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DESCRIPTION

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] Modified-IgG antibodies, each comprising a first, a second, a third and a fourth polypeptide chain, which exhibit high affinity and avidity to Transforming Growth Factor- β 1 (TGF β 1) but not to TGF β 2 or TGF β 3. Compositions comprising the modified-IgG antibodies and methods of using the same for treatment of diseases involving TGF β 1 activity are provided.

[0002] Many severe diseases are linked to malfunctions of the TGF β -induced signaling pathway. For instance, an increased tissue level of TGF β is believed to be a factor in the development of idiopathic pulmonary fibrosis and myocardial fibrosis. Furthermore, high local tissue levels of TGF β may allow the maintenance and progression of some types of cancer cells. Down-regulation of TGF β signaling therefore may reduce the viability of such tumor cells.

[0003] TGF β isoforms are ~25 kDa homodimeric molecules with a similar structural framework in which two monomers are covalently linked via a disulfide bridge. The mammalian isoforms share a sequence identity of 70-82%, but have non-overlapping activities in vascular development and the regulation of immune cell function. Three TGF β isoforms have been reported in humans: TGF β 1, TGF β 2, and TGF β 3 (Swiss Prot accession numbers P01137, P08112, and P10600, respectively). TGF β 1 and TGF β 3 trigger a cellular signaling cascade upon binding to the extracellular domains of two transmembrane receptors, known as TGF β receptor types I and II. TGF β 2 may bind to TGF β receptor types I and II, as well as TGF β receptor type III.

[0004] Antibodies that can bind human TGF β 1, TGF β 2, and TGF β 3 have been tested for clinical use. For instance, Grütter et al. disclosed GC1008, a human IgG4 monoclonal antibody (Mab; i.e., GC1008) in clinical development for treating malignancy and fibrotic diseases. Proc. Nat'l Acad. Sci. USA 105(51): 20251-56 (2008). GC1008 is a "pan-specific" TGF β neutralizing antibody, because it can neutralize all three human TGF β isoforms. Antibodies that selectively neutralize TGF β 1 are disclosed, for example, in U.S. Patent No. 6,492,497 and U.S. Patent No. 7,151,169. Metelimumab, also known as CAT192 (IgG4), is a human IgG4 monoclonal antibody that selectively neutralizes TGF- β 1. See e.g., U.S. Patent No. 6,492,497. Metelimumab was tested for the treatment of diffuse cutaneous systemic sclerosis, also known as scleroderma, but demonstrated insufficient efficacy. WO 00/66631 discloses an anti-TGF- β 1 antibody (SL15A) comprising four polypeptide chains (2 heavy and 2 light chains), wherein the VH and the VL domain comprise the following CDRs: SYGMH (HCDR1), VISYDGSIKYYADSVKG (HCDR2), TGEYSGYDTPQYS (HCDR3), RASQGIGDDL (LCDR1), GTSTLQS (LCDR2) and LQDSNYPL T (LCDR3). The sequence linking VL and CL is therefore LEIKTVA, and the sequence linking VH and CH1 is TVSSAST.

BRIEF SUMMARY OF THE INVENTION

[0005] The present disclosure provides an isolated binding protein that binds TGF β 1 according to claim 1. Particularly provided are TGF β 1-binding modified-IgG antibodies that are capable of selectively binding and neutralizing human TGF β 1. The disclosed modified-IgG antibodies are derived from metelimumab. The VH and VL domains of the modified-IgG antibodies exhibit a TGF β 1-binding affinity and avidity and TGF β 1 neutralizing capability similar to those of metelimumab. In many cases, the disclosed antibodies offer improved affinity, avidity and neutralization capacity over metelimumab. The modified-IgG antibodies contain two polypeptide chains each comprising a VL domain linked to a CL domain, and two polypeptide chains each comprising a VH domain linked to a CH1 domain, a hinge and a Fc region. Further aspects and embodiments of the present invention are disclosed in the accompanying claims.

[0006] The modified-IgG antibodies of the present invention comprise a variable domain that is capable of binding TGF β 1. The disclosed modified-IgG antibodies may comprise a binding protein which exhibits a K_d for human TGF β 1 at least about 50% lower than the K_d of the same binding protein for human TGF β 2, as measured by surface plasmon resonance.

[0007] The first and second polypeptide chains may have the formula of, from N-terminal to C-terminal:

(VL domain)-(linker1)-(CL domain),

wherein the VL domain comprises a variable light complementarity determining region 1 (LCDR1), a variable light complementarity determining region 2 (LCDR2), and a variable light complementarity determining region 3 (LCDR3). Alternatively, the third and fourth polypeptide chains have the formula of, from N-terminal to C-terminal:

(VH domain)-(linker2)-(CH1 domain)-(hinge)-(Fc region),

wherein the VH domain comprises a variable heavy complementarity determining region 1 (HCDR1), a variable heavy complementarity determining region 2 (HCDR2), and a variable heavy complementarity determining region 3 (HCDR3)

[0008] If present, linker1 and linker2 may comprise an amino acid residue or amino acid sequence selected from the group consisting of G, GG, GGS, GGGS, and GGGGS.

[0009] The VH domain comprises the amino acid sequence as set forth in SEQ ID No. 1 or SEQ ID No. 2.

[0010] The VL domain of the disclosed binding protein comprises the human Vk domain sequences set forth in SEQ ID No. 3 or SEQ ID No. 4. Preferably, each polypeptide of the dimer may comprise the VH domain set forth in SEQ ID NO: 1 and the Vk domain set forth in SEQ ID No. 3, which are the VH and VL domains present in metelimumab, respectively.

[0011] The Fc region is connected to the CH1 domain by a hinge. The hinge may comprise amino acid sequences derived from a human IgG1 or IgG4 hinge region. For example, the hinge may comprise the amino acid sequence PKSCDKTHTCPPCPAPELLGPP (SEQ ID No. 5), or a variant

thereof having up to five amino acid modifications. In one embodiment, the hinge length may vary from 1-15 amino acids.

[0012] Site-directed mutagenesis can be performed on CAT192 Fab elbow regions to improve TGF β 1 binding affinity. One to five amino acids (G, GG, GGS, GGGS and GGGGS) are inserted to the light chain elbow region to increase the flexibility of the hinge that may be required to present a functional binding paratope from the two chains. Conditioned media from an Expi293 transfection show good expression and significant improvement in binding to TGF β 1 by Octet. The mutants are purified by Ni-NTA and high TGF β binding affinities are confirmed using Biacore. The CAT192 mutants with 1 to five amino acids inserted into the LC elbow region re-gain the high affinity binding of scFv to TGF β 1. These engineered elbow insertion mutants also retain isoform-selectivity and may serve as TGF β 1 specific antagonists.

[0013] The VH domain may contain a variable heavy complementarity determining region 1 (HCDR1) having the amino acid sequence of SEQ ID No. 7, a variable heavy complementarity determining region 2 (HCDR2) having the amino acid sequence of SEQ ID No. 8, and a variable heavy complementarity determining region 3 (HCDR3) having the amino acid sequence selected from the group consisting of SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, and SEQ ID No. 15.

[0014] The VL domain may contain a variable light complementarity determining region 1 (LCDR1) having the amino acid sequence of SEQ ID No. 12, a variable light complementarity determining region 2 (LCDR2) having the amino acid sequence of SEQ ID No. 13, and a variable light complementarity determining region 3 (LCDR3) having the amino acid sequence of SEQ ID No. 14.

[0015] The disclosed TGF β 1-binding IgG molecule preferably selectively binds TGF β 1, but does not bind TGF β 2 or TGF β 3 to a significant extent.

[0016] In another embodiment, an isolated polynucleotide is disclosed which may comprise a nucleotide sequence encoding the modified IgG antibodies disclosed herein. The isolated polynucleotide may be a cDNA, a recombinant DNA or a synthetic DNA. A host cell may comprise the isolated nucleic acid. The host cell may be a human cell, such as a Human Embryonic Kidney 293 (HEK293) cell and cell lines derived therefrom, or it may be a Chinese Hamster Ovary (CHO) cell. A method of making the modified IgG antibodies may include culturing the host cell under suitable conditions to produce the modified IgG antibodies. The modified IgG antibodies may be purified. The degree of purity may be 90%, 95%, 99%, 99.5% or more.

[0017] In certain embodiments, the modified IgG antibodies of the present invention may be an element of a pharmaceutical composition. The pharmaceutical composition may comprise a therapeutically effective amount of the modified IgG antibodies. The composition may further comprise one or more biologically active components, excipients, or diluents.

[0018] Also disclosed is a method of treating a disease or condition resulting directly or indirectly from TGF β 1 activity in a human comprising administering a pharmaceutical composition comprising a therapeutically effective amount of the modified IgG antibodies. The disease or condition may be selected from the group consisting of a fibrotic disease, cancer, or an immune-mediated disease, e.g., diffuse cutaneous systemic sclerosis, bone remodeling disease, kidney disease and/or a

combination thereof. The modified IgG antibodies may be used in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of a fibrotic disease, cancer, or an immune-mediated disease, e.g., diffuse cutaneous systemic sclerosis, bone remodeling disease, kidney disease and/or a combination thereof. The treatment of the disease or disorder may comprise neutralizing TGF β 1 or inhibiting TGF β 1 signaling. The treatment of the disease or disorder may comprise inhibiting TGF β 1-mediated fibronectin production, vascular endothelial growth factor (VEGF) production, epithelial cell proliferation, endothelial cell proliferation, smooth muscle cell proliferation, and/or immunosuppression. The treatment of the disease or disorder may comprise increasing natural killer cell activity.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0019] The drawings presented herein are for purpose of illustration and are not to be used to limit the scope of the present invention.

Figure 1 depicts the results of a Biacore TGF β 1 binding assay which showed the loss of affinity when the scFv (CAT191) was converted into a full length IgG4 (CAT192) molecule.

Figure 2 depicts structural elements of a scFv, a Fab, an IgG molecule, and the elbow regions that were engineered to restore affinity.

Figure 3 shows the results of an SDS-PAGE gel of purified IgG variants with additional amino acids in the heavy and light chain elbow regions. The SDS-PAGE shows the purity of the purified IgG variants under reducing and non-reducing conditions.

Figure 4 shows a Biacore binding assay of purified IgG variants with additional amino acids in the heavy and light chain elbow regions. The Biacore assay result demonstrates the isoform-selective and high affinity binding by the variants.

Figure 5 shows an A549 cell bioassay of purified IgG variants with additional amino acids in the light chain elbow regions. The A549 assay compares the inhibitory effects by various antibody constructs on TGF β 1-stimulated IL-11 production, showing the elbow engineered variants are highly potent in this cell-based potency assay.

Figure 6 depicts a Biacore TGF β 1 binding assay which shows high affinity binding is regained when additional amino acids are inserted into the elbow region of both the heavy and light chain of CAT192 Fab.

Figure 7 shows results of a Differential Scanning Fluorimetry (DSF) analysis of the thermostability of the CAT192 Fab mutants.

Figure 8 shows results of a Differential Scanning Fluorimetry (DSF) analysis of the thermostability of the CAT192 IgG4 mutants.

Figure 9 shows the crystal structures solved for the CAT192 Fab variants.

DETAILED DESCRIPTION OF THE INVENTION

[0020] The disclosed modified IgG antibodies bind and neutralize TGF β 1 selectively and with high affinity and avidity. The modified IgG antibodies may be composed of the same VH and VL domains as in metelimumab. The modified IgG antibodies advantageously show greater efficacy in neutralizing TGF β 1 than when the variable domains are used in other formats.

[0021] As used herein, a first element "and/or" a second element means a specific disclosure of the first or second element separately, or the first and second elements in combination. The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

[0022] An "isolated" polynucleotide (or nucleic acid) or protein is removed and/or altered from its natural form using genetic engineering technologies. A "purified" nucleic acid or protein may be substantially pure, e.g., at least 90% pure, or in homogeneous form.

[0023] "Selective binding", or "binding selectively" to human TGF β 1, means that the binding protein (e.g., scFv-Fc dimer) is capable of binding human TGF β 1 with a higher affinity than binding to human TGF β 2 or human TGF β 3, e.g., with a dissociation constant with human TGF β 1 at least 50% lower than its dissociation constant with human TGF β 2 or human TGF β 3, as measured by surface plasmon resonance.

[0024] The present modified IgG antibodies' variable domains may comprise complementarity determining regions (CDRs) from the CDRs disclosed in U.S. Patent No. 6,492,497 (e.g., SEQ ID Nos. 11-19 of U.S. Patent No. 6,492,497). The CDR regions are listed below:

HCDR1	SYGMH	SEQ ID No. 7
HCDR2	VISYDGSIKYYADSVKG	SEQ ID No. 8
HCDR3	TGEYSGYDTSGVEL	SEQ ID No. 9
	TGEYSGYDTPQYS	SEQ ID No. 10
	TGFYSGYDTPASPD	SEQ ID No. 11
LCDR1	RASQGIGDDL	SEQ ID No. 12
LCDR2	GTSTLQS	SEQ ID No. 13
LCDR3	LQDSNYPLT	SEQ ID No. 14

[0025] Surprisingly, a consensus HCDR3 binding motif is revealed, having the sequence:

HCDR3	TGX ₁ YSGYDTX ₂ X ₃ X ₄ X ₅ X ₆	SEQ ID No. 15
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Wherein: X₁ may be any amino acid (preferably E, or F), or absent,

X₂ may be any amino acid (preferably S, D, or P), or absent,

X₃ may be any amino acid (preferably G, P, or A), or absent,

X₄ may be any amino acid (preferably V, Q, or S), or absent,

X₅ may be any amino acid (preferably E, Y, or P), or absent,

X₆ may be any amino acid (preferably L, S, or D), or absent.

[0026] The VH domain of each of the third and fourth polypeptide chains comprises a HCDR1 having the sequence of SEQ ID No. 7, a HCDR2 having the sequence of SEQ ID No. 8, and a HCDR3 having a sequence selected from the group consisting of

[0027] SEQ ID No. 9, SEQ ID No. 10, SEQ ID No. 11, and SEQ ID No. 15. The CDR sequences may be separated by anywhere from one to four framework regions, in order from the N-terminal: FW1 - CDR1 - FW2 - CDR2 - FW3 - CDR3 - FW4. The framework regions of the VH domain may be selected from a variable heavy germline sequence. The FW region sequences may be selected from the same human variable heavy germline sequence. The framework regions of the VL domain may be selected from a variable lambda or kappa germline sequence, e.g., from the same human variable lambda or kappa germline sequence. At present, about 40 variable heavy germline sequences are known in the art, as are about 40 variable kappa germline sequences and about 30 variable lambda germline sequences, e.g., V_H3, V_k1, V_H 1-69, and V_H 1-e.

[0028] Composite VH or VL domains may be generated by using the CDR sequences disclosed herein. For example, crystal structures of the VH or VL domains may be used as a guidance to generate composite domain using CDR sequences from one antibody and using the germline FW regions from another antibody. More details can be found in U.S. Patent Application Publication No. 20020099179; and Homes and Foote, J Immunol. 1997 Mar 1; 158(5):2192-201.

[0029] The present modified IgG antibodies may be composed of the same VH and VL domains as in metelimumab, having the sequences set forth in SEQ ID No. 1 and SEQ ID No. 3, respectively. The VH domain may be replaced by the VH domain having the sequences set forth in SEQ ID No. 2; the VL domain may be replaced by the VL domain having the sequences set forth in SEQ ID No. 4. These VH and VL domains are disclosed in U.S. Patent No. 6,492,497 (e.g., SEQ ID Nos. 4, 6, 8, and 10 of U.S. Patent No. 6,492,497).

[0030] A "variable domain" (VD) refers to a hypervariable binding domain of an immunoglobulin, or a ligand binding domain of a receptor, involved in antigen/ligand binding as is known by persons skilled in the art. Variable domains are routinely referred to by their location or origin within an immunoglobulin; e.g., variable domains of the light chain of an immunoglobulin (VL), variable domains of the heavy chain of an immunoglobulin (VH), variable domains of the heavy chain of a camelid immunoglobulin (VHH).

[0031] A "variant" variable domain comprises amino acid additions, substitutions, and/or deletions, compared to the reference sequence. A "variant" of the VH or VL domains may have up to four such amino acid modifications. For example, one of the two domains may comprise an amino acid substitution, while the other domain is unmodified, or both of the domains may comprise amino acid substitutions. Modifications that add or delete amino acid residues may be made at the N-terminus

or C-terminus of the VH or VL domain. For example, the N-terminal residue of the VH domain may be deleted.

For purpose of this disclosure, the terms "between," "from," "to," and "at least" are inclusive. For example, an integer "from 0 to 5" means any integer equal to or greater than 0 but equal to or smaller than 5.

[0032] Up to five amino acid substitutions may be made to de-immunize the modified IgG antibodies. De-immunization may be performed according to the method of Harding et al. (2010) mAbs 2: 256-265, for example.

[0033] Framework residues of the VH and/or VL domains, for example, may be substituted to increase the stability of the modified IgG antibodies and/or decrease their tendency to aggregate. Poor stability can affect the ability of the expressed modified IgG antibodies to fold properly when recombinantly expressed, resulting in a fraction of the expressed antibodies being non-functional. Low stability antibodies also may be prone to forming potentially immunogenic aggregates or may have impaired avidity or shelf-life. Framework amino acid substitutions that are expected to increase the stability and/or decrease the tendency to aggregate of a VH and/or VL domain, e.g., in a modified IgG antibody, are disclosed in WO 2007/109254, for example. Substitutions in corresponding residues in the present VH and VL domains are expected similarly to increase stability and/or decrease the tendency of modified IgG antibodies to aggregate.

[0034] Substitutions that can be tolerated are expected to include those that would replace an amino acid of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, or SEQ ID No. 4 with a corresponding amino acid that occurs in another human VH or VL domain germline sequence. A substitution of a framework amino acid with an amino acid occurring in any of these germline sequences may be tolerated. For example, a residue of a VH domain of SEQ ID No. 1 may be substituted with an amino acid appearing in a corresponding position in any VH germline sequence, e.g., the germline sequence from DP-10 (V_H 1-69) or DP-88 (V_H 1-e). Corresponding positions in this case are determined by a sequence alignment between the various germline sequences, using alignment techniques well known in the art, e.g., ClustalW.

[0035] Additional substitutions that are expected to be tolerated are those made to an amino acid with most of its side chain exposed to the solvent, as determined by analysis of the three co-crystal structures. The solvent-accessible surface area of a residue may be estimated using techniques well known in the art. Further, it is expected that substitutions to amino acids buried within the variable domains will be better tolerated if the side chain of the amino acid does not create steric hindrance with adjoining residues. For this reason, buried amino acids generally are substituted with amino acids with side chains of similar or smaller size. For example, a substitution of a buried Ile residue with a Leu, Val, Ala, or Gly is expected to be tolerated. Possible steric hindrance created by a substitution can be predicted by analysis of the three co-crystal structures. Further substitutions that are expected to be tolerated are those maintaining existing electrostatic interactions within the variable domains, e.g., dipole-dipole interactions, induced dipole interactions, hydrogen bonds, or ionic bonds.

[0036] Additional amino acid substitutions of variable domains include those expected to confer new useful properties to the antibodies or antigen-binding fragments thereof. For example, putative

N-glycosylation sites in the VH and/or VL domains can be removed to prevent or reduce the formation of N-glycoforms. The amino-terminal residue can be substituted with a Gln residue to cause pyroglutamylation, which can decrease the number of charge variants. Amino acid substitutions can be used to lower the isoelectric point, which can decrease the rate of elimination of IgG polypeptide antibodies, for example.

[0037] Surface residues of variable domains can be substituted with Cys or Lys residues, for example, which then can be covalently modified and coupled to molecules conferring useful characteristics to the antibodies or antigen-binding fragments thereof, e.g., a detectable label, toxin, targeting moiety, or protein. For example, Cys residue can be coupled to a cytotoxic drug to form a drug conjugate. Cys residues also can be coupled to molecules that increase the serum half-life, e.g., polyethylene glycol (PEG) or serum albumin. Such amino acid modifications are reviewed in Beck et al. (2010) Nature 10: 345-52, for example.

[0038] Detectable labels include radiolabels such as ^{131}I or ^{99}Tc , which may be attached to antibodies or antigen-binding fragments thereof using methods known in the art. Labels also include enzyme labels such as horseradish peroxidase. Labels further include chemical moieties such as biotin which may be detected via binding to a specific cognate detectable moiety, e.g., labeled avidin. Other moieties can be attached that facilitate purification. For example, antibodies or antigen-binding fragments thereof can be His-tagged using well-known methods of recombinant modification and expression.

[0039] The VL domains of the modified IgG antibodies are linked to the CL domains by a linker, termed Linker1 herein. The VH domains of the modified IgG antibodies are optionally linked to the CH1 domains by a second linker, termed Linker2 herein. Linkers suitable for making modified IgG antibodies are well known in the art. See, e.g., Bird et al. (1988) Science, 242: 423-426; Huston et al. (1988) Proc. Nat'l Acad. Sci. USA 85: 5879-5883. This can be accomplished by fusing the encoding nucleic acids in-frame and expressing the fusion protein in a suitable host cell, for example.

[0040] Linker1 and Linker2 when present comprise the amino acid residue or amino acid sequence selected from the group consisting of G, GS, GGS, GGGS, and GGGGS.

[0041] A hinge is inserted between the CH1 domain and Fc region of the modified IgG antibodies. The hinge region can be a flexible domain that optionally joins the CH1 portion to the Fc region. The flexibility of the hinge region in IgG molecules may allow the Fab arms to adopt a wide range of angles, permitting binding to epitopes spaced variable distances apart. A suitable hinge region may include, for example, the human IgG1 hinge region having the amino acid sequence PKSCDKHTHTCPPCPAPELLGGP (SEQ ID No. 5). This sequence corresponds to a portion of the human IgG1 upper hinge, the middle hinge, and an N-terminal portion of the CH₂ domain, as disclosed in FIG. 4B of U.S. Patent No. 8,048,421, for example.

[0042] Suitable Fc regions of the modified IgG antibodies contain two or three constant regions. Fc regions may include those from human IgG1, as set forth in SEQ ID No. 6, or IgG4, as set forth in the CH₂ and CH₃ domains of SEQ ID No. 17. The Fc region of an antibody mediates its serum half-life and effector functions, such as complement-dependent cytotoxicity (CDC), antibody-dependent

cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP).

[0043] Modifications can be made to the hinge and Fc region to improve various properties of the modified IgG antibodies. For example, one, two, three, four, five or up to ten amino acids of a naturally occurring human Fc region can be modified, in addition to modifications of the hinge region. For example, the Fc region can be modified to increase the serum half-life of the modified IgG antibodies. The half-life of an IgG depends on its pH-dependent binding to the receptor FcRn. FcRn, which is expressed on the surface of endothelial cells, binds the IgG in a pH-dependent manner and protects it from degradation. Mutations located at the interface between the CH₂ and CH₃ domains, for example, have been shown to increase the binding affinity to FcRn and the half-life of IgG1 *in vivo*. Such modifications are reviewed in Strohl WR., 2009. Optimization of Fc-mediated effector functions of monoclonal antibodies. *Curr Opin Biotechnol.* 20(6):685-91; and Vaccaro C. et al., 2005. Engineering the Fc region of immunoglobulin G to modulate *in vivo* antibody levels. *Nat Biotechnol.* 23(10): 1283-8, for example.

[0044] Other modifications to the hinge and/or Fc region can increase or reduce effector functions. The four human IgG isotypes bind the activating Fcγ receptors (FcγRI, FcγRIIa, FcγRIIIa), the inhibitory FcγRIIb receptor, and the first component of complement (C1q) with different affinities, resulting in different effector functions. Binding of IgG to the FcγRs or C1q, for example, depends on residues located in the IgG hinge region and CH₂ domain. Single or multiple amino acid substitutions of these residues can affect effector function by modulating the IgG interaction with FcγRs or C1q. Other substitutions are known to affect effector function. These modifications are reviewed in Strohl (2009) "Optimization of Fc-mediated effector functions of monoclonal antibodies," *Curr. Opin. Biotechnol.* 20:685-91, for example.

[0045] Representative modifications of the hinge and/or Fc region are summarized in Table 1.

Table 1: Representative Hinge and Fc Region Modifications

Isotype	Species	Substitutions	FcR/C1q Binding	Effector Function	Refs
IgG1	Human	T250Q/M428L	Increased binding to FcRn	Increased half-life	1
IgG1	Human	1M252Y/S254T/T256E + H433K/N434F	Increased binding to FcRn	Increased half-life	2
IgG1	Human	E233P/L234V/L235A/G236 + A327G/A330S/P331S	Reduced binding to FcγRI	Reduced ADCC and CDC	3,4
IgG1	Human	E333A	Increased binding to FcγRIIIa	Increased ADCC and CDC	5,6
IgG1	Human	S239D/A330L/I332E	Increased binding to FcγRIIIa	Increased ADCC	7, 8
IgG1	Human	P257I/Q311	Increased binding to FcRn	Unchanged half-life	9
IgG1	Human	K326W/E333S	Increased	Increased CDC	10

Isotype	Species	Substitutions	FcR/C1q Binding	Effector Function	Refs
			binding to C1q		
IgG1	Human	S239D/I332E/G236A	Increased FcγRIIIa/FcγRIIb ratio	Increased macrophage phagocytosis	11
IgG1	Human	K322A	Reduced binding to C1q	Reduced CDC	5
IgG4	Human	S228P	-	Reduced Fab-arm exchange	12
IgG2a	Mouse	L235E + E318A/K320A/K322A	Reduced binding to FcγRI and C1q	Reduced ADCC and CDC	10

1. 1. Hinton et al. (2004) J. Biol. Chem. 279(8):6213-16.
2. 2. Vaccaro et al. (2005) Nature Biotechnol. 23(10): 1283-88.
3. 3. Armour et al. (1999) Eur. J. Immunol. 29(8):2613-24.
4. 4. Shields et al. (2001) J. Biol. Chem. 276(9):6591-604.
5. 5. Idusogie et al. (2000) J. Immunol. 164(8):4178-84.
6. 6. Idusogie et al. (2001) J. Immunol. 166(4):2571-75.
7. 7. Lazar et al. (2006) Proc. Nat'l Acad. Sci. USA 103(11): 4005-10.
8. 8. Ryan et al. (2007) Mol. Cancer Ther. 6: 3009-18.
9. 9. Datta-Mannan et al. (2007) DrugMetab. Dispos. 35: 86-94.
10. 10. Steurer et al. (1995) J. Immunol. 155(3):1165-74.
11. 11. Richards et al. (2008) Mol. Cancer Ther. 7(8):2517-27.
12. 12. Labrijn et al. (2009) Nature Biotechnol. 27(8):767-71.

[0046] Further, recombinant amino acid modifications can be used to decrease structural homogeneity of the expressed polypeptides. A representative example is Peters et al. (2012) J. Biol. Chem. 287(29): 24525-33, which discloses Cys to Ser substitutions in the IgG4 hinge region that reduce the disulfide bond heterogeneity and increase Fab domain thermal stability. Similarly, Zhang et al. (2010) Anal. Chem. 82: 1090-99 disclose engineering the IgG2 hinge region to limit disulfide bond scrambling and the formation of structural isomers in therapeutic applications. Amino acid modifications to a CH3 domain also can be used to delete carboxy-terminal Lys residues to decrease the number of charge variants. Amino acid modifications also can be used to improve the pharmacological function of recombinant antibodies or antigen-binding fragments thereof. For example, amino acid modifications can be used to increase complement activation, enhance antibody-dependent cellular cytotoxicity (ADCC) by increasing FcγRIIIA binding or decreasing FcγRIIIB binding, and/or increase serum half-life by increasing FcRn binding. Such amino acid modifications are reviewed in Beck et al. (2010) Nature 10: 345-52, for example.

Nucleic Acids and Methods of Making Modified IgG Antibodies

[0047] A further aspect of the present invention provides nucleic acids encoding the modified IgG antibodies of the invention. The isolated nucleic acid may be a synthetic DNA, a non-naturally occurring mRNA, or a cDNA, for example. Examples include the nucleic acids encoding the VH and VL domains set forth in SEQ ID NOS: 3, 5, 7, and 9 of U.S. Patent No. 6,492,497. A recombinant host cell may comprise one or more constructs above. Methods of preparing modified IgG antibodies comprise expressing the encoding nucleic acid in a host cell under conditions to produce the modified IgG antibodies, and recovering the antibodies. The process of recovering the antibodies may comprise isolation and/or purification of the antibodies. The method of production may comprise formulating the antibodies into a composition including at least one additional component, such as a pharmaceutically acceptable excipient.

[0048] The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which exogenous DNA has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Preferably host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. Preferred eukaryotic cells include protist, fungal, plant and animal cells. Most preferably host cells include but are not limited to the prokaryotic cell line *E. Coli*; mammalian cell lines CHO, HEK 293 and COS; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

[0049] Suitable vectors comprising a nucleic acid encoding modified IgG antibodies can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, phage, phagemids, adenoviral, AAV, lentiviral, for example. Techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells, and gene expression, are well known in the art.

[0050] The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. Such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions, are also disclosed.

[0051] Introducing such nucleic acids into a host cell can be accomplished using techniques well known in the art. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection, and transduction using retroviruses or other viruses, for example. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation, and transfection using bacteriophage. The introduction may be followed by causing or allowing expression from the nucleic acid, e.g. by culturing host cells under conditions for expression of the gene. For example, the nucleic acid of the invention can be integrated into the genome, e.g., chromosome, of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques.

[0052] Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, plant cells, insect cells, fungi, yeast and transgenic plants and animals. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, mouse melanoma cells, rat myeloma cells, human embryonic kidney cells, e.g., HEK293 cells, human embryonic retina cells, and many others. The expression of antibodies and antibody fragments in prokaryotic cells, such as *E. coli*, is well established in the art. For a review, see for example, Plückthun *Bio/Technology* 9: 545-551 (1991). Expression in cultured eukaryotic cells is also available to those skilled in the art, as reviewed in Andersen et al. (2002) *Curr. Opin. Biotechnol.* 13: 117-23, for example.

[0053] The disclosed modified IgG antibodies may be glycosylated, either naturally or the choice of expression host, e.g., CHO, HEK293, or NSO (ECACC 85110503) cells, or they may be unglycosylated, for example if produced by expression in a prokaryotic cell. Glycosylation may also be intentionally altered, for example by inhibiting fucosylation, in order to increase ADCC activity of the resulting modified IgG antibodies.

Methods of Using Antibodies or Antigen-Binding Fragments Thereof

[0054] The modified IgG antibodies may be used in a method of treatment or diagnosis of the human or animal body, such as a method of treatment (which may include prophylactic treatment) of a disease or disorder in a human patient, which comprises administering an effective amount to treat the patient. Treatable conditions include any in which TGF β 1 plays a role, e.g., a fibrotic disease, cancer, an immune-mediated disease, and wound healing, e.g., diffuse systemic sclerosis, bone remodeling disease, kidney disease and/or a combination thereof.

[0055] Antibodies specific for human TGF β 1 have been shown to be effective in animal models for the treatment of TGF β 1 glomerulonephritis (Border et al. (1990) *Nature* 346: 371-374), neural scarring (Logan et al. (1994) *Eur. J. Neurosci.* 6: 355-363), dermal scarring (Shah et al. (1992) *Lancet* 339: 213-214; Shah et al. (1994) *J. Cell Science* 107: 1137-1157; Shah et al. (1995) *J. Cell Science* 108: 985-1002), and pulmonary fibrosis (Giri et al. (1993) *Thorax* 48: 959-966). Further, antibodies to TGF β 1, 2, and 3 have been shown to be effective in models of lung fibrosis, radiation induced fibrosis (U.S. Patent No. 5,616,561), myelofibrosis, burns, Dupuytren's contracture, gastric

ulcers, and rheumatoid arthritis (Wahl et al. (1993) Exp. Medicine 177: 225-230).

[0056] The modified IgG antibodies are useful to treat a disease and condition resulting directly or indirectly from TGF β 1 activity. The modified IgG antibodies may selectively inhibit the activity of a human TGF β 1 isoform *in vitro* or *in vivo*. Activities of TGF β 1 isoforms include, but are not limited to, TGF β -mediated signaling, extracellular matrix (ECM) deposition, inhibiting epithelial and endothelial cell proliferation, promoting smooth muscle proliferation, inducing Type III collagen expression, inducing TGF- β , fibronectin, VEGF, and IL-11 expression, binding Latency Associated Peptide, tumor-induced immunosuppression, promotion of angiogenesis, activating myofibroblasts, promotion of metastasis, and inhibition of NK cell activity. For example, the modified IgG antibodies are useful to treat focal segmental glomerulosclerosis (FSGS), hepatic fibrosis (HF), acute myocardial infarction (AMI), idiopathic pulmonary fibrosis (IPF), scleroderma (SSc), and Marfan Syndrome.

[0057] The modified IgG antibodies are useful to treat diseases and conditions including, but not limited to, fibrotic diseases (such as glomerulonephritis, neural scarring, dermal scarring, pulmonary fibrosis, lung fibrosis, radiation induced fibrosis, hepatic fibrosis, myelofibrosis), burns, immune mediated diseases, inflammatory diseases (including rheumatoid arthritis), transplant rejection, cancer, Dupuytren's contracture, and gastric ulcers. The modified IgG antibodies are also useful for treating, preventing and reducing the risk of occurrence of renal insufficiencies including but not limited to: diabetic (type I and type II) nephropathy, radiation-induced nephropathy, obstructive nephropathy, diffuse systemic sclerosis, pulmonary fibrosis, allograft rejection, hereditary renal disease (e.g., polycystic kidney disease, medullary sponge kidney, horseshoe kidney), glomerulonephritis, nephrosclerosis, nephrocalcinosis, systemic lupus erythematosus, Sjogren's syndrome, Berger's disease, systemic or glomerular hypertension, tubulointerstitial nephropathy, renal tubular acidosis, renal tuberculosis, and renal infarction. In particular, the modified IgG antibodies are useful when combined with antagonists of the renin-angiotensin-aldosterone system including, but not limited to: renin inhibitors, angiotensin-converting enzyme (ACE) inhibitors, Ang II receptor antagonists (also known as "Ang II receptor blockers"), and aldosterone antagonists. By way of example, methods for using modified IgG antibodies in combination with such antagonists are set forth in WO 2004/098637.

[0058] The modified IgG antibodies also are useful to treat diseases and conditions associated with the deposition of ECM, including, systemic sclerosis, postoperative adhesions, keloid and hypertrophic scarring, proliferative vitreoretinopathy, glaucoma drainage surgery, corneal injury, cataract, Peyronie's disease, adult respiratory distress syndrome, cirrhosis of the liver, post myocardial infarction scarring, post angioplasty restenosis, scarring after subarachnoid hemorrhage, multiple sclerosis, fibrosis after laminectomy, fibrosis after tendon and other repairs, scarring due to tattoo removal, biliary cirrhosis (including sclerosing cholangitis), pericarditis, pleurisy, tracheostomy, penetrating central nervous system injury, eosinophilic myalgic syndrome, vascular restenosis, veno-occlusive disease, pancreatitis and psoriatic arthropathy.

[0059] The modified IgG antibodies further are useful to promote re-epithelialization in diseases and conditions such as venous ulcers, ischaemic ulcers (pressure sores), diabetic ulcers, graft sites, graft donor sites, abrasions and burns, diseases of the bronchial epithelium, such as asthma, ARDS, diseases of the intestinal epithelium, such as mucositis associated with cytotoxic treatment,

esophageal ulcers (reflux disease), stomach ulcers, small intestinal and large intestinal lesions (inflammatory bowel disease).

[0060] The modified IgG antibodies also may be used to promote endothelial cell proliferation, for example, in stabilizing atherosclerotic plaques, promoting healing of vascular anastomoses, or to inhibit smooth muscle cell proliferation, such as in arterial disease, restenosis and asthma.

[0061] The modified IgG antibodies are useful to enhance the immune response to macrophage-mediated infections. They are also useful to reduce immunosuppression caused, for example, by tumors, AIDS, or granulomatous diseases. The modified IgG antibodies are useful to treat hyperproliferative diseases, such as cancers including, but not limited to, breast, prostate, ovarian, stomach, renal, pancreatic, colorectal, skin, lung, cervical and bladder cancers, glioma, mesothelioma, as well as various leukemias and sarcomas, such as Kaposi's sarcoma, and are useful to treat or prevent recurrences or metastases of such tumors. Modified IgG antibodies also are useful to inhibit cyclosporin-mediated metastases.

[0062] In the context of cancer therapy, "treatment" includes any medical intervention resulting in the slowing of tumor growth or reduction in tumor metastases, as well as partial remission of the cancer in order to prolong life expectancy of a patient.

[0063] Methods of treatment comprise administering a modified IgG antibody or pharmaceutical compositions comprising the modified IgG antibody. The modified IgG antibodies may be used in the manufacture of a medicament for administration. For example, a method of making a medicament or pharmaceutical composition comprises formulating a modified IgG antibody with a pharmaceutically acceptable excipient. A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

[0064] Administration is preferably in a "therapeutically effective amount" sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom of a particular disease or condition. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease or condition being treated. Prescription of treatment, e.g., decisions on dosage etc., may be determined based on preclinical and clinical studies the design of which is well within the level of skill in the art.

[0065] The precise dose will depend upon a number of factors, including whether the modified IgG antibody is for diagnosis or for treatment, the size and location of the area to be treated, and the nature of any detectable label or other molecule attached to the modified IgG antibody. A typical dose of a modified IgG antibody, for example, can be in the range 100 µg to 1 gram for systemic applications, and 1 µg to 1 mg for topical applications. The dose for a single treatment of an adult patient may be adjusted proportionally for children and infants. Treatments may be repeated at daily, twice-weekly, weekly, monthly or other intervals, at the discretion of the physician. Treatment may be periodic, and the period between administrations is about two weeks or more, preferably about three weeks or more, more preferably about four weeks or more, or about once a month.

[0066] Dose levels of about 0.1, 0.3, 1, 3, 10, 15 mg, or 20 mg of the disclosed antibodies per kg

body weight of the patient may be useful and safe in humans. For example, 0.5-5 mg/kg in rat and mouse has been an effective dose in an acute setting. Therefore, for long-term dosing, 0.3-10 mg/kg may be administered to humans, based on an expected half-life of 21 days. Doses may be sufficient for efficacy, while low enough to facilitate optimal administration. For example, a dose of less than 50 mg facilitates subcutaneous administration. Intravenous administration may be used as the route of delivery for severe diseases, where high doses and the long dosing intervals may be required. Subcutaneous injection can increase the potential immune response to a product. Local administration for localized disease can reduce the amount of administered product and increase the concentration at the site of action, which can improve safety.

[0067] Modified IgG antibodies may be administered by injection, for example, subcutaneously, intravenously, intracavity (e.g., after tumor resection), intralesionally, intraperitoneally, or intramuscularly. Modified IgG antibodies also may be delivered by inhalation or topically (e.g., intraocular, intranasal, rectal, into wounds, on skin), or orally.

[0068] A modified IgG antibody will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the modified IgG antibody. Thus pharmaceutical compositions may comprise a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. Such materials could include, for example, any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic, and absorption delaying agents. Some examples of pharmaceutically acceptable carriers are water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or auxiliary substances, such as emulsifying agents, preservatives, or buffers, which increase the shelf life or effectiveness.

[0069] The precise nature of the carrier or other material will depend on the route of administration. For intravenous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pK, isotonicity, and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as sodium chloride injection, Ringer's injection, and lactated Ringer's injection. Preservatives, stabilizers, buffers, antioxidants, and/or other additives may be included.

[0070] A modified IgG antibody may be formulated in liquid, semi-solid, or solid forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, powders, liposomes, and suppositories. The preferred form depends on the intended mode of administration, the therapeutic application, the physicochemical properties of the molecule, and the route of delivery. Formulations may include excipients, or a combination of excipients, for example: sugars, amino acids and surfactants. Liquid formulations may include a wide range of modified IgG antibody concentrations and pH. Solid formulations may be produced by lyophilization, spray drying, or drying by supercritical fluid technology, for example.

[0071] Therapeutic compositions can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the modified IgG antibody in an appropriate solvent with one or a combination of ingredients enumerated above, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by using a coating such as lecithin, by maintaining the particle size of a dispersion, or by using surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[0072] The active compound may be prepared with a carrier that will protect the modified IgG antibody against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

[0073] A method of using a modified IgG antibody may comprise causing or allowing binding to TGF β . Such binding may take place *in vivo*, e.g., following administration of a modified IgG antibody to a patient, or it may take place *in vitro*, e.g., in ELISA, Western blotting, immunocytochemistry, immunoprecipitation, affinity chromatography, or cell based assays, or in *ex vivo* based therapeutic methods, e.g., methods in which cells or bodily fluids are contacted *ex vivo* with a modified IgG antibody and then administered to a patient.

[0074] A kit comprising a modified IgG antibody is provided. The modified IgG antibody may be labeled to allow its reactivity in a sample to be determined. Kits may be employed in diagnostic analysis, for example. A kit may contain instructions for use of the components. Ancillary materials to assist in or to enable performing such a method may be included within the kit.

[0075] The reactivity of a modified IgG antibody in a sample may be determined by any appropriate means, e.g., radioimmunoassay (RIA). Radioactively labeled antigen may be mixed with unlabeled antigen (the test sample) and allowed to bind to the modified IgG antibody. Bound antigen is physically separated from unbound antigen and the amount of radioactive antigen bound to the modified IgG antibody is determined. A competitive binding assay also may be used with non-radioactive antigen, using an antigen or an analogue linked to a reporter molecule. The reporter molecule may be a fluorochrome, phosphor, or dye. Suitable fluorochromes include fluorescein, rhodamine, phycoerythrin and Texas Red. Suitable chromogenic dyes include diaminobenzidine.

[0076] Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are colored, magnetic or paramagnetic, and biologically or chemically active agents that can directly or indirectly cause detectable signals to be visually observed, electronically detected or otherwise recorded. These molecules may be enzymes that catalyze reactions that

develop or change colors or cause changes in electrical properties, for example. They may be molecularly excitable, such that electronic transitions between energy states result in characteristic spectral absorptions or emissions. They may include chemical entities used in conjunction with biosensors. Biotin/avidin or biotin/streptavidin and alkaline phosphatase detection systems may be employed. The signals generated by antibody-reporter conjugates may be used to derive quantifiable absolute or relative data of the relevant antibody binding in samples.

[0077] Also disclosed is the use of a modified IgG antibody for measuring antigen levels in a competition assay. The modified IgG antibody can be linked to a reporter molecule so that a physical or optical change occurs on binding, for example. The reporter molecule may directly or indirectly generate detectable, and preferably measurable, signals. The reporter molecules may be linked directly or indirectly, covalently, e.g., via a peptide bond or non-covalently. The modified IgG antibody and a protein reporter may be linked by a peptide bond and recombinantly expressed as a fusion protein.

[0078] Further aspects of the present invention will be apparent to those skilled in the art in the light of the present disclosure, including the following experimental exemplification.

Examples

Example 1: Modified IgG4 Antibody with Additional Amino Acids in the Light Chain Elbow Region

[0079] CAT192 is a TGF β 1-specific antibody, but most of its binding affinity was lost when it was converted from a scFv into a full-length IgG4 (Figure 1). Antibody subtype and Fc formats alone do not explain this phenomenon because both IgG1 and IgG4 Fab displayed very low affinity to TGF β 1. The tight binding of scFv to TGF β 1 may be due to the high flexibility resulting from the long (GGGGS)₃ linker connecting the heavy and light chain Fv domains. This high flexibility may have been lost during conversion of the scFv to Fab or IgG versions. The low affinity of CAT192 was characterized by a very slow on-rate but also a very slow off-rate. The slow on-rate and off-rate suggested that the binding between CAT192 and TGF β 1 may require a potential conformational change which was limited by unfavorable amino acids in CAT192(IgG4). Described here are experiments designed to increase flexibility/affinity of the Fab or IgG versions of the scFv by adding additional amino acids in the light chain elbow region, which links the antibody Fv domain to the CH1 domain. More specifically, mutants were designed to add one glycine (G), two glycines (GG), two glycines and one serine (GGS), three glycines and one serine (GGGS), and four glycines and one serine (GGGGS) sequences into the wild-type light chain elbow region as shown below in Table 2, with the added amino acids underlined.

Table 2: Modified-IgG4 Light Chain Elbow Region Insertion Mutants

Name	Position	Amino acid sequence
WT	Light chain elbow region	LEIKRTVA (SEQ ID No. 21)
LC+G	Light chain elbow region	LEIK <u>G</u> RTVA (SEQ ID No. 22)

Name	Position	Amino acid sequence
LC+GG	Light chain elbow region	LEIKGGRTVA (SEQ ID No. 23)
LC+GGs	Light chain elbow region	LEIKGGsRTVA (SEQ ID No. 24)
LC+GGGS	Light chain elbow region	LEIKGGGSRTVA (SEQ ID No. 25)
LC+GGGGS	Light chain elbow region	LEIKGGGGSRTVA (SEQ ID No. 26)
A25S	Light chain Fv	Ala25→Ser25

[0080] The light chain amino acid #25 is an Ala in scFv, but was changed to Ser when converted to IgG4. Therefore, an additional A25S mutant was included as a control to test whether changing the Ala to Ser affects the affinity of the scFv to TGFβ1. The wild-type CAT192 and the mutant DNA and amino acid sequences are listed below.

SEQ ID No. 38: Amino acid sequence of CAT192 IgG1 Wild-Type LC with the elbow region underlined:

EIVLTQSPSSLSASVGDRTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
 QSGVPSRFSGSGSGTDFLTINSLPEDFATYYCLQDSNYPLTFGGGTRLEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
 ESVTEQDSKDSTYLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGE
 C

SEQ ID No. 27: Coding sequence of CAT192 (IgG1) Light Chain

atgggctggctcctgcatcactctgtttctgggtggccacagccaccggcgtgcacagcGAGATCGTGCTGACA
 CAGAGCCCCAGCAGCCTGTCTGCCAGCGTGGGCGACAGAGTGACCATCAC
 CTGTAGAGCCAGCCAGGGCATCGGCGACGACCTGGGATGGTATCAGCAGA
 AGCCTGGCAAGGCCCCCATCCTGCTGATCTACGGCACCAGCACACTGCAG
 AGCGGCGTGCCCTCCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACC
 CTGACCATCAACAGCCTGCAGCCCAGGACTTCGCCACCTACTACTGTCTG
 CAAGACAGCAACTACCCCTGACCTTCGGCGGAGGCACCCGGCTGGAAAT
 CAAGCGTACGGTGGCCGCTCCTTCCGTGTTTCATCTTCCCTCCCTCCGACGA
 GCAGCTGAAGTCCGGCACCGCCTCCGTGGTGTGTCTGCTGAACAACTTCTA
 CCCTCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCG
 GCAACTCCCAGGAGTCCGTCACCGAGCAGGACTCCAAGGACAGCACCTAC
 TCCCTGTCTCTCCACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCACAA
 GGTGTACGCCTGTGAGGTGACCCACCAGGGCCTGTCCAGCCCTGTGACCA
 AGTCCTTCAACCGGGGCGAGTGCTGA

CAT192LC+G (LEIKGRTVA)

Forward	5'-ggctggaaatcaagggccgtacgggtggccgc-3' (SEQ ID No. 28)
Complement	5'-gcgccaccgtacggcccttgattccagcc-3' (SEQ ID No. 29)

CAT192LC+GG. (LEIKGGRTVA)

Forward	5'-ggctggaaatcaagggcgccgtacgggtggccgc-3' (SEQ ID No. 30)
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Complement	5'-gcggccaccgtacggcgcccttgattccagcc-3' (SEQ ID No. 31)
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CAT 192LC+GGS. (LEIKGGSRTVA)

Forward	5'-ggctggaaatcaagggcgccagccgtacggtggccgc-3' (SEQ ID No. 32)
Complement	5'-gcggccaccgtacggctgccgccccttgattccagcc-3' (SEQ ID No. 33)

CAT192LC+GGGS. (LEIKGGGSRTVA)

Forward	5'-ggctggaaatcaagggcgccgcccagccgtacggtggccgc-3' (SEQ ID No. 34)
Complement	5'-gcggccaccgtacggctgccgccccttgattccagcc-3' (SEQ ID No. 35)

CAT 192LC+GGGGS. (LEIKGGGGSRTVA)

Forward	5'-ggctggaaatcaagggcgccgcccagccgtacggtggccgc-3' (SEQ ID No. 36)
Complement	5'-gcggccaccgtacggctgccgccccttgattccagcc-3' (SEQ ID No. 37)

[0081] The five CAT192 LC mutants, along with the A25S mutant and WT LC were co-expressed with His-tagged CAT192 HC Fab using the Expi293F transfection system (Life Technologies) in a 24-well plate format (4 x 1mL). Conditioned media (CM) was harvested 4 days post-transfection and the Octet QK384 instrument was used to calculate expression level and TGF β 1 binding in a single assay. Purified CAT192 Fab-His was used as a standard curve (diluted 2-fold from 100 to 3.125 μ g/mL). The CAT192 Fab CM was diluted 1:10 in diluent and GC1008 Fab CM was included as positive control. Binding to anti-Fab-CH1 biosensors was measured for 2 min at 1000 rpm and 30°C plate temperature for quantitation and capture. The sensors were then moved into wells containing 200 nM of TGF β 1 for binding assessment.

[0082] The TGF β 1 binding result showed that each additional amino acid insertion increased the binding affinity of the CAT192 Fab as compared to the WT counterpart. Addition of at least two glycines increased the binding affinity to a level comparable to that of the GC1008 Fab antibody. The A25S mutant showed weak TGF β 1 binding affinity, similar to the affinity of the WT and the purified recombinant CAT192 Fab. This result was qualitative because the amount of Fab captured to the sensors was not normalized for concentration or buffer subtracted.

[0083] The CAT192 LC Fab variants were then purified using PureSpeed IMAC tips from Rainin in order to accurately assess the affinity to TGF β 1 and confirm isoform-specificity using surface plasmon resonance. The conditioned media for each sample was split into four wells (~800 μ L each) and ~200 μ L equilibration buffer was added to each in order to use two 1 mL purification tips (20 μ L resin) for each sample. The samples were buffer exchanged using Amicon Ultra filters into Gibco PBS pH 7.2 after the elution step to remove the imidazole. The concentrations were measured by A280 and 3.5 μ g was loaded onto a non-reducing 4-20% Tris-Glycine SDS-PAGE gel and Coomassie stained to check the purity. The overall yield ranged from 12-42% of the starting material in CM.

[0084] The Biacore T200 instrument was used to assess the TGF β binding affinity of purified

CAT192 WT and LC mutant Fabs. TGF β 1, TGF β 2, and TGF β 3 (124, 125, and 112 RU) were immobilized to a CM5 series S chip using amine chemistry. A wide concentration range was used to account for both the low and high affinity binders. The Fabs were diluted 3-fold from 270 to 0.37nM in HBS-EP+ buffer. Each sample was injected in duplicate. The K_D was determined using the typical concentration range with 30nM as the top concentration. The Biacore binding results were in accordance with the Octet results described above, namely, addition of amino acids in the elbow region of CAT192 LC improved the binding affinity to TGF β 1. A step-wise improvement with insertion of each additional residue was demonstrated (Figure 6). None of the CAT192 Fab LC mutants bound to TGF β 2 or TGF β 3 under the same conditions, demonstrating that the mutants retained the isoform-selectivity, while significantly increasing TGF β 1 binding. Therefore, the elbow engineered mutants were a set of novel variants with isoform-selectivity and high affinity binding to TGF β 1.

Table 3: K_D of Modified Fabs

Sample	K_D (nM)
WT Fab	n/a
LC+G Fab	n/a
LC+GG Fab	3.32
LC+GGG Fab	3.76
A25S Fab	n/a

Example 2: Modified IgG4 Antibody with Additional Amino Acids in the Heavy Chain Elbow Region

[0085] As a follow up to the light chain mutants in the elbow region which has shown high affinity and TGF β 1-selective binding, mutants were also designed to increase flexibility/affinity by inserting additional amino acids in the heavy chain elbow region, which linked the antibody Fv domain to the CH1 domain. More specifically, mutants were designed to add one glycine (G), two glycines (GG), and four glycines and a serine (GGGGS) sequence into the wild-type heavy chain elbow region as shown below in Table 4, with the added amino acids underlined.

Table 4: Modified-IgG4 Heavy Chain Elbow Insertion Mutants

Name	Position	Amino acid sequence
WT	Heavy chain elbow region	TVTSSAS (SEQ ID No. 44)
HC+G	Heavy chain elbow region	TVTSSAS (SEQ ID No. 45)
HC+GG	Heavy chain elbow region	TVTSSAS (SEQ ID No. 46)
HC+GG-ST	Heavy chain elbow region	TVTSSAS (SEQ ID No. 47)
HC+GGGGS	Heavy chain elbow region	TVTSSAS (SEQ ID No. 48)

[0086] The HC+GG-ST was an unexpected by-product from the PCR mutagenesis process, which added the two glycines in the elbow as designed but also had two amino acids deleted at the end of

the elbow region, as confirmed by DNA sequencing. This mutant had the same number of amino acids in the heavy chain elbow region but different amino acids composition in the elbow linker. It was included as a control for characterization and affinity comparisons.

CAT192 HC+G primers

Forward 5'-ccaccgtgacagtgtctggccagcgccagc-3' (SEQ ID No. 50)

Complement 5'-gctggcgctgcccagacactgtcacggtgg-3' (SEQ ID No. 51)

CAT192 HC+GG-ST primers

Forward 5'-ccaccgtgacagtgtctggcgccagcgccagc-3' (SEQ ID No. 52)

Complement 5'-gctggcgctgccgccagacactgtcacggtgg-3' (SEQ ID No. 53)

CAT192 HC+GGGS primers

Forward 5'-caccaccgtgacagtgtctggcggcgcagcgccagca-3' (SEQ ID No. 54)

Complement 5'-tgctggcgctgctgccgcccgccagacactgtcacggtggtg-3' (SEQ ID No. 55)

CAT192 HC+GG primers

Forward 5'- caccaccgtgacagtgtctggcggcagcgccagca -3' (SEQ ID No. 529)

Complement 5'- tgctggcgctgccgcccagacactgtcacggtggtg -3' (60)

[0087] These CAT 192 HC mutants were co-expressed with CAT 192 LC Fab using the Expi293F transfection system (Life Technologies) in a 24-well plate format (4 x 1mL). Conditioned media was harvested 4 days post-transfection and then purified using PureSpeed IMAC tips from Rainin in order to accurately assess the affinity to TGFβ1.

[0088] The Biacore T200 instrument was used to assess the TGFβ binding affinity of purified CAT192 mutant Fabs as described in Example 1. The results shown in Figure 6 suggested that, like the mutants in the light chain elbow region, addition of amino acids in the elbow region of CAT192 heavy chain also improved the binding affinity to TGFβ1. For example, the CAT192 HC+GGGS mutant showed very high affinity binding to TGFβ1.

Example 3: Heavy and Light Chain Combination Mutants

[0089] Combination CAT192 mutants were created by co-transfection DNAs harboring mutants at the elbow regions of both heavy and light chains using the Expi293F transfection system (Life Technologies) in a 24-well plate format (4 x 1mL). The different combinations are listed in Table 5.

Table 5: Various Heavy and Light Chain Combination Mutants

WT HC LC	WT HC LC+G	WT HC LC+GG	WT HC LC+GGS	WT HC LC+GGGS	WT HC LC+GGGS
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HC+G WT LC	HC+G LC+G	HC+G LC+GG	HC+G LC+GGS	HC+G LC+GGGS	HC+G LC+GGGGS
HC+GG WT LC	HC+GG LC+G	HC+GG LC+GG	HC+GG LC+GGS	HC+GG LC+GGGS	HC+GG LC+GGGGS
HC+GG-ST WT LC	HC+GG-ST LC+G	HC+GG-ST LC+GG	HC+GG-ST LC+GGS	HC+GG-ST LC+GGGS	HC+GG-ST LC+GGGGS
HC+GGGGS WT LC	HC+GGGGS LC+G	HC+GGGGS LC+GG	HC+GGGGS LC+GGS	HC+GGGGS LC+GGGS	HC+GGGGS LC+GGGGS

[0090] Conditioned media was harvested 4 days post-transfection and then purified using PureSpeed IMAC tips from Rainin in order to accurately assess the affinity to TGFβ1.

[0091] The Biacore T200 instrument was used to assess the TGFβ binding affinity of purified CAT192 mutant Fabs as described in Example 1. The results shown in Figure 3 suggested that the combination mutants restored the high affinity binding to TGFβ1 of CAT192. The binding affinity (KD) by these mutant Fabs are listed in Table 6.

Table 6: TGFβ1-Binding Affinity (KD) of the Heavy and Light Chain Combination Mutants Determined by Biacore

Fabs	Antigen	k_a ($\times 10^5/\text{Ms}$)	k_d ($\times 10^{-4}/\text{s}$)	K_D nM)
WT HC/WT LC	TGFβ1	n/d	n/d	>100
WT HC/LC+G	TGFβ1	0.16	10.6	66.5
WT HC/LC+GG	TGFβ1	0.70	5.69	8.1
WT HC/LC+GGS	TGFβ1	0.68	5.95	8.8
WT HC/LC+GGGS	TGFβ1	0.89	5.87	6.6
WT HC/LC+GGGGS	TGFβ1	1.18	5.83	5.0
HC+G/WT LC	TGFβ1	n/d	n/d	n/d
HC+G/LC+G	TGFβ1	1.51	6.32	4.2
HC+G/LC+GG	TGFβ1	2.36	6.64	2.8
HC+G/LC+GGS	TGFβ1	2.27	6.96	3.1
HC+G/LC+GGGS	TGFβ1	2.54	6.93	2.7
HC+G/LC+GGGGS	TGFβ1	2.62	6.90	2.6
HC+GG/WT LC	TGFβ1	0.2	5.3	31.7
HC+GG/LC+G	TGFβ1	1.1	4.9	4.4
HC+GG/LC+GG	TGFβ1	2.2	3.8	1.8
HC+GG/LC+GGS	TGFβ1	1.8	4.3	2.3
HC+GG/LC+GGGS	TGFβ1	2.4	3.7	1.5
HC+GG/LC+GGGGS	TGFβ1	2.2	3.9	1.8
HC+GG-ST/WT LC	TGFβ1	0.85	8.11	9.6
HC+GG-ST/LC+G	TGFβ1	1.31	5.87	4.5

Fabs	Antigen	$k_a (\times 10^5/\text{Ms})$	$k_d (\times 10^{-4}/\text{s})$	$K_D \text{ nM}$
HC+GG-ST/LC+GG	TGF β 1	2.04	6.64	3.3
HC+GG-ST/LC+GGS	TGF β 1	2.11	6.67	3.2
HC+GG-ST/LC+GGGS	TGF β 1	1.47	6.31	4.3
HC+GG-ST/LC+GGGGS	TGF β 1	2.50	7.17	2.9
HC+GGGGS/WT LC	TGF β 1	0.68	5.79	8.5
HC+GGGGS/LC+G	TGF β 1	2.21	6.77	3.1
HC+GGGGS/LC+GG	TGF β 1	2.65	7.27	2.7
HC+GGGGS/LC+GGS	TGF β 1	2.63	7.09	2.7
HC+GGGGS/LC+GGGS	TGF β 1	2.78	7.16	2.6
HC+GGGGS/LC+GGGGS	TGF β 1	2.64	7.44	2.8
GC1008 Fab	TGF β 1	7.11	20.80	2.9
n/d = none detected				

[0092] Fewer amino acids insertions were required when the elbow region of both heavy chain and light chain were engineered. For example, combination of "HC+G" and "LC+G" mutant showed very high affinity binding to TGF β 1.

Example 4: Affinity and Potency Characterization of Full Length IgG4 Variants

[0093] Mutants in IgG4 format were generated to determine if the regained affinity by Biacore can be confirmed in the A549 cell-based potency assay. The CAT192 HC Fab was cloned into the heavy chain S228P IgG4 backbone to minimize the half-antibody formation, and Expi293F cells were then co-transfected with CAT192 IgG4 S228P HC and the LC insertion mutant.

[0094] A 30mL transfection was performed on the full length CAT192 HC and LC insertion mutants in order to obtain enough material for the bioassay. Expi293F cells were transfected with 30 μ g DNA (15 μ g LC + 15 μ g HC). Conditioned media was collected 4 days after the transfection and analyzed by Octet using Protein A biosensors to have around 200 μ g/mL expression. The CM was then purified using Hi-Trap Protein A HP columns with a peristaltic pump. CM was loaded onto each column at 0.5 mL/min, washed with 25 column volumes (CV) of 50 mM NaPi, 25 mM NaCl pH 7.1 (2 mL/min), washed with 25 CV of 10 mM sodium succinate pH 6.0 (2 mL/min), and eluted in 3 x 2 mL fractions with 10 mM sodium succinate pH 3.75 at 1 mL/min (labeled #1,#2,#3). The protein A eluates were neutralized with 0.2 M NaOH, and 0.2 M NaCl was added for a final concentration of 40 mM NaCl. The samples were then concentrated and buffer exchanged into 50 mM NaPi, 25 mM NaCl pH 7.1. These CAT192 IgG4 S228P HC and LC insertion mutant protein A eluates were then run on a 4-20% Tris Glycine gel (Figure 3) and compared by Biacore for TGF β 1/ TGF β 2/ TGF β 3 binding (Figure 4). Biacore results showed that the purified CAT192 IgG4 mutants indeed regained TGF β 1 binding. None of the mutants bound to TGF β 2 or TGF β 3 (Figure 4).

Table 7 TGF β 1-Binding Affinity (KD) of the Full-length IgG4 Variants as Determined by Biacore

Sample	$k_a (\times 10^5/\text{Ms})$	$k_d (\times 10^{-4}/\text{s})$	$K_D (\text{nM})$
CAT192 IgG4 S228P	n/d	n/d	>100
CAT192 IgG4 S228P LC+G	0.15	5.26	36.1
CAT192 IgG4 S228P LC+GG	1.0	0.2	0.2
CAT192 IgG4 S228P LC+GGS	0.8	2.2	2.7
CAT192 IgG4 S228P LC+GGGS	0.6	0.6	1.0
CAT192 IgG4 S228P LC+GGGGS	0.6	1.5	2.6
CAT192 IgG4 S228P HC+G	0.5	2.1	4.1
CAT192 IgG4 S228P HC+GG	1.0	2.0	2.2
CAT192 IgG4 S228P HC+GGGGS	1.1	1.5	1.3
CAT192 IgG4 S228P HC+GG/LC+GG	2.4	0.5	0.2
n/d = none detected			

[0095] The CAT192 IgG S228P LC insertion mutants were then characterized in a A549 cell potency assay (Rapoza et al., 2006, J Immunol Methods, Vol316, pp18). The results (Figure 5) showed that the CAT192 insertion mutants neutralized TGF β 1 activity, as demonstrated by the inhibitory effects by the mutants on TGF β 1-stimulated IL-11 production. It appeared that two glycines added to the light chain elbow were sufficient to for CAT192 to regain efficacy, as observed in the Biacore binding experiment.

Example 5: Thermostability Study

[0096] Differential Scanning Fluorimetry (DSF) was performed on the elbow-insertion mutants to determine how the additional amino acids at the hinge region of heavy chain and light chain affected the thermostability of the CAT192 Fab insertion mutants. The basic principle of DSF is that as the temperature increases, a fluorescent dye binds to the hydrophobic regions of the protein as it unfolds providing an increase in signal. This method can be performed with limited sample and can be used to get relative stability of samples in a high throughput manner. Sypro orange was used as the fluorescent dye. The conditions used were 0.1 mg/mL protein, a 1:4000 dye ratio and a total volume of 10 μ L. The results showed that relative stability of the CAT192 Fab insertion mutants decreased slightly with the addition of glycines in the elbow, with the least stable mutants having the longest addition (Figure 7). The T_m values are summarized in Figure 7. The T_m values of some of the longer chain mutants were not calculated due to their unfolding pattern. The slight decrease was also observed when some of the light chain mutants were converted from Fab into IgG4 format (Figure 8).

Example 6: Crystal Structure Determination of CAT192 Fab Variants

[0097] The protein structures of CAT192 Fab WT and 3 variants were solved to provide the structural explanation as to how the high affinity was restored with the increased flexibility of the variable domains.

[0098] A 150 mL transfection was performed on the CAT192 HC and LC Fab insertion mutants to obtain enough material for the structure studies. Expi293F cells were transfected with 150 µg DNA (75 µg LC + 75 µg HC). Conditioned media was collected 5 days after the transfection. The CM was then purified using His-Trap Excel columns equilibrated with 20 mM NaPi pH 7.4, 500 mM NaCl, 5mM imidazole. Fab protein was eluted with 20 mM NaPi pH 7.4, 500 mM NaCl, 500 mM imidazole and immediately buffer exchanged into 20 mM HEPES pH 7.0, 50 mM NaCl using a size exclusion chromatography column (Superdex 200 10/300). The Fabs were then concentrated to 20 mg/mL and sparse matrix screens were set-up at both room temperature and 4°C. All crystals used for structure determination were obtained at 4°C in a 1:1 protein to crystallization condition ratio. Wild-type protein and the lower binding affinity mutants crystallized in a P21 space group in similar PEG conditions (WT: 12% PEG 8K/ 0.1 M sodium cacodylate pH 6/0.2 M MgCl₂, CAT192 WT HC/LC+G: 12% PEG 20K/ 0.1 M MES pH 6.5, CAT192 HC+GGGG/WT LC: 12% PEG 20K, 0.1 M MES pH 5.75). The high binding affinity mutant (HC+GG/LC+GG) crystallized in 2 M ammonium sulfate, 0.1 M sodium acetate pH 4.6 (space group: C2).

[0099] The low/moderate binding affinity variants and the wild type Fab structures (HC+WT/LC+G and HC+GGGG/LC+WT) were nearly identical (Figure 9, part A). WT HC/LC+G and HC+GGGG/WT LC superimposed with WT CAT192 with a R.M.S.D of 0.516Å and 0.538Å respectively. In each of these structures, the electron density for the CDRH3 region was missing for all molecules in the asymmetric unit. This implied that this CDR was highly flexible for the low/moderate binding affinity mutants. In contrast, the high binding affinity mutant (HC+GG/LC+GG) displayed large conformational changes in the variable domains compared to the other CAT192 Fab structures (Figure 9, part A). While the constant domains between all four Fabs superimposed nicely, the variable domains in CAT192 HC+GG/LC+GG shifted significantly compared to the other structures (Figure 9, part B). Furthermore, the HC CDR3 region was completely structured in the high binding affinity structure and was stabilized by interacting with the LC CDR3 (<3Å). These four structures, in accordance with the Biacore results, suggested that large conformational rearrangement was required to restore the high binding affinity of CAT192.

SEQUENCE LISTING

[0100]

SEQ ID No. 1: Human IgG1 VH domain Clone SL15 (SQN4 US6492497)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
GYDTPQYSWGQGTTTVTVSS

SEQ ID No. 2: Human IgG1 VH domain Clone JT182 (SQN10 US6492497)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
GYDTPASPDWGQGTTTVTVSS

SEQ ID No. 3: Human IgG1 Vk domain Clone SL15A: (SQN6 US6492497)

EIVLTQSPSSLSASVGDRVITTCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFSGSGSGTDFLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIK

SEQ ID No. 4: Human IgG1 Vk domain Clone SL15S: (SQN8 US6492497)

EIVLTQSPSSLSASVGDRVITTCRSSQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFSGSGSGTDFLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIK

SEQ ID No. 5: Human IgG1 Hinge Region PKSCDKTHTCPPCPAPELLGGP**SEQ ID No. 6: Human IgG1 Fc Region**

SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP
PVLDSGDSFFLYSKLTVDKSRWQQGNVSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID No. 7 SYGMH**SEQ ID No. 8 VISYDGSIKYYADSVKG****SEQ ID No. 9 TGEYSGYDTSGVEL****SEQ ID No. 10 TGEYSGYDTPQYS****SEQ ID No. 11 TGFYSGYDTPASPD****SEQ ID No. 12 RASQGIGDDLGL****SEQ ID No. 13 GTSTLQS****SEQ ID No. 14 LQDSNYPLT****SEQ ID No. 15 TGX₁YSGYDTX₂X₃X₄X₅X₆****SEQ ID No. 16: CAT192 (IgG4) Light Chain**

EWLTQSPSSLSASVGDRVITTCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFSGSGSGTDFLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C

SEQ ID No. 17: CAT192 (IgG4) Heavy Chain

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
GYDTPQYSWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYF
PEPVTWNSWGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVD
HKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSDQEDPEVFQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSR
WQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID No. 18: CAT192 (IgG4) S228P Heavy Chain

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
GYDTPQYSWGQGTITVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYF
DEQVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYTCNVD

FEFYIVSWNSUALISQVHHTFAVLQSSQLISESSVYIVFSSSELRITITCNVD
 HKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT
 CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ
 DWLNGKEYKCKVSNKGLPSSIEKTIISKAKGQPREPQVYTLPPSQEEMTKNQV
 SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSR
 WQEGNVFSCSVMEALHNHYTQKSLSLGLK

SEQ ID No. 19: CAT191 (scFv)

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
 SYDGSIKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARTGEYS
 GYDTPQYSWGQGTTVTVSSSGGGSGGGSGGGGSEIVLTQSPSSLSASVGD
 RVTITCRSSQGIGDDLGWYQQKPGKAPILLIYGTSTLQSGVPSRFSGSGSGTDF
 TLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIK

SEQ ID No. 20: Human TGFβ1

ALDTNYCFSSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYTW
 SLDTQYSKVLALYNQHNP GASAAPCCVPQALEPLIVYYVGRKPKVEQLSNM
 IVRSCKCS

SEQ ID No. 21: CAT192 IgG4 Wild-type LC Elbow Region LEIKRTVA**SEQ ID No. 22: Mutant LC Elbow Region with 1 Additional Amino Acid Inserted
LEIKGRTVA****SEQ ID No. 23: Mutant LC Elbow Region with 2 Additional Amino Acids Inserted
LEIKGGRTVA****SEQ ID No. 24: Mutant LC Elbow Region with 3 Additional Amino Acids Inserted
LEIKGGGRTVA****SEQ ID No. 25: Mutant LC Elbow Region with 4 Additional Amino Acids Inserted
LEIKGGGGSRTVA****SEQ ID No. 26: Mutant LC Elbow Region with 5 Additional Amino Acids Inserted
LEIKGGGGGSRTVA****SEQ ID No. 27: Coding sequence of CAT192 (IgG1) Light Chain**

atgggctggtcctgcatcatcctgtttctggtggccacagccaccggcgtgcacagcGAGATCGTGCTGACA
 CAGAGCCCCAGCAGCCTGTCTGCCAGCGTGGGCGACAGAGTGACCATCAC
 CTGTAGAGCCAGCCAGGGCATCGGCGACGACCTGGGATGGTATCAGCAGA
 AGCCTGGCAAGGCCCCCATCCTGCTGATCTACGGCACCAGCACACTGCAG
 AGCGGCGTGCCCTCCAGATTTTCTGGCAGCGGCTCCGGCACCGACTTCACC
 CTGACCATCAACAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGTCTG
 CAAGACAGCAACTACCCCTGACCTTCGGCGGAGGCACCCGGCTGGAAAT
 CAAGCGTACGGTGGCCGCTCCTTCCGTGTTTCATCTTCCCTCCCTCCGACGA
 GCAGCTGAAGTCCGGCACCGCCTCCGTGGTGTGTCTGCTGAACAACCTTCTA
 CCCTCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCG
 GCAACTCCCAGGAGTCCGTCACCGAGCAGGACTCCAAGGACAGCACCTAC
 TCCCTGTCTCCACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCACAA
 GGTGTACGCCTGTGAGGTGACCCACCAGGGCCTGTCCAGCCCTGTGACCA
 AGTCCTTCAACCGGGGCGAGTGCTGA

SEQ ID No. 28: CAT192LC+G (LEIKGRTVA), Forward 5'-ggctggaaatcaagggccgtacggtggccgc-3'**SEQ ID No. 29: CAT192LC+G (LEIKGRTVA), Complement 5'-gcggccaccgtacggcccttgattccagcc-3'**

SEQ	ID	No.	30:	CAT192LC+GG	(LEIKGGRTVA),	Forward	5'-
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ggctggaaatcaagggcgccgctacggtggccgc-3'

SEQ ID No. 31: CAT192LC+GG (LEIKGGRTVA), Complement 5'-
gcgccaccgtacggcgcccttgattccagcc-3'

SEQ ID No. 32: CAT192LC+GGS (LEIKGGSRTVA), Forward 5'-
ggctggaaatcaagggcgccgctacggtggccgc-3'

SEQ ID No. 33: CAT192LC+GGS (LEIKGGSRTVA), Complement 5'-
gcgccaccgtacggcgcccttgattccagcc-3'

SEQ ID No. 34: CAT192LC+GGGS (LEIKGGGSRRTVA), Forward 5'-
ggctggaaatcaagggcgccgctacggtggccgc-3'

SEQ ID No. 35: CAT192LC+GGGS (LEIKGGGSRRTVA), Complement 5'-
gcgccaccgtacggcgcccttgattccagcc-3'

SEQ ID No. 36: CAT192LC+GGGGS (LEIKGGGGSRRTVA), Forward 5'-
ggctggaaatcaagggcgccgctacggtggccgc-3'

SEQ ID No. 37: CAT192LC+GGGGS (LEIKGGGGSRRTVA), Complement 5'-
gcgccaccgtacggcgcccttgattccagcc-3'

SEQ ID No. 38: CAT192 IgG1 Wild-Type LC

EIVLTQSPSSLSASVGDRVTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFGSGSGTDFTLTINSIQPEDFATYYCLQDSNYPLTFGGGTRLEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C

SEQ ID No. 39: Mutant Light Chain with 1 Additional Amino Acid Inserted

EIVLTQSPSSLSASVGDRVTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFGSGSGTDFTLTINSIQPEDFATYYCLQDSNYPLTFGGGTRLEIKG
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG
EC

SEQ ID No. 40: Mutant Light Chain with 2 Additional Amino Acids Inserted

EIVLTQSPSSLSASVGDRVTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFGSGSGTDFTLTINSIQPEDFATYYCLQDSNYPLTFGGGTRLEIKG
GRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGN
SQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

SEQ ID No. 41: Mutant Light Chain with 3 Additional Amino Acids Inserted

EIVLTQSPSSLSASVGDRVTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFGSGSGTDFTLTINSIQPEDFATYYCLQDSNYPLTFGGGTRLEIKG
GSRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSG
NSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGE

SEQ ID No. 42: Mutant Light Chain with 4 Additional Amino Acids Inserted

EIVLTQSPSSLSASVGDRVTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
QSGVPSRFGSGSGTDFTLTINSIQPEDFATYYCLQDSNYPLTFGGGTRLEIKG
GGSRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS
GNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGE

SEQ ID No. 43: Mutant Light Chain with 5 Additional Amino Acids Inserted

EIVLTQSPSSLSASVGDRTTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL
 QSGVPSRFSGSGSGTDFLTINSIQPEDFATYYCLQDSNYPLTFGGGTRLEIKG
GGGSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
 SGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
 FNRGEC

SEQ ID No. 44: CAT192 IgG4 Wild-Type HC Elbow Region TVTVSSAS**SEQ ID No. 45: Mutant HC Elbow Region with 1 Additional Amino Acid Inserted**

TVTVSGSAS

SEQ ID No. 46: Mutant HC Elbow Region with 2 Additional Amino Acids Inserted

TVTVSGGSAS

SEQ ID No. 47: Mutant HC Elbow Region with 2 Additional Amino Acids Inserted and one Amino Acid Deleted

TVTVSGGSA

SEQ ID No. 48: Mutant HC Elbow Region with 5 Additional Amino Acids InsertedTVTVSGGGGSSAS**SEQ ID No. 49: Coding Sequence of CAT192 IgG4 Wild-Type HC**

ATGGGCTGGTCCTGCATCCTGTTTCTGGTGCCACCGCCACCGGCGTG
 CACTCTGAAGTGCAGCTGGTGGAATCTGGCGGCGGAGTGGTGCAGCCTGG
 CAGAAGCCTGAGACTGAGCTGTGCCGCCAGCGGCTTCACCTTCAGCAGCT
 ACGGAATGCACTGGGTGCGCCAGGCCCTGGCAAAGAACTGGAATGGGT
 GGCCGTGATCAGCTACGACGGCAGCATCAAGTACTACGCCGACAGCGTGA
 AGGGCCGGTTTACCATCTCCCGGACAAACAGCAAGAACACCCCTGTACCTG
 CAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGTGCTAG
 AACCGGCGAGTACAGCGGCTACGACACCGACCCCTCAGTACTCTTGGGGCC
 AGGGCACCAACCGTGACAGTGTCTAGCGCCAGCACCAAGGGCCCAAGCGTG
 TTCCCTCTGGCCCCCTTGACGACAGAAGCACCAGCGAATCTACAGCCGCCCT
 GGGCTGCCTCGTGAAGGACTACTTTCCCGAGCCCGTGACAGTGTCTGGA
 ACTCTGGCGCCCTGACCAGCGGAGTGCATACCTTTCCAGCCGTGCTGCAG
 AGCAGCGGCCTGTACTCTCTGAGCAGCGTCTGACTGTGCCAGCAGCTC

TCTGGGCACCAAGACCTACACCTGTAACGTGGACCACAAGCCCAGCAACA
 CCAAGGTGGACAAGAGAGTGCATCACCACCACCATCAC

SEQ ID No. 50: CAT192HC+G (TVTVSGSAS), Forward 5'-ccaccgtgacagtgtctggcagcgccagc-3'**SEQ ID No. 51: CAT192HC+G (TVTVSGSAS), Complement 5'-gctggcgctgccagacactgtcacgggtgg-3'**

SEQ ID No. 52: CAT192HC+GG-ST (TVTVSGGSA), Forward 5'-
 ccaccgtgacagtgtctggcggcagcgccagc-3'

SEQ ID No. 53: CAT192HC+GG-ST (TVTVSGGSA), Complement 5'-
 gctggcgctgccgagacactgtcacgggtgg-3'

SEQ ID No. 54: CAT192HC+GGGS (TVTVSGGGGSSAS), Forward 5'-
 caccaccgtgacagtgtctggcggcggcggcagcgccagca-3'

SEQ ID No. 55: CAT192HC+GGGS (TVTVSGGGGSSAS), Complement 5'-
 tctggcgctgtgccgcccagacactgtcacgggtgg-3'

SEQ ID No. 56: Mutant Heavy Chain with 1 Additional Amino Acid Inserted

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
 GYDTPQYSWGQGTTVTVSGSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD
 YFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
 NHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
 TPETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVL
 TVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
 VKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

SEQ ID No. 57: Mutant Heavy Chain with 2 Additional Amino Acids Inserted and 2 Amino Acids Deleted

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
 GYDTPQYSWGQGTTVTVSGSAKGPSVFPLAPSSKSTSGGTAALGCLVKDY
 FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNV
 HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT

ETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
 VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
 VKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

SEQ ID No. 58: Mutant Heavy Chain with 5 Additional Amino Acids Inserted

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
 GYDTPQYSWGQGTTVTVSGGGGSSASTKGPSVFPLAPSSKSTSGGTAALGC
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
 TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
 LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
 VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
 SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSPGK

SEQ ID No. 59: CAT192HC+GG (TVTVSGGSAS), Forward 5'-
 caccaccgtgacagtgtctgcccgcagccagca-3'

SEQ ID No. 60: CAT192HC+GG (TVTVSGGSAS), Complement 5'-
 tgctggcgctgccgcagacactgtcacgggtgtg-3'

SEQ ID No. 61: Mutant Heavy Chain with 2 Additional Amino Acids Inserted

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS
 GYDTPQYSWGQGTTVTVSGSASTKGPSVFPLAPSSKSTSGGTAALGCLVK
 DYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
 VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
 RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
 LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL
 TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT
 VKSRWQQGNV FSCSV MHEALHNHYTQKSLSLSP

SEQUENCE LISTING

[0101]

<110> Qiu, Huawei Bird, Julie

<120> MODIFIED-IgG ANTIBODIES THAT BIND TRANSFORMING GROWTH FACTOR-Betal WITH HIGH AFFINITY, AVIDITY AND SPECIFICITY

<130> 578941

<150> 62/128,149 <151> 2015-03-04

<160> 61

<170> PatentIn version 3.5

<210> 1

<211> 123

<212> PRT

<213> Homo sapiens

<400> 1

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
          20           25           30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
          35           40           45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
          50           55           60

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

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
          100          105          110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
          115          120

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<210> 2

<211> 123

<212> PRT

<213> Homo sapiens

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
50 55 60

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

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Pro Ala Ser Pro Asp
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 3

<211> 107

<212> PRT

<213> Homo sapiens

<400> 3

Glu Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly Asp Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Ile Leu Leu Ile
35 40 45

Tyr Gly Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Ser Asn Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys
100 105

<210> 4

<211> 107

<212> PRT

<213> Homo sapiens

<400> 4

Glu Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ser Ser Gln Gly Ile Gly Asp Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Ile Leu Leu Ile
35 40 45

Tyr Gly Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Ser Asn Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys
100 105

<210> 5

<211> 22

<212> PRT

<213> Homo sapiens

<400> 5

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
1 5 10 15

Glu Leu Leu Gly Gly Pro
20

<210> 6

<211> 209

<212> PRT

<213> Homo sapiens

<400> 6

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
1 5 10 15

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
20 25 30

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
35 40 45

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
50 55 60

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
65 70 75 80

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
85 90 95

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 100 105 110

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 115 120 125

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 130 135 140

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 145 150 155 160

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 165 170 175

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 180 185 190

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 195 200 205

Lys

<210> 7

<211> 5

<212> PRT

<213> Homo sapiens

<400> 7

Ser Tyr Gly Met His
 1 5

<210> 8

<211> 17

<212> PRT

<213> Homo sapiens

<400> 8

Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

<210> 9

<211> 14

<212> PRT

<213> Homo sapiens

<400> 9

Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Ser Gly Val Glu Leu
 1 5 10

<210> 10

<211> 14

<212> PRT

<213> Homo sapiens

<400> 10

Thr	Gly	Glu	Tyr	Ser	Gly	Tyr	Asp	Thr	Asp	Pro	Gln	Tyr	Ser
1				5					10				

<210> 11

<211> 14

<212> PRT

<213> Homo sapiens

<400> 11

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

<210> 12

<211> 11

<212> PRT

<213> Homo sapiens

<400> 12

Arg	Ala	Ser	Gln	Gly	Ile	Gly	Asp	Asp	Leu	Gly
1				5					10	

<210> 13

<211> 7

<212> PRT

<213> Homo sapiens

<400> 13

Gly	Thr	Ser	Thr	Leu	Gln	Ser
1				5		

<210> 14

<211> 9

<212> PRT

<213> Homo sapiens

<400> 14

Leu	Gln	Asp	Ser	Asn	Tyr	Pro	Leu	Thr
1				5				

<210> 15

<211> 14

<212> PRT

<213> Homo sapiens

<220>

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<223> Xaa can be any naturally occurring amino acid

<220>

<221> misc_feature

<222> (10)..(14)

<223> Xaa can be any naturally occurring amino acid

<400> 15

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

<210> 16

<211> 213

<212> PRT

<213> Homo sapiens

<400> 16

Glu	Trp	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp
1				5					10					15	

Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Gly	Ile	Gly	Asp	Asp	Leu
			20					25					30		

Gly	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Ile	Leu	Leu	Ile	Tyr
		35					40					45			

Gly	Thr	Ser	Thr	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	Ser
	50					55					60				

Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Asn	Ser	Leu	Gln	Pro	Glu
65					70					75					80

Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Leu	Gln	Asp	Ser	Asn	Tyr	Pro	Leu	Thr
				85					90					95	

Phe	Gly	Gly	Gly	Thr	Arg	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> 17

<211> 450

<212> PRT

<213> Homo sapiens

<400> 17

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20           25           30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
35           40           45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
50           55           60

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

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
100          105          110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
115          120          125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
130          135          140

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

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

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
180          185          190

Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val
195          200          205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys
210          215          220

Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly
225          230          235          240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245          250          255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
260          265          270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275          280          285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg

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      290      295      300
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305      310      315      320
Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
      325      330      335
Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
      340      345      350
Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
      355      360      365
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
      370      375      380
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385      390      395      400
Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp
      405      410      415
Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
      420      425      430
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
      435      440      445
Gly Lys
450
<210> 18
<211> 450
<212> PRT
<213> Homo sapiens

<400> 18
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1      5      10      15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20      25      30
Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
35      40      45
Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
50      55      60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65      70      75      80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85      90      95

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Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
 100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
 130 135 140

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

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

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 180 185 190

Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val
 195 200 205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys
 210 215 220

Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Leu Gly Gly
 225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
 245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu
 260 265 270

Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
 275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg
 290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
 305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu
 325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
 340 345 350

Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu
 355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
 370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
 385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp

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      405      410      415
Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His
      420      425      430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu
      435      440      445

Gly Lys
      450

<210> 19
<211> 245
<212> PRT
<213> Homo sapiens

<400> 19
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1          5          10          15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
      20          25          30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
      35          40          45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
      50          55          60

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

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
      100          105          110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ser Gly Gly Gly Ser
      115          120          125

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Ile Val Leu Thr Gln
      130          135          140

Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr
      145          150          155          160

Cys Arg Ser Ser Gln Gly Ile Gly Asp Asp Leu Gly Trp Tyr Gln Gln
      165          170          175

Lys Pro Gly Lys Ala Pro Ile Leu Leu Ile Tyr Gly Thr Ser Thr Leu
      180          185          190

Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
      195          200          205

Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr
      210          215          220

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Tyr	Cys	Leu	Gln	Asp	Ser	Asn	Tyr	Pro	Leu	Thr	Phe	Gly	Gly	Gly	Thr
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Arg Leu Glu Ile Lys
245

<210> 20

$\langle 211 \rangle$ 112

<212> PRT

<213> Homo sapiens

<400> 20

Ala Leu Asp Thr Asn Tyr Cys Phe Ser Ser Thr Glu Lys Asn Cys Cys
1 5 10 15

Val Arg Gln Leu Tyr Ile Asp Phe Arg Lys Asp Leu Gly Trp Lys Trp
20 25 30

Ile His Glu Pro Lys Gly Tyr His Ala Asn Phe Cys Leu Gly Pro Cys
35 40 45

Pro Tyr Ile Trp Ser Leu Asp Thr Gln Tyr Ser Lys Val Leu Ala Leu
50 55 60

Tyr Asn Gln His Asn Pro Gly Ala Ser Ala Ala Pro Cys Cys Val Pro
65 70 75 80

Gln Ala Leu Glu Pro Leu Pro Ile Val Tyr Tyr Val Gly Arg Lys Pro
85 90 95

Lys Val Glu Gln Leu Ser Asn Met Ile Val Arg Ser Cys Lys Cys Ser
100 105 110

<210> 21

$\langle 211 \rangle$ 8

<212> PRT

<213> Homo sapiens

<400> 21

Leu Glu Ile Lys Arg Thr Val Ala
1 5

<210> 22

<211> 9

<212> PRT

<213> Homo sapiens

<400> 22

Leu Glu Ile Lys Gly Arg Thr Val Ala
1 5

<210> 23

$\langle 211 \rangle$ 10

<212> PRT

<213> Homo sapiens

<400> 23

Leu Glu Ile Lys Gly Gly Arg Thr Val Ala
 1 5 10

<210> 24

<211> 11

<212> PRT

<213> Homo sapiens

<400> 24

Leu Glu Ile Lys Gly Gly Ser Arg Thr Val Ala
 1 5 10

<210> 25

<211> 12

<212> PRT

<213> Homo sapiens

<400> 25

Leu Glu Ile Lys Gly Gly Gly Ser Arg Thr Val Ala
 1 5 10

<210> 26

<211> 13

<212> PRT

<213> Homo sapiens

<400> 26

Leu Glu Ile Lys Gly Gly Gly Gly Ser Arg Thr Val Ala
 1 5 10

<210> 27

<211> 702

<212> DNA

<213> Homo sapiens

<400> 27

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atcgtgctga cacagagccc cagcagcctg tctgccagcg tgggcgacag agtgaccatc	120
acctgtagag ccagccaggg catcggcgac gacctgggat ggtatcagca gaagcctggc	180
aaggccccc tctgctgat ctacggcacc agcacactgc agagcggcgt gccctccaga	240
ttttctggca gcggtccgg caccgacttc accctgacca tcaacagcct gcagcccgag	300
gacttcgcca cctactactg tctgcaagac agcaactacc ccctgacctt cggcggaggc	360
acccggctgg aaatcaagcg tacgggtggc gtccttccg tgttcattctt ccctccctcc	420
gacgagcagc tgaagtccgg caccgcctcc gtggtgtgtc tgctgaacaa cttctaccct	480
cgggaggcca aggtgcagtg gaaggtggac aacgccctgc agtccggcaa ctcccaggag	540
tccgtcaccg agcaggactc caaggacagc acctactccc tgtcctccac cctgacctg	600
tccaaggccg actacgagaa gcacaagggtg tacgcctgtg aggtgacctc ccagggcctg	660
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			20					25					30		

Leu	Gly	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Ile	Leu	Leu	Ile
		35					40					45			

Tyr	Gly	Thr	Ser	Thr	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
	50					55					60				

Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Asn	Ser	Leu	Gln	Pro
65					70					75				80	

Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Leu	Gln	Asp	Ser	Asn	Tyr	Pro	Leu
				85					90					95	

Thr Phe Gly Gly Gly Thr Arg 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
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Phe Asn Arg Gly Glu Cys
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<211> 215

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<213> Homo sapiens

<400> 39

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly Asp Asp
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Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Ile Leu Leu Ile
35 40 45

Tyr Gly Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Ser Asn Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys Gly Arg Thr Val Ala
100 105 110

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

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

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

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

Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val
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Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys
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Ser Phe Asn Arg Gly Glu Cys
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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly Asp Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Ile Leu Leu Ile
35 40 45

Tyr Gly Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Ser Asn Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys Gly Gly Arg Thr Val
100 105 110

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

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

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

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

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

Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr

195 200 205

Lys Ser Phe Asn Arg Gly Glu Cys
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35 40 45
Tyr Gly Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Ser Asn Tyr Pro Leu
85 90 95
Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys Gly Gly Ser 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
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<210> 42
<211> 218
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<213> Homo sapiens

<400> 42

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly Asp Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Ile Leu Leu Ile
35 40 45

Tyr Gly Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Ser Asn Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys Gly Gly Gly Ser 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> 43

<211> 219

<212> PRT

<213> Homo sapiens

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Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Gly Asp Asp
20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Ile Leu Leu Ile
35 40 45

Tyr Gly Thr Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Asp Ser Asn Tyr Pro Leu
 85 90 95
 Thr Phe Gly Gly Gly Thr Arg Leu Glu Ile Lys Gly Gly Gly Gly Ser
 100 105 110
 Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 130 135 140
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
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<212> PRT

<213> Homo sapiens

<400> 44

Thr Val Thr Val Ser Ser Ala Ser
 1 5

<210> 45

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<212> PRT

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<400> 45

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<400> 46

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<210> 47

<211> 9

<212> PRT

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<400> 47

Thr Val Thr Val Ser Gly Gly Ser Ala
 1 5

<210> 48

<211> 13

<212> PRT

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<211> 738

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<213> Homo sapiens

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tgtgccgccca gcggcttcac cttcagcagc tacggaatgc actgggtgcg ccaggcccct	180
ggcaaagaac tggaatgggt ggccgtgatc agctacgacg gcagcatcaa gtactacgcc	240
gacagcgtga agggcccggt caccatctcc cgggacaaca gcaagaacac cctgtacctg	300
cagatgaaca gcctgcgggc cgaggacacc gccgtgtact actgtgctag aaccggcgag	360
tacagcggct acgacaccga ccctcagtac tcttggggcc agggcaccac cgtgacagtg	420
tctagcgcca gcaccaaggg cccaagcgtg ttccctctgg cccttgagcagaagaagcacc	480
agcgaatcta cagccgcctt gggctgcctc gtgaaggact actttcccga gcccgtagaca	540
gtgtcctgga actctggcgc cctgaccagc ggagtgcata cctttccagc cgtgctgcag	600
agcagcggcc tgtactctct gacgagcgtc gtgactgtgc ccagcagctc tctgggcacc	660
aagacctaca cctgtaacgt ggaccacaag cccagcaaca ccaagggtgga caagagagtg	720
catcaccacc accatcac	738

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<211> 29

<212> DNA

<213> Homo sapiens

<400> 51

gctggcgctg ccagacactg tcacggtgg 29

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<211> 32

<212> DNA

<213> Homo sapiens

<400> 52

ccaccgtgac agtgtctggc ggcagcgcca gc 32

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<211> 32

<212> DNA

<213> Homo sapiens

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<210> 54

<211> 44

<212> DNA

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<210> 55

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<400> 55

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<210> 56

<211> 454

<212> PRT

<213> Homo sapiens

<400> 56

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Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr
			20					25					30		

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
 35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
 50 55 60

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

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
 100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Gly Ser Ala Ser Thr Lys
 115 120 125

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

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

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

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
 180 185 190

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

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

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
 225 230 235 240

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 245 250 255

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 260 265 270

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 305 310 315 320

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 325 330 335

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu

Ala Phe Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Phe Arg Glu
340 345 350

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
385 390 395 400

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
405 410 415

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
420 425 430

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
435 440 445

Ser Leu Ser Pro Gly Lys
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<210> 57

<211> 453

<212> PRT

<213> Homo sapiens

<400> 57

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
50 55 60

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

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Gly Gly Ser Ala Lys Gly
115 120 125

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

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

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

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 180 185 190

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

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

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
 225 230 235 240

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
 245 250 255

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
 260 265 270

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
 275 280 285

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
 290 295 300

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
 305 310 315 320

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
 325 330 335

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
 340 345 350

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
 355 360 365

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
 370 375 380

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
 385 390 395 400

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
 405 410 415

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
 420 425 430

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
 435 440 445

Leu Ser Pro Glu Lys

Leu Ser His Gly Lys
450

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<211> 458

<212> PRT

<213> Homo sapiens

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Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
50 55 60

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

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Gly Gly Gly Gly Ser Ser
115 120 125

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

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
145 150 155 160

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

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
180 185 190

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
195 200 205

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
210 215 220

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
225 230 235 240

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
245 250 255

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
260 265 270

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 275 280 285
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 290 295 300
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 305 310 315 320
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 325 330 335
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 340 345 350
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
 355 360 365
 Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 370 375 380
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 385 390 395 400
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 405 410 415
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 420 425 430
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 435 440 445
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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<210> 59

<211> 35

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<212> DNA

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<210> 61

<211> 455

<212> PRT

<213> Homo sapiens

<400> 61

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20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Glu Leu Glu Trp Val
35 40 45

Ala Val Ile Ser Tyr Asp Gly Ser Ile Lys Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

65 70 75 80

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

Ala Arg Thr Gly Glu Tyr Ser Gly Tyr Asp Thr Asp Pro Gln Tyr Ser
100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Gly Gly Ser Ala Ser Thr
115 120 125

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

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

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

Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
180 185 190

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

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

Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
225 230 235 240

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
245 250 255

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
260 265 270

Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
275 280 285

Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 290 295 300
 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 305 310 315 320
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 325 330 335
 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 340 345 350
 Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
 355 360 365
 Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 370 375 380
 Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 385 390 395 400
 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 405 410 415
 Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 420 425 430
 Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 435 440 445
 Leu Ser Leu Ser Pro Gly Lys
 450 455

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Patentkrav

1. Isoleret bindingsprotein, der binder TGF β 1, hvor det isolerede bindingsprotein er et IgG, der omfatter en første
5 polypeptidkæde, en anden polypeptidkæde, en tredje polypeptidkæde og en fjerde polypeptidkæde, der er kendetegnet ved, at enten
- (a) en linker er tilføjet til den første og anden polypeptidkæde, som hver herefter har formlen fra N-terminalen til C-terminalen:
10 (VL-domæne)-(linker1)-(CL-domæne), eller
- (b) en linker er tilføjet til den tredje og fjerde polypeptidkæde, som hver herefter har formlen fra N-terminalen til C-terminalen: (VH-domæne)-(linker2)-(CH1-domæne)-(hængsel)-
(Fc-region), eller
- 15 (c) både (a) og (b);
hvor:
- (i) VL-domænet af hver af den første og anden polypeptidkæde omfatter aminosyresekvensen ifølge SEQ ID NO: 3 eller SEQ ID NO: 4; og
- 20 (ii) VH-domænet af hver af den tredje og fjerde polypeptidkæde omfatter aminosyresekvensen ifølge SEQ ID NO: 1 eller SEQ ID NO: 2; og
- hvor linker1, når den er til stede, og linker2, når den er til stede, omfatter aminosyreresten eller aminosyresekvensen valgt
25 fra listen, der består af G, GG, GGS, GGGS og GGGGS.
2. Isoleret bindingsprotein ifølge krav 1, hvor IgG er et humant IgG4.
- 30 3. Isoleret bindingsprotein ifølge krav 2, hvor IgG4 omfatter en S228P-mutation (EU-nummerering).
4. Isoleret bindingsprotein ifølge krav 1, hvor IgG er en human IgG1-isotype.
- 35 5. Isoleret bindingsprotein ifølge krav 1, hvor hver af den første og anden polypeptidkæde uafhængigt omfatter aminosyresekvensen ifølge SEQ ID NO: 16, 38, 39, 40, 41, 42

eller 43.

6. Isoleret bindingsprotein ifølge et hvilket som helst af kravene 1-3 og 5, hvor hver af den tredje og fjerde polypeptidkæde uafhængigt omfatter aminosyresekvensen ifølge SEQ ID NO: 17, 18, 56, 57, 58 eller 61.

7. Farmaceutisk sammensætning, som omfatter det isolerede bindingsprotein ifølge et hvilket som helst af kravene 1-6 og et farmaceutisk acceptabelt hjælpestof.

8. Isoleret bindingsprotein ifølge et hvilket som helst af kravene 1-6 eller farmaceutisk sammensætning ifølge krav 7 til anvendelse til behandling af en sygdom eller tilstand, der direkte eller indirekte skyldes TGF β 1-aktivitet, hvor sygdommen er

- (i) en fibrosesygdom, cancer eller en immunmedieret sygdom;
- (ii) knogleremodelleringssygdom;
- (iii) nyresygdom; eller
- (iv) diffus kutan systemisk sklerose.

9. Isoleret polynukleotid, som omfatter en nukleotidsekvens, der koder for det isolerede bindingsprotein ifølge et hvilket som helst af kravene 1-6.

25

10. Vektor, som omfatter polynukleotidet ifølge krav 9.

11. Værtscelle, som omfatter vektoren ifølge krav 10.

30 12. Fremgangsmåde til fremstilling af det isolerede bindingsprotein ifølge et hvilket som helst af kravene 1-6, som omfatter dyrkning af værtscellen ifølge krav 11 under egnede betingelser til produktion af bindingsproteinet.

DRAWINGS

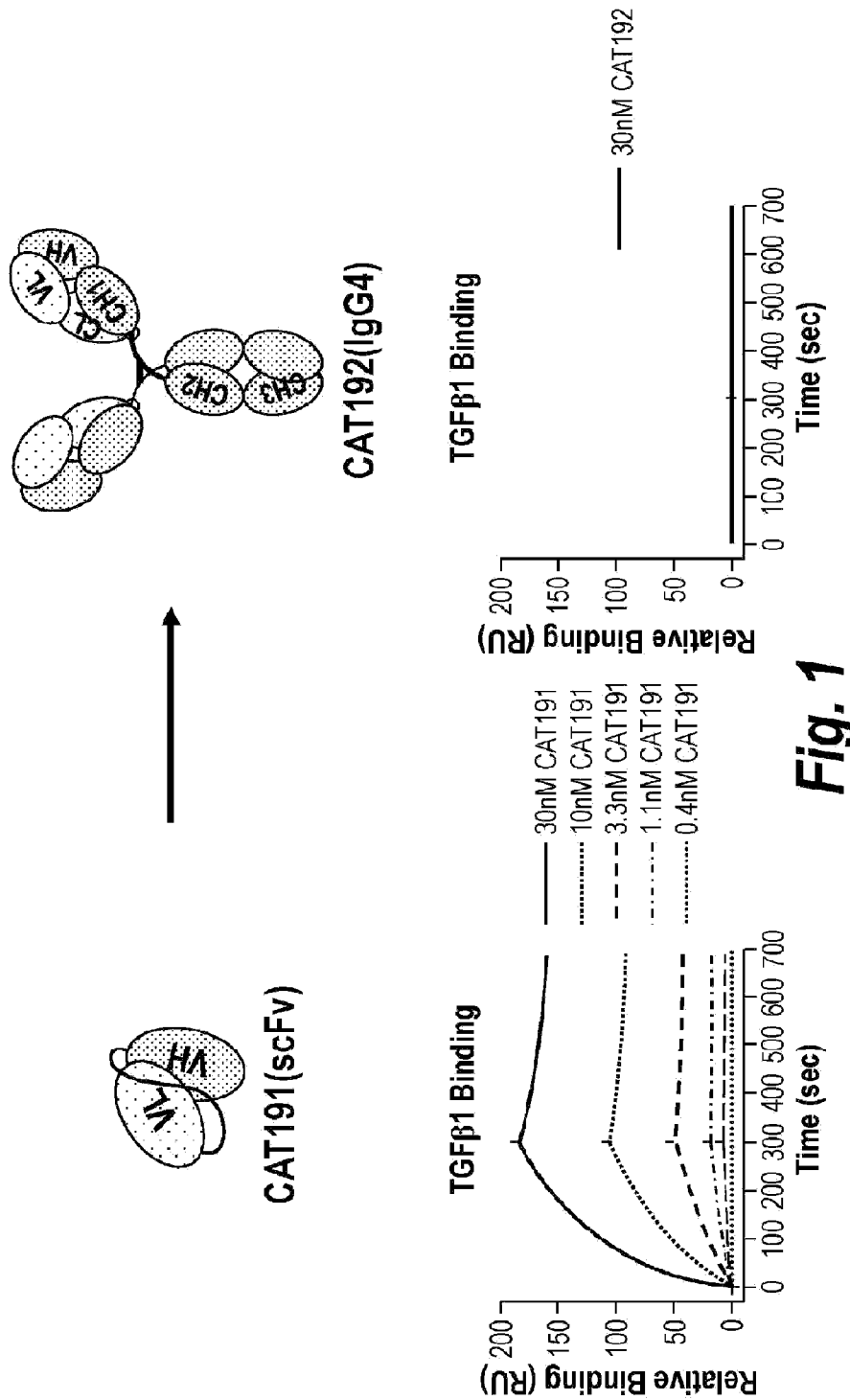
Comparison of TGF β 1 Affinity between scFv (CAT191) and IgG4 (CAT192)

Fig. 1

Structural Elements of scFv, Fab, and IgG Comparisons

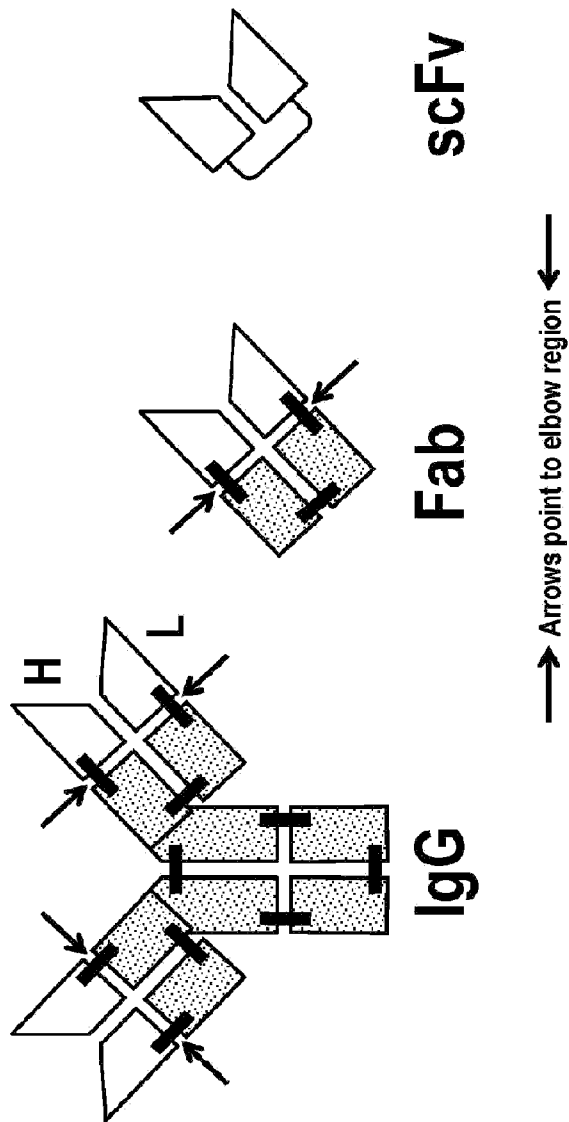
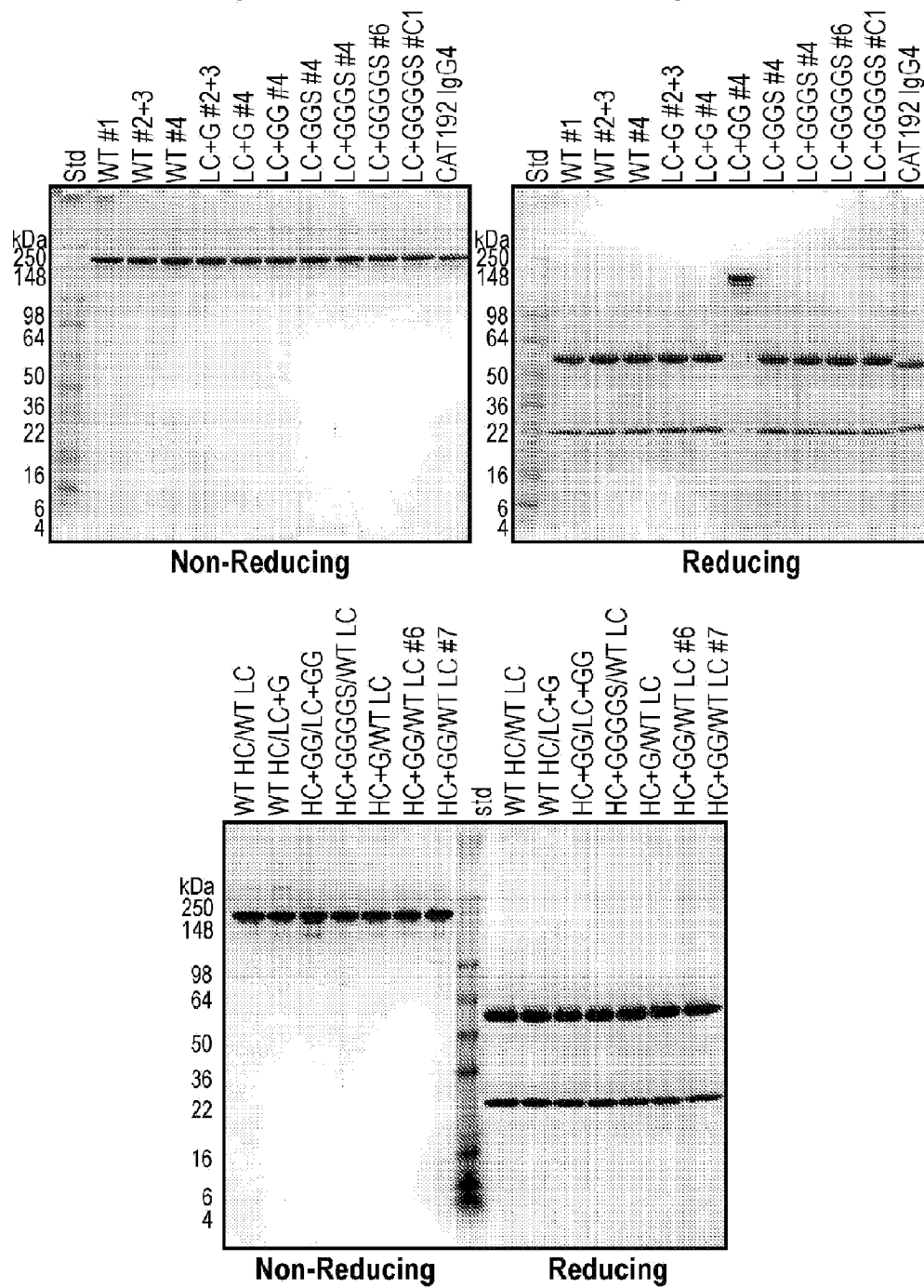
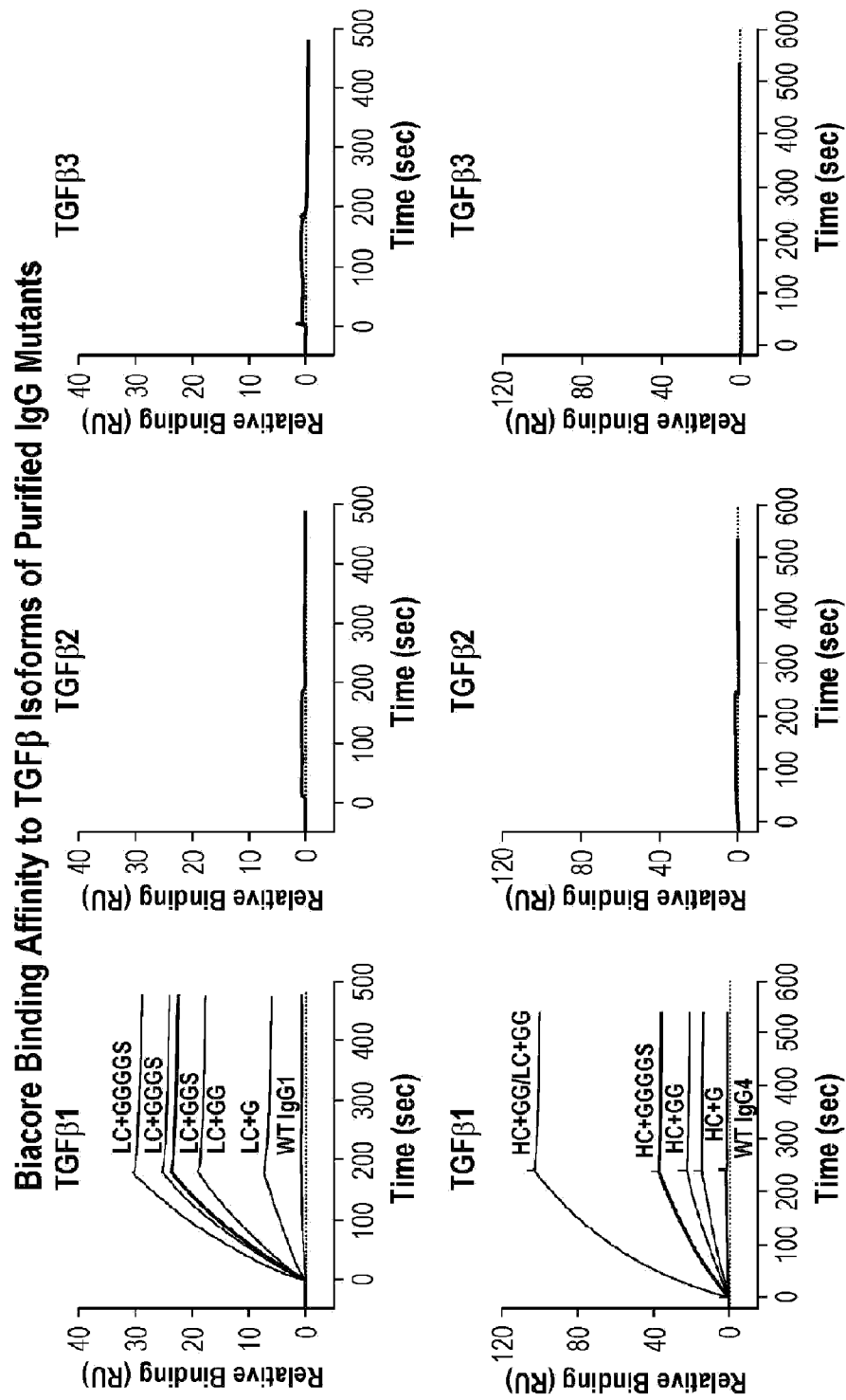


Fig. 2

SDS Page Gel Electrophoresis of Purified IgG Mutants

**Fig. 3**

**Fig. 4**

A549 Cell Potency Assay of Purified IgG Mutants

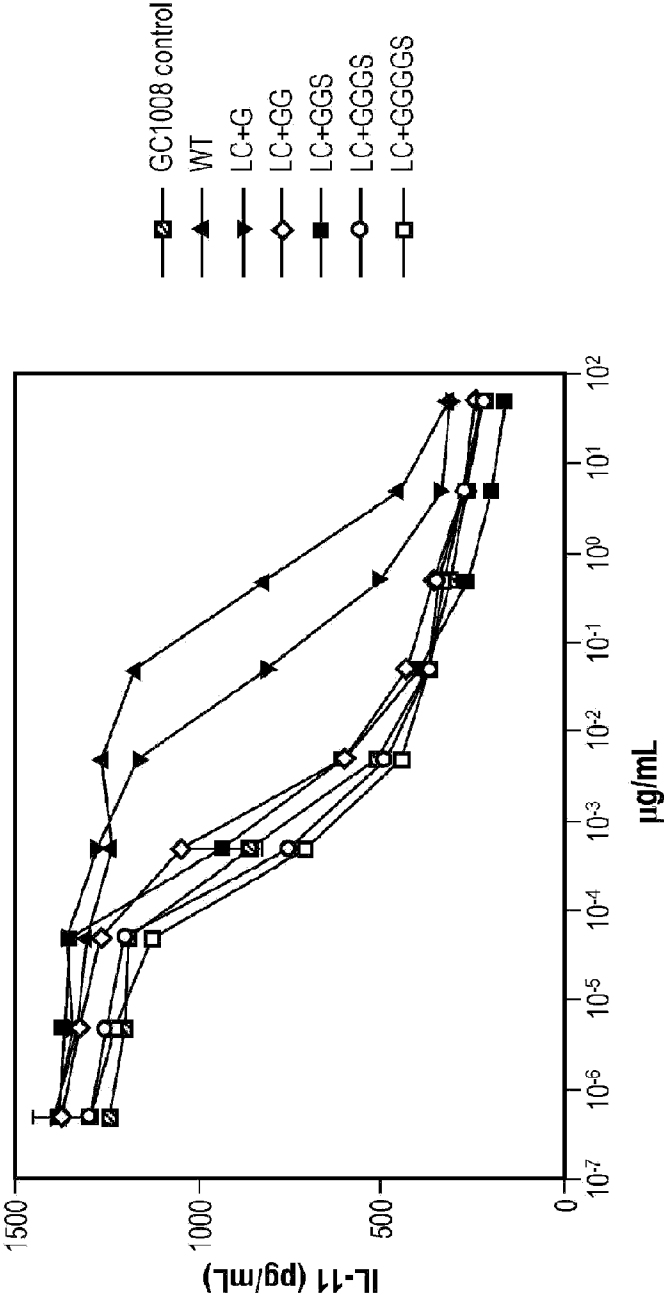


Fig. 5

Biacore TGFβ1 Binding Assay Results of Purified Fab Variants

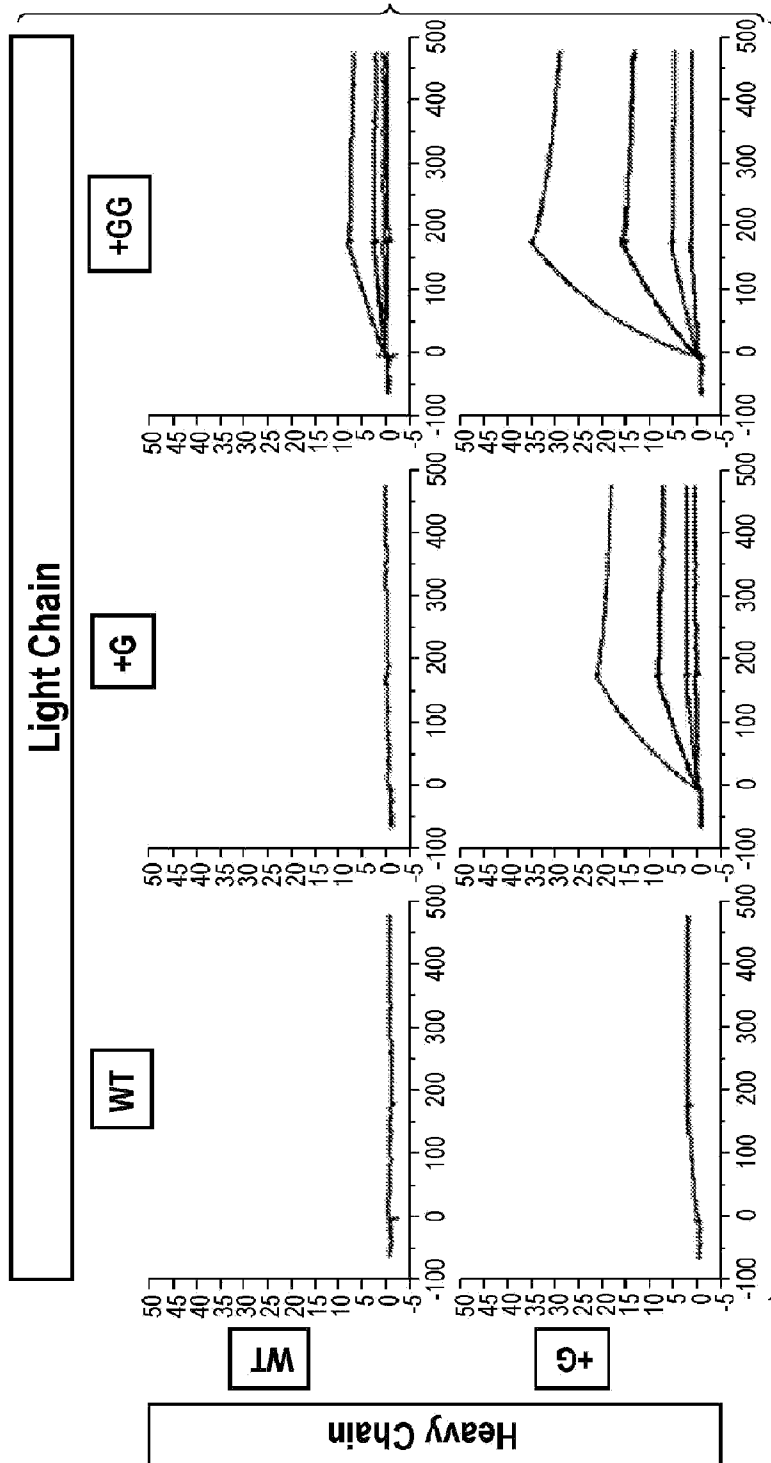


Fig. 6

Continue to Sheet 8 of 17

Continue to Sheet 7 of 17

Biacore TGFβ1 Binding Assay Results of Purified Fab Variants

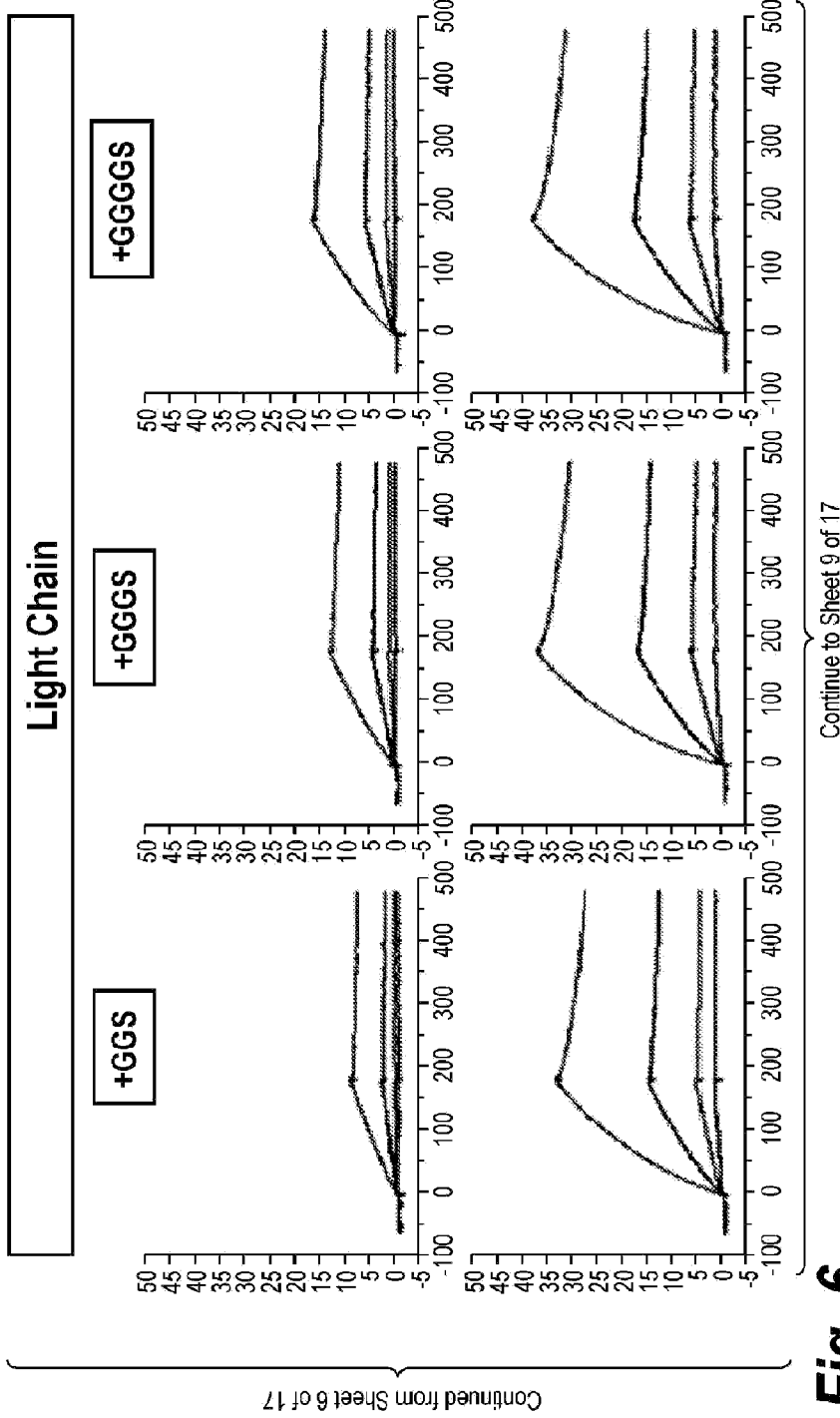


Fig. 6
(Continued)

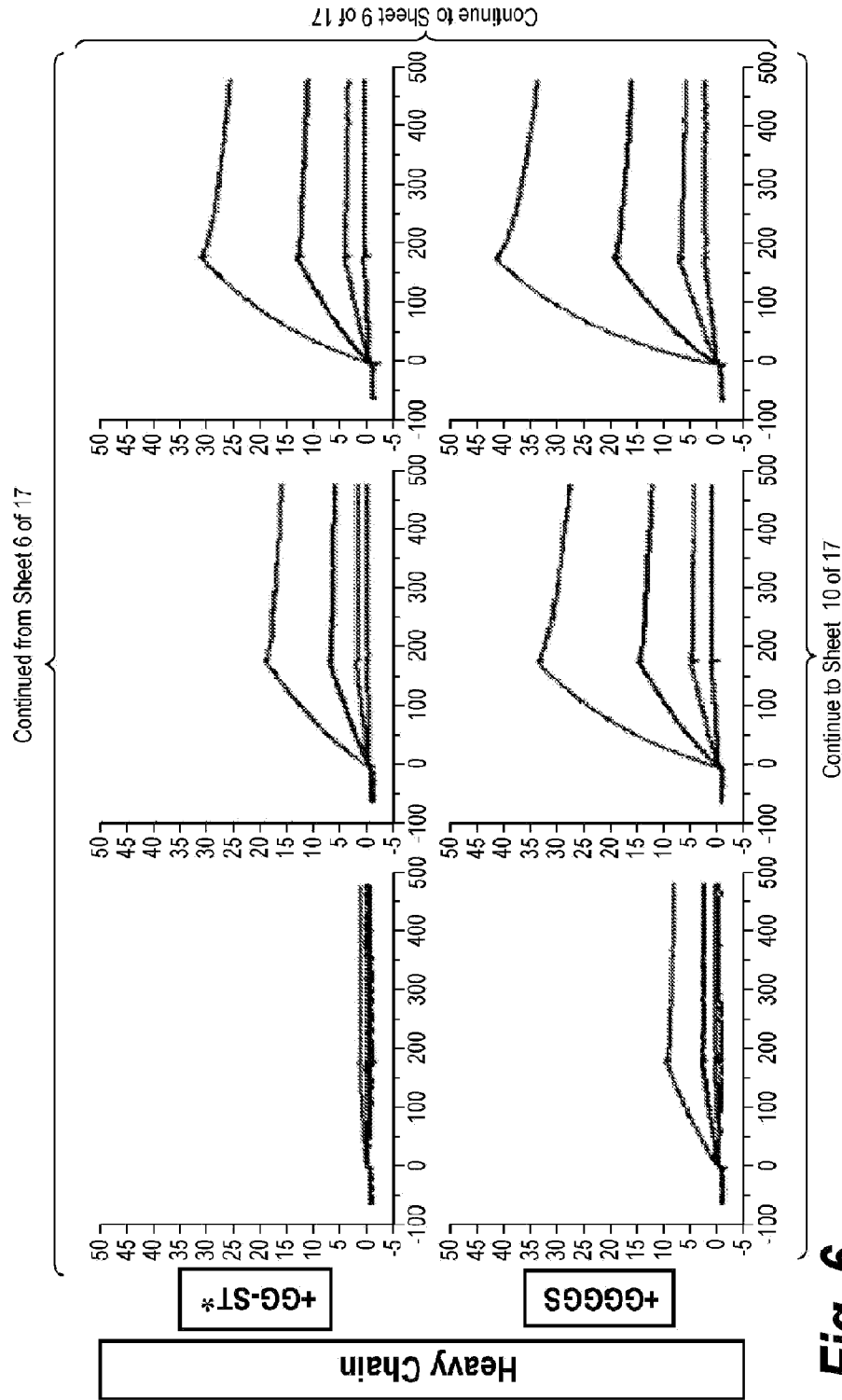
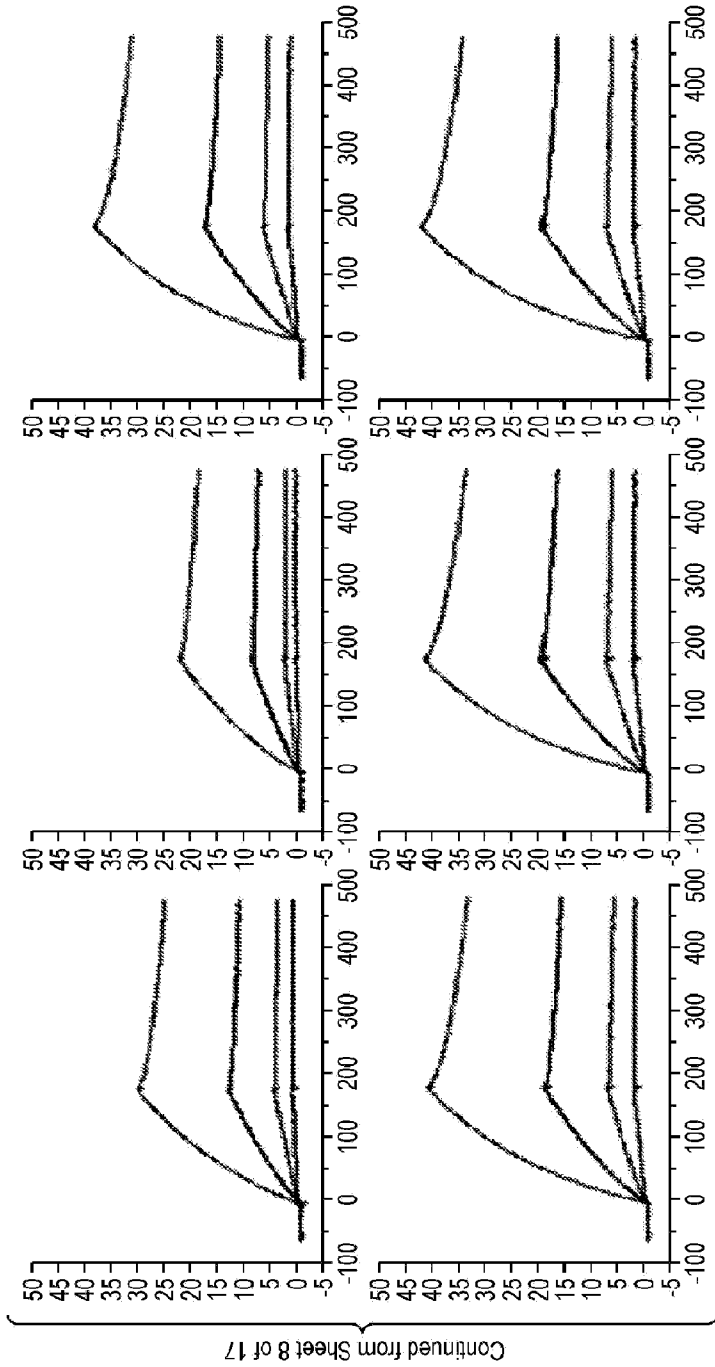


Fig. 6
(Continued)

Continued from Sheet 7 of 17

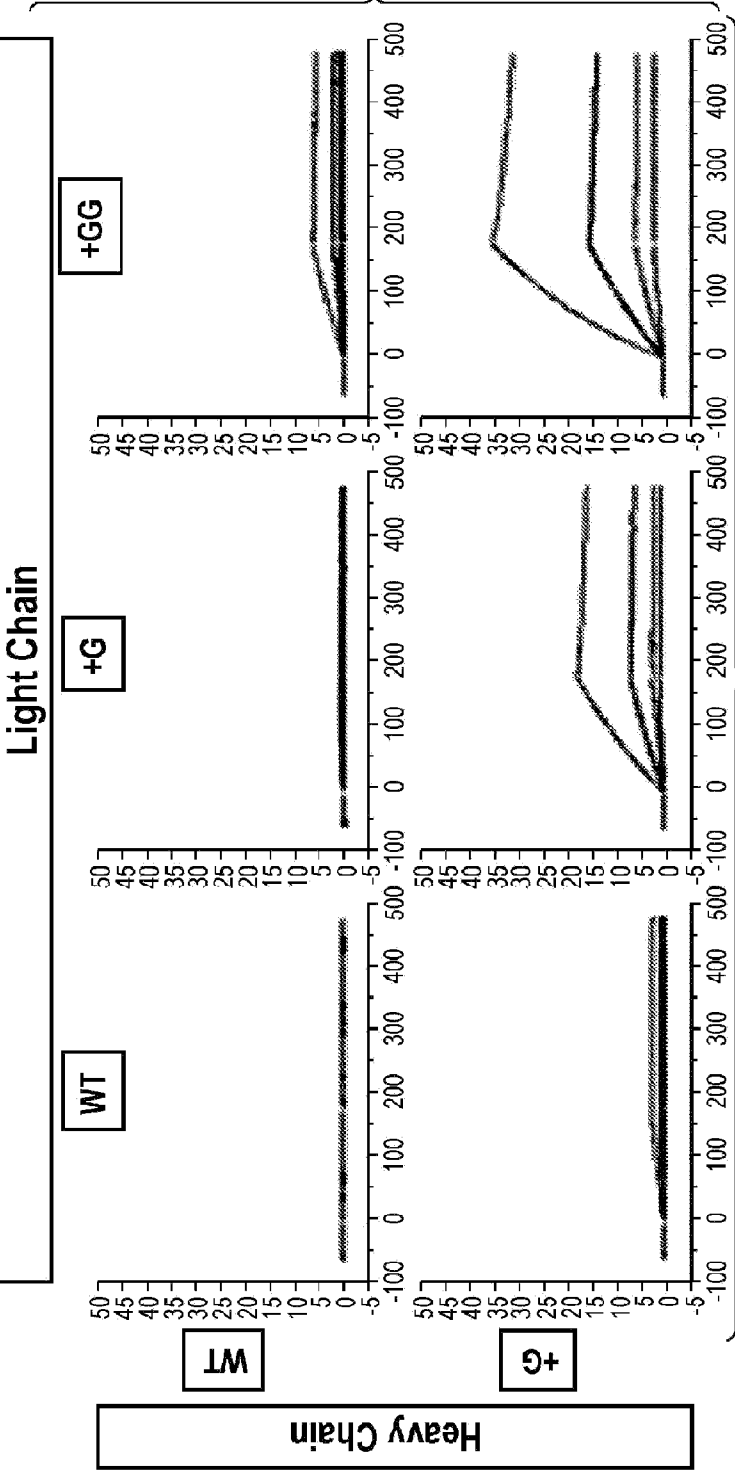


Continue to Sheet 11 of 17

Fig. 6
(Continued)

Continued from Sheet 8 of 17

Biacore TGFβ1 Binding Assay Results of Purified Fab Variants



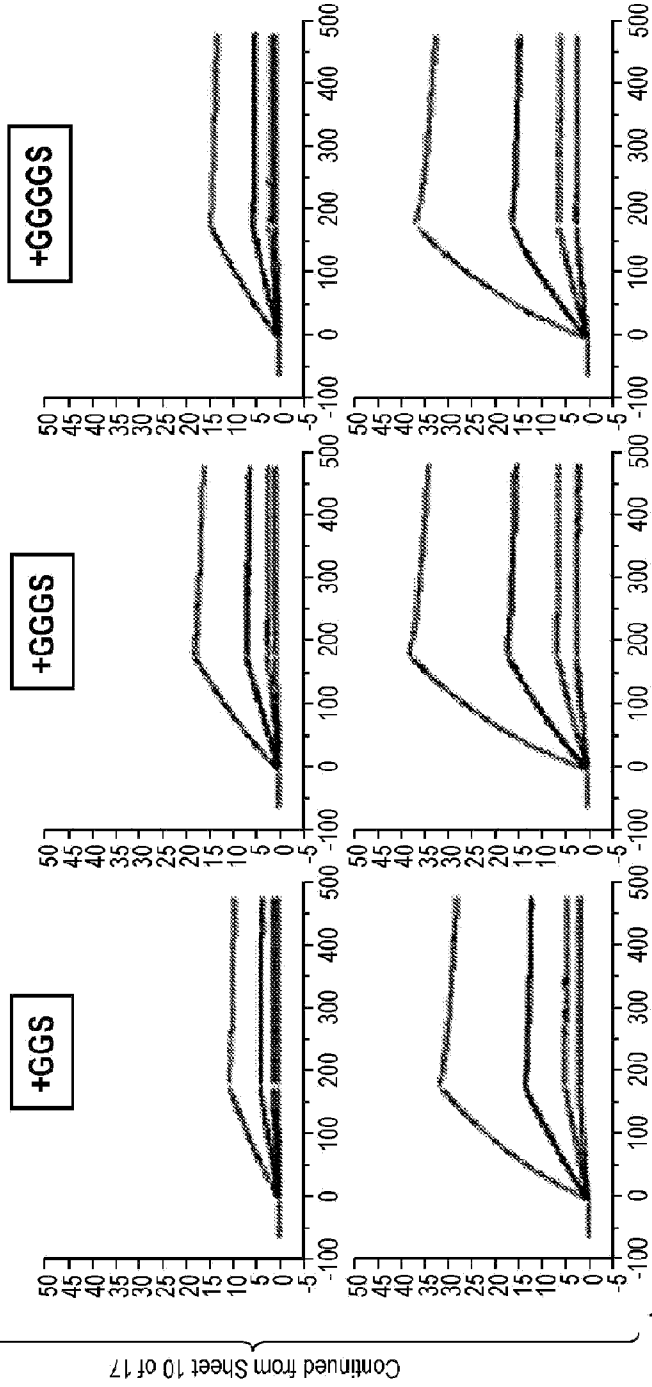
Continue to Sheet 12 of 17

Fig. 6
(Continued)

Continued from Sheet 9 of 17

Biacore TGFβ1 Binding Assay Results of Purified Fab Variants

Light Chain



Continued from Sheet 10 of 17

Continue to Sheet 13 of 17

Fig. 6
(Continued)

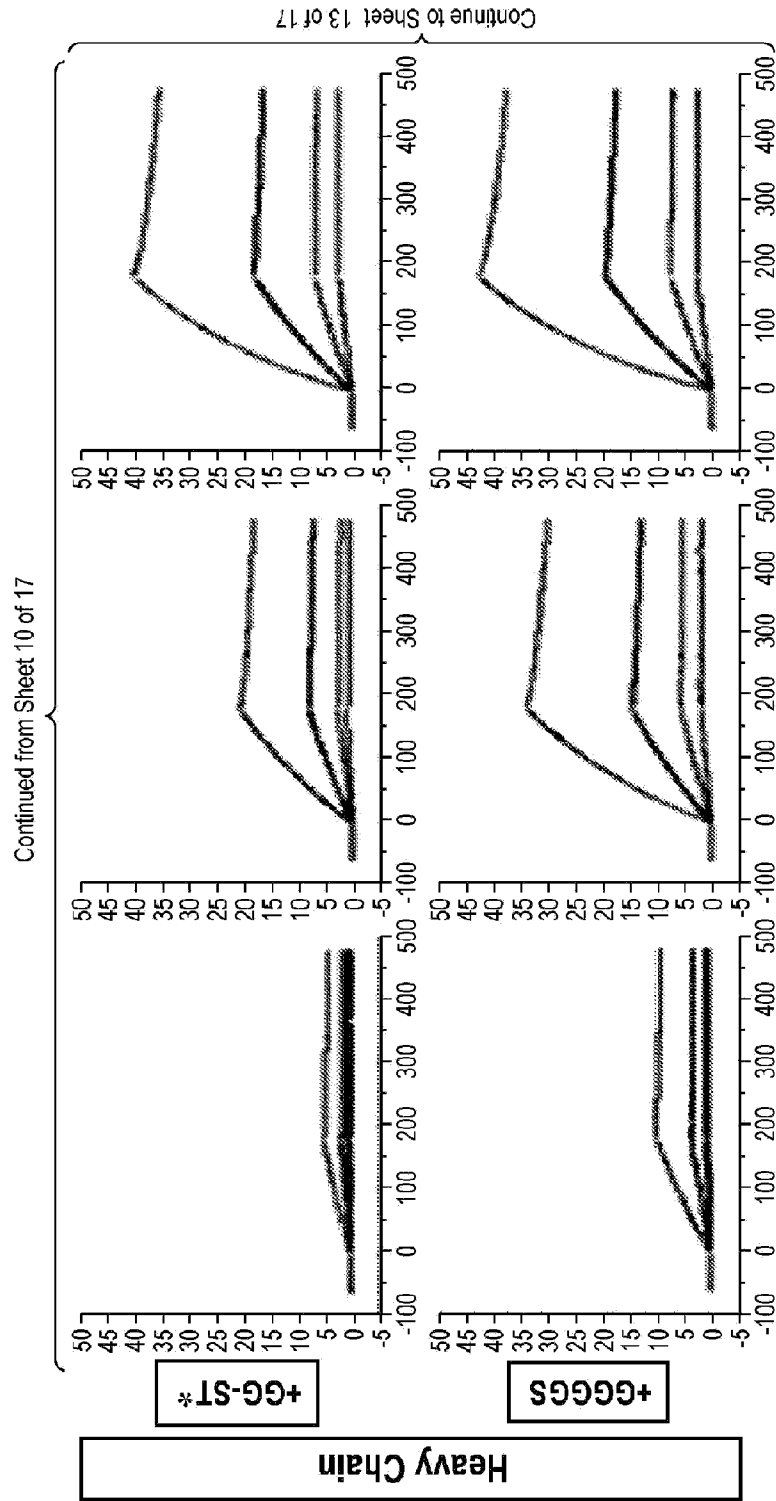


Fig. 6
(Continued)

Continued from Sheet 11 of 17

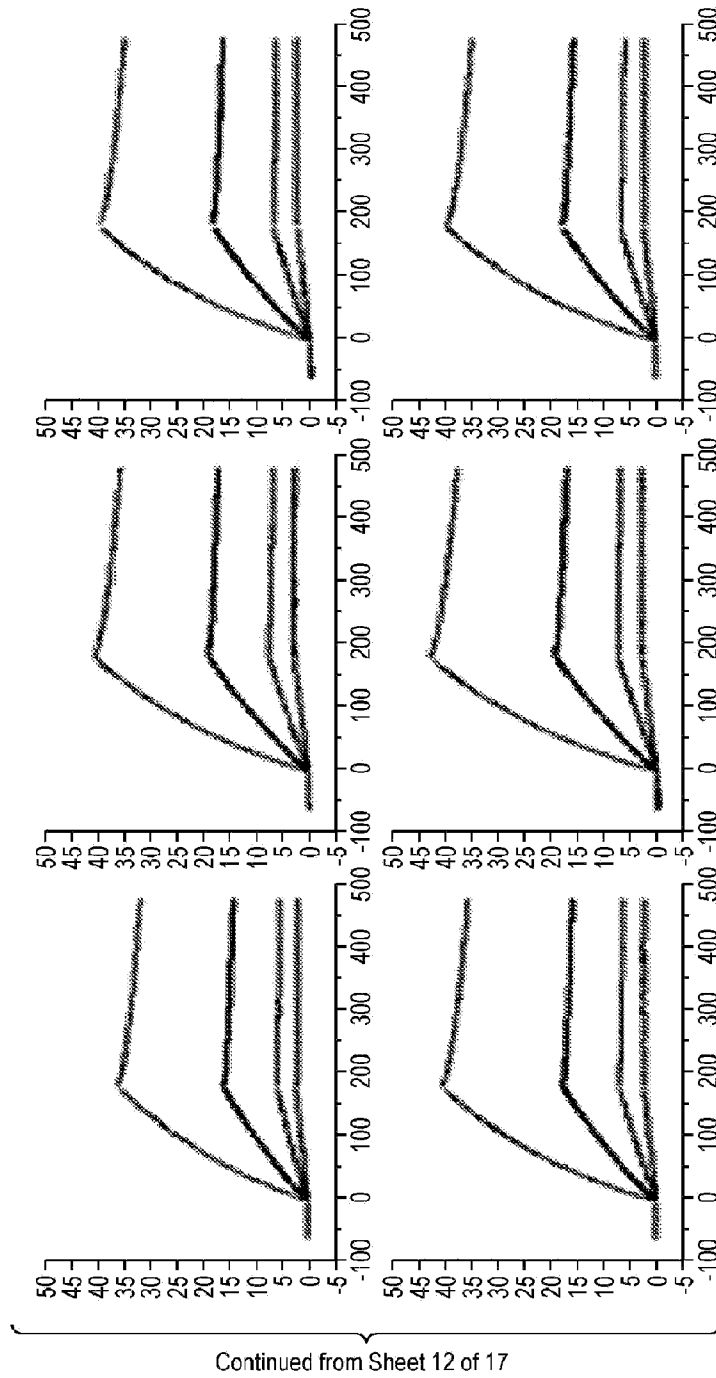


Fig. 6
(Continued)

Summary of DSF Test Results for CAT192 Fab Mutants

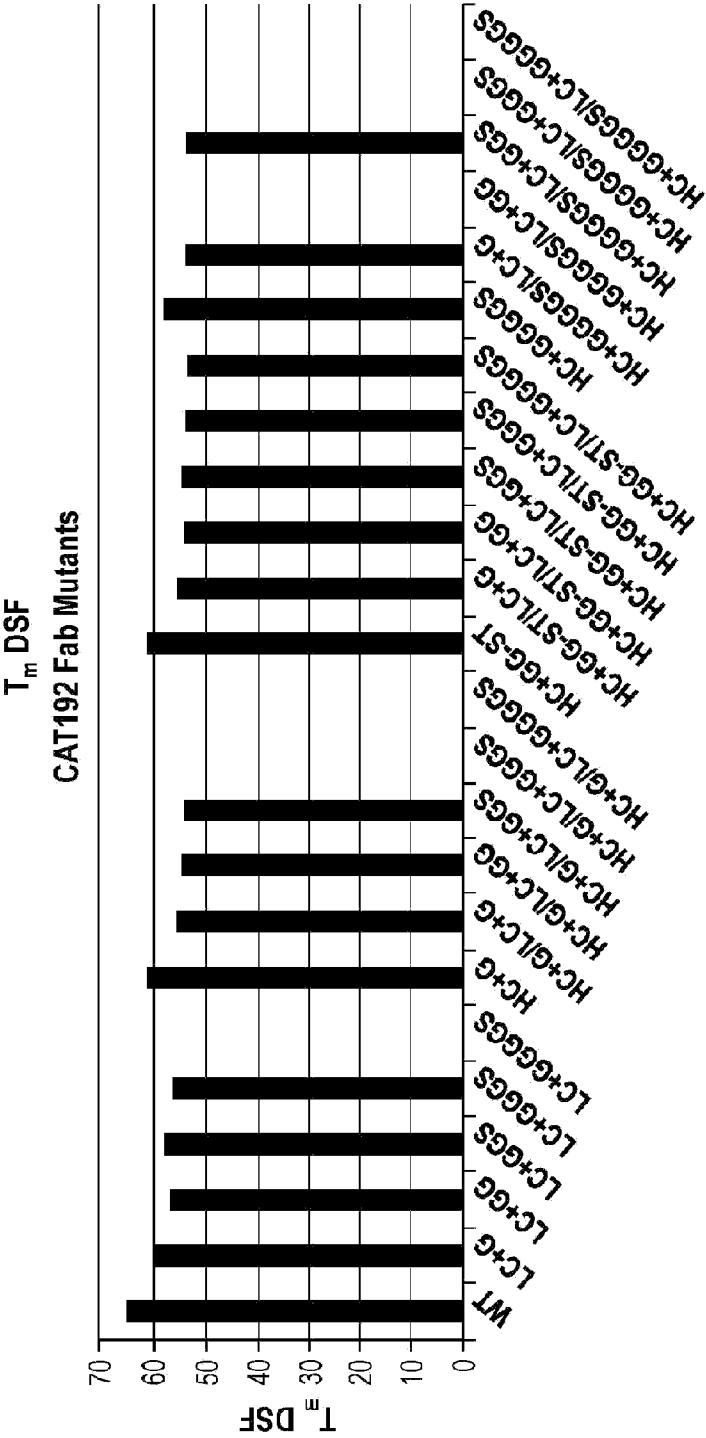
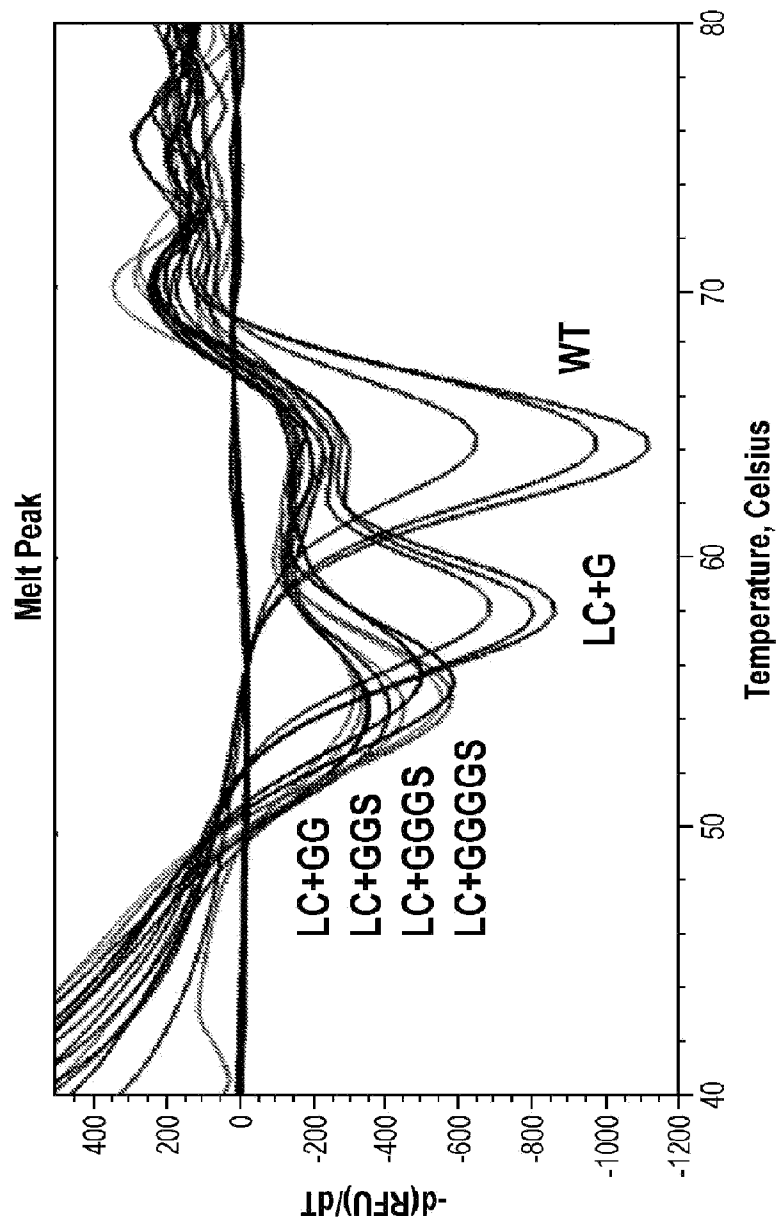
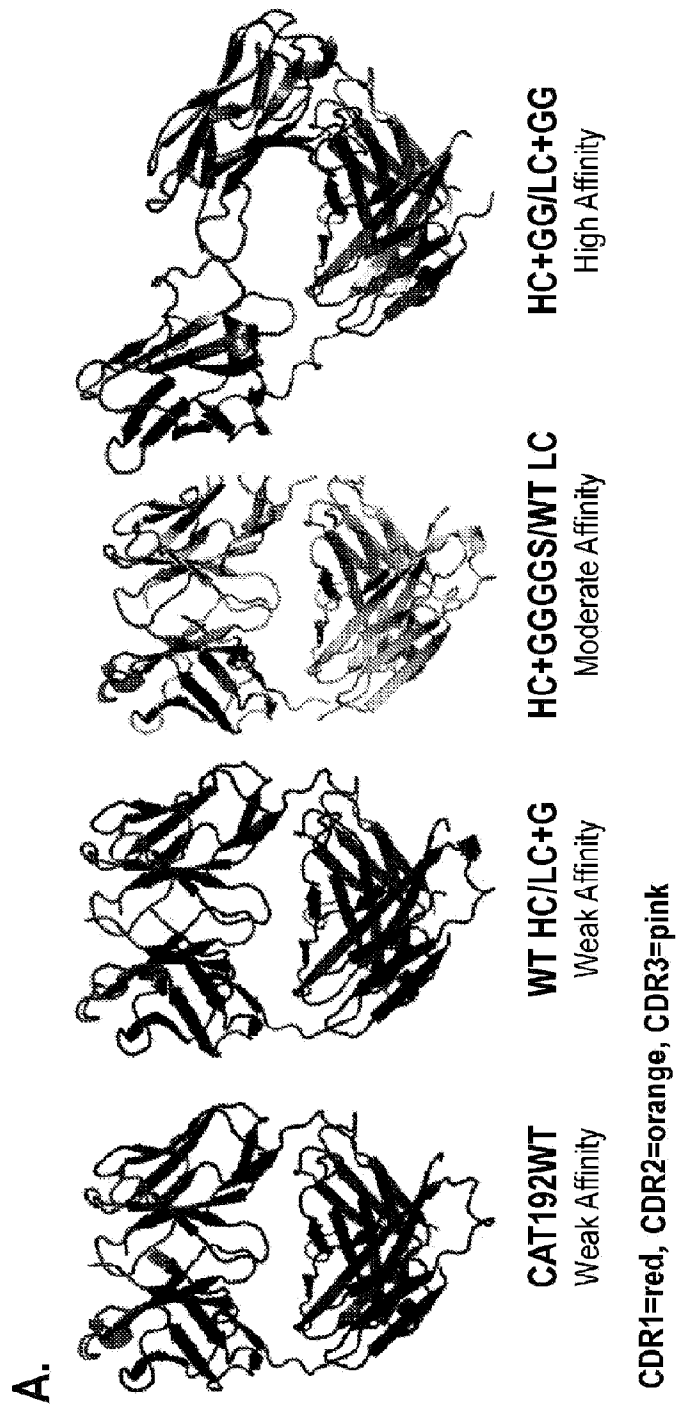


Fig. 7

DSF Test Results for CAT192 IgG4 Mutants

**Fig. 8**

**Fig. 9**

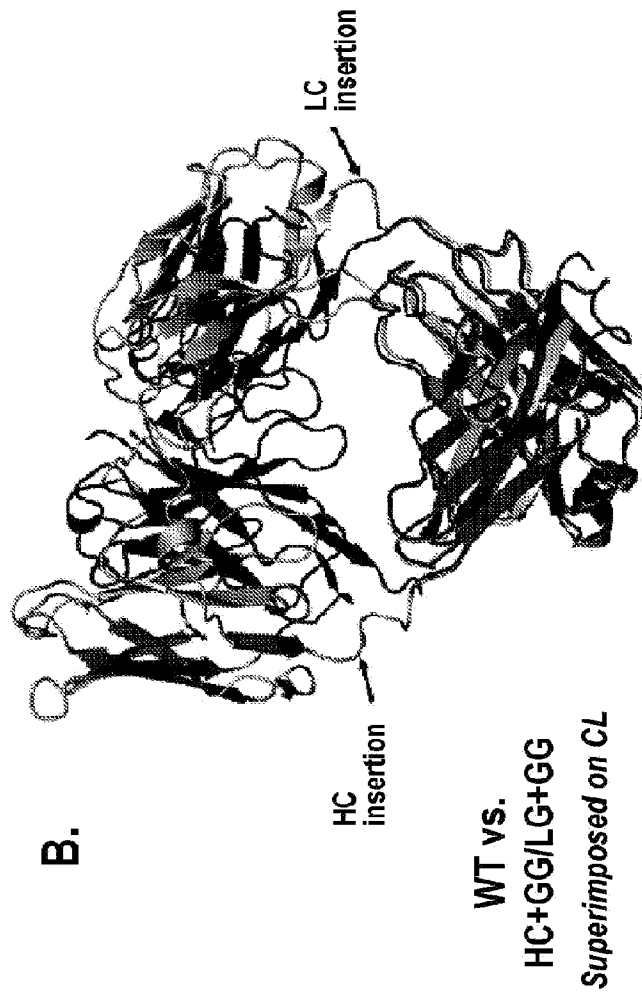


Fig. 9
(Continued)