-MB-453 Cells
Measured by ^{51}Cr Release (24 h)
5:1 T cells to MDA-MB-453 cells

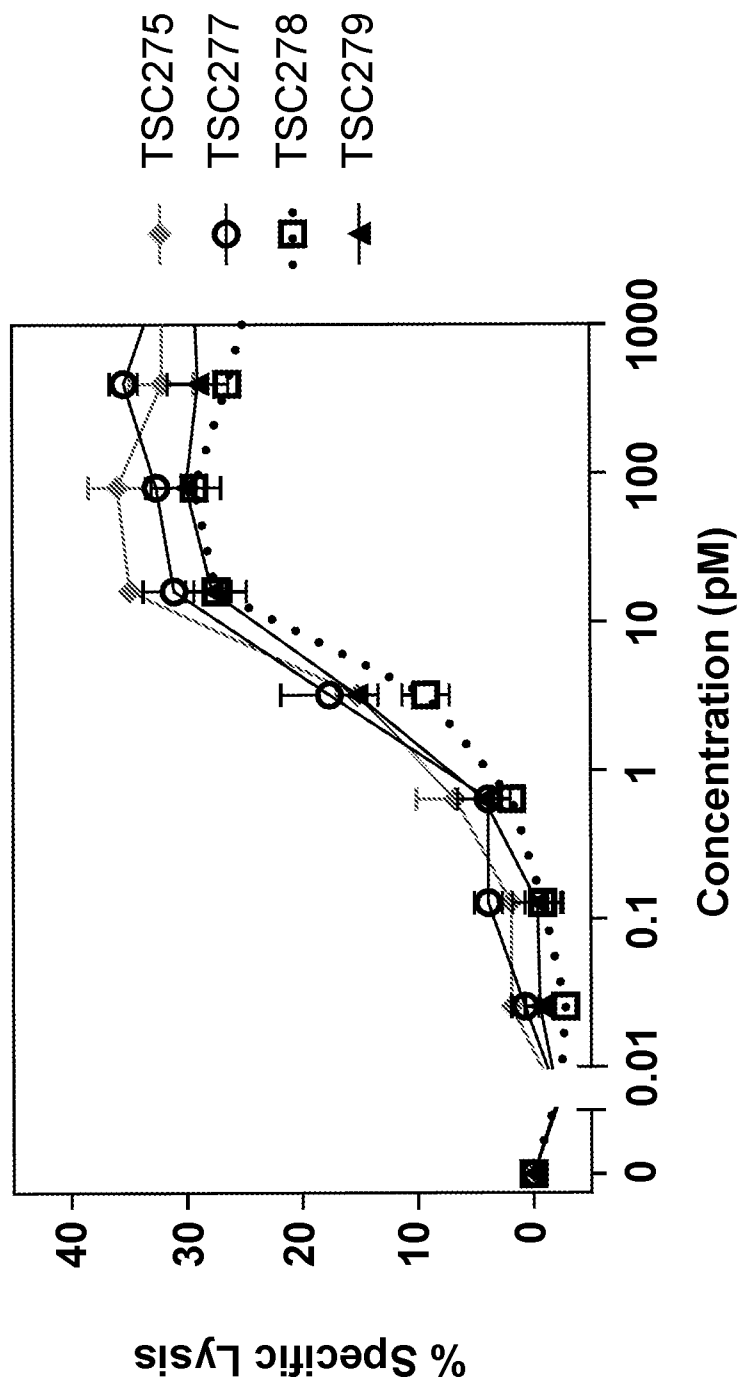


FIG. 24

Binding to CD3 on Jurkat cells
(flow cytometry)

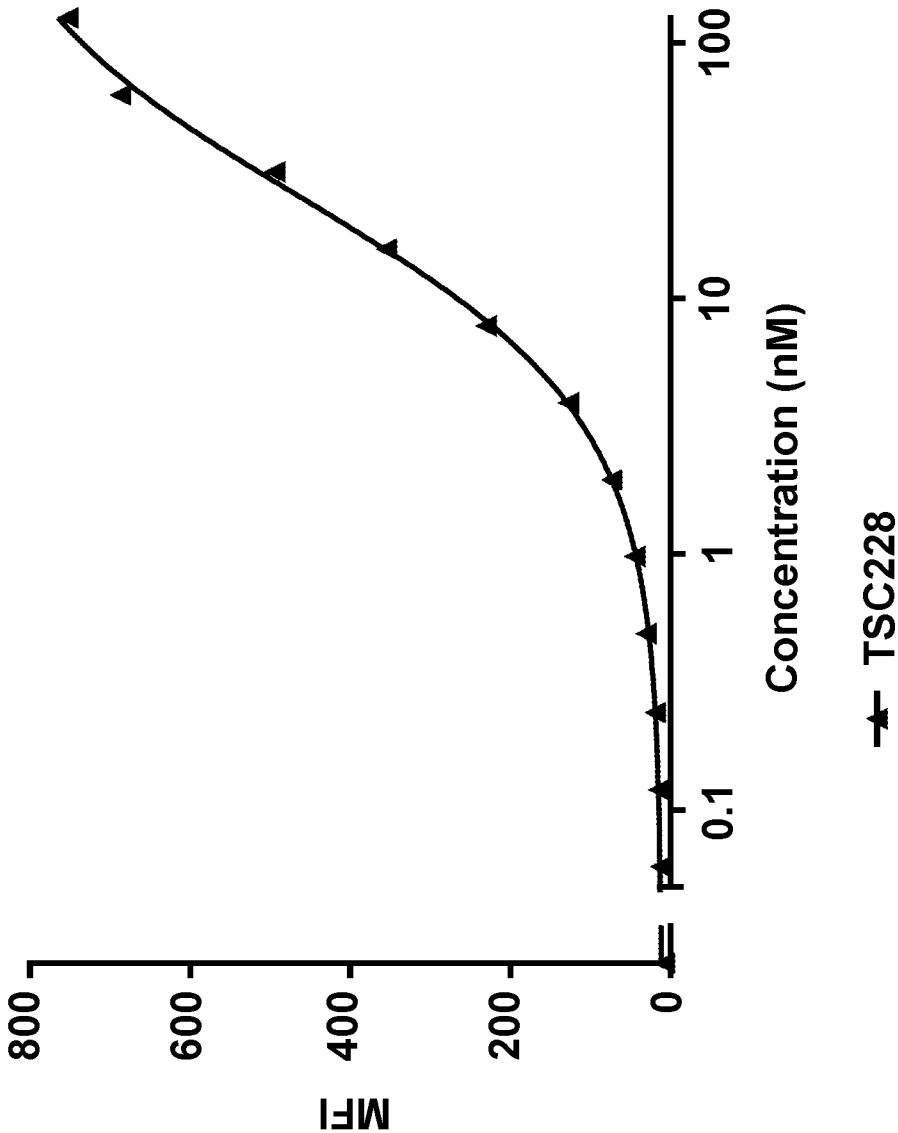


FIG. 25A

Binding to CD3 on Jurkat T cells
(flow cytometry)

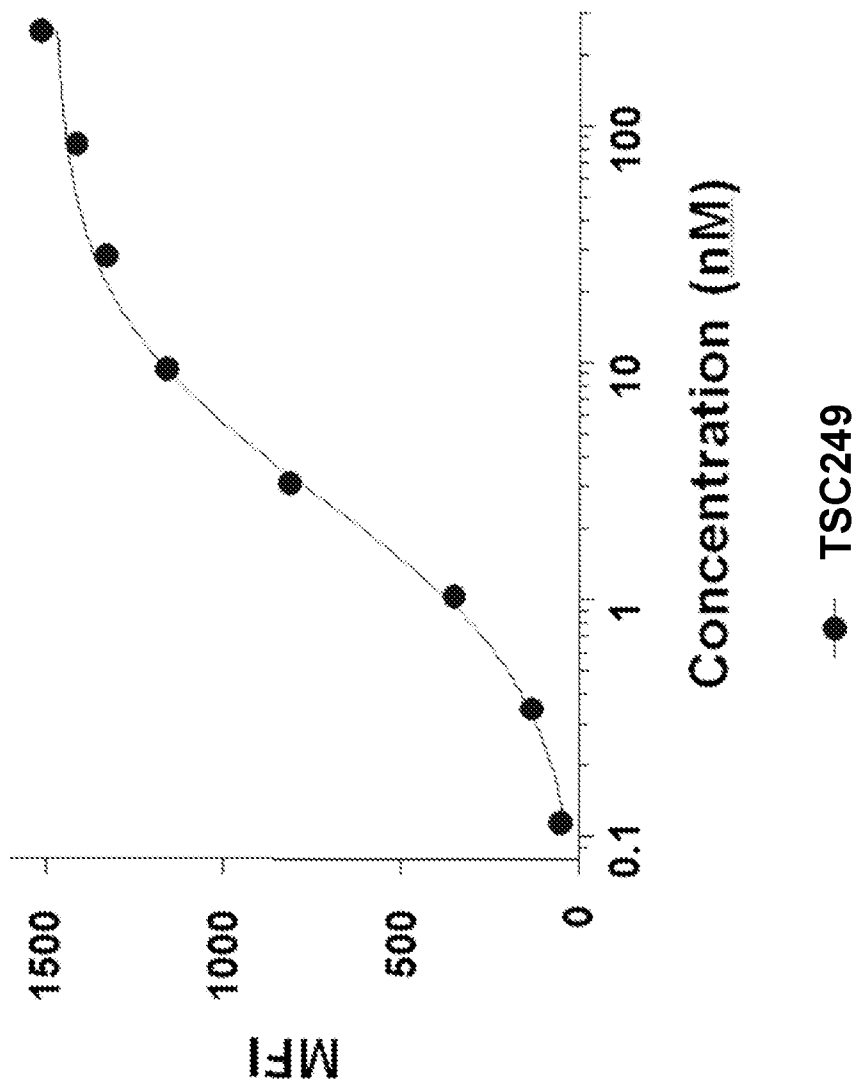


FIG. 25B

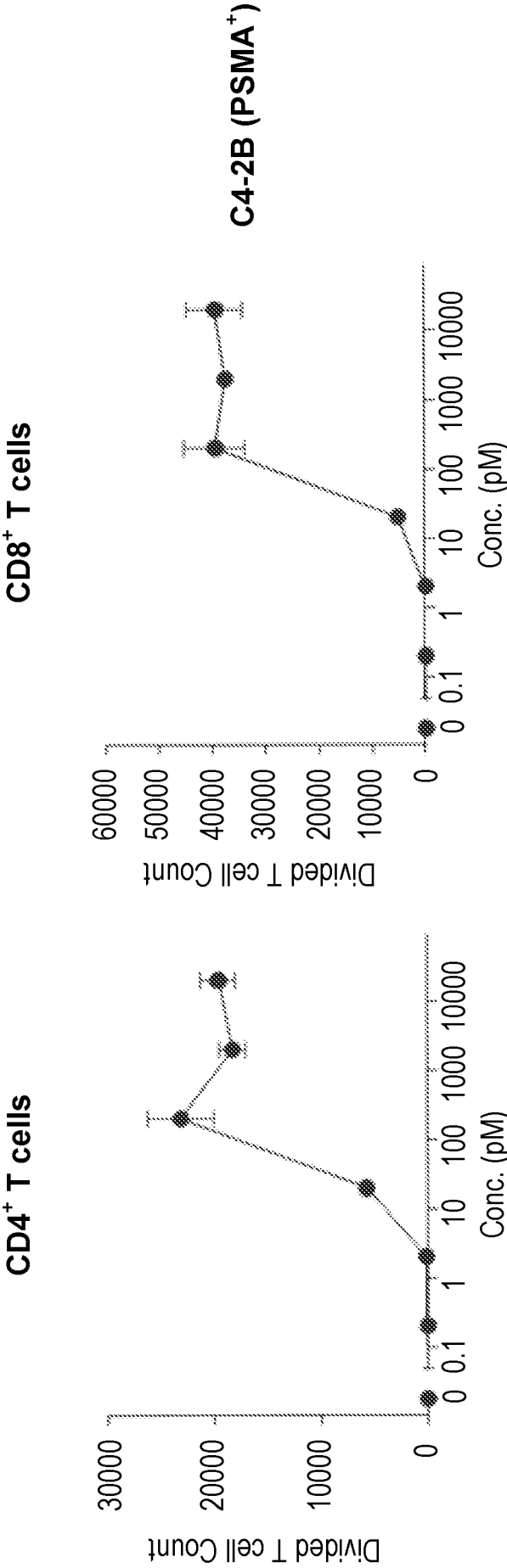


FIG. 26A

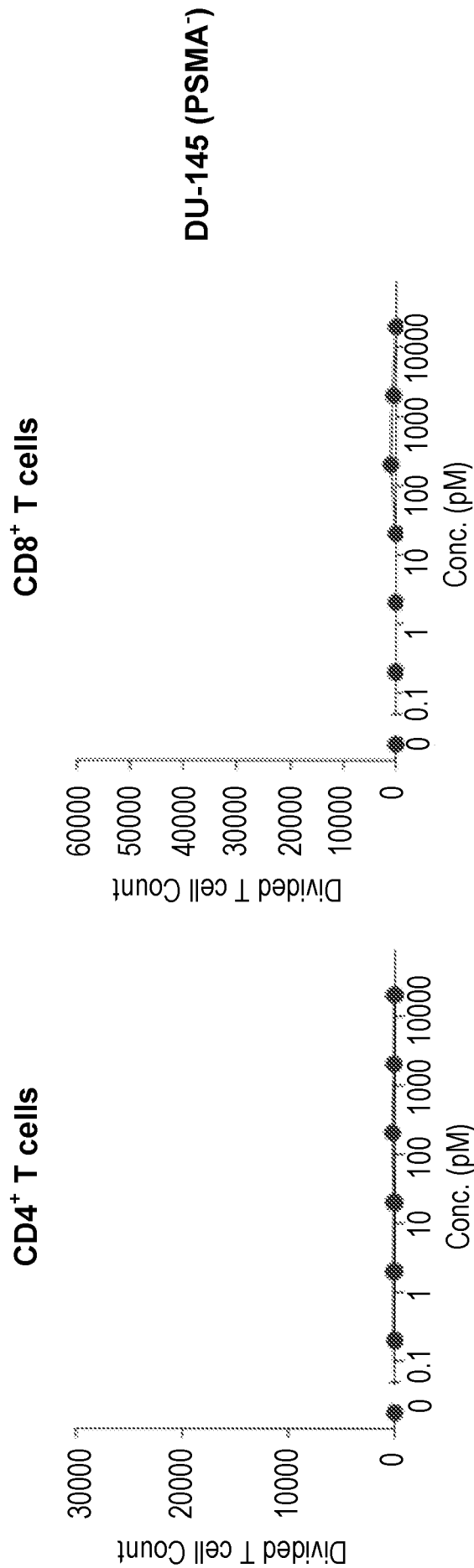


FIG. 26B

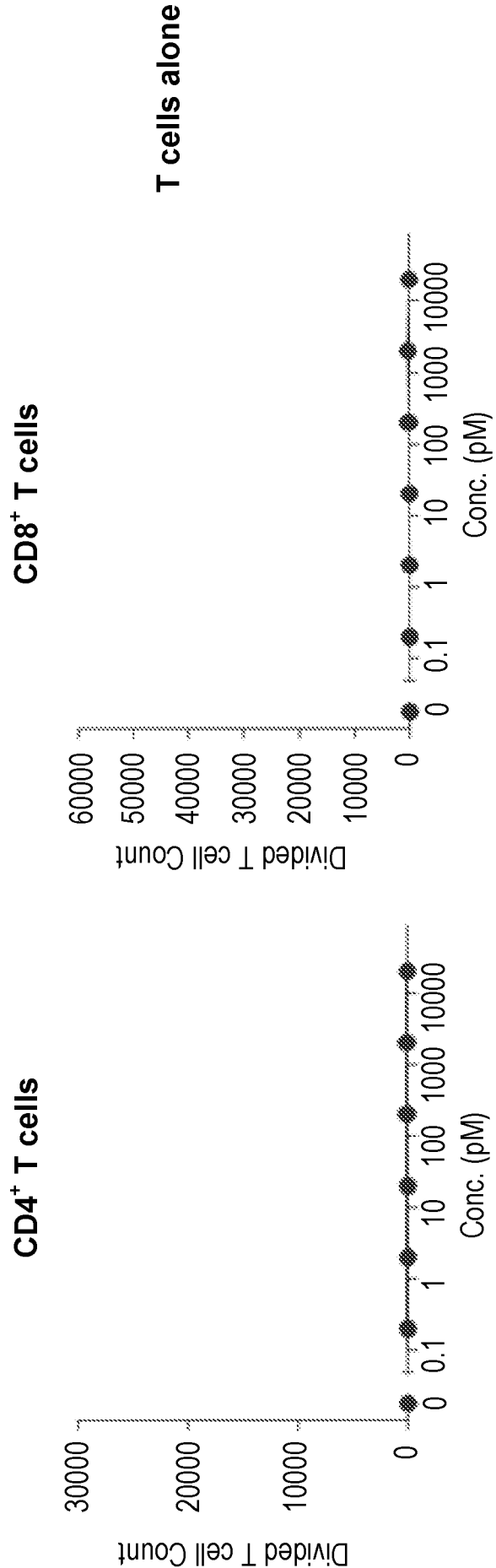


FIG. 26C

RTCC on C4-2 cells
Measured by ^{51}Cr release (4 hr)
5:1 ratio of T cells to C4-2 cells

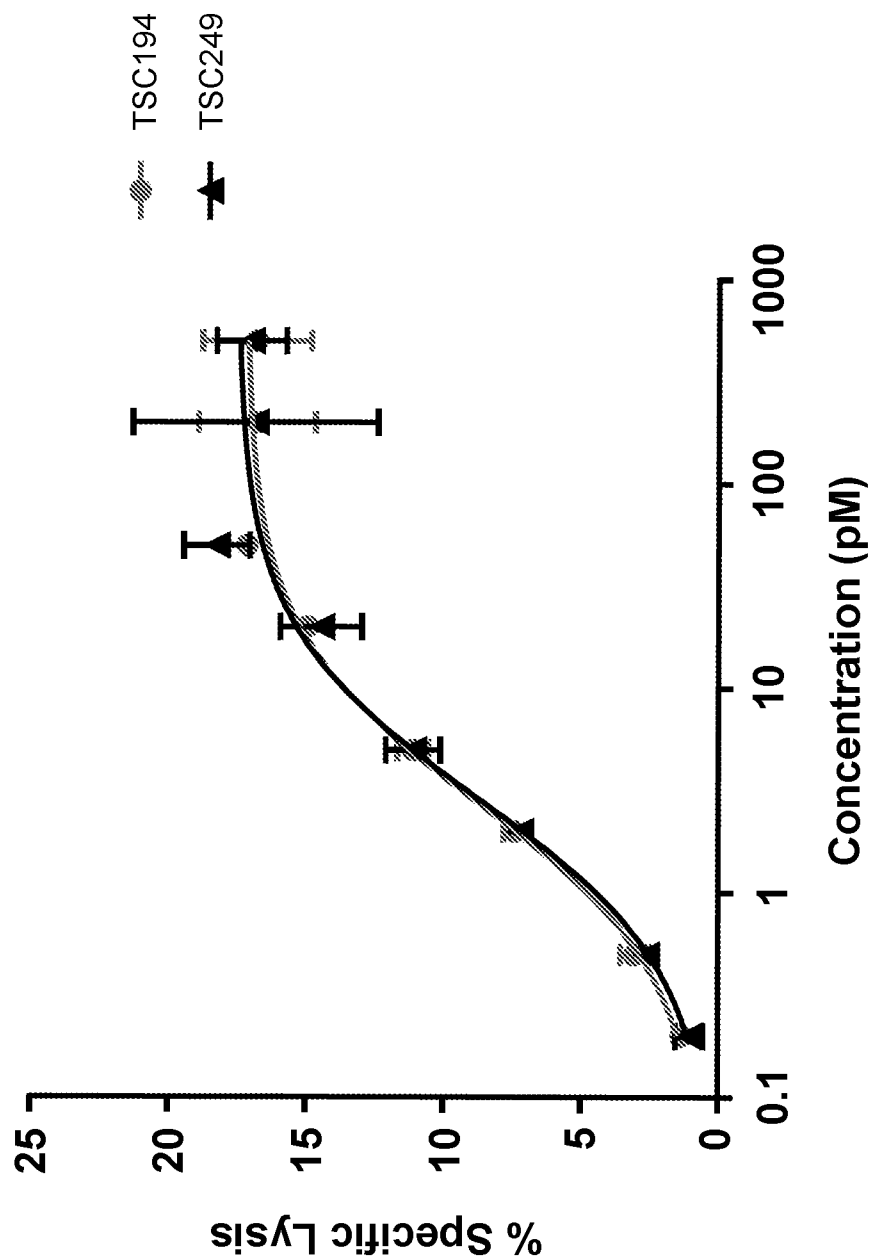


FIG. 27

PATENT COOPERATION TREATY

PCT

DECLARATION OF NON-ESTABLISHMENT OF INTERNATIONAL SEARCH REPORT

(PCT Article 17(2)(a), Rules 13^{ter}.1(c) and (d) and 39)

Applicant's or agent's file reference 2479.113PC03	IMPORTANT DECLARATION	Date of mailing (day/month/year) 05 JUL 2013
International application No. PCT/US13/37135	International filing date (day/month/year) 18 April 2013	(Earliest) Priority Date (day/month/year) 20 April 2012
International Patent Classification (IPC) or both national classification and IPC C12P 21/06 (2009.01); 514/2		
Applicant EMERGENT PRODUCT DEVELOPMENT SEATTLE, LLC		

This International Searching Authority hereby declares, according to Article 17(2)(a), that **no international search report will be established** on the international application for the reasons indicated below.

1. ☐ The subject matter of the international application relates to:
 - a. ☐ scientific theories
 - b. ☐ mathematical theories
 - c. ☐ plant varieties
 - d. ☐ animal varieties
 - e. ☐ essentially biological processes for the production of plants and animals, other than microbiological processes and the products of such processes
 - f. ☐ schemes, rules or methods of doing business
 - g. ☐ schemes, rules or methods of performing purely mental acts
 - h. ☐ schemes, rules or methods of playing games
 - i. ☐ methods for treatment of the human body by surgery or therapy
 - j. ☐ methods for treatment of the animal body by surgery or therapy
 - k. ☐ diagnostic methods practised on the human or animal body
 - l. ☐ mere presentations of information
 - m. ☐ computer programs for which this International Searching Authority is not equipped to search prior art
2. ☐ The failure of the following parts of the international application to comply with prescribed requirements prevents a meaningful search from being carried out:

☐ the description
☐ the claims
☐ the drawings
3. ☒ A meaningful search could not be carried out without the sequence listing; the applicant did not, within the prescribed time limit:

☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
☒ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13^{ter}.1(a) or (b).
4. ☐ A meaningful search could not be carried out without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-bis of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.
5. Further comments: Applicant failed to submit a valid electronic seq. listing in response to the ISA/225.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774



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代理人 孟锐

权利要求书4页 说明书53页

序列表344页 附图29页

(54) 发明名称

CD3 结合多肽

(57) 摘要

本发明涉及与 CD3 特异性结合或相互作用的单特异性和多特异性多肽。这些多肽可以是,但不限于:抗体、其片段、scFv、Fab、双 scFv 单域抗体、双链抗体、双可变域结合蛋白和含有抗体或抗体片段的多肽。在一个实施方案中,多特异性多肽结合 T 细胞上的 T 细胞受体复合物和肿瘤抗原以诱导靶依赖性 T 细胞细胞毒性、活化和增殖。

1. 一种 CD3 结合多肽,其包含与具有氨基酸序列 SEQ ID NO:41 的结合域相比具有降低的等电点的 CD3 结合域。

2. 如权利要求 1 所述的 CD3 结合多肽,其中所述结合域包含 VH 区和 VL 区,且其中所述 VH 区和所述 VL 区包含构架区。

3. 如权利要求 2 所述的 CD3 结合多肽,其中可以通过用中性氨基酸取代带正电的氨基酸和 / 或用带负电的氨基酸取代中性氨基酸在 VH 和 / 或 VL 的构架区中对两个或更多个氨基酸进行修饰。

4. 如权利要求 3 所述的 CD3 结合多肽,其中所述构架区的经修饰氨基酸被经对应的人生殖细胞系 IgG 序列内所含的氨基酸取代。

5. 如权利要求 4 所述的 CD3 结合多肽,其中所述人生殖细胞系 IgG 序列包括 SEQ ID NO:43

6. 如权利要求 4 所述的 CD3 结合多肽,其中所述人生殖细胞系 IgG 序列包括 SEQ ID NO:44

7. 如权利要求 1 到 3 所述的 CD3 结合多肽,还包括前铰链区。

8. 如权利要求 7 所述的 CD3 结合多肽,其中所述前铰链区与具有 RRT 前铰链区的 CD3 结合多肽相比具有降低的等电点。

9. 一种 CD3 结合多肽,其包含具有构架区、前铰链区和铰链区的 CD3 结合域,

其中所述 CD3 结合多肽与 SEQ ID NO:42 相比具有三个或更多个经修饰的氨基酸,所述修饰是在所述构架区和 / 或前铰链区中用中性氨基酸取代带正电的氨基酸和 / 或用带负电的氨基酸取代中性氨基酸。

10. 如权利要求 9 所述的 CD3 结合多肽,其中可以通过用中性氨基酸取代带正电的氨基酸和 / 或用带负电的氨基酸取代中性氨基酸而在 VH 和 / 或 VL 的构架区中对至少两个或更多个氨基酸进行修饰。

11. 如权利要求 3 和 9 所述的 CD3 结合多肽,其中可以通过用中性氨基酸取代带正电的氨基酸和 / 或用带负电的氨基酸取代中性氨基酸而在 VH 和 / 或 VL 的构架区中对至少四个或更多个氨基酸进行修饰。

12. 如权利要求 3 和 9 所述的 CD3 结合多肽,其中可以通过用中性氨基酸取代带正电的氨基酸和 / 或用带负电的氨基酸取代中性氨基酸而在 VH 和 / 或 VL 的构架区中对三到五个氨基酸进行修饰。

13. 如权利要求 3 和 9 所述的 CD3 结合多肽,其中可以通过用中性氨基酸取代带正电的氨基酸和 / 或用带负电的氨基酸取代中性氨基酸而在 VH 和 / 或 VL 的构架区中对三到十个氨基酸进行修饰。

14. 如权利要求 8 到 9 所述的 CD3 结合多肽,其中所述 CD3 结合多肽前铰链区包含氨基酸序列 SSS 或 SST。

15. 如权利要求 1 到 8 所述的 CD3 结合多肽,其中所述 CD3 结合多肽还包括铰链区和恒定区。

16. 如权利要求 1 到 15 所述的 CD3 结合多肽,其中所述 CD3 结合多肽具有比多肽 SEQ ID NO:4 小至少 1 个 pI 单位的经验等电点。

17. 如权利要求 1 所述的 CD3 结合多肽,其中所述 CD3 结合多肽是人源化抗体、单链

Fv(scFv) 或 SMIP。

18. 如权利要求 15 所述的 CD3 结合多肽,其中所述 CD3 结合多肽从氨基末端到羧基末端包含 CD3 结合域、铰链区和恒定区。

19. 如权利要求 15 所述的 CD3 结合多肽,其中所述结合多肽从氨基末端到羧基末端包含恒定区、铰链区和 CD3 结合域。

20. 如权利要求 15 所述的 CD3 结合多肽,其中所述结合多肽还包含第二结合域。

21. 如权利要求 20 所述的 CD3 结合多肽,其中所述结合多肽从氨基末端到羧基末端包含 CD3 结合域、铰链区、恒定区和第二结合域。

22. 如权利要求 20 所述的 CD3 结合多肽,其中所述 CD3 结合多肽从氨基末端到羧基末端包含第二结合域、铰链区、恒定区和 CD3 结合域。

23. 如权利要求 15 所述的 CD3 结合多肽,其中所述 CD3 结合多肽还包含异源二聚域。

24. 如权利要求 23 所述的 CD3 结合多肽,其中所述 CD3 结合多肽从氨基末端到羧基末端包含 CD3 结合域、铰链区、恒定区和异源二聚域。

25. 如权利要求 1 到 24 所述的 CD3 结合多肽,其中所述 CD3 结合多肽还包含第二结合域。

26. 如权利要求 25 所述的 CD3 结合多肽,其中所述第二结合域结合靶分子或与其相互作用,且所述 CD3 结合多肽诱导 T 细胞细胞毒性。

27. 如权利要求 26 所述的 CD3 结合多肽,其中所述第二结合域结合肿瘤相关抗原或与其相互作用。

28. 如权利要求 27 所述的 CD3 结合多肽,其中所述 CD3 结合多肽诱导 T 细胞溶解肿瘤细胞。

29. 如权利要求 27 所述的 CD3 结合多肽,其中所述 CD3 结合多肽在肿瘤附近诱导多克隆 T 细胞活化和扩增。

30. 如权利要求 27 到 29 所述的 CD3 结合多肽,其中所述肿瘤相关抗原选自 RON、c-Met、CEACAM-6、PSMA、EpCAM、CEA、PCTA-1、STEAP-1、STEAP-2、PSCA、PSA、PAP、ALCAM(CD166)、PECAM-1、EphA2、CD151、CA-125/MUC16、MUC-1、MAGE-1、TROP2、IGF1R、TGFBR2、GHRHR、GHR、IL-6R、gp130、TNFR2、OSMR β 、Patched-1、Frizzled、Robo1、LT β R、CD25、CD26、CD27、CD30、CD33、CD44、CD44v6、CD63、CD80、CD81、CD86、CD100、CD151、CXCR4、CCR5、HER-2/ErbB1、HER-3/ErbB3、HER-4/ErbB4、EGFR/ErbB1、EGFRvIII 同种型、MUC2、MUC3、MUC4、MUC5_{AC}、MUC5_b、MUC7、 β hCG、Lewis-Y、神经节苷脂 GD3、9-O-乙酰基-GD3、GM2、Globo H、岩藻糖酰 GM1、Poly SA、GD2、碳酸酐酶 IX(MN/CA IX)、音猬因子(Shh)、Wue-1、浆细胞抗原、(膜结合) IgE、黑色素瘤硫酸软骨素蛋白多糖(MCSP)、CCR8、TNF- α 前体、间皮素、A33 抗原、Ly-6 β 、桥粒核心糖蛋白 4、E-钙粘附蛋白新表位、胎儿乙酰胆碱受体、CA19-9 标记物、苗勒氏抑制物质(MIS)受体 II 型、sTn(唾液酸化 Tn 抗原;TAG-72)、FAP(成纤维细胞活化抗原)、内皮唾液酸蛋白、LG、SAS、BCMA、TWEAKR/Fn14、FGFR4、VEGFR1、VEGFR2、SSX1 和 SSX2。

31. 如权利要求 1 到 30 所述的 CD3 结合多肽,其中所述 CD3 结合域结合 T 细胞上的 T 细胞受体复合物的 CD3 ϵ 亚单位或与其相互作用。

32. 如权利要求 1 到 31 所述的 CD3 结合多肽,其中所述 CD3 结合多肽诱导 T 细胞受体复合物内化。

33. 如权利要求 1 到 32 所述的 CD3 结合多肽, 其中所述 CD3 结合域是来源于鼠类 Cris-7 单克隆抗体或 HuM291 的人源化 CD3 结合域。

34. 如权利要求 33 所述的 CD3 结合多肽, 其中所述 CD3 结合域包含鼠类 Cris-7 抗体或 HuM291 的重链 CDR1、CDR2 和 CDR3 以及轻链 CDR1、CDR2 和 CDR3。

35. 如权利要求 1 所述的 CD3 结合多肽, 其中所述 CD3 结合域包含选自 SEQ ID NO:28、SEQ ID NO:30、SEQ ID NO:32、SEQ ID NO:38 和 SEQ ID NO:40 的 VH 区序列或 VL 区序列。

36. 如权利要求 1 所述的 CD3 结合多肽, 其中所述 CD3 结合域包含选自 SEQ ID NO:22、SEQ ID NO:24、SEQ ID NO:26、SEQ ID NO:28、SEQ ID NO:30 和 SEQ ID NO:32 的 VH 区和选自 SEQ ID NO:38 和 SEQ ID NO:40 的 VL 区。

37. 如权利要求 1 所述的 CD3 结合多肽, 其中所述 CD3 结合域包含选自 SEQ ID NO:28、SEQ ID NO:30 和 SEQ ID NO:32 的 VH 区和选自 SEQ ID NO:34、SEQ ID NO:36、SEQ ID NO:38 和 SEQ ID NO:40 的 VL 区。

38. 如权利要求 2 所述的 CD3 结合多肽, 其中所述 VH 区包含 SEQ ID NO:28 且所述 VL 区包含 SEQ ID NO:34。

39. 如权利要求 2 所述的 CD3 结合多肽, 其中所述 VH 区包含 SEQ ID NO:28 且所述 VL 区包含 SEQ ID NO:38。

40. 如权利要求 2 所述的 CD3 结合多肽, 其中所述 VH 区包含 SEQ ID NO:26 且所述 VL 区包含 SEQ ID NO:38。

41. 如权利要求 9 和 14 所述的 CD3 结合多肽, 其中所述 VH 区包含 SEQ ID NO:26 且所述 VL 区包含 SEQ ID NO:34。

42. 如权利要求 1 所述的 CD3 结合多肽, 其中所述 CD3 结合多肽包含选自 SEQ ID NO:6、SEQ ID NO:8、SEQ ID NO:10、SEQ ID NO:12、SEQ ID NO:14、SEQ ID NO:16、SEQ ID NO:18 和 SEQ ID NO:20 的氨基酸序列。

43. 如权利要求 1 所述的 CD3 结合多肽, 其中所述 CD3 结合域包含重链 CDR1、CDR2 和 CDR3 以及轻链 CDR1、CDR2 和 CDR3, 且其中所述重链 CDR3 包含 SEQ ID NO:51。

44. 如权利要求 43 所述的 CD3 结合多肽, 其中重链 CDR1 包含 SEQ ID NO:49, CDR2 包含 SEQ ID NO:50 且 CDR3 包含 SEQ ID NO:51, 并且轻链 CDR1 包含 SEQ ID NO:52, CDR2 包含 SEQ ID NO:53, 且 CDR3 包含 SEQ ID NO:54。

45. 如权利要求 15 所述的 CD3 结合多肽, 其中恒定区包含 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD 或其任何组合的 CH2 和 CH3 域; IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE、IgM 或其任何组合的免疫球蛋白 CH3 域; 或 IgE、IgM 或其组合的免疫球蛋白 CH3 和 CH4 域。

46. 如权利要求 15 所述的 CD3 结合多肽, 其中所述恒定区包含 CH2 和 CH3。

47. 如权利要求 15 所述的 CD3 结合多肽, 其中所述恒定区实质上由 CH2 域和 CH3 域组成。

48. 如权利要求 45 到 47 所述的 CD3 结合多肽, 其中所述恒定区经修饰以减少或消除效应因子功能。

49. 如权利要求 45 到 47 所述的 CD3 结合多肽, 其中所述恒定区经修饰以便不固定补体。

50. 如权利要求 45 到 47 所述的 CD3 结合多肽,其中所述恒定区经修饰以便不结合 Fc γ 受体。

51. 如权利要求 50 所述的 CD3 结合多肽,其中所述 Fc γ 受体选自 CD16、CD32 和 CD64。

52. 如权利要求 15 所述的 CD3 结合多肽,其中所述恒定区包含氨基酸序列 SEQ ID NO:55。

53. 一种核酸,其编码任一种如权利要求 1 到 52 所述的多肽。

54. 一种分离的核酸,包括选自 SEQ ID NO:5、SEQ ID NO:7、SEQ ID NO:9、SEQ ID NO:11、SEQ ID NO:13、SEQ ID NO:15、SEQ ID NO:17、SEQ ID NO:19、SEQ ID NO:27 和 SEQ ID NO:37 的核酸。

55. 一种表达载体,包含如权利要求 53 或 54 所述的核酸。

56. 一种重组宿主细胞,包含如权利要求 32 所述的表达载体。

57. 一种组合物,包含如权利要求 1 到 52 中任一项所述的 CD3 结合多肽和药学上可接受的载剂、稀释剂或赋形剂。

58. 一种用于治疗癌症或自体免疫病症的方法,包括向有需要的患者施用治疗有效量的如权利要求 57 所述的组合物或如权利要求 1 到 52 所述的 CD3 结合多肽。

59. 如权利要求 1 到 52 所述的 CD3 结合多肽,其中所述 CD3 结合多肽形成二聚体。

60. 如权利要求 59 所述的 CD3 结合多肽,其中所述二聚体是同源二聚体或异源二聚体。

61. 如权利要求 3 所述的 CD3 结合多肽,其中与 Cris-7VL J κ (J κ) 区小鼠序列 LQIT (SEQ ID NO:252) 相比,至少一个氨基酸修饰是在 VL 区的 J κ 区中。

62. 如权利要求 61 所述的 CD3 结合多肽,其中所述 J κ 区包含氨基酸序列 VEIK (SEQ ID NO:253)。

63. 如权利要求 25 所述的 CD3 结合多肽,其中所述第二结合域包含与选自以下的氨基酸序列具有至少 90%、至少 95%或 100%同一性的氨基酸序列:SEQ ID NO:212 的氨基酸 1-107 和 124-243;SEQ ID NO:226 的氨基酸 1-107 和 124-243;SEQ ID NO:216 的氨基酸 1-107 和 124-243;和 SEQ ID NO:196 的 1-111 和 128-251。

64. 如权利要求 1 所述的 CD3 结合多肽,其中所述结合多肽包含与 SEQ ID NO:57、59、61、63、65、67、69、71、186、190、192、194、196、198、200、202、204 或 206 具有至少 90%、至少 95%或 100%同一性的氨基酸序列。

65. 如权利要求 1 所述的 CD3 结合多肽,其中所述多肽是异源二聚体的一部分。

66. 如权利要求 65 所述的 CD3 结合多肽,其中所述异源二聚体包含一对单链多肽,其中所述单链对包含与选自以下的配对具有至少 90%、至少 95%或 100%同一性的氨基酸序列:SEQ ID NO:210 和 247、SEQ ID NO:210 和 218、SEQ ID NO:210 和 220、SEQ ID NO:208 和 249、SEQ ID NO:208 和 222、或 SEQ ID NO:208 和 224、SEQ ID NO:212 和 218、SEQ ID NO:216 和 222、SEQ ID NO:228 和 226、或 SEQ ID NO:214 和 218。

67. 如权利要求 1 所述的 CD3 结合多肽,其中所述 CD3 结合域与 SEQ ID NO:41 具有约 90%到约 99%同一性。

CD3 结合多肽

[0001] 相关申请

[0002] 本发明涉及 2012 年 4 月 20 日提交的标题为“Prostate-Specific Membrane Antigen Binding Proteins And Related Compositions And Methods”的 PCT 申请 PCT/US12/034575, 所述 PCT 申请要求 2011 年 4 月 22 日提交的美国临时专利申请 61/478, 449 的优先权, 且还涉及美国申请号 61/636, 557, 该三个文献全部以全文引用的方式并入。

发明领域

[0003] 本发明涉及结合 CD3 或与其相互作用的单特异性和多特异性蛋白治疗剂。这包括抗体、其片段、scFv、Fab、双 scFv 单域抗体和含有抗体或抗体片段的多肽。

[0004] 随附序列列表

[0005] 与本文一起以电子形式提交的文本文件(名称:“2479_113PC03_SequenceListing_ascii.txt”, 大小:459,722 字节;创建日期:2013 年 4 月 16 日)的内容以全文引用的方式并入本文中。

[0006] 背景

[0007] 用抗 CD3 单克隆抗体靶向人 T 细胞上的 TCR 复合物已经用于或提议用于治疗自体免疫疾病和相关病症, 诸如用于治疗器官同种异体移植排斥。对人 CD3 特异性的小鼠单克隆抗体, 诸如 OKT3 (Kung 等 (1979) Science 206:347-9) 是所述治疗的第一代。尽管 OKT3 具有强免疫抑制效能, 但与其免疫原性和促有丝分裂潜力相关的严重副作用妨碍了其临床使用 (Chatenoud (2003) Nature Reviews 3:123-132)。它诱导抗球蛋白反应, 从而促进其自身快速清除和中和 (Chatenoud 等 (1982) Eur. J. Immunol. 137:830-8)。另外, OKT3 在体外诱导 T 细胞增殖和细胞因子产生, 且在体内导致细胞因子的大规模释放 (Hirsch 等 (1989) J. Immunol. 142:737-43, 1989)。细胞因子释放 (也称为“细胞因子风暴”又导致以发烧、寒战、头疼、恶心、呕吐、腹泻、呼吸窘迫、无菌性脑膜炎和血压过低为特征的“流感样”综合征 (Chatenoud, 2003)。所述严重副作用限制了 OKT3 更广泛地用于移植中以及将其用途扩展到其它临床领域, 诸如自体免疫性 (同上)。

[0008] 为了减少第一代抗 CD3 单克隆抗体的副作用, 已经不仅通过鼠类抗 CD3 单克隆抗体的将互补性决定区 (CDR) 移植到人 IgG 序列中, 而且通过将非 FcR 结合突变引入到 Fc 中以减少细胞因子风暴的发生而开发出了第二代经基因工程改造的抗 CD3 单克隆抗体 (Cole 等 (1999) Transplantation 68:563; Cole 等 (1997) J. Immunol. 159:3613)。还参见 PCT 公布号 W02010/042904, 该文献以全文引用的方式并入本文中。

[0009] 除了靶向 CD3 的单特异性治疗剂以外, 选择性地结合 T 细胞和肿瘤细胞的多特异性多肽也能提供重定向 T 细胞细胞毒性以针对肿瘤细胞和治疗癌症的机制。然而, 设计双特异性或多特异性 T 细胞募集抗体的一个问题在于维持特异性而同时通过多个调整路径而越过 T 细胞活化规则。

[0010] 仍然需要改良的抗 CD3 单特异性和多特异性分子。尽管先前已经对 CD3 靶向分子的 Fc 部分进行了改良以降低细胞因子风暴的可能性, 但仍需要与现有技术分子相比具有

增加的半衰期、可以有效地制造、展现改良的 T 细胞结合和 / 或改良的重定向 T 细胞细胞毒性（在设计用于治疗癌症的多特异性分子的情况下）的抗 CD3 治疗剂。

[0011] 发明概述

[0012] 在一个方面,本发明包括一种 CD3 结合多肽,其包含与具有氨基酸序列 SEQ ID NO:41 的结合域相比具有降低的等电点的 CD3 结合域。在某些实施方案中,所述 CD3 结合域包含 VH 和 VL 区,VH 和 VL 区各自含有构架区。为了降低所述结合域和 / 或多肽的等电点 (pI),可以通过用中性氨基酸取代带正电的氨基酸和 / 或用带负电的氨基酸取代中性氨基酸来修饰构架区中的两个或更多个氨基酸。举例来说,可以用 S 取代 R 和 T,可以用 E 取代 Q,可以用 F 取代 Y,且可以用 V 取代 T。在一个实施方案中,例如,为了降低免疫原性的风险,取代到序列中的氨基酸是人生殖细胞系 IgG 序列中普遍存在的或包含在人生殖细胞系序列中的相同或邻近的位置,例如在对应的人生殖细胞系 IgG 序列中。在一些实施方案中,所述人生殖细胞系 IgG 序列包含 SEQ ID NO:43 或 44。

[0013] 在本发明的另一个实施方案中,所述 CD3 结合多肽在前较链区 (prehinge region) 中包含能降低所述前较链区或整个多肽的 pI 的修饰。所述前较链区处于所述结合域与较链区之间的接合处。举例来说,可以用序列 SSS 置换具有序列 RRT 的三氨基酸前较链区以降低等电点。在一些实施方案中,前较链区与具有 RRT 前较链区的 CD3 结合多肽相比具有降低的等电点。

[0014] 可以设计 CD3 结合多肽以便例如通过使 VH 和 / 或 VL 区和 / 或前较链区的构架区突变来降低等电点。等电点可以降低 0.5 到 2.5 或更多个单位。

[0015] 在本发明的一个实施方案中,所述 CD3 结合多肽是抗体,例如人源化抗体。在另一个实施方案中,它是一种小模块式免疫药物蛋白 (SMIP)。在一些实施方案中,CD3 结合多肽包含单链可变片段 (scFv)。

[0016] 在另一个实施方案中,它是多特异性或双特异性多肽。举例来说,所述 CD3 结合多肽可以包含 CD3 结合域和肿瘤抗原结合域。在一个实施方案中,所述双特异性或多特异性分子重定向 T 细胞细胞毒性以针对肿瘤细胞。

[0017] 在本发明的另一个实施方案中,所述 CD3 多肽作为同源二聚体或异源二聚体存在。

[0018] 附图简述

[0019] 图 1 是描绘先前产生的人源化 Cris-7SMIP 多肽的理论 pI 与约 200 种其它 SMIP 多肽的理论 pI 相比较的图。

[0020] 图 2A 和 2B 说明了包含比本发明的 CD3 多肽高的 pI 的 CD3 结合 SMIP 多肽的剪接。图 2A 是能定量经过纯化的蛋白质的低分子量剪接段含量的 SDS 毛细管电泳。图 2B 含有剪接型 CD3SMIP 分子的说明。图 2B 中所示的序列对应于 SEQ ID NO:4 的氨基酸 252-260、SEQ ID NO:4 的 243-252 和 SEQ ID NO:240 的 251-260。

[0021] 图 3 是 Cris-7 小鼠 VH 与衍生的人源化 VH 序列的氨基酸序列比对。Cris7VH 小鼠、H1、H2、H3、H4、H5、H6 和共有序列分别对应于 SEQ ID NO:45、22、24、26、28、30、32 和 229。

[0022] 图 4 是 Cris-7 小鼠 VL 与衍生的人源化 VL 序列的氨基酸序列比对。Cris7VL 小鼠、L1、L2、L3、L4 和共有序列分别对应于 SEQ IDNO:46、34、36、38、40 和 230。

[0023] 图 5 是 H3 与生殖细胞系序列 IGHV1-69*01 和 IGHV3-30*01 的氨基酸序列比对。

H3、IGHV1-69*01、IGHV3-30*01 和共有序列分别对应于 SEQ ID NO:26、43、44 和 331。

[0024] 图 6 是生殖细胞系序列的 VL 区的 J κ 区 IGKV1-5*01 与 L1 的氨基酸序列比对。所述 VL 区的 J κ 区 (IGKVI-5*01)、L1 链和共有序列分别对应于 SEQ ID NO:232、34 和 233。所列出的 IGKJ1*01、IGKJ2*01、IGKJ3*01、IGKJ4*01 和 IGKJ5*01 序列分别对应于 SEQ ID NO:234-238。

[0025] 图 7A 和 7B 描绘了与 DRA209 相比具有降低的理论 pI 的各种 CD3 结合多肽 SMIP 分子的氨基酸序列比对。针对 DRA209、DRA219、DRA222、DRA221、DRA223、DRA224、DRA225 和共有序列所列出的序列分别对应于 SEQ ID NO:4 的氨基酸 1-423、SEQ ID NO:6 的氨基酸 1-423、SEQ ID NO:8 的氨基酸 1-423、SEQ ID NO:10 的氨基酸 1-423、SEQ ID NO:12 的氨基酸 1-423、SEQ ID NO:14 的氨基酸 1-426、SEQ ID NO:16 和 SEQ ID NO:239 的氨基酸 1-426。

[0026] 图 8 是 pI 变体 DRA233 和 DRA234 与 DRA161 相比的氨基酸序列比对。针对 DRA161、DRA233、DRA234 和共有序列所列出的序列分别对应于 SEQ ID NO:240、18、20 和 241。

[0027] 图 9 是如何确定经验 pI 值的说明。

[0028] 图 10 是显示 DRA209SMIP 和 scFv 的经验 pI 的图。

[0029] 图 11 是显示 DRA161SMIP 与 pI 变体 DRA233 和 DRA234 相比的经验 pI 的图。

[0030] 图 12 是显示用与 DRA209 和 DRA161 相同 (DRA227 除外) 的方式结合 T 细胞的 SMIP pI 变体的图。DRA227SMIP 用作对照且它不含 CD3 或 T 细胞结合域。各分子名称之后的字母“a”指示特定分子是在 CHO 细胞中产生且四位数是批次编号。

[0031] 图 13 描绘了非还原型 SDS-PAGE 的图像, 显示 CD3 结合 SMIP 构建体 DRA233 和 DRA234 与 SMIP DRA161 (它与 DRA209 共享相同的 VH 和 VL) 相比不那么倾向于破碎。

[0032] 图 14 描绘了还原型 SDS-PAGE 的图像, 显示 CD3 结合 SMIP 构建体 DRA233 和 DRA234 与 SMIP DRA161 (它与 DRA209 共享相同的 VH 和 VL) 相比不那么倾向于破碎。

[0033] 图 15 是显示 pI 变体 DRA233 和 DRA234 主要是完整表达 (例如, 不进行剪接) 的图和表。

[0034] 图 16 是显示 DRA161 (经过纯化) 的非还原型 CE-SDS 的图像。

[0035] 图 17 显示了 DRA234 和 DRA233 的 PK 研究的数据。

[0036] 图 18 提供了来自于如实施例 14 中所描述的 DRA233 和 DRA234 的 WinNonLin 分析的数据。

[0037] 图 19 是 DRA233 和 DRA234 的 PK 估计值的表格。

[0038] 图 20 是 DRA161 与 DRA233 (存在和不存在 504 小时时间点) 和 DRA234 相比的 PK 参数的表格。

[0039] 图 21 描绘了得自于使用不同的靶向 CD19 的同源二聚体双特异性多肽分子的靶依赖性 T 细胞增殖测定的结果。图 21A 和 21B 分别示出了如实施例 10 中所描述的 CD4+T 细胞增殖和 CD8+T 细胞增殖的结果。

[0040] 图 22 描绘了得自于使用不同的靶向 CD19 的异源二聚体双特异性多肽分子的靶依赖性 T 细胞增殖测定的结果。图 22A 和 22B 分别示出了如实施例 10 中所描述的 CD8+T 细胞增殖和 CD4+T 细胞增殖的结果。

[0041] 图 23 描绘了得自于使用不同的靶向 CD19 的多肽异源二聚体和同源二聚体的重定向 T 细胞细胞毒性测定的结果。图 23A 示出了同源二聚双特异性多肽 TSC129a、TSC233 和

TSC234 的结果。图 23B 示出了异源二聚双特异性多肽 TSC127、TSC227 和 TSC228 的结果。

[0042] 图 24 描绘了得自于使用靶向 RON 的不同的双特异性多肽同源二聚体 TSC275、TSC277、TSC278 和 TSC279 的重定向 T 细胞细胞毒性测定的结果。

[0043] 图 25 描绘了双特异性多肽异源二聚体和同源二聚体的 T 细胞结合的结果。图 25A 和 25B 示出了双特异性分子 TSC228(异源二聚体)和 TSC249(同源二聚体)与 Jurkat 细胞的剂量依赖性结合。

[0044] 图 26 描绘了靶向 PSMA 的多肽同源二聚体的靶依赖性 T 细胞增殖的结果。图 26A 示出了在用 TSC249 处理后表达靶 PSMA 抗原的 C4-2B 细胞的结果。图 26B 示出了在用 TSC249 处理后不表达靶 PSMA 抗原的 DU-145 细胞的结果。图 26C 示出了当用 TSC249 处理经分离的 T 细胞时的结果。

[0045] 图 27 描绘了得自于使用靶向 PSMA 的同源二聚双特异性多肽 TSC194 和 TSC249 的重定向 T 细胞细胞毒性测定的结果。

[0046] 发明详述

[0047] 本发明提供了与现有技术 CD3 结合多肽相比具有改良的特征的 CD3 结合多肽。已经通过修饰重链和/或轻链框架区和/或前铰链前铰链区中的氨基酸以降低分子的等电点而设计了本发明的分子以展现降低的等电点。在一个实施方案中,修饰是在 VL 区的 J_κ 区中进行。在一些实施方案中,修饰是通过用具有中性电荷的氨基酸取代具有正电荷的氨基酸和/或用具有负电荷的氨基酸取代具有中性电荷的氨基酸来进行。

[0048] 在一些实施方案中,本发明的 CD3 结合多肽的理论 pI 比抗 CD3DRA209SMIP(SEQ ID NO:4) 低约 0.5 到 2.5 个单位。举例来说,DRA209 的理论 pI 是 9,而 pI 变体 DRA219(SEQ ID NO:6) 的理论 pI 是 8.4, DRA221(SEQ ID NO:10) 的理论 pI 是 8.2, DRA222(SEQ ID NO:8) 的理论 pI 是 7.5, DRA223(SEQ ID NO:12) 的理论 pI 是 7.2,且 DRA224(SEQ ID NO:14) 的理论 pI 是 6.8。在一些实施方案中,CD3 结合多肽的经验等电点比多肽 SEQ ID NO:4 低至少 1 个 pI 单位。

[0049] 令人惊讶的是,pI 变体展现了改良的半衰期且在 CHO 细胞中表达得比 DRA209 更好,例如,参见以下实施例 4,所述实施例描述了用于测量半衰期的方法。此外,与具有诸如 DRA209 结合域等其它抗 CD3 结合域的类似构建体相比,包含 DRA222scFv 的多特异性构建体展现了改良的性质(参见相关 PCT 公布号 W02012/145714)。

[0050] 本文中所使用的章节标题仅用于组织目的,而不应被视为限制所描述的主题。本文中所引用的所有文献或文献的部分,包括但不限于专利、专利申请、论文、书籍和专著,都以全文引用的方式明确并入在此以用于任何目的。在所并入的文献或文献的部分中的一者或多者的术语定义与本申请中的术语定义相矛盾的情况下,以本申请中出现的定义为准。

[0051] 除非另外指出,否则在本发明描述中,任何浓度范围、百分比范围、比率范围或整数范围都应理解为包括所述范围内的任何整数值,且当适当时,包括其分数(诸如整数的十分之一和百分之一)。除非另外指出,否则如本文中所使用,“约”意指所指示范围、值或结构的 ±20%。应理解,除非另外指出,否则如本文中所使用的术语“一个”和“一种”是指所枚举的组分中的“一个(种)或多个(种)”。使用代替性用词(例如“或”)应理解为意指代替物之一、两者或其任何组合。如本文中所使用,术语“包括”和“包含”以同义使用。另外,应理解,本申请对包含本文中所描述的组分(例如域或区)和取代基的各种组合的多

肽的公开程度就如同个别地阐述各多肽。因而,个别多肽的特定组分的选择在本公开的范围之内。

[0052] 如本文中所使用,术语“结合域”或“结合区”是指能够特异性识别和结合诸如抗原、配体、受体、底物或抑制剂(例如 CD3 或肿瘤相关抗原,诸如 RON、CD19、CD37 或 PSMA)等靶分子的蛋白质、多肽、寡肽或肽的域、区、部分或位点。示例性结合域包括单链抗体可变区(例如域抗体、sFv、scFv 和 scFab)、受体胞外域和配体(例如细胞因子、趋化因子)。在某些实施方案中,结合域包含以下各项或由以下各项组成:抗原结合位点(例如包含来自于置于交替构架区(FR)(例如任选地包含一个或多个氨基酸取代的人 FR)中的抗体的可变重链序列和可变轻链序列或三个轻链互补决定区(CDR)和三个重链 CDR)。已知多种用于鉴别本公开的特异性结合特定靶的结合域的测定,包括 Western 印迹、ELISA、噬菌体展示文库筛选和 **BIACORE®** 互相作用分析。如本文中所使用,CD3 结合多肽可以具有“CD3 结合域”和任选地存在的“第二结合域”。CD3 结合域可以位于氨基或羧基末端。在某些实施方案中,CD3 结合域包含来源于小鼠单克隆抗体(例如 Cris-7 或 HuM291)的人源化 scFv。举例来说,CD3 结合域可以由源自于小鼠单克隆抗体的 VH 区和 VL 区构成,其中用诸如 $(\text{Gly}_4\text{Ser})_3$ (SEQ ID NO:76) 连接子等连接子接合 VH 区与 VL 区。在其它实施方案中,CD3 结合域实质上由或由源自于小鼠单克隆抗体的人源化 scFv 组成。在一些实施方案中,本发明的 CD3 结合域包含鼠类 Cris-7 抗体或 HuM291 的重链 CDR1、CDR2 和 CDR3 以及轻链 CDR1、CDR2 和 CDR3。

[0053] 如果结合域以等于或大于 10^5M^{-1} 的亲合力或 K_a (即,特定结合相互作用的平衡缔合常数,单位是 $1/\text{M}$) 结合靶标而不显著结合测试样品中存在的其它组分,则它“特异性结合”靶标。结合域可以分为“高亲合力”结合域和“低亲合力”结合域。“高亲合力”结合域是指具有至少 10^7M^{-1} 、至少 10^8M^{-1} 、至少 10^9M^{-1} 、至少 10^{10}M^{-1} 、至少 10^{11}M^{-1} 、至少 10^{12}M^{-1} 或至少 10^{13}M^{-1} 的 K_a 的那些结合域。“低亲合力”结合域是指具有最多 10^7M^{-1} 、最多 10^6M^{-1} 、最多 10^5M^{-1} 的 K_a 的那些结合域。或者,亲合力可以定义为特定结合相互作用的平衡解离常数 (K_d),单位为 M (例如 10^{-5}M 到 10^{-13}M)。根据本公开的结合域多肽和单链多肽的亲合力可以使用常规技术容易地测定(参见例如 Scatchard 等 (1949) Ann. N. Y. Acad. Sci. 51:660 ;和美国专利号 5, 283, 173、5, 468, 614 或等效物)。

[0054] 本领域中已知“CD3”为六个链的多蛋白复合物(参见例如 Abbas 和 Lichtman, 2003 ;Janeway 等, 第 172 和 178 页, 1999),所述六个链是 T 细胞受体复合物的亚单元。在哺乳动物中,T-细胞受体复合物的 CD3 亚单元是 CD3 γ 链、CD3 δ 链、两个 CD3 ϵ 链和 CD3 ζ 链同源二聚体。CD3 γ 、CD3 δ 和 CD3 ϵ 链是含有单个免疫球蛋白域的免疫球蛋白超家族的高度相关细胞表面蛋白。CD3 γ 、CD3 δ 和 CD3 ϵ 链的跨膜区带负电,这是允许这些链与带正电的 T 细胞受体链缔合的特征。CD3 γ 、CD3 δ 和 CD3 ϵ 链的细胞内尾部各自含有称为基于免疫受体酪氨酸的活化基元或 ITAM 的单个保守基元,而各 CD3 ζ 链具有三个。相信 ITAM 对于 TCR 复合物的信号传导能力是重要的。如本公开中所使用的 CD3 可以来自于不同的动物物种,包括人、猴子、小鼠、大鼠或其它哺乳动物。

[0055] 如本文中所使用,“保守取代”在本领域中被公认为一个氨基酸取代具有相似性质的另一个氨基酸。示例性保守取代在本领域是众所周知的(参见例如 1997 年 3 月 13 日公开的 W097/09433, 第 10 页 ;Lehninger, Biochemistry, 第二版 ;Worth Publishers, Inc. NY:NY(1975), 第 71-77 页 ;Lewin, Genes IV, Oxford University Press, NY and Cell

Press, Cambridge, MA (1990), 第 8 页)。在某些实施方案中,保守取代包括亮氨酸对丝氨酸取代。

[0056] 如本文中所使用,术语“衍生物”是指在存在或不存在酶的情况下通过化学或生物学手段,例如通过糖基化、烷基化、酰化、酯形成或酰胺形成对肽的一个或多个氨基酸残基的修饰。

[0057] 如本文中所使用,“源自于”指定多肽或蛋白质的多肽或氨基酸序列是指多肽的来源。在一个实施方案中,源自于特定序列的多肽或氨基酸序列具有实质上与该序列或其一部分同一的氨基酸序列,其中所述部分由至少 10-20 个氨基酸、至少 20-30 个氨基酸或至少 30-50 个氨基酸或至少 50-150 个氨基酸组成;或本领域技术人员可用其它方式鉴别它,因为在序列中具有其来源。在一个实施方案中,人源化或嵌合结合域源自于由另一个动物产生的抗体的 VH 和 / 或 VL。举例来说,人源化结合域或嵌合结合域可能源自于鼠类抗体的 VH 和 / 或 VL 区。举例来说,可以通过用本领域中已知的方法修饰构架区来进行鼠类抗体的人源化。

[0058] 源自于另一个多肽的多肽相对于起始多肽可能具有一个或多个突变,例如其一个或多个氨基酸残基经另一个氨基酸残基取代或其具有一个或多个氨基酸残基插入或缺失。多肽可能包含非天然存在的氨基酸序列。所述变体与起始多肽必然具有小于 100% 的序列同一性或相似性。在一个实施方案中,变体的氨基酸序列与起始多肽的氨基酸序列将具有约 60% 到小于 100% 的氨基酸序列同一性或相似性。在另一个实施方案中,变体的氨基酸序列与起始多肽的氨基酸序列将具有约 75% 到小于 100%、约 80% 到小于 100%、约 85% 到小于 100%、约 90% 到小于 100%、约 95% 到小于 100% 的氨基酸序列同一性或相似性。

[0059] 如本文中所使用,除非另外规定,否则氨基酸残基在免疫球蛋白分子的可变区中的位置是根据 Kabat 编号规定 (Kabat, Sequences of Proteins of Immunological Interest, 第 5 版, Bethesda, MD: Public Health Service, National Institutes of Health (1991)) 进行编号,且氨基酸残基在免疫球蛋白分子的恒定区中的位置是根据 EU 命名法 (Ward 等, 1995 Therap. Immunol. 2:77-94) 进行编号。

[0060] 如本文中所使用,术语“二聚体”是指由经由一种或多种形式的分子内力 (包括共价键,例如二硫键;和其它相互作用,例如静电相互作用、盐桥、氢键合和疏水性相互作用) 彼此缔合的两个亚单元组成且在适当条件 (例如,在生理条件下,在适合表达、纯化和 / 或存储重组蛋白质的水溶液中,或在用于非变性和 / 或非还原电泳的条件下) 下稳定的生物学实体。如本文中所使用的“异源二聚体”或“异源二聚体蛋白”是指由两个不同的多肽形成的二聚体。异源二聚体不包括由四个多肽 (即,两个轻链和两个重链) 形成的抗体。在本发明的一个实施方案中,使用包含异源二聚域的 Emergent 的 Interceptor 平台产生异源二聚体。如本文中所使用的“同源二聚体”或“同源二聚体蛋白”是指由两个同一的多肽形成的二聚体。在本发明的某些实施方案中,使用 Emergent 的 SMIP、PIMS 或 Scorpion 平台产生同源二聚体。

[0061] 如本文中所使用,“铰链区”或“铰链”是指源自于以下的多肽:(a) 跨膜蛋白 (例如 I 型跨膜蛋白) 的域间区;或 (b) II 型 C-凝集素的茎部区 (stalk region)。举例来说,铰链区可以源自于免疫球蛋白超家族成员的域间区;这个特定类别内的合适的铰链区包括 (i) 免疫球蛋白铰链区 (举例来说,由上部区和核心区组成) 或其功能变体,包括野生型和

改变的免疫球蛋白铰链；和(ii)连接免疫球蛋白V样或免疫球蛋白C样结构域的区域（或其功能变体）。

[0062] “野生型免疫球蛋白铰链区”是指在抗体重链中发现的插入在CH1与CH2域之间且连接CH1与CH2域（对于IgG、IgA和IgD来说）或插入在CH1与CH3域之间且连接CH1与CH3域（对于IgE和IgM来说）的天然存在的上部和中间铰链氨基酸序列。在某些实施方案中，野生型免疫球蛋白铰链区序列是人的，且可以包含人IgG铰链区。

[0063] “改变的野生型免疫球蛋白铰链区”或“改变的免疫球蛋白铰链区”是指(a)具有最多30%氨基酸变化（例如，最多25%、20%、15%、10%或5%氨基酸取代或缺失）的野生型免疫球蛋白铰链区，或(b)野生型免疫球蛋白铰链区中长度为约5个氨基酸（例如约5、6、7、8、9、10、11、12、13、14、15、16、17、18、19或20个氨基酸）到最多约120个氨基酸（例如，长度为约10到约40个氨基酸或约15到约30个氨基酸或约15到约20个氨基酸或约20到约25个氨基酸），具有最多约30%氨基酸变化（例如，最多约25%、20%、15%、10%、5%、4%、3%、2%或1%氨基酸取代或缺失或其组合）且具有如PCT公布W02011/090762和W02011/090754中所公开的IgG核心铰链区的部分。

[0064] 如本文中所使用，术语“人源化”是指通过使用基因工程技术使抗体或免疫球蛋白结合蛋白和源自于非人物种（例如小鼠或大鼠）的多肽对人具有较弱免疫原性，同时仍保留原始抗体的抗原结合性质的过程。在一些实施方案中，将抗体或免疫球蛋白结合蛋白和多肽（例如，轻链和重链可变区、Fab、scFv）的结合域人源化。可以使用称为CDR移植的技术及其变型将非人结合域人源化（Jones等，Nature 321:522(1986)），所述变型包括“改型”（Verhoeyen等，1988Science239:1534-1536；Riechmann等，1988Nature 332:323-337；Tempest等，Bio/Technol 19919:266-271）、“高嵌合”（Queen等，1989Proc Natl Acad Sci USA 86:10029-10033；Co等，1991Proc Natl Acad Sci USA88:2869-2873；Co等，1992J Immunol 148:1149-1154）和“饰面”（Mark等，“Derivation of therapeutically active humanized and veneered anti-CD18antibodies.”Metcalf BW,Dalton BJ编，Cellular adhesion:molecular definition to therapeutic potential.New York:Plenum Press,1994:291-312）。如果源自于非人来源，则抗体或免疫球蛋白结合蛋白和多肽的其它区，诸如铰链区和恒定区结构域，也可以进行人源化。

[0065] “免疫球蛋白恒定区”或“恒定区”是本文中定义的术语，它是指对应于或源自于部分或所有的一个或多个恒定区结构域的肽或多肽序列。在一个实施方案中，恒定区含有源抗体的所有恒定区结构域。在一个实施方案中，恒定区包含IgG CH2和CH3域，例如IgG1CH2和CH3域。在一些实施方案中，恒定区不包含CH1域。在某些实施方案中，组成恒定亚区的恒定区结构域是人的。在一些实施方案中，本公开的融合蛋白的恒定区结构域缺乏或具有极少抗体依赖性细胞介导的细胞毒性（ADCC）和补体活化和补足依赖性细胞毒性（CDC）的效应因子功能，而保留结合一些Fc受体（诸如FcRn，即新生儿Fc受体）的能力且在体内保留相对较长的半衰期。

[0066] 在其它变化方案中，本公开的融合蛋白包括保留ADCC和CDC之一或两者的所述效应因子功能的恒定域。在某些实施方案中，本公开的结合域与人IgG恒定区（例如IgG1）融合，其中所述恒定区具有一个或多个以下突变的氨基酸：位置234上的亮氨酸（L234）、位置235上的亮氨酸（L235）、位置237上的甘氨酸（G237）、位置318上的谷氨酸酯（E318）、

位置 320 上的赖氨酸 (K320)、位置 322 上的赖氨酸 (K322) 或其任何组合 (根据 EU 进行编号)。举例来说,可以将这些氨基酸中的任何一个或多个变成丙氨酸。在另一个实施方案中, IgG Fc 域 (诸如 IgG1) 中的 L234、L235、G237、E318、K320 和 K322 (根据 EU 编号) 各自突变成例如丙氨酸 (即,分别为 L234A、L235A、G237A、E318A、K320A 和 K322A) 和任选地存在的 N297A 突变 (即,实质上消除 CH2 域的糖基化)。

[0067] 如这里所使用,术语“小模块式免疫药物蛋白”或“SMIP”用于指总体上如例如美国专利申请公布号 2003/0133939、2003/0118592 和 2005/0136049 中所公开的蛋白质支架,所述文献以全文引用的方式并入本文中。在本文中,实施例和公开内容全文中所描述的“SMIP 分子”应理解为包含 SMIP 支架,例如按照从氨基到羧基末端的顺序包含第一结合域、铰链区和免疫球蛋白恒定区的结合蛋白。“CD3 特异性 SMIP 分子”应理解为包含 SMIP 支架的 CD3 结合蛋白。

[0068] 如这里所使用,术语“PIMS”用于指总体上如例如美国专利申请公布号 2009/0148447 中所公开的蛋白质支架,所述文献以全文引用的方式并入本文中。在本文中,实施例和公开内容全文中所描述的“PIMS 分子”应理解为包含 PIMS 支架,例如按照从氨基到羧基末端的顺序包含免疫球蛋白恒定区、铰链区和第一结合域的结合蛋白。“CD3 特异性 PIMS 分子”应理解为包含 PIMS 支架的 CD3 结合蛋白。

[0069] “Fc 区”或“Fc 域”是指对应于或源自于源抗体中负责结合细胞上的抗体受体和补体上的 C1q 组分的部分的多肽序列。Fc 代表“结晶片段”,即容易形成蛋白质晶体的抗体片段。最初通过蛋白水解消化描述的独特蛋白质片段可以定义免疫球蛋白的总体通用结构。如文献中最初所定义, Fc 片段由二硫化物连接的重链铰链区、CH2 和 CH3 域组成。然而,近来该术语已被应用于由 CH3、CH2 和足以与第二个这样的链形成二硫化物连接的二聚体的铰链的至少一部分组成的单链。关于免疫球蛋白结构和功能的回顾,参见 Putnam, *The Plasma Proteins*, 第 V 卷 (Academic Press, Inc., 1987), 第 49-140 页;和 Padlan, *Mol. Immunol.* 31:169-217, 1994。如本文中所使用,术语 Fc 包括天然存在的序列的变体。

[0070] 如本文中所使用,“等电点”或 pI 是净电荷为零时的 pH 值。

[0071] 如本文中所使用,术语“Interceptor”是用于指总体上如 PCT 公布 W02011/090762 和 W02011/090754 中所公开的单特异性或多特异性异源二聚蛋白质支架。本文中所描述的 Interceptor 分子应理解为包含两个非同源多肽链的 CD3 结合蛋白,各多肽链包含免疫球蛋白异源二聚域。界面免疫球蛋白异源二聚域是不同的。在一个实施方案中,免疫球蛋白异源二聚域包含 CH1 域或其衍生物。在另一个实施方案中,免疫球蛋白异源二聚域包含 CL 域或其衍生物。在一个实施方案中,CL 域是 C κ 或 C λ 同种型或其衍生物。

[0072] 如本文中所使用,II 型 C-凝集素的“茎部区”是指 II 型 C-凝集素的细胞外域中位于 C-型凝集素样结构域 (CTL D;例如,类似于自然杀伤细胞受体的 CTL D) 与跨膜域之间的部分。举例来说,在人 CD94 分子 (GenBank 登录号 AAC50291.1, PRI, 1995 年 11 月 30 日) 中,细胞外域对应于氨基酸残基 34-179,而 CTL D 对应于氨基酸残基 61-176。因此,人 CD94 分子的茎部区包括氨基酸残基 34-60,据发现它处在膜和 CTL D 之间 (参见 Boyington 等, *Immunity* 10:75, 1999;关于其它茎部区的描述,也参见 Beavil 等, *Proc. Nat'l. Acad. Sci. USA* 89:753, 1992;和 Figdor 等, *Nature Rev. Immunol.* 2:77, 2002)。这些 II 型 C-凝集素还可以在茎部区与跨膜区或 CTL D 之间具有 6 到 10 个接合氨基酸。在另一个实施例中,

233 个氨基酸的人 NKG2A 蛋白 (GenBank 登录号 P26715.1, PRI, 2010 年 6 月 15 日) 具有在氨基酸 71-93 范围内的跨膜区和在氨基酸 94-233 范围内的细胞外域。CTLD 由氨基酸 119-231 构成, 且茎部区包含氨基酸 99-116, 两侧由五个和两个氨基酸连接。其它 II 型 C-凝集素以及其细胞外配体结合域、域间或茎部区, 和 CTLD 在本领域中是已知的 (关于人 CD23、CD69、CD72、NKG2A 和 NKG2D 和其描述, 分别参见例如 GenBank 登录号 NP_001993.2; AAH07037.1, PRI, 2006 年 7 月 15 日; NP_001773.1, PRI, 2010 年 6 月 20 日; AAL65234.1, PRI, 2002 年 1 月 17 日; 和 CAA04925.1, PRI, 2006 年 11 月 14 日)。

[0073] 如本文中所使用, 跨膜蛋白 (例如 I 型跨膜蛋白) 的“域间区”是指跨膜蛋白的细胞外域中位于两个相邻结构域之间的部分。域间区的实例包括连接免疫球蛋白超家族成员的相邻 Ig 域的区域 (例如, 来自于 IgG、IgA、IgD 或 IgE 的免疫球蛋白铰链区; 连接 CD2 的 IgV 域与 IgC2 域的区域; 或连接 CD80 或 CD86 的 IgV 域与 IgC 域的区域)。域间区的另一个例子是连接 CD22 (一种结合 I 型唾液酸的 Ig 样凝集素) 的非 Ig 域与 IgC2 域的区域。

[0074] “源自于” II 型 C-凝集素茎部区或“源自于”跨膜蛋白域间区 (例如, 免疫球蛋白铰链区) 的多肽区是指约 5 到约 150 个氨基酸序列, 其整体或一部分包括 (i) 野生型茎部区或域间区序列; (ii) 野生型茎部区或域间区序列的片段; (iii) 与 (i) 或 (ii) 具有至少 80%、85%、90% 或 95% 氨基酸序列同一性的多肽; 或 (iv) 其中一、二、三、四或五个氨基酸具有缺失、插入、取代或其任何组合的 (i) 或 (ii), 例如一个或多个变化是取代, 或一个或多个突变仅包括一个缺失。在一些实施方案中, 与野生型茎部区序列, 诸如源自于 NKG2A、NKG2D、CD23、CD64、CD72 或 CD94 的约 8 到约 20、约 10 到约 25 或约 15 到约 25 个氨基酸的那些序列相比, 茎部区衍生物对蛋白水解裂解更具抗性。

[0075] 如本文中所使用, 术语“接合氨基酸”或“接合氨基酸残基”是指多肽的两个相邻区域或结构域之间, 诸如铰链与相邻免疫球蛋白恒定亚区之间, 或铰链与相邻结合域之间, 或连接两个免疫球蛋白可变域的肽连接子与相邻免疫球蛋白可变域之间的一个或多个 (例如约 2-10 个) 氨基酸残基。接合氨基酸可以由多肽构建体设计产生 (例如, 由在构建编码多肽的核酸分子期间使用限制酶位点产生的氨基酸残基)。结合域与铰链区之间的接合氨基酸在本文中称为前铰链区, 例如, 可由添加限制性位点到编码核苷酸序列中而产生。在一个实施方案中, 前铰链区由约 1、2、3、4、5、6、7、8、9、10 或 15 个氨基酸组成。DRA209 和 DRA161 的前铰链区序列不是鼠类 Cris-7 或人免疫球蛋白生殖细胞系序列的一部分。DRA209 和 DRA161 的前铰链区是氨基酸序列 RRT, 而对于 DRA219、DRA221、DRA222、DRA223、DRA224、DRA225、DRA228、DRA229、DRA233、DRA234、TSC249、TSC250、TSC251、TSC252、TSC295、TSC296、TSC301 和 TSC302, 它是 SSS。本发明的结合分子可能包括或可能不包括前铰链区。

[0076] 如本文中所使用, 短语“CH3 与 CH1 或 CL 之间的连接子”是指 CH3 域 (例如野生型 CH3 或突变型 CH3) 的 C 末端与 CH1 域或 CL 域 (例如 Ck) 的 N 末端之间的一个或多个 (例如约 2-12 个) 氨基酸残基。

[0077] 如本文中所使用, 术语“有需要的患者”是指有风险罹患或正罹患能用本文中所提供的 CD3 结合蛋白或多肽或其组合物治疗或改善的疾病、病症或病状的患者。

[0078] 如本文中所使用, 术语“肽连接子”是指连接重链可变区与轻链可变区且提供与两个亚结合域的相互作用相容的间隔物功能以使得所得多肽保留对作为包含相同轻链和重链可变区的抗体的相同靶分子的特异性结合亲和力的氨基酸序列。在某些实施方案中, 连

接子由 5 到约 35 个氨基酸,例如约 15 到约 25 个氨基酸构成。

[0079] 如本文中所使用,术语“药学上可接受的”是指当使用本领域中众所周知的途径施用分子实体和组成在大多数受试者中不会产生过敏性或其它严重有害反应。联邦或州政府的管理机构批准的或美国药典或其它一般公认的药典中登记的供动物且更确切地说供人使用的分子实体和组成被视为是“药学上可接受的”。

[0080] 如本文中所使用,术语“启动子”是指涉及结合 RNA 聚合酶以起始转录的 DNA 区域。

[0081] 如本文中所使用,术语“核酸”、“核酸分子”或“聚核苷酸”是指呈单链或双链形式的脱氧核糖核苷酸或核糖核苷酸和其聚合物。除非有具体限制,否则该术语涵盖与参考核酸具有相似结合性质且以类似于天然存在的核苷酸的方式代谢的含有天然核苷酸类似物的核酸。除非另外指出,否则特定核酸序列还暗含其经保守修饰(例如简并密码子取代)的变体和互补序列以及明确指示的序列。具体地说,简并密码子取代可以通过产生一个或多个选定(或所有)密码子的第三位置经混合碱基和/或脱氧肌苷残基取代的序列来实现(Batzer 等(1991)*Nucleic Acid Res.* 19:5081;Ohtsuka 等(1985)*J. Biol. Chem.* 260:2605-2608;Cassol 等(1992);Rossolini 等(1994)*Mol. Cell. Probes* 8:91-98)。术语核酸与基因、cDNA 和由基因编码的 mRNA 可互换使用。如本文中所使用,术语“核酸”、“核酸分子”或“聚核苷酸”意在包括 DNA 分子(例如 cDNA 或基因组 DNA)、RNA 分子(例如 mRNA)、使用核苷酸类似物产生的 DNA 或 RNA 类似物和其衍生物、片段和同源物。

[0082] 术语“表达”是指由核酸编码的产物的生物合成。举例来说,在编码相关多肽的核酸区段的情况下,表达涉及将核酸区段转录到 mRNA 中和将 mRNA 翻译成一种或多种多肽。

[0083] 术语“表达单元”和“表达盒”在本文中可互换使用,且表示编码相关多肽且能够在宿主细胞中表达核酸区段的核酸区段。表达单元典型地包含转录启动子、编码相关多肽的开放阅读框和转录终止子,全部呈可操作的构型。除转录启动子和终止子以外,表达单元可以进一步包括其它核酸区段,诸如增强子或聚腺苷酸化信号。

[0084] 如本文中所使用,术语“表达载体”是指线性或环形核酸分子,其包含一个或多个表达单元。除一个或多个表达单元以外,表达载体还可以包括其它核酸区段,诸如一个或多个复制起点或一个或多个可选标记物。表达载体一般源自于质粒或病毒 DNA,或可以含有两者的元件。

[0085] 如本文中所使用,Scorpion 是用于指多特异性结合蛋白支架的术语。举例来说,PCT 申请公布号 WO 2007/146968、美国专利申请公布号 2006/0051844、PCT 申请公布号 WO 2010/040105、PCT 申请公布号 WO2010/003108 和美国专利号 7,166,707 中公开了多特异性结合蛋白和多肽。Scorpion 多肽包含两个结合域(所述结构域可以设计用于特异性结合相同或不同的靶标)、两个连接子和一个免疫球蛋白恒定亚区。PCT 申请公布号 WO2010/003108 中描述了用于 Scorpion 分子的连接子。在一些实施方案中,连接子序列为约 2-45 个氨基酸或 2-38 个氨基酸或 5-45 个氨基酸。在一些实施方案中,连接子是抗体铰链区(例如来自于 IgG)或 C-凝集素茎部区。Scorpion 蛋白是包含两个同一的由二硫化物键合的 Scorpion 多肽的同源二聚蛋白。

[0086] 如本文中所使用,术语“序列同一性”是指两个或更多个聚核苷酸序列之间或者两个或更多个多肽序列之间的关系。当一个序列中的位置被比较序列的对应位置上的相同核酸碱基或氨基酸残基占据时,将所述序列称为在那个位置上“同一”。“序列同一性”百分比

是通过确定两个序列中存在同一的核酸碱基或氨基酸残基的位置数以产生“同一的”位置数来计算。然后将“同一的”位置数除以比较窗中的总位置数并乘以 100,以产生“序列同一性”百分比。通过在比较窗上比较两个最佳比对的序列来确定“序列同一性”百分比。核酸序列的比较窗的长度可以是例如至少 20、30、40、50、60、70、80、90、100、110、120、130、140、150、160、170、180、190、200、300、400、500、600、700、800、900 或 1000 个或更多个核酸。多肽序列的比较窗的长度可以是例如至少 20、30、40、50、60、70、80、90、100、110、120、130、140、150、160、170、180、190、200、300 个或更多个氨基酸。为了最佳比对序列以供比较,比较窗中的聚核苷酸或多肽序列部分可以包含称为空位的添加或缺失,而参考序列保持恒定。最佳比对是即使有空位的情况下也能在参考序列与比较序列之间产生最大的可能“同一的”位置数的比对。两个序列之间的“序列同一性”百分比可以使用程序版本“BLAST 2Sequences”确定,该程序版本可得自国家生物技术信息中心(截止到 2004 年 9 月 1 日),该程序并入了程序 BLASTN(用于核苷酸序列比较)和 BLASTP(用于多肽序列比较),这两个程序是基于 Karlin 和 Altschul (Proc. Natl. Acad. Sci. USA90(12):5873-5877, 1993) 的算法。当利用“BLAST 2Sequences”时,参数(即,截止到 2004 年 9 月 1 日的缺省参数)可以用于字长(3)、开放空位罚分(11)、延伸空位罚分(1)、空位下降(50)、期望值(10)和任何其它所需参数,包括但不限于基质选项。如果两个核苷酸或氨基酸序列相对于彼此具有至少 80%、至少 85%、至少 90%、至少 95%、至少 96%、至少 97%、至少 98%或至少 99%序列同一性,则这两个序列被视为具有“基本上相似的序列同一性”或“基本的序列同一性”。

[0087] 如本文中所使用,“多肽”或“多肽链”是共价连接的氨基酸的单一线性连续排列。它不包括以非线性方式,诸如经由链间二硫键连接在一起的两个多肽链(例如,轻链与重链经由二硫键连接的半免疫球蛋白分子)。多肽可以具有或形成一个或多个链内二硫键。关于如本文中所描述的多肽,提到对应于由 SEQ ID NO 说明的那些氨基酸残基的氨基酸残基包括所述残基的翻译后修饰。

[0088] “蛋白质”是包含一个或多个多肽链的大分子。蛋白质还可以包含非肽组分,诸如碳水化合物基团。碳水化合物和其它非肽取代基可以由产生蛋白质的细胞添加到蛋白质,并且将随细胞类型而变化。蛋白质在本文中是依据其氨基酸主链结构进行定义;一般不说明诸如碳水化合物基团等取代基,但仍然可能存在。

[0089] 术语“氨基末端”(N 末端)和“羧基末端”(C 末端)在本文中用于表示多肽内的位置。在上下文允许的情况下,这些术语是参考特定序列或多肽部分使用以表示邻近或相对位置。举例来说,多肽内相对于参考序列位于羧基末端的某些序列位于参考序列的羧基末端附近,但未必在完整多肽的羧基末端上。

[0090] “T 细胞受体”(TCR)是在 T 细胞表面上发现的分子,一般与 CD3 一起负责识别与主要组织相容性复合物(MHC)分子结合的抗原。在大部分 T 细胞中,它由高度可变 α 和 β 链的二硫化物连接的异源二聚体组成。在其它 T 细胞中,表达由可变 γ 和 δ 链组成的代替受体。TCR 的各链是免疫球蛋白超家族的成员,并且拥有一个 N 末端免疫球蛋白可变域、一个免疫球蛋白恒定域、跨膜区和 C 末端的短胞质尾(参见 Abbas 和 Lichtman, Cellular and Molecular Immunology(第 5 版),编者:Saunders, Philadelphia, 2003; Janeway 等, Immunobiology: The Immune System in Health and Disease, 第 4 版, Current Biology Publications, 第 148、149 和 172 页, 1999)。如本公开中所使用的 TCR 可以来自

于各种动物物种,包括人、小鼠、大鼠或其它哺乳动物。

[0091] 如本文中所使用的“TCR 复合物”是指由 CD3 链与其它 TCR 链缔合形成的复合物。举例来说,TCR 复合物可以由 CD3 γ 链、CD3 δ 链、两个 CD3 ϵ 链、CD3 ζ 链的同源二聚体、TCR α 链和 TCR β 链构成。或者,TCR 复合物可以由 CD3 γ 链、CD3 δ 链、两个 CD3 ϵ 链、CD3 ζ 链的同源二聚体、TCR γ 链和 TCR δ 链构成。

[0092] 如本文中所使用的“TCR 复合物的组分”是指 TCR 链(例如 TCR α 、TCR β 、TCR γ 或 TCR δ)、CD3 链(例如 CD3 γ 、CD3 δ 、CD3 ϵ 或 CD3 ζ)或由两个或更多个 TCR 链或 CD3 链形成的复合物(例如 TCR α 与 TCR β 的复合物、TCR γ 与 TCR δ 的复合物、CD3 ϵ 与 CD3 δ 的复合物、CD3 γ 与 CD3 ϵ 的复合物,或 TCR α 、TCR β 、CD3 γ 、CD3 δ 和两个 CD3 ϵ 链的亚 TCR 复合物)。

[0093] 如本文中所使用的“抗体依赖性细胞介导的细胞毒性”和“ADCC”是指细胞介导的过程,其中表达 Fc γ R 的非特异性细胞毒性细胞(例如,单核细胞,诸如自然杀伤(NK)细胞和巨噬细胞)识别靶细胞上的结合抗体(或能够结合 Fc γ R 的其它蛋白质)且随后导致靶细胞溶解。原则上,具有活化 Fc γ R 的任何效应细胞都可能被触发而介导 ADCC。介导 ADCC 的原代细胞是 NK 细胞,其仅表达 Fc γ RIII,而单核细胞取决于它们的活化状态、定位或分化可以表达 Fc γ RI、Fc γ RII 和 Fc γ RIII。关于造血细胞上的 Fc γ R 表达的回顾,参见例如 Ravetch 等,1991, *Annu. Rev. Immunol.*, 9:457-92。

[0094] 如本文中提到多肽或蛋白质时使用的术语“具有 ADCC 活性”意指多肽或蛋白质(例如,包含具有 CH2 和 CH3 域的免疫球蛋白铰链区和免疫球蛋白恒定亚区,诸如源自于 IgG 的多肽或蛋白质(例如 IgG1))能够通过结合表达 Fc 受体的细胞溶解型免疫效应细胞(例如 NK 细胞)上的细胞溶解型 Fc 受体(例如 Fc γ RIII)来介导抗体依赖性细胞介导的细胞毒性(ADCC)。

[0095] 如本文中所使用的“补体依赖性细胞毒性”和“CDC”是指正常血清中的组分(“补体”)与抗体或与靶抗原结合的其它 C1q 补体结合蛋白一起展现表达靶抗原的靶细胞的溶解的过程。补体由一组协同作用且有序排列以发挥其作用的血清蛋白组成。

[0096] 如本文中所使用的术语“经典补体路径”和“经典补体系统”是同义的,并且是指活化补体的特定路径。经典路径需要抗原-抗体复合物以便以有序方式起始和参与九种主要蛋白质组分(指定为 C1 到 C9)的活化。对于活化过程中的若干个步骤,产物是能催化后续步骤的酶。这一级联用相对较小的起始信号扩增并活化了大量补体。

[0097] 如本文中关于多肽或蛋白质使用的术语“具有 CDC 活性”意指所述多肽或蛋白质(例如,包含具有 CH2 和 CH3 域的免疫球蛋白铰链区和免疫球蛋白恒定亚区,诸如源自于 IgG 的多肽或蛋白质(例如 IgG1))能够通过结合 C1q 补体蛋白和活化经典补体系统来介导补体依赖性细胞毒性(CDC)。

[0098] 如本文中所使用的“重定向 T 细胞细胞毒性”和“RTCC”是指 T 细胞介导的过程,其中使用能够特异性结合细胞毒性 T 细胞和靶细胞两者,且借此引发针对靶细胞的靶依赖性细胞毒性 T 细胞反应的多特异性蛋白将细胞毒性 T 细胞募集到靶细胞。

[0099] 如本文中所使用,术语“治疗(treatment)”、“治疗(treating)”或“缓解”是指治疗性治疗或防范性/预防性治疗。若接受治疗的个体中的至少一种疾病症状有改善或治疗可以延迟个体中的进行性疾病的恶化或防止其它相关疾病发作,则治疗为治疗性的。

[0100] 如本文中所使用,术语“转化”、“转染”和“转导”是指将核酸(例如核苷酸聚合物)转移到细胞中。如本文中所使用,术语“遗传转化”是指将 DNA,尤其是重组 DNA 转移和并入到细胞中。转移的核酸可以经由表达载体而引入到细胞中。

[0101] 如本文中所使用,术语“变体”是指不同于参考核酸或多肽,但保留其基本性质的核酸或多肽。一般来说,变体总体上密切类似于参考核酸或多肽且在许多区域中与参考核酸或多肽是同一的。举例来说,与活性部分或全长参考核酸或多肽相比,变体可以展现至少约 70%、至少约 80%、至少约 90%、至少约 95%、至少约 96%、至少约 97%、至少约 98%或至少约 99%序列同一性。

[0102] 术语“轻链可变区”(也称为“轻链可变域”或“VL”)和“重链可变区”(也称为“重链可变域”或“VH”)分别是指来自于抗体轻链和重链的可变结合区。可变结合区由称为“互补性决定区”(CDR)和“构架区”(FR)的离散的明确的亚区组成。在一个实施方案中,FR 被人源化。术语“CL”是指“免疫球蛋白轻链恒定区”或“轻链恒定区”,即,来自于抗体轻链的恒定区。术语“CH”是指“免疫球蛋白重链恒定区”或“重链恒定区”,取决于抗体同种型,它还可以进一步分为 CH1、CH2 和 CH3(IgA、IgD、IgG)或 CH1、CH2、CH3 和 CH4 域(IgE、IgM)。“Fab”(抗原结合片段)是抗体中结合抗原的部分且包括重链中经由链内二硫键与轻链连接的可变区和 CH1 域。

[0103] 本公开尤其提供了包含结合域,确切地说,包含特异性结合 CD3 的第一结合域的多肽和蛋白质。本公开的包含结合域的多肽和蛋白质可以包含以下各项中的一个或多个:免疫球蛋白恒定亚区、连接肽、铰链区、免疫球蛋白异源二聚域、免疫球蛋白二聚域、一个或多个接合氨基酸、标签等等。本文中进一步详细描述所公开的多肽和蛋白质的这些组分。

[0104] 另外,本文中公开的 CD3 结合多肽和蛋白质可以呈多种不同形式中任一种的抗体或融合蛋白的形式(例如,融合蛋白可以呈 SMIP 蛋白、PIMS 蛋白、Scorpion 蛋白或 Interceptor 蛋白形式)。

[0105] 在一些实施方案中,CD3 结合多肽包含第二结合域。在一些实施方案中,CD3 结合多肽从氨基到羧基末端包含 CD3 结合域、铰链区、恒定区和第二结合域。在其它实施方案中,CD3 结合多肽从氨基到羧基末端包含第二结合域、铰链区、恒定区和 CD3 结合域。

[0106] 根据本发明的 CD3 结合蛋白一般包括(a)至少一个包含如本文中所述的 CD3 结合域的 CD3 结合多肽链。在某些变化方案中,CD3 结合多肽还包括(b)羧基末端连接到 CD3 结合域的铰链区和(c)免疫球蛋白恒定区(例如 SMIP 多肽)。在其它变化方案中,CD3 结合多肽还包括(d)羧基末端连接到免疫球蛋白恒定亚区的第二铰链区,和(e)羧基末端连接到第二铰链区的第二结合域(例如 Scorpion 多肽)。在一些实施方案中,CD3 结合多肽包含具有构架区、前铰链区和铰链区的 CD3 结合域。

[0107] 在一些实施方案中,CD3 结合多肽包含三个或更多个与 SEQ ID NO:42 相比经修饰的氨基酸,举例来说,所述修饰是用中性氨基酸取代带正电的氨基酸和/或用带负电的氨基酸取代中性氨基酸,例如在构架区和/或前铰链区中。在一些实施方案中,通过用中性氨基酸取代带正电的氨基酸和/或用带负电的氨基酸取代中性氨基酸在 VH 和/或 VL 的构架区中对至少 2 个、至少 3 个、至少 4 个、3-5 个或 3-10 个氨基酸进行修饰。

[0108] 在本发明的一些实施方案中,CD3 结合多肽在前铰链区中包含例如能降低前铰链区或整个多肽的 pI 的修饰。前铰链区处于结合域与铰链区之间的接合处。举例来说,可以

用序列 SSS 或 SST 置换具有序列 RRT 的三个氨基酸的前铰链区以降低等电点。在一些实施方案中,前铰链区与具有 RRT 前铰链区的 CD3 结合多肽相比具有降低的等电点。

[0109] 在一个实施方案中,为了降低免疫原性的风险,将人生殖细胞系 IgG 序列中普遍存在的或人生殖细胞系序列中相同或邻近的位置上,例如在对应的人生殖细胞系 IgG 序列中含有的氨基酸取代到序列中。在一些实施方案中,所述人生殖细胞系 IgG 序列包含 SEQ ID NO:43 或 44。

[0110] 在一些实施方案中,与 Cris-7VL J κ (Jk) 区小鼠序列 LQIT (SEQ ID NO:252) 相比,至少一个氨基酸修饰在 VL 区的 Jk 区内。在一些实施方案中,Jk 区包含氨基酸序列 VEIK (SEQ ID NO:253),例如代替 LQIT (SEQ ID NO:252)。

[0111] 在一些实施方案中,CD3 结合多肽包含铰链区和恒定区,举例来说,CD3 结合多肽可以包含 (a) 氨基末端连接到 CD3 结合域的铰链区,和 (b) 氨基末端连接到铰链区的免疫球蛋白恒定区 (例如,PIMS 多肽)。在一些实施方案中,CD3 结合多肽从氨基到羧基末端包含 CD3 结合域、铰链区和恒定区。在一些实施方案中,CD3 结合多肽从氨基到羧基末端包含恒定区、铰链区和 CD3 结合域。

[0112] 典型地,上述形式的 CD3 结合多肽 (SMIP、Scorpion 或 PIMS) 能够进行同源二聚,典型地通过二硫键,经由免疫球蛋白恒定区和 / 或铰链区 (例如,经由包含 IgG CH2 和 CH3 域的免疫球蛋白恒定区和 IgG 铰链区)。因而,在本发明的某些方面,两个同一的 CD3 结合多肽进行同源二聚,以形成二聚 CD3 结合蛋白。

[0113] 在其它实施方案中,CD3 结合多肽还包括能够与第二非同一多肽链中的不同的异源二聚域进行异源二聚的异源二聚域。在某些变化方案中,用于异源二聚的第二多肽链包括第二结合域。因此,在本发明的某些方面,两个非同一多肽链 (一个包含 CD3 结合域且第二个任选地包含第二结合域) 二聚以形成具有 1 到 4 个结合域的异源二聚蛋白质。在一些实施方案中,CD3 结合多肽从氨基到羧基末端包含 CD3 结合域、铰链区、恒定区、异源二聚域和任选地存在的第二结合域。

[0114] 在一些实施方案中,第二结合域结合靶分子或与其相互作用,且 CD3 结合多肽诱导 T 细胞细胞毒性。这可以呈非二聚体、异源二聚体或同源二聚体形式。在一些实施方案中,第二结合域结合肿瘤相关抗原或与其相互作用。在一些实施方案中,CD3 结合多肽在肿瘤附近诱导 T 细胞溶解肿瘤细胞和 / 或诱导多克隆 T 细胞活化和扩增。

[0115] 包含本发明的抗 CD3 结合域的多肽可以是或者包含抗体或包括有保留结合特异性的功能抗体片段或片段衍生物的抗体衍生物。在一些实施方案中,本发明包括含有可变重链域和 / 或轻链域的融合蛋白和其它多肽。

[0116] 在本发明的一些实施方案中,CD3 结合多肽包含了包含有选自 SEQ ID NO:28、30、32、38 和 40 的 VH 区序列或 VL 区序列的 CD3 结合域。在一些实施方案中,CD3 结合域包含选自 SEQ ID NO:22、24、26、28、30 和 32 的 VH 区和选自 SEQ ID NO:38 和 40 的 VL 区。在一些实施方案中,CD3 结合域包含选自 SEQ ID NO:28、30 和 32 的 VH 区和选自 SEQ ID NO:34、36、38 和 40 的 VL 区。在一些实施方案中,VH 区包含 SEQ ID NO:28 且 VL 区包含 SEQ ID NO:34;VH 区包含 SEQ ID NO:28 且 VL 区包含 SEQ ID NO:38;VH 区包含 SEQ ID NO:26 且 VL 区包含 SEQ ID NO:38;或 VH 区包含 SEQ ID NO:26 且 VL 区包含 SEQ ID NO:34。在一些实施方案中,CD3 结合多肽包含有包含重链 CDR1、CDR2 和 CDR3 以及轻链 CDR1、CDR2 和 CDR3,且

其中重链 CDR3 包含 SEQ ID NO:51 的 CD3 结合域。在一些实施方案中,CD3 结合多肽包括有包含 SEQ ID NO:49 的重链 CDR1、包含 SEQ ID NO:50 的 CDR2 和包含 SEQ ID NO:51 的 CDR3, 以及包含 SEQ ID NO:52 的轻链 CDR1、包含 SEQ ID NO:53 的 CDR2 和包含 SEQ ID NO:54 的 CDR3。

[0117] 在一些实施方案中,CD3 结合多肽包含选自 SEQ ID NO:6、8、10、12、14、16、18 和 20 的氨基酸序列。

[0118] 本发明包括有包含二聚的单链多肽的多特异性结合蛋白,各单链多肽从氨基到羧基末端包含第一结合域、N 末端连接子、免疫球蛋白恒定区、C 末端连接子和本发明的 CD3 结合域。同样,本发明包括有包含二聚的单链多肽的多特异性结合蛋白,各单链多肽从氨基到羧基末端包含本发明的 CD3 结合域、N 末端连接子、免疫球蛋白恒定区、C 末端连接子和第一结合域。在一些实施方案中,N 末端连接子可以包含免疫球蛋白铰链区或可能实质上由其组成。

[0119] 在本发明的另一个方面,多特异性结合蛋白包含单链多肽,所述单链多肽从氨基到羧基末端包含第一结合域、N 末端连接子、免疫球蛋白恒定区、C 末端连接子和 CD3 结合域。同样,本发明包括多特异性结合蛋白,其从氨基到羧基末端包含 CD3 结合域、N 末端连接子、免疫球蛋白恒定区、C 末端连接子和第一结合域。

[0120] 本发明包括多特异性结合蛋白,所述多特异性结合蛋白包含经由连接子域与第二结合域连接的第一结合域(例如,经由连接子与另一个 scFv 连接的 scFv)。举例来说,本发明包括多特异性结合蛋白,其包含经由肽连接子域与 CD3 结合域(呈 V_H -连接子- V_L 或 V_L -连接子- V_H 取向)连接的第一结合域(呈 V_H -连接子- V_L 或 V_L -连接子- V_H 取向)。在一些实施方案中,呈 scFv-连接子-scFv 形式的双特异性蛋白质可以包含源自于针对 T 细胞抗原(诸如 CD3)的抗体的可变重域和可变轻域,包括但不限于本文中所公开的可变域。隔开 scFv 域的连接子可以包含 $((Gly_4)Ser)_n$, 其中 $n = 1-5$ (SEQ ID NO:74-78)。在一个实施方案中,连接子是 $((Gly_4)Ser)_3$ (SEQ ID NO:76)。连接子还可以包含约 8-12 个氨基酸。在一些实施方案中,呈这种形式(“双特异性单链抗体”)的本发明蛋白质不包含 Fc 区,且因此不具有 Fc 相关的效应功能。

[0121] 本发明实质上包括任何类型的含有如本文中所描述的结合域的多肽。这包括抗体、其片段、scFv、Fab、双 scFv 单域抗体、双链抗体、双可变域结合蛋白和含有抗体或抗体片段的多肽。本文中描述了本发明中所包括的其它类型多肽。

[0122] 在一些实施方案中, V_H 和 V_L 域被用作 scFv,且以允许 scFv 和 V_H/V_L 结合靶标的方式与其它多肽或氨基酸序列连接/融合。在一些实施方案中,如本文中所描述的 V_H 和 V_L 域用于抗体或其片段中。举例来说,将所述 V_H 和 V_L 域并入到 V_H 和 V_L 域在抗体中的天然位置。在一些实施方案中,本发明的 CD3 结合多肽是抗体,例如具有如本文中所描述的 V_L 链和 V_H 链。

[0123] 在本发明的一个实施方案中,多特异性蛋白质是 scFv 二聚体或双链抗体,而不是完整抗体。双链抗体和 scFv 二聚体可以仅使用可变域构建而不具有 Fc 区。双链抗体是二价双特异性抗体,其中 V_H 和 V_L 域被表达在单一多肽链上,但所使用的肽连接子过短而无法允许同一链上的两个结构域配对,从而迫使所述结构域与另一个链的互补域配对且产生两个抗原结合位点(参见例如 Holliger, P. 等 (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448 ;

Poljak, R. J. 等 (1994) Structure 2:1121-1123)。所述抗体结合部分在本领域中是已知的 (Kontermann 和 Dubel 编, Antibody Engineering (2001) Springer-Verlag, New York, 790pp. (ISBN 3-540-41354-5))。

[0124] 在本发明的一个实施方案中,多特异性蛋白质是经二硫化物稳定的双链抗体。举例来说,多特异性抗体可以包含两个独特的多肽,它们共表达以产生对 2 个特异性中的每一个都具有一个结合位点的共价连接的异源二聚复合物。在这个实施方案中,各 Fv 是通过呈 $V_{LA}-V_{HB}$ (第一链) 和 $V_{LB}-V_{HA}$ (第二链) 构型的一个链上的 V_L 伴侣与第二个链上的 V_H 伴侣缔合而形成。这可以通过两个代替羧基末端异源二聚域中的任一个加以稳定:一个链上的 VEPKSC (SEQ ID NO:254) 与另一个链上的 FNRGEC (SEQ ID NO:255) 配对,或带相反电荷的卷曲螺旋域的配对。参见例如 Moore 等, 2011, Blood. 117:4542-4551。在这个实施方案中,多特异性结合蛋白可以包含具有与第一结合域 V_L 连接的 CD3 结合域 V_H 的第一链,且第二链包含与第一结合域 V_H 连接的 CD3 结合域 V_L ,并且两个链经由二硫键在 C 末端连接。可以使用源自于已知抗体的可变重链和轻链,包括例如本文中所公开的可变重链和轻链来设计经二硫化物稳定的双链抗体。

[0125] 在另一个实施方案中,多特异性结合蛋白是能够特异性结合第一结合位点 (例如肿瘤抗原) 和 TCR 复合物的双可变域结合蛋白。在这个实施方案中,重组蛋白包含多肽链,其中所述多肽链包含 $VD1-(X1)_n-VD2-C-(X2)_n$, 其中 $VD1$ 是第一可变域, $VD2$ 是第二可变域, C 是恒定域, $X1$ 是连接子 (例如,约 10 到 20 个氨基酸长的多肽连接子), $X2$ 代表 Fc 区且 n 是 0 或 1。参见例如美国专利号 8,258,268。

[0126] 在本发明的一个实施方案中,多特异性结合蛋白包含一个、两个、三个或更多个多肽链。举例来说,本发明包括多特异性蛋白质,其具有包含 $V_{H1}-V_{L2}$ 的第一链,包含 $CH2-CH3-V_{L1}-V_{H2}$ 的第二链和包含 $CH2-CH3$ 的第三链。在这个实施方案中, V_{H1} 和 V_{L1} 可以对应于第一结合域的可变域,而 V_{H2} 和 V_{L2} 可以对应于抗 CD3 可变域。或者, V_{H1} 和 V_{L1} 可以对应于抗 CD3 可变域,而 V_{H2} 和 V_{L2} 可以对应于第一结合域的可变域。

[0127] 在其它实施方案中,多特异性结合蛋白由包含不同的单链多肽的二聚单链多肽链 (异源二聚体) 构成。在这种形式 (“多特异性异源二聚体”) 中,各多肽链包含结合域、N 末端连接子、恒定区、C 末端连接子和任选地存在的另一个结合域且还包括异源二聚域。在某些变化方案中,用于异源二聚的第二多肽链包括其它结合域。因此,在本发明的某些实施方案中,异源二聚重组蛋白可以含有两个、三个或四个不同的结合域。关于一些例子和相关方法,参见 PCT 公布号 W02011/090762。

[0128] 如上文所指出,本公开的 CD3 结合多肽包含特异性结合 CD3 的结合域。在一些变化方案中,CD3 结合域能够竞争结合氨基酸序列如 SEQ ID NO:41 中所示的具有单链 Fv (scFv) 的 CD3。在特定的实施方案中,CD3 结合域包含 (i) 包含 CDR HCDR1、HCDR2 和 HCDR3 的免疫球蛋白重链可变区 (V_H), 和 (ii) 包含 CDR LCDR1、LCDR2 和 LCDR3 的免疫球蛋白轻链可变区 (V_L)。合适的 CD3 结合域包括具有源自于鼠类单克隆抗体 Cris-7 的 V_L 和 V_H 区的 CD3 结合域 (Reinherz, E. L. 等 (编), Leukocyte typing II., Springer Verlag, New York, (1986))。在一些这样的实施方案中, LCDR3 具有 SEQ ID NO:54 中所述的氨基酸序列和 / 或 HCDR3 具有 SEQ ID NO:51 中所述的氨基酸序列;且 LCDR1 和 LCDR2 分别任选地具有如 SEQ ID NO:52 和 SEQ ID NO:53 中所述的氨基酸序列,且 HCDR1 和 HCDR2 分别任选地具有如 SEQ ID NO:49 和

SEQ ID NO:50 中所述的氨基酸序列。在一些实施方案中,举例来说,LCDR1、LCDR2 和 LCDR3 具有分别如 SEQ ID NO:52、53 和 54 中所示的氨基酸序列;和/或 HCDR1、HCDR2 和 HCDR3 具有分别如 SEQ ID NO:49、50 和 51 中所示的氨基酸序列。

[0129] 可以得到本公开的结合域的示例性抗 CD3 抗体包括 Cris-7 单克隆抗体 (Reinherz, E. L. 等 (编), Leukocyte typing II., Springer Verlag, New York, (1986)

[0130] ($V_L =$ QVVLTSQSPAIMSAFPGEKVTMTCSASSSVSYMNWYQQKSGTSPKRWIYDSSKLASGVPARFSGSGSGTSYSLTISSMETEDAATYYCQQWSRPPTFGGGTKLQITR (SEQ ID NO:46) 且

[0131] $V_H =$ QVQLQQSGAELARPGASVKMSCKASGYTFTRSTMHWVKQRPGQGLEWIGYINPSSAYTNYNQKFKDKATLTADKSSSTAYMQLSSLTSEDSAVYYCASPQVHYDYNQFPYWGQGTLLTVSA (SEQ ID NO:45); HuM291 (Chau 等 (2001) Transplantation 71:941-950 ($V_L =$ DIQMTQSPSSLSASVGDRTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKV EIK (SEQ ID NO:72) 且

[0132] $V_H =$ QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCARSAYDYDGFAYWGQGTLLTVSS (SEQ ID NO:73); BC3 单克隆抗体 (Anasetti 等 (1990) J. Exp. Med. 172:1691); OKT3 单克隆抗体 (Ortho muliicener Transplant Study Group (1985) N. Engl. J. Med. 313:337) 及其衍生物, 诸如 OKT3ala-ala (也称为 OKT3AA-FL 或 OKT3FL), 即在位置 234 和 235 上具有丙氨酸取代的人源化 Fc 变体 (Herold 等 (2003) J. Clin. Invest. 11:409); 维西珠单抗 (Carpenter 等 (2002) Blood 99:2712); G19-4 单克隆抗体 (Ledbetter 等, 1986, J. Immunol. 136:3945) 和 145-2C11 单克隆抗体 (Hirsch 等 (1988) J. Immunol. 140:3766)。示例性抗 TCR 抗体是 BMA031 单克隆抗体 (Borst 等 (1990) Human Immunology 29:175-188)。

[0133] 在一些实施方案中,结合域是包含相关靶特异性 V_H 和 V_L 区的单链 F_v 片段 (scFv)。在某些实施方案中, V_H 和 V_L 区是人的。

[0134] 在本发明的一个实施方案中, CD3 结合多肽的经验和/或理论 pI 比 DRA209SMIP 的小至少 0.25、0.5、0.75、1、1.25、1.5、2、2.5 或更多个单位。在一些实施方案中,本发明的 CD3 结合多肽的经验和/或理论 pI 比 DRA209SMIP 的小约 0.25 到约 2.5 个、0.5 到约 2.5 个、约 0.5 到约 2.0 个、约 0.5 到约 1.5 个、约 0.5 到约 1.25 个、约 0.5 到约 1.0 个、约 0.5 到约 0.75 个、约 0.75 到约 1.0 个、约 0.75 到约 1.25 个、约 0.75 到约 1.5 个、约 0.75 到约 2.0 个、约 1.0 到约 1.5 个、约 1.0 到约 2.0 个或约 1.0 到约 2.5 个单位。在一些实施方案中,本发明的 CD3 多肽的经验或理论 pI 小于约 8.9、8.8、8.7、8.6、8.5、8.4、8.3、8.2、8.1、8.75、7.25、7.0 或 6.75。在一些实施方案中,本发明的 CD3 多肽的经验或理论 pI 为约 8.9、8.8、8.7、8.6、8.5、8.4、8.3、8.2、8.1、8.75、7.25、7.27、0.6、8 或 6.75。在一些实施方案中,本发明的 CD3 多肽的经验或理论 pI 为约 7.9 到约 8.7、7.9 到约 8.6、8.0 到约 8.6、8.0 到约 8.5、8.0 到约 8.4、8.0 到约 8.3、8.0 到约 8.2、8.0 到约 8.1、约 8.1 到约 8.6、约 8.2 到约 8.6、约 8.3 到约 8.6、约 8.4 到约 8.6、约 8.5 到约 8.6、约 8.5 到约 8.3、约 8.4 到约 8.2 或约 8.3 到约 8.1。

[0135] 在某些实施方案中, CD3 结合域包含或是与 SEQ ID NO:41 的 scFv 相比 pI (经验和/或理论) 降低至少约 0.25、0.5、1、1.25、1.5、2、2.5 或更多个单位的 scFv。在一些实施方案中, CD3 结合域包含或是 pI (经验和/或理论) 比 SEQ ID NO:41 的降低约 0.25 到约 2.5、

0.5 到约 2.5、约 0.5 到约 2.0、约 0.5 到约 1.5、约 0.5 到约 1.25、约 0.5 到约 1.0、约 0.5 到约 0.75、约 0.75 到约 1.0、约 0.75 到约 1.25、约 0.75 到约 1.5、约 0.75 到约 2.0、约 1.0 到约 1.5、约 1.0 到约 2.0 或约 1.0 到约 2.5 个单位的 scFv。这样的 scFv 包括 SEQ ID NO:6、8、10、12、14、16、18 和 20 内所含的 scFv,或与 SEQ ID NO:6、8、10、12、14、16、18 和 20 内所含的 scFv 的氨基酸序列具有至少约 90%、至少约 91%、至少约 92%、至少约 93%、至少约 94%、至少约 95%、至少约 96%、至少约 97%、至少约 98%、至少约 99%、至少约 99.5% 或 100% 同一性的 scFv。

[0136] 在一些实施方案中,本发明的 CD3 结合域与 SEQ ID NO:41 具有约 80%到约 99%、约 82%到约 99%、约 84%到约 99%、约 86%到约 99%、约 88%到约 99%、约 90%到约 99%、约 92%到约 99%、约 94%到约 99%、约 96%到约 99%、约 97%到约 99%、约 98%到约 99%、约 80%到约 85%、约 85%到约 90%、约 85%到约 95%、约 90%到约 97%、约 90%到约 95%、约 90%到约 93%、约 90%到约 91%、约 95%到约 96%、约 96%到约 97%、约 97%到约 98%、约 96%到约 97%或约 96%到约 98% 的同一性。

[0137] 在一些实施方案中,本发明的 CD3 结合域是 SEQ ID NO:41 的变体,其中已经使氨基酸序列中的一个或多个氨基酸突变以降低结合域的 pI。突变包括用一个氨基酸取代另一个氨基酸以降低结合域的 pI、插入氨基酸以降低结合分子的 pI 和 / 或缺失氨基酸以降低结合域的 pI。在一个实施方案中,突变是取代。在一些实施方案中,突变在可变链的构架区中。在一些实施方案中,变体中有 1、2、3、4、5、6、7、8、9、10 或更多个氨基酸突变。

[0138] 在一个实施方案中,CD3 结合域包含或是包含选自 SEQ ID NO:28、30、32、38 和 40 的重链或轻链的 scFv。在另一个实施方案中,CD3 结合域包含选自 SEQ ID NO:22、24、26、28、30 和 32 的 VH 区和选自 SEQ ID NO:38 和 40 的 VL 区。在另一个实施方案中,CD3 结合域包含选自 SEQ ID NO:28、30 和 32 的 VH 区和选自 SEQ ID NO:34、36、38 和 40 的 VL 区。在另一个实施方案中,CD3 结合域包含 SEQ ID NO:28 和 34。在另一个实施方案中,CD3 结合域包含 SEQ ID NO:28 和 38。在另一个实施方案中,CD3 结合域包含 SEQ ID NO:26 和 38。在另一个实施方案中,CD3 结合域包含 SEQ ID NO:26 和 34。在一些实施方案中,CD3 结合域包含选自 SEQ ID NO:38 和 40 的 VL 区和选自 SEQ ID NO:22、24、26、28、30 和 32 的 VH 区。在其它实施方案中,与来自于特异性结合相关靶标的单克隆抗体或其片段或衍生物(例如 CD3)的 CDR 相比,各 CDR 包含不超过一个、两个或三个取代、插入或缺失。

[0139] 在一些实施方案中,CD3 结合域结合 T 细胞上的 T 细胞受体复合物的 CD3 ϵ 亚单位或与其相互作用。在一些实施方案中,CD3 结合域与 Cris-7 或 HuM291 单克隆抗体竞争结合 CD3 ϵ 。

[0140] 在一些实施方案中,CD3 结合多肽诱导 T 细胞受体复合物内在化。

[0141] 在一些变化方案中,结合域是包含由肽连接子接合的免疫球蛋白 V_L区和 V_H区的单链 Fv(scFv)。用于接合 V_L区和 V_H区的肽连接子的使用在本领域中是众所周知的,且这一特定领域内存在许多公布。一种广泛使用的肽连接子是由 3 个重复的 Gly-Gly-Gly-Gly-Ser(SEQ ID NO:74) 氨基酸序列 (Gly₄Ser)₃(EQ ID NO:76) 组成的 15 聚体。在一些实施方案中,肽连接子包含 SEQ ID NO:74-78 中的任一个或由其组成。已经使用了其它连接子,且已经使用噬菌体展示技术以及选择性感染性噬菌体技术使连接子序列多样化和选择适当的连接子序列(Tang 等, J. Biol. Chem. 271, 15682-15686, 1996 ;Hennecke

等, Protein Eng. 11, 405-410, 1998)。在某些实施方案中, V_H 区和 V_L 区是通过具有包含式 $(Gly_4Ser)_n$ 的氨基酸序列的肽连接子相连, 其中 $n = 1-5$ (分别是 SEQ ID NO: 74-78)。可以通过随机突变发生优化简单连接子 (例如 $(Gly_4Ser)_n$) 来获得其它合适的连接子。

[0142] 在特定的实施方案中, 结合域包含人源化免疫球蛋白 V_L 和 / 或 V_H 区。用于使免疫球蛋白 V_L 区和 V_H 区人源化的技术在本领域中是已知的, 且论述于例如美国专利申请公布号 2006/0153837 中。

[0143] 期望“人源化”产生免疫原性较弱、完全保留原始分子的抗原结合性质的抗体。为了保留原始抗体的所有抗原结合性质, 将再产生其抗原结合位点结构的“人源化”型式。这可以通过在保留或不保留重要构架残基的情况下仅将非人 CDR 移植到人可变构架域和恒定区上 (Jones 等, Nature 321:522(1986); Verhoeyen 等, Science 239:1539(1988)) 或通过重组整个非人可变域 (以保留配体结合性质) 但通过审慎地置换暴露的残基用人样表面将其“掩蔽” (以降低抗原性) (Padlan, Molec. Immunol. 28:489(1991)) 来实现。

[0144] 实质上, 通过 CDR 移植进行人源化涉及仅将非人抗体的 CDR 重组到人可变区构架和人恒定区上。理论上, 这将显著降低或消除免疫原性 (除非存在同种异型或个体基因型差异)。然而, 已经报告也可能需要保留原始抗体的一些构架残基 (Reichmann 等, Nature, 332:323(1988); Queen 等, Proc. Natl. Acad. Sci. USA, 86:10, 029(1989))。

[0145] 需要保留的构架残基适于通过计算机模拟来鉴别。或者, 可以通过比较已知的抗原结合位点结构来潜在地鉴别重要的构架残基 (Padlan, Molec. Immunol., 31(3):169-217(1994), 以引用的方式并入本文中)。

[0146] 潜在地影响抗原结合的残基分为若干组。第一组包含具有抗原位点表面的连续残基, 它们因此可以与抗原直接接触。这些残基包括氨基末端残基和与 CDR 相邻的残基。第二组包括可能通过接触抗体中的 CDR 或另一个肽链而改变 CDR 的结构或相对排列的残基。第三组包含具有可能影响可变域的结构完整性的隐藏侧链的氨基酸。这些组中的残基通常是在相同的位置上发现的 (Padlan, 1994, 同上), 但取决于编号系统, 它们在鉴别时的位置可能不同 (参见 Kabat 等, “Sequences of proteins of immunological interest, 第 5 版, 发行号 91-3242, U. S. Dept. Health&Human Services, NIH, Bethesda, Md., 1991)。

[0147] 尽管本文中描述的一些例子涉及 scFv、SMIP、Scorpion 和 Interceptor 分子而不是抗体的人源化, 但本领域中关于人源化抗体的知识适用于根据本发明的多肽。

[0148] 在某些实施方案中, 铰链是野生型人免疫球蛋白铰链区。在某些其它实施方案中, 可以在野生型免疫球蛋白铰链区的氨基或羧基末端上添加一个或多个氨基酸残基作为融合蛋白构建体设计的一部分。举例来说, 铰链氨基末端的其它接合氨基酸残基可以是“RT”、“RSS”、“TG”或“T”, 或在铰链羧基末端可以是“SG”, 或可将铰链缺失与添加组合, 诸如在羧基末端添加具有“SG”的 ΔP 。

[0149] 在某些实施方案中, 铰链是改变的免疫球蛋白铰链, 其中野生型免疫球蛋白铰链区中的一个或多个半胱氨酸残基经一个或多个其它氨基酸残基 (例如丝氨酸或丙氨酸) 取代。

[0150] 示例性改变的免疫球蛋白铰链包括但不限于存在野生型人 IgG1 铰链中发现的一个、两个或三个半胱氨酸残基被一个、两个或三个不同的氨基酸残基 (例如丝氨酸或丙氨酸) 取代的免疫球蛋白人 IgG1 铰链区。改变的免疫球蛋白铰链可能另外存在脯氨酸被另

一个氨基酸（例如丝氨酸或丙氨酸）取代。举例来说，上述改变的人 IgG1 铰链可以另外存在位于羧基末端的连接到野生型人 IgG1 铰链区的三个半胱氨酸的脯氨酸被另一个氨基酸残基（例如丝氨酸、丙氨酸）取代。在一个实施方案中，核心铰链区的脯氨酸未经取代。

[0151] 在某些实施方案中，铰链多肽包含或者是与诸如野生型人 IgG1 铰链、野生型人 IgG2 铰链或野生型人 IgG4 铰链等野生型免疫球蛋白铰链区具有至少 80%、至少 81%、至少 82%、至少 83%、至少 84%、至少 85%、至少 86%、至少 87%、至少 88%、至少 89%、至少 90%、至少 91%、至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98% 或至少 99% 同一性的序列。

[0152] 在其它实施方案中，CD3 结合多肽中存在的铰链可以是并非基于或源自于免疫球蛋白铰链的铰链（即，非野生型免疫球蛋白铰链或改变的免疫球蛋白铰链）。这样的铰链的例子包括源自于跨膜蛋白的域间区或 II 型 C-凝集素的茎部区的具有约 5 到约 150 个氨基酸的肽，例如具有约 8 到 25 个氨基酸的肽和具有约 7 到 18 个氨基酸的肽。

[0153] 在某些实施方案中，域间区或茎部区铰链具有 7 到 18 个氨基酸，且可以形成 α -螺旋卷曲螺旋结构。在某些实施方案中，域间区或茎部区铰链含有 0、1、2、3 或 4 个半胱氨酸。示例性域间区或茎部区铰链是域间区或茎部区的肽片段，诸如来自于 CD69、CD72、CD94、NKG2A 和 NKG2D 的茎部区的 10 到 150 个氨基酸的片段。

[0154] 在某些实施方案中，铰链序列具有约 5 到 150 个氨基酸、5 到 10 个氨基酸、10 到 20 个氨基酸、20 到 30 个氨基酸、30 到 40 个氨基酸、40 到 50 个氨基酸、50 到 60 个氨基酸、5 到 60 个氨基酸、5 到 40 个氨基酸、8 到 20 个氨基酸或 10 到 15 个氨基酸。铰链可以主要地是挠性的，但也可以提供较多刚性特征，或可以主要含有 α -螺旋结构与极少 β -片状结构。铰链的长度或序列可能影响铰链直接或间接（经由另一个区或域，诸如异源二聚域）连接的结合域的结合亲和力以及铰链直接或间接连接的 Fc 区部分的一种或多种活性。

[0155] 在某些实施方案中，铰链序列在血浆和血清中是稳定的且能抵抗蛋白水解裂解。可以使 IgG1 上铰链区中的第一赖氨酸突变以将蛋白水解裂解减到最少，举例来说，赖氨酸可以经甲硫氨酸、苏氨酸、丙氨酸或甘氨酸取代或缺失。

[0156] 在本发明的一些实施方案中，CD3 结合多肽能够与第二多肽链形成异源二聚体且包含如下铰链区：(a) 氨基末端紧邻免疫球蛋白恒定区（例如，氨基末端连接到 CH2 域，其中免疫球蛋白恒定区包括 CH2 和 CH3 域，或氨基末端连接到 CH3 域，其中免疫球蛋白区包括 CH3 和 CH4 域）；(b) 插入在结合域（例如 scFv）与免疫球蛋白异源二聚域之间且连接结合域与免疫球蛋白异源二聚域；(c) 插入在免疫球蛋白异源二聚域与免疫球蛋白恒定区（例如，其中免疫球蛋白恒定区包括 CH2 和 CH3 域或 CH3 和 CH4 域）之间且连接免疫球蛋白异源二聚域与免疫球蛋白恒定区；(d) 插入在免疫球蛋白恒定区与结合域之间且连接免疫球蛋白恒定区与结合域；(e) 在多肽链的氨基末端；或 (f) 在多肽链的羧基末端。包含如本文中所描述的铰链区的多肽链能够与不同的多肽链缔合以形成本文中所提供的同源二聚或异源二聚蛋白，且所形成的二聚体将含有保留了其靶特异性和 / 或其特异性靶结合亲和力的结合域。

[0157] 在某些实施方案中，能与另一个多肽链形成异源二聚体的多肽中存在的铰链可以是免疫球蛋白铰链，诸如野生型免疫球蛋白铰链区或其改变的免疫球蛋白铰链区。在某些实施方案中，异源二聚蛋白的一个多肽链的铰链与所述异源二聚体的另一个多肽链的对应

铰链同一。在某些其它实施方案中,一个链的铰链不同于另一个链的铰链(例如,在其长度和/或序列方面)。不同的链中的不同的铰链允许对铰链所连接的结合域的结合亲和力进行不同的控制,使得异源二聚体能够结合一个结合域的靶标而略过另一个结合域的靶标。举例来说,在某些实施方案中,异源二聚蛋白在一个链中具有 CD3 结合域或 TCR 结合域,且在另一个链中具有第二结合域(诸如肿瘤抗原结合域)。在两个链中具有两个不同的铰链可以允许异源二聚体首先结合肿瘤抗原,然后结合 CD3 或其它 TCR 组分。因而,异源二聚体可以将 CD3⁺T 细胞募集到表达肿瘤抗原的细胞(例如,表达 PSMA 的肿瘤细胞),这又可以破坏或毁坏表达肿瘤抗原的细胞。

[0158] 适合根据本发明使用的示例性铰链区示于以下表 1 和 2 中。

[0159] 表 1:示例性铰链区

[0160]

铰链区	氨基酸序列
sss(s)-hlgG1 铰链	EPKSSDKTHTSPPSS (SEQ ID NO:112)
csc(s)-hlgG1 铰链	EPKSCDKTHTSPPCS (SEQ ID NO:113)
ssc(s)-hlgG1 铰链	EPKSSDKTHTSPPCS (SEQ ID NO:114)
scc(s)-hlgG1 铰链	EPKSSDKTHTCPPCS (SEQ ID NO:115)
css(s)-hlgG1 铰链	EPKSCDKTHTSPPSS (SEQ ID NO:116)
scs(s)-hlgG1 铰链	EPKSSDKTHTCPPSS (SEQ ID NO:117)
ccc(s)-hlgG1 铰链	EPKSCDKTHTSPPCS (SEQ ID NO:118)
ccc(p)-hlgG1 铰链	EPKSCDKTHTSPPCP (SEQ ID NO:119)
sss(p)-hlgG1 铰链	EPKSSDKTHTSPPSP (SEQ ID NO:120)
csc(p)-hlgG1 铰链	EPKSCDKTHTSPPCP (SEQ ID NO:121)
ssc(p)-hlgG1 铰链	EPKSSDKTHTSPPCP (SEQ ID NO:122)
scc(p)-hlgG1 铰链	EPKSSDKTHTCPPCP (SEQ ID NO:123)
css(p)-hlgG1 铰链	EPKSCDKTHTSPPSP (SEQ ID NO:124)
scs(p)-hlgG1 铰链	EPKSSDKTHTCPPSP (SEQ ID NO:125)
Scppcp	SCPPCP (SEQ ID NO:126)
STD1	NYGGGGSGGGGSGGGGSGNS (SEQ ID NO:127)
STD2	NYGGGGSGGGGSGGGGSGNYGGGGSGGGGSGGGGSGNS (SEQ ID NO:128)
H1	NS (SEQ ID NO:129)
H2	GGGGSGNS (SEQ ID NO:130)
H3	NYGGGGSGNS (SEQ ID NO:131)
H4	GGGGSGGGGSGNS (SEQ ID NO:132)
H5	NYGGGGSGGGGSGNS (SEQ ID NO:133)
H6	GGGGSGGGGSGGGGSGNS (SEQ ID NO:134)
H7	GCPPCPNS (SEQ ID NO:135)

[0161]

H9	GSPPSPNS (SEQ ID NO:136)
(G4S)3	GGGGSGGGGSGGGGS (SEQ ID NO:137)
H105	SGGGGSGGGGSGGGGS (SEQ ID NO:138)
(G4S)4	GGGGSGGGGSGGGGSGGGGS (SEQ ID NO:139)
H75 (NKG2A四倍突变体)	QRHNNSSLNTGTQMAGHSPNS (SEQ ID NO:140)
H83 (NKG2A衍生的)	SSLNTGTQMAGHSPNS (SEQ ID NO:141)
H106 (NKG2A衍生的)	QRHNNSSLNTGTQMAGHS (SEQ ID NO:142)
H81 (NKG2D衍生的)	EVQIPLTESYSPNS (SEQ ID NO:143)
H91 (NKG2D衍生的)	NSLANQEVQIPLTESYSPNS (SEQ ID NO:144)
H94	SGGGGSGGGGSGGGGSPNS (SEQ ID NO:145)

[0162] 表 2 : 示例性铰链区 (得自于 H7 铰链, II 型 C- 凝集素的茎部区, 或 I 型跨膜蛋白的域间区)

[0163]

铰链区	氨基酸序列	由其得到的 分子和/或铰链
H16	LSVKADFLTPSIGNS (SEQ ID NO:146)	CD80
H17	LSVKADFLTPSISCPNPCNS (SEQ ID NO:147)	CD80 + H7
H18	LSVLANSQPEIGNS (SEQ ID NO:148)	CD86
H19	LSVLANSQPEISCPNPCNS (SEQ ID NO:149)	CD86 + H7
H20	LKIQERVSKPKISNS (SEQ ID NO:150)	CD2
H21	LKIQERVSKPKISCPNPCNS (SEQ ID NO:151)	CD2 + H7
H22	LNVSERPFPPHIQNS (SEQ ID NO:152)	CD22
H23	LDVSERPFPPHIQSCPPNPCNS (SEQ ID NO:153)	CD22 + H7
H24	REQLAEVTLCLKANS (SEQ ID NO:154)	CD80
H25	REQLAEVTLCLKACPPNPCNS (SEQ ID NO:155)	CD80 + H7
H26	RIHQMNSELSVLANS (SEQ ID NO:156)	CD86
H27	RIHQMNSELSVLACPPNPCNS (SEQ ID NO:157)	CD86 + H7
H28	DTKGKNVLEKIFSNS (SEQ ID NO:158)	CD2
H30	LPPETQESQEVTLNS (SEQ ID NO:159)	CD22
H32	RIHLNVSERPFPPNS (SEQ ID NO:160)	CD22
H33	RIHLNVSERPFPPCPNPCNS (SEQ ID NO:161)	CD22 + H7
H36	GCPPCPGGGGSNS (SEQ ID NO:162)	H7
H40	GCPPCPANS (SEQ ID NO:163)	H7
H41	GCPPCPANS (SEQ ID NO:164)	H7
H42	GCPPCPNS (SEQ ID NO:165)	H7
H44	GGGASCPPCPGNS (SEQ ID NO:166)	H7
H45	GGGASCPPCAGNS (SEQ ID NO:167)	H7
H46	GGGASCPPCANS (SEQ ID NO:168)	H7
H47	LSVKADFLTPSIGNS (SEQ ID NO:169)	CD80
H48	ADFLTPSIGNS (SEQ ID NO:170)	CD80

[0164]

H50	LSVLNFSQPEIGNS (SEQ ID NO:171)	CD86
H51	LSVLNFSQPEIGNS (SEQ ID NO:172)	CD86
H52	SQPEIVPISNS (SEQ ID NO:173)	CD86
H53	SQPEIVPISCPPCPNS (SEQ ID NO:174)	CD86 + H7
H54	SVLANFSQPEISCPPCPNS (SEQ ID NO:175)	CD86 + H7
H55	RIHQMNSELSVLANS (SEQ ID NO:176)	CD86
H56	QMNSELSVLANS (SEQ ID NO:177)	CD86
H57	VSERPFPPNS (SEQ ID NO:178)	CD22
H58	KPFFTCGSADTCPNS (SEQ ID NO:179)	CD72
H59	KPFFTCGSADTCPNS (SEQ ID NO:180)	CD72
H60	QYNCPGQYTF SMPNS (SEQ ID NO:181)	CD69
H61	EPAFTPGPNIELQKSDCPNS (SEQ ID NO:182)	CD94
H62	QRHNNSSLNTRTQKARHCPNS (SEQ ID NO:183)	NKG2A
H63	NSLFNQEVQIPLTESYCPNS (SEQ ID NO:184)	NKG2D

[0165] 在某些实施方案中,本发明的 CD3 结合多肽或蛋白质可以包含“免疫球蛋白二聚域”或“免疫球蛋白异源二聚域”。

[0166] 如本文中所使用的“免疫球蛋白二聚域”或“免疫球蛋白异源二聚域”是指与第二多肽链的第二免疫球蛋白域相互作用或缔合的多肽链的免疫球蛋白域,其中第一和第二免疫球蛋白异源二聚域的相互作用基本上有助于或能有效促进第一和第二多肽链的异源二聚(即,在两个不同的多肽链之间形成二聚体,这也称为“异源二聚体”或“异源二聚蛋白”)。如果在不存在第一多肽链的免疫球蛋白异源二聚域和/或第二多肽链的免疫球蛋白异源二聚域的情况下,第一和第二多肽链的二聚存在统计上显著的减少,则免疫球蛋白异源二聚域之间的相互作用“基本上有助于或能有效促进”第一和第二多肽链的异源二聚。在某些实施方案中,当第一和第二多肽链共表达时,至少 60%、至少约 60%到约 70%、至少约 70%到约 80%、至少 80%到约 90%、91%、92%、93%、94%、95%、96%、97%、98%或 99% 的第一和第二多肽链与彼此形成异源二聚体。代表性免疫球蛋白异源二聚域包括免疫球蛋白 CH1 域、免疫球蛋白 CL 域(例如,C κ 或 C λ 同种型)或其衍生物,包括野生型免疫球蛋白 CH1 和 CL 域和改变(或突变)的免疫球蛋白 CH1 和 CL 域,诸如本文中所提供的。

[0167] 在需要由两个非同源的多肽链形成异源二聚体的情况下可以使用二聚/异源二聚域,其中一个或两个多肽链包含结合域。在某些实施方案中,本文中所描述的某些异源二聚体的一个多肽链成员不含结合域。如上文所指出,本公开的异源二聚蛋白在各多肽链中包含免疫球蛋白异源二聚域。异源二聚体的多肽链中的免疫球蛋白异源二聚域彼此不同,且因而可以差异性地修饰以促进两个链的异源二聚和将任一链的同源二聚减到最少。如 PCT 公布号 W02011/090762 中所示,本文中所提供的免疫球蛋白异源二聚域允许不同的多肽之间发生有效的异源二聚且促进所得异源二聚蛋白的纯化。

[0168] 如本文中所提供,可用于促进根据本公开的两个不同的单链多肽(例如,一个短一个长)发生异源二聚的免疫球蛋白异源二聚域包括免疫球蛋白 CH1 和 CL 域,例如人 CH1

和 CL 域。在某些实施方案中,免疫球蛋白异源二聚域是野生型 CH1 域,诸如野生型 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE 或 IgM CH1 域。在其它实施方案中,免疫球蛋白异源二聚域是分别如 PCT 公布 No. W02011/090762 的 SEQ ID NO:114、186-192 和 194(所述序列以引用的方式并入本文中)中所述的野生型人 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE 或 IgM CH1 域。在某些实施方案中,免疫球蛋白异源二聚域是如 W02011/090762 的 SEQ ID NO:114(所述序列以引用的方式并入本文中)中所述的野生型人 IgG1CH1 域,所述序列与本申请的 SEQ ID NO:80 相同。

[0169] 在其它实施方案中,免疫球蛋白异源二聚域是改变的免疫球蛋白 CH1 域,诸如改变的 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE 或 IgM CH1 域。在某些实施方案中,免疫球蛋白异源二聚域是改变的人 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE 或 IgM CH1 域。在其它实施方案中,在改变的免疫球蛋白 CH1 域中,与野生型免疫球蛋白 CL 域(例如人 CL)一起参与形成二硫键的野生型 CH1 域(例如人 CH1)的半胱氨酸残基缺失或被取代,使得改变的 CH1 域与野生型 CL 域之间不形成二硫键。

[0170] 在某些实施方案中,免疫球蛋白异源二聚域是野生型 CL 域,诸如野生型 C κ 域或野生型 C λ 域。在特定的实施方案中,免疫球蛋白异源二聚域是分别如 W02011/090762 的 SEQ ID NO:112 和 113(所述序列以引用的方式并入本文中)中所述的野生型人 C κ 或人 C λ 域,所述序列分别与本申请的 SEQ ID NO:81 和 82 相同。在其它实施方案中,免疫球蛋白异源二聚域是改变的免疫球蛋白 CL 域,诸如改变的 C κ 或 C λ 域,例如,改变的人 C κ 或人 C λ 域。

[0171] 在某些实施方案中,在改变的免疫球蛋白 CL 域中,与野生型免疫球蛋白 CH1 域(例如人 CH1)一起参与形成二硫键的野生型 CL 域(例如人 CL)的半胱氨酸残基缺失或被取代。所述改变的 CL 域还可以在其氨基末端包含氨基酸缺失。示例性 C κ 域阐述于 W02011/090762 的 SEQ ID NO:141(所述序列以引用的方式并入本文中,它是本申请的 SEQ ID NO:83)中,其中野生型人 C κ 域的第一个精氨酸和最后一个半胱氨酸都缺失。在某些实施方案中,在改变的 C κ 域中,仅野生型人 C κ 域的最后一个半胱氨酸缺失,这是因为第一个精氨酸从野生型人 C κ 域中缺失可以由羧基末端具有精氨酸且连接改变的 C κ 域的氨基末端与另一个结构域(例如,免疫球蛋白亚区,诸如包含免疫球蛋白 CH2 和 CH3 域的亚区)的连接子提供。示例性 C λ 域阐述于 W02011/090762 的 SEQ ID NO:140(所述序列以引用的方式并入本文中,它是本申请的 SEQ ID NO:84)中,其中野生型人 C λ 域的第一个精氨酸缺失且与 CH1 域中的半胱氨酸一起参与形成二硫键的半胱氨酸被丝氨酸取代。

[0172] 在其它实施方案中,免疫球蛋白异源二聚域是与野生型 C κ 域相比在 C κ -C κ 界面上可能参与形成链间氢键网络的位置上含有一个或多个氨基酸取代的改变的 C κ 域。举例来说,在某些实施方案中,免疫球蛋白异源二聚域是在位置 N29、N30、Q52、V55、T56、S68 或 T70 上有一个或多个氨基酸被不同的氨基酸取代的改变的人 C κ 域。氨基酸的编号是基于其在如 W02011/090762 的 SEQ ID NO:141(所述序列以引用的方式并入本文中,它是本申请的 SEQ ID NO:83)中所述的改变的人 C κ 序列中的位置。在某些实施方案中,免疫球蛋白异源二聚域是在位置 N29、N30、V55 或 T70 上有一个、两个、三个或四个氨基酸取代的改变的人 C κ 域。用作上述位置上的取代物的氨基酸可以是丙氨酸或具有本体侧链部分的氨基酸残基,诸如精氨酸、色氨酸、酪氨酸、谷氨酸、谷氨酰胺或赖氨酸。可用于取代野生

型人 C κ 序列中在上述位置（例如 N30）上的氨基酸残基的其它氨基酸残基包括天冬氨酸、甲硫氨酸、丝氨酸和苯丙氨酸。示例性改变的人 C κ 域阐述于 W02011/090762 的 SEQ ID NO:142-178 中（所述序列以引用的方式并入本文中）。改变的人 C κ 域是促进与 CH1 域进行异源二聚，但将与另一个 C κ 域的同源二聚减到最少的 C κ 域。代表性改变的人 C κ 域阐述于 W02011/090762 的 SEQ ID NO:160 (N29W V55A T70A)、161 (N29Y V55A T70A)、202 (T70E N29A N30A V55A)、167 (N30RV55A T70A)、168 (N30K V55A T70A)、170 (N30E V55A T70A)、172 (V55R N29A N30A)、175 (N29W N30Y V55A T70E)、176 (N29Y N30YV55A T70E)、177 (N30E V55A T70E)、178 (N30Y V55A T70E)、838 (N30D V55A T70E)、839 (N30M V55A T70E)、840 (N30S V55A T70E) 和 841 (N30F V55A T70E) 中（所述序列以引用的方式并入本文中）。

[0173] 在某些实施方案中，除本文中所描述的 C κ 域中的突变以外或作为其代替物，多肽异源二聚体的免疫球蛋白异源二聚域（例如免疫球蛋白 CH1 和 CL 域）具有突变，使得所得免疫球蛋白异源二聚域在突变位点上的氨基酸残基之间形成盐桥（即，离子相互作用）。举例来说，多肽异源二聚体的免疫球蛋白异源二聚域可以是突变的 CH1 域与突变的 C κ 域的组合。在突变的 CH1 域中，野生型人 CH1 域的位置 68 上的缬氨酸 (V68) 被具有负电荷的氨基酸残基（例如天冬氨酸或谷氨酸）取代，而第一个精氨酸和最后一个半胱氨酸缺失的突变的人 C κ 域的位置 29 上的亮氨酸 (L29) 被具有正电荷的氨基酸残基（例如赖氨酸、精氨酸或组氨酸）取代。所得突变 CH1 域中具有负电荷的氨基酸残基与所得突变 C κ 域中具有正电荷的氨基酸残基之间的电荷-电荷相互作用形成盐桥，所述盐桥能使突变的 CH1 和 C κ 域之间的异源二聚界面稳定。或者，野生型 CH1 的 V68 可以被具有正电荷的氨基酸残基取代，而第一个精氨酸和最后一个半胱氨酸缺失的突变人 C κ 域的 L29 可以被具有负电荷的氨基酸残基取代。V68 被具有正电荷或负电荷的氨基酸取代的示例性突变 CH1 序列阐述于 W02011/090762 的 SEQ ID NO:844 和 845 中（所述序列以引用的方式并入本文中）。L29 被具有正电荷或负电荷的氨基酸取代的示例性突变 C κ 序列阐述于 W02011/090762 的 SEQ ID NO:842 和 843 中（所述序列以引用的方式并入本文中）。

[0174] 除 CH1 域的 V68 和 C κ 域的 L29 中的突变以外或作为其代替方案，除人 CH1 域的 V68 和人 C κ 域的 L29 以外的位置也可以被具有相反电荷的氨基酸取代以便在氨基酸之间产生离子相互作用。所述位置可以通过任何合适的方法来鉴别，包括随机突变发生；分析 CH1-C κ 配对的晶体结构，这是用于鉴别 CH1-C κ 界面上的氨基酸残基且使用一组标准（例如，参与离子相互作用的倾向性、与潜在伴侣残基的邻近程度等等）在 CH1-C κ 界面上的氨基酸残基中进一步鉴别合适的位置。

[0175] 在某些实施方案中，本公开的多肽异源二聚体仅含有一对免疫球蛋白异源二聚域。举例来说，多肽异源二聚体的第一个链可以包含 CH1 域作为免疫球蛋白异源二聚域，而第二个链可以包含 CL 域（例如 C κ 或 C λ ）作为免疫球蛋白异源二聚域。或者，第一个链可以包含 CL 域（例如 C κ 或 C λ ）作为免疫球蛋白异源二聚域，而第二个链可以包含 CH1 域作为免疫球蛋白异源二聚域。如本文中所述，第一个和第二个链的免疫球蛋白异源二聚域能够缔合以形成本公开的异源二聚蛋白。

[0176] 在某些其它实施方案中，本公开的异源二聚蛋白可以具有两对免疫球蛋白异源二聚域。举例来说，异源二聚体的第一个链可以包含两个 CH1 域，而第二个链可以具有两个 CL 域，该两个 CL 域与第一个链的两个 CH1 域缔合。或者，第一个链可以包含两个 CL 域，而第

二个链可以具有两个 CH1 域,该两个 CH1 域与第一个链的两个 CL 域缔合。在某些实施方案中,第一个多肽链包含 CH1 域和 CL 域,而第二个多肽链包含 CL 域和 CH1 域。该 CL 域和 CH1 域分别与第一个多肽链的 CH1 域和 CL 域缔合。

[0177] 在异源二聚蛋白仅包含一个异源二聚配对(例如,各链中有一个免疫球蛋白异源二聚域)的实施方案中,各链的免疫球蛋白异源二聚域可以位于该链的免疫球蛋白恒定亚区的氨基末端。或者,各链中的免疫球蛋白异源二聚域可以位于该链的免疫球蛋白恒定亚区的羧基末端。

[0178] 在异源二聚蛋白包含两个异源二聚配对(例如,各链中有两个免疫球蛋白异源二聚域)的实施方案中,各链中的两个免疫球蛋白异源二聚域都可以位于该链的免疫球蛋白恒定亚区的氨基末端。或者,各链中的两个免疫球蛋白异源二聚域可以都位于该链的免疫球蛋白恒定亚区的羧基末端。在其它实施方案中,各链中的一个免疫球蛋白异源二聚域可以位于该链的免疫球蛋白恒定亚区的氨基末端,而另一个免疫球蛋白异源二聚域可以位于该链的免疫球蛋白恒定亚区的羧基末端。换句话说,在那些实施方案中,免疫球蛋白恒定亚区是插入在各链的两个免疫球蛋白异源二聚域之间。

[0179] 如本文中所指示,在某些实施方案中,本公开的 CD3 结合多肽(例如小模块式免疫药物蛋白)(SMIP)、同源二聚体双特异性治疗剂(例如,Scorpion)、异源二聚体单特异性和多特异性治疗剂(例如,Interceptor)在各多肽链中包含免疫球蛋白恒定区。术语“恒定区”与恒定亚区和免疫球蛋白恒定区在本文中可互换使用。包括免疫球蛋白恒定区可以减缓由两个 CD3 结合多肽链形成的同源二聚和异源二聚蛋白在施用到受试者之后从循环中清除。

[0180] 通过突变或其它改变,免疫球蛋白恒定区还使得能够相对容易地调节二聚多肽效应功能(例如,ADCC、ADCP、CDC、补体固定和 Fc 受体结合),如本领域中已知和本文中所描述,所述二聚多肽效应功能可以取决于所治疗的疾病而增加或降低。

[0181] 在某些实施方案中,本公开的多肽同源二聚体和异源二聚体的多肽链之一或两者的免疫球蛋白恒定区应能够介导这些效应功能中的一种或多种。

[0182] 在其它实施方案中,与对应的野生型免疫球蛋白恒定区相比,在本公开的多肽同源二聚体和异源二聚体的多肽链之一或两者的免疫球蛋白恒定区中,这些效应功能中的一种或多种被降低或缺乏。举例来说,单特异性 CD3 结合多肽以及包含第二结合域(例如,设计用于引发重定向 T 细胞毒性(RTCC))的 CD3 结合多肽的免疫球蛋白恒定区相对于对应的野生型免疫球蛋白恒定区具有降低的效应功能或无效应功能是特别有用的。在一个实施方案中,恒定区经修饰以便不固定补体。恒定区还可以经修饰以便不结合一种或多种 Fc γ 受体,诸如 CD16、CD32 和 CD64。

[0183] 本公开的 CD3 结合多肽中存在的免疫球蛋白恒定亚区可以由以下各项构成或源自于以下各项的一部分或全部:CH2 域、CH3 域、CH4 域或其任何组合。举例来说,免疫球蛋白恒定亚区可以包含 CH2 域、CH3 域、CH2 域和 CH3 域两者、CH3 域和 CH4 域两者、两个 CH3 域、CH4 域、两个 CH4 域和 CH2 域及 CH3 域的一部分。在一些实施方案中,恒定区包含 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD 或其任何组合的 CH2 和 CH3 域;IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE、IgM 或其任何组合的免疫球蛋白 CH3 域;或 IgE、IgM 或其组合的免疫球蛋白 CH3 和 CH4 域。在一些实施方案中,恒定区实质上由 CH2 域和 CH3 域组成。

[0184] 可以形成本公开的 CD3 结合多肽的免疫球蛋白恒定区的 CH2 域可以是来自于某些免疫球蛋白类别或子类（例如 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2 或 IgD）和来自于各种的物种（包括人、小鼠、大鼠和其它哺乳动物）的野生型免疫球蛋白 CH2 域或其改变的免疫球蛋白 CH2 域。在一些实施方案中，恒定区经修饰以降低或消除效应功能、减少或不固定补体和 / 或减少结合或不结合 Fc γ 受体。在一些实施方案中，Fc γ 受体选自 CD16、CD32 和 CD64。

[0185] 在某些实施方案中，CH2 域是野生型人免疫球蛋白 CH2 域，诸如人 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2 或 IgD 的野生型 CH2 域，分别如 PCT 公布 W02011/090762 的 SEQ ID NO:115、199-201 和 195-197（所述序列以引用的方式并入本文中）中所述。在某些实施方案中，CH2 域是如 W02011/090762 的 SEQ ID NO:115 中所述的野生型人 IgG1CH2 域（所述序列以引用的方式并入本文中，它是本申请的 SEQ ID NO:85）。在一些实施方案中，恒定区包含 SEQ ID NO:55 的氨基酸序列或由其组成。

[0186] 在某些实施方案中，CH2 域是在位置 297 的天冬酰胺上包含氨基酸取代（例如，天冬酰胺变成丙氨酸）的改变的免疫球蛋白 CH2 区（例如，改变的人 IgG1CH2 域）。这样的氨基酸取代减少或消除这个位点上的糖基化且取消 Fc γ R 与 C1q 的有效 Fc 结合。在位置 297 上具有 Asn 变成 Ala 取代的改变的人 IgG1CH2 域的序列阐述于 W02011/090762 的 SEQ ID NO:324 中（所述序列以引用的方式并入本文中，它是本申请的 SEQ ID NO:86）。

[0187] 在某些实施方案中，CH2 域是在位置 234 到 238 上包含至少一个取代或缺失的改变的免疫球蛋白 CH2 区（例如，改变的人 IgG1CH2 域）。举例来说，免疫球蛋白 CH2 区可以在位置 234、235、236、237 或 238、位置 234 和 235、位置 234 和 236、位置 234 和 237、位置 234 和 238、位置 234-236、位置 234、235 和 237、位置 234、236 和 238、位置 234、235、237 和 238、位置 236-238 上或位置 234-238 上的两个、三个、四个或五个氨基酸的任何其它组合包含取代。另外或作为代替方案，改变的 CH2 区可以在位置 234-238 上，例如在位置 236 或位置 237 之一上包含一个或多个（例如两个、三个、四个或五个）氨基酸缺失，而在另一个位置上经取代。上述突变能降低或消除包含改变的 CH2 域的多肽异源二聚体的抗体依赖性细胞介导的细胞毒性 (ADCC) 活性或 Fc 受体结合能力。在某些实施方案中，已经用一个或多个丙氨酸残基置换位置 234-238 中的一个或多个上的氨基酸残基。在其它实施方案中，位置 234-238 上的氨基酸残基中仅一个缺失，而位置 234-238 上的其余氨基酸中的一个或多个可以经另一个氨基酸（例如丙氨酸或丝氨酸）取代。

[0188] 在某些其它实施方案中，CH2 域是在位置 253、310、318、320、322 和 331 上包含一个或多个氨基酸取代的改变的免疫球蛋白 CH2 区（例如，改变的人 IgG1CH2 域）。举例来说，免疫球蛋白 CH2 区可以在位置 253、310、318、320、322 或 331、位置 318 和 320、位置 318 和 322、位置 318、320 和 322 上或位置 253、310、318、320、322 和 331 上的两个、三个、四个、五个或六个氨基酸的任何其它组合包含取代。上述突变能降低或消除包含改变的 CH2 域的多肽异源二聚体的补体依赖性细胞毒性 (CDC)。

[0189] 在某些其它实施方案中，除位置 297 上的氨基酸取代以外，改变的 CH2 区（例如，改变的人 IgG1CH2 域）还可以在位置 234-238 上包含一个或多个（例如，两个、三个、四个或五个）其它取代。举例来说，免疫球蛋白 CH2 区可以在位置 234 和 297、位置 234、235 和 297、位置 234、236 和 297、位置 234-236 和 297、位置 234、235、237 和 297、位置 234、236、

238 和 297、位置 234、235、237、238 和 297、位置 236-238 和 297 或除位置 297 以外的位置 234-238 上的两个、三个、四个或五个氨基酸的任何组合包含取代。另外或作为代替方案,改变的 CH2 区可以在位置 234-238 上,诸如在位置 236 或位置 237 上包含一个或多个(例如两个、三个、四个或五个)氨基酸缺失。其它突变能降低或消除包含改变的 CH2 域的多肽异源二聚体的抗体依赖性细胞介导的细胞毒性(ADCC)活性或 Fc 受体结合能力。在某些实施方案中,已经用一个或多个丙氨酸残基置换位置 234-238 中的一个或多个上的氨基酸残基。在其它实施方案中,位置 234-238 上的氨基酸残基中仅一个缺失,而位置 234-238 上的其余氨基酸中的一个或多个可以经另一个氨基酸(例如丙氨酸或丝氨酸)取代。

[0190] 在某些实施方案中,除位置 234-238 上的一个或多个(例如,2、3、4 或 5 个)氨基酸取代以外,本公开的融合蛋白中的突变的 CH2 区(例如,改变的人 IgG1CH2 域)可以在一个或多个参与补体固定的位置上(例如,在位置 I253、H310、E318、K320、K322 或 P331 上)含有一个或多个(例如,2、3、4、5 或 6 个)其它氨基酸取代(例如经丙氨酸取代)。突变的免疫球蛋白 CH2 区的例子包括在位置 234、235、237(如果存在)、318、320 和 322 上具有丙氨酸取代的人 IgG1、IgG2、IgG4 和小鼠 IgG2a CH2 区。示例性突变的免疫球蛋白 CH2 区是在 L234、L235、G237、E318、K320 和 K322 上具有丙氨酸取代的小鼠 IGHG2c CH2 区。

[0191] 在其它实施方案中,除位置 297 上的氨基酸取代和位置 234-238 上的其它缺失或取代以外,改变的 CH2 区(例如,改变的人 IgG1CH2 域)还可以在位置 253、310、318、320、322 和 331 上包含一个或多个(例如,两个、三个、四个、五个或六个)其它取代。举例来说,免疫球蛋白 CH2 区可以包含(1)位置 297 上的取代,(2)位置 234-238 上的一个或多个取代或缺失或其组合,和位置 I253、H310、E318、K320、K322 和 P331 上的一个或多个(例如,2、3、4、5 或 6 个)氨基酸取代,诸如位置 E318、K320 和 K322 上的一个、两个、三个取代。上述位置上的氨基酸可以被丙氨酸或丝氨酸取代。

[0192] 在某些实施方案中,免疫球蛋白 CH2 区多肽包含:(i)位置 297 的天冬酰胺上的氨基酸取代和位置 234、235、236 或 237 上的一个氨基酸取代;(ii)位置 297 的天冬酰胺上的氨基酸取代和位置 234-237 中的两个上的氨基酸取代;(iii)位置 297 的天冬酰胺上的氨基酸取代和位置 234-237 中的三个上的氨基酸取代;(iv)位置 297 的天冬酰胺上的氨基酸取代、位置 234、235 和 237 上的氨基酸取代和位置 236 上的氨基酸缺失;(v)位置 234-237 中的三个上的氨基酸取代和位置 318、320 和 322 上的氨基酸取代;或(vi)位置 234-237 中的三个上的氨基酸取代、位置 236 上的氨基酸缺失和位置 318、320 和 322 上的氨基酸取代。

[0193] 在位置 297 的天冬酰胺上具有氨基酸取代的示例性改变的免疫球蛋白 CH2 区包括:在 L234、L235、G237 和 N297 上具有丙氨酸取代和在 G236 上具有缺失的人 IgG1CH2 区(WO2011/090762 的 SEQ ID NO:325,所述序列以引用的方式并入本文中)、在 V234、G236 和 N297 上具有丙氨酸取代的人 IgG2CH2 区(WO2011/090762 的 SEQ ID NO:326,所述序列以引用的方式并入本文中)、在 F234、L235、G237 和 N297 上具有丙氨酸取代和在 G236 上具有缺失的人 IgG4CH2 区(WO2011/090762 的 SEQ ID NO:322,所述序列以引用的方式并入本文中)、在 F234 和 N297 上具有丙氨酸取代的人 IgG4CH2 区(WO2011/090762 的 SEQ ID NO:343,所述序列以引用的方式并入本文中)、在 L235 和 N297 上具有丙氨酸取代的人 IgG4CH2 区(WO2011/090762 的 SEQ ID NO:344,所述序列以引用的方式并入本文中)、在 G236 和 N297 上具有丙氨酸取代的人 IgG4CH2 区(WO2011/090762 的 SEQ ID NO:345,所述序列以引用的

方式并入本文中)、和在 G237 和 N297 上具有丙氨酸取代的人 IgG4CH2 区 (W02011/090762 的 SEQ ID NO:346, 所述序列以引用的方式并入本文中)。

[0194] 在某些实施方案中,除上述氨基酸取代以外,改变的 CH2 区(例如,改变的人 IgG1CH2 域)可能在除上述位置以外的一个或多个位置上含有一个或多个其它氨基酸取代。所述氨基酸取代可以是保守或非保守氨基酸取代。举例来说,在某些实施方案中,在改变的 IgG2CH2 区中,P233 可以变成 E233(参见例如 W02011/090762 的 SEQ ID NO:326, 所述序列以引用的方式并入本文中)。另外或作为代替方案,在某些实施方案中,改变的 CH2 区可以含有一个或多个氨基酸插入、缺失或两者。插入、缺失或取代可以位于免疫球蛋白 CH2 区中的任何地方,诸如在野生型免疫球蛋白 CH2 区的 N 或 C 末端,从而经由铰链连接 CH2 区与另一个区(例如,结合域或免疫球蛋白异源二聚域)。

[0195] 在某些实施方案中,本公开的多肽中的改变的 CH2 区包含或者是与野生型免疫球蛋白 CH2 区,诸如野生型人 IgG1、IgG2 或 IgG4 或小鼠 IgG2a(例如,IGHG2c)的 CH2 区具有至少 90%、至少 91%、至少 92%、至少 93%、至少 94%、至少 95%、至少 96%、至少 97%、至少 98%或至少 99%同一性的序列。

[0196] 本公开的 CD3 结合多肽中的改变的免疫球蛋白 CH2 区可以源自于各种物种(包括人、小鼠、大鼠和其它哺乳动物)的各种免疫球蛋白同种型,诸如 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2 和 IgD 的 CH2 区。在某些实施方案中,本公开的融合蛋白中的改变的免疫球蛋白 CH2 区可以源自于人 IgG1、IgG2 或 IgG4 或小鼠 IgG2a(例如 IGHG2c)的 CH2 区,它们的序列阐述于 W02011/090762 的 SEQ ID NO:115、199、201 和 320 中(所述序列以引用的方式并入本文中)。

[0197] 在某些实施方案中,改变的 CH2 域是在位置 235、318、320 和 322 上具有丙氨酸取代(即,具有 L235A、E318A、K320A 和 K322A 取代的人 IgG1CH2 域)(W02011/090762 的 SEQ ID NO:595, 所述序列以引用的方式并入本文中)和任选地存在的 N297 突变(例如突变成丙氨酸)的人 IgG1CH2 域。在某些其它实施方案中,改变的 CH2 域是在位置 234、235、237、318、320 和 322 上具有丙氨酸取代(即,具有 L234A、L235A、G237A、E318A、K320A 和 K322A 取代的人 IgG1CH2 域)(W02011/090762 的 SEQ ID NO:596, 所述序列以引用的方式并入本文中)和任选地存在的 N297 突变(例如突变成丙氨酸)的人 IgG1CH2 域。

[0198] 在某些实施方案中,改变的 CH2 域是具有本领域中已知的能增强诸如 ADCC、ADCP、CDC、补体固定、Fc 受体结合或其任何组合等免疫学活性的突变的改变的人 IgG1CH2 域。

[0199] 可以形成本公开的 CD3 结合多肽的免疫球蛋白恒定区的 CH3 域可以是来自于各种物种(包括人、小鼠、大鼠和其它哺乳动物)的某些免疫球蛋白类别或子类(例如 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE、IgM)的野生型免疫球蛋白 CH3 域或其改变的免疫球蛋白 CH3 域。在某些实施方案中,CH3 域是野生型人免疫球蛋白 CH3 域,诸如人 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE 或 IgM 的野生型 CH3 域,分别如 W02011/090762 的 SEQ ID NO:116、208-210、204-207 和 212 中所述(所述序列以引用的方式并入本文中)。在某些实施方案中,CH3 域是如 W02011/090762 的 SEQ ID NO:116(所述序列以引用的方式并入本文中)中所述的野生型人 IgG1CH3 域。在某些实施方案中,CH3 域是改变的人免疫球蛋白 CH3 域,诸如基于或源自于人 IgG1、IgG2、IgG3、IgG4、IgA1、IgA2、IgD、IgE 或 IgM 抗体的野生型 CH3 域的改变的 CH3 域。举例来说,改变的 CH3 域可以是在位置 H433 和 N434 上具有一

个或两个突变的人 IgG1CH3 域（位置是根据 EU 编号法进行编号）。所述位置上的突变可能参与补体固定。在某些其它实施方案中，改变的 CH3 域可以是人 IgG1CH3 域，但在位置 F405 或 Y407 上具有一个或两个氨基酸取代。所述位置上的氨基酸参与了与另一个 CH3 域的相互作用。在某些实施方案中，改变的 CH3 域可以是最后一个赖氨酸缺失的改变的人 IgG1CH3 域。这种改变的 CH3 域的序列阐述于 W02011/090762 的 SEQ ID NO:761 中（所述序列以引用的方式并入本文中）。

[0200] 在某些实施方案中，形成多肽异源二聚体的 CD3 结合多肽包含一个 CH3 对，其包含所谓的“钮入孔”突变（参见 Marvin 和 Zhu, *Acta Pharmacologica Sinica* 26:649-58, 2005 ; Ridgway 等, *Protein Engineering* 9:617-21, 1996）。更具体地说，突变可以引入到各多肽链的两个 CH3 域的每一个中，使得 CH3/CH3 缔合所需的空间互补性强制这两个 CH3 域与彼此配对。举例来说，多肽异源二聚体的一个单链多肽中的 CH3 域可以含有 T366W 突变（“钮”突变，其用较大的氨基酸取代小氨基酸），且多肽异源二聚体的另一个单链多肽中的 CH3 域可以含有 Y407A 突变（“孔”突变，其用较小的氨基酸取代大氨基酸）。其它示例性钮入孔突变包括（1）一个 CH3 域中的 T366Y 突变和另一个 CH3 域中的 Y407T，和（2）一个 CH3 域中的 T366W 突变和另一个 CH3 域中的 T366S、L368A 和 Y407V 突变。

[0201] 可以形成本公开的 CD3 结合多肽的免疫球蛋白恒定区的 CH4 域可以是来自于 IgE 或 IgM 分子的野生型免疫球蛋白 CH4 域或其改变的免疫球蛋白 CH4 域。在某些实施方案中，CH4 域是野生型人免疫球蛋白 CH4 域，诸如分别如 W02011/090762 的 SEQ ID NO:213 和 214（所述序列以引用的方式并入本文中）中所述的人 IgE 和 IgM 分子的野生型 CH4 域。在某些实施方案中，CH4 域是改变的人免疫球蛋白 CH4 域，诸如基于或源自于人 IgE 或 IgM 分子的 CH4 域的改变的 CH4 域，所述分子具有能增加或降低已知与 IgE 或 IgM Fc 区相关的免疫活性的突变。

[0202] 在某些实施方案中，本公开的 CD3 结合多肽的免疫球蛋白恒定区包含 CH2、CH3 或 CH4 域的组合（即，一个以上选自 CH2、CH3 和 CH4 的恒定区域）。举例来说，免疫球蛋白恒定亚区可以包含 CH2 和 CH3 域或 CH3 和 CH4 域。在某些其它实施方案中，免疫球蛋白恒定区可以包含两个 CH3 域且不包含 CH2 或 CH4 域（即，仅两个或更多个 CH3）。形成免疫球蛋白恒定亚区的多个恒定区结构域可以基于或源自于相同的免疫球蛋白分子，或者相同类别或子类的免疫球蛋白分子。在某些实施方案中，免疫球蛋白恒定亚区是 IgG CH2CH3（例如 IgG1CH2CH3、IgG2CH2CH3 和 IgG4CH2CH3），且可以是人（例如，人 IgG1、IgG2 和 IgG4）CH2CH3。举例来说，在某些实施方案中，免疫球蛋白恒定亚区包含（1）野生型人 IgG1CH2 和 CH3 域、（2）具有 N297A 取代的人 IgG1CH2（即，CH2(N297A)）和野生型人 IgG1CH3，或（3）人 IgG1CH2(N297A) 和最后一个赖氨酸缺失的改变的人 IgG1CH3。

[0203] 或者，多个恒定区结构域可以基于或源自于不同的免疫球蛋白分子或者不同类别或子类的免疫球蛋白分子。举例来说，在某些实施方案中，免疫球蛋白恒定亚区包含人 IgM CH3 域和人 IgG1CH3 域。形成免疫球蛋白恒定亚区的多个恒定区结构域可以直接连接在一起，或可以经由一个或多个（例如约 2-10 个）氨基酸彼此连接。

[0204] 示例性免疫球蛋白恒定亚区阐述于 W02011/090762 的 SEQ ID NO:305-309、321、323、341、342 和 762 中（所述序列以引用的方式并入本文中）。

[0205] 在某些实施方案中，同源二聚体或异源二聚体的两个 CD3 结合多肽链的免疫球蛋白

白恒定区彼此同一。在某些其它实施方案中,异源二聚蛋白的一个多肽链的免疫球蛋白恒定亚区与所述异源二聚体的另一个多肽链的免疫球蛋白恒定亚区不同。举例来说,异源二聚蛋白的一个免疫球蛋白恒定亚区可以含有具有“钮”突变的 CH3 域,而所述异源二聚蛋白的另一个免疫球蛋白恒定亚区可以含有具有“孔”突变的 CH3 域。

[0206] 本发明的一些实施方案涉及使用如下的双特异性或多特异性结合分子:(i) 靶向人 T 细胞上的 TCR 复合物(例如 CD3)和(ii)细胞表面蛋白,例如以重定向 T 细胞细胞毒性针对具有所述细胞表面蛋白的细胞,例如针对表达肿瘤抗原的肿瘤细胞以便治疗癌症。

[0207] 在某些实施方案中,CD3 结合蛋白可以包含一个或多个结合除 CD3 以外的靶标的其它结合域(例如第二结合域)。这些其它靶分子可以包含例如肿瘤相关抗原。在本发明的一个实施方案中,一个或多个其它结合域结合一个或多个以下肿瘤抗原或与其相互作用:RON、c-Met、CEACAM-6、PSMA、EpCAM、CEA、PCTA-1、STEAP-1、STEAP-2、PSCA、PSA、PAP、ALCAM(CD166)、PECAM-1、EphA2、CD151、CA-125/MUC16、MUC-1、MAGE-1、TROP2、IGF1R、TGFB2、GHRHR、GHR、IL-6R、gp130、TNFR2、OSMR β 、Patched-1、Frizzled、Robo1、LT β R、CD19、CD25、CD26、CD27、CD30、CD33、CD44、CD44v6、CD63、CD80、CD81、CD86、CD100、CD151、CXCR4、CCR5、HER-2/ErbB1、HER-3/ErbB3、HER-4/ErbB4、EGFR/ErbB1、EGFRvIII 同种型、MUC2、MUC3、MUC4、MUC5_{AC}、MUC5_b、MUC7、 β hCG、Lewis-Y、神经节苷脂 GD3、9-O-乙酰基-GD3、GM2、Globo H、岩藻糖酰 GM1、Poly SA、GD2、碳酸酐酶 IX(MN/CA IX)、音猬因子(Sonic Hedgehog)(Shh)、Wue-1、浆细胞抗原、(膜结合)IgE、黑色素瘤硫酸软骨素蛋白多糖(MCSP)、CCR8、TNF- α 前体、间皮素、A33 抗原、Ly-6;桥粒核心糖蛋白 4、E-钙粘附蛋白新表位、胎儿乙酰胆碱受体、CA19-9 标记物、苗勒氏抑制物质(MIS)受体 II 型、sTn(唾液酸化 Tn 抗原;TAG-72)、FAP(成纤维细胞活化抗原)、内皮唾液酸蛋白、LG、SAS、BCMA、TWEAKR/Fn14、FGFR4、VEGFR1、VEGFR2、SSX1 和 SSX2。

[0208] 在某些实施方案中,CD3 结合域结合 TCR 结合域以便将 T 细胞募集到表达肿瘤抗原的靶细胞。在某些实施方案中,CD3 结合多肽可以包含特异性结合 TCR 复合物或其组分(例如 TCR α 、TCR β 、CD3 γ 、CD3 δ 和 CD3 ϵ)的 CD3 结合域和特异性结合肿瘤相关抗原的另一个结合域。

[0209] PSMA 是一种高度受限的前列腺癌相关细胞膜抗原。在前列腺癌细胞中,PSMA 的表达是正常前列腺上皮细胞上的 1000 倍(Su 等,Cancer Res. 199544:1441-1443)。PSMA 的表达随着前列腺癌进展而增加,并且典型地在转移性疾病、激素难治性病例和较高等级病变中最高(Israeli 等,Cancer Res. 1994,54:1807-1811;Wright 等,Urologic Oncology:Seminars and Original Investigations 19951:18-28;Wright 等,Urology 199648:326-332;Sweat 等,Urology 199852:637-640)。另外,PSMA 丰富地表达于多种其它实性肿瘤(包括膀胱癌、胰腺癌、黑素瘤、肺癌和肾癌)的新生血管上而不是正常的新生血管上(Chang 等,Urology 200157:801-805;Divgi 等,Clin. Cancer Res. 19984:2729-3279)。靶向 PSMA 的结合域包括但不限于 PCT 公布号 W02012/145714 中所描述的那些,该文献以全文引用的方式并入本文中。

[0210] 在一些实施方案中,PSMA 结合域包含与选自以下的氨基酸序列具有至少 90%、至少 95%或 100%同一性的氨基酸序列:(i)SEQ ID NO:212 的氨基酸 1-107 和 124-243;(ii)SEQ ID NO:226 的氨基酸 1-107 和 124-243;或(iii)SEQ ID NO:216 的氨基酸 1-107 和

124-243。在一些实施方案中,本发明的蛋白质或分子包含选自 SEQ ID NO:212、214、216 和 226 的氨基酸序列。可根据本发明使用的 PSMA 结合域还描述于 PCT 公布号 W02012/145714 中,包括了包含 W02012/145714 的氨基酸序列 SEQ ID NO:19、21、30、31、34 或 35 的 PSMA 结合域,或包含有包含选自 W02012/145714 的 SEQ ID NO:5 和 23 的氨基酸序列的 VL 链和包含选自 W02012/145714 的 SEQ ID NO:2、25 和 27 的氨基酸序列的 VH 链。

[0211] RON(受体酪氨酸激酶,也称为 MST1R)是胚胎发育所必需的受体型蛋白酪氨酸激酶且在发炎反应中也起着重要作用(Camp 等,Ann. Surg. Oncol. 12:273-281(2005))。RON 主要表达于上皮衍生的细胞类型中,并且已经提出,RON 如同许多其它受体型酪氨酸激酶一样可能在恶性上皮癌进展中起作用(Wang 等,Carcinogenesis 23:1291-1297(2003))。RON 的活化引发牵涉到诸如细胞增殖、细胞凋亡抑制和细胞运动性等肿瘤发生活性的下游信号传导路径。尤其是,RON 由于其信号传导性质和 / 或 RON 在结肠直肠癌、乳腺癌、卵巢癌和胰腺癌中的过表达而代表了上皮癌的治疗靶标。

[0212] 用于靶向 RON 的结合域包括但不限于实施例部分中所描述的那些和 PCT 公布 W02011/090761 中所描述的那些,该文献以全文引用的方式并入本文中。在一些实施方案中,抗 RON 结合域包含 (a) VL 域,其包含 i. CDR1 氨基酸序列 SEQ ID NO:87、CDR2 氨基酸序列 SEQ ID NO:88 和 CDR3 氨基酸序列 SEQ ID NO:89 ;或 ii. CDR1 氨基酸序列 SEQ ID NO:90、CDR2 氨基酸序列 SEQ ID NO:91 和 CDR3 氨基酸序列 SEQ ID NO:92 ;或 (b) VH 域,其包含 i. CDR1 氨基酸序列 SEQ ID NO:93、CDR2 氨基酸序列 SEQ ID NO:94 和 CDR3 氨基酸序列 SEQ ID NO:95 ;或 ii. CDR1 氨基酸序列 SEQ ID NO:96、CDR2 氨基酸序列 SEQ ID NO:97 和 CDR3 氨基酸序列 SEQ ID NO:98 ;或 (c) (a) 的 VL 和 (b) 的 VH。在一个实施方案中,VL 域包含 SEQ ID NO:99 或 100 中任一者的氨基酸序列,且 VH 域包含 SEQ ID NO:101、102 和 103 中任一者的氨基酸序列。在另一个实施方案中,VL 和 VH 域被人源化。在某些实施方案中,人源化 VL 包含 SEQ ID NO:104、105 和 106 中任一者的氨基酸序列,且人源化 VH 域包含 SEQ ID NO:107-111 中任一者的氨基酸序列。在一些实施方案中,抗 RON 结合域包含有包含 SEQ ID NO:99 和 100 中任一者的氨基酸序列的 VL 域,和包含 SEQ ID NO:101、102 和 103 中任一者的氨基酸序列的 VH 域。在一些实施方案中,抗 RON 结合域包含有包含 SEQ ID NO:104、105 和 106 中任一者的氨基酸序列的 VL 域,和包含 SEQ ID NO:107-111 中任一者的氨基酸序列的 VH 域。

[0213] 在一些实施方案中,RON 结合域包含与 SEQ ID NO:187 具有至少 90%、92%、94%、96%、97%、98%、99% 或 100% 同一性的氨基酸序列和 / 或包含与 SEQ ID NO:188 具有至少 90%、92%、94%、96%、97%、98%、99% 或 100% 同一性的氨基酸序列。在一些实施方案中,RON 结合域包含 (i) SEQ ID NO:187,其中氨基酸 43(丙氨酸)经另一个氨基酸取代,例如经赖氨酸(A43K)或苏氨酸(A43T)取代;(ii) SEQ ID NO:188,其中氨基酸 38(谷氨酰胺)和 / 或 113(谷氨酰胺)经另一个氨基酸取代,例如经谷氨酸和 / 或精氨酸取代,诸如 Q38R 和 / 或 Q113E ;或 (iii) 其任何组合。在一些实施方案中,抗 RON/ 抗 CD3 双特异性分子包含与 SEQ ID NO:186、190、192 或 194 中的任一者具有至少 90%、92%、94%、96%、97%、98%、99% 或 100% 同一性的氨基酸序列或由其组成。在一些实施方案中,本发明提供了一种编码抗 RON/ 抗 CD3 双特异性分子的核酸,其中所述核酸包含与 SEQ ID NO:185、189、191 或 193 中的任一者具有至少 90%、92%、94%、96%、97%、98%、99% 或 100% 同一性的核苷

酸序列。

[0214] CD19 是在 B 细胞,包括大部分恶性 B 细胞的表面上发现的。靶向 CD19 可用于抑制和治疗 B 细胞相关疾病、白血病和淋巴瘤。

[0215] 在一些实施方案中,CD19 结合域包含与 SEQ ID NO:196 的氨基酸 1-111 具有至少 90%、92%、94%、96%、97%、98%、99%或 100%同一性的氨基酸序列和 / 或包含与 SEQ ID NO:196 的氨基酸 128-251 具有至少 90%、92%、94%、96%、97%、98%、99%或 100%同一性的氨基酸序列。在一些实施方案中,抗 CD19/ 抗 CD3 双特异性分子包含与 SEQ ID NO:196、198、200、202、204 或 206 中任一者具有至少 90%、92%、94%、96%、97%、98%、99%或 100%同一性的氨基酸序列或由其组成。在一些实施方案中,本发明提供了一种编码抗 CD19/ 抗 CD3 双特异性分子的核酸,其中所述核酸包含与 SEQ ID NO:195、197、199、201、203 或 206 中的任一者具有至少 90%、92%、94%、96%、97%、98%、99%或 100%同一性的核苷酸序列。

[0216] HER2 也称为人 ErbB2。在乳腺癌、卵巢癌和其它癌瘤(包括胃癌、子宫内膜癌、唾液腺癌、肺癌、肾癌、结肠癌、甲状腺癌、胰腺癌和膀胱癌)中也观察到了 HER2 的过表达(往往但不总是由于基因扩增)。靶向 HER2 的结合域包括但不限于 PCT 公布 W02009/055074 中所描述的那些,该文献以全文引用的方式并入本文中。

[0217] 在一些实施方案中,CD3 结合多肽包含与 SEQ ID NO:57、59、61、63、65、67、69、71、186、190、192、194、196、198、200、202、204 或 206 具有至少 90%、至少 95%或 100%同一性的氨基酸序列。

[0218] 在一些实施方案中,CD3 结合多肽是异源二聚体的一部分。在一些实施方案中,异源二聚体包含一对单链多肽,其中所述单链对包含与选自以下的配对具有至少 90%、至少 95%或 100%同一性的氨基酸序列:SEQ ID NO:210 和 247、SEQ ID NO:210 和 218、SEQ ID NO:210 和 220、SEQ ID NO:208 和 249、SEQ ID NO:208 和 222 或 SEQ ID NO:208 和 224、SEQ ID NO:212 和 218、SEQ ID NO:216 和 222、SEQ ID NO:228 和 226 或 SEQ ID NO:214 和 218。

[0219] 本发明还包括编码如本文中所描述的 CD3 结合多肽,或如本文中所描述的二聚或异源二聚 CD3 结合蛋白的一个或多个多肽链的核酸(例如 DNA 或 RNA)。本发明的核酸包括具有与如 SEQ ID NO:5、7、9、11、13、15、17、19、27、29、31、37 和 39 中所提供的聚核苷酸基本上同一的区域的核酸或包含编码相同或相似多肽的编码区的核酸。在某些实施方案中,根据本发明的核酸与如 SEQ ID NO:5、7、9、11、13、15、17、19、27、29、31、37 和 39 中所提供的多肽编码聚核苷酸具有至少 80%、典型地至少约 90%且更典型地至少约 95%或至少约 98%同一性。本发明的核酸还包括互补核酸。在一些情况下,序列在比对时将完全互补(无错配)。在其它情形下,序列中可能存在最多约 20%错配。在本发明的一些实施方案中提供了编码本发明的异源二聚 CD3 结合蛋白的第一和第二多肽链两者的核酸。本文中所提供的核酸序列可以使用密码子优化、简并序列、沉默突变和其它 DNA 技术加以利用以优化在特定宿主中的表达,且本发明涵盖所述序列修饰。

[0220] 通过将所述分子放在载体中对包含所要聚核苷酸序列的聚核苷酸分子进行增殖。本发明还包括含有本发明核酸和 / 或表达本发明多肽的表达载体。使用病毒和非病毒载体,包括质粒。质粒的选择将取决于需要增殖的细胞类型和增殖目的。某些载体适用于扩增和制造大量的所需 DNA 序列。其它载体适合于在培养中的细胞中进行表达。其它载体适

合于在整个动物或人的细胞中进行转移和表达。适当载体的选择完全在本领域技术人员的掌控之内。许多这样的载体可以得自市面。典型地借助于 DNA 连接酶连接到载体中裂解的限制酶位点来将部分或全长聚核苷酸插入载体中。或者,可以通过体内同源重组来插入所要的核苷酸序列。典型地,这伴随有将同源性区域连接到载体中所要核苷酸序列的两侧上。举例来说,通过寡核苷酸连接或通过聚合酶链反应,使用包含同源性区域和所要核苷酸序列的一部分的引物加入同源性区域。

[0221] 对于表达来说,可以采用表达盒或系统。为了表达编码本文中所公开的多肽的核酸,将编码所述多肽的核酸分子引入到宿主细胞中,所述核酸分子可操作地连接到能控制在表达载体中的转录表达的调节序列。本发明包括了包含本发明的核酸和 / 或表达载体,例如编码本发明多肽的核酸的宿主细胞。除诸如启动子和增强子等转录调节序列以外,表达载体可以包括翻译调节序列和适合于选择携带表达载体的细胞的标记基因。本发明聚核苷酸所编码的基因产物表达于任何适宜的表达式中,包括例如细菌、酵母、昆虫、两栖动物和哺乳动物系统。在表达载体中,在适当时将多肽编码聚核苷酸连接到调节序列以获得所要的表达性质。这些可以包括启动子、增强子、终止子、操纵子、抑制子和诱导物。启动子可以是调节性的(例如来自于类固醇诱导性 pIND 载体 (Invitrogen) 的启动子)或组成性的(例如来自于 CMV、SV40、延伸因子或 LTR 序列的启动子)。使用如上文关于连接到载体所述的技术将这些连接到所要的核苷酸序列。可以使用本领域中已知的任何技术。因此,表达载体一般将提供转录和翻译起始区,它可以是诱导性的或组成性的,其中在转录起始区及转录和翻译结束区的转录控制下可操作地连接编码区。

[0222] 可以将表达盒(“表达单元”)引入到多种载体中,例如质粒、BAC、YAC、噬菌体(诸如 λ 、P1、M13 等)、植物或动物病毒载体(例如,基于逆转录病毒的载体、腺病毒载体)等等,其中载体正常以选择包含表达载体的细胞的能力为特征。载体可以提供染色体外维持,特别是作为质粒或病毒,或用于整合到宿主染色体中。在需要染色体外维持的情况下,提供起点序列以便复制质粒,它可以是低或高拷贝数。多种标记物可用于选择,确切地说防止毒素,更确切的说是防止抗生素的那些标记物。根据宿主的性质来选择所选的特定标记物,其中在一些情况下,可以用营养缺陷型宿主进行补充。DNA 构建体的引入可以使用任何适宜的方法,包括例如结合、细菌转化、钙沉淀的 DNA、电穿孔、融合、转染、用病毒载体感染、生物弹法等等。

[0223] 因此,本发明内使用的蛋白质可以根据常规技术在经基因工程改造的宿主细胞中产生。合适的宿主细胞为可以用外源性 DNA 转化或转染且在培养基中生长的细胞类型,并且包括细菌、真菌细胞和培养的高级真核细胞(包括多细胞生物体的培养细胞),特别是培养的哺乳动物细胞。以下文献公开了用于操控克隆的 DNA 分子和将外源性 DNA 引入到多种宿主细胞中的技术: Sambrook 和 Russell, *Molecular Cloning: A Laboratory Manual* (第 3 版, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2001); 和 Ausubel 等, *Short Protocols in Molecular Biology* (第 4 版, John Wiley & Sons, 1999)。

[0224] 举例来说,为了重组表达包含两个同一的如本文中所描述的 CD3 结合多肽链的同源二聚 CD3 结合蛋白,表达载体一般将包括可操作地连接到启动子的编码 CD3 结合多肽的核酸区段。为了重组表达包含不同的第一和第二多肽链的异源二聚 CD3 结合蛋白,第一和第二多肽链可以由宿主细胞中单独的载体共表达以便表达整个异源二聚蛋白。或者,为了

表达异源二聚 CD3 结合蛋白,第一和第二多肽链可以由宿主细胞中的同一载体中单独的表达单元共表达以便表达整个异源二聚蛋白。通过常规技术将表达载体转移到宿主细胞,然后通过常规技术培养经过转染的细胞以产生编码多肽,从而产生对应的 CD3 结合蛋白。

[0225] 为了将重组蛋白引导到宿主细胞的分泌路径中,在表达载体中提供分泌信号序列(也称为前导序列)。分泌信号序列可以是重组蛋白的天然形式的分泌信号序列,或可以源自于另一个分泌蛋白或重新合成。将分泌信号序列可操作地连接到编码多肽的 DNA 序列,即,将两个序列在正确的阅读框中接合且进行定位以便将新合成的多肽引导到宿主细胞的分泌路径中。分泌信号序列通常相对于编码相关多肽的 DNA 序列位于 5',但某些信号序列可以位于相关 DNA 序列中的其它地方(参见例如 Welch 等,美国专利号 5,037,743;Holland 等,美国专利号 5,143,830)。在特定变化方案中,根据本发明使用的分泌信号序列具有氨基酸序列 MEAPAQLLFLLLLWLPDTTG(SEQ ID NO:79)。

[0226] 培养的哺乳动物细胞是适合于产生在本发明内使用的重组蛋白的宿主。用于将外源性 DNA 引入到哺乳动物宿主细胞中的方法包括磷酸钙介导的转染(Wigler 等,Cell 14:725,1978;Corsaro 和 Pearson, Somatic Cell Genetics 7:603,1981;Graham 和 Van der Eb, Virology 52:456,1973)、电穿孔(Neumann 等,EMBO J. 1:841-845,1982)、DEAE 右旋糖酐介导的转染(Ausubel 等,同上)和脂质体介导的转染(Hawley-Nelson 等,Focus 15:73,1993;Ciccarone 等,Focus 15:80,1993)。举例来说,以下文献中公开了在培养的哺乳动物细胞中产生重组多肽:Levinson 等,美国专利号 4,713,339;Hagen 等,美国专利号 4,784,950;Palmiter 等,美国专利号 4,579,821;和 Ringold, 美国专利号 4,656,134。合适的哺乳动物宿主细胞的例子包括非洲绿猴肾细胞(Vero;ATCC CRL 1587)、人胚肾细胞(293-HEK;ATCC CRL 1573)、幼仓鼠肾细胞(BHK-21、BHK-570;ATCC CRL 8544、ATCC CRL10314)、犬肾细胞(MDCK;ATCC CCL 34)、中国仓鼠卵巢细胞(CHO-K1;ATCC CCL61;CHO DG44;CHO DXB11(Hyclone, Logan, UT);也参见例如 Chasin 等, Som. Cell. Molec. Genet. 12:555,1986)、大鼠垂体细胞(GH1;ATCC CCL82)、HeLa S3 细胞(ATCC CCL2.2)、大鼠肝细胞癌细胞(H-4-II-E;ATCC CRL 1548)、经 SV40 转化的猴肾细胞(COS-1;ATCC CRL 1650)和鼠胚细胞(NIH-3T3;ATCC CRL1658)。其它合适的细胞系在本领域中是已知的,并且可得自诸如弗吉尼亚州马纳萨斯的美国典型培养物保藏中心等公共保藏中心。可以使用强转录启动子,诸如得自于 SV-40 或巨细胞病毒的启动子。参见例如美国专利号 4,956,288。其它合适的启动子包括来自于金属硫蛋白基因的启动子(美国专利号 4,579,821 和 4,601,978)和腺病毒主要晚期启动子。

[0227] 药物选择一般用于选择出其中已插入外来 DNA 的培养的哺乳动物细胞。所述细胞通常称为“转基因细胞”。在选择剂存在下培养且能够将相关基因传递给其后代的细胞称为“稳定转基因细胞”。示例性选择标记物包括对抗生素新霉素具有抗性的编码基因,它允许在诸如 G-418 等新霉素类型药物存在下进行选择;针对黄嘌呤-鸟嘌呤磷酸核糖基转移酶的 gpt 基因,它允许宿主细胞在霉酚酸/黄嘌呤存在下生长;和提供针对博来霉素、争光霉素、灭瘟素和潮霉素的抗性的标记物(参见例如 Gatignol 等, Mol. Gen. Genet. 207:342,1987;Drocourt 等, Nucl. Acids Res. 18:4009,1990)。选择系统还可以用于增加相关基因的表达水平,这一过程称为“扩增”。通过在低水平选择剂存在下培养转基因细胞,然后增加选择剂的量直到选择出能产生所引入基因的高水平产物的细胞来进行扩

增。示例性可扩增选择标记物是二氢叶酸还原酶,它能赋予针对氨甲蝶呤的抗性。还可以使用其它抗药性基因(例如潮霉素抗性、多药物抗性、嘌呤霉素乙酰转移酶)。

[0228] 还可以使用其它高级真核细胞作为宿主,包括昆虫细胞、植物细胞和禽类细胞。Sinkar 等, *J. Biosci. (Bangalore)* 11:47-58, 1987 已经回顾了使用发根农杆菌作为在植物细胞中表达基因的载体。以下文献中公开了转化昆虫细胞和在其中产生外来多肽:Guarino 等, 美国专利号 5,162,222;和 WIPO 公布 WO 94/06463。

[0229] 可以用通常源自于苜蓿银纹夜蛾核多角体病毒 (AcNPV) 的重组杆状病毒感染昆虫细胞。参见 King 和 Possee, *The Baculovirus Expression System: A Laboratory Guide* (Chapman & Hall, London); O'Reilly 等, *Baculovirus Expression Vectors: A Laboratory Manual* (Oxford University Press, New York 1994); 和 *Baculovirus Expression Protocols. Methods in Molecular Biology* (Richardson 编, Humana Press, Totowa, NJ, 1995)。还可以通过使用 Luckow 等 (*J. Virol.* 67:4566-4579, 1993) 描述的基于转座子的系统来产生重组杆状病毒。这个系统利用转移载体,可以呈试剂盒形式得自市面 (BAC-T0-BAC 试剂盒;Life Technologies, Gaithersburg, MD)。转移载体(例如 PFASTBAC1;Life Technologies)含有用于将编码相关蛋白质的 DNA 移动到呈大质粒(称为“杆粒”)形式维持于大肠杆菌中的杆状病毒基因组中的 Tn7 转座子。参见 Hill-Perkins 和 Possee, *J. Gen. Virol.* 71:971-976, 1990;Bonning 等, *J. Gen. Virol.* 75:1551-1556, 1994;和 Chazenbalk 和 Rapoport, *J. Biol. Chem.* 270:1543-1549, 1995。另外,转移载体可以包括如上文所公开与编码多肽延伸或亲和力标签的 DNA 进行框内融合。使用本领域中已知的技术,将含有蛋白质编码 DNA 序列的转移载体转化到大肠杆菌宿主细胞中,且针对含有指示重组杆状病毒的中断 lacZ 基因的杆粒筛选所述细胞。使用常用技术分离含有重组杆状病毒基因组的杆粒 DNA,且用于转染草地贪夜蛾细胞,诸如 Sf9 细胞。随后产生表达相关蛋白质的重组病毒。通过本领域中通常使用的方法制造重组病毒储备液。

[0230] 对于蛋白质产生来说,使用重组病毒感染宿主细胞,典型地为源自于草地粘虫、草地贪夜蛾(例如 Sf9 或 Sf21 细胞)或粉纹夜蛾(例如 HIGH FIVE 细胞;Invitrogen, Carlsbad, CA)的细胞系。一般参见 Glick 和 Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA* (ASM Press, Washington, D. C., 1994)。还参见美国专利号 5,300,435。使用无血清培养基来生长和维持所述细胞。合适的培养基制剂在本领域中是已知的且可以获自市面上的供应商。使所述细胞从大约 $2-5 \times 10^5$ 个细胞的接种密度生长到 $1-2 \times 10^6$ 个细胞的密度,此时加入感染复数 (MOI) 为 0.1 到 10,更典型地为接近 3 的重组病毒储备液。所使用的程序一般描述于可用的实验室手册中(参见例如 King 和 Possee, 同上;O'Reilly 等, 同上;Richardson, 同上)。

[0231] 本发明内也可以使用真菌细胞,包括酵母细胞。在这方面特别相关的酵母种类包括酿酒酵母、巴斯德毕赤氏酵母和甲醇毕赤酵母。举例来说,以下文献公开了用外源性 DNA 转化酿酒酵母细胞和由其产生重组多肽的方法:Kawasaki, 美国专利号 4,599,311;Kawasaki 等, 美国专利号 4,931,373;Brake, 美国专利号 4,870,008;Welch 等, 美国专利号 5,037,743;和 Murray 等, 美国专利号 4,845,075。通过由通常具有抗药性或能够在不存在特定营养(例如亮氨酸)的情况下生长的选择标记物确定的表型来选择经过转化的细胞。用于酿酒酵母的示例性载体系统是 Kawasaki 等(美国专利号 4,931,373)所公

开的 POT1 载体系统,它允许通过在含葡萄糖的培养基中生长来选择经转化的细胞。用于酵母的合适的启动子和终止子包括来自于糖解酶基因(参见例如 Kawasaki, 美国专利号 4,599,311;Kingsman 等, 美国专利号 4,615,974;和 Bitter, 美国专利号 4,977,092)和醇脱氢酶基因的那些。还参见美国专利号 4,990,446、5,063,154、5,139,936 和 4,661,454。用于其它酵母,包括多形汉逊酵母、粟酒裂殖酵母、乳酸克鲁维酵母、脆壁克鲁维酵母、玉米黑粉菌、巴斯德毕赤氏酵母、甲醇毕赤酵母、季也蒙毕赤酵母和麦芽糖假丝酵母的转化系统在本领域中是已知的。参见例如 Gleeson 等, *J. Gen. Microbiol.* 132:3459-3465, 1986;Cregg, 美国专利号 4,882,279;和 Raymond 等, *Yeast* 14:11-23, 1998。可以根据 McKnight 等, 美国专利号 4,935,349 的方法利用曲霉细胞。Sumino 等, 美国专利号 5,162,228 公开了用于转化产黄头孢的方法。Lambowitz, 美国专利号 4,486,533 公开了用于转化脉孢菌的方法。美国专利号 5,716,808、5,736,383、5,854,039 和 5,888,768 中公开了在甲醇毕赤酵母中产生重组蛋白。

[0232] 原核宿主细胞,包括细菌大肠杆菌、芽胞杆菌属和其它菌属的菌株,也是本发明内可用的宿主细胞。用于转化这些宿主和表达克隆于其中的外来 DNA 序列的技术在本领域中是众所周知的(参见例如 Sambrook 和 Russell, 同上)。当在诸如大肠杆菌等细菌中表达重组蛋白时,蛋白可以保留在细胞质中,典型地呈不溶性颗粒形式,或可以被细菌分泌序列引导到壁膜间隙。在前一种情况下,细胞被溶解,且颗粒被回收并且使用例如异硫氰酸胍或尿素使其变性。然后,变性蛋白可以再折叠且通过稀释变性剂来进行二聚,诸如通过针对尿素溶液及还原型和氧化型谷胱甘肽的组合进行透析,随后针对缓冲生理盐水溶液进行透析。在替代方案中,蛋白质可以呈溶解形式从细胞质中回收且在不使用变性剂的情况下分离。蛋白质是在例如磷酸盐缓冲生理盐水中呈水性提取物形式从细胞中回收。为了俘获相关蛋白质,将提取物直接应用于色谱介质,诸如固定的抗体或肝素-琼脂糖凝胶柱。可以通过破坏细胞(例如,通过超声处理或渗透性冲击)以释放壁膜间隙的内含物且回收蛋白质,从而避免对变性和再折叠的需要而从壁膜间隙回收呈可溶解和功能形式的分泌蛋白。可以根据已知的方法在细菌宿主细胞中产生抗体,包括单链抗体。参见例如 Bird 等, *Science* 242:423-428, 1988;Huston 等, *Proc. Natl. Acad. Sci. USA* 85:5879-5883, 1988;和 Pantoliano 等, *Biochem.* 30:10117-10125, 1991。

[0233] 根据常规程序在含有营养和所选宿主细胞生长所需的其它组分的培养基中培养经过转化或转染的宿主细胞。多种合适的培养基,包括确定成分培养基和复合型培养基,是本领域中已知的,且一般包括碳源、氮源、必需氨基酸、维生素和矿物质。培养基还可以视需要含有诸如生长因子或血清等组分。一般将通过例如药物选择或必需营养素缺乏来选择用于含有外源添加 DNA 的细胞的生长培养基,所述必需营养素是通过表达载体上携带的或共同转染到宿主细胞中的选择标记物来补充。

[0234] 可以利用常规蛋白质纯化方法,典型地通过色谱技术的组合来纯化 CD3 结合蛋白。一般参见 *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988);Scopes, *Protein Purification: Principles and Practice* (Springer-Verlag, New York 1994)。可以通过在固定的蛋白 A 或蛋白 G 上进行亲和色谱法来纯化包含免疫球蛋白 Fc 区的蛋白质。可以使用诸如凝胶过滤等其它纯化步骤来获得所要的纯度水平或进行脱盐、缓冲液交换等等。

[0235] 本发明还包括了包含本发明的 CD3 结合多肽和药学上可接受的载剂、稀释剂和/或赋形剂的组合物。

[0236] 在本发明的一个实施方案中,将单特异性 CD3 结合多肽施用到罹患诸如变形性关节炎等自体免疫疾病的患者。在本发明的另一个实施方案中,将本发明的单特异性 CD3 结合多肽施用到将要进行器官移植的受试者。

[0237] 在另一个方面,本发明提供了一种用于治疗诸如癌症等以肿瘤抗原过表达为特征的病症的方法。一般来说,所述方法包括向需要所述治疗的受试者施用治疗有效量的如本文中所描述的包含能结合肿瘤抗原的第二结合域的 CD3 结合蛋白。在一些实施方案中,CD3 结合蛋白诱导重定向 T 细胞细胞毒性 (RTCC) 针对受试者中表达肿瘤抗原的细胞。

[0238] 在所述方法的某些变化方案中,所述病症是癌症。在其它变化方案中,所述病症是自体免疫疾病。本发明还提供了用于治疗癌症或自体免疫病症的方法,包括向有需要的患者施用治疗有效量的本文中所描述的组合物或 CD3 结合多肽。

[0239] 在本文中所描述的治疗方法的各实施方案中,以符合与寻求治疗的疾病或病症的处置相关的常规方法的方式递送 CD3 结合蛋白。根据本文中的公开内容,将有效量的 CD3 结合蛋白施用给需要该治疗的受试者持续足以预防或治疗所述疾病或病症的时间或在足以预防或治疗所述疾病或病症的条件下施用。

[0240] 为了施用,将 CD3 结合蛋白配制为药物组合物。包含 CD3 结合蛋白的药物组合物可以根据已知的方法来配制以制备药学上有用的组合物,其中组合治疗分子,呈与药学上可接受的载剂的混合物的形式。如果组合物的施用能被受体患者耐受,则将其称为“药学上可接受的载剂”。无菌磷酸盐缓冲生理盐水是药学上可接受的载剂的一个例子。其它合适的载剂对于本领域技术人员是众所周知的。(参见例如 Gennaro(编), Remington's Pharmaceutical Sciences(Mack Publishing Company, 第 19 版,1995)。)制剂还可以包括一种或多种赋形剂、防腐剂、增溶剂、缓冲剂、白蛋白,以防止小瓶表面上的蛋白质损失等。

[0241] 将包含 CD3 结合蛋白的药物组合物以治疗有效量施用给受试者。根据本发明的方法,CD3 结合蛋白可以通过多种施用模式施用给受试者,包括例如通过肌肉内、皮下、静脉内、心房内、关节内、肠胃外、鼻内、肺内、经皮、胸膜内、鞘内和口服施用途径。出于预防和治疗目的,施用给受试者可以例如以单次快速推注递送方式、经由延长时间段内的连续递送(例如连续经皮递送)或以重复施用方案(例如,以每小时、每日或每周计)。

[0242] 分子或组合物的“治疗有效量(或剂量)”或“有效量(或剂量)”是能在缓解病症的一种或多种症状方面产生统计上显著的效果(诸如统计上显著地减缓疾病进展或统计上显著地改良器官功能)的量。确切剂量将由临床医师根据公认标准,考虑欲治疗的病症的性质和严重程度、患者特性等来确定。剂量的确定在本领域技术人员的水平内。当提到组合时,治疗有效剂量是指产生治疗效果的活性成分的组合量,无论是依次施用还是同时施用(例如,在同一制剂中或在单独的制剂中同时施用)。

[0243] 有效剂量的决定在本文中典型地是基于动物模型研究和随后的人临床试验,且通过确定能在模型受试者中显著减少受试者病症的发生或严重程度的有效剂量和施用方案加以指导。本发明组合物的有效剂量取决于许多不同的因素而变化,包括施用手段、目标部位、患者生理状态、患者是人还是动物、施用的其它药物处理、治疗是预防性的还是治疗性的,以及组合物本身的特定活性和其在个体中引出所要反应的能力。通常,患者是人,但在

一些疾病中,患者可以是非人哺乳动物。典型地,调节给药方案以提供最佳治疗反应,例如,以优化安全性和功效。

实施例

[0244] 实施例 1:设计和构建理论 pI 降低的抗 CD3SMIP 分子

[0245] L1、L2、H1、H2 和 H3(参见图 3 和 4) 是先前构建的人源化 Cris-7 的重链和轻链,并且针对结合和活性进行分析。使用 H3L1 制造 SMIPDRA209(SEQ ID NO:3 和 4;IgG4AA ADCC-CDC null Fc) 和 SMIP DRA161(IgG4AA N297A Fc)。虽然这些构建体与 OKT3 和现有技术中的其它抗 CD3 治疗剂相比展现了改良的特征,但当表达于 CHO 细胞中(参见图 2A 和 2B) 时,与其它 SMIP 分子相比,所述构建体展现出显著较短的半衰期、较高的理论 pI(参见图 1) 和处于结合域或前铰链区的“剪接”。

[0246] 设计抗 CD3pI 变体以降低结合域和 / 或前铰链区的 pI。为了制造抗 CD3pI 变体,通过 Blue Heron 对新的重链和轻链 (L3、L4、H4、H5 和 H6) 进行排序和合成。参见表 3 及图 3 和 4。

[0247] 表 3:CD3 可变重链和轻链

[0248]

名称	SEQ ID NO:
H1	SEQ ID NO:21 核酸 (na)
	SEQ ID NO:22 氨基酸 (aa)
H2	SEQ ID NO:23 (na)
	SEQ ID NO:24 (aa)
H3	SEQ ID NO:25 (na)
	SEQ ID NO:26 (aa)
H4	SEQ ID NO:27 (na)
	SEQ ID NO:28 (aa)
H5	SEQ ID NO:29 (na)
	SEQ ID NO:30 (aa)
H6	SEQ ID NO:31 (na)
	SEQ ID NO:32 (aa)
L1	SEQ ID NO:33 (na)
	SEQ ID NO:34 (aa)
L2	SEQ ID NO:35 (na)
	SEQ ID NO:36 (aa)
L3	SEQ ID NO:37 (na)
	SEQ ID NO:38 (aa)
L4	SEQ ID NO:39 (na)
	SEQ ID NO:40 (aa)

[0249] H4 与 H3 相比具有 2 个点突变。一个突变置换了正电荷残基 (K 变成 Q), 而另一个突变引入了负电荷 (Q 变成 E)。两种变化都意在降低蛋白质的 pI (或是通过去除正电荷,或是通过引入负电荷)。在构架序列上进行突变以避免损失亲和力,且所引入的新残基经验证存在于生殖细胞系序列中可避免免疫原性风险的位置。通过使用生殖细胞系序列作为引导序列以降低已经人源化的结合域的 pI, 可以进行氨基酸取代以添加生殖细胞系序列中普遍

存在的氨基酸。这些氨基酸可以在生殖细胞系序列中的同一位置或邻近位置。参见图 5。

[0250] 通过以产生比 DRA209 和 DRA161 低的 pI 的方式对重链序列进行“再人源化”(与 DRA209 和 DRA161 相比)来设计 H5。这是通过设计与 IGHV1-3*01 重链生殖细胞系序列的 VH1 构架相当的重链来进行。

[0251] 通过以产生比 DRA209 和 DRA161 低的 pI 的方式对重链序列进行“再人源化”(与 DRA209 和 DRA161 相比)来设计 H6。换句话说,用于设计 H6 的起始序列是生殖细胞系序列而不是先前人源化的序列。具体地说,这是通过使用 IGHV3-21*01 重链序列的 VH3 构架设计重链来进行。

[0252] 与 L1 相比, L3 具有 6 个点突变,其中使 RW 突变(即,置换正电荷残基, R 变成 L)成 LL(使用 LL 是因为 LL 在生殖细胞系序列中比 LW 更常见),且用 Jk4 序列(VEIK;SEQ ID NO:253)置换 J κ (Jk) 区的一部分(LQIT;SEQ ID NO:252)。Jk4 是基于 Cris-7H_CDR3 序列的更佳匹配的 Jk 区。参见图 6。再次说明,仅在构架区中进行突变,且所引入的新残基经验证存在于生殖细胞系序列中可避免免疫原性风险的位置。

[0253] 通过以产生比 DRA209 和 DRA161 低的 pI 的方式对轻链序列进行“再人源化”(与 DRA209 和 DRA161 相比)来设计 L4。使用 IGKV3-11*01 轻链序列的 Vk3 的构架作为引导序列对 L4 进行再人源化。

[0254] 基于理论计算,预期所有新的轻链和重链都具有较低 pI。使用载体 NTI(Invitrogen)帮助将这些序列放在一起(连接这些序列)。生殖细胞系序列是获自 NCBI 网站(<http://www.ncbi.nlm.nih.gov/igblast/>)。

[0255] 构建以其他方式与 DRA209 和 DRA161 同一的 SMIP 分子的前铰链区中包含经取代的氨基酸的变体。前铰链区是连接结合域与铰链区的短氨基酸序列,例如,可以由添加限制性位点到编码核苷酸序列中而产生。

[0256] 所制造的一种变体涉及通过 PCR 突变发生用 SSS 置换 DRA209 的前铰链区中的 RRT 序列。有意这样做以去除两个带正电的残基(精氨酸)。实验显示,这个变化被翻译为蛋白质的 pI 降低。这个构建体仍具有 H3L1 组合且给予其新名称 DRA219。除了去除带正电的残基(两个精氨酸)以外,这种 SSS 突变还引入了允许简单组装较新的 pI 变体构建体的 XhoI 限制位点。

[0257] 使用标准分子生物学技术,使用相同的 Fc 尾部作为 DRA209,使用 H 与 L 的以下组合来构建 SMIP 构建体:a)H4L1(具有 IgG4AA ADCC-CDC null Fc;在本文中也称为 DRA222;SEQ ID NO:7 和 8)、b)H4L3(具有 IgG4AA ADCC-CDC null Fc;在本文中也称为 DRA221;SEQ ID NO:9 和 10)、c)H3L3(具有 IgG4AA ADCC-CDC null Fc;在本文中也称为 DRA223;SEQ ID NO:11 和 12)、d)H5L4(具有 IgG4AA ADCC-CDC null Fc;在本文中也称为 DRA224;SEQ ID NO:13 和 14)和 e)H6L4(具有 IgG4AA ADCC-CDC null Fc;在本文中也称为 DRA225;SEQ ID NO:15 和 16)。参见图 7A 和 7B。

[0258] 将所有这些构建体转染到 HEK 细胞中以产生蛋白质以便用于体外结合研究。所有构建体都表达所要蛋白质,除了 DRA225 构建体,它不具有明显表达。发现所有其它构建体都(i)以不同程度结合 CD3(DRA222 在一定程度上具有最佳结合)和(ii)具有比母体分子 DRA209 低的 pI。测量 pI 可在表 4 中获得。

[0259] 表 4:测量 pI

[0260]

构建体	测量 pI
DRA209	9.0
DRA219	8.4
DRA221	8.2
DRA222	7.5
DRA223	7.2
DRA224	6.8

[0261] DRA219、DRA221、DRA222、DRA223 和 DRA224 都抑制人混合的淋巴细胞反应 (MLR ; 数据未示出), 且抑制的量与所测量的其 CD3 结合能力相关。

[0262] DRA219(H3L1IgG4AA ADCC-CDC null FC ;SEQ ID NO :5 和 6) 和 DRA222(SEQ ID NO : 7 和 8) 都保持结合 CD3 且具有较低的测量 pI, 且将其进一步克隆到编码不同的 Fc 尾部的 pEE12.4 载体 (IgG4AAN297A) 中以便再产生两个构建体, 分别是 DRA233(H3L1 ;SEQ ID NO : 17 和 18) 和 DRA234(H4L1 ;SEQ ID NO :19 和 20)。此外, 将 DRA219 和 DRA222 克隆到 pEE12.4 载体中以便在 CHO 中表达, 且分别命名为 DRA228 和 DRA229。实质上, DRA219 等效于 DRA228 且 DRA222 等效于 DRA229。随后将所有构建体 (DRA228、DRA229、DRA233 和 DRA234) 都转染到 CHO 细胞中以产生蛋白质用于结合研究、蛋白质表征和 PK 研究。参见图 8。

[0263] 通过蛋白 A 亲和色谱法, 使用 AKTA 纯化器 HPLC 系统从细胞上清液中纯化出蛋白质。将预填充 POROS A 柱 (Life Technologies) 用于体外制剂, 且将预填充 MabSuRe 蛋白 A 柱 (GE Healthcare) 用于体内材料。在 PBS 存在下装载样品且用柠檬酸盐缓冲液洗提。用 TRIS 缓冲液中和洗提液。

[0264] 在 Beckman PA 800 仪器上进行毛细管等电聚焦且用于测定纯化蛋白质的等电点 (pI)。根据标准 Beckman cIEF 方案进行分析。在 3M 尿素于 cIEF 凝胶 (Beckman) 中的溶液中制备样品, 该溶液补充有 Pharmalyte 3-10 两性电解质 (GE Healthcare)、阴极和阳极稳定剂及合成肽 pI 标记物 (Beckman)。将样品引入到中性毛细管 (Beckman) 中, 然后将毛细管入口浸没在阳极电解液中、出口浸没在阴极电解液中且对毛细管应用 25kV 电位持续 15 分钟进行聚焦。在聚焦之后, 通过化学手段使样品流通以使所有分离的组分都通过测量 280nm 下的吸收率的固定波长检测器。根据由合成肽 pI 标记物的结果构建的标准曲线计算所有样品的等电点 (pI)。参见图 9-11。构建体具有以下测量 pI :DRA228 = 8.4 ; DRA229 = 7.5 ;DRA233 = 8.6 ;DRA234 = 8.0, 而 DRA161 = 9.2 且 DRA209 = 8.8。因此, 表达的所有 pI 变体都具有比其对应的母体构建体 DRA161 或 DRA209 低的 pI。

[0265] 实施例 2 :抗 CD3 SMIP 分子结合 T 细胞

[0266] 使用标准密度梯度离心从新鲜全血中分离人周围血液单核细胞 (PBMC), 且在含 0.2% BSA 的 PBS 中洗涤三次。将细胞以 200,000 个 PBMC/ 孔接种在 96 孔 U 形底板中且在冰上用 50ul 抗体以如图 12 中所指示的 nM 浓度标记 30 分钟。将板洗涤三次且用含有最佳

浓度的抗 CD5-APC 加 F(ab')₂ 山羊抗 hu IgG Fc-PE 的 50 μ l 抗体混合液标记 30 分钟。将板洗涤三次且在 120 μ l 含 1% 聚甲醛的 PBS 中在 4℃ 下固定过夜。所有抗体孵育和洗涤都是在冰上用冷 PBS、0.2% BSA、2nM EDTA 进行。通过流式细胞术,使用 BD LSRII 分析器分析样品。从各孔中收集 60 微升。使用 Flowjo 软件,通过闸选 T 细胞 (FCS 对 SSC, CD5+ 细胞) 来分析所收集的样品。PE 通道上的平均荧光强度 (MFI) 示于图中。DRA227SMIP 仅用作对照且不含 CD3 或 T 细胞结合域,并且未进行用于降低 pI 的修饰。参见图 12。结合 CD3 的所有测试 pI 变体及 DRA228 和 DRA229 都保留结合活性,非常类似于它们的母构建体。

[0267] 实施例 3:具有降低的 pI 的抗 CD3 SMIP 分子在 CHO 细胞中表达得更好

[0268] 尺寸排除色谱法:使用尺寸排除色谱法评估经纯化蛋白质的高分子量聚集物含量。HPLC 系统是装备有四元泵、溶剂脱气器、DAD 和温度控制自动取样器的 Agilent 1200 系列。使用在 200mM 磷酸钾和 250mM 氯化钾的运作缓冲液 (pH 7.2) 中平衡的 Tosoh TSKgel G3000SWx1 柱进行色谱分离。溶剂流速是 1mL/ 分钟且样品注入质量是 50 μ g。用 Agilent DAD 检测器在 280nm 波长下监测柱流出物。

[0269] SDS-PAGE:使用变性十二烷基硫酸钠聚丙烯酰胺凝胶电泳 (SDS-PAGE) 来评估经纯化蛋白质的完整性。用 Novex 样品缓冲液 (Invitrogen) 制备样品,且在装载到 4-20% Novex Tris- 甘氨酸凝胶 (Invitrogen) 中之前,在 85℃ 下加热 3 分钟以确保完全变性。在加热步骤之前,任选地通过添加 Novex 还原剂还原样品。通过在填充有 Tris- 甘氨酸缓冲液 (Invitrogen, 来自于 10X 储备液) 的电泳室中在凝胶剂上应用 125V 持续 110 分钟来使蛋白质流通并加以分离。通过用 SimplyBlue Safe Stain (Invitrogen) 染色来观察蛋白质谱带。参见图 13-14。测试 pI 变体显示与其母体分子 DRA161 相比有所减少的剪接。

[0270] CE-SDS:在 Beckman PA 800 仪器上进行在 SDS 中的毛细管电泳,且用来对经纯化蛋白质的低分子量剪接含量进行定量。在 50% SDS 样品缓冲液 (Beckman) 中制备 1mg/mL 浓度的样品,且在分析之前,在 85℃ 下加热 3 分钟以确保完全变性。将样品电动 (10kV, 20s) 注入到填充有 SDS 凝胶缓冲液 (Beckman) 的裸熔融二氧化硅毛细管中,且通过在毛细管 (反极性) 上应用 15kV 电压持续 30 分钟进行分离。在检测器窗上测量 220nm 和 280nm 下的吸收率。参见图 15-16。这显示 pI 变体蛋白质的品质优于母体分子,例如,由于较低表观蛋白水解,在本文中有时称为“剪接”。

[0271] 实施例 4:抗 CD3SMIP pI 变体分子展现增加的半衰期

[0272] 在时间 0 点对雌性 BALB/c 小鼠经静脉内 (IV) 注射含有 200 μ g DRA234 或 DRA233 的 200 μ L PBS。在各时间点对每组三只小鼠进行注射。在注射之后,在所指示的时间点经由心脏穿刺对麻醉的小鼠进行抽血,且如下文所描述收集血清。使用酶联免疫吸附测定 (ELISA) 测定 DRA234 和 DRA233 的血清浓度。通过非区隔分析,使用 WinNonlin™ 专业软件 (v5.3) 且应用针对 IV 快速推注施用和稀疏取样的预编译模型 201 来估计各蛋白质的药物动力学配置参数。

[0273] 在时间 0 点,对小鼠经静脉内注射含有 200 μ g (约 10mg/kg) DRA233 或 DRA234 的 200 μ L PBS。在各时间点对每组三只小鼠进行注射。经由心脏穿刺对麻醉的小鼠进行抽血,且在注射之后在 t = 15 分钟和 2、6、24、48、72、96、168、336 和 504 小时收集血清。使用特异性夹心 ELISA 测定各蛋白质的血清浓度。结果表达为随时间变化平均血清浓度 (μ g/mL) $\pm$ SD。以线性 (A) 和对数 (B) 形式显示数据。参见图 17。

[0274] 使用夹心 ELISA 检测抗药物抗体 (ADA), 其中将 DRA161 (DRA233 和 DRA234 的原始形式) 涂布在板上以俘获 ADA, 且抗小鼠 IgG (H&L) HRP 用于检测结合的 ADA。使用过氧化物酶底物测量结合的复合物, 在荧光板读数器上读取结果。结果表达为平均荧光单位 (FU) 相对于血清稀释度。对于 DRA233, 504 小时处的 ADA 水平清楚可见, 而可能的低水平起始于 336 小时; 然而在任何 DRA234 样品中都未出现 ADA。(数据未示出)。

[0275] 在有和无 504 小时的数据的情况下对所有 DRA233PK 时间点绘制平均血清浓度相对于时间的曲线。DRA234 的血清浓度相对于时间的曲线在图 18C 中可见。结果表达为观测数据集, 且通过 WinNonLin™ 软件计算预计值。Rs_q 值和 Rs_q 调节值是调节估计 HL_{Lambda} z 所使用的点数前后的最终消除阶段的拟合优度统计量。仅使用无可检测水平的抗药物抗体的时间点时, DRA233 的近似半衰期是 95 小时, 且 DRA234 的是 84 小时。参见图 18。

[0276] 来自于 WinNonLin 程序的 PK 估计值 (呈表格形式): 通过使用 WinNonlin™ 专业软件 (v5.3), 应用非区隔分析, 使用针对 IV 快速推注施用的预编译模型 201 (均匀加权) 来进行药物动力学分析。使用线性梯形法则, 利用线性 / 对数内插算法来估计时间 0 点到最后一个可测量浓度 (T_{最后}) 的血清浓度 - 时间曲线下面积 (AUC)。估计以下药物动力学配置参数: 最大观测血清浓度 (C_{max})、最后可测量浓度 (C_{最后}) 下的浓度、达到最大观测血清浓度的时间 (T_{max})、表观最终消除半衰期 (HL_{Lambda} z)、从给药时间到最后一个可测量浓度的 AUC (AUC_总)、从给药时间开始随时间推移到无穷大的 AUC (AUC_{INF_obs})、血清清除率 (Cl_{obs})、基于最终阶段的分布体积 (V_{z_obs}) 和平均滞留时间 (MRT)。参见图 19 和 20。本实施例显示, 测试 pI 变体在小鼠中与母体分子 DRA161 相比具有较长体内血清半衰期和较慢清除率。

[0277] 实施例 5: 双特异性同源二聚体分子 - 抗 CD3 和抗 PSMA

[0278] 使用标准技术构建结合 CD3 和 PSMA 的双特异性同源二聚体。这些构建体, 即 TSC249、TSC250、TSC251、TSC252、TSC295、TSC296、TSC301 和 TSC302 描述于表 5 中。通过用限制酶 HindIII 和 XhoI 或 AgeI 和 XhoI 消化母体模板和 scFv 插入物来实现插入 N 末端 scFv 结合域, 鉴别所要的片段, 且通过琼脂糖凝胶纯化进行分离, 并且连接。通过用限制酶 EcoRI 和 NotI 消化母体模板和 scFv 插入物来实现插入 C 末端 scFv 结合域, 鉴别所要的片段, 且通过琼脂糖凝胶纯化进行分离, 并且连接。

[0279] 表 5: 结合多肽序列和组分

[0280]

名称	SEQ ID NO
人源化TSC249 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv)	SEQ ID NO:56 核酸 (na) SEQ ID NO:57 氨基酸 (aa)
人源化TSC250 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有H81连接子)	SEQ ID NO:58 (na) SEQ ID NO:59 (aa)
人源化 TSC251 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有H83 连接子)	SEQ ID NO:60 (na) SEQ ID NO:61 (aa)
人源化TSC252 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有H91连接子)	SEQ ID NO:62 (na) SEQ ID NO:63 (aa)
人源化TSC295 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有H9连接子)	SEQ ID NO:64 (na) SEQ ID NO:65 (aa)
人源化TSC296 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv 具有H94连接子)	SEQ ID NO:66 (na) SEQ ID NO:67 (aa)
人源化TSC301 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有H105连接子)	SEQ ID NO:68 (na) SEQ ID NO:69 (aa)
人源化 TSC302 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有H106连接子)	SEQ ID NO:70 (na) SEQ ID NO:71 (aa)

[0281] 实施例 6 :双特异性同源二聚体分子 - 抗 CD3 和抗 RON

[0282] 使用标准技术构建具有结合 CD3 和 RON 两者的同源二聚体的双特异性分子。这些构建体,即 TSC275、TSC277、TSC278 和 TSC279,描述于表 6 中。TSC275 含有 hu4C04 的 VL 和 VH 链,分别为 SEQ ID NO:187 和 188,这些链是 RON 结合域的一部分。除了如表 6 中所指示的突变 / 取代以外,TSC277、TSC278 和 TSC279 与 TSC275 相同。

[0283] 表 6 :结合多肽序列和互补序列

[0284]

名称	简单描述	核酸序列 (SEQ ID NO)	氨基酸序列 (SEQ ID NO)
TSC275	hu4C04 x DRA222 null2	185	186
TSC277	hu4C04 (A43K Q240E) x DRA222 null2	189	190
TSC278	hu4C04 (A43T) x DRA222 null2	191	192
TSC279	hu4C04 (Q165R) x DRA222 null2	193	194

[0285] 实施例 7 :双特异性同源二聚体分子 - 抗 CD3 和抗 CD19

[0286] 使用标准技术构建具有结合 CD3 和 CD19 两者的同源二聚体的双特异性分子,例如,参见 PCT 公布号 W02007/146968。这些构建体,即 TSC233、TSC234、TSC235、TSC240、TSC241 和 TSC242,描述于表 7 中。TSC233 含有 HD37 链的 VL (SEQ ID NO:196 的氨基酸 1-111) 和 VH (SEQ ID NO:196 的氨基酸 128-251),这些链是 CD19 结合域的一部分。

[0287] 表 7 :结合多肽序列和组分

[0288]

名称	简单描述	核酸序列 (SEQ ID NO)	氨基酸序列 (SEQ ID NO)
TSC129a	HD37 x Cris7 scorpion, H75 连接子	244	245
TSC233	HD37 x DRA221 scorpion, H75连接子	195	196
TSC234	HD37 x DRA222 scorpion, H75 连接子	197	198
TSC235	HD37 x DRA224 scorpion, H75 连接子	199	200
TSC240	HD37 x DRA222 scorpion, H81连接子	201	202
TSC241	HD37 x DRA222 scorpion, H83连接子	203	204
TSC242	HD37 x DRA222 scorpion, H91连接子	205	206

[0289] 实施例 8 : 双特异性异源二聚体分子 - 抗 CD3 和抗 CD19

[0290] 以类似于如 PCT 公布 W02011/090762 中所描述的方式, 通过对编码如表 8 中所指示的氨基酸序列的核酸进行共同转染来构建包含 CD19 结合域和 CD3 结合域的不同的异源二聚双特异性分子。通过共表达两个不同的多肽链来制造异源二聚体, 一个多肽链包含免疫球蛋白 CH1 异源二聚域且另一个多肽链包含免疫球蛋白 CL 异源二聚域。

[0291] 表 8 : 用于抗 CD3 和抗 CD19 异源二聚体构建的共同转染

[0292]

异源二聚体 名称	各异源二聚体中的 结合域	用于共转染的构建体 (na 和 aa 序列)	
TSC127	Cris 7 scFv x HD37	TSC096 SEQ ID NO : 209 和 210	TSC125 SEQ ID NO : 242 和 243
TSC224	DRA221 scFv* x HD37	TSC096 SEQ ID NO : 209 和 210	TSC218 SEQ ID NO : 246 和 247
TSC225	DRA222 scFv** x HD37	TSC096 SEQ ID NO : 209 和 210	TSC219 SEQ ID NO : 217 和 218
TSC226	DRA224 scFv*** x HD37	TSC096 SEQ ID NO : 209 和 210	TSC220 SEQ ID NO : 219 和 220
TSC227	HD37 X DRA221 scFv*	TSC049 SEQ ID NO : 207 和 208	TSC221 SEQ ID NO : 248 和 249
TSC228	HD37 X DRA222 scFv**	TSC049 SEQ ID NO : 207 和 208	TSC222 SEQ ID NO : 221 和 222
TSC229	HD37 X DRA224 scFv***	TSC049 SEQ ID NO : 207 和 208	TSC223 SEQ ID NO : 223 和 224

[0293] *SEQ ID NO:10 的氨基酸 1-244

[0294] **SEQ ID NO:8 的氨基酸 1-244

[0295] ***SEQ ID NO:14 的氨基酸 1-247

[0296] TSC049 从它的氨基末端到羧基末端包含 :HD37 (抗 CD19) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 CH1。TSC096 从它的氨基末端到羧基末端包含 :HD37 (抗 CD19) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 C κ (YAE)。TSC125 从它的氨基末端到羧基末端包含 :Cris7 (抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 CH1。TSC218 从它的氨基末端到羧基末端包含 :DRA221 (抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 CH1。TSC219 从它的氨基末端到羧基末端包含 :DRA222 (抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 CH1。TSC220 从它的氨基末端

到羧基末端包含 :DRA224(抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 CH1。TSC221 从它的氨基末端到羧基末端包含 :DRA221(抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 C κ (YAE)。TSC222 从它的氨基末端到羧基末端包含 :DRA222(抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 C κ (YAE)。TSC223 从它的氨基末端到羧基末端包含 :DRA224(抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 C κ (YAE)。

[0297] 实施例 9 :双特异性异源二聚体分子 - 抗 CD3 和抗 PSMA

[0298] 以类似于如 PCT 公布 W02011/090762 中所描述的方式,通过对编码如表 9 中所指示的氨基酸序列的核酸进行共同转染来构建包含 PSMA 结合域和 CD3 结合域的不同的异源二聚双特异性分子。通过共表达两个不同的多肽链来制造异源二聚体,一个多肽链包含免疫球蛋白 CH1 异源二聚域且另一个多肽链包含免疫球蛋白 CL 异源二聚域。PCT 公布号 W02011/090761 中描述了一些 PSMA 结合域。

[0299] 表 9 :用于抗 CD3 和抗 PSMA 异源二聚体构建的共转染

[0300]

异源二聚体名称	各异源二聚体中的结合域	用于共转染的构建体 (na 和 aa 序列)	
TSC236	DRA222 scFv* x 人源化 107-1A4 VL- VH#2 scFv**	TSC192 SEQ ID NO:211和212	TSC219 SEQ ID NO:217和218
TSC237	人源化 107-1A4 scFv** x DRA222 scFv*	TSC195 SEQ ID NO:215和216	TSC222 SEQ ID NO:221和222
TSC264	人源化 107-1A4 VL- VH#1 scFv*** x DRA222 scFv*	TSC258 SEQ ID NO:227和228	TSC254 SEQ ID NO:225和226
TSC265	DRA222 scFv* x 人源化 107-1A4 VL- VH#1 scFv**	TSC193 SEQ ID NO:213和214	TSC219 SEQ ID NO:217和218

[0301] *SEQ ID NO:8 的氨基酸 1-244

[0302] **SEQ ID NO:216 的氨基酸 1-243

[0303] ***SEQ ID NO:226 的氨基酸 1-243

[0304] “人源化 107-1A4VL-VH#_2scFv”和“人源化 107-1A4VL-VH#_1scFv”是基于抗人 PSMA 107-1A4 单克隆抗体的人源化 scFv,并且也描述于 PCT 公布号 W02011/090761 中。“人源化 107-1A4VL-VH#2scFv”和“人源化 107-1A4VL-VH#1scFv”分别对应于 SEQ ID NO:216 和 226 的氨基酸 1-243。

[0305] TSC192 从它的氨基末端到羧基末端包含 :人源化 107-1A4(抗 PSMA)VL-VH#2scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 C κ (YAE)。TSC195 从它的氨基末端到羧基末端包含 :人源化 107-1A4(抗 PSMA)VL-VH#2scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 CH1。TSC193 从它的氨基末端到羧基末端包含 :人源化 107-1A4(抗 PSMA)VL-VH#1scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 C κ (YAE)。

[0306] TSC219 从它的氨基末端到羧基末端包含 :DRA222(抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 CH1。TSC222 从它的氨基末端到羧基末端包含 :DRA222(抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3 和人 C κ (YAE)。TSC254 从它的氨基末端到羧基末端包含 :人源化 107-1A4(抗 PSMA)VL-VH#1scFv、人 IgG1SCC-P 铰链、人

IgG1CH2、人 IgG1CH3、连接子和人 CH1。TSC258 从它的氨基末端到羧基末端包含 :DRA222 (抗 CD3) scFv、人 IgG1SCC-P 铰链、人 IgG1CH2、人 IgG1CH3、连接子和人 C κ (YAE)。

[0307] 实施例 10 :靶向 CD19 的多肽异源二聚体和同源二聚体所致的靶依赖性 T 细胞增殖

[0308] 为了比较不同的双特异性多肽异源二聚体分子在诱导靶依赖性 T 细胞活化和增殖方面的效力,比较了三种不同的同源二聚双特异性分子 (TSC129a、TSC233 和 TSC234 (分别是 SEQ ID NO:245、196 和 198)),它们具有共同的抗 CD19 结合域 (HD37) 和三个不同的抗 CD3 结合域 (TSC129a 的是 Cris7, TSC233 的是来自于 DRA221 的 scFv 结合域 (SEQ ID NO:10 的氨基酸 1-244, TSC234 的是来自于 DRA222 的 scFv 结合域 (SEQ ID NO:8 的氨基酸 1-244))。另外,比较了三种不同的异源二聚双特异性分子 (TSC127、TSC227、TSC228),它们具有三个不同的抗 CD3 结合域 (TSC127 的是 Cris7, TSC227 的是来自于 DRA221 的 scFv 结合域 (SEQ ID NO:10 的氨基酸 1-244),且 TSC228 的是来自于 DRA222 的 scFv 结合域 (SEQ ID NO:8 的氨基酸 1-244))。

[0309] 从 ATCC (Manassas, VA) 获得 Daudi 伯基特氏淋巴瘤细胞 (CD19+) 且根据所提供的方案进行培养。使用标准聚蔗糖梯度从人血液分离周围血液单核细胞 (PBMC)。在生理盐水缓冲液中洗涤分离的细胞。另外使用得自于 Miltenyi Biotec (Bergisch Gladbach, Germany) 的淘选 T 细胞分离试剂盒 II,使用制造商方案从这些 PBMC 中分离 T 细胞。

[0310] 通过用羧基荧光素二乙酸琥珀酰亚胺酯 (CFSE) 标记分离的 T 细胞群体来评估增殖。将经 CFSE 标记的 T 细胞以 100,000 个 T 细胞 / 孔与 33,000 个肿瘤细胞 / 孔接种在 U 形底 96 孔板中,以实现 T 细胞对肿瘤细胞比率为大约 3:1。将浓度在 1nM 到 5fM 范围内的测试分子加入到总共 200 μ L / 孔补充有 10% 人或牛血清、丙酮酸钠和非必需氨基酸的 RPMI 1640 培养基中的细胞混合物中。在湿润孵育器中在 37 $^{\circ}$ C、5% CO $_2$ 下对板进行孵育。3 天之后,用抗体标记细胞以便进行流式细胞术分析。标记细胞且在其原板中洗涤以便将转移期间的细胞损失最小化,且在含 0.2% 牛血清白蛋白的生理盐水缓冲液中进行所有标记。首先,在室温下将细胞与 100 μ g/ml 人 IgG 一起预孵育 15 分钟。随后,将细胞与经以下染料标记的抗体的混合物 (总体积 50 μ L) 一起孵育 :CD5-PE、CD4-APC、CD8-太平洋蓝、CD25-PE-Cy7,以及 7-氨基放线菌素 D (此后为 7AAD),持续 40 分钟。将板洗涤两次,再悬浮于 80 到 120 μ L 体积中且立即在 BD LSRII 流式细胞仪上运作以获取各孔的 80% 内含物。使用 FlowJo 软件分析样品文件以便根据其 CFSE 曲线通过按顺序筛选活化的活 CD4 $^{+}$ 或 CD8 $^{+}$ T 细胞 (分别是 7AAD $^{-}$ 、CD5+CD25+CD4 $^{+}$ 或 7AAD-CD5+CD25+CD8 $^{+}$) 来计算进行了至少一次细胞分裂的细胞的百分比和数目。使用 Microsoft Excel 软件计算平均值和标准偏差。使用 Microsoft Excel 或 Graphpad Prism 绘制曲线图。

[0311] 对来自于经完整 T 细胞处理的 Daudi 细胞的活 CD4 $^{+}$ 和 CD8 $^{+}$ 群体的分析显示,在展示靶 CD19 抗原的 Daudi 细胞存在下,细胞总数和增殖细胞百分比都显著增加 (图 21 和 22)。

[0312] 对于同源二聚双特异性多肽 (TSC129a、TSC233、TSC234),在 CD4 $^{+}$ 和 CD8 $^{+}$ 细胞之间的增殖相当 (图 21)。TSC129a 和 TSC234 诱导的增殖在 CD4 $^{+}$ 和 CD8 $^{+}$ 细胞之间相当 (图 21),表明来自于 DRA222 的 pI 变体抗 CD3scFv 结合域与母体 Cris7 结合域具有实质上等效的活性。TSC233 诱导的 T 细胞增殖曲线与 TSC129a 和 TSC234 稍微不同,其中诱导的 CD4 $^{+}$

增殖减少(图 21A)而诱导的 CD8+ 增殖稍微增加(图 21B)。这表明来自于 DRA221 的 pI 变体抗 CD3scFv 结合域在活性方面与母体 Cris7 结合域不完全等效。

[0313] 对于异源二聚双特异性多肽(TSC127、TSC227、TSC228),在 Daudi 细胞存在下,CD8+T 细胞的增殖高于 CD4+T 细胞(图 22)。TSC127 和 TSC228 诱导的增殖在 CD4+ 和 CD8+ 细胞之间相当(图 22),表明来自于 DRA222 的 pI 变体抗 CD3scFv 结合域与母体 Cris7 结合域具有实质上等效的活性。TSC227 诱导的 T 细胞增殖曲线与 TSC127 和 TSC228 稍微不同,其中诱导的 CD4+ 增殖减少而诱导的 CD8+ 增殖相当。再次,这表明来自于 DRA221 的 pI 变体抗 CD3scFv 结合域在活性方面与母体 Cris7 结合域不完全等效。

[0314] 实施例 11:靶向 CD19 的多肽异源二聚体和同源二聚体所致的重定向 T 细胞细胞毒性

[0315] 为了比较不同的双特异性多肽异源二聚体分子在诱导靶依赖性 T 细胞细胞毒性方面的效力,比较了三种不同的同源二聚双特异性分子(TSC129a、TSC233 和 TSC234(分别是 SEQ ID NO:245、196 和 198)),它们具有共同的抗 CD19 结合域(HD37)和三个不同的抗 CD3 结合域(TSC129a 的是 Cris7, TSC233 的是来自于 DRA221 的 scFv 结合域(SEQ ID NO:10 的氨基酸 1-244, TSC234 的是来自于 DRA222 的 scFv 结合域(SEQ ID NO:8 的氨基酸 1-244))。另外,比较了三种不同的异源二聚双特异性分子(TSC127、TSC227、TSC228),它们具有三个不同的抗 CD3 结合域(TSC127 的是 Cris7, TSC227 的是来自于 DRA221 的 scFv 结合域(SEQ ID NO:10 的氨基酸 1-244),且 TSC228 的是来自于 DRA222 的 scFv 结合域(SEQ ID NO:8 的氨基酸 1-244))。

[0316] 从 ATCC(Manassas, VA) 获得 Daudi 伯基特氏淋巴瘤细胞(CD19+)且根据所提供的方案进行培养。使用标准蔗糖梯度从人血液中分离周围血液单核细胞(PBMC)且在生理盐水缓冲液中洗涤。另外使用得自于 Miltenyi Biotec(Bergisch Gladbach, Germany) 的淘选 T 细胞分离试剂盒 II,使用制造商方案从 PBMC 中分离 T 细胞。

[0317] 通过 ^{51}Cr 释放测定评估细胞毒性。用 0.3mCi ^{51}Cr 处理大约 5×10^6 个 Daudi 细胞,且在 37°C 下孵育 75 分钟。在 75 分钟之后,用培养基(RPMI+10% FBS)将细胞洗涤 3 次,且再悬浮于 11.5mL 培养基中。由这一悬浮液以每孔 50 μL 分配到 96 孔 U 形底板中(大约 20,000 个细胞/孔)。将浓度在 500pM 到 0.1pM 范围内的双特异性分子加入 Daudi 细胞,使总体积达到 100 μL /孔。在室温下将靶细胞孵育 15 分钟。然后加入 100 μL 分离的 T 细胞(大约 200,000 个)以使 T 细胞对靶细胞比率达到 10:1。将 50 μL 0.8% NP-40 加入含有靶细胞的对照孔,静置 15 分钟,然后加入 100 μL 培养基以提供完全溶解对照。

[0318] 将板孵育 4 小时,然后以 1500rpm 旋转 3 分钟,且从各孔转移 25 μL 上清液到 96 孔 Luma 样品板的对应孔中。允许样品板在化学安全罩中风干 18 小时,然后在 Topcount 闪烁计数器上使用标准方案读取放射性。

[0319] 对细胞毒性数据的分析示出了在 T 细胞和抗 CD19 定向双特异性分子存在下用 Daudi 细胞得到的 T 细胞定向细胞毒性(图 23)。

[0320] 对于同源二聚双特异性多肽(TSC129a、TSC233、TSC234),TSC129a 与 TSC234 之间的观测细胞毒性是相当的(图 23A),表明该 DRA222pI 变体与母体 Cris7 结合域具有等效活性。在 TSC233 存在下观测到细胞毒性稍微下降,表明该 DRA221pI 变体的活性低于母体 Cris7 结合域。

[0321] 对于异源二聚双特异性多肽 (TSC127、TSC227、TSC228), TSC127 与 TSC228 之间的观测细胞毒性是相当的 (图 23B), 表明该 DRA222scFv 结合域 pI 变体与母体 Cris7 结合域具有等效活性。在 TSC227 存在下观测到细胞毒性稍微下降, 表明该 DRA221scFv 结合域 pI 变体的活性低于母体 Cris7 结合域。

[0322] 实施例 12: 靶向 RON 的多肽同源二聚体所致的重定向 T 细胞细胞毒性

[0323] 为了比较不同的双特异性多肽异源二聚体分子在诱导靶依赖性 T 细胞细胞毒性方面的效力, 比较了四种不同的同源二聚双特异性分子 (TSC275、TSC277、TSC278 和 TSC279), 它们具有共同的抗 CD3 结合域 (来自于 DRA222 的 scFv 结合域 (SEQ ID NO:8 的氨基酸 1-244)) 和同一抗 RON 结合域的四个变体 (TSC275 的是 hu4C04scFv, TSC277 的是 hu4C04scFv (A43K/Q240E), TSC278 的是 hu4C04scFv (A43T), 且 TSC279 的是 hu4C04scFv (Q165R))。

[0324] 从 ATCC (Manassas, VA) 获得 MDA-MB-453 乳腺癌细胞 (RON+) 且根据 ATCC 所提供的方案进行培养。使用标准聚蔗糖梯度从人血液中分离周围血液单核细胞 (PBMC) 且在生理盐水缓冲液中洗涤。另外使用得自于 Miltenyi Biotec (Bergisch Gladbach, Germany) 的淘选 T 细胞分离试剂盒 II, 使用制造商方案从 PBMC 中分离 T 细胞。

[0325] 通过 ^{51}Cr 释放测定评估细胞毒性。用 $0.3\text{mCi } ^{51}\text{Cr}$ 处理大约 5×10^6 个 Daudi 细胞, 且在 37°C 下孵育 75 分钟。在 75 分钟之后, 用培养基 (RPMI+10% FBS) 将细胞洗涤 3 次, 且再悬浮于 11.5mL 培养基中。由这一悬浮液以每孔 $50\ \mu\text{L}$ 分配到 96 孔 U 形底板中 (大约 20,000 个细胞/孔)。将浓度在 10nM 到 0.005fM 范围内的双特异性分子加入 Daudi 细胞, 使总体积达到 $100\ \mu\text{L}$ /孔。在室温下将靶细胞孵育 15 分钟。然后加入 $100\ \mu\text{L}$ 分离的 T 细胞 (大约 100,000 个) 以使 T 细胞对靶细胞比率达到 5:1。将 $50\ \mu\text{L}$ 0.8% NP-40 加入含有靶细胞的对照孔, 静置 15 分钟, 然后加入 $100\ \mu\text{L}$ 培养基以提供完全溶解对照。

[0326] 将板孵育 24 小时, 然后以 1500rpm 旋转 3 分钟, 且从各孔转移 $25\ \mu\text{L}$ 上清液到 96 孔 Luma 样品板的对应孔中。允许样品板在化学安全罩中风干 18 小时, 然后在 Topcount 闪烁计数器上使用标准方案读取放射性。

[0327] 对细胞毒性数据的分析示出了在 T 细胞和抗 RON 定向双特异性分子存在下用 MDA-MB-453 细胞得到的 T 细胞定向细胞毒性 (图 24)。

[0328] 对于同源二聚双特异性多肽 (TSC275、TSC277、TSC278 和 TSC279), 观测细胞毒性一般相当 (图 24), 表明 ORN196 结合域内的突变不显著影响总体活性。然而, 针对 TSC278 观测到的最大溶解稍微低于其它三种变体 (图 24)。在 10pM 与 10nM 的浓度之间观测到了最大溶解。

[0329] 实施例 13: 双特异性多肽异源二聚体和同源二聚体的 T 细胞结合

[0330] 为了比较以 pI 变体 CD3 结合域 (DRA222 中发现的 scFv (SEQ ID NO:8 的氨基酸 1-244)) 为特征的双特异性多肽分子在结合 T 细胞方面的效力, 在两个独立的实验中比较了抗 CD19 $\times$ 抗 CD3 双特异性多肽异源二聚体 TSC228 (如实施例 8, 表 8 中所描述) 的细胞上结合特征与抗 PSMA $\times$ 抗 CD3 双特异性多肽同源二聚体 TSC249 (如实施例 5, 表 5 中所描述) 的细胞上结合特征。

[0331] 从 ATCC (Manassas, VA) 获得 Jurkat (CD3 $^{+}$) T 细胞白血病细胞且根据 ATCC 提供的方案进行培养。通过在 4°C 下将 5×10^5 个 Jurkat 细胞与连续稀释的双特异性分子 TSC228 或

TSC249 一起以 250nM 到 0.125nM(TSC249) 或 125nM 到 1pM(TSC228) 的浓度孵育 30 分钟来评估结合。将细胞洗涤三次,然后与山羊抗人 IgG-FITC(1:200 稀释度)一起在 4℃下再孵育 30 分钟。然后将细胞再洗涤三次,在 1%聚甲醛中固定且在 FACS-Calibur 仪器上读数。

[0332] 在 FlowJo v7.5(Tree Star, Inc., Ashland, OR) 中对高 FSC、高 SSC 子集进行的分析显示了双特异性分子 TSC228 和 TSC249 与 Jurkat 细胞的剂量依赖性结合(分别是图 25A 和 B)。仅拥有一个抗 CD3 结合域的 TSC228 双特异性多肽异源二聚体在结合时的表观亲和力($EC_{50} = 24.5nM$) 低于拥有两个抗 CD3 结合域的 TSC249 双特异性多肽同源二聚体($EC_{50} = 1.9nM$)。这表明 TSC249 双特异性多肽异源二聚体具有一定的结合亲合力。

[0333] 实施例 14:靶向 PSMA 的双特异性多肽同源二聚体所致的靶依赖性 T 细胞增殖

[0334] 为了测定不同的双特异性多肽异源二聚体分子在诱导靶依赖性 T 细胞活化和增殖方面的效力,在不存在靶细胞的情况下,在表达 PSMA 的前列腺癌细胞系、不表达 PSMA 的前列腺癌细胞系和 T 细胞上测试具有抗 PSMA 结合域(hu107-1A4)和抗 CD3 结合域(来自于 DRA222 的 scFv(SEQ ID NO:8 的氨基酸 1-244))的同源二聚双特异性分子(TSC249,参见实施例 5,表 5)。

[0335] 从马里兰安德森癌症中心(Houston, TX)获得 C4-2B 前列腺癌细胞(PSMA+)且根据所提供的方案进行培养。从 ATCC(Manassas, VA)获得 DU-145 前列腺癌细胞(PSMA-)且根据所提供的方案进行培养。使用标准聚蔗糖梯度从人血液中分离周围血液单核细胞(PBMC)且进一步在生理盐水缓冲液中洗涤。然后使用得自于 Miltenyi Biotec(Bergisch Gladbach, Germany)的淘选 T 细胞分离试剂盒 II,使用制造商方案从这些 PBMC 中分离 T 细胞。

[0336] 通过用羧基荧光素二乙酸琥珀酰亚胺酯(CFSE)标记分离的 T 细胞群体来评估增殖。同时建立三个单独的实验。将经 CFSE 标记的 T 细胞仅与 TSC249 分子混合;将经 CFSE 标记的 T 细胞与 DU-145 细胞和 TSC249 分子混合;和将经 CFSE 标记的 T 细胞与 C4-2B 细胞和 TSC249 分子混合。将经 CFSE 标记的 T 细胞以 100,000 个细胞/孔与 33,000 个肿瘤细胞/孔接种在 U 形底 96 孔板中,以实现 T 细胞对肿瘤细胞比率大约为 3:1。将浓度在 20nM 到 5fM 范围内的测试分子加入到总共 200uL/孔的补充有 10%人或牛血清、丙酮酸钠和非必需氨基酸的 RPMI 1640 培养基中的细胞混合物中。在湿润孵育器中在 37℃、5% CO₂下对板进行孵育。3 天之后,用抗体标记细胞以便进行流式细胞术分析。标记细胞且在其原板中洗涤以便将转移期间的细胞损失最小化,且在含 0.2%牛血清白蛋白的生理盐水缓冲液中进行所有标记。首先,在室温下将细胞与 100ug/ml 人 IgG 一起预孵育 15 分钟。随后,将细胞与经以下染料标记的抗体的混合物(总体积 50uL)一起孵育:CD5-PE、CD4-APC、CD8-太平洋蓝、CD25-PE-Cy7,以及 7-氨基放线菌素 D(此后为 7AAD),持续 40 分钟。将板洗涤两次,再悬浮于 80 到 120uL 体积中且立即在 BD LSRII 流式细胞仪上运作以获取各孔的 80%内含物。使用 FlowJo 软件分析样品文件以便根据其 CFSE 曲线通过按顺序筛选活化的活 CD4+ 或 CD8+T 细胞(分别是 7AAD-、CD5+CD25+CD4+ 或 7AAD-CD5+CD25+CD8+)来计算进行了至少一次细胞分裂的细胞的百分比和数目。使用 Microsoft Excel 软件计算平均值和标准偏差。使用 Microsoft Excel 或 Graphpad Prism 绘制曲线图。

[0337] 对来自于经完整 T 细胞处理的 C4-2B 细胞的活 CD4+ 和 CD8+ 群体的分析显示,在用 TSC249 处理后表达靶 PSMA 抗原的 C4-2B 细胞存在下,细胞总数显著增加(图 26A)。在

用 TSC249 处理之后不表达靶 PSMA 抗原的 DU-145 细胞存在下未见到这种增加（图 26B）。最后，当用 TSC249 处理分离的 T 细胞时也未见到这种增加（图 26C）。这证实了 TSC249 在表达靶抗原的肿瘤细胞存在下诱导的 T 细胞增殖的靶依赖性。

[0338] 实施例 15：体内测试 CD3 和 PSMA 的双特异性靶向

[0339] 为了证实抗 CD3 和抗 PSMA 双特异性分子在体内抑制肿瘤生长方面的效力，可以使用以下任何一者或多者来评估 PSMA 定向分子。

[0340] 对皮下肿瘤的肿瘤移植的预防性处理或预防：将所培养的表达式 PSMA 的肿瘤细胞系（诸如 LNCaP、LNCaP C4-2、LNCaP C4-2B、VCaP、CWR22Rv1、LAPC4、MDA-PCa-2b、LuCaP 23.1、LuCaP 58、LuCaP 70、LuCaP 77）与人淋巴细胞（人周围血液单核细胞或纯化的 T 细胞）混合，且经皮下注射到免疫缺陷小鼠（诸如 SCID、NOD/SCID 等）中。在注射当天和随后的若干天经静脉内注射抗 PSMA 双特异性分子。如通过肿瘤体积所评估，对肿瘤长出的剂量依赖性抑制指示对应的分子对体内表达式 PSMA 的肿瘤具有功效。

[0341] 对先前确定的皮下肿瘤的治疗性治疗或消退：将所培养的表达式 PSMA 的肿瘤细胞系（诸如 LNCaP、LNCaP C4-2、LNCaP C4-2B、VCaP、CWR22Rv1、LAPC4、MDA-PCa-2b、LuCaP 23.1AI、LuCaP 58、LuCaP 70、LuCaP 77）经皮下注射到免疫缺陷小鼠（诸如 SCID、NOD/SCID 等）中。监测肿瘤生长，且当肿瘤显示明确生长（典型地，体积为约 200mm³）的迹象时开始研究。在注射当天，经静脉内注射人淋巴细胞（人周围血液单核细胞或纯化的 T 细胞）以及抗 PSMA 双特异性分子。随后的若干天注射抗 PSMA 双特异性分子。如通过肿瘤体积所评估，对肿瘤生长的剂量依赖性抑制指示对应的分子对体内表达式 PSMA 的肿瘤具有功效。

[0342] 对胫骨内肿瘤的肿瘤移植的预防性治疗或预防：将所培养的表达式 PSMA 的肿瘤细胞系（诸如 LNCaP C4-2、LNCaP C4-2B、VCaP、CWR22Rv1、LAPC4、MDA-PCa-2b、LuCaP 23.1、LuCaP 58、LuCaP70、LuCaP 77）与人淋巴细胞（人周围血液单核细胞或纯化的 T 细胞）混合，且经胫骨内注入免疫缺陷小鼠（诸如 SCID、NOD/SCID 等）中。在注射当天和随后的若干天经静脉内注射抗 PSMA 双特异性分子。如通过血清生物标记物、射线照相术、荧光成像、体重损失和肿瘤体积的其它委托测量值所评估，对肿瘤生长的剂量依赖性抑制指示对应的分子对体内表达式 PSMA 的肿瘤具有功效。

[0343] 对先前确定的胫骨内肿瘤的治疗性治疗或消退：将所培养的表达式 PSMA 的肿瘤细胞系（诸如 LNCaP C4-2、LNCaP C4-2B、VCaP、CWR22Rv1、LAPC4、MDA-PCa-2b、LuCaP 23.1AI、LuCaP 58、LuCaP70、和 LuCaP 77）经胫骨内注射到免疫缺陷小鼠（诸如 SCID、NOD/SCID 等）中。监测肿瘤生长，且当肿瘤显示明确生长（典型地，体积为约 200mm³）的迹象时开始研究。在注射当天，经静脉内注射人淋巴细胞（人周围血液单核细胞或纯化的 T 细胞）以及抗 PSMA 双特异性分子。随后的若干天注射抗 PSMA 双特异性分子。如通过血清生物标记物、射线照相术、荧光成像、体重损失和肿瘤体积的其它委托测量值所评估，对肿瘤生长的剂量依赖性抑制指示对应的分子对体内表达式 PSMA 的肿瘤具有功效。

[0344] 实施例 16：靶向 PSMA 的多肽异源二聚体和同源二聚体所致的重定向 T 细胞细胞毒性

[0345] 为了比较不同的双特异性多肽异源二聚体分子在诱导靶依赖性 T 细胞细胞毒性方面的效力，比较了两种不同的同源二聚双特异性分子（TSC194、TSC249），它们具有共同的抗 PSMA 结合域（hu107-1A4）和两个不同的抗 CD3 结合域（TSC194 的是 Cris7scFV，TSC249

的是来自于 DRA222 的 scFv)。TSC194 是同源二聚双特异性的 (huVL-VH#2107-1A4scFv-Fc-Cris7scFv) (SEQ ID NO:250 和 251)。

[0346] 从马里兰安德森获得 C4-2B 细胞 (PSMA+) 且根据公开的培养条件在 RPMI-1640 培养基 (Life Technologies, Carlsbad, CA) 加 10% FBS 中进行培养。使用标准聚蔗糖梯度从人血液中分离周围血液单核细胞 (PBMC) 且在生理盐水缓冲液中洗涤。另外使用得自于 Miltenyi Biotec (Bergisch Gladbach, Germany) 的淘选 T 细胞分离试剂盒 II, 使用制造商方案从 PBMC 中分离 T 细胞。

[0347] 通过 ^{51}Cr 释放测定评估细胞毒性。用 0.3mCi ^{51}Cr 处理大约 5×10^6 个 C4-2 细胞, 且在 37°C 下孵育 75 分钟。在 75 分钟之后, 用培养基 (RPMI+10% FBS) 将细胞洗涤 3 次, 且再悬浮于 11.5mL 培养基中。由这一悬浮液以每孔 50 μL 分配到 96 孔 U 形底板中 (大约 20,000 个细胞 / 孔)。将浓度在 500pM 到 0.2pM 范围内的双特异性分子加入 C4-2 细胞, 使总体积达到 100 μL / 孔。在室温下将靶细胞孵育 15 分钟。然后加入 100 μL 分离的 T 细胞 (大约 200,000 个) 以使 T 细胞对靶细胞比率达到 10:1。将 50 μL 0.8% NP-40 加入含有靶细胞的对照孔, 静置 15 分钟, 然后加入 100 μL 培养基以提供完全溶解对照。

[0348] 将板孵育 4 小时, 然后以 1500rpm 旋转 3 分钟, 且从各孔转移 25 μL 上清液到 96 孔 Luma 样品板的对应孔中。允许样品板在化学安全罩中风干 18 小时, 然后再 Topcount 闪烁计数器上使用标准方案读取放射性。

[0349] 对细胞毒性数据的分析示出了在 T 细胞和抗 PSMA 定向双特异性分子存在下用 C4-2 细胞得到的 T 细胞定向细胞毒性 (图 27)。对于同源二聚双特异性多肽 (TSC194、TSC249), TSC194 与 TSC249 之间的观测细胞毒性是相当的 (表 10), 表明该 DRA222 抗 CD3scFv 结合域 pI 变体与母体 Cris7 结合域具有等效活性。

[0350] 表 10 : 观测到的细胞毒性

[0351]

	RTCC 的 EC_{50}
TSC194	$2.8 \pm 0.6\text{pM}$
TSC249	$2.9 \pm 1.0\text{pM}$

[0001]

序列表

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Natarajan, Sateesh K
Tan, Philip H
McMahan, Catherine J

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<150> US 61/718,635

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tccitgaagg cttctggcta cacctttact agatctacga tgcactgggt aaggcaggcc 120
cctggaaagg gtctggaatg gattggatac attaatccta gcagtgetta tactaattac 180
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[0003]

```

ggcagtgggt ctgggaccga ctataccctc acaatcagca gcctgcagcc cgaagatttc      660
gccacttatt actgccagca gtggagtcgt aaccaccca cgttcggagg ggggaccaag      720
ctacaaattt cagcagcaac tgagcccaaa tcttctgaca aaactcaaac atgccaccgc      780
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1              5              10              15

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Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser

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[0004]

20	25	30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile		
35	40	45
Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe		
50	55	60
Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe		
65	70	75
80		
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys		
85	90	95
Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly		
100	105	110
Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly		
115	120	125
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln		
130	135	140
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr		
145	150	155
160		
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys		
165	170	175
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala		
180	185	190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr		

[0005]

195	200	205
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210	215	220
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225	230	235 240
Leu Gln Ile Thr Arg Arg Thr Glu Pro Lys Ser Ser Asp Lys Thr His		
245	250	255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe		
260	265	270
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro		
275	280	285
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val		
290	295	300
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr		
305	310	315 320
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val		
325	330	335
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys		
340	345	350
Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser		
355	360	365
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro		

[0006]

370	375	380
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val		
385	390	395 400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly		
	405	410 415
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp		
	420	425 430
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp		
	435	440 445
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His		
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caggtccagc tgggtcagtc tgggggcgga gtggtgcagc ctgggcggtc actgaggetg 60

tcttgcaagg ctctggeta cacttttact agatctacga tgcactgggt aaggcaggcc 120

cetggaaagg gtctggaatg gattggatac attaatecta gcagtgetta tactaattac 180

aatcagaaat tcaaggacag gttcacaatc agcgcagaca aatccaagag cacagccttc 240

[0007]

ctgcagatgg acagcctgag gcccaggagac accggcgctct atttctgtgc acggccccaa	300
gtccactatg attacaacgg gtttccttac tggggccaag ggactcccggt cactgtctct	360
agcggtggeg gagggtcttg gggtagcgga tccggagggt gtagctctgc acaagacatc	420
cagatgaccc agtctccaag cagcctgtct gcaagcgtag gggacagggt caccatgacc	480
tgcagtcca getcaagtgt aagttacatg aactggtagc agcagaagcc gggcaaggcc	540
cccaaaagat ggatttatga ctcatccaaa ctggctcttg gagtccctgc tcgtttcagt	600
ggcagtgggt ctgggaccga ctatacctc acaatcagca gcctgcagcc cgaagatttc	660
gccacttatt actgccagca gtggagtcgt aaccaccca cgttcggagg ggggaccaag	720
ctacaaatta catctcgag cgagcccaaa tcttctgaca aaetcacac atgcccaccg	780
tgccagcac ctgaagcgc agctccgtca gtcttctct tcccccaaa acccaaggac	840
acctcatga tctcccgac cctgaggtc acgtgcgtg tggtagcgt gagccaggaa	900
gaccccgagg tccagttcaa ctggtacgtg gatggcgtag aggtgcataa tgccaagaca	960
aagccgggg aggagcagtt caacagcacg taccgtgtg tcagcgtcct caccgtcctg	1020
caccaggact ggtgaacgg caaggcatac gcctgcgtg tctccaaca aggctcccg	1080
tcctccatg agaaaacct ctccaaagcc aaagggcagc cccgagagcc acagggtgac	1140
acctgcctc catccagga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc	1200
aaaggttct accccagcga catcgccgtg gtagggaga gcaatgggca gccggagaac	1260
aactacaaga ccaagcctcc cgtgctggac tccagggct ctttcttct ctacagcagg	1320
ctaaccgtg acaagagccg gtggcaggag gggaatgtt tctcatgctc cgtgatgcat	1380
gaggtctctg acaaccacta cacacagaag agcctctccc tgtctccggg taaa	1434

<210> 6

<211> 478

<212> PRT

[0008]

<213> 人工序列

<220>

<223> DRA219 (H3 L1 IgG4 AA ADCC-CDC null Fc)

<400> 6

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr

[0009]

145	150	155	160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys			
165	170	175	
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala			
180	185	190	
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr			
195	200	205	
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr			
210	215	220	
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys			
225	230	235	240
Leu Gln Ile Thr Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His			
245	250	255	
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe			
260	265	270	
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro			
275	280	285	
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val			
290	295	300	
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr			
305	310	315	320
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val			

[0010]

	325	330	335
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys			
	340	345	350
Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser			
	355	360	365
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro			
	370	375	380
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val			
385	390	395	400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly			
	405	410	415
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp			
	420	425	430
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp			
	435	440	445
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His			
	450	455	460
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys			
465	470	475	
<210>	7		
<211>	1434		
<212>	DNA		
<213>	人工序列		

[0011]

<220>

<223> DRA222 (H4 L1 具有 IgG4 AA ADCC-CDC null Fc)

<400> 7

caggteccagc tgggtggagtc tgggggcgga gtggtgcagc ctgggcggtc actgaggetg	60
tcctgcaagg ctcttggeta cacctttact agatctacga tgcactgggt aaggcaggec	120
cctggacaag gtctggaatg gattggatac attaatceta gcagtgetta tactaattac	180
aatcagaaat tcaaggacag gttcacaatc agcgcagaca aatccaagag cacagccttc	240
ctgcagatgg acagcctgag gcccgaggac accggcgtct attcttgtgc acggccccaa	300
gtccactatg attacaacgg gtttctttac tggggccaag ggactccctg cactgtctct	360
agcgggtggcg gaggtcttgg ggggtggcgga tccggaggtg gtggctctgc acaagacatc	420
cagatgaccc agtctccaag cagcctgtct gcaagcgtgg gggacagggt caccatgacc	480
tgcagtgcc a gctcaagtgt aagttacatg aactggfacc agcagaagcc gggcaaggcc	540
cccaaaagat ggatttatga ctctccaaa ctggcttctg gagtccctgc tgccttcagt	600
ggcagtgggt ctgggaccga ctataccctc acaatcagca gcctgcagcc cgaagatttc	660
gccacttatt actgccagca gtggagtcgt aaccacacca cgttcggagg ggggaccaag	720
ctacaaatta cctctcagag cgagcccaaa tcttctgaca aaactcacac atgcccaccg	780
tgcccagcac ctgaagccgc agtccctca gtcttctct tcccccaaa acccaaggac	840
accctcatga tctcccgac cctgaggtc acgtgcgtgg tgggtgaagt gagccaggaa	900
gaccccgagg tccagttcaa ctggtacgtg gatggcgtgg aggtgcataa tgccaagaca	960
aagccgcggg aggagcagtt caacagcacg taccgtgtgg tcagcgctct caccgtcttg	1020
caccaggact ggctgaacgg caaggcatac gcctgcgtct tctccaacaa aggcctcccc	1080
tcctccatcg agaaaacat ctccaaagcc aaagggcagc cccgagagcc acaggtgtac	1140
accctgcccc catcccagga ggagatgacc aagaaccagg tcagcctgac ctgcctggte	1200

[0012]

```

aaaggtttct accccagcga catcgccgtg gagtgggaga gcaatgggca gccggagaac      1260
aactacaaga ccacgcctec cgtgctggac tccgacgget ccttcttcct ctacagcagg      1320
ctaaccgtgg acaagagcgg gtggcaggag gggaatgtct tctcatgctc cgtgatgcat      1380
gaggtctctgc acaaccacta cacacagaag agcctctccc tgtctccggg taaa          1434

```

<210> 8

<211> 478

<212> PRT

<213> 人工序列

<220>

<223> DRA222 (H4 L1 具有 IgG4 AA ADCC-CDC null Fc)

<400> 8

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1              5              10              15

```

```

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
          20              25              30

```

```

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
          35              40              45

```

```

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
          50              55              60

```

```

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65              70              75              80

```

```

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
          85              90              95

```

```

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly

```

[0013]

100	105	110
Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly		
115	120	125
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln		
130	135	140
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr		
145	150	155 160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys		
165	170	175
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala		
180	185	190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr		
195	200	205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr		
210	215	220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys		
225	230	235 240
Leu Gln Ile Thr Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His		
245	250	255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe		
260	265	270
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro		

[0014]

275	280	285
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val		
290	295	300
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr		
305	310	315 320
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val		
325	330	335
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys		
340	345	350
Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser		
355	360	365
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro		
370	375	380
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val		
385	390	395 400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly		
405	410	415
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp		
420	425	430
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp		
435	440	445
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His		

[0015]

450

455

460

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys

465

470

475

<210> 9

<211> 1434

<212> DNA

<213> 人工序列

<220>

<223> DRA221 (H4 L3 具有 IgG4 AA ADCC-CDC null Fc)

<400> 9

caggtcacgc tggtagagtc tgggggcgga gtggtgcagc ctgggcggtc actgaggctg 60

tcctgcaagg ctcttggtta cacccttact agatctacga tgcactgggt aaggcaggcc 120

cctggacaag gtctggaatg gattggatac attaatccta gcagtgccta tactaattac 180

aatcagaaat tcaaggacag gtteacaate agcgcagaca aatccaagag cacagccttc 240

ctgcagatgg acagcctgag gcccgaggac accggcgtct atttctgtgc acggccccc 300

gtccactatg attacaacgg gtctccttac tggggccaag ggactccgt cactgtctct 360

agcgggtggcg gaggtcttgg ggttggcgga tccggaggtg gtggtcttgc acaagacatc 420

cagatgacc agtctccaag cagcctgtct gcaagcgtgg gggacagggt caccatgacc 480

tgcagtgcga gctcaagtgt aagttacatg aactggtacc agcagaagcc gggcaaggcc 540

cccaaaactc tcatttatga ctcatccaaa ctggtctctg gactccctgc tgccttcagt 600

ggcagtgggt ctgggaccga ctataccctc acaatcagca gctgcagcc cgaagatttc 660

gccacttatt actgccagca gtggagtcgt aaccaccca ctctcggcgg agggaccaag 720

gtggagatca aatctctgag cgagcccaaa tcttctgaca aaatcacac atgcccaccg 780

tgcccagcac ctgaagccgc agctccgtca gtcttctct tcccccaaa acccaaggac 840

[0016]

```

acctcatga tctcccgac cctgaggtc acgtgcgtgg tggtagacgt gagccaggaa      900
gaccccgagg tccagttcaa ctggtacgtg gatggcgtgg aggtgcataa tgccaagaca      960
aagccgcggg aggagcagtt caacagcacg taccgtgtgg tcagcgtcct caccgtectg     1020
caccaggact ggctgaacgg caaggcatac gcctgcgtcg tctccaacaa aggcctcccg     1080
tcttccatcg agaaaacat ctccaaagcc aaagggcagc cccgagagcc acaggtgtac     1140
accttgcctc catcccagga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc     1200
aaaggcttct accccagcga catcgccgtg gagtgggaga gcaatgggca gccggagAAC     1260
aactacaaga ccacgectcc cgtgctggac tccgacggct ccttcttctt ctacagcagg     1320
ctaaccgtgg acaagagccg gtggcaggag gggaatgtct tctcatgctc cgtgatgcat     1380
gaggctctgc acaaccaata cacacagaag agcctctccc tgtctccggg taaa          1434

```

<210> 10

<211> 478

<212> PRT

<213> 人工序列

<220>

<223> DRA221 (H4 L3 具有 IgG4 AA ADCC-CDC null Fc)

<400> 10

```

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1              5              10              15

```

```

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
              20              25              30

```

```

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
              35              40              45

```

```

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe

```

[0017]

50	55	60
Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe		
65	70	75 80
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys		
	85	90 95
Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly		
	100	105 110
Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly		
	115	120 125
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln		
	130	135 140
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr		
145	150	155 160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys		
	165	170 175
Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Ser Ser Lys Leu Ala		
	180	185 190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr		
	195	200 205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr		
210	215	220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys		

[0018]

225	230	235	240
Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His			
245	250	255	
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe			
260	265	270	
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro			
275	280	285	
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val			
290	295	300	
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr			
305	310	315	320
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val			
325	330	335	
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys			
340	345	350	
Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser			
355	360	365	
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro			
370	375	380	
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val			
385	390	395	400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly			

[0019]

	405	410	415
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp			
420	425	430	
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp			
435	440	445	
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His			
450	455	460	
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys			
465	470	475	

<210> 11

<211> 1434

<212> DNA

<213> 人工序列

<220>

<223> DRA223 (H3 L3 具有 IgG4 AA ADCC-CDC null Fc)

<400> 11

caggteccagc tgggtgcagtc tgggggcgga gtggtgcagc ctgggcgggc actgaggetg	60
tectgcaagg cttetggcta cacctttact agatctacga tgcactgggt aaggeaggcc	120
cctggaaagg gtctggaatg gattggatac attaattcta gcagtgetta tactaattac	180
aatcagaaat tcaaggacag gttcacaate agcgcagaca aatccaagag cacagecttc	240
ctgcagatgg acagcctgag gcccgaggac accggcgtct atttctgtgc acggccccaa	300
gtccactatg attacaacgg gtttccttac tggggccaag ggactcccggt cactgtctct	360
agcgggtggcg gagggctctgg gggtagcgga tccggagggt gtggctctgc acaagacatc	420
cagatgacct agtctccaag cagcctgtct gcaagcgtgg gggacagggt caccatgacc	480

[0020]

tgcagtgcc a gctcaagtgt aagttacatg aacitgtacc agcagaagcc gggcaaggcc	540
cccaaactcc tcatttatga ctcatccaaa ctggcttctg gagtcctgc tcgcttcagt	600
ggcagtgggt ctgggaccga ctataccctc acaatcagea gctgcagcc cgaagatttc	660
gccacttatt actgccagea gtggagtcgt aaccacacca ctctcgccgg agggaccaag	720
gtggagatca aatctctgag cgagcccaaa tcttctgaca aaactcacac atgcccaccg	780
tgccagcac ctgaagccgc agctccgtca gtcttctct tcccccaaa acccaaggac	840
acctcatga tctcccgac cctgaggtc acgtgcgtgg tggtagcgt gagccaggaa	900
gaccccgagg tccagttcaa ctggtagctg gatggcgtgg aggtgcataa tgccaagaca	960
aagcccgagg aggagcagtt caacagcacg taccgtgtgg tcagcgtct caccgtctg	1020
caccaggact ggctgaacgg caaggcatat gcctgcgtg tctccaaca aggctcccg	1080
tcttccatcg agaaaacct ctccaaagcc aaagggcagc ccgagagcc acaggtgtac	1140
acctgcccc catcccagga ggagatgacc aagaaccagg tcagcctgac ctgcttggtc	1200
aaaggtttct accccagega catcgccgtg gagtgggaga gcaatgggca gccggagaa	1260
aactacaaga ccacgcctc cgtgtggac tccagcggt cctcttctc ctacagcagg	1320
ctaaccgtgg acaagagccg gtggcaggag gggaatgtct tctcatgtc cgtgatgat	1380
gaggtctgc acaaccacta cacacagaag agcctctccc tgtctccggg taaa	1434

<210> 12

<211> 478

<212> PRT

<213> 人工序列

<220>

<223> DRA223 (H3 L3 具有 IgG1 AA ADCC-CDC null Fc)

<400> 12

Gln Val Gln Leu Val Gln Ser Gly Gly Val Val Gln Pro Gly Arg

[0021]

1	5	10	15
Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser			
20	25	30	
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile			
35	40	45	
Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe			
50	55	60	
Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe			
65	70	75	80
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys			
85	90	95	
Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly			
100	105	110	
Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly			
115	120	125	
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln			
130	135	140	
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr			
145	150	155	160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys			
165	170	175	
Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Ser Ser Lys Leu Ala			

[0022]

	180		185		190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr					
	195		200		205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr					
	210		215		220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys					
225		230		235	240
Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His					
	245		250		255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe					
	260		265		270
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro					
	275		280		285
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val					
	290		295		300
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr					
305		310		315	320
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val					
	325		330		335
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys					
	340		345		350
Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser					

[0023]

	355		360		365
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro					
370		375		380	
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val					
385		390		395	400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly					
	405		410		415
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp					
	420		425		430
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp					
	435		440		445
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His					
	450		455		460
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys					
465		470		475	

<210> 13

<211> 1443

<212> DNA

<213> 人工序列

<220>

<223> DRA224 (H5 L4 具有 IgG4 AA ADCC-CDC null Fc)

<400> 13

caggctccagc ttgtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaagggt 60

tcttgcaagg cttctggata caccttcact agatctacga tgcactgggt gcgccaggcc 120

[0024]

cccgacaaa ggcttgagtg gatgggatac attaatccia gcagtgccta tactaattac	180
aatcagaaat tcaaggacag agtcaccatt accagggaca catccgcgag cacagcctac	240
atggagctga gcagccfgag atctgaagac acggctgtgt attactgtgc gagaccccaa	300
gtccactatg attacaacgg gtttccttac tggggccaag gaacctggt caccgtctcc	360
tcaggatggag gcggttcagg cggaggtgga tccggcgggt gcggatcggg tggcggcgga	420
tctgaaattg tgttgacaca gtctccagcc acctgtctt tgtctccagg ggaaagagcc	480
acctctctct gcagtgcag ctcaagtga agttacatga actggtaeca acagaaacct	540
ggccaggtct ccaggtctct catctatgac tcatccaaac tggtttcttg catccagcc	600
aggttcagtg gcagtgggtc tgggacagac ttactctca ccatcagcag cctagagcct	660
gaagattttg cagtttatta ctgtcagcag tggagtcgta acccaccac ttccggcgga	720
gggaccaagg tggagatcaa atctcagagc gagcccaaat ctctgacaa aactcacaca	780
tgcccaccgt gccagcacc tgaagccgca gctccgtcag tcttctctt cccccaaaa	840
cccaaggaca cctcatgat ctcccgacc cctgaggta cgtgcgtggt ggtggacgtg	900
agccaggaag accccgaggt ccagttcaac tggtagctgg atggcgtgga ggtgcataat	960
gccaagacaa agcccgggga ggagcagttc aacagcacgt accgtgtggt cagcgtctc	1020
accgtctctg accaggactg gctgaacggc aaggcatacg cctgcgctgt ctccaacaaa	1080
ggctctccgt cctcatcga gaaaaccate tccaaagcca aagggcagcc ccgagagcca	1140
caggtgtaca ccctgcccc atcccaggag gagatgacca agaaccaggt cagcctgacc	1200
tgcctggta aagcttcta cccagcgac atcgccgtgg agtgggagag caatgggcag	1260
ccggagaaca actacaagac cagcctccc gtgttggaact ccgacggctc ctctctctc	1320
tacagcaggc taacctgga caagagccgg tggcaggagg ggaatgtctt ctcatgtctc	1380
gtgatgcatg aggtctctga caaccactac acacagaaga gctctctct gtctccgggt	1440

[0025]

aaa

1443

<210> 14

<211> 481

<212> PRT

<213> 人工序列

<220>

<223> DRA224 (H5 L4 具有 IgG4 AA ADCC-CDC null Fc)

<400> 14

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

[0026]

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val
130 135 140

Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala
145 150 155 160

Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr
165 170 175

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ser Ser
180 185 190

Lys Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
195 200 205

Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala
210 215 220

Val Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly
225 230 235 240

Gly Thr Lys Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp
245 250 255

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro
260 265 270

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
275 280 285

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp
290 295 300

[0027]

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
305 310 315 320

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val
325 330 335

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala
340 345 350

Tyr Ala Cys Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
355 360 365

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
370 375 380

Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
385 390 395 400

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
405 410 415

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
420 425 430

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys
435 440 445

Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu
450 455 460

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
465 470 475 480

[0028]

Lys

<210> 15

<211> 1443

<212> DNA

<213> 人工序列

<220>

<223> DRA225 (H6 L4 具有 IgG4 AA ADCC-CDC null Fc)

<400> 15

gaggtgcagc tgggtggagtc tgggggagge ctggtcaagc ctgggggggc cctgagaetc	60
tectgtgcag cctctggatt caccctcagt agatctacga tgcactgggt ccgccagget	120
ccagggaagg ggctggagtg ggtctctatc attaatccta gcagtgccta tactaattac	180
aatcagaaat tcaaggaccg attcaccatc tccagagaca acgccaagaa ctcactgtat	240
ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagaccccaa	300
gtccactatg attacaacgg gtttccttac tggggccaag gaacctgggt caccgtctcc	360
tcaggtggag gcggttcagg cggaggtgga tccggcgggt gcggatcggg tggcggcgga	420
tctgaaattg tgttgacaca gtctccagcc accctgtctt tgtctccagg ggaaagagcc	480
accctctcct gcagtgccag ctcaagtgtg agttacatga actggtacca acagaaacct	540
ggccaggctc ccaggtcct catctatgac tcatccaaac tggtctctgg catcccagcc	600
aggttcagtg gcagtgggtc tgggacagac ttactctca ccatcagcag cctagagcct	660
gaagattttg cagtttatta ctgtcagcag tggagtcgta acccaccac tttcggcgga	720
gggaccaagg tggagatcaa atcctcgagc gagcccaaat cttctgacaa aactcacaca	780
tgcccaccgt gcccagcacc tgaagccgca gctccgtcag tcttctcttt cccccaaaa	840
cccaggaca cctcatgat ctcceggacc cctgaggtea cgtgcgtggg ggtggacgtg	900

[0029]


```

agccaggaag accccgaggt ccagttcaac tggtagtgg atggcgtgga ggtgcataat      960
gccaagacaa agcccgggga ggagcagttc aacagcacgt accgtgtggt cagcgtctc      1020
accgtccitge accaggactg getgaacggc aaggcaiaeg cctgcgtgt cttcaacaaa      1080
ggcctcccgt cctccatega gaaaaccatc tccaaagcca aagggcagcc ccgagagcca      1140
caggtgtaca cctgcccc atcccaggag gagatgacca agaaccaggt cagcctgacc      1200
tgcttggtca aaggtttcta ccccagcgac atgcctgtg agtgggagag caatgggcag      1260
ccggagaaca actacaagac cagccctccc gtgtggact ccgacggctc cttcttctc      1320
tacagcaggc taaccgtgga caagagccgg tggcaggagg ggaatgtctt ctcctgctcc      1380
gtgatgcatt aggtcttgea caaccactac acacagaaga gcctctccct gtctccgggt      1440
aaa                                                                    1443

```

<210> 16

<211> 481

<212> PRT

<213> 人工序列

<220>

<223> DRA225 (H6 L4 具有 IgG4 AA ADCC-CDC null Fc)

<400> 16

```

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1           5           10          15

```

```

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Ser
          20          25          30

```

```

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
          35          40          45

```

```

Ser Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe

```

[0030]

50	55	60
Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr		
65	70	75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys		
	85	90 95
Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly		
	100	105 110
Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly		
	115	120 125
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val		
	130	135 140
Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala		
	145	150 155 160
Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr		
	165	170 175
Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ser Ser		
	180	185 190
Lys Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly		
	195	200 205
Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala		
	210	215 220
Val Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly		

[0031]

225	230	235	240
Gly Thr Lys Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp			
245	250	255	
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro			
260	265	270	
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser			
275	280	285	
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp			
290	295	300	
Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn			
305	310	315	320
Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val			
325	330	335	
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala			
340	345	350	
Tyr Ala Cys Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys			
355	360	365	
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr			
370	375	380	
Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr			
385	390	395	400
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu			

[0032]

	405	410	415
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu			
	420	425	430
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys			
	435	440	445
Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu			
	450	455	460
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly			
	465	470	475
			480

Lys

<210> 17

<211> 1434

<212> DNA

<213> 人工序列

<220>

<223> DRA233 (H3 L1, 具有 IgG4 AA N297A 突变 - Fc 尾不同于顶部构筑体)

<400> 17

caggtccagc tgggtgcagtc tgggggcgga gtggtgcagc ctgggcggtc actgaggetg	60
tcctgcaagg cttctggcta cacctttact agatctacga tgcactgggt aaggcaggcc	120
cctggaaagg gtciggaatg gattggatac attaatccta gcagtgetta tactaattac	180
aatcagaaat tcaaggacag gttcacaatc agcgcagaca aatccaagag cacagccttc	240
ctgcagatgg acagcctgag gcccgaggac accggcgtct atttctgtgc acggccccaa	300
gtccactatg attacaacgg gtttccttac tggggccaag ggactcccg t cactgtctct	360

[0033]

agcgggtggcg gaggggtctgg gggtagcgga tccggaggtg gtggetctgc acaagacatc	420
cagatgaccc agtciccaaag cagcctgtct gcaagcgigg gggacagggt caccatgacc	480
tgcagtgcca gctcaagtgt aagttacatg aactggtagc agcagaagcc gggcaaggcc	540
cccaaaagat ggattttatga ctcatccaaa ctggcttctg gagtccttgc tcgcttcagt	600
ggcagtgggt ctgggacoga ctataccctc acaatcagca gccctgcagcc cgaagatttc	660
gccacttatt actgccagca gtggagtcgt aaccaccca cgttcggagg ggggaccaag	720
ctacaaatta catccctgag cgagcccaaa lcttctgaca aaactcacac atgccacccg	780
tgcccagcac ctgaagccgc agctccgtca gtcttctctt tcccccaaa acccaaggac	840
accctcatga tctcccgac cctgaggtc acgtgcgtgg tggtagcgt gagccaggaa	900
gaccccgagg tccagttaa ctggtacgtg gatggcgtgg aggtgcataa tgccaagaca	960
aagccgcggg aggagcagtt cggcagcacg taccgtgigg tcagcgtcct caccgtctg	1020
caccaggact ggetgaacgg caaggagtac aagtgcagg tctccaacaa aggcctcccg	1080
tcttccatcg agaaaaccat ctccaaagcc aaagggcage cccgagagcc acaggtgtac	1140
accctgcccc catcccagga ggagatgacc aagaaccagg tcagcctgac ctgcctggtc	1200
aaaggcttct accccagcga catcgccgtg gactgggaga gcaatgggca gccggagAAC	1260
aactacaaga ccacgcctcc cgtgcctggac tccgacggt ctttcttct ctacagcagg	1320
ctaaccgtgg acaagagccg gtggcaggag gggaatgtct tctcatgtct cgtgatgcac	1380
gaggetctgc acaaccaata cacacagaag agcctctccc tgtctccggg taaa	1434

<210> 18

<211> 478

<212> PRT

<213> 人工序列

<220>

<223> DRA233 (H3 L1, 具有 IgG4 AA N297A 突变 - Fc 尾不同于顶部构筑体)

[0034]

<400> 18

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr
145 150 155 160

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys

[0035]

	165		170		175
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala					
	180		185		190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr					
	195		200		205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr					
	210		215		220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys					
225		230		235	240
Leu Gln Ile Thr Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His					
	245		250		255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe					
	260		265		270
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro					
	275		280		285
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val					
	290		295		300
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr					
305		310		315	320
Lys Pro Arg Glu Glu Gln Phe Ala Ser Thr Tyr Arg Val Val Ser Val					
	325		330		335
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys					

[0036]

340	345	350
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser		
355	360	365
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro		
370	375	380
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val		
385	390	395
		400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly		
	405	410
		415
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp		
	420	425
		430
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp		
	435	440
		445
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His		
	450	455
		460
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		
465	470	475
<210> 19		
<211> 1434		
<212> DNA		
<213> 人工序列		
<220>		
<223> DRA234 (H4 L1, 具有 IgG4 AA N297A 突变 - Fc 尾与 DRA233 相同)		
<400> 19		

[0037]

cagggtccagc tgggtggagtc tgggggcgga gtggtgcagc ctgggcggtc actgaggctg	60
tcctgcaagg ctcttggeta cacettctact agatctacga tgcactgggt aaggcaggcc	120
cctggacaag gtctggaatg gattggatac attaatccta gcagtgetta tactaattac	180
aatcagaaat tcaaggacag gttcacaatc agcgcagaca aatccaagag cacagccttc	240
ctgcagatgg acagcctgag gcccgaggac accggcgtct atttctgtgc acggccccaa	300
gtccactatg attacaacgg gtttccctac tggggccaag ggactcccggt cactgtctct	360
agcggtaggc gagggctctg gggtagcgga tccggaggig gtggctctgc acaagacatc	420
cagatgaccc agtctccaag cagcctgtct gcaagcgtgg gggacagggt caccatgacc	480
tgcagtgcga gtccaagtgt aagttacatg aactggtacc agcagaagcc gggcaaggcc	540
cccaaaagat ggatttatga ctatecaaaa ctggcttctg gagtccctgc tcgcttcagt	600
ggcagtgggt ctgggaccga ctataccctc acaatcagca gcctgcagcc cgaagatttc	660
gccacttatt actgccagca gtggagtcgt aaccaccca cgttcggagg ggggaccaag	720
ctacaaatta catectcgag cgagcccaaa tcttctgaca aaactcacac atgcccaccg	780
tgcccagcac ctgaagccgc agctccgtca gtcttctct tcccccaaa acccaaggac	840
accctcatga tctcccgac cctgaggtc acgtgcgtgg tggtagcgt gagccaggaa	900
gaccccgagg tccagttcaa ctggtacgtg gatggcgtgg aggtgcataa tgccaagaca	960
aagccgcggg aggagcagtt cgcagcacg taccgtgtgg tcagcgtctt caccgtctg	1020
caccaggact ggctgaacgg caaggagtac aagtgaagg tctccaacaa aggcctccc	1080
tcctccatcg agaaaaccat ctccaaagcc aaagggcagc cccgagagcc acagggttac	1140
acctgcccc catcccagga ggagatgacc aagaaccagg tcagcctgac ctgcttggtc	1200
aaaggcttct accecagcga catcgccgtg gagggggaga gcaatgggca gccggagaac	1260
aactacaaga ccacgcctcc cgtgctggac tccgacggct ccttcttct ctacagcagg	1320

[0038]

ctaaccgtgg acaagagccg gtggcaggag gggaatgtct tctcatgctc cgtgatgcac 1380

gaggctctgc acaaccacta cacacagaag agcctctccc tgtctccggg taaa 1434

<210> 20

<211> 478

<212> PRT

<213> 人工序列

<220>

<223> DRA234 (H4 L1, 具有 IgG4 AA N297A 突变 - Fc 尾与 DRA233 相同)

<400> 20

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly

[0039]

115	120	125
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln		
130	135	140
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr		
145	150	155
		160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys		
165	170	175
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala		
180	185	190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr		
195	200	205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr		
210	215	220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys		
225	230	235
		240
Leu Gln Ile Thr Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His		
245	250	255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe		
260	265	270
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro		
275	280	285
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val		

[0040]

290	295	300
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr		
305	310	315 320
Lys Pro Arg Glu Glu Gln Phe Ala Ser Thr Tyr Arg Val Val Ser Val		
325	330	335
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys		
340	345	350
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser		
355	360	365
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro		
370	375	380
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val		
385	390	395 400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly		
405	410	415
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp		
420	425	430
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp		
435	440	445
Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His		
450	455	460
Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		

[0041]

465	470	475
<210> 21		
<211> 363		
<212> DNA		
<213> 人工序列		
<220>		
<223> H1 链		
<400> 21		
cagggtccagc tggatgcagtc tggggctgaa gtgaagaagc ctggggcctc agtgaagggtg		60
tcttgcaagg cttctggcta cacctttact agatctacga tgcactgggt aaaacaggcc		120
cctggacagg gtctggaatg gattggatac attaatccta gcagtgetta tactaattac		180
aatcagaaat tcaaggacaa ggccacattg actgcagaca aatcctccag tacagcctac		240
atgcaactga gtagcctgag gtctgaggac accgcagtct attactgtgc acggccccaa		300
gtccactatg attacaacgg gtttccttac tggggccaag ggactctggt cactgtctct		360
agc		363
<210> 22		
<211> 121		
<212> PRT		
<213> 人工序列		
<220>		
<223> H1 链		
<400> 22		
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala		
1 5 10 15		
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser		
20 25 30		

[0042]

Thr Met His Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
 50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 23

<211> 363

<212> DNA

<213> 人工序列

<220>

<223> H2 链

<400> 23

cagggtccagc tgggtgcagtc tgggggcgga gtggtgcagc ctgggcggtc actgaggctg 60

tcctgcaagg cttctggcta caccitttact agatctacga tgcactgggt aaggcaggcc 120

cctggaaaagg gtctggaatg gatiggatac attaatecta gcagtgttta tactaattac 180

aatcagaaat tcaaggacaa ggccacattg actgcagaca aatccaagaa cacagcctac 240

atggagctga gtagectgag gtctgaggac accgcagtct attactgtgc acggccceaa 300

[0043]

gtccactatg attacaacgg gtttccttac tggggccaag ggactctggc cactgctctc 360

agc 363

<210> 24

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> H2 链

<400> 24

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Lys Asn Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser

[0044]

115

120

<210> 25

<211> 363

<212> DNA

<213> 人工序列

<220>

<223> H3 链

<400> 25

cagggtccagc tgggtgcagtc tgggggcgga gtgggtgcagc ctgggcggtc actgaggetg 60

tcctgcaagg cttctggcta caccittact agatctacga tgcactgggt aaggcaggcc 120

cctggaaagg gtctggaatg gattggatac attaatecta gcagtgccta tactaattac 180

aatcagaaat tcaaggacag gttcacaatc agcgcagaca aatccaagag cacagccttc 240

ctgcagatgg acagectgag gcccgaggac accggcgtct atttetgtgc acggccccaa 300

gtccactatg attacaacgg gtctecttac tggggccaag ggactcccgt cactgtctct 360

agc 363

<210> 26

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> H3 链

<400> 26

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg

1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser

20 25 30

[0045]

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser
115 120

<210> 27

<211> 363

<212> DNA

<213> 人工序列

<220>

<223> H4 链

<400> 27

caggteccagc tgggtggagtc tgggggcgga gtggtgcagc ctgggcgggc actgaggctg 60

tcctgcaagg ettcaggcta caccittact agatctacga tgcactgggt aaggcaggcc 120

cctggacaag gtctggaatg gattggatac attaatecta gcagtgetta tactaattac 180

aatcagaaat tcaaggacag gttcacaatc agcgcagaca aatccaagag cacagccttc 240

ctgcagatgg acagcctgag gcccgaggac accggcgtct atttctgtgc acggccccaa 300

[0046]

gtccactatg attacaacgg gtttccttac tggggccaag ggactcccg t cactgctct 360

agc 363

<210> 28

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> H4 链

<400> 28

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser

[0047]

115

120

<210> 29

<211> 363

<212> DNA

<213> 人工序列

<220>

<223> H5 链

<400> 29

cagggtccagc ttgtgcagtc tggggctgag gtgaagaagc ctggggccctc agtgaagggt 60

tcctgcaagg cttctggata cacccttact agatctacga tgcactgggt gcgccaggcc 120

cccggacaaa ggcttgagtg gatgggatac attaatccta gcagtgccta tactaattac 180

aatcagaaat tcaaggacag agtcaccatt accagggaca catccgcgag cacagcctac 240

atggagctga gcagcctgag atctgaagac acggetgtgt attactgtgc gagaccccaa 300

gtccactatg attacaacgg gtctcttacc tggggccaag gaaccttggt caccgtctcc 360

tca 363

<210> 30

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> H5 链

<400> 30

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala

1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser

20 25 30

[0048]

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
 35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
 50 55 60

Lys Asp Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 31

<211> 363

<212> DNA

<213> 人工序列

<220>

<223> H6 链

<400> 31

gaggtgcagc tgggtggagtc tggggggaggc ctgggtcaagc ctgggggggtc cctgagactc 60

tcctgtgcag cctctggatt cacccttcagt agatctacga tgcactgggt cegccagget 120

ccagggaagg ggctggagtg ggtctcatac attaatecta gcagtgtta tactaattac 180

aatcagaaat tcaaggaccg attcaccatc tccagagaca acgccaagaa ctcaactgtat 240

ctgcaaatga acagcctgag agccgaggac acggctgtgt attactgtgc gagaccccaa 300

[0049]

gtccactatg attacaacgg gtttccttac tggggccaag gaacctggt caccgtctcc 360

tca 363

<210> 32

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> H6 链

<400> 32

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser

[0050]

115

120

<210> 33

<211> 324

<212> DNA

<213> 人工序列

<220>

<223> L1 链

<400> 33

gcacaagaca tccagatgac ccagtcacca agcagcctgt ctgcaagcgt gggggacagg 60

gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgaactggta ccagcagaag 120

ccgggcaagg cccccaaaag atggatttat gactcatcca aactggettc tggagtcctt 180

gtctgcttca gtggcagtgg gtctgggacc gactataccc tcacaatcag cagcctgcag 240

cccgaagatt tcgccactta ttactgccag cagtggagtc gtaaccacc cagcttcgga 300

ggggggacca agctacaaat taca 324

<210> 34

<211> 108

<212> PRT

<213> 人工序列

<220>

<223> L1 链

<400> 34

Ala	Gln	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser
1					5				10					15	

Val	Gly	Asp	Arg	Val	Thr	Met	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val	Ser
				20				25						30	

Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Arg	Trp

[0051]

45

109

<213> 人工序列

<220>

<223> L2 链

<400> 36

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
1 5 10 15

Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser
20 25 30

Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp
35 40 45

Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro
85 90 95

Pro Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
100 105

<210> 37

<211> 324

<212> DNA

<213> 人工序列

<220>

<223> L3 链

<400> 37

[0053]


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gcacaagaca tccagatgac ccagtcctcca agcagcctgt ctgcaagcgt gggggacagg      60
gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgaactggta ccagcagaag      120
ccgggcaagg cccccaaet cctcatttat gactcatcca aactggcttc tggagtcct      180
gtctgcttca gtggcagtgg gtctgggacc gactataccc tcacaatcag cagcctgcag      240
cccgaagatt tcgccactta ttactgccag cagtggagtc gtaaccacc cactttcggc      300
ggagggacca aggtggagat caaa      324

```

<210> 38

<211> 108

<212> PRT

<213> 人工序列

<220>

<223> L3 链

<400> 38

```

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
1           5           10           15

```

```

Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
          20           25           30

```

```

Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu
          35           40           45

```

```

Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
          50           55           60

```

```

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65           70           75           80

```

```

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro

```

[0054]

85

90

95

Pro Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys

100

105

<210> 39

<211> 318

<212> DNA

<213> 人工序列

<220>

<223> L4 链

<400> 39

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 60

ctctctctgca gtgccagetc aagtgttaagt tacatgaact ggtaccaaca gaaacctggc 120

caggtccca ggctctctcat ctatgactca tccaaactgg cttctggcat cccagccagg 180

ttcagtggca gtgggtctgg gacagacttc actctcacca tcagcagcct agagcctgaa 240

gattttgcag ttattactg tcagcagtgg agtcgtaacc caccacttt cggcggaggg 300

accaaggtgg agatcaaa 318

<210> 40

<211> 106

<212> PRT

<213> 人工序列

<220>

<223> L4 链

<400> 40

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly

1

5

10

15

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met

[0055]

	20	25	30
Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr			
	35	40	45
Asp Ser Ser Lys Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser			
	50	55	60
Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu			
	65	70	75 80
Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr			
	85	90	95
Phe Gly Gly Gly Thr Lys Val Glu Ile Lys			
	100	105	

<210> 41
<211> 244
<212> PRT
<213> 人工序列

<220>
<223> DRA209 结合域

<400> 41

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg			
1	5	10	15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser			
20	25	30	

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile			
35	40	45	

[0056]

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr
145 150 155 160

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
165 170 175

Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala
180 185 190

Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
195 200 205

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
210 215 220

[0057]

Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys
225 230 235 240

Leu Gln Ile Thr

<210> 42

<211> 247

<212> PRT

<213> 人工序列

<220>

<223> DRA209 加交接序列

<400> 42

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly

[0058]

100	105	110
Gln Gly Thr Pro Val Thr Val	Ser Ser Gly Gly Gly Gly Ser Gly Gly	
115	120	125
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln		
130	135	140
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr		
145	150	155 160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys		
165	170	175
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala		
180	185	190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr		
195	200	205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr		
210	215	220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys		
225	230	235 240
Leu Gln Ile Thr Arg Arg Thr		
245		
<210> 43		
<211> 98		
<212> PRT		
<213> 人工序列		

[0059]

<220>

<223> IGHV1-69*1

<400> 43

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly	Ser
1				5					10					15	

Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Gly	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Ala	Ile	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
			35				40					45			

Gly	Gly	Ile	Ile	Pro	Ile	Phe	Gly	Thr	Ala	Asn	Tyr	Ala	Gln	Lys	Phe
		50				55					60				

Gln	Gly	Arg	Val	Thr	Ile	Thr	Ala	Asp	Glu	Ser	Thr	Ser	Thr	Ala	Tyr
65					70					75				80	

Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
					85				90				95		

Ala Arg

<210> 44

<211> 98

<212> PRT

<213> 人工序列

<220>

<223> IGHV3-30*01

<400> 44

Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg

[0060]

1	5	10	15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr			
20	25	30	
Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val			
35	40	45	
Ala Val Ile Ser Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val			
50	55	60	
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
65	70	75	80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys			
85	90	95	

Ala Arg

<210> 45
<211> 115
<212> PRT
<213> 人工序列

<220>
<223> CRIS7 VH

<400> 45

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala			
1	5	10	15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser			
20	25	30	

[0061]

Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr
115

<210> 46

<211> 107

<212> PRT

<213> 人工序列

<220>

<223> CRIS7 VL

<400> 46

Gln Val Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Phe Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr

[0062]

	35	40	45
Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser			
50	55	60	
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Thr Glu			
65	70	75	80
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr			
	85	90	95
Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr Arg			
	100	105	
<210> 47			
<211> 121			
<212> PRT			
<213> 人工序列			
<220>			
<223> DRA161 VH			
<400> 47			
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg			
1	5	10	15
Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser			
	20	25	30
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile			
	35	40	45
Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe			
50	55	60	

[0063]

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser
115 120

<210> 48

<211> 107

<212> PRT

<213> 人工序列

<220>

<223> DRA161 VL

<400> 48

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu

[0064]

⟨210⟩ 51

122

<211> 11
<212> PRT
<213> 人工序列

<220>
<223> CRIS7 VHCDR3

<400> 51

Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr
1 5 10

<210> 52
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> CRIS7 VL CDR1

<400> 52

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn
1 5 10

<210> 53
<211> 7
<212> PRT
<213> 人工序列

<220>
<223> CRIS7 VL CDR2

<400> 53

Asp Ser Ser Lys Leu Ala Ser
1 5

<210> 54
<211> 9
<212> PRT

[0066]

<213> 人工序列

<220>

<223> CRIS7 VL CDR3

<400> 54

Gln Gln Trp Ser Arg Asn Pro Pro Thr
1 5

<210> 55

<211> 216

<212> PRT

<213> 人工序列

<220>

<223> DRA161 修饰的 Fc 结构域

<400> 55

Ala Pro Glu Ala Ala Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
1 5 10 15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
20 25 30

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
35 40 45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
50 55 60

Phe Ala Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
65 70 75 80

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
85 90 95

[0067]

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
100 105 110

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr
115 120 125

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
130 135 140

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
145 150 155 160

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
165 170 175

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe
180 185 190

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
195 200 205

Ser Leu Ser Leu Ser Pro Gly Lys
210 215

<210> 56

<211> 2289

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC249 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv)

<400> 56

atggaagcac cagcgcagct tetcttctct ctgtactct ggtctccaga taccaccggt

60

[0068]

gatatccaga tgacccagtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc	120
atcacttgcc gggcgagtaa gagcattage aaatatttag cctggtttca gcagaaacca	180
gggaaagttc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccatct	240
cggttcagtg gcagtggate tgggacagaa ttactctca ccatcagcag cctgcagcct	300
gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gtteggccaa	360
gggaccaagg tggaaatcaa acgaggtggc ggaggtctg ggggtggcgg atccggaggt	420
ggtggetctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca	480
gtgaaggctt cctgcaaggc ttctggatac acattcacig actactacat gcactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatectta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcacatga ccaggacac gtctatcagc	660
acagcctaca tggagctgag cagcctgaga tctgacgaca cggccgtgta ttactgtgca	720
agatcggatg gttactacga tgetatggac tactggggte aaggaaccac agtcaccgtc	780
tcctcgagtg ageccaaatc ttctgacaaa actcacacat gccacccgtg cccagcacct	840
gaagccgcgg gtgcaccgtc agtcttcctc tccccccaa aaccaagga caccctcatg	900
atctcccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagttea actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtect gcaccaggac	1080
tggtgtaatg gcaaggcgta cgcgtgcgcg gtctccaaca aagccctccc agccccatc	1140
gagaaaacca tctccaaagc caaagggcag ccccgagaac cacaggtgta caccctgccc	1200
ccateccggg atgagetgac caagaaccag gtcagcctga cctgcctggt caaaggett	1260
tatccaagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
accacgcctc ccgtgctgga ctccgacggc tccttcttcc tctacagcaa gctcaccgtg	1380

[0069]

gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg	1440
cacaaccact acacgcagaa gagectctcc ctgtctccgg gtcagaggca caacaattct	1500
tccctgaata caggaactca gatggcaggt cattctccga attctcaggt ccagctggtg	1560
gagtctgggg gcggagtggg gcagcctggg cggteactga ggctgtcctg caaggettct	1620
ggctacacct ttactagatc tacgatgcae tgggtaaggc aggeccctgg acaaggctctg	1680
gaatggattg gatacattaa tcttagcagt gcttatacta attacaatca gaaattcaag	1740
gacaggttca caatcagcgc agacaaatcc aagagcacag ccttctgca gatggacagc	1800
ctgaggeccg aggacacccg cgtctatttc tgtgcacggc cccaagteca ctatgattac	1860
aacgggttcc cttaactggg ccaagggaact cccgtcactg tctctagcgg tggcggaggg	1920
tctgggggtg gcggatccgg aggtgggtggc tctgcacaag acatccagat gaccagctct	1980
ccaagcagcc tgtctgaag cgtgggggac agggtcacca tgacctgcag tgccagctca	2040
agtgttaagt acatgaactg gtaccagcag aagccgggca agggcccca aagatggatt	2100
taigactcat ccaaactggc ttctggagtc cctgctcgtt tcagtggcag tgggtctggg	2160
accgactata cctcacaat cagcagcctg cagccagaag atttcgccac ttattactgc	2220
cagcagtgga gtctgaaccc acccacgttc ggagggggga ccaagctaca aattacatcc	2280
tccagctaa	2289

<210> 57

<211> 742

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC249 Scorpion (huVL-VH#2 107-1A1 scFv-Fc-DRA222 scFV)

<400> 57

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly

[0070]

1	5	10	15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr	20	25	30
Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile	35	40	45
His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly	50	55	60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro	65	70	75
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp	85	90	95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly	100	105	110
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val	115	120	125
Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser	130	135	140
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val	145	150	155
Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro	165	170	175
Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr			

[0071]

180	185	190
Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser		
195	200	205
Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly		
210	215	220
Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val		
225	230	235 240
Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro		
245	250	255
Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro		
260	265	270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr		
275	280	285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn		
290	295	300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg		
305	310	315 320
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val		
325	330	335
Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser		
340	345	350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys		

[0072]

355	360	365
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp		
370	375	380
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe		
385	390	395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu		
405	410	415
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe		
420	425	430
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly		
435	440	445
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr		
450	455	460
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gln Arg His Asn Asn Ser		
465	470	475 480
Ser Leu Asn Thr Gly Thr Gln Met Ala Gly His Ser Pro Asn Ser Gln		
485	490	495
Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser		
500	505	510
Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr		
515	520	525
Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly		

[0073]

530	535	540
Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe Lys		
545	550	555 560
Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe Leu		
565	570	575
Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala		
580	585	590
Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln		
595	600	605
Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly		
610	615	620
Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser		
625	630	635 640
Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys		
645	650	655
Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro		
660	665	670
Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser		
675	680	685
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr		
690	695	700
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys		

[0074]

705	710	715	720
Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu			
	725	730	735

Gln Ile Thr Ser Ser Ser
740

<210> 58

<211> 2268

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC250 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有 H81 连接子)

<400> 58

atggaagcac cagcgcagct tctcttctctc ctgtactctt ggctccaga taccaccggt	60
gatatccaga tgaccacgtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc	120
atcacttgcc gggcgagtaa gagcattagc aaatatttag cctggtttca gcagaaacca	180
gggaaagttc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccatct	240
cggttcagtg gcagtggatc tgggacagaa ttactctca ccatcagcag cctgcgcct	300
gaagatttig caacttatta ctgtcaacag catattgaat acccgtggac gtctggccaa	360
gggaccaagg tggaaatcaa acgaggtggc ggaggtctg ggggtggcgg atccggaggt	420
ggtggctctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca	480
gtgaaggctt cctgcaaggc ttctggatac acattcactg actactacat gcactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatectta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcaccaiga ccagggacac gctatcagc	660
acagcctaca tggagctgag cagcctgaga tctgacgaca cggccgtgta ttactgtgca	720

[0075]

agatcggatg gttactacga tgctatggac tactggggtc aaggaaccac agtcaccgtc	780
tcctcgagtg agcccaaate ttctgacaaa actcacacat gccaccgtg cccagcacct	840
gaagccgcgg gtgcaccgtc agtcttctc tccccccaa aaccaagga caccctcatg	900
atctccccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtct gcaccaggac	1080
tggtgaatg gcaaggcgta cgcgtgcgcg gtctccaaca aagccctccc agccccate	1140
gagaaaacca tctccaaagc caaagggcag ccccgagAAC cacaggtgta caccctgccc	1200
ccatccccgg atgagctgac caagaaccag gtcagcctga cctgcctggt caaaggttc	1260
tatccaagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
accacgcctc ccgtgtgga ctccgacggc tctttcttc tctacagaa gtcaccgtg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggetctg	1440
cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtgaagtcca aattcccttg	1500
accgaaagtt acagcccga ttctcaggtc cagctggtag agtctggggg cggagtgggt	1560
cagcctgggc ggtcactgag gtgtctctgc aaggtctctg gctacacctt tactagatct	1620
acgatgcact gggtaaggca ggcctctgga caaggtctgg aatggattgg atacattaat	1680
cctagcagtg cttatactaa ttacaatcag aaattcaagg acaggttcac aatcagcga	1740
gacaaatcca agagcacagc cttcctgcag atggacagcc tgaggeccga ggacaccggc	1800
gtctatttct gtgcacggcc ccaagtcac tatgattaca acgggtttcc ttactggggc	1860
caagggaact ccgtcactgt ctctagcggg gccggagggt ctgggggtgg cggatccgga	1920
ggtgggtggct ctgcacaaga catccagatg acccagctct caagcagcct gtctgcaagc	1980
gtgggggaca gggtcacat gacctgcagt gccagctcaa gtgtaagtta catgaactgg	2040

[0076]

taccagcaga agccgggcaa ggcccccaaa agatggattt atgactcate caaactggct 2100
tctggagtcc ctgctcgctt cagtggcagt gggctctggga cegactatac cctcacaate 2160
agcagcctgc agccccaaga tttegecaet tattactgcc agcagtggag tcgtaaceca 2220
cccacgttcg gaggggggac caagetacaa attacatcct ccagctaa 2268

<210> 59

<211> 1477

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC250 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv,
具具有 H81 连接子)

<400> 59

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

[0077]

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val
115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser
130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
145 150 155 160

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr
180 185 190

Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

[0078]

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

[0079]

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gln Arg His Asn Asn Ser
465 470 475 480

Ser Leu Asn Thr Gly Thr Gln Met Ala Gly His Ser Pro Asn Ser Gln
485 490 495

Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser
500 505 510

Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr
515 520 525

Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly
530 535 540

Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe Lys
545 550 555 560

Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe Leu
565 570 575

Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala
580 585 590

Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln
595 600 605

Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly
610 615 620

[0080]

Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser
625 630 635 640

Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys
645 650 655

Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro
660 665 670

Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser
675 680 685

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr
690 695 700

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
705 710 715 720

Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu
725 730 735

Gln Ile Thr Ser Ser Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ala
740 745 750

Met Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser
755 760 765

Lys Ser Ile Ser Lys Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys
770 775 780

Val Pro Lys Leu Arg Ile His Ser Gly Ser Thr Leu Gln Ser Gly Val
785 790 795 800

[0081]

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr
805 810 815

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
820 825 830

His Ile Glu Tyr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
835 840 845

Lys Arg Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
850 855 860

Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
865 870 875 880

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp
885 890 895

Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
900 905 910

Met Gly Tyr Phe Asn Pro Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys
915 920 925

Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala
930 935 940

Tyr Met Glu Leu Ser Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr
945 950 955 960

Cys Ala Arg Ser Asp Gly Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln
965 970 975

[0082]

Gly Thr Thr Val Thr Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys
980 985 990

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro
995 1000 1005

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
1010 1015 1020

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
1025 1030 1035

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
1040 1045 1050

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
1055 1060 1065

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
1070 1075 1080

Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu
1085 1090 1095

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
1100 1105 1110

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
1115 1120 1125

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
1130 1135 1140

[0083]

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
1145 1150 1155

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
1160 1165 1170

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
1175 1180 1185

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
1190 1195 1200

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Glu Val
1205 1210 1215

Gln Ile Pro Leu Thr Glu Ser Tyr Ser Pro Asn Ser Gln Val Gln
1220 1225 1230

Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu
1235 1240 1245

Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr
1250 1255 1260

Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
1265 1270 1275

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys
1280 1285 1290

Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr
1295 1300 1305

[0084]

Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val
1310 1315 1320

Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe
1325 1330 1335

Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
1340 1345 1350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln
1355 1360 1365

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
1370 1375 1380

Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
1385 1390 1395

Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg
1400 1405 1410

Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg
1415 1420 1425

Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser
1430 1435 1440

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
1445 1450 1455

Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile
1460 1465 1470

[0085]

Thr Ser Ser Ser
1475

<210> 60

<211> 2274

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC251 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具具有 H83 连接子)

<400> 60

atggaagcac cagcgagct tctcttcttc ctgctactct ggtcccaga taccacgggt	60
gatatccaga tgaccagtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc	120
atcacttgcc gggcgagtaa gagcattagc aaatatttag cctggtttca gcagaaacca	180
gggaaagtgc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccatct	240
cggttcagtg gcagtggatc tgggacagaa ttactctca ccatcagcag cctgcagcct	300
gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gtccggccaa	360
gggaccaagg tggaaatcaa acgaggtggc ggaggtcttg ggggtggcgg atccggaggt	420
ggtggctctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca	480
gtgaaggtct cctgcaaggc ttctggatac acattcactg actactacat gcaactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatcctta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcaccatga ccagggacac gtctatcagc	660
acagcctaca tggagctgag cagcctgaga tctgacgaca cgcccggtga ttactgtgca	720
agateggatg gttactacga tctatggac tactggggtc aaggaaccac agtcaccgtc	780
tctcgagtgc agcccaaate ttctgacaaa actcacacat gccaccgtg cccagcacct	840
gaagccgcgg gtgcaccgtc agtcttcttc ttcccccctaa aaccaagga caccctcatg	900

[0086]

atctcccga cccctgaggt cacatgctg gtggtggacg tgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac	1080
tggetgaatg gcaaggcgta cgcgtgcgcg gtetccaaca aagccctccc ageccccate	1140
gagaaaacca tctccaaagc caaagggcag ccccagaaac cacaggtgta caccctgccc	1200
ccatcccggg atgagctgac caagaaccag gtcagcctga cctgcctggt caaagcttc	1260
tatccaagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
aecacgcctc ccgtgctgga ctccgaeggc tccttcttcc tetacagaa gctcacgtg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg	1440
cacaaccact acacgcagaa gagccctccc ctgtctccgg gtctctccct gaatacagga	1500
actcagatgg caggtcattc tccgaattct caggtccagc tggtaggagtc tggggcgga	1560
gtggtgcagc ctgggcggtc actgaggctg tectgcaagg cttctggcta caectttact	1620
agatctacga tgcactgggt aaggcaggcc cctggacaag gtctggaatg gattggatac	1680
attaatccta gcagtgttta tactaattac aatcagaaat tcaaggacag gttcacaaac	1740
agcgcagaca aatccaagag cacagccttc ctgcagatgg acagcctgag gcccgaggac	1800
accggcgtct atttctgtgc acggccccaa gtccactatg attacaacgg gtttccttac	1860
tggggccaag ggactccgt cactgtctct agcggtaggc gagggctctg gggtagcgga	1920
tccggaggtg gtggctctgc acaagacatc cagatgacce agtctccaag cagcctgtct	1980
gcaagcgtgg gggacagggt caccatgacc tgcagtgcc agetcaagtgt aagttacatg	2040
aactgggtacc agcagaagcc gggcaaggcc cccaaaagat ggatttatga ctcatcmeta	2100
ctggcttctg gagtccctgc tcgttcagtc ggagtggtg ctgggaccga ctataccctc	2160
acaatcagca gctgcagcc cgaagatttc gccacttatt actgccagca gtggagtcgt	2220

[0087]

aaccaccca cgttcggagg ggggaccaag ctacaaatta catcctccag ctaa

2274

<210> 61

<211> 737

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC251 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv,
具具有 H83 连接子)

<400> 61

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val

[0088]

115	120	125
Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser		
130	135	140
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val		
145	150	155 160
Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro		
165	170	175
Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr		
180	185	190
Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser		
195	200	205
Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly		
210	215	220
Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val		
225	230	235 240
Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro		
245	250	255
Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro		
260	265	270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr		
275	280	285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn		

[0089]

290	295	300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg		
305	310	315 320
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val		
325	330	335
Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser		
340	345	350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys		
355	360	365
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp		
370	375	380
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe		
385	390	395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu		
405	410	415
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe		
420	425	430
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly		
435	440	445
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr		
450	455	460
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ser Ser Leu Asn Thr Gly		

[0090]

465	470	475	480
Thr Gln Met Ala Gly His Ser Pro Asn Ser Gln Val Gln Leu Val Glu			
	485	490	495
Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys			
	500	505	510
Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp Val Arg			
	515	520	525
Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser			
	530	535	540
Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile			
545	550	555	560
Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu			
	565	570	575
Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His			
	580	585	590
Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr			
	595	600	605
Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly			
	610	615	620
Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser			
625	630	635	640
Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser			

[0091]

	645	650	655
Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys			
660	665	670	
Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg			
675	680	685	
Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser			
690	695	700	
Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg			
705	710	715	720
Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser			
725	730	735	

Ser

<210> 62

<211> 2286

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC252 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有 H91 连接器)

<400> 62

atggaagcac cagcgagct tctcttcctc ctgctactct ggctcccaga taccaccggt 60

gatatccaga tgaccacgac tccatccgcc atgtctgeat ctgtaggaga cagagtcacc 120

atcacttgcc gggcgagtaa gagcattagc aaatatttag cctggttica gcagaaacca 180

gggaaagtgc ctaagctecg catccattct ggatctactt tgcaatcagg ggtcccatct 240

[0092]

cggttcagtg gcagtggtac tgggacagaa ttactctca ccatcagcag cctgcagcct	300
gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gticggccaa	360
gggaccaagg tggaaatcaa acgaggtggc ggaggtcttg ggggtggcgg atccggaggt	420
ggtggctctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggettca	480
gtgaaggtct cctgcaaggc ttctggatac acattcactg actactacat gcactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatectta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcacatga ccagggacac gtctatcage	660
acagcctaca tggagctgag cagcctgaga tctgaagaca egccctgta ttaetgtgca	720
agatcggatg gttactacga tgetatggac tactggggtc aaggaaccac agtcaccgtc	780
tcctcgagtg agcccaaate ttctgacaaa actcacacat gcccaaccgtg cccageacct	840
gaagcccggg gtgcaccgtc agtcttctc tccccccaa aaccaagga caccctcatg	900
atctcccgga cccctgaggt cacatgcgtg ggggtggacg lgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcggtg gaggtgcata atgccaagac aaagcccggg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac	1080
tggctgaatg gcaaggcgta cgcgtgcgcg gtctccaaca aagccctccc agccccate	1140
gagaaaacca tctccaaage caaagggcag ccccgagaac cacaggtgta caccctgccc	1200
ccatcccggg atgagctgac caagaaccag gtcagcctga cctgectggt caaaggettc	1260
tatccaagcg acatgcctgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
accacgctc cegtgtctga ctccgaegge tctttcttc tetacagcaa gctcaccgtg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct cegtgatgca tgaggetctg	1440
cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtaactcatt agcaaaccaa	1500
gaagttcaaa ttcccttgac cgaaagttae agcccgatc ctcaggteca gctggtggag	1560

[0093]

tctgggggcg gagtgggtgca gcctgggdcgg tcactgagge tgccttgcaa ggctttctgce	1620
tacaccttta ctagatctac gatgcactgg gtaaggcagg cccctggaca aggtetggaa	1680
tggattggat acattaatcc tagcagtgct tatactaatt acaatcagaa attcaaggac	1740
aggttcacaa tcagcgcaga caaatccaag agcacagcct tectgcagat ggacagcctg	1800
aggcccagagg acaccggcgt ctattttctgt gcacggcccc aagtcacta tgattacaac	1860
gggtttctctt actggggcca agggactccc gtcactgtct ctagcgggtg cggagggtct	1920
gggggtggcg gatccggagg tggfggctct gcacaagaca tccagatgac ccagttcca	1980
agcagcctgt ctgcaagcgt gggggacagg gtcaccatga cctgcagtgc cagctcaagt	2040
gtaagttaca tgaactggta ccagcagaag ccgggcaagg cccccaaaag atggatttat	2100
gactcateca aactggettc tggagtcct gctcgttca gtggcagtgg gtctgggacc	2160
gactatacce tcacaatcag cagcctgcag ccgaagatt tcgccactta ttaactgccag	2220
cagtggagtc gtaaccacac cagttcggga ggggggacca agctacaaat tacatctcc	2280
agctaa	2286

<210> 63

<211> 741

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC252 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv,
具具有 H91 连接器)

<400> 63

Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ala	Met	Ser	Ala	Ser	Val	Gly
1				5				10						15	

Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Lys	Ser	Ile	Ser	Lys	Tyr

[0094]

20	25	30
Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile		
35	40	45
His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly		
50	55	60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro		
65	70	75
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp		
85	90	95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly		
100	105	110
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val		
115	120	125
Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser		
130	135	140
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val		
145	150	155
Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro		
165	170	175
Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr		
180	185	190
Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser		

[0095]

195	200	205
Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly		
210	215	220
Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val		
225	230	235 240
Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro		
245	250	255
Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro		
260	265	270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr		
275	280	285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn		
290	295	300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg		
305	310	315 320
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val		
325	330	335
Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser		
340	345	350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys		
355	360	365
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp		

[0096]

370	375	380
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe		
385	390	395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu		
	405	410 415
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe		
	420	425 430
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly		
	435	440 445
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr		
	450	455 460
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Asn Ser Leu Ala Asn Gln		
465	470	475 480
Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr Ser Pro Asn Ser Gln Val		
	485	490 495
Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu		
	500	505 510
Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met		
	515	520 525
His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr		
530	535	540
Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp		

[0097]

545	550	555	560
Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln			
565	570	575	
Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg			
580	585	590	
Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly			
595	600	605	
Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly			
610	615	620	
Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro			
625	630	635	640
Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys Ser			
645	650	655	
Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly			
660	665	670	
Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly			
675	680	685	
Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu			
690	695	700	
Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln			
705	710	715	720
Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln			

[0098]

725

730

735

Ile Thr Ser Ser Ser

740

<210> 64

<211> 2250

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC295 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具具有 H9 连接子)

<400> 64

atggaagcac cagcgcagct tctcttcctc ctgtctactct ggctcccaga taccaccggt 60

gatatccaga tgaccacgct tccatccgcc atgtctgcat ctgtaggaga cagagtcacc 120

atcaattgcc gggcgagtaa gagcattage aaatatttag cctggtttca gcagaaacca 180

gggaaagttc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccatct 240

cggttcagtg gcagtgagtc tgggacagaa ttactctca ccatcagcag cctgcagcct 300

gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gttcggccaa 360

gggaccaagg tggaaatcaa acgaggtagc ggagggtctg ggggtggcgg atccggaggt 420

ggtggetctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca 480

gtgaaggctc cctgcaaggc ttctggatac acattcactg actactacat gcaetgggtg 540

cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatcetta taatgattat 600

actagatacg cacagaagtt ccagggcaga gtcacatga ccagggacac gtctatcagc 660

acagcetaca tggagctgag cagcctgaga tctgacgaca cggccgtgta ttactgtgca 720

agatcggatg gttactacga tgctatggac tactggggtc aaggaaccac agtcaccgte 780

tctctgagtg agcccaaacc ttctgacaaa actcacacat gccccaccgtg cccagcacct 840

[0099]

gaagccgcgg gtgcaccgtc agtcttcctc tccccccaa aaccaagga caccctcatg	900
atctcccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac	1080
tggctgaatg gcaaggcgta cgcgtgcgcg gtctccaaca aagccctccc agccccatc	1140
gagaaaacca tctccaaagc caaagggcag ccccagaaac cacaggtgta caccctgccc	1200
ccatcccggg atgagctgac caagaaccag gtcagcctga cctgcttggg caaaggett	1260
tatccaagcg acatgcctgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
accacgcctc ccgtgctgga ctccgacggc tectttcttc tctacagcaa gtcaccgtg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca lgaggctcig	1440
cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtgggagccc accttcaccg	1500
aattctcagg tccagctggg ggagtctggg ggcggagtgg tgcagcctgg gcggtcactg	1560
aggetgtcct gcaaggett	1620
tggttacacc ttactagat ctacgatgca ctgggtaagg	1620
caggcccctg gacaaggctt ggaatggatt ggatacatta atcctagcag tgettatact	1680
aattacaatc agaaattcaa ggacaggctt acaatcagcg cagacaaatc caagagcaca	1740
gccttctctg agatggacag cctgaggccc gaggacaccg gcgtctatct ctgtgcacgg	1800
ccccaaagtc actatgatta caacgggttt ccttactggg gccaaaggac tcccgctact	1860
gtctctagcg gtggcggagg gtctgggggt ggcggtaccg gaggtggtgg ctctgcacaa	1920
gacatccaga tgaccagtc tccaagcagc ctgtctgcaa gcgtggggga cagggtcacc	1980
atgacctgca gtgccagctc aagtgttaagt tacatgaact ggtaccagca gaagccgggc	2040
aagccccca aaagatggat ttatgactca tccaaactgg cttctggagt cctgtctcgc	2100
ttcagtggca gtgggtctgg gaccgaetat accctcacia teagcagcct gcagcccgaa	2160

[0100]

gatttcgccca cttattactg ccagcagtgg agtcgtaacc caccacggtt cggagggggg 2220

accaagctac aaattacatc ctccagctaa 2250

<210> 65

<211> 729

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC295 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv,
具具有 H9 连接子)

<400> 65

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

[0101]

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val
115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser
130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
145 150 155 160

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr
180 185 190

Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

[0102]

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

[0103]

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gly Ser Pro Pro Ser Pro
465 470 475 480

Asn Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro
485 490 495

Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
500 505 510

Arg Ser Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu
515 520 525

Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln
530 535 540

Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr
545 550 555 560

Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr
565 570 575

Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr
580 585 590

Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser
595 600 605

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met
610 615 620

Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
625 630 635 640

[0104]

Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln
645 650 655

Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys
660 665 670

Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr
675 680 685

Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
690 695 700

Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly
705 710 715 720

Thr Lys Leu Gln Ile Thr Ser Ser Ser
725

<210> 66

<211> 2283

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC296 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv,
具具有 H94 连接子)

<400> 66

atggaagcac cagcgcagct tctcttctct cttgctactct ggtctccaga taccaccggt 60

gatatccaga tgaccagtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc 120

atcaattgcc gggcgagtaa gagcattagc aaatatttag cctggtttca gcagaaacca 180

gggaaagtgc ctaagctccg catccattct ggtctactt tgcaatcagg ggtcccatct 240

cggttcagtg gcagtggatc tgggacagaa ttactctca ccatcagcag cctgcagcct 300

[0105]

gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gticggccaa	360
gggaccaagg tggaaatcaa acgaggtggc ggaggtcttg ggggtggcgg atccggaggt	420
ggtggctctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca	480
gtgaaggctc cctgcaaggc ttctggatac acattcactg actactacat gcactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatcetta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcacatga ccagggacac gtctatcagc	660
acagcctaca tggagctgag cagcctgaga tetgacgaca cgccctgtga ttactgtgca	720
agatcggatg gttactacga tctatggac taetggggtc aaggaaccac agtcacgtc	780
tcctcgagtg agcccaaato ttctgacaaa actcacacat gccaccgtg cccagcacct	840
gaagccggg gtcaccgic agcttctc tccccccaa aaccaagga caccctcag	900
atctcccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccggg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagegtcc tcaccgtcct gcaccaggac	1080
tggctgaatg gcaaggcgtg cgggtgcgcg gtctccaaca aagccctccc agccccate	1140
gagaaaacca tctccaaagc caaagggcag ccccgagaac cacaggtgta caccctgccc	1200
ccatcccggg atgagctgac caagaaccag gtcagectga cctgcctggt caaaggettc	1260
tatccaagcg acatgccgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
accacgctc cctgctgga ctccgacgce tctttcttc tctacagca gtcaccgtg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct cctgatgca tgaggctcig	1440
cacaaccact acacgcagaa gagcctctcc ctgtctccgg gttctggtgg aggcggttca	1500
ggcggaggtg gctccggcgg tggcgatcg ccgaattctc aggtccagct ggtggagtct	1560
ggggcgagg tggtgcagcc tggcggtca ctgaggtgt cctgcaagcc ttctggctac	1620

[0106]

acctttacta gatctacgat gcactgggta aggcaggccc ctggacaagg tctggaatgg 1680
 attggataca ttaatcctag cagtgttat actaattaca atcagaaatt caaggacagg 1740
 ttcacaatca ggcagacaa atccaagage acagecttcc tgcagatgga cagcctgagg 1800
 ccagaggaca ccggcgtcta tttctgtgca cgcccccaag tccactatga ttacaacggg 1860
 tttccttact ggggcccaagg gactcccgtc actgtctcta gcggtggcgg agggctctggg 1920
 ggtggcggat ccggaggtgg tggctctgca caagacatcc agatgaccca gtctccaagc 1980
 agcctgtctg caagcgtggg ggacagggtc accatgaect gcagtccag ctcaagtgtg 2040
 agttacatga actggtacca gcagaagccg ggcaaggccc ccaaaagatg gatttatgac 2100
 tcatccaaac tggcttctgg agtccctgct cgttcagtgc gcagtgggtc tgggaccgac 2160
 tataacctca caatcagcag cctgcagccc gaagatttcg ccacttatta ctgccagcag 2220
 tggagtcgta acccaccacac gtccggaggg gggaccaagc tacaaattac atcctccagc 2280
 taa 2283

<210> 67

<211> 740

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC296 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具具有 H94 连接子)

<400> 67

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
 20 25 30

[0107]

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val
115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser
130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
145 150 155 160

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr
180 185 190

Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

[0108]

Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

[0109]

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ser Gly Gly Gly Gly Ser
465 470 475 480

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Pro Asn Ser Gln Val Gln
485 490 495

Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg
500 505 510

Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His
515 520 525

Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile
530 535 540

Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg
545 550 555 560

[0110]

Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln Met
565 570 575

Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro
580 585 590

Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr
595 600 605

Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
610 615 620

Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser
625 630 635 640

Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala
645 650 655

Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys
660 665 670

Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val
675 680 685

Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr
690 695 700

Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
705 710 715 720

Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile
725 730 735

[0111]

Thr Ser Ser Ser
740

<210> 68

<211> 2274

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC301 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具具有 H105 连接子)

<400> 68

atggaagcac cagcgagct tctcttctc ctgctactct ggtcccaga taccacggg	60
gatatccaga tgaccagtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc	120
atcacttgcc gggcgagtaa gagcattagc aaatatttag cctggtttca gcagaaacca	180
gggaaagtgc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccatct	240
cggttcagtg gcagtggatc tgggacagaa ttactctca ccacagcag cctgcagcct	300
gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gttcggccaa	360
gggaccaagg tggaaatcaa acgaggtggc ggaggtctg ggggtggcgg atccggaggt	420
ggtggctctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca	480
gtgaaggtct cctgcaaggc ttctggatac acattcactg actactacat gcaactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatcctta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcaccatga ccagggacac gtctatcagc	660
acagcctaca tggagctgag cagcctgaga tctgacgaca cgcccggtga ttactgtgca	720
agategggatg gttactacga tctatggac tactggggtc aaggaaccac agtcaccgtc	780
tctcgagtgc agcccaaate ttctgacaaa actcacacat gccaccgtg cccagcacct	840
gaagccgcgg gtgcaccgtc agtcttctc tccccccaa aaccaagga caccctcatg	900

[0112]

atctcccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac	1080
tggetgaatg gcaaggcgta cgcgtgcgcg gtctccaaca aagccctccc agecccccate	1140
gagaaaacca tctccaaagc caaagggcag ccccagaaac cacaggtgta caccctgccc	1200
ccatcccggg atgagctgac caagaaccag gtcagcctga cctgccttgt caaaggttcc	1260
tatccaagcg acatgcctgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
aecacgcctc ccgtgctgga ctccgaeggc tccttcttcc ttacagcaa gctcacctg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggctctg	1440
cacaaccact acacgcagaa gagccctccc ctgtctccgg gtctcgttgg aggcggttca	1500
ggcggaggtg gctccggcgg tggcggatcg caggtccagc tggtaggagc tgggggcgga	1560
gtggtgcagc ctgggcggtc actgaggctg tectgcaagg cttctggcta caectttact	1620
agatctacga tgcactgggt aaggcaggcc cctggacaag gtctggaatg gattggatac	1680
attaatccta gcagtgttta tactaattac aatcagaaat tcaaggacag gttcacaatc	1740
agcgcagaca aatccaagag cacagccttc ctgcagatgg acagcctgag gcccgaggac	1800
accggcgtct atttctgtgc acggcccaca gtccactatg attacaacgg gtttcettac	1860
tggggccaag ggactccgtt cactgtctct agcggtaggc gagggctctg gggtagcgga	1920
tccggaggtg gtggctctgc acaagacatc cagatgacce agtctccaag cagcctgtct	1980
gcaagcgtgg gggacagggt caccatgacc tgcagtgcc agetcaagtgt aagttacatg	2040
aactgggtacc agcagaagcc gggcaaggcc cccaaaagat ggatttatga ctcatccaaa	2100
ctggcttctg gagtccctgc tcgtttcagt ggtagtgggt ctgggaccga ctataccctc	2160
acaatcagca gectgcagcc cgaagatttc gccacttatt actgccagca gtggagtcgt	2220

[0113]

aaccaccca cgttcggagg ggggaccaag ctacaaatta catcctccag ctaa

2274

<210> 69

<211> 737

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC301 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv,
具具有 H105 连接子)

<400> 69

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val

[0114]

115	120	125
Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser		
130	135	140
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val		
145	150	155 160
Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro		
165	170	175
Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr		
180	185	190
Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser		
195	200	205
Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly		
210	215	220
Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val		
225	230	235 240
Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro		
245	250	255
Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro		
260	265	270
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr		
275	280	285
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn		

[0115]

290	295	300
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg		
305	310	315 320
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val		
325	330	335
Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser		
340	345	350
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys		
355	360	365
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp		
370	375	380
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe		
385	390	395 400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu		
405	410	415
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe		
420	425	430
Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly		
435	440	445
Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr		
450	455	460
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ser Gly Gly Gly Ser		

[0116]

465	470	475	480
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu			
485	490	495	
Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys			
500	505	510	
Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp Val Arg			
515	520	525	
Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser			
530	535	540	
Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile			
545	550	555	560
Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu			
565	570	575	
Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His			
580	585	590	
Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr			
595	600	605	
Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly			
610	615	620	
Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser			
625	630	635	640
Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser			

[0117]

	645	650	655
Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys			
660	665	670	
Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg			
675	680	685	
Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser			
690	695	700	
Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg			
705	710	715	720
Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser			
725	730	735	

Ser

<210> 70

<211> 2280

<212> DNA

<213> 人工序列

<220>

<223> 人类化 TSC302 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有 H106 连接子)

<400> 70

atggaagcac cagcgagct tctcttcctc ctgctactct ggctcccaga taccaccggt	60
gatatccaga tgaccaccgtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc	120
atcacttgcc gggcgagtaa gagcattagc aaatatttag cctggtttca gcagaaacca	180
gggaaagtgc ctaagctecg catccattct ggatctactt tgcaatcagg ggtcccatct	240

[0118]

cggttcagtg gcagtggtac tgggacagaa ttactctca ccatcagcag cctgcagcct	300
gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gticggccaa	360
gggaccaagg tggaaatcaa acgaggtggc ggaggtcttg ggggtggcgg atccggaggt	420
ggtggctctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggettca	480
gtgaaggtct cctgcaaggc ttctggatac acattcactg actactacat gcactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatectta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcacatga ccagggacac gtctatcage	660
acagcctaca tggagctgag cagcctgaga tctgaagaca egcccggtga ttactgtgca	720
agatcggatg gttactacga tgetatggac tactggggtc aaggaaccac agtcaccgtc	780
tcctcgagtg agcccaaate ttctgacaaa actcacacat gcccaaccgtg cccageacct	840
gaagcccggg gtgcaccgtc agtcttctc tccccccaa aaccaagga caccctcatg	900
atctcccgga cccctgaggt cacatgcgtg ggggtggacg lgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcggtg gaggtgcata atgccaagac aaagcccggg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac	1080
tggctgaatg gcaaggcgta cgcgtgcgcg gtctccaaca aagccctccc agccccate	1140
gagaaaacca tctccaaagc caaagggcag ccccgagaac cacaggtgta caccctgccc	1200
ccatcccggg atgagctgac caagaaccag gtcagcctga cctgectggt caaaggettc	1260
tatccaagcg acatgcctg ggagtgggag agcaatgggc agccggagaa caactacaag	1320
accacgctc cegtgtctga ctccgaegge tctttcttc tetacagcaa gctcaccgtg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct cegtgatgca tgaggetctg	1440
cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtcagaggca caacaattct	1500
tcctgaata caggaaactca gatggcaggt cattctcagg tccagctggt ggagtctggg	1560

[0119]

ggcgagtggtg tgcagcctgg gcggtcactg aggctgtcct gcaaggttc tggctacacc 1620
 ttactagat ctacgatgca ctgggtaagg caggccccctg gacaaggtct ggaatggatt 1680
 ggatacatta atcctagcag tgcctatact aattacaate agaaattcaa ggacaggttc 1740
 acaatcagcg cagacaaatc caagageaca gccttcctgc agatggacag cctgaggccc 1800
 gaggacaccg gcgtctatct ctgtgcacgg cccaagtcc actatgatta caacgggttt 1860
 ccttactggg gccaaaggac tcccgctact gtctctagcg gtggcggagg gtctgggggt 1920
 ggcgatccg gagtggtgg ctctgcacaa gacatccaga tgaccagtc tccaagcagc 1980
 ctgtctgcaa gcgtggggga cagggtcacc atgacctgca gtgccagctc aagtgttaagt 2040
 tacatgaact ggtaccagca gaagccgggc aaggccccca aaagatggat ttatgactca 2100
 tccaaactgg ctcttgaggt ccctgtctgc ttcatggca gtgggtctgg gaccgactat 2160
 accctcaca tccagcagct gcagcccgaa gatttcgcca cttattactg ccagcagtg 2220
 agtcgtaacc caccacgtt cggagggggg accaagctac aaattacatc ctccagctaa 2280

<210> 71

<211> 739

<212> PRT

<213> 人工序列

<220>

<223> 人类化 TSC302 Scorpion (huVL-VH#2 107-1A4 scFv-Fc-DRA222 scFv, 具有 HI06 连接子)

<400> 71

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
 20 25 30

[0120]

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val
115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser
130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
145 150 155 160

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr
180 185 190

Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

[0121]

Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

[0122]

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Gln Arg His Asn Asn Ser
465 470 475 480

Ser Leu Asn Thr Gly Thr Gln Met Ala Gly His Ser Gln Val Gln Leu
485 490 495

Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu
500 505 510

Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp
515 520 525

Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn
530 535 540

Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe
545 550 555 560

[0123]

Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln Met Asp
565 570 575

Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro Gln
580 585 590

Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Pro
595 600 605

Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
610 615 620

Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
625 630 635 640

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser
645 650 655

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala
660 665 670

Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro
675 680 685

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile
690 695 700

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp
705 710 715 720

Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr
725 730 735

[0124]

Ser Ser Ser

<210> 72

<211> 106

<212> PRT

<213> 人工序列

<220>

<223> HuM291 VL

<400> 72

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80

Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 73

[0125]

<211> 120
<212> PRT
<213> 人工序列

<220>
<223> HuM29I VH

<400> 73

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ile Ser Tyr
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Tyr Ile Asn Pro Arg Ser Gly Tyr Thr His Tyr Asn Gln Lys Leu
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Ala Tyr Tyr Asp Tyr Asp Gly Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 74
<211> 5
<212> PRT

[0126]

<213> 人工序列

<220>

<223> 连接子

<400> 74

Gly Gly Gly Gly Ser

1 5

<210> 75

<211> 10

<212> PRT

<213> 人工序列

<220>

<223> 连接子

<400> 75

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser

1 5 10

<210> 76

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> 连接子

<400> 76

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser

1 5 10 15

<210> 77

<211> 20

<212> PRT

<213> 人工序列

[0127]

<220>

<223> 连接子

<400> 77

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20

<210> 78

<211> 25

<212> PRT

<213> 人工序列

<220>

<223> 连接子

<400> 78

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25

<210> 79

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> 分泌信号序列

<400> 79

Met Glu Ala Pro Ala Gln Leu Leu Phe Leu Leu Leu Trp Leu Pro
1 5 10 15

[0128]

Asp Thr Thr Gly
20

<210> 80

<211> 98

<212> PRT

<213> 人工序列

<220>

<223> 野生型人类 IgG1 CH1 结构域

<400> 80

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val

<210> 81

[0129]

<211> 107

<212> PRT

<213> 人工序列

<220>

<223> 野生型人类 CK 结构域

<400> 81

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 82

<211> 106

<212> PRT

<213> 人工序列

<220>

<223> 野生型人类 CA 结构域

[0130]

<400> 82

Gly Gln Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser
1 5 10 15

Glu Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp
20 25 30

Phe Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro
35 40 45

Val Lys Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn
50 55 60

Lys Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys
65 70 75 80

Ser His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val
85 90 95

Glu Lys Thr Val Ala Pro Thr Glu Cys Ser
100 105

<210> 83

<211> 105

<212> PRT

<213> 人工序列

<220>

<223> CK 结构域

<400> 83

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

[0131]

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
65 70 75 80

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
85 90 95

Val Thr Lys Ser Phe Asn Arg Gly Glu
100 105

<210> 84

<211> 105

<212> PRT

<213> 人工序列

<220>

<223> CA 结构域

<400> 84

Gln Pro Lys Ala Asn Pro Thr Val Thr Leu Phe Pro Pro Ser Ser Glu
1 5 10 15

Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe
20 25 30

Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Gly Ser Pro Val

[0132]

	35	40	45
Lys Ala Gly Val Glu Thr Thr Lys Pro Ser Lys Gln Ser Asn Asn Lys			
50	55	60	
Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser			
65	70	75	80
His Arg Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu			
	85	90	95
Lys Thr Val Ala Pro Thr Glu Ser Ser			
	100	105	
<210>	85		
<211>	110		
<212>	PRT		
<213>	人工序列		
<220>			
<223>	野生型人类 IgG1 CH2 结构域		
<400>	85		
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys			
1	5	10	15
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val			
	20	25	30
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr			
35	40	45	
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu			
50	55	60	

[0133]

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
85 90 95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
100 105 110

<210> 86

<211> 110

<212> PRT

<213> 人工序列

<220>

<223> 野生型人类 IgG1 CH2 结构域

<400> 86

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
1 5 10 15

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
20 25 30

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
35 40 45

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
50 55 60

Gln Tyr Ala Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
65 70 75 80

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys

[0134]

85

90

95

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
100 105 110

<210> 87

<211> 13

<212> PRT

<213> 人工序列

<220>

<223> R0N-e01 VL CDR1

<400> 87

Arg Ala Ser Gln Asp Ile Gly Ser Ser Leu Ser Trp Leu
1 5 10

<210> 88

<211> 11

<212> PRT

<213> 人工序列

<220>

<223> R0N-e01 VL CDR2

<400> 88

Arg Leu Ile Tyr Ser Thr Ser Ser Leu Asp Ser
1 5 10

<210> 89

<211> 9

<212> PRT

<213> 人工序列

<220>

<223> R0N-e01 VL CDR3

<400> 89

[0135]

Leu Gln Tyr Ala Ser Ser Pro Trp Thr
1 5

<210> 90
<211> 13
<212> PRT
<213> 人工序列

<220>
<223> RON-f01 VL CDR1

<400> 90

Leu Ala Ser Gln Thr Ile Gly Thr Trp Leu Ala Trp Tyr
1 5 10

<210> 91
<211> 11
<212> PRT
<213> 人工序列

<220>
<223> RON-f01 VL CDR2

<400> 91

Leu Leu Ile Tyr Ala Ala Thr Ser Leu Ala Asp
1 5 10

<210> 92
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> RON-f01 VL CDR3

<400> 92

Gln Gln Leu Tyr Asn Thr Pro Trp Thr

[0136]

1 5

<210> 93

<211> 10

<212> PRT

<213> 人工序列

<220>

<223> RON-e01 VH CDR1

<400> 93

Gly Tyr Thr Phe Thr Ser Tyr Trp Ile His

1 5 10

<210> 94

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> RON-e01 VH CDR2

<400> 94

Trp Ile Gly Tyr Ile Tyr Pro Thr Thr Gly Tyr Thr Glu Ser Asn Gln

1 5 10 15

Lys Phe Lys Asp

20

<210> 95

<211> 10

<212> PRT

<213> 人工序列

<220>

<223> RON-e01 VH CDR3

<400> 95

[0137]

Phe Leu Leu Arg Pro Trp Tyr Phe Asp Val
1 5 10

<210> 96

<211> 10

<212> PRT

<213> 人工序列

<220>

<223> RON-f01 VH CDR1

<400> 96

Asp Tyr Thr Leu Thr Asp Phe Tyr Met Asn
1 5 10

<210> 97

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> RON-f01 VH CDR2

<400> 97

Trp Ile Gly Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu
1 5 10 15

Lys Phe Arg Asp
20

<210> 98

<211> 14

<212> PRT

<213> 人工序列

<220>

<223> RON-f01 VH CDR3

[0138]

<400> 98

Ala Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr
1 5 10

<210> 99

<211> 107

<212> PRT

<213> 人工序列

<220>

<223> R0N-e01 鼠类 VL

<400> 99

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln Asp Ile Gly Ser Ser
20 25 30

Leu Ser Trp Leu Gln Gln Glu Pro Asp Gly Thr Ile Lys Arg Leu Ile
35 40 45

Tyr Ser Thr Ser Ser Leu Asp Ser Gly Val Pro Lys Arg Phe Ser Gly
50 55 60

Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu Ser
65 70 75 80

Glu Asp Phe Val Asp Tyr Tyr Cys Leu Gln Tyr Ala Ser Ser Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

[0139]

<210> 100
<211> 107
<212> PRT
<213> 人工序列

<220>
<223> RON-f01 鼠类 VL

<400> 100

Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Gln Ser Ala Ser Leu Gly
1 5 10 15

Glu Ser Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ser Pro Gln Leu Leu Ile
35 40 45

Tyr Ala Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Ala
50 55 60

Ser Gly Ser Gly Thr Lys Phe Ser Phe Lys Ile Asp Ser Leu Gln Ser
65 70 75 80

Glu Asp Phe Val Ser Tyr Tyr Cys Gln Gln Leu Tyr Asn Thr Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 101
<211> 117
<212> PRT
<213> 人工序列

[0140]

<220>

<223> RON-e01 鼠类 VH

<400> 101

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ser Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Ile His Trp Val Lys Glu Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Tyr Pro Thr Thr Gly Tyr Thr Glu Ser Asn Gln Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Phe Leu Leu Arg Pro Trp Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr
100 105 110

Val Thr Val Ser Ser
115

<210> 102

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> RON-f01 鼠类 VH

[0141]

<400> 102

Gln Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Thr
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Asp Tyr Thr Leu Thr Asp Phe
20 25 30

Tyr Met Asn Trp Val Arg Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe
50 55 60

Arg Asp Lys Ala Ile Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Ser Val Thr Val Ser Leu
115 120

<210> 103

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> RON-f01 鼠类 VH 变体

<400> 103

[0142]

Gln Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Thr
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Asp Tyr Thr Leu Thr Asp Phe
20 25 30

Tyr Met Asn Trp Val Arg Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe
50 55 60

Arg Asp Lys Ala Ile Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Phe Cys
85 90 95

Ala Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Ser Val Thr Val Ser Ser
115 120

<210> 104

<211> 107

<212> PRT

<213> 人工序列

<220>

<223> RON-e01h6 人类化 VL

<400> 104

Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly

[0143]

1	5	10	15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Gly Ser Ser			
20	25	30	
Leu Ser Trp Leu Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile			
35	40	45	
Tyr Ser Thr Ser Ser Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly			
50	55	60	
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro			
65	70	75	80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Tyr Ala Ser Ser Pro Trp			
85	90	95	
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys			
100	105		

<210> 105

<211> 107

<212> PRT

<213> 人工序列

<220>

<223> RON-e01h7 人类化 VL

<400> 105

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly			
1	5	10	15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile Gly Ser Ser			
20	25	30	

[0144]

Leu Ser Trp Leu Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
35 40 45

Tyr Ser Thr Ser Ser Leu Asp Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Tyr Ala Ser Ser Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105

<210> 106

<211> 107

<212> PRT

<213> 人工序列

<220>

<223> RON-f01h2 人类化 VL

<400> 106

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly

[0145]

50	55	60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro		
65	70	75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Tyr Asn Thr Pro Trp		
	85	90 95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys		
	100	105
<210> 107		
<211> 117		
<212> PRT		
<213> 人工序列		
<220>		
<223> R0N-e01h8 人类化 VH		
<400> 107		
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala		
1	5	10 15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr		
	20	25 30
Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile		
	35	40 45
Gly Tyr Ile Tyr Pro Thr Thr Gly Tyr Thr Glu Ser Asn Gln Lys Phe		
	50	55 60
Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr		
65	70	75 80

[0146]

85

90

95

Phe Leu Leu Arg Pro Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr
 100 105 110

Val Thr Val Ser Ser
 115

<210> 109

<211> 117

<212> PRT

<213> 人工序列

<220>

<223> RON-e01h10 人类化 VH

<400> 109

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

Trp Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Ile Tyr Pro Thr Thr Gly Tyr Thr Glu Ser Asn Gln Lys Phe
 50 55 60

Lys Asp Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

[0148]

Phe Leu Leu Arg Pro Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Thr
 100 105 110

Val Thr Val Ser Ser
 115

<210> 110

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> RON-f01h4 人类化 VH

<400> 110

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Thr Val Lys Ile Ser Cys Lys Val Ser Asp Tyr Thr Leu Thr Asp Phe
 20 25 30

Tyr Met Asn Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe
 50 55 60

Arg Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly

[0149]

	100	105	110
Gln Gly Thr Thr Val Thr Val Ser Ser			
	115	120	
<210>	111		
<211>	121		
<212>	PRT		
<213>	人工序列		
<220>			
<223>	RON-f01h5 人类化 VH		
<400>	111		
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser			
1	5	10	15
Ser Val Lys Val Ser Cys Lys Ala Ser Asp Tyr Thr Leu Thr Asp Phe			
	20	25	30
Tyr Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile			
	35	40	45
Gly Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe			
	50	55	60
Arg Asp Arg Val Thr Ile Thr Ala Asp Lys Ser Thr Ser Thr Ala Tyr			
65	70	75	80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys			
	85	90	95
Ala Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly			
	100	105	110

[0150]

Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 112
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> sss(s)-hlgG1 铰链

<400> 112

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Ser
1 5 10 15

<210> 113
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> csc(s)-hlgG1 铰链

<400> 113

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys Ser
1 5 10 15

<210> 114
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> ssc(s)-hlgG1 铰链

[0151]

<400> 114

Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Cys	Ser
1				5					10					15

<210> 115

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> scc(s)-hlg1 铰链

<400> 115

Glu	Pro	Lys	Ser	Ser	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Ser
1				5					10					15

<210> 116

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> css(s)-hlg1 铰链

<400> 116

Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Ser	Pro	Pro	Ser	Ser
1				5					10					15

<210> 117

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> scs(s)-hlg1 铰链

[0152]

<400> 117

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Ser Ser
1 5 10 15

<210> 118

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> ccc(s)-hlgG1 铰链

<400> 118

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys Ser
1 5 10 15

<210> 119

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> ccc(p)-hlgG1 铰链

<400> 119

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys Pro
1 5 10 15

<210> 120

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> sss(p)-hlgG1 铰链

<400> 120

[0153]

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Ser Pro
1 5 10 15

<210> 121

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> csc(p)-hlgG1 铰链

<400> 121

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Cys Pro
1 5 10 15

<210> 122

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> ssc(p)-hlgG1 铰链

<400> 122

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Ser Pro Pro Cys Pro
1 5 10 15

<210> 123

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> scc(p)-hlgG1 铰链

<400> 123

[0154]

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro
1 5 10 15

<210> 124

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> css(p)-hlgG1 铰链

<400> 124

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Ser Pro Pro Ser Pro
1 5 10 15

<210> 125

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> scs(p)-hlgG1 铰链

<400> 125

Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro Ser Pro
1 5 10 15

<210> 126

<211> 6

<212> PRT

<213> 人工序列

<220>

<223> Scppcp

<400> 126

[0155]

Ser Cys Pro Pro Cys Pro
1 5

<210> 127

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> STD1

<400> 127

Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15

Ser Gly Asn Ser
20

<210> 128

<211> 38

<212> PRT

<213> 人工序列

<220>

<223> STD2

<400> 128

Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15

Ser Gly Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
20 25 30

Gly Gly Ser Gly Asn Ser
35

[0156]

<210> 129
<211> 2
<212> PRT
<213> 人工序列

<220>
<223> H1

<400> 129

Asn Ser
1

<210> 130
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> H2

<400> 130

Gly Gly Gly Gly Ser Gly Asn Ser
1 5

<210> 131
<211> 10
<212> PRT
<213> 人工序列

<220>
<223> H3

<400> 131

Asn Tyr Gly Gly Gly Gly Ser Gly Asn Ser
1 5 10

<210> 132
<211> 13

[0157]

<212> PRT

<213> 人工序列

<220>

<223> H4

<400> 132

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Asn Ser
1 5 10

<210> 133

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H5

<400> 133

Asn Tyr Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Asn Ser
1 5 10 15

<210> 134

<211> 18

<212> PRT

<213> 人工序列

<220>

<223> H6

<400> 134

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Asn Ser

[0158]

<210> 135

<211> 8

<212> PRT

<213> 人工序列

<220>

<223> H7

<400> 135

Gly Cys Pro Pro Cys Pro Asn Ser

1 5

<210> 136

<211> 8

<212> PRT

<213> 人工序列

<220>

<223> H9

<400> 136

Gly Ser Pro Pro Ser Pro Asn Ser

1 5

<210> 137

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> (G4S)3

<400> 137

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser

1 5 10 15

<210> 138

<211> 16

[0159]

<212> PRT

<213> 人工序列

<220>

<223> H105

<400> 138

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

<210> 139

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> (G4S)4

<400> 139

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20

<210> 140

<211> 21

<212> PRT

<213> 人工序列

<220>

<223> H75 (NKG2A 四倍突变体)

<400> 140

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly
1 5 10 15

[0160]

His Ser Pro Asn Ser
20

<210> 141
<211> 16
<212> PRT
<213> 人工序列

<220>
<223> H83 (NKG2A 衍生的)

<400> 141

Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly His Ser Pro Asn Ser
1 5 10 15

<210> 142
<211> 18
<212> PRT
<213> 人工序列

<220>
<223> H106 (NKG2A 衍生的)

<400> 142

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly
1 5 10 15

His Ser

<210> 143
<211> 14
<212> PRT
<213> 人工序列

<220>
<223> H81 (NKG2D 衍生的)

[0161]

<400> 143

Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr Ser Pro Asn Ser
1 5 10

<210> 144

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> H91 (NKG2D 衍生的)

<400> 144

Asn Ser Leu Ala Asn Gln Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr
1 5 10 15

Ser Pro Asn Ser
20

<210> 145

<211> 19

<212> PRT

<213> 人工序列

<220>

<223> H94

<400> 145

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10 15

Pro Asn Ser

<210> 146

<211> 15

[0162]

<212> PRT

<213> 人工序列

<220>

<223> H16

<400> 146

Leu	Ser	Val	Lys	Ala	Asp	Phe	Leu	Thr	Pro	Ser	Ile	Gly	Asn	Ser
1				5					10					15

<210> 147

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> H17

<400> 147

Leu	Ser	Val	Lys	Ala	Asp	Phe	Leu	Thr	Pro	Ser	Ile	Ser	Cys	Pro	Pro
1				5					10					15	

Cys	Pro	Asn	Ser
			20

<210> 148

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H18

<400> 148

Leu	Ser	Val	Leu	Ala	Asn	Phe	Ser	Gln	Pro	Glu	Ile	Gly	Asn	Ser
1				5					10					15

[0163]

<210> 149
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> H19

<400> 149

Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Ser Cys Pro Pro
1 5 10 15

Cys Pro Asn Ser
20

<210> 150
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> H20

<400> 150

Leu Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Asn Ser
1 5 10 15

<210> 151
<211> 20
<212> PRT
<213> 人工序列

<220>
<223> H21

<400> 151

Leu Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Cys Pro Pro
1 5 10 15

[0164]

Cys Pro Asn Ser
20

<210> 152
<211> 15
<212> PRT
<213> 人工序列

<220>
<223> H22

<400> 152

Leu Asn Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Asn Ser
1 5 10 15

<210> 153
<211> 21
<212> PRT
<213> 人工序列

<220>
<223> H23

<400> 153

Leu Asp Val Ser Glu Arg Pro Phe Pro Pro His Ile Gln Ser Cys Pro
1 5 10 15

Pro Cys Pro Asn Ser
20

<210> 154
<211> 15
<212> PRT
<213> 人工序列

<220>

[0165]

<223> H24

<400> 154

Arg Glu Gln Leu Ala Glu Val Thr Leu Ser Leu Lys Ala Asn Ser
1 5 10 15

<210> 155

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> H25

<400> 155

Arg Glu Gln Leu Ala Glu Val Thr Leu Ser Leu Lys Ala Cys Pro Pro
1 5 10 15

Cys Pro Asn Ser
20

<210> 156

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H26

<400> 156

Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala Asn Ser
1 5 10 15

<210> 157

<211> 20

<212> PRT

<213> 人工序列

[0166]

<220>

<223> H27

<400> 157

Arg	Ile	His	Gln	Met	Asn	Ser	Glu	Leu	Ser	Val	Leu	Ala	Cys	Pro	Pro
1				5				10						15	

Cys	Pro	Asn	Ser
			20

<210> 158

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H28

<400> 158

Asp	Thr	Lys	Gly	Lys	Asn	Val	Leu	Glu	Lys	Ile	Phe	Ser	Asn	Ser
1				5				10						15

<210> 159

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H30

<400> 159

Leu	Pro	Pro	Glu	Thr	Gln	Glu	Ser	Gln	Glu	Val	Thr	Leu	Asn	Ser
1				5				10						15

<210> 160

<211> 15

[0167]

<212> PRT

<213> 人工序列

<220>

<223> H32

<400> 160

Arg	Ile	His	Leu	Asn	Val	Ser	Glu	Arg	Pro	Phe	Pro	Pro	Asn	Ser
1				5					10				15	

<210> 161

<211> 20

<212> PRT

<213> 人工序列

<220>

<223> H33

<400> 161

Arg	Ile	His	Leu	Asn	Val	Ser	Glu	Arg	Pro	Phe	Pro	Pro	Cys	Pro	Pro
1				5					10				15		

Cys	Pro	Asn	Ser
			20

<210> 162

<211> 13

<212> PRT

<213> 人工序列

<220>

<223> H36

<400> 162

Gly	Cys	Pro	Pro	Cys	Pro	Gly	Gly	Gly	Gly	Ser	Asn	Ser
1				5					10			

[0168]

<210> 163
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> H40

<400> 163

Gly Cys Pro Pro Cys Pro Ala Asn Ser
1 5

<210> 164
<211> 9
<212> PRT
<213> 人工序列

<220>
<223> H41

<400> 164

Gly Cys Pro Pro Cys Pro Ala Asn Ser
1 5

<210> 165
<211> 8
<212> PRT
<213> 人工序列

<220>
<223> H42

<400> 165

Gly Cys Pro Pro Cys Pro Asn Ser
1 5

<210> 166
<211> 13

[0169]

<212> PRT

<213> 人工序列

<220>

<223> H44

<400> 166

Gly Gly Gly Ala Ser Cys Pro Pro Cys Pro Gly Asn Ser
1 5 10

<210> 167

<211> 13

<212> PRT

<213> 人工序列

<220>

<223> H45

<400> 167

Gly Gly Gly Ala Ser Cys Pro Pro Cys Ala Gly Asn Ser
1 5 10

<210> 168

<211> 12

<212> PRT

<213> 人工序列

<220>

<223> H46

<400> 168

Gly Gly Gly Ala Ser Cys Pro Pro Cys Ala Asn Ser
1 5 10

<210> 169

<211> 15

<212> PRT

<213> 人工序列

[0170]

<220>

<223> H47

<400> 169

Leu	Ser	Val	Lys	Ala	Asp	Phe	Leu	Thr	Pro	Ser	Ile	Gly	Asn	Ser
1				5					10				15	

<210> 170

<211> 11

<212> PRT

<213> 人工序列

<220>

<223> H48

<400> 170

Ala	Asp	Phe	Leu	Thr	Pro	Ser	Ile	Gly	Asn	Ser
1				5					10	

<210> 171

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H50

<400> 171

Leu	Ser	Val	Leu	Ala	Asn	Phe	Ser	Gln	Pro	Glu	Ile	Gly	Asn	Ser
1				5					10				15	

<210> 172

<211> 15

<212> PRT

<213> 人工序列

<220>

[0171]

<223> H51

<400> 172

Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Gly Asn Ser
1 5 10 15

<210> 173

<211> 11

<212> PRT

<213> 人工序列

<220>

<223> H52

<400> 173

Ser Gln Pro Glu Ile Val Pro Ile Ser Asn Ser
1 5 10

<210> 174

<211> 16

<212> PRT

<213> 人工序列

<220>

<223> H53

<400> 174

Ser Gln Pro Glu Ile Val Pro Ile Ser Cys Pro Pro Cys Pro Asn Ser
1 5 10 15

<210> 175

<211> 19

<212> PRT

<213> 人工序列

<220>

<223> H54

[0172]

<400> 175

Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Ser Cys Pro Pro Cys
1 5 10 15

Pro Asn Ser

<210> 176

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H55

<400> 176

Arg Ile His Gln Met Asn Ser Glu Leu Ser Val Leu Ala Asn Ser
1 5 10 15

<210> 177

<211> 12

<212> PRT

<213> 人工序列

<220>

<223> H56

<400> 177

Gln Met Asn Ser Glu Leu Ser Val Leu Ala Asn Ser
1 5 10

<210> 178

<211> 10

<212> PRT

<213> 人工序列

<220>

[0173]

<223> H57

<400> 178

Val Ser Glu Arg Pro Phe Pro Pro Asn Ser
1 5 10

<210> 179

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H58

<400> 179

Lys Pro Phe Phe Thr Cys Gly Ser Ala Asp Thr Cys Pro Asn Ser
1 5 10 15

<210> 180

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H59

<400> 180

Lys Pro Phe Phe Thr Cys Gly Ser Ala Asp Thr Cys Pro Asn Ser
1 5 10 15

<210> 181

<211> 15

<212> PRT

<213> 人工序列

<220>

<223> H60

[0174]

<400> 181

Gln Tyr Asn Cys Pro Gly Gln Tyr Thr Phe Ser Met Pro Asn Ser
1 5 10 15

<210> 182

<211> 21

<212> PRT

<213> 人工序列

<220>

<223> H61

<400> 182

Glu Pro Ala Phe Thr Pro Gly Pro Asn Ile Glu Leu Gln Lys Asp Ser
1 5 10 15

Asp Cys Pro Asn Ser
20

<210> 183

<211> 21

<212> PRT

<213> 人工序列

<220>

<223> H62

<400> 183

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys Ala Arg
1 5 10 15

His Cys Pro Asn Ser
20

<210> 184

<211> 20

[0175]

<212> PRT

<213> 人工序列

<220>

<223> H63

<400> 184

Asn Ser Leu Phe Asn Gln Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr

1

5

10

15

Cys Pro Asn Ser

20

<210> 185

<211> 2307

<212> DNA

<213> TSC275 (hu4C04 x DRA222 null2 Scorpion)

<400> 185

atggaagcac cagcgcagct tetcttcctc ctgtactct ggctcccaga taccaccgt 60

gacatccaga tgacccagtc tcaatctctc ctgtctgcat ctgtaggaga cagagtcacc 120

atcacttgcc tggcaagtca gaccattggt acatggtag catggtatca gcagaaacca 180

gggaaagccc ctaagctcct gatttatgct gcaaccagct tggeagatgg ggtcccatca 240

aggttcagtg gcagtggatc tgggacagat ttactctca ccatcagcag tctgcaacct 300

gaagattttg caacttacta ctgtcaacaa ctttacaata ctccgtggac gtteggcgga 360

gggaccaagg tggagatcaa aggggggtgga ggctctggtg gcggtggctc tggcggaggt 420

ggatccggcg ggggtggctc tgaggtccag ctggtacagt ctggggctga ggtgaagaag 480

cctggggcta cagtgaataat ctctgcaag gttctgact acacactcac tgacttctat 540

atgaactggg tgcaacaggc ccctggaaaa gggcttgagt ggattgggag gatttatcct 600

ggaaccgata aaactagata caatgagaaa ticagggaca gagtcacat aaecgcggac 660

[0176]

acgtctacag acacagccta catggagctg agcagcctga gatctgagga cacggccgtg	720
tattactgtg caagatccgc ctactatggt aactacgttg ctatggacta ctgggggcaa	780
gggaccacgg tcaccgtctc ctcgagttag cccaaatctt ctgacaaaae tcacacatgc	840
ccaccgtgac cagcacctga agccgcgggt gcaccgtcag tcttctcttt cccccaaaa	900
cccgaaggaca ccttcattgat ctcccggaac cctgagggtc catgcgtggg ggtggacgtg	960
agccacgaag accctgaggt caagttcaac tggtagctgg acggcgtgga ggtgcataat	1020
gccaagacaa agccgcggga ggagcagtac aacagcacgt accgtgtggg cagcgtcttc	1080
aecgtcttgc accaggactg gctgaatggc aaggcgtacg cgtgcgggtt ctccaacaaa	1140
gccctcccag ccccatctga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca	1200
cagggtgtaca ccttgcctcc atcccggtat gagctgacca agaaccaggt cagcctgacc	1260
tgccttggtc aaggcttcta tccaagcgac atcgccgtgg agtgggagag caatgggcag	1320
ccggagaaca actacaagac cagcctctcc gtgctggact ccgacggctc ctctctcttc	1380
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgtctc	1440
gtgatgcatg aggtcttgca caaccactac acgcagaaga gcctctccct gtctccgggt	1500
cagaggcaca acaattcttc cctgaataca ggaactcaga tggcaggtca ttctccgaat	1560
tctcaggtec agctgggtgga gtctgggggc ggagtgggtc agcctgggag gtcactgagg	1620
ctgtcctgea aggettctgg ctacaccttt actagatcta cgatgcactg ggtaaggcag	1680
gccccctggac aaggtctgga atggattgga tacattaatc ctagcagtgc ttataactaat	1740
tacaatcaga aattcaagga caggttcaca atcagcgcag acaaatccaa gagcacagcc	1800
tctctgcaga tggacagcct gaggcceagag gacaccggcg tctattttctg tgcacggccc	1860
caagtcact atgattacaa cgggtttcct tactggggcc aagggaactc cgteactgtc	1920
tctagcgggtg gcggagggtc tgggggtggc ggcacggag gtggtggctc tgcacaagac	1980

[0177]

atccagaiga cccagtcctc aagcagcctg tctgcaagcg tgggggacag ggtcaccatg 2040
acctgcagtg ccagctcaag tgtaagttac atgaactggg accagcagaa gccgggcaag 2100
gcccccaaaa gatggattta tgactcatcc aaactggett ctggagtccc tgetcgttc 2160
agtggcagtg ggtctgggac cgactatacc ctcacaatca gcagcctgca gcccgagat 2220
ttcgccaatt attactgcca gcagtggagt cgtaaccac ccacgttcgg aggggggacc 2280
aagctacaaa ttacatcctc cagctaa 2307

<210> 186

<211> 748

<212> PRT

<213> 人工序列

<220>

<223> TSC275 (hu4C04 x DRA222 null2 Scorpion)

<400> 186

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Tyr Asn Thr Pro Trp

[0178]

	85	90	95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser			
	100	105	110
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu			
	115	120	125
Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr			
	130	135	140
Val Lys Ile Ser Cys Lys Val Ser Asp Tyr Thr Leu Thr Asp Phe Tyr			
	145	150	155
			160
Met Asn Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly			
	165	170	175
Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe Arg			
	180	185	190
Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr Met			
	195	200	205
Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala			
	210	215	220
Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly Gln			
	225	230	235
			240
Gly Thr Thr Val Thr Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys			
	245	250	255
Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro			

[0179]

	260		265		270
Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser					
275		280		285	
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp					
290		295		300	
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn					
305		310		315	320
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val					
	325		330		335
Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala					
	340		345		350
Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys					
355		360		365	
Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr					
370		375		380	
Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr					
385		390		395	400
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu					
	405		410		415
Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu					
	420		425		430
Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys					

[0180]

435	440	445
Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu		
450	455	460
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly		
465	470	475 480
Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly		
485	490	495
His Ser Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val		
500	505	510
Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr		
515	520	525
Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala Pro Gly Gln		
530	535	540
Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn		
545	550	555 560
Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser		
565	570	575
Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr		
580	585	590
Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly		
595	600	605
Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly		

[0181]

610	615	620
Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp		
625	630	635 640
Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp		
	645	650 655
Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn		
	660	665 670
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp		
	675	680 685
Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly		
	690	695 700
Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp		
	705	710 715 720
Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe		
	725	730 735
Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser Ser		
	740	745
<210> 187		
<211> 107		
<212> PRT		
<213> 人工序列		
<220>		
<223> hu4C04 VL		
<400> 187		

[0182]

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Tyr Asn Thr Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105

<210> 188

<211> 121

<212> PRT

<213> 人工序列

<220>

<223> hu4C04 VH

<400> 188

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Val Ser Asp Tyr Thr Leu Thr Asp Phe

[0183]

20	25	30
Tyr Met Asn Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile		
35	40	45
Gly Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe		
50	55	60
Arg Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr		
65	70	75
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys		
85	90	95
Ala Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly		
100	105	110
Gln Gly Thr Thr Val Thr Val Ser Ser		
115	120	

<210> 189

<211> 2307

<212> DNA

<213> TSC277 (hu4C04 (A43K Q240E) x DRA222 null2 Scorpion)

<400> 189

atggaagcac cagcgcagct tctcttcctc ctgtactctt ggtcccaga taccaceggt	60
gacatccaga tgaccacagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc	120
atcacttgcc tggcaagtca gaccattggt acatggttag catggtatca gcagaaacca	180
gggaaaaagc ctaagctcct gatttatgct gcaaccagct tggcagatgg ggtcccatca	240
aggctcagtg gcagtggaac tgggacagat ttcactctca ccatcagcag tctgcaacct	300
gaagattttg caacttacta ctgtaacaa ctttacaata ctccgtggac gttcggcgga	360

[0184]

gggaccaagg tggagatcaa aggggggtgga ggctctgggtg gcgggtggctc tggcggaggt	420
ggatccggcg ggggtggctc tgaggiccag ctggtacagt ctggggctga ggtgaagaag	480
cctggggcta cagtgaaaat ctctgcaag gtttctgact acacactcac tgacttctat	540
atgaactggg tgcaacaggc ccttgaaaaa gggettgagt ggattgggag gatttatect	600
ggaaccgata aaactagata caatgagaaa ttcagggaca gattcaccat aaccgcggac	660
acgtctacag acacagccta catggagctg agcagcciga gatctgagga caeggccgtg	720
tattactgtg caagatccgc ctactatggt aactacgttg ctatggacta ctggggggag	780
gggaccaagg tcaccgtctc ctogagtgag eccaaatctt ctgacaaaaa tcacacatgc	840
ccaccgtgcc cagcacctga agccgcgggt gcaccgtcag tcttctcttt cccccaaaa	900
cccaaggaca cctcatgat ctcccgacc cctgaggta catgcgtggt ggtggacgtg	960
agccacgaag accctgaggt caagtccaac tggtagctgg acggcgtgga ggtgcataat	1020
gccaaagaaa agccgcggga ggagcagtac aacagcacgt accgtgtggt cagcgtctc	1080
accgtctgc accaggactg gctgaatggc aaggcgtacg cgtgcgcggt ctccaacaaa	1140
gccctcccag ccccatcga gaaaaccate tccaaagcca aagggcagcc ccgagaacca	1200
caggtgtaca cctgcccc atccgggat gagctgacca agaaccaggt cagcctgacc	1260
tgcctggtea aaggttctta tccaagcgac atcgcctgg agtgggagag caatgggcag	1320
ccggagaaca actacaagac cagccctccc gtgctggact ccgaaggctc ctcttctctc	1380
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc	1440
gtgatgcatt aggtctctga caaccactac acgcagaaga gcctctccct gtctccgggt	1500
cagaggcaca acaattcttc cctgaataca ggaactcaga tggcaggtea ttctccgaat	1560
ctcaggctcc agctgggtgga gtctgggggc ggagtggctc agcctgggag gtcactgagg	1620
ctgtcctgca aggtctctgg ctacaccttt actagatcta cgatgcactg ggtaaggcag	1680

[0185]

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geccctggac aaggtctgga atggattgga tacattaate ctagcagtgc ttataactaat      1740

tacaatcaga aattcaagga caggttcaca atcagegcag acaaatccaa gagcacagcc      1800

ttcctgcaga tggacagcct gagggccgag gacaccggcg tctattttctg tgcacggccc      1860

caagtcact atgattacaa cgggtttcct tactggggcc aagggaetcc cgtcactgtc      1920

tctagcggtg geggagggtc tgggggtggc ggatccggag gtggtggctc tgcacaagac      1980

atccagatga cccagtctcc aagcagcctg tctgcaagcg tgggggacag ggtaaccatg      2040

acctgcagtg ccagctcaag tgtaagttac atgaactggt accagcagaa gccgggcaag      2100

geccccaaaa gatggattta tgactcatcc aaactggett ctggagtecc tgetcgettc      2160

agtggcagtg ggtctgggac cgactatacc ctcacaatca gcagcctgca gcccgagat      2220

ttcgccactt attactgcca gcagtggagt cgtaaccac ccacgttcgg agggggggacc      2280

aagctacaaa ttacatcctc cagctaa      2307

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<210> 190

<211> 748

<212> PRT

<213> 人工序列

<220>

<223> TSC277 (hu4C04 (A43K Q240E) x DRA222 null2 Scorpion)

<400> 190

```

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15

```

```

Asp Arg Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
          20           25           30

```

```

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Lys Pro Lys Leu Leu Ile
          35           40           45

```

[0186]

Tyr Ala Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Tyr Asn Thr Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser
100 105 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
115 120 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr
130 135 140

Val Lys Ile Ser Cys Lys Val Ser Asp Tyr Thr Leu Thr Asp Phe Tyr
145 150 155 160

Met Asn Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly
165 170 175

Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe Arg
180 185 190

Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr Met
195 200 205

Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
210 215 220

[0187]

Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly Glu
225 230 235 240

Gly Thr Thr Val Thr Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys
245 250 255

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro
260 265 270

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
275 280 285

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
290 295 300

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
305 310 315 320

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
325 330 335

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala
340 345 350

Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
355 360 365

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
370 375 380

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
385 390 395 400

[0188]

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
405 410 415

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
420 425 430

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
435 440 445

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
450 455 460

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
465 470 475 480

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly
485 490 495

His Ser Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val
500 505 510

Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr
515 520 525

Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala Pro Gly Gln
530 535 540

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn
545 550 555 560

Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser
565 570 575

[0189]

Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr
580 585 590

Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly
595 600 605

Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
610 615 620

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp
625 630 635 640

Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
645 650 655

Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn
660 665 670

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp
675 680 685

Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly
690 695 700

Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
705 710 715 720

Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe
725 730 735

Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser Ser
740 745

[0190]

<210> 191
 <211> 2307
 <212> DNA
 <213> TSC278 (hu4C04 (A43T) x DRA222 null2 Scorpion)

 <400> 191
 atggaagcac cagcgagct tctcttctc ctgctactct ggctcccaga taccaaccgt 60
 gacatccaga tgacccagtc tccatctctc ctgtctgcat ctgtaggaga cagagtcacc 120
 atcacttgcc tggcaagtc gaccattggt acatggttag catggtatca gcagaaacca 180
 gggaaaaccc ctaagctctt gatttatgct gcaaccagct tggcagatgg ggccccatca 240
 aggttcagtg gcagtggatc tgggacagat ttactctca ccatcagcag tetgcaacct 300
 gaagattttg caacttacta ctgtcaacaa ctttacaata ctccgtggac gtccggcgga 360
 gggaccaagg tggagatcaa aggggggtgga ggctctggtg gcggtggctc tggcggaggt 420
 ggatccggcg ggggtggctc tgaggctcag ctggtacagt ctggggctga ggtgaagaag 480
 cctggggcta cagtgaat ctctgcaag gtttctgaet acacactcac tgacttctat 540
 atgaactggg tgcaacaggc ccttgaaaaa gggttgagt ggattgggag gatttatctt 600
 ggaaccgata aaactagata caatgagaaa ttcagggaca gattcaccat aaccgaggac 660
 acgtctacag acacagccta catggagctg agcagcctga gatctgagga caccggcctg 720
 tattactgtg caagatccgc ctactatggt aactacgttg ctatggacta ctgggggcaa 780
 gggaccacgg tcaccgtctc ctgagtgag ccgaatctt ctgacaaaac tcacacatgc 840
 ccaccgtgcc cagcactga agccggggt gcaccgtcag tcttctctt cccccaaaa 900
 cccaaggaca cctcatgat ctccggacc cctgaggtea catgcgtggt ggtggacgtg 960
 agccacgaag acctgaggt caagtccaac tggtagctgg acggcgtgga ggtgcataat 1020
 gccaaagaaa agccgcggga ggagcagtac aacagcagct accgtgtggt cagcgtcctc 1080

[0191]

accgtcctgc accaggactg gctgaatggc aaggegtacg cgtgcgcggt ctccaacaaa	1140
gccctcccag ccccatcgga gaanaaccatc tccaaagcca nagggcagcc ccgagaacca	1200
caggtgtaca ccttgcctcc atcccgggat gagctgacca agaaccaggt cagcctgacc	1260
tgcctggtea aaggetteta tccaagegac atgcgcgtgg agtgggagag caatgggcag	1320
ccggagaaca actacaagac cagccctccc gtgctggact ccgaecggtc cttcttctc	1380
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgtccc	1440
gtgatgeatg aggetetgca caaccactac acgcagaaga gcctctccct gtctccgggt	1500
cagaggcaca acaattcttc cctgaafaca ggaactcaga tggcaggtca ttctccgaat	1560
tctcaggtec agctgggtga gtctgggggc ggagtgggtc agcctgggcg gtcactgagg	1620
cgtctctgca agcttcttgg ctacaccttt actagatcta cgatgcactg ggtaaggcag	1680
gccccggac aaggcttggga atggattgga tacattaatc ctacgagtgc ttatacta	1740
tacaatcaga aattcaagga caggttcaca atcagcgcag acaaatccaa gagcacagcc	1800
ttcttgcaga tggacagcct gagggccgag gacaccggcg tctatttctg tgcacggccc	1860
caagtccact atgattacaa cgggtttcct tactggggcc aagggactcc cgtcactgtc	1920
tctagcggtg gcggagggtc tgggggtggc ggatecggag gtggtgggtc tgcacaagac	1980
atccagatga cccagtctcc aagcagcctg tctgcaagcg tgggggacag ggtaaccatg	2040
acctgcagtg ccagctcaag tgtaagttac atgaactggg accagcagaa gccgggcaag	2100
gccccaaaa gatggattta tgactcatcc aaactggctt ctggagtccc tgctcgcttc	2160
agtggcagtg ggcttgggac cgactatacc ctcaaatca gcagcctgca gcccgagat	2220
ttcgccactt attactgcca gcagtggagt cgtaaccac ccacgttcgg aggggggacc	2280
aagctacaaa ttacatcttc cagctaa	2307

<210> 192

[0192]

<211> 748

<212> PRT

<213> 人工序列

<220>

<223> TSC278 (hu4C04 (A43T) x DRA222 null2 Scorpion)

<400> 192

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Thr Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Tyr Asn Thr Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser
100 105 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
115 120 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr
130 135 140

[0193]

Val Lys Ile Ser Cys Lys Val Ser Asp Tyr Thr Leu Thr Asp Phe Tyr
145 150 155 160

Met Asn Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly
165 170 175

Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe Arg
180 185 190

Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr Met
195 200 205

Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
210 215 220

Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly Gln
225 230 235 240

Gly Thr Thr Val Thr Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys
245 250 255

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro
260 265 270

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
275 280 285

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
290 295 300

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
305 310 315 320

[0194]

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
325 330 335

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala
340 345 350

Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
355 360 365

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
370 375 380

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
385 390 395 400

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
405 410 415

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
420 425 430

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
435 440 445

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
450 455 460

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
465 470 475 480

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly
485 490 495

[0195]

His Ser Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val
500 505 510

Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr
515 520 525

Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala Pro Gly Gln
530 535 540

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn
545 550 555 560

Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser
565 570 575

Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr
580 585 590

Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly
595 600 605

Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
610 615 620

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp
625 630 635 640

Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
645 650 655

Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn
660 665 670

[0196]

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp
675 680 685

Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly
690 695 700

Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
705 710 715 720

Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe
725 730 735

Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser Ser
740 745

<210> 193

<211> 2307

<212> DNA

<213> TSC279 (hu4C04 (Q165R) x DRA222 null2 Scorpion)

<400> 193

atggaagcac cagcgcagct tctcttctc ctgctactct ggetcccaga taccaccggt 60

gacatccaga tgaccagtc tccatctctc ctgtctgeat ctgtaggaga cagagtcacc 120

atcacttgcc tggcaagtca gaccattggt acatggttag catggatca gcagaaacca 180

gggaaagecc ctaagctcct gatttatgct gcaaccagct tggcagatgg ggteccatca 240

aggttcagtg gcagtggatc tgggacagat ttcactctca ccatcagcag tctgcaacct 300

gaagattttg caacttacta ctgtcaacaa ctttacaata ctccgtggac gticggcgga 360

gggaaccaagg tggagatcaa agggggtgga ggctctggtg gcggtggctc tggcggaggt 420

ggatccggcg ggggtggctc tgaggtccag ctggtacagt ctggggctga ggtgaagaag 480

cctggggcta cagtgaanaa ctcttgaag gtttctgact acacactcac tgacttctat 540

[0197]

atgaactggg tgagacagge ccttggaana gggcttgagt ggattgggag gatttatcct	600
ggaaccgata aaactagata caatgagaaa ttcagggaca gagtcacat aaccgcggac	660
acgtetacag acacagccta catggagctg agcagcctga gatctgagga caggccctg	720
tattactgtg caagatccgc ctactatggt aactacgttg ctatggacta ctgggggcaa	780
gggaccacgg tcaccgtctc ctgagtgag cccaaatctt ctgacaaaac tcacacatgc	840
ccaccgtgcc cagcacctga agccgcgggt gcaccgtcag tcttctctt cccccaaaa	900
cccaaggaca cctcatgat ctcccgacc cctgaggta catgcgtggt ggtggacgtg	960
agccacgaag accctgaggt caagtccaac tggtaegtgg accgcgtgga ggtgcataat	1020
gccaaagaaa agccgcggga ggagcagta aacagcagct accgtgtggt cagcgtctc	1080
accgtctctg accaggactg gctgaatggc aaggcgtacg cgtgcgcggf ctccaacaaa	1140
gccctcccag ccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca	1200
caggltgaca cctgcccc atcccggaat gagctgacca agaaccaggt cagcctgacc	1260
tgcctggta aaggttctc tccaagcagc atcgccgtgg agtgggagag caatgggcag	1320
ccggagaaca actacaagac cagcctccc gtgctggact ccgacggctc cttcttctc	1380
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgtcc	1440
gtgatgatg aggtctctga caaccactac acgcagaaga gcctctcct gtctecgggt	1500
cagaggcaca acaattcttc cctgaataca ggaactcaga tggcaggta ttctccgaat	1560
tctcaggtcc agctgggtga gtctgggggc ggagtgggtc agcctgggcg gtcactgagg	1620
ctgtcttga aggtctctg ctacacctt actagatcta cgtgcactg ggtaaggcag	1680
gccccggac aaggtctgga atggattgga tacattaatc ctgacagtgc ttatactaat	1740
tacaatcaga aaticaagga caggttcaca atcagcgcag acaaatccaa gagcacagcc	1800
tctctgcaga tggacagcct gagggccgag gacaccggcg tctatttttg tgcaaggccc	1860

[0198]

caagtccact atgattacaa cgggtttcct tactggggcc aagggactcc cgtcactgtc 1920
tctagcggtg gcggagggtc tgggggtggc ggatccggag gtggtggctc tgcacaagac 1980
atccagatga cccagtctcc aagcagcctg tctgcaagcg tgggggacag ggtcaccatg 2040
acctgcagtg ccagctcaag tgtaagttac atgaactggc accagcagaa gcggggcaag 2100
gcccccaaaa gatggattta tgactcatcc aaactggctt ctggagtcce tgcctgcttc 2160
agtggcagtg ggtctgggac cgactatacc ctcaaatca gcagcctgca gcccgagat 2220
ttcgccactt attactgcca gcagtggagt cgtaacccac ccacgttcgg aggggggacc 2280
aagctacaaa ttacatcttc cagctaa 2307

<210> 194

<211> 748

<212> PRT

<213> 人工序列

<220>

<223> TSC279 (hu4C04 (Q165R) x DRA222 null2 Scorpion)

<400> 194

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Leu Ala Ser Gln Thr Ile Gly Thr Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ala Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

[0199]

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Leu Tyr Asn Thr Pro Trp
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Ser
100 105 110

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu
115 120 125

Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr
130 135 140

Val Lys Ile Ser Cys Lys Val Ser Asp Tyr Thr Leu Thr Asp Phe Tyr
145 150 155 160

Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly
165 170 175

Arg Ile Tyr Pro Gly Thr Asp Lys Thr Arg Tyr Asn Glu Lys Phe Arg
180 185 190

Asp Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr Met
195 200 205

Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
210 215 220

Arg Ser Ala Tyr Tyr Gly Asn Tyr Val Ala Met Asp Tyr Trp Gly Gln
225 230 235 240

[0200]

Gly Thr Thr Val Thr Val Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys
245 250 255

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro
260 265 270

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
275 280 285

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
290 295 300

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
305 310 315 320

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
325 330 335

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala
340 345 350

Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
355 360 365

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
370 375 380

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
385 390 395 400

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
405 410 415

[0201]

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
420 425 430

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
435 440 445

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
450 455 460

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
465 470 475 480

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly
485 490 495

His Ser Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val
500 505 510

Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr
515 520 525

Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala Pro Gly Gln
530 535 540

Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn
545 550 555 560

Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser
565 570 575

Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr
580 585 590

[0202]

Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly
595 600 605

Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
610 615 620

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp
625 630 635 640

Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
645 650 655

Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn
660 665 670

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp
675 680 685

Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly
690 695 700

Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
705 710 715 720

Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe
725 730 735

Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser Ser
740 745

<210> 195

<211> 2316

<212> DNA

<213> TSC233 (HD37 x DRA221 scorpion, H75 连接器)

[0203]

<400> 195	
atggaagcac cagcgagct tctcttctc ctgctactct ggctcccaga taccaccggt	60
gacattgtgc tgaccaatc tccagettct ttggctgtgt ctctaggga gagggccacc	120
atctcctgca aggccagcca aagtggtgat tatgatggtg atagttatct gaactggtag	180
caacagattc caggacagcc acccaaacct ctcattatg atgcatcaa tctagttct	240
gggatccac ccaggtttag tggcagtggg tctgggacag acttcacct caacatccat	300
cctgtggaga aggtggatgc tgcaacctat cactgccagc aaagtactga ggatccgtg	360
acgttcggtg gaggcaccac gctggaaatc aaaggtggcg gtggttcggg cgggtggtgg	420
tcgggtggcg gggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg	480
cctgggtcct cagtgaagat ttctgcaag gcttctggct atgcattcag tagctactgg	540
atgaactggg tgaagcagag gcctggacag ggtcttgagt ggattggaca gatttgacct	600
ggagatggtg ataactaact caatggaaag ttcaagggtc aagccactct gactgcagac	660
gaatcctcca gcacagccta catgcaactc agcagcctag catctgagga ctctgcggtc	720
tatttctgtg caagacggga gactacgacg gtaggccgtt attactatgc tatggactac	780
tgggtcaag gaacctcagt caccgtctcc tcgagtgage ccaaactctc tgacaaaact	840
cacacatgcc caccgtgccc agcacctgaa gccgcgggtg caccgtcagt ctctctctc	900
ccccaaaac ccaaggacac cctcatgac tcccgaccc ctgaggtcac atgcgtggtg	960
gtggacgtga gccacgaaga cctgagggtc aagttcaact ggtacgtgga cggcgtggag	1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	1080
agcgtctcca ccgtctgca ccaggactgg ctgaatggca aggcgtacgc gtgcgcggtc	1140
tccaacaaag cctcccagc ccccatcgag aaaacctct ccaaagccaa agggcagccc	1200
cgagaaccac aggtgtacac cctgccccca tcccggtatg agctgaccaa gaaccaggtc	1260

[0204]

agcctgacct gcctgggtcaa aggcttctat ccaagcgaca tcgccgtgga gtgggagagc	1320
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgctggactc cgacggctcc	1380
tcttctctct acagcaaget caccgtggac aagagcaggt ggcagcaggg gaacgtcttc	1440
tcattgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg	1500
tctccgggtc agaggcacaa caattcttcc ctgaatacag gaactcagat ggcaggctcat	1560
tctccgaatt ctacaggtcca gctgggtggag tctgggggcg gattgggtgca gcctgggcgg	1620
tcactgaggc tgctctgcaa ggtctctggc tacaccttta ctagatctac gatgcactgg	1680
gtaaggeagg cccctggaca aggtctggaa tggattggat acattaatcc tagcagtgt	1740
tatactaatt acaatcagaa attcaaggac aggttcacaa tcagcgcaga caaatccaag	1800
agcacagcct tcctgcagat ggacagcctg aggcccgagg acaccggcgt ctatttctgt	1860
gcacggcccc aagtcacta tgattacaac gggtttctct actggggcca agggactccc	1920
gtcactgtct ctagcggtag cggagggtct gggggtagcg gatccggagg tggtagctct	1980
gcacaagaca tccagatgac ccagtctcca agcagcctgt ctgcaagcgt gggggacagg	2040
gtcaccatga cctgcagtgc cagctcaagt gtaagttaca tgaactggtc ccagcagaag	2100
ccgggcaagg cccccaaact cctcatctat gactcatcca aactggcttc tggagtccct	2160
gtctgcttca gtggcagtgg gtctgggacc gactataccc tcacaatcag cagcctgcag	2220
cccgaagatt tcgccactta ttactgccag cagtggagtc gtaaccacc cacttctggc	2280
ggagggacca aggtggagat caaatcctcc agctaa	2316

<210> 196

<211> 751

<212> PRT

<213> 人工序列

<220>

<223> TSC233 (HD37 x DRA221 scorpion, H75 连接子)

[0205]

<400> 196

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly

[0206]

	165	170	175
Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys			
	180	185	190
Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met			
	195	200	205
Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala			
	210	215	220
Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr			
225	230	235	240
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser			
	245	250	255
Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala			
	260	265	270
Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu			
	275	280	285
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser			
290	295	300	
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu			
305	310	315	320
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr			
	325	330	335
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn			

[0207]

	340		345		350
Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro					
355		360		365	
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln					
370		375		380	
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val					
385		390		395	400
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val					
	405		410		415
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro					
	420		425		430
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr					
	435		440		445
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val					
	450		455		460
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu					
465		470		475	480
Ser Pro Gly Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln					
	485		490		495
Met Ala Gly His Ser Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly					
	500		505		510
Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala					

[0208]

	515	520	525
Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala			
530	535	540	
Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala			
545	550	555	560
Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala			
	565	570	575
Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro			
	580	585	590
Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp			
595	600	605	
Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser			
610	615	620	
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser			
625	630	635	640
Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser			
	645	650	655
Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser			
	660	665	670
Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu			
675	680	685	
Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser			

[0209]

690	695	700	
Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln			
705	710	715	720
Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro			
	725	730	735
Pro Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Ser Ser Ser			
	740	745	750
<210> 197			
<211> 2316			
<212> DNA			
<213> TSC234 (HD37 x DRA222 scorpion, H75 连接子)			
<400> 197			
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gacattgtgc tgaccaate tecagettct ttggctgtgt ctctagggea gagggccacc			120
atctcttgcaggccagcca aagtgltgat tatgatggcg atagttattt gaactgggtac			180
caacagattc caggacagcc acccaaactc ctcatctatg atgcatccaa tctagtttct			240
gggatcccac ccaggttttag tggcagtggt tetgggacag acttcacct caacatccat			300
ctgttgagga aggtggatgc tgcaacctat cactgccagc aaagtactga ggatecgtgg			360
acgttcgggtg gaggcaccaa gctggaaatc aaaggtggcg gtggttcggg cgggtggtgg			420
tcgggtggcg gaggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg			480
cttgggtcct cagtgaagat ttcttgcagg gcttctggt atgcattcag tagctactgg			540
atgaactggg tgaagcagag gcttgacag ggtcttgagt ggattggaca gatttggcct			600
ggagatggcg atactaacta caatggaaag ttcaaggga aagccactct gactgcagac			660
gaatctcca gcacagccta catgcaactc agcagcctag catctgagga ctctcggtc			720

[0210]

tatttctgtg caagacggga gactacgacg gtaggccgtt attactatgc tatggactac	780
tggggtaag gaacctcagt caccgtctcc tcgagtgage ccaaatcttc tgacaaaact	840
cacacatgcc caccgtgccc agcacctgaa gccgcgggtg caccgtcagt ctctctcttc	900
ccccaaaaac ccaaggacac cctcatgac tcceggaccc ctgaggteac atgcgtggtg	960
gtggacgtga gccacgaaga ccctgaggtc aagttcaact ggtacgtgga cggcgtggag	1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	1080
agcgtctca ccgtctgca ccaggactgg ctgaatggca aggcgtacgc gtgcgcggtc	1140
tccaacaaag cctctccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc	1200
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccagtc	1260
agcctgacct gcctgggtcaa aggtctctat ccaagcgaca tcgccgtgga gtgggagagc	1320
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgcctggactc cgacggctcc	1380
ttctctctct acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc	1440
tcatgtctcg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg	1500
tctccgggtc agaggcacia caattcttcc ctgaatacag gaactcagat ggcaggtcac	1560
tctccgaatt ctacaggtcca gctggtggag tctgggggag gagtggtgca gcctgggcgg	1620
tcactgagge tgctctgcaa ggtctctggc tacacctta ctgatctac gatgcactgg	1680
gtaaggcagg cccctggaca aggtctggaa tggattggat acattaatcc tagcagtget	1740
tatactaatt acaatcagaa attcaaggac aggttcacaa tcagcgcaga caaatccaag	1800
agcacagcct tctgcagat ggacagcctg aggcccgagg acaccggcgt ctatttctgt	1860
gcacggcccc aagtccacta tgattacaac gggtttcctt actggggcca agggactccc	1920
gtcactgtct ctacgggtgg cggagggtct ggggggtggc gatccggagg tgggtgctct	1980
gcacaagaca tccagatgac ccagtctcca agcagcctgt ctgcaagcgt gggggacagg	2040

[0211]

gtcaccatga cctgcagtcg cagctcaagt gtaagttaca tgaactggta ccagcagaag 2100
ccgggcaagg cccccaaaag atggatttat gactcatcca aactggcttc tggagtcctt 2160
gtctgcttca gtggeagttg gtcctgggacc gactataccc tcacaatcag cagcctgcag 2220
cccgaagatt tcgccactta ttactgccag cagtggagtc gtaacccacc caggttcgga 2280
ggggggacca agctacaaat tacatcctcc agctaa 2316

<210> 198

<211> 751

<212> PRT

<213> 人工序列

<220>

<223> TSC234 (HD37 x DRA222 scorpion, H75 连接子)

<400> 198

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

[0212]

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
165 170 175

Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
180 185 190

Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met
195 200 205

Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
210 215 220

Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr
225 230 235 240

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser
245 250 255

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
260 265 270

[0213]

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
275 280 285

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
290 295 300

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
305 310 315 320

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
325 330 335

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
340 345 350

Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro
355 360 365

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
370 375 380

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
385 390 395 400

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
405 410 415

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
420 425 430

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
435 440 445

[0214]

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
450 455 460

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
465 470 475 480

Ser Pro Gly Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln
485 490 495

Met Ala Gly His Ser Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly
500 505 510

Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala
515 520 525

Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala
530 535 540

Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala
545 550 555 560

Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala
565 570 575

Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro
580 585 590

Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp
595 600 605

Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser
610 615 620

[0215]

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
625 630 635 640

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
645 650 655

Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
660 665 670

Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp
675 680 685

Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
690 695 700

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
705 710 715 720

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro
725 730 735

Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser Ser
740 745 750

<210> 199

<211> 2325

<212> DNA

<213> TSC235 (HD37 x DRA224 scorpion, H75 连接子)

<400> 199

atggaagcac cagegcagct tctcttctctc ctgctactct ggetcccaga taccaccggt 60

gacattgtgc tgaccaatc tccagettct tiggctgtgt ctctagggca gagggccacc 120

[0216]

atctcctgca aggccagcca aagtgttgat tatgatggtg atagttattt gaactggtac	180
caacagattc caggacagcc acccaaacte ctcatctatg atgcatccaa tctagtttct	240
gggatccac ccaggtttag tggcagtggg tetgggacag acttcaccct caacatccat	300
cctgtggaga aggtggatgc tgcaacctat cactgccage aaagtactga ggatccgtgg	360
acgttcggtg gaggcaccaa gctggaaatc aaaggtggcg gtggttcggg cgggtggtgg	420
tcgggtggcg gcggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg	480
cctgggtcct cagtgaagat ttcttgaag gcttctggct atgcattcag tagctactgg	540
atgaactggg tgaagcagag gcctggacag ggtcttgagt ggattggaca gatttggcct	600
ggagatggtg atactaacta caatggaaag ttcaagggtc aagccactct gactgcagac	660
gaatcctcca gcacagccta catgcaactc agcagcctag catctgagga ctctgcggtc	720
tatttctgtg caagacggga gactacgacg gtaggccgtt attactatgc tatggactac	780
tggggctcaag gaacctcagt caccgtctcc tcgagtgage ccaaattctc tgacaaaact	840
cacacatgcc caccgtgccc agcacctgaa gccgcgggtg caccgtcagt ctctctcttc	900
ccccaaaac ccaaggacac cctcatgac tcccggaccc ctgaggtcac atgcgtggtg	960
gtggacgtga gccacgaaga cctgaggtc aagttcaact ggtacgtgga cggcgtggag	1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	1080
agcgtcctca cegtcctgca ccaggactgg ctgaatggca aggcgtacgc gtgcgcggtc	1140
tccaacaaag ccctcccage ccccatcgag aaaacctct ccaaagccaa agggcagccc	1200
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc	1260
agcctgacct gcctggtcaa aggtttctat ccaagcaca tcgccgtgga gtgggagagc	1320
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgetggactc cgaaggctcc	1380
ttcttctctt acageaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc	1440

[0217]

tcattgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg 1500

tctccgggtc agaggcacia caattcttcc ctgaatacag gaactcagat ggcaggtcat 1560

tctccgaatt ctcaggtcca gcttctgcag tctggggctg aggtgaagaa gcctggggcc 1620

tcagtgaagg ttctctgcaa ggcttctgga tacaccttca ctagatctac gatgcactgg 1680

gtgcgccagg ccccccagaca aaggcttgag tggatgggat acattaatcc tagcagtgt 1740

tatactaatt acaatcagaa attcaaggac agagtcacca ttaccaggga cacatccgcg 1800

agcacagcct acatggagct gacgagcctg agatctgaag acacggctgt gtattactgt 1860

gcgagacccc aagtccacta tgattacaac gggtttctt actggggcca aggaacctg 1920

gtcacctgtt cctcaggtgg aggcgggttca ggcggaggtg gatccggcgg tggcggatcg 1980

ggtggcggcg gatctgaaat tgtgttgaca cagtctccag ccacctgtc ttgtctcca 2040

ggggaaagag ccacctctc ctgcagtgc agctcaagt taagttacat gaactggtac 2100

caacagaaac ctggccagge tccaggtc ctcctctatg actcatcaa actggtctt 2160

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actttcggcg gagggaccaa ggtggagatc aaatcttcca gctaa 2325

<210> 200

<211> 754

<212> PRT

<213> 人工序列

<220>

<223> TSC235 (HD37 x DRA224 scorpion, H75 连接子)

<400> 200

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

[0218]

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
165 170 175

Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
180 185 190

[0219]

Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met
195 200 205

Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
210 215 220

Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr
225 230 235 240

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser
245 250 255

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
260 265 270

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
275 280 285

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
290 295 300

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
305 310 315 320

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
325 330 335

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
340 345 350

Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro
355 360 365

[0220]

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
370 375 380

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
385 390 395 400

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
405 410 415

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
420 425 430

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
435 440 445

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
450 455 460

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
465 470 475 480

Ser Pro Gly Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln
485 490 495

Met Ala Gly His Ser Pro Asn Ser Gln Val Gln Leu Val Gln Ser Gly
500 505 510

Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala
515 520 525

Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala
530 535 540

[0221]

Pro Gly Gln Arg Leu Glu Trp Met Gly Tyr Ile Asn Pro Ser Ser Ala
545 550 555 560

Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Val Thr Ile Thr Arg
565 570 575

Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser
580 585 590

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Pro Gln Val His Tyr Asp
595 600 605

Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser
610 615 620

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
625 630 635 640

Gly Gly Gly Gly Ser Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu
645 650 655

Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser
660 665 670

Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
675 680 685

Arg Leu Leu Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Ile Pro Ala
690 695 700

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
705 710 715 720

[0222]

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser
725 730 735

Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Ser
740 745 750

Ser Ser

<210> 201

<211> 2295

<212> DNA

<213> TSC240 (HD37 x DRA222 scorpion, H81 连接器)

<400> 201

atggaagcac cagcgcagct tctcttcctc ctgctactct ggtcccaga taccaccgt	60
gacattgtgc tgaccaatc tccagcttct ttggtgtgt ctctagggca gagggccacc	120
atctctgca aggccagcca aagtgtgat tatgatggtg atagttattt gaactggtac	180
caacagattc caggacagcc acccaaacte ctcattatg atgcattcaa tctagtttct	240
gggateccac ccaggtttag tggcagtggg tetgggacag acttcacct caacatccat	300
cctgtggaga aggtggatgc tgcaacctat cactgccage aaagtactga ggatccgtgg	360
acgttcggtg gaggeaccaa gtgggaaatc aaaggtggcg gtggttcggg cgggtggtggg	420
tgggtggcg gggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg	480
cctgggtect cagtgaagat ttctgcaag gcttctggt atgcattcag tagctaetgg	540
atgaactggg tgaagcagag gcctggacag ggtcttgagt ggattggaca gatttggcct	600
ggagatggtg atactaacta caatggaaag ttcaagggt aagccactct gactgcagac	660
gaatcctcca gcacagccta catgcaactc agcagcctag catctgagga ctctgcggtc	720
tatttctgtg caagacggga gactacgacg gtaggccgtt attactatgc tatggactac	780

[0223]

tggggtaag gaacctcagt caccgtctcc tcgagtgage ccaaatcttc tgacaaaact	840
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ccccaaaaac ccaaggacac cctcatgate tcccggacce ctgaggteac atgcgtggig	960
gtggaegtga gccacgaaga ccctgaggte aagttcaact ggtacgtgga cggcgtggag	1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	1080
agcgtctctca ccgtctgca ccaggactgg ctgaatggca aggcgtacgc gtgcgcggtc	1140
tccaacaaag cctctccage ccccatcgag aaaacctct ccaaagccaa agggcagccc	1200
cgagaaccac aggtgtacac cctgcccaca tcccgggatg agctgaccaa gaaccaggtc	1260
agcctgacct gcctggtaaa agcttctat ccaagcaca tcgccgtgga gtgggagagc	1320
aatgggcagc cggagaacaa ctacaagacc acgctcccg tgcctggact cgacggctcc	1380
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ggagggtctg ggggtggcgg atccggaggt ggtggctctg cacaagacat ccagatgacc	1980
cagtctceaa gcagcctgtc tgcaagcgtg ggggacaggg tcaccatgac ctgcagtgcc	2040
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[0224]

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tetgggaccg actataccct cacaatcage agcctgcage ccgaagattt cgcacttat 2220
tactgccage agtggagtcg taacccaccc acgttcggag gggggaccaa gctacaaatt 2280
acatecteca gctaa 2295

<210> 202

<211> 744

<212> PRT

<213> 人工序列

<220>

<223> TSC240 (HD37 x DRA222 scorpion, H81 连接子)

<400> 202

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

[0225]

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
165 170 175

Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
180 185 190

Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met
195 200 205

Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
210 215 220

Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr
225 230 235 240

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser
245 250 255

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
260 265 270

[0226]

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
275 280 285

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
290 295 300

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
305 310 315 320

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
325 330 335

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
340 345 350

Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro
355 360 365

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
370 375 380

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
385 390 395 400

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
405 410 415

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
420 425 430

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
435 440 445

[0227]

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
450 455 460

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
465 470 475 480

Ser Pro Gly Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr Ser Pro Asn
485 490 495

Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly
500 505 510

Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg
515 520 525

Ser Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
530 535 540

Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys
545 550 555 560

Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala
565 570 575

Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe
580 585 590

Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp
595 600 605

Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
610 615 620

[0228]

Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr
625 630 635 640

Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met
645 650 655

Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
660 665 670

Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu
675 680 685

Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
690 695 700

Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
705 710 715 720

Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr
725 730 735

Lys Leu Gln Ile Thr Ser Ser Ser
740

<210> 203

<211> 2301

<212> DNA

<213> TSC241 (HD37 x DRA222 scorpion, H83 连接子)

<400> 203

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gacattgtgc tgaccaatc tccagcttct ttggtgtgt ctctaggga gagggccacc 120

atctctgca aggccagcca aaggttgat tatgatgtg atagttatt gaactgttac 180

[0229]

caacagattc caggacagcc acccaaaactc ctcattctatg atgcatccaa tctagtttct	240
gggatccac ccaggttttag tggcagtggg tctgggacag acttcaccct caacatccat	300
cctgtggaga aggtggatgc tgcaacctat cactgccagc aaagtactga ggatccgtgg	360
acgttcggtg gaggcaccaa gctggaaatc aaaggtggcg gtggttcggg cgggtgtggg	420
tgggtggcg gcggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg	480
cctgggtcct cagtgaagat ttcctgcaag gcttctggct atgcattcag tagctactgg	540
atgaactggg tgaagcagag gcttgacag ggtcttgagt ggattggaca gatttggcct	600
ggagatggtg atactaacta caatggaaag ttcaagggtg aagccactct gactgcagac	660
gaatcctcca gcacagccta catgcaactc agcagcctag catctgagga ctctgcggtc	720
tatttctgtg caagacggga gactacgacg gtaggcggtt attactatgc tatggactac	780
tgggtcaag gaacctcagt caccgtctcc tcgagtgage ccaaattctc tgacaaaact	840
cacacatgcc caccgtgcc agcacctgaa gccgcgggtg caccgtcagt ctctctcttc	900
ccccaaaac ccaaggacac cctcatgac tcccggaccc ctgaggtcac atgcgtggtg	960
gtggacgtga gccacgaaga cctgaggtc aagtccaact ggtacgtgga cggcgtggag	1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	1080
agcgtctcea ccgtcctgca ccaggactgg ctgaatggca aggcgtaegc gtgcgcggtc	1140
tccaacaaag ccttcccage ccccatcgag aaaaccatct ccaaagccaa agggcagccc	1200
cgagaaccac aggtgtacac cctgcccaca tcccgggatg agctgaccaa gaaccaggtc	1260
agcctgacct gcctgggtcaa agcttctat ccaagcgaca tcgccgtgga gtgggagagc	1320
aatgggcage cggagaacaa ctacaagacc acgcctcccg tgetggactc cgacggetcc	1380
ttcttctctt acagcaagct caccgtggac aagagcaggt ggcagcaggg gaacgtcttc	1440
tcatgctccg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg	1500

[0230]

tctccgggtt cttccctgaa tacaggaact cagatggcag gtcattctcc gaattctcag	1560
gtccagctgg tggagtctgg gggcggagtg gtgcagcctg ggcggctact gaggctgtcc	1620
tgcaaggctt ctggetacac ctttactaga tctacgatgc actgggtaag gcaggeccct	1680
ggacaaggtc tggaatggat tggatcatt aatctagca gtgettatac taattacaat	1740
cagaaattca aggacaggtt cacaatcagc gcagacaaat ccaagagcac agccttcctg	1800
cagatggaca gcctgaggcc cgaggacacc ggcgtctatt tctgtgcacg gccccaagtc	1860
cactatgatt acaaegggtt tcttactagg ggccaaggga ctcccgtcac tgtctctage	1920
ggtggcggag ggtctggggg tggeggatcc ggaggtggtg gctctgcaca agacatecag	1980
atgacceagt ctccaageag cctgtctgca agcgtggggg acagggtcac catgacctgc	2040
agtgccagct caagtgttag ttacatgaac tgggtaccagc agaagccggg caaggccccc	2100
aaaagatgga tttatgactc atccaaactg gcttctggag tccctgctcg cttcagtggc	2160
agtgggtctg ggaccgacta taccctcaca atcagcagcc tgcagcccca agatttegcc	2220
acttattact gccagcagtg gagtcgtaac ccaccacgt tcggaggggg gaccaagcta	2280
caaattacat cctccagcta a	2301

<210> 204

<211> 746

<212> PRT

<213> 人工序列

<220>

<223> TSC241 (HD37 x DRA222 scorpion, H83 连接子)

<400> 204

Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Ala	Ser	Leu	Ala	Val	Ser	Leu	Gly
1				5				10						15	

Gln	Arg	Ala	Thr	Ile	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp

[0231]

20	25	30
Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro		
35	40	45
Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro		
50	55	60
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His		
65	70	75
80		
Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr		
85	90	95
Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly		
100	105	110
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln		
115	120	125
Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser		
130	135	140
Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp		
145	150	155
160		
Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly		
165	170	175
Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys		
180	185	190
Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met		

[0232]

195	200	205
Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala		
210	215	220
Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr		
225	230	235 240
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser		
245	250	255
Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala		
260	265	270
Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
275	280	285
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
290	295	300
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
305	310	315 320
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
325	330	335
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
340	345	350
Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro		
355	360	365
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		

[0233]

370	375	380
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val		
385	390	395
400		
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
405	410	415
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
420	425	430
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
435	440	445
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
450	455	460
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
465	470	475
480		
Ser Pro Gly Ser Ser Leu Asn Thr Gly Thr Gln Met Ala Gly His Ser		
485	490	495
Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln		
500	505	510
Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe		
515	520	525
Thr Arg Ser Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu		
530	535	540
Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn		

[0234]

545	550	555	560
Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser			
565	570	575	
Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val			
580	585	590	
Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro			
595	600	605	
Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly			
610	615	620	
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln			
625	630	635	640
Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val			
645	650	655	
Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr			
660	665	670	
Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser			
675	680	685	
Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly			
690	695	700	
Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala			
705	710	715	720
Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly			

[0235]

	725	730	735
	Gly Thr Lys Leu Gln Ile Thr Ser Ser Ser		
	740	745	
<210>	205		
<211>	2313		
<212>	DNA		
<213>	TSC242 (HD37 x DRA222 scorpion, H91 连接子)		
<400>	205		
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	gacattgtgc tgaccaate tccagcttct ttggtgtgt ctctaggga gagggccacc		120
	atctctgca aggccagcca aagtgtgat tatgatggg atagttatt gaactggtac		180
	caacagattc caggacagcc acccaaacct ctcctctatg atgcattcaa tctagtttct		240
	gggatccac ccaggtttag tggcagtggg tctgggacag acttcacct caacatccat		300
	cctgtggaga aggtggatgc tgcaacctat cactgccagc aaagtactga ggatccgtgg		360
	acgttcggtg gaggcaccaa gctggaaatc aaaggtggcg gtggttcggg cgggtgtggg		420
	tgggtggcg gggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg		480
	cctgggtcct cagtgaagat ttcttgcaag gcttctgget atgcattcag tagctactgg		540
	atgaactggg tgaagcagag gcctggacag ggtcttgagt ggattggaca gatttgcct		600
	ggagatggtg atactaacta caatggaaag ttcaagggtc aagccactct gactgcagac		660
	gaatctcca gcacagcta catgcaactc agcagcctag catctgagga ctctcggtc		720
	tatttctgtg caagacggga gactacgacg gtaggcggtt attactatgc tatggactac		780
	tgggtcaag gaacctcagt caccgtctcc tegtgtgagc ccaaatcttc tgacaaaact		840
	cacacatgcc caccgtgcc agcacctgaa gccgcgggtg caccgtcagt ctctctcttc		900
	ccccaaaaac ccaaggacac cctcatgata tcccgacccc ctgaggtcac atgcgtggtg		960

[0236]

gtggacgtga gccacgaaga ccctgaggtc aagtccaact ggtacgtgga cggcgtggag	1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtggtc	1080
agegtctctca cegtcctgca ccaggactgg ctgaatggca aggcgtaacg gtgcgcggtc	1140
tccaacaaag ccctcccagc ccccatcgag aaaaccatct ccaaagccaa agggcagccc	1200
cgagaaccac aggtgtacac cctgccccca tcccgggatg agctgaccaa gaaccaggtc	1260
agcctgacct gecttggtcaa agcttcttat ccaagcgaca tcgccgtgga gtgggagagc	1320
aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgcctggactc cgacggctcc	1380
ttcttctctt acagcaagct caacgtggac aagagcaggt ggcagcaggg gaacgtcttc	1440
tcattgctcg tgatgcatga ggctctgcac aaccactaca cgcagaagag cctctccctg	1500
tctccgggta actcattagc aaaccaagaa gtccaattc ccttgaccga aagttacagc	1560
ccgaattctc aggtccagct ggtggagtct gggggcggag tggtcagcc tgggcggta	1620
ctgaggctgt cctgcaagge ttctggctac acccttacta gatctacgat gcactgggta	1680
aggcaggecc ctggacaagg tctggaatgg attgatata ttaatectag cagtgtctat	1740
actaattaca atcagaaatt caaggacagg ttcaaatca gcgcagacaa atccaagagc	1800
acagccttcc tgcagatgga cagcctgagg cccgaggaca ccggcgteta ttctctgtca	1860
cggccccaag tccactatga ttacaacggg ttctctact ggggccaagg gactccgctc	1920
actgtcteta gcggtggcgg agggctctgg ggtggcggat ccggaggtgg tggctctgca	1980
caagacatcc agatgacca gtctccaagc agcctgtctg caagcgtggg ggacagggtc	2040
accatgacct gcagtgcag ctcaagigta agttacatga actggtacca gcagaagccg	2100
ggcaaggecc ccaaaagatg gatttatgac tcatecaaac tggtctctgg agtccctgct	2160
cgttcagtg gcagtgggtc tgggaccgac tataccctca caatcagcag cctgcagccc	2220
gaagatttcg ccacttatta ctgccagcag tggagtcgta acccaccac gtccggaggg	2280

[0237]

gggaccaagc tacaaattac atcctccagc taa

2313

<210> 206

<211> 750

<212> PRT

<213> 人工序列

<220>

<223> TSC242 (HD37 x DRA222 scorpion, H9I 连接子)

<400> 206

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
1 5 10 15

Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

[0238]

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
165 170 175

Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
180 185 190

Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met
195 200 205

Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
210 215 220

Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr
225 230 235 240

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser
245 250 255

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
260 265 270

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
275 280 285

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
290 295 300

[0239]

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
305 310 315 320

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
325 330 335

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
340 345 350

Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro
355 360 365

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
370 375 380

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
385 390 395 400

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
405 410 415

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
420 425 430

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
435 440 445

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
450 455 460

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
465 470 475 480

[0240]

Ser Pro Gly Asn Ser Leu Ala Asn Gln Glu Val Gln Ile Pro Leu Thr
 485 490 495

Glu Ser Tyr Ser Pro Asn Ser Gln Val Gln Leu Val Glu Ser Gly Gly
 500 505 510

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
 515 520 525

Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala Pro
 530 535 540

Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala Tyr
 545 550 555 560

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala Asp
 565 570 575

Lys Ser Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
 580 585 590

Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp Tyr
 595 600 605

Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser
 610 615 620

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala
 625 630 635 640

Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
 645 650 655

[0241]

Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr
660 665 670

Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp Ile
675 680 685

Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly
690 695 700

Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
705 710 715 720

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro
725 730 735

Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr Ser Ser Ser
740 745 750

<210> 207

<211> 1815

<212> DNA

<213> 人工序列

<220>

<223> TSC049

<400> 207

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gacattgtgc tgaccaaatc tccagcttct ttggetgtgt ctctagggea gagggccacc 120

atctectgca aggccagcca aagtgttgat tatgatgggtg atagttattt gaactggtac 180

caacagattc caggacagcc acccaaacte ctcatctatg atgcaccaa tctagtttct 240

gggatccac ccaggttttag tggcagtggg tctgggacag acttcaccct caacatccat 300

[0242]

cctgtggaga aggtggatgc tgcaacctat cactgccagc aaagtactga ggatccgtgg	360
acgttcggtg gaggcaccaa gctggaaatc aaaggtggcg gtggttcggg cgggtgtggg	420
tcgggtggcg geggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg	480
cctgggtccf cagtgaagat ttcttgcaag gcttctggct atgcattcag tagctactgg	540
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ggagatggtg atactaacta caatggaaag ttcaagggtg aagccactct gaetgcagac	660
gaatccctca gcacagccta catgcaactc agcagcctag catctgagga ctctgcggtc	720
tatttctgtg caagacggga gactacgacg gtaggcogtt attactatgc tatggaetac	780
tggggtaag gaacctcagt caccgtctcc tegagtgagc ccaaattctc tgacaaaact	840
cacacatgcc caccgtgcc agcaccigaa gccgcgggag caccgtcagt ctctctctc	900
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tccaagagca ctcttggggg cacagcggcc ctgggctgcc tggtaagga ctacttcccc	1620

[0243]

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agcttgggca cccagacctc catctgcaac gtgaatcaca agcccagcaa caccaaggtg 1800
gacaagaaag tttaga 1815

<210> 208
<211> 584
<212> PRT
<213> 人工序列

<220>
<223> TSC049

<400> 208

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
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Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

[0244]

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
165 170 175

Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
180 185 190

Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met
195 200 205

Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
210 215 220

Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr
225 230 235 240

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser
245 250 255

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
260 265 270

[0245]

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
275 280 285

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
290 295 300

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
305 310 315 320

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
325 330 335

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
340 345 350

Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro
355 360 365

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
370 375 380

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
385 390 395 400

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
405 410 415

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
420 425 430

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
435 440 445

[0246]

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
450 455 460

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
465 470 475 480

Ser Pro Gly Lys Ser Arg Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
485 490 495

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
500 505 510

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
515 520 525

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
530 535 540

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
545 550 555 560

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
565 570 575

Asn Thr Lys Val Asp Lys Lys Val
580

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<211> 1836

<212> DNA

<213> 人工序列

<220>

<223> TSC096

[0247]

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atctctgca aggccagca aagtgtgat tatgatgtg atagttatt gaactggta	180
caacagattc caggacagcc acccaaacct ctcattatg atgcatcaa tctagtttct	240
gggatccac ccaggcttag tggcagtggt tctgggacag acttcacct caacatccat	300
cctgtggaga aggtggatgc tgcaacctat cactgccagc aaagtactga ggatecgtg	360
acgttcgggt gaggcaccaa gctggaatc aaaggtggcg gtggttcggg cgtgtgtgg	420
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gaatctcca gcacagcta catgcaactc agcagctag catctgagga ctctcggtc	720
tattctgtg caagacggga gactacgacg gtagccgtt attactatgc tatggactac	780
tgggtcaag gaacctcagt caccgtctcc tcgagtgcg ccaaatcttc tgacaaaact	840
cacacatgcc caccgtgcc agcactgaa gccgcgggtg caccgtcagt ctctctcttc	900
ccccaaaac ccaaggacac cctcatgac tccggaccc ctgaggtcac atgcgtgtg	960
gtggacgtga gccacgaaga ccctgaggtc aagtccaact ggtacgtgga cggegtggag	1020
gtgcataatg ccaagacaaa gccgcgggag gagcagtaca acagcacgta ccgtgtgtc	1080
agcgtctca ccgtctgca ccaggactgg ctgaatggca aggcgtacgc gtgcggtc	1140
tccaacaaag cctcccagc ccccatcgag aaaacctct ccaaagccaa agggcagccc	1200
cgagaaccac aggtgtacac cctgcccaca tccgggatg agctgaccaa gaaccagtc	1260
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[0248]

aatgggcagc cggagaacaa ctacaagacc acgcctcccg tgcctggactc cgacggctcc 1380
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gaggccaaag tacagtggaa ggtggataac gccctccaat cgggtaactc ccaggagagt 1680
gccacagagc aggacagcaa ggacagcacc tacagctcca gcagcgagct gacgtgagc 1740
aaagcagact acgagaaaca caaagtctac gcctgcgaag tcacccatca gggcctgagc 1800
tcgcccgtca caaagagctt caacagggga gattga 1836

<210> 210

<211> 591

<212> PRT

<213> 人工序列

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<223> TSC096

<400> 210

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
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Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

[0249]

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
165 170 175

Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
180 185 190

Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met
195 200 205

Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
210 215 220

Arg Arg Glu Thr Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr
225 230 235 240

[0250]

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser
245 250 255

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
260 265 270

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
275 280 285

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
290 295 300

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
305 310 315 320

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
325 330 335

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
340 345 350

Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro
355 360 365

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
370 375 380

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
385 390 395 400

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
405 410 415

[0251]

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
420 425 430

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
435 440 445

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
450 455 460

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
465 470 475 480

Ser Pro Gly Lys Ser Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
485 490 495

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
500 505 510

Leu Leu Asn Tyr Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
515 520 525

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Ala Thr Glu Gln
530 535 540

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Glu Leu Thr Leu Ser
545 550 555 560

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
565 570 575

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu
580 585 590

[0252]

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<213>	人工序列	
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<223>	TSC192	
<400>	211	
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gatatccaga tgaccagtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc		120
atcaattgcc gggcgagtaa gagcattage aaatatttag cctggtttca gcagaaacca		180
gggaaagttc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccatct		240
cggttcagtg gcagtggtc tgggacagaa ttactctca ccatcagcag cctgcagcct		300
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actagatacg cacagaagtt ccagggcaga gtcaccatga ccagggacac gtctatcage		660
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tcctegagtg agcccaaata ttctgacaaa actcacacat gccaccgtg cccagcacct		840
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atctcccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag		960
gtcaagtta actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg		1020

[0253]

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gttgtgtgcc tgctgaatta ctctatccc agagaggcca aagtacagtg gaagggtgat 1620
aacgccctcc aatcgggtaa ctcccaggag agtgccacag agcaggacag caaggacagc 1680
acctacagcc tcagcagcga gctgacgctg agcaaagcag actacgagaa acacaaagtc 1740
tacgcctgcg aagtcaccca tcagggcctg agctcgcccg tcacaaagag ctccaacagg 1800
ggagagtga 1809

<210> 212

<211> 582

<212> PRT

<213> 人工序列

<220>

<223> TSC192

<400> 212

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
1 5 10 15

[0254]

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
 20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
 35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
 100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val
 115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser
 130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
 145 150 155 160

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
 165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr
 180 185 190

[0255]

Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

[0256]

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser Arg Thr Val Ala
465 470 475 480

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
485 490 495

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Tyr Phe Tyr Pro Arg Glu
500 505 510

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
515 520 525

Gln Glu Ser Ala Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
530 535 540

[0257]

Ser Ser Glu Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
545 550 555 560

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
565 570 575

Ser Phe Asn Arg Gly Glu
580

<210> 213

<211> 1809

<212> DNA

<213> 人工序列

<220>

<223> TSC193

<400> 213

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atcacttgcc gggcgagtaa gagcattagc aaatatttag cctggtttca gcagaaacca 180

gggaaagttc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccatct 240

cggttcagtg gcagtggatc tgggacagaa ttactctca ccatcagcag cctgcagcct 300

gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gttcggccaa 360

gggaccaagg tggaaatcaa acgaggtggc ggagggtctg ggggtggcgg atccggaggt 420

ggtggctctg aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggctaca 480

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caacaggccc ctggaaaagg gcttgagtgg atgggatatt ttaatectta taatgattat 600

actagatacg cagagaagtt ccagggcaga gtcaccataa ccggcgacac gtctacagac 660

acagcctaca tggagctgag cagcctgaga tctgaggaca cggccgtgia ttactgtgca 720

[0258]

agatcggatg gttactacga tgctatggac tactggggtc aaggaaccac agtcaccgtc	780
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gaagccgcgg gtgcaccgtc agtcttcttc tttccccaa aaccaagga caccctcatg	900
atctccccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccgcgg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtctt gcaccaggac	1080
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ggagagtga	1809

<210> 214

<211> 582

<212> PRT

<213> 人工序列

[0259]

<220>

<223> TSC193

<400> 214

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
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Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val
115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser
130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
145 150 155 160

[0260]

Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Glu Lys Phe Gln Gly Arg Val Thr
180 185 190

Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

[0261]

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser Arg Thr Val Ala
465 470 475 480

Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
485 490 495

Gly Thr Ala Ser Val Val Cys Leu Leu Asn Tyr Phe Tyr Pro Arg Glu
500 505 510

[0262]

Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser
515 520 525

Gln Glu Ser Ala Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu
530 535 540

Ser Ser Glu Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
545 550 555 560

Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
565 570 575

Ser Phe Asn Arg Gly Glu
580

<210> 215

<211> 1788

<212> DNA

<213> 人工序列

<220>

<223> TSC195

<400> 215

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gatatccaga tgaccagtc tccatccgcc atgtctgcat ctgtaggaga cagagtcacc 120

atcacttgcc gggcgagtaa gacattagc aaatatttag cctggtttca gcagaaacca 180

gggaaagttc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccattt 240

cggttcagtg gcagtggatc tgggacagaa ttactctca ccatcagcag cctgcagcct 300

gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gttcggccaa 360

gggaccaagg tggaatcaa acgaggtggc ggagggtctg ggggtggcgg atccggaggt 420

[0263]

ggtggctctc aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca	480
gtgaaggtct cctgcaaggc ttctggatac acattcacig actactacat gcactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatectta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcaccatga ccagggacac gtctatcage	660
acagcctaca tggagctgag cagcctgaga tctgacgaca cgcccggtga ttactgtgca	720
agatcggatg gttactacga tgctatggac tactggggtc aaggaaccac agtcaccgtc	780
tcctcgagtg ageccaaatc ttctgacaaa actcacacat gccacacgtg cccagcacct	840
gaagccgggg gtgcacgctc agtcttctc ttcccccaa aaccaagga caccctcatg	900
atctcccga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagttca actggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccggcg	1020
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tggctgaatg gcaaggcgta cgcgtgcgag gtctccaaca aagccctccc agccccatc	1140
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ccatcccggg atgagctgac caagaaccag gtcagcctga cctgcctggt caaaggttc	1260
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gacaagagca ggtggcagca ggggaacgct ttctcatgct ccgtgatgca tgaggctctg	1440
cacaaccact acaegcagaa gagctctcc ctgtctccg gtaaattctag agcctccacc	1500
aagggeccat cggctctccc cctggcaccc tcctccaaga gcacctctgg gggcacagcg	1560
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ggcgcctga ccagcggcgt gcacaccttc ccgctgtcc tacagtcctc aggactctac	1680
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[0264]

aacgtgaatc acaagcccag caacaccaag gtggacaaga aagtttga

1788

<210> 216

<211> 575

<212> PRT

<213> 人工序列

<220>

<223> TSC195

<400> 216

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Gln Val Gln Leu Val
115 120 125

[0265]

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser
130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
145 150 155 160

Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr
180 185 190

Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

[0266]

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser Arg Ala Ser Thr
465 470 475 480

[0267]

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
 485 490 495

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 500 505 510

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 515 520 525

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 530 535 540

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
 545 550 555 560

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
 565 570 575

<210> 217

<211> 1806

<212> DNA

<213> 人工序列

<220>

<223> TSC219

<400> 217

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aggctgtcct gcaaggettc tggtacacc ttactagat ctacgatgca ctgggtaagg 180

caggeccctg gacaaggctt ggaatggatt ggatacatta atcctagcag tgcttatact 240

aattacaatc agaaattcaa ggacaggctt acaatcagcg cagacaaatc caagagcaca 300

[0268]

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gtctctagcg gtggcggagg gcttgggggt ggcggtaccg gaggtggtgg ctctgcacaa	480
gacatccaga tgaaccagtc tccaagcage ctgtctgcaa gcgtggggga cagggtcacc	540
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aaggccccca aaagaaggat ttatgaetca tccaaactgg cttctggagt cctgtctcgc	660
ttcagtggca gtgggtctgg gaccgaactat accctcacia tcagcagcct gcagcccgaa	720
gatttcgcca cttattactg ccagcagtggt agtcgtaacc cactcacgtt cggagggggg	780
accaagctac aaattacatc ctcgagttag cccaaatctt ctgacaaaac tcacacatgc	840
ccaccgtgcc cagcacciga agccgcgggt gcaccgtcag tcttctctct cccccaaaa	900
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gcccctccag ccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca	1200
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gtgatgcatg aggtctctga caaccactac acgcagaaga gcctctccct gtctccgggt	1500
aaatctagag cctccaccaa gggcccatcg gtcttcccc ttggcaacctc ctccaagagc	1560
acctctgggg gcacagcggc cctgggtctg ctggtcaagg actacttccc cgagccggtg	1620

[0269]

acgggtgtcgt ggaactcagg cgccctgacc agcggcgtgc acacctccc ggetgtccta 1680
cagtctcag gactctactc cctcagcagc gtggtgaccg tgccctccag cagcttgggc 1740
accagacct acatctgcaa cgtgaatcac aagcccagea acaccaaggt ggacaagaaa 1800
gtttga 1806

<210> 218
<211> 579
<212> PRT
<213> 人工序列

<220>
<223> TSC219

<400> 218

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

[0270]

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly		
100	105	110
Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly		
115	120	125
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln		
130	135	140
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr		
145	150	155
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys		
165	170	175
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala		
180	185	190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr		
195	200	205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr		
210	215	220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys		
225	230	235
Leu Gln Ile Thr Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His		
245	250	255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val		
260	265	270

[0271]

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
275 280 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
290 295 300

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305 310 315 320

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
325 330 335

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala
340 345 350

Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
355 360 365

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
370 375 380

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385 390 395 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
405 410 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
420 425 430

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
435 440 445

[0272]

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
450 455 460

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser
465 470 475 480

Arg Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
485 490 495

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
500 505 510

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
515 520 525

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
530 535 540

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
545 550 555 560

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
565 570 575

Lys Lys Val

<210> 219

<211> 1809

<212> DNA

<213> 人工序列

<220>

<223> TSC220

[0273]

<400> 219	
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tcctgcaagg ctcttgata caccctcact agatetaaga tgcaetgggt gcgccaggec	180
ccggacaaa ggtttgagt gatgggatac attaatccta gcagtgetta tactaattac	240
aalcagaaat tcaaggacag agtcaccatt accagggaca catccgcgag cacagcctac	300
atggagctga gcagcctgag atctgaagac aeggtgtgtt attactgtgc gagaccccaa	360
gtccactatg attacaacgg gtttcttacc tggggccaag gaacctggt caccgtctcc	420
tcaggtggag gcggttcagg cggagggtga tccggcgggt gcggatcggg tggcggcgga	480
tctgaaattg tgttgacaca gtctccagcc accctgtctt tgtctccagg ggaaagagcc	540
acctctctct gcagtgcag ctcaagtgt agttacatga actggtacca acagaaacct	600
ggccaggctc ccaggctctt catctatgac tcatccaaac tggtttctgg catccagcc	660
aggttcagtg gcagtgggtc tgggacagac tteactctca ccacagcag cctagagcct	720
gaagattttg cagtttatta ctgtcagcag tggagtcgta acccaccac ttccggcgga	780
gggaccaagg tggagatcaa atctctgagt gageccaaat ctctgacaa aactcacaca	840
tgcccaccgt gccagcacc tgaagccgcg ggtgcaccgt cagtcttctt ctccccca	900
aaacccaagg acacctcat gatctcccg accctgagg tcacatgcgt ggtggtggac	960
gtgagccacg aagacctga ggtcaagttc aactggtacg tggacggcgt ggaggtgcat	1020
aatgccaaga caaagccgcg ggaggagcag tacaacagca cgtaccgtgt ggtcagcgtc	1080
ctcaccgtcc tgcaccagga ctggtgaat ggcaaggcgt acgcgtgcgc ggtctccaac	1140
aaagccctcc cagccccat cgagaaaacc atctccaaag ccaaagggca gccccagaa	1200
ccacaggtgt acacctgce ccctcccg gatgagctga ccaagaacca ggtcagcctg	1260
acctgcctgg tcaaaggett ctatccaagc gacatgccg tggagtggga gagcaatggg	1320

[0274]

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 tccgtgatgc atgaggctct gcacaaccac tacacgcaga agagcctctc cctgtctccg 1500
 ggtaaataeta gagcctccac caagggccca teggtcttcc ccctggcacc ctccctcaag 1560
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 ctacagtctt caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcagcttg 1740
 ggcaccacaga cctacatctg caacgtgaat cacaagccca gcaacaccaa ggtggacaag 1800
 aaagtttga 1809

<210> 220

<211> 582

<212> PRT

<213> 人工序列

<220>

<223> TSC220

<400> 220

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
 20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
 35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
 50 55 60

[0275]

Lys Asp Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val
130 135 140

Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala
145 150 155 160

Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr
165 170 175

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ser Ser
180 185 190

Lys Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
195 200 205

Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala
210 215 220

Val Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly
225 230 235 240

[0276]

Gly Thr Lys Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp
245 250 255

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala
260 265 270

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350

Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415

[0277]

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480

Gly Lys Ser Arg Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
485 490 495

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
500 505 510

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
515 520 525

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
530 535 540

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
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Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
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Lys Val Asp Lys Lys Val
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[0278]

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aggetgtcct gcaaggcttc tgctacacc ttactagat ctacgatgca ctgggtaagg		180
caggccccctg gacaaggctt ggaatggatt ggatacatta atcctagcag tgcttatact		240
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aagccccca aaagatggat ttatgactca tccaaactgg cttctggagt cctgtctgc		660
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ccaccgtgcc cagcacctga agcccggggt gcaccgtcag tcttctcttt cccccaaaa		900
cccaaggaca cctcatgat ctcccgacc cctgaggta catgcgtggg ggtggacgtg		960
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[0279]

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[0280]

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Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr
145 150 155 160

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
165 170 175

Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala
180 185 190

[0281]

Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
195 200 205

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
210 215 220

Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys
225 230 235 240

Leu Gln Ile Thr Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His
245 250 255

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val
260 265 270

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
275 280 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
290 295 300

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305 310 315 320

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
325 330 335

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala
340 345 350

Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
355 360 365

[0282]

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
370 375 380

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385 390 395 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
405 410 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
420 425 430

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
435 440 445

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
450 455 460

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser
465 470 475 480

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
485 490 495

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Tyr Phe
500 505 510

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
515 520 525

Ser Gly Asn Ser Gln Glu Ser Ala Thr Glu Gln Asp Ser Lys Asp Ser
530 535 540

[0283]

Thr Tyr Ser Leu Ser Ser Glu Leu Thr Leu Ser Lys Ala Asp Tyr Glu
545 550 555 560

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
565 570 575

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu
580 585

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<211> 1830

<212> DNA

<213> 人工序列

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aatcagaaat tcaaggacag agtcaccatt accagggaca catccgcgag cacagcctac 300

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<212> PRT

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[0285]

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Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Val Thr Ile Thr Arg Asp Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val
130 135 140

Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala
145 150 155 160

[0286]

Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr
165 170 175

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ser Ser
180 185 190

Lys Leu Ala Ser Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly
195 200 205

Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala
210 215 220

Val Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly
225 230 235 240

Gly Thr Lys Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp
245 250 255

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala
260 265 270

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
275 280 285

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
290 295 300

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
305 310 315 320

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
325 330 335

[0287]

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
340 345 350

Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
355 360 365

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
370 375 380

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
385 390 395 400

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
405 410 415

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
420 425 430

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
435 440 445

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
450 455 460

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
465 470 475 480

Gly Lys Ser Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
485 490 495

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
500 505 510

[0288]

Asn Tyr Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
515 520 525

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Ala Thr Glu Gln Asp Ser
530 535 540

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Glu Leu Thr Leu Ser Lys Ala
545 550 555 560

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
565 570 575

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu
580 585

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atcacttgcc gggcgagtaa gagcattage aaatatttag cctggtttca gcagaaacca 180

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tga 1803

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<211> 580

<212> PRT

<213> 人工序列

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<400> 226

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly
100 105 110

[0291]

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val
115 120 125

Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Thr Val Lys Ile Ser
130 135 140

Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val
145 150 155 160

Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro
165 170 175

Tyr Asn Asp Tyr Thr Arg Tyr Ala Glu Lys Phe Gln Gly Arg Val Thr
180 185 190

Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr Met Glu Leu Ser Ser
195 200 205

Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly
210 215 220

Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val
225 230 235 240

Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro
245 250 255

Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro
260 265 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
275 280 285

[0292]

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
290 295 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305 310 315 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
325 330 335

Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser
340 345 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
355 360 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp
370 375 380

Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385 390 395 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
405 410 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
420 425 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
435 440 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
450 455 460

[0293]

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ser Ser Leu Asn Thr Pro
465 470 475 480

Asn Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
485 490 495

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
500 505 510

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
515 520 525

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu
530 535 540

Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr
545 550 555 560

Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val
565 570 575

Asp Lys Lys Val
580

<210> 227

<211> 1845

<212> DNA

<213> 人工序列

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<223> TSC258

<400> 227

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[0295]

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<210> 228

<211> 592

<212> PRT

<213> 人工序列

<220>

<223> TSC258

<400> 228

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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
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Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
          20           25           30

```

```

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
          35           40           45

```

```

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
          50           55           60

```

```

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
          65           70           75           80

```

[0296]

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr
145 150 155 160

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
165 170 175

Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala
180 185 190

Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
195 200 205

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
210 215 220

Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys
225 230 235 240

Leu Gln Ile Thr Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His
245 250 255

[0297]

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val
260 265 270

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
275 280 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
290 295 300

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
305 310 315 320

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
325 330 335

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala
340 345 350

Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
355 360 365

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
370 375 380

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385 390 395 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
405 410 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
420 425 430

[0298]

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
435 440 445

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
450 455 460

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Ser Ser
465 470 475 480

Leu Asn Thr Pro Asn Ser Arg Thr Val Ala Ala Pro Ser Val Phe Ile
485 490 495

Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val
500 505 510

Cys Leu Leu Asn Tyr Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys
515 520 525

Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Ala Thr Glu
530 535 540

Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Glu Leu Thr Leu
545 550 555 560

Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr
565 570 575

His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu
580 585 590

<210> 229

<211> 121

[0299]

<212> PRT

<213> 人工序列

<220>

<223> 来自于图 3 的共具有序列

<220>

<221> misc_feature

<223> Xaa 为不是图 3 中的共具有序列的位置, 其中 Ala 和 Phe
在那个位置上同样普遍且七分之一的序列中存在 Val

<220>

<221> misc_feature

<222> (68)..(68)

<223> Xaa 可以是任何天然存在的氨基酸

<400> 229

Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Gly	Gly	Val	Val	Lys	Pro	Gly	Ala
1			5						10					15	

Ser	Leu	Arg	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Arg	Ser
			20						25					30	

Thr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile
			35					40						45	

Gly	Tyr	Ile	Asn	Pro	Ser	Ser	Ala	Tyr	Thr	Asn	Tyr	Asn	Gln	Lys	Phe
		50					55							60	

Lys	Asp	Arg	Xaa	Thr	Ile	Thr	Ala	Asp	Lys	Ser	Lys	Ser	Thr	Ala	Tyr
	65						70				75				80

Met	Gln	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
						85					90				95

Ala	Arg	Pro	Gln	Val	His	Tyr	Asp	Tyr	Asn	Gly	Phe	Pro	Tyr	Trp	Gly
										100				105	110

[0300]

Gln Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 230

<211> 108

<212> PRT

<213> 人工序列

<220>

<223> 来自于图 4 的共具有序列

<400> 230

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
1 5 10 15

Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
20 25 30

Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp
35 40 45

Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
50 55 60

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
65 70 75 80

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro
85 90 95

Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr
100 105

[0301]

- <210> 231
<211> 98
<212> PRT
<213> 人工序列
- <220>
<223> 来自于图 5 的共具有序列
- <220>
<221> misc_feature
<223> 这是来自于图 5 的共具有序列。X 表示 null 关紧要，所以 X 同样可以是其它序列中的那个位置上所示的氨基酸之一。
- <220>
<221> misc_feature
<222> (50).. (50)
<223> Xaa 可以是任何天然存在的氨基酸
- <220>
<221> misc_feature
<222> (52).. (52)
<223> Xaa 可以是任何天然存在的氨基酸
- <220>
<221> misc_feature
<222> (54).. (55)
<223> Xaa 可以是任何天然存在的氨基酸
- <220>
<221> misc_feature
<222> (57).. (58)
<223> Xaa 可以是任何天然存在的氨基酸
- <220>
<221> misc_feature
<222> (74).. (74)
<223> Xaa 可以是任何天然存在的氨基酸
- <220>
<221> misc_feature
<222> (84).. (84)
<223> Xaa 可以是任何天然存在的氨基酸

[0302]

<400> 231

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Xaa Ile Xaa Pro Xaa Xaa Ala Xaa Xaa Asn Tyr Ala Gln Lys Phe
50 55 60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Xaa Ser Lys Ser Thr Ala Tyr
65 70 75 80

Leu Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 232

<211> 95

<212> PRT

<213> 人工序列

<220>

<223> IGKV1-5*01

<400> 232

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly
1 5 10 15

[0303]

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp
20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Ser
85 90 95

<210> 233

<211> 91

<212> PRT

<213> 人工序列

<220>

<223> 来自于图 6 的共具有序列

<220>

<221> misc_feature

<223> 这是来自于图 6 的共具有序列。X 表示 null 关紧要，所以 X 同样可以是其它序列中的那个位置上所示的氨基酸之一。

<220>

<221> misc_feature

<222> (24)..(24)

<223> Xaa 可以是任何天然存在的氨基酸

<220>

<221> misc_feature

<222> (27)..(27)

<223> Xaa 可以是任何天然存在的氨基酸

[0304]

<220>
<221> misc_feature
<222> (31)..(31)
<223> Xaa 可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (34)..(34)
<223> Xaa 可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (46)..(47)
<223> Xaa 可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (53)..(53)
<223> Xaa 可以是任何天然存在的氨基酸

<220>
<221> misc_feature
<222> (55)..(55)
<223> Xaa 可以是任何天然存在的氨基酸

<400> 233

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Xaa Ala Ser Xaa Ser Ile Ser Xaa Trp
20 25 30

Leu Xaa Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Xaa Xaa Ile
35 40 45

Tyr Asp Ala Ser Xaa Leu Xaa Ser Gly Val Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro

[0305]

65	70	75	80
Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp			
	85	90	
<210> 234			
<211> 12			
<212> PRT			
<213> 人工序列			
<220>			
<223> IGKJ1*01			
<400> 234			
Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys			
1	5	10	
<210> 235			
<211> 12			
<212> PRT			
<213> 人工序列			
<220>			
<223> IGKJ2*01			
<400> 235			
Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys			
1	5	10	
<210> 236			
<211> 12			
<212> PRT			
<213> 人工序列			
<220>			
<223> IGKJ3*01			
<400> 236			

[0306]

Phe Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys
1 5 10

<210> 237

<211> 12

<212> PRT

<213> 人工序列

<220>

<223> IGKJ4*01

<400> 237

Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
1 5 10

<210> 238

<211> 12

<212> PRT

<213> 人工序列

<220>

<223> IGKJ5*01

<400> 238

Ile Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys
1 5 10

<210> 239

<211> 423

<212> PRT

<213> 人工序列

<220>

<223> 图 7 共具有序列

<400> 239

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg

[0307]

1	5	10	15
Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser			
20	25	30	
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile			
35	40	45	
Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe			
50	55	60	
Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe			
65	70	75	80
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys			
85	90	95	
Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly			
100	105	110	
Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly			
115	120	125	
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln			
130	135	140	
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr			
145	150	155	160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys			
165	170	175	
Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Ser Ser Lys Leu Ala			

[0308]

	180		185		190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr					
	195		200		205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr					
	210		215		220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys					
225		230		235	240
Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His					
	245		250		255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe					
	260		265		270
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro					
	275		280		285
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val					
	290		295		300
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr					
305		310		315	320
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val					
	325		330		335
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys					
	340		345		350
Ala Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser					

[0309]

355	360	365
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro		
370	375	380
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val		
385	390	395 400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly		
405	410	415
Gln Pro Glu Asn Asn Tyr Lys		
420		
<210> 240		
<211> 478		
<212> PRT		
<213> 人工序列		
<220>		
<223> DRA161		
<400> 240		
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg		
1 5 10 15		
Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser		
20 25 30		
Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile		
35 40 45		
Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe		
50 55 60		

[0310]

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr
145 150 155 160

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
165 170 175

Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala
180 185 190

Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
195 200 205

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
210 215 220

Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys
225 230 235 240

[0311]

Leu Gln Ile Thr Arg Arg Thr Ser Pro Lys Ser Ser Asp Lys Thr His
245 250 255

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe
260 265 270

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
275 280 285

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
290 295 300

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
305 310 315 320

Lys Pro Arg Glu Glu Gln Phe Ala Ser Thr Tyr Arg Val Val Ser Val
325 330 335

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
340 345 350

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
355 360 365

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
370 375 380

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
385 390 395 400

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
405 410 415

[0312]

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 420 425 430

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
 435 440 445

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
 450 455 460

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 465 470 475

<210> 241

<211> 478

<212> PRT

<213> 人工序列

<220>

<223> 图 8 的共具有序列

<400> 241

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
 20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
 50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe

[0313]

[0314]

	245	250	255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Ala Pro Ser Val Phe			
260	265	270	
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro			
275	280	285	
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val			
290	295	300	
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr			
305	310	315	320
Lys Pro Arg Glu Glu Gln Phe Ala Ser Thr Tyr Arg Val Val Ser Val			
325	330	335	
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys			
340	345	350	
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser			
355	360	365	
Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro			
370	375	380	
Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val			
385	390	395	400
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly			
405	410	415	
Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp			

[0315]

	420	425	430
Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp			
435	440	445	

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
450 455 460

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 242
 <211> 1800
 <212> DNA
 <213> 人工序列

<220>
 <223> TSC125

<400> 242	
atggaagcac cagcgcagct tctcttctctc ctgctactctt ggctcccaga taccacccgt	60
caggctccagc tgggtgcagtc tgggggcgga gtggtgcagc ctgggcggtc actgaggctg	120
tcttgcaagg cttctggcta cacccttact agatctacga tgcactgggt aaggcaggcc	180
cctggaaagg gtctggaatg gattggatac attaatccta gcagtgttta tactaattac	240
aatcagaaat tcaaggacag gttcacaatc agcgcagaca aatccaagag cacagccttc	300
ctgcagatgg acagcctgag gcccgaggac accggcgctt atttctgtgc acggccccaa	360
gtccactatg attacaacgg gtttccttac tggggccaag ggaactccgt cactgtctct	420
agcgggtggcg gagggctctgg ggggtggcgga tccggaggtg gtggctctgc acaagacatc	480
cagatgaacc agtctccaag cagcctgtct gcaagcgtgg gggacagggt caccatgacc	540
tgcagtgcc a gctcaagtgt aagttacatg aactggtacc agcagaagcc cggcaaggcc	600

[0316]

cccaaaagat ggatttatga ctcatccaaa ctggtctctg gagtcctgc tcgttcagt	660
ggcagtgggt ctgggaccga ctataccctc acaatcagca gcctgcagcc cgaagatttc	720
gccacttatt actgccagca gtggagtcgt aaccaccca cgttcggagg ggggaccaag	780
ctacaaatta cacgctegag tgagcccaaa tcttctgaca aaactcaaac atgcccaccg	840
tgccagcac ctgaagccgc gggcgcaccg tcagtcttc tcttccccc aaaacccaag	900
gacaccctca tgatctcccg gacccctgag gtcacatgcg tggcgtgga cgtgagccac	960
gaagaccctg aggtcaagtt caactggtac gtggacggcg tggaggtgca taatgccaag	1020
acaaagccgc gggaggagca gtacaacagc acgtaccgtg tggcagcgt cctcaccgtc	1080
ctgcaccagg actggctgaa tggcaaggcg tacgcgtgcg cggctctcaa caaagccctc	1140
ccagccccc tcgagaaaac catctccaaa gccaaagggc agccccgaga accacaggtg	1200
tacaccctgc cccateccg ggatgagctg accaagaacc aggtcagcct gacctgcctg	1260
gtcaaaggct tctatccaag cgacatgcc gtggagtggt agagcaatgg gcagccggag	1320
aacaactaca agaccagcc tcccgctcg gactccgacg gctccttctt cctctacagc	1380
aagctcaccg tggacaagag caggtggcag cagggaacg tcttctcatg ctccgtgatg	1440
catgaggctc tgcacaacca ctacacgcag aagagcctct cctgtcttc gggtaaattc	1500
agagcctcca ccaagggecc atcggctctt cccctggcac cctctccaa gagcacctct	1560
gggggcacag cgccctggg ctgcctggtc aaggactact tccccagcc ggtgacggcg	1620
tcgtggaact caggcgcct gaccagcggc gtgcaacct tcccggctgt cctacagtcc	1680
tcaggactct actccctcag cagcgtggcg accgtgccct ccagcagctt gggcaccag	1740
acctacatct gcaacgtgaa tcacaagccc agcaacacca aggtggacaa gaaagtttga	1800

<210> 243

<211> 579

<212> PRT

[0317]

<213> 人工序列

<220>

<223> TSC125 成熟蛋白质

<400> 243

Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr

[0318]

145	150	155	160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys			
	165	170	175
Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala			
	180	185	190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr			
	195	200	205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr			
	210	215	220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys			
225	230	235	240
Leu Gln Ile Thr Arg Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His			
	245	250	255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val			
	260	265	270
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr			
	275	280	285
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu			
	290	295	300
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys			
305	310	315	320
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser			

[0319]

	325		330		335
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala					
	340		345		350
Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile					
	355		360		365
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro					
	370		375		380
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu					
	385		390		395
					400
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn					
	405		410		415
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser					
	420		425		430
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg					
	435		440		445
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu					
	450		455		460
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser					
	465		470		475
					480
Arg Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser					
	485		490		495
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp					

[0320]

	500	505	510
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr			
515	520	525	
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr			
530	535	540	
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln			
545	550	555	560
Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp			
565	570	575	
Lys Lys Val			

<210> 244

<211> 2310

<212> DNA

<213> 人工序列

<220>

<223> TSC129a DNA

<400> 244

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atctcctgca aggccagcca aagtgtgat tatgatggtg atagtatttt gaactggtac	180
caacagattc caggacagcc acccaaaact ctcctctatg atgcatccaa tctagtttct	240
gggataccac ccaggttttag tggcagtggg tctgggacag actteaccct caacatccat	300
cctgtggaga aggtggatgc tgcaacctat cactgccagc aaagtactga ggatccgtgg	360

[0321]

acgttcggtg gaggcaccaa gctggaaatc aaaggtggcg gtggttcggg cgggtgtggg	420
tccggtggcg gcggagctag ccaggttcag ctgcagcagt ctggggctga gctggtgagg	480
cctgggtcct cagtgaagat ttctgcaag gcttctggct atgcattcag tagctactgg	540
atgaactggg tgaagcagag gcctggacag ggtcttgagt ggattggaca gatttggcct	600
ggagatggtg atactaacta caatggaaag ttcaagggtg aagccactct gactgcagac	660
gaatcctcca gcacagccta catgcaactc agcagcctag catctgagga ctctgcggtc	720
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cacacatgcc caccgtgccc agcacctgaa gccgcgggtg caccgtcagt ctctctcttc	900
cccccaaac ccaaggacac cctcatgata tcccggaccc ctgaggtcac atgcgtggtg	960
gtggacgtga gccacgaaga ccttgaggtc aagtccaact ggtacgtgga cggcgtggag	1020
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agcctgacct gcctggfcaa aggtttctat ccaagcgaca tcgccgtgga gtgggagagc	1320
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tctccgggtc agaggcacia caattcttcc ctgaatacag gaactcagat ggcaggtcac	1560
tctccgaatt ctccaggtcca gctgggtcag tctgggggag gactgggtga gcctgggcgg	1620
tcactgaggc tgtcttcaa ggttcttggc tacaccttta ctgatctac gatgcactgg	1680

[0322]

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tataactaatt acaatcagaa attcaaggac aggttcacaa tcagcgcaga caaatccaag 1800
agcacagcct tccctgcagat ggacagcctg aggccccgagg acaccggcgt ctatttctgt 1860
gcacggcccc aagtccacta tgattacaac gggtttcctt actggggcca agggactccc 1920
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cccggaagg cccccaaaag atggatttat gactcatcca aactggcttc tggagtcct 2160
gtctgcttea gtggcagttg gtctgggacc gactataccc tcacaatcag cagcctgcag 2220
cccgaagatt tcgccactta ttactgccag cagtggagtc gtaaccacc cacttcgga 2280
ggggggacca agctacaaat tacacgataa 2310

<210> 245

<211> 749

<212> PRT

<213> 人工序列

<220>

<223> TSC129a 成熟蛋白质

<400> 245

Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
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Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp
20 25 30

Gly Asp Ser Tyr Leu Asn Trp Tyr Gln Gln Ile Pro Gly Gln Pro Pro
35 40 45

[0323]

Lys Leu Leu Ile Tyr Asp Ala Ser Asn Leu Val Ser Gly Ile Pro Pro
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His
65 70 75 80

Pro Val Glu Lys Val Asp Ala Ala Thr Tyr His Cys Gln Gln Ser Thr
85 90 95

Glu Asp Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Gly
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala Ser Gln
115 120 125

Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ser Ser
130 135 140

Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ser Ser Tyr Trp
145 150 155 160

Met Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly
165 170 175

Gln Ile Trp Pro Gly Asp Gly Asp Thr Asn Tyr Asn Gly Lys Phe Lys
180 185 190

Gly Lys Ala Thr Leu Thr Ala Asp Glu Ser Ser Ser Thr Ala Tyr Met
195 200 205

Gln Leu Ser Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Phe Cys Ala
210 215 220

[0324]

Arg Arg Glu Thr Thr Val Gly Arg Tyr Tyr Tyr Ala Met Asp Tyr
225 230 235 240

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ser Glu Pro Lys Ser
245 250 255

Ser Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala
260 265 270

Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
275 280 285

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
290 295 300

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
305 310 315 320

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
325 330 335

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
340 345 350

Gly Lys Ala Tyr Ala Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro
355 360 365

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
370 375 380

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
385 390 395 400

[0325]

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
405 410 415

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
420 425 430

Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
435 440 445

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
450 455 460

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
465 470 475 480

Ser Pro Gly Gln Arg His Asn Asn Ser Ser Leu Asn Thr Gly Thr Gln
485 490 495

Met Ala Gly His Ser Pro Asn Ser Gln Val Gln Leu Val Gln Ser Gly
500 505 510

Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala
515 520 525

Ser Gly Tyr Thr Phe Thr Arg Ser Thr Met His Trp Val Arg Gln Ala
530 535 540

Pro Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Ser Ala
545 550 555 560

Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Ala
565 570 575

[0326]

Asp Lys Ser Lys Ser Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro
580 585 590

Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Pro Gln Val His Tyr Asp
595 600 605

Tyr Asn Gly Phe Pro Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser
610 615 620

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
625 630 635 640

Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
645 650 655

Val Gly Asp Arg Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser
660 665 670

Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Trp
675 680 685

Ile Tyr Asp Ser Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser
690 695 700

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln
705 710 715 720

Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Arg Asn Pro
725 730 735

Pro Thr Phe Gly Gly Gly Thr Lys Leu Gln Ile Thr Arg
740 745

[0327]

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<212>	DNA	
<213>	人工序列	
<220>		
<223>	TSC218 DNA	
<400>	246	
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aggetgtcct gcaaggcttc tggtacacac ttactagat ctacgatgca ctgggtaagg	180	
caggccccctg gacaaggctt ggaatggatt ggatacatta atcctagcag tgcctatact	240	
aattacaatc agaaattcaa ggacaggctt acaatcagcg cagacaaatc caagagcaca	300	
gccttcctgc agatggacag cctgaggccc gaggacaccg gcgtctatct ctgtgcacgg	360	
ccccaagtcc actatgatta caacggggtt ccttactggg gccaaaggac tcccgtaact	420	
gtctctagcg gtggcggagg gtctgggggt ggccggtccg gaggtaggtg ctctgcacaa	480	
gacatccaga tgaccagtc tccaagcagc ctgtctgcaa gcgtggggga cagggtcacc	540	
atgacctgca gtgccagctc aagtgtagt tacatgaact ggtaccagca gaagccgggc	600	
aaggccccca aactctcat ttatgactca tccaaactgg cttctggagt cctgtctgc	660	
ttcagtggca gtgggtctgg gaccgactat accctcaca tcagcagcct gcagcccgaa	720	
gatttcgcca cttattactg ccagcagtg agtcgtaacc caccacttt cggcggaggg	780	
accaaggtgg agatcaaatc ctgagtgag cccaaatctt ctgacaaaac tcacacatgc	840	
ccaccgtgcc cagcacciga agccgggggt gcaccgtcag tcttcctctt cccccaaaa	900	
cccaaggaca cctcatgat ctcccgacc cctgaggta catgcgtggg ggtggacgtg	960	
agccacgaag accctgaggt caagttcaac tggtagctgg acggcgtgga ggtgcataat	1020	

[0328]

gccaagacaa agccgcggga ggagcagtac aacagcacgt accgtgtggt cagcgtcctc	1080
accgtccctgc accaggactg gctgaatgga aaggcgtacg cgtgcgcggt ctccaacaaa	1140
gccctcccag ccccatcga gaaaaccatc tccaaagcca aagggcagcc ccgagaacca	1200
caggtgtaca cctgcccc atcecggtat gagctgacca agaaccaggt cagcctgacc	1260
tgcctggta aaggcttcta tccaagcgac atcgccgtgg agtgggagag caatgggcag	1320
ccggagaaca actacaagac cagccctccc gtgctggact ccgacggctc cttcttcctc	1380
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgctcc	1440
gtgatgcatt aggtcttgea caaccactac acgcagaaga gcctctccct gtctccgggt	1500
aaatctagag cctccaccaa ggcccctcgt gtcttcccc tggcaccctc ctccaagagc	1560
acctctgggg gcacagcggc cctgggctgc ctggteaagg actacttccc cgagccggtg	1620
acggtgtcgt ggaactcagg cgccttgacc agcggcgtgc acaccttccc ggctgtccta	1680
cagtctcag gactctactc cctcagcage gtggtgaccg tgcctctcag cagcttgggc	1740
accagacct acatctgcaa cgtgaatcac aagccagca acaccaaggt ggacaagaaa	1800
gtttga	1806

<210> 247

<211> 579

<212> PRT

<213> 人工序列

<220>

<223> TSC218 成熟蛋白质

<400> 247

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser

[0329]

20	25	30
Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile		
35	40	45
Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe		
50	55	60
Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe		
65	70	75 80
Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys		
85	90	95
Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly		
100	105	110
Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly		
115	120	125
Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln		
130	135	140
Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr		
145	150	155 160
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys		
165	170	175
Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Ser Ser Lys Leu Ala		
180	185	190
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr		

[0330]

195	200	205
Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr		
210	215	220
Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys		
225	230	235 240
Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His		
245	250	255
Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val		
260	265	270
Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr		
275	280	285
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu		
290	295	300
Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys		
305	310	315 320
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser		
325	330	335
Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala		
340	345	350
Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile		
355	360	365
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro		

[0331]

370	375	380
Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu		
385	390	395 400
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn		
	405	410 415
Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser		
	420	425 430
Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg		
	435	440 445
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu		
	450	455 460
His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser		
	465	470 475 480
Arg Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser		
	485	490 495
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp		
	500	505 510
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr		
	515	520 525
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr		
	530	535 540
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln		

[0332]

545	550	555	560
Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp			
	565	570	575

Lys Lys Val

<210> 248

<211> 1827

<212> DNA

<213> 人工序列

<220>

<223> TSC221 DNA

<400> 248

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aggetgtcct gcaaggttc tggetacacc ttactagat ctacgatgca ctgggtaagg	180
caggccccctg gacaaggtct ggaatggatt ggatacatt atcctagcag tgcttatact	240
aattacaatc agaaattcaa ggacaggttc acaatcagcg cagacaaatc caagagcaca	300
gccttcctgc agatggacag cctgaggecc gaggacaccg gcgtctatct ctgtgcacgg	360
ccccaaagtc actatgatta caacgggttt ccttactggg gccaaaggac tcccgtcact	420
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gacatcaga tgaccagtc tccaagcagc ctgtctgcaa gcgtggggga cagggtcacc	540
atgacctgca gtgccagctc aagtgttaagt tacatgaact ggtaccagca gaagccgggc	600
aaggccccca aactcctcat ttatgactca tccaaactgg cttctggagt ccctgctcgc	660
ttcagtggca gtgggtctgg gaccgactat accctcaca tcagcagcct gcagcccga	720

[0333]

gatttcgcca cttattactg ccagcagtggt agtcgtaacc caccacttt cggcggaggg	780
accaaggtgg agatcaaate ctgagtgag cccaaatctt ctgacaaaac tcacacatgc	840
ccaccgtgcc cagcacetga agccgcgggt gcaccgtcag tcttctcttt cccccaaaa	900
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agccacgaag accctgaggt caagttcaac tggtagctgg acggcgtgga ggtgcataat	1020
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accgtctgc accaggactg gctgaatggt aaggcgtacg cgtgcgggt ctccaacaaa	1140
gccctcccag ccccatcga gaaaaccatc tccaaagcca aagggeagcc ccgagaacca	1200
caggtgtaca ccctgcccc atcccgggat gagctgacca agaaccaggt cagcctgacc	1260
tgcctggtea aaggtttcta tccaagcgac atgccgtgg agtgggagag caatgggcag	1320
ccggagaaca actacaagac cagcctccc gtgctggact ccgacggctc cttcttcctc	1380
tacagcaagc tcaccgtgga caagagcagg tggcagcagg ggaacgtctt ctcatgetcc	1440
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aaatctagaa ctgtggctgc accatctgtc ttcatcttcc cgccatctga tgagcagtig	1560
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gtacagtgga aggtggataa cgccctccaa teggtaact ccaggagag tgccacagag	1680
caggacagca aggacagcac ctacagctc agcagcgagc tgacgtgag caaagcagac	1740
tacgagaaac acaaagtcta cgctgcgaa gtcaccatc agggcctgag ctgcgccgtc	1800
acaaagagct tcaacagggg agagtga	1827

<210> 249

<211> 586

<212> PRT

<213> 人工序列

[0334]

<220>

<223> TSC221 成熟蛋白质

<400> 249

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Ser
20 25 30

Thr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Tyr Ile Asn Pro Ser Ser Ala Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Arg Phe Thr Ile Ser Ala Asp Lys Ser Lys Ser Thr Ala Phe
65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly
100 105 110

Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln
130 135 140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr
145 150 155 160

[0335]

Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys
 165 170 175

Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Ser Ser Lys Leu Ala
 180 185 190

Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr
 195 200 205

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
 210 215 220

Cys Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys
 225 230 235 240

Val Glu Ile Lys Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His
 245 250 255

Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val
 260 265 270

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
 275 280 285

Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu
 290 295 300

Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
 305 310 315 320

Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser
 325 330 335

[0336]

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala
340 345 350

Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
355 360 365

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
370 375 380

Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
385 390 395 400

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
405 410 415

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
420 425 430

Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
435 440 445

Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
450 455 460

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys Ser
465 470 475 480

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
485 490 495

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Tyr Phe
500 505 510

[0337]

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
515 520 525

Ser Gly Asn Ser Gln Glu Ser Ala Thr Glu Gln Asp Ser Lys Asp Ser
530 535 540

Thr Tyr Ser Leu Ser Ser Glu Leu Thr Leu Ser Lys Ala Asp Tyr Glu
545 550 555 560

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
565 570 575

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu
580 585

<210> 250

<211> 2283

<212> DNA

<213> 人工序列

<220>

<223> TSC194 DNA

<400> 250

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atcacttgcc gggcgagtaa ggcattagc aaatatttag cctggtttca gcagaaacca 180

gggaaagttc ctaagctccg catccattct ggatctactt tgcaatcagg ggtcccattt 240

cggttcagtg gcagtggatc tgggacagaa ttactctca ccatcagcag cctgcagcct 300

gaagattttg caacttatta ctgtcaacag catattgaat acccgtggac gttcggccaa 360

gggaccaagg tggaatcaa acgaggtgge ggagggtctg ggggtggcgg atccggaggt 420

[0338]

gtgggtctct aggtccagct ggtacagtct ggggctgagg tgaagaagcc tggggcttca	480
gtgaaggtct cctgcaaggc ttctggatac acattcactg actactacat gcactgggtg	540
cgacaggccc ctggacaagg gcttgagtgg atgggatatt ttaatcetta taatgattat	600
actagatacg cacagaagtt ccagggcaga gtcacatga ccagggaac gtctatcagc	660
acagcctaca tggagctgag cagcctgaga tctgacgaca cggccgtgta ttactgtgca	720
agatcggatg gttactacga tgctatggac tcttggggtc aaggaaccac agtcaccgtc	780
tctctgagtg agccccaaatc ttctgacaaa actcacacat gccaccgtg cccagcacct	840
gaagccgggg gtgcaccgtc agtcttctc tttccccaa aaccaagga caccctcatg	900
atctcccgga cccctgaggt cacatgcgtg gtggtggacg tgagccacga agaccctgag	960
gtcaagtcca actgggtacgt ggacggcgtg gaggtgcata atgccaagac aaagccggcg	1020
gaggagcagt acaacagcac gtaccgtgtg gtcagcgtcc tcaccgtcct gcaccaggac	1080
tggctgaatg gcaaggcgla cgcgtgcgcg gcttccaaca aagccctccc agccccatc	1140
gagaaaacca tctccaaagc caaagggcag ccccgagaac cacaggtgta caccctgccc	1200
ccatcccggg atgagctgac caagaaccag gtcagcctga cctgcctggc caaaggcttc	1260
tatccaagcg acatcgccgt ggagtgggag agcaatgggc agccggagaa caactacaag	1320
accacgcctc ccgtgctgga ctccgacggc tctttcttc tetacagca gctcacctg	1380
gacaagagca ggtggcagca ggggaacgtc ttctcatgct ccgtgatgca tgaggtctctg	1440
cacaaccact acacgcagaa gagcctctcc ctgtctccgg gtcagaggca caacaattct	1500
tcctgaata caggaactca gatggcaggt cattctcga attctcaggt ccagctgggtg	1560
cagtctgggg gcggagtggc gcagcctggg cggtaactga ggtgtctctg caaggtctct	1620
ggctacacct ttactagatc tacgatgcac tgggtaagge aggcccttgg aaagggtctg	1680
gaatggattg gatacattaa tcctagcagt gcttatacta attacaatca gaaattcaag	1740

[0339]

gacaggttca caatcagcgc agacaaatcc aagagcacag ccttcctgca gatggacagc 1800
 ctgaggcccg aggacaccgg cgtctatttc tgtgcacggc cccaagtcca ctatgattac 1860
 aacgggtttc ctactggggg ccaagggact cccgtcactg tctctagcgg tggcggaggg 1920
 tctgggggtg gcgatccgg aggtgggtggc tetgcacaag acatccagat gaccagctct 1980
 ccaagcagcc tgtctgcaag cgtgggggac agggtcacca tgacctgcag tgccagctca 2040
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 tatgactcat ccaaactggc ttctggagtc cctgctcgtt tcagtggcag tgggtctggg 2160
 accgactata cctcacaat cagcagcctg cagccccaag atttcgccac ttattactgc 2220
 cagcagtgga gtctgaaccc acccacttcc ggagggggga ccaagctaca aattacacga 2280
 taa 2283

<210> 251

<211> 740

<212> PRT

<213> 人工序列

<220>

<223> TSC194 成熟蛋白质

<400> 251

Asp Ile Gln Met Thr Gln Ser Pro Ser Ala Met Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Lys Ser Ile Ser Lys Tyr
 20 25 30

Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Arg Ile
 35 40 45

His Ser Gly Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly

[0340]

50	55	60
Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro		
65	70	75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Ile Glu Tyr Pro Trp		
85	90	95
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Gly Gly Gly Gly		
100	105	110
Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val		
115	120	125
Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser		
130	135	140
Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Tyr Met His Trp Val		
145	150	155 160
Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Tyr Phe Asn Pro		
165	170	175
Tyr Asn Asp Tyr Thr Arg Tyr Ala Gln Lys Phe Gln Gly Arg Val Thr		
180	185	190
Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Ser		
195	200	205
Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Ser Asp Gly		
210	215	220
Tyr Tyr Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val		

[0341]

225	230	235	240
Ser Ser Ser Glu Pro Lys Ser Ser Asp Lys Thr His Thr Cys Pro Pro			
245	250	255	
Cys Pro Ala Pro Glu Ala Ala Gly Ala Pro Ser Val Phe Leu Phe Pro			
260	265	270	
Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr			
275	280	285	
Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn			
290	295	300	
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg			
305	310	315	320
Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val			
325	330	335	
Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Ala Cys Ala Val Ser			
340	345	350	
Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys			
355	360	365	
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp			
370	375	380	
Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe			
385	390	395	400
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu			

[0342]

405										410										415									
Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe														
420										425										430									
Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly														
435										440										445									
Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr														
450										455										460									
Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Gln	Arg	His	Asn	Asn	Ser														
465										470										475									
Ser	Leu	Asn	Thr	Gly	Thr	Gln	Met	Ala	Gly	His	Ser	Pro	Asn	Ser	Gln														
485										490										495									
Val	Gln	Leu	Val	Gln	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg	Ser														
500										505										510									
Leu	Arg	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Arg	Ser	Thr														
515										520										525									
Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Ile	Gly														
530										535										540									
Tyr	Ile	Asn	Pro	Ser	Ser	Ala	Tyr	Thr	Asn	Tyr	Asn	Gln	Lys	Phe	Lys														
545										550										555									
Asp	Arg	Phe	Thr	Ile	Ser	Ala	Asp	Lys	Ser	Lys	Ser	Thr	Ala	Phe	Leu														
565										570										575									
Gln	Met	Asp	Ser	Leu	Arg	Pro	Glu	Asp	Thr	Gly	Val	Tyr	Phe	Cys	Ala														

[0343]

	580		585		590
Arg Pro Gln Val His Tyr Asp Tyr Asn Gly Phe Pro Tyr Trp Gly Gln					
595		600		605	
Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly					
610		615		620	
Gly Ser Gly Gly Gly Gly Ser Ala Gln Asp Ile Gln Met Thr Gln Ser					
625		630		635	640
Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Met Thr Cys					
645		650		655	
Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro					
660		665		670	
Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Ser Ser Lys Leu Ala Ser					
675		680		685	
Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr					
690		695		700	
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys					
705		710		715	720
Gln Gln Trp Ser Arg Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu					
725		730		735	
Gln Ile Thr Arg					
740					
<210>	252				

[0344]

<211> 4
<212> PRT
<213> 小家鼠

<400> 252

Leu Gln Ile Thr
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<210> 253
<211> 4
<212> PRT
<213> 人工序列

<220>
<223> VEIK

<400> 253

Val Glu Ile Lys
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<210> 254
<211> 6
<212> PRT
<213> 智人

<400> 254

Val Glu Pro Lys Ser Cys
1 5

<210> 255
<211> 6
<212> PRT
<213> 智人

<400> 255

Phe Asn Arg Gly Glu Cys
1 5

先前人源化的抗 CD3 SMIP 分子与其它 SMIP 分子相比的理论 pI

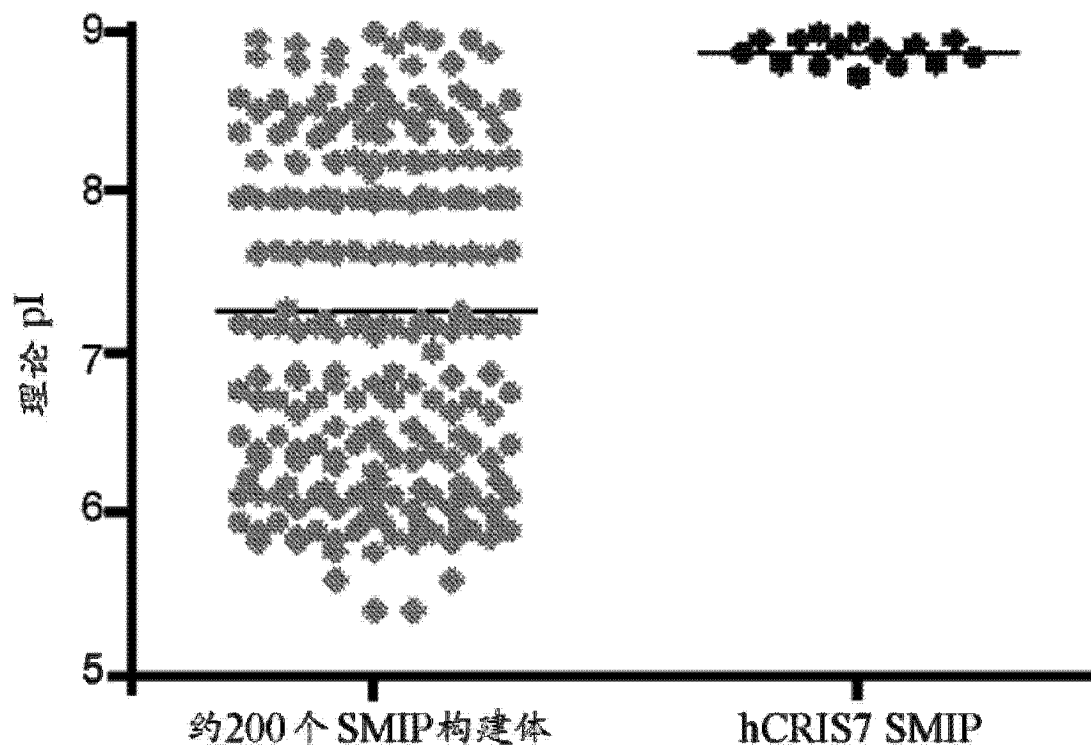


图 1

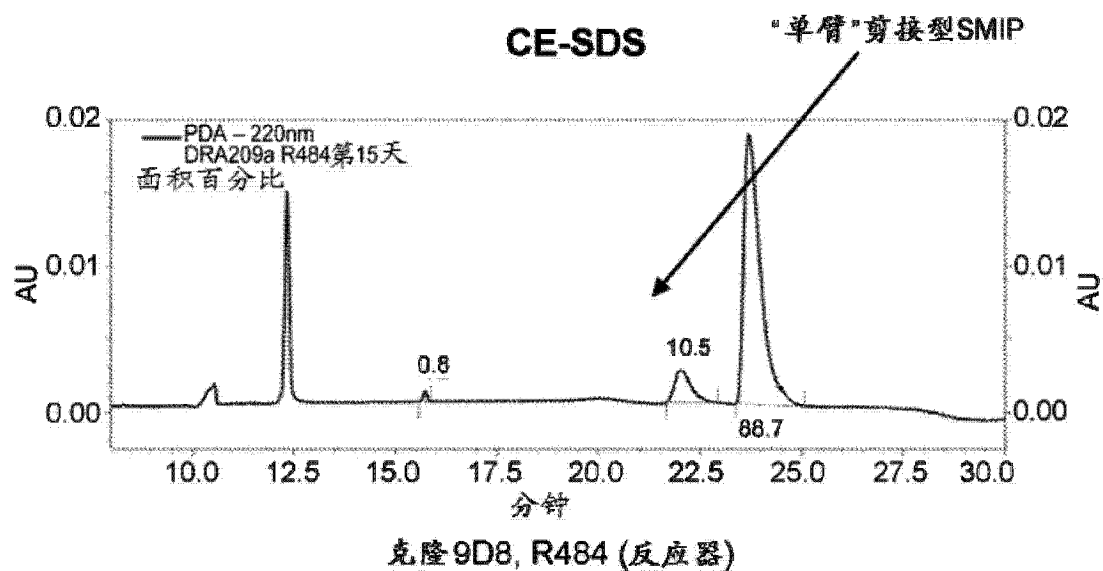


图 2A

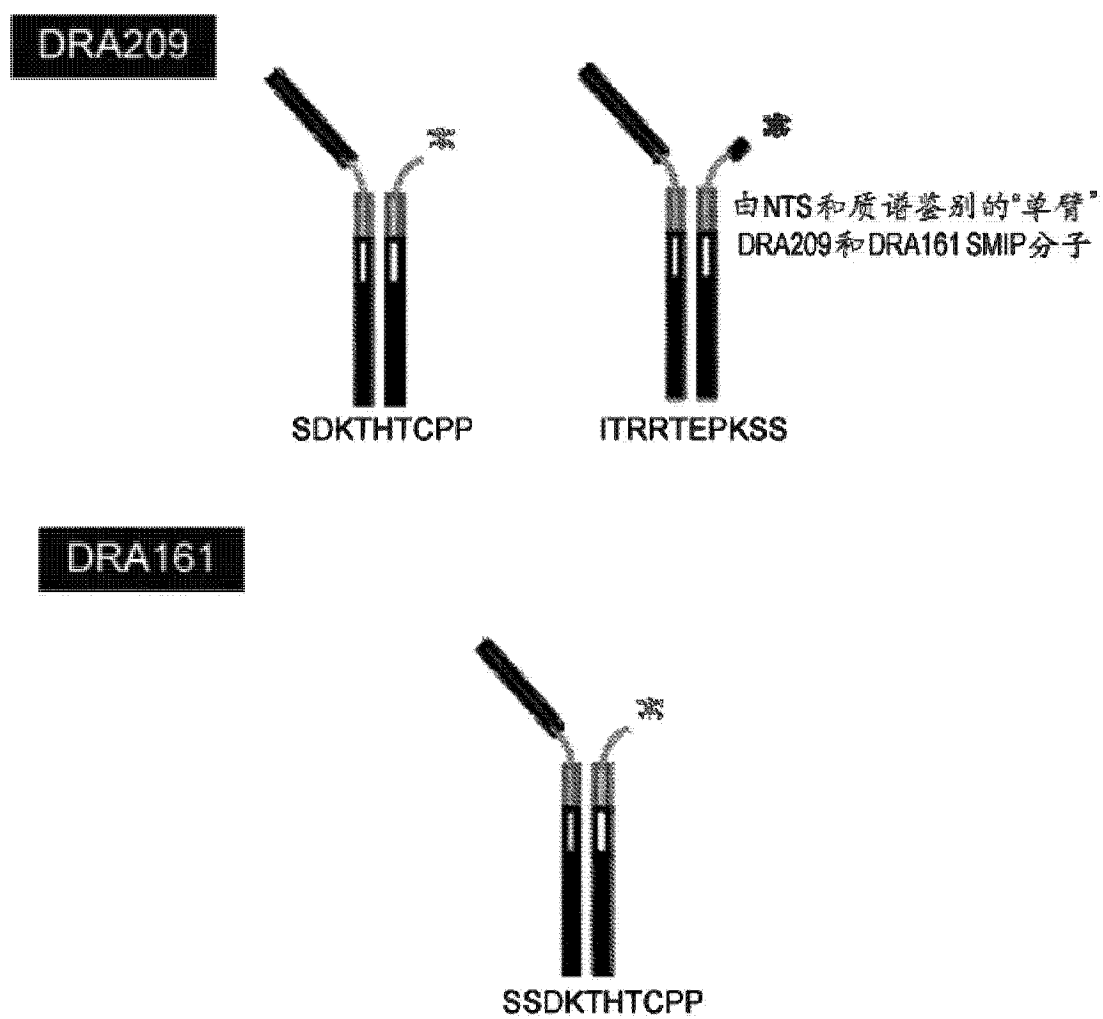


图 2B

图 3

		第1部分						
		(1)	1	10	20	30	40	50
On17 VL 小鼠	(1)	AQCIVLTQSPKINSAFFGKRTMTCSASSSVSYNNWYQQKFGTSPKAIYDQ						
L4 链	(1)	--EIVLTQSPATIELEPGERATIECSASSSVSYNNWYQQKFGTSPKAIYDQ						
L1 链	(1)	AQCIVLTQSPKINSAFFGKRTMTCSASSSVSYNNWYQQKFGTSPKAIYDQ						
L2 链	(1)	AQCIVLTQSPKINSAFFGKRTMTCSASSSVSYNNWYQQKFGTSPKAIYDQ						
L3 链	(1)	AQCIVLTQSPKINSAFFGKRTMTCSASSSVSYNNWYQQKFGTSPKAIYDQ						
共有序列	(1)	AQCIVLTQSPKINSAFFGKRTMTCSASSSVSYNNWYQQKFGTSPKAIYDQ						
		第2部分						
		(53)	53	60	70	80	90	104
On17 VL 小鼠	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCQQWSRNPPTFGGGTF						
L4 链	(51)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCQQWSRNPPTFGGGTF						
L1 链	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCQQWSRNPPTFGGGTF						
L2 链	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCQQWSRNPPTFGGGTF						
L3 链	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCQQWSRNPPTFGGGTF						
共有序列	(53)	SKLASGVPARFSGSGSGTDYTLTISSSLQPEDFATYYCQQWSRNPPTFGGGTF						
		第3部分						
		(105)	105	108				
On17 VL 小鼠	(105)	EQIIE						
L4 链	(103)	QPIIK						
L1 链	(105)	EQIIE						
L2 链	(105)	EQIIE						
L3 链	(105)	QPIIK						
共有序列	(105)	LQIT						

图 4

		第 1 部分						
		(1)	1	10	20	30	40	53
H3 链	(1)	QVQLVQSGGGGVVQPGRLSLRLSCKASGGTFPSSYAMHWVRQAPGKGLNWIGYINP						
IGHV1-69*01	(1)	QVQLVQSGAEVKRFGSSVKSCKASGGTFPSSYALSWVRQAPGKGLNWIGGIIP						
IGHV3-30*01	(1)	QVQLVSSGGGVVQPGRLSLRLSCKAASGGTFPSSYAMHWVRQAPGKGLNWIVVISY						
共有序列	(1)	QVQLVQSGGGGVVQPGRLSLRLSCKASGGTFPSSYAMHWVRQAPGKGLNWIG I P						
		第 2 部分						
		(54)	54	60	70	80	90	106
H3 链	(54)	SSAYTNYNQRFKDRFTISADKSKSTAYLQMDSLRAEDTAVYYCARPQVHYDYN						
IGHV1-69*01	(54)	IFATANLAKKFGRTITADESTAYMKLSSLRSEDATVYYCAR-----						
IGHV3-30*01	(54)	DGSNRYLADSVKGRFTISRDNSENTLYLQMNSLRSEDATVYYCAR-----						
共有序列	(54)	A NYAQKFKGRFTISAD SKSTAYLQMD SLRAEDTAVYYCAR						
		第 3 部分						
		(107)	107	121				
H3 链	(107)	GFPYNGQGTFVTVES						
IGHV1-69*01	(99)	-----						
IGHV3-30*01	(99)	-----						
共有序列	(107)							

图 5

[illegible]

图 6

[illegible]

图 7A

第4部分										第5部分										第6部分									
(214)	214	220	230	240	250	260	270	284		(285)	285	290	300	310	320	330	340	355		(356)	356	370	380	390	400	410	426		
DRA209的翻译	(210)	T193LQPEDFANVTCQ	QUS3RNPPTT	TG6GTEKQIT	TRRCEYK33DXTHT	CFP3AP	EAAAP	SVNLT	PPPPKNT		LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC								
DRA219的翻译	(211)	T193LQPEDFANVTCQ	QUS3RNPPTT	TG6GTEKQIT	SS3EP	KS3DXTHT	CFP3AP	EAAAP	SVNLT	PPPPKNT		LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC							
DRA222的翻译	(211)	T193LQPEDFANVTCQ	QUS3RNPPTT	TG6GTEKQIT	SS3EP	KS3DXTHT	CFP3AP	EAAAP	SVNLT	PPPPKNT		LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC							
DRA221的翻译	(211)	T193LQPEDFANVTCQ	QUS3RNPPTT	TG6GTEKQIT	SS3EP	KS3DXTHT	CFP3AP	EAAAP	SVNLT	PPPPKNT		LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC							
DRA223的翻译	(211)	T193LQPEDFANVTCQ	QUS3RNPPTT	TG6GTEKQIT	SS3EP	KS3DXTHT	CFP3AP	EAAAP	SVNLT	PPPPKNT		LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC							
DRA224的翻译	(214)	T193LQPEDFANVTCQ	QUS3RNPPTT	TG6GTEKQIT	SS3EP	KS3DXTHT	CFP3AP	EAAAP	SVNLT	PPPPKNT		LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC							
DRA225的翻译	(214)	T193LQPEDFANVTCQ	QUS3RNPPTT	TG6GTEKQIT	SS3EP	KS3DXTHT	CFP3AP	EAAAP	SVNLT	PPPPKNT		LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC							
共有序列 (214)										共有序列 (285)										共有序列 (356)									
DRA209的翻译	(282)	LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC																	
DRA219的翻译	(282)	LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC																	
DRA222的翻译	(282)	LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC																	
DRA221的翻译	(282)	LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC																	
DRA223的翻译	(282)	LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC																	
DRA224的翻译	(285)	LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC																	
DRA225的翻译	(285)	LH13RTP	EVTCVWVWVVSQ	ED3FVQ	FWWVQ	QWVWVH	AKT3P3EE	QFNST	YAVV3VL	TVL	RGQDL	MGKAYAC																	
共有序列 (285)										共有序列 (356)										共有序列 (356)									
DRA209的翻译	(353)	AV3NKG	CLP33IENT	ISNAK	GQ3RNPPTT	TL3P3	QFNTENQ	VS3LT	C3WNG	ITP3DI	AVE	NE	SS3G	QPE	NTXK														
DRA219的翻译	(353)	AV3NKG	CLP33IENT	ISNAK	GQ3RNPPTT	TL3P3	QFNTENQ	VS3LT	C3WNG	ITP3DI	AVE	NE	SS3G	QPE	NTXK														
DRA222的翻译	(353)	AV3NKG	CLP33IENT	ISNAK	GQ3RNPPTT	TL3P3	QFNTENQ	VS3LT	C3WNG	ITP3DI	AVE	NE	SS3G	QPE	NTXK														
DRA221的翻译	(353)	AV3NKG	CLP33IENT	ISNAK	GQ3RNPPTT	TL3P3	QFNTENQ	VS3LT	C3WNG	ITP3DI	AVE	NE	SS3G	QPE	NTXK														
DRA223的翻译	(353)	AV3NKG	CLP33IENT	ISNAK	GQ3RNPPTT	TL3P3	QFNTENQ	VS3LT	C3WNG	ITP3DI	AVE	NE	SS3G	QPE	NTXK														
DRA224的翻译	(356)	AV3NKG	CLP33IENT	ISNAK	GQ3RNPPTT	TL3P3	QFNTENQ	VS3LT	C3WNG	ITP3DI	AVE	NE	SS3G	QPE	NTXK														
DRA225的翻译	(356)	AV3NKG	CLP33IENT	ISNAK	GQ3RNPPTT	TL3P3	QFNTENQ	VS3LT	C3WNG	ITP3DI	AVE	NE	SS3G	QPE	NTXK														
共有序列 (356)										共有序列 (356)										共有序列 (356)									

图 7B

图 8

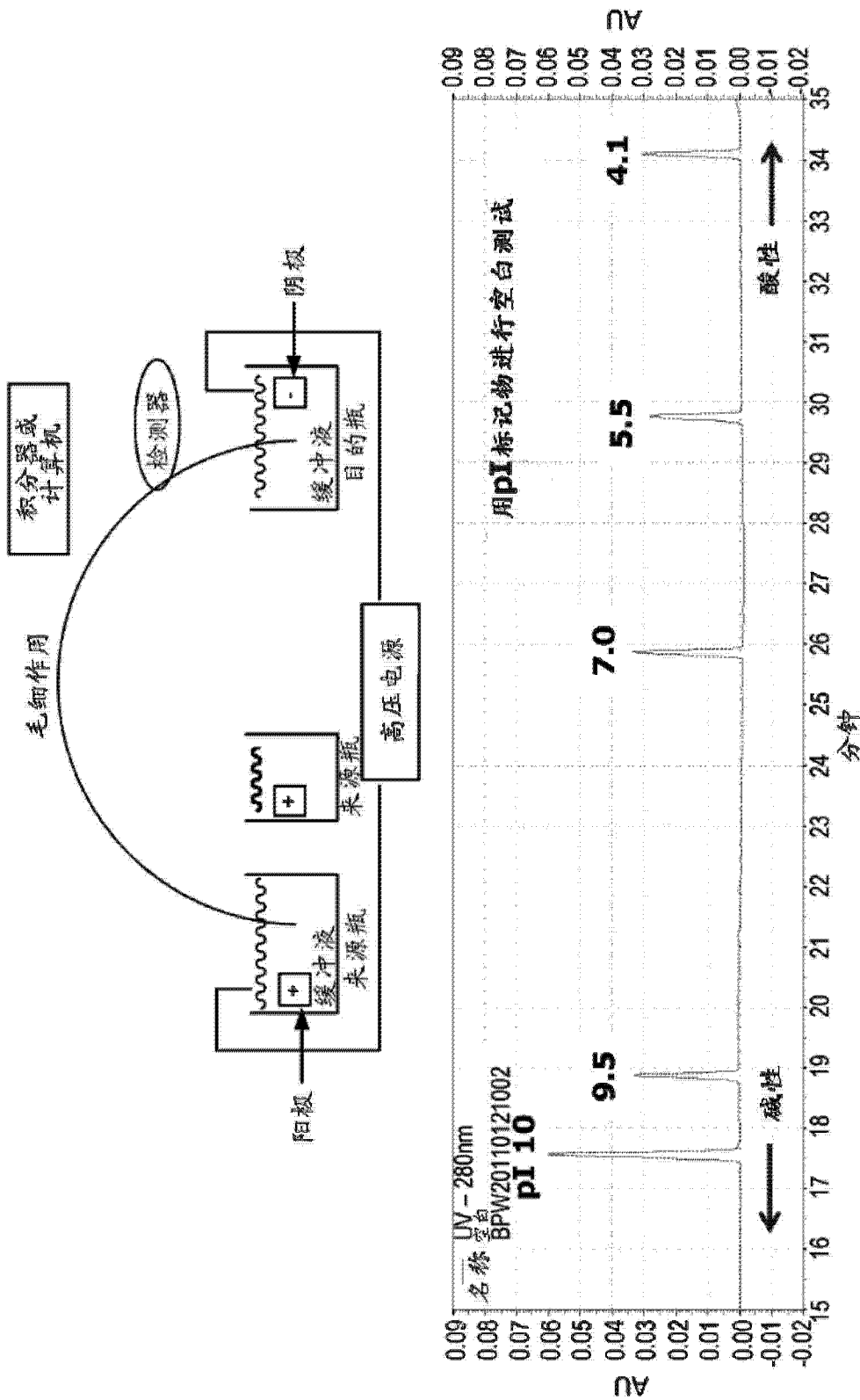


图 9

clEF: SMIP和scFv的经验pl甚至高于理论pl

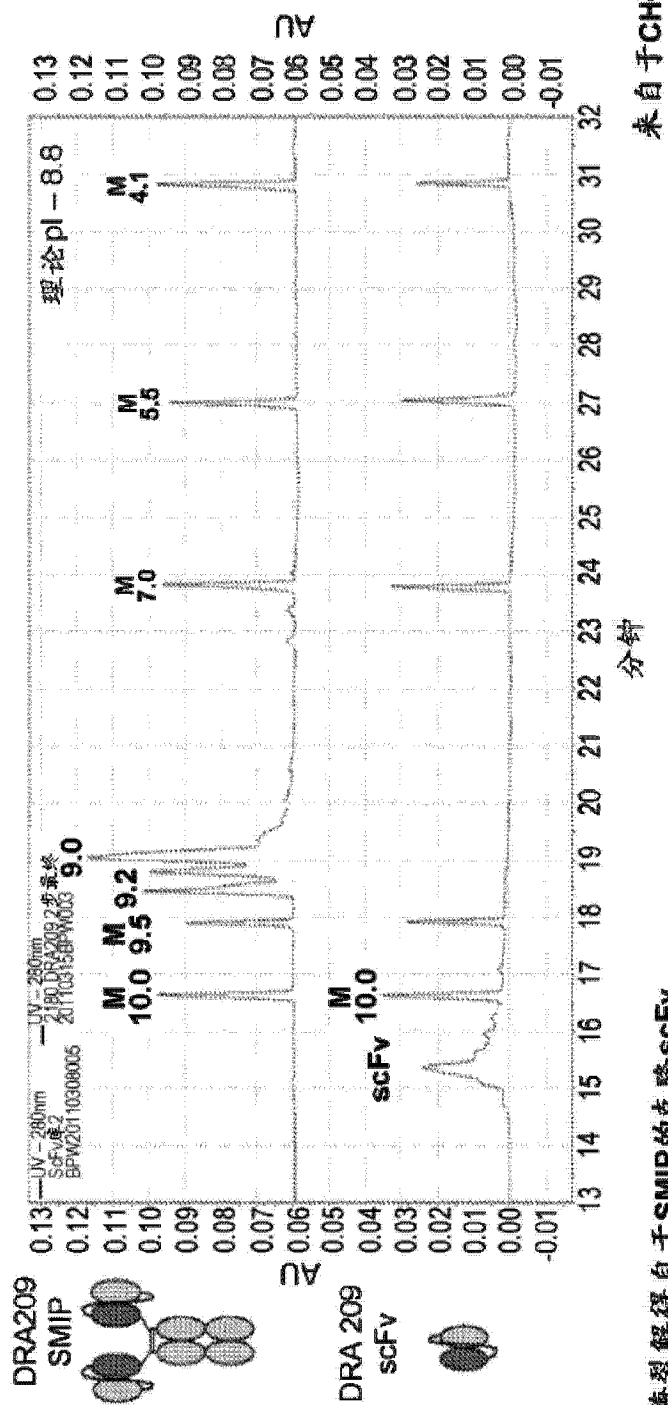
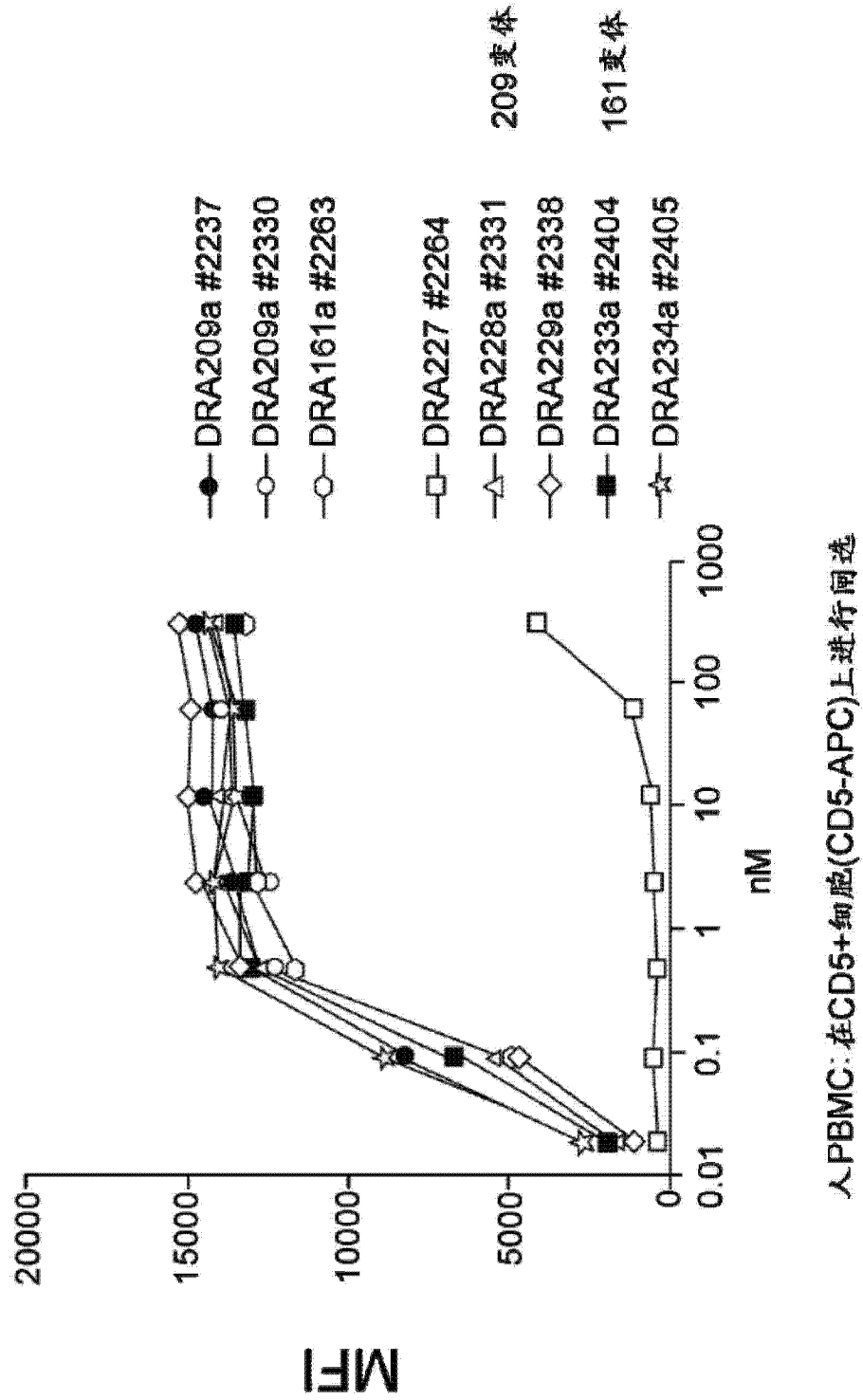


图 10



表达于CHO细胞中的DRA233a和DRA234a

图 11



人PBMC: 在CD5+细胞(CD5-APC)上进行筛选

图 12

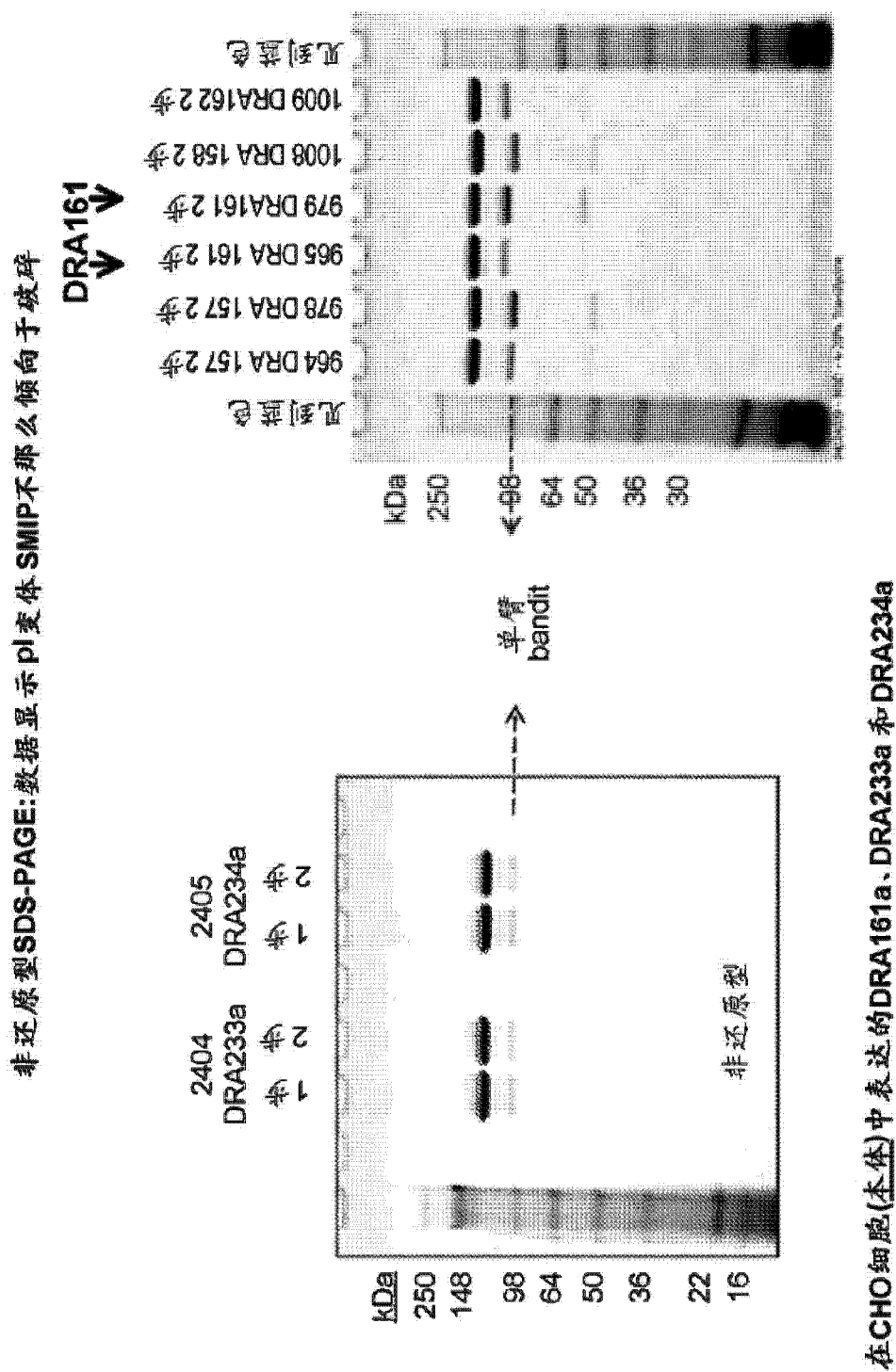
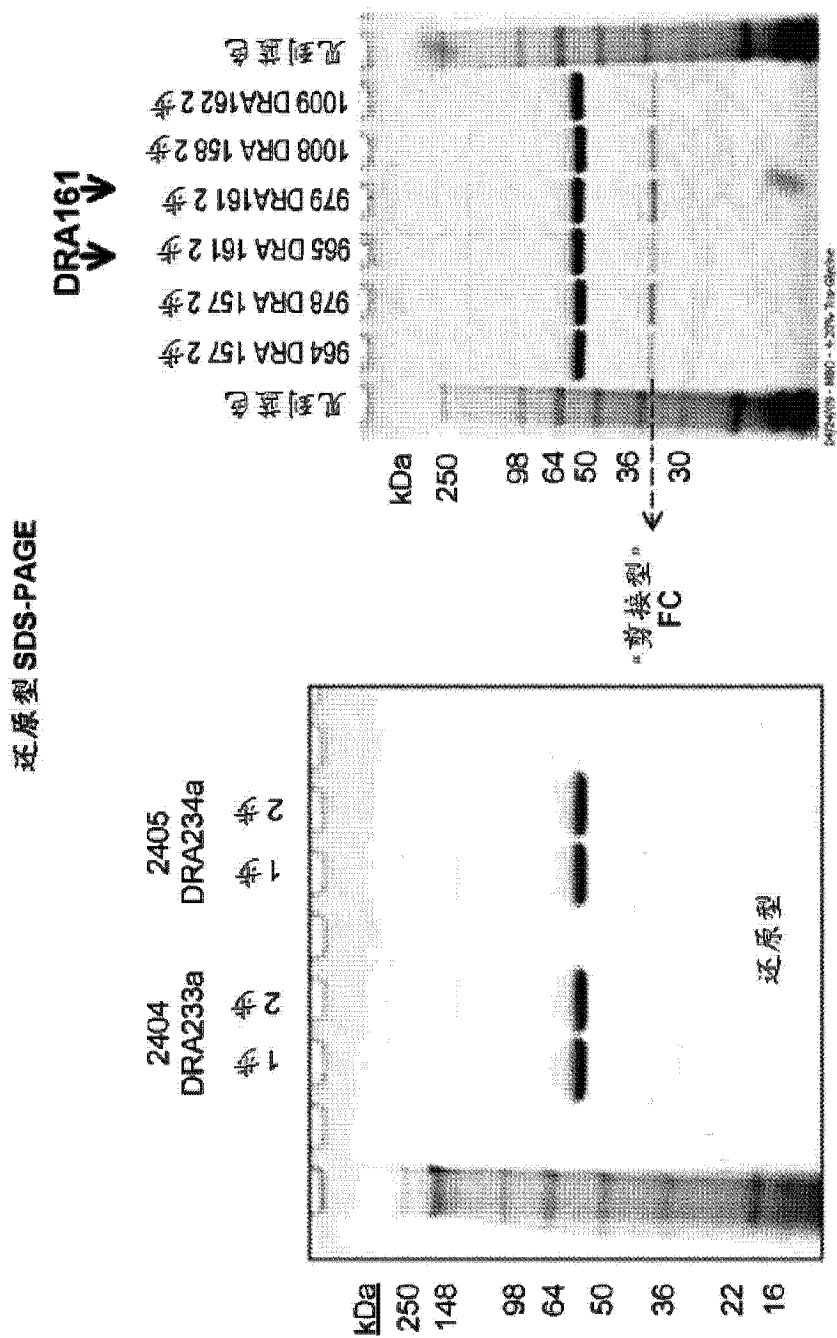


图 13



在CHO细胞(本体)中表达的DRA161a、DRA233a 和 DRA234a

图 14

PI 变体 SMIP 的非还原型 CE-SDS

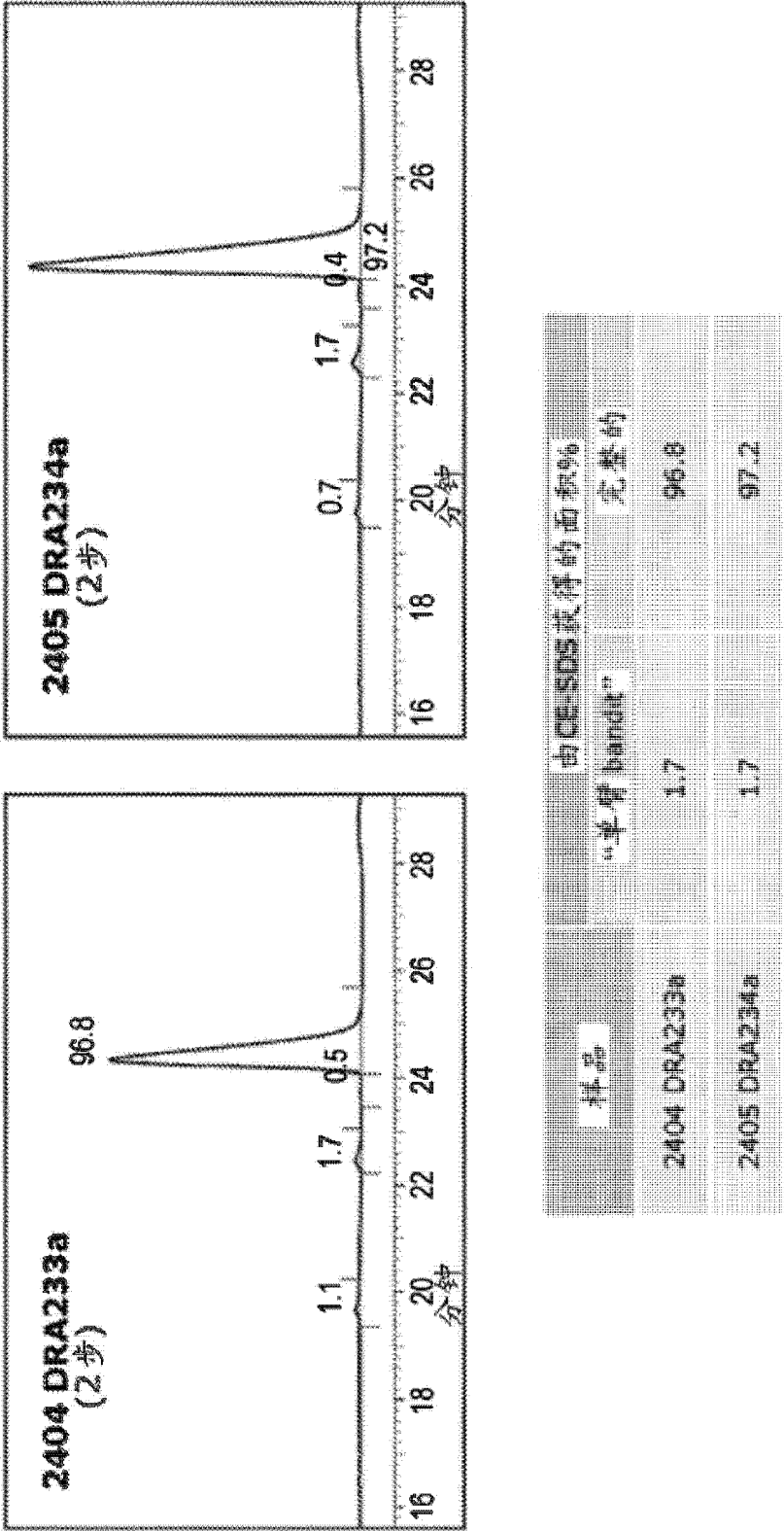


图 15

**DRA 161 的非还原型CE-SDS(本体)
通过PAD以大规模进行纯化**

**DRA161/209的
PI变体**

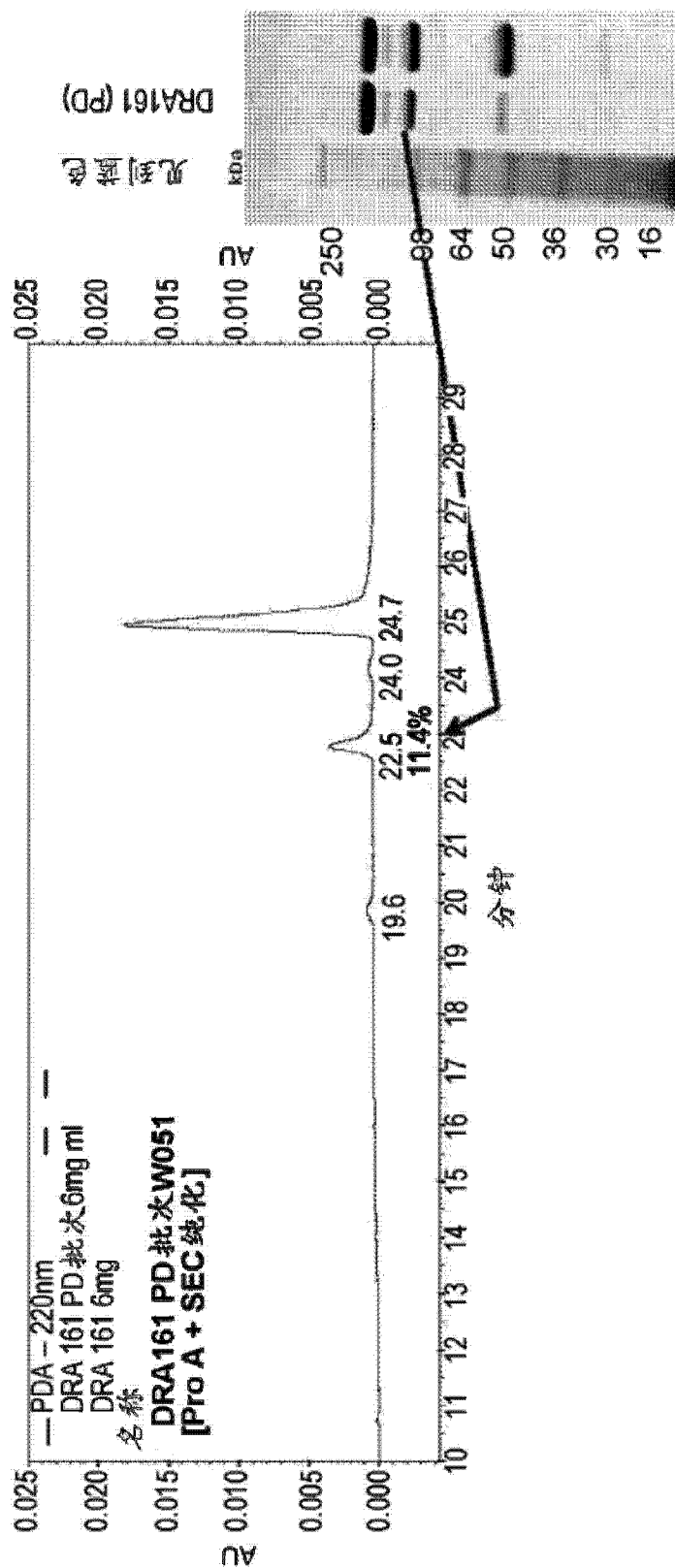


图 16

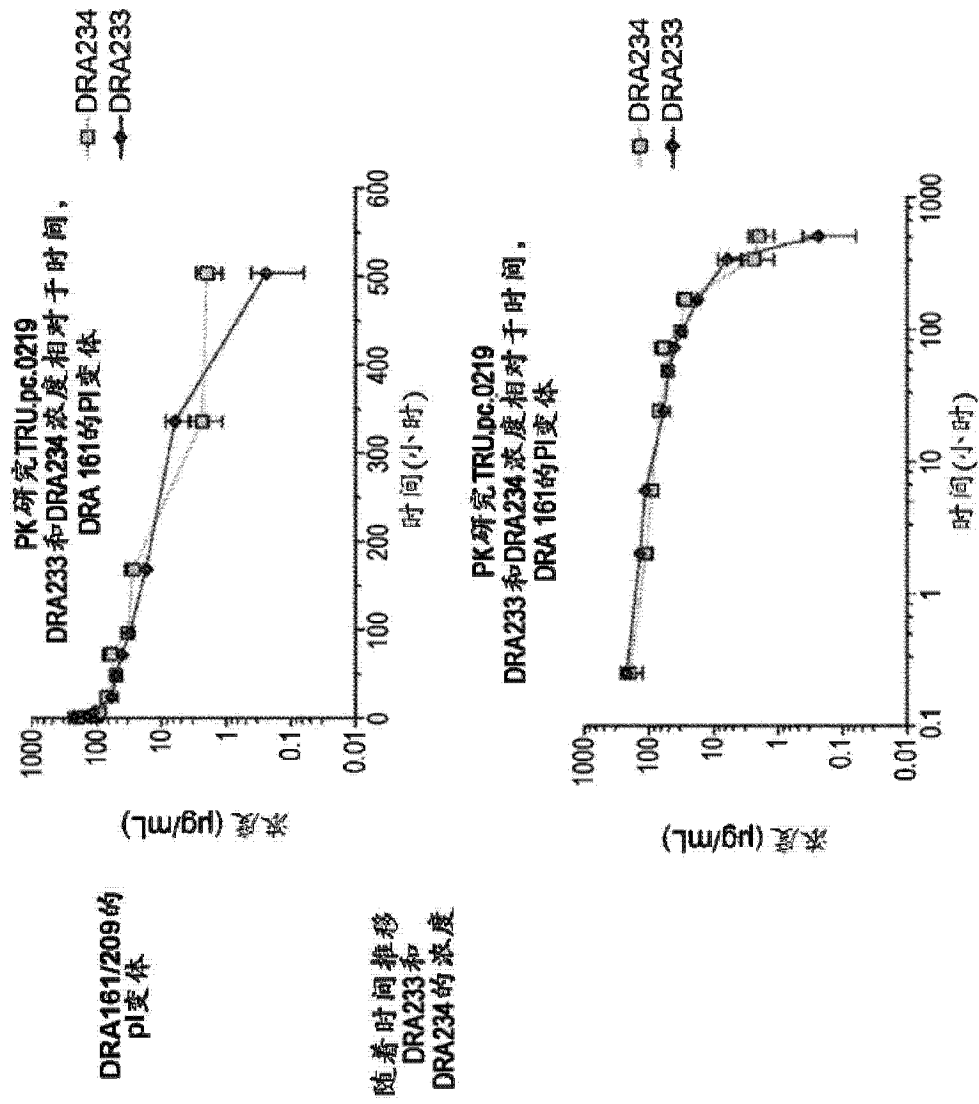


图 17

WinNonLin 分析

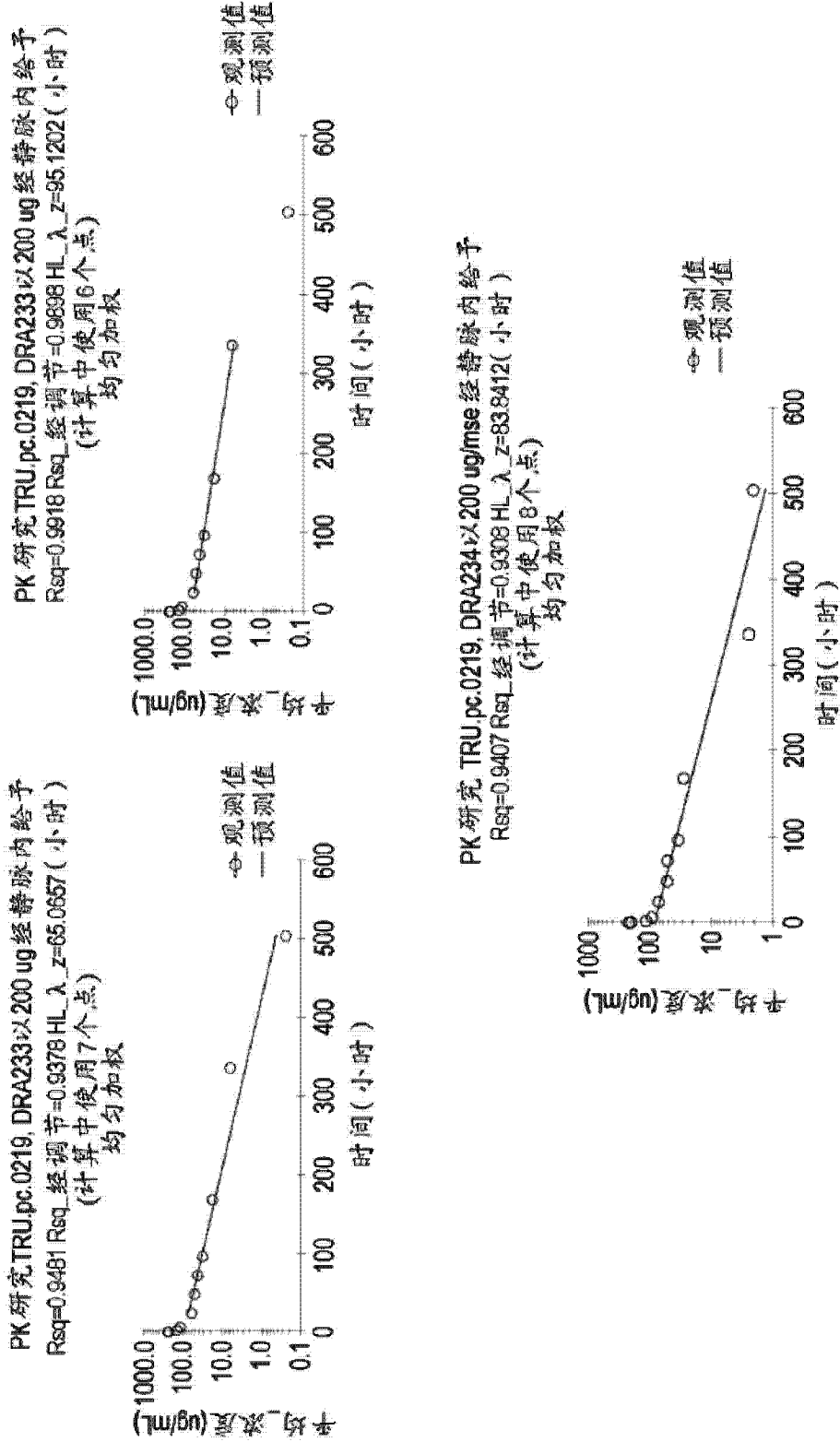


图 18

DRA 161/209 的 PI 变体	估算值	单位	DRA233 w/o 504 小时	DRA233	DRA234
	Rs _q		0.9918	0.9481	0.9407
	Rs _q 经调节		0.9998	0.9378	0.9308
	Corr _{XY}		-0.9959	-0.9737	-0.9899
	无点 λ _z		8	7	8
	λ _z	1/小时	0.0073	0.0107	0.0083
	λ _z 较低的	小时	24	24	6
	λ _z 较高的	小时	336	504	504
	HL _{λ_z}	小时	95.12	66.07	83.84
	T _{max}	小时	0.25	0.25	0.25
	C _{max}	ug/mL	223.67	223.67	198.91
	SE _{C_{max}}	ug/mL	14.44	14.44	42.40
	C _{max_D}	kg*ug/mL/mg	20.98	20.98	18.04
	C ₀	ug/mL	239.3	239.3	215.5
	T _{最后}	小时	504	504	504
	C _{最后}	ug/mL	0.2412	0.2412	2.0063
DRA233 和 DRA234 的 PK 参数	AUC _{最后}	小时*ug/mL	10029	10029	10891
	SE _{AUC} 最后	小时*ug/mL	363.4	363.4	278.1
	AUC _{全部}	小时*ug/mL	10029	10029	10891
	SE _{AUC} 全部	小时*ug/mL	363.4	363.4	278.1
	AUCINF _{obs}	小时*ug/mL	10062	10051	11234
	AUCINF _{D_obs}	小时*kg*ug/mL/mg	943.9	942.9	1063.8
	AUC _{%外推_obs}	%	0.329	0.225	2.160
	AUC _{%反向_外推_obs}	%	0.575	0.576	0.4612
	V _{z_obs}	mL/kg	145.39	99.55	113.7
	Cl _{obs}	mL/小时/kg	1.059	1.061	0.94
	AUMC _{最后}	小时*小时*ug/mL	1027660	1027660	1122005
	AUMCINF _{obs}	小时*小时*ug/mL	1048981	1041185	1273870
	AUMC _{%外推_obs}	%	2.023	1.300	11.91
	MRT _{最后}	小时	102.47	102.47	102.00
	MRTINF _{obs}	小时	104.24	103.59	113.38
	V _{ss_obs}	mL/kg	110.44	109.68	108.58
	平均剂量	mg/kg	10.68	10.68	10.56

图 19

DRA161/209的
PI变体

人源化CRIS7构建体: PK参数的比较

参数	估算值			
	DRA161	DRA233 w/o 504 小时*	DRA233	DRA234
经验PI	9.2	8.6	8.6	8.0
(半衰期, 小时)	59.3	95.1	65.1	83.8
(清除率, mL/小时/kg)	2.15	1.1	1.1	0.9

*不包括
最后一个
时间点

DRA 234显示了显著高于DRA 161的半衰期

图 20

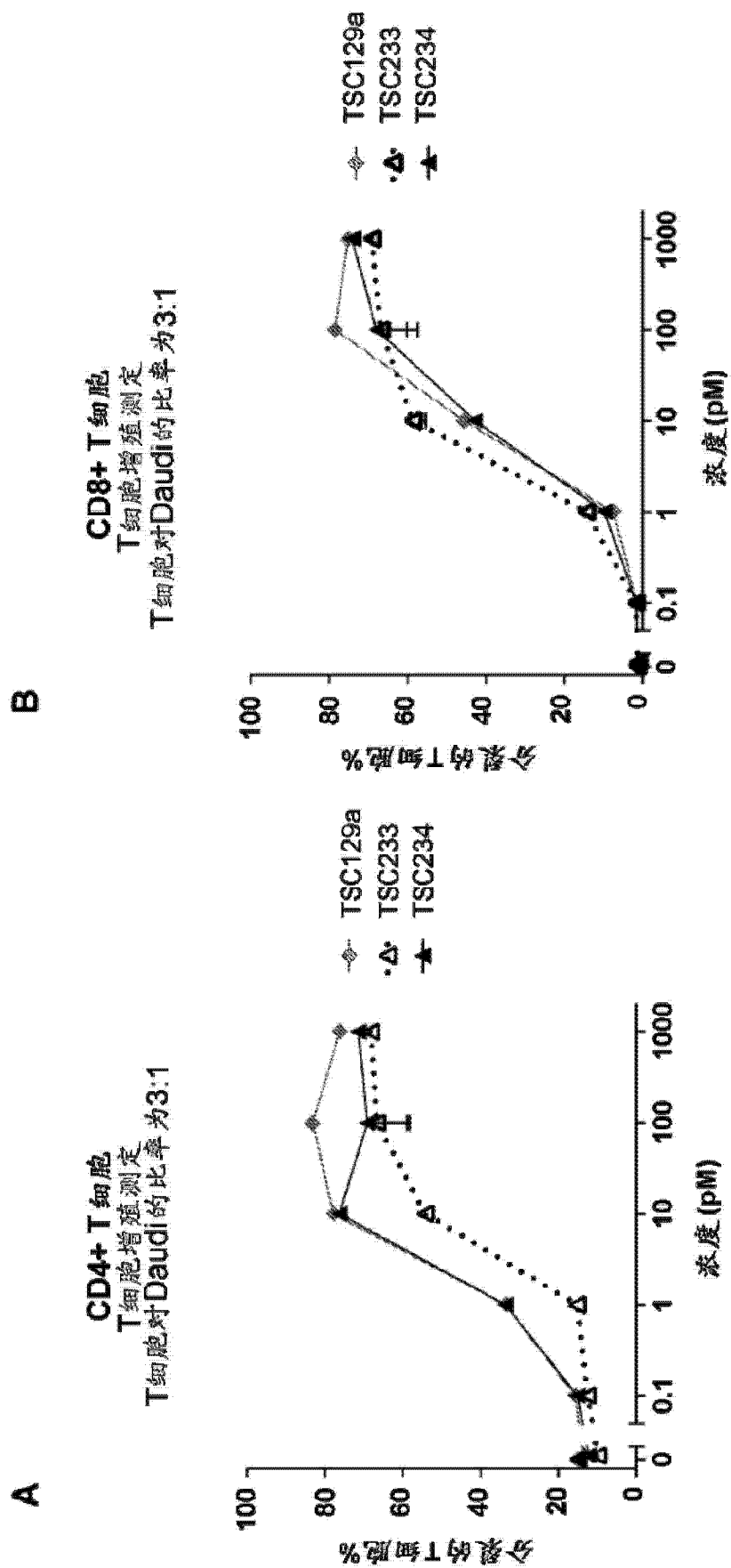


图 21

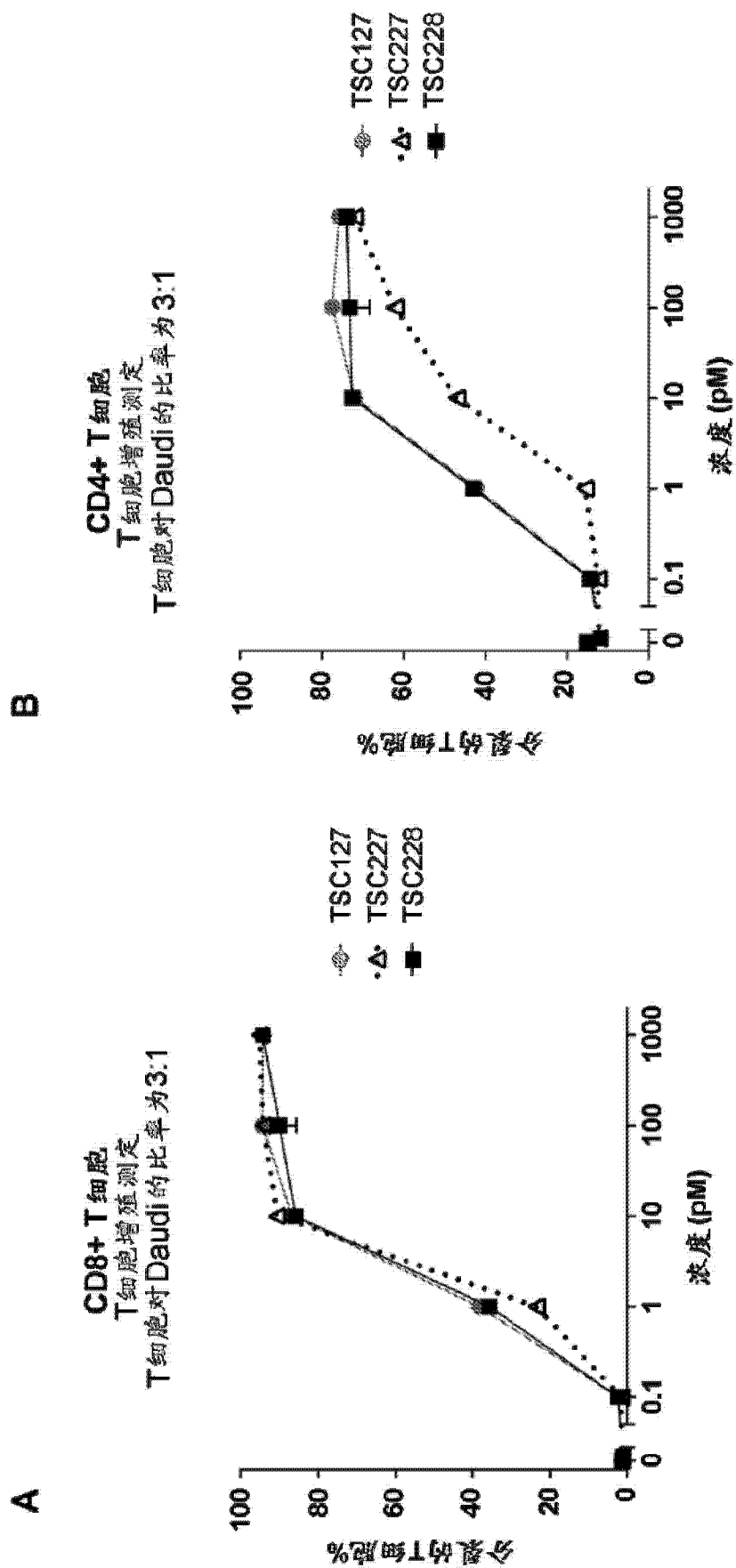


图 22

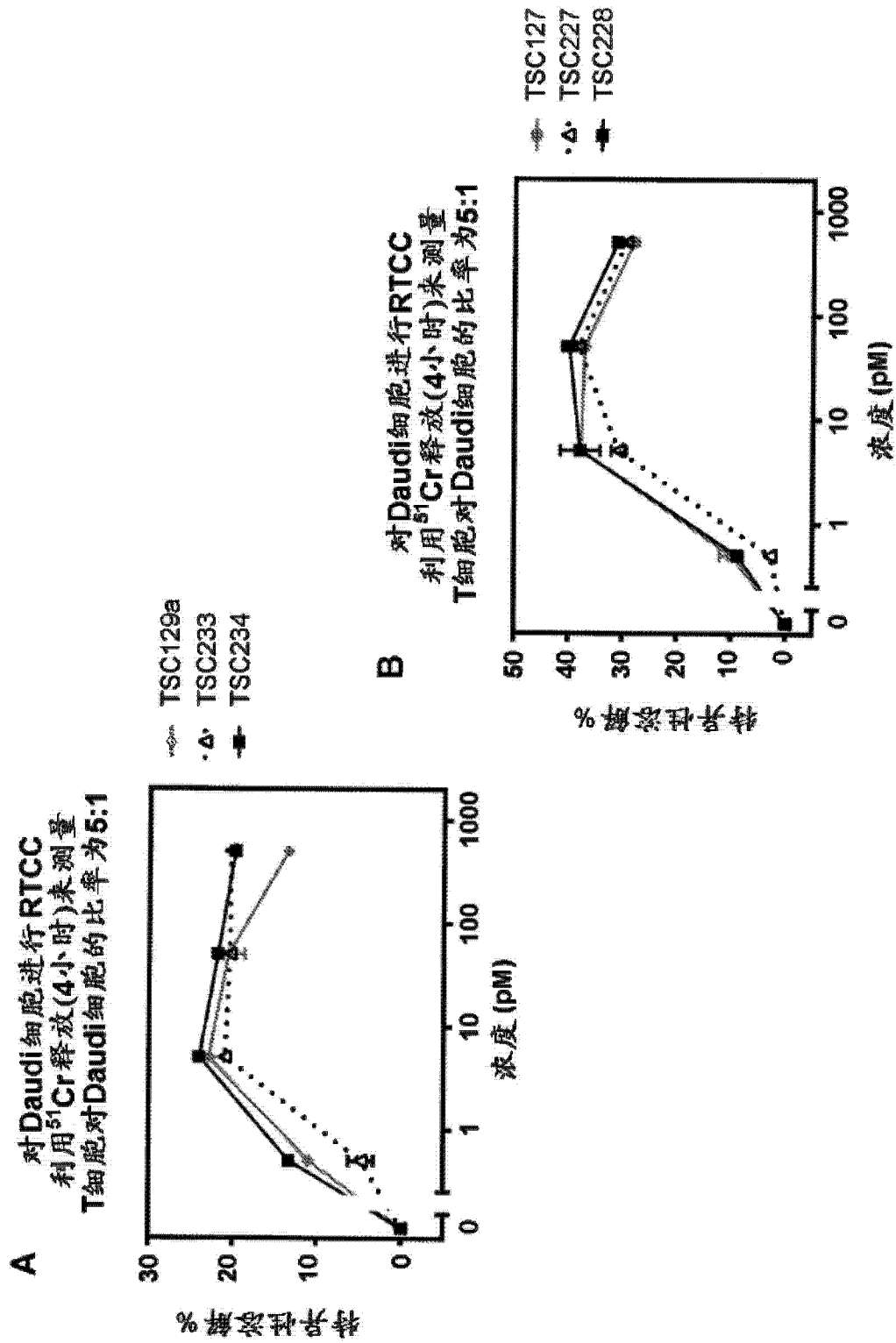


图 23

对MDA-MB-453细胞进行RTCC
利用 ^{51}Cr 释放(24小时)来测量
T细胞对MDA-MB-453细胞的比率为5:1

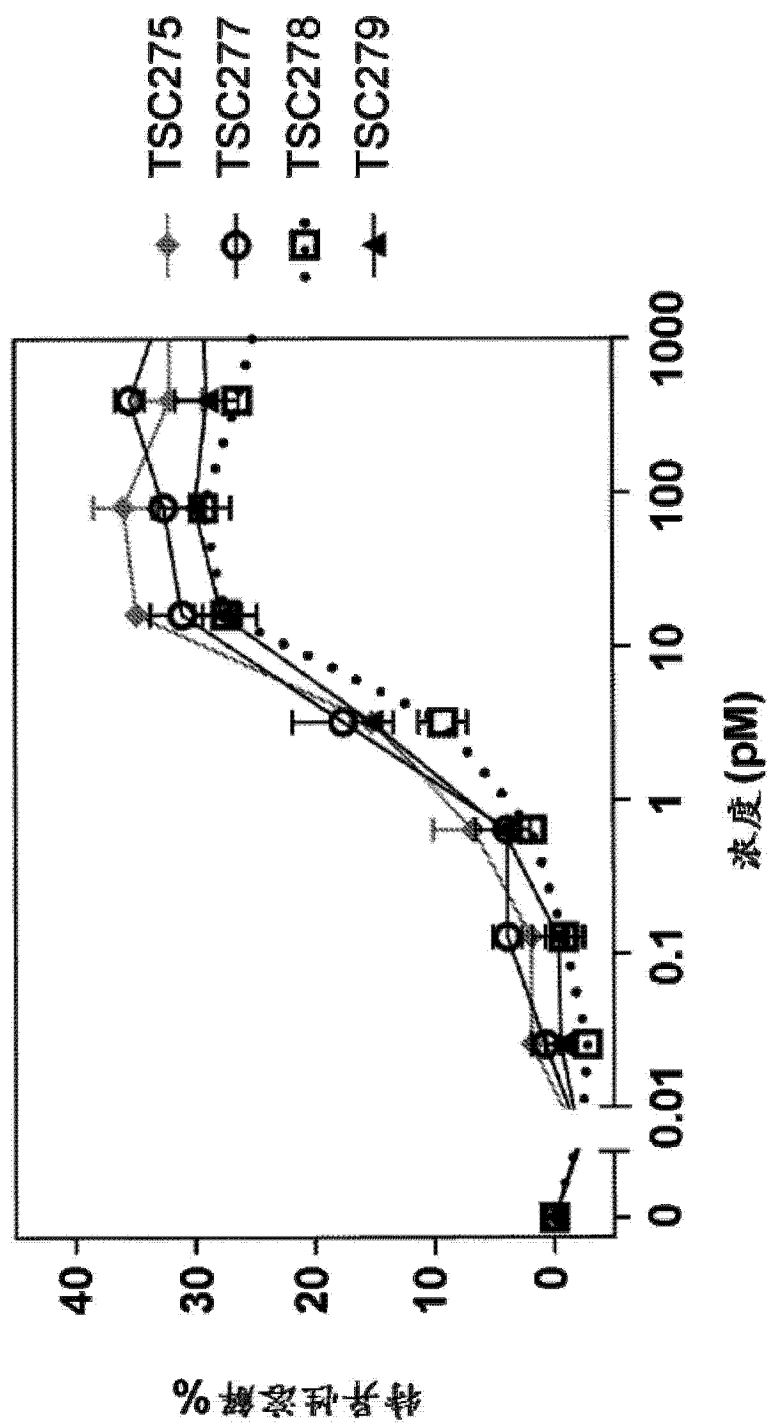


图 24

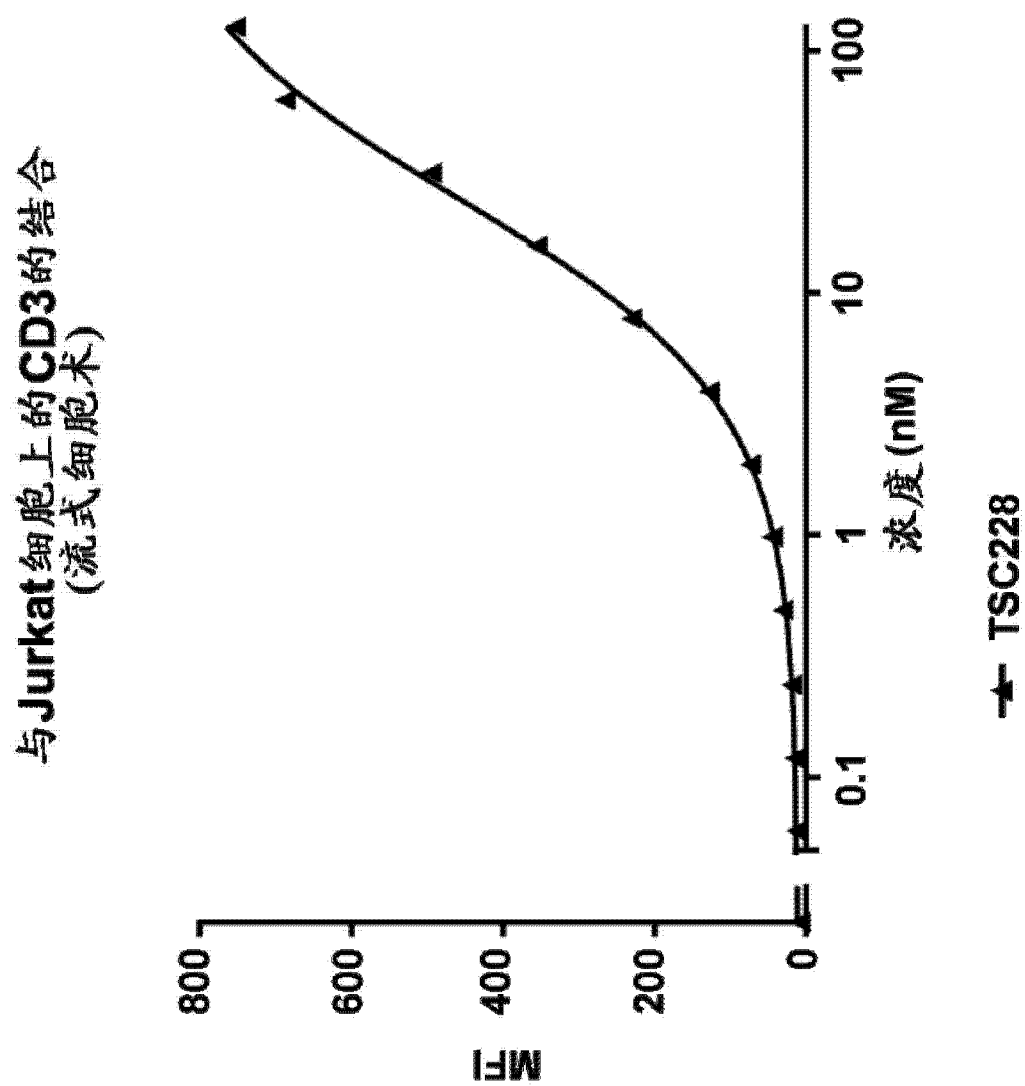


图 25A

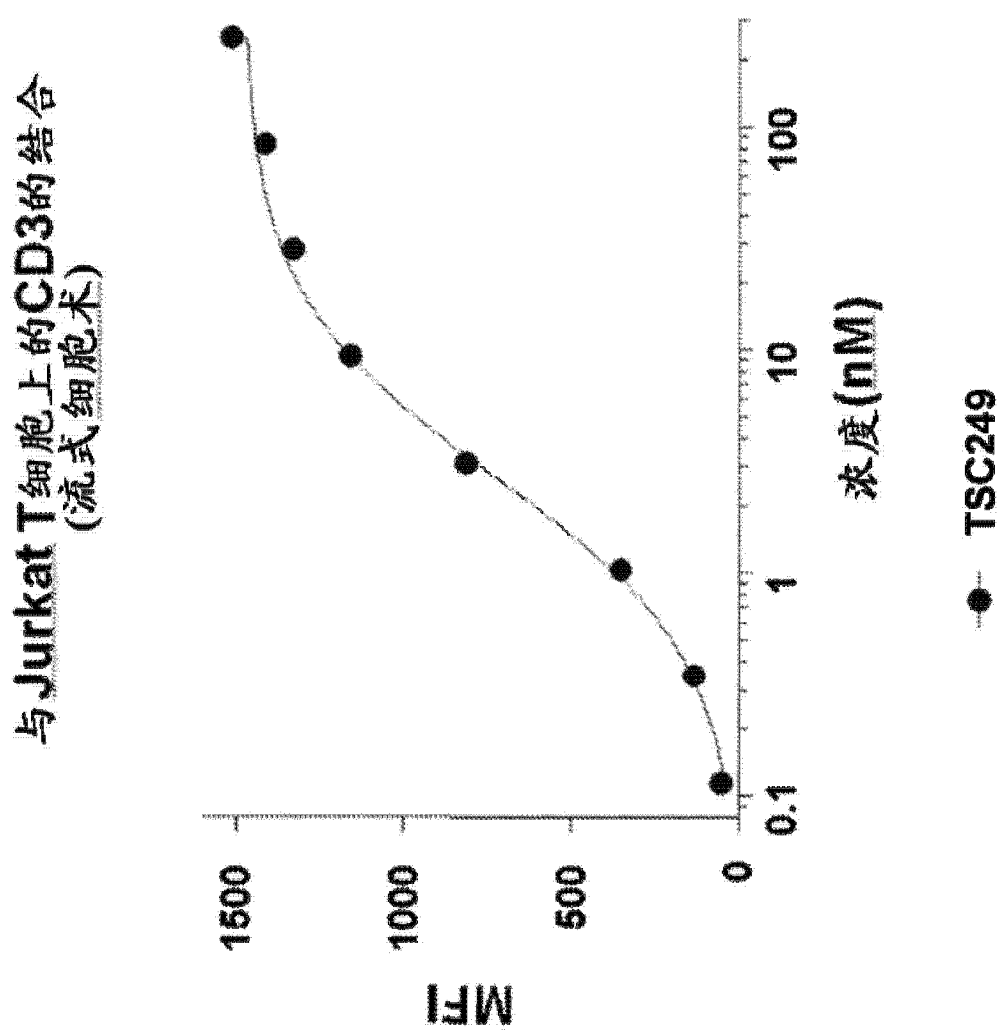


图 25B

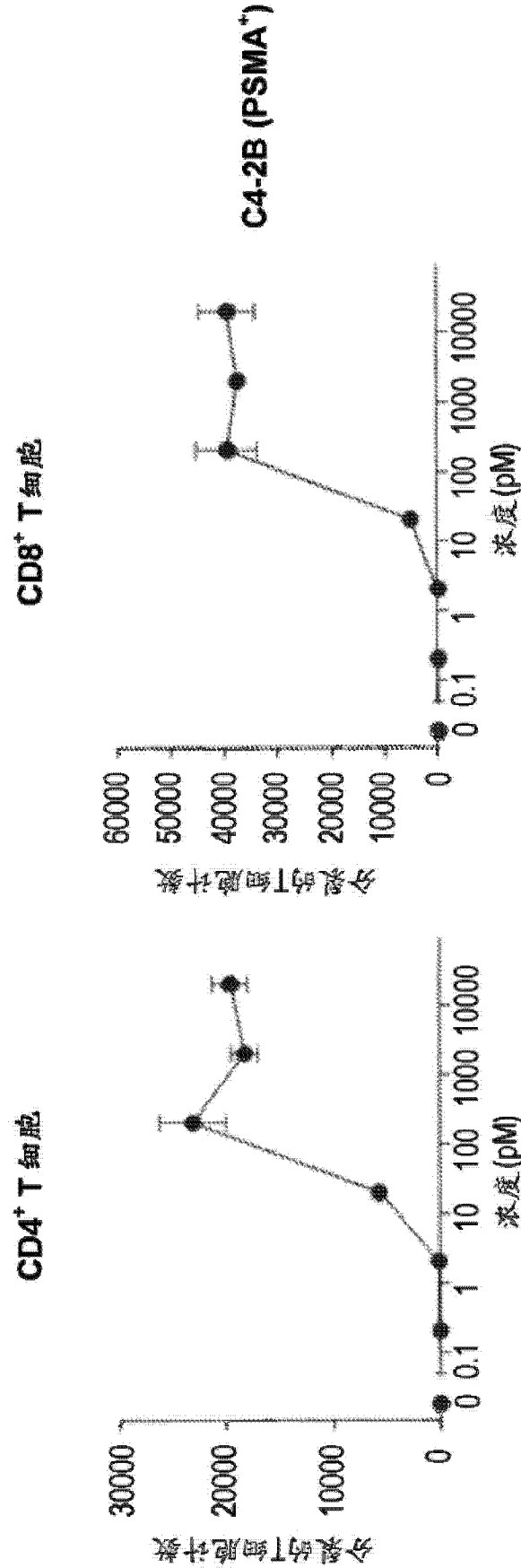


图 26A

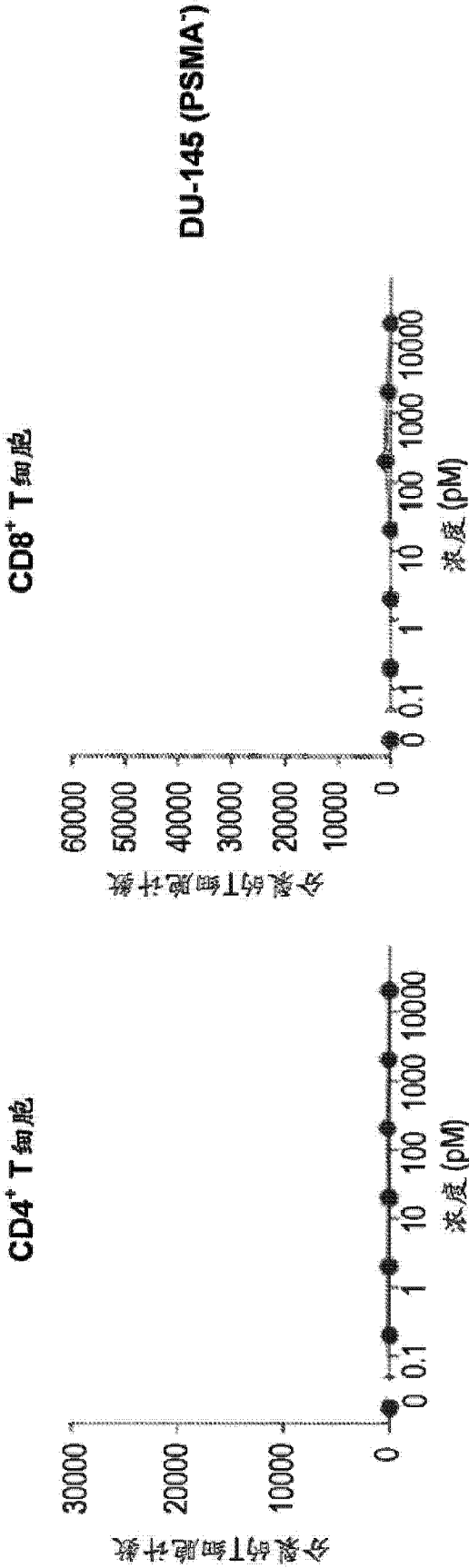


图 26B

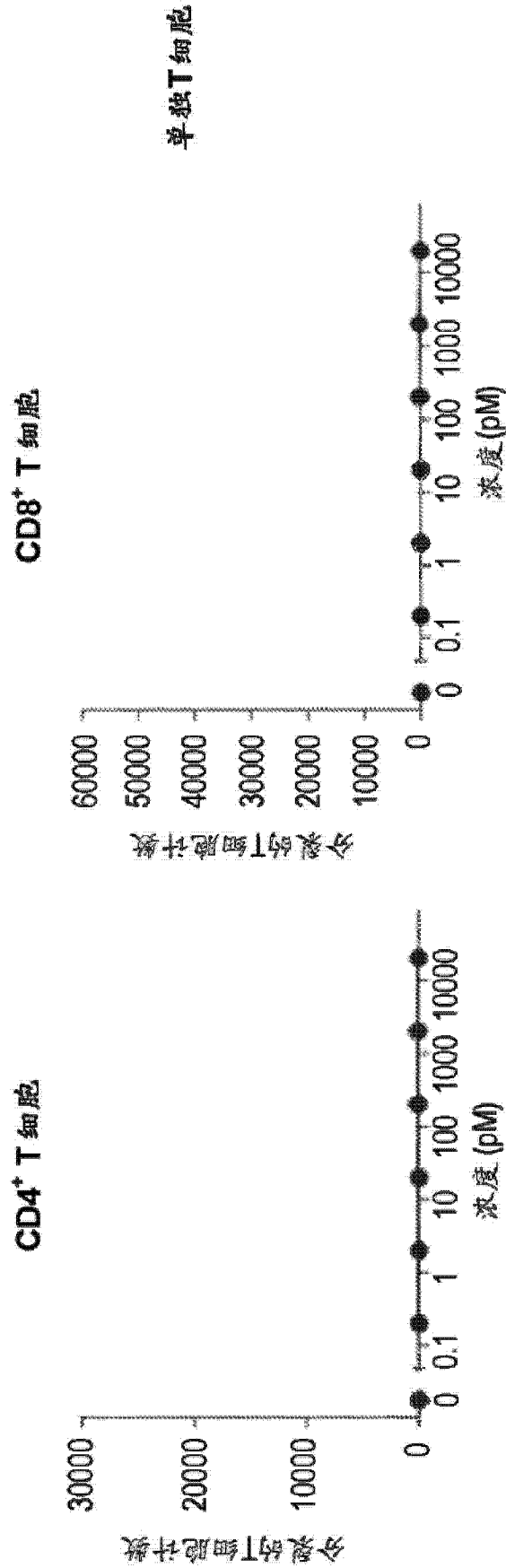


图 26C

对C4-2细胞进行RTCC
利用⁵¹Cr释放(4小时)来测量
T细胞对C4-2细胞的比率为5:1

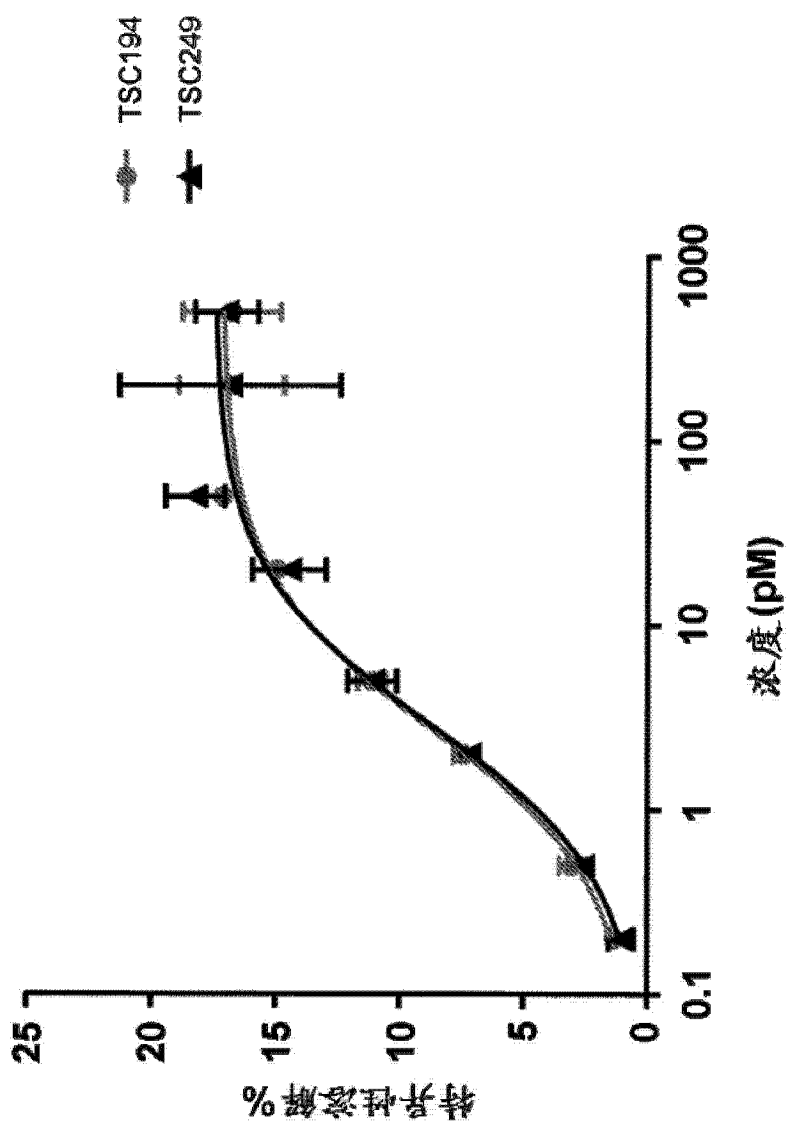


图 27

Abstract

The present invention relates to mono-specific and multi-specific polypeptides that specifically bind or interact with CD3. These polypeptides can be, but are not limited to, antibodies, fragments thereof, scFvs, Fabs, di-scFvs single domain antibodies, diabodies, dual variable domain binding proteins and polypeptides containing an antibody or antibody fragments. In one embodiment, a multi-specific polypeptide binds both a T-cell receptor complex on T-cells and a tumor antigen to induce target-dependent T-cell cytotoxicity, activation and proliferation.