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(71) Demandeur/Applicant:  
GENZYME CORPORATION, US  
(72) Inventeurs/Inventors:  
QIU, HUAWEI, US;  
PAN, CLARK, US;  
BIRD, JULIE, US  
(74) Agent: ROBIC

(54) Titre : DIMERES SCFV-FC QUI SE LIENT AU FACTEUR DE CROISSANCE TRANSFORMANT  $\beta 1$  AVEC UNE  
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(54) Title: SCFV-FC DIMERS THAT BIND TRANSFORMING GROWTH FACTOR- $\beta 1$  WITH HIGH AFFINITY, AVIDITY  
AND SPECIFICITY

(57) **Abrégé/Abstract:**

An scFv-Fc dimer binds and neutralizes TGF $\beta 1$  selectively and with high affinity and avidity. The scFv region may comprise the same VH and VL domains or CDR regions as metelimumab. The unique combination of their smaller size, high selectivity, potency against TGF $\beta 1$ , and long in vivo half-life makes the scFv-Fc dimers ideal candidates for therapeutic applications.

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(71) Applicant: GENZYME CORPORATION [US/US]; 500  
Kendall Street, Cambridge, MA 02142 (US).

(72) Inventors: QIU, Huawei; c/o Sanofi, 55 Corporate Drive,  
Mail Code: 55A-505A, Bridgewater, NJ 08807 (US). PAN,  
Clark; 353 Dutton Road, Sudbury, MA 01776 (US).  
BIRD, Julie; c/o Sanofi, 55 Corporate Drive, Mail Code:  
55A-505A, Bridgewater, NJ 08807 (US).

(74) Agents: VELEMA, James, H. et al.; Lathrop & Gage  
LLP, 28 State Street, Boston, MA 02109 (US).

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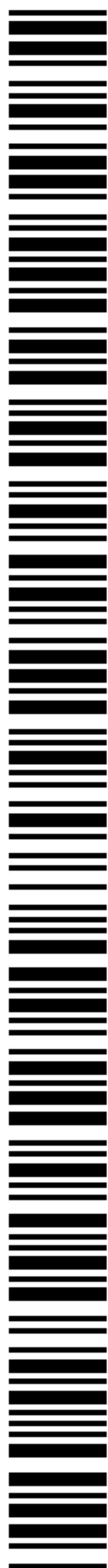
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(57) Abstract: An scFv-Fc dimer binds and neutralizes TGFβ1 selectively and with high affinity and avidity. The scFv region may  
comprise the same VH and VL domains or CDR regions as metelimumab. The unique combination of their smaller size, high se-  
lectivity, potency against TGFβ1, and long *in vivo* half-life makes the scFv-Fc dimers ideal candidates for therapeutic applications.



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## TITLE OF THE INVENTION

**scFv-Fc DIMERS THAT BIND TRANSFORMING GROWTH  
FACTOR- $\beta$ 1 WITH HIGH AFFINITY, AVIDITY AND  
SPECIFICITY**

5

## RELATED APPLICATIONS

This patent application claims the benefit of U.S. Provisional Patent Application 62/128,133, filed March 4, 2015, which is incorporated herein by reference in its entirety.

## 10 BACKGROUND OF THE INVENTION

*Technical Field*

An antigen-binding dimer having two polypeptide monomers, each comprising a single-chain fragment variable molecule (scFv), a hinge, and an Fc molecule, exhibits high affinity and avidity to Transforming Growth Factor- $\beta$ 1 (TGF $\beta$ 1) but not to TGF $\beta$ 2 or to TGF $\beta$ 3. Compositions comprising the antigen-binding dimer and methods of using the same for treatment of diseases involving TGF $\beta$ 1 activity are provided.

*Background*

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

TGF $\beta$  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 $\beta$  isoforms have been reported in humans: TGF $\beta$ 1, TGF $\beta$ 2, and TGF $\beta$ 3 (Swiss



Prot accession numbers P01137, P08112, and P10600, respectively). TGF $\beta$ 1 and TGF $\beta$ 3 trigger a cellular signaling cascade upon binding to the extracellular domains of two transmembrane receptors, known as TGF $\beta$  receptor types I and II. TGF $\beta$ 2 may bind to TGF $\beta$  receptor types I and II, as well as TGF $\beta$  receptor type III.

5           Antibodies that can bind human TGF $\beta$ 1, TGF $\beta$ 2, and TGF $\beta$ 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 $\beta$  neutralizing antibody, because it can  
10       neutralize all three human TGF $\beta$  isoforms. Antibodies that selectively neutralize TGF $\beta$ 1 are disclosed, for example, in U.S. Patent No. 6,492,497 and U.S. Patent No. 7,151,169, which are incorporated by reference into this disclosure. Metelimumab, also known as CAT192 (IgG4), is a human IgG4 monoclonal antibody that selectively neutralizes TGF- $\beta$ 1. See e.g., U.S. Patent No. 6,492,497. Metelimumab was tested  
15       for the treatment of diffuse cutaneous systemic sclerosis, also known as scleroderma, but demonstrated insufficient efficacy.

#### BRIEF SUMMARY OF THE INVENTION

          The present disclosure provides TGF $\beta$ 1-binding scFv-Fc dimers that are capable of selectively neutralizing human TGF $\beta$ 1. In one embodiment, the scFv-Fc  
20       dimers are formatted as scFv-Fc fusion proteins comprised of two polypeptide monomers, each monomer comprising a single-chain Fv region (scFv), a hinge, and an Fc region. The VH and VL domains of the scFv-Fc dimer exhibit a higher affinity and avidity to TGF $\beta$ 1 and more effectively neutralize TGF $\beta$ 1 than when used in the IgG1 or IgG4 format.

25           In one embodiment, the scFv component may be composed of the same VH and VL domains as the VH and VL domains of metelimumab. The variable domains in the scFv component may be linked together by a linker, e.g., a [G4S]<sub>3</sub>-type linker. Each of the scFv components of the scFv-Fc dimers may be fused via a hinge region, e.g., a human IgG1 or IgG4 hinge region, to an Fc region. The monomers of the  
30       dimer may be covalently linked by a disulfide bond between cysteine residues in the hinge region. In another embodiment, the scFv-Fc dimers may have structural dissimilarities to metelimumab, most notably the absence of CH<sub>1</sub> and CL domains and the presence of a linker between the VH and VL domains. Advantageously, the scFv-

Fc dimers display an apparent affinity toward TGF $\beta$ 1 nearly two orders of magnitude greater than that of an scFv comprising the same VH and VL domains

(CAT191(scFv), shown in SEQ ID NO: 12) in an A549 cell potency bioassay.

Further, the scFv-Fc dimers display an apparent affinity toward TGF $\beta$ 1 over three  
 5 orders of magnitude greater than that of an IgG-formatted antibody comprising the same VH and VL domains (*e.g.*, CAT192) in the A549 cell bioassay. The scFv-Fc dimers also display desirable stability and pharmacokinetic properties. Because of their relatively small size and extended half-life in serum, the scFv-Fc dimers are particularly useful for therapeutic applications.

10 Accordingly, the present invention is directed to an isolated binding protein comprising a variable domain that is capable of binding TGF $\beta$ 1, wherein the binding protein exhibits a K<sub>d</sub> for human TGF $\beta$ 1 at least about 50% lower than the K<sub>d</sub> of the same binding protein for human TGF $\beta$ 2, as measured by surface plasmon resonance.

In another embodiment, the present invention is directed to an isolated binding  
 15 protein comprising a variable domain that is capable of binding TGF $\beta$ 1, wherein the binding protein exhibits a K<sub>d</sub> for human TGF $\beta$ 1 at least about 50% lower than the K<sub>d</sub> of the same binding protein for human TGF $\beta$ 3, as measured by surface plasmon resonance.

In a further embodiment, the present invention is directed to an isolated  
 20 binding protein comprising a variable domain that is capable of binding TGF $\beta$ 1, wherein the binding protein exhibits a K<sub>d</sub> for human TGF $\beta$ 1 at least about 50% lower than the K<sub>d</sub> of the same binding protein for human TGF $\beta$ 2, and at least about 50% lower than the K<sub>d</sub> of the same binding protein for human TGF $\beta$ 3, as measured by surface plasmon resonance.

25 In a further embodiment, the present invention is directed to an isolated binding protein that binds TGF $\beta$ 1, wherein the binding protein comprises a first polypeptide chain and a second polypeptide chain, the first and the second polypeptide chains each having the formula of:

$$(VD_1)-(linker1)_n-(VD_2)-(linker2)_m-(hinge)_p-(Fc\ region),$$

30 wherein VD<sub>1</sub> comprises a first variable domain selected from the group consisting of a VL domain isolated from an antibody capable of binding TGF $\beta$ 1, and a VH domain isolated from an antibody capable of binding TGF $\beta$ 1, and VD<sub>2</sub>



comprises a second variable domain selected from the group consisting of a VL domain isolated from an antibody capable of binding TGF $\beta$ 1, and a VH domain isolated from an antibody capable of binding TGF $\beta$ 1; and wherein, n is 0 or 1, m is 0 or 1, and p is 0 or 1.

5 In one embodiment, the present invention is directed to an isolated TGF $\beta$ 1-binding scFv-Fc dimer that selectively binds TGF $\beta$ 1. The scFv-Fc dimer may comprise two polypeptide monomers, each having the following formula, from N-terminal to C-terminal: (VH domain)-(linker)-(VL domain)-(hinge)-(Fc region). In another embodiment, an isolated binding protein that binds TGF $\beta$ 1 is disclosed, which  
10 comprises a first polypeptide chain and a second polypeptide chain. The first and the second polypeptide chains may both have the formula of, from N-terminal to C-terminal: (VH domain)-(linker1)<sub>n</sub>-(VL domain)-(linker2)<sub>m</sub>-(hinge)<sub>p</sub>-(Fc region). p may be 0 or 1, n may be 0 or 1, and m may be 0 or 1. In one aspect, the first and second polypeptide chains may be identical and may form a dimer.

15 In another embodiment, the disclosed TGF $\beta$ 1 binding protein may comprise a polypeptide chain having the formula of, from N-terminal to C-terminal: (VH domain)-(linker1)<sub>n</sub>-(VL domain)-(linker2)<sub>m</sub>-(hinge)<sub>p</sub>-(Fc region), wherein p may be 0 or 1, n may be 0 or 1, and m may be 0 or 1.

The VH domain of the disclosed binding protein may comprise a variable  
20 heavy complementarity determining region 1 (HCDR1), a variable heavy complementarity determining region 2 (HCDR2), and a variable heavy complementarity determining region 3 (HCDR3). In one aspect, the HCDR1 may have the amino acid sequence of SEQ ID NO: 22, The HCDR2 may have the amino acid sequence of SEQ ID NO: 23, and the HCDR3 may have the amino acid sequence  
25 of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 30.

The framework regions of the VH domain may be selected from a variable heavy germline sequence. The VH domain may be selected, for example, from the human VH domain sequences set forth in SEQ ID NO: 1 or SEQ ID NO: 2, or a variant thereof having modifications in up to four amino acids.

30 The VL domain of the disclosed binding protein may comprise 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). In one aspect, the LCDR1 may have the amino acid sequence of SEQ ID NO: 27, the LCDR2 may have the amino acid sequence of SEQ ID NO: 28, and the LCDR3 may have the amino acid sequence of SEQ ID NO: 29.

The framework regions of the VL domain may be selected from a variable  
 5 lambda or kappa germline sequence. The VL domain may be selected, for example, from the human Vk domain sequences set forth in SEQ ID NO: 5 or SEQ ID NO: 6, or a variant thereof having modifications of up to four amino acids. In one embodiment, 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: 5, which are the VH and  
 10 VL domains present in metelimumab, respectively.

In one embodiment, the variable domains in the scFv component may be linked by a flexible linker about 15 amino acids in length. "About" in this context means the linker can vary by up to plus or minus four amino acids in length. For optimal flexibility, the linker is composed predominantly of glycine and serine  
 15 residues. For example, the linker may be a [G<sub>4</sub>S]<sub>3</sub>-type linker. The linker may have the amino acid sequence SGGGSGGGGSGGGGS (SEQ ID NO: 3), the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 4), or a variant thereof having up to four amino acid modifications. For purpose of this disclosure, "having up to x amino acids modifications" means that the up to x number of amino acids may be changed to  
 20 different amino acids by one of skill in the art without significantly altering the structure and function of the polypeptide.

In another embodiment, p is 1 and the scFv component is connected to an Fc region 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  
 25 acid sequence PKSCDKTHTCPPCPAPPELLGGP (SEQ ID NO: 7), or a variant thereof having up to four amino acid modifications. In one embodiment, the hinge length may vary from 3-15 amino acids. When the hinge is from a human IgG1, it may comprise the amino acid sequence CPPCP (SEQ ID NO: 21). Further, the variant of the hinge of SEQ ID NO: 7, which is also a human IgG1 hinge, may  
 30 comprise the amino acid sequence CPPCP (SEQ ID NO: 21).

In another embodiment, m is 1 and a linker2 is present between scFv component and the hinge. In one aspect, linker2 may comprise the amino acid



sequence GGSG (SEQ ID NO: 20), or a variant thereof having up to 2 amino acid modifications.

The Fc region may comprise two or three constant domains, e.g., a CH<sub>2</sub> domain and CH<sub>3</sub> domain. The Fc region may be obtained from a human IgG1, a human IgG4, or a variant of a human IgG1 or IgG4 having up to ten amino acid modifications, for example. In one embodiment, each polypeptide of the dimer has the sequence set forth in SEQ ID NO: 9. The structure of the scFv-Fc dimer of SEQ ID NO: 9 is shown in FIG. 2. The scFv-Fc dimer may bind TGFβ1 selectively. The scFv-Fc dimer may show an apparent dissociation constant less than 1 nM or even less than 0.1 nM. The apparent dissociation constant may be measured by using an A549 bioassay or by surface plasmon resonance, for example.

In another embodiment, an isolated polynucleotide is disclosed which may comprise a nucleotide sequence encoding the scFv-Fc dimer. 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 scFv-Fc dimer may include culturing the host cell under suitable conditions to produce the scFv-Fc dimer. The scFv-Fc dimer may be purified. The degree of purity may be 90%, 95%, 99%, 99.5% or more.

The scFv-Fc dimer of the present invention may be an element of a composition. The composition may be a pharmaceutical composition. The pharmaceutical composition may comprise a therapeutically effective amount of the scFv-Fc dimer. The composition may further comprise one or more biologically active components, excipients, or diluents.

A method of treating a disease or condition resulting directly or indirectly from TGFβ1 activity in a human may comprise administering a pharmaceutical composition comprising a therapeutically effective amount of the scFv-Fc dimer. The disease or condition may be selected from the group consisting of a fibrotic disease, cancer, an immune-mediated disease, e.g., diffuse cutaneous systemic sclerosis, bone remodeling disease, kidney disease and/or combinations thereof. The scFv-Fc dimer 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, an immune-mediated disease, e.g., diffuse cutaneous systemic sclerosis, bone remodeling disease, kidney disease and/or combinations thereof. The treatment of the disease or disorder may comprise neutralizing TGF $\beta$ 1 or inhibiting TGF $\beta$ 1 signaling. The treatment of

5 the disease or disorder may comprise inhibiting TGF $\beta$ 1-mediated fibronectin production, vascular endothelial growth factor (VEGF) production, epithelial cell proliferation, endothelial cell proliferation, smooth muscle cell proliferation, or immunosuppression. The treatment of the disease or disorder may comprise increasing natural killer cell activity.

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

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

FIG. 1 depicts the general structures of the various formats.

FIG. 2 depicts the results of a Biacore TGF $\beta$ 1 binding assay which showed the

15 loss of affinity when the scFv(CAT191) was converted into a full length IgG4 (CAT192) molecule.

FIG. 3 shows the results of an A549 cell bioassay comparing the inhibitory effects by various antibody constructs on TGF $\beta$ 1-stimulated IL-11 production: scFv diabody 5aa (SEQ ID NO: 14); CAT191(scFv) (SEQ ID NO: 12); CAT191(scFv-Fc) (SEQ ID NO: 9); and CAT192(IgG4) (light chain SEQ ID NO: 10 and heavy chain

20 SEQ ID NO: 11).

FIG. 4 depicts the results of pharmacokinetic tests to determine the half-life of CAT191 (scFv-Fc) following intravenous (IV) administration.

FIG. 5 depicts the results of pharmacokinetic tests to determine the half-life of

25 CAT191 (scFv-Fc) following intraperitoneal (IP) administration.

FIG. 6 shows the TGF $\beta$ 1-specific binding results of CAT191(scFv-Fc) prepared from CHO cells.

FIG. 7 shows the the cell-based potency assay results of CAT191(scFv-Fc) prepared from CHO cells.

## DETAILED DESCRIPTION OF THE INVENTION

The disclosed scFv-Fc dimers bind and neutralize TGF $\beta$ 1 selectively and with high affinity and avidity. The scFv regions may be composed of the same VH and VL domains as in metelimumab. scFv-Fc dimers advantageously show greater efficacy in neutralizing TGF $\beta$ 1 than when the variable domains are used in other formats. Because of their relatively small size and extended half-life in serum, the present scFv-Fc dimers are ideal candidates for therapeutic applications.

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.

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.

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

*scFv-Fc Dimers*

In one embodiment, the present scFv-Fc dimer variable domains 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), incorporated herein by reference. The CDR regions are listed below:

	HCDR1	SYGMH	SEQ ID No. 22
	HCDR2	VISYDGSIKYYADSVKG	SEQ ID No. 23
	HCDR3	TGEYSGYDTSGVEL	SEQ ID No. 24
30		TGEYSGYDTPQYS	SEQ ID No. 25
		TGFYSGYDTPASPD	SEQ ID No. 26
	LCDR1	RASQGIGDDL	SEQ ID No. 27



LCDR2 GTSTLQS SEQ ID No. 28

LCDR3 LQDSNYPLT SEQ ID No. 29

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

5 HCDR3 TGX<sub>1</sub>YSGYDTX<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub> SEQ ID No. 30

Wherein: X<sub>1</sub> may be any amino acid (preferably E, or F), or absent,  
 X<sub>2</sub> may be any amino acid (preferably S, D, or P), or absent,  
 X<sub>3</sub> may be any amino acid (preferably G, P, or A), or absent,  
 X<sub>4</sub> may be any amino acid (preferably V, Q, or S), or absent,  
 10 X<sub>5</sub> may be any amino acid (preferably E, Y, or P), or absent,  
 X<sub>6</sub> may be any amino acid (preferably L, S, or D), or absent.

The VH domain comprises the HCDR1 of SEQ ID No. 22, the HCDR2 of SEQ ID No. 23, and one of the HCDR3s selected from the group consisting of SEQ ID No. 24, SEQ ID No. 25, SEQ ID No. 26, and SEQ ID No. 30. The CDR  
 15 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. In one embodiment, the FW region sequences may be selected from the same human variable heavy germline sequence. The VL domain comprises  
 20 the LCDR1 of SEQ ID NO: 7, the LCDR2 of SEQ ID NO: 28, and the LCDR3 of SEQ ID NO: 29. 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  
 25 and about 30 variable lambda germline sequences, e.g., V<sub>H</sub>3, V<sub>κ</sub>1, V<sub>H</sub> 1-69, and V<sub>H</sub> 1-e.

In another embodiment, 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  
 30 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, both of which are hereby incorporated into this disclosure by reference.

The present scFv-Fc dimers 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: 5, 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: 6. 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), incorporated herein by reference.

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).

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.

Up to four amino acid substitutions may be made to de-immunize the scFv-Fc dimer, for example. De-immunization can be performed according to the method of Harding et al. (2010) *mAbs* 2: 256-265, for example.

Framework residues of the VH and/or VL domains, for example, may be substituted to increase the stability of the scFv-Fc dimers and/or decrease their tendency to aggregate. Poor stability can affect the ability of the expressed scFv-Fc dimers 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. scFv polypeptides in particular may demonstrate problems with



stability, solubility, expression, aggregation, breakdown products, and overall manufacturability in both bacterial and mammalian expression systems. 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 an scFv polypeptide, 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 scFv-Fc dimers to aggregate.

Substitutions that can be tolerated are expected to include those that would replace an amino acid of SEQ ID NO: 1, 2, 5, or 6 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 could 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<sub>H</sub> 1-69) or DP-88 (V<sub>H</sub> 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.

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.

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  
 5 substitutions can be used to lower the isoelectric point, which can decrease the rate of elimination of IgG polypeptide antibodies, for example.

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  
 10 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.

15 Detectable labels include radiolabels such as  $^{131}\text{I}$  or  $^{99}\text{Tc}$ , 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  
 20 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.

The VH and VL domains of the scFv-Fc dimers are linked together by a linker, termed Linker1 herein. Linkers suitable for making an scFv fragment are well  
 25 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. Suitable linkers include those of the  $[\text{G}_4\text{S}]_3$ -type. The  $[\text{G}_4\text{S}]_3$ -type linkers are composed of repeating units of glycine and serine residues.  
 30 Such linkers may have a sequence of SGGGSGGGGSGGGGS (SEQ ID NO: 3) or GGGGSGGGGSGGGGS (SEQ ID NO: 4) or a variant thereof having up to four amino acid modifications, for example. Modifications can include deletions or insertions that change the linker length, or amino acid substitutions, preferably from



Gly to Ser or vice versa. [G<sub>4</sub>S]<sub>3</sub>-type linkers have been widely used to link variable domains in an scFv structure, because the linkers are hypoallergenic and causes minimal conformational distortions to the variable domains. *See, e.g.*, Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85: 5879-83.

5           In the scFv-Fc dimers, a short linker sequence, termed Linker2 herein, is optionally inserted between the VL domain and the hinge. This linker sequence increases the flexibility of the scFv component with respect to the Fc component. In one embodiment, Linker2 has the sequence of GGSG (SEQ ID NO: 20). Suitable modifications to the GGSG linker include altering its length by one to four amino  
10       acids or substituting one to two amino acids, preferably from Gly to Ser or vice versa.

          The hinge region is a flexible domain that joins the scFv portion to the Fc region. The flexibility of the hinge region in IgG and IgA molecules allows the Fab arms to adopt a wide range of angles, permitting binding to epitopes spaced variable distances apart. A suitable hinge region includes, for example, the human IgG1 hinge  
15       region having the amino acid sequence PKSCDKTHTCPPCPAPPELLGGP (SEQ ID NO: 7). This sequence corresponds to a portion of the human IgG1 upper hinge, the middle hinge, and an N-terminal portion of the CH<sub>2</sub> domain, as disclosed in FIG. 4B of U.S. Patent No. 8,048,421, for example. The hinge from a human IgG1 contains two Cys residues, which can form disulfide bonds with the Cys residues of the hinge  
20       on the corresponding monomer. The human IgG1 hinge portion that forms the disulfide bonds contains the amino acid sequence CPPCP (SEQ ID NO: 21). Variants of a human IgG1 hinge may comprise this sequence.

          The scFv component is fused in frame to an Fc region, which forms the Fc component of the dimer. Suitable Fc regions contain two or three constant regions.  
25       Fc regions include those from human IgG1, as set forth in SEQ ID NO: 8, or IgG4, as set forth in the CH<sub>2</sub> and CH<sub>3</sub> domains of SEQ ID NO: 11. 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).

30           Modifications can be made to the hinge and Fc region to improve various properties of the scFv-Fc dimers. In one embodiment, 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 scFv-Fc dimer. 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  
5 protects it from degradation. Mutations located at the interface between the CH<sub>2</sub> and CH<sub>3</sub> 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  
10 region of immunoglobulin G to modulate in vivo antibody levels. *Nat Biotechnol.* 23(10):1283-8, for example.

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  
15 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 CH<sub>2</sub> 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  
20 modifications are reviewed in Strohl (2009) "Optimization of Fc-mediated effector functions of monoclonal antibodies," *Curr. Opin. Biotechnol.* 20:685-91, for example.

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 binding to C1q	Increased CDC	10
IgG1	Human	S239D/I332E/G236A	Increased FcγRIIa/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. Hinton et al. (2004) *J. Biol. Chem.* 279(8):6213-16.
2. Vaccaro et al. (2005) *Nature Biotechnol.* 23(10):1283-88.
3. Armour et al. (1999) *Eur. J. Immunol.* 29(8):2613-24.
4. Shields et al. (2001) *J. Biol. Chem.* 276(9):6591-604.
5. Idusogie et al. (2000) *J. Immunol.* 164(8):4178-84.
6. Idusogie et al. (2001) *J. Immunol.* 166(4):2571-75.
7. Lazar et al. (2006) *Proc. Nat'l Acad. Sci. USA* 103(11): 4005-10.
8. Ryan et al. (2007) *Mol. Cancer Ther.* 6: 3009-18.
9. Datta-Mannan et al. (2007) *Drug Metab. Dispos.* 35: 86-94.
10. Steurer et al. (1995) *J. Immunol.* 155(3):1165-74.
11. Richards et al. (2008) *Mol. Cancer Ther.* 7(8):2517-27.
12. Labrijn et al. (2009) *Nature Biotechnol.* 27(8):767-71.

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  
5 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  
10 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.  
15 (2010) *Nature* 10: 345-52, for example.

#### *Nucleic Acids and Methods of Making scFv-Fc Dimers*

A further aspect of the present invention provides nucleic acids encoding scFv-Fc dimers. The isolated nucleic acid may be a synthetic DNA, a non-naturally occurring mRNA, or a cDNA, for example. Examples include the nucleic acids  
20 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. Additional nucleic acids include the sequence set forth in SEQ ID NO: 13 of the present invention, which encodes the diabody-5aa set forth in SEQ ID NO: 14, and the sequence set forth in SEQ ID NO: 15, which encodes the leucine zipper peptide-derived dimer having the amino acid sequence set forth in SEQ ID  
25 NO: 16. Additional nucleic acids include the sequence set forth in SEQ ID NO: 17, which encodes CAT191(scFv-Fc), which has the amino acid sequence set forth in SEQ ID NO: 9. The nucleic acid may be inserted within a plasmid, vector, or transcription or expression cassette. The nucleic acids encoding the scFv-Fc dimers may be made and the expressed antibodies may be tested using conventional  
30 techniques well known in the art, such as disclosed in Borsi et al. (2002) *Int. J. Cancer* 102: 75-85.

A recombinant host cell may comprise one or more constructs above. Methods of preparing scFv-Fc dimers comprise expressing the encoding nucleic acid



in a host cell under conditions to produce the scFv-Fc dimers, 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  
5 a pharmaceutically acceptable excipient.

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  
10 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  
15 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*.

Suitable vectors comprising a nucleic acid encoding scFv-Fc dimers can be chosen or constructed, containing appropriate regulatory sequences, including  
20 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  
25 expression, are well known in the art.

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  
30 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. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adena-associated viruses), which serve equivalent functions.

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. In one embodiment, the nucleic acid of the invention is 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.

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.



scFv-Fc dimers 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  
 5 fucosylation, in order to increase ADCC activity of the resulting scFv-Fc dimer.

*Methods of Using Antibodies or Antigen-Binding Fragments Thereof*

The scFv-Fc dimers 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  
 10 administering an effective amount to treat the patient. Treatable conditions include any in which TGF $\beta$ 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 combinations thereof.

Antibodies specific for human TGF $\beta$ 1 have been shown to be effective in  
 15 animal models for the treatment of TGF $\beta$ 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  
 20 TGF $\beta$ 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).

The scFv-Fc dimers are useful to treat a disease and condition resulting  
 25 directly or indirectly from TGF $\beta$ 1 activity. The scFv-Fc dimers may selectively inhibit the activity of a human TGF $\beta$ 1 isoform *in vitro* or *in vivo*. Activities of TGF $\beta$ 1 isoforms include, but are not limited to, TGF $\beta$ -mediated signaling, extracellular matrix (ECM) deposition, inhibiting epithelial and endothelial cell proliferation, promoting smooth muscle proliferation, inducing Type III collagen  
 30 expression, inducing TGF- $\beta$ , 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 scFv-Fc dimers are useful to treat focal segmental

glomerulosclerosis (FSGS), hepatic fibrosis (HF), acute myocardial infarction (AMI), idiopathic pulmonary fibrosis (IPF), scleroderma (SSc), and Marfan Syndrome.

The scFv-Fc dimers are useful to treat diseases and conditions including, but not limited to, a 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. They 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, they 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. Methods for using scFv-Fc dimers in combination with such antagonists are set forth in WO 2004/098637, for example.

The scFv-Fc dimers 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.

The scFv-Fc dimers 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  
5 bowel disease).

The scFv-Fc dimers 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.

10 The scFv-Fc dimers 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 scFv-Fc dimers are useful to treat hyperproliferative diseases, such as cancers including, but not limited to, breast, prostate, ovarian, stomach, renal, pancreatic, colorectal, skin,  
15 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. The scFv-Fc dimers of the invention also are useful to inhibit cyclosporin-mediated metastases.

In the context of cancer therapy, "treatment" includes any medical  
20 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.

Methods of treatment comprise administering a scFv-Fc dimer or pharmaceutical compositions comprising the scFv-Fc dimer. The scFv-Fc dimers  
25 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 scFv-Fc dimer 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.

30 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.

The precise dose will depend upon a number of factors, including whether the scFv-Fc dimer 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 scFv-Fc dimer. A typical dose of a scFv-Fc dimer, 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.

Dose levels of about 0.1, 0.3, 1, 3, 10, or 15 mg per kg body weight of the patient are expected to be useful and safe. 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.

The scFv-Fc dimers of the invention may be administered by injection, for example, subcutaneously, intravenously, intracavity (e.g., after tumor resection), intralesionally, intraperitoneally, or intramuscularly. ScFv-Fc dimers also may be delivered by inhalation or topically (e.g., intraocular, intranasal, rectal, into wounds, on skin), or orally.

A scFv-Fc dimer will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the scFv-Fc dimer. 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  
5 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.

10 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,  
15 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.

A scFv-Fc dimer may be formulated in liquid, semi-solid, or solid forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or  
20 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 combinations of excipients, for example: sugars, amino acids and surfactants. Liquid formulations may include a wide range of scFv-Fc dimer  
25 concentrations and pH. Solid formulations may be produced by lyophilization, spray drying, or drying by supercritical fluid technology, for example.

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 scFv-Fc dimer in an  
30 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.

In certain embodiments, the active compound may be prepared with a carrier that will protect the scFv-Fc dimer 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.

A method of using a scFv-Fc dimer may comprise causing or allowing binding to TGF $\beta$ . Such binding may take place *in vivo*, e.g., following administration of a scFv-Fc dimer 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 scFv-Fc dimer and then administered to a patient.

A kit comprising a scFv-Fc dimer is provided. The scFv-Fc dimer 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.

The reactivity of a scFv-Fc dimer 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 scFv-Fc dimer. Bound antigen is physically separated from unbound antigen and the amount of radioactive antigen bound to the scFv-Fc dimer 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.

Other reporters include macromolecular colloidal particles or particulate material such as latex beads that are colored, magnetic or paramagnetic, and  
5 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  
10 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.

15 The present invention also provides the use of a scFv-Fc dimer for measuring antigen levels in a competition assay. The scFv-Fc dimer 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,  
20 covalently, e.g., via a peptide bond or non-covalently. The scFv-Fc dimer and a protein reporter may be linked by a peptide bond and recombinantly expressed as a fusion protein.

Further aspects and embodiments of the present invention will be apparent to those skilled in the art in the light of the present disclosure, including the following  
25 experimental exemplification.

### *Examples*

#### **Example 1: Affinity and Potency of scFv and IgG4 Antibody**

CAT192(IgG4) (metelimumab) is a human IgG4 monoclonal antibody that selectively neutralizes TGF- $\beta$ 1. TGF $\beta$ 1 (20-600RU) was immobilized to a CM5 chip  
30 on Biacore using NHS/EDC chemistry. Various amounts of CAT192(IgG4) were injected over the surface to monitor the binding to TGF $\beta$ 1 determined by surface plasmon resonance. The data were analyzed with a 1:1 binding model to determine binding constants. CAT192(IgG4) was found to bind TGF $\beta$ 1 with relatively low

affinity as determined by surface plasmon resonance, when compared to the binding by the parental CAT191scFv as shown in FIG.2. CAT192(IgG4) also showed a relatively low efficacy ( $IC_{50} = \sim 10$  nM) in an A549 cell-based potency assay, which measured inhibition of TGF $\beta$ 1-stimulated IL-11 production. Representative results of an A549 assay are shown in FIG. 3. The A549 assay was conducted according to the procedure disclosed in Rapoza et al. (2006) "Development of an in vitro potency assay for therapeutic TGF $\beta$  antagonists: the A549 cell bioassay," *J. Immunol. Methods* 316: 18-26. While an apparent dissociation constant of  $\sim 10$  nM showed specific binding to TGF $\beta$ 1, therapeutic applications of CAT192 (IgG4) would benefit from a higher relative potency.

### Example 2: Modified IgG1 Antibody

CAT192(IgG4) affinity can be slightly enhanced by certain denaturing conditions, suggesting that antibody folding may have caused the loss of affinity during the conversion of scFv to IgG4. IgG4 folding has been proposed to be unique (Aalberse and Schuurman "IgG4 breaking the rules", *Immunology* 105:9-19). The Fab arm exchange in IgG4 and the interaction of Fabs with Fc CH2 domain may possibly explain this loss of affinity by CAT192(IgG4). Therefore, CAT192 was remodeled to produce the IgG1 version by replacing IgG4 Fc (CH1, CH2 and CH3 domains) with the consensus IgG1 sequence. The DNA coding the CAT192 (IgG1) was synthesized from GeneArt and subcloned into expression vector pCEP4(-E+I)Dest.

CAT192(IgG1) was produced from HEK293 transfection and purified with Protein A column. Remodeling CAT192 from IgG4 to IgG1, however, did not increase its affinity. Fab fragments generated from the IgG1 and IgG4 did not increase its affinity either. It was concluded that the high affinity of CAT191(scFv) (SEQ ID NO: 12) was lost during conversion to a full-length antibody format, whether it was a IgG1 or IgG4. This was unexpected, because scFv components obtained from a library are often engineered to a full-length IgG format for therapeutic development.

### Example 3: Various Dimer Designs

CAT191(scFv) (SEQ ID NO: 12) was found to bind TGF $\beta$ 1 with high affinity, using surface plasmon resonance, but CAT191(scFv) lacked the avidity needed for effective neutralization of TGF $\beta$ 1. Accordingly, various other formats were tested,



using the scFv component as a basic building block. General formats of antibody fragments, including the tested formats, are depicted in FIG. 1.

Tested formats included a diabody, a peptide-derived dimer (*e.g.*, a leucine zipper peptide-derived dimer), and an scFv-Fc dimer. scFv CAT191 diabody had the (Gly4Ser)<sub>3</sub>-type linker replaced with a short 5aa linker (GSSGG) (SEQ ID NO: 19) to create a non-covalent divalent binder (diabody dimer). Each monomer had the sequence set forth in SEQ ID NO: 14. Each monomer of the leucine zipper peptide-derived dimer had the sequence set forth in SEQ ID NO: 16. Finally, each monomer of the scFv-Fc dimer had the sequence set forth in SEQ ID NO: 9. The diabody and the peptide derived dimer were expressed in *E. Coli* and the scFv-Fc was expressed in HEK293 cells.

The leucine zipper peptide-derived dimer was difficult to express, and the partially purified dimer only showed intermediate affinity, as measured by surface plasmon resonance. The diabody (scFv 5aa) only showed intermediate affinity, but no avidity. By contrast, a scFv-Fc dimer produced from transient HEK293 transfection was found to bind to TGFβ1 specifically with high affinity and avidity. The binding results expressed as apparent dissociation constants obtained with surface plasmon resonance are summarized below in Table 2.

**Table 2: Binding Results for scFv-Fc Dimer**

Sample	24 RU TGFβ1 K <sub>d</sub> (nM)	105 RU TGFβ1 K <sub>d</sub> (nM)	544 RU TGFβ1 K <sub>d</sub> (nM)	
scFv-Fc	0.5	0.2	0.09	Avidity
CAT191 scFv	1.7	1.6	1.3	No Avidity
scFv Saa	4.1	3.9	4.8	

20

The TGFβ1 neutralizing potency of various formats was also compared in the A549 cell-based bioassay. FIG. 3 shows the A549 bioassay results for the diabody (“scFv diabody 5aa”), CAT191(scFv) (“scFv”), the scFv-Fc dimer (“CAT191(scFv-Fc)”), and CAT192(IgG4) (“CAT192”). As seen in FIG. 3, the scFv-Fc dimer demonstrated an apparent dissociation constant in this assay over four orders of magnitude lower than CAT192 (~10<sup>-3</sup> nM versus ~10<sup>1</sup> nM).

25

#### Example 4: scFv-Fc Clone

CAT191(scFv-Fc) was cloned and produced in larger scale in CHO cells. CAT191 scFv-Fc coding sequence was PCR amplified from a pCEP4 based

30

expression vector using a gene specific forward and reverse primer set. As part of the PCR amplification the following changes were introduced to the CAT191 scFv-Fc coding sequence: 1) addition of endonuclease sites at the 5' and 3' ends, 2) addition of Kozak consensus sequence immediately upstream of the start codon, 3) change of the "TAG" stop codon to "TAA", and 4) mutation of the thymidine 4 nucleotides upstream of the stop codon to a guanosine thus eliminating an endogenous splice donor site. The splice donor site mutation did not result in an amino acid change.

The PCR amplified CAT191 coding sequence was subcloned into a shuttle vector to facilitate sequence verification and molecular cloning. After sequence verification, the CAT191 coding sequence was cloned into Genzyme expression vectors pGZ600 and pGZ620. Both vectors used the hamster  $\beta$ -actin promoter to drive expression of the CAT191 transgene. They also contained the DHFR selectable marker that was driven by a separate promoter (SV40) to enable selection in CHO cells. CHO-8D6 host cell line was transfected with either the pGZ600-CAT191 or pGZ620-CAT191 expression plasmid. Following a brief recovery period, the transfected cells were placed into nucleotide-deficient growth medium for selection to generate pools of stable transfectants. After pools recovered from selection, a second round of selection was performed in the presence of 20nM methotrexate. The CHO pools selected this way was scaled up and the conditioned media was used for purification using Protein A column.

The CHO cell-produced protein was characterized by SDS-PAGE, Biacore binding, SEC-HPLC, and the A549 cell potency assay. The results confirmed that the scFv-Fc dimer had a higher affinity and potency, and it specifically neutralized TGF $\beta$ 1. The potency compared favorably to the pan-specific GC1008 antibody (FIG. 6 and FIG. 7).

### **Example 5: Circulation Half-Life**

The circulation half-life of CAT191(scFv-Fc) was tested in a mouse model using the study design depicted in Table 3.



Table 3: Circulation Half-Life of scFv-Fc Dimer

Group	Animal #'s	Test Article	Dose (mg/kg)	Dose Route	Time Points
1	1-8	scFv-Fc	1.0	IP	2, 6, 24, 72, 144, 240, and 336 hours post-dose
2	9-16	scFv-Fc	1.0	IV	0.25, 6, 24, 72, 144, 240, and 336 hours post-dose

Blood was drawn from the retro-orbital plexus at the specified times after intraperitoneal (IP) or intravenous (IV) administration. Approximately 60  $\mu$ L of whole blood was collected into hematocrit tubes and processed for serum. All samples were stored at -80°C until analysis. The CAT191(scFv-Fc) concentration was determined by ELISA. The results of this pharmacokinetic study are depicted in FIG. 4 and FIG. 5. The results suggested a circulation half-life of 1.5-2.0 days, much longer than that for a typical scFv molecule, which is several hours.

Example 6: scFv-Fc Dimer Stability

The stability of CAT191(scFv-Fc) stored at -80°C was monitored for a year by SEC-HPLC, Biacore TGF $\beta$ 1 binding, and the A549 potency assay. No change in aggregation, affinity, or potency was observed during the test period. Material stored at 4°C displayed a slight but steady increase in aggregation over 1 year. The unique combination of the smaller size, high selectivity, potency against TGF $\beta$ 1, and long *in vivo* half-life made CAT191(scFv-Fc) an ideal candidate for therapeutic applications.

All documents cited throughout this disclosure, including but not limited to scientific publications, patents and publication of patent applications, are hereby incorporated by reference in this disclosure as if the full contents are reproduced herein.

SEQUENCE LISTING

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

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI  
SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS  
GYDTPQYSWGQGTTVTVSS

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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI  
SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS  
GYDTPASPDWGQGTTVTVSS

**SEQ ID No. 3: Synthetic linker**

SGGGSGGGGSGGGGS

**SEQ ID No. 4: Synthetic linker**

5 GGGGSGGGGSGGGGS

**SEQ ID No. 5: Human IgG1 Vk domain Clone SL15A: (SQN6 US6492497)**

10 EIVLTQSPSSLSASVGDRVTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL  
QSGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIK

**SEQ ID No. 6: Human IgG1 Vk domain Clone SL15S: (SQN8 US6492497)**

EIVLTQSPSSLSASVGDRVTITCRSSQGIGDDLGWYQQKPGKAPILLIYGTSTL  
QSGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIK

**15 SEQ ID No. 7: Human IgG1 hinge region**

PKSCDKTHTCPPCPAPELLGGP

**SEQ ID No. 8: Human IgG1 Fc region**

20 SVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK  
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ  
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTP  
PVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

**SEQ ID No. 9: CAT191(scFv-Fc)**

25 EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI  
SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS  
GYDTPQYSWGQGTTVTVSSSGGGSGGGGSGGGGSEIVLTQSPSSLSASVGD  
RVTITCRSSQGIGDDLGWYQQKPGKAPILLIYGTSTLQSGVPSRFSGSGSGTDF  
TLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIKGGSGPKSCDKTHTCPPCP  
30 APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV  
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE  
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ  
PENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT  
QKSLSLSPGK  
35

**SEQ ID No. 10: CAT192 (IgG4) Light Chain**

EWLTQSPSSLSASVGDRVTITCRASQGIGDDLGWYQQKPGKAPILLIYGTSTL  
QSGVPSRFSGSGSGTDFTLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIKR  
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ  
40 ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE  
C



**SEQ ID No. 11: CAT192 (IgG4) Heavy Chain**

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI  
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS  
 GYDTPQYSWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYF  
 5 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTCTYTCNVD  
 HKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVT  
 CVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLTQH  
 DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV  
 SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSR  
 10 WQEGNVFSCSVMEALHNHYTQKSLSLSLGK

**SEQ ID No. 12: CAT191(scFv)**

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI  
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS  
 15 GYDTPQYSWGQGTTVTVSSSGGGSGGGGSGGGGSEIVLTQSPSSLSASVGD  
 RVTITCRSSQGIGDDL GWYQQKPGKAPILLIYGTSTLQSGVPSRFSGSGSGTDF  
 TLTINSLQPEDFATYYCLQDSNYPLTFGGGTTRLEIK

**SEQ ID No. 13: Diabody-5aa encoding nucleic acid**

20 atgaccatgattacgccaa gctttggagcctttttttggagatttcaacgtgaaaaattattatcgaattccttagttgttcc  
 tttctatgcggcccagccggccatggccgaggtgcagctggtggagtctgggggagggcgtggtccagcctgggaggtcc  
 ctgagactctcctgtgcagcctctggattcacctcagtagctatggcatgcactgggtccgccaggtccaggcaaggag  
 ctggagtgggtggcagttatatcatatgatggaagtattaaataactatgcagactccgtgaagggccgattcaccatctccag  
 agacaattccaagaacacgctgtatctgcaaatgaacagcctgagagctgaggacacggctgtgtattactgtgcgcgaac  
 25 tggatgaatatagtggctacgatacggacccccagtactcctgggggcaagggaccacggtcaccgtctcctcaggttctc  
 tggcgggtgaaattgtgctgactcagctcctcctcctgtctgcatctgtaggagacagagtcaccatcacttgcgggtcaa  
 gtcagggcattggagatgatttgggtggtatcagcagaagccagggaagccctatcctcctgatctatggtacatccac  
 ttacaaagtgggggtcccgtcaagggtcagcggcagtggtatctggcacagatttactctcaccatcaacagcctgcagcct  
 gaagattttgcaactattactgtctacaagattccaattacccgctcacttctggcggaggggacacgactggagattaaacgt  
 30 gcggccgcacatcatcaccatcacggggccgcagaacaaaactcatctcagaagaggatctgaatggggccgcac  
 agtagctcgagatcaaacgggctagccagccagaactcggcccggaagaccccgaggatgtcgagcaccaccaccac  
 ac

**SEQ ID No. 14: Diabody-5aa**

35 EVQLVESGGGVVQPGRSLRLSCAASGFTESSYGMHWVRQAPGKELEWVAVI  
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS  
 GYDTPQYSWGQGTTVTVSSGSSGGEIVLTQSPSSLSASVGDRVTITCRSSQGI  
 GDDL GWYQQKPGKAPILLIYGTSTLQSGVPSRFSGSGSGTDFTLTINSLQPEDF  
 ATYYCLQDSNYPLTFGGGTTRLEIKRAAAHHHHHHHGAAEQKLISEEDLNGAA  
 40

**SEQ ID No. 15: Leucine zipper peptide-derived dimer encoding nucleic acid**

gaggtgcagctggtggagtctgggggagggcgtggtccagcctgggaggtccctgagactctcctgtgcagcctctggatt  
 cacttcagtagctatggcatgcactgggtccgccaggtccaggcaaggagctggagtgggtggcagttatatcatatga  
 tggaaagtattaaataactatgcagactccgtgaagggccgattcaccatctccagagacaattccaagaacacgctgtatctg  
 45 caaatgaacagcctgagagctgaggacacggctgtgtattactgtgcgcgaactggtgaatatagtggctacgatacggac  
 cccagctactcctgggggcaagggaccacggtcaccgtctcctcaagtggagggcgttcaggcggaggtggcagcggc



ggtggcggatcggaattgtgctgactcagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccg  
 gtcaagtcagggcattggagatgatttgggctggtatcagcagaagccagggaaagcccctatcctcctgatctatgttaca  
 tccactttacaaagtgggggtcccgtcaaggttcagcggcagtgatctggcacagatttactctcaccatcaacagcctgc  
 agcctgaagattttgcaacttattactgtctacaagattccaattacccgctcactttcggcggaggacacgactggagatta  
 5 aacgtgcggccgcacatcatcatcaccatcacggggccgcagaacaaaaactcatctcagaagaggatctgaatggggc  
 cgcacccaagcccagtagccccccaggttcttcaggcgaactggaagaactgctgaaacatctgaaagaactgctgaaag  
 gcccgcgtaaaggcgaactggaagaactgctgaaacatctgaaagaactgctgaaaggcgggtgcgccgggcgggtcatc  
 atcatcaccatcat

#### 10 SEQ ID No. 16: Leucine zipper peptide-derived dimer

EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKELEWVAVI  
 SYDGSIKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARTGEYS  
 GYDTPQYSWGQGTTVTVSSSGGGSGGGGSGGGGSEIVLTQSPSSLSASVGD  
 RVTITCRSSQGIGDDL GWYQQKPGKAPILLIYGTSTLQSGVPSRFSGSGSGTDF  
 15 TLTINSLQPEDFATYYCLQDSNYPLTFGGGTRLEIKRAAAHHHHHHHGAAEQK  
 LISEEDLNGAAPKPSTPPGSSGELEELLKHLKELLKGPRKGELEELLKHLKELL  
 KGGAPGGHHHHHH

#### SEQ ID No. 17: CAT191(scFv-Fc) encoding nucleic acid

20 gaggtgcagctggtggagtctgggggagggcgtggtccagcctgggaggtccctgagactctcctgtgcagcctctggatt  
 cacttcagtagctatggcatgcactgggtccgccaggctccaggcaaggagctggagtgggtggcagttatatcatatga  
 tggaagtattaaatactatgcagactccgtgaaggggcggattcaccatctccagagacaattccaagaacacgctgtatctg  
 caaatgaacagcctgagagctgaggacacggctgtgtattactgtgcgcgaactggtgaatatagtggctacgatacggac  
 cccagtagtctctgggggcaaggggaccacggtcaccgtctcctcaagtggagggcgggtcaggcggaggtggcagcggc  
 25 ggtggcggatcggaattgtgctgactcagtctccatcctccctgtctgcatctgtaggagacagagtcaccatcacttgccg  
 gtcaagtcagggcattggagatgatttgggctggtatcagcagaagccagggaaagcccctatcctcctgatctatgttaca  
 tccactttacaaagtgggggtcccgtcaaggttcagcggcagtgatctggcacagatttactctcaccatcaacagcctgc  
 agcctgaagattttgcaacttattactgtctacaagattccaattacccgctcactttcggcggaggacacgactggagatta  
 aaggtggcagcggacctaatacttgtgacaaaactcacacatgccaccgtgccagcactgaactcctgggggggacc  
 30 gtcagtcttctcttccccccaaaacccaaggacaccctcatgatctcccggaccctgaggtcacatgcgtggtggtggac  
 gtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgc  
 gggaggagcagtagaacagcaggtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactgggtgaatggcaagga  
 gtacaagtgaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaagggcagccccga  
 gaaccacaggtgtacaccctgccccatcccgggatgagctgaccaagaaccaggtcagcctgacgtgcctggtcaaag  
 35 gcttctatcccagcagatcgccgtggagtgggagagcaatgggcagccggagaacaactacaagaccacgcctcccgt  
 gctggactccgacggctccttcttctctacagcaagctcaccgtggacaagagcagatggcagcaggggaacgtcttctc  
 atgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatagtag

#### SEQ ID No. 18: Human TGFβ1

40 ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIW  
 SLDTQYSKVLALYNQHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNM  
 IVRSCKCS

#### SEQ ID No. 19

45 GSSGG



**SEQ ID No. 20**

GGSG

**SEQ ID No. 21**

5 CPPCP

**SEQ ID No. 22**

SYGMH

**SEQ ID No. 23**

10 VISYDGSIKYYADSVKG

**SEQ ID No. 24**

TGEYSGYDTSGVEL

15

**SEQ ID No. 25**

TGEYSGYDTPQYS

**SEQ ID No. 26**

20 TGFYSGYDTPASPD

**SEQ ID No. 27**

RASQGIGDDLQ

**SEQ ID No. 28**

25 GTSTLQS

**SEQ ID No. 29**

LQDSNYPLT

30

**SEQ ID No. 30**TGX<sub>1</sub>YSGYDTX<sub>2</sub>X<sub>3</sub>X<sub>4</sub>X<sub>5</sub>X<sub>6</sub>

## CLAIMS

1. An isolated binding protein that binds TGF $\beta$ 1, wherein said binding protein comprises a first polypeptide chain and a second polypeptide chain, the first and the second polypeptide chains both having the formula of, from N-terminal to C-terminal:  

$$(\text{VH domain})-(\text{linker1})_n-(\text{VL domain})-(\text{linker2})_m-(\text{hinge})_p-(\text{Fc region}),$$
wherein the VH domain of each of the first and second polypeptide chains 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), said HCDR1 comprising the amino acid sequence of SEQ ID NO: 22; said HCDR2 comprising the amino acid sequence of SEQ ID NO: 23; and said HCDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 30;  
wherein the VL domain of each of the first and second polypeptide chains 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), said LCDR1 comprising the amino acid sequence of SEQ ID NO: 27, said LCDR2 comprising the amino acid sequence of SEQ ID NO: 28, and said LCDR3 comprising the amino acid sequence of SEQ ID NO: 29; and  
wherein p is 0 or 1, n is 0 or 1, and m is 0 or 1.
2. The binding protein of claim 1, wherein the first and second polypeptide chains are identical and form a dimer.
3. The binding protein of any of claims 1-2, wherein the framework regions of the VH domain of the first or second polypeptide chain are selected from the same variable heavy germline sequence, and the framework regions of the VL domain of the same polypeptide chain are selected from the same variable lambda or kappa germline sequence.
4. The binding protein of any of claims 1-3, wherein the VH domain of the first or second polypeptide chain comprises the human VH domain sequences set



forth in SEQ ID NO: 1 or SEQ ID NO: 2, or a variant thereof having up to four amino acid modifications; and

wherein the VL domain of the same polypeptide chain comprises the human V $\kappa$  domain sequences set forth in SEQ ID NO: 5 or SEQ ID NO: 6, or a variant thereof having up to four amino acid modifications.

5. The binding protein of any of claims 1-4, wherein the VH domain of the first or second polypeptide chain comprises the sequence set forth in SEQ ID NO: 1 and the VL domain of the same polypeptide chain comprises the sequence set forth in SEQ ID NO: 5.
6. The binding protein of any of claims 1-5, wherein the first polypeptide chain or the second polypeptide chain comprises the sequence set forth in SEQ ID NO: 9.
7. The binding protein of any of claims 1-6, wherein the binding protein selectively binds TGF $\beta$ 1.
8. The binding protein of any of claims 1-7, wherein the binding protein has an IC50 to human TGF $\beta$ 1 of less than 1 nM in an A549 bioassay.
9. The binding protein of any of claims 1-8, wherein the binding protein has an IC50 to human TGF $\beta$ 1 of less than 0.1 nM in an A549 bioassay.
10. The binding protein of any of claims 1-9, wherein n is 1 and the linker1 is about 15 amino acids in length.
11. The binding protein of any of claims 1-10, wherein n is 1 and the linker1 comprises the amino acid sequence SGGGSGGGGSGGGGS (SEQ ID NO: 3), the amino acid sequence GGGGSGGGGSGGGGS (SEQ ID NO: 4), or a variant thereof having up to four amino acid modifications.
12. The binding protein of any of claims 1-11, wherein p is 1, and the hinge comprises an amino acid sequence from a human IgG1 or IgG4 hinge region.

13. The binding protein of any of claims 1-12, wherein the hinge comprises the amino acid sequence PKSCDKTHTCPPCPAPPELLGGP (SEQ ID NO: 7), or a variant thereof having up to four amino acid modifications.
14. The binding protein of any of claims 1-12, wherein the hinge comprises the amino acid sequence CPPCP (SEQ ID NO: 21).
15. The binding protein of any of claims 1-14, wherein m is 1 and the linker2 comprises the amino acid sequence GGSG (SEQ ID NO: 20), or a variant thereof having up to 2 amino acid modifications.
16. The binding protein of any of claims 1-15, wherein the Fc region comprises constant domains CH2 and CH3.
17. The binding protein of any of claims 1-16, wherein the Fc region is derived from a human IgG1, a human IgG4, or a variant of a human IgG1 or IgG4 wherein up to ten amino acid may be modified.
18. An isolated binding protein that binds TGF $\beta$ 1, wherein said binding protein comprises a polypeptide chain having the formula of, from N-terminal to C-terminal:  

(VH domain)-(linker1)<sub>n</sub>-(VL domain)-(linker2)<sub>m</sub>-(hinge)<sub>p</sub>-(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), said HCDR1 having the amino acid sequence of SEQ ID NO: 22; said HCDR2 having the amino acid sequence of SEQ ID NO: 23; and said HCDR3 having the amino acid sequence selected from the group consisting of SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 30;  
 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), said LCDR1 having the amino acid sequence of SEQ ID NO: 27, said LCDR2 having the amino acid sequence of SEQ ID NO: 28, and said LCDR3 having the amino acid sequence of SEQ ID NO: 29; and



wherein p is 0 or 1, n is 0 or 1, and m is 0 or 1.

19. An isolated binding protein comprising a variable domain that is capable of binding TGF $\beta$ 1, wherein said binding protein exhibits a K<sub>d</sub> for human TGF $\beta$ 1 at least about 50% lower than said binding protein's K<sub>d</sub> for human TGF $\beta$ 2 as measured by surface plasmon resonance.
20. An isolated binding protein comprising a variable domain that is capable of binding TGF $\beta$ 1, wherein said binding protein exhibits a K<sub>d</sub> for human TGF $\beta$ 1 at least about 50% lower than said binding protein's K<sub>d</sub> for human TGF $\beta$ 3 as measured by surface plasmon resonance.
21. An isolated binding protein comprising a variable domain that is capable of binding TGF $\beta$ 1, wherein said binding protein exhibits a K<sub>d</sub> for human TGF $\beta$ 1 at least about 50% lower than said binding protein's K<sub>d</sub> for human TGF $\beta$ 2 and at least about 50% lower than said binding protein's K<sub>d</sub> for human TGF $\beta$ 3 as measured by surface plasmon resonance.
22. An isolated binding protein that binds TGF $\beta$ 1, wherein said binding protein comprises a first polypeptide chain and a second polypeptide chain, the first and the second polypeptide chains each having the formula of:  

$$(VD_1)-(linker1)_n-(VD_2)-(linker2)_m-(hinge)_p-(Fc\ region),$$
 wherein VD<sub>1</sub> comprises a first variable domain selected from the group consisting of a VL domain isolated from an antibody capable of binding TGF $\beta$ 1, and a VH domain isolated from an antibody capable of binding TGF $\beta$ 1, and VD<sub>2</sub> comprises a second variable domain selected from the group consisting of a VL domain isolated from an antibody capable of binding TGF $\beta$ 1, and a VH domain isolated from an antibody capable of binding TGF $\beta$ 1; and  
 wherein, n is 0 or 1, m is 0 or 1, and p is 0 or 1.
23. An isolated binding protein that binds TGF $\beta$ 1, wherein said binding protein comprises a first polypeptide chain and a second polypeptide chain, the first and the second polypeptide chains both having the formula of, from N-terminal to C-terminal:

$$(VH\ domain)-(linker1)_n-(VL\ domain)-(linker2)_m-(hinge)_p-(Fc\ region),$$

wherein p is 0 or 1, n is 0 or 1, and m is 0 or 1, and wherein said binding protein selectively binds TGF $\beta$ 1.

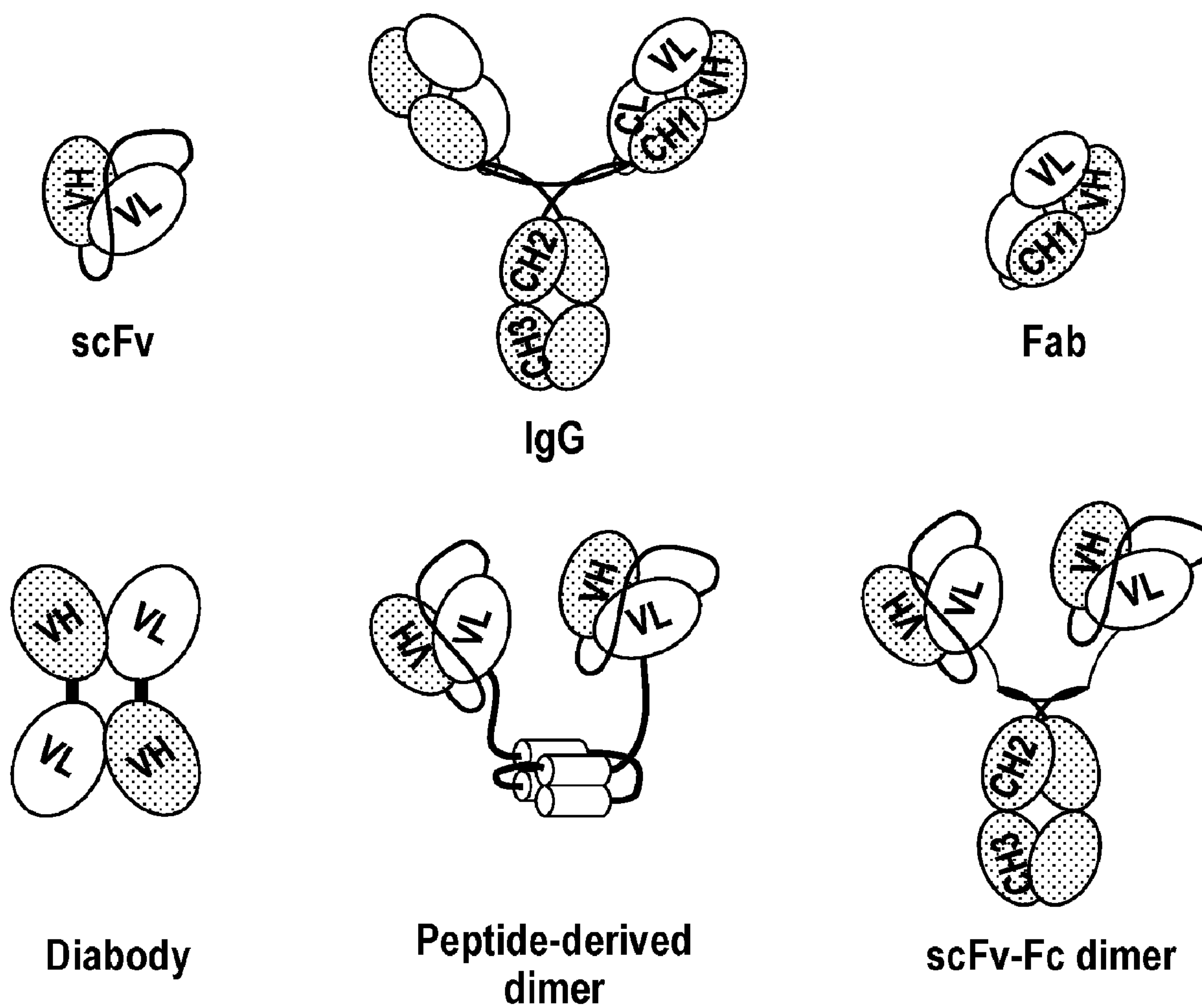
24. An isolated polynucleotide comprising a nucleotide sequence encoding the binding protein of any of claims 1-23.
25. The isolated polynucleotide of claim 24 comprising the nucleotide sequence set forth in SEQ ID NO: 17.
26. A vector comprising the polynucleotide of any of claims 24-25.
27. A host cell comprising the polynucleotide of any of claims 24-25.
28. The host cell of claim 27, wherein the host cell is a human cell.
29. The host cell of any of claims 27-28, wherein the host cell is a Human Embryonic Kidney 293 (HEK293) cell.
30. The host cell of claim 27, wherein the host cell is a Chinese Hamster Ovary cell.
31. A method of making the binding protein of any of claims 1-23, comprising culturing the host cell of claim 27 under suitable conditions to produce the binding protein.
32. The method of claim 31, further comprising purifying the binding protein.
33. A composition comprising the binding protein of any of claims 1-23.
34. The composition of claim 33, wherein the composition is a pharmaceutical composition comprising a therapeutically effective amount of the binding protein.
35. The composition of any of claims 33-34, further comprising one or more biologically active components, excipients, or diluents.
36. A method of treating a disease or condition resulting directly or indirectly from TGF $\beta$ 1 activity in a human comprising administering to a human a



pharmaceutical composition comprising a therapeutically effective amount of the binding protein of any of claims 1-23.

37. The method of claim 36, wherein the disease or condition is selected from the group consisting of a fibrotic disease, cancer, an immune-mediated disease, and a combination thereof.
38. The method of any of claims 36-37, where the disease is diffuse cutaneous systemic sclerosis.
39. Use of the binding protein of any of claims 1-23 in the manufacture of a medicament for treatment of a disease or disorder selected from the group consisting of a fibrotic disease, cancer, an immune-mediated disease, and a combination thereof.
40. The use of claim 39, wherein the disease is diffuse cutaneous systemic sclerosis.
41. The use of claim 39, wherein the disease is bone remodeling disease
42. The use of claim 39, wherein the disease is kidney disease.

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**Fig. 1**



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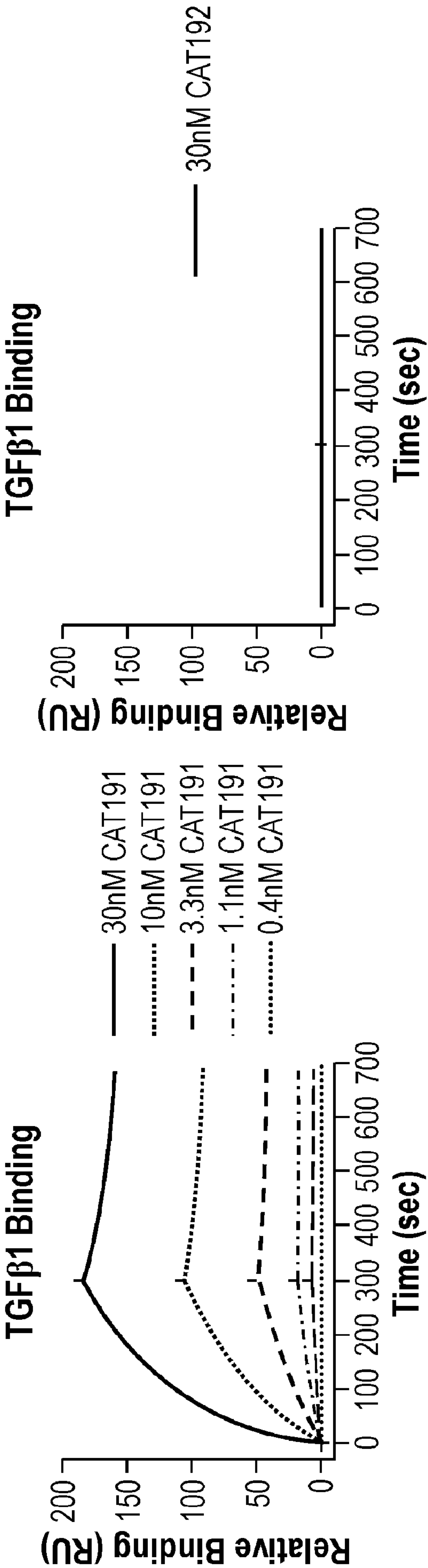
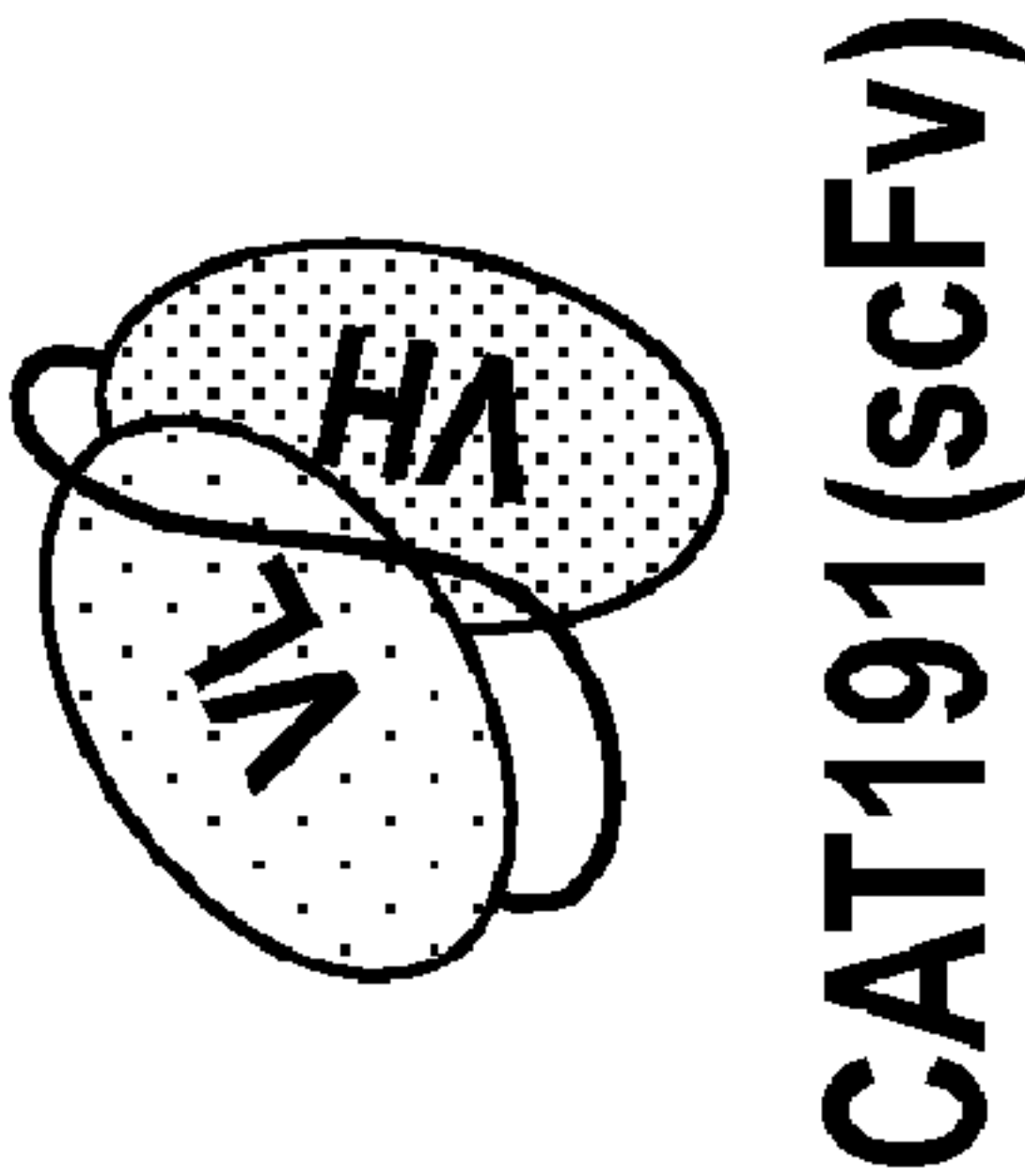
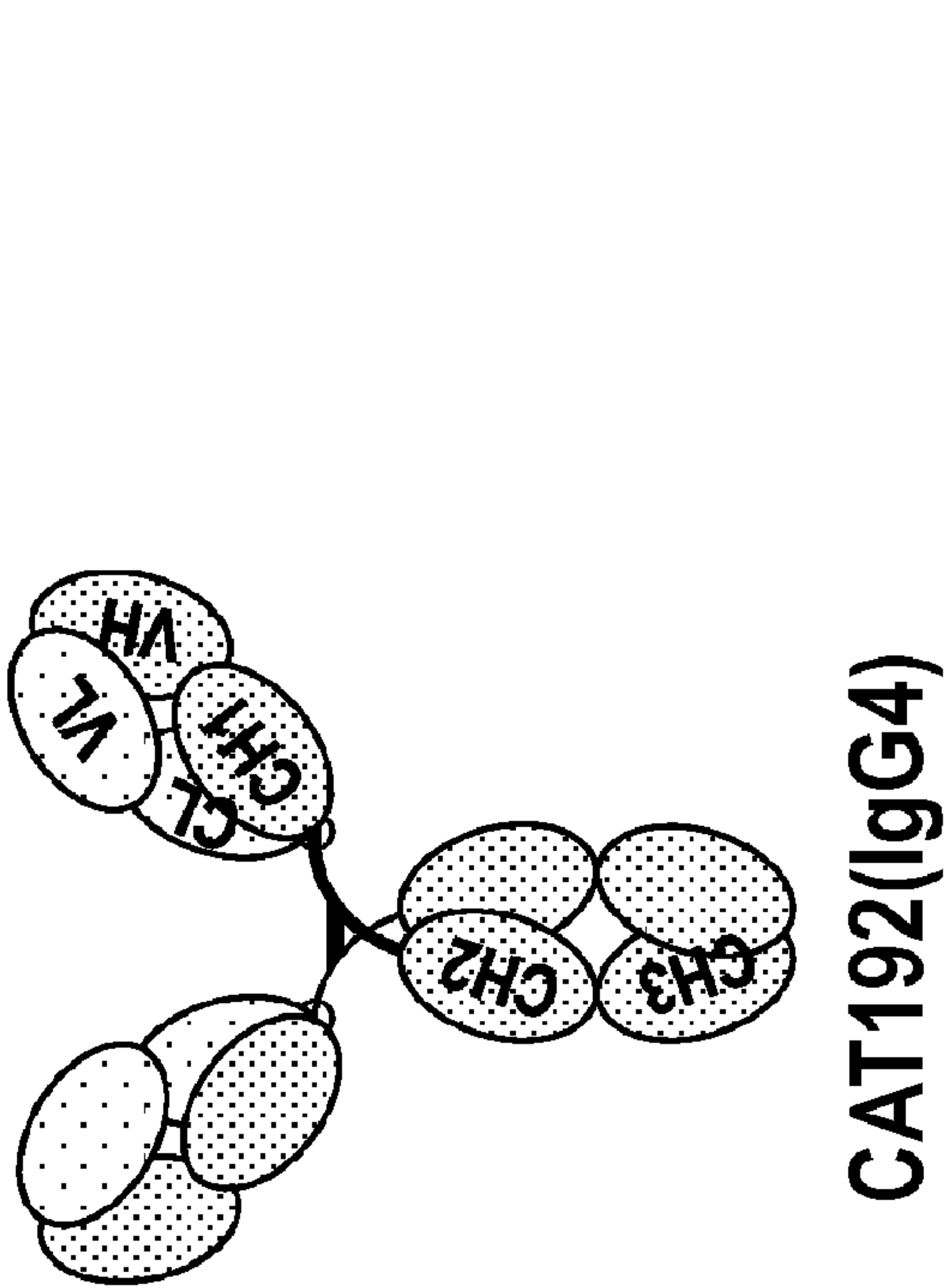
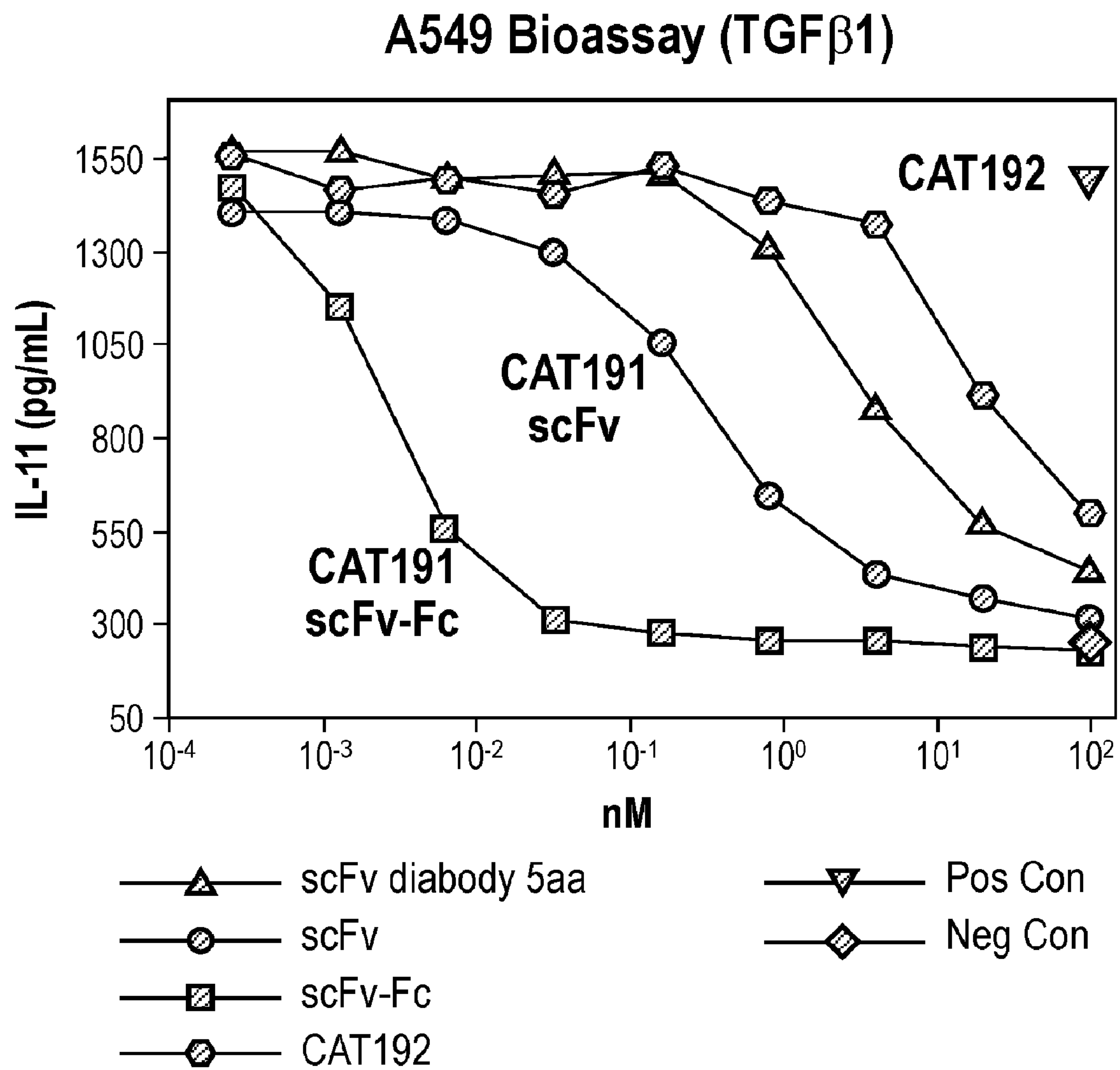


Fig. 2

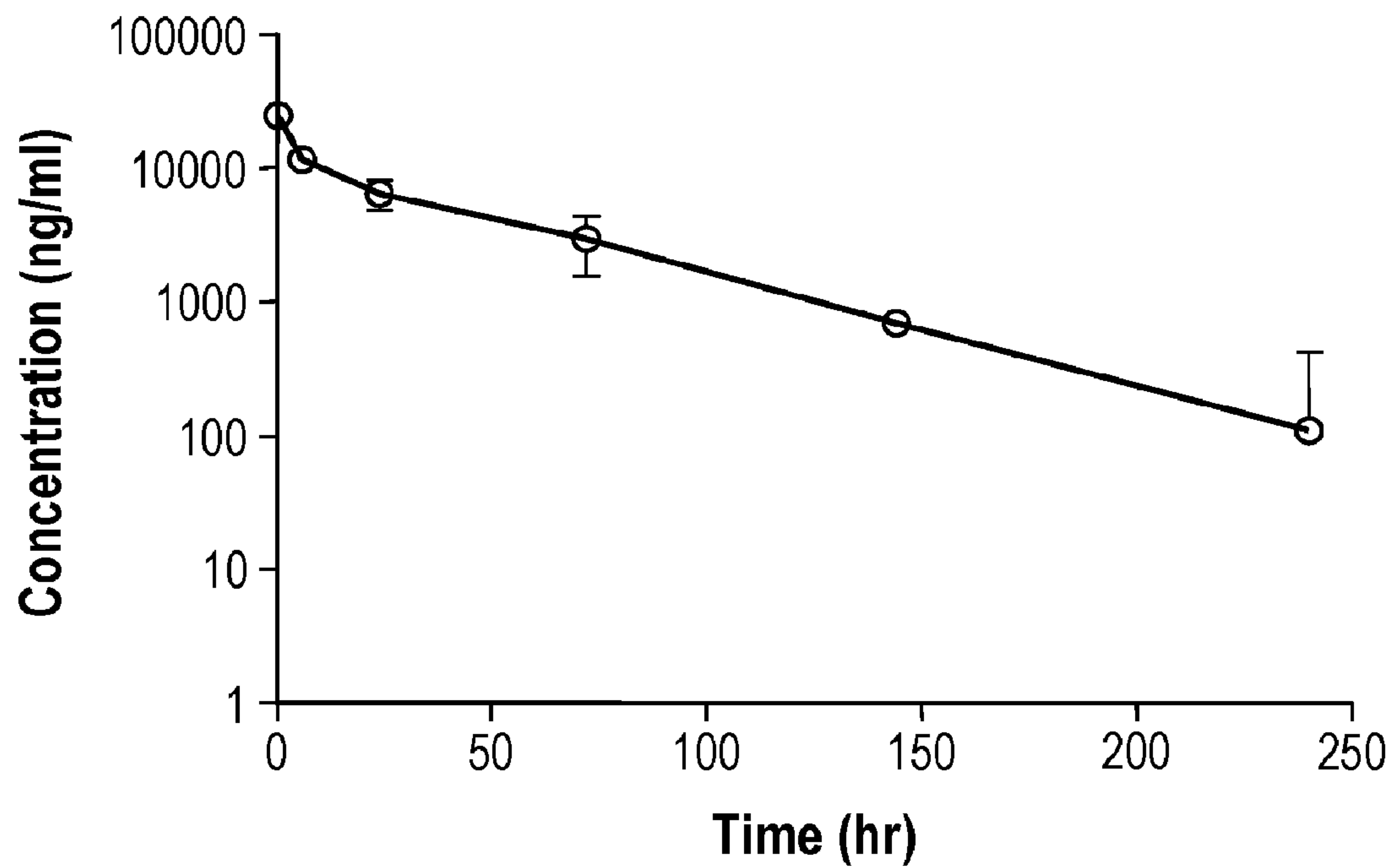
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**Fig. 3**

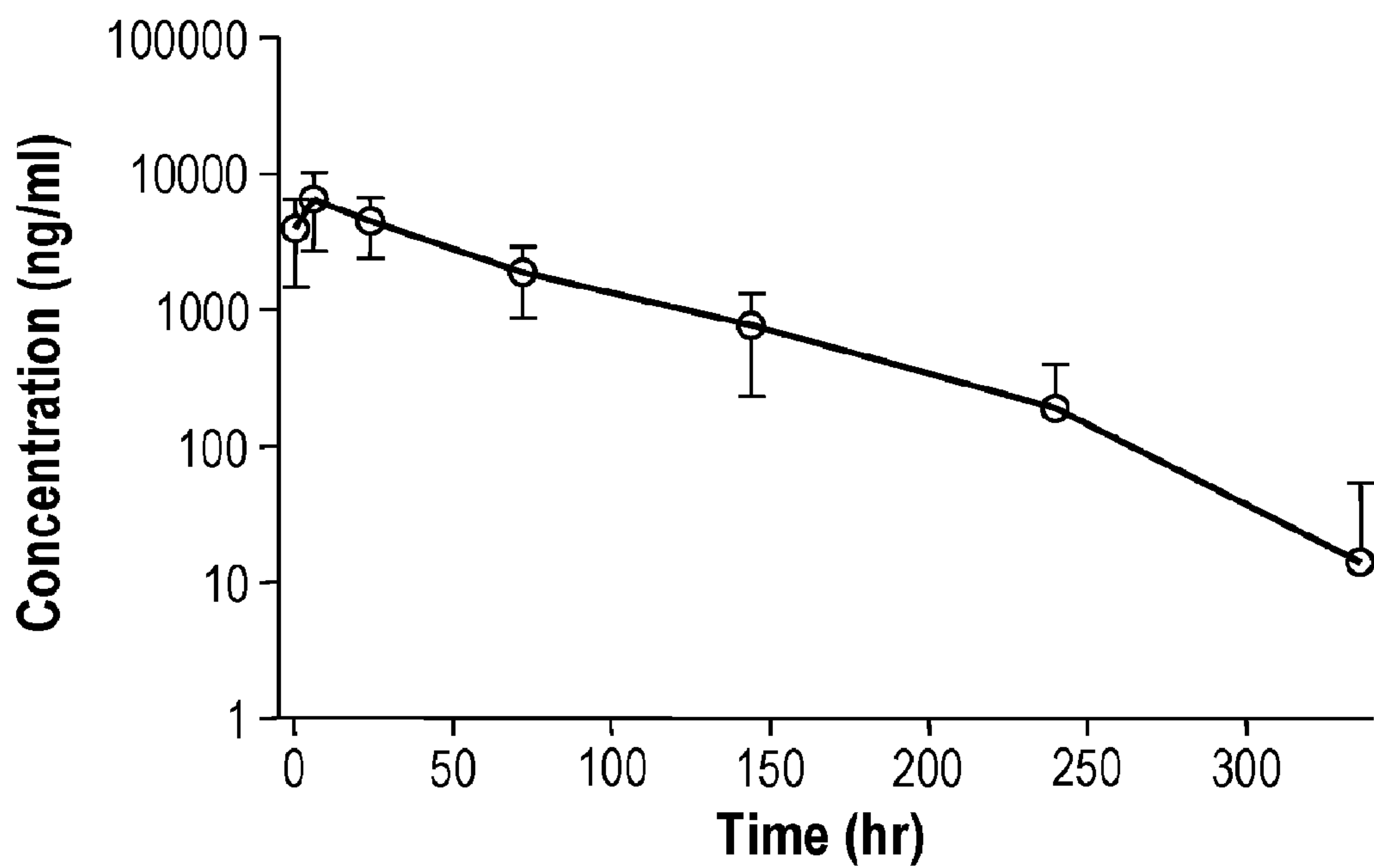


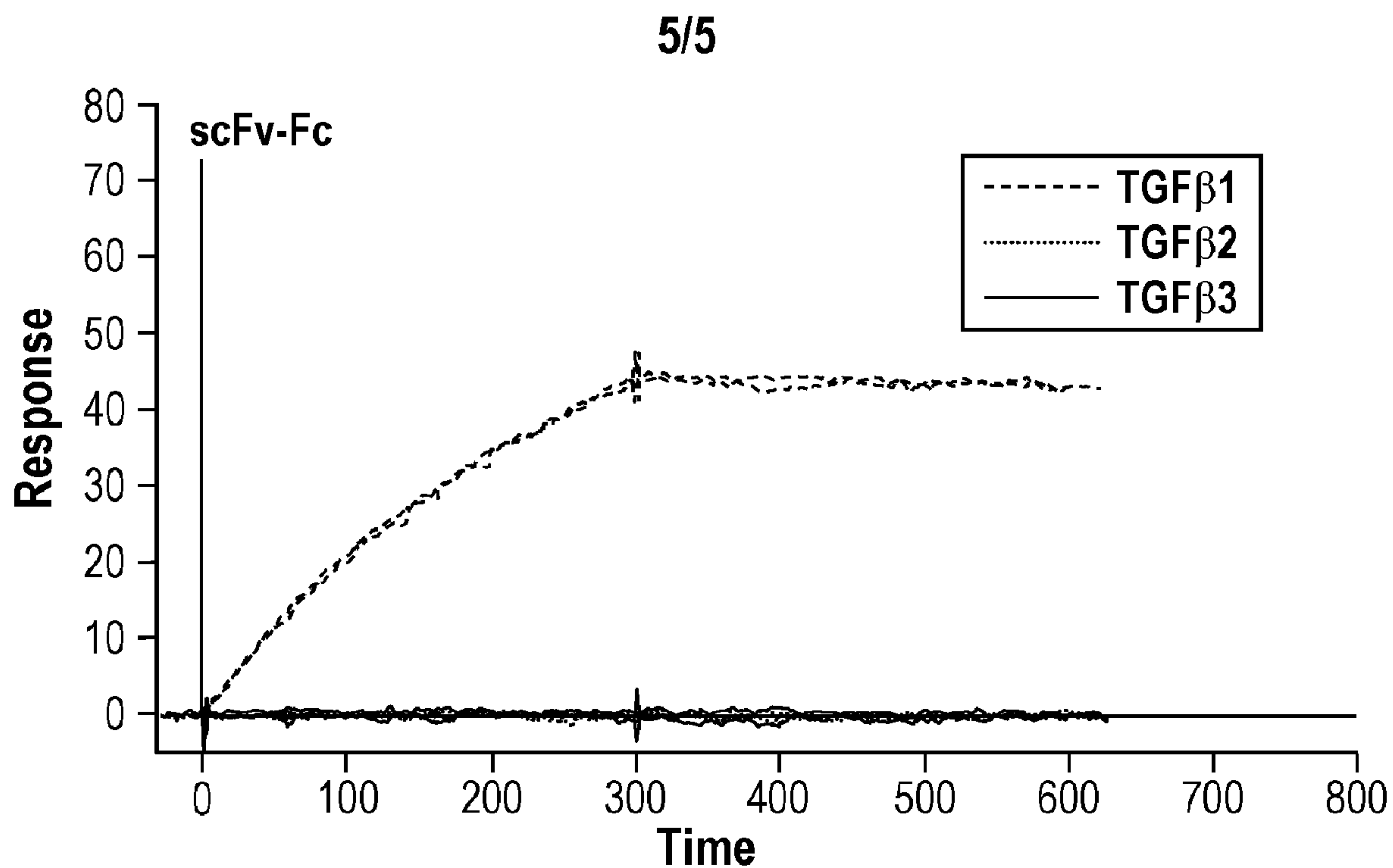
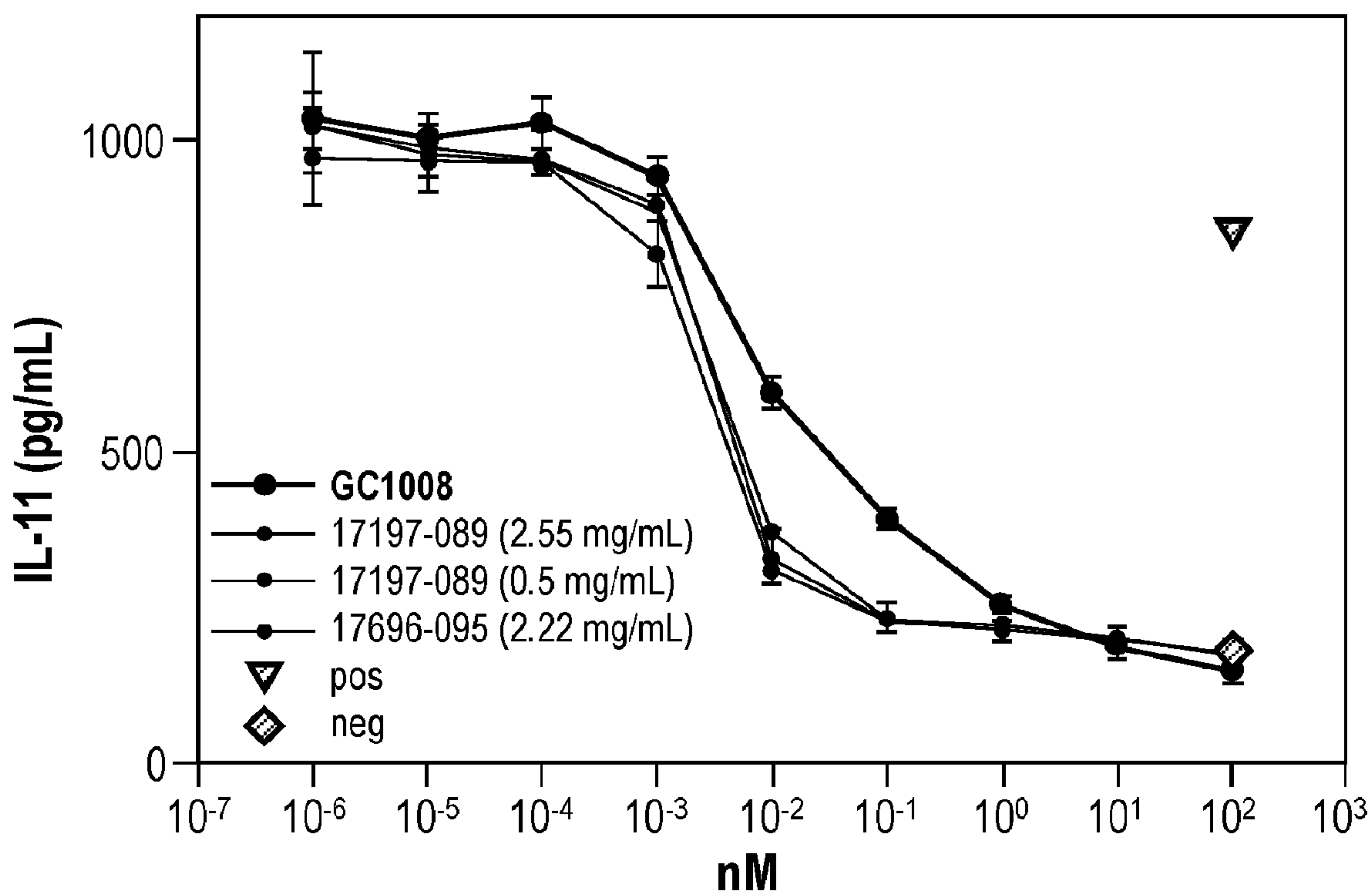
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IV

**Fig. 4**

IP

**Fig. 5**

**Fig. 6****Inhibition of TGFbeta1 by scFv-Fc fusions****Fig. 7**