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(56) Related Art  
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**Kepler, Thomas B., et al. "Immunoglobulin gene insertions and deletions in the affinity maturation of HIV-1 broadly reactive neutralizing antibodies." Cell host & microbe 16.3 (2014): 304-313.**



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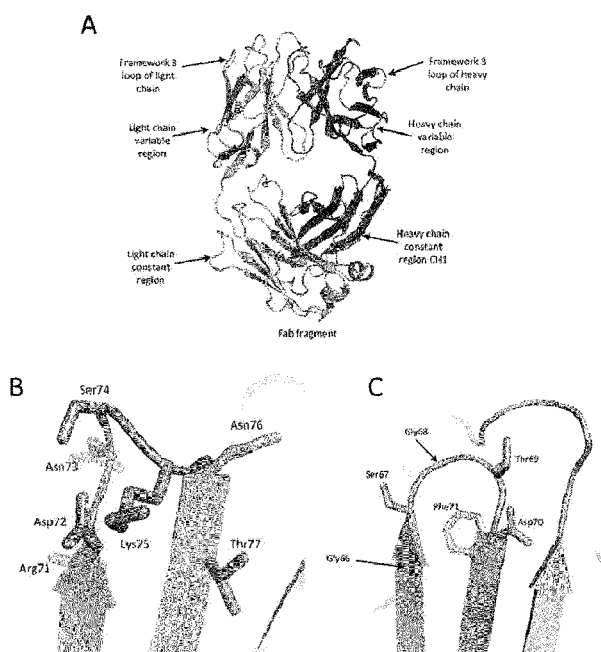
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(54) Title: ANTIBODIES COMPRISING A POLYPEPTIDE INSERTED IN FRAMEWORK 3 REGION

(57) Abstract: The present invention provides antibodies comprising a variable (V) domain and an insert polypeptide, wherein the insert polypeptide is within the framework 3 region of the V domain.

FIGURE 1



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## ANTIBODIES COMPRISING A POLYPEPTIDE INSERTED IN FRAMEWORK 3 REGION

### FIELD OF THE INVENTION

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The present invention relates to antibodies comprising a variable (V) domain and an insert polypeptide within the framework 3 region of the V domain. Such antibodies may provide improved functionality and/or pharmacokinetics for the insert polypeptide.

### 10 BACKGROUND OF THE INVENTION

The high specificity and affinity of antibodies makes them ideal diagnostic and therapeutic agents. Advances in the field of recombinant antibody technology have resulted in the production of antibody fragments, such as Fv, Fab, Fab' and F(ab')<sub>2</sub> fragments. These smaller  
15 molecules retain the antigen binding activity of whole antibodies and can also exhibit improved tissue penetration and pharmacokinetic properties in comparison to whole immunoglobulin molecules. Indeed, antibody fragments are proving to be versatile therapeutic agents. Whilst such fragments appear to exhibit a number of advantages over whole immunoglobulins, they also suffer from an increased rate of clearance from serum since they lack the Fc domain that  
20 imparts a long lifetime *in vivo* (Medasan *et al.*, 1997, J. Immunol. 158:2211-2217). Previous approaches to improving the serum half-life of antibody molecules include conjugation to agents or domains which interact with the FcRn receptor, such as human serum albumin (HSA). PEG has also been shown to improve serum half-life.

25 Antibodies with dual specificity, i.e. which bind to two different antigens have been previously described (for reviews, see Segal *et al.*, 1999, Curr. Opin. Immunol. 11:558-562; Pluckthun & Pack, 1997, Immunotechnology, 3:83-105; Fischer and Leger, 2007, Pathobiology, 74: 3-14). Previous approaches to making hetero-bispecific antibody-based molecules have generally employed chemical cross-linking or protein engineering techniques. Chemical cross-linking  
30 suffers from poor yields of hetero- and homo- dimer formation, and the requirement for their subsequent chromatographic separation. Protein engineering approaches have either been highly elaborate (e.g. knobs-into- holes engineering; Ridgway *et al.*, 1996, Protein Eng. 9(7):617-621) or have used molecules with inappropriate stability characteristics (e.g. diabodies, scFv). In some cases, bispecific antibodies can also suffer from steric hindrance

problems such that both antigens cannot bind simultaneously to each antibody arm. Single variable domain antibodies also known as single domain antibodies or sdAbs, correspond to the variable regions of either the heavy (VH) or light (VL) chain of an antibody. Murine single-domain antibodies were described by Ward *et al.*, 1989, Nature, 341, 544-546. Human and 'camelised' human single domain antibodies have also been described (Holt *et al.*, 2003, Trends in Biotechnology, 21, 484-490). Single domain antibodies have also been obtained from the camelids (camels and llamas) and cartilaginous fish (wobbegong and nurse sharks). These organisms have evolved high affinity single V-like domains (called VHH in camelids and V-NAR in sharks), mounted on an Fc-equivalent constant domain framework as an integral and crucial component of their immune system (see Holliger & Hudson, for a review; 2005, Nature Biotechnology, 23(9):1126-1136).

As such, there remains a need to provide further engineered antibodies such as fusion proteins comprising an antibody portion which exhibits one or more desired properties, such as increased functionality (*e.g.* multi-valent binding) or improved pharmacokinetics (*e.g.* increased half-life in serum). The present invention relates to a novel engineered antibody format which exhibit increased functionality (*e.g.* increased binding to its antigen, bi-specific binding, or multi-valent binding) or improved pharmacokinetics (*e.g.* increased half-life in serum). In particular, the present invention relates to a novel bispecific antibody format, in particular stable and capable of simultaneously binding two antigens.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art in Australia or any other country.

## SUMMARY OF THE INVENTION

In one aspect, the present invention provides an antibody comprising a variable (V) domain and an insert polypeptide, wherein the insert polypeptide is within the framework 3 region of the V domain, and wherein the insert polypeptide is heterologous to the antibody comprising a V domain and wherein both the antibody comprising a V domain and the insert polypeptide are functional and/or retain functionality when the insert polypeptide is present.

In some embodiments, the V domain is a VH domain, and the antibody may further comprise a VL domain.

In some embodiments, the V domain is a VL domain, and the antibody may further comprise a VH domain.

In some embodiments, the insert polypeptide is between amino acid residues 73 and 76 of the VH domain. For example, the insert polypeptide may be in the VH domain: (i) between amino acid residues 73 and 74; (ii) between amino acid residues 74 and 75; or (iii) between amino acid residues 75 and 76.

In some embodiments, one or more amino acid residues between 73 and 76 of the VH domain are replaced by the insert polypeptide.

In some embodiments, the insert polypeptide is between amino acid residues 67 and 70 of the VL domain. For example, the insert polypeptide may be in the VL domain: (i) between amino acid residues 67 and 68; (ii) between amino acid residues 68 and 69; or (iii) between amino acid residues 69 and 70.

In some embodiments, one or more amino acid residues between 67 and 70 of the VL domain are replaced by the insert polypeptide.

In some embodiments, the antibody of the invention further comprises a linker sequence, preferably of at least one, two, three, four, five, six, seven, eight, nine or ten amino acids joining the N- and/or C-terminal end of the insert polypeptide to the framework 3 region.

In some embodiments, the antibody of the invention is a full-length antibody or a binding fragment thereof. In some embodiments, the antibody is a full-length IgG or a binding fragment thereof. In some embodiments, the antibody is a Fab, Fab', F(ab')<sub>2</sub>, VHH, or scFv, in particular dsscFv.

In some embodiments, the antibody of the invention is humanised.

In some embodiments, the antibody of the invention comprises a V domain which binds to human serum albumin (HSA). In one embodiment, the antibody comprises a VL domain comprising CDR-L1 having the sequence SEQ ID NO: 3; CDR-L2 having the sequence SEQ ID NO: 4; CDR-L3 having the sequence SEQ ID NO: 5. In one embodiment, the antibody

comprises a VL domain comprising SEQ ID NO: 2. In one embodiment, the antibody comprises a light (L) chain comprising SEQ ID NO: 1. In one embodiment, the antibody comprises a VH domain comprising CDR-H1 having the sequence SEQ ID NO: 8; CDR-H2 having the sequence SEQ ID NO: 9; CDR-H3 having the sequence SEQ ID NO: 10. In one embodiment, the antibody comprises a VH domain comprising SEQ ID NO: 7. In one embodiment, the antibody comprises a heavy (H) chain comprising SEQ ID NO: 6.

In some embodiments, the insert polypeptide is a cytokine, preferably wherein the cytokine is selected from IL-10, IL-15, IL-2, G-CSF, GM-CSF and EPO. In other embodiments, the insert polypeptide is sclerostin. In other embodiments, the insert polypeptide is a second antibody, preferably wherein the second antibody is an scFv, in particular a dsscFv, or a domain antibody such as a VH or VL or VHH. The VHH may comprise a sequence selected from SEQ ID NOs: 67, 69 and 89. In such embodiments, the antibody may bind its cognate antigen by said V domain with a binding affinity which is at least 50%, 60%, 70%, 80% or 90% when compared to the antibody without the insert polypeptide present.

In some embodiments, the second antibody binds to the same antigen as the V domain. Preferably, the second antibody binds to a different epitope as the V domain. In such embodiments, the antibody may bind its cognate antigen with a binding affinity which is greater than when compared to the antibody without the second antibody present. In some embodiments, the second antibody binds to a different antigen to the first antibody, i-e the antibody comprising a V domain. In some embodiments, the second antibody binds to IL-17 and the first antibody, i-e the antibody comprising a V domain, binds to HSA. In some embodiments, the second antibody binds to CD3 and the first antibody, i-e the antibody comprising a V domain, binds to CD28. In some embodiments, the second antibody binds to CD28 and the first antibody, i-e the antibody comprising a V domain binds to CD3.

A second aspect provides at least one isolated DNA molecule encoding the antibody according to the first aspect.

A third aspect provides at least one cloning or expression vector encoding the antibody according to the first aspect.

A fourth aspect provides an isolated or non-human host cell comprising the at least one DNA molecule according to the second aspect or the at least one cloning or expression vector according to the third aspect.

Also disclosed is an isolated polynucleotide(s), such as a DNA molecule(s), and cloning or expression vector(s) which encodes an antibody disclosed herein. Also disclosed is a host cell comprising said polynucleotide(s) or vector(s).

A fifth aspect provides a process for producing the antibody of the first aspect, comprising culturing the host cell of the fourth aspect and isolating the antibody.

A sixth aspect provides an antibody when produced by the process according to the fifth aspect.

A seventh aspect provides a pharmaceutical composition comprising the antibody according to the first or sixth aspect in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

Also disclosed is a pharmaceutical composition comprising an antibody comprising a V domain and an insert polypeptide as disclosed herein in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier. Also provided is said antibody or said pharmaceutical composition for use in therapy.

An eighth aspect provides a method of therapy comprising administering a therapeutically effective amount of the antibody of the first or sixth aspect or the pharmaceutical composition of the seventh aspect to a subject in need of the therapy.

A ninth aspect provides use of the antibody of the first or sixth aspect in the manufacture of a medicament.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: (A) Typical Fab structure, highlighting framework 3 loop region; (B) highlighting certain residues in the heavy chain variable domain framework 3 region; (C) highlighting certain residues in the light chain variable domain framework 3 region.

Figure 2: (A) SPR sensorgram showing CA645/IL-15<sub>Fwk3</sub> binding to IL-15 receptor chains; (B) Modelled structure showing the IL-15 insert into framework 3 of the CA645 VH domain.

Figure 3: (A) SPR sensorgram showing CA645/IL-2<sub>Fwk3</sub> binding to IL-2 receptor  $\beta$  chain with and without added IL-15; (B) Modelled structure showing the IL-2 insert into framework 3 of the CA645 VH domain.

Figure 4: (A) SPR sensorgram showing CA645/G-CSF<sub>Fwk3</sub> binding to HSA and/or G-CSF receptor; (B) Modelled structure showing the G-CSF insert into framework 3 of the CA645 VH domain.

Figure 5: (A) SPR sensorgram showing CA645/GM-CSF<sub>Fwk3</sub> (human) binding to GM-CSF receptor, with comparison to a CA645/GM-CSF<sub>CHI</sub> (human) construct; (B) Modelled structure showing the GM-CSF insert into framework 3 of the CA645 VH domain.

Figure 6: SPR sensorgram showing CA645/GM-CSF<sub>Fwk3</sub> (murine) binding to GM-CSF receptor, with comparison to a CA645/GM-CSF<sub>CHI</sub> (murine) construct.

Figure 7: (A) SPR sensorgram showing CA645/EPO<sub>Fwk3</sub> binding to EPO receptor with and without added EPO; (B) Modelled structure showing the EPO insert into framework 3 of the CA645 VH domain.

Figure 8: (A) SPR sensorgram showing CA645/VHH6<sub>Fwk3</sub> binding to IL-6, HSA, IL-6:gp80 with or without HSA; (B) Modelled structure showing the VHH6 insert into framework 3 of the CA645 VH domain.

Figure 9: (A) SPR sensorgram showing CA645/VHH15<sub>Fwk3</sub> binding to HSA and/or IL-6; (B) Modelled structure showing the VHH15 insert into framework 3 of the CA645 VH domain.

Figure 10: (A) SPR sensorgram showing CA645/Sclerostin<sub>Fwk3</sub> (core or full-length) binding to LRP6 extracellular domain with various linker constructs; (B) Modelled structure showing the sclerostin core insert into framework 3 of the CA645 VH domain.

Figure 11: SPR sensorgram showing CA645/Sclerostin<sub>Fwk3</sub> (core or full-length) binding to LRP4 extracellular domain with various linker constructs.

Figure 12: (A) SPR sensorgram showing CA645/(IL-10<sub>Fwk3</sub>)<sub>2</sub> having different linker sequences binding to IL-10 receptor; (B) Modelled structure showing the IL-10 dimer insert into framework 3 of the CA645 VH domain.

Figure 13: SPR sensorgram showing CA497/IL-15<sub>Fwk3</sub> binding to IL-2 receptor  $\beta$  chain with and without added IL-15.

Figure 14: SPR sensorgram showing CA240/IL-15<sub>Fwk3</sub> binding to IL-2 receptor  $\beta$  chain with and without added IL-15.

Figure 15: SPR sensorgram showing dilution series for HyHEL5/VHH D3L11<sub>Fwk3</sub> binding to lysozyme.

Figure 16: SPR sensorgram showing dilution series for HyHEL5/VHH15<sub>Fwk3</sub> binding to lysozyme.

Figure 17: SPR sensorgram showing dilution series for CA645/VHH D3L11<sub>Fwk3</sub> binding to lysozyme.

Figure 18: Representation of potential binding schematic of HyHEL5/VHH D3L11<sub>Fwk3</sub> to lysozyme.

Figure 19: SPR sensorgram showing CA645/IL-15<sub>Fwk3</sub> having different linker sequences binding to IL-15 receptor chain.

Figure 20: DSC curves (solid line: CA645 Fab; dotted line: CA497 dsscFv; inverted triangle: CA645 Fab- Fwk3 CA497 dsscFv (vLvH); triangle: CA645 Fab- Fwk3 CA497 dsscFv (vHvL)).

Figure 21: Inhibition curves of IL-17A in absence (solid circle) or in presence (open circle) of HSA; (A) CA497 IgG4; (B) CA645 Fab- Fwk3 CA497 dsscFv (vHvL); (C) CA645 Fab- Fwk3 CA497 dsscFv (vLvH).

Figure 22: SPR analysis showing the binding of IL-17A and HSA to immobilized CA645 IgG4P comprising an anti-IL-17 CA497 dsscFv (vHvL) inserted into Fwk3 region.

Figure 23: DSC curves. (A) IgG4P anti-CD28 comprising a dsscFv anti-CD3 within the Fwk3 region (solid line); IgG4P anti-CD3 comprising a dsscFv anti-CD3 within the Fwk3 region (dotted line). (B) Fab anti-CD28 comprising a dsscFv anti-CD3 within the Fwk3 region (solid line); Fab anti-CD3 comprising a dsscFv anti-CD3 within the Fwk3 region (dotted line).

Figure 24: T cell activation assay in PBMC. Open triangle: anti-CD28 Fab Fwk3-anti-CD3 dsscFv; solid circle: anti-CD3 Fab; solid inverted triangle: anti-CD28 Fab; solid circle: anti-CD3 Fab Fwk3-anti-CD3 dsscFv; open square: anti-CD28 IgG4P Fwk3-anti-CD3 dsscFv; solid triangle: anti-CD3 IgG4P-Fwk3 anti-CD3 dsscFv; \* symbol: OKT3; open circle: Cells

only control. (A) CD4 T cell activation (patient 1) (B) CD4 T cell activation (patient 2) (C) CD8 T cell activation (patient 1) (D) CD8 T cell activation (patient 2).

## DETAILED DESCRIPTION

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### Antibody

The present invention provides an antibody comprising a variable (V) domain and an insert polypeptide, wherein the insert polypeptide is within the framework 3 (Fwk3) region of the V domain.

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The antibody of the present invention is typically constructed by incorporating the insert polypeptide into the Fwk3 region of a V domain of an antibody. The V domain may for example be a V<sub>H</sub> domain or a V<sub>L</sub> domain. The antibody into which the insert polypeptide is incorporated may be considered a scaffold antibody and/or where the insert polypeptide is also an antibody, the antibody into which the insert polypeptide is incorporated may alternatively be termed the first antibody while the insert polypeptide is termed the second antibody. The terms “scaffold antibody” and “first antibody” can be used interchangeably in the context of the present disclosure to refer to the antibody into which the insert polypeptide is incorporated.

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In some embodiments, the antibody of the invention is a full-length antibody or a binding fragment thereof. In some embodiments, the antibody is a full-length IgG or a binding fragment thereof. In some embodiments, the antibody is a full-length IgG1. In some embodiments, the antibody is a full-length IgG4. In some embodiments, the antibody is a full-length IgG4P, wherein the serine at position 241 (numbered according to the Kabat numbering system) has been changed to proline. In some embodiments, the antibody is a Fab, Fab', F(ab')<sub>2</sub>, VHH, or scFv. In some embodiments, the antibody is a disulphide stabilised scFv or “dsScFv”. “Disulphide-stabilised single chain variable fragment” or “dsScFv” as employed herein refer to a single chain variable fragment which is stabilised by a peptide linker between the V<sub>H</sub> and V<sub>L</sub> variable domain and also includes an inter-domain disulphide bond between V<sub>H</sub> and V<sub>L</sub>. (see for example, Weatherill et al., Protein Engineering, Design & Selection, 25 (321-329), 2012; WO2007109254. In some embodiments, the inter-domain disulphide bond between V<sub>H</sub> and V<sub>L</sub> is formed between positions V<sub>H</sub>44 and V<sub>L</sub>100 (numbering according to Kabat numbering system).

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The term ‘antibody’ as used herein generally relates to full length (intact, whole) antibodies, *i.e.* comprising elements of two heavy chains and two light chains in the case of an IgG antibody.

- 5 The terms “antibody construct”, “engineered antibody”, “antibody fusion protein” can be used interchangeably in the context of the present invention to refer to an antibody of the invention.

Binding fragments of antibodies generally comprise at least one variable light (VL) or variable heavy (VH) domain and include: single chain antibodies (*e.g.* a full length heavy chain or light chain), Fab, modified Fab, Fab’, modified Fab’, F(ab’)<sub>2</sub>, Fv, Fab-Fv, Fab-dsFv, single domain antibodies (sdAb, *e.g.* VH or VL or VHH), scFv, dsscFv, Bis-scFv, diabodies, tribodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody binding fragments are well known in the art (see for example Verma *et al.*, 1998, Journal of Immunological Methods, 216, 165-181). For example, antibody binding fragments may be obtained from any whole antibody, especially a whole monoclonal antibody, using any suitable enzymatic cleavage and/or digestion techniques, *e.g.* treatment with pepsin. Alternatively, the antibody starting material may be prepared by the use of recombinant DNA techniques involving the manipulation and re-expression of DNA encoding antibody variable and/or constant regions. Standard molecular biology techniques may be used to modify, add or delete amino acids or domains as desired. Any alterations to the variable or constant regions are still encompassed by the terms ‘variable’ and ‘constant’ regions as used herein. The antibody fragment starting material may be obtained from any species including, for example, mouse, rat, rabbit, hamster, camel, llama, goat or human. Parts of the antibody fragment may be obtained from more than one species; for example, the antibody fragments may be chimeric. In one example, the constant regions are from one species and the variable regions from another. The antibody fragment starting material may also be modified. In another example, the variable region of the antibody fragment has been created using recombinant DNA engineering techniques. Such engineered versions include those created for example from natural antibody variable regions by insertions, deletions or changes in or to the amino acid sequences of the natural antibodies. Particular examples of this type include those engineered variable region domains containing at least one CDR and, optionally, one or more framework amino acids from one antibody and the remainder of the variable region domain from a second

antibody. The Fab-Fv format was first disclosed in WO2009/040562, and the disulphide stabilised version thereof, the Fab-dsFv, was first disclosed in WO2010/035012. Other antibody fragments include the Fab and Fab' fragments described in International patent applications WO2005/003169, WO2005/003170 and WO2005/003171. Multi-valent  
5 antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO 92/22583 and WO05/113605). One such example of the latter is a Tri-Fab (or TFM) as described in WO92/22583. Another multi-specific format is described in WO2015/197772.

10 A typical Fab' molecule comprises a heavy and a light chain pair in which the heavy chain comprises a variable region VH, a constant domain CH1 and a natural or modified hinge region and the light chain comprises a variable region VL and a constant domain CL.

A binding domain of an antibody typically comprises 6 CDRs, three from a heavy chain and  
15 three from a light chain. In such an antibody, the CDRs are in a framework and together form a variable region. Thus, in one embodiment the antibody comprises a binding domain specific for an antigen comprising a light chain variable region and a heavy chain variable region. In other embodiments, for example wherein the antibody is a single domain antibody, the binding domain may only comprise the 3 CDRs from a single V domain of either a heavy chain or a  
20 light chain. Thus, in one embodiment the antibody comprises a binding domain specific for an antigen comprising one of a light chain variable region (VL domain) or a heavy chain variable region (VH or VHH domain). It will be appreciated that the V domain which binds the antigen may do so alone, or co-operatively with another V domain, for example as a complementary VH/VL pair. Accordingly, the antibody comprising a V domain may bind its antigen co-  
25 operatively with a complementary V (VH or VL) domain. In one embodiment, the V domain is a VH domain, and the antibody of the invention further comprises a VL domain, wherein the VH domain binds its antigen co-operatively with the VL domain as a VH/VL pair, i-e the antibody of the invention comprises a VH domain and binds its antigen co-operatively with a complementary VL domain. In one embodiment, the V domain is a VL domain, and the  
30 antibody of the invention further comprises a VH domain, wherein the VL binds its antigen co-operatively with the VH domain as a VH/VL pair, i-e the antibody of the invention comprises a VL domain and binds its antigen co-operatively with a complementary VH domain.

The binding domain of the antibody of the invention typically refers to the VH, VL, or pair of VH/VL which binds the antigen.

Antibodies contain generally one or more binding domains. For example, an unmodified Fab fragment comprises one binding domain formed by a VH/VL pair, and a whole IgG comprises two binding domains, each one being formed by a VH/VL pair, wherein the two binding domains are identical (monospecific antibody) or different (bispecific or bivalent antibody). In one example, the antibody of the invention may be a Fab fragment comprising a VH domain and an insert polypeptide within the Fwk3 region of the VH domain, and further comprising a VL domain which forms a complementary pair with the VH domain. In one example, the VL domain may be unmodified (i-e the antibody of the invention is a Fab fragment comprising a single insert polypeptide within the Fwk3 region of the VH domain) or may comprise an insert polypeptide in its Fwk3 region (i-e the antibody of the invention is a Fab fragment comprising two insert polypeptides, identical, similar or different, one inserted within the Fwk3 region of the VH domain and the other one inserted within the Fwk3 region of the VL domain). In one example, the antibody of the invention may be a Fab fragment comprising a VL domain and an insert polypeptide within the Fwk3 region of the VL domain, and further comprising a VH domain which forms a complementary pair with the VL domain. In one example, the VH domain may be unmodified (i-e the antibody of the invention is a Fab fragment comprising a single insert polypeptide within the Fwk3 region of the VL domain) or may comprise an insert polypeptide in its Fwk3 region (i-e the antibody of the invention is a Fab fragment comprising two insert polypeptides, identical, similar or different, one inserted within the Fwk3 region of the VL domain and the other one inserted within the Fwk3 region of the VH domain). In another example, the antibody of the invention is a full IgG comprising a VH domain and an insert polypeptide within the Fwk3 region of the VH domain, and further comprising a VL domain which forms a complementary pair with the VH domain. In one example, the VL domain may be unmodified (i-e the antibody of the invention is an IgG comprising two insert polypeptides, each one being inserted into one of the two VH domains of the IgG) or may comprise an insert polypeptide in its Fwk3 region (i-e the antibody of the invention is an IgG comprising four insert polypeptides, identical, similar or different, each of them being inserted into the Fwk3 region of each one of its two VH and two VL domains). In another example, the antibody of the invention is a full IgG comprising a VL domain and an insert polypeptide within the Fwk3 region of the VL domain, and further comprising a VH domain which forms a complementary pair with the VL. The VH domain may be unmodified (i-e the antibody of the invention is an IgG comprising two insert polypeptides, each one being inserted into one of the two VL domains of the IgG) or may comprise an insert polypeptide in its Fwk3 region (i-e the antibody of the invention is an IgG comprising four insert polypeptides, identical, similar or different,

each of them being inserted into the Fwk3 region of each one of its two VL and two VH domains).

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA. This numbering system is used in the present specification except where otherwise indicated.

The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a “standard” Kabat numbered sequence by methods known in the art.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system. In addition to the CDR loops, a fourth loop exists between CDR-2 and CDR-3 which is formed by framework 3 (Fwk3). Figure 1A provides a representation of the framework 3 loop in the context of a Fab having a light and a heavy chain. Figure 1B and 1C also shows representative residues from these loops. The Kabat numbering system defines framework 3 as positions 66-94 in a heavy chain and positions 57-88 in a light chain.

Thus, in one embodiment, an antibody of the present invention comprises a variable (V) domain and an insert polypeptide, wherein the insert polypeptide is within positions 66-94 when the V domain is a VH domain, or positions 57-88 when the V domain is a VL domain, in accordance with the Kabat numbering system.

In some embodiments, the insert polypeptide is between amino acid residues 73 and 76 of the VH domain, in accordance with the Kabat numbering system. Preferably, the insert polypeptide may be between amino acid residues 73 and 74, 74 and 75, or 75 and 76 of the VH

domain, in accordance with the Kabat numbering system. In some embodiments, the insert polypeptide is between amino acid residues 76 and 77, or 77 and 78 of the VH domain, in accordance with the Kabat numbering system. In some embodiments, one or more amino acid residues of the VH domain are replaced by the insert polypeptide.

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In some embodiments, the insert polypeptide is between amino acid residues 67 and 70 of the VL domain, in accordance with the Kabat numbering system. Preferably, the insert polypeptide may be between amino acid residues 67 and 68, 68 and 69, or 69 and 70 of the VL domain, in accordance with the Kabat numbering system. In some embodiments, one or more amino acid residues of the VL domain are replaced by the insert polypeptide.

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The antibody of the present invention may further comprise constant region domains. The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when antibody effector functions are not required. It will be appreciated that sequence variants of these constant region domains may also be used. For example, IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal *et al.* Molecular Immunology, 1993, 30 (1), 105-108 may be used. It will also be understood by one skilled in the art that antibodies may undergo a variety of post-translational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. Journal of Chromatography 705:129-134, 1995).

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Antibodies for use in the present invention may be fully human antibodies, humanised antibodies, or chimeric antibodies.

In one embodiment, the antibody of the present invention is not a murine antibody. The term "murine antibody" refers to an antibody having heavy and light chains derived exclusively from murine B cells. This antibody is thus constituted of murine amino acid sequences, whatever the origin of the cell which enables the production thereof.

5 In one embodiment, the antibody of the present invention is a chimeric antibody. The term "chimeric antibody " refers to an antibody or antigen-binding fragment thereof wherein each light chain and/or each heavy chain sequence of which it is constituted comprises or consists of a hybrid sequence derived from at least two different animals, notably mammals, in particular, an antibody that combines the rabbit, murine, rat, or cynomolgus variable region  
10 with the human constant region.

In one embodiment, the antibody of the present invention is a humanised antibody. Humanised antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule (see, for example, US 5,585,089). In  
15 particular, the term "humanised " refers to an antibody or antigen-binding fragment thereof wherein the heavy and/or light chain contains one or more CDRs from a non-human antibody (such as a rabbit, murine, rat, cynomolgus or llama monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody such as a human antibody. Rather than the entire CDR being transferred, only one or more of the specificity determining  
20 residues from any one of the CDRs described herein above can be transferred to the human antibody framework (see for example, Kashmiri et al., 2005, Methods, 36, 25-34). Selected framework residues of the humanised antibody can be substituted with corresponding residues (so-called donor residues) from a non-human antibody (e.g. the antibody from which the CDRs or the specificity determining residues are derived) e.g. to restore or improve the antibody  
25 specificity or affinity.

Fully human antibodies are those antibodies in which the variable region(s) and the constant region(s) (where present) of both the heavy and/or the light chains are all of human origin, or substantially identical to sequences of human origin, not necessarily from the same antibody.  
30 Examples of fully human antibodies may include antibodies produced for example by phage display methods and antibodies produced by mice in which the murine immunoglobulin variable and/or constant region genes have been replaced by their human counterparts, e.g. as

described in general terms in EP0546073 B1, US 5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,661,016, US 5,770,429, EP 0438474 B1 and EP0463151 B1.

The antibodies comprising a V domain and an insert polypeptide as disclosed herein may be further modified, for example chemically conjugated to further compounds which may affect e.g. its pharmacokinetic properties or antigen binding.

In one example the antibodies of the present invention are attached to poly(ethyleneglycol) (PEG) moieties. Antibodies linked to PEG may display further increased serum half-life, for example. In one particular example the antibody is an antibody fragment and the PEG molecules may be attached through any available amino acid side-chain or terminal amino acid functional group located in the antibody fragment, for example any free amino, imino, thiol, hydroxyl or carboxyl group. Such amino acids may occur naturally in the antibody fragment or may be engineered into the fragment using recombinant DNA methods (see for example US 5,219,996; US 5,667,425; WO98/25971, WO2008/038024). In one example, the antibody of the present invention is a modified Fab fragment wherein the modification is the addition to the C-terminal end of its heavy chain of one or more amino acids to allow the attachment of an effector molecule. Suitably, the additional amino acids form a modified hinge region containing one or more cysteine residues to which the effector molecule may be attached. Multiple sites can be used to attach two or more PEG molecules.

Suitably PEG molecules may be covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the antibody fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond. Where a thiol group is used as the point of attachment, appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an  $\alpha$ -halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures.

Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

Preferably, the antibody of the present invention has a binding affinity (as measured by its dissociation constant  $K_D$ ) for its cognate antigen of  $10^{-5}$  M or less,  $10^{-6}$  M or less,  $10^{-7}$  M or less,  $10^{-8}$  M or less,  $10^{-9}$  M or less,  $10^{-10}$  M or less, or  $10^{-11}$  M or less. Affinity can be measured by known techniques such as surface plasmon resonance techniques including Biacore™. Affinity may be measured at room temperature, 25°C or 37°C. Affinity may be measured at physiological pH, i.e. at about pH 7.4.

It will be appreciated that the affinity of antibodies provided by the present invention may be altered using any suitable method known in the art. The present invention therefore also relates to variants of the antibody molecules of the present invention, which have an improved affinity for albumin. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al.*, J. Mol. Biol, 254. 392-403, 1995), chain shuffling (Marks *et al.*, Bio/Technology, 10, 779-783, 1992), use of mutator strains of *E. coli* (Low *et al.*, J. Mol. Biol., 260. 359-368, 1996), DNA shuffling (Patten *et al.*, Curr. Opin. Biotechnol, 8, 724-733, 1997), phage display (Thompson *et al.*, J. Mol. Biol, 256. 77-88, 1996) and sexual PCR (Cramer *et al.*, Nature, 391, 288-291, 1998).

## **Human Serum Albumin-binding antibody**

In one embodiment, the antibody of the invention comprises a V domain which binds to human serum albumin (HSA). In such an embodiment, the antibody may be useful in extending the serum half-life of the insert polypeptide. It will be appreciated that the V domain which binds albumin may do so alone, or co-operatively with another V domain, for example as a complementary VH/VL pair. Accordingly, in some embodiments, the antibody comprising a V domain binds albumin co-operatively with a complementary VH or VL domain. In one embodiment, the V domain is a VH domain, and the antibody of the invention further comprises a VL domain, wherein the VH binds albumin co-operatively with the VL domain as a VH/VL pair, i.e. the antibody of the invention comprises a VH domain and binds to albumin co-operatively with a complementary VL. In one embodiment, the V domain is a VL domain, and the antibody of the invention further comprises a VH domain, wherein the VL binds albumin

co-operatively with the VH domain as a VH/VL pair, i.e the antibody of the invention comprises a VL domain and binds to albumin co-operatively with a complementary VH. Where the antibody of the invention binds albumin, the VH/VL is typically termed “albumin binding domain”. As such, the albumin binding domain of the antibody of the invention refers to the  
5 VH, VL, or pair of VH/VL which binds to albumin.

In one embodiment, the antibody of the present invention binds cynomolgus serum albumin, murine serum albumin and/or rat serum albumin.

10 In one embodiment, the antibody of the present invention comprising a V domain which binds to human serum albumin is humanised. In one embodiment, the antibody of the present invention comprising an albumin binding domain is a humanised antibody wherein the heavy and/or light chain contains one or more CDRs from a rabbit, murine, or rat monoclonal antibody grafted into a heavy and/or light chain variable region framework of a human  
15 antibody. In one embodiment, the antibody of the present invention comprising an albumin binding domain is a humanised antibody wherein the heavy and/or light chain contains one or more CDRs from a rabbit antibody grafted into a heavy and/or light chain variable region framework of a human antibody, and wherein the heavy and/or light chain variable region framework comprises at least one amino acid substitution, such as an amino acid which is a  
20 donor residue.

In one embodiment, the antibody of the present invention, comprising a V domain which binds to human serum albumin, comprises a light and/or heavy chain sequence as shown below. (Variable domain underlined; CDRs in ***bold and italics***.) In one embodiment, the antibody of  
25 the present invention, comprising a V domain which binds to human serum albumin, comprises a light and/or heavy chain sequence selected from below. In one embodiment, the antibody of the present invention, comprising a V domain which binds to human serum albumin, comprises a light and/or heavy chain variable domain sequence selected from below. In one embodiment, the antibody of the present invention, comprising a V domain which binds to human serum  
30 albumin, comprises at least one of CDR-L1, CDR-L2, and CDR-L3 sequence and/or at least one of CDR-H1, CDR-H2, and CDR-H3 sequence selected from below. In one embodiment, the antibody of the present invention, comprising a V domain which binds to human serum albumin, comprises a light and/or heavy chain sequence; and/or a light and/or heavy chain

variable domain sequence; and/or at least one of CDR-L1, CDR-L2, and CDR-L3 sequence; and/or at least one of CDR-H1, CDR-H2, and CDR-H3 sequence selected from below.

CA645 Light Chain (gL5)

5

DIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSG  
VPSRFGSGSGTDFTLTISSLQPEDFATYYCGGGYSSISDTTFGGGKVEIKRTVAAPS  
 VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS  
 TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 1)

10

VL domain (gL5):

DIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTS  
 GVPSRFGSGSGTDFTLTISSLQPEDFATYYCGGGYSSISDTTFGGGKVEIK (SEQ ID  
 NO: 2)

15

CDR-L1: QSSPSVWSNFLS (SEQ ID NO: 3)

CDR-L2: EASKLTS (SEQ ID NO: 4)

CDR-L3: GGGYSSISDTT (SEQ ID NO: 5)

20 CA645 Heavy Chain (gH5)

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQ  
GTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG  
 VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ  
 ID NO: 6)

VH domain (gH5):

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGT  
 30 TFYATWAKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDL  
 WGQGTLVTVSS (SEQ ID NO: 7)

CDR-H1: GIDLSNYAIN (SEQ ID NO: 8)

CDR-H2: IIWASGTTFYATWAKG (SEQ ID NO: 9)

CDR-H3: TVPGYSTAPYFDL (SEQ ID NO: 10)

In some embodiments, the antibody of the present invention comprises variants of the VL and VH domains which bind human serum albumin as described above (SEQ ID NO: 2 and 7 respectively) that comprise an additional cysteine residue such that a disulphide bond may be formed between the VL and VH domains. The additional cysteine-containing variants may have the following sequences (wherein the additional cysteine residues are underlined):

CA645-Cys VL domain (gL5):

10 DIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSG  
VPSRFSGSGSGTDFTLTISLQPEDFATYYCGGGYSSISDTTFGCGTKVEIK (SEQ ID  
NO: 102)

CA645-Cys VH domain (gH5):

15 EVQLLES GGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKCLEWIGIIWASGTTF  
YATWAKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQ  
GTLVTVSS (SEQ ID NO: 103)

Additional examples of VL and VH domains which bind human serum albumin, and which  
20 may be used in the antibody of the invention comprise SEQ ID NO: 105, 106, 107, 108 and  
109 as described below (CDRs in ***bold and italics***).

CA645 VH domain (gH1):

25 EVQLLES GGGLVQPGGSLRLSCAVS***GIDLSNYAINWVRQAPGKGLEWIGIIWASGTT***  
***FYATWAKGRFTISR***DSTTVYLQMNSLRAEDTAVYYCART***VPGYSTAPYFDL***WGQGT  
LTVSS (SEQ ID NO: 105)

CA645 VH domain (gH37):

30 EVQLLES GGGLVQPGGSLRLSCAVS***GIDLSNYAINWVRQAPGKGLEWIGIIWASGTTA***  
***YATWAKGRFTI***SRDN SKNTV YLQMNSLRAEDTAVYYCART***VPGYSTAPYFDL***WGQ  
GTLVTVSS (SEQ ID NO: 106)

CA645 VH domain (gH47):

EVQLLESGGGLVQPGGSLRLSCAVS**GIDLSNYAINWVRQAPGKGLEWIGIIWASGTT**  
**FYATWAKGRFTISRDNSKNTVYLQMNSLRAEDAVYYCARTVPGYSAAPYFDLWGQ**  
 GTLVTVSS (SEQ ID NO: 107)

5 CA645 VL domain (gL1):

DIVMTQSPSSVSASVGDRVTITC**QSSPSVWSNFL**SWYQQKPGKAPKLLIYE**ASKLTS**  
 GVPSRFKSGSGTDFTLTISSLQPEDFATYYC**GGGYSSISD**TTFGGGTKVEIK (SEQ ID  
 NO: 108)

10 CA645 VL domain (gL4):

DIQMTQSPSSVSASVGDRVTITC**QSSPSVWSNFL**SWYQQKPGKAPKLLIYE**ASKLTS**  
 GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC **GGGYSSISD** TTFGGG**TKVEIK**  
 (SEQ ID NO: 109)

15 In some embodiments, the antibody of the present invention comprises variants of the VL and  
 VH domains which bind human serum albumin as described above (SEQ ID NO: 105 to SEQ  
 ID NO: 109) that comprise an additional cysteine residue such that a disulphide bond may be  
 formed between the VL and VH domains. The additional cysteine-containing variants may  
 have the following sequence (wherein the additional cysteine residue is underlined):

20

CA645-Cys VL (gL4):

DIQMTQSPSSVSASVGDRVTITC**QSSPSVWSNFL**SWYQQKPGKAPKLLIYE**ASKLTS**G  
 VPSRFSGSGSGTDFTLTISSLQPEDFATYYC**GGGYSSISD**TTFGCGTKVEIKRT(SEQ ID  
 NO: 110)

25 In some embodiments, the VH framework of the albumin binding domain is human (for  
 example VH3, such as VH3 1-3 3-23), and comprises for example 1, 2, 3, 4, 5 or 6 amino acid  
 substitutions, such as amino acids which are donor residues. In such embodiments, the VH may  
 have a sequence shown in SEQ ID NO: 7, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO:  
 106, SEQ ID NO: 107 or a variant of any one of the same with at least 95, 96, 97, 98 or 99%  
 30 similarity of identity.

In some embodiments, the VL framework of the albumin binding domain is human (for example V $\kappa$ 1, such as 2-1- (1) L5), and comprises for example 1, 2, 3, 4, 5 or 6 amino acid substitutions, such as amino acids which are donor residues. In such embodiments, the VL may have a sequence shown in SEQ ID NO: 2, SEQ ID NO: 102, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, or a variant of any one of the same with at least 95, 96, 97, 98 or 99% similarity of identity.

In some embodiments, the albumin binding domain comprises VH and VL sequences selected from the combinations SEQ ID NO: 7&2, 7&102, 7&108, 7&109, 7&110, 103&2, 103&102, 103&108, 103&109, 103&110, 105&2, 105&102, 105&108, 105&109, 105&110, 106&2, 106&102, 106&108, 106&109, 106&110, 107&2, 107&102, 107&108, 107&109, 107&110 or a variant or variants of any of the same with at least 95, 96, 97, 98 or 99% similarity or identity.

In some embodiments, the VL and VH sequences of the albumin binding domain are SEQ ID NO:102 and SEQ ID NO: 103, respectively. In some embodiments, the VL and VH sequences of the albumin binding domain are SEQ ID NO:2 and SEQ ID NO: 7, respectively.

In one embodiment, the antibody comprising a V domain which binds human serum albumin is a Fab or scFv. The VL and VH domains of SEQ ID NOs: 2 and 7 are preferred for the Fab format. In one embodiment, the antibody comprising a V domain which binds human serum albumin is a scFv which binds albumin cooperatively with a complementary V (VL or VH) domain. In such embodiment, the scFv may comprise any one of the VH domains and any one of the VL domains as described above, linked in the VH-VL or VL-VH orientation by a (G4S)<sub>4</sub> linker (SEQ ID NO:16). The VL and VH domains of SEQ ID NOs: 2 and 7 are preferred for the scFv format. The VL domain of SEQ ID NO: 2 may comprise the additional two amino acids RT at the C-terminus. In one embodiment, the antibody comprising a V domain which binds human serum albumin is a scFv which comprises the VL and VH domains of SEQ ID NOs: 2 and 7 separated by a (G4S)<sub>4</sub> linker. In one example, the scFv is VH-VL oriented. In another example, the scFv is VL-VH oriented. In one embodiment, the antibody comprising a V domain which binds human serum albumin is a scFv which comprises or has the SEQ ID NO: 143 as shown below. In one particular embodiment, the antibody comprising a V domain which binds human serum albumin is a dsScFv. In one embodiment, the antibody comprising a V domain which binds human serum albumin is a dsScFv which binds albumin cooperatively with a complementary V (VL or VH) domain. In such embodiment, the dsScFv may comprise

any one of the VH domains and any one of the VL domains as described above, comprising, if not already present, an additional cysteine residue such that a disulphide bond may be formed between the VL and VH domains, wherein the VL and VH domains are linked in the VH-VL or VL-VH orientation by a (G4S)<sub>4</sub> linker. The VL and VH domains of SEQ ID NOs: 102 and 103 are preferred for the dsscFv format. The VL domain of SEQ ID NO: 102 may comprise the additional two amino acids RT at the C-terminus. In one embodiment, the antibody comprising a V domain which binds human serum albumin is a dsscFv which comprises the VL and VH domains of SEQ ID NOs: 102 and 103 separated by a (G4S)<sub>4</sub> linker (SEQ ID NO:16). In one example, the dsscFv is VH-VL oriented. In another example, the dsscFv is VL-VH oriented. In one embodiment, the antibody comprising a V domain which binds human serum albumin is a dsscFv which comprises or has the SEQ ID NO: 144 as shown below.

*CA645 scFv (VH-VL):*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWG  
QGTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVITITCQSSPS  
VWSNFLSWYQQKPGKAPKLLIYEASKLTSGVPSRFSGSGSGTDFTLTISSLQPEDFAT  
YYCGGGYSSISDTTFGQGTKVEIK (SEQ ID NO: 143)

*CA645 dsscFv (VH-VL) wherein the additional cysteine residues are underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKCLEWIGIIWASGTTF  
YATWAKGRFTISRDNSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQ  
GTLVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVITITCQSSPSV  
WSNFLSWYQQKPGKAPKLLIYEASKLTSGVPSRFSGSGSGTDFTLTISSLQPEDFATY  
YCGGGYSSISDTTFGCGTKVEIK (SEQ ID NO: 144)

In another embodiment, the antibody comprising a V domain which binds human serum albumin is a full-length IgG. The VL and VH domains of SEQ ID NOs: 2 and 7 are preferred for the IgG format. In one embodiment, the antibody comprising a V domain which binds human serum albumin is an IgG1. In one embodiment, the antibody comprising a V domain which binds human serum albumin is an IgG4. In one embodiment, the antibody comprising a V domain which binds human serum albumin is an IgG4P.

It will be appreciated that one or more (for example 1, 2, 3 or 4) amino acid substitutions, additions and/or deletions may be made to the CDRs or other sequences (e.g. variable domains)

provided by the present invention without significantly altering the ability of the antibody to bind to its cognate antigen (e.g. HSA). The effect of any amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art. For example, one or more (for example 1, 2, 3 or 4) amino acid substitutions, additions and/or deletions may be made to the framework region employed in the antibody or fragment provided by the present invention, wherein binding affinity to the cognate antigen is retained or increased. In some embodiments, the amino acid at position 72 of the framework 3 of the VH domain is wild type amino acid, naturally present at position 72 of said VH domain.

As such, the present invention also includes antibodies which comprise sequences which are at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% similar or identical to a sequence given herein. "Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains).

Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. *et al.*, 1990, J. Mol. Biol. 215:403-410; Gish, W. & States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.*, 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.*, 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656).

The antibody of the present invention may bind to albumin with a binding affinity sufficient to extend the half-life of the insert polypeptide, such as a therapeutic protein or a second antibody, *in vivo*. In one embodiment, the insert polypeptide binds to HSA. In such embodiment, the insert polypeptide may bind to albumin with a binding affinity sufficient to extend the half-life of the scaffold antibody. It has been reported that a  $K_D$  for albumin of less than or equal to 2.5  $\mu$ M will extend half-life *in vivo* (Nguyen, A. *et al* (2006) Protein Engineering, Design & Selection, 19(7), 291-297). In one example the antibody of the present invention may bind to albumin with a high binding affinity which is nanomolar or micromolar, for example a  $K_D$  equal to or less than 3 nM. Affinity may be measured using any suitable method known in the art, including surface plasmon resonance using natural or recombinant serum albumin.

In some embodiments, the insert polypeptide is an albumin binding peptide. Examples of albumin binding peptides are provided in WO2007/106120 and include:

| SEQ ID NO: | SEQUENCE                |
|------------|-------------------------|
| 111        | DLCLRDWGCLW             |
| 112        | DICLPRWGCLW             |
| 113        | MEDICLPRWGCLWGD         |
| 114        | QRLMEDICLPRWGCLWEDDE    |
| 115        | QGLIGDICLPRWGCLWGRSV    |
| 116        | QGLIGDICLPRWGCLWGRSVK   |
| 117        | EDICLPRWGCLWEDD         |
| 118        | RLMEDICLPRWGCLWEDD      |
| 119        | MEDICLPRWGCLWEDD        |
| 120        | MEDICLPRWGCLWED         |
| 121        | RLMEDICLARWGCLWEDD      |
| 122        | EVRSFCTRWP AEK SCK PLRG |
| 123        | RAPESFVCYWETICFERSEQ    |
| 124        | EMCYFPGICWM             |

In one embodiment, the insert polypeptide is a second antibody which binds to HSA. In such embodiment, the insert polypeptide may have the features described above for the antibody comprising a V domain, i.e the scaffold antibody. In some embodiments, the insert polypeptide may comprise at least one of the CDRs, at least one of the VL or VH domains, or at least one of the light or heavy chains which bind albumin, as described above. In one embodiment, the insert polypeptide comprises at least one of the CDR-L1, CDR-L2 and CDR-L3 of sequence

SEQ ID NO: 3, SEQ ID NO: 4 and SEQ ID NO: 5 respectively and/or one of the CDR-H1, CDR-H2 and CDR-H3 of sequence SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10 respectively.

## 5 **Insert polypeptide**

In accordance with the present invention, the present disclosure is directed to antibodies comprising an insert polypeptide within a framework 3 region in a V domain. The V domain may be a VL or a VH domain. In one example, the antibody of the invention may comprise an insert polypeptide within a framework 3 region of both VL and VH, i.e the antibody of the invention may comprise two insert polypeptides, wherein one insert polypeptide is inserted into the framework 3 region of a VL and one insert polypeptide is inserted into the framework 3 region of a VH, and wherein the insert polypeptides are identical, similar or different. In one embodiment, the insert polypeptide is heterologous to the antibody. As used herein, “heterologous” generally means derived from a genotypically distinct entity from the rest of the entity to which it is being compared. In this context, it means that the inserted polypeptide would not normally be present in the native antibody sequence. In one embodiment, the insert polypeptide is a full-length protein. In one embodiment, the insert polypeptide is a fragment of a full-length protein. In one embodiment, the insert polypeptide is a functional fragment of a full-length protein. In one embodiment, the insert polypeptide is functional when expressed on its own. In one embodiment, the insert polypeptide is functional and/or retains functionality when present in the framework 3 region in a V domain of the antibody of the invention. Thus, in one embodiment, the present invention provides an antibody comprising a variable (V) domain and an insert polypeptide, wherein the insert polypeptide is within the framework 3 (Fwk3) region of the V domain and wherein the insert polypeptide is functional and/or retains functionality when present in the framework 3 region in the V domain of the antibody of the invention.

In one embodiment, the insert polypeptide comprises at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, or 200 amino acids. In one embodiment, the insert polypeptide comprises less than 250, 300 or 350 amino acids. In one embodiment, the insert polypeptide comprises less than 250 amino acids. In one embodiment, the insert polypeptide comprises at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 150, or 200 amino acids and less than 250 amino acids. In one embodiment, the insert polypeptide

comprises between 50 and 250, preferably 100 and 250, more preferably between 150 and 250 amino acids.

In one embodiment, the insert polypeptide may be a therapeutic or diagnostic polypeptide.

5 Suitable therapeutic polypeptides may include, for example, receptor agonists or antagonists, enzyme inhibitors, metal chelators, anti-viral agents, anti-fungal agents, cardiovascular drugs and chemotherapeutic drugs. In some embodiments, the insert polypeptide is a cytokine. The cytokine may be selected from, for example, IL-10, IL-15, IL-2, G-CSF, GM-CSF, EPO. In other embodiments, the insert polypeptide is sclerostin. In some embodiments, the insert  
10 polypeptide comprises more than one polypeptide sequence such as two copies of the same polypeptide, optionally linked. Advantageously in such embodiment, the insert polypeptide may form a multimer, in particular a functional multimer. In some embodiments the insert polypeptide comprises two, three or four polypeptide sequences and is able to form a dimer, trimer or tetramer of the polypeptide. In some embodiments. the insert polypeptide forms a  
15 homodimer, a homotrimer or a homotetramer. In some embodiments, the insert polypeptide comprises at least two cytokines. In one embodiment, the insert polypeptide comprises two human IL-10 sequences, optionally separated by a peptide linker, e.g. (G4S)<sub>3</sub> (SEQ ID NO: 15), i-e two human IL-10 sequences are inserted into the Fwk3 region of a V domain of the antibody of the invention. In such embodiment, the two human IL-10 sequences form an  
20 homodimer of IL-10 incorporated into the antibody of the invention.

In some embodiments, the insert polypeptide is a chemokine. The chemokine may be selected from, for example, CCLs (C-C motif chemokine ligands), CXCLs (CXC motif chemokine ligands). In some embodiments, the insert polypeptide is a hormone. The hormone may be selected from, for example, insulin or leptin. In some embodiments, the insert polypeptide is a  
25 growth factor.

It has been surprisingly found by the present inventors that insertion of a polypeptide into an antibody in the framework 3 loop region of a V domain does not significantly hinder the functionality of either the antibody or the insert polypeptide. In particular, the examples herein  
30 show that specific and cognate binding to the respective binding partners of both the antibody and the insert polypeptide is seen for several tested fusion protein constructs.

In some embodiments, the antibody of the invention binds its cognate antigen by said V domain with a binding affinity which is at least 50%, 60%, 70%, 80% or 90% when compared to the

antibody without the insert polypeptide present. It will be appreciated that the V domain which binds the antigen may do so alone, or co-operatively with another V domain depending on the antibody format, for example as a complementary VH/VL pair. Accordingly, the antibody comprising a V domain may bind its antigen co-operatively with a complementary V (VH or VL) domain. In some embodiments, the antibody of the invention binds its cognate antigen with a binding affinity which is at least 50%, 60%, 70%, 80% or 90% when compared to the antibody without the insert polypeptide present. In some embodiments, the antibody of the invention comprises a VH domain and binds its antigen co-operatively with a complementary VL with a binding affinity which is at least 50%, 60%, 70%, 80% or 90% when compared to the antibody without the insert polypeptide present. In some embodiments, the antibody of the invention comprises a VL domain and binds its antigen co-operatively with a complementary VH with a binding affinity which is at least 50%, 60%, 70%, 80% or 90% when compared to the antibody without the insert polypeptide present. In some embodiments, the insert polypeptide part of the antibody binds its cognate binding partner with a binding affinity which is at least 50%, 60%, 70%, 80% or 90% when compared to the free polypeptide when not linked to the antibody.

Therefore, in one embodiment, the antibody of the invention comprises a variable (V) domain and an insert polypeptide, wherein the insert polypeptide is within the framework 3 (Fwk3) region of the V domain and wherein both the antibody comprising a V domain and the insert polypeptide are functional and/or retain functionality when the insert polypeptide is present. In such embodiments, the antibody of the invention has functionality of both the scaffold antibody and the insert polypeptide. Advantageously, the antibody of the invention may exercise simultaneously the functionality of both the scaffold antibody and the insert polypeptide.

The antibody comprising a variable (V) domain is considered functional and/or retaining functionality when the insert polypeptide is present in the framework 3 region of the V domain, where the antibody of the invention exhibits at least 50%, 60%, 70%, 80% or 90% of at least one of the functions of the antibody comprising a variable (V) domain without the insert polypeptide present. The insert polypeptide is considered functional and/or retaining functionality when it is present in the framework 3 region of the V domain, where the insert polypeptide exhibits at least 50%, 60%, 70%, 80% or 90% of at least one of the functions of the insert polypeptide when expressed on its own, i-e when compared to the free polypeptide when not linked to the antibody.

Functional or functionality of the antibody and/or the insert polypeptide refers to at least one biological activity of said antibody and/or insert polypeptide including for example target binding (e.g. antigen binding), binding affinity, cross-reactivity, neutralisation, half-life in serum, activation of cell surface proteins, e.g. T cell surface receptors. Methods for measuring functionality, e.g. binding affinity, neutralisation and activation of target cells are well known in the art and include for example the methods described in the Examples provided herein. For example, standard assays such as Scatchard analysis, or surface plasmon resonance technique (e.g. using BIACORE®) may be used to determine target binding (e.g. antigen binding) and binding affinity.

Surprisingly, the V domain (VL or VH domain) of the antibody of the invention can still form a functional binding domain with its corresponding or complementary V domain (VH or VL), where present.

In some embodiments, the insert polypeptide may be a second antibody, or binding fragment thereof. In such embodiments, the insert polypeptide may have any of the features of antibodies as described above. In particular, in some embodiments, the insert polypeptide is a scFv, a dsScFv, a single domain antibody such as a VH or VL or VHH. In some embodiments, the insert polypeptide may be a fully human, humanised, or chimeric antibody. The insert polypeptide may be comprised of one or more CDRs or may consist of one or more CDRs. For example, the insert polypeptide may comprise or consist of at least one of CDR-H1, CDR-H2, CDR-H3 and/or CDR-L1, CDR-L2, CDR-L3, optionally linked.

It will be appreciated by those skilled in the art that this would function as a bivalent antibody, or bispecific antibody. Thus, in one aspect, the invention provides a novel bivalent or bispecific antibody format, capable of simultaneous binding two different epitopes. In some aspects, the epitopes are distinct epitopes present on the same antigen. In another aspect, the invention provides a novel bispecific antibody format capable of simultaneous binding two different antigens.

In one embodiment, an antigen of interest bound by the first or the second antibody or antibody fragment may be a cell-associated protein, for example a cell surface protein on cells such as bacterial cells, yeast cells, T-cells, endothelial cells or tumour cells, or it may be a soluble

protein. Antigens of interest may also be any medically relevant protein such as those proteins upregulated during disease or infection, for example receptors and/or their corresponding ligands. Particular examples of cell surface proteins include adhesion molecules, for example integrins such as  $\beta$ 1 integrins e.g. VLA-4, E-selectin, P selectin or L-selectin, CD2, CD3, CD4, CD5, CD7, CD8, CD11a, CD11b, GD1S, CD19, CD20, CD23, CD25, CD28, CD33, CD38, CD40, CD45, CDW52, CD69, CD 134 (OX40), ICOS, BCMP7, CD 137, CD27L, CDCP1, DPCR1, DPCR1, dudulin2, FLJ20584, FLJ40787, HEK2, KIAA0634, KIAA0659, KIAA1246, KIAA1455, FTBP2, FTK, MAF2, MRP2, nectin-like2, NKCC1, PTK7, RAIG1, TCAM1, SC6, BCMP101, BCMP84, BCMP11, DTD, carcinoembryonic antigen (CEA), human milk fat globulin (HMFG1 and 2), MHC Class I and MHC Class II antigens, and VEGF, and where appropriate, receptors thereof. Soluble antigens include interleukins such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-12, IL-16 or IL-17, viral antigens for example respiratory syncytial virus or cytomegalovirus antigens, immunoglobulins, such as IgE, interferons such as interferon  $\alpha$ , interferon  $\beta$  or interferon  $\gamma$ , tumour necrosis factor- $\alpha$ , tumour necrosis factor- $\beta$ , colony stimulating factors such as G-CSF or GM-CSF, and platelet derived growth factors such as PDGF- $\alpha$ , and PDGF- $\beta$  and where appropriate receptors thereof. Other antigens include bacterial cell surface antigens, bacterial toxins, viruses such as influenza, EBV, Hep A, B and C, bioterrorism agents, radionuclides and heavy metals, and snake and spider venoms and toxins.

In some embodiments, neither the scaffold antibody nor the insert polypeptide binds the hapten (4-hydroxy-3-nitrophenyl) acetyl (NP) or hapten (4-hydroxy-5-iodo-3-nitrophenyl) acetyl (NIP). In some embodiments, the antibody is different from BI-8 murine antibody.

In one embodiment, the second antibody binds to a different antigen to the V domain of the antibody.

In other embodiments, the second antibody binds to the same antigen as the V domain of the antibody. Preferably, the second antibody binds to a different epitope compared to the V domain of the antibody. In such embodiments, the antibody may bind its cognate antigen with a binding affinity which is greater than when compared to the antibody without the second antibody present.

In one embodiment, the insert polypeptide is a second antibody which binds IL-17, in particular IL-17A. In one embodiment, the insert polypeptide is a second antibody which binds IL-17AA, IL-17AF, and IL-17FF. In one embodiment, the second antibody which binds IL-17 is a scFv. In one embodiment, the second antibody which binds IL-17 is a dsScFv. In one embodiment, the antibody of the present invention is a Fab fragment that binds to both HSA and IL-17, in particular the antibody comprising a V domain binds to HSA and the insert polypeptide is a dsScFv which binds IL-17 i.e. the scaffold antibody is a Fab fragment which binds HSA and the second antibody inserted into the Fwk3 region of the V domain of the scaffold antibody is a dsScFv which binds IL-17. In another embodiment, the antibody of the invention is a whole IgG that binds to both HSA and IL-17, in particular the antibody comprising a V domain binds to HSA and the insert polypeptide is a dsScFv which binds IL-17 i.e. the scaffold antibody is a full IgG which binds HSA and the second antibody inserted into the Fwk3 region of the V domain of the scaffold antibody is a dsScFv which binds IL-17. In one embodiment, the antibody of the invention is a Fab fragment which comprises or has the heavy and light chains of SEQ ID NO: 129 (CA645 Fab heavy chain with CA497 dsScFv (vHvL) inserted into framework 3) and SEQ ID NO: 1 respectively, or a variant or variants of any of the same with at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% similarity or identity provided that it retains its functionality, e.g. binding to HSA and/or IL-17. In one embodiment, the antibody of the invention is a Fab fragment which comprises or has the heavy and light chains of SEQ ID NO: 130 (CA645 Fab heavy chain with CA497 dsScFv (vLvH) inserted into framework 3) and SEQ ID NO: 1 respectively or a variant or variants of any of the same with at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% similarity or identity provided that it retains its functionality e.g. binding to HSA and/or IL-17. In one embodiment, the antibody of the invention is a whole IgG which comprises or has the heavy and light chains of SEQ ID NO: 131 (CA645 IgG4P heavy chain with CA497 dsScFv (vHvL) inserted into framework 3) and SEQ ID NO: 1 respectively, or a variant or variants of any of the same with at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% similarity or identity provided that it retains its functionality, e.g. binding to HSA and/or IL-17. In one embodiment, the antibody of the invention is a whole IgG which comprises or has the heavy and light chains of SEQ ID NO: 132 (CA645 IgG4P heavy chain with CA497 dsScFv (vLvH) inserted into framework 3) and SEQ ID NO: 1 respectively, or a variant or variants of any of the same with at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% similarity or identity provided that it retains its functionality, e.g. binding to HSA and/or IL-17.

In one embodiment, the antibody of the invention binds to both CD3 and CD28. In one embodiment, the antibody of the invention is a Fab fragment that binds to both CD3 and CD28, in particular the antibody comprising a V domain binds to CD3 and the insert polypeptide is a dsScFv which binds CD28 i.e. the scaffold antibody is a Fab fragment which binds CD3 and the second antibody inserted into the Fwk3 region of the V domain of the scaffold antibody is a dsScFv which binds CD28. In one embodiment, the antibody of the invention is a Fab fragment that binds to both CD3 and CD28, in particular the antibody comprising a V domain binds to CD28 and the insert polypeptide is a dsScFv which binds CD3 i.e. the scaffold antibody is a Fab fragment which binds CD28 and the second antibody inserted into the Fwk3 region of the V domain of the scaffold antibody is a dsScFv which binds CD3. In another embodiment, the antibody of the invention is a whole IgG that binds to both CD3 and CD28, in particular the antibody comprising a V domain binds to CD3 and the insert polypeptide is a dsScFv which binds CD28 i.e. the scaffold antibody is a full IgG which binds CD3 and the second antibody inserted into the Fwk3 region of the V domain of the scaffold antibody is a dsScFv which binds CD28. In another embodiment, the antibody of the invention is a whole IgG that binds to both CD3 and CD28, in particular the antibody comprising a V domain binds to CD28 and the insert polypeptide is a dsScFv which binds CD3 i.e. the scaffold antibody is a full IgG which binds CD28 and the second antibody inserted into the Fwk3 region of the V domain of the scaffold antibody is a dsScFv which binds CD3. In one embodiment, the antibody of the invention is a Fab fragment which comprises or has the heavy and light chains of SEQ ID NO: 139 (anti-CD28 Fab heavy chain with anti-CD3 dsScFv inserted into framework 3) and SEQ ID NO: 136 respectively, or a variant or variants of any of the same with at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% similarity or identity provided that it retains its functionality, e.g. binding to CD3 and/or CD28. In another embodiment, the antibody of the invention is a whole IgG which comprises or has the heavy and light chains of SEQ ID NO: 141 (anti-CD28 IgG4P heavy chain with anti-CD3 dsScFv inserted into framework 3) and SEQ ID NO: 136 respectively or a variant or variants of any of the same with at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% similarity or identity provided that it retains its functionality e.g. binding to CD3 and/or CD28.

Example sequences for use in the present invention include SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76,

SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, SEQ ID NO: 96, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 125, SEQ ID NO: 126, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, SEQ ID NO: 133, SEQ ID NO: 134, SEQ ID NO: 135, SEQ ID NO: 136, SEQ ID NO: 137, SEQ ID NO: 138, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 143 and SEQ ID NO: 144. Example combinations of heavy and light chains for use in the Fab fragments of the present invention include SEQ ID NO: 54 and SEQ ID NO: 1, SEQ ID NO: 56 and SEQ ID NO: 1, SEQ ID NO: 58 and SEQ ID NO: 1, SEQ ID NO: 60 and SEQ ID NO: 1, SEQ ID NO: 61 and SEQ ID NO: 1, SEQ ID NO: 63 and SEQ ID NO: 1, SEQ ID NO: 64 and SEQ ID NO: 1, SEQ ID NO: 66 and SEQ ID NO: 1, SEQ ID NO: 68 and SEQ ID NO: 1, SEQ ID NO: 70 and SEQ ID NO: 1, SEQ ID NO: 73 and SEQ ID NO: 1, SEQ ID NO: 74 and SEQ ID NO: 1, SEQ ID NO: 75 and SEQ ID NO: 1, SEQ ID NO: 76 and SEQ ID NO: 1, SEQ ID NO: 93 and SEQ ID NO: 1, SEQ ID NO: 94 and SEQ ID NO: 1, SEQ ID NO: 95 and SEQ ID NO: 1, SEQ ID NO: 101 and SEQ ID NO: 1, SEQ ID NO: 129 and SEQ ID NO: 1, SEQ ID NO: 130 and SEQ ID NO: 1, SEQ ID NO: 139 and SEQ ID NO: 136, SEQ ID NO: 140 and SEQ ID NO: 133. Example combinations of heavy and light chains for use in the whole IgG of the present invention include SEQ ID NO: 131 and SEQ ID NO: 1, SEQ ID NO: 132 and SEQ ID NO: 1, SEQ ID NO: 141 and SEQ ID NO: 136, SEQ ID NO: 142 and SEQ ID NO: 133.

### Linker sequence

25

In some embodiments, the insert polypeptide is linked directly to the framework 3 region of the antibody. Linked directly as employed herein is intended to refer to the fact that the N- and C-terminal amino acids of the heterologous insert polypeptide would be linked by a peptide bond to the framework 3 region of the V domain of the antibody of the present invention.

30

In some embodiments, the antibody of the invention further comprises a linker sequence. In such embodiments, the linker sequence is preferably heterologous to both the antibody and insert polypeptide, that is the linker sequence would not normally be present in the native antibody sequence or insert polypeptide at that position.

In some embodiments, the linker sequence comprises at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty or thirty-one amino acids joining the N- and/or C-terminal end of the insert polypeptide to the framework 3 region. In some embodiments, the linker sequence comprises between one and thirty-one, or between five and thirty-one, or between six and twenty-eight, or between ten and twenty-six, or between fifteen and twenty-six, or between ten and twenty-one or between five and ten amino acids joining the N- and/or C-terminal end of the insert polypeptide to the framework 3 region.

In some embodiments, the linker sequence comprises at most thirty amino acids joining the N- and/or C-terminal end of the insert polypeptide to the framework 3 region.

In some embodiments, the linker at the N-terminus of the insert polypeptide is different in composition and/or length to the linker at the C-terminal end of the insert polypeptide. In some embodiments, the linker at the N-terminus of the insert polypeptide is identical or similar in composition and/or length to the linker at the C-terminal end of the insert polypeptide. In some embodiments, the linker sequence is derived from a human sequence. In some embodiments, the linker is a human linker. In some embodiments, the linker sequence is derived from a non-human sequence. In some embodiments, the linker is derived from a bovine sequence. In some embodiments, the linker is a bovine linker.

Examples of suitable linker regions for linking the insert polypeptide to the framework 3 region of a V domain include, but are not limited to, flexible linker sequences and rigid linker sequences. Flexible linker sequences include those disclosed in Huston *et al.*, 1988, PNAS 85:5879-5883; Wright & Deonarain, Mol. Immunol., 2007, 44(11):2860-2869; Alftan *et al.*, Prot. Eng., 1995, 8(7):725-731; Luo *et al.*, J. Biochem., 1995, 118(4):825-831; Tang *et al.*, 1996, J. Biol. Chem. 271(26):15682-15686; and Turner *et al.*, 1997, JIMM 205, 42-54 (see Table 1 below for representative examples).

**Table 1: Examples of Flexible Linker Sequences**

| SEQ ID NO: | SEQUENCE |
|------------|----------|
| 11         | SGGGGSE  |

|    |                                    |
|----|------------------------------------|
| 12 | DKTHTS                             |
| 13 | (S)GGGS                            |
| 14 | (S)GGGSGGGS                        |
| 15 | (S)GGGSGGGSGGGS                    |
| 16 | (S)GGGSGGGSGGGSGGGS                |
| 17 | (S)GGGSGGGSGGGSGGGSGGGS            |
| 18 | AAAGSG-GASAS                       |
| 19 | AAAGSG-XGGGS-GASAS                 |
| 20 | AAAGSG-XGGGSXGGGS-GASAS            |
| 21 | AAAGSG- XGGGSXGGGSXGGGS -GASAS     |
| 22 | AAAGSG- XGGGSXGGGSXGGGSXGGGS-GASAS |
| 23 | AAAGSG-XS-GASAS                    |
| 24 | PGGNRGT TTTTRRPATTTGSSPGPTQSHY     |
| 25 | ATTTGSSPGPT                        |
| 26 | ATTTGS                             |
| -  | GS                                 |
| 27 | EPSGPISTINSPPSKESHKSP              |
| 28 | GTVAAPS VFIFPPSD                   |
| 29 | GGGGIAPSMVGGGS                     |
| 30 | GGGGKVEGAGGGGS                     |
| 31 | GGGSMKSHDGGGS                      |
| 32 | GGGGLITIVGGGS                      |
| 33 | GGGGVVPSLPGGGGS                    |
| 34 | GGEKSIPGGGS                        |
| 35 | RPLSYRPPFPFGFPSVRP                 |
| 36 | YPRSIYIRRRHPSPSLTT                 |
| 37 | TPSHLSHILPSFGLPTEN                 |
| 38 | RPVSPFTFPRLSNWLPA                  |
| 39 | SPAAHFPRSIPRPGPIRT                 |
| 40 | APGPSAPSHRSLPSRAFG                 |
| 41 | PRNSIHFLHPLLVA PLGA                |
| 42 | MPSLSGVLQVRYLSPPDL                 |
| 43 | SPQYPSPLTLTLPPHPSL                 |
| 44 | NPSLNPPSYLHRAPSRIS                 |
| 45 | LPWRTSLLPSLPLRRRP                  |
| 46 | PPLFAKGPVGLLSRSFPP                 |
| 47 | VPPAPVVSLRSAHARPPY                 |
| 48 | LRPTPPRVRSYTCCPTP-                 |
| 49 | PNVAHVLP LLTVPWDNLR                |
| 50 | CNPLLPLCARSPAVRTFP                 |
| 77 | KYQSGGSGMG                         |
| 78 | SGGSYTY                            |
| 79 | GGSGGGG                            |
| 80 | GSSSSGS                            |
| -  | GGG                                |
| 81 | SGGGG                              |
| -  | S                                  |

(S) is optional in sequences 13 to 17. X represents any residue.

In particular embodiments, the antibody comprises the linker sequences SEQ ID NOs: 106 and 107.

5

Examples of rigid linkers include the peptide sequences GAPAAPAPA (SEQ ID NO:51), PPPP (SEQ ID NO:52), PPP, IPFTV (SEQ ID NO: 82) and EYHGLQ (SEQ ID NO: 104). Further examples include sequences derived from the elongated stalk of CDRH3 of bovine antibodies (Wang *et al.*, Cell, 2013, 153:1379-1393); preferable sequences include long linker sequences such as TSVHQETKKYQS (SEQ ID NO: 96) and SYTYNYEWHVDV (SEQ ID NO: 97) (comprising both solvent exposed and buried residues from a bovine CDH3 stalk); and short linker sequences such as ETKKYQS (SEQ ID NO: 98) and SYTYNYE (SEQ ID NO: 99) (comprising only the solvent exposed residues from a bovine CDH3 stalk).

10

15 In some embodiments, variants of any of the linker sequences disclosed herein may be used, for example, variants where in one, two, three, four or more amino acid residues have been substituted, inserted, or removed with respect to the original linker sequence. Variants of linker sequences may also include repeat variants, where the linker sequence is elongated by repeating the linker sequence. In one embodiment, a preferred linker sequence to be repeated is SEQ ID NO: 13, leading to the motif: (G<sub>m</sub>S)<sub>n</sub>, where m is 4 and n can be any integer (for example 1, 2 or 3). In other embodiments, m can be a different integer from 1 to 10.

20

Whether or not the insert polypeptide is linked directly or via a linker sequence, in some embodiments, one or more amino acids in the framework 3 region may be deleted as a result of linking the polypeptide insert. In some embodiments, the insert polypeptide is between amino acid residues 73 and 75 of the VH domain, in accordance with the Kabat numbering system and the amino acid in position 74 is deleted.

25

### **Pharmaceutical compositions and methods of use**

30

Antibodies of the invention and as described herein are useful in the treatment of diseases or disorders including inflammatory diseases and disorders, immune diseases and disorders, fibrotic disorders and cancers.

The term “inflammatory disease” or “disorder” and “immune disease or disorder” includes rheumatoid arthritis, psoriatic arthritis, still's disease, Muckle Wells disease, psoriasis, Crohn's disease, ulcerative colitis, SLE (Systemic Lupus Erythematosus), asthma, allergic rhinitis, atopic dermatitis, multiple sclerosis, vasculitis, Type I diabetes mellitus, transplantation and graft-versus-host disease.

The term “fibrotic disorder” includes idiopathic pulmonary fibrosis (IPF), systemic sclerosis (or scleroderma), kidney fibrosis, diabetic nephropathy, IgA nephropathy, hypertension, end-stage renal disease, peritoneal fibrosis (continuous ambulatory peritoneal dialysis), liver cirrhosis, age-related macular degeneration (ARMD), retinopathy, cardiac reactive fibrosis, scarring, keloids, burns, skin ulcers, angioplasty, coronary bypass surgery, arthroplasty and cataract surgery.

The term “cancer” includes a malignant new growth that arises from epithelium, found in skin or, more commonly, the lining of body organs, for example: breast, ovary, prostate, lung, kidney, pancreas, stomach, bladder or bowel. Cancers tend to infiltrate into adjacent tissue and spread (metastasise) to distant organs, for example: to bone, liver, lung or the brain.

Thus, according to a further aspect of the invention, there is provided a pharmaceutical composition which comprises an antibody of the invention in association with one or more pharmaceutically acceptable carriers, excipients or diluents.

Also provided is the use of an antibody of the invention for the manufacture of a medicament for the treatment of a disease or disorder. Most preferably, the disease or disorder is an inflammatory disease or disorder.

Pharmaceutical compositions according to the invention may, for example, take a form suitable for parenteral, subcutaneous, or ophthalmic administration. Where appropriate, for example if the antibody binds to albumin via its V domain, it may be desirable to pre-formulate the antibody of the invention with human or recombinant serum albumin, using any suitable method known in the art.

Where the pharmaceutical formulation is a liquid, for example a solution or suspension, then the formulation may further comprise albumin, for example human serum albumin, in

particular recombinant albumin such as recombinant human serum albumin. Suitable amounts may be in the range of less than 2% w/w of the total formulation, in particular less than 1, 0.5, or 0.1% w/w. This may assist in stabilizing the antibody component in the formulation. The pharmaceutical composition may be lyophilized for reconstitution later, with an aqueous solvent. In one embodiment there is provided a unit dose container, such as a vial, comprising a lyophilized antibody according to the invention.

The antibodies of the invention may be formulated for parenteral administration by injection, e.g. by bolus injection or infusion. Formulations for injection may be presented in unit dosage form, e.g. in glass ampoules or multi-dose containers, e.g. glass vials. The compositions for injection may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising, preserving and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack or dispensing device may be accompanied by instructions for administration.

The antibody formats of the invention can be delivered dispersed in a solvent, e.g., in the form of a solution or a suspension. It can be suspended in an appropriate physiological solution, e.g., saline or other pharmacologically acceptable solvent or a buffered solution. Buffered solutions known in the art may contain 0.05 mg to 0.15 mg disodium edetate, 8.0 mg to 9.0 mg NaCl, 0.15 mg to 0.25 mg polysorbate, 0.25 mg to 0.30 mg anhydrous citric acid, and 0.45 mg to 0.55 mg sodium citrate per 1 ml of water so as to achieve a pH of about 4.0 to 5.0. A suspension can employ, for example, lyophilised antibody.

The therapeutic suspensions or solution formulations can also contain one or more excipients. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent/solution used for the formulation, aseptic suspension of the antibody in the sterile buffered solvent solution, and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

The quantity of an antibody of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the antibody and the condition to be treated. In general, however, daily dosages may range from around 10 ng/kg to 1000 mg/kg, from 100 ng/kg to 100 mg/kg, or from 0.1 mg/kg to 10 mg/kg body weight.

The present invention also provides for methods and compositions for the delivery of the antibodies as described herein by gene therapy, particularly by adeno-associated virus (AAV) vector.

Hence, the present invention provides for a pharmaceutical composition comprising a viral vector having a viral capsid and an artificial genome comprising an expression cassette flanked by inverted terminal repeats (ITRs) wherein the expression cassette comprises a transgene comprising a polynucleotide sequence encoding the antibody as described herein. The ITRs sequences may be used for packaging the artificial genome comprising the polynucleotide sequences encoding the antibody as described herein into the virion of the viral vector.

The transgene in the expression cassette is operably linked to expression control elements such as promoters that will control expression of the transgene in human cells.

The viral vector is preferably AAV based viral vectors. A variety of AAV capsids have been described in the art. Methods of generating AAV vectors have also been described extensively in the literature (e.g., WO 2003/042397; WO 2005/033321, WO 2006/110689; U.S. Pat. No. 7,588,772 B2). The source of AAV capsids may be selected from an AAV which targets a desired tissue. For example, suitable AAV may include, e.g., AAV9 (U.S. Pat. No. 7,906,111; US 2011-0236353-A1), rh10 (WO 2003/042397) and/or hu37 (US 7,906,111B2; US20110236353). However, other AAV, including, e.g., AAV1, AAV2, AAV-TT, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8 and AAV.PHP.B (or variants thereof) and others may also be selected.

Methods for generating and isolating AAV viral vectors suitable for delivery to a subject are known in the art (US7,790,449B2; US7,282,199B2; WO 2003/042397; WO 2005/033321, WO 2006/110689; and US7,588,772 B2).

The pharmaceutical composition comprising a viral vector having a viral capsid and an artificial genome according to the invention is designed to ensure that both the heavy and light chains are expressed. The polynucleotide sequences of the heavy and light chain of the antibodies according to the present invention may be engineered in a single construct and separated by, for example, a cleavable linker or internal ribosome entry site (IRES) element so that the heavy and light chains are separately expressed. In some embodiments, the cleavable linker is a self-cleaving furin/F2A linker (Fang et al., Nature Biotechnology 23: 584-590, 2005 and Fang et al. Mol Ther 15: 1153-9, 2007). For example, a furin/F2A linker may be incorporated into an expression cassette to separate the heavy and light chain polynucleotide coding sequences.

In some other embodiments, the polynucleotide sequence of the heavy and light chains of the antibodies according to the present invention are connected via a flexible, non-cleavable linker. In some embodiments such linker may be a flexible peptide linker such as those encoding scFv (composed of Gly and Ser) so that the heavy and light chain domains are free to move relative to one another.

The invention will now be described with reference to the following examples, which are merely illustrative and should not in any way be construed as limiting the scope of the present invention.

In the claims which follow and in the description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

## EXAMPLES

### General Methodology

#### *Framework 3 insertion*

In addition to three CDR loops, antibody light and heavy chains, both conventional and single-chain camelid VHH, have a fourth loop which is formed by framework 3 (Figure 1A). The Kabat numbering system defines framework 3 as positions 66-94 in a heavy chain and positions 57-88 in a light chain.

*Gene design and transient cell expression*

Genes encoding light and heavy chain V-regions, including variants with a framework 3 insertion, were designed and constructed by an automated synthesis approach (ATUM).

The genes were cloned into expression vectors containing DNA encoding human C $\kappa$  region (Km3 allotype) and heavy chain  $\gamma$ 1 CH1 region, respectively. Heavy and light chain expression vectors were then co-transfected into HEK293 cells and the recombinant antibody/Fab fusion protein molecules screened using an SPR binding assay.

The light V-region or light chain of the CA645 Fab fusion proteins described in the following Examples comprised or had SEQ ID NO: 2 (CA645 VL domain (gL5)) or SEQ ID NO: 1 (CA645 light chain gL5) respectively. Alternative light chains or light V-regions may be used, for example light V-regions comprising the VL domain of SEQ ID NO: 108 or SEQ ID NO: 109.

*Determination of antibody/Fab fusion protein binding potency by Surface Plasmon Resonance*

All surface plasmon resonance (SPR) experiments were carried out at 25°C on a Biacore 3000 system using a pH 7.4 running buffer containing 10 mM HEPES, 150 mM NaCl, EDTA 2 mM and 0.005% (v/v) P20 (HBS-EP buffer).

Polyclonal goat F(ab)<sub>2</sub> fragment anti-human F(ab)<sub>2</sub>, (Jackson Labs product code #109-006-097) was immobilized to the surface of CM5 sensor chips (GE Healthcare) by amine coupling. Briefly, the carboxymethyl dextran surface was activated with a fresh 1:1 (v/v) mixture of 50 mM N-hydroxysuccinimide and 200 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide for 5 minutes at a flow rate of 10  $\mu$ L/min. The anti-human F(ab)<sub>2</sub> in 10 mM acetate pH 5.0 buffer was covalently coupled to chips at 50  $\mu$ g/mL in a 10 minute pulse at the same flow rate. Finally the surface was deactivated with a 10 minute pulse of 1 M ethanolamine.HCl pH 8.5. A reference flow cell was prepared on the chip by omitting the protein from the above procedure.

Supernatants containing Fab-loop insert constructs, wild type Fab or control supernatants were diluted 1 in 5 in HPS-EP buffer prior to testing for binding of their respective ligand or receptor.

Human serum albumin (HSA) was supplied by Jackson ImmunoResearch (catalog #009-000-051) and was diluted in HBS-EP buffer to 1000 nM. The following cytokines and receptors were obtained from R&D Systems Ltd.: IL-15, IL-15-R $\alpha$ -hFc, IL-2, IL-2-sR $\beta$ , G-CSF, G-CSF-R $\alpha$ , GM-CSF, GM-CSF-R $\alpha$ , EPO, EPO-R, IL-6, LRP4(ecd)-hFc, LRP6(ecd)-hFc, Lysozyme,

KLK5. These proteins were reconstituted at typically 100 µg/mL and stored at -20°C. For experimental purposes they were freshly diluted in HBS-EP buffer to typically 50, 100 or 200 nM.

5 A typical SPR cycle comprised a flow rate of 10 µL/min with HBS-EP as running buffer and diluted supernatant was injected for 1 min to capture the antibody/Fab fusion protein. This was followed by a 30 µl injection of the diluted cognate ligand or receptor or buffer control. The subsequent dissociation phase was monitored for at least 3 min. In some experiments an additional 30 µL injection of the relevant cognate antigen of the antibody portion was included,  
10 HSA in the case of fusion protein constructs using CA645 Fab as the antibody polypeptide portion. In some experiments a specificity control cycle was included where the cognate receptor / ligand (the insert portion of the antibody/Fab fusion protein) was pre-mixed with a 2-fold molar excess of its binding partner. Each cycle terminated with a regeneration step comprising two 10 µL injections of 40 mM HCl interspersed with a 5 µL injection of 5 mM  
15 NaOH.

Report points were collected 10 s before supernatant and ligand / receptor injections for measuring fusion protein construct capture and at 5 s before and 15 s after the end of the ligand / receptor injection for assessing binding of cognate ligand / receptor. Report point and  
20 sensorgram data collected represented the difference in resonance units (RU) between the polyclonal capture flow cell and the reference flow cell.

Report point and sensorgram data were processed by subtracting the respective control cycle from the ligand / receptor cycle to account for any slow dissociation of the fusion protein  
25 construct from the capture surface. In every experiment the respective wild type Fab supernatant was included to check for any non-specific binding of ligand / receptor. If significant this was subtracted from the above.

In examples where dissociation constants ( $K_D$ ) were estimated, the above method was modified  
30 by increasing the flow rate to 30 µl/min. Following capture of the Fab construct, 90 µl of the cognate ligand or buffer control was injected followed by a dissociation phase of 180 sec. A ligand binding over a range of concentrations (0.1, 0.2, 0.5, 1, 2, 5, & 10 nM) was tested. Sensorgrams were corrected for drift by subtraction of a zero ligand sensorgram and for non-specific interaction by subtraction of the wild type Fab corrected sensorgram. Affinity

constants were calculated from separate dissociation and association rate constants that were fitted to the corrected sensorgrams using the Langmuir model of the BIAevaluation software (version 4.1.1).

## 5 EXAMPLE 1 - CA645 Fab with IL-15 insert polypeptide

Interleukin 15 (IL-15), is a cytokine which plays a role in the immune response, and functions to promote the proliferation of T cells and natural killer (NK) cells. It is structurally similar to IL-2, and its receptor shares common chains with the IL-2 receptor.

10

A fusion protein was generated and expressed in accordance with the above methods to insert the human IL-15 sequence into the framework 3 region of the CA645 Fab construct, as shown below.

### 15 *Human IL-15 sequence:*

NWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCCKVTAMKCFLELQVISLESGDAS  
IHDTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQMFIN (SEQ ID  
NO: 53)

### 20 *CA645 Fab heavy chain with human IL-15 (bold and italics) inserted into framework 3, linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNKYQSGGSGMG***NWVNVISDLKKIEDLIQSMHIDATLYTESDV***  
***HPSCCKVTAMKCFLELQVISLESGDASI******HDTVENLIILANNSLSSNGNVTESGCKECE***  
25 ***ELEEKNIKEFLQSFVHIVQMFIN******SGGSYTY***KNTVYLQMNSLRAEDTAVYYCARTVP  
GYSTAPYFDLWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP  
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK  
VDKKVEPKSC (SEQ ID NO: 54)

30 Figure 2A shows the Biacore analysis of binding potency for this CA645/IL-15 antibody fusion protein when tested with 100 nM IL-2-R $\beta$ , IL-15-R $\alpha$  (both of which are binding partners for IL-15) and 1000 nM HSA (ligand for CA645). As is clearly seen in Figure 2A, binding was seen to all three ligands showing that functionality is retained in both the CA645 Fab portion

as well as the IL-15 graft in the framework 3 region. Figure 2B shows a modelled representation of the CA645/IL-15<sub>Fwk3</sub> protein.

## EXAMPLE 2 - CA645 Fab with IL-2 insert polypeptide

5

Interleukin 2 (IL-2) is a pluripotent cytokine produced primarily by activated CD4<sup>+</sup> T cells, which plays a crucial role in generating a normal immune response. IL-2 promotes proliferation and expansion of activated T lymphocytes, potentiates B cell growth, and activates monocytes and natural killer cells.

10

A fusion protein was generated and expressed in accordance with the above methods to insert the human IL-2 sequence into the framework 3 region of the CA645 Fab construct, as shown below.

15 *Human IL-2 sequence:*

APTSSSTKKTQLQLEHLLLDLQMILNGINNYKNPKLTRMLTFKFYMPKKATELKHLQ  
CLEEELKPLEEVLNLAQSKNFHLRPRDLISNINVIVLELKGSETTFMCEYADETATIVE  
FLNRWITFCQSIISTLT (SEQ ID NO: 55)

20 *CA645 Fab heavy chain with human IL-2 (bold and italics) inserted into framework 3, linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSSGGSGGGG***APTSSSTKKTQLQLEHLLLDLQMILNGINNYK***  
***NPKLTRMLTFKFYMPKKATELKHLQCLEEELKPLEEVLNLAQSKNFHLRPRDLISNI***  
25 ***NVIVLELKGSETTFMCEYADETATIVEFLNRWITFCQSIISTLT***GGGKNTVYLQMNSL  
RAEDTAVYYCARTVPGYSTAPYFDLWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG  
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT  
QTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 56)

30 Figure 3A shows the Biacore analysis of binding potency for this CA645/IL-2 antibody fusion protein when tested with 100 nM IL-2-R $\beta$  (binding partner for IL-2), with and without prior incubation with 200 nM IL-15 (a competing ligand). As is shown in Figure 3A, binding seen to IL-2-R $\beta$  is abolished in the presence of the competing ligand in molar excess, showing that the binding seen to the IL-2 portion in framework 3 of the fusion protein is a genuine functional

interaction and not non-specific. Figure 3B shows a modelled representation of the CA645/IL-2<sub>Fwk3</sub> protein.

### EXAMPLE 3 - CA645 Fab with G-CSF insert polypeptide

5

Granulocyte-colony stimulating factor (G-CSF) is a cytokine produced by a number of different cell types (e.g. endothelium, macrophages) which acts to simulate the production of mature granulocytes from bone marrow.

10 A fusion protein was generated and expressed in accordance with the above methods to insert the human G-CSF sequence into the framework 3 region of the CA645 Fab construct, as shown below.

*Human G-CSF sequence:*

15 ATPLGPASSLPQSFLKCLEQVRKIQGDGAALQEKLVSECATYKLCHPEELVLLGHSL  
GIPWAPLSSCPSQALQLAGCLS~~QLHSGFLYQGLL~~QALEGISPELGPTLDTLQLDVAD  
FATTIWQQMEELGMAPALQPTQGAMPAFASAFQRRAGGVLVASHLQSFLEVSYRVL  
RHLAQP (SEQ ID NO: 57)

20 *CA645 Fab heavy chain with human G-CSF (bold and italics) inserted into framework 3, Gly-Ser linkers underlined:*

EVQLLES~~GGGLVQPGGSLRL~~SCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDN~~SGGGGS~~***ATPLGPASSLPQSFLKCLEQVRKIQGDGAALQEK***  
***LVSECATYKLCHPEELVLLGHSLGIPWAPLSSCPSQALQLAGCLS~~QLHSGFLYQGLL~~***  
25 ***QALEGISPELGPTLDTLQLDVADFATTIWQQMEELGMAPALQPTQGAMPAFASAFQ***  
***RRAGGVLVASHLQSFLEVSYRVLRLHAQPSGGGGKNTVYLQMNSLRAEDTAVYYC***  
ARTVPGYSTAPYFDLWGQGT~~LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD~~  
YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP  
SNTKVDKKVEPKSC (SEQ ID NO: 58)

30

Figure 4A shows the Biacore analysis of binding potency for this CA645/G-CSF antibody fusion protein when tested with 100 nM G-CSF-R $\alpha$  (binding partner for G-CSF), 1000 nM HSA (ligand for CA645), and both G-CSF-R $\alpha$  and HSA. Figure 4A shows in line with the other Examples, both the G-CSF and Fab portions of the fusion protein are functionally active

and are able to bind their respective binding partners. Figure 4A further shows that in the presence of both binding partners, the signal seen from the SPR trace is additive from the single-binder traces, showing that binding to both functional portions can occur simultaneously. Figure 4B shows a modelled representation of the CA645/G-CSF<sub>Fwk3</sub> protein.

5

#### EXAMPLE 4 – CA645 Fab with GM-CSF insert polypeptide

GM-CSF is a cytokine which stimulates stem cells to produce granulocytes (neutrophils, eosinophils, and basophils) and monocytes.

10

A fusion protein was generated and expressed in accordance with the above methods to insert the human and the murine GM-CSF sequences into the framework 3 region of the CA645 Fab construct, as shown below.

15 *Human GM-CSF (hGM-CSF) sequence:*

APARSPSPSTQPWEHVNAIQEARRLLNLSRD~~TAAEMNETVEVISEMFDLQEPTCLQT~~  
RLELYKQGLRGSLTKLKGPLTMMASHYKQHCPPTPETS~~CATQIITFESFKENLKDFLL~~  
VIPFDCWEPVQE (SEQ ID NO: 59)

20 *CA645 Fab heavy chain with human GM-CSF (bold and italics) inserted into framework 3, Gly-Ser linkers underlined:*

EVQLLES~~GGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT~~  
FYATWAKGRFTISRDN~~SGGGSGGGG~~***APARSPSPSTQPWEHVNAIQEARRLLNLSRDT***  
***AAEMNETVEVISEMFDLQEPTCLQTRLELYKQGLRGSLTKLKGPLTMMASHYKQHC***  
25 ***PPTPETS******CATQIITFESFKENLKDFLLVIPFDCWEPVQE***~~GGGKNTVYLQMNSLRAED~~  
TAVYYCARTVPGYSTAPYFDLWGQGT~~LVTVSSASTKGPSVFPLAPSSKSTSGGTAAL~~  
GCLVKDYFPEPVT~~VSWSNGALTS~~GVHTFPAVLQSSGLYSLSSV~~VTVPSSSLGTQTYIC~~  
NVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 60)

30 *CA645 Fab heavy chain with human GM-CSF (bold and italics) at C-terminus of CH1, Gly-Ser linker underlined (for comparison purposes):*

EVQLLES~~GGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT~~  
FYATWAKGRFTISRDN~~SKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWG~~  
QGT~~LVTVSSASTKGPSVFPLAPSSKSTSGGTAAL~~GCLVKDYFPEPVT~~VSWSNGALTS~~

GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCGG  
GSGGGG***APARSPSPSTQPWEHVNAIQEARRLLNLSRDTAAEMNETVEVISEMFDLQ***  
***EPTCLQTRLELYKQGLRGS�TKLKGPLTMMASHYKQHCPPTPETSCATQHITFESFKE***  
***NLKDFLLVIPFDCWEPVQE*** (SEQ ID NO: 61)

5

*Murine GM-CSF (mGM-CSF) sequence:*

APTRSPITVTRPWKHVEAIKEALNLLDDMPVTLNEEEVVSNEFSFKKLTCVQTRLKI  
 FEQGLRGNF TKLKGALNMTASYQTYCPPTPETDCETQVTTYAD FIDSLKTFLTDIPF  
 ECKKPGQK (SEQ ID NO: 62)

10

*CA645 Fab heavy chain with murine GM-CSF (bold and italics) inserted into framework 3, Gly-Ser linkers underlined:*

EVQLLES GGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNSGGGSGGGG***APTRSPITVTRPWKHVEAIKEALNLLDDMPVT***  
***LNEEEVVSNEFSFKKLTCVQTRLKIFEQGLRGNF TKLKGALNMTASYQTYCPPTP***  
***ETDCETQVTTYAD FIDSLKTFLTDIPFECKKPGQK***GGGKNTVYLQMNSLRAEDTAV  
 YYCARTVPGYSTAPYFDLWGQGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN  
 HKPSNTKVDKKVEPKSC (SEQ ID NO: 63)

20

*CA645 Fab heavy chain with murine GM-CSF (bold and italics) at C-terminus of CH1, Gly-Ser linkers underlined (for comparison purposes):*

EVQLLES GGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDN SKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWG  
 QGT LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALT  
 GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHNKPSNTKVDKKVEPKSCGG  
GSGGGG***APTRSPITVTRPWKHVEAIKEALNLLDDMPVTLNEEEVVSNEFSFKKLTC***  
***VQTRLKIFEQGLRGNF TKLKGALNMTASYQTYCPPTPETDCETQVTTYAD FIDSLKT***  
***FLTDIPFECKKPGQK*** (SEQ ID NO: 64)

30

Figures 5A and 6 shows the Biacore analysis of binding potency for the CA645/hGM-CSF and CA645/mGM-CSF antibody fusion proteins (respectively) when tested with the cognate GM-CSF receptor (at 100 nM). The results demonstrate that the binding to the GM-CSF receptor is specific and dependent upon the presence of the insert polypeptide. The Figures also show

a separate trace for a constant domain (CH1) fusion of the GM-CSF peptide, showing that the framework 3 insert functions in a comparable manner. Figure 5B shows a modelled representation of the CA645/GM-CSF<sub>Fwk3</sub> protein.

## 5 EXAMPLE 5 - CA645 Fab with EPO insert polypeptide

Erythropoietin (EPO) is a cytokine released by the kidney in response to cellular hypoxia which stimulates red blood cell production in the bone marrow.

- 10 A fusion protein was generated and expressed in accordance with the above methods to insert the human EPO sequence into the framework 3 region of the CA645 Fab construct, as shown below.

*Human EPO sequence:*

- 15 APPRLICDSRVLERYLLEAKEAENITTGCAEHCSLNENITVPDTKVNIFYAWKRMEVG  
QQAWEVWQGLALLSEAVLRGQALLVNSSQPWEPLQLHVDKAVSGLRSLTTLRLALG  
AQKEAISPPDAASAAPLRTITADTFRKLFRVYSNFLRGKCLKLYTGEACRTGDR (SEQ  
ID NO: 65)

- 20 *CA645 Fab heavy chain with human EPO (bold and italics) inserted into framework 3, Gly-Ser linkers underlined:*

- EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSSGGSGGGG***APPRLICDSRVLERYLLEAKEAENITTGCAEH***  
***CSLNENITVPDTKVNIFYAWKRMEVGQQAWEVWQGLALLSEAVLRGQALLVNSSQPW***  
25 ***EPLQLHVDKAVSGLRSLTTLRLALGAQKEAISPPDAASAAPLRTITADTFRKLFRVYS***  
***NFLRGKCLKLYTGEACRTGDR***GGGKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAP  
YFDLWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP  
KSC (SEQ ID NO: 66)

30

Figure 7A shows the Biacore analysis of binding potency for this CA645/EPO antibody fusion protein when tested with 50 nM EPO-R (binding partner for EPO), with and without prior incubation with 500 nM soluble EPO at (competing ligand). Figure 7A shows in line with the other Examples, the EPO portion of the fusion protein is functionally active and is able to bind

its cognate receptor. Furthermore, the decrease in signal seen with the prior incubation of EPO shows the specificity of the interaction, while the unmodified CA645 Fab acts as a further control to demonstrate that the binding seen is derived from the EPO graft. Figure 7B shows a modelled representation of the CA645/EPO<sub>Fwk3</sub> protein.

5

#### EXAMPLE 6 - Antibody/Fab fusion protein with CA645 Fab with VHH6 insert

VHH6 is an engineered single-domain heavy-chain camelid antibody which binds a junctional epitope in that it is able to bind the IL-6:gp80 complex, but not the individual components alone.

10

A fusion protein was generated and expressed in accordance with the above methods to insert the VHH6 sequence into the framework 3 region of the CA645 Fab construct, as shown below.

15 *Camelid VHH6 sequence:*

DVQFVESGGGSVHAGGSLRLNCATSGYIYSTYCMGWFRQAPGKEREGVAHIYTNSG  
RTYYADSVKGRFTISQDNAKNTVYLMQNSLKPEDTAIYYCAARPSIRCASFSALEYK  
DWGQGTQVTVSS (SEQ ID NO: 67)

20 *CA645 Fab heavy chain with VHH6 (bold and italics) inserted into framework 3, Gly-Ser linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSGGGGSGGGGS***DVQFVESGGGSVHAGGSLRLNCATSGYIYS***  
***TYCMGWFRQAPGKEREGVAHIYTNSGR******RTYYADSVKGRFTISQDNAKNTVYLMQNSL***  
25 ***KPEDTAIYYCAARPSIRCASFSALEYKDWGQGTQVTVSS******SGGGGSGGGGS******KNTVYLMQ***  
***NSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGT******LVTVSSASTKGPSVFPLAPSSKST***  
***SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS***  
***SSSLGTQTYICNVNHKPSNTKVDKKVEPKSC*** (SEQ ID NO: 68)

30 Figure 8A shows the Biacore analysis of binding potency for this CA645/VHH6 antibody fusion protein when tested with 100 nM IL-6 (negative control for VHH6 as VHH6 solely binds the complex), 100 nM IL-6/gp80 fusion (binding partner for VHH6), 1000 nM HSA (binding partner for CA645) and both the IL-6 fusion and HSA together. Figure 8A shows in line with the other Examples, both the VHH6 and CA645 parts of the antibody fusion protein

are functionally active as binding fragments and are able to bind their respective binding partners. Figure 8A further shows that in the presence of both binding partners, the signal seen from the SPR trace is additive from the single-binder traces, showing that binding to both functional portions can occur simultaneously. Figure 8B shows a modelled representation of the CA645/VHH6<sub>Fwk3</sub> protein.

#### EXAMPLE 7 - Antibody/Fab fusion protein with CA645 Fab with VHH15 insert

VHH15 is an engineered single domain heavy-chain camelid antibody which recognises and binds to IL-6.

A fusion protein was generated and expressed in accordance with the above methods to insert the VHH15 sequence into the framework 3 region of the CA645 Fab construct, as shown below.

*Camelid VHH15 sequence:*

QVQLVESGGGSVQAGGSLRLSCVAASGYTGCTYDMRWYRQAPGKEREFVSGIDSD  
GRATYADSVKGRFTISQSNAKIAVYLQMDSLKLEDTAMYYCNLQCLRYPGEYYWG  
QGTQVTVSS (SEQ ID NO: 69)

*CA645 Fab heavy chain with VHH15, an anti-IL-6 camelid domain antibody (bold and italics), inserted into framework 3, Gly-Ser linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSSGGGGSGGGGS***QVQLVESGGGSVQAGGSLRLSCVAASGYT***  
***GCTYDMRWYRQAPGKEREFVSGIDSDGRATYADSVKGRFTISQSNAKIAVYLQMDSL***  
***KLEDTAMYYCNLQCLRYPGEYYWGQGTQVTVSS***GGGGSGGGGSKNTVYLQMNSLR  
AEDTAVYYCARTVPGYSTAPYFDLWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGT  
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQ  
TYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 70)

Figure 9A shows the Biacore analysis of binding potency for this CA645/VHH15 antibody fusion protein when tested with 100 nM IL-6 (ligand for VHH15), 1000 nM HSA (ligand for CA645), and both IL-6 and HSA. Figure 9A shows in line with the other Examples, both the VHH15 and Fab portions of the antibody fusion protein are functionally active as binding fragments and are able to bind their respective binding partners. Figure 9A further shows that

in the presence of both binding partners, the signal seen from the SPR trace is additive from the single-binder traces, showing that binding to both functional portions can occur simultaneously. Figure 9B shows a modelled representation of the CA645/VHH15<sub>Fwk3</sub> protein.

## 5 EXAMPLE 8 - CA645 Fab with Sclerostin insert polypeptide

Sclerostin is the product of the SOST gene in humans and is involved in the process of bone formation, being largely expressed in osteocytes. Sclerostin functions as a regulatory protein in the Wnt signalling pathway through binding to the LRP-type co-receptors.

10

Fusion proteins were generated and expressed in accordance with the above methods to insert the core or the whole Sclerostin sequence into the framework 3 region of the CA645 Fab construct, as shown below.

15 *Human mature full-length sclerostin sequence:*

QGWQAFKNDATETIPELGEYPEPPPELENNKTMNRAENGGRPPHHPFETKDVSEYSC  
RELHFTRYVTDGPCRSAKPVTELVCSGQCQGPAPLLPNAIGRGKWWRPSGPDFRCIPD  
RYRAQRVQLLCPGGEAPRARKVRLVASCKCKRLTRFHNQSELKDFGTEAARPQKGR  
KPRPRARSAKANQAELENAY (SEQ ID NO: 71)

20

*Sequence of core domain of human sclerostin:*

CRELHFTRYVTDGPCRSAKPVTELVCSGQCQGPAPLLPNAIGRGKWWRPSGPDFRCIP  
DRYRAQRVQLLCPGGEAPRARKVRLVASCKC (SEQ ID NO: 72)

25 *CA645 Fab heavy chain with human sclerostin core domain (bold and italics) inserted into framework 3, single S linkers underlined:*

EVQLLESQGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSSCRELHFTRYVTDGPCRSAKPVTELVCSGQCQGPAPLLPNA  
IGRGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGEAPRARKVRLVASCKCSKNTVYL  
30 QMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLLTVSSASTKGPSVFPLAPSS  
KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP  
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 73)

*CA645 Fab heavy chain with human sclerostin core domain (bold and italics) inserted into framework 3, Gly-Ser (2X G<sub>4</sub>S) linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNSGGGGSGGGGS***CRELHFTRYVTDGPCRSAKPVTELVCSGQ***  
 5 ***CGPARLLPNAIGRGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGEAPRARKVRLVASC***  
***KCSGGGGSGGGGSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTL***  
 VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF  
 PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID  
 NO: 74)

10

*CA645 Fab heavy chain with human sclerostin core domain (bold and italics) inserted into framework 3, rigid linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNIPFTV***CRELHFTRYVTDGPCRSAKPVTELVCSGQC******CGPARLL***  
 15 ***PNAIGRGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGEAPRARKVRLVASC******KCEYHG***  
***LQKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTL******VTVSSASTKGPS***  
 VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS  
 LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 75)

20 *CA645 Fab heavy chain with human mature full-length sclerostin (bold and italics) inserted into framework 3, Gly-Ser (1X G<sub>4</sub>S) linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNSGGGGS***QGWQAFKNDTEIPELGEYPEPPPELENNKTMN***  
***RAENGGRPPHHPFETKDVSEYSCRELHFTRYVTDGPCRSAKPVTELVCSGQC******CGPARL***  
 25 ***LPNAIGRGKWWRPSGPDFRCIPDRYRAQRVQLLCPGGEAPRARKVRLVASC******KCKRLT***  
***RFHNQSELKDFGTEAARPQKGRKPRPRARSAKANQAEL******ENAYSGGGGSKNTVYLQ***  
 MNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLVTVSSASTKGPSVFPLAPSSKS  
 TSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS  
 SLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 76)

30

Figures 10A and 11 show the Biacore analysis of potency for this CA645/sclerostin antibody fusion protein when tested as 1 in 5 diluted supernatant for binding to immobilized extracellular domains of LRP6 and LRP4 respectively (binding partners for Sclerostin). This experiment also investigated the effects of different linker region constructs. It was noted that all of the

tested constructs showed binding to LRP6 and LRP4. Figure 10B shows a modelled representation of the CA645/Sclerostin<sub>core-Fwk3</sub> protein.

### EXAMPLE 9 - CA645 Fab with IL-10 insert polypeptide (IL-10 dimer)

5

Interleukin 10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine. It functions generally as a homodimer.

A fusion protein was generated and expressed in accordance with the above methods to insert  
10 two human IL-10 sequences into the framework 3 region of the CA645 Fab construct, as shown below.

*Human IL-10 monomer sequence:*

NCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWTEEDVNDNTLFKWMIFNGGA  
15 PNCIPCKEPRVCAPDCSNITWKGPCGLDGKTYRNECALLKARCKEQPELEVQYQ  
GRCKKTCRDVFCPGSSTCVVDQTNNAVCVTCNRICPEPASSEQYLCGNDGVITYSSAC  
HLRKATCLLGRSIGLAGEYK (SEQ ID NO: 100)

*CA645 Fab heavy chain with two human IL-10 (bold and italics) monomers inserted into  
20 framework 3, linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCA VSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSGGGGSGS***NCWLRQAKNGRCQVLYKTELSKEECCSTGRLS***  
***TSWTEEDVNDNTLFKWMIFNGGAPNCIPCKEPRVCAPDCSNITWKGPCGLDGKT***  
***YRNECALLKARCKEQPELEVQYQGRCKKTCRDVFCPGSSTCVVDQTNNAVCVTCNRI***  
25 ***CPEPASSEQYLCGNDGVITYSSACHLRKATCLLGRSIGLAGEYK***GGGGSGGGGSGGGG  
***SNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWTEEDVNDNTLFKWMIFNGGA***  
***PNCIPCKEPRVCAPDCSNITWKGPCGLDGKTYRNECALLKARCKEQPELEVQYQG***  
***RCKKTCRDVFCPGSSTCVVDQTNNAVCVTCNRICPEPASSEQYLCGNDGVITYSSACH***  
***LRKATCLLGRSIGLAYE***GGGGSGSKNTVYLQMNSLRAEDTAVYYCARTVPGYSTAP  
30 YFDLWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
SGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEP  
KSC (SEQ ID NO: 101)

Figure 12A shows the Biacore analysis of binding potency for this CA645/IL-10 antibody fusion protein when tested with 100 nM IL-10 receptor (binding partner for IL-10), with and without prior incubation with 200 nM IL-10 (competing ligand). As is shown in Figure 12A, binding seen to IL-10 receptor is abolished in the presence of the competing ligand in molar excess, showing that the binding seen to the IL-10 portion in framework 3 of the fusion protein is a genuine functional interaction and not non-specific. This result also suggests that the inserted IL-10 monomers are also able to form a functional dimer within the framework 3 construct. Figure 12B shows a modelled representation of the CA645/IL-10<sub>Fwk3</sub> protein.

### EXAMPLE 10 – Antibody-framework 3 insertion constructs with other Fabs

Fusion proteins were generated and expressed in accordance with the above methods to insert the human IL-15 sequence into the framework 3 region of the CA497 and CA240 Fab constructs, as shown below.

*Anti-IL-17A CA497 Fab heavy chain sequence (Fab, CH1 from IgG1; Variable domain underlined; CDRs in bold and italics.)*

EVQLVESGGGLVKPGGSLRLSCAASGVIFSDYYMAWVRQAPGKGLEWVASINFNAD  
ISYYRESVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTDANRQNYDWFAYWG  
 QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS  
 GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC  
 (SEQ ID NO: 125)

*Anti-IL-17A CA497 Fab heavy chain with human IL-15 (bold and italics) inserted into framework 3, Gly-Ser linkers underlined:*

EVQLVESGGGLVKPGGSLRLSCAASGVIFSDYYMAWVRQAPGKGLEWVASINFNAD  
 ISYYRESVKGRFTISRDDSGGGSGGGGNWVNVISDLKKIEDLIQSMHIDATLYTESDV  
 HPSCKVTAMKCFLELQVISLESGDASIHDVTENLIILANNSLSSNGNVTESGCKECE  
ELEEKNIKEFLQSFVHIVQMFINGGGKNTLYLQMNSLKTEDTAVYYCTTDANRQNY  
 DWFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS  
 WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK  
 VEPKSC (SEQ ID NO: 83)

*Anti-IL-17A CA497 Fab light chain sequence:*

AIQLTQSPSSLSASVGDRVITITCKASESVSSSMYSYMHWYQQKPGKAPKLLIYRASN  
 LESGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSWTAPRTFGQGTKVEIKRTVA  
 APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS  
 5 KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 84)

*Anti-IL-6 CA240 Fab heavy chain sequence (Fab, CH1 from IgG1; Variable domain underlined; CDRs in bold and italics):*

EVQLVESGGGLVQPGGSLRLSCAAS***GFNFNDYFMNWVRQAPGKGLEWVAQMRNK***  
 10 ***NYOYGTYYAESLEGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCARESYYGFTSYWG***  
***QGT***LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS  
 GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC  
 (SEQ ID NO: 126)

15 *Anti-IL-6 CA240 Fab heavy chain with human IL-15 (bold and italics) inserted into framework 3, Gly-Ser linkers underlined:*

EVQLVESGGGLVQPGGSLRLSCAASGFNFNDYFMNWVRQAPGKGLEWVAQMRNK  
 NYQYGTYYAESLEGRFTISRDDSGGGSGGGG***NWVNVISDLKKIEDLIQSMHIDATLY***  
***TESDVHPSCKVTAMKCFLELQVISLES***GDASI***HDTVENLIILANNSLSSNGNV***TESG  
 20 ***CKECEELEEKNIKEFLQSFVHIVQMFINGGG***KNSLYLQMNSLKTEDTAVYYCARES  
 YYGFTSYWGQGT***LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV***  
 SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 KVEPKSC (SEQ ID NO: 85)

25 *Anti-IL-6 CA240 Fab light chain sequence:*

DIQMTQSPSSLSASVGDRVITITCQASQDIGISLSWYQQKPGKAPKLLIYNANNLADGV  
 PSRFSGSGSGTDFTLTISLQPEDFATYYCLQHNSAPYTFGQGTKLEIKRTVAAPS VFIF  
 PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS  
 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 86)

30

Figures 13 and 14 show the Biacore analysis of binding potency for the CA497/IL-15 and CA240/IL-15 (respectively) antibody fusion proteins when tested with 100 nM IL-2-R $\beta$  (binding partner for IL-15), with and without prior incubation with IL-15 (a competing ligand).

As is shown in both experiments, binding seen to IL-2-R $\beta$  is abolished in the presence of the competing ligand in molar excess, showing that the binding seen to the IL-15 portion in framework 3 of the fusion proteins is a genuine functional interaction and not non-specific.

## 5 EXAMPLE 11 – Fab constructs with a VHH inserted into framework 3

HyHEL5 is an anti-lysozyme Fab with the following sequences:

*HyHEL5 heavy chain:*

10 EVQLQQSGAELMKPGASVKISCKASGYTFS<sub>1</sub>SDYWIEWVKQRP<sub>1</sub>GHGLEWIG<sub>1</sub>EILPGSGS<sub>1</sub>  
 TNYHERFKGKATFTADTSSSTAYMQLNSLTSEDSGVYYCLHGN<sub>1</sub>YDFD<sub>1</sub>GWGQGTTLT<sub>1</sub>  
 VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT<sub>1</sub>TVSWNSGALTSGVHTFPA<sub>1</sub>  
 VLQSSGLYSLSSV<sub>1</sub>TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO:  
 87)

15

*HyHEL5 light chain:*

DIVLTQSPA<sub>1</sub>IMASAPGKVTMTCSASSSVNYMYWYQQKSGTSPKRWIYDTSKLASG<sub>1</sub>  
 VPVRFSGSGSGTSYSLTISSMETEDAATYYCQQWGRNPTFGGGTKLEIKRTVAAPSV<sub>1</sub>  
 FIFPPSDEQLKSGTASV<sub>1</sub>VCLLN<sub>1</sub>NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST<sub>1</sub>  
 20 YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 88)

D3L11 is an anti-lysozyme VHH with the following sequence:

*D3L11 :*

25 DVQLVESGGGSVQAGGSLRLSCAASGSTD<sub>1</sub>SI<sub>1</sub>EYMTWFRQAPGKAREGVAALYTHTG<sub>1</sub>  
 NTYYTDSVKGRFTISQDKAKNMAYLRMDSVKSEDTAIYTCGATRKYPV<sub>1</sub>RFALDQS<sub>1</sub>  
 SYDYWGQGTQVTVSS (SEQ ID NO: 89)

30 The following fusion constructs were generated to test whether inserting a second antibody molecule into the framework 3 region of a first antibody where the antibodies bound the same target antigen would result in a greater affinity for the antigen than either of the individual antibodies.

*Anti-lysozyme HyHEL5 light chain with anti-lysozyme VHH D3L11 (bold) inserted into framework 3, Gly-Ser linkers underlined:*

DIVLTQSPAIMASAPGEKVTMTCSASSSVNYMYWYQQKSGTSPKRWIYDTSKLAGS  
 VPVRFSGSGSGSGGGGSDVQLVESGGGSVQAGGSLRLSCAASGSTDSEIYMTWFR  
 5 **QAPGKAREGVAALY**THTGNTYYTDSVKGRFTISQDKAKNMAYLRMDSVKSED  
**TAIYTCGATRKYVPVRFALDQSSYDYWGQGTQVTVSS**GGGGSGGGGSGGGGSTS  
 YSLTISSMETEDAATYYCQQWGRNPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGT  
 ASVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKAD  
 YEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 90)

10

*Anti-lysozyme HyHEL5 light chain with anti-IL-6 VHH15 (bold) inserted into framework 3, Gly-Ser linkers underlined:*

DIVLTQSPAIMASAPGEKVTMTCSASSSVNYMYWYQQKSGTSPKRWIYDTSKLAGS  
 VPVRFSGSGSGSGGGGSQVQLVESGGGSVQAGGSLRLSCVAASGYTGCTYDMR  
 15 **WYRQAPGKEREFVSGIDSDGRATYADSVKGRFTISQ**NAKIAVYLQMDSLKLED  
**TAMYYCNLQCLRYPGEYYWGQGTQVTVSS**GGGGSGGGGSGGGGSTSYSLTISSM  
 ETEDAATYYCQQWGRNPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLL  
 NNFYPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKADYEKHKVY  
 ACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 91)

20

*CA645 light chain with anti-lysozyme VHH D3L11 (bold) inserted into framework 3, Gly-Ser linkers underlined:*

DIQMTQSPSSVSASVGDRVTITCQSSPSVWSNFLSWYQQKPGKAPKLLIYEASKLTSG  
 VPSRFSGSGSGSGGGGSDVQLVESGGGSVQAGGSLRLSCAASGSTDSEIYMTWFR  
 25 **QAPGKAREGVAALY**THTGNTYYTDSVKGRFTISQDKAKNMAYLRMDSVKSED  
**TAIYTCGATRKYVPVRFALDQSSYDYWGQGTQVTVSS**GGGGSGGGGSGGGGSTD  
 FTLTISSLQPEDFATYYCGGGYSSISDTTFGGGTVKVEIKRTVAAPSVFIFPPSDEQLKSG  
 TASVVCLLNFPYAPREKQVQWKVDNALQSGNSQESVTEQDSKDYSLSSSTLTLSKA  
 DYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 92)

30

The SPR traces from the dilution series conducted for each of the constructs when tested for binding to lysozyme is shown in Figures 15 to 17. From the subsequent analysis, using the methods described above, the following table reveals the binding affinity for lysozyme calculated for each construct:

**Table 2 – Binding affinities for antibody constructs**

| construct                                             | Fab specificity     | VHH specificity | Binding Affinity for lysozyme ( $K_D$ ) |
|-------------------------------------------------------|---------------------|-----------------|-----------------------------------------|
| Antibody: HyHEL5 Fab<br>Framework 3 Insert: VHH D3L11 | lysozyme            | lysozyme        | $2.6 \times 10^{-11}$ M                 |
| Antibody: HyHEL5 Fab<br>Framework 3 Insert: VHH15     | lysozyme            | IL-6            | $3.1 \times 10^{-10}$ M                 |
| Antibody: CA645 Fab<br>Framework 3 Insert: VHH D3L11  | Human serum albumin | lysozyme        | $4.8 \times 10^{-10}$ M                 |

As is clearly seen from the above results, the binding affinity for lysozyme increases (i.e. a decrease is seen in the  $K_D$ ) for the construct where the Fab and the VHH insert both bound to lysozyme. Figure 18 illustrates how this may happen at a molecular level. This also hypothesises that the linker length may be important to enable multivalent interactions on the same target molecule in such antibodies.

#### EXAMPLE 12 - CA645 Fab with IL-15 insert polypeptide using bovine linkers

Variants were generated on the CA645/IL-15<sub>Fwk3</sub> protein described in Example 1, where the linkers were changed to other types of linkers including bovine linkers, and their effects on binding tested. As well as the original heavy chain construct (SEQ ID NO: 54), further constructs were generated as shown below:

*CA645 Fab heavy chain with human IL-15 (bold and italics) inserted into framework 3 with bovine long linker (underlined)*

EVQLLES GGG L V Q P G G S L R L S C A V S G I D L S N Y A I N W V R Q A P G K G L E W I G I I W A S G T T  
FYATWAKGRFTISRDNSTSVHQETKKYQS NWVNVISDLKKIEDLIQSMHIDATLYTES  
***DVHPSCKVTAMKCFLLELQVISLES GDASIHD TVENLIHLANN SLSSNGNVTESGC KE***  
***CEELEEKNIKEFLQSFVHIVQMFINSYTYNYEWHVDVKNTVYLQMNSLRAEDTAV***  
YYCARTVPGYSTAPYFDLWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL  
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN  
HKPSNTKVDKKVEPKSC (SEQ ID NO: 93)

*CA645 Fab heavy chain with human IL-15 (bold and italics) inserted into framework 3 with bovine short linker (underlined)*

EVQLLES GGGLVQP GGS LRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNSETKKYQSNWVN***VISDLKKIEDLIQSMHIDATLYTESDVHPS***  
 5 ***CKVTAMKCFLLELQVISLES GDASI HDTVENLIILANNSLSSNGNVTESGCKECEELE***  
***EKNIKEFLQSFVHIVQMFINSYTYNYEKNTVY LQMNSLRAEDTAVYYCARTVPGYS***  
 TAPYFDLWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV  
 SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 KVEPKSC (SEQ ID NO: 94)

10

*CA645 Fab heavy chain with human IL-15 (bold and italics) inserted into framework 3 with 2xG4S linker (underlined)*

EVQLLES GGGLVQP GGS LRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNSSGGGGSGGGGSNWVN***VISDLKKIEDLIQSMHIDATLYTESD***  
 15 ***VHPSCVTAMKCFLLELQVISLES GDASI HDTVENLIILANNSLSSNGNVTESGCKE***  
***EELEEKNIKEFLQSFVHIVQMFINGGGGGSGGGGSKNTVY LQMNSLRAEDTAVYYC***  
 ARTVPGYSTAPYFDLWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD  
 YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP  
 SNTKVDKKVEPKSC (SEQ ID NO: 95)

20

Figure 19 shows the Biacore binding analysis of the above CA645/IL-15<sub>Fwk3</sub> constructs when tested with 100 nM IL-2-R $\beta$ , and compared against the construct in Example 1 (original linker) and the wild type CA645 Fab, which has no IL-15 insert. It was noted that all of the tested constructs showed successful binding.

25

### **EXAMPLE 13 – CA645 Fab fragment with a dsscFv inserted into framework 3**

#### **Gene design**

Two antibodies of the invention were generated and expressed in accordance with the above methods to insert an anti-IL17A CA497 dsscFv (vHvL, or vLvH) into the framework 3 region of the CA645 Fab fragment (scaffold antibody), as shown below. The resulting antibodies may be termed CA645 Fab-Fwk3 CA497 dsscFv (vHvL) or CA645 Fab-Fwk3 CA497 dsscFv (vLvH) respectively. The CA497 dsscFv and the CA645 Fab were generated as controls.

30

Genes encoding light and heavy chain V-regions, including variants with a framework 3 insertion, were designed and constructed by an automated synthesis approach (ATUM). Light

chain V-region genes (e.g, coding for the CA645 VL domain of SEQ ID NO: 2) were cloned into expression vectors containing DNA encoding human Cκ region (Km3 allotype). Heavy chain V-region genes were cloned into expression vectors containing DNA encoding IgG1 CH1 region.

5

*CA497 dsscFv (vHvL)*

EVQLVESGGGLVKPGGSLRLSCAASGVIFSDYYMAWVRQAPGKCLEWVASINFNAD  
ISYYRESVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTDANRQNYDWFAYW  
GQGTLLTVSSGGGGSGGGGSGGGGSAIQLTQSPSSLSASVGDRVTITCKASESVSSSM  
10 YSYMHWYQQKPGKAPKLLIYRASNLESGVPSRFSGSGSGTDFTLTISLQPEDFATYY  
CQQSWTAPRTFGCGTKVEIK (SEQ ID NO: 127)

*CA497 dsscFv (vLvH)*

AIQLTQSPSSLSASVGDRVTITCKASESVSSSMYSYMHWYQQKPGKAPKLLIYRASN  
15 LESGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQSWTAPRTFGCGTKVEIKGGGG  
SGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGVIFSDYYMAWVRQAPGKC  
LEWVASINFNADISYYRESVKGRFTISRDDSKNTLYLQMNSLKTEDTAVYYCTTDAN  
RQNYDWFAYWGQGTLLTVSS (SEQ ID NO: 128)

20 *CA645 Fab heavy chain with CA497 dsscFv (vHvL) (bold and italics) inserted into framework  
3, linkers underlined*

EVQLLESGGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGVIFS  
***DYYMAWVRQAPGKCLEWVASINFNADISYYRESVKGRFTISRDDSKNTLYLQMNSLK***  
25 ***TEDTAVYYCTTDANRQNYDWFAYWGQGTLLTVSSGGGGSGGGGSGGGGSAIQLTQS***  
***PSSLSASVGDRVTITCKASESVSSSMYSYMHWYQQKPGKAPKLLIYRASNLESGVPSR***  
***FSGSGSGTDFTLTISLQPEDFATYYCQQSWTAPRTFGCGTKVEIKGGGGSGGGGSK***  
NTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGTLLTVSSASTKGPSVFP  
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS  
30 VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 129)

*CA645 Fab heavy chain with CA497 dsscFv (vLvH) (bold and italics) inserted into framework  
3, linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCA VSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNSSGGGSGGGGS*AIQLTQSPSSLSASVGDRVITITCKASESVSSS*  
*MYSYMHWYQQKPGKAPKLLIYRASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFATY*  
*YCQQSWTAPRTFGCGTKVEIKGGGSGGGGSGGGGSEVQLVESGGGLVKPGGSLRL*  
 5 *SCAASGVIFSDYYMAWVRQAPGKCLEWVASINFNADISYYRESVKGRFTISRDDSKNT*  
*LYLQMNSLKTEDTAVYYCTTDANRQNYDWFAYWGQGLVTVSSGGGSGGGGSKN*  
 TVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGLVTVSSASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV  
 VTPVSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 130)

10

### Transient cell expression

Engineered Fab fragments were transiently expressed in either HEK-293 cells using  
 ExpiFectamine 293 transfection kit (Life Technologies, according to the manufacturer's  
 instructions) or CHO-S XE cells, a CHO-K1 derived cell line (Cain K et al, A CHO cell line  
 15 engineered to express XBP1 and ERO1- $\alpha$  has increased levels of transient protein expression.  
 Biotechnology Progress 2013; 29: 697-706), using ExpiFectamine CHO transfection kit (Life  
 Technologies, according to the manufacturer's instructions). HEK-293 cells were used for  
 small scale expression (2 ml) to prepare antibody constructs for direct SPR analysis. CHO-S  
 XE cells were used for large scale expression (200 ml) to prepare antibody constructs for  
 20 purification prior to further characterization.

### Purification

Affinity chromatography was used to purify Fab fragments from culture supernatants.  
 Supernatants containing the antibodies were passed over a HiTrap Protein G column (GE  
 25 Healthcare). Following a washing step with phosphate buffered saline (PBS) pH 7.4, the bound  
 material was eluted with a gradient of PBS pH 7.4 versus an increasing percentage by volume  
 of 0.1M phosphate citrate buffer pH 2.6. The eluate was neutralized with 2 M Tris-HCl (pH 8).  
 Fractions were pooled, quantified by absorbance at 280 nm, and concentrated using Amicon  
 Ultra centrifugal filters (Merck Millipore). To isolate the monomeric fractions of the  
 30 constructs, size exclusion chromatography over a HiLoad 16/60, Superdex 200 column (GE  
 Healthcare) equilibrated with PBS pH 7.4, was used. Fractions containing monomeric Fab were  
 pooled, quantified, concentrated and stored at 4 °C.

UPLC (Ultra-high-performance liquid chromatography):

2µg of sample was injected onto a SEC BEH200 column, pre-equilibrated in PBS, pH7.4 and run for 10 mins. Fluorescence intensity was recorded against elution time and peak areas picked manually in EPower software.

The UPLC elution profiles were obtained and analysed. Peaks appeared >98% monomeric (99,42% for CA645 Fab-Fwk3 CA497 dsscFv (vLvH), 99,51% for CA645 Fab-Fwk3 CA497 dsscFv (vHvL)). The controls CA497 dsscFv and CA645 Fab showed respectively 99,99% and 98.43% of multimeric forms (data not shown). Accordingly, the purified proteins were homogenous and lacked multimeric forms or aggregates.

## 10 Molecular stability

The molecular stability was measured by melting temperature (T<sub>m</sub>) (measure of unfolding) analysis by Differential Scanning Calorimetry (DSC) method. Thermograms were acquired on an automated MicroCal VP DSC (Malvern Panalytical) from 0-100°C, using a step rate of 1°C/min, with a pre-incubation scan time of 15 min, a filtering period of 5s and feedback set to passive. The data was buffer subtracted before a manual baseline subtraction and concentration correction. The thermograms were fitted to a non-2-state model in Origin7.0.

The results are shown in figure 20 (DSC curves; vertical axis: (C<sub>p</sub>) specific heat; horizontal axis: temperature in Degrees C (°C)) and in the table 3 below. CA497 dsscFv and CA645 Fab showed single transitions of 58.40°C and 83.57°C respectively. When combined in the Fab-Fwk3 inserted dsscFv format, the CA497 dsscFv T<sub>m</sub> is almost identical (difference of 2-3 °C) and the CA645 Fab has a variation of T<sub>m</sub> of about 4-5°C. Accordingly, the pairing of CA497 dsscFv and CA645 Fab in this antibody format according to the invention does not impact significantly the conformational stability of either domain.

**Table 3 -Unfolding transition temperatures**

| Sample                                                            | T <sub>m</sub> 1 (°C) | T <sub>m</sub> 2 (°C) |
|-------------------------------------------------------------------|-----------------------|-----------------------|
| CA645 Fab                                                         | 83.57                 |                       |
| CA497 dsscFv                                                      | 58.40                 |                       |
| CA645 Fab heavy chain with CA497 dsscFv (vLvH) inserted into Fwk3 | 55.59                 | 78.64                 |

|                                                                                  |              |              |
|----------------------------------------------------------------------------------|--------------|--------------|
| <b>CA645 Fab heavy chain<br/>with CA497 dsscFv (vHvL)<br/>inserted into Fwk3</b> | <b>55.41</b> | <b>78.73</b> |
|----------------------------------------------------------------------------------|--------------|--------------|

### Neutralisation assay

The potency of antibody CA497 IgG4, CA645 Fab-Fwk3 CA497 dsscFv (vHvL) and CA645 Fab-Fwk3 CA497 dsscFv (vLvH) against human recombinant IL-17A in HeLa cells was tested, in presence or in absence of HSA. HeLa cells were obtained from the cell bank at ATCC (ATCC CCL-2). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum, penicillin, gentamycin and glutamine.  $1 \times 10^4$  cells were plated out into 96 well flat bottomed tissue culture plates. Cells were incubated overnight and washed once in assay buffer. Human IL-17A ( $25 \text{ ng ml}^{-1}$ ) was incubated in the presence of a fixed concentration of human TNF- $\alpha$ , and this mixture was preincubated with the tested antibody. The cytokines and antibody were then added to the HeLa cells to stimulate the cells which were incubated overnight. In the HeLa cell line, IL-17A synergises with TNF- $\alpha$  to induce the production of IL-6 which can be quantified, e.g. by ELISA. The production of IL-6 in the cell culture supernatant is proportionate to the amount of IL-17A added to the cells. Human IL-6 levels were measured by ELISA and quantified by comparison with known standard concentrations of human IL-6. In this assay, the inhibition of the secretion of IL-6 is correlated with the inhibition of IL-17A.

The inhibition curves are shown in Figure 21 (horizontal axis: antibody (inhibitor) concentration in ng/ml, vertical axis: inhibition of the secretion of IL-6 (%)) and indicate that the parent antibody CA497 IgG4 and both antibody fusion constructs potently neutralised human recombinant IL-17A. In addition, the results indicate that the dsscFv anti-IL-17 retained functionality when inserted into the Fwk3 region of the Fab anti-HSA. The EC<sub>50</sub> values calculated from these experiments (table 4 below) indicated that antibody CA645 Fab-Fwk3 CA497 dsscFv (vLvH) and CA645 Fab-Fwk3 CA497 dsscFv (vHvL) gave an EC<sub>50</sub> of 10.5 ng/ml and 10.6 ng/ml respectively against human recombinant IL-17A ( $25 \text{ ng ml}^{-1}$ ) and that the neutralisation potency was not affected by the presence of HSA (EC<sub>50</sub> of 10.8 and 8.7 ng/ml respectively, in presence of HSA). Also, the CA645 Fab-Fwk3 CA497 dsscFv constructs had a neutralisation potency comparable to the one of the parent CA497 IgG4 antibody.

**Table 4-EC50 values for the neutralisation of IL-17A in presence or absence of HSA**

|                                    | HSA (2,5%) | IL-17A EC50 (ng/ml) |
|------------------------------------|------------|---------------------|
| CA497 IgG4                         | -          | 8.1                 |
| CA497 IgG4                         | +          | 7.6                 |
| CA645 Fab-Fwk3 CA497 dsscFv (vLvH) | -          | 10.5                |
| CA645 Fab-Fwk3 CA497 dsscFv (vLvH) | +          | 10.8                |
| CA645 Fab-Fwk3 CA497 dsscFv (vHvL) | -          | 10.6                |
| CA645 Fab-Fwk3 CA497 dsscFv (vHvL) | +          | 8.7                 |

#### 5 **EXAMPLE 14 – Full CA645 IgG with a dsscFv inserted into framework 3**

A fusion protein was generated and expressed in accordance with the above methods to insert CA497 dsscFv (vH-vL) or CA497 dsscFv (vL-vH) into the framework 3 region of the CA645 IgG4P construct, as shown below. The resulting antibody fusion proteins may be termed CA645 IgG4P Fwk3-CA497 dsscFv (vHvL) and CA645 IgG4P Fwk3-CA497 dsscFv (vLvH).

#### 10 **Gene design**

Genes encoding light and heavy chain V-regions, including variants with a framework 3 insertion, were designed and constructed by an automated synthesis approach (ATUM).

Light chain V-region genes (e.g. coding for the CA645 VL domain of SEQ ID NO: 2) were cloned into expression vectors containing DNA encoding human C $\kappa$  region (Km3 allotype).

15 Heavy chain V-region genes were cloned into expression vectors containing DNA encoding IgG4P constant regions for full-length antibody.

*CA645 IgG4P heavy chain with CA497 dsscFv (vHvL) (bold and italics) inserted into framework 3, linkers underlined:*

20 EVQLLES GGGLVQPGGSLRLSCAVSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
FYATWAKGRFTISRDNSSGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGVIFS  
DYMAWVRQAPGKCLEWVASINFNADISYYRESVKGRFTISRDDSKNTLYLQMNSLK

***TEDTAVYYCTTDANRQNYDWFAYWGQGLVTVSSGGGGSGGGGSGGGGSAIQLTQS***  
***PSSLSASVGDRVITITCKASESVSSSMYSYMHWYQQKPGKAPKLLIYRASNLESGVPSR***  
***FSGSGSGTDFTLTISLQPEDFATYYCQQSWTAPRTFGCGTKVEIKGGGGSGGGGSK***  
 NTVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGLVTVSSASTKGPSVFP  
 5 LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSS  
 VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFL  
 FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS  
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR  
 DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV  
 10 DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 131)

*CA645 IgG4P heavy chain with CA497 dsscFv (vLvH) (bold and italics) inserted into framework 3, linkers underlined:*

EVQLLESGGGLVQPGGSLRLSCA VSGIDLSNYAINWVRQAPGKGLEWIGIIWASGTT  
 FYATWAKGRFTISRDNSGGGGSGGGGSAIQLTQSPSSLSASVGDRVITITCKASESVSSS  
 15 ***MYSYMHWYQQKPGKAPKLLIYRASNLESGVPSRFSGSGSGTDFTLTISLQPEDFATY***  
***YCQQSWTAPRTFGCGTKVEIKGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGSLRL***  
***SCAASGVIFSDYYMAWVRQAPGKCLEWVASINFNADISYYRESVKGRFTISRDDSKNT***  
***LYLQMNSLKTEDTAVYYCTTDANRQNYDWFAYWGQGLVTVSSGGGGSGGGGSKN***  
 TVYLQMNSLRAEDTAVYYCARTVPGYSTAPYFDLWGQGLVTVSSASTKGPSVFPL  
 20 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV  
 VVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLF  
 PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST  
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD  
 ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV  
 25 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 132)

### **Transient cell expression**

Engineered antibodies were transiently expressed in either HEK-293 cells using  
 ExpiFectamine 293 transfection kit (Life Technologies, according to the manufacturer's  
 30 instructions) or CHO-S XE cells, a CHO-K1 derived cell line, using ExpiFectamine CHO  
 transfection kit (Life Technologies, according to the manufacturer's instructions). HEK-293  
 cells were used for small scale expression (2 ml) to prepare antibody constructs for direct SPR

analysis. CHO-S XE cells were used for large scale expression (200 ml) to prepare antibody constructs for purification prior to further characterization.

### **Purification**

5 Affinity chromatography was used to purify IgG from culture supernatants. Supernatants containing IgG were passed over a MabSelect SuRe column (GE Healthcare). Following a washing step with phosphate buffered saline (PBS) pH 7.4, the bound material was eluted with a gradient of PBS pH 7.4 versus an increasing percentage by volume of 0.1M phosphate citrate buffer pH 2.6. The eluate was neutralized with 2 M Tris-HCl (pH 8). Fractions were pooled,  
10 quantified by absorbance at 280 nm, and concentrated using Amicon Ultra centrifugal filters (Merck Millipore). To isolate the monomeric fractions of IgG-based constructs, size exclusion chromatography over a HiLoad 16/60, Superdex 200 column (GE Healthcare) equilibrated with PBS pH 7.4, was used. Fractions containing monomeric IgG constructs were pooled, quantified, concentrated and stored at 4 °C.

15

### **Simultaneous binding to IL-17 and HSA**

In order to test simultaneous binding of IL-17A and HSA to CA645 IgG4P Fwk3-CA497 dsscFv (vHvL), the latter was immobilised on a Biacore chip in place of polyclonal goat F(ab)<sub>2</sub> in the same procedure as previously. Three concentrations of both IL-17A and HSA were  
20 selected to give low, medium and high levels of binding to the immobilized antibody, specifically these corresponded to stoichiometric ratios of ligand to antibody in the ranges 0.15 to 0.3, 0.4 to 0.6 and 0.65 to 0.75 respectively. In the case of IL-17A concentrations were 4, 20 and 100 nM and for HSA concentrations were 40, 200 and 1000 nM respectively. The above low, medium and high concentrations, were tested by SPR as previously described in individual  
25 cycles either as separate ligands or as IL17A / HSA mixtures and report points corrected by subtraction of buffer control cycles. The results are shown in Figure 22 and show that the CA645 IgG4P Fwk3-CA497 dsscFv (vHvL) bind both IL-17A and HSA, in a concentration dependent manner. Figure 22 further shows that in the presence of both binding partners, the signal is additive, showing that binding to both IL-17A and HSA can occur simultaneously.

### **30 EXAMPLE 15- Antibodies of the invention which bind to both CD3 and CD28**

#### **Gene design**

The antibodies were generated and expressed in accordance with the methods described above to insert anti-CD3 dsscFv (vH-vL) into the framework 3 region of the anti-CD3 or anti-CD28

scaffold antibody (IgG4P or Fab fragment) as shown below in Table 5. The resulting antibody constructs according to the invention may be termed anti-CD28 Fab Fwk3-anti-CD3 dsscFv (LC of SEQ ID NO: 136, HC of SEQ ID NO: 139), anti-CD3 Fab Fwk3-anti-CD3 dsscFv (LC of SEQ ID NO: 133, HC of SEQ ID NO: 140), anti-CD28 IgG4P Fwk3-anti-CD3 dsscFv (LC of SEQ ID NO: 136, HC of SEQ ID NO: 141), anti-CD3 IgG4P Fwk3-anti-CD3 dsscFv (LC of SEQ ID NO: 133, HC of SEQ ID NO: 142).

**Table 5 – Antibody Constructs**

| Scaffold antibody | Antigen specificity (V-domain of the scaffold) | Antigen specificity (dsscFv inserted into Fwk3) | Sequence                                                       |
|-------------------|------------------------------------------------|-------------------------------------------------|----------------------------------------------------------------|
| Fab               | CD3                                            | none                                            | Light chain of SEQ ID NO: 133<br>Heavy chain of SEQ ID NO: 134 |
| Fab               | CD28                                           | none                                            | Light chain of SEQ ID NO: 136<br>Heavy chain of SEQ ID NO: 137 |
| Fab               | CD28                                           | CD3                                             | Light chain of SEQ ID NO: 136<br>Heavy chain of SEQ ID NO: 139 |
| Fab               | CD3                                            | CD3                                             | Light chain of SEQ ID NO: 133<br>Heavy chain of SEQ ID NO: 140 |
| IgG4P             | CD3                                            | none                                            | Light chain of SEQ ID NO: 133<br>Heavy chain of SEQ ID NO: 135 |
| IgG4P             | CD28                                           | none                                            | Light chain of SEQ ID NO: 136<br>Heavy chain of SEQ ID NO: 138 |
| IgG4P             | CD28                                           | CD3                                             | Light chain of SEQ ID NO: 136<br>Heavy chain of SEQ ID NO: 141 |
| IgG4P             | CD3                                            | CD3                                             | Light chain of SEQ ID NO: 133<br>Heavy chain of SEQ ID NO: 142 |

10

*Anti-CD3 Light chain, muromonab (humanised OKT3) (Variable domain underlined; CDRs in bold and italics):*

DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKLLIYDTSKLASGVP  
SRFSGSGSGTDYFTFTISLQPEDATYYCQOWSSNPFTFGQGTKLEIKRTVAAPSVFIFP  
 PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL  
 SSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 133)

- 5 *Anti-CD3 Heavy chain Fab, CH1 from IgG1, muromonab (humanised OKT3) (Variable domain underlined; CDRs in bold and italics):*

QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRG  
YTNYNOKFKDRFTISRDN SKNTAFLQMDSL RPEDTGVYFCARYYDDHYCLDYWGQG  
TPVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH  
 10 TFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID  
 NO: 134)

*Anti-CD3 Heavy chain IgG4P, muromonab (humanised OKT3) (Variable domain underlined; CDRs in bold and italics):*

- QVQLVQSGGGVVPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGYINPSRG  
 15 YTNYNOKFKDRFTISRDN SKNTAFLQMDSL RPEDTGVYFCARYYDDHYCLDYWGQG  
TPVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH  
 TFP AVLQSSGLYSLSSVVTVPSSSLGTQTYTCNV DHKPSNTKVDKR VESKYGP PCPPC  
 PAPEFLGGPSVFLFPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHN  
 AKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQP  
 20 REPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD S  
 DGSFFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHYTQKSLSLSLGK (SEQ ID NO:  
 135)

*Anti-CD28 Light chain, theralizumab (Variable domain underlined; CDRs in bold and italics):*

- DIQMTQSPSSLSASVGDRVTITCHASONIYVWLNWYQQKPGKAPKLLIYKASNLHTG  
 25 VPSRFSGSGSGTDFTLTISLQPEDFATYYCQOGOTYPYTFGGGTKVEIKRTVAAPSVF  
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY  
 SLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 136)

*Anti-CD28 Heavy chain Fab, CH1 from IgG1, theralizumab (Variable domain underlined; CDRs in bold and italics):*

QVQLVQSGAEVKKPGASVKVSCKAS***GYTFTSY******YIHWVRQAPGQGLEWIGCIYPGNV***  
***NTNYNEKFKDRATLTVDTSISTAYMEL***SRLRSDDTAVYFCTR***SHYGLDWNFDVWGQ***  
 GTT***TVTVSS***ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG  
 VHTFPAVLQSSGLYSLSSV***TVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC*** (SEQ  
 5 ID NO: 137)

*Anti-CD28 Heavy chain IgG4P, theralizumab (Variable domain underlined; CDRs in bold and italics):*

QVQLVQSGAEVKKPGASVKVSCKAS***GYTFTSY******YIHWVRQAPGQGLEWIGCIYPGNV***  
***NTNYNEKFKDRATLTVDTSISTAYMEL***SRLRSDDTAVYFCTR***SHYGLDWNFDVWGQ***  
 10 ***GTTTVTVSS***ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV  
 HTFPAVLQSSGLYSLSSV***TVTPSSSLG***TKTYTCNV***DKHPSNTKVDKRVESKYGP***PCPP  
 CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSQEDPEVQFNWYVDGVEVH  
 NAKTKPREEQFNSTYRVVSVLT***TVLH***QDWLN***GKEYKCKVSNKGLPSSIEKTISKAKG***  
 QPREPQVYTLPPS***QEEMTKNQVSLTCLVKGF***YPSDIAVEWESNGQPENNYK***TTTPVL***  
 15 ***DSDGSFFLYSRLTVDKSRWQEGNVFSCSV***MHEALHNHYTQKSLSLSLGK (SEQ ID  
 NO: 138)

*Anti-CD28 Fab heavy chain with anti-CD3 dsscFv (vHvL) (bold and italics) inserted into framework 3, linkers underlined:*

QVQLVQSGAEVKKPGASVKVSC***KASGYTFTSY******YIHWVRQAPGQGLEWIGCIYPGNV***  
 20 ***NTNYNEKFKDRATLTVDTS***SGGGGSGGGGS***QVQLVQSGGGVVQPGRSLRLSCKASGY***  
***TFTRYTMHWVRQAPGKCLEWIGYINPSRGYTNYNQKFKDRFTISRDN***SKNTA***FLQM***  
***DSLRPEDTGVYFCARYYDDHYCLDYWGQ***GP***TVTVSSGGGGSGGGGSGGGGSGGGG***  
***SDIQMTQSPSSLSASV***GDRVTITCSASSSVSYMNWY***QQTPGKAPKLLIYDTSKLASGVP***  
***SRFSGSGSGTDYFT***ISS***LQPED***ATYYC***QQWSSNPFTFGCGTKLEIKGGGGSGGGGS***  
 25 ***ISTAYMEL***SRLRSDDTAVYFCTR***SHYGLDWNFDVWGQ***GTT***TVTVSS***ASTKGPSVFPL  
 APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV  
 VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC (SEQ ID NO: 139)

*Anti-CD3 Fab heavy chain with anti-CD3 dsscFv (vHvL) (bold and italics) inserted into framework 3, linkers underlined:*

30 QVQLVQSGGGVV***QPGRSLRLSCKASGYTFTRYTMHWVRQAPGK***GLEWIGYINPSRG  
 YTNYN***QKFKDRFTISRDN***SGGGGSGGGGS***QVQLVQSGGGVVQPGRSLRLSCKASGY***

***TFTRYTMHWVRQAPGKCLEWIGYINPSRGYTNYNQKFKDRFTISRDN SKNTAFLQM***  
***DSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSGGGGSGGGGSGGGGSGGGG***  
***SDIQMTQSPSSLSASVGDRVITITCSASSSVSYMNWYQQTPGKAPKLLIYDTSKLASGVP***  
***SRFSGSGSGTDYFTISSLQPEDATYYCQQWSSNPFTFGCGTKLEIKGGGGSGGGGS***  
5 ***KNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSASTKGPSVFPLA***  
***PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV***  
***TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC*** (SEQ ID NO: 140)

*Anti-CD28 IgG4P heavy chain with anti-CD3 dsscFv (vHvL) (bold and italics) inserted into framework 3, linkers underlined:*

10 ***QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYIHVVRQAPGQGLEWIGCIYPGNV***  
***NTNYNEKFKDRATLTVDTS******GGGGSGGGGS******QVQLVQSGGGVVQPGRSLRLSCKASGY***  
***TFTRYTMHWVRQAPGKCLEWIGYINPSRGYTNYNQKFKDRFTISRDN SKNTAFLQM***  
***DSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSGGGGSGGGGSGGGGSGGGG***  
***SDIQMTQSPSSLSASVGDRVITITCSASSSVSYMNWYQQTPGKAPKLLIYDTSKLASGVP***  
15 ***SRFSGSGSGTDYFTISSLQPEDATYYCQQWSSNPFTFGCGTKLEIKGGGGSGGGGS***  
***ISTAYMELSRLRSDDTAVYFCTRSHYGLDWNFDVWGQTTVTVSSASTKGPSVFPL***  
***APCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV***  
***TVPSSSLGTQTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKP***  
***KDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVV***  
20 ***SVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTK***  
***NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRW***  
***QEGNVFSCSVMHEALHNHYTQKSLSLGLK*** (SEQ ID NO: 141)

*Anti-CD3 IgG4P heavy chain with anti-CD3 dsscFv (vHvL) (bold and italics) inserted into framework 3, linkers underlined:*

25 ***QVQLVQSGGGVVQPGRSLRLSCKASGYTFTTRYTMHWVRQAPGKCLEWIGYINPSRG***  
***YTNYNQKFKDRFTISRDN******SGGGSGGGGS******QVQLVQSGGGVVQPGRSLRLSCKASGY***  
***TFTRYTMHWVRQAPGKCLEWIGYINPSRGYTNYNQKFKDRFTISRDN SKNTAFLQM***  
***DSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSGGGGSGGGGSGGGGSGGGG***  
***SDIQMTQSPSSLSASVGDRVITITCSASSSVSYMNWYQQTPGKAPKLLIYDTSKLASGVP***  
30 ***SRFSGSGSGTDYFTISSLQPEDATYYCQQWSSNPFTFGCGTKLEIKGGGGSGGGGS***  
***KNTAFLQMDSLRPEDTGVYFCARYYDDHYCLDYWGQGTPVTVSSASTKGPSVFPLA***  
***PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT***

VPSSSLGTKTYTCNVDPKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPK  
 DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVS  
 VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKN  
 QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ  
 5 EGNVFSCSVMHEALHNHYTQKSLSLGLK (SEQ ID NO: 142)

UPLC (Ultra-high-performance liquid chromatography):

2µg of sample was injected onto a SEC BEH200 column, pre-equilibrated in PBS, pH7.4 and run for 10 mins. Fluorescence intensity was recorded against elution time and peak areas picked manually in EMpower software.

- 10 The UPLC elution profiles were obtained and analysed. Peaks appeared >98% monomeric (anti-CD3 IgG4P Fwk3-anti-CD3 dsscFv: 99,02%, anti-CD3 Fab Fwk3-anti-CD3 dsscFv: 99,79%, anti-CD28 Fab Fwk3-anti-CD3 dsscFv: 99,48%) with the exception of a lower molecular weight shoulder of 12.12% in anti-CD28 IgG4P Fwk3-anti-CD3 dsscFv. There was no evidence of multimerization of any format.

15

### Molecular Stability

The molecular stability was measured by melting temperature (T<sub>m</sub>) (measure of unfolding) analysis by Differential Scanning Calorimetry (DSC) method as described above. The DSC curves are shown in Figure 23 (A) and (B), and the T<sub>m</sub> values presented in Table 6 and 7  
 20 below.

**Table 6- Fab-Fwk3-dsscFv**

| Antibody of the invention          | T <sub>m</sub> 1 (°C) | T <sub>m</sub> 2 (°C) |
|------------------------------------|-----------------------|-----------------------|
| Anti-CD28 Fab Fwk3-anti-CD3 dsscFv | 70.16                 | 74.73                 |
| Anti-CD3 Fab Fwk3-anti-CD3 dsscFv  | 70.04                 |                       |

- 25 The DSC curves for anti-CD3 Fab Fwk3-anti-CD3 dsscFv showed a single unfolding transition at 70.04°C. In contrast, the unfolding of the construct containing anti-CD28 cannot be accurately described by a single transition but fit better to a non-2-state, two transition model; the lowest of which can be attributed to the dsscFv component. The results notably show that

inserting the Fab in the framework 3 position resulted in stable constructs, as the values 65°C -75°C are in line with that of a typical IgG.

**Table 7- IgG4P-Fwk3-dsscFv**

| Antibody of the invention            | Tm1 (°C) | Tm2 (°C) |
|--------------------------------------|----------|----------|
| Anti-CD28 IgG4P Fwk3-anti-CD3 dsscFv | 68.97    | 73.28    |
| Anti-CD3 IgG4P Fwk3-anti-CD3 dsscFv  | 68.55    |          |

5

The DSC curves for anti-CD3 IgG4P Fwk3-anti-CD3 dsscFv showed a single unfolding transition at 68.55°C. In contrast, the unfolding of constructs containing anti-CD28 cannot be accurately described by a single transition but fit better to a non-2-state, two transition model; the lowest of which can be attributed to the dsscFv component. The results notably show that inserting the IgG in the framework 3 position resulted in stable constructs, as the Tm values of 65°C -75°C are typical of IgG-like molecules.

10

#### **Evaluation of Fab and IgG4P constructs with framework 3 inserts in a human functional PBMC assay**

15

Constructs were tested for their ability to stimulate T cell activation in a culture of peripheral blood mononuclear cells (PBMC) according to the method described below.

#### Reagents

20

Antibodies used to detect CD4 and CD8 T cells, distinguish live and dead cells and measure the activation marker CD69 were obtained from BD Biosciences (Anti-CD4 antibody, anti-CD8, anti-CD69 antibody and the antibody distinguishing Live/dead cells were coupled respectively to the fluorophores FITC, PE, BV421 and Near Infrared. All cell culture was performed in RPMI1640 (Thermo) plus 5% heat-inactivated human AB serum (Sigma) and 2mM glutamine (Thermo). All flow cytometry staining was performed using a standard flow cytometry buffer comprising Dulbecco's PBS (Thermo) plus 1% bovine serum albumin (Sigma), 2mM EDTA (Sigma) and 0.1% sodium azide (Sigma). Human OKT3 (Ebioscience) and T cell Transact (Miltenyi) a colloidal suspension of anti-CD3 and CD28. All flow cytometry data was acquired using an iQUEplus (Sartorius). T cells were identified using

25

Forecyt software (Sartorius) and data metrics imported into GraphPad Prism 7 (GraphPad) for data transformations and graphical representation.

### Method

Antibody constructs in table 5 were diluted down from 25µg/ml in eight 1 in 5 dilution steps. In the post analysis step all these values were converted into nM concentrations to allow equal comparisons of all constructs. Into 96 well 'U' bottomed tissue culture plates  $1.5 \times 10^5$  PBMC (from two different donors anonymised as donor 1 and 2) were mixed with the diluted antibodies. OKT3 (5µg/ml), Transact (CD3/CD28 diluted 1 in 100) and media alone were reference controls for the experiment. After mixing, plates were incubated at 37 degrees Celsius for 24 hours. After this time, plates were cooled on ice and spun (all subsequent spins were at 500g for 5 mins at 4 degrees Celsius) and supernatant removed from each well. Plates were washed again, supernatant aspirated and 20µl of an antibody cocktail added to each well (all antibodies were diluted 1:20 in flow cytometry buffer and 1µl of live/dead stain added per 1ml of cocktail). Plates were left on ice in the dark for 1 hour. After this time plates were washed a further two times and supernatant removed from all wells. Plates were finally resuspended in 20µl of flow cytometry buffer and at least 10,000 cells per well acquired.

### Results

The results are shown in Figure 24 (A-D; the vertical axis represents the level of expression of CD69 (MFI) and the horizontal axis represents the concentration of antibody construct in nM). The experiment was deemed successful since the positive control (OKT3) induced the early activation marker, CD69, on both CD4 and CD8 T cells. The minimum (cells only) level of activation was determined by the cells only control. As can be seen from figure 24(A) and (B), the induction of CD69 on CD4 T cells for the tested constructs was consistent between donors.

The antibodies of the invention containing CD3 and CD28 (either in the Fab or IgG4P format) were the most potent activators of CD4 T cells. As expected in this assay constructs containing an Fc can also bring in added activity via Fc receptors on other (non) T cells, in particular monocytes, which give enhanced costimulatory signals. In this respect the anti-CD3 IgG4P Fwk3-anti-CD3dsccFv also showed activity in this assay (as does the OKT3 control which is a whole IgG anti-CD3 antibody). This data demonstrates that antibodies of the invention which bind to both CD3 and CD28 are functional activators of human CD4 T cells in a mixed PBMC assay, and as such are capable of exercising the function of a bispecific antibody.

In addition, activation of CD8 T cells was also measured in the same assay. As described previously all positive and negative controls performed as expected. As can be seen from figure 24 (C) and (D), the induction of CD69 on CD8 T cells for the tested constructs was consistent between donors. The antibodies of the invention containing CD3 and CD28 (either in the Fab or IgG4P format) were the most potent activators of CD8 T cells. The anti-CD3 IgG4P Fwk3 anti-CD3dsscFv also showed activity in this assay (as does the OKT3 control which is a whole IgG anti-CD3 antibody). This data demonstrates that antibodies of the invention which bind to both CD3 and CD28 are functional activators of human CD8 T cells in a mixed PBMC assay, and as such are capable of exercising the function of a bispecific antibody.

10 The results show that antibodies according to the invention have the functionality of both the scaffold antibody (e.g. anti-CD28 Fab or IgG4P), and the insert polypeptide, i.e. the second antibody (e.g. anti-CD3 dsscFv) when the insert polypeptide is present in the Fwk3 region of the scaffold antibody.

15 Comprising in the context of the present specification is intended to mean including.

Where technically appropriate, embodiments of the invention may be combined. Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

20

It will of course be understood that, although the present invention has been described by way of example, the examples are in no way meant to be limiting, and modifications can be made within the scope of the claims hereinafter. Preferred features of each embodiment of the invention are as for each of the other embodiments *mutatis mutandis*. All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication was specifically and individually indicated to be incorporated by reference herein.

25

# CLAIMS

1. An antibody comprising a variable (V) domain and an insert polypeptide, wherein the insert polypeptide is within the framework 3 region of the V domain, and wherein the insert polypeptide is heterologous to the antibody comprising a V domain and wherein both the antibody comprising a V domain and the insert polypeptide are functional and/or retain functionality when the insert polypeptide is present.
2. The antibody according to claim 1, wherein the V domain is a VH domain.
3. The antibody according to claim 2, which further comprises a VL domain.
4. The antibody according to claim 2, wherein the insert polypeptide is between amino acid residues 73 and 76 of the VH domain.
5. The antibody according to claim 4, wherein the insert polypeptide is in the VH domain: (i) between amino acid residues 73 and 74; (ii) between amino acid residues 74 and 75; or (iii) between amino acid residues 75 and 76.
6. The antibody according to claim 4 or 5, wherein one or more amino acid residues between 73 and 76 of the VH domain are replaced by the insert polypeptide.
7. The antibody according to claim 1, wherein the V domain is a VL domain.
8. The antibody according to claim 7, which further comprises a VH domain.
9. The antibody according to claim 7, wherein the insert polypeptide is between amino acid residues 67 and 70 of the VL domain.
10. The antibody according to claim 9, wherein the insert polypeptide is in the VL domain: (i) between amino acid residues 67 and 68; (ii) between amino acid residues 68 and 69; or (iii) between amino acid residues 69 and 70.

11. The antibody according to claim 9 or 10, wherein one or more amino acid residues between 67 and 70 of the VL domain are replaced by the insert polypeptide.

12. The antibody according to any one of claims 1 to 11, further comprising a linker sequence of at least one amino acid joining the N- and/or C-terminal end of the insert polypeptide to the framework 3 region.

13. The antibody according to claim 12, wherein the linker sequence is at least two, three, four, five, six, seven or eight or more amino acids at the N-terminal end of the insert polypeptide portion.

14. The antibody according to claim 12 or 13, wherein the linker sequence is at least two, three, four, five, six, seven or eight or more amino acids at the C-terminal end of the insert polypeptide portion.

15. The antibody according to any one of claims 1 to 14, which is a full-length antibody or an antigen-binding fragment thereof.

16. The antibody according to any one of claims 1 to 15, which is a full-length IgG or an antigen-binding fragment thereof.

17. The antibody according to any one of claims 1 to 15, which is a Fab, Fab', F(ab')<sub>2</sub>, VHH, or scFv, optionally dsScFv.

18. The antibody according to claim 17, which is a Fab.

19. The antibody according to any one of claims 1 to 18, which is humanised.

20. The antibody according to any one of claims 1 to 19, wherein said V domain binds to human serum albumin.

21. The antibody according to claim 20, which comprises a VL domain comprising CDR-L1 having the sequence SEQ ID NO: 3; CDR-L2 having the sequence SEQ ID NO: 4; CDR-L3 having the sequence SEQ ID NO: 5.

22. The antibody according to claim 21, which comprises a VL domain comprising SEQ ID NO: 2 or SEQ ID NO: 102.

23. The antibody according to claim 22, which comprises a L chain comprising SEQ ID NO: 1.

24. The antibody according to any one of claims 20 to 23, which comprises a VH domain comprising CDR-H1 having the sequence SEQ ID NO: 8; CDR-H2 having the sequence SEQ ID NO: 9; CDR-H3 having the sequence SEQ ID NO: 10.

25. The antibody according to claim 24, which comprises a VH domain comprising SEQ ID NO: 7 or SEQ ID NO: 103.

26. The antibody according to claim 25, which comprises a H chain comprising SEQ ID NO: 6.

27. The antibody according to any one of claims 1 to 26, wherein the insert polypeptide is a cytokine.

28. The antibody according to claim 27, wherein the cytokine is selected from IL-10, IL-15, IL-2, G-CSF, GM-CSF, and EPO.

29. The antibody according to any one of claims 1 to 26, wherein the insert polypeptide is a second antibody.

30. The antibody according to claim 29, wherein the second antibody is an scFv, optionally a dsScFv, or a domain antibody, optionally a VH or VL or VHH.

31. The antibody according to claim 30, wherein the VHH comprises a sequence selected from SEQ ID NOs: 67, 69, and 89.

32. The antibody according to claim 30, wherein the second antibody binds to the same antigen as the V domain.

33. The antibody according to claim 32, wherein the second antibody binds to a different epitope compared to the V domain.

34. The antibody according to claim 30, wherein the second antibody binds to a different antigen to the antibody comprising a V domain.

35. The antibody according to claim 34, wherein the second antibody binds to IL-17 and the antibody comprising a V domain binds to human serum albumin.

36. The antibody according to claim 34, wherein the second antibody binds to CD3 and the antibody comprising a V domain binds to CD28.

37. The antibody according to claim 34, wherein the second antibody binds to CD28 and the antibody comprising a V domain binds to CD3.

38. The antibody according to any one of claims 1 to 37, which binds its cognate antigen by said V domain with a binding affinity which is at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% when compared to the antibody without the insert polypeptide present.

39. The antibody according to claim 32 or 33, which binds its cognate antigen with a binding affinity which is greater than when compared to the antibody without the second antibody present.

40. At least one isolated DNA molecule encoding the antibody according to any one of claims 1 to 39.

41. At least one cloning or expression vector encoding the antibody according to any one of claims 1 to 39.

42. An isolated or non-human host cell comprising the at least one DNA molecule according to claim 40 or the at least one cloning or expression vector according to claim 41.

43. A process for producing the antibody according to any one of claims 1 to 39, comprising culturing the host cell of claim 42 and isolating the antibody.

44. An antibody when produced by the process according to claim 43.
45. A pharmaceutical composition comprising the antibody according to any one of claims 1 to 39 or 44 in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.
46. A method of therapy comprising administering a therapeutically effective amount of the antibody according to any one of claims 1 to 39 or 44 or the pharmaceutical composition according to claim 45 to a subject in need of the therapy.
47. Use of the antibody according to any one of claims 1 to 39 or 44 or the pharmaceutical composition according to claim 45 in the manufacture of a medicament.

FIGURE 1

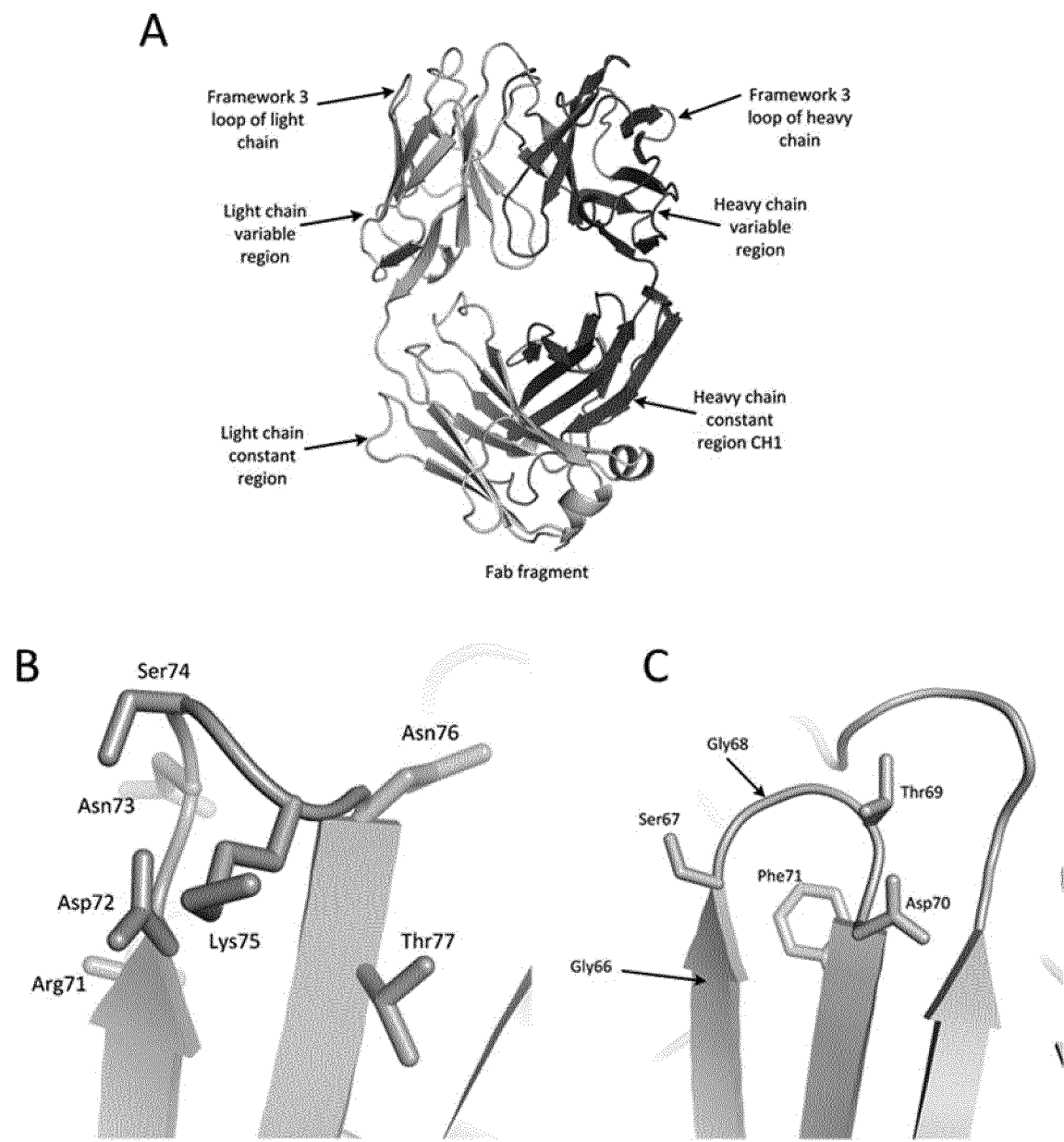
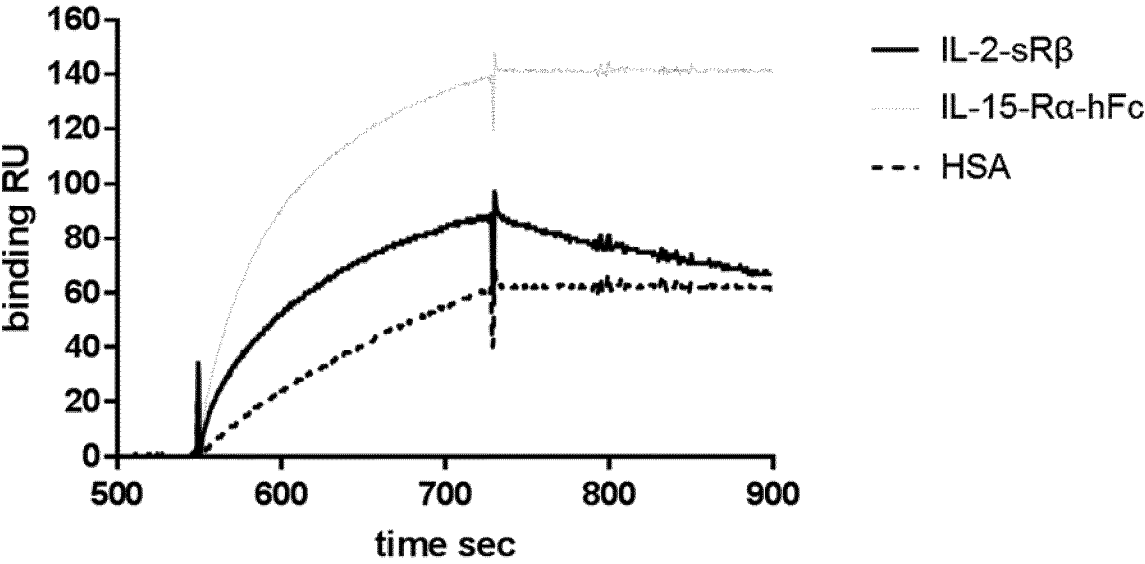


FIGURE 2

A



B

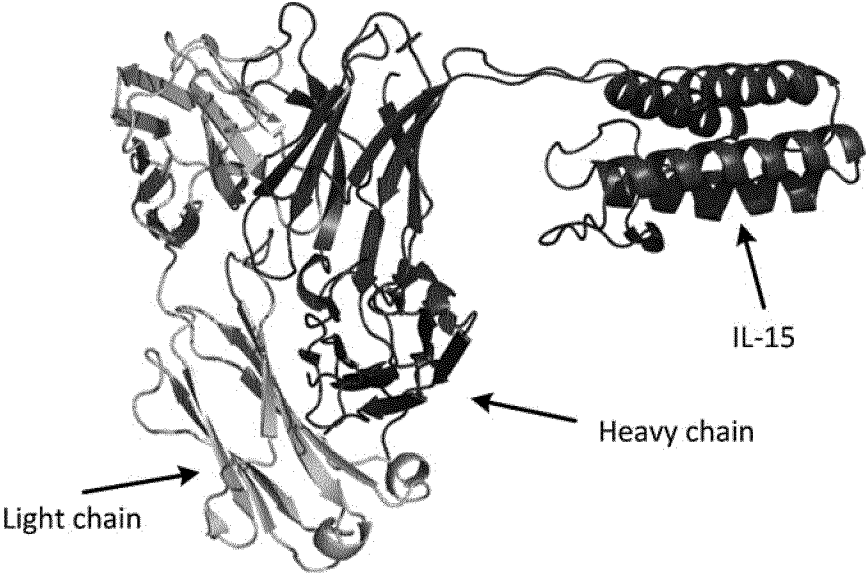


FIGURE 3

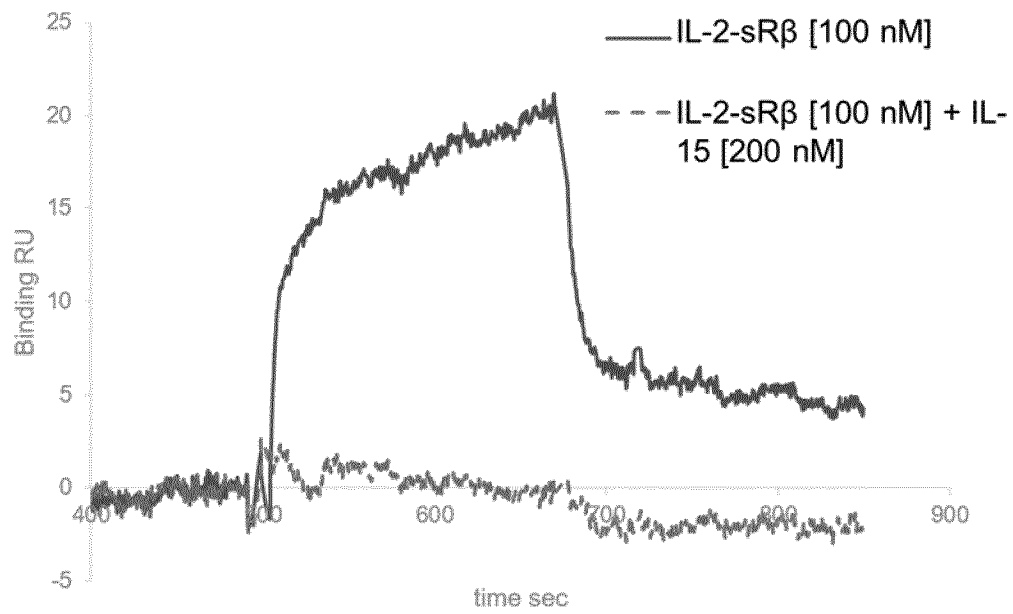
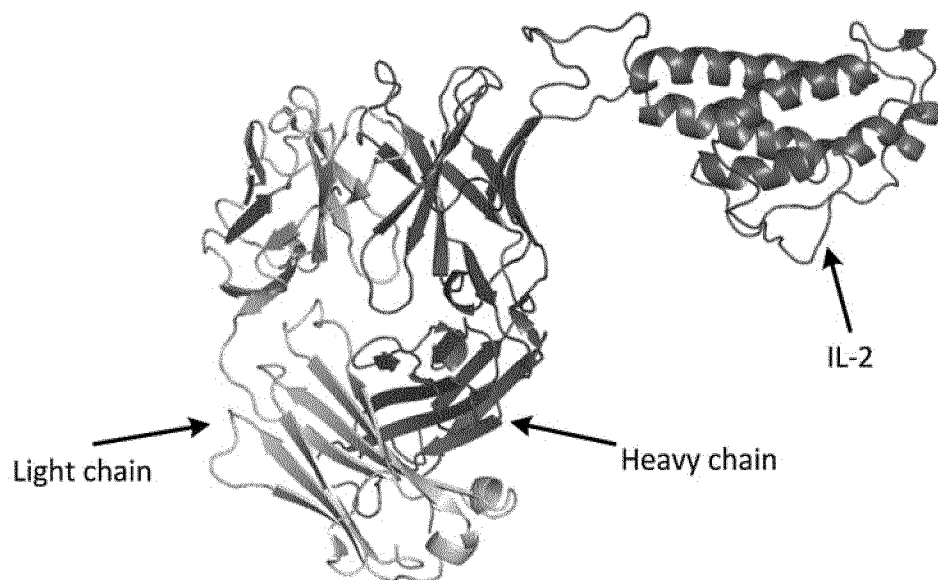
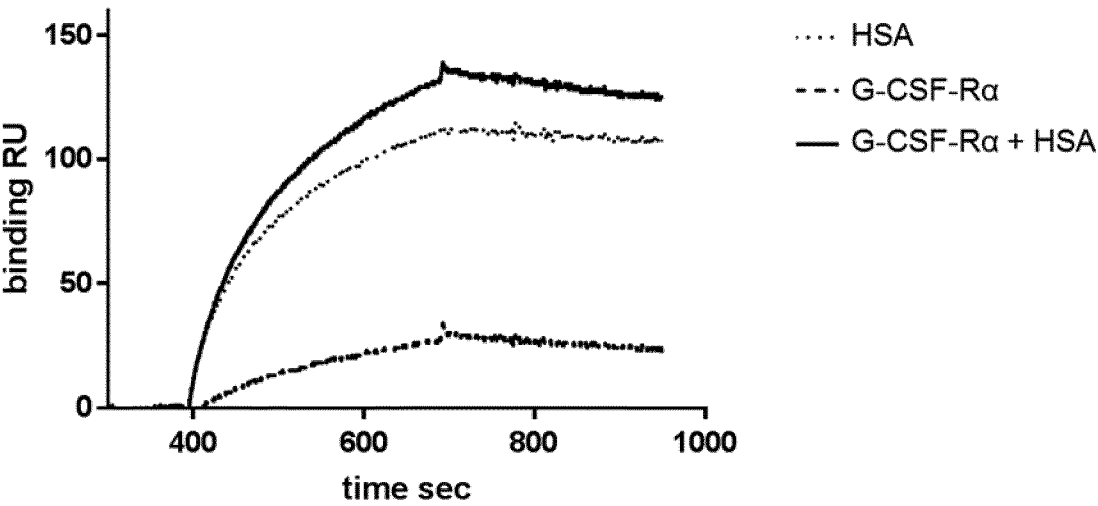
**A****B**

FIGURE 4

A



B

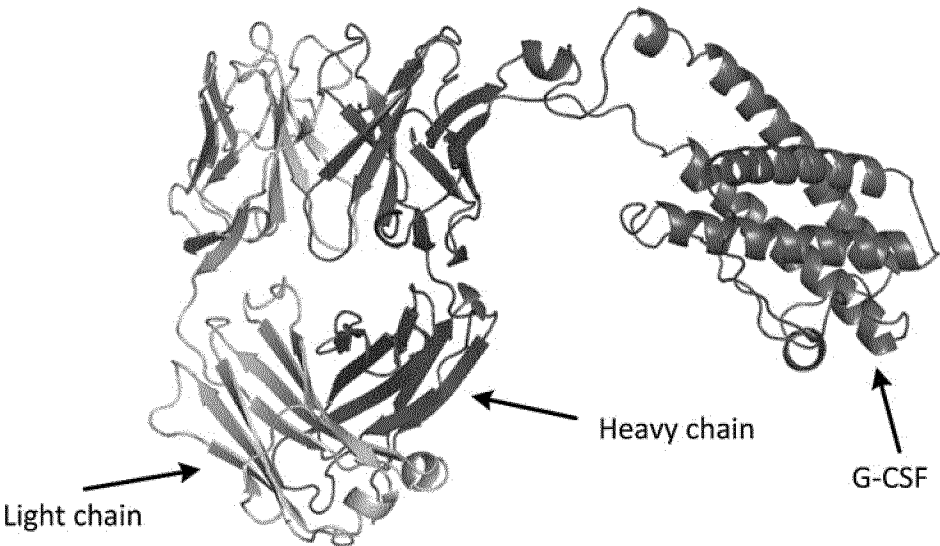
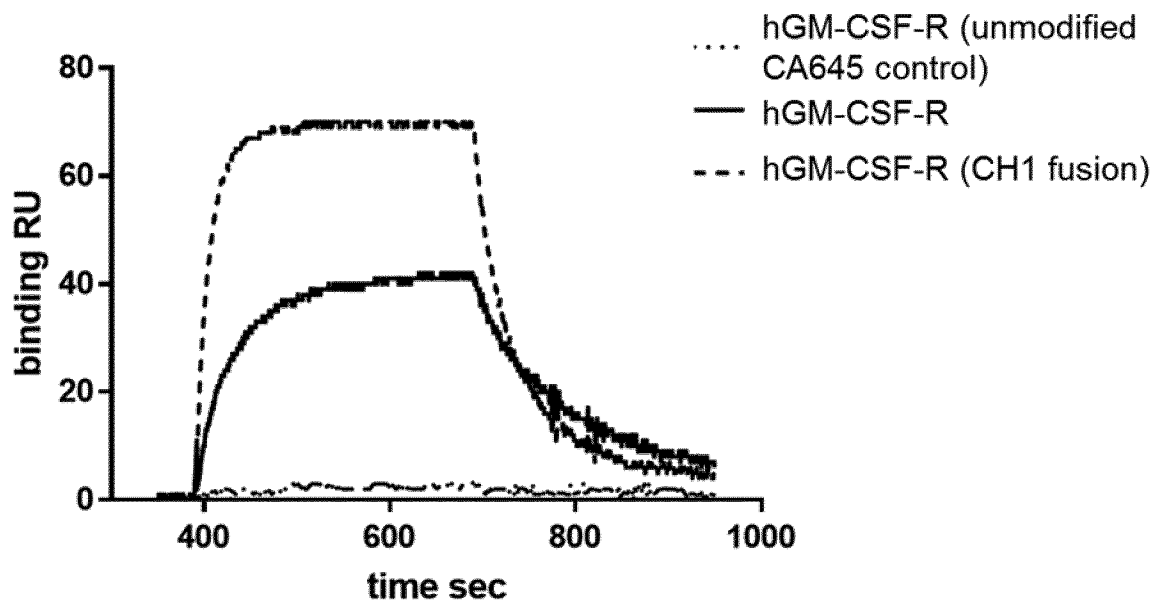


FIGURE 5

A



B

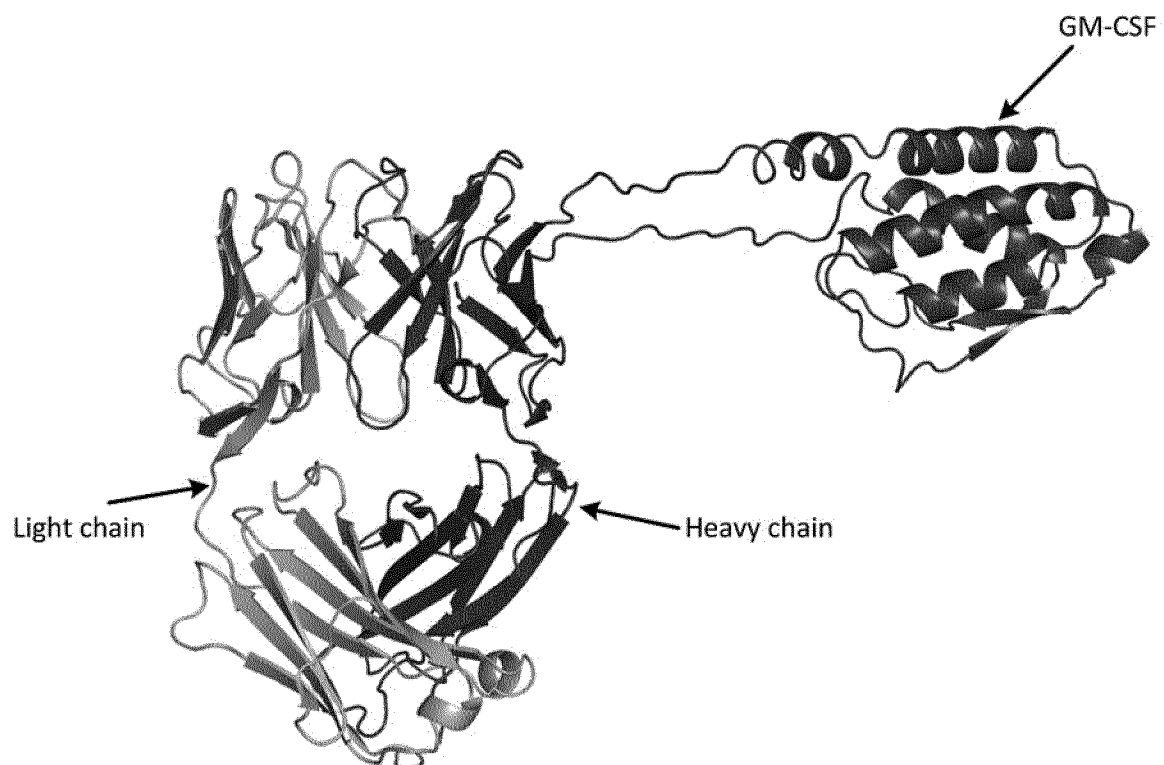


FIGURE 6

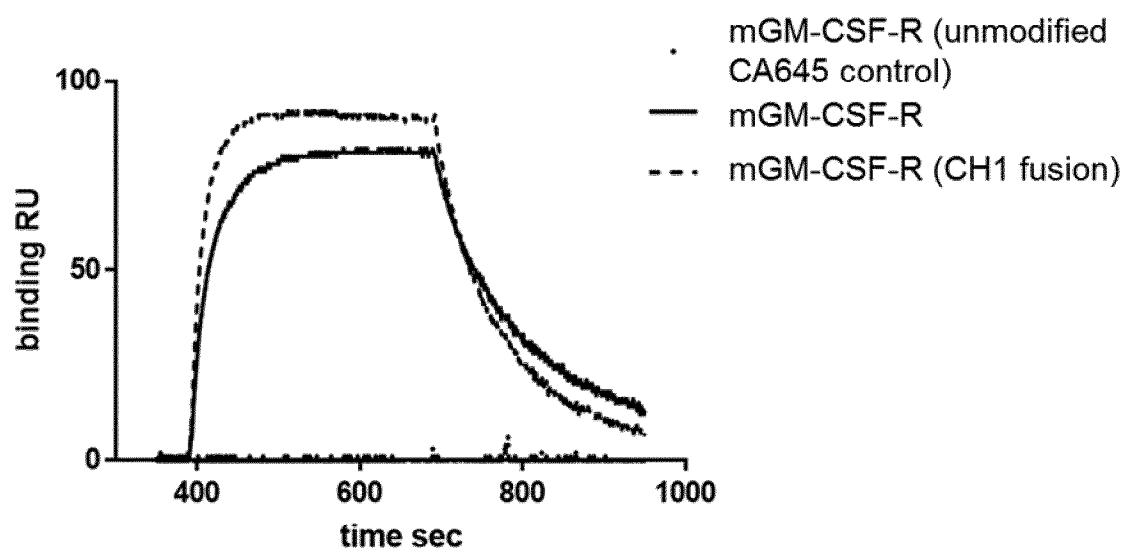
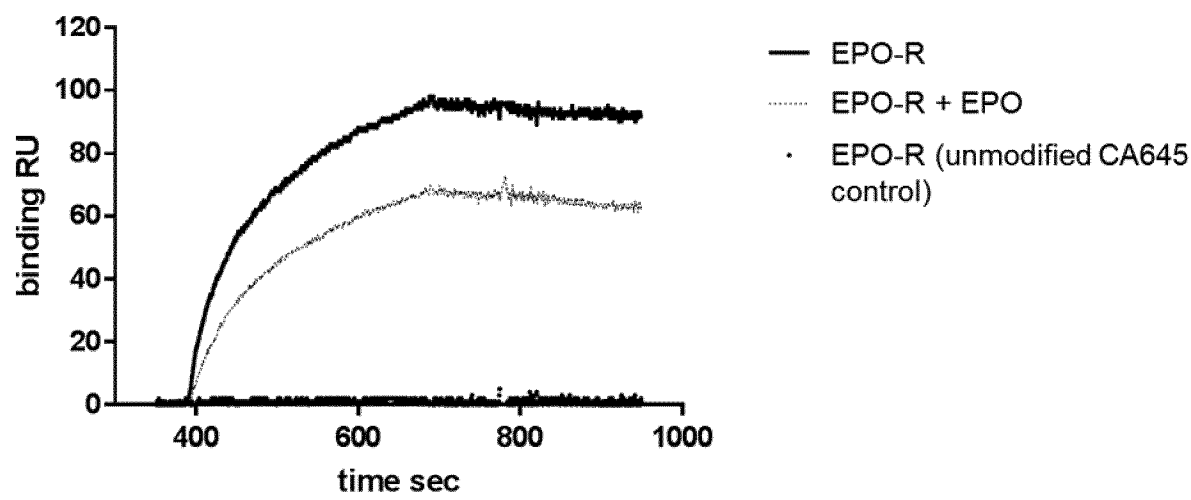


FIGURE 7

A



B

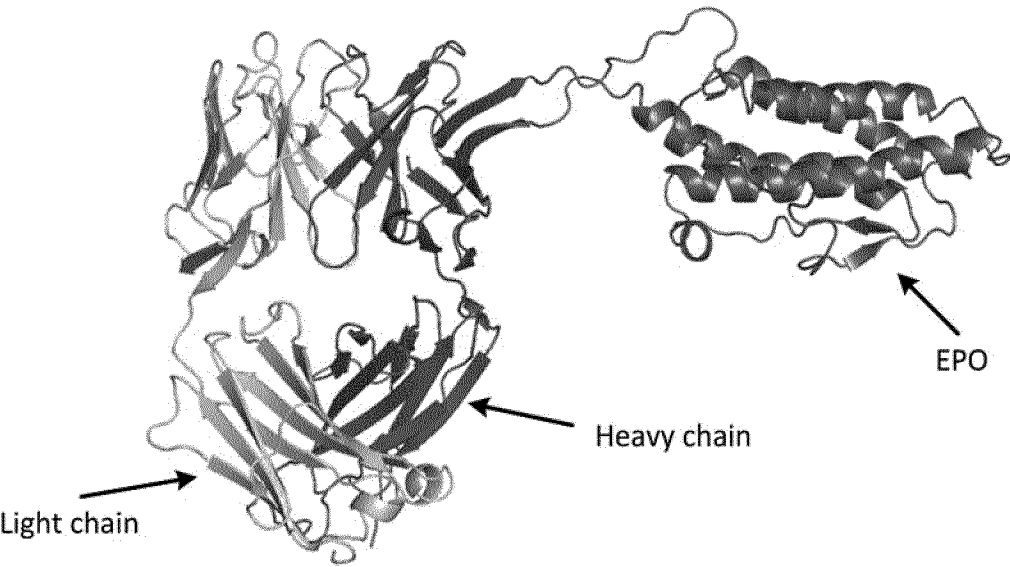


FIGURE 8

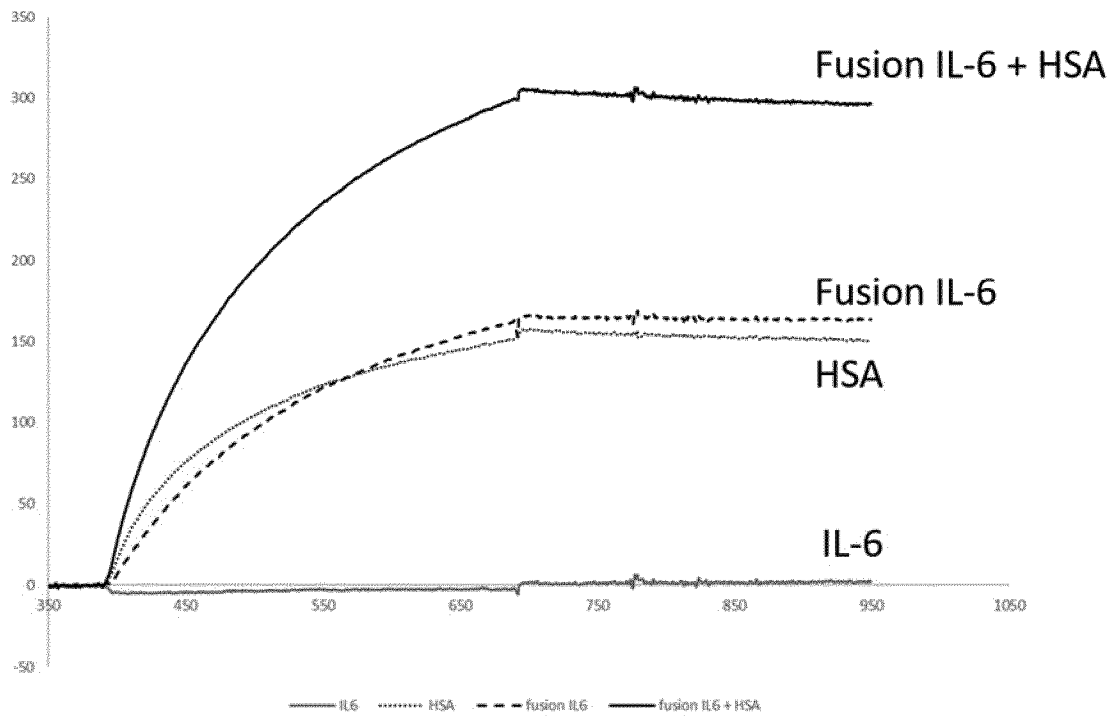
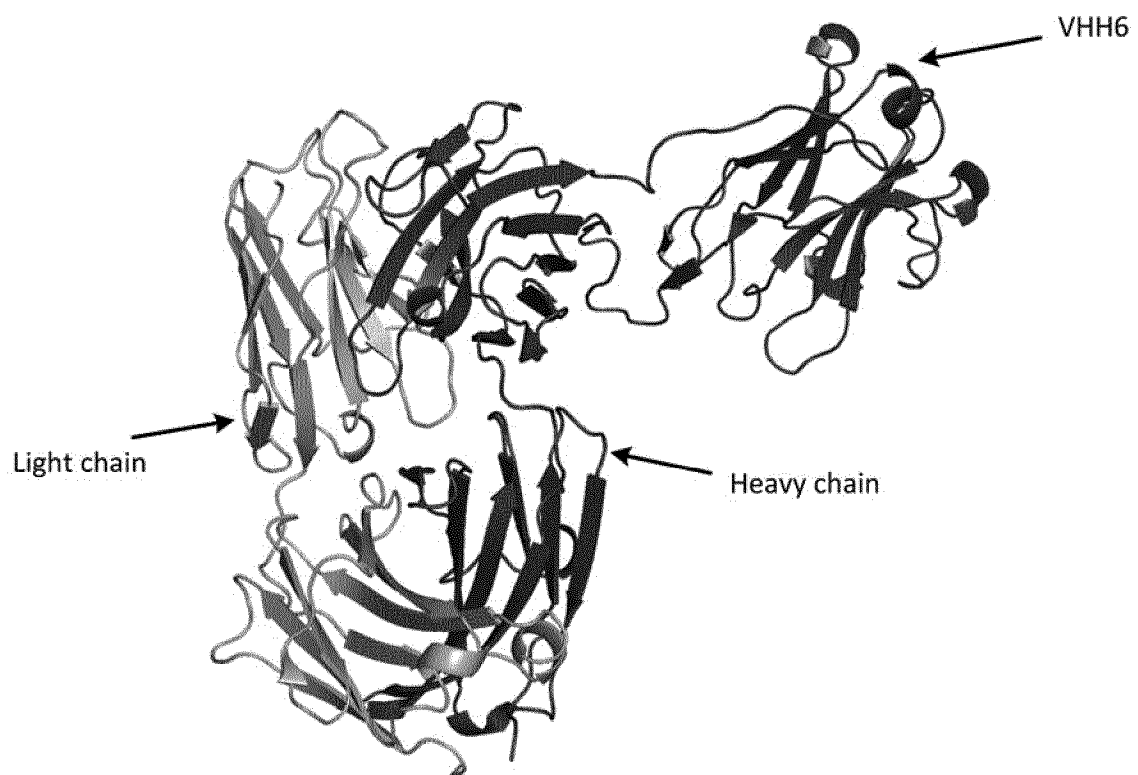
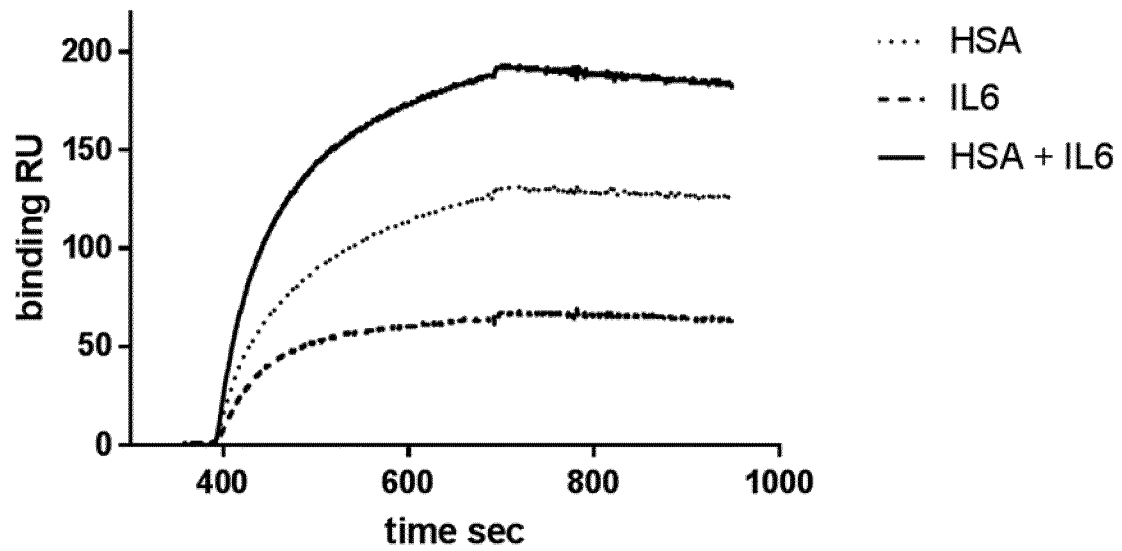
**A****B**

FIGURE 9

A



B

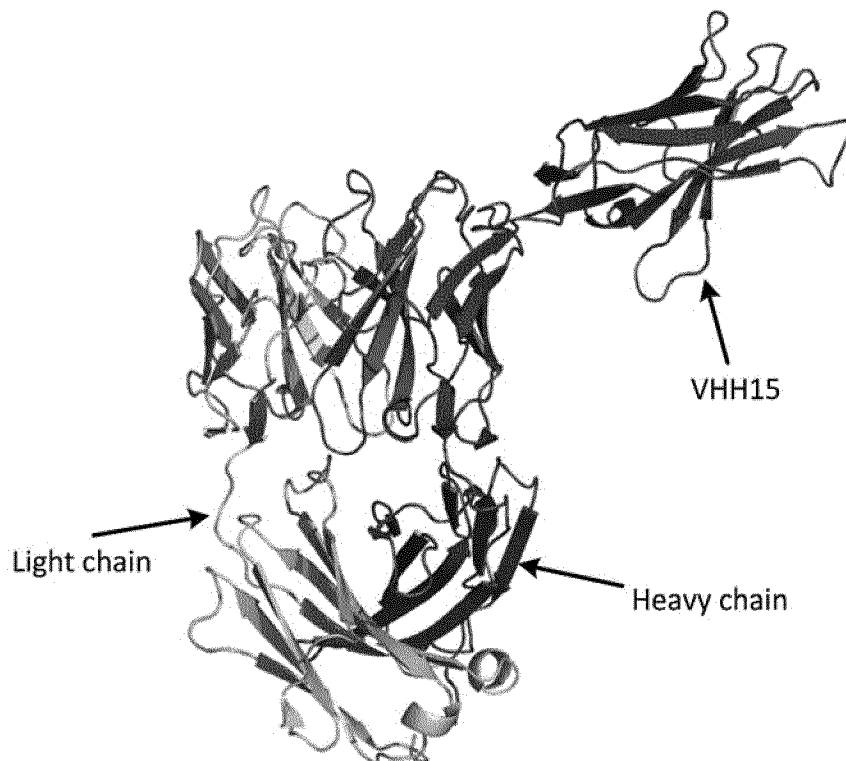
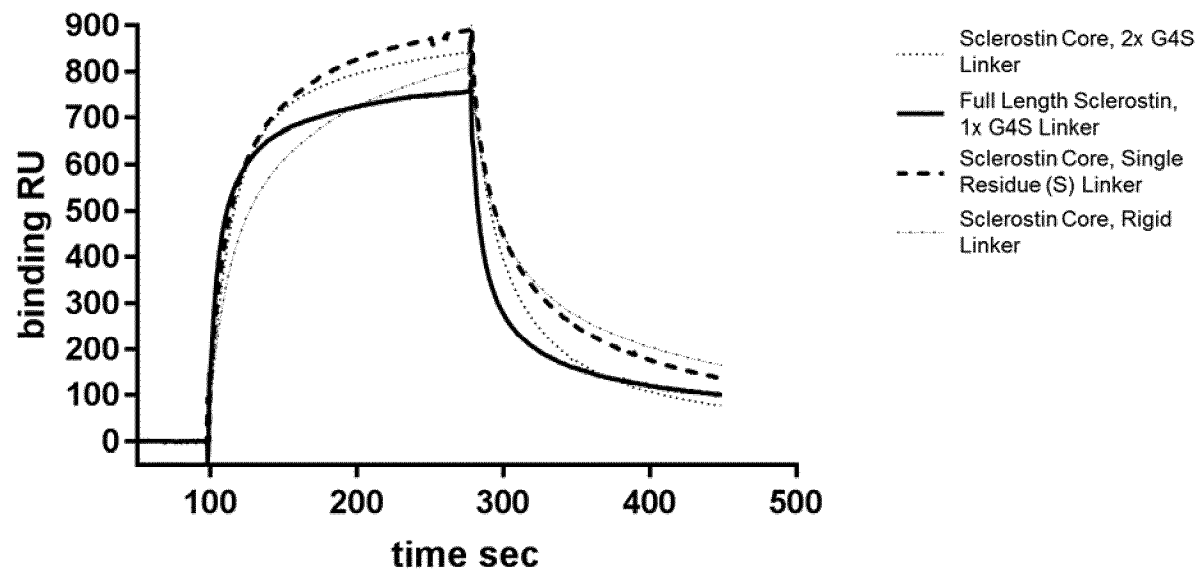


FIGURE 10

A



B

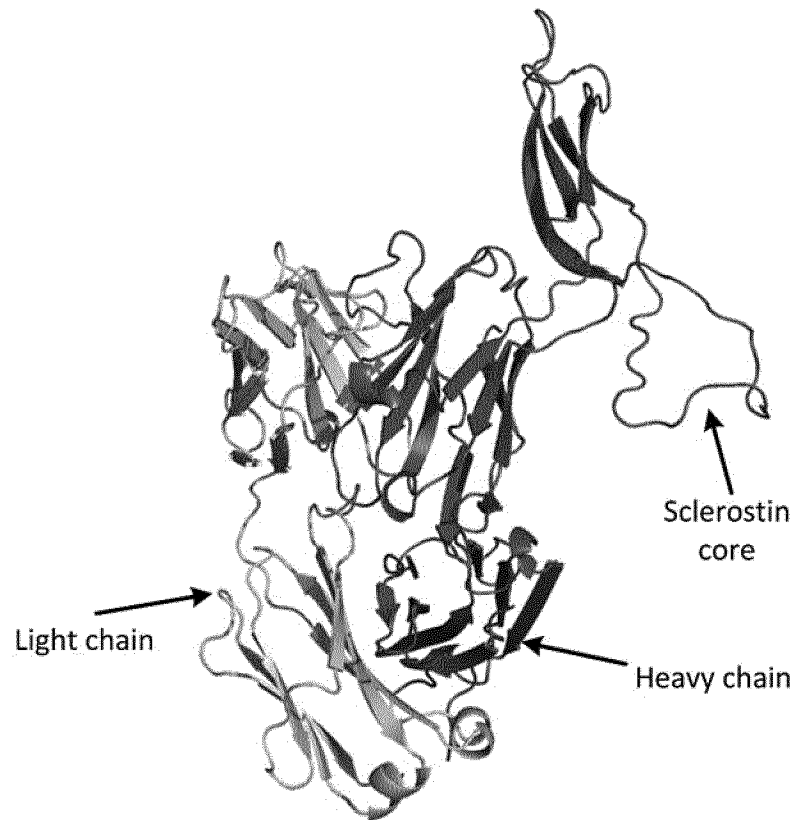


FIGURE 11

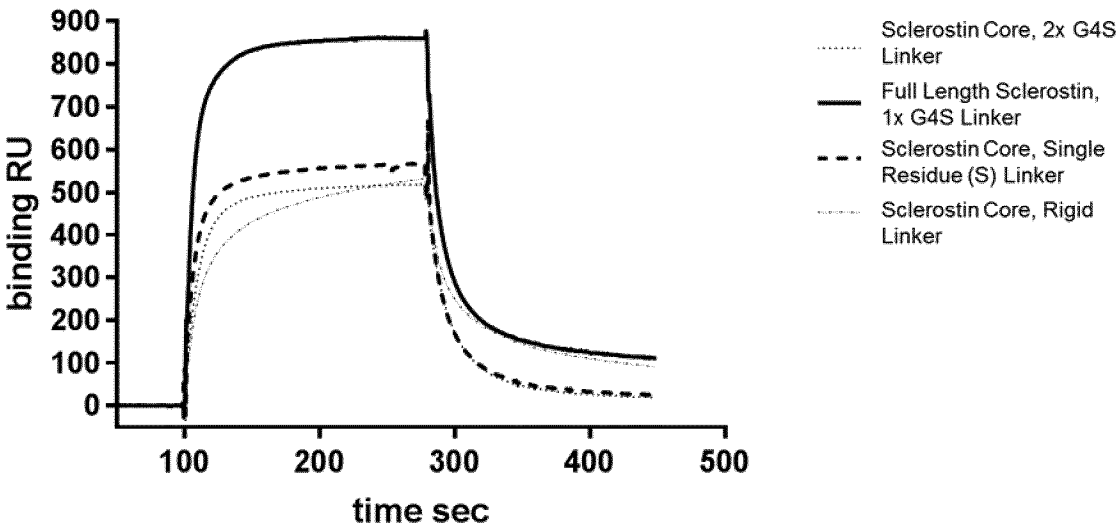
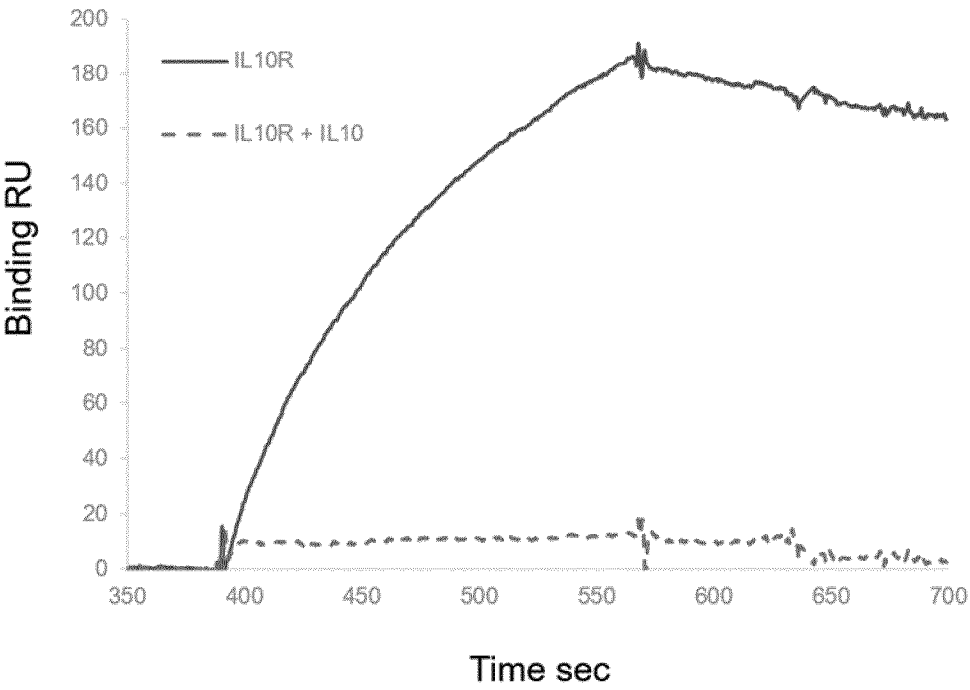


FIGURE 12

A



B

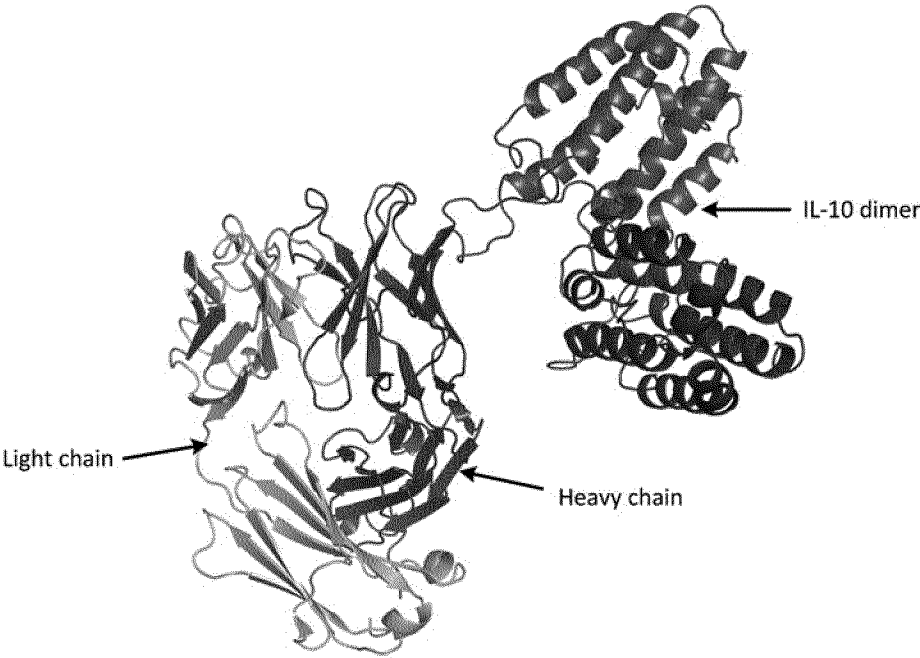


FIGURE 13

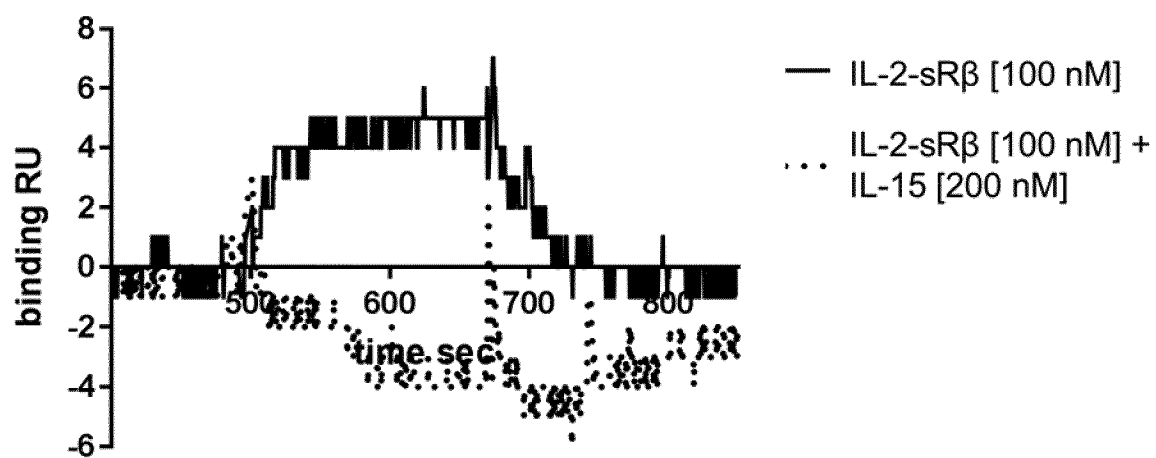


FIGURE 14

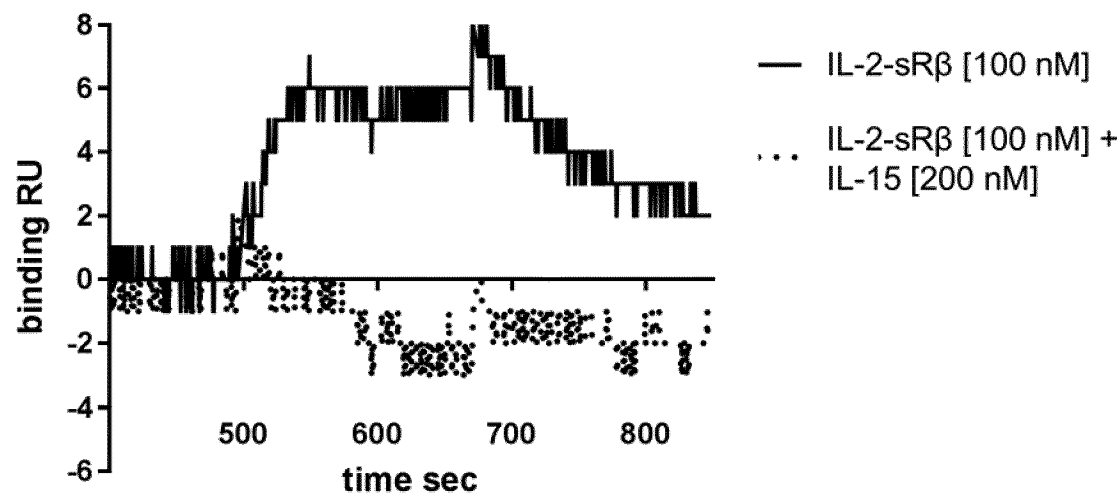


FIGURE 15

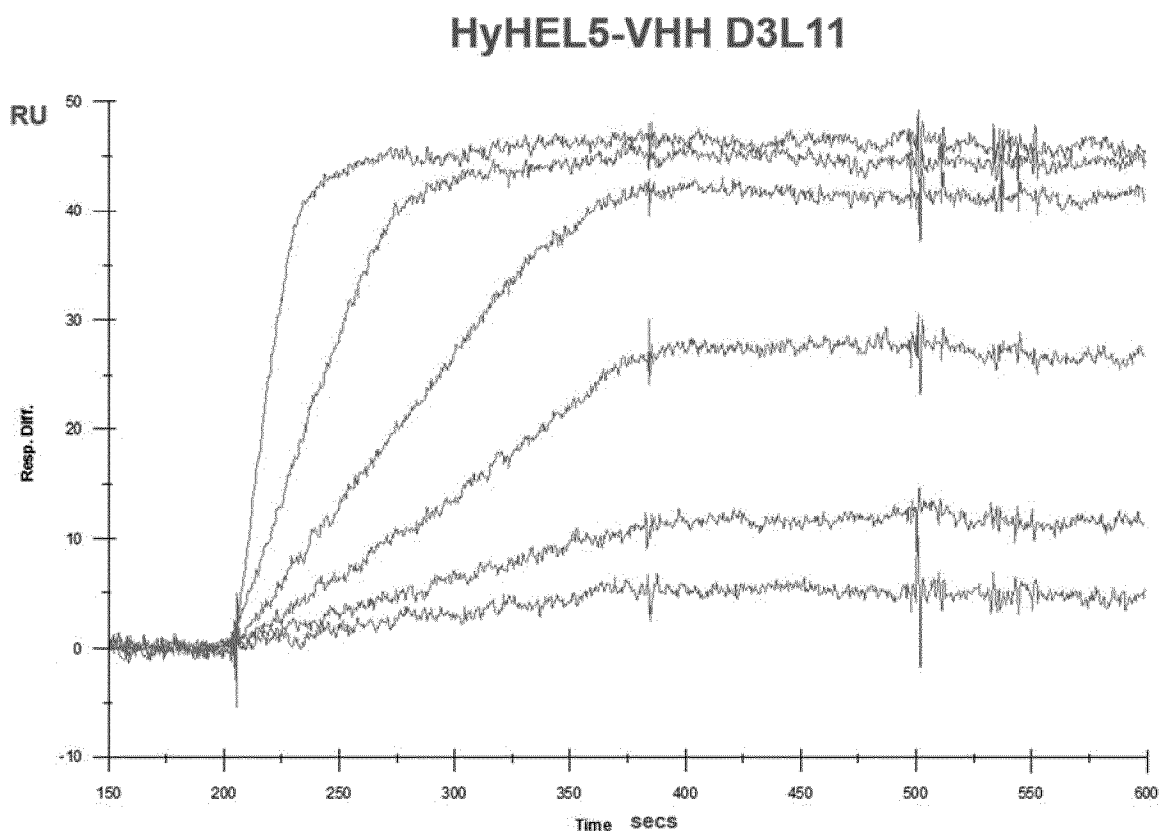


FIGURE 16

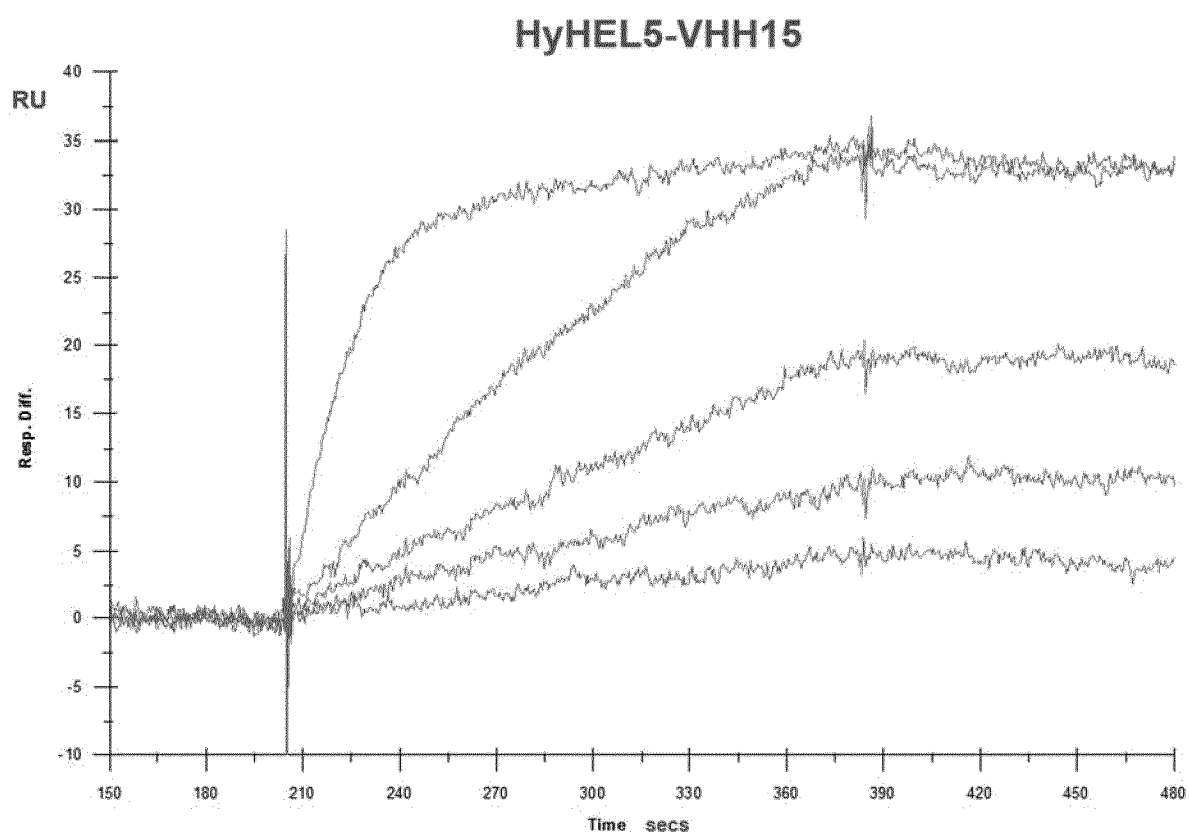


FIGURE 17

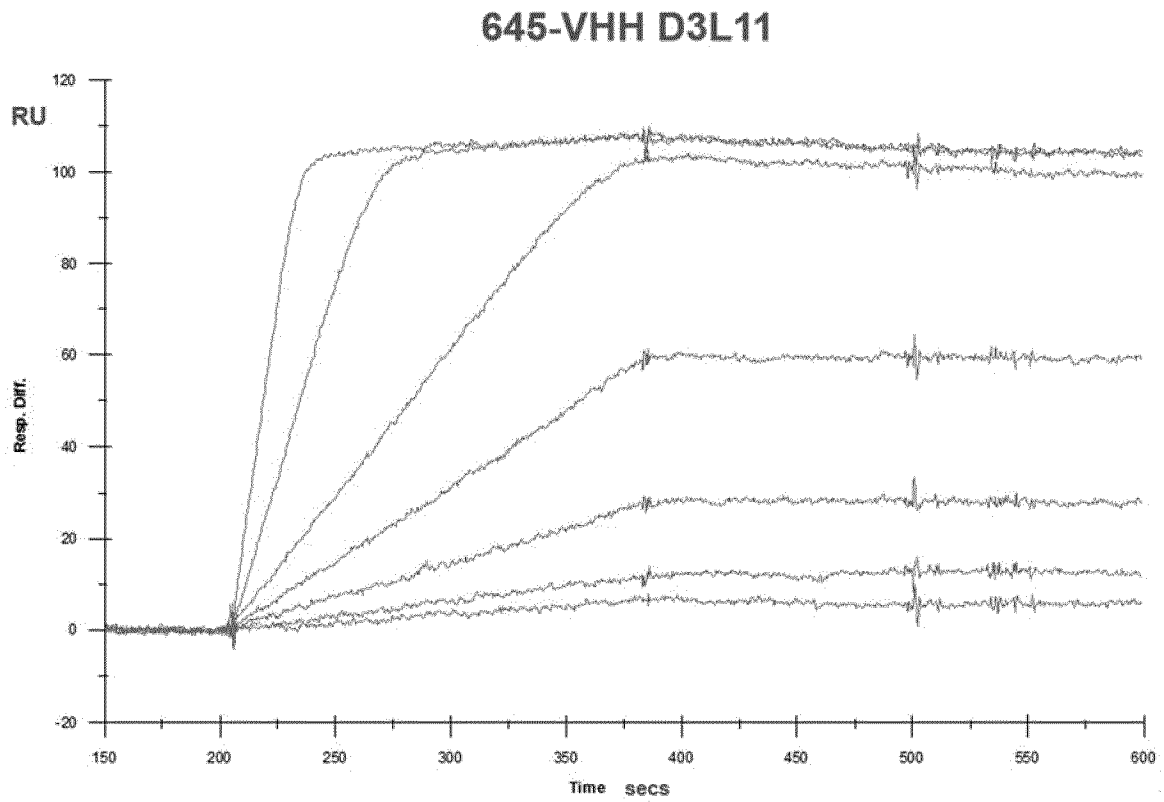


FIGURE 18

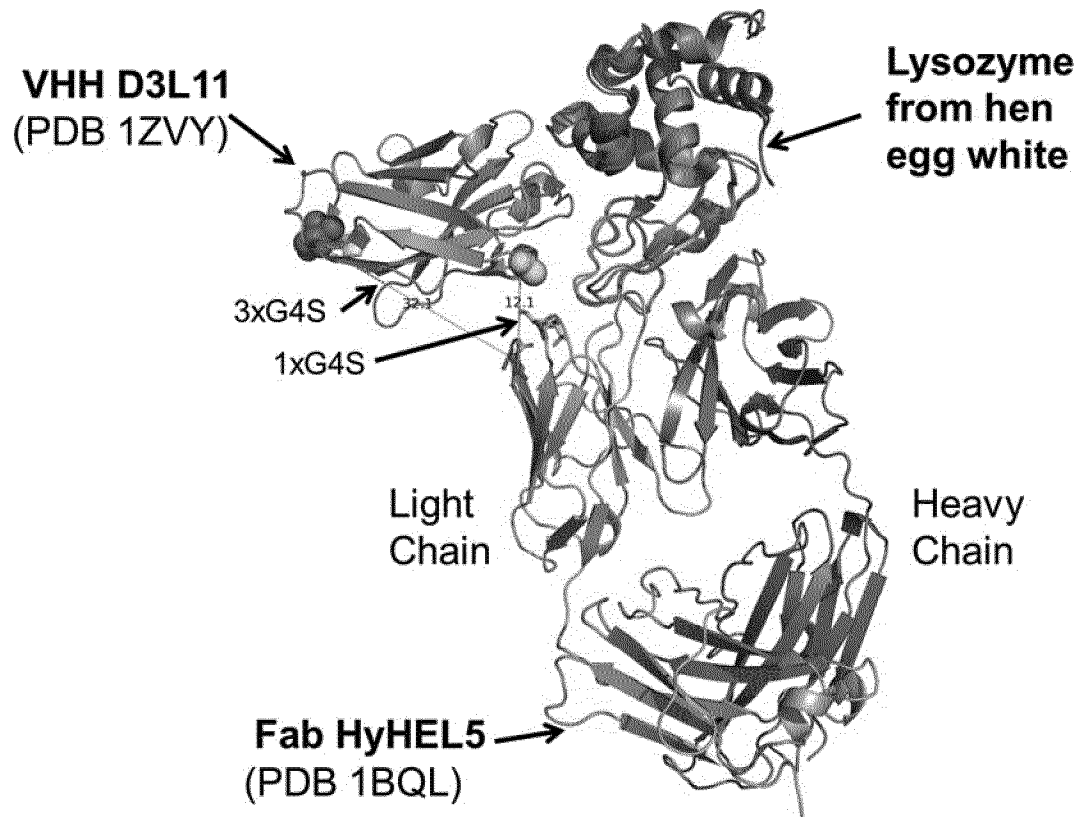


FIGURE 19

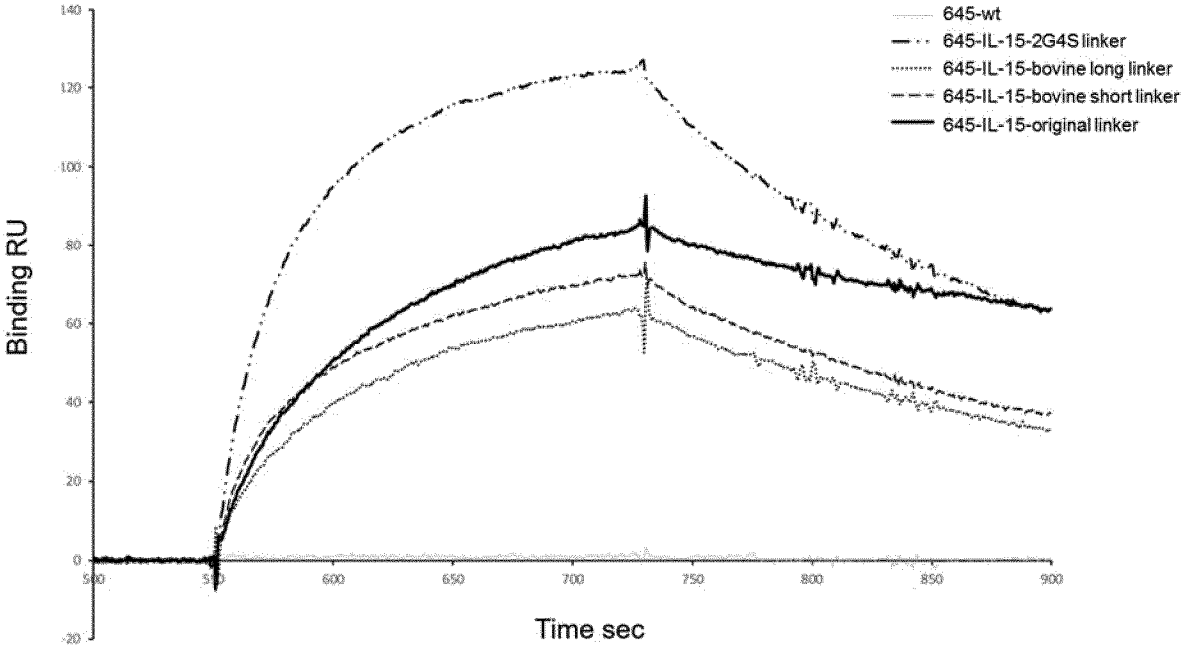


FIGURE 20

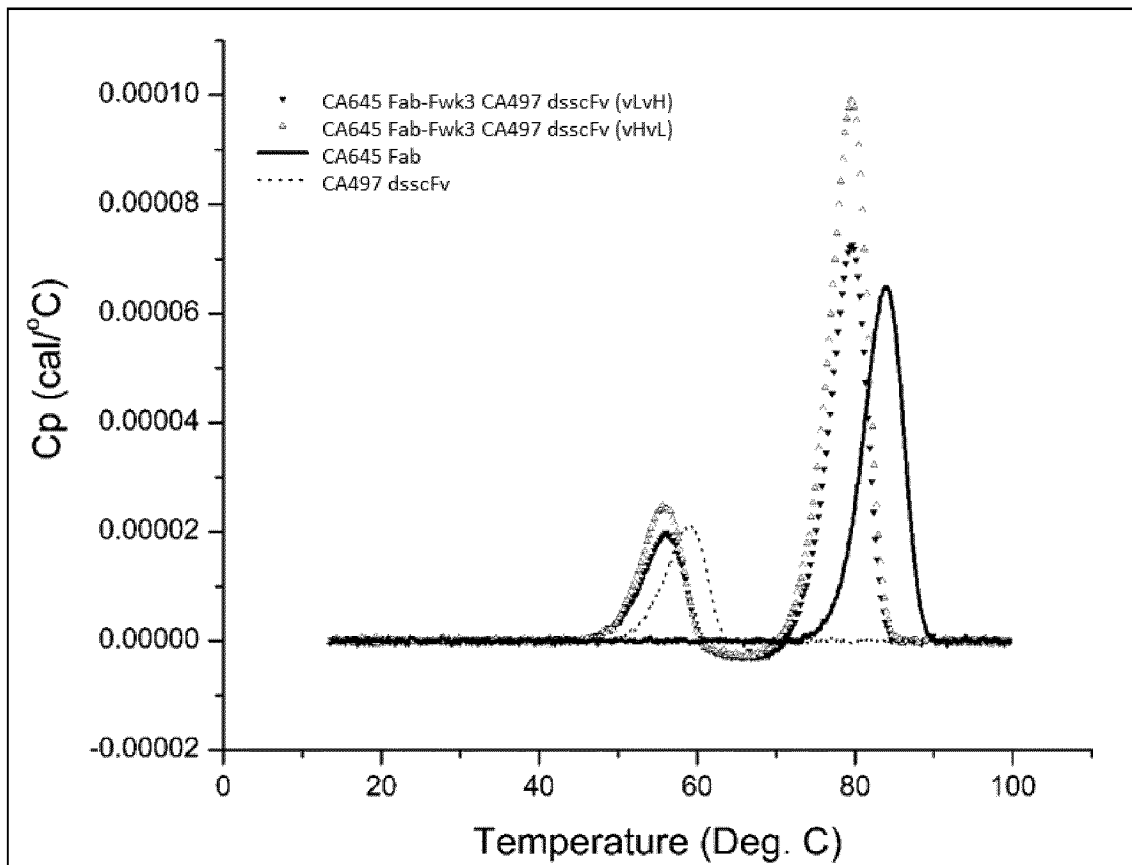
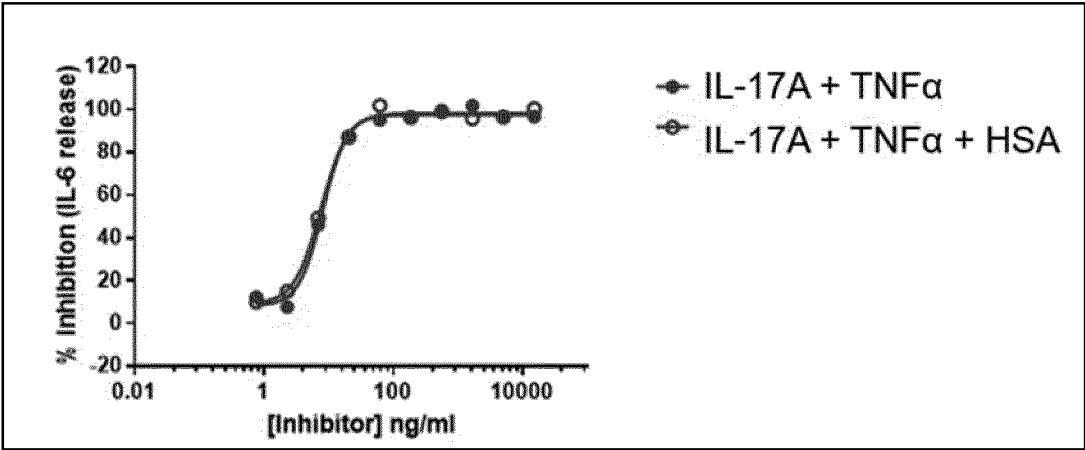
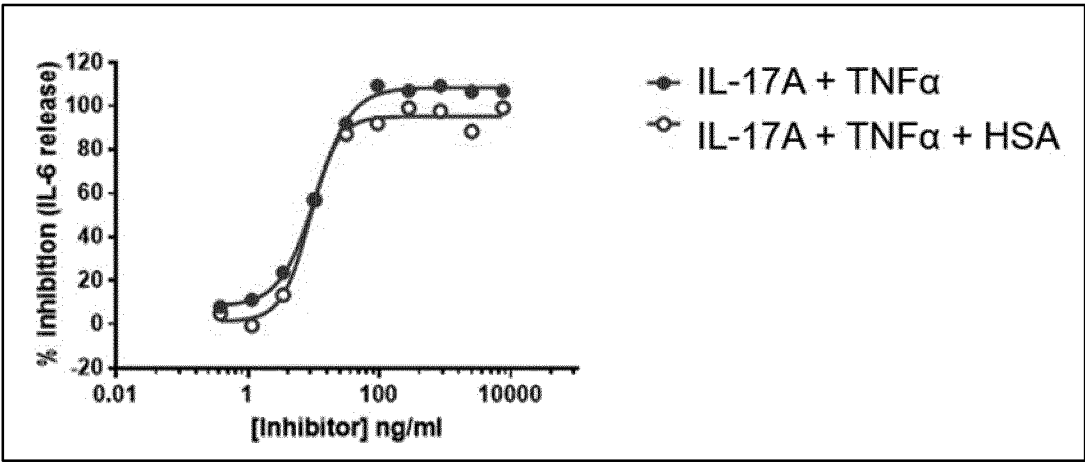


FIGURE 21

A



B



C

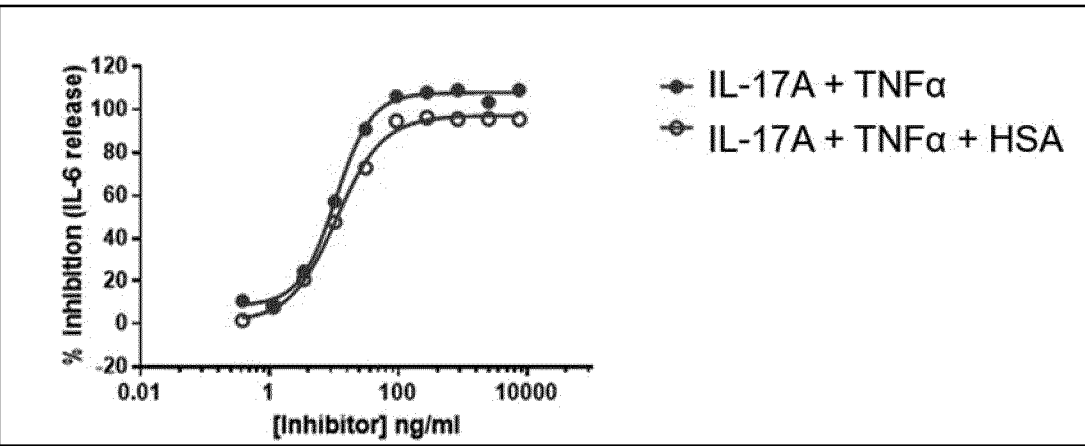


FIGURE 22

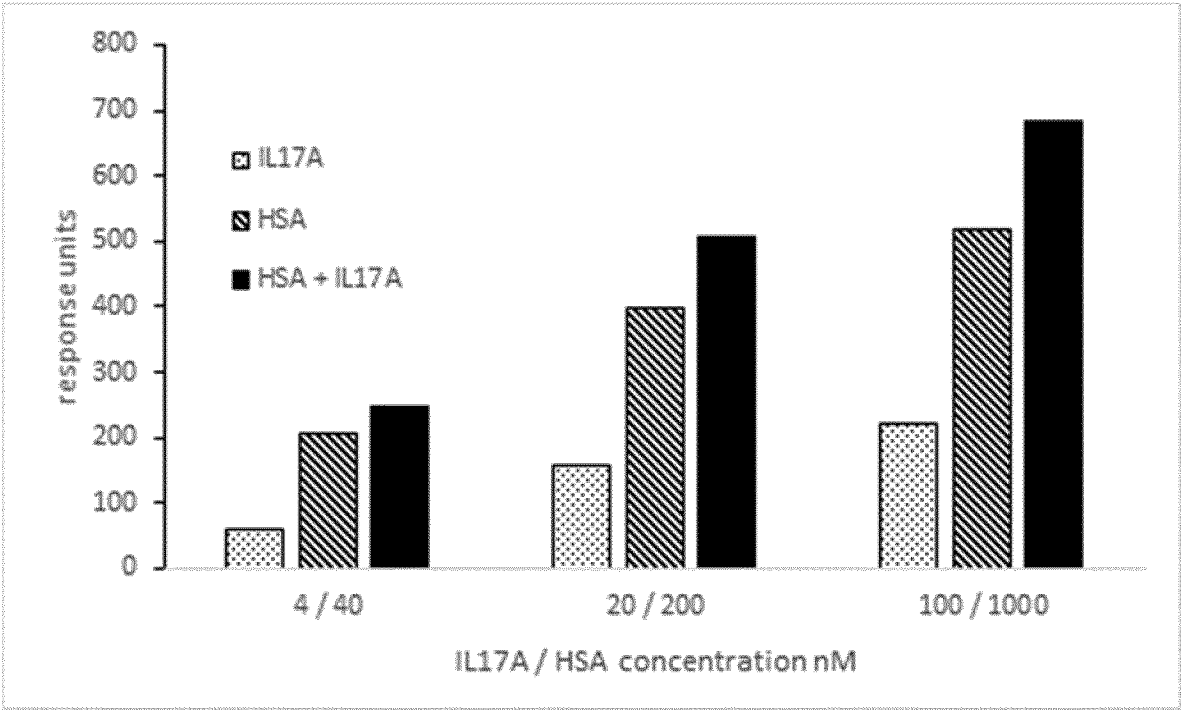


FIGURE 23

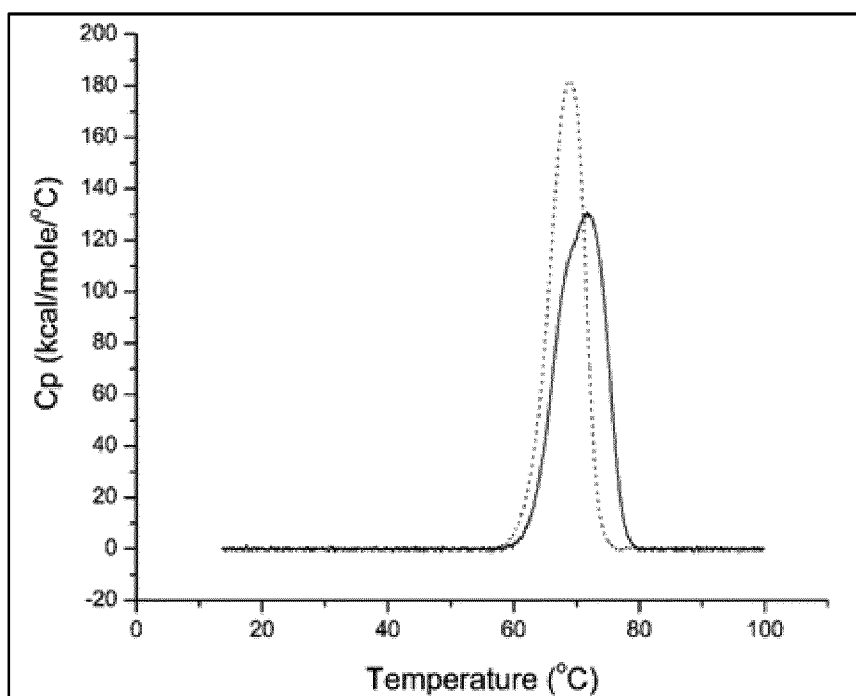
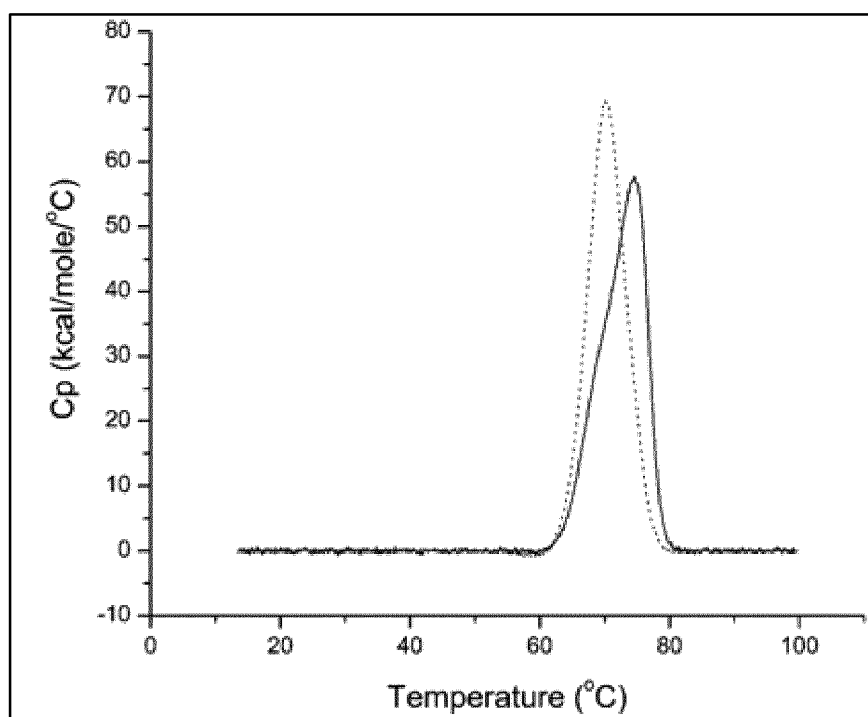
**A****B**

FIGURE 24

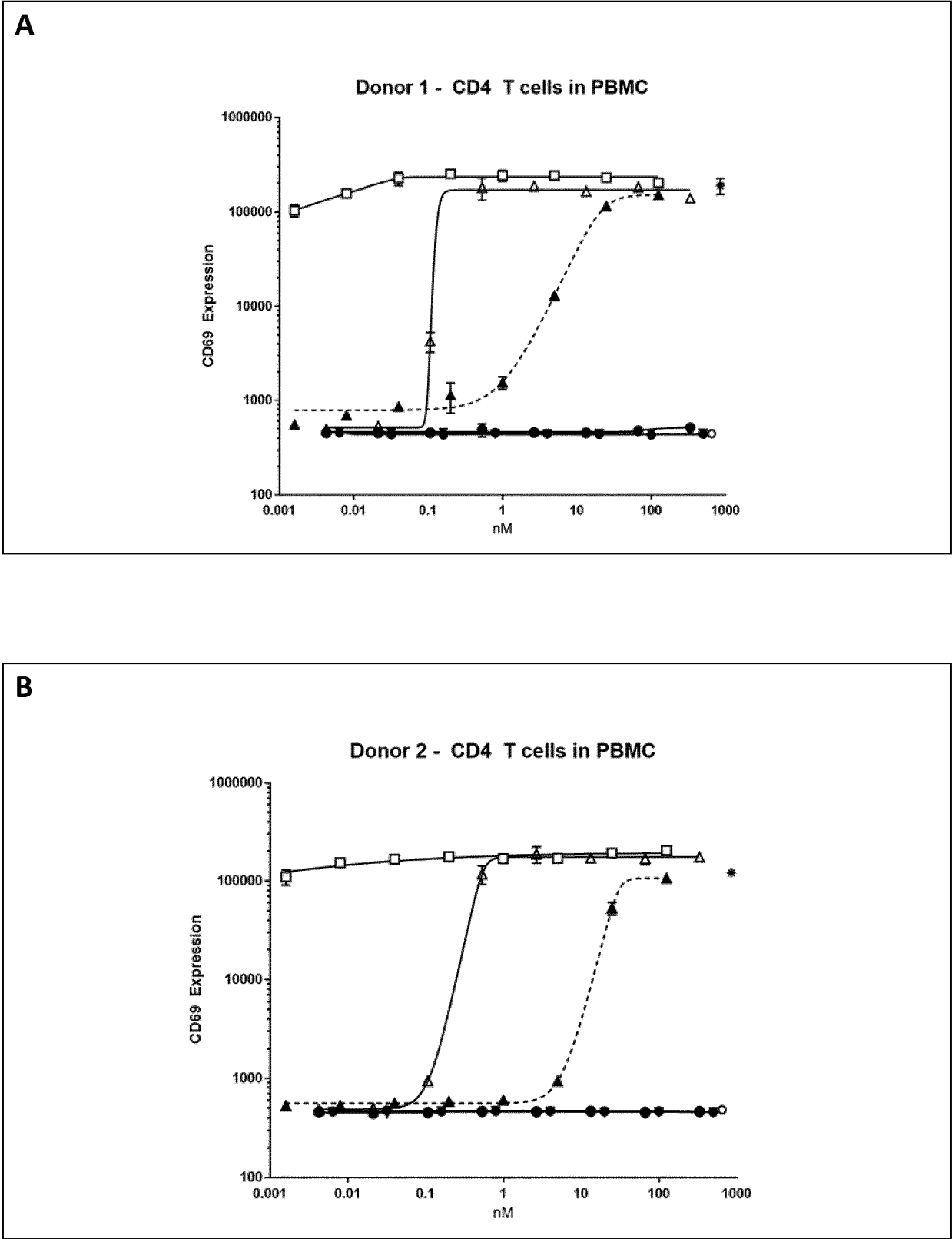
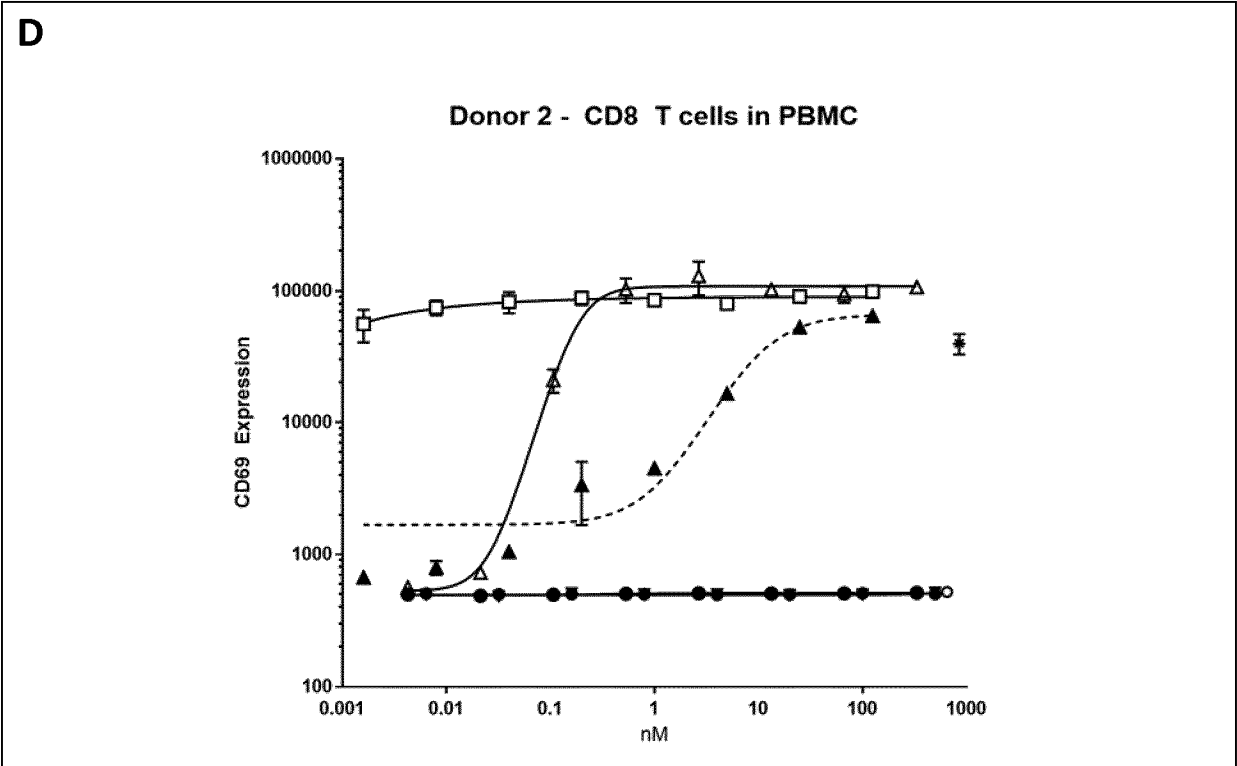
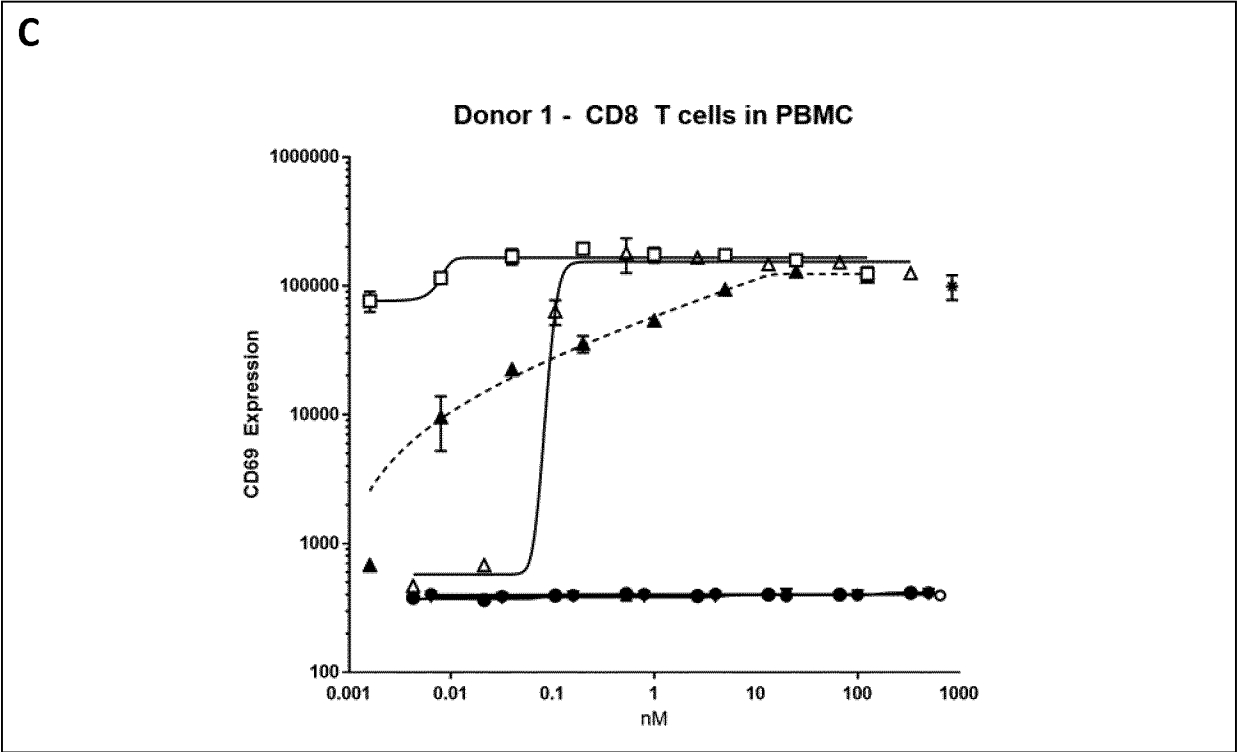


FIGURE 24 CONTINUED



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<223> CA645 Light Chain gL5

<400> 1

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Ile | Gln | Met | Thr | Gln | Ser | Pro | Ser | Ser | Val | Ser | Ala | Ser | Val | Gly |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asp | Arg | Val | Thr | Ile | Thr | Cys | Gln | Ser | Ser | Pro | Ser | Val | Trp | Ser | Asn |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Phe | Leu | Ser | Trp | Tyr | Gln | Gln | Lys | Pro | Gly | Lys | Ala | Pro | Lys | Leu | Leu |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Tyr | Glu | Ala | Ser | Lys | Leu | Thr | 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 | Phe | Ala | Thr | Tyr | Tyr | Cys | Gly | Gly | Gly | Tyr | Ser | Ser | Ile |
|     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Asp | Thr | Thr | Phe | Gly | Gly | Gly | Thr | Lys | Val | Glu | Ile | Lys | Arg | Thr |
|     |     |     | 100 |     |     |     |     | 105 |     |     |     |     |     | 110 |     |

Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu  
115 120 125

Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro  
130 135 140

Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly  
145 150 155 160

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr  
165 170 175

Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His  
180 185 190

Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val  
195 200 205

Thr Lys Ser Phe Asn Arg Gly Glu Cys  
210 215

<210> 2  
<211> 110  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 VL domain (gL5)

<400> 2

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn  
20 25 30

Phe Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu  
35 40 45

Ile Tyr Glu Ala Ser Lys Leu Thr 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 Phe Ala Thr Tyr Tyr Cys Gly Gly Gly Tyr Ser Ser Ile  
85 90 95

Ser Asp Thr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105 110

<210> 3  
<211> 12  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 CDR L1

<400> 3

Gln Ser Ser Pro Ser Val Trp Ser Asn Phe Leu Ser  
1 5 10

<210> 4  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 CDR L2

<400> 4

Glu Ala Ser Lys Leu Thr Ser  
1 5

<210> 5  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 CDR L3

<400> 5

Gly Gly Gly Tyr Ser Ser Ile Ser Asp Thr Thr  
1 5 10

<210> 6  
 <211> 224  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 Heavy Chain gH5

<400> 6

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
 85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
210 215 220

<210> 7  
<211> 121  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 VH domain (gH5)

<400> 7

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 8  
<211> 10  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 CDR H1

<400> 8

Gly Ile Asp Leu Ser Asn Tyr Ala Ile Asn  
1 5 10

<210> 9  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 CDR H2

<400> 9

Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys Gly  
1 5 10 15

<210> 10  
<211> 13  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 CDR H3

<400> 10

Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu  
1 5 10

<210> 11  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 11

Ser Gly Gly Gly Gly Ser Glu  
1 5

<210> 12  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 12

Asp Lys Thr His Thr Ser  
1 5

<210> 13  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 13

Ser Gly Gly Gly Gly Ser  
1 5

<210> 14  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 14

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10

<210> 15  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 15

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

<210> 16  
<211> 21  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 16

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

Gly Gly Gly Gly Ser  
20

<210> 17  
<211> 26  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 17

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
1 5 10 15

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
20 25

<210> 18  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 18

Ala Ala Ala Gly Ser Gly Gly Ala Ser Ala Ser  
1 5 10

<210> 19  
<211> 16  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<220>  
<221> misc\_feature  
<222> (7)..(7)  
<223> Xaa can be any naturally occurring amino acid

<400> 19

Ala Ala Ala Gly Ser Gly Xaa Gly Gly Gly Ser Gly Ala Ser Ala Ser  
1 5 10 15

<210> 20  
<211> 21  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<220>  
<221> misc\_feature  
<222> (7)..(7)  
<223> Xaa can be any naturally occurring amino acid

<220>  
<221> misc\_feature  
<222> (12)..(12)  
<223> Xaa can be any naturally occurring amino acid

<400> 20

Ala Ala Ala Gly Ser Gly Xaa Gly Gly Gly Ser Xaa Gly Gly Gly Ser  
1 5 10 15

Gly Ala Ser Ala Ser  
20

<210> 21  
<211> 26  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<220>  
<221> misc\_feature  
<222> (7)..(7)  
<223> Xaa can be any naturally occurring amino acid

<220>  
<221> misc\_feature  
<222> (12)..(12)  
<223> Xaa can be any naturally occurring amino acid

<220>  
<221> misc\_feature  
<222> (17)..(17)  
<223> Xaa can be any naturally occurring amino acid

<400> 21

Ala Ala Ala Gly Ser Gly Xaa Gly Gly Gly Ser Xaa Gly Gly Gly Ser  
1 5 10 15

Xaa Gly Gly Gly Ser Gly Ala Ser Ala Ser  
20 25

<210> 22  
<211> 31  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<220>  
<221> misc\_feature  
<222> (7)..(7)  
<223> Xaa can be any naturally occurring amino acid

<220>  
<221> misc\_feature  
<222> (12)..(12)  
<223> Xaa can be any naturally occurring amino acid

<220>  
<221> misc\_feature  
<222> (17)..(17)  
<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc\_feature  
<222> (22)..(22)  
<223> Xaa can be any naturally occurring amino acid

<400> 22

Ala Ala Ala Gly Ser Gly Xaa Gly Gly Gly Ser Xaa Gly Gly Gly Ser  
1 5 10 15

Xaa Gly Gly Gly Ser Xaa Gly Gly Gly Ser Gly Ala Ser Ala Ser  
20 25 30

<210> 23  
<211> 13  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<220>  
<221> misc\_feature  
<222> (7)..(7)  
<223> Xaa can be any naturally occurring amino acid

<400> 23

Ala Ala Ala Gly Ser Gly Xaa Ser Gly Ala Ser Ala Ser  
1 5 10

<210> 24  
<211> 28  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 24

Pro Gly Gly Asn Arg Gly Thr Thr Thr Thr Arg Arg Pro Ala Thr Thr  
1 5 10 15

Thr Gly Ser Ser Pro Gly Pro Thr Gln Ser His Tyr  
20 25

<210> 25  
<211> 11

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 25

Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr  
1 5 10

<210> 26  
<211> 6  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 26

Ala Thr Thr Thr Gly Ser  
1 5

<210> 27  
<211> 21  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 27

Glu Pro Ser Gly Pro Ile Ser Thr Ile Asn Ser Pro Pro Ser Lys Glu  
1 5 10 15

Ser His Lys Ser Pro  
20

<210> 28  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 28

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Thr | Val | Ala | Ala | Pro | Ser | Val | Phe | Ile | Phe | Pro | Pro | Ser | Asp |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

<210> 29  
 <211> 15  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Flexible linker

<400> 29

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gly | Gly | Gly | Ile | Ala | Pro | Ser | Met | Val | Gly | Gly | Gly | Gly | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

<210> 30  
 <211> 15  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Flexible linker

<400> 30

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gly | Gly | Gly | Lys | Val | Glu | Gly | Ala | Gly | Gly | Gly | Gly | Gly | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

<210> 31  
 <211> 15  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Flexible linker

<400> 31

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gly | Gly | Gly | Gly | Ser | Met | Lys | Ser | His | Asp | Gly | Gly | Gly | Gly | Ser |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

<210> 32  
 <211> 15  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Flexible linker

<400> 32

Gly Gly Gly Gly Asn Leu Ile Thr Ile Val Gly Gly Gly Gly Ser  
1 5 10 15

<210> 33

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Flexible linker

<400> 33

Gly Gly Gly Gly Val Val Pro Ser Leu Pro Gly Gly Gly Gly Ser  
1 5 10 15

<210> 34

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Flexible linker

<400> 34

Gly Gly Glu Lys Ser Ile Pro Gly Gly Gly Gly Ser  
1 5 10

<210> 35

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Flexible linker

<400> 35

Arg Pro Leu Ser Tyr Arg Pro Pro Phe Pro Phe Gly Phe Pro Ser Val  
1 5 10 15

Arg Pro

<210> 36

<211> 18

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 36

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Pro | Arg | Ser | Ile | Tyr | Ile | Arg | Arg | Arg | His | Pro | Ser | Pro | Ser | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Thr Thr

<210> 37  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 37

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Pro | Ser | His | Leu | Ser | His | Ile | Leu | Pro | Ser | Phe | Gly | Leu | Pro | Thr |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Glu Asn

<210> 38  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 38

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Pro | Val | Ser | Pro | Phe | Thr | Phe | Pro | Arg | Leu | Ser | Asn | Ser | Trp | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Pro Ala

<210> 39  
<211> 18

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 39

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Pro | Ala | Ala | His | Phe | Pro | Arg | Ser | Ile | Pro | Arg | Pro | Gly | Pro | Ile |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Arg Thr

<210> 40  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 40

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ala | Pro | Gly | Pro | Ser | Ala | Pro | Ser | His | Arg | Ser | Leu | Pro | Ser | Arg | Ala |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Phe Gly

<210> 41  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 41

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Arg | Asn | Ser | Ile | His | Phe | Leu | His | Pro | Leu | Leu | Val | Ala | Pro | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Gly Ala

<210> 42  
<211> 18

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 42

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Pro | Ser | Leu | Ser | Gly | Val | Leu | Gln | Val | Arg | Tyr | Leu | Ser | Pro | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Asp Leu

<210> 43  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 43

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Pro | Gln | Tyr | Pro | Ser | Pro | Leu | Thr | Leu | Thr | Leu | Pro | Pro | His | Pro |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Ser Leu

<210> 44  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexiible linker

<400> 44

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Pro | Ser | Leu | Asn | Pro | Pro | Ser | Tyr | Leu | His | Arg | Ala | Pro | Ser | Arg |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Ile Ser

<210> 45  
<211> 17

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 45

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Pro | Trp | Arg | Thr | Ser | Leu | Leu | Pro | Ser | Leu | Pro | Leu | Arg | Arg | Arg |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |     |

Pro

<210> 46  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 46

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Pro | Leu | Phe | Ala | Lys | Gly | Pro | Val | Gly | Leu | Leu | Ser | Arg | Ser | Phe |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |     |

Pro Pro

<210> 47  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 47

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Val | Pro | Pro | Ala | Pro | Val | Val | Ser | Leu | Arg | Ser | Ala | His | Ala | Arg | Pro |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |     |

Pro Tyr

<210> 48  
<211> 17

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 48

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Arg | Pro | Thr | Pro | Pro | Arg | Val | Arg | Ser | Tyr | Thr | Cys | Cys | Pro | Thr |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Pro

<210> 49  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 49

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Asn | Val | Ala | His | Val | Leu | Pro | Leu | Leu | Thr | Val | Pro | Trp | Asp | Asn |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Leu Arg

<210> 50  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 50

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Asn | Pro | Leu | Leu | Pro | Leu | Cys | Ala | Arg | Ser | Pro | Ala | Val | Arg | Thr |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

Phe Pro

<210> 51  
<211> 11

<212> PRT  
<213> Artificial Sequence

<220>  
<223> Rigid linker

<400> 51

Gly Ala Pro Ala Pro Ala Ala Pro Ala Pro Ala  
1 5 10

<210> 52  
<211> 4  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Rigid linker

<400> 52

Pro Pro Pro Pro  
1

<210> 53  
<211> 112  
<212> PRT  
<213> Homo sapiens

<400> 53

Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu Ile  
1 5 10 15

Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val His  
20 25 30

Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu Gln  
35 40 45

Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val Glu  
50 55 60

Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn Val  
65 70 75 80

Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn Ile

85

90

95

Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile Asn  
 100 105 110

<210> 54

<211> 352

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab HC Fwk3 IL-15

<400> 54

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Lys Tyr Gln Ser Gly Gly Ser  
 65 70 75 80

Gly Met Gly Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu  
 85 90 95

Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser  
 100 105 110

Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu  
 115 120 125

Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp  
 130 135 140

Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 145 |     | 150 |     | 155 |     | 160 |     |     |     |     |     |     |     |     |     |
| Gly | Asn | Val | Thr | Glu | Ser | Gly | Cys | Lys | Glu | Cys | Glu | Glu | Leu | Glu | Glu |
|     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |
| Lys | Asn | Ile | Lys | Glu | Phe | Leu | Gln | Ser | Phe | Val | His | Ile | Val | Gln | Met |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |
| Phe | Ile | Asn | Ser | Gly | Gly | Ser | Tyr | Thr | Tyr | Lys | Asn | Thr | Val | Tyr | Leu |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |
| Gln | Met | Asn | Ser | Leu | Arg | Ala | Glu | Asp | Thr | Ala | Val | Tyr | Tyr | Cys | Ala |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |
| Arg | Thr | Val | Pro | Gly | Tyr | Ser | Thr | Ala | Pro | Tyr | Phe | Asp | Leu | Trp | Gly |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |
| Gln | Gly | Thr | Leu | Val | Thr | Val | Ser | Ser | Ala | Ser | Thr | Lys | Gly | Pro | Ser |
|     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |
| Val | Phe | Pro | Leu | Ala | Pro | Ser | Ser | Lys | Ser | Thr | Ser | Gly | Gly | Thr | Ala |
|     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |
| Ala | Leu | Gly | Cys | Leu | Val | Lys | Asp | Tyr | Phe | Pro | Glu | Pro | Val | Thr | Val |
|     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |
| Ser | Trp | Asn | Ser | Gly | Ala | Leu | Thr | Ser | Gly | Val | His | Thr | Phe | Pro | Ala |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Val | Leu | Gln | Ser | Ser | Gly | Leu | Tyr | Ser | Leu | Ser | Ser | Val | Val | Thr | Val |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |
| Pro | Ser | Ser | Ser | Leu | Gly | Thr | Gln | Thr | Tyr | Ile | Cys | Asn | Val | Asn | His |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Lys | Pro | Ser | Asn | Thr | Lys | Val | Asp | Lys | Lys | Val | Glu | Pro | Lys | Ser | Cys |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |

<210> 55  
 <211> 133  
 <212> PRT

<213> Homo sapiens

<400> 55

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His  
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys  
20 25 30

Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met Pro Lys  
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu Leu Lys  
50 55 60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu  
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu  
85 90 95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala  
100 105 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln Ser Ile  
115 120 125

Ile Ser Thr Leu Thr  
130

<210> 56

<211> 368

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab HC Fwk3 IL-2

<400> 56

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Ser Gly Gly  
65 70 75 80

Gly Gly Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu  
85 90 95

Glu His Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn  
100 105 110

Tyr Lys Asn Pro Lys Leu Thr Arg Met Leu Thr Phe Lys Phe Tyr Met  
115 120 125

Pro Lys Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Glu  
130 135 140

Leu Lys Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe  
145 150 155 160

His Leu Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu  
165 170 175

Glu Leu Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu  
180 185 190

Thr Ala Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Cys Gln  
195 200 205

Ser Ile Ile Ser Thr Leu Thr Gly Gly Gly Lys Asn Thr Val Tyr Leu  
210 215 220

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
225 230 235 240

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly  
245 250 255

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
260 265 270

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
275 280 285

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
290 295 300

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
305 310 315 320

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
325 330 335

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
340 345 350

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
355 360 365

<210> 57  
<211> 178  
<212> PRT  
<213> Homo sapiens

<400> 57

Ala Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu  
1 5 10 15

Lys Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu  
20 25 30

Gln Glu Lys Leu Val Ser Glu Cys Ala Thr Tyr Lys Leu Cys His Pro  
35 40 45

Glu Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro

50

55

60

Leu Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser  
65 70 75 80

Gln Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu  
85 90 95

Glu Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu  
100 105 110

Asp Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu  
115 120 125

Gly Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe  
130 135 140

Ala Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His  
145 150 155 160

Leu Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala  
165 170 175

Gln Pro

<210> 58

<211> 412

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab HC Fwk3 G-CSF

<400> 58

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile

35

40

45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Ala  
 65 70 75 80

Thr Pro Leu Gly Pro Ala Ser Ser Leu Pro Gln Ser Phe Leu Leu Lys  
 85 90 95

Cys Leu Glu Gln Val Arg Lys Ile Gln Gly Asp Gly Ala Ala Leu Gln  
 100 105 110

Glu Lys Leu Val Ser Glu Cys Ala Thr Tyr Lys Leu Cys His Pro Glu  
 115 120 125

Glu Leu Val Leu Leu Gly His Ser Leu Gly Ile Pro Trp Ala Pro Leu  
 130 135 140

Ser Ser Cys Pro Ser Gln Ala Leu Gln Leu Ala Gly Cys Leu Ser Gln  
 145 150 155 160

Leu His Ser Gly Leu Phe Leu Tyr Gln Gly Leu Leu Gln Ala Leu Glu  
 165 170 175

Gly Ile Ser Pro Glu Leu Gly Pro Thr Leu Asp Thr Leu Gln Leu Asp  
 180 185 190

Val Ala Asp Phe Ala Thr Thr Ile Trp Gln Gln Met Glu Glu Leu Gly  
 195 200 205

Met Ala Pro Ala Leu Gln Pro Thr Gln Gly Ala Met Pro Ala Phe Ala  
 210 215 220

Ser Ala Phe Gln Arg Arg Ala Gly Gly Val Leu Val Ala Ser His Leu  
 225 230 235 240

Gln Ser Phe Leu Glu Val Ser Tyr Arg Val Leu Arg His Leu Ala Gln  
 245 250 255

Pro Ser Gly Gly Gly Gly Lys Asn Thr Val Tyr Leu Gln Met Asn Ser  
260 265 270

Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro  
275 280 285

Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu  
290 295 300

Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu  
305 310 315 320

Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys  
325 330 335

Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser  
340 345 350

Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser  
355 360 365

Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser  
370 375 380

Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn  
385 390 395 400

Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
405 410

<210> 59  
<211> 127  
<212> PRT  
<213> Homo sapiens

<400> 59

Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu His Val  
1 5 10 15

Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg Asp Thr  
20 25 30

Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met Phe Asp  
35 40 45

Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr Lys Gln  
50 55 60

Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr Met Met  
65 70 75 80

Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr Ser Cys  
85 90 95

Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu Lys Asp  
100 105 110

Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln Glu  
115 120 125

<210> 60  
<211> 362  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 Fab HC Fwk3 GM-CSF

<400> 60

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Ser Gly Gly  
65 70 75 80

Gly Gly Ala Pro Ala Arg Ser Pro Ser Pro Ser Thr Gln Pro Trp Glu  
85 90 95

His Val Asn Ala Ile Gln Glu Ala Arg Arg Leu Leu Asn Leu Ser Arg  
100 105 110

Asp Thr Ala Ala Glu Met Asn Glu Thr Val Glu Val Ile Ser Glu Met  
115 120 125

Phe Asp Leu Gln Glu Pro Thr Cys Leu Gln Thr Arg Leu Glu Leu Tyr  
130 135 140

Lys Gln Gly Leu Arg Gly Ser Leu Thr Lys Leu Lys Gly Pro Leu Thr  
145 150 155 160

Met Met Ala Ser His Tyr Lys Gln His Cys Pro Pro Thr Pro Glu Thr  
165 170 175

Ser Cys Ala Thr Gln Ile Ile Thr Phe Glu Ser Phe Lys Glu Asn Leu  
180 185 190

Lys Asp Phe Leu Leu Val Ile Pro Phe Asp Cys Trp Glu Pro Val Gln  
195 200 205

Glu Gly Gly Gly Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg  
210 215 220

Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly Tyr  
225 230 235 240

Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val Thr  
245 250 255

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro  
260 265 270

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val  
275 280 285

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala  
 290 295 300

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly  
 305 310 315 320

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly  
 325 330 335

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys  
 340 345 350

Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 355 360

<210> 61  
 <211> 359  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 Fab HC with GM-CSF at C-terminus of CH1

<400> 61

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
 85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
210 215 220

Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Ala Arg Ser Pro Ser Pro  
225 230 235 240

Ser Thr Gln Pro Trp Glu His Val Asn Ala Ile Gln Glu Ala Arg Arg  
245 250 255

Leu Leu Asn Leu Ser Arg Asp Thr Ala Ala Glu Met Asn Glu Thr Val  
260 265 270

Glu Val Ile Ser Glu Met Phe Asp Leu Gln Glu Pro Thr Cys Leu Gln  
275 280 285

Thr Arg Leu Glu Leu Tyr Lys Gln Gly Leu Arg Gly Ser Leu Thr Lys  
290 295 300

Leu Lys Gly Pro Leu Thr Met Met Ala Ser His Tyr Lys Gln His Cys  
305 310 315 320

Pro Pro Thr Pro Glu Thr Ser Cys Ala Thr Gln Ile Ile Thr Phe Glu  
 325 330 335

Ser Phe Lys Glu Asn Leu Lys Asp Phe Leu Leu Val Ile Pro Phe Asp  
 340 345 350

Cys Trp Glu Pro Val Gln Glu  
 355

<210> 62  
 <211> 124  
 <212> PRT  
 <213> Murine

<400> 62

Ala Pro Thr Arg Ser Pro Ile Thr Val Thr Arg Pro Trp Lys His Val  
 1 5 10 15

Glu Ala Ile Lys Glu Ala Leu Asn Leu Leu Asp Asp Met Pro Val Thr  
 20 25 30

Leu Asn Glu Glu Val Glu Val Val Ser Asn Glu Phe Ser Phe Lys Lys  
 35 40 45

Leu Thr Cys Val Gln Thr Arg Leu Lys Ile Phe Glu Gln Gly Leu Arg  
 50 55 60

Gly Asn Phe Thr Lys Leu Lys Gly Ala Leu Asn Met Thr Ala Ser Tyr  
 65 70 75 80

Tyr Gln Thr Tyr Cys Pro Pro Thr Pro Glu Thr Asp Cys Glu Thr Gln  
 85 90 95

Val Thr Thr Tyr Ala Asp Phe Ile Asp Ser Leu Lys Thr Phe Leu Thr  
 100 105 110

Asp Ile Pro Phe Glu Cys Lys Lys Pro Gly Gln Lys  
 115 120

<210> 63

<211> 359  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 Fab HC Fwk3 mGM-CSF

<400> 63

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Ser Gly Gly  
 65 70 75 80

Gly Gly Ala Pro Thr Arg Ser Pro Ile Thr Val Thr Arg Pro Trp Lys  
 85 90 95

His Val Glu Ala Ile Lys Glu Ala Leu Asn Leu Leu Asp Asp Met Pro  
 100 105 110

Val Thr Leu Asn Glu Glu Val Glu Val Val Ser Asn Glu Phe Ser Phe  
 115 120 125

Lys Lys Leu Thr Cys Val Gln Thr Arg Leu Lys Ile Phe Glu Gln Gly  
 130 135 140

Leu Arg Gly Asn Phe Thr Lys Leu Lys Gly Ala Leu Asn Met Thr Ala  
 145 150 155 160

Ser Tyr Tyr Gln Thr Tyr Cys Pro Pro Thr Pro Glu Thr Asp Cys Glu  
 165 170 175

Thr Gln Val Thr Thr Tyr Ala Asp Phe Ile Asp Ser Leu Lys Thr Phe

180

185

190

Leu Thr Asp Ile Pro Phe Glu Cys Lys Lys Pro Gly Gln Lys Gly Gly  
 195 200 205

Gly Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp  
 210 215 220

Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly Tyr Ser Thr Ala  
 225 230 235 240

Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser  
 245 250 255

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys  
 260 265 270

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
 275 280 285

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
 290 295 300

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
 305 310 315 320

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr  
 325 330 335

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys  
 340 345 350

Lys Val Glu Pro Lys Ser Cys  
 355

&lt;210&gt; 64

&lt;211&gt; 356

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; CA645 Fab HC with mGM-CSF at C-terminus of CH1

<400> 64

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly  
100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 210 215 220

Gly Gly Gly Ser Gly Gly Gly Gly Ala Pro Thr Arg Ser Pro Ile Thr  
 225 230 235 240

Val Thr Arg Pro Trp Lys His Val Glu Ala Ile Lys Glu Ala Leu Asn  
 245 250 255

Leu Leu Asp Asp Met Pro Val Thr Leu Asn Glu Glu Val Glu Val Val  
 260 265 270

Ser Asn Glu Phe Ser Phe Lys Lys Leu Thr Cys Val Gln Thr Arg Leu  
 275 280 285

Lys Ile Phe Glu Gln Gly Leu Arg Gly Asn Phe Thr Lys Leu Lys Gly  
 290 295 300

Ala Leu Asn Met Thr Ala Ser Tyr Tyr Gln Thr Tyr Cys Pro Pro Thr  
 305 310 315 320

Pro Glu Thr Asp Cys Glu Thr Gln Val Thr Thr Tyr Ala Asp Phe Ile  
 325 330 335

Asp Ser Leu Lys Thr Phe Leu Thr Asp Ile Pro Phe Glu Cys Lys Lys  
 340 345 350

Pro Gly Gln Lys  
 355

<210> 65  
 <211> 166  
 <212> PRT  
 <213> Homo sapiens

<400> 65

Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg Tyr Leu  
 1 5 10 15

Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala Glu His  
 20 25 30

Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val Asn Phe  
35 40 45

Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu Val Trp  
50 55 60

Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln Ala Leu  
65 70 75 80

Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His Val Asp  
85 90 95

Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg Ala Leu  
100 105 110

Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser Ala Ala  
115 120 125

Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe Arg Val  
130 135 140

Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly Glu Ala  
145 150 155 160

Cys Arg Thr Gly Asp Arg  
165

<210> 66  
<211> 401  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 Fab HC Fwk3 EPO

<400> 66

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Ser Gly Gly  
65 70 75 80

Gly Gly Ala Pro Pro Arg Leu Ile Cys Asp Ser Arg Val Leu Glu Arg  
85 90 95

Tyr Leu Leu Glu Ala Lys Glu Ala Glu Asn Ile Thr Thr Gly Cys Ala  
100 105 110

Glu His Cys Ser Leu Asn Glu Asn Ile Thr Val Pro Asp Thr Lys Val  
115 120 125

Asn Phe Tyr Ala Trp Lys Arg Met Glu Val Gly Gln Gln Ala Val Glu  
130 135 140

Val Trp Gln Gly Leu Ala Leu Leu Ser Glu Ala Val Leu Arg Gly Gln  
145 150 155 160

Ala Leu Leu Val Asn Ser Ser Gln Pro Trp Glu Pro Leu Gln Leu His  
165 170 175

Val Asp Lys Ala Val Ser Gly Leu Arg Ser Leu Thr Thr Leu Leu Arg  
180 185 190

Ala Leu Gly Ala Gln Lys Glu Ala Ile Ser Pro Pro Asp Ala Ala Ser  
195 200 205

Ala Ala Pro Leu Arg Thr Ile Thr Ala Asp Thr Phe Arg Lys Leu Phe  
210 215 220

Arg Val Tyr Ser Asn Phe Leu Arg Gly Lys Leu Lys Leu Tyr Thr Gly  
225 230 235 240

Glu Ala Cys Arg Thr Gly Asp Arg Gly Gly Gly Lys Asn Thr Val Tyr  
245 250 255

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
260 265 270

Ala Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp  
275 280 285

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro  
290 295 300

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr  
305 310 315 320

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr  
325 330 335

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro  
340 345 350

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr  
355 360 365

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn  
370 375 380

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser  
385 390 395 400

Cys

<210> 67  
<211> 125  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> VHH6

<400> 67

Asp Val Gln Phe Val Glu Ser Gly Gly Gly Ser Val His Ala Gly Gly  
1 5 10 15

Ser Leu Arg Leu Asn Cys Ala Thr Ser Gly Tyr Ile Tyr Ser Thr Tyr  
20 25 30

Cys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu Arg Glu Gly Val  
35 40 45

Ala His Ile Tyr Thr Asn Ser Gly Arg Thr Tyr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys Asn Thr Val Tyr  
65 70 75 80

Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala Ile Tyr Tyr Cys  
85 90 95

Ala Ala Arg Pro Ser Ile Arg Cys Ala Ser Phe Ser Ala Thr Glu Tyr  
100 105 110

Lys Asp Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser  
115 120 125

<210> 68  
<211> 369  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 Fab HC Fwk3 VHH6

<400> 68

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
65 70 75 80

Gly Gly Gly Ser Asp Val Gln Phe Val Glu Ser Gly Gly Gly Ser Val  
85 90 95

His Ala Gly Gly Ser Leu Arg Leu Asn Cys Ala Thr Ser Gly Tyr Ile  
100 105 110

Tyr Ser Thr Tyr Cys Met Gly Trp Phe Arg Gln Ala Pro Gly Lys Glu  
115 120 125

Arg Glu Gly Val Ala His Ile Tyr Thr Asn Ser Gly Arg Thr Tyr Tyr  
130 135 140

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln Asp Asn Ala Lys  
145 150 155 160

Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Lys Pro Glu Asp Thr Ala  
165 170 175

Ile Tyr Tyr Cys Ala Ala Arg Pro Ser Ile Arg Cys Ala Ser Phe Ser  
180 185 190

Ala Thr Glu Tyr Lys Asp Trp Gly Gln Gly Thr Gln Val Thr Val Ser  
195 200 205

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Lys Asn Thr Val Tyr  
210 215 220

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
225 230 235 240

Ala Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp  
245 250 255

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro  
260 265 270

Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr  
275 280 285

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr  
290 295 300

Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro  
305 310 315 320

Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr  
325 330 335

Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn  
340 345 350

His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser  
355 360 365

Cys

<210> 69  
<211> 119  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> VHH15

<400> 69

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Val Ala Ala Ser Gly Tyr Thr Gly Cys Thr  
20 25 30

Tyr Asp Met Arg Trp Tyr Arg Gln Ala Pro Gly Lys Glu Arg Glu Phe  
35 40 45

Val Ser Gly Ile Asp Ser Asp Gly Arg Ala Thr Tyr Ala Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Ser Asn Ala Lys Ile Ala Val Tyr  
65 70 75 80

Leu Gln Met Asp Ser Leu Lys Leu Glu Asp Thr Ala Met Tyr Tyr Cys  
85 90 95

Asn Leu Gln Cys Leu Arg Tyr Pro Gly Glu Tyr Tyr Trp Gly Gln Gly  
100 105 110

Thr Gln Val Thr Val Ser Ser  
115

<210> 70

<211> 363

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab HC Fwk3 VHH15

<400> 70

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
65 70 75 80

Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val  
85 90 95

Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Val Ala Ala Ser Gly Tyr  
100 105 110

Thr Gly Cys Thr Tyr Asp Met Arg Trp Tyr Arg Gln Ala Pro Gly Lys  
115 120 125

Glu Arg Glu Phe Val Ser Gly Ile Asp Ser Asp Gly Arg Ala Thr Tyr  
130 135 140

Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Gln Ser Asn Ala Lys  
145 150 155 160

Ile Ala Val Tyr Leu Gln Met Asp Ser Leu Lys Leu Glu Asp Thr Ala  
165 170 175

Met Tyr Tyr Cys Asn Leu Gln Cys Leu Arg Tyr Pro Gly Glu Tyr Tyr  
180 185 190

Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser  
195 200 205

Gly Gly Gly Gly Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu  
210 215 220

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly  
225 230 235 240

Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val  
245 250 255

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
260 265 270

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
275 280 285

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
290 295 300

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
305 310 315 320

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
325 330 335

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
340 345 350

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
355 360

<210> 71  
<211> 190  
<212> PRT  
<213> Homo sapiens

<400> 71

Gln Gly Trp Gln Ala Phe Lys Asn Asp Ala Thr Glu Ile Ile Pro Glu  
1 5 10 15

Leu Gly Glu Tyr Pro Glu Pro Pro Pro Glu Leu Glu Asn Asn Lys Thr  
20 25 30

Met Asn Arg Ala Glu Asn Gly Gly Arg Pro Pro His His Pro Phe Glu  
35 40 45

Thr Lys Asp Val Ser Glu Tyr Ser Cys Arg Glu Leu His Phe Thr Arg  
50 55 60

Tyr Val Thr Asp Gly Pro Cys Arg Ser Ala Lys Pro Val Thr Glu Leu  
65 70 75 80

Val Cys Ser Gly Gln Cys Gly Pro Ala Arg Leu Leu Pro Asn Ala Ile  
85 90 95

Gly Arg Gly Lys Trp Trp Arg Pro Ser Gly Pro Asp Phe Arg Cys Ile  
100 105 110

Pro Asp Arg Tyr Arg Ala Gln Arg Val Gln Leu Leu Cys Pro Gly Gly  
115 120 125

Glu Ala Pro Arg Ala Arg Lys Val Arg Leu Val Ala Ser Cys Lys Cys  
130 135 140

Lys Arg Leu Thr Arg Phe His Asn Gln Ser Glu Leu Lys Asp Phe Gly  
 145 150 155 160

Thr Glu Ala Ala Arg Pro Gln Lys Gly Arg Lys Pro Arg Pro Arg Ala  
 165 170 175

Arg Ser Ala Lys Ala Asn Gln Ala Glu Leu Glu Asn Ala Tyr  
 180 185 190

<210> 72  
 <211> 88  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Core domain of human sclerostin

<400> 72

Cys Arg Glu Leu His Phe Thr Arg Tyr Val Thr Asp Gly Pro Cys Arg  
 1 5 10 15

Ser Ala Lys Pro Val Thr Glu Leu Val Cys Ser Gly Gln Cys Gly Pro  
 20 25 30

Ala Arg Leu Leu Pro Asn Ala Ile Gly Arg Gly Lys Trp Trp Arg Pro  
 35 40 45

Ser Gly Pro Asp Phe Arg Cys Ile Pro Asp Arg Tyr Arg Ala Gln Arg  
 50 55 60

Val Gln Leu Leu Cys Pro Gly Gly Glu Ala Pro Arg Ala Arg Lys Val  
 65 70 75 80

Arg Leu Val Ala Ser Cys Lys Cys  
 85

<210> 73  
 <211> 314  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 Fab heavy chain with human sclerostin core domain inserted

into framework 3, single S linkers

<400> 73

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Ser Cys Arg Glu Leu His  
65 70 75 80

Phe Thr Arg Tyr Val Thr Asp Gly Pro Cys Arg Ser Ala Lys Pro Val  
85 90 95

Thr Glu Leu Val Cys Ser Gly Gln Cys Gly Pro Ala Arg Leu Leu Pro  
100 105 110

Asn Ala Ile Gly Arg Gly Lys Trp Trp Arg Pro Ser Gly Pro Asp Phe  
115 120 125

Arg Cys Ile Pro Asp Arg Tyr Arg Ala Gln Arg Val Gln Leu Leu Cys  
130 135 140

Pro Gly Gly Glu Ala Pro Arg Ala Arg Lys Val Arg Leu Val Ala Ser  
145 150 155 160

Cys Lys Cys Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg  
165 170 175

Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly Tyr  
180 185 190

Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val Thr  
195 200 205

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro  
 210 215 220

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val  
 225 230 235 240

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala  
 245 250 255

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly  
 260 265 270

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly  
 275 280 285

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys  
 290 295 300

Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 305 310

<210> 74  
 <211> 333  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 Fab heavy chain with human sclerostin core domain inserted  
 into framework 3, Gly-Ser (2X G4S) linkers

<400> 74

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys

50

55

60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
65 70 75 80

Gly Gly Gly Ser Cys Arg Glu Leu His Phe Thr Arg Tyr Val Thr Asp  
85 90 95

Gly Pro Cys Arg Ser Ala Lys Pro Val Thr Glu Leu Val Cys Ser Gly  
100 105 110

Gln Cys Gly Pro Ala Arg Leu Leu Pro Asn Ala Ile Gly Arg Gly Lys  
115 120 125

Trp Trp Arg Pro Ser Gly Pro Asp Phe Arg Cys Ile Pro Asp Arg Tyr  
130 135 140

Arg Ala Gln Arg Val Gln Leu Leu Cys Pro Gly Gly Glu Ala Pro Arg  
145 150 155 160

Ala Arg Lys Val Arg Leu Val Ala Ser Cys Lys Cys Ser Gly Gly Gly  
165 170 175

Gly Ser Gly Gly Gly Gly Ser Lys Asn Thr Val Tyr Leu Gln Met Asn  
180 185 190

Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val  
195 200 205

Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr  
210 215 220

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro  
225 230 235 240

Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly  
245 250 255

Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn  
260 265 270

Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln  
275 280 285

Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser  
290 295 300

Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser  
305 310 315 320

Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
325 330

<210> 75

<211> 323

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab heavy chain with human sclerostin core domain inserted  
into framework 3, rigid linkers

<400> 75

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Ile Pro Phe Thr Val Cys  
65 70 75 80

Arg Glu Leu His Phe Thr Arg Tyr Val Thr Asp Gly Pro Cys Arg Ser  
85 90 95

Ala Lys Pro Val Thr Glu Leu Val Cys Ser Gly Gln Cys Gly Pro Ala  
100 105 110

Arg Leu Leu Pro Asn Ala Ile Gly Arg Gly Lys Trp Trp Arg Pro Ser  
115 120 125

Gly Pro Asp Phe Arg Cys Ile Pro Asp Arg Tyr Arg Ala Gln Arg Val  
130 135 140

Gln Leu Leu Cys Pro Gly Gly Glu Ala Pro Arg Ala Arg Lys Val Arg  
145 150 155 160

Leu Val Ala Ser Cys Lys Cys Glu Tyr His Gly Leu Gln Lys Asn Thr  
165 170 175

Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr  
180 185 190

Tyr Cys Ala Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp  
195 200 205

Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
210 215 220

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
225 230 235 240

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
245 250 255

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
260 265 270

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
275 280 285

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
290 295 300

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
305 310 315 320

Lys Ser Cys

<210> 76  
<211> 425  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 Fab heavy chain with human mature full-length sclerostin  
inserted into framework 3, Gly-Ser (1X G4S) linkers

<400> 76

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gln  
65 70 75 80

Gly Trp Gln Ala Phe Lys Asn Asp Ala Thr Glu Ile Ile Pro Glu Leu  
85 90 95

Gly Glu Tyr Pro Glu Pro Pro Pro Glu Leu Glu Asn Asn Lys Thr Met  
100 105 110

Asn Arg Ala Glu Asn Gly Gly Arg Pro Pro His His Pro Phe Glu Thr  
115 120 125

Lys Asp Val Ser Glu Tyr Ser Cys Arg Glu Leu His Phe Thr Arg Tyr  
130 135 140

Val Thr Asp Gly Pro Cys Arg Ser Ala Lys Pro Val Thr Glu Leu Val  
145 150 155 160

Cys Ser Gly Gln Cys Gly Pro Ala Arg Leu Leu Pro Asn Ala Ile Gly  
165 170 175

Arg Gly Lys Trp Trp Arg Pro Ser Gly Pro Asp Phe Arg Cys Ile Pro  
180 185 190

Asp Arg Tyr Arg Ala Gln Arg Val Gln Leu Leu Cys Pro Gly Gly Glu  
195 200 205

Ala Pro Arg Ala Arg Lys Val Arg Leu Val Ala Ser Cys Lys Cys Lys  
210 215 220

Arg Leu Thr Arg Phe His Asn Gln Ser Glu Leu Lys Asp Phe Gly Thr  
225 230 235 240

Glu Ala Ala Arg Pro Gln Lys Gly Arg Lys Pro Arg Pro Arg Ala Arg  
245 250 255

Ser Ala Lys Ala Asn Gln Ala Glu Leu Glu Asn Ala Tyr Ser Gly Gly  
260 265 270

Gly Gly Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala  
275 280 285

Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly Tyr Ser  
290 295 300

Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val Thr Val  
305 310 315 320

Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser  
325 330 335

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys  
340 345 350

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu  
355 360 365

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu

370

375

380

Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr  
 385 390 395 400

Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val  
 405 410 415

Asp Lys Lys Val Glu Pro Lys Ser Cys  
 420 425

<210> 77  
 <211> 10  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Flexible linker

<400> 77

Lys Tyr Gln Ser Gly Gly Ser Gly Met Gly  
 1 5 10

<210> 78  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Flexible linker

<400> 78

Ser Gly Gly Ser Tyr Thr Tyr  
 1 5

<210> 79  
 <211> 8  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Flexible linker

<400> 79

Gly Gly Gly Ser Gly Gly Gly Gly

1

5

<210> 80  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 80

Gly Ser Ser Ser Ser Gly Ser  
1 5

<210> 81  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Flexible linker

<400> 81

Ser Gly Gly Gly Gly  
1 5

<210> 82  
<211> 5  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Rigid linker

<400> 82

Ile Pro Phe Thr Val  
1 5

<210> 83  
<211> 347  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA497 Fab heavy chain with human IL-15 inserted into framework 3,  
Gly-Ser linkers

<400> 83

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 Val Ile Phe Ser Asp Tyr  
20 25 30

Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ala Ser Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr Arg Glu Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gly Gly Gly Ser Gly  
65 70 75 80

Gly Gly Gly Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu  
85 90 95

Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser  
100 105 110

Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu  
115 120 125

Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp  
130 135 140

Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn  
145 150 155 160

Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu  
165 170 175

Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met  
180 185 190

Phe Ile Asn Gly Gly Gly Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser  
195 200 205

Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr Asp Ala Asn  
210 215 220

Arg Gln Asn Tyr Asp Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val  
225 230 235 240

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
245 250 255

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
260 265 270

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
275 280 285

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
290 295 300

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
305 310 315 320

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
325 330 335

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
340 345

<210> 84  
<211> 218  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA497 Fab light chain

<400> 84

Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Ser Val Ser Ser Ser  
20 25 30

Met Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro  
35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Ser  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Trp  
85 90 95

Thr Ala Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg  
100 105 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln  
115 120 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr  
130 135 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser  
145 150 155 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
165 170 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys  
180 185 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro  
195 200 205

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
210 215

<210> 85

<211> 346

<212> PRT

<213> Artificial Sequence

<220>

<223> CA240 Fab heavy chain with human IL-15 inserted into framework 3,  
Gly-Ser linkers

<400> 85

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Phe Asn Asp Tyr  
20 25 30

Phe Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ala Gln Met Arg Asn Lys Asn Tyr Gln Tyr Gly Thr Tyr Tyr Ala Glu  
50 55 60

Ser Leu Glu Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Gly Gly Gly  
65 70 75 80

Ser Gly Gly Gly Gly Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys  
85 90 95

Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr  
100 105 110

Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe  
115 120 125

Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile  
130 135 140

His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser  
145 150 155 160

Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu  
165 170 175

Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val  
180 185 190

Gln Met Phe Ile Asn Gly Gly Gly Lys Asn Ser Leu Tyr Leu Gln Met

195

200

205

Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu  
 210 215 220

Ser Tyr Tyr Gly Phe Thr Ser Tyr Trp Gly Gln Gly Thr Leu Val Thr  
 225 230 235 240

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro  
 245 250 255

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val  
 260 265 270

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala  
 275 280 285

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly  
 290 295 300

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly  
 305 310 315 320

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys  
 325 330 335

Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 340 345

&lt;210&gt; 86

&lt;211&gt; 214

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; CA240 Fab light chain

&lt;400&gt; 86

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 Gln Ala Ser Gln Asp Ile Gly Ile Ser

20

25

30

Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 35 40 45

Tyr Asn Ala Asn Asn 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 Leu Gln His Asn Ser Ala Pro Tyr  
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala  
 100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
 115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
 130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
 145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
 165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
 180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser  
 195 200 205

Phe Asn Arg Gly Glu Cys  
 210

<210> 87

<211> 219

<212> PRT

<213> Artificial Sequence

<220>

<223> HyHEL5 heavy chain

<400> 87

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala  
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Asp Tyr  
20 25 30

Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile  
35 40 45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr His Glu Arg Phe  
50 55 60

Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Ser Thr Ala Tyr  
65 70 75 80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Gly Val Tyr Tyr Cys  
85 90 95

Leu His Gly Asn Tyr Asp Phe Asp Gly Trp Gly Gln Gly Thr Thr Leu  
100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
115 120 125

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
180 185 190

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
195 200 205

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
210 215

<210> 88  
<211> 212  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> HyHEL5 light chain

<400> 88

Asp Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly  
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Asn Tyr Met  
20 25 30

Tyr Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr  
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Val 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 Gly Arg Asn Pro Thr Phe  
85 90 95

Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser  
100 105 110

Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala  
115 120 125

Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val  
130 135 140

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser  
145 150 155 160

Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr  
165 170 175

Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys  
180 185 190

Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn  
195 200 205

Arg Gly Glu Cys  
210

<210> 89  
<211> 127  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> D3L11

<400> 89

Asp Val Gln Leu Val Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Ser Thr Asp Ser Ile Glu  
20 25 30

Tyr Met Thr Trp Phe Arg Gln Ala Pro Gly Lys Ala Arg Glu Gly Val  
35 40 45

Ala Ala Leu Tyr Thr His Thr Gly Asn Thr Tyr Tyr Thr Asp Ser Val  
50 55 60

Lys Gly Arg Phe Thr Ile Ser Gln Asp Lys Ala Lys Asn Met Ala Tyr  
65 70 75 80

Leu Arg Met Asp Ser Val Lys Ser Glu Asp Thr Ala Ile Tyr Thr Cys  
85 90 95

Gly Ala Thr Arg Lys Tyr Val Pro Val Arg Phe Ala Leu Asp Gln Ser  
100 105 110

Ser Tyr Asp Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser  
115 120 125

<210> 90

<211> 360

<212> PRT

<213> Artificial Sequence

<220>

<223> Anti-lysozyme HyHEL5 light chain with anti-lysozyme VHH D3L11  
inserted into framework 3, Gly-Ser linkers

<400> 90

Asp Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly  
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Asn Tyr Met  
20 25 30

Tyr Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr  
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser  
50 55 60

Gly Ser Gly Ser Gly Gly Gly Gly Ser Asp Val Gln Leu Val Glu Ser  
65 70 75 80

Gly Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Ala  
85 90 95

Ala Ser Gly Ser Thr Asp Ser Ile Glu Tyr Met Thr Trp Phe Arg Gln  
100 105 110

Ala Pro Gly Lys Ala Arg Glu Gly Val Ala Ala Leu Tyr Thr His Thr  
115 120 125

Gly Asn Thr Tyr Tyr Thr Asp Ser Val Lys Gly Arg Phe Thr Ile Ser  
130 135 140

Gln Asp Lys Ala Lys Asn Met Ala Tyr Leu Arg Met Asp Ser Val Lys  
 145 150 155 160

Ser Glu Asp Thr Ala Ile Tyr Thr Cys Gly Ala Thr Arg Lys Tyr Val  
 165 170 175

Pro Val Arg Phe Ala Leu Asp Gln Ser Ser Tyr Asp Tyr Trp Gly Gln  
 180 185 190

Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly  
 195 200 205

Gly Ser Gly Gly Gly Gly Ser Thr Ser Tyr Ser Leu Thr Ile Ser Ser  
 210 215 220

Met Glu Thr Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Gly Arg  
 225 230 235 240

Asn Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val  
 245 250 255

Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys  
 260 265 270

Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg  
 275 280 285

Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn  
 290 295 300

Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser  
 305 310 315 320

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys  
 325 330 335

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr  
 340 345 350

Lys Ser Phe Asn Arg Gly Glu Cys  
 355 360

<210> 91  
 <211> 352  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Anti-lysozyme HyHEL5 light chain with anti-IL-6 VHH15 inserted  
 into framework 3, Gly-Ser linkers

<400> 91

Asp Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly  
 1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Asn Tyr Met  
 20 25 30

Tyr Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr  
 35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser  
 50 55 60

Gly Ser Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Glu Ser  
 65 70 75 80

Gly Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser Cys Val  
 85 90 95

Ala Ala Ser Gly Tyr Thr Gly Cys Thr Tyr Asp Met Arg Trp Tyr Arg  
 100 105 110

Gln Ala Pro Gly Lys Glu Arg Glu Phe Val Ser Gly Ile Asp Ser Asp  
 115 120 125

Gly Arg Ala Thr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser  
 130 135 140

Gln Ser Asn Ala Lys Ile Ala Val Tyr Leu Gln Met Asp Ser Leu Lys  
 145 150 155 160

Leu Glu Asp Thr Ala Met Tyr Tyr Cys Asn Leu Gln Cys Leu Arg Tyr  
165 170 175

Pro Gly Glu Tyr Tyr Trp Gly Gln Gly Thr Gln Val Thr Val Ser Ser  
180 185 190

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Thr  
195 200 205

Ser Tyr Ser Leu Thr Ile Ser Ser Met Glu Thr Glu Asp Ala Ala Thr  
210 215 220

Tyr Tyr Cys Gln Gln Trp Gly Arg Asn Pro Thr Phe Gly Gly Gly Thr  
225 230 235 240

Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe  
245 250 255

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys  
260 265 270

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val  
275 280 285

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln  
290 295 300

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser  
305 310 315 320

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His  
325 330 335

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
340 345 350

<210> 92

<211> 365

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 light chain with anti-lysozyme VHH D3L11 inserted into framework 3, Gly-Ser linkers

<400> 92

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn  
20 25 30

Phe Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu  
35 40 45

Ile Tyr Glu Ala Ser Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser  
50 55 60

Gly Ser Gly Ser Gly Ser Gly Gly Gly Gly Ser Asp Val Gln Leu Val  
65 70 75 80

Glu Ser Gly Gly Gly Ser Val Gln Ala Gly Gly Ser Leu Arg Leu Ser  
85 90 95

Cys Ala Ala Ser Gly Ser Thr Asp Ser Ile Glu Tyr Met Thr Trp Phe  
100 105 110

Arg Gln Ala Pro Gly Lys Ala Arg Glu Gly Val Ala Ala Leu Tyr Thr  
115 120 125

His Thr Gly Asn Thr Tyr Tyr Thr Asp Ser Val Lys Gly Arg Phe Thr  
130 135 140

Ile Ser Gln Asp Lys Ala Lys Asn Met Ala Tyr Leu Arg Met Asp Ser  
145 150 155 160

Val Lys Ser Glu Asp Thr Ala Ile Tyr Thr Cys Gly Ala Thr Arg Lys  
165 170 175

Tyr Val Pro Val Arg Phe Ala Leu Asp Gln Ser Ser Tyr Asp Tyr Trp  
180 185 190

Gly Gln Gly Thr Gln Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly  
195 200 205

Gly Gly Gly Ser Gly Gly Gly Gly Ser Thr Asp Phe Thr Leu Thr Ile  
210 215 220

Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gly Gly Gly  
225 230 235 240

Tyr Ser Ser Ile Ser Asp Thr Thr Phe Gly Gly Gly Thr Lys Val Glu  
245 250 255

Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser  
260 265 270

Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn  
275 280 285

Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala  
290 295 300

Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys  
305 310 315 320

Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp  
325 330 335

Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu  
340 345 350

Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
355 360 365

<210> 93

<211> 360

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab heavy chain with human IL-15 inserted into framework 3  
with bovine long linker

<400> 93

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Thr Ser Val His Gln Glu  
 65 70 75 80

Thr Lys Lys Tyr Gln Ser Asn Trp Val Asn Val Ile Ser Asp Leu Lys  
 85 90 95

Lys Ile Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr  
 100 105 110

Thr Glu Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys  
 115 120 125

Phe Leu Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser  
 130 135 140

Ile His Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu  
 145 150 155 160

Ser Ser Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu  
 165 170 175

Leu Glu Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile  
 180 185 190

Val Gln Met Phe Ile Asn Ser Tyr Thr Tyr Asn Tyr Glu Trp His Val  
 195 200 205

Asp Val Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu

210

215

220

Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly Tyr Ser Thr  
 225 230 235 240

Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser  
 245 250 255

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser  
 260 265 270

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp  
 275 280 285

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr  
 290 295 300

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr  
 305 310 315 320

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln  
 325 330 335

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp  
 340 345 350

Lys Lys Val Glu Pro Lys Ser Cys  
 355 360

&lt;210&gt; 94

&lt;211&gt; 350

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> CA645 Fab heavy chain with human IL-15 inserted into framework 3  
 with bovine short linker

&lt;400&gt; 94

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Glu Thr Lys Lys Tyr Gln  
65 70 75 80

Ser Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile Glu Asp Leu  
85 90 95

Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu Ser Asp Val  
100 105 110

His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu Leu Glu Leu  
115 120 125

Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His Asp Thr Val  
130 135 140

Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser Asn Gly Asn  
145 150 155 160

Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu Glu Lys Asn  
165 170 175

Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln Met Phe Ile  
180 185 190

Asn Ser Tyr Thr Tyr Asn Tyr Glu Lys Asn Thr Val Tyr Leu Gln Met  
195 200 205

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr  
210 215 220

Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly  
225 230 235 240

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe  
245 250 255

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu  
260 265 270

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp  
275 280 285

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu  
290 295 300

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser  
305 310 315 320

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro  
325 330 335

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
340 345 350

<210> 95

<211> 356

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab heavy chain with human IL-15 inserted into framework 3  
with 2xG4S linker

<400> 95

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys

50

55

60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
65 70 75 80

Gly Gly Gly Ser Asn Trp Val Asn Val Ile Ser Asp Leu Lys Lys Ile  
85 90 95

Glu Asp Leu Ile Gln Ser Met His Ile Asp Ala Thr Leu Tyr Thr Glu  
100 105 110

Ser Asp Val His Pro Ser Cys Lys Val Thr Ala Met Lys Cys Phe Leu  
115 120 125

Leu Glu Leu Gln Val Ile Ser Leu Glu Ser Gly Asp Ala Ser Ile His  
130 135 140

Asp Thr Val Glu Asn Leu Ile Ile Leu Ala Asn Asn Ser Leu Ser Ser  
145 150 155 160

Asn Gly Asn Val Thr Glu Ser Gly Cys Lys Glu Cys Glu Glu Leu Glu  
165 170 175

Glu Lys Asn Ile Lys Glu Phe Leu Gln Ser Phe Val His Ile Val Gln  
180 185 190

Met Phe Ile Asn Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Lys Asn  
195 200 205

Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val  
210 215 220

Tyr Tyr Cys Ala Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe  
225 230 235 240

Asp Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr  
245 250 255

Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser  
260 265 270

Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu  
 275 280 285

Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His  
 290 295 300

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser  
 305 310 315 320

Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys  
 325 330 335

Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu  
 340 345 350

Pro Lys Ser Cys  
 355

<210> 96  
 <211> 12  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Linker

<400> 96

Thr Ser Val His Gln Glu Thr Lys Lys Tyr Gln Ser  
 1 5 10

<210> 97  
 <211> 12  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Linker

<400> 97

Ser Tyr Thr Tyr Asn Tyr Glu Trp His Val Asp Val  
 1 5 10

<210> 98

<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Linker

<400> 98

Glu Thr Lys Lys Tyr Gln Ser  
1 5

<210> 99  
<211> 7  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Linker

<400> 99

Ser Tyr Thr Tyr Asn Tyr Glu  
1 5

<210> 100  
<211> 188  
<212> PRT  
<213> Homo sapiens

<400> 100

Asn Cys Trp Leu Arg Gln Ala Lys Asn Gly Arg Cys Gln Val Leu Tyr  
1 5 10 15

Lys Thr Glu Leu Ser Lys Glu Glu Cys Cys Ser Thr Gly Arg Leu Ser  
20 25 30

Thr Ser Trp Thr Glu Glu Asp Val Asn Asp Asn Thr Leu Phe Lys Trp  
35 40 45

Met Ile Phe Asn Gly Gly Ala Pro Asn Cys Ile Pro Cys Lys Glu Pro  
50 55 60

Arg Cys Val Cys Ala Pro Asp Cys Ser Asn Ile Thr Trp Lys Gly Pro  
65 70 75 80

Val Cys Gly Leu Asp Gly Lys Thr Tyr Arg Asn Glu Cys Ala Leu Leu  
85 90 95

Lys Ala Arg Cys Lys Glu Gln Pro Glu Leu Glu Val Gln Tyr Gln Gly  
100 105 110

Arg Cys Lys Lys Thr Cys Arg Asp Val Phe Cys Pro Gly Ser Ser Thr  
115 120 125

Cys Val Val Asp Gln Thr Asn Asn Ala Tyr Cys Val Thr Cys Asn Arg  
130 135 140

Ile Cys Pro Glu Pro Ala Ser Ser Glu Gln Tyr Leu Cys Gly Asn Asp  
145 150 155 160

Gly Val Thr Tyr Ser Ser Ala Cys His Leu Arg Lys Ala Thr Cys Leu  
165 170 175

Leu Gly Arg Ser Ile Gly Leu Ala Gly Glu Tyr Lys  
180 185

<210> 101

<211> 627

<212> PRT

<213> Artificial Sequence

<220>

<223> CA645 Fab heavy chain with two human IL-10 monomers inserted into  
framework 3

<400> 101

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
65 70 75 80

Ser Asn Cys Trp Leu Arg Gln Ala Lys Asn Gly Arg Cys Gln Val Leu  
85 90 95

Tyr Lys Thr Glu Leu Ser Lys Glu Glu Cys Cys Ser Thr Gly Arg Leu  
100 105 110

Ser Thr Ser Trp Thr Glu Glu Asp Val Asn Asp Asn Thr Leu Phe Lys  
115 120 125

Trp Met Ile Phe Asn Gly Gly Ala Pro Asn Cys Ile Pro Cys Lys Glu  
130 135 140

Pro Arg Cys Val Cys Ala Pro Asp Cys Ser Asn Ile Thr Trp Lys Gly  
145 150 155 160

Pro Val Cys Gly Leu Asp Gly Lys Thr Tyr Arg Asn Glu Cys Ala Leu  
165 170 175

Leu Lys Ala Arg Cys Lys Glu Gln Pro Glu Leu Glu Val Gln Tyr Gln  
180 185 190

Gly Arg Cys Lys Lys Thr Cys Arg Asp Val Phe Cys Pro Gly Ser Ser  
195 200 205

Thr Cys Val Val Asp Gln Thr Asn Asn Ala Tyr Cys Val Thr Cys Asn  
210 215 220

Arg Ile Cys Pro Glu Pro Ala Ser Ser Glu Gln Tyr Leu Cys Gly Asn  
225 230 235 240

Asp Gly Val Thr Tyr Ser Ser Ala Cys His Leu Arg Lys Ala Thr Cys  
245 250 255

Leu Leu Gly Arg Ser Ile Gly Leu Ala Gly Glu Tyr Lys Gly Gly Gly  
260 265 270

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asn Cys Trp Leu

| 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Arg | Gln | Ala | Lys | Asn | Gly | Arg | Cys | Gln | Val | Leu | Tyr | Lys | Thr | Glu | Leu |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |
| Ser | Lys | Glu | Glu | Cys | Cys | Ser | Thr | Gly | Arg | Leu | Ser | Thr | Ser | Trp | Thr |
| 305 |     |     |     | 310 |     |     |     |     |     | 315 |     |     |     |     | 320 |
| Glu | Glu | Asp | Val | Asn | Asp | Asn | Thr | Leu | Phe | Lys | Trp | Met | Ile | Phe | Asn |
|     |     |     |     | 325 |     |     |     |     | 330 |     |     |     |     | 335 |     |
| Gly | Gly | Ala | Pro | Asn | Cys | Ile | Pro | Cys | Lys | Glu | Pro | Arg | Cys | Val | Cys |
|     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |
| Ala | Pro | Asp | Cys | Ser | Asn | Ile | Thr | Trp | Lys | Gly | Pro | Val | Cys | Gly | Leu |
|     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Asp | Gly | Lys | Thr | Tyr | Arg | Asn | Glu | Cys | Ala | Leu | Leu | Lys | Ala | Arg | Cys |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
| Lys | Glu | Gln | Pro | Glu | Leu | Glu | Val | Gln | Tyr | Gln | Gly | Arg | Cys | Lys | Lys |
| 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
| Thr | Cys | Arg | Asp | Val | Phe | Cys | Pro | Gly | Ser | Ser | Thr | Cys | Val | Val | Asp |
|     |     |     | 405 |     |     |     |     |     | 410 |     |     |     |     | 415 |     |
| Gln | Thr | Asn | Asn | Ala | Tyr | Cys | Val | Thr | Cys | Asn | Arg | Ile | Cys | Pro | Glu |
|     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |
| Pro | Ala | Ser | Ser | Glu | Gln | Tyr | Leu | Cys | Gly | Asn | Asp | Gly | Val | Thr | Tyr |
|     |     | 435 |     |     |     |     | 440 |     |     |     |     | 445 |     |     |     |
| Ser | Ser | Ala | Cys | His | Leu | Arg | Lys | Ala | Thr | Cys | Leu | Leu | Gly | Arg | Ser |
|     | 450 |     |     |     |     | 455 |     |     |     |     | 460 |     |     |     |     |
| Ile | Gly | Leu | Ala | Tyr | Glu | Gly | Gly | Gly | Gly | Ser | Gly | Ser | Lys | Asn | Thr |
| 465 |     |     |     |     | 470 |     |     |     |     | 475 |     |     |     |     | 480 |
| Val | Tyr | Leu | Gln | Met | Asn | Ser | Leu | Arg | Ala | Glu | Asp | Thr | Ala | Val | Tyr |
|     |     |     |     | 485 |     |     |     |     | 490 |     |     |     |     | 495 |     |

Tyr Cys Ala Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp  
500 505 510

Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
515 520 525

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
530 535 540

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
545 550 555 560

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
565 570 575

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
580 585 590

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
595 600 605

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro  
610 615 620

Lys Ser Cys  
625

<210> 102  
<211> 110  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645-Cys VL domain (gL5)

<400> 102

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn  
20 25 30

Phe Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu  
35 40 45

Ile Tyr Glu Ala Ser Lys Leu Thr 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 Phe Ala Thr Tyr Tyr Cys Gly Gly Gly Tyr Ser Ser Ile  
85 90 95

Ser Asp Thr Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys  
100 105 110

<210> 103  
<211> 121  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645-Cys VH domain (gH5)

<400> 103

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120

<210> 104  
 <211> 6  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Rigid linker

<400> 104

Glu Tyr His Gly Leu Gln  
 1 5

<210> 105  
 <211> 119  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 VH domain (gH1)

<400> 105

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Ser Thr Thr Val Tyr Leu Gln Met  
 65 70 75 80

Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr

85

90

95

Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly  
 100 105 110

Thr Leu Val Thr Val Ser Ser  
 115

<210> 106  
 <211> 121  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 VH domain (gH37)

<400> 106

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Ala Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
 85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser  
 115 120

<210> 107

<211> 120  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 VH domain (gH47)

<400> 107

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Ala Val Tyr Tyr Cys Ala Arg  
85 90 95

Thr Val Pro Gly Tyr Ser Ala Ala Pro Tyr Phe Asp Leu Trp Gly Gln  
100 105 110

Gly Thr Leu Val Thr Val Ser Ser  
115 120

<210> 108  
<211> 110  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 VL domain (gL1)

<400> 108

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn  
20 25 30

Phe Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu  
35 40 45

Ile Tyr Glu Ala Ser Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Lys  
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 Phe Ala Thr Tyr Tyr Cys Gly Gly Gly Tyr Ser Ser Ile  
85 90 95

Ser Asp Thr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105 110

<210> 109  
<211> 110  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 VL domain (gL4)

<400> 109

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn  
20 25 30

Phe Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu  
35 40 45

Ile Tyr Glu Ala Ser Lys Leu Thr 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 Phe Ala Thr Tyr Tyr Cys Gly Gly Gly Tyr Ser Ser Ile  
85 90 95

Ser Asp Thr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys  
100 105 110

<210> 110  
<211> 112  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645-Cys VL domain (gL4)

<400> 110

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn  
20 25 30

Phe Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu  
35 40 45

Ile Tyr Glu Ala Ser Lys Leu Thr 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 Phe Ala Thr Tyr Tyr Cys Gly Gly Gly Tyr Ser Ser Ile  
85 90 95

Ser Asp Thr Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Arg Thr  
100 105 110

<210> 111  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Albumin binding peptide

<400> 111

Asp Leu Cys Leu Arg Asp Trp Gly Cys Leu Trp  
1 5 10

<210> 112

<211> 11

<212> PRT

<213> Artificial Sequence

<220>

<223> Albumin binding peptide

<400> 112

Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp  
1 5 10

<210> 113

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Albumin binding peptide

<400> 113

Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Gly Asp  
1 5 10 15

<210> 114

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Albumin binding peptide

<400> 114

Gln Arg Leu Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp  
1 5 10 15

Glu Asp Asp Glu  
20

<210> 115

<211> 20  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Albumin binding peptide

<400> 115

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Gly | Leu | Ile | Gly | Asp | Ile | Cys | Leu | Pro | Arg | Trp | Gly | Cys | Leu | Trp |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |
|-----|-----|-----|-----|
| Gly | Arg | Ser | Val |
|     |     |     | 20  |

<210> 116  
<211> 21  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Albumin binding peptide

<400> 116

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Gly | Leu | Ile | Gly | Asp | Ile | Cys | Leu | Pro | Arg | Trp | Gly | Cys | Leu | Trp |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |
|-----|-----|-----|-----|-----|
| Gly | Arg | Ser | Val | Lys |
|     |     |     |     | 20  |

<210> 117  
<211> 15  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Albumin binding peptide

<400> 117

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Asp | Ile | Cys | Leu | Pro | Arg | Trp | Gly | Cys | Leu | Trp | Glu | Asp | Asp |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

<210> 118  
<211> 18  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Albumin binding peptide

<400> 118

Arg Leu Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu  
1 5 10 15

Asp Asp

<210> 119

<211> 16

<212> PRT

<213> Artificial Sequence

<220>

<223> Albumin binding peptide

<400> 119

Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu Asp Asp  
1 5 10 15

<210> 120

<211> 15

<212> PRT

<213> Artificial Sequence

<220>

<223> Albumin binding peptide

<400> 120

Met Glu Asp Ile Cys Leu Pro Arg Trp Gly Cys Leu Trp Glu Asp  
1 5 10 15

<210> 121

<211> 18

<212> PRT

<213> Artificial Sequence

<220>

<223> Albumin binding peptide

<400> 121

Arg Leu Met Glu Asp Ile Cys Leu Ala Arg Trp Gly Cys Leu Trp Glu  
1 5 10 15

Asp Asp

<210> 122  
<211> 20  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Albumin binding peptide

<400> 122

Glu Val Arg Ser Phe Cys Thr Arg Trp Pro Ala Glu Lys Ser Cys Lys  
1 5 10 15

Pro Leu Arg Gly  
20

<210> 123  
<211> 20  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Albumin binding peptide

<400> 123

Arg Ala Pro Glu Ser Phe Val Cys Tyr Trp Glu Thr Ile Cys Phe Glu  
1 5 10 15

Arg Ser Glu Gln  
20

<210> 124  
<211> 11  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Albumin binding peptide

<400> 124

Glu Met Cys Tyr Phe Pro Gly Ile Cys Trp Met  
1 5 10

<210> 125  
 <211> 224  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA497 Fab Heavy Chain

<400> 125

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 Val Ile Phe Ser Asp Tyr  
 20 25 30

Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45

Ala Ser Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr Arg Glu Ser Val  
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr  
 65 70 75 80

Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Thr Thr Asp Ala Asn Arg Gln Asn Tyr Asp Trp Phe Ala Tyr Trp Gly  
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala  
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His  
195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
210 215 220

<210> 126  
<211> 223  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA240 Fab Heavy chain

<400> 126

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Phe Asn Asp Tyr  
20 25 30

Phe Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
35 40 45

Ala Gln Met Arg Asn Lys Asn Tyr Gln Tyr Gly Thr Tyr Tyr Ala Glu  
50 55 60

Ser Leu Glu Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Ser  
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr  
85 90 95

Tyr Cys Ala Arg Glu Ser Tyr Tyr Gly Phe Thr Ser Tyr Trp Gly Gln  
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
 130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser  
 145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
 165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
 180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
 195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 210 215 220

<210> 127  
 <211> 247  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA497 dsscFv (vHvL)

<400> 127

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 Val Ile Phe Ser Asp Tyr  
 20 25 30

Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val  
 35 40 45

Ala Ser Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr Arg Glu Ser Val  
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr  
 65 70 75 80

Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95

Thr Thr Asp Ala Asn Arg Gln Asn Tyr Asp Trp Phe Ala 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 Ala Ile Gln Leu Thr Gln Ser Pro  
130 135 140

Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys  
145 150 155 160

Ala Ser Glu Ser Val Ser Ser Ser Met Tyr Ser Tyr Met His Trp Tyr  
165 170 175

Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Arg Ala Ser  
180 185 190

Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly  
195 200 205

Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala  
210 215 220

Thr Tyr Tyr Cys Gln Gln Ser Trp Thr Ala Pro Arg Thr Phe Gly Cys  
225 230 235 240

Gly Thr Lys Val Glu Ile Lys  
245

<210> 128  
<211> 247  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA497 dsscFv (vLvH)

<400> 128

Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Ser Val Ser Ser Ser  
20 25 30

Met Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro  
35 40 45

Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Ser  
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser  
65 70 75 80

Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Trp  
85 90 95

Thr Ala Pro Arg Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Gly  
100 105 110

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Val  
115 120 125

Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu  
130 135 140

Arg Leu Ser Cys Ala Ala Ser Gly Val Ile Phe Ser Asp Tyr Tyr Met  
145 150 155 160

Ala Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Val Ala Ser  
165 170 175

Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr Arg Glu Ser Val Lys Gly  
180 185 190

Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr Leu Tyr Leu Gln  
195 200 205

Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr Tyr Cys Thr Thr

210

215

220

Asp Ala Asn Arg Gln Asn Tyr Asp Trp Phe Ala Tyr Trp Gly Gln Gly  
 225 230 235 240

Thr Leu Val Thr Val Ser Ser  
 245

<210> 129  
 <211> 491  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 Fab HC Fwk3 CA497 dsscFv (vHvL)

<400> 129

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
 65 70 75 80

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val  
 85 90 95

Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ile  
 100 105 110

Phe Ser Asp Tyr Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Cys  
 115 120 125

Leu Glu Trp Val Ala Ser Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr

130

135

140

Arg Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys  
145 150 155 160

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala  
165 170 175

Val Tyr Tyr Cys Thr Thr Asp Ala Asn Arg Gln Asn Tyr Asp Trp Phe  
180 185 190

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly  
195 200 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ile Gln Leu  
210 215 220

Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr  
225 230 235 240

Ile Thr Cys Lys Ala Ser Glu Ser Val Ser Ser Ser Met Tyr Ser Tyr  
245 250 255

Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
260 265 270

Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
275 280 285

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
290 295 300

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Trp Thr Ala Pro Arg  
305 310 315 320

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser  
325 330 335

Gly Gly Gly Gly Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu  
340 345 350

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly  
355 360 365

Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val  
370 375 380

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
385 390 395 400

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
405 410 415

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
420 425 430

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
435 440 445

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
450 455 460

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
465 470 475 480

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
485 490

<210> 130  
<211> 491  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 Fab HC Fwk3 CA497 dsscFv (vLvH)

<400> 130

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
 65 70 75 80

Gly Gly Gly Ser Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser  
 85 90 95

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Ser  
 100 105 110

Val Ser Ser Ser Met Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro  
 115 120 125

Gly Lys Ala Pro Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser  
 130 135 140

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
 145 150 155 160

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys  
 165 170 175

Gln Gln Ser Trp Thr Ala Pro Arg Thr Phe Gly Cys Gly Thr Lys Val  
 180 185 190

Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly  
 195 200 205

Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro  
 210 215 220

Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ile Phe Ser  
 225 230 235 240

Asp Tyr Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu

|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 245                                                             | 250 | 255 |
| Trp Val Ala Ser Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr Arg Glu |     |     |
| 260                                                             | 265 | 270 |
| Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr |     |     |
| 275                                                             | 280 | 285 |
| Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr |     |     |
| 290                                                             | 295 | 300 |
| Tyr Cys Thr Thr Asp Ala Asn Arg Gln Asn Tyr Asp Trp Phe Ala Tyr |     |     |
| 305                                                             | 310 | 315 |
| Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser |     |     |
| 325                                                             | 330 | 335 |
| Gly Gly Gly Gly Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu |     |     |
| 340                                                             | 345 | 350 |
| Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly |     |     |
| 355                                                             | 360 | 365 |
| Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val |     |     |
| 370                                                             | 375 | 380 |
| Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala |     |     |
| 385                                                             | 390 | 395 |
| Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu |     |     |
| 405                                                             | 410 | 415 |
| Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly |     |     |
| 420                                                             | 425 | 430 |
| Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser |     |     |
| 435                                                             | 440 | 445 |
| Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu |     |     |
| 450                                                             | 455 | 460 |

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 465 470 475 480

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 485 490

<210> 131  
 <211> 718  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 IgG4P HC Fwk3 CA497 dsscFv (vHvL)

<400> 131

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
 65 70 75 80

Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val  
 85 90 95

Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ile  
 100 105 110

Phe Ser Asp Tyr Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Cys  
 115 120 125

Leu Glu Trp Val Ala Ser Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr  
 130 135 140

Arg Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys  
 145 150 155 160

Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala  
 165 170 175

Val Tyr Tyr Cys Thr Thr Asp Ala Asn Arg Gln Asn Tyr Asp Trp Phe  
 180 185 190

Ala Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly  
 195 200 205

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ala Ile Gln Leu  
 210 215 220

Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr  
 225 230 235 240

Ile Thr Cys Lys Ala Ser Glu Ser Val Ser Ser Ser Met Tyr Ser Tyr  
 245 250 255

Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
 260 265 270

Tyr Arg Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly  
 275 280 285

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro  
 290 295 300

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Trp Thr Ala Pro Arg  
 305 310 315 320

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys Gly Gly Gly Gly Ser  
 325 330 335

Gly Gly Gly Gly Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu  
 340 345 350

Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly

355

360

365

Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val  
 370 375 380

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala  
 385 390 395 400

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu  
 405 410 415

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly  
 420 425 430

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser  
 435 440 445

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu  
 450 455 460

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 465 470 475 480

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 485 490 495

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
 500 505 510

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 515 520 525

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 530 535 540

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 545 550 555 560

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 565 570 575

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
580 585 590

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
595 600 605

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
610 615 620

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
625 630 635 640

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
645 650 655

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
660 665 670

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp  
675 680 685

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
690 695 700

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
705 710 715

<210> 132  
<211> 718  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 IgG4P HC Fwk3 CA497 dsscFv (vLvH)

<400> 132

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser Gly  
65 70 75 80

Gly Gly Gly Ser Ala Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser  
85 90 95

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Glu Ser  
100 105 110

Val Ser Ser Ser Met Tyr Ser Tyr Met His Trp Tyr Gln Gln Lys Pro  
115 120 125

Gly Lys Ala Pro Lys Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Ser  
130 135 140

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
145 150 155 160

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys  
165 170 175

Gln Gln Ser Trp Thr Ala Pro Arg Thr Phe Gly Cys Gly Thr Lys Val  
180 185 190

Glu Ile Lys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly  
195 200 205

Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro  
210 215 220

Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Val Ile Phe Ser  
225 230 235 240

Asp Tyr Tyr Met Ala Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu

|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 245                                                             | 250 | 255 |
| Trp Val Ala Ser Ile Asn Phe Asn Ala Asp Ile Ser Tyr Tyr Arg Glu |     |     |
| 260                                                             | 265 | 270 |
| Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asp Ser Lys Asn Thr |     |     |
| 275                                                             | 280 | 285 |
| Leu Tyr Leu Gln Met Asn Ser Leu Lys Thr Glu Asp Thr Ala Val Tyr |     |     |
| 290                                                             | 295 | 300 |
| Tyr Cys Thr Thr Asp Ala Asn Arg Gln Asn Tyr Asp Trp Phe Ala Tyr |     |     |
| 305                                                             | 310 | 315 |
| Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser |     |     |
| 325                                                             | 330 | 335 |
| Gly Gly Gly Gly Ser Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu |     |     |
| 340                                                             | 345 | 350 |
| Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Thr Val Pro Gly |     |     |
| 355                                                             | 360 | 365 |
| Tyr Ser Thr Ala Pro Tyr Phe Asp Leu Trp Gly Gln Gly Thr Leu Val |     |     |
| 370                                                             | 375 | 380 |
| Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala |     |     |
| 385                                                             | 390 | 395 |
| Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu |     |     |
| 405                                                             | 410 | 415 |
| Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly |     |     |
| 420                                                             | 425 | 430 |
| Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser |     |     |
| 435                                                             | 440 | 445 |
| Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu |     |     |
| 450                                                             | 455 | 460 |

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr  
 465 470 475 480

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr  
 485 490 495

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe  
 500 505 510

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 515 520 525

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val  
 530 535 540

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
 545 550 555 560

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val  
 565 570 575

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
 580 585 590

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser  
 595 600 605

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
 610 615 620

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
 625 630 635 640

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
 645 650 655

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
 660 665 670

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp

675

680

685

Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
 690 695 700

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
 705 710 715

&lt;210&gt; 133

&lt;211&gt; 213

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Anti-CD3 Light chain

&lt;400&gt; 133

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 Thr Pro Gly Lys Ala Pro Lys Leu 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 Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
 65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr  
 85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro  
 100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr  
 115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys

130

135

140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu  
 145 150 155 160

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser  
 165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala  
 180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe  
 195 200 205

Asn Arg Gly Glu Cys  
 210

<210> 134  
 <211> 222  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Anti-CD3 Fab HC

<400> 134

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 Tyr  
 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 Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn 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 Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe  
 115 120 125

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu  
 130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp  
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu  
 165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser  
 180 185 190

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro  
 195 200 205

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
 210 215 220

<210> 135

<211> 446

<212> PRT

<213> Artificial Sequence

<220>

<223> Anti-CD3 IgG4P HC

<400> 135

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 Tyr  
 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 Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
 50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn 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 Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly  
 100 105 110

Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe  
 115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu  
 130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp  
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu  
 165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser  
 180 185 190

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro  
 195 200 205

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro  
 210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val Phe  
 225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro  
 245 250 255

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val  
260 265 270

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr  
275 280 285

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val  
290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys  
305 310 315 320

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser  
325 330 335

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro  
340 345 350

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val  
355 360 365

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly  
370 375 380

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp  
385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp  
405 410 415

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His  
420 425 430

Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
435 440 445

<210> 136  
<211> 214  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Anti-CD28 Light Chain

<400> 136

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 His Ala Ser Gln Asn Ile Tyr Val Trp  
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45

Tyr Lys Ala Ser Asn Leu His Thr 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 Gly Gln Thr Tyr Pro Tyr  
85 90 95

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala  
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly  
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala  
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln  
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser  
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr  
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser

195

200

205

Phe Asn Arg Gly Glu Cys  
210

<210> 137  
<211> 223  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Anti-CD28 Fab HC

<400> 137

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

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Cys Ile Tyr Pro Gly Asn Val Asn Thr Asn Tyr Asn Glu Lys Phe  
50 55 60

Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Ile Ser Thr Ala Tyr  
65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Phe Cys  
85 90 95

Thr Arg Ser His Tyr Gly Leu Asp Trp Asn Phe Asp Val Trp Gly Gln  
100 105 110

Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val  
115 120 125

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala  
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser

145                      150                      155                      160  
 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val  
                                  165                                   170                                   175  
 Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro  
                                  180                                   185                                   190  
 Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys  
                                  195                                   200                                   205  
 Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys  
                                  210                                   215                                   220  
  
 <210> 138  
 <211> 447  
 <212> PRT  
 <213> Artificial Sequence  
  
 <220>  
 <223> Anti-CD28 IgG4P HC  
  
 <400> 138  
  
 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  
  
 Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
                                  35                                   40                                   45  
  
 Gly Cys Ile Tyr Pro Gly Asn Val Asn Thr Asn Tyr Asn Glu Lys Phe  
                                  50                                   55                                   60  
  
 Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Ile Ser Thr Ala Tyr  
 65                                   70                                   75                                   80  
  
 Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Phe Cys  
                                  85                                   90                                   95  
  
 Thr Arg Ser His Tyr Gly Leu Asp Trp Asn Phe Asp Val Trp Gly Gln

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|--|
|     | 100 |     | 105 |     | 110 |     |     |     |     |     |     |     |     |     |     |  |  |  |  |
| Gly | Thr | Thr | Val | Thr | Val | Ser | Ser | Ala | Ser | Thr | Lys | Gly | Pro | Ser | Val |  |  |  |  |
|     | 115 |     |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |  |  |  |
| Phe | Pro | Leu | Ala | Pro | Cys | Ser | Arg | Ser | Thr | Ser | Glu | Ser | Thr | Ala | Ala |  |  |  |  |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |  |  |
| Leu | Gly | Cys | Leu | Val | Lys | Asp | Tyr | Phe | Pro | Glu | Pro | Val | Thr | Val | Ser |  |  |  |  |
| 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     |     | 160 |  |  |  |  |
| Trp | Asn | Ser | Gly | Ala | Leu | Thr | Ser | Gly | Val | His | Thr | Phe | Pro | Ala | Val |  |  |  |  |
|     |     |     | 165 |     |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |  |  |
| Leu | Gln | Ser | Ser | Gly | Leu | Tyr | Ser | Leu | Ser | Ser | Val | Val | Thr | Val | Pro |  |  |  |  |
|     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |  |  |
| Ser | Ser | Ser | Leu | Gly | Thr | Lys | Thr | Tyr | Thr | Cys | Asn | Val | Asp | His | Lys |  |  |  |  |
|     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |  |  |
| Pro | Ser | Asn | Thr | Lys | Val | Asp | Lys | Arg | Val | Glu | Ser | Lys | Tyr | Gly | Pro |  |  |  |  |
|     | 210 |     |     |     |     | 215 |     |     |     |     | 220 |     |     |     |     |  |  |  |  |
| Pro | Cys | Pro | Pro | Cys | Pro | Ala | Pro | Glu | Phe | Leu | Gly | Gly | Pro | Ser | Val |  |  |  |  |
| 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     |     | 240 |  |  |  |  |
| Phe | Leu | Phe | Pro | Pro | Lys | Pro | Lys | Asp | Thr | Leu | Met | Ile | Ser | Arg | Thr |  |  |  |  |
|     |     |     | 245 |     |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |  |  |
| Pro | Glu | Val | Thr | Cys | Val | Val | Val | Asp | Val | Ser | Gln | Glu | Asp | Pro | Glu |  |  |  |  |
|     |     | 260 |     |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |  |  |
| Val | Gln | Phe | Asn | Trp | Tyr | Val | Asp | Gly | Val | Glu | Val | His | Asn | Ala | Lys |  |  |  |  |
|     | 275 |     |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |  |  |
| Thr | Lys | Pro | Arg | Glu | Glu | Gln | Phe | Asn | Ser | Thr | Tyr | Arg | Val | Val | Ser |  |  |  |  |
|     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |  |  |
| Val | Leu | Thr | Val | Leu | His | Gln | Asp | Trp | Leu | Asn | Gly | Lys | Glu | Tyr | Lys |  |  |  |  |
| 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     |     | 320 |  |  |  |  |

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile  
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro  
340 345 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu  
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn  
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser  
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg  
405 410 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu  
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
435 440 445

<210> 139  
<211> 488  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Anti-CD28 Fab HC Fwk3 anti-CD3 dsscFv (vHvL)

<400> 139

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

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Cys Ile Tyr Pro Gly Asn Val Asn Thr Asn Tyr Asn Glu Lys Phe  
50 55 60

Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Gly Gly Gly Gly Ser  
65 70 75 80

Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val  
85 90 95

Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr  
100 105 110

Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Lys  
115 120 125

Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn  
130 135 140

Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser  
145 150 155 160

Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr  
165 170 175

Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp  
180 185 190

Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly  
195 200 205

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
210 215 220

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
225 230 235 240

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met  
245 250 255

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr

|                                                                 |     |     |
|-----------------------------------------------------------------|-----|-----|
| 260                                                             | 265 | 270 |
| Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser |     |     |
| 275                                                             | 280 | 285 |
| Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu |     |     |
| 290                                                             | 295 | 300 |
| Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr |     |     |
| 305                                                             | 310 | 315 |
| Phe Gly Cys Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly |     |     |
| 325                                                             | 330 | 335 |
| Gly Gly Gly Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg |     |     |
| 340                                                             | 345 | 350 |
| Ser Asp Asp Thr Ala Val Tyr Phe Cys Thr Arg Ser His Tyr Gly Leu |     |     |
| 355                                                             | 360 | 365 |
| Asp Trp Asn Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser |     |     |
| 370                                                             | 375 | 380 |
| Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser |     |     |
| 385                                                             | 390 | 395 |
| Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp |     |     |
| 405                                                             | 410 | 415 |
| Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr |     |     |
| 420                                                             | 425 | 430 |
| Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr |     |     |
| 435                                                             | 440 | 445 |
| Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln |     |     |
| 450                                                             | 455 | 460 |
| Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp |     |     |
| 465                                                             | 470 | 475 |
|                                                                 |     | 480 |

Lys Lys Val Glu Pro Lys Ser Cys  
485

<210> 140  
<211> 487  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Anti-CD3 Fab HC Fwk3 anti-CD3 dsscFv (vHvL)

<400> 140

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 Tyr  
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 Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser  
65 70 75 80

Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val  
85 90 95

Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr  
100 105 110

Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Lys  
115 120 125

Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn  
130 135 140

Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser  
145 150 155 160

Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr  
165 170 175

Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp  
180 185 190

Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly  
195 200 205

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
210 215 220

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
225 230 235 240

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met  
245 250 255

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
260 265 270

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
275 280 285

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
290 295 300

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr  
305 310 315 320

Phe Gly Cys Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly  
325 330 335

Gly Gly Gly Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg  
340 345 350

Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His  
355 360 365

Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser

370

375

380

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys  
385 390 395 400

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
405 410 415

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
420 425 430

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
435 440 445

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr  
450 455 460

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys  
465 470 475 480

Lys Val Glu Pro Lys Ser Cys  
485

<210> 141  
<211> 712  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Anti-CD28 IgG4P HC Fwk3 anti-CD3 dsscFv (vHvL)

<400> 141

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

Tyr Ile His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile  
35 40 45

Gly Cys Ile Tyr Pro Gly Asn Val Asn Thr Asn Tyr Asn Glu Lys Phe

50

55

60

Lys Asp Arg Ala Thr Leu Thr Val Asp Thr Ser Gly Gly Gly Gly Ser  
65 70 75 80

Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val  
85 90 95

Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr  
100 105 110

Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Lys  
115 120 125

Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn  
130 135 140

Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser  
145 150 155 160

Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr  
165 170 175

Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp  
180 185 190

Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly  
195 200 205

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
210 215 220

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
225 230 235 240

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met  
245 250 255

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
260 265 270

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
275 280 285

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
290 295 300

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr  
305 310 315 320

Phe Gly Cys Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly  
325 330 335

Gly Gly Gly Ser Ile Ser Thr Ala Tyr Met Glu Leu Ser Arg Leu Arg  
340 345 350

Ser Asp Asp Thr Ala Val Tyr Phe Cys Thr Arg Ser His Tyr Gly Leu  
355 360 365

Asp Trp Asn Phe Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser  
370 375 380

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser  
385 390 395 400

Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp  
405 410 415

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr  
420 425 430

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr  
435 440 445

Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys  
450 455 460

Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp  
465 470 475 480

Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala

485

490

495

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
 500 505 510

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
 515 520 525

Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val  
 530 535 540

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
 545 550 555 560

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln  
 565 570 575

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly  
 580 585 590

Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro  
 595 600 605

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr  
 610 615 620

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser  
 625 630 635 640

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr  
 645 650 655

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr  
 660 665 670

Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe  
 675 680 685

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys  
 690 695 700

Ser Leu Ser Leu Ser Leu Gly Lys  
705 710

<210> 142  
<211> 711  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Anti-CD3 IgG4P HC Fwk3 anti-CD3 dsscFv (vHvL)

<400> 142

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 Tyr  
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 Arg Gly Tyr Thr Asn Tyr Asn Gln Lys Phe  
50 55 60

Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser Gly Gly Gly Gly Ser  
65 70 75 80

Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val  
85 90 95

Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Tyr  
100 105 110

Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro Gly Lys  
115 120 125

Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn  
130 135 140

Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp Asn Ser  
145 150 155 160

Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr  
165 170 175

Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp  
180 185 190

Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly  
195 200 205

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser  
210 215 220

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
225 230 235 240

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met  
245 250 255

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr  
260 265 270

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser  
275 280 285

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu  
290 295 300

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr  
305 310 315 320

Phe Gly Cys Gly Thr Lys Leu Glu Ile Lys Gly Gly Gly Gly Ser Gly  
325 330 335

Gly Gly Gly Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg  
340 345 350

Pro Glu Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His  
355 360 365

Tyr Cys Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser

370

375

380

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg  
 385 390 395 400

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr  
 405 410 415

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser  
 420 425 430

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser  
 435 440 445

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr  
 450 455 460

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys  
 465 470 475 480

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro  
 485 490 495

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys  
 500 505 510

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val  
 515 520 525

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp  
 530 535 540

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe  
 545 550 555 560

Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp  
 565 570 575

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu  
 580 585 590

Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg  
595 600 605

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys  
610 615 620

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp  
625 630 635 640

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys  
645 650 655

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser  
660 665 670

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser  
675 680 685

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser  
690 695 700

Leu Ser Leu Ser Leu Gly Lys  
705 710

<210> 143  
<211> 251  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> CA645 scFv (VH-VL)

<400> 143

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu 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 Asp Ile Gln  
130 135 140

Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly Asp Arg Val  
145 150 155 160

Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn Phe Leu Ser  
165 170 175

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Glu  
180 185 190

Ala Ser Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly  
195 200 205

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp  
210 215 220

Phe Ala Thr Tyr Tyr Cys Gly Gly Gly Tyr Ser Ser Ile Ser Asp Thr  
225 230 235 240

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys  
245 250

<210> 144

<211> 251  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> CA645 dsscFv (VH-VL)

<400> 144

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Val Ser Gly Ile Asp Leu Ser Asn Tyr  
 20 25 30

Ala Ile Asn Trp Val Arg Gln Ala Pro Gly Lys Cys Leu Glu Trp Ile  
 35 40 45

Gly Ile Ile Trp Ala Ser Gly Thr Thr Phe Tyr Ala Thr Trp Ala Lys  
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Val Tyr Leu  
 65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
 85 90 95

Arg Thr Val Pro Gly Tyr Ser Thr Ala Pro Tyr Phe Asp Leu 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 Asp Ile Gln  
 130 135 140

Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly Asp Arg Val  
 145 150 155 160

Thr Ile Thr Cys Gln Ser Ser Pro Ser Val Trp Ser Asn Phe Leu Ser  
 165 170 175

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Glu

180

185

190

Ala Ser Lys Leu Thr Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly  
 195 200 205

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp  
 210 215 220

Phe Ala Thr Tyr Tyr Cys Gly Gly Gly Tyr Ser Ser Ile Ser Asp Thr  
 225 230 235 240

Thr Phe Gly Cys Gly Thr Lys Val Glu Ile Lys  
 245 250