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(57) **Abrégé/Abstract:**

The present disclosure relates to TGF-beta antibodies and binding fragments thereof, DNA encoding the same, host cells comprising said DNA and methods of expressing the antibody or binding fragment in a host cell. The disclosure also extends to pharmaceutical compositions comprising the antibody or a binding fragment thereof and use of the antibody, binding fragment and compositions comprising the same in treatment of various diseases including fibrosis.

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(57) **Abstract:** The present disclosure relates to TGF-beta antibodies and binding fragments thereof, DNA encoding the same, host cells comprising said DNA and methods of expressing the antibody or binding fragment in a host cell. The disclosure also extends to pharmaceutical compositions comprising the antibody or a binding fragment thereof and use of the antibody, binding fragment and compositions comprising the same in treatment of various diseases including fibrosis.

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ANTAGONIST ANTIBODIES THAT T BIND TO HUMAN TGFB1 , TGFB2 AND TO TGFB3 AND THEIR USE FOR THE TREATMENT OF LUNG FIBROSIS

BACKGROUND OF THE INVENTION

There are 3 TGF-beta isoforms present in humans, TGF-beta 1, TGF-beta 2 and TGF-beta 3. The isoforms are homologous and share ~70% sequence identity. They are all synthesised and secreted as a latent complex in which TGF-beta is complexed with two other polypeptides, latent TGF-beta binding protein (LTBP) and latency-associated peptide (LAP) (a protein derived from the N-terminal region of the TGF-beta gene product). Serum proteinases such as plasmin catalyze the release of active mature TGF-beta from the complex.

In their active forms, TGF-beta isoforms exist as a ~25KDa homodimeric protein. All 3 isoforms signal via the same transmembrane receptors TbetaRI and TbetaRII. TGF-beta first binds to TbetaRII which then forms a heterotetrameric complex with TbetaRI, leading to phosphorylation of TbetaRI and activation of subsequent signalling pathways (see Derynck & Miyazono (eds), 2008, The TGF-beta Family, Cold Spring Harbor Press). Despite signalling via the same receptor complex, distinct non-overlapping functions of the 3 isoforms have been noted which is exemplified by mice containing genetic deletions of the individual isoforms each having different phenotypes (Shull *et al.*, 1992, Nature 359: 693-699; Sanford *et al.*, 1997, Development 124: 2659-2670; Proetzel *et al.*, 1995, Nature Genet., 11: 409-414).

TGF-beta is a pleotropic molecule involved in a range of biological processes. TGF-beta inhibits the proliferation of many cell types, including epithelial, endothelial, haematopoietic and immune cells. The effector functions of immune cells are also responsive to TGF-beta and TGF-beta suppresses Th1 and Th2 cell differentiation whilst stimulating Treg cells, thus TGF-beta has a predominantly immunosuppressive function (Li *et al.*, 2006, Ann Rev Immunol., 24: 99-146; Rubtsov & Rudensky, 2007, Nat Rev Immunol., 7: 443-453). TGF-beta expression is highly regulated and involved in maintenance of tissue homeostasis. However chronic over expression of TGF-beta is linked with driving disease progression in disease states such as cancer and fibrosis.

Due to the role of human TGF-beta in a variety of human disorders, therapeutic strategies have been designed to inhibit or counteract TGF-beta activity. In particular, antibodies that bind to, and neutralize, TGF-beta have been sought as a means to inhibit TGF-beta activity.

Antibodies to TGF-beta are known in the art. A systemically administered anti-TGF-beta1 antibody (CAT-192) was evaluated in a Phase I/II trial in systemic sclerosis patients, with no evidence of efficacy with doses up to 10mg/kg (Denton *et al.*, 2007, *Arthritis Rheum*, 56: 323-333). A humanised antibody (TbetaM1) optimised for activity against TGF-beta1 was assessed in a Phase1 trial in patients with metastatic cancer, but no anti-tumor effect was noted (Cohn *et al.*, 2014, *Int J Oncol.*, 45: 2221-2231). A human TGF-beta2 antibody (CAT-152) was evaluated for prevention of scarring after trabeculectomy, but no difference from placebo was noted (CAT-152 0102 Trabeculectomy Study Group, 2007, *Ophthalmology*, 114: 1822-1830). A systemically administered full length IgG specific for TGF-beta1, 2 and 3 (Fresolimumab, GC1008) has been investigated for the treatment of certain cancers and fibrotic disease. However, side effects have been reported including skin lesions that appear to be associated with systemic delivery of the antibody (Lacouture *et al.*, 2015, *Cancer Immunol Immunother.*, 64: 437-446).

Fibrosis is an aberrant response to wound healing wherein excess fibrous connective tissue is formed in an organ or tissue. In the remodelling phase during normal wound healing, synthesis of new collagen exceeds the rate at which it is degraded, resulting in scar formation. The final process of normal wound healing is scar resolution which occurs through a combination of reduced collagen synthesis and increased collagen degradation, a process controlled by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPS) produced by granulocytes, macrophages, epidermal cells and myofibroblasts. Thus wound healing involves a shift in metabolic equilibrium from stimulation of deposition followed by resolution. Any disruption in this equilibrium may result in excessive deposition of matrix components resulting in hardening and scarring of tissues and destruction of normal tissue architecture and a compromise in tissue function; this disruption is termed fibrosis.

Abnormal epithelial-mesenchymal interactions, altered fibroblast phenotypes, exaggerated fibroblast proliferation, and excessive deposition of collagen and extracellular matrix are all the key processes which contribute to fibrotic disease. A key cell type in this process is the myofibroblast. Activation of myofibroblasts results in their increased secretion of types I, III and IV collagen, fibronectin, laminin and proteoglycans. Other cell types considered to play a prominent role in fibrosis include epithelial cells and macrophages. TGF-beta is considered to be

a master regulator of fibrosis and contributes to the fibrotic process via actions on several cell types including macrophages and fibroblasts (Leask & Abraham, 2004, FASEB J., 18: 816-827). Key profibrotic activities include the stimulation of fibroblast migration and the transformation of fibroblasts to myofibroblasts, stimulating excessive ECM deposition. TGF-beta is also involved in macrophage migration and stimulates the production of mesenchymal growth factors from macrophages such as PDGF, as well as inhibiting ECM degradation through the increased expression of protease inhibitors such as TIMP3.

Fibrotic diseases are a leading cause of morbidity and mortality and can affect many tissue and organ systems. Included in this group of diseases are interstitial lung diseases. Idiopathic pulmonary fibrosis (IPF) is the most common form of interstitial lung diseases and is one of seven distinct groups of idiopathic interstitial pneumonias (IIP). The interstitium is the microscopic space between the basement membranes of the alveolar epithelium and capillary endothelium, and forms part of the blood-gas barrier. IIPs are characterised by expansion of the interstitial compartment by inflammatory cells, with associated fibrosis particularly noted for IPF.

IPF patients present with progressive exertional dyspnoea and cough with progressive pulmonary parenchymal fibrosis, resulting in pulmonary restriction and hypoxemia. The diagnosis of IPF is established using a combination of clinical, radiographic and pathological criteria and is associated with a characteristic pathological pattern called usual interstitial pneumonia (UIP).

IPF can be diagnosed at any age, but is most prevalent in those aged over 50 years and prevalence is higher in men than women. IPF has a mortality rate higher than many neoplastic diseases, with a 3 year survival rate of 50% and a 5 year survival rate of only 20%. The cause of IPF is unknown, but it is hypothesised that there are multiple episodes of epithelial cell activation from as yet unidentified exogenous and endogenous stimuli, which if left untreated leads to progressive lung injury and ultimately fibrosis. Disruption of the alveolar epithelium is followed by migration, proliferation and activation of mesenchymal cells, resulting in the formation of fibroblastic/myofibroblastic foci with excessive accumulation of ECM.

TGF-beta expression is increased in the fibrotic lungs of IPF patients (Broekelmann et al., 1991, PNAS, 88: 6642-6646; Khalil et al., 1991, Am J Respir Cell Mol Biol, 5: 155-162) and together with the well-established role of TGF-beta in driving fibrotic mechanisms the inhibition of TGF-beta should be considered as an effective mechanism for the treatment of IPF patients.

There is no effective therapy available for IPF patients. Anti-inflammatory agents, including corticosteroids, cyclophosphamide and azothiaprime have proved to be of little benefit for patients and have associated side effects. Recently two small molecule drugs, pirfenidone and nintedanib, have been approved for the treatment of IPF. Both drugs have been shown to slow the progression of disease, but neither cures the disease and many patients continue to decline. In addition treatment-related adverse events such as gastrointestinal events, rash and photosensitivity are evident (Cottin and Maher, 2015, Eur Respir Rev, 24: 58-64; Mazzei *et al.*, 2015, Ther Adv Respir Dis.) To date, no targeted therapies and no antibody therapies have been approved for fibrotic indications.

Furthermore, TGF-beta is also associated with pulmonary hypertension, such as pulmonary arterial hypertension (PAH). Increased expression of TGF-beta in patients with pulmonary hypertension has been shown by immunohistochemistry (Botney et al., 1994, Am J Pathol, 144: 286-295) and also noted in blood and lung homogenates from pulmonary hypertension patients (Selimovic et al., 2009, Eur Respir J, 34: 662-668; Gore et al., PLOS One (2014) 9(6):e100310). A TbetaRI kinase inhibitor has also been shown to inhibit the monocrotaline-induced model of pulmonary hypertension (Zaiman et al., 2008, Am J Respir Crit Care Med, 177: 896-905). Pulmonary hypertension is a well-recognised complication of IPF, and these data support the hypothesis that IPF patients whose symptoms are driven by both interstitial fibrosis and pulmonary hypertension could be a sub-population of patients for whom anti-TGF-beta therapies could potentially be even more effective.

Therefore, there exists a need in the art for suitable and/or improved antibodies capable of binding and inhibiting all three isoforms of TGF-beta suitable for therapeutic applications. Such antibodies may also be more effective for treating pulmonary indications and/or have fewer side effects if delivered by inhalation.

BRIEF SUMMARY OF THE INVENTION

This invention pertains to novel TGF-beta specific antibodies and binding fragments thereof, in particular antagonistic antibodies and fragments.

In one aspect there is provided an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 comprising a heavy chain, wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:4 for CDR-H1, a CDR having the sequence given in SEQ ID NO:5 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3.

In one aspect there is provided an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3.

The disclosure also extends to a polynucleotide, such as DNA, encoding an antibody or fragment as described herein.

Also provided is a host cell comprising said polynucleotide.

Methods of expressing an antibody or binding fragment thereof are provided herein.

The present disclosure also relates to pharmaceutical compositions comprising said antibodies or binding fragments thereof.

In one embodiment there is provided a method of treatment comprising administering a therapeutically effective amount of an antibody, fragment or composition as described herein.

The present disclosure also extends to an antibody, binding fragment or composition according to the present disclosure for use in treatment, particularly in the treatment of cancer and/or fibrotic disease.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1 A-I show certain antibody amino acid and polynucleotide sequences of the disclosure.

Figure 1A provides CDR sequences from antibody 4856 (SEQ ID NOs:1-9).

Figure 1B provides rabbit sequences for antibody 4856 (SEQ ID NOs:10-25).

Figure 1C provides murinised sequences for antibody 4856 (SEQ ID NOs: 26-33) as well as murine acceptor sequences (SEQ ID NOs: 34-37).

Figure 1D provides light chain (SEQ ID NOs: 45-51) and variable region sequences (SEQ ID NO:38-44) for antibody 4856 gL3.

Figure 1E provides Fab heavy chain (SEQ ID NOs: 59-65) and variable region sequences (SEQ ID NO:52-58) for antibody 4856 gH13.

Figure 1F provides Fab heavy chain (SEQ ID NOs: 73-79) and variable region sequences (SEQ ID NO:66-72) for antibody 4856 gH20.

Figure 1G provides Fab heavy chain (SEQ ID NOs: 87-93) and variable region sequences (SEQ ID NO:80-86) for antibody 4856 gH23.

Figure 1H provides Fab heavy chain (SEQ ID NOs:101-107) and variable region sequences (SEQ ID NO:94-100) for antibody 4856 gH29.

Figure 1I provides human acceptor framework sequences (SEQ ID NOs:108-111).

Figure 2 shows alignments of the amino acid sequences of various light chain (Figure 2A) and heavy chain (Figure 2B) of antibody 4856 and acceptor sequences.

Figure 3A shows the amino acid sequence of human Latency-associated Peptide and TGF-beta 1

Figure 3B shows the amino acid sequence of mature human TGF-beta 1

Figure 3C shows the amino acid sequence of human Latency-associated Peptide and TGF-beta 2

Figure 3D shows the amino acid sequence of mature human TGF-beta 2

Figure 3E shows the amino acid sequence of human Latency-associated Peptide and TGF-beta 3

Figure 3F shows the amino acid sequence of mature human TGF-beta 3

Figures 4A, B and C show the effect of rabbit antibody 4856 Fab in the (A) TGF-beta1, (B) TGF-beta2 and (C) TGF-beta3 HEK-Blue-TGF-beta reporter gene assay

Figure 5 shows the effect of 4856 rabbit Fab in the endogenous BxPC3-HEK-Blue TGF-beta reporter gene co-culture assay

Figure 6 shows images of ECM deposition by HRMCs in response to 10nM Adriamycin and in the presence of increasing concentrations of 4856 Fab grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29 or control Fab

Figures 7A, B and C show the effect of 4856 Fab grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29 on the deposition of (A) fibronectin, (B) collagen I and III and (C) collagen IV from HRMCs treated with Adriamycin

Figure 8 shows images of ECM deposition by SAEpCs and IPF fibroblasts co-cultures in the presence of increasing concentrations of 4856 Fab graft gL3gH13 and a control Fab

Figures 9A, B and C show the effect of 4856 Fab graft gL3gH13 and a control Fab on the deposition of (A) fibronectin, (B) collagen I and III and (C) collagen IV from SAEpCs and IPF fibroblasts co-cultures

Figures 10A, B and C show the effect of 4856 Fab graft gL3gH13 on the inhibition of (A)TGF-beta 1, (B) TGF-beta 2 and (C) TGF-beta 3 induced fibronectin deposition from a mono-culture of human renal proximal tubular epithelial cells

Figure 10D shows the effect of 4856 Fab graft gL3gH13 on the inhibition of fibronectin deposition from a co-culture of human renal proximal tubular epithelial cells and human renal fibroblasts

Figures 11A and B the effect of 4856 Fab graft gL3gH13 on the inhibition of TGF-beta 1 induced (A) collagen I and III, (B) collagen V deposition from a mono-culture of human renal proximal tubular epithelial cells

Figure 12 Comparison of the effect of intranasal administration of the indicated 4856 Fabs on the expression of PAI-1 in mice at day 7 after challenge with bleomycin.

Figure 13 Dose comparison of intranasally administered 4856 gL3gH13 Fab on the expression of PAI-1 in mice at day 7 after challenge with bleomycin

Figures 14A- B The effect of intranasally administered 4856 gL3gH13 Fab from day 1-28 on A) bleomycin-induced collagen deposition (PSR stain) and (B) hydroxyproline content in the lung.

Figures 15A- B The effect of intranasally administered 4856 gL3gH13 Fab from day 13-28 on A) bleomycin-induced collagen deposition (PSR stain) and B) hydroxyproline content in the lung.

Figures 16A- B The effect of intranasally administered 4856 gL3gH13 Fab from day A) 1-28 or B) 13-28 on bleomycin-induced myofibroblast differentiation in the lung.

Figures 17A- B The effect of intranasally administered 4856 gL3gH13 Fab from A) day 1-28 or B) 13-28 on bleomycin-induced pSmad2/3 expression in type 1 collagen expressing cells.

Figure 18A shows the sequence of mature human TGF-beta 1 (SEQ ID NO:114) with the residues involved in interaction with 4856 Fab gL3gH13 (underlined) and residues critical for interaction with TbetaRI and TbetaRII (bold) using crystallographic data at 4Å resolution.

Figure 18B shows the sequence of mature human TGF-beta 1 (SEQ ID NO:114) with the residues involved in interaction with 4856 Fab gL3gH13 (underlined) and residues critical for interaction with TbetaRI and TbetaRII (bold) using crystallographic data at 5Å resolution.

Figure 18C shows the sequence of mature human TGF-beta 2 (SEQ ID NO:116).

DETAILED DESCRIPTION

The antibodies of the present disclosure bind TGF-beta 1, TGF-beta 2 and TGF-beta 3. In one embodiment the antibodies of the present disclosure bind all three isoforms of mature TGF-beta, mature TGF-beta 1 (SEQ ID NO:114), mature TGF-beta 2 (SEQ ID NO:115) and mature TGF-beta 3 (SEQ ID NO:118). In one embodiment the antibodies of the present disclosure bind the homodimer of each of the three isoforms of mature TGF-beta, the homodimer of mature TGF-beta 1 (SEQ ID NO:114), the homodimer of mature TGF-beta 2 (SEQ ID NO:115) and the homodimer of mature TGF-beta 3 (SEQ ID NO:118). In one embodiment the antibodies of the present disclosure do not bind the latent forms of TGF-beta 1, TGF-beta 2 and TGF-beta 3 comprising the latency-associated peptide (LAP), as shown in SEQ ID NO:113, SEQ ID NO:115 and SEQ ID NO:117.

In one embodiment the antibodies described herein are antagonistic. As used herein, the term 'antagonistic antibody' describes an antibody that is capable of inhibiting and/or neutralising the

biological signalling activity of TGF-beta 1, TGF-beta 2 and TGF-beta 3, for example by blocking binding or substantially reducing binding of TGF-beta 1, TGF-beta 2 and TGF-beta 3 to TbetaRI and/or TbetaRII and thus inhibiting the formation and activation of the TGF-beta receptor complex.

Assays suitable for determining the ability of an antibody to inhibit and/or neutralise the biological signalling activity of TGF-beta 1, TGF-beta 2 and TGF-beta 3 are described in the Examples herein, for example the HEK-Blue TGF-beta reporter gene assay using recombinant TGF-beta 1, 2 and/or 3 described in Example 1 and Example 2, or the BxPC3 and HEK-Blue TGF-beta reporter gene co-culture assay driven by the production of TGF-beta by BvPC3 cells described in Example 3.

In one embodiment, the antibody molecules of the present invention have inhibitory activity in the recombinant TGF-beta 1, TGF-beta 2 or TGF-beta 3 HEK-Blue TGF-beta reporter gene assay, wherein the antibody inhibits human TGF-beta 1 activity with an IC₅₀ of 0.5nM or better, inhibits human TGF-beta 2 activity with an IC₅₀ of 0.05nM or better and inhibits human TGF-beta 3 activity with an IC₅₀ of 2nM or better. In one embodiment the antibody inhibits TGF-beta in the endogenous TGF-beta HEK-Blue TGF-beta reporter gene assay with an IC₅₀ of 10nM or better.

The antibody molecules of the present invention suitably have a high binding affinity. Affinity may be measured using any suitable method known in the art, including techniques such as surface plasmon resonance, for example BIAcore, as described in the Examples herein, using isolated natural or recombinant TGF-beta 1, TGF-beta 2 and TGF-beta 3 or a suitable fusion protein/polypeptide. In one embodiment, the antibody molecules of the present invention have the following order of binding affinity of highest for human TGF-beta 1, followed by human TGF-beta 2 and the lowest binding affinity for human TGF-beta 3. In one embodiment, the antibody molecules of the present invention have a binding affinity for human TGF-beta 1 that is 10 to 30 times, such as 15 to 25 times, higher than the binding affinity for human TGF-beta 3. In one embodiment, the antibody molecules of the present invention have a binding affinity for human TGF-beta 2 that is 2 to 20 times, such as 5 to 15 times, higher than the binding affinity for human TGF-beta 3.

Suitably the antibody molecules of the present invention have a binding affinity for isolated human TGF-beta 1, TGF-beta 2 and TGF-beta 3 of about 2000pM or less than 2000pM. In one embodiment the antibody molecule of the present invention has a binding affinity for human TGF-beta 1 of 500pM or lower, such as 200pM or lower or 100pM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity for human TGF-beta 2 of 500pM or lower, such as 300pM or lower, 200pM or lower. In one embodiment the antibody molecule of the present invention has a binding affinity for human TGF-beta 3 of 3000pM or lower, such as 2500pM or lower, 2000pM or lower.

In one embodiment, the antibody of the present invention has a binding affinity for human TGF-beta 1 of 100pM or lower, a binding affinity for human TGF-beta 2 of 200pM or lower and a binding affinity for human TGF-beta 3 of 2000pM or better.

The lower the numerical value of the affinity the higher the affinity of the antibody or fragment for the TGF-beta isoform.

The present inventors have provided new anti-TGF-beta antibodies, including humanised antibodies. The antibodies were generated from immunisation of rabbits with mature TGF-beta 1 and mature TGF-beta 2.

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

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

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M., J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue 26 to residue 32. Thus unless indicated otherwise 'CDR-H1' as employed herein is intended to refer to residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia's topological loop definition.

The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and residues 89-97 (CDR-L3) according to the Kabat numbering system.

Antibodies for use in the present disclosure may be obtained using any suitable method known in the art. The TGF-beta polypeptide/protein including fusion proteins, cells (recombinantly or naturally) expressing the polypeptide can be used to produce antibodies which specifically recognise TGF- beta. The polypeptide may be the 'mature' polypeptide of TGF-beta 1, TGF-beta 2 and TGF-beta 3 as shown in SEQ ID NOs: 113, 115 and 117 or a biologically active fragment or derivative thereof. Polypeptides, for use to immunize a host, may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems or they may be recovered from natural biological sources. In the present application, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified. The TGF- beta polypeptide may in some instances be part of a larger protein such as a fusion protein for example fused to an affinity tag, leader sequence, or other sequence.

Antibodies generated against the TGF-beta polypeptide may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, preferably a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable.

Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, *Nature*, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, 1983, *Immunology Today*, 4:72) and the EBV-hybridoma technique (Cole *et al.*, 1985, *Monoclonal Antibodies and Cancer Therapy*, pp77-96, Alan R Liss, Inc.).

Antibodies may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by, for example, the methods described by Babcook, J. *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93:7843-7848; WO92/02551; WO04/051268 and International Patent Application number WO04/106377.

Screening for antibodies can be performed using assays to measure binding to human TGF-beta and/or assays to measure the ability to block ligand binding to the receptor. Examples of suitable assays are described in the Examples herein.

'Specific' as employed herein is intended to refer to an antibody that only recognises the antigen to which it is specific or an antibody that has significantly higher binding affinity to the antigen to which it is specific compared to binding to antigens to which it is non-specific, for example at least 5, 6, 7, 8, 9, 10 times higher binding affinity.

The amino acid sequences and the polynucleotide sequences of certain antibodies according to the present disclosure are provided in Figures 1 and 2.

In one aspect of the invention the antibody is an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 comprising a heavy chain, wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:4 for CDR-H1, a CDR having the sequence given in SEQ ID NO:5 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3. Preferably the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3.

In a second aspect of the invention the antibody is an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3. Preferably the variable domain of the light chain comprises the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3.

The antibody molecules of the present invention suitably comprise a complementary light chain or a complementary heavy chain, respectively.

In one embodiment the antibody of the invention is an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 comprising a heavy chain as defined above and additionally comprising a light chain wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO: 1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3. The variable domain of the light chain preferably comprises the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3.

In one embodiment the antibody of the invention is antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 comprising a heavy chain and a light chain, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3; and wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3.

It will be appreciated that one or more amino acid substitutions, additions and/or deletions may be made to the CDRs provided by the present invention without significantly altering the ability of the antibody to bind to TGF-beta 1, TGF-beta 2 and TGF-beta 3 and to neutralise TGF-

beta 1, TGF-beta 2 and TGF-beta 3 activity. The effect of any amino acid substitutions, additions and/or deletions can be readily tested by one skilled in the art, for example by using the methods described herein, particularly those illustrated in the Examples, to determine TGF-beta 1, TGF-beta 2 and TGF-beta 3 binding and inhibition of the TGF-beta 1, TGF-beta 2 and TGF-beta 3 and receptor interaction. In one embodiment, at least one amino acid is replaced with a conservative substitution in one or more CDRs selected from the group consisting independently of:

any one of CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, CDR-L3;

any one of the combinations CDR-H1 and H2, CDR-H1 and H3, CDR-H1 and L1, CDR-H1 and L2, CDR-H1 and L3, CDR-H2 and H3, CDR-H2 and L1, CDR-H2 and L2, CDR-H2 and L3, CDR-H3 and L1, CDR-H3 and L2, CDR-H3 and L3, CDR-L1 and L2, CDR-L1 and L3, CDR-L2 and L3;

CDR-H1, H2 and H3, CDR-H1, H2 and L1, CDR-H1, H2 and L2, CDR-H1, H2 and L3, CDR-H2, H3 and L1, CDR-H2, H3 and L2, CDR-H2, H3 and L3, CDR-H3, L1 and L2, CDR-H3, L1 and L3, CDR-L1, L2, L3;

any one of the combinations CDR-H1, H2, H3 and L1, CDR-H1, H2, H3 and L2, CDR-H1, H2, H3 and L3, CDR-H2, H3, L1 and L2, CDR-H2, H3, L2 and L3, CDR-H3, L1, L2 and L3, CDR-L1, L2, L3 and H1, CDR-L1, L2, L3 and H2, CDR-L1, L2, L3 and H3, CDR-L2, L3, H1 and H2,

CDR-H1, H2, H3, L1 and L2, CDR-H1, H2, H3, L1 and L3, CDR-H1, H2, H3, L2 and L3, CDR-L1, L2, L3, H1 and H2, CDR-L1, L2, L3, H1 and H3, CDR-L1, L2, L3, H2 and H3; and the combination CDR-H1, H2, H3, L1, L2 and L3.

Accordingly, the present invention provides an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 comprising one or more CDRs selected from CDRH-1 (SEQ ID NO:4), CDRH-2 (SEQ ID NO:5), CDRH-3 (SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9), CDRL-1 (SEQ ID NO:1), CDRL-2 (SEQ ID NO:2) and CDRL-3 (SEQ ID NO:3) in which one or more amino acids in one or more of the CDRs has been substituted with another amino acid, for example a similar amino acid as defined herein below.

In one embodiment, the present invention provides an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 comprising CDRH-1 (SEQ ID NO:4), CDRH-2 (SEQ ID NO:5), CDRH-3 (SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9), CDRL-1 (SEQ ID NO:1), CDRL-2 (SEQ ID NO:2) and CDRL-3 (SEQ ID NO:3), for example in which one or more amino acids in one or more of the CDRs has been substituted with another amino acid, such as a similar amino acid as defined herein below.

In one embodiment, a domain of the heavy chain disclosed herein includes the sequence with 1, 2, 3 or 4 conservative amino acid substitutions, for example wherein the substitutions are in the framework.

In one embodiment, the framework of the heavy chain variable region comprises 1, 2, 3, or 4 amino acids which have been inserted, deleted, substituted or a combination thereof. In one embodiment, the substituted amino acid is a corresponding amino acid from the donor antibody.

In one embodiment, a light variable region disclosed herein includes the sequence with 1, 2, 3 or 4 conservative amino acid substitutions, for example wherein the substitutions are in the framework.

In one embodiment, the framework of the light chain variable region comprises 1, 2, 3 or 4 amino acid which have been inserted, deleted substituted or a combination thereof. In one embodiment the substituted amino is a corresponding amino acid form a donor antibody.

In one aspect of the present invention, there is provided an anti-TGF-beta antibody or binding fragment thereof, wherein the variable domain of the heavy chain comprises three CDRs and the sequence of CDR-H1 has at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identity or similarity to the sequence given in SEQ ID NO:4, the sequence of CDR-H2 has at least 60%, 70%, 80%, 90% or 95% identity or similarity to the sequence given in SEQ ID NO:5 and the sequence of CDR-H-3 has at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identity or similarity to the sequence given in SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9. Preferably, the anti-TGF-beta antibody or binding fragment thereof, additionally comprising a light chain, wherein the variable domain of the light chain comprises three CDRs and the sequence of CDR-L1 has at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identity or similarity to the sequence given in SEQ ID NO:1, the

sequence of CDR-L2 has at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identity or similarity to the sequence given in SEQ ID NO:2 and the sequence of CDR-L3 has at least 60% identity or similarity to the sequence given in SEQ ID NO:3.

In one embodiment a variable region is provided with at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or more identity or similarity to a variable region sequence disclosed herein.

In one embodiment the present invention provides an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 which contacts a sequence on that is at least 90% identical to amino acids 24-35 of SEQ ID NO:114 and optionally at least one of amino acids 90-95 of SEQ ID NO:114. In a further embodiment, the antibody contacts a sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:114. In a one embodiment the antibody further contacts at least one of amino acids 60, 97 and 101 of SEQ ID NO:114. In a further embodiment, the antibody also contacts amino acids outside the amino acids provided herein. By 'contacts' or 'contacting' it is meant that an interaction can be detected using standard X-ray crystallography techniques at a suitable resolution, such as 5 Å or 4 Å.

In another embodiment there is provided an anti-TGF-beta antibody which competes with the binding of an antibody or fragment of the invention for binding to TbetaRI and/or TbetaRII.

In one embodiment there is provided an anti-TGF-beta antibody which cross-blocks the binding of an antibody comprising a the 6 CDRs given in sequence SEQ ID NO:1 for CDR-L1, SEQ ID NO:2 for CDR-L2, SEQ ID NO:3 for CDR-L3, SEQ ID NO:4 for CDR-H1, SEQ ID NO:5 for CDR-H2 and SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3, in particular wherein the cross blocking is allosteric.

In one embodiment there is provided an anti-TGF-beta antibody which cross-blocks the binding of an antibody comprising the 6 CDRs given in sequence SEQ ID NO:1 for CDR-L1, SEQ ID NO:2 for CDR-L2, SEQ ID NO:3 for CDR-L3, SEQ ID NO:4 for CDR-H1, SEQ ID NO:5 for CDR-H2 and SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3, in particular wherein the antibody cross-blocks the binding by binding the same epitope as the antibody which it blocks.

In one embodiment, the antibody or binding fragment is from a mouse, rat, rabbit, camelid or other mammalian species. For example, the antibody or binding fragment may be from a rabbit. Examples of variable regions for such antibodies are provided in SEQ ID NOs:10-17.

In one embodiment, the antibody or binding fragments is chimeric. Generally, chimeric antibodies or binding fragments comprise elements from two or more species while retaining certain characteristics of that species. For example, a chimeric antibody or binding fragment may have a variable region from one species, such as from a mouse, rat, rabbit or other mammalian species and all or part of a constant region from another species, such as human.

In one embodiment the antibody or binding fragments according to the invention is humanised.

As used herein, the term 'humanised antibody' refers to an antibody or antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody) (see, e.g. US 5,585,089; WO91/09967). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998. In one embodiment rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri *et al.*, 2005, Methods, 36:25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are transferred to the human antibody framework. In another embodiment only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework. When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions.

Suitably, the humanised antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs provided specifically herein. Thus, provided in one embodiment is a humanised antibody which binds

human TGF-beta 1, TGF-beta 2 and TGF-beta 3 wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.

Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al.*, *supra*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: www2.mrc-lmb.cam.ac.uk/vbase or at www.imgt.org, both last accessed 07 January 2016.

In a humanised antibody of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

In one embodiment a human framework comprises 1, 2, 3, or 4 amino acid substitutions, additions or deletions, for example 1, 2, 3 or 4 conservative substitutions or substitutions of donor residues.

In one embodiment the sequence employed as a human framework is 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more similar or identical to a sequence disclosed herein.

A suitable framework region for the heavy chain of the humanised antibody of the present invention is derived from the human sub-group VH3 sequence IGHV3-21 together with JH5 (SEQ ID NO:111).

A suitable framework region for the light chain of the humanised antibody of the present invention is derived from the human sub-group VK1 sequence IGKV1-5 sequence together with JK4 (SEQ ID NO:109).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 4 for CDR-H1, the sequence given in SEQ ID NO: 5 for CDR-H2 and the sequence given in SEQ ID NO:6 or SEQ ID NO:7 or SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3, wherein the heavy chain framework region is derived from the human subgroup VH3 sequence IGHV3-21 together with JH5 (SEQ ID NO:111).

In one example the heavy chain variable domain of the antibody comprises the sequence given in SEQ ID NO:52, SEQ ID NO:66, SEQ ID NO:80 or SEQ ID NO:94.

A suitable framework region for the light chain of the humanised antibody of the present invention is derived from the human germline sub-group VK1 sequence IGKV1-5 sequence together with JK4 (SEQ ID NO:109).

Accordingly, in one example there is provided a humanised antibody comprising the sequence given in SEQ ID NO: 1 for CDR-L1, the sequence given in SEQ ID NO: 2 for CDR-L2 and the sequence given in SEQ ID NO: 3 for CDR-L3, wherein the light chain framework region is derived from the human subgroup VK1 sequence IGKV1-5 sequence together with JK4 (SEQ ID NO:109).

In one example the light chain variable domain of the antibody comprises the sequence given in SEQ ID NO: 38.

In a humanised antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann *et al.*, 1998, Nature, 332:323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO91/09967.

Donor residue as employed herein refers to a residue from the non-human antibody (e.g. murine or rabbit antibody) which donated the CDRs.

In one embodiment there is provided a humanised antibody wherein the heavy chain variable domain does not contain any donor residues.

Similarly, in one embodiment there is provided an antibody or binding fragment that is 'murinised'. Such an antibody or binding fragment may have a rabbit donor and a murine acceptor. Examples of such antibodies are provided in SEQ ID NOs: 26-33. Examples of murine acceptor sequences are provided in SEQ ID NOs: 34-37.

In a particular embodiment, the present invention provides an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 having a heavy chain comprising the heavy chain variable domain sequence given in SEQ ID NO:52, SEQ ID NO:66,

SEQ ID NO:80 or SEQ ID NO:94 and a light chain comprising the light chain variable domain sequence given in SEQ ID NO: 38.

In one embodiment the disclosure provides an antibody sequence which is 80% similar or identical to a sequence disclosed herein, for example 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or more over part or whole of the relevant sequence. In one embodiment the relevant sequence is SEQ ID NO:52, SEQ ID NO:66, SEQ ID NO:80 or SEQ ID NO:94. In one embodiment the relevant sequence is SEQ ID NO: 38.

"Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

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

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

The antibody molecules of the present invention may comprise a complete antibody molecule having full length heavy and light chains or a binding fragment thereof and may be, but are not

limited to Fab, modified Fab, Fab', modified Fab', F(ab')₂, Fv, single domain antibodies (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibodies, Bis-scFv, diabodies, triabodies, tetrabodies and epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). The methods for creating and manufacturing these antibody fragments are well known in the art (see for example Verma *et al.*, 1998, Journal of Immunological Methods, 216:165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in International patent applications WO05/003169, WO05/003170 and WO05/003171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO92/22853, WO05/113605, WO2009/040562 and WO2010/035012).

Binding fragment of an antibody as employed herein refers to a fragment capable of binding an antigen with affinity to characterise the fragment as specific for the antigen.

In one embodiment the antibody according to the present disclosure is provided as TGF-beta binding antibody fusion protein which comprises an immunoglobulin moiety, for example a Fab or Fab' fragment, and one or two single domain antibodies (dAb) linked directly or indirectly thereto, for example as described in WO2009/040562, WO2010/035012, WO2011/030107, WO2011/061492 and WO2011/086091 all incorporated herein by reference.

In one embodiment the fusion protein comprises two domain antibodies, for example as a variable heavy (VH) and variable light (VL) pairing, optionally linked by a disulphide bond.

In one embodiment the Fab or Fab' element of the fusion protein has the same or similar specificity to the single domain antibody or antibodies. In one embodiment the Fab or Fab' has a different specificity to the single domain antibody or antibodies, that is to say the fusion protein is multivalent. In one embodiment a multivalent fusion protein according to the present invention has an albumin binding site, for example a VH/VL pair therein provides an albumin binding site.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region

domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses and antibody effector functions are required.

Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required e.g. for simply blocking TGF-beta activity.

It will be appreciated that sequence variants of these constant region domains may also be used. For example IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal *et al.*, 1993, *Molecular Immunology*, 1993, 30:105-108 may be used. Accordingly, in the embodiment where the antibody is an IgG4 antibody, the antibody may include the mutation S241P.

It will also be understood by one skilled in the art that antibodies may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. *Journal of Chromatography* 705:129-134, 1995). However, there is no C-terminal Lysine on either heavy or light chain of Ab4856 embodiment of the invention.

In one example one or more CDRs provided herein may be modified to remove undesirable residues or sites, such as cysteine residues or aspartic acid (D) isomerisation sites or asparagine (N) deamidation sites.

For example one or more cysteine residues in any one of the CDRs may be substituted with another amino acid, such as serine.

In one example an Asparagine deamidation site may be removed from one or more CDRs by mutating the asparagine residue (N) and/or a neighbouring residue to any other suitable amino acid. In one example an asparagine deamidation site such as NG or NS may be mutated, for example to NA or NT.

In one example an Aspartic acid isomerisation site may be removed from one or more CDRs by mutating the aspartic acid residue (D) and/or a neighbouring residue to any other suitable amino acid. In one example an aspartic acid isomerisation site such as DG or DS may be mutated, for example to EG, DA or DT.

In one example an N-glycosylation site such as NLS may be removed by mutating the asparagine residue (N) to any other suitable amino acid, for example to SLS or QLS. In one example an N-glycosylation site such as NLS may be removed by mutating the serine residue (S) to any other residue with the exception of threonine (T).

In one embodiment the antibody heavy chain comprises a CH1 domain, a CH2 domain and a CH3 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

In one embodiment the antibody heavy chain comprises a CH1 domain and the antibody light chain comprises a CL domain, either kappa or lambda.

In one embodiment the antibody provided by the present invention is an antagonistic antibody having specificity for human TGF-beta in which the heavy chain constant region comprises a modified hinge region. Accordingly, the present invention provides an antibody in which the heavy chain comprises or consists of the sequence given in SEQ ID NO:59, SEQ ID NO:73, SEQ ID NO:87 or SEQ ID NO:101.

The present invention also provides an antibody in which the light chain comprises or consists of the sequence given in SEQ ID NO:45.

An antibody provided by the present invention has a heavy chain comprising the sequence given in SEQ ID NO:59, SEQ ID NO:73, SEQ ID NO:87 or SEQ ID NO:101 and a light chain comprising the sequence given in SEQ ID NO: 45.

Also provided is an anti-TGF-beta antibody or binding fragment thereof, in which the heavy and light chains are at least 80% (preferably 85%, 90%, 95%, 96%, 97%, 98%, 99% or more) identical or similar to a heavy chain comprising the sequence given in SEQ ID NO:59, SEQ ID NO:73, SEQ ID NO:87 or SEQ ID NO:101 and a light chain comprising the sequence given in SEQ ID NO: 45. In one embodiment, the light chain has or consists of the sequence given in SEQ ID NO: 45 and the heavy chain has or consists of the sequence given in SEQ ID NO:59, SEQ ID NO:73, SEQ ID NO:87 or SEQ ID NO:101. In another embodiment, the light chain has

or consists of the sequence of SEQ ID NO: 45 and the heavy chain has or consists of the sequence of SEQ ID NO: 59.

Also provided by the present invention is a specific region or epitope of human TGF-beta 1, 2 or 3 which is bound by an antibody provided by the present invention, in particular an antibody 4856 comprising the heavy chain sequence gH13 (SEQ ID NO: 59) and/or the light chain sequence gL3 (SEQ ID NO:45).

This specific region or epitope of the human TGF-beta 1, 2, or 3 polypeptide can be identified by any suitable epitope mapping method known in the art in combination with any one of the antibodies provided by the present invention. Examples of such methods include screening peptides of varying lengths derived from TGF-beta for binding to the antibody of the present invention with the smallest fragment that can specifically bind to the antibody containing the sequence of the epitope recognised by the antibody (for example a peptide in the region of about 5 to 20, preferably about 7 amino acids in length). The TGF-beta peptides may be produced synthetically or by proteolytic digestion of the TGF-beta polypeptide. Peptides that bind the antibody can be identified by, for example, mass spectrometric analysis. In another example, NMR spectroscopy or X-ray crystallography can be used to identify the epitope bound by an antibody of the present invention. Once identified, the epitopic fragment which binds an antibody of the present invention can be used, if required, as an immunogen to obtain additional antibodies which bind the same epitope.

Antibodies which cross-block the binding of an antibody according to the present invention in particular, an antibody comprising the heavy chain sequence (SEQ ID NO:59) and the light chain sequence (SEQ ID NO:45) may be similarly useful in antagonising TGF-beta 1, 2 and 3 activity. Accordingly, the present invention also provides an antagonistic antibody having specificity for human TGF-beta 1, 2 and 3, which cross-blocks the binding of any one of the antibodies described above to human TGF-beta 1, 2 and/or 3 and/or is cross-blocked from binding TGF-beta 1, 2 and/or 3 by any one of those antibodies. In one embodiment, such an antibody binds to the same epitope as an antibody described herein above. In another embodiment the cross-blocking neutralising antibody binds to an epitope which borders and/or overlaps with the epitope bound by an antibody described herein above. In another embodiment

the cross-blocking neutralising antibody of this aspect of the invention does not bind to the same epitope as an antibody of the present invention or an epitope that borders and/or overlaps with said epitope.

Cross-blocking antibodies can be identified using any suitable method in the art, for example by using competition ELISA or BIAcore assays where binding of the cross blocking antibody to human TGF-beta 1, 2 and/or 3 prevents the binding of an antibody of the present invention or *vice versa*.

In one embodiment there is provided an antagonistic antibody having specificity for human TGF-beta 1, 2 and 3, which cross-blocks the binding of an antibody whose heavy chain comprises the sequence shown in SEQ ID NO: 59 and whose light chain comprises the sequence shown in SEQ ID NO: 45 to human TGF-beta 1, 2 and 3. In one embodiment the cross-blocking antibodies provided by the present invention inhibit the binding of an antibody comprising the heavy chain sequence shown in SEQ ID NO:59 and the light chain sequence shown in SEQ ID NO:45 by greater than 80%, for example by greater than 85%, such as by greater than 90%, in particular by greater than 95%, 96%, 97%, 98%, 99% or more.

Alternatively or in addition, antagonistic antibodies according to this aspect of the invention may be cross-blocked from binding to human TGF-beta 1, 2 and 3 by an antibody comprising the heavy chain sequence shown in SEQ ID NO:59 and the light chain sequence shown in SEQ ID NO: 45. Also provided therefore is an antagonistic antibody molecule having specificity for human TGF-beta 1, 2 and 3 which is cross-blocked from binding human TGF-beta 1, 2 and 3 by an antibody comprising the heavy chain sequence shown in SEQ ID NO: 59 and the light chain sequence shown in SEQ ID NO: 45. In one embodiment the antagonistic antibodies provided by this aspect of the invention are inhibited from binding human TGF-beta 1, 2 and 3 by an antibody comprising the heavy chain sequence shown in SEQ ID NO: 59 and the light chain sequence shown in SEQ ID NO: 45 by greater than 80%, for example by greater than 85%, such as by greater than 90%, in particular by greater than 95%, 96%, 97%, 98%, 99% or more.

In one embodiment the cross-blocking antibodies provided by the present invention are fully human. In one embodiment the cross-blocking antibodies provided by the present invention are humanised. In one embodiment the antibodies of the present invention are suitable for inhaled

delivery, for example, by nebulisation. In one example the physical properties of the antibodies of the present invention e.g. binding affinity and potency are not substantially altered by nebulisation. In one example the antibodies of the present invention are highly stable. One measure of antibody stability is melting temperature (T_m). Melting temperature may be determined by any suitable method known in the art, for example using Thermofluor (Ericsson *et al*, Analytical Biochemistry 357 (2006) 289-298) or DSC (differential scanning calorimetry). Preferably the antibodies provided by the present invention have a high melting temperature (T_m), typically of at least 75°C. In one example the antibody of the present invention has a T_m of at least 75°C. In one example the antibody of the present invention has a T_m of at least 77°C. In one example the antibody of the present invention has a T_m of at least 79°C.

Biological molecules, such as antibodies or fragments, contain acidic and/or basic functional groups, thereby giving the molecule a net positive or negative charge. The amount of overall “observed” charge will depend on the absolute amino acid sequence of the entity, the local environment of the charged groups in the 3D structure and the environmental conditions of the molecule. The isoelectric point (pI) is the pH at which a particular molecule or solvent accessible surface thereof carries no net electrical charge. In one example, the TGF-beta antibody and fragments of the invention may be engineered to have an appropriate isoelectric point. This may lead to antibodies and/or fragments with more robust properties, in particular suitable solubility and/or stability profiles and/or improved purification characteristics.

Thus in one aspect the invention provides a humanised TGF-beta antibody engineered to have an isoelectric point different to that of the originally identified antibody. The antibody may, for example be engineered by replacing an amino acid residue such as replacing an acidic amino acid residue with one or more basic amino acid residues. Alternatively, basic amino acid residues may be introduced or acidic amino acid residues can be removed. Alternatively, if the molecule has an unacceptably high pI value acidic residues may be introduced to lower the pI, as required. It is important that when manipulating the pI care must be taken to retain the desirable activity of the antibody or fragment. Thus in one embodiment the engineered antibody or fragment has the same or substantially the same activity as the “unmodified” antibody or fragment.

Programs such as ** ExPASy www.expasy.ch/tools/pi_tool.html (accessed 21 December 2015) may be used to predict the isoelectric point of the antibody or fragment.

In one embodiment the cross-blocking antibody has an isoelectric point of at least 7, for example at least 8, such as 8.5, 8.6, 8.7, 8.8 or 8.9 or at least 9, such as 9.0, 9.1, 9.2, 9.3 or 9.4.

It will be appreciated that the affinity of antibodies provided by the present invention may be altered using any suitable method known in the art. The present invention therefore also relates to variants of the antibody molecules of the present invention, which have an improved affinity for TGF-beta. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al.*, 1995, J. Mol. Biol., 254:392-403), chain shuffling (Marks *et al.*, 1992, Bio/Technology, 10:779-783), use of mutator strains of *E. coli* (Low *et al.*, 1996, J. Mol. Biol., 250:359-368), DNA shuffling (Patten *et al.*, 1997, Curr. Opin. Biotechnol., 8:724-733), phage display (Thompson *et al.*, J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Cramer *et al.*, 1998, Nature, 391:288-291). Vaughan *et al.* (*supra*) discusses these methods of affinity maturation.

If desired an antibody for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies of the present invention. Where it is desired to obtain an antibody fragment linked to an effector molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom *et al.*, Controlled Drug Delivery, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-53; Thorpe *et al.*, 1982, Immunol. Rev., 62:119-58 and Dubowchik *et al.*, 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO93/06231, WO92/22583, WO89/00195, WO89/01476 and WO03/031581. Alternatively, where the effector molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO86/01533 and EP0392745.

The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, other antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups, such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to (*e.g.* kills) cells. Examples, include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

Effector molecules also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.* vincristine and vinblastine).

Other effector molecules may include chelated radionuclides such as ^{111}In and ^{90}Y , Lu^{177} , Bismuth²¹³, Californium²⁵², Iridium¹⁹² and Tungsten¹⁸⁸/Rhenium¹⁸⁸; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin.

Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as

insulin, tumour necrosis factor, α -interferon, beta-interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), nerve growth factor (NGF) or other growth factor and immunoglobulins.

Other effector molecules may include detectable substances useful, for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I , ^{131}I , ^{111}In and ^{99}Tc .

In another example the effector molecule may increase the half-life of the antibody *in vivo*, and/or reduce immunogenicity of the antibody and/or enhance the delivery of an antibody across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin binding proteins or albumin binding compounds such as those described in WO05/117984.

In one embodiment a half-life provided by an effector molecule which is independent of TGF-beta is advantageous.

Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, *e.g.* a homo- or hetero- polysaccharide.

Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups.

Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol), such as methoxypoly(ethyleneglycol) or derivatives thereof.

Specific naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof.

In one embodiment the polymer is albumin or a fragment thereof, such as human serum albumin or a fragment thereof.

“Derivatives” as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, for example from 5000 to 40000Da, such as from 20000 to 40000Da. The polymer size may in particular be selected on the basis of the intended use of the product, for example ability to localize to certain tissues such as tumors or extend circulating half-life (for review see Chapman, 2002, Advanced Drug Delivery Reviews, 54, 531-545). Thus, for example, where the product is intended to leave the circulation and penetrate tissue, for example for use in the treatment of a tumour, it may be advantageous to use a small molecular weight polymer, for example with a molecular weight of around 5000Da. For applications where the product remains in the circulation, it may be advantageous to use a higher molecular weight polymer, for example having a molecular weight in the range from 20000Da to 40000Da.

Suitable polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 15000Da to about 40000Da.

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

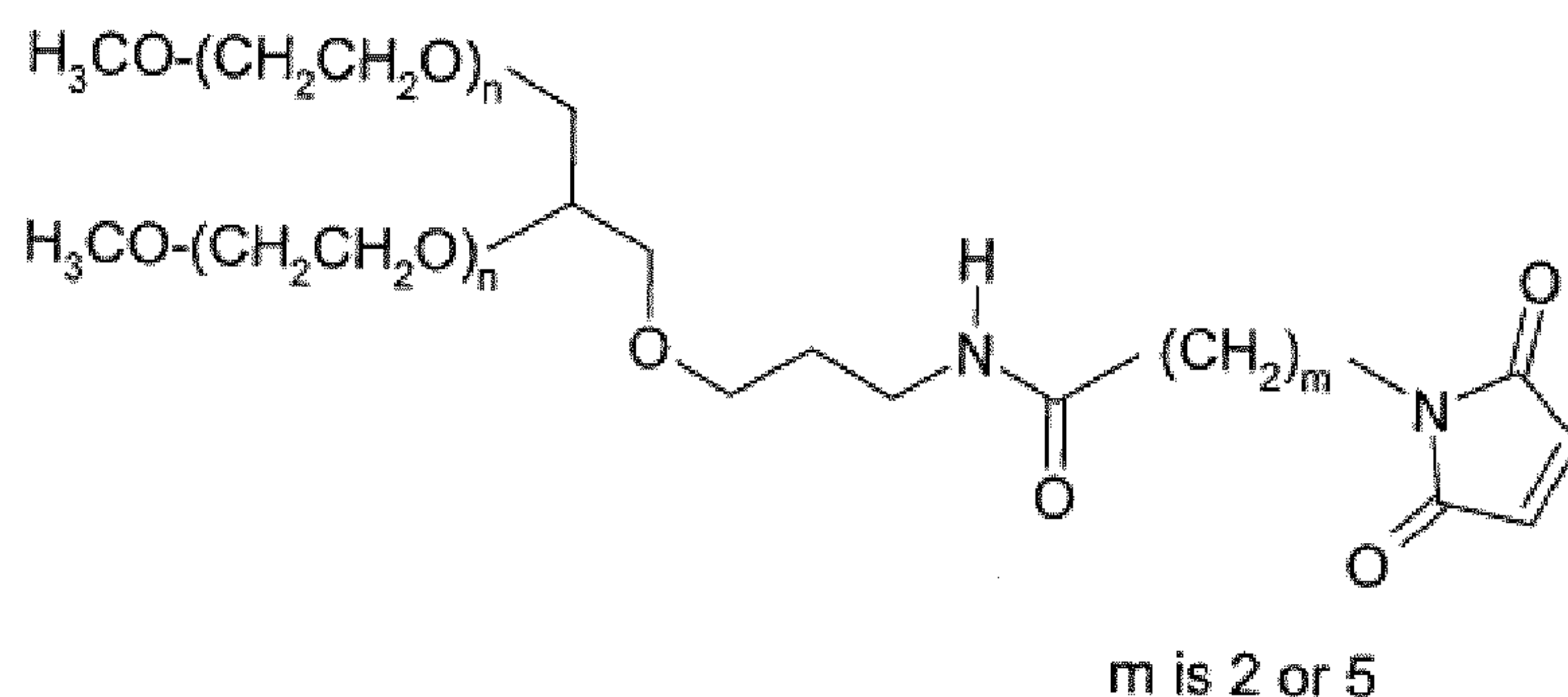
Suitably PEG molecules are covalently linked through a thiol group of at least one cysteine residue located in the antibody fragment. Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond. Where a thiol group is used as the point of attachment appropriately activated effector molecules, for example thiol selective derivatives such as maleimides and cysteine derivatives may be used. An activated polymer may be used as the starting material in the preparation of polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α -halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Nektar, formerly Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting materials using conventional chemical procedures. Particular PEG molecules include 20K methoxy-PEG-amine (obtainable from Nektar, formerly Shearwater; Rapp Polymere; and SunBio) and M-PEG-SPA (obtainable from Nektar, formerly Shearwater).

In one embodiment, the antibody is a modified Fab fragment, Fab' fragment or diFab which is PEGylated, *i.e.* has PEG (poly(ethyleneglycol)) covalently attached thereto, *e.g.* according to

the method disclosed in EP 0948544 or EP1090037 [see also "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York; Chapman, A. 2002, Advanced Drug Delivery Reviews 2002, 54:531-545]. In one example PEG is attached to a cysteine in the hinge region. In one example, a PEG modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue may be covalently linked to the maleimide group and to each of the amine groups on the lysine residue may be attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000Da. The total molecular weight of the PEG attached to the Fab fragment may therefore be approximately 40,000Da.

Particular PEG molecules include 2-[3-(N-maleimido)propionamido]ethyl amide of N,N'-bis(methoxypoly(ethylene glycol) MW 20,000) modified lysine, also known as PEG2MAL40K (obtainable from Nektar, formerly Shearwater).

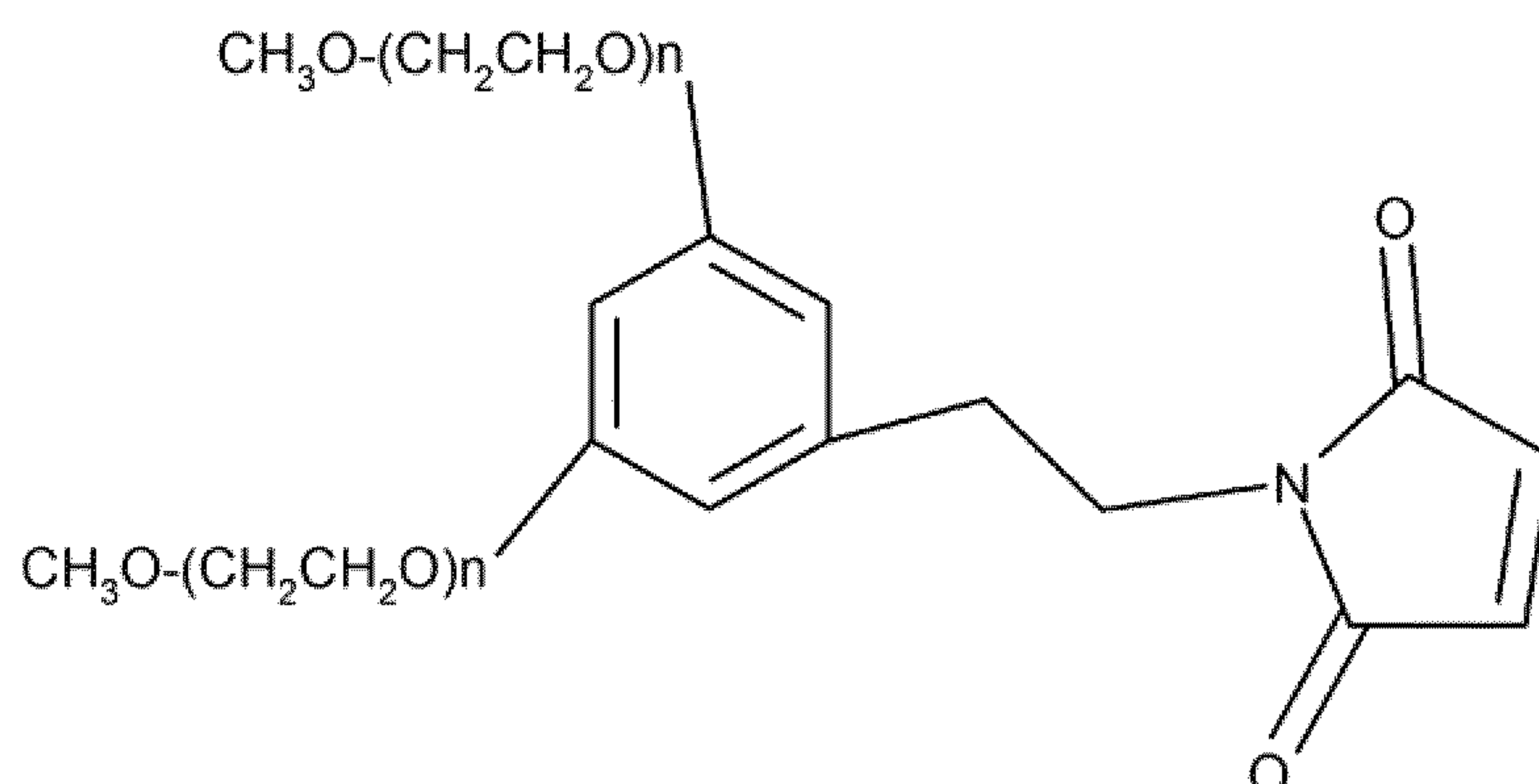
Alternative sources of PEG linkers include NOF who supply GL2-400MA3 (wherein m in the structure below is 5) and GL2-400MA (where m is 2) and n is approximately 450:



That is to say each PEG is about 20,000Da.

Thus in one embodiment the PEG is 2,3-Bis(methylpolyoxyethylene-oxy)-1-[[3-(6-maleimido-1-oxohexyl)amino]propyloxy} hexane (the 2 arm branched PEG, -CH₂)₃NHCO(CH₂)₅-MAL, Mw 40,000 known as SUNBRIGHT GL2-400MA3.

Further alternative PEG effector molecules of the following type:



In one embodiment there is provided an antibody, such as a full length antibody, which is PEGylated (for example with a PEG described herein), attached through a cysteine amino acid residue at or about amino acid 226 in the chain, for example amino acid 226 of the heavy chain (by sequential numbering). In one embodiment, PEG is attached to Cys 226 of SEQ ID NO:101.

In one embodiment the present disclosure provides a Fab-PEG molecule comprising one or more PEG polymers, for example 1 or 2 polymers such as a 40kDa polymer or polymers.

Fab-PEG molecules according to the present disclosure may be particularly advantageous in that they have a half-life independent of the Fc fragment.

In one embodiment there is provided a scFv conjugated to a polymer, such as a PEG molecule, a starch molecule or an albumin molecule.

In one embodiment the antibody or fragment is conjugated to a starch molecule, for example to increase the half-life. Methods of conjugating start to a protein as described in US 8,017,739 incorporated herein by reference.

A reporter molecule as employed herein is a molecule which is capable of being detected, for example a fluorescent dye, radiolabel or other detectable entity.

The present invention also provides an isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody molecule of the present invention. Suitably, the DNA sequence encodes the heavy or the light chain of an antibody molecule of the present invention. The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

DNA sequences which encode an antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

Examples of suitable DNA sequences are provided in Figure 1.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Accordingly, provided is a cloning or expression vector comprising one or more DNA sequences encoding an antibody of the present invention. In one embodiment the vector comprises a light chain DNA sequences given in SEQ ID NO:46 or SEQ ID NO:47 and/or a heavy chain DNA sequence given in SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:102 or SEQ ID NO:103. Suitably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, preferably SEQ ID NO: 47 and SEQ ID NO: 61, respectively and suitable signal sequences. In one example the vector comprises an intergenic sequence between the heavy and the light chains (see WO03/048208).

General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

Also provided is a host cell comprising one or more cloning or expression vectors comprising one or more DNA sequences encoding an antibody of the present invention. Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody

molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used or eukaryotic, for example mammalian, host cell expression systems may also be used. Suitable mammalian host cells include HEK, CHO, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell containing a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfect the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

The antibodies and fragments according to the present disclosure are expressed at good levels from host cells. Thus the properties of the antibodies and/or binding fragments are suitable for expression on a commercial scale.

Thus there is provided a process for culturing a host cell and expressing an antibody or fragment thereof, isolating the latter and optionally purifying the same to provide an isolated antibody or fragment. In one embodiment the process further comprises the step of conjugating an effector molecule to the isolated antibody or fragment, for example conjugating to a PEG polymer in particular as described herein.

In one embodiment there is provided a process for purifying an antibody (in particular an antibody or fragment according to the invention) comprising performing anion exchange chromatography in non-binding mode such that the impurities are retained on the column and the antibody is eluted.

In one embodiment the purification employs affinity capture on a TGF-beta column.

In one embodiment the purification employs cibacron blue or similar for purification of albumin fusion or conjugate molecules.

Suitable ion exchange resins for use in the process include Q.FF resin (supplied by GE-Healthcare). The step may, for example be performed at a pH about 8.

The process may further comprise an initial capture step employing cation exchange chromatography, performed for example at a pH of about 4 to 5, such as 4.5. The cation exchange chromatography may, for example employ a resin such as CaptoS resin or SP sepharose FF (supplied by GE-Healthcare). The antibody or fragment can then be eluted from the resin employing an ionic salt solution such as sodium chloride, for example at a concentration of 200mM.

Thus the chromatography step or steps may include one or more washing steps, as appropriate.

The purification process may also comprise one or more filtration steps, such as a diafiltration step.

Thus in one embodiment there is provided a purified anti-TGF-beta antibody or fragment, for example a humanised antibody or fragment, in particular an antibody or fragment according to the invention, in substantially purified form, in particular free or substantially free of endotoxin and/or host cell protein or DNA.

Purified form as used *supra* is intended to refer to at least 90% purity, such as 91, 92, 93, 94, 95, 96, 97, 98, 99% w/w or more pure.

Substantially free of endotoxin is generally intended to refer to an endotoxin content of 1 EU per mg antibody product or less such as 0.5 or 0.1 EU per mg product.

Substantially free of host cell protein or DNA is generally intended to refer to host cell protein and/or DNA content 400µg per mg of antibody product or less such as 100µg per mg or less, in particular 20µg per mg, as appropriate.

The present invention also provides an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 (or pharmaceutical compositions comprising same) according to the disclosure for use as a medicament. The present invention also provides an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 (or pharmaceutical compositions comprising same) according to the disclosure for use in the

treatment or prophylaxis of a pathological disorder that is mediated by TGF-beta 1, 2 and/or 3 or that is associated with an increased level of TGF-beta 1, 2 and/or 3.

The present invention also provides the use of an antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 according to the disclosure in the manufacture of a medicament for the treatment or prophylaxis of a pathological disorder that is mediated by TGF-beta 1, 2 and/or 3 or that is associated with an increased level of TGF-beta 1, 2 and/or 3.

The present invention also provides method for the treatment of a human subject suffering from or at risk of a pathological disorder that is mediated by TGF-beta 1, 2 and/or 3 or that is associated with an increased level of TGF-beta 1, 2 or 3, the method comprising administering to the subject an effective amount of an antibody according to the disclosure. In the present application, the pathological disorder that is mediated by TGF-beta 1, 2 and/or 3 or that is associated with an increased level of TGF-beta 1, 2 and/or 3 may be any suitable disorder. In one embodiment the pathological disorder is selected from the group consisting of: pulmonary fibrosis such as idiopathic pulmonary fibrosis, pulmonary hypertension such as pulmonary arterial hypertension.

The antibody according to the disclosure may be used in the treatment of pulmonary diseases including pulmonary arterial hypertension.

The antibody according to the disclosure may be used in the treatment patients suffering from idiopathic pulmonary fibrosis and pulmonary arterial hypertension.

The present invention also provides an antagonistic antibody Fab or Fab' fragment which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 for use in the treatment or prophylaxis by inhaled administration of a pathological disorder that is mediated by TGF-beta 1, 2 or 3 and/or that is associated with an increased level of TGF-beta 1, 2 and/or 3.

The present invention also provides an antagonistic antibody Fab or Fab' fragment which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 in the manufacture of a medicament for the treatment or prophylaxis by inhaled administration of a pathological disorder that is mediated by TGF-beta 1, 2 and/or 3 or that is associated with an increased level of TGF-beta 1, 2 and/or 3.

The present invention also provides a method for the treatment of a human subject suffering from or at risk of a pathological disorder that is mediated by TGF-beta 1, 2 and/or 3 or that is associated with an increased level of TGF-beta 1, 2 and/or 3, the method comprising administering to the subject an effective amount of an antagonistic antibody Fab or Fab' fragment which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 by inhaled administration.

The pathological disorder suitable for treatment by inhaled administration may be any pulmonary disease that is mediated by TGF-beta 1, 2 and/or 3 or that is associated with an increased level of TGF-beta 1, 2 and/or 3 for example diseases selected from the group consisting of: pulmonary fibrosis such as idiopathic pulmonary fibrosis (IPF), for example mild, moderate and/or severe IPF, and cystic fibrosis and pulmonary hypertension such as pulmonary arterial hypertension (PAH). In another embodiment the antibody may be used to treat mild IPF, such as mild IPF associated with pulmonary hypertension, particularly PAH or disproportionate pulmonary hypertension. In one embodiment, the antibody may be used to treat a patient suffering IPF and pulmonary hypertension, such as IPF and PAH. In another embodiment, the antibody may be used to treated systemic sclerosis. In a further embodiment, the antibody may be used to treat systemic sclerosis associated with at least one of the following: pulmonary fibrosis (SSc-ILD); pulmonary hypertension, for example connective tissue disease-associated pulmonary hypertension; or both IPF and pulmonary hypertension.

The use of an inhaled antibody that binds to human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 may reduce the risk of side-effects by local administration to the lungs compared to systemic administration of the antibody.

The antibodies and fragments according to the present disclosure may be employed in treatment or prophylaxis.

The antibody molecule of the present invention may also be used in diagnosis, for example in the *in vivo* diagnosis and imaging of disease states involving TGF-beta.

As the antibodies of the present invention are useful in the treatment and/or prophylaxis of a pathological condition, the present invention also provides a pharmaceutical or diagnostic composition comprising an antibody molecule of the present invention in combination with one

or more of a pharmaceutically acceptable excipient, diluent or carrier. Accordingly, provided is the use of an antibody of the invention for the manufacture of a medicament. The composition will usually be supplied as part of a sterile, pharmaceutical composition that will normally include a pharmaceutically acceptable carrier. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically-acceptable adjuvant.

The present invention also provides a process for preparation of a pharmaceutical or diagnostic composition comprising adding and mixing the antibody molecule of the present invention together with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

The antibody molecule may be the sole active ingredient in the pharmaceutical or diagnostic composition. Alternatively, the antibody may be administered in combination, e.g. simultaneously, sequentially or separately, with one or more other therapeutically active ingredients. According the antibody molecule in the pharmaceutical or diagnostic composition may be accompanied by other active ingredients including other antibody ingredients, for example epidermal growth factor receptor family (EGFR, HER-2), vascular endothelial growth factor receptors (VEGFR), platelet derived growth factor receptor (PDGFR) antibodies, or non-antibody ingredients such as imatinib, dasatinib, nilotinib, basutinib, gefitinib, erlotinib, temsirolimus, vandetanib, vemurafenib, crizotinib, vorinostat, romidepsin, bortezomib, sorafenib, sunitinib, pazopanib, regorafenib, cabozantinib, perfenidone, nintedanib, steroids or other drug molecules, in particular drug molecules whose half-life is independent of TGF-beta binding. In a particular embodiment, the antibody is administered with nintedanib, for example for the treatment of IPF.

Active ingredient as employed herein refers to an ingredient with a pharmacological effect, such as a therapeutic effect, at a relevant dose.

The pharmaceutical compositions suitably comprise a therapeutically effective amount of the antibody of the invention. The term “therapeutically effective amount” as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic, pharmacological or preventative effect. For any antibody, the therapeutically effective amount can be estimated initially either in cell culture

assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 500 mg/kg, for example 0.1 mg/kg to 200 mg/kg, such as 100mg/kg. In particular, the therapeutically effective amount will be between 0.001 to 100 mg/kg.

Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose.

Therapeutic doses of the antibodies according the present disclosure show no apparent or limited toxicology effects *in vivo*.

Compositions may be administered individually to a patient or may be administered in combination (*e.g.* simultaneously, sequentially or separately) with other agents, drugs or hormones.

The antibodies to be used to treat various inflammatory diseases can be used alone or combined with various other anti-inflammatory agents.

The antibodies to be used to treat various fibrotic diseases can be used alone or combined with various other anti-fibrotic agents. Example of such agents are Pirfenidone and/or Nintedanib.

The dose at which the antibody molecule of the present invention is administered depends on the nature of the condition to be treated, the severity of the condition present and on whether the antibody molecule is being used prophylactically or to treat an existing condition.

The frequency of dose will depend on the half-life of the antibody molecule and the duration of its effect. If the antibody molecule has a short half-life (*e.g.* 2 to 10 hours) it may be necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long

half-life (e.g. 2 to 15 days) and/or long lasting pharmacodynamics (PD) profile it may only be necessary to give a dosage once per day, once per week or even once every 1 or 2 months.

Half-life as employed herein is intended to refer the duration of the molecule in circulation, for example in serum/plasma.

Pharmacodynamics as employed herein refers to the profile and in particular duration of the biological action of the molecule according the present disclosure.

The pharmaceutically acceptable carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

Suitable forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, in one or more embodiments the compositions are adapted for administration to human subjects.

Suitably in formulations according to the present disclosure, the pH of the final formulation is not similar to the value of the isoelectric point of the antibody or fragment, for example if the pH of the formulation is 7 then a pI of from 8-9 or above may be appropriate. Whilst not wishing to be bound by theory it is thought that this may ultimately provide a final formulation with improved stability, for example the antibody or fragment remains in solution.

In one example the pharmaceutical formulation at a pH in the range of 4.0 to 7.0 comprises: 1 to 200mg/mL of an antibody according to the present disclosure, 1 to 100mM of a buffer, 0.001 to 1% of a surfactant, a) 10 to 500mM of a stabiliser, b) 10 to 500mM of a stabiliser and 5 to 500 mM of a tonicity agent, or c) 5 to 500 mM of a tonicity agent.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Preferably the antibody molecules of the present invention are administered subcutaneously, by inhalation or topically. For example, the antibody may be administered intranasally or orally, such as by inhalation.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

In one embodiment the formulation is provided as a formulation for topical administrations including inhalation.

Suitable inhalable preparations include inhalable powders, metering aerosols containing propellant gases or inhalable solutions free from propellant gases (such as nebulisable solutions or suspensions). Inhalable powders according to the disclosure containing the active substance may consist solely of the abovementioned active substances or of a mixture of the abovementioned active substances with physiologically acceptable excipient.

These inhalable powders may include monosaccharides (e.g. glucose or arabinose), disaccharides (e.g. lactose, saccharose, maltose), oligo- and polysaccharides (e.g. dextrans), polyalcohols (e.g. sorbitol, mannitol, xylitol), salts (e.g. sodium chloride, calcium carbonate) or mixtures of these with one another. Mono- or disaccharides are suitably used, the use of lactose or glucose, particularly but not exclusively in the form of their hydrates.

Particles for deposition in the lung require a particle size less than 10 microns, such as 1-9 microns for example from 0.1 to 5 μm , in particular from 1 to 5 μm . The particle size of the active ingredient (such as the antibody or fragment) is of primary importance as it is thought to correlate with deposition of in areas of the lung suitable for treatment with the antibody or binding fragment of the invention. For example, particles that are 10 μm or less, such as 0.1 to 5 μm , in particular from 1 to 5 μm , are more likely to deposit in the alveolar structures of the lung.

The propellant gases which can be used to prepare the inhalable aerosols are known in the art. Suitable propellant gases are selected from among hydrocarbons such as n-propane, n-butane or isobutane and halohydrocarbons such as chlorinated and/or fluorinated derivatives of methane, ethane, propane, butane, cyclopropane or cyclobutane. The abovementioned propellant gases may be used on their own or in mixtures thereof.

Particularly suitable propellant gases are halogenated alkane derivatives selected from among TG 11, TG 12, TG 134a and TG227. Of the abovementioned halogenated hydrocarbons, TG134a (1,1,1,2-tetrafluoroethane) and TG227 (1,1,1,2,3,3,3-heptafluoropropane) and mixtures thereof are particularly suitable.

The propellant-gas-containing inhalable aerosols may also contain other ingredients such as cosolvents, stabilisers, surface-active agents (surfactants), antioxidants, lubricants and means for adjusting the pH. All these ingredients are known in the art.

The propellant-gas-containing inhalable aerosols according to the invention may contain up to 5 % by weight of active substance. Aerosols according to the invention contain, for example, 0.002 to 5 % by weight, 0.01 to 3 % by weight, 0.015 to 2 % by weight, 0.1 to 2 % by weight, 0.5 to 2 % by weight or 0.5 to 1 % by weight of active ingredient.

Alternatively topical administrations to the lung may also be by administration of a liquid solution or suspension formulation, for example employing a device such as a nebuliser, for example, a nebuliser connected to a compressor.

In one embodiment the formulation is provided as discrete ampoules containing a unit dose for delivery by nebulisation.

In one embodiment the antibody is supplied in lyophilised form, for reconstitutions or alternatively as a suspension formulation.

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

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

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

Nebulisable formulation according to the present disclosure may be provided, for example, as single dose units (e.g., sealed plastic containers or vials) packed in foil envelopes. Each vial contains a unit dose in a volume, e.g., 2 mL, of solvent/solution buffer.

The antibodies disclosed herein are thought to be suitable for delivery via nebulisation.

It is also envisaged that the antibody of the present invention may be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and light chains of the antibody molecule under the control of appropriate DNA components are introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled *in situ*.

In one embodiment the present disclosure comprises use of antibodies or fragments thereof as a reagent or diagnosis, for example conjugated to a reporter molecule. Thus there is provided antibody or fragment according to the disclosure which is labelled. In one aspect there is provided a column comprising an antibody or fragment according to the disclosure.

Thus there is provided an anti-TGF-beta antibody or fragment for use as a reagent for such uses as:

- 1) purification of TGF-beta protein (or binding fragment thereof) – being conjugated to a matrix and used as an affinity column, or (as a modified form of anti-TGF-beta) as a precipitating agent (e.g. as a form modified with a domain recognised by another molecule, which may be modified), which is optionally precipitated by an anti-Fc reagent)
- 2) detection and/or quantification of TGF-beta on cells or in cells, live or fixed (cells *in vitro* or in tissue or cell sections). Uses for this may include quantification of TGF-beta as a biomarker, to follow the effect of anti-TGF-beta treatment. For these purposes, the candidate might be used in a modified form (e.g. by addition another moiety, as a genetic

fusion protein or chemical conjugate, such as addition of a reporter molecule, for example a fluorescent tag used for the purposes of detection).

- 3) purification or sorting of TGF-beta-bearing cells labeled by binding to candidate modified by ways exemplified in (1) and (2).

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

Where technically appropriate, embodiments of the invention may be combined.

Embodiments are described herein as comprising certain features/elements. The disclosure also extends to separate embodiments consisting or consisting essentially of said features/elements.

Technical references such as patents and applications are incorporated herein by reference.

Any embodiments specifically and explicitly recited herein may form the basis of a disclaimer either alone or in combination with one or more further embodiments.

The present invention is further described by way of illustration only in the following examples:

EXAMPLES

In the following Examples, the use of the terms TGF-beta 1, TGF-beta 2 and TGF-beta 3 refer to the mature sequences of TGF-beta 1, TGF-beta 2 and TGF-beta 3 as shown in Figures 3b, 3d and 3f respectively.

Example 1 – Immunization and Primary and Secondary Screening of B Cell Culture Supernatants

4 female Half-Lop rabbits (>2kg) were immunised sub-cutaneously with 250ug human TGF-beta1 (Figure 3b) protein mixed with 250ug human TGF-beta2 protein (Figure 3d) to give a total dose of 500ug per rabbit emulsified in an equal volume of complete Freund's adjuvant (CFA) by vigorously mixing with a syringe. Rabbits were given booster injections at 21 day intervals using incomplete Freund's adjuvant (IFA) with bleeds taken, from the ear, 14 days post immunisation. 3 doses were administered of the isoform1/2 mix before a final dose of human TGF-beta2 protein only (500ug). Termination occurred 14 days after the final boost with single

cell suspensions of spleen, bone marrow and peripheral blood mononuclear cells prepared and frozen in 10% DMSO/FCS at -80°C.

Immune responses generated were determined by ELISA. Nunc-Immuno™ 1 Maxisorp™ 96 well microtitre plates were coated with either human TGF-beta1 protein (Peprotech; #100-21C) at 2µg/ml in PBS, human TGF-beta2 protein (R&D systems; 302-B2-010/CF) or human TGF-beta-3 protein (R&D systems; 243-B3-010/CF) at 0.5µg/ml in PBS and incubated overnight at 4°C. Plates were washed after each layer (automated, 4x200ml washes with PBS + 0.05% Tween). Wells were blocked with 1% (w/v) casein (VWR Chemicals; 440203H) in PBS by incubation at room temperature (RT) for 1hr. Sera, log dilutions from 1/100 in 1% casein, were added and incubated for 1 ½ hours at RT. 100µl of goat anti-rabbit IgG Fc specific horseradish peroxidase antibody (Jackson; 111-036-046) at a 1/3000 dilution in 1% (w/v) casein in PBS was added to each well and incubated for 1 hour at RT. Substrate, 100µl of TMB (3,3',5,5' Tetramethylbenzidine, soluble), was added and reaction was stopped with 50µl 2.5% sodium fluoride solution in dH2O. Optical densities (ODs) were determined at 610nm using an ELISA reader.

B cell cultures were prepared using a method similar to that described by Zubler *et al.* (1985). Briefly, peripheral blood mononuclear cell (PBMC)-derived B cells from immunized rabbits, were cultured at a density of approximately 5000 cells per well in bar-coded 96-well tissue culture plates with 200 µl/well RPMI 1640 medium (Gibco BRL) supplemented with 10% FCS (Sigma Aldrich), 2% HEPES (Sigma Aldrich), 2% L-Glutamine (Gibco BRL), 1% penicillin/streptomycin solution (Gibco BRL), 0.1% beta-mercaptoethanol (Gibco BRL), 0.2% Normocin (Invivogen), 1% activated human peripheral blood mononuclear cell (PBMC) supernatant and gamma-irradiated mutant EL4 murine thymoma cells (5×10^4 /well) for seven days at 37°C in an atmosphere of 5% CO₂.

Primary Screen for TGF-beta1 binding:

The presence of TGF-beta1 protein-specific antibodies in B cell culture supernatants was determined using a homogeneous fluorescence-based binding assay using Superavidin™ beads (Bangs Laboratories) coated with biotinylated TGF-beta1 (Peprotech). TGF-beta1 protein was

biotinylated using a Lightning-Link® Biotinylation kit (Innova Biosciences) according to manufacturer's instructions.

10 ul of B cell culture supernatant was transferred from barcoded 96-well tissue culture plates into barcoded 384-well black-walled assay plates containing TGF-beta1 immobilised on beads (10ul/well) using a Bravo automated liquid handler (Agilent). Binding was revealed with a goat anti-rabbit IgG Fcy-specific FITC conjugate (Jackson ImmunoResearch). Plates were read on a TTP Labtech mirrorball® detection system.

Following primary screening, positive supernatants for TGF-beta1 binding were consolidated onto 96-well bar-coded master plates using a Beckman hit-picking robot and B cells in cell culture plates frozen at -80°C.

Secondary Screen for binding to TGF-beta 1, 2 and 3:

To determine the ability of the antibodies to bind different isoforms of TGF-beta, B-cell supernatant in these master plates was screened in an ELISA assay on the 3 different isoforms of TGF-beta. The ELISA assay involved the coating of different TGF-beta isoforms 1, 2, 3 (Peprotech) onto 384-well Maxisorp™ plates (ThermoScientific/Nunc) at 2ug/ml in PBS. Plates were blocked with 1% BSA in PBS and then incubated with 10ul/well of B cell culture supernatant. Secondary HRP-conjugated goat anti-rabbit IgG fc antibody (Jackson ImmunoResearch) was added to plates, followed by visualisation of binding with TMB substrate (3,3',5,5'-Tetramethylbenzidine, from EMD Millipore; 10ul/well). The optical density was measured at 630nm using BioTek Synergy 2 microplate reader.

Results from Primary Screen and Secondary Screen

79 Mice, rats and rabbits were immunized with only human TGF-beta1 (SEQ ID NO:114; Figure 3b) and screened for TGF-beta 1 binding with varying levels of positive TGF-beta 1 binders. From these 79 different immunized rats, mice and rabbit animals 2656 anti-human TGF-beta 1 binders were identified. However, only 831 of these wells showed binding to all three isoforms in the secondary screen.

As described above, 4 rabbits were immunized with both human TGF-beta 1 and human TGF-beta 2. In total sera from these 4 rabbits were analysed in a primary screen and showed 1367 positive wells for TGF-beta 1 binding and were then screened in the secondary screen for binding to all three TGF-beta isoforms 1, 2 and 3 which resulted in 1026 wells with binding to all three isoforms of TGF-beta.

Following Primary and Secondary screening, B cell wells demonstrating binding to all three isoforms were then assayed for blocking activity.

HEK-Blue TGF-beta reporter gene assay using recombinant TGF-beta 1

A reporter gene assay was developed using HEK-Blue TGF-beta cells (HEK-Blue TGF-beta cell line; Invivogen). The HEK-Blue TGF-beta cell line responds to the presence of TGF-beta by expression of the SEAP which is detected with a colourimetric detection reagent. Antibodies able to neutralise TGF-beta will cause a reduction in the signal generated in the reporter cell line. The ability of test agents to neutralise TGF-beta 1 was assessed.

Antibodies were titrated 3-fold or added at a single concentration and incubated with human TGF-beta isoform 1, 2 or 3 (50 pg/ml TGF-beta isoform) in test medium (DMEM, 4.5 g/l glucose, 10% (v/v) fetal bovine serum, 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normoci, 2 mM L-glutamine) for 30 minutes prior to the addition of 50,000 HEK-Blue TGF-beta cells, and incubated for 16 hours at 37°C. SEAP produced by the cells in response to activation by TGF-beta was detected by addition of Quanti Blue (Invivogen) reagent for 1 hour at 37°C and detection by absorbance at 630 nm. The maximum signal was generated from wells containing HEK-Blue TGF-beta cells and TGF-beta and the minimum signal was generated using an excess of TGF-beta-neutralising antibody.

B cell culture supernatant containing BSN.4856 was assayed in the single point TGF-beta1 reporter gene assay (Master plate 3142, from well D012). The antibody exhibited 80 % inhibition of TGF-beta1. The percent inhibition from concentration response assays was calculated based on the maximum and minimum signals in the assay plate.

Example 2 - Cloning of variable region genes from B cells and expression and characterisation of recombinant Fab activity in *in vitro* assays

Data from binding ELISAs and the blocking reporter gene assays in Example 1 allowed selection of wells for variable region recovery. To recover antibody variable region genes from wells of interest, a deconvolution step had to be performed to enable identification of the antigen-specific B cells in a given well because a heterogeneous population of B cells is present. This was achieved using the fluorescent foci method (Clargo et al., mAbs Vol. 6, Iss. 1, 2014). Briefly, immunoglobulin-secreting B cells from a positive well were mixed with streptavidin beads (New England Biolabs) coated with biotinylated TGF-beta1 and a 1:1200 final dilution of a goat anti-rabbit Fcγ fragment-specific FITC conjugate (Jackson ImmunoResearch). After static incubation at 37°C for 1 hour, antigen-specific B cells could be identified due to the presence of a fluorescent halo surrounding that B cell. These individual B cells, identified using an Olympus microscope, were then picked with an Eppendorf micromanipulator and deposited into a PCR tube.

Antibody variable region genes were recovered from single cells by reverse transcription (RT)-PCR using heavy and light chain variable region-specific primers. Two rounds of PCR were performed, with the nested 2° PCR incorporating restriction sites at the 3' and 5' ends allowing cloning of the variable region into a rabbit Fab no hinge (VH) or rabbit kappa (VL) mammalian expression vector. Heavy and light chain constructs were co-transfected into Expi-293 cells using ExpiFectamine™ 293 (Invitrogen) and recombinant antibody expressed in a 48 deep well block in a volume of 1 ml or in a conical flask at 30 mL scale. After 7 days expression, unpurified transient supernatants were harvested and tested again for binding by ELISA and blocking in the reporter gene assay as described in Example 1. Binding to all 3 isoforms was confirmed with the recombinant 4856 rabbit Fab in an ELISA. Sequences are provided in Figure 1B.

The expressed 4856 rabbit Fab molecule was purified by affinity capture using a small scale vacuum based purification system. Briefly, supernatant from the 30 ml cell culture was 0.22μm sterile filtered before 1 ml of GammaBind Plus™ beads (GE Healthcare) were added. The supernatant/bead mixture was then tumbled for an hour before supernatant was removed by

applying vacuum. The beads were washed with PBS before elution with 0.1M glycine pH 2.7. The eluted fractions were neutralized and buffer exchanged into PBS before being 0.22 μ m sterile filtered. The final analysis consisted of concentration determination by A280; purity by SEC-UPLC (BEH200 column, Waters); and endotoxin by PTS-Endosafe™ cartridge system (Charles River).

Example 3 - Characterisation of recombinant rabbit Fab activity in *in vitro* assays

HEK-SEAP-SBE reporter gene assay using recombinant TGF-beta 1, 2 and 3:

The purified rabbit Fab was then tested (n=2) in the TGF-beta reporter gene assay as described in Example 1, in 10 point dose response against TGF-beta 1, 2 and 3. TGF-beta isoforms 1, 2 and 3 were added at 50 pg/ml and the ability of antibody to neutralise TGF-beta 1, 2 and 3 was assessed.

The data was fitted using a 4 parameter logistical fit (Figure 4a, 4b and 4c). The IC₅₀ was calculated based on the inflexion point of the curve (Table 1).

Identifier	Geomean IC ₅₀ (nM)		
	TGF-beta1	TGF-beta2	TGF-beta3
BSN.4856.rbFab. (n=2, of 5 separate samples)	1.77 (0.04nM)	0.30 (0.01nM)	24.51 (0.54nM)

Table 1. IC₅₀ values of purified 4856 rabbit Fab in the HEK-Blue TGF-beta (Invivogen) reporter gene assay.

Purified rabbit Fab 4856 inhibited the TGF-beta1-, TGF-beta2- and TGF-beta3-driven HEK-Blue TGF-beta reporter gene assay with an IC₅₀s of 0.04, 0.01, and 0.54 nM respectively.

Endogenous TGF-beta BxPC3 and HEK-Blue TGF-beta reporter gene co-culture assay:

A co-culture system was developed consisting of Bx-PC3 cells (ATCC) and the HEK-Blue TGF-beta cell line (Invivogen). The BxPC-3 cells constitutively produce and activate TGF-beta. The HEK-Blue TGF-beta cell line responds to the presence of active TGF-beta by expression of the SEAP which is detected with a colourimetric detection reagent. Antibodies able to neutralise TGF-beta will cause a reduction in the signal generated in the reporter cell line.

HEK-Blue TGF-beta cells were plated out at 100000 cells per well in DMEM with 10% FCS and incubated for 90 minutes at 37°C. Test agents were titrated 3-fold in serum-free DMEM containing 0.2% (w/v) BSA and added to the HEK-Blue TGF-beta cells. BxPC3 cells were added in serum-free DMEM containing 0.2% (w/v) BSA at 50000 cells per well and incubated for 18 hours at 37°C. The maximum signal was generated from wells containing both Bx-PC3 and HEK-Blue TGF-beta cells and the minimum signal was generated using an excess of TGF-beta-neutralising antibody. SEAP was detected by addition of QuantiBlue reagent for 1 hour at 37°C and measuring absorbance at 630 nm.

Purified 4856 rabbit Fab was assayed in the BxPC3- HEK-Blue TGF-beta reporter gene co-culture assay (n=3). The percent inhibition from concentration response assays was calculated based on the maximum and minimum signals in the assay and the data fitted using 4 parameter logistical fit (Figure 5). The IC50 was calculated based on the inflexion point of the curve (Table 2).

Table 2. Potency results of 4856 rabbit Fab in the BxPC3- HEK-Blue TGF-beta reporter gene co-culture assay. Five different samples of 4856 rabbit Fab were each tested in three independent experiments.

Identifier	n	Geomean IC50 (nM) range
BSN.4856.rbFab	3	3.7; 0.8-16

BSN.4856.rbFab inhibits the BxPC3- HEK-Blue TGF-beta reporter gene co-culture assay with an IC50 of 3.7 nM.

Affinity of 4856 rabbit Fab

The affinity of 4856 rabbit Fab against TGF- β isoforms 1, 2, and 3 was determined by Surface Plasmon Resonance using a Biacore™ T200 (GE Healthcare).

TGF- β isoforms 1, 2, and 3 (Peprotech) were immobilised on CM5 Series S chip via amine coupling chemistry on Flowcell 2, 3 and 4 (respectively) to a level of approximately 150RU. HBS-EP buffer (10mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05 % surfactant P20; GE Healthcare) was used as the running buffer. 4856 rabbit Fab was titrated over all three isoforms at various concentrations (200nM to 12.5nM) at a flow rate of 30 μ L/min. The surface was regenerated by 2 x 10 μ L injection of 10 mM HCl at a flowrate of 10uL/min.

Background subtraction binding curves were analysed using the Biacore™ T200 evaluation software (version 1.0) following standard procedures. Kinetic parameters for 4856 rabbit Fab were determined using the ‘heterogeneous ligand fitting’ algorithm with RI=0. Kinetic parameters are summarized in Table 3.

The immobilisation of each isoform of TGF β to the Biacore™ sensor chip via lysine residues is believed to have occluded the binding of the antibody to one binding domain and resulted in a secondary weak interaction (KD1). The data has been fitted to a heterogeneous model to account for the two independent binding events. The higher affinity component (KD2) is believed to represent the non-occluded interaction and therefore the most representative affinity measurement of the test antibody.

Table 3. Affinity for 4856 rabbit Fab determined using a Biacore affinity assay, n=5 for each group.

	Ka1 (1/Ms)	Kd1 (1/s)	KD1 (M)	ka2 (1/Ms)	kd2 (1/s)	KD2 (M)
TGFb1	5.05E+04	3.18E-04	6.30E-09	4.55E+05	5.08E-05	1.12E-10
TGFb2	3.87E+04	6.55E-05	1.69E-09	6.32E+05	3.83E-04	6.06E-10
TGFb3	6.33E+04	7.82E-04	1.24E-08	8.73E+05	1.44E-03	1.65E-09

Example 4 – Generation of Chimeric and Humanised grafts of Antibody 4856

The antibody Fab 4856 was selected for further optimisation based on its excellent inhibitory activity in both the HEK-Blue™-SBE reporter gene assay and the BxPC3-HEK-Blue™-SBE reporter gene co-culture assay combined with its ability to bind all three isoforms of TGF-beta with high affinity.

Chimeric Antibody 4856

The variable regions of antibody 4856 were cloned into separate heavy- and light-chain expression vectors and were expressed as a human Fab (no hinge) fragment.

The VH gene (SEQ ID NO: 17) was cloned into vector pMhFab-HIS₆, which contains DNA encoding the human gamma-1 CH1 constant region (G1m17 allotype) with a truncated hinge and a C-terminal tag of six histidine residues. The VL gene (kappa) (SEQ ID NO: 13) was cloned into vector pMhCK, which contains DNA encoding the human kappa constant region (K1m3 allotype).

Antibodies were expressed by transient co-transfection of heavy- and light-chain vectors into Expi293F™ cells.

Humanised Antibody 4856

Antibody 4856 was humanised by grafting the CDRs from the rabbit antibody V-region onto human germline antibody V-region frameworks.

In order to recover the activity of the antibody, a number of framework residues from the rabbit V-region were also retained in the humanised sequence. These residues were selected using the protocol outlined by Adair et al. (1991) (Humanised antibodies. WO91/09967). Alignments of the rabbit antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences are shown in Figures 2a and 2b, together with the designed humanised sequences.

The CDRs grafted from the donor to the acceptor sequence are as defined by Kabat *et al.* (*supra*), with the exception of CDRH1 where the combined Chothia/Kabat definition is used (see Adair *et al.* *supra*).

Genes encoding a number of variant heavy and light chain V-region sequences were designed and constructed by an automated synthesis approach by DNA2.0 Inc. Further variants of both heavy and light chain V-regions were created by modifying the VH and VK genes by oligonucleotide-directed mutagenesis, including, in some cases, mutations within CDRs to modify potential Aspartic acid isomerisation sites.

These genes were cloned into a number of vectors to enable expression of humanised 4856 Fab antibody in *E. coli* and mammalian cells. The variant chains, and combinations thereof, were assessed for their potency relative to the parent antibody, their biophysical properties and suitability for downstream processing, leading to the selection of the gL3 light chain graft and gH13 heavy chain graft.

Human V-region IGKV1-5 plus JK4 J-region (Figure 1I, also available from IMGT®, www.imgt.org, last accessed 05 January 2016) was chosen as the acceptor for antibody 4856 light chain CDRs. The light chain framework residues in graft gL3 are all from the human germline gene, with the exception of residues 1, 2, 3 and 71 (Kabat numbering), where the donor residues Alanine (A1), Tyrosine (Y2), Aspartic acid (D3) and Tyrosine (Y71) were retained, respectively. Retention of residues A1, Y2, D3 and Y71 was essential for full potency of the humanised antibody.

Human V-region IGHV3-21 plus JH5 J-region (Figure 1I, also available from IMGT®, www.imgt.org, last accessed 05 January 2016) was chosen as the acceptor for the heavy chain CDRs of antibody 4856. In common with many rabbit antibodies, the VH gene of antibody 4856 is shorter than the selected human acceptor. When aligned with the human acceptor sequence, framework 1 of the VH region of antibody 4856 lacks the N-terminal residue, which is retained in the humanised antibody (Figure 2b). Framework 3 of the 4856 rabbit VH region also lacks two residues (75 and 76) in the loop between beta sheet strands D and E: in graft gH13 the gap is filled with the corresponding residues (Lysine 75, K75; Asparagine 76, N76) from the selected human acceptor sequence (Figure 2b). The heavy chain framework residues in grafts gH13,

gH23 and gH29 are all from the human germline gene, with the exception of residues 48, 49, 73 and 78 (Kabat numbering), where the donor residues Isoleucine (I48), Glycine (G49), Serine (S73) and Valine (V78) were retained, respectively. Retention of residues E1, V2, Q3, I48, G49, S73 and V78 was essential for full potency of the humanised antibody.

In graft gH20, framework residues are all from the human germline gene, with the exception of residues 48, 69, 71, 73 and 78 (Kabat numbering), where the donor residues Isoleucine (I48), Methionine (M69), Lysine (K71), Serine (S73) and Valine (V78) were retained, respectively.

Residue 98 in CDRH3 of grafts gH13, gH20, gH23 and gH29 was mutated from a Glycine (G98) to an Alanine (A98) residue, thus removing a potential Aspartic acid isomerization site from the gH13, gH20, gH23 and gH29 sequences.

A potential Asparagine deamidation site at residues N100e and G100f (Figure 2b), was removed in the gH23 graft by mutating G100f to A100f and was removed in the gH29 graft by mutating N100e to D100e.

Expression of humanised 4856 Fab

The original 4856 Fab fragments were constructed and tested as mammalian expression vectors. In order to achieve the highest yield the codon usage of the grafts was changed to suit *E. coli* periplasmic expression. The grafts were aligned with the previous humanized Fabs which historically gave consistently high yields and corresponding codons altered to match the framework sequences.

For expression of humanised 4856 Fab in *E. coli*, the humanised heavy chain V-region gene (SEQ ID NO: 54, SEQ ID NO: 68, SEQ ID NO:82 or SEQ ID NO: 96) and light chain V-region gene (SEQ ID NO:40) were cloned into the UCB expression vector pTTOD, which contains DNA encoding the human C-kappa constant region (K1m3 allotype) and the human gamma-1 CH1 region (G1m17 allotype). The *E.coli* fkpA and dsbC genes were also introduced into the expression plasmid, as co-expression of these chaperone proteins was found to improve the yield of the humanised Fab in *E. coli* strain MXE016 during batch-fed fermentation, using IPTG to induce Fab expression. The 4856 Fab light and heavy chains and FkpA and DsbC polypeptides

were all expressed from a single multi-cistron under the control of the IPTG-inducible tac promoter.

Expression of the Fab was tested in the *E. coli* production strain MXE016 using a 5ml auto induction method. The combination of FkpA and DsbC chaperones increased the yield of Fab obtained substantially.

For expression of humanised 4856 Fab in mammalian cells, the humanised light chain V-region gene was joined to a DNA sequence encoding the human C-kappa constant region (K1m3 allotype), to create a contiguous light chain gene (SEQ ID NO:41). The humanised heavy chain V-region gene was joined to a DNA sequence encoding the human gamma-1 CH1 region (G1m17 allotype), to create a contiguous heavy chain gene (SEQ ID NO: 55, SEQ ID NO: 69, SEQ ID NO: 83 or SEQ ID NO: 97). The heavy and light chain genes were cloned into the mammalian expression vector pMXE692 Cellca vector DGV 4856 gL3 gH13 VL VH.

Biacore affinity determination of E.coli derived 4856 Fab gL3gH13

Antibody 4856 gL3gH13 produced in *E. coli* according to the method described above was tested for affinity against TGF- β isoforms 1, 2, and 3 determined by Surface Plasmon Resonance using a Biacore T200 (GE Healthcare). Human TGF- β isoforms 1, 2, and 3 (Peprotech) were immobilised on CM5 Series S chip via amine coupling chemistry on Flowcell 2, 3 and 4 (respectively) to a level of approximately 20RU. HBS-EP buffer (10mM HEPES pH 7.4, 0.15 M NaCl, 3 mM EDTA, 0.05 % Surfactant P20, GE Healthcare) was used as the running buffer. Antibody 4856 gL3gH13Fab was titrated over all three isoforms at various concentrations (200nM to 1.56nM) at a flow rate of 30 μ L/min. The surface was regenerated by 2 x 10 μ L injection of 10 mM HCl at a flowrate of 10uL/min.

Background subtraction binding curves were analysed using the T200evaluation software (version 1.0; GE Healthcare) following standard procedures. Kinetic parameters were determined using heterogeneous fitting algorithm with RI=0), as described in Example 3, and the values are provided in Table 4. As before with the rabbit version of the Fab in Table 3, the KD2 of Table 4 is believed to represent the unoccluded binding value for antibody 4856 gL3gH13Fab.

Table 4: Affinity for 4856 humanised Fab determined using a Biacore affinity assay, n=4 for each group.

TGFb isoform	ka1 (1/Ms)	kd1 (1/s)	KD1 (M)	ka2 (1/Ms)	kd2 (1/s)	KD2 (M)
TGFb1	7.49E+04	7.09E-04	9.48E-09	7.04E+05	5.56E-05	8.08E-11
TGFb2	5.77E+04	2.11E-04	3.67E-09	9.04E+05	1.70E-04	1.96E-10
TGFb3	9.32E+04	1.46E-03	1.57E-08	2.00E+06	3.39E-03	1.72E-09

Analytical gel filtration was performed to determine whether the antibody graft 4856 gL3gH13 binds to the full length sequence of TGF-beta 1 including the latency associated peptide. The data showed that antibody 4856 gL3gH13 does not bind full length TGF-beta 1 including the latency associated peptide (data not shown).

Example 5 - *In Vitro* Inhibitory Activity of Humanised Grafts

Inhibitory Activity in HEK-Blue TGF-beta reporter gene assay using recombinant TGF-beta 1, 2 and 3:

The inhibitory activity of 4856 humanised grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29, was analysed in the HEK-Blue TGF-beta reporter gene assay using recombinant TGF-beta 1, 2 and 3, as described in Example 1, in 10 point dose response against TGF-beta 1, 2 and 3. TGF-beta isoforms 1, 2 and 3 were added at 50 pg/ml and the ability of antibody to neutralise TGF-beta 1, 2 and 3 was assessed. The data was fitted using a 4 parameter logistical fit. The IC50 was calculated based on the inflexion point of the curve (Table 5). It can be seen from Table 5 that the humanized grafts, particularly gL3gH13 and gL3gH20 were effective in neutralizing TGF-beta 1, 2 and 3 activity.

Table 5: Inhibition of exogenous TGF-beta isoforms 1, 2 and 3.

TGF-beta1	N	Geometric mean (nM)	95% CI (range where N=<4)
4856gL3gH29	9	0.255	0.173-0.376
4856gL3gH23	8	0.41	0.313-0.538
4856gL3gH13	11	0.074	0.053-0.104
4856gL3gH20	12	0.038	0.024-0.062
TGF-beta 2	N	Geometric mean (nM)	95% CI (range where N=<4)
4856gL3gH29	3	0.016	0.013-0.018 (range)
4856gL3gH23	3	0.017	0.015-0.022 (range)
4856gL3gH13	3	0.01	0.007-0.019 (range)
4856gL3gH20	5	0.011	0.010-0.027
TGF-beta 3	N	Geometric mean (nM)	95% CI (range where N=<4)
4856gL3gH29	3	1.954	1.6-2.46 (range)
4856gL3gH23	4	1.987	1.21-1.88 (range)
4856gL3gH13	4	0.475	0.248-0.909
4856gL3gH20	5	0.302	0.141-0.647

Inhibitory Activity in Endogenous TGF-beta BxPC3 and HEK-Blue TGF-beta reporter gene co-culture assay:

The inhibitory activity of 4856 humanized grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29, was analysed in the BxPC3 and HEK-Blue TGF-beta reporter gene co-culture assay as described in Example 3. The percent inhibition from concentration response assays was calculated based on the maximum and minimum signals in the assay and the data fitted using 4 parameter logistical fit. The IC₅₀ was calculated based on the inflexion point of the curve and

the results are shown in Table 6. It can be seen from Table 6 that the humanized grafts, particularly gL3gH13, gL3gH20 and gL3gH29 were effective in neutralizing TGF-beta expressed and activated by cells in this assay.

Table 6: Inhibition of TGF-beta expressed by BxPC3 cells in a co-culture assay with HEK-Blue TGF-beta cells.

Endogenous assay	N	Geometric mean (nM)	95% CI (range where N=<4)
4856gL3gH23	4	9.104	3.89-21.31
4856gL3gH29	4	3.773	0.71-20.05
4856gL3gH13	4	3.949	2.52-6.19
4856gL3gH20	3	4.626	3.01-6.41 (range)

Example 6 - Inhibitory Activity of Humanised 4856 Grafts in an Adriamycin-Induced In Vitro Model of Kidney Fibrosis

Adriamycin-induced nephropathy is a well characterised rodent model of acquired kidney fibrosis with pathological features similar to human glomerulosclerosis and tubulointerstitial fibrosis. Mesangial cells are one of the main cell types involved in the fibrotic phenotype in response to Adriamycin. A human in vitro model for the fibrotic response to Adriamycin treatment was established and the ability of TGF-beta-neutralizing grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29 of antibody 4856 Fab to modulate the deposition of extracellular matrix (ECM) components in this system was assessed.

Primary human renal mesangial cells (HRMCs, Innoprot) were plated at 1.6×10^4 cells/cm² in the presence of 10nM Adriamycin and of test 4856 Fab grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29 and a control Fab, (3-fold sequential dilutions ranging from 0.2 to 6000 nM). Cells were incubated for 6 days at 37°C, 5%CO₂, then lysed in 0.25M NH₄OH/25mM Tris (30min at 37°C) and the deposited ECM fixed in ice-cold Methanol (30min at -20°C). Deposition of the

individual ECM components was detected by high content imaging after immunostaining for Fibronectin (AlexaFluor488-conjugated Ebioscience 53-9869-82), Collagens I and III (Millipore rabbit polyclonal antibodies AB745 and AB747), and Collagen type IV (Efluor660-conjugated Ebioscience 50-9871-80). Images were acquired and the fluorescence intensity detected by a Cellomics Arrayscan. The maximum signal was generated from wells containing Adriamycin-treated cells in the absence of Fab and the minimum signal was obtained in wells where cells had not been exposed to Adriamycin.

The percent inhibition from concentration response assays was calculated based on the maximum and minimum fluorescence intensities in the assay and the data fitted using 4 parameter logistical fit. Images and plots shown are representative of three replicate experiments.

Figure 6 shows representative images of ECM deposition by HRMCs in response to 10nM Adriamycin and in the presence of the indicated concentrations of TGF-beta-neutralising 4856 Fab grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29 or control Fab.

Figure 7 shows representative concentration response curves for 4856 Fab grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29 in the Adriamycin in vitro assay.

The four tested TGF-beta-neutralising Fab grafts inhibited Adriamycin-induced ECM deposition by HRMCs (Table 7).

Table 7: Inhibition of ECM deposition by 4856 Fab grafts gL3gH13, gL3gH20, gL3gH23 and gL3gH29 in the Adriamycin-Induced Model of Kidney Fibrosis (Geomean from 3 replicate experiments).

4856 Fab graft	Fibronetin		Collagen I&III		Collagen IV	
	Geomean IC50 (nM)	range n=3	Geomean IC50 (nM)	range n=3	Geomean IC50 (nM)	range n=3
gL3gH29	105.96	49.3-190.1	90.10	40.2-178.1	22.93	6.0-50.7
gL3gH23	227.29	40.6-1387.7	198.35	110.4-540.2	44.86	24.8-99.8
gL3gH13	57.30	32.9-118.6	57.94	31.0-87.7	10.39	9.2-11.8
gL3gH20	104.84	52.1-329.4	33.86	16.8-60.2	16.65	8.3-51.0

Note, the upper and lower asymptotes of some curves were fixed at minimum or maximum values.

Example 7 - Inhibitory Activity of Humanized 4856 Graft in an In Vitro Model of Human Lung Interstitial Fibrosis

Epithelial damage and fibroblast activation are crucial events leading to ECM accumulation during the fibrotic process. In order to establish an in-vitro model of lung interstitial fibrosis, an assay was developed using primary human small airway epithelial cells (SAEpCs, ATCC) and lung fibroblasts (ATCC) isolated from an IPF patient. The co-culture of these two cell types in epithelial cell media induces significant ECM deposition even in the absence of additional stimulus, allowing the study of anti-fibrotic agents.

1.8×10^4 primary human small airway epithelial cells (SAEpCs) and equal number of IPF lung fibroblasts were plated per cm^2 (total of 3.6×10^4 cells/ cm^2) and co-cultured for 7 days at 37°C , $5\%\text{CO}_2$. 4856 Fab graft gL3gH13, and a control Fab were titrated 3-fold within the range of 0.03 to 1000nM. After the 7 day co-culture, cell viability was assessed with Presto Blue, the cells were then lysed in 0.25M NH_4OH /25mM Tris (30min at 37°C) and the deposited ECM fixed in ice-cold Methanol (30min at -20°C). Deposition of the individual ECM components was detected by high content imaging after immunostaining for Fibronectin and Collagens type I, III,

IV and V. Images were acquired and the fluorescence intensity detected by a Cellomics Arrayscan. The maximum signal was generated in untreated co-cultures and the minimum signal was generated using excess Fab.

The percent inhibition from concentration response assays was calculated based on the maximum and minimum signals in the assay and the data fitted using 4 parameter logistical fit. The IC₅₀ was calculated based on the inflexion point of the curve. Images and plots shown are representative of three replicate experiments.

Figure 8 shows images of ECM deposition by SAEpCs and IPF fibroblasts co-cultures in the presence of the indicated concentrations of 4856 Fab graft gL3gH13 and a control Fab.

Figure 9 shows concentration response curves of the 4856 Fab graft gL3gH13 and a control Fab in the human lung co-culture assay.

Table 7 shows the potency results for the inhibition of ECM deposition by 4856 Fab graft gL3gH13 in the lung co-culture assay (Geomean from 3 replicate experiments)

ECM protein	Geomean IC ₅₀ (nM) (range) n=3
	4856 gL3gH13
Fibronectin	2.42 (1.6-3.9)
Collagen I & III	2.66 (2.3-3.2)
Collagen IV	2.91 (2.1-5.5)
Collagen V	2.70 (1.8-4.5)

ECM deposition in co-cultures of SAEpCs and IPF fibroblasts was inhibited by 4856 Fab graft gL3gH13.

Example 8 - Inhibitory Activity of Humanized 4856 Graft in an In Vitro Model of Human Kidney Fibrosis

The capacity of 4856 Fab gL3gH13 to inhibit fibronectin and collagen deposition in human primary kidney cells was assessed using extra cellular matrix (ECM) accumulation assays on a mono-culture of human renal proximal tubular epithelial cells (RPTEC) stimulated with TGF-beta 1, 2 or 3 and a co-culture of human renal proximal tubular epithelial cells (RPTEC) with human renal fibroblasts (HRF) (no stimulation).

Human renal proximal tubular epithelial cells (RPTEC, Innoprot) and human renal fibroblasts (HRF, InnoProt) were seeded at 2,000 cells per well (ratio 1:1 in the co-culture) in a 384-well black clear-bottomed plate (Corning) in the presence of 0.1 to 100 µg/mL (0.2-2000 nM) of anti-TGF-beta antibody (gL3gH13 Fab) or control Fab and 10 ng/ml TGF-beta1 (Peprotech), TGF-beta2 (R&D) or TGF-beta3 (R&D) for the mono-culture of RPTEC, or no exogenous TGF-beta (no stimulation) for the co-culture of RPTEC and HRF, in a final volume of 50 µL in Renal Epithelial Cell Basal medium + 0.5% Fcs and supplements (ATCC).

After 7 days incubation at 37°C 5% CO₂, cells were washed in PBS and lysed with 20 µl 0.25 M NH₄OH / 25 mM Tris for 15 min at 37°C. Matrix was washed 3 times in PBS, fixed in 40 µl 100% methanol for 30 min at -20°C and washed 3 times in PBS before being stained using anti-Fibronectin (eBiosciences), anti-Collagen I (Millipore), anti-Collagen III (Millipore), anti-Collagen IV (eBiosciences) and anti-Collagen V (Abcam) antibodies. Plates were scanned on the Cellomics Arrayscan HC reader using a 3-channel protocol under the “Cellomics CellHealth” profiling bioapplication and a 10x objective (new X1 camera) with 2x2 binning (1104x1104 pixels/field).

Although data was generated for the Collagen IV readout, the results have been excluded due to unacceptable assay windows and variability.

Results are shown in Table 9 and Figures 10a, b, c and d and Figures 11a and b which shows that gL3gH13 Fab is able to inhibit TGF-beta1, 2 and 3 induced accumulation of Fibronectin and Collagen I, III and V in the RPTEC monoculture system, and by endogenously produced TGF-beta in the RPTEC and HRF co-culture system.

Table 9: IC50s and Geometric Mean IC50s (nM) (N=3) for gL3gH13:

Marker	Antibody	Mono-culture + TGFB1				Mono-culture + TGFB2				Mono-culture + TGFB3				Co-culture		
		N=1	N=2	N=3	Geo Mean	N=1	N=2	N=3	Geo Mean	N=1	N=2	N=3	Geo Mean	N=1	N=2	Geo Mean
Fibronectin	gL3gH13	12.6	57.4	9.8	19.2	0.16	0.76	0.22	0.30	51.5	140.0	76.8	82.1	32.6	27.1	29.7
Collagen I &III	gL3gH13	5.1	13.8	19.8	11.2	<2	0.60	0.13	0.30	64.3	123.8	67.2	81.2	22.4	48.0	32.8
Collagen V	gL3gH13	13.1	92.1	69.4	43.7	0.52	10.5	5.29	3.1	140.8	336.3	146.6	190.8	38.9	81.6	56.3

Example 9 - In Vivo murine model of lung fibrosis

i) 7 day challenge

The acute bleomycin-induced model of lung injury involves the local administration of the glycopeptide bleomycin directly to the lungs of mice. This induces an inflammatory response associated with an increase in Plasminogen Activator Inhibitor-1 (PAI-1) and ultimately results in pulmonary fibrosis. PAI-1 is transcriptionally regulated by TGF-beta and can act as a potent fibrogenic mediator inducing the recruitment of inflammatory cells and the deposition of extracellular matrix (ECM) (Ghosh and Vaughan, 2012, J Cell Physiol, 227: 493-507).

Any effect on test anti-TGF-beta Fabs to limit fibrogenesis such as PAI-1 inhibition provides supporting evidence that a pan-specific anti-TGF-beta blocking Fab delivered directly to the lung is a viable therapeutic for pulmonary fibrosis in humans.

4856 Fab grafts (humanised) were locally administered directly to the lungs of mice via the intranasal (i.n) route. C57/BL6 mice were administered with 4856gL3gH13 or 4856gL3gH29 (i.n; 200µg/mouse) 1 hour before bleomycin challenge (o.p; 0.05U/mouse) and 6 hours after. Mice subsequently received 4856gL3gH13 or 4856gL3gH29 (i.n; 200µg/mouse) every 12 hours until they were terminated on day 7. Immediately after termination bronchoalveolar lavage fluid (BAL) was collected and total PAI-1 concentrations were determined by ELISA. Statistical analysis performed by one-way ANOVA versus bleomycin treated control group.

The results of this administration of the two 4856Fabs (4856gL3gH13 and 4856gL3gH29) is shown in Figure 12. Figure 12 demonstrates that mice challenged with bleomycin had greatly elevated PAI-1 levels in the BAL compared to saline challenged control mice and that humanised Fabs 4856gL3gH13 and 4856gL3gH29 were capable of inhibiting bleomycin-induced PAI-1 by 49% and 64% respectively when delivered directly to the lung.

In a second study C57/BL6 mice were administered humanised 4856gL3gH13 (i.n; 20, 60, 200µg/mouse) 1 hour before bleomycin challenge (o.p; 0.05U/mouse) and 6 hours after. Mice subsequently received 4856gL3gH13 every 12 hours until they were terminated on day 7. Immediately after termination BAL was collected and total PAI-1 concentrations were determined by ELISA. Statistical analysis performed by one-way ANOVA versus bleomycin treated control group. * $p \leq 0.05$, ** $p \leq 0.005$, *** $p \leq 0.0005$, **** $p \leq 0.00005$.

In order to demonstrate superior efficacy of 4856gL3gH13 in this system, Figure 13 illustrates the effect of 4856gL3gH13 on bleomycin-induced PAI-1 at different doses. As previously shown, bleomycin induces an increase in BAL PAI-1 levels that can be significantly inhibited up to 76% using 200µg/mouse i.n 4856gL3gH13 and up to 45% using 60µg/mouse i.n 4856gL3gH13.

This demonstrates that locally delivered (i.n) 4856gL3gH13 significantly inhibits acute bleomycin-induced PAI-1 and that it is possible to locally inhibit TGF-beta in the lung, potentially avoiding unwanted systemic events.

ii) 28 day challenge

The longer term effects of bleomycin challenge results in pulmonary fibrosis, and a similar study was therefore performed in mice for 28 days with murinised 4856gL3gH13 (called 4856 hereafter) dosed prophylactically from day 1 as well as from day 13 of the bleomycin challenge. This later administration of 4856 allows fibrosis to become more fully established in the lung before treatment is started.

The impact of 4856 on bleomycin-induced pulmonary fibrosis was assessed by the attenuation of ECM depositon and myofibroblast differentiation in the lung. ECM deposition was determined histologically in paraffin-embedded lung tissue by staining for collagen using Picro Sirius Red (PSR). This was supported by more quantitative analysis of hydroxyproline levels in digested lung tissue. Hydroxyproline is a major component of collagen and can be used to estimate the amount of collagen in tissues. In addition, the number of myofibroblasts; the predominant cell type believed to be responsible for collagen deposition in the lung, was determined using immunohistochemical (IHC) staining for α -Smooth Muscle Actin (α -SMA). Furthermore, inhibition of phosphorylated-Mothers against decapentaplegic homolog 2 and 3 (p-Smad2/3) was also determined by IHC to demonstrate specific inhibition of the TGF β signaling pathway by 4856. All statistics were determined using unpaired t-test against the assigned bleomycin challenged control group. *p=0.05; **p=0.01; ***p=0.001; ****p=0.0001.

a) 4856 ameliorates bleomycin-induced collagen deposition in the lungs

Treatment with 4856 from day 1-28 Mice (Male c57BL/6; n=8/group) were treated with saline (i.t, 50µL) or bleomycin (i.t, 50µL; 0.5mg/mL) for 28 days. In addition mice were treated with either vehicle (i.n, 25 µL) or 4856 (i.n, 25µL; 400µg/mouse) twice daily from day 1-28.

Treatment with 4856 from day 13-28 Mice (Male c57BL/6; n=8/group) were treated with saline (i.t, 50µL) or bleomycin (i.t, 50µL; 0.5mg/mL) for 12 or 28 days. In addition mice were treated with either vehicle (i.n, 25 µL) or 4856 (i.n, 25µL; 400µg/mouse) twice daily from day 13-28.

Assay The entire left lobe was fixed in 4 % formalin for 6 h and embedded in paraffin. 5 µm sections were cut and stained with PSR. Images were captured using a Nikon Eclipse 80i microscope (Nikon, Badhoevedorp, Netherlands) and the fibrotic area in a minimum of four fields per mouse was analysed using ImageJ (V. 1.42q, National Institutes of Health, USA). The amount of collagen protein in the three lower lobes of the right lung (azygous lobe, cardiac lobe and diaphragmatic lobe) was determined via hydroxyproline assay. After digestion in 6 M HCl for three hours at 120 °C, the pH of the samples was adjusted to 6 with 6 M NaOH. Afterwards, 0.06 M chloramine T was added to each sample and incubated for 20 min at room temperature. Next, 3.15 M perchloric acid and 20 % p-dimethylaminobenzaldehyde were added and samples were incubated for additional 20 min at 60 °C. The absorbance was determined at 557 nm with a Spectra MAX 190 microplate spectrophotometer.

Results Intratracheal (i.t) instillation of bleomycin using a micro-sprayer (50µL; 0.5mg/mL) induced prominent pulmonary fibrosis compared to control mice treated with i.t instillation of saline (0.9% NaCl, the solvent of bleomycin). This is demonstrated by enhanced PSR staining and elevated hydroxyproline content in the lung (Figures 14 and 15). In addition, these fibrotic changes were more pronounced after 28 days than after 12 days (Figure 15) suggesting that the severity of fibrosis progressed over time.

Treatment with 4856 from day 1-28 (25µL i.n; 400µg/mouse; twice daily) resulted in a significant reduction in both PSR (figure 14A) and hydroxyproline content in the lung (figure 14B). This suggests that 4856 can prevent bleomycin-induced pulmonary fibrosis. Furthermore, administration of 4856 to bleomycin challenged mice from day 13-28 significantly limited the progressive increase in PSR (figure 15A) and hydroxyproline (figure 15B) observed at day 28

when compared to vehicle treated mice. This suggests that 4856 is capable of limiting the progression of disease when given after fibrosis is already evident.

b) 4856 ameliorates bleomycin-induced myofibroblast differentiation in the lungs

Mice (Male c57BL/6; n=8/group) were treated with saline (i.t, 50 μ L) or bleomycin (i.t, 50 μ L; 0.5mg/mL) for 12 or 28 days. In addition mice were treated with either vehicle (i.n, 25 μ L) or 4856 (i.n, 25 μ L; 400 μ g/mouse) twice daily from day 1-28 or 13-28. Myofibroblasts are characterized by the expression of α -smooth muscle actin (α -SMA). Fibroblasts positive for α -SMA were detected by incubation with monoclonal anti- α SMA antibodies (clone 1A4, Sigma-Aldrich, Steinheim, Germany). The expression was visualized with horseradish peroxidase labeled secondary antibodies and 3,3-diaminobenzidine tetrahydrochloride (DAB) (Sigma-Aldrich). Monoclonal mouse IgG antibodies (Calbiochem, San Diego, CA, USA) were used for isotype controls. Four different fields were evaluated per mouse.

The administration of bleomycin also induced an increase in myofibroblast differentiation in the lungs characterized by the expression of α -SMA by IHC (figure 16). Figure 16A illustrates that bleomycin-induced myofibroblast differentiation in the lung was inhibited by i.n administration of 4856 from day 1-28. Additionally, although there was not a significant increase in α -SMA expression between day 12 and 28 of bleomycin treatment, administration of 4856 from day 13-28 also caused a significant attenuation in myofibroblast differentiation. Furthermore, this was reduced to below the level of α -SMA expression observed after bleomycin treatment alone for 12 days suggesting a possible reversal of fibrotic processes at this time point (figure 16B).

c) 4856 inhibits bleomycin-induced TGF- β signaling in the lungs.

Mice (Male c57BL/6; n=8/group) were treated with saline (i.t, 50 μ L) or bleomycin (i.t, 50 μ L; 0.5mg/mL) for 12 or 28 days. In addition mice were treated with either vehicle (i.n, 25 μ L) or 4856 (i.n, 25 μ L; 400 μ g/mouse) twice daily from day 1-28 or 13-28. Lung sections were stained with goat anti-pSmad2/3 antibodies (Santa Cruz Biotechnology, Heidelberg, Germany) and type I collagen antibodies (Abcam, Cambridge, UK). HRP-conjugated- or Alexa Fluor antibodies (Life Technologies, Darmstadt, Germany) were used as secondary antibodies. Irrelevant isotype matched antibodies served as controls. Nuclei were stained using DAPI (Santa Cruz Biotechnology). Staining was visualized using a Nikon Eclipse 80i microscope (Nikon,

Badhoevedorp, Netherlands). The expression of pSmad2/3 in type 1 collagen positive cells was assessed in three different fields per mouse.

Bleomycin-induced lung fibrosis was associated with an increase in pSmad2/3 expression in type 1 collagen positive cells at both day 12 and to a greater extent at day 28 compared to saline treated controls (figure 17B). Treatment with 4856 from day 1-28 (figure 17A) and from day 13-28 (figure 17B) significantly inhibited pSmad2/3 expression to below that of saline treated control mice, suggesting that TGF β -dependent Smad2/3 phosphorylation was completely blocked by 4856. In addition, 4856 dosed from day 13-28 can reverse the bleomycin-induced increase in pSmad2/3 expression observed after 12 days of bleomycin challenge plus vehicle, which correlates with the effect seen on myofibroblast differentiation.

d) Summary

4856 exerted potent anti-fibrotic effects in a murine model of bleomycin-induced pulmonary fibrosis and ameliorated histological changes in collagen deposition (PSR staining), collagen accumulation (hydroxyproline assay), myofibroblast differentiation (α -SMA expression), and activation of canonical TGF- β signaling (pSmad2/3 expression). Furthermore, 4856 proved efficacious when dosed either prophylactically from day 1-28 or as an intervention from day 13-28 after fibrotic changes were already evident. This supports the hypothesis that it is possible to locally inhibit TGF β in the lung, potentially avoiding unwanted systemic events.

Example 10 - Biophysical Analysis of Humanized 4856 Fab grafts

The humanized grafts of antibody 4856 : gL3gH13, gL3gH20, gL3gH23, gL3gH29 were subjected to a series of biochemical and biophysical analyses to screen and select the most robust molecule for development and administration stability. The analyses included comparison of characteristics such as T_m (melting temperature at mid-point of unfolding); experimental pI, and aggregation stability at an air-liquid interface (mimic of shear stress in manufacture and nebulization stability); and deamidation propensity.

Thermal stability measurement (T_m)

A fluorescence-based thermal shift assay (also referred to as the thermofluor assay) was performed to obtain the T_m (temperature at the mid-point of unfolding) to assess the thermal stabilities of purified molecules. The reaction mix contained 5 μ l of 30x SYPRO® Orange dye

(Invitrogen), diluted with PBS from 5000X stock solution and 45 μl of sample at 0.12 mgml^{-1} , (in PBS pH 7.4). 10 μl of the mix was dispensed in quadruplicate into a 384 PCR optical well plate and was run on a 7900HT Fast Real-Time PCR System (Applied Biosystems). The PCR system heating device was set at 20 $^{\circ}\text{C}$ to 99 $^{\circ}\text{C}$ with a ramp rate of 1.1 $^{\circ}\text{C min}^{-1}$. A charge-coupled device monitored fluorescence changes in the wells. Intensity increases were plotted, the inflection point of the slope(s) was used to generate the T_m .

T_m (the temperature at the midpoint of unfolding) was determined by the thermofluor assay. In this method, SYPRO orange (fluorescent dye) is used to monitor the unfolding process by binding to hydrophobic regions that become exposed during thermal ramping. A higher T_m value equates to a greater molecular stability and robustness to developability and nebulisation stress.

One unfolding domain, as expected, was observed for all molecules, equivalent to the Fab unfolding domain. The results are summarised in Table 10.

It was possible to rank the molecules based on their melting temperature: gL3gH13 was shown to have the highest melting temperature, the substitution of N109G with D109G in the HC CDR3 (gL3gH29) resulted in a 2 $^{\circ}\text{C}$ decrease in the melting temperature and both gL3gH23 and gL3gH20 exhibited a further 2 $^{\circ}\text{C}$ decrease in the melting temperature. gL3gH13 had the highest melting temperature of 79 $^{\circ}\text{C}$ which makes it an excellent candidate for use in local delivery to the lung via nebulization where the Fab has to retain sufficient biological activity following nebulization.

Table 10 : T_m Analysis : Thermofluor Assay : gL3gH13>gL3gH29>gL3gH20=gL3gH23

Antibody 4856 Fab graft	T_m $^{\circ}\text{C}$
gL3gH13	79.0
gL3gH20	75.3
gL3gH23	75.7
gL3gH29	77.0

Experimental pI and analysis of charge variants

Purified samples were analysed using whole-capillary imaged cIEF ICE3 system (ProteinSimple). Samples were prepared by mixing the following: 30µl sample (from a 1mg/ml stock in HPLC grade water), 35µl of 1% methylcellulose solution (Protein Simple), 4µl pH3-10 ampholytes (Pharmalyte), 0.5 µl of 4.65 and 0.5 µl 9.77 synthetic pI markers (ProteinSimple), 12.5 µl of 8M urea solution (Sigma-Aldrich). HPLC grade water was used to make up the final volume to 100 µl. The mixture was vortexed briefly to ensure complete mixing and centrifuged at 10,000 rpm for 3 minutes to remove air bubbles before analysis. Samples were focused for 1 min at 1.5 kV, followed by 5 min at 3 kV, and A_{280} images of the capillary were taken using the ProteinSimple software. The resulting electropherograms were first analysed using iCE3 software and pI values were assigned (linear relationship between the pI markers). The calibrated electropherograms were then integrated using Empower software (Waters).

The pI of all lead candidates was high (see Table 11) such that it is unlikely that the molecules would have approximately zero overall molecular charge (where there is increased risk of aggregation) at the expected formulation pH. The experimental pI was found to be similar for all molecules and so could not discriminate between them.

Table 11: Experimental pI

	Experimental pI
gL3gH13	9.34
gL3gH20	9.25
gL3gH23	9.34
gL3gH29	9.10

Aggregation Propensity at an Air-Liquid Interface

Purified samples (3 x 250µl aliquots) in PBS pH 7.4 at 1mg ml⁻¹ were vortexed at 1400rpm at 25°C in 1.5ml eppendorfs using an Eppendorf Mixmate. Samples were analysed for turbidity generation at various time-points post vortexing by obtaining absorption at 595nm using a spectrophotometer (Varian). The data was plotted versus time.

All lead candidates were subjected to stress by vortexing to provide information on aggregation stability at an air-liquid interface. This served to mimic shear during manufacture as

well as potential stability during nebulisation. Aggregation stability was monitored by measurement of turbidity at 595 nm up to 144 hours. The rate of aggregation was slow (low absorbance values) and no difference was observed between the grafts.

Analysis of Deamidation Status Asn(N)109 (Heavy Chain CDR3).

A deamidation motif in the heavy chain CDR3 for gL3gH13, gL3gH20 (both N109G) and gL3gH23 (N109A) is present. Chemical instability at this site could result in product heterogeneity and immunogenicity. Graft gL3gH29 has D109G at this site (the deamidated product) to test suitability as a candidate whilst minimising the risk of deamidation.

The basal percent deamidation at N109 in the heavy chain CDR3 for gL3gH13, gL3gH20 and gL3gH23 immediately post purification was determined by (i) tryptic digestion/peptide mapping/ mass spectrometry and (ii) capillary imaged isoelectric focussing (ICE3). The rate/propensity for the N109 site to deamidate (loss of ammonia; generation of acidic species) was determined by subjecting the three molecules to accelerated stress conditions known to promote deamidation (pH 8, 37°C).

(i) Mass spectrometry

Aliquots (50µg) were reduced with DTT, alkylated with iodoacetamide then digested with trypsin (1:20w/w) overnight at room temperature. The digest (~2µg) was analysed by injection onto a C18 column (1 x 150mm BEH-300) equilibrated with 0.2% formic acid/ water. The resultant peptides were eluted at 20µL/minute with an acetonitrile gradient into a Thermo Fusion mass spectrometer operated in +ve-ion mode. Data dependent acquisition (DDA) consisted of an orbitrap full scan (120000 resolution) followed by HCD fragmentation and ion-trap measurement of the most intense precursors. MS data was analysed using Thermo Pepfinder to match acquired spectra against the expected sequence of the antibody.

The percent basal level of deamidation (ammonia loss) at site N109G (heavy chain CDR3) in gL3gH13 and gL3gH20 was similar (~4%) , whereas, no deamidation was noted at N109A for gL3gH23.

After accelerated stress (pH 8 for 2 weeks at 4°C and 37°C), there was no change in the levels of deamidation for any Fab graft (Table 12).

Overall, substitution of N109G with N109A resulted in gL3gH23 having a potentially lower deamidation risk. However, since the basal level was low for all and there was no increased propensity post accelerated stress, all molecules have a deamidation level that would be suitable for a therapeutic candidate.

Table 12: Percent deamidation pre and post accelerated stress (pH 8).

	gL3gH13	gL3gH20	gL3gH23	gL3gH29
Site (Heavy Chain CDR3)	N109G	N109G	N109A	D109G
T0 (basal level)	4.70%	4.10%	not detected	not detected
2 weeks/ 4°C	4.40%	4.00%	not detected	not detected
2 weeks/ 37°C	3.40%	2.40%	not detected	not detected

(ii) Capillary imaged isoelectric focussing (ICE3)

ICE3 was performed as described above for pI measurement and the results showed no significant differences between the gL3gH13, gL3gH20, gL3gH23 and gL3gH29 grafts in % charged Species Pre and Post Accelerated Stress (pH 8).

Example 11 – Nebulization Study of Humanized 4856 Fab graft gL3gH13

The aggregation stability to the shear forces exerted by nebulisation of Fab 4856 graft gL3gH13 (referred to hereafter as 4856) was determined utilising an investigational PARI eFlow® nebulizer (PARI Pharma GmbH, Grafeling, Germany) *E.coli* expressed Fab 4856 at ~100mg/mL in a pH 6.0 buffer diluted to nominal concentrations 20mg/mL, 50mg/mL and 80mg/mL and a pH 7.4 buffer diluted to nominal concentrations 20mg/mL and 50mg/mL.

Pre-filtered /sterile samples of the 4856 samples (~1.0mL) at different concentrations and buffers were nebulised using the eFlow® nebuliser.

(i) Effect of Nebulisation on Concentration

Diluted samples (<1AU at 280nm) were measured using a Varian Cary 50-Bio UV/Vis Spectrophotometer. Concentration was calculated using an extinction coefficient of 1.72AU (280 nm, 1 mg/mL, and 1cm path length).

No difference in concentration (mg/mL) was observed between the pre and post nebulised samples.

(ii) Size exclusion HPLC (SEC HPLC)

This analysis monitored the generation of soluble aggregates and fragmented material.

Samples were diluted to 1mg/mL (25 μ L injection volume) or 5mg/mL (20 μ L injection volume). Analysis was performed using using a TSK G3000SW (7.7mm I.Dx30.0cm) column connected to an Agilent 1100 system, eluted isocratically using 0.2 M sodium phosphate, pH 7 at 1.0 mL/min for 17 minutes, 30°C . The peaks were monitored at 280nm.

No difference was observed in the percent of high molecular weight species (HMW) present in the pre- and post-nebulised material. Hence nebulisation did not result in the generation of soluble aggregate. There was no evidence for any low molecular weight material hence no fragmentation observed as a consequence of nebulisation.

(iii) Dynamic light scattering (DLS)

This analysis monitored the generation of large molecular weight species (insoluble particulate material that would be filtered by SEC HPLC column matrix). Fab 4856 in buffer comprising histidine and sodium chloride, at pH 6.0 was tested by dynamic light scattering using a Malvern Nano ZS instrument.

No difference was observed in the main peak intensity (%) from the intensity size distribution profile (SDP) for 4856 up to 50mg/mL in the pre and post nebulization samples. With regards to percent poly-dispersity (%PD), the intensity distribution is heavily weighted to larger molecular weight material (scattering is proportional to the square of the molecular weight) and when this is converted to volume distribution describing the relative proportion of multiple species present, minimal insignificant changes were observed up to 50mg/mL.

(iv) SDS PAGE (Non-Reducing and Reducing conditions)

This analysis monitored the generation of aggregation/fragmentation in buffer comprising histidine and NaCl, at pH 6.0.

For non-reducing conditions, 10 μ L of sample at 1mg/mL was mixed with 10 μ L of Tris/Glycine SDS Sample Buffer (2x, Invitrogen) and 2 μ L of 100mM N-ethylmaleimide; heated at 100°C for 2 minutes.

For reducing conditions, 10 μ L of sample at 1mg/mL was mixed with 10 μ L of Tris/Glycine SDS Sample Buffer (2x, Invitrogen) and 2 μ L of DTT (10x Invitrogen); heated at 100°C for 3 minutes.

After centrifugation, 10 μ L (4.5 μ g) of each sample was loaded onto a Novex Tris/Glycine (4-20%) gel (Invitrogen) and electrophoresed at 125mV (constant voltage) for 100 minutes. The bands were visualised by Coomassie Blue.

No difference was observed between pre and post nebulisation samples up to 20mg/mL.

(vi) Functional *in vitro* assay.

This analysis was performed in histidine pH 6.0, NaCl, at 20, 50 and 80 mg/mL.

HEK-Blue TGF-beta reporter gene assay using recombinant TGF-beta 1 was carried out as described in Example 3.

No significant differences in IC₅₀ were observed between pre and post nebulised samples at any concentration.

(v) Aerosol characterisation of the nebulised antibody in a breath simulation

This analysis monitored the droplet size as well as delivered dose and nebulisation times of Fab 4856 at a concentration of 50mg/mL (pH 6.0) using the PARI eFlow® nebuliser. Briefly, the nebuliser was connected to a sinus pump and aerosol droplets containing the nebulised material was collected on an inspiratory collection filter. An adult breathing pattern was used, with a tidal volume of 500 ml at 15 breaths per minute and an inhalation:exhalation ratio of 50:50. After completion, the collection filter was washed to extract the nebulised material, which was analysed by HPLC (Table 13).

Table 13. Data from the breath simulation experiments using two different nebuliser heads.

Mass Median Diameter (MMD)		2.9 μm		3.7 μm	
		Mean	SD (n=6)	Mean	SD (n=6)
Nebulization time	min	14.10* After back flushing: 5.75	5.00	3.62	0.34
DD	mg	71.5	5.5	66.4	3.5
DD	%	68.0	5.1	63.5	3.4
Residue	mg	14.7	3.2	22.9	2.1
Residue	%	14.0	3.1	21.9	2.0
RD<5 μm	mg	66.6	5.0	52.1	4.3
RD<5 μm	%	63.4	4.6	49.8	4.2
RD<3.3 μm	mg	45.2	4.9	25.7	3.1
RD<3.3 μm	%	43.1	4.7	24.6	2.9
DDR	mg/ min	2.2	1.2	20.8	3.3
DDR	%/min	2.1	1.1	19.9	3.2

Delivered Dose (DD) was determined by breath simulation experiments. The DD [%] was 68% (2.9 μm MMD) and 64% (3.7 μm MMD), which is judged to be a typical good result as DD values are rarely over 70%. The residue was 14% (2.9 μm MMD) and 21.9% (3.7 μm MMD), which is also in the typical range. The Respirable Doses < 5 μm (RD<5 μm) are 63% (2.9 μm MMD) vs. 50% (3.7 μm MMD). Particle size affects delivery to the different regions of the respiratory pathway, and RD<5 μm corresponds to alveolar delivery.

These data confirm the suitability of the 4856 Fab for delivery by nebulisation.

Example 12 – X-ray crystallography structure

The antibody 4856 was crystallised with TGF-beta1 to determine the amino acid residues that the antibody contacts.

i) Sample preparation

2 ml of mature TGF-beta 1 at 2.25mg/ml in 5mM HCl was adjusted to pH7 by addition of 30ul 2M Tris pH8. After incubation at RT for 1hr the precipitate was removed by centrifugation leaving 2ml at 2.1mg/ml. To this was added 0.46ml Fab4856gL3gH13 at 32mg/ml (14.7mg) an approximate molar ratio of 1:1.1 TGF-beta 1:Fab. This was left at RT for 1hr then loaded onto an S200 16/160 column pre-equilibrated in 50 mM NaCl, 25 mM Tris, 5% glycerol, pH 7.5. Peak fractions of the complex were pooled and concentrated to 10mg/ml for crystallography.

ii) Crystallization

Crystallization experiment type	Sitting drop, vapour diffusion		
Crystallization condition	0.1M Sodium phosphate citrate pH4.2, 40% PEG300		
Protein concentration	10mg/ml	Drop volume/ratio	0.4ul Protein + 0.4ul Reservoir
Crystal growth time	8-21 days		
Cryoprotection	Crystals were harvested from the drop and flash-frozen in liquid nitrogen (-180°C) within 10 seconds.		

Screening was performed using the various conditions that were available from Qiagen (approximately 2000 conditions). The incubation and imaging was performed by Formulatrix RockImager 1000 (for a total incubation period of 21 days).

iii) Data collection and structure refinement

X-ray source	Diamond synchrotron		
Experiment Type	oscillation	Wavelength	0.92819Å
Processing Software	Mosflm		
Resolution Limits	40-2.48	Space group	P6 ₅ 22
Unit Cell parameters	a = 114.0 Å	b = 114.0 Å	c = 289.5 Å
	α = 90 °	β = 90 °	γ = 120 °
Completeness	99.9 (100.0)	Multiplicity	18.3 (19.0)

$\ \sigma(I)$	20.1 (3.7)	R_{merge}	11.2% (83.2 %)
Number of reflections	741045 (109123)	Number of unique reflections	40392 (5758)
Comments			

Structure determination method	Molecular Replacement	Program(s) used	Phaser
Structure template	internal human Fab, 3KFD		
Refinement program	Refmac	Resolution limits	40-2.48
R factor	22.18%	Free R factor	25.14%
Number of non-hydrogen atoms	4175		
RMSD bond length	0.0205 Å	RMSD bond angle	2.057°
Ramachandran favoured	515 (94.5%)	Ramachandran outliers	2 (0.37%)
Comments	rebuilt using Coot		

TGF-beta 1 residues interacting with 4856gL3gH13 Fab are given in Figures 18A and 18B. 4856gL3gH13 Fab binds in a region that overlays with the receptor binding region suggesting that receptor blockade by 4856gL3gH13 Fab is by competition, the anti- TGF-beta 1 being effective by superior affinity. A variant of 4856gL3H13 containing an asparagine substitution for threonine in the light chain (position 5 of SEQ ID NO:45) was also tested and found to cause no change in the structure.

Similarly, the single chain antibody format of antibody 4856 (scFv 4856, SEQ ID NO:108) was crystallised with TGF-beta 2 (SEQ ID NO:116) to determine the amino acid residues that the antibody contacts. The TGF-beta 2 residues interacting with scFv 4856 are given in Figure 18C, showing binding in the same region as shown for the Fab binding of TGF-beta 1.

What is claimed is:

1. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 comprising a heavy chain, wherein the variable domain of the heavy chain comprises at least one of a CDR having the sequence given in SEQ ID NO:4 for CDR-H1, a CDR having the sequence given in SEQ ID NO:5 for CDR-H2 and a CDR having the sequence given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3.

2. An antibody according to claim 1, wherein the variable domain of the heavy chain comprises the sequence given in SEQ ID NO:4 for CDR-H1, the sequence given in SEQ ID NO:5 for CDR-H2 and the sequence given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3.

3. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3.

4. An antibody according to claim 1 or claim 2, additionally comprising a light chain, wherein the variable domain of the light chain comprises at least one of a CDR having the sequence given in SEQ ID NO:1 for CDR-L1, a CDR having the sequence given in SEQ ID NO:2 for CDR-L2 and a CDR having the sequence given in SEQ ID NO:3 for CDR-L3.

5. An antibody according to claim 3 or claim 4 wherein the variable domain of the light chain comprises the sequence given in SEQ ID NO:1 for CDR-L1, the sequence given in SEQ ID NO:2 for CDR-L2 and the sequence given in SEQ ID NO:3 for CDR-L3.

6. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, wherein the variable domain of the heavy chain comprises three CDRs and the sequence of CDR-H1 has at least 80% identity or similarity to the sequence given in SEQ ID NO:4, the sequence of CDR-H2 has at least 80% identity or similarity to the sequence given in SEQ ID NO:5 and the sequence of CDR-H3 has at least 80% identity or similarity to the sequence given in SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9.

7. An antibody, according to claim 6, additionally comprising a light chain, wherein the variable domain of the light chain comprises three CDRs and the sequence of CDR-L1 has at least 80% identity or similarity to the sequence given in SEQ ID NO:1, the sequence of CDR-L2 has at least 80% identity or similarity to the sequence given in SEQ ID NO:2 and the sequence of CDR-L3 has at least 80% identity or similarity to the sequence given in SEQ ID NO:3.

8. An antibody according to any one claims 1 to 7, wherein the antibody comprises a heavy chain comprising the sequence given in SEQ ID NO:52, SEQ ID NO:66, SEQ ID NO:80 or SEQ ID NO:94.

9. An antibody according to any one of claims 1 to 8, wherein the antibody comprises a light chain comprising the sequence given in SEQ ID NO:38.

10. An antibody according to any one of claims 1 to 9, wherein the antibody molecule is selected from the group consisting of: a complete antibody molecule having full length heavy and light chains or a fragment thereof, for example selected from the group comprising a Fab, modified Fab', Fab', F(ab')₂, Fv, VH, VL and scFv fragment.

11. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, having a heavy chain comprising the sequence given in SEQ ID NO:52, SEQ ID NO:66, SEQ ID NO:80 or SEQ ID NO:94 and a light chain comprising the sequence given in SEQ ID NO:38.

12. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, wherein the variable domain of the light chain comprises a sequence having at least 80% identity or similarity to the light chain variable domain of the antibody of claim 11 and wherein the variable domain of the heavy chain comprises a sequence having at least 80% identity or similarity to the heavy chain variable domain of the antibody of claim 11.

13. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 having a heavy chain comprising the sequence given in SEQ ID NO:59, SEQ ID NO:73, SEQ ID NO:87 or SEQ ID NO:101 and a light chain comprising the sequence given in SEQ ID NO:45.

14. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, in which the heavy and light chains are at least 80% identical or similar to the corresponding heavy and light chains of the antibody of claim 13.

15. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3, wherein the antibody contacts a sequence 90% identical to amino acids 25-34 of SEQ ID NO:114 and optionally at least one of amino acids 90-95 of SEQ ID NO:114.

16. An antibody of claim 15, wherein the antibody further contacts at least one of amino acids 60, 97 and 101 of SEQ ID NO:114.

17. An antibody according to any one of claims 1 to 16, having an effector or a reporter molecule attached to it.

18. An antagonistic antibody which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 which cross-blocks the binding of an antibody comprising the CDRs given in sequence SEQ ID NO:1 for CDR-L1, SEQ ID NO:2 for CDR-L2, SEQ ID NO:3 for CDR-L3, SEQ ID NO:4 for CDR-H1, SEQ ID NO:5 for CDR-H2 and SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:9 for CDR-H3.

19. An antibody according to claim 18, wherein the antibody cross-blocks the binding by binding the same epitope as the antibody which it blocks.

20. An antibody according to any one of claims 1 to 19, having a binding affinity for human TGF-beta 1 of 200pM or better, a binding affinity for human TGF-beta 2 of 300pM or better and a binding affinity for human TGF-beta 3 of 2500pM or better.

21. An antibody according to claim 20, having a binding affinity for human TGF-beta 1 of 100pM or better, a binding affinity for human TGF-beta 2 of 200pM or better and a binding affinity for human TGF-beta 3 of 2000pM or better.

22. An antibody according to any one of claims 1 to 21, wherein in the recombinant TGF-beta 1, TGF-beta 2 or TGF-beta 3 HEK-SEAP-SBE reporter gene assay the antibody inhibits human TGF-beta 1 activity with an IC₅₀ of 0.5nM or better, inhibits human TGF-beta 2 activity with an IC₅₀ of 0.05nM or better and inhibits human TGF-beta 3 activity with an IC₅₀ of 2nM or better.

23. An antibody according to any one of claims 1 to 22, wherein in the endogenous TGF-beta HEK-SEAP-SBE reporter gene assay the antibody inhibits human TGF-beta with an IC₅₀ of 10nM or better.

24. An antibody according to any preceding claim, wherein said antibody or binding fragment thereof is a monoclonal humanized antibody.

25. An isolated DNA sequence encoding the heavy and/or light chain(s) of an antibody according to any one of claims 1 to 24.

26. A cloning or expression vector comprising one or more DNA sequences according to claim 26.

27. A vector according to claim 26, wherein the vector comprises a light chain DNA sequences given in SEQ ID NO:45 or SEQ ID NO:46 and/or a heavy chain DNA sequence given in SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:102 or SEQ ID NO:103.

28. A host cell comprising one or more cloning or expression vectors according to claim 27.

29. A process for the production of the antibody of any one of claims 1 to 24, comprising culturing the host cell of claim 22 and isolating the antibody.

30. A pharmaceutical composition comprising an antibody according to any one of claims 1 to 24 in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

31. A pharmaceutical composition according to claim 30, additionally comprising other active ingredients.

32. A pharmaceutical composition of claim 30 or 31, wherein said pharmaceutical composition is suitable for inhaled administration, for example by nebulisation, aerosol and/or dry powder.

33. An antibody according to any one of claims 1 to 24 or a composition according to claims 30 to 32 for use in the treatment or prophylaxis of a pathological disorder that is mediated by TGF-beta 1, 2 or 3 or that is associated with an increased level of TGF-beta 1, 2 or 3.

34. The use of an antibody according to any one of claims 1 to 24 in the manufacture of a medicament for the treatment or prophylaxis of a pathological disorder that is mediated by TGF-beta 1, 2 or 3 or that is associated with an increased level of TGF-beta 1, 2 or 3.

35. A method for the treatment of a human subject suffering from or at risk of a pathological disorder that is mediated by TGF-beta 1, 2 or 3 or that is associated with an increased level of TGF-beta 1, 2 or 3, the method comprising administering to the subject an effective amount of an antibody according to any one of claims 1 to 24.

36. An antagonistic antibody Fab or Fab' fragment which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 for use in the treatment or prophylaxis of a pulmonary disorder that is mediated by TGF-beta 1, 2 or 3 or that is associated with an increased level of TGF-beta 1, 2 or 3 by inhaled administration, for example by nebulisation, aerosol and/or dry powder.

37. The use of an antagonistic antibody Fab or Fab' fragment which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 in the manufacture of a medicament for the treatment or prophylaxis of a pulmonary disorder that is mediated by TGF-beta 1, 2 or 3 or that is associated with an increased level of TGF-beta 1, 2 or 3 by inhaled administration, for example by nebulisation, aerosol and/or dry powder.

38. A method for the treatment of a human subject suffering from or at risk of a pulmonary disorder that is mediated by TGF-beta 1, 2 or 3 or that is associated with an increased level of TGF-beta 1, 2 or 3, the method comprising administering to the subject an effective amount of an antagonistic antibody Fab or Fab' fragment which binds human TGF-beta 1, human TGF-beta 2 and human TGF-beta 3 by inhaled administration, for example by nebulisation, aerosol and/or dry powder.

39. The antibody according to claim 36, the use according to claim 37 or the method according to claim 38, wherein the antibody is as defined in any one of claims 1 to 24.

40. The antibody according to claim 36, the use according to claim 37 or the method according to claim 38, wherein the pulmonary disorder is selected from the group consisting of:

pulmonary fibrosis such as idiopathic pulmonary fibrosis, cystic fibrosis or secondary to systemic sclerosis, and pulmonary hypertension such as pulmonary arterial hypertension.

FIGURE 1A**Ab 4856****CDRL1**

QASESIYSGLA SEQ ID NO: 1

CDRL2

AASDLAS SEQ ID NO: 2

CDRL3

QQTWTDGGIDNP SEQ ID NO: 3

CDRH1

GFSLSSYDMS SEQ ID NO: 4

CDRH2

IIYGGSGSTWYASWAKG SEQ ID NO: 5

CDRH3

GRDGGAGGSRNGYSL SEQ ID NO: 6

CDRH3 variant 1 – present in gH13 and gH20

GRDAGAGGSRNGYSL SEQ ID NO: 7

CDRH3 variant 2 – present in gH23

GRDAGAGGSRNAYSL SEQ ID NO: 8

CDRH3 variant 3 – present in gH29

GRDAGAGGSRDGYSL SEQ ID NO: 9

FIGURE 1B

Rabbit Ab 4856 VL region SEQ ID NO: 10

AYDMTQTPAS VEVAVGGTVT IKCQASESIY SGLAWYQQTP GQRPKLLIYA
ASDLASGVPS RFKGS GSGTE YTLTISGVEC ADAATYYCQQ TWDGGIDNP
FGGGTEVVVK

Rabbit Ab 4856 VL region SEQ ID NO: 11

gcctatgata tgacccagac tccagcctct gtggaggtag ctgtgggagg
cacagtcacc atcaagtgcc aggccagtga gagcatttac agtgggtttgg
cctggtatca gcagacacca gggcagcgtc ccaagctcct gatctatgct
gcatccgatac tggcatctgg ggtcccatcg cgattcaaag gcagtggatc
tgggacagag tacactctca ccatcagcgg cgtggagtgt gccgatgctg
ccacttacta ctgtcaacag acttggactg atggtggtat tgataatcct
ttcggcggag ggaccgaggt ggtggtcaaa

Rabbit Ab 4856 VL region with signal sequence underlined and italicised SEQ ID NO: 12

MNMRAPTQLL GLLLLWLPGA RCAYDMTQTP ASVEVAVGGT VTIKCQASES
IYSGLAWYQQ TPGQRPKLLI YAASDLASGV PSRFKGS GSG TEYTLTISGV
ECADAATYYC QQTWDGGID NPFGGGTEVV VK

Rabbit Ab 4856 VL region with signal sequence underlined and italicised SEQ ID NO: 13

atgaacatga gggccccac tcagctgctg gggctcctgc tgctctggct
cccagggtgcc agatgtgcct atgatatgac ccagactcca gcctctgtgg
aggtagctgt gggaggcaca gtcaccatca agtgccaggc cagtgagagc
atttacagtg gtttggcctg gtatcagcag acaccagggc agcgtcccaa
gctcctgatac tatgctgcat ccgatctggc atctggggtc ccategcgat
tcaaaggcag tggatctggg acagagtaca ctctcaccat cagcggcgtg
gagtgtgccg atgctgccac ttactactgt caacagactt ggactgatgg
tggtattgat aatcctttcg gcggagggac cgagggtggtg gtcaaa

Rabbit Ab 4856 VH region SEQ ID NO: 14

QSLEESGGRL VTPGTPLTLT CTASGFSLS YDMSWVRQAP GKGLEWIGII
YGGSGSTWYA SWAKGRFTMS KTSTTVDLKI TSPTTEDMAT YFCARGRDGG
AGGSRNGYSL WGQGTLVTVS S

Rabbit Ab 4856 VH region SEQ ID NO: 15

cagtcgctgg aggagtccgg gggtcgcctg gtcacgcctg ggacaccct

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gacactcacc tgcacagcct ctggattctc cctcagcagc tacgacatga
 gctgggtccg ccaggctcca gggaaggggc tggaatggat cggaatcatt
 tatggtggta gtggtagcac atggtacgcg agctgggcga aaggccgatt
 caccatgtcc aaaacgtcga ccacgggtgga tctgaaaatc accagtccga
 cgaccgagga catggccacc tatttctgtg ccagaggacg ggatgggtgg
 gctggtggtt ctcgtaatgg ctattccttg tggggccaag gcaccctggt
 caccgtctcg agt

Rabbit Ab 4856 VH region with signal sequence underlined and italicised SEQ ID NO: 16

METGLRWLLL VAVLKGVCQ SLEESGGRLV TPGTPLTLTC TASGFSLSY
 DMSWVRQAPG KGLEWIGIIY GSGSTWYAS WAKGRFTMSK TSTVLDLKIT
 SPTTEDMATY FCARGRDGGA GSRNGYSLW GQGLVTVSS

Rabbit Ab 4856 VH region with signal sequence underlined and italicised SEQ ID NO: 17

atggagactg ggctgcgctg gcttctcctg gtcgctgtgc tcaaaggtgt
ccagtgtcag tcgctggagg agtccggggg tcgcctggtc acgcctggga
 caccctgac actcacctgc acagcctctg gattctccct cagcagctac
 gacatgagct ggggccgcca ggctccaggg aaggggctgg aatggatcgg
 aatcatttat ggtggtagtg gtagcacatg gtacgcgagc tgggcgaaag
 gccgattcac catgtccaaa acgtcgacca cggatggatct gaaaatcacc
 agtccgacga ccgaggacat ggccacctat ttctgtgcca gaggacggga
 tgggtggtgct ggtggttctc gtaatggcta ttccttgtgg ggccaaggca
 ccctggtcac cgtctcgagt

Rabbit Ab 4856 light chain (V + constant) SEQ ID NO: 18

AYDMTQTPAS VEVAVGGTVT IKQASESIY SGLAWYQQTP GQRPKLLIYA
 ASDLASGVPS RFKSGSGSTE YTLTISGVEC ADAATYYCQQ TWDGGIDNP
 FGGGTEVVVK RTPVAPTVLI FPPAADQVAT GTVTIVCVAN KYFPDVTVTW
 EVDGTTQTTG IENSKTPQNS ADCTYNLSST LTLTSTQYNS HKEYTCKVTQ
 GTTSVVQSFN RGDC

Rabbit Ab 4856 light chain (V + constant) SEQ ID NO: 19

gcctatgata tgacccagac tccagcctct gtggaggtag ctgtgggagg
 cacagtcacc atcaagtgcc aggccagtga gagcatttac agtggtttgg
 cctggtatca gcagacacca gggcagcgtc ccaagctcct gatctatgct
 gcatccgac tgatcatctg ggtcccatcg cgattcaaag gcagtggatc
 tgggacagag tacactctca ccatcagcgg cgtggagtgt gccgatgctg
 ccacttacta ctgtcaacag acttggactg atggtgggtat tgataatcct
 ttcggcgagg ggaccgagg ggtggtcaaa cgtacgccag ttgcacctac
 tgcctcatc tttccaccag ctgctgatca ggtggcaact ggaacagtca
 ccatcgtgtg tgtggcgaat aaatactttc ccgatgtcac cgtcacctgg
 gaggtggatg gcaccacca aacaactggc atcgagaaca gtaaaacacc

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gcagaattct gcagattgta cctacaacct cagcagcact ctgacactga
 ccagcacaca gtacaacagc cacaagagat acacctgcaa ggtgacccag
 ggcacgacct cagtcgtcca gagcttcaat aggggtgact gt

Rabbit Ab 4856 light chain with signal sequence underlined and italicized SEQ ID NO: 20

MNMRAPTQLL *GLLLLWLPGA* RCAYDMTQTP ASVEVAVGGT VTIKCQASES
 IYSGLAWYQQ TPGQRPKLLI YAASDLASGV PSRFKSGSGS TEYTLTISGV
 ECADAATYYC QQTWTDGGID NPFGGGTEVV VKRTPVAPTV LIFPPAADQV
 ATGTVTIIVCV ANKYFPDVTV TWEVDGTTQT TGIENSKTPQ NSADCTYNLS
 STLTLTSTQY NSHKEYTCKV TQGTTSVVQS FNRGDC

Rabbit Ab 4856 light chain with signal sequence underlined and italicized SEQ ID NO: 21

atgaacatga *gggccccac* *tcagctgctg* *gggctcctgc* *tgctctggct*
cccaggtgcc *agatgtgcct* atgatatgac ccagactcca gcctctgtgg
 aggtagctgt gggaggcaca gtcaccatca agtgccaggc cagtgaagagc
 atttacagtg gtttggcctg gtatcagcag acaccagggc agcgtcccaa
 gctcctgac tatgctgcat ccgatctggc atctggggtc ccatcgcgat
 tcaaaggcag tggatctggg acagagtaca ctctcaccat cagcggcgtg
 gagtgtgccg atgctgccac ttactactgt caacagactt ggactgatgg
 tggatttgat aatcctttcg gcggaggggc cgagggtggg gtcaaacgta
 cgccagttgc acctactgtc ctcatcttcc caccagctgc tgatcagggtg
 gcaactggaa cagtcaccat cgtgtgtgtg gcgaataaat actttcccga
 tgtcaccgtc acctgggagg tggatggcac caccacaaca actggcatcg
 agaacagtaa aacaccgcag aattctgcag attgtacct caacctcagc
 agcactctga cactgaccag cacacagtac aacagccaca aagagtacac
 ctgcaagggtg acccagggca cgacctcagt cgtccagagc ttcaataggg
 gtgactgt

Rabbit Ab 4856 Fab heavy chain SEQ ID NO: 22

QSLEESGGRL VTPGTPLTLT CTASGFSLS YDMSWVRQAP GKGLEWIGII
 YGGSGSTWYA SWAKGRFTMS KTSTTVDLKI TSPTTEDMAT YFCARGRDGG
 AGGSRNGYSL WGQGTLVTVS SGQPKAPSVF PLAPCCGDTP SSTVTLGCLV
 KGYLPEPVTW TWNSGTLTNG VRTFPSVRQS SGLYSLSSVV SVTSSSQPVT
 CNVAHPATNT KVDKTV

Rabbit Ab 4856 Fab heavy chain SEQ ID NO: 23

cagtcgctgg aggagtccgg gggtcgcctg gtcacgcctg ggacaccct
 gacactcacc tgcacagcct ctggattctc cctcagcagc tacgacatga
 gctgggtccg ccaggctcca ggggaagggc tggaatggat cggaatcatt
 tatgggtgga gtggtagcac atgggtacgc agctgggcca aaggccgatt
 caccatgtcc aaaacgtcga ccacgggtgga tctgaaaatc accagtccga

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cgaccgagga catggccacc tatttctgtg ccagaggacg ggatggtggt
gctggtggtt ctcgtaatgg ctattccttg tggggccaag gcaccctggt
cacggtctcg agtgggcaac ctaaggctcc atcagtcttc ccactggccc
cctgctgcgg ggacacaccc agctccacgg tgaccctggg ctgcctggtc
aaaggctacc tcccggagcc agtgaccgtg acctggaact cgggcaccct
caccaatggg gtacgcacct tcccgtccgt ccggcagtcc tcaggcctct
actcgctgag cagcgtggtg agcgtgacct caagcagcca gcccgtcacc
tgcaacgtgg cccacccagc caccaacacc aaagtggaca agaccgtt

Rabbit Ab 4856 Fab heavy chain with signal sequence underlined and italicized SEQ ID NO: 24

METGLRWLLL VAVLKGVQCQ SLEESGGRLV TPGTPLTLTC TASGFSLSY
DMSWVRQAPG KGLEWIGIIY GGSGSTWYAS WAKGRFTMSK TSTTVDLKIT
SPTTEDMATY FCARGRDGGA GGSRNGYSLW GQGTILTVSS GQPKAPSVFP
LAPCCGDTPS STVTLGCLVK GYLPEPVTVT WNSGTLINGV RTFPSVRQSS
GLYSLSSVVS VTSSSQPVTC NVAHPATNTK VDKTV

Rabbit Ab 4856 Fab heavy chain with signal sequence underlined and italicized SEQ ID NO: 25

atggagactg ggctgcgctg gcttctcctg gtcgctgtgc tcaaaggtgt
ccagtgtcag tcgctggagg agtccggggg tcgcctggtc acgcctggga
caccctgac actcacctgc acagcctctg gattctccct cagcagctac
gacatgagct gggtcgccca ggctccaggg aaggggctgg aatggatcgg
aatcatttat ggtggtagt gtagcacatg gtacgcgagc tgggcgaaag
gccgattcac catgtccaaa acgtcgacca cgggtgatct gaaaatcacc
agtccgacga ccgaggacat ggccacctat ttctgtgcc aaggacggga
tggtggtgct ggtggttctc gtaatggcta ttccttgtgg ggccaaggca
ccctggtcac cgtctcgagt gggcaacct aagctccatc agtcttcca
ctggccccct gctgcgggga cacaccagc tccacggtga ccctgggctg
cctggtcaaa ggctacctcc cggagccagt gaccgtgacc tggaactcgg
gcaccctcac caatggggta cgcaccttcc cgtccgtccg gcagtcctca
ggcctctact cgctgagcag cgtggtgagc gtgacctcaa gcagccagcc
cgtcacctgc aacgtggccc acccagccac caacaccaa gtggacaaga
ccgtt

FIGURE 1C**Murinised 4856 mL1.1 V-region SEQ ID NO:26**

AYDMNQSPSS LSASLGDTIT ITCQASESIY SGLAWYQQKP GNIPKLLIYA
 ASDLASGVPS RFKGS GSGTE YTLTISSLQP EDIATYYCQQ TWDGGIDNP
 FGGGTKLEIK

Murinised 4856 mL1.1 V-region SEQ ID NO:27

gcctacgaca tgaaccagtc gccatcaagc ctgagcgcct cccttggcga
 caccatcacc attacttgcc aagcctccga aagcatctac tccggactcg
 cctggtatca gcagaaaccg gggaacattc ccaagctcct gatctacgcc
 gcttccgact tggcatcggg agtgccgtca cggttcaagg ggtccggatc
 gggaaccgag tacaccctga ctatctctc cctgcaaccc gaggatattg
 cgacctacta ctgtcagcag acttggacgg atggtggaat cgacaaccct
 ttcggtggcg gcaccaagct ggaaatcaag

Murinised 4856 mL1.1 V-region with signal sequence underlined and italicized SEQ ID NO:28

MSVPTQVLGL LLLWLTDARC AYDMNQSPSS LSASLGDTIT ITCQASESIY
 SGLAWYQQKP GNIPKLLIYA ASDLASGVPS RFKGS GSGTE YTLTISSLQP
 EDIATYYCQQ TWDGGIDNP FGGGTKLEIK

Murinised 4856 mL1.1 V-region with signal sequence underlined and italicized SEQ ID NO:29

atgtctgtcc ccaccaagt cctcggactc ctgctactct ggcttacaga
tgccagatgc gcctacgaca tgaaccagtc gccatcaagc ctgagcgcct
 cccttggcga caccatcacc attacttgcc aagcctccga aagcatctac
 tccggactcg cctggtatca gcagaaaccg gggaacattc ccaagctcct
 gatctacgcc gcttccgact tggcatcggg agtgccgtca cggttcaagg
 ggtccggatc gggaaccgag tacaccctga ctatctctc cctgcaaccc
 gaggatattg cgacctacta ctgtcagcag acttggacgg atggtggaat
 cgacaaccct ttcggtggcg gcaccaagct ggaaatcaag

Murinised 4856 mH2.1 V-region SEQ ID NO:30

EVKLLES GGG LVQPGGSLKL SCTASGFSL S YDMSWVRQA PGKGLEWIGI
 IYGGSGSTWY ASWAKGKFIM SKDSAKNTVY LQMSKVRSED MATYFCARGR
 DGGAGGSRNG YSLWGQGTLV TVSS

Murinised 4856 mH2.1 V-region SEQ ID NO:31

gaggtcaagc tgctggaatc ggggggaggt ctggtgcagc cgggcggatc
 tctgaagctg tcatgcaccg catccgggtt tagcctttcg tcctacgaca
 tgtcctgggt gcgccaggcc cccggaaagg gattggaatg gatcggcatt
 atctacgggg gctccggttc cacttggtac gcgagctggg ccaaggggaa
 gttcatcatg tcgaaggact ccgctaagaa caccgtgtac ctccaaatga
 gcaaagtccg gagcgaggat atggccacct atttctgcgc ccggggaagg
 gacggaggag ccggcggttc cagaaacggc tactcactgt ggggacaggg
 caccctcgtg actgtctcga gt

Murinised 4856 mH2.1 V-region with signal sequence underlined and italicized SEQ ID NO: 32

MEWSWVFLFF *LSVTTGVHSE* VKLLESGGGL VQPGGSLKLS CTASGFSLSS
YDMSWVRQAP GKGLEWIGII YGGSGSTWYA SWAKGKFIMS KDSAKNTVYL
QMSKVRSEDM ATYFCARGRD GGAGGSRNGY SLWGQGTLVT VSS

Murinised 4856 mH2.1 V-region with signal sequence underlined and italicized SEQ ID NO: 33

atggaatgga *gctgggtctt* *tctcttcttc* *ctgtcagtaa* *ctacaggagt*
ccattctgag gtcaagctgc tggaaatcggg gggaggtctg gtgcagccgg
gcggatctct gaagctgtca tgcaccgcat ccgggttttag cctttcgtcc
tacgacatgt cctgggtgcg ccaggccccc ggaaagggat tggaatggat
cggcattatc tacgggggct ccggttccac ttggtacgcg agctgggcca
aggggaagtt catcatgtcg aaggactccg ctaagaacac cgtgtacctc
caaatgagca aagtccggag cgaggatatg gccacctatt tctgcgcccg
gggaaggac ggaggagccg gcggttccag aaacggctac tcaactgtggg
gacagggcac cctcgtgact gtctcgagt

Mouse IGKV15-103 JK1 acceptor framework SEQ ID NO: 34

DIQMNQSPSS LSASLGDTIT ITCHASQNIN VWLSWYQQKP GNIPKLLIYK
ASNLHTGVPS RFSGSGSGTG FTLTISSLQP EDIATYYCQQ GQSYPWTFGG
GTKLEIK

Mouse IGKV15-103 JK1 acceptor framework SEQ ID NO: 35

gacatccaga tgaaccagtc tccatccagt ctgtctgcat cccttggaga
cacaattacc atcacttgcc atgccagtc gaacattaat gtttggttaa
gctggtacca gcagaaacca ggaaatatc ctaaactatt gatctataag
gcttccaact tgcacacagg cgtcccatca aggttttagtg gcagtggatc
tggaacaggt ttcacattaa ccatcagcag cctgcagcct gaagacattg
ccacttacta ctgtcaacag ggtcaaagtt atccttggac gttcgggtgga
ggcaccaagc tggaaatcaa a

Mouse IGHV4-S1 JH3 acceptor framework SEQ ID NO: 36

EVKLESGGG LVQPGGSLKL SCAASGFDFS RYWMSWVRQA PGKGLEWIGE
INPDSSTINY TPSLKDKFII SRDNAKNTLY LQMSKVRSED TALYYCARWF
AYWGQGTLVT VSA

Mouse IGHV4-S1 JH3 acceptor framework SEQ ID NO: 37

gaggtgaagc ttctcgagtc tggaggtggc ctggtgcagc ctggaggatc
cctgaaactc tcctgtgcag cctcaggatt cgatttttagt agatactgga
tgagttgggt ccggcaggct ccagggaag ggctagaatg gattggagaa
attaatccag atagcagtac gataaactat acgccatctc taaaggataa
attcatcatc tccagagaca acgcaaaaaa tacgctgtac ctgcaaatga
gcaaagtgag atctgaggac acagcccttt attactgtgc aagatggttt
gcttactggg gccaaaggac tctggtcact gtctctgca

FIGURE 1D**4856 gL3 V-region** SEQ ID NO:38

AYDMTQSPST LSASVGDRVT ITCQASESIY SGLAWYQQKP GKAPKLLIYA
 ASDLASGVPS RFSGSGSGTE YTLTISSLQP DDFATYYCQQ TWDGGIDNP
 FGGGTKVEIK

4856 gL3 V-region (mammalian expression) SEQ ID NO:39

gcctacgaca tgacccagtc cccctcaacc ctctccgctt ccgtgggaga
 tcgcgtgacc atcacttgcc aagcctccga atcgatctac tcgggtctgg
 cctggtatca gcagaagcca gggaaggcac ctaagctggt gatctacgcg
 gcctcagacc tggccagcgg agtgcccagc cggttctccg gctccggaag
 cggcactgag tacaccctga ccatttcctc gcttcaaccg gatgacttcg
 cgacctacta ctgtcagcag acttggacgg acgggggcat cgacaacccg
 ttggtggag gcaccaaagt cgagattaag

4856 gL3 V-region (*E. coli* expression) SEQ ID NO:40

gcgtatgata tgacccagag tccaagcacc ctctccgcca gcgtaggcga
 tcgtgtgact attacctgtc aggccagtga aagcatctat agcggcctgg
 cgtggtatca gcaaaaaccg ggcaaagccc cgaagctgct catctatgcg
 gcgtccgata tggcgagcgg tgtgccaagc cgtttcagtg gcagcggcag
 cggcaccgaa tataccctca caatttcgtc tctccagccg gatgatttcg
 ccacttacta ttgtcagcaa acctggaccg atggcggcga tgataacccg
 ttcggtggcg gcactaaagt agaaatcaaa

4856 gL3 V-region with signal sequence underlined and Italicized (mammalian expression)

SEQ ID NO:41

MKWVTFISLL *FLFSSAYSAY* DMTQSPSTLS ASVGDRVTIT CQASESIYSG
 LAWYQQKPGK APKLLIYAAS DLASGVPSRF SSGSGSGTEYT LTISSLQPDD
 FATYYCQQTW TDGGIDNPFQ GGTKVEIK

4856 gL3 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:42

atgaagtggg *tcaccttcat* *ctccctgctg* *tttctgttct* *cctccgccta*
ctccgcctac gacatgacct agtccccctc aaccctctcc gcttccgtgg
 gagatcgctg gaccatcact tgccaagcct ccgaatcgat ctactcgggt
 ctggcctggt atcagcagaa gccagggaag gcacctaaagc tgttgatcta
 cgcggcctca gacctggcca gcggagtgcc cagccggttc tccggctccg
 gaagcggcac tgagtacacc ctgaccattt cctcgcttca accggatgac
 ttcgcgacct actactgtca gcagacttgg acggacgggg gcacgcacaa
 cccgtttggt ggaggcacca aagtcgagat taag

4856 gL3 V-region with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 43

*MKKT**AI**AI**AV* *ALAGFATVAQ* *AAYDMTQSPS* *TLSASVGDRV* *TITCQASESI*
YSGLAWYQQK *PGKAPKLLIY* *AASDLASGVP* *SRFSGSGSGT* *EYTLTISSLQ*
PDDFATYYCQ *QTWIDGGIDN* *PFGGGTKVEI* K

4856 gL3 V-region with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 44

atgaaaaaga *cagctatcgc* *aattgcagtg* *gccttggctg* *gtttcgctac*
cgtagcgcaa *gctgcgtatg* *atatgacca* *gagtccaagc* *accctctccg*
ccagcgtagg *cgatcgtgtg* *actattacct* *gtcaggccag* *tgaaagcatc*
tatagcggcc *tggcgtggta* *tcagcaaaaa* *ccgggcaaag* *ccccgaagct*
gctcatctat *gcggcgctccg* *atctggcgag* *cggtgtgcca* *agccgtttca*
gtggcagcgg *cagcggcacc* *gaatataccc* *tcacaatttc* *gtctctccag*
ccggatgatt *tcgccactta* *ctattgtcag* *caaacctgga* *ccgatggcgg*
cattgataac *ccgttcggtg* *gcggcactaa* *agtagaaatc* *aaa*

4856 gL3 light chain (V + constant) SEQ ID NO: 45

AYDMTQSPST *LSASVGDRV* *ITCQASESIY* *SGLAWYQQKP* *GKAPKLLIYA*
ASDLASGVPS *RFSGSGSGTE* *YTLTISSLQP* *DDFATYYCQQ* *TWIDGGIDNP*
FGGGTKVEIK *RTVAAPSVFI* *FPPSDEQLKS* *GTASVVCLLN* *NFYPPREAKVQ*
WKVDNALQSG *NSQESVTEQD* *SKDSTYSLSS* *TLTLKADYE* *KHKVYACEVT*
HQGLSSPVTK *SFNRGEC*

4856 gL3 light chain (V + constant) (mammalian expression) SEQ ID NO: 46

gcctacgaca *tgacccagtc* *cccctcaacc* *ctctccgctt* *ccgtgggaga*
tcgcgtgacc *atcacttgcc* *aagcctccga* *atcgatctac* *tcgggtctgg*
cctggtatca *gcagaagcca* *gggaaggcac* *ctaagctgtt* *gatctacgcg*
gcctcagacc *tggccagcgg* *agtgccccagc* *cggttctccg* *gctccggaag*
cggcactgag *tacaccctga* *ccatttcctc* *gcttcaaccg* *gatgacttcg*
cgacctacta *ctgtcagcag* *acttggacgg* *acggggggcat* *cgacaacccg*
tttgggtggag *gcaccaaagt* *cgagattaag* *cgtacggtag* *cggccccatc*
tgtcttcac *ttcccgccat* *ctgatgagca* *gttgaaatct* *ggaactgcct*
ctgttgtgtg *cctgctgaat* *aacttctatc* *ccagagaggc* *caaagtacag*
tggaagggtg *ataacgccct* *ccaatcgggt* *aactcccagg* *agagtgtcac*
agagcaggac *agcaaggaca* *gcacctacag* *cctcagcagc* *accctgacgc*
tgagcaaagc *agactacgag* *aaacacaaag* *tctacgcctg* *cgaagtcacc*
catcagggcc *tgagctcgcc* *cgtcacaaag* *agcttcaaca* *ggggagagtg*
t

4856 gL3 light chain (V + constant) (*E. coli* expression) SEQ ID NO: 47

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gcgtatgata tgacccagag tccaagcacc ctctccgcca gcgtaggcga
 tcgtgtgact attacctgtc aggccagtga aagcatctat agcggcctgg
 cgtggtatca gcaaaaaccg ggcaaagccc cgaagctgct catctatgcg
 gcgtccgata tggcgagcgg tgtgccaagc cgtttcagtg gcagcggcag
 cggcaccgaa tataccctca caatttcgtc tctccagccg gatgatttcg
 ccacttacta ttgtcagcaa acctggaccg atggcggcgcat tgataaaccg
 ttcggtggcg gcactaaagt agaaatcaaa cgtacggtag cggccccatc
 tgtcttcata ttcccgccat ctgatgagca gttgaaatct ggaactgcct
 ctgttggtgtg cctgctgaat aacttctatc ccagagaggc caaagtacag
 tggaagggtg ataacgccct ccaatcgggt aactcccagg agagtgtcac
 agagcaggac agcaaggaca gcacctacag cctcagcagc accctgacgc
 tgagcaaagc agactacgag aaacacaaag tctacgcctg cgaagtcacc
 catcagggcc tgagctcacc agtaacaaaa agttttaata gaggggagtg
 t

4856 gL3 light chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 48

MKWVTFISLL FLFSSAYSAY DMTQSPSTLS ASVGDRVITIT CQASESIYSG
 LAWYQQKPGK APKLLIYAAS DLASGVPSRF SGSGSGTEYT LTISSLQPDD
 FATYYCQQTW TDGGIDNPFQ GGTKVEIKRT VAAPSVFIFP PSDEQLKSGT
 ASVVCLLNNF YPREAKVQWK VDNALQSGNS QESVTEQDSK DSTYSLSSTL
 TLSKADYEKH KVYACEVTHQ GLSSPVTKSF NRGEC

4856 gL3 light chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 49

atgaagtggg tcaccttcat ctccctgctg tttctgttct cctccgccta
ctccgcctac gacatgaccc agtccccctc aacctctctc gcttccgtgg
 gagatcgctg gaccatcact tgccaagcct ccgaatcgat ctactcgggt
 ctggcctggg atcagcagaa gccaggggaag gcacctaaagc tgttgatcta
 cgcggcctca gacctggcca gcggagtgcc cagccgggtc tccggctccg
 gaagcggcac tgagtacacc ctgaccattt cctcgcttca accggatgac
 ttcgcgacct actactgtca gcagacttgg acggacgggg gcatcgacaa
 cccgtttggg ggaggcacca aagtcgagat taagcgtacg gtagcggccc
 catctgtctt catcttcccg ccatctgatg agcagttgaa atctggaact
 gcctctgttg tgtgcctgct gaataacttc tatcccagag aggccaaagt
 acagtggaaag gtggataacg ccctccaatc gggtaactcc caggagagtg
 tcacagagca ggacagcaag gacagcacct acagcctcag cagcaccctg
 acgctgagca aagcagacta cgagaaacac aaagtctacg cctgcgaagt
 caccatcag ggcctgagct cgcccgtcac aaagagcttc aacaggggag
 agtgt

4856 gL3 light chain with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 50

MKKTAIAlAV ALAGFATVAQ AAYDMTQSPS TLSASVGDRV TITCQASESI
 YSGLAWYQQK PGKAPKLLIY AASDLASGVP SRFSGSGSGT EYTLTISSLQ

11/47

PDDFATYYCQ QTWIDGGIDN PFGGGTKVEI KRTVAAPSVF IFPPSDEQLK
SGTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSL
STLTLSKADY EKHKVYACEV THQGLSSPVT KSFNRGEC

4856 gL3 light chain with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 51

atgaaaaaga cagctatcgc aattgcagtg gccttggctg gtttcgctac
cgtagcgcaa gctgcgtatg atatgacca gagtccaagc accctctccg
ccagcgtagg cgatcgtgtg actattacct gtcaggccag tgaaagcatc
tatagcggcc tggcgtggta tcagcaaaaa ccgggcaaag ccccgaaagct
gtcatcttat gcggcgtccg atctggcgag cgggtgtgcca agccgtttca
gtggcagcgg cagcggcacc gaatataccc tcacaatttc gtctctccag
ccggatgatt tcgccactta ctattgtcag caaacctgga ccgatggcgg
cattgataac ccgttcggtg gcggcactaa agtagaaatc aaacgtacgg
tagcggcccc atctgtcttc atcttcccgc catctgatga gcagttgaaa
tctggaactg cctctgttgt gtgcctgctg aataacttct atcccagaga
ggccaaagta cagtggaagg tggataacgc cctccaatcg ggtaactccc
aggagagtgt cacagagcag gacagcaagg acagcaccta cagcctcagc
agcaccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc
ctgcgaagtc acccatcagg gcctgagctc accagtaaca aaaagtttta
atagagggga gtgt

FIGURE 1E

4856gH13 V-region SEQ ID NO: 52

EVQLVESGGG LVKPGGSLRL SCAASGFSLS SYDMSWVRQA PGKGLEWIGI
 IYGGSGSTWY ASWAKGRFTI SRDSAKNSVY LQMNSLRAED TAVYYCARGR
 DAGAGGSRNG YSLWGQGLTV TVSS

4856gH13 V-region (mammalian expression) SEQ ID NO: 53

gaagtgcaac tgggtggaag cggaggaggt ttgggtgaaac cgggagggtc
 actgcggctc tcctgtgccg cctccggatt cagcctctcg tcctacgaca
 tgagctgggt cagacaggcc cccgggaagg gcctggagtg gattggtatc
 atctacggcg gctccggctc gacttggtac gcttcgtggg ccaagggacg
 gttcaccatc tcccgcgatt ccgcgaagaa cagcgtgtat ctgcagatga
 actctctgcg ggccgaggac accgcagtgt actactgcgc gagggggcgc
 gacgccggcg ccgggggata acgcaacggg tactcccttt ggggacaggg
 aaccctggtc actgtctcca gc

4856gH13 V-region (*E. coli* expression) SEQ ID NO: 54

gaggttcagc tggtcgagtc tggaggcggg cttgtcaaac ctggagggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gattggcatt
 atttatggcg gcagcggcag cacatggtac gcgagctggg cgaagggccg
 tttcaccatc tcccgggaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcaacggc tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gc

4856gH13 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 55

MKWVTFISLL *FLFSSAYSEV* QLVESGGGLV KPGGSLRLSC AASGFSLSY
 DMSWVRQAPG KGLEWIGIIY GGSGSTWYAS WAKGRFTISR DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRNGYS LWGQGLTVTV SS

4856gH13 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 56

atgaagtggg *tcaccttcat* *ctccctgctg* *tttctgttct* *ccagcgccta*
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctcgtcctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggattgg
 tatcatctac ggcggctccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catctcccgc gattccgcga agaacagcgt gtatctgcag

atgaactctc tgcgggccga ggacaccgca gtgtactact gcgcgagggg
gcgcgacgcc ggcgccgggg gatcacgcaa cggttactcc ctttggggac
agggaaccct ggtcactgtc tccagc

4856gH13 V-region with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 57

MKKTAIAlAV *ALAGFATVAQ* *AEVQLVESGG* GLVKPGGSLR LSCAASGFSL
SSYDMSWVRQ APGKGLEWIG IIYGGSGSTW YASWAKGRFT ISRDSAKNSV
YLQMNSLRAE DTAVYYCARG RDAGAGGSRN GYSLWGQGTL VTVSS

4856gH13 V-region with signal sequence underlined and italicized (*E. coli* expression) SEQ ID NO: 58

atgaagaaga *ctgctatagc* *aattgcagtg* *gcgctagctg* *gtttcgccac*
cgtggcgcaa *gctgaggttc* agctggtcga gtctggaggc gggcttgtca
aacctggagg gagcctgcgt ctctcttggt cagcaagcgg cttcagcctg
tcctcttacg atatgtcctg ggtgcgccag gcacctggga agggcctgga
gtggattggc attatttatg gcggcagcgg cagcacatgg tacgcgagct
gggcgaaggg ccgtttcacc atctcccggg acagcgcaaa gaatagcgtg
tacctccaga tgaactctct ccgcgcagag gacacagcag tctattactg
tgcgcggggc cgcgatgcag gcgcggggcg cagccgcaac ggctatagcc
tgtggggaca ggggaccctt gtgacagtct cgagc

4856 gH13 Fab heavy chain (V + human gamma-1 CH1) SEQ ID NO: 59

EVQLVESGGG LVKPGGSLRL SCAASGFSL S YDMSWVRQA PGKGLEWIGI
IYGGSGSTWY ASWAKGRFTI SRDSAKNSVY LQMNSLRAED TAVYYCARGR
DAGAGGSRNG YSLWGQGTLV TVSSASTKGP SVFPLAPSSK STSGGTAALG
CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS SVVTVPSSSL
GTQTYICNVN HKPSNTKVDK KVEPKSC

4856 gH13 Fab heavy chain (V + human gamma-1 CH1) (mammalian expression) SEQ ID NO: 60

gaagtgcac tgggtgaaag cggaggaggt ttggtgaaac cgggagggtc
actgcggctc tcctgtgccg cctccggtt cagcctctcg tcctacgaca
tgagctgggt cagacaggcc cccgggaagg gcctggagtg gattggatc
atctacggcg gctccggctc gacttggtac gcttcgtggg ccaagggacg
gttcaccatc tcccgcgatt ccgcgaagaa cagcgtgtat ctgcagatga
actctctgcg ggccgaggac accgcagtgt actactgcgc gagggggcgc
gacgccggcg ccgggggatc acgcaacggt tactcccttt ggggacaggg
aaccctggtc actgtctcca gcgcttctac aaagggccca tcggtcttcc
ccctggcacc ctctccaag agcacctctg ggggcacagc ggccctgggc
tgcttggtca aggactactt ccccgaaccg gtgacgggtg cgtggaactc
aggcgccctg accagcggcg tgcacacctt cccggctgtc ctacagtcct
caggactcta ctccctcagc agcgtgggtga ccgtgccctc cagcagcttg
ggcaccaga cctacatctg caacgtgaat cacaagccca gcaacaccaa
ggtggacaag aaagttgagc ccaaattcttg t

4856 gH13 Fab heavy chain (V + human gamma-1 CH1) (*E. coli* expression) SEQ ID NO:61

gaggttcagc tggtcgagtc tggaggcggg cttgtcaaac ctggagggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gattggcatt
 atttatggcg gcagcggcag cacatggtac gcgagctggg cgaagggccg
 tttcaccatc tcccgggaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcaacggc tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gcgcttctac aaagggccca tcggtcttcc
 ccctggcacc ctctccaag agcacctctg ggggcacagc ggccctgggc
 tgcttggtca aggactactt ccccgaaccg gtgacggtgt cgtggaactc
 aggcgccttg accagcggcg tgcacacctt cccggctgtc ctacagtcct
 caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcagcttg
 ggcacccaga cctacatctg caacgtgaat cacaagccca gcaacaccaa
 ggtcgacaag aaagttgagc ccaaattctt t

4856 gH13 Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:62

MKWVTFISLL FLESSAYSEV QLVESGGGLV KPGGSLRLSC AASGFSLSY
 DMSWVRQAPG KGLEWIGIIY GSGSTWYAS WAKGRFTISR DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRNGYS LWGQGLVTV SSASTKGPSV
 FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ
 SSGLYSLSSV VTPVSSSLGT QTYICNVNHK PSNTKVDKKV EPKSC

4856 gH13 Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:63

atgaagtggg tcaccttcat ctccctgctg tttctgttct ccagcgccta
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctcgtcctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggattgg
 tatcatctac ggcggctccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catctcccgc gattccgcga agaacagcgt gtatctgcag
 atgaactctc tgcgggcca ggacaccgca gtgtactact gcgcgagggg
 gcgcgacgcc ggcgccgggg gatcacgcaa cggttactcc ctttggggac
 aggaaccct ggtcactgtc tccagcgtt ctacaaagg cccatcggtc
 tcccccttg caccctctc caagagcacc tctgggggca cagcggccct
 gggctgcctg gtcaaggact acttccccga accggtgacg gtgtcgtgga
 actcaggcgc cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag
 tcctcaggac tctactccct cagcagcgtg gtgaccgtgc cctccagcag
 cttgggcacc cagacctaca tctgcaacgt gaatcacaag cccagcaaca
 ccaaggtgga caagaaagtt gagcccaaat cttgt

4856 gH13 Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:64

*MKKTAI**IAIAV* *ALAGFATVAQ* AEVQLVESGG GLVKPGGSLR LSCAASGFSL
SSYDMSWVRQ APGKGLEWIG IIYGGSGSTW YASWAKGRFT ISRDSAKNSV
YLQMNSLRAE DTAVYYCARG RDAGAGGSRN GYSLWGQGTL VTVSSASTKG
PSVFPLAPSS KSTSGGTAAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA
VLQSSGLYSL SSVVTVPSST LGTQTYICNV NHKPSNTKVD KKVEPKSC

4856 gH13 Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:65

atgaagaaga ctgctatagc aattgcagtg gcgctagctg gtttcgccac
cgtggcgcaa gctgaggttc agctggtcga gtctggaggc gggcttgtca
aacctggagg gagcctgcgt ctctcttgtg cagcaagcgg cttcagcctg
tcctcttacg atatgtcctg ggtgcgccag gcacctggga agggcctgga
gtggattggc attatttatg gcggcagcgg cagcacatgg tacgcgagct
gggcgaaggg ccgtttcacc atctcccggg acagcgcaaa gaatagcgtg
tacctccaga tgaactctct ccgcgcagag gacacagcag tctattactg
tgcgcggggc cgcgatgcag gcgcggggcg cagccgcaac ggctatagcc
tgtggggaca ggggaccctt gtgacagtct cgagcgcttc taaaagggc
ccatcgggtct tccccctggc accctcctcc aagagcacct ctgggggcac
agcggccctg ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg
tgtcgtggaa ctcaggcgcc ctgaccagcg gcgtgcacac cttcccggct
gtcctacagt cctcaggact ctactccctc agcagcgtgg tgaccgtgcc
ctccagcagc ttgggcaccc agacctacat ctgcaacgtg aatcacaagc
ccagcaacac caaggtcgac aagaaagttg agcccaaata ttgt

FIGURE 1F

4856gH20 V-region SEQ ID NO:66

EVQLVESGGG LVKPGGSLRL SCAASGFSLS SYDMSWVRQA PGKGLEWISI
 IYGGSGSTWY ASWAKGRFTM SKDSAKNSVY LQMNSLRAED TAVYYCARGR
 DAGAGGSRNG YSLWGQGLTV TVSS

4856gH20 V-region (mammalian expression) SEQ ID NO:67

gaagtgcaac tgggtggaag cggaggaggt ttgggtgaaac cgggagggtc
 actgcggctc tcctgtgccg cctccggatt cagcctctcg tcctacgaca
 tgagctgggt cagacaggcc cccgggaagg gcctggagtg gatttccatc
 atctacggcg gctccggctc gacttggtac gcttcgtggg ccaagggacg
 gttcaccatg tccaaggatt ccgcgaagaa cagcgtgtat ctgcagatga
 actctctgcg ggccgaggac accgcagtgt actactgcgc gagggggcgc
 gacgccggcg ccgggggata acgcaacggt tactcccttt ggggacaggg
 aaccctggtc actgtctcca gc

4856gH20 V-region (*E. coli* expression) SEQ ID NO:68

gaggttcagc tggtcgagtc tggaggcggg cttgtcaaac ctggagggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gatttctatt
 atttatggcg gcagcggcag cacatggtac gcgagctggg cgaagggccg
 tttcaccatg tccaaagaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcaatggg tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gc

4856gH20 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:69

MKWVTFISLL *FLFSSAYSEV* QLVESGGGLV KPGGSLRLSC AASGFSLSY
 DMSWVRQAPG KGLEWISIIY GSGSTWYAS WAKGRFTMSK DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRNGYS LWGQGLTVTV SS

4856gH20 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:70

atgaagtggg *tcaccttcat* *ctccctgctg* *tttctgttct* *ccagcgccta*
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctcgtcctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggatttc
 catcatctac ggcggtcccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catgtccaag gattccgcga agaacagcgt gtatctgcag
 atgaactctc tgcggggccga ggacaccgca gtgtactact gcgcgagggg
 gcgcgacgcc ggcgccgggg gatcacgcaa cggttactcc ctttggggac

agggaaccct ggtcactgtc tccagc

4856gH20 V-region with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:71

MKKTAIAlAV ALAGFATVAQ AEVQLVESGG GLVKPGGSLR LSCAASGFSL
SSYDMSWVRQ APGKGLEWIS IIYGGSGSTW YASWAKGRFT MSKDSAKNSV
YLQMNSLRAE DTAIVYYCARG RDAGAGGSRN GYSLWGQGTL VTVSS

4856gH20 V-region with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:72

atgaagaaga ctgctatagc aattgcagtg gcgctagctg gtttcgccac
cgtggcgcaa gctgagggtc agctggtcga gtctggaggc gggcttgtca
aacctggagg gagcctgcgt ctctcttgtg cagcaagcgg cttcagcctg
tcctcttacg atatgtcctg ggtgcgccag gcacctggga agggcctgga
gtggatttct attatttatg gcggcagcgg cagcacatgg tacgcgagct
gggcgaaggg ccgtttcacc atgtccaaag acagcgcaaa gaatagcgtg
tacctccaga tgaactctct ccgcgcagag gacacagcag tctattactg
tgcgcggggc cgcgatgcag gcgcggggcg cagccgcaat gggatatagc
tgtggggaca ggggaccctt gtgacagtct cgagc

4856 gH20 Fab heavy chain (V + human gamma-1 CH1) SEQ ID NO:73

EVQLVESGGG LVKPGGSLRL SCAASGFSLI SYDMSWVRQA PGKGLEWISI
IIYGGSGSTWY ASWAKGRFTM SKDSAKNSVY LQMNSLRAED TAVYYCARGR
DAGAGGSRNG YSLWGQGTLV TVSSASTKGP SVFPLAPSSK STSGGTAALG
CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS SVVTVPSSSL
GTQTYICNVN HKPSNTKVDK KVEPKSC

4856 gH20 Fab heavy chain (V + human gamma-1 CH1) (mammalian expression) SEQ ID NO:74

gaagtgcaac tgggtgaaag cggaggaggt ttggtgaaac cgggagggtc
actgcggctc tcctgtgccg cctccgatt cagcctctcg tectacgaca
tgagctgggt cagacaggcc cccgggaagg gcctggagtg gatttccatc
atctacggcg gctccggctc gacttggtag gcttcgtggg ccaagggacg
gttcaccatg tccaaggatt ccgcgaagaa cagcgtgtat ctgcagatga
actctctgcg ggccgaggac accgcagtgt actactgcgc gagggggcgc
gacgccggcg ccgggggagc acgcaacggt tactcccttt ggggacaggg
aaccctggtc actgtctcca gcgcttctac aaagggccca tcggtcttcc
ccctggcacc ctccctcaag agcacctctg ggggcacagc ggccctgggc
tgcttggtca aggactactt ccccgaaccg gtgacgggtg cgtggaactc
aggcgccctg accagcggcg tgcacacctt cccggctgtc ctacagtct
caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcagcttg
ggcaccacga cctacatctg caacgtgaat cacaagccca gcaacaccaa
ggtggacaag aaagttgagc ccaaattctg t

4856 gH20 Fab heavy chain (V + human gamma-1 CH1) (*E. coli* expression) SEQ ID NO:75

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gaggttcagc tggtcgagtc tggagggcggg cttgtcaaac ctggagggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gatttctatt
 atttatggcg gcagcggcag cacatggtac gcgagctggg cgaagggccg
 tttcaccatg tccaaagaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcaatggg tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gcgcttctac aaagggccca tcggtcttcc
 ccctggcacc ctccccaag agcacctctg ggggcacagc ggccctgggc
 tgccctggta aggactactt ccccgaaccg gtgacgggtg cgtggaactc
 aggcgccctg accagcggcg tgcacacctt cccggctgtc ctacagtcct
 caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcagcttg
 ggcaccaga cctacatctg caacgtgaat cacaagccca gcaacaccaa
 ggtcgacaag aaagttgagc ccaaattctg t

4856 gH2O Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:76

MKWVTFISLL *FLFSSAYSEV* QLVESGGGLV KPGGSLRLSC AASGFSLSY
 DMSWVRQAPG KGLEWISIIY GSGSTWYAS WAKGRFTMSK DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRNGYS LWGQGLTVTV SSASTKGPSV
 FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ
 SSGLYSLSSV VTPVSSSLGT QTYICNVNHN PSNTKVDKKV EPKSC

4856 gH2O Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:77

atgaagtggg *tcaccttcat* *ctccctgctg* *tttctgttct* *ccagcgccta*
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctcgtcctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggatttc
 catcatctac ggcggctccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catgtccaag gattccgcga agaacagcgt gtatctgcag
 atgaactctc tgcgggccga ggacaccgca gtgtactact gcgcgagggg
 gcgcgacgcc ggcgccgggg gatcacgcaa cggttactcc ctttggggac
 agggaaacct ggtcactgtc tccagcgtt ctacaaaggg cccatcggtc
 ttccccctgg caccctcctc caagagcacc tctgggggca cagcggccct
 gggctgcctg gtcaaggact acttccccga accggtgacg gtgtcgtgga
 actcaggcgc cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag
 tcctcaggac tctactccct cagcagcgtg gtgaccgtgc cctccagcag
 cttgggcacc cagacctaca tctgcaacgt gaatcacaag cccagcaaca
 ccaaggtgga caagaaagtt gagcccaaat cttgt

4856 gH2O Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:78

*MKKTALAI**AV* *ALAGFATVAQ* *AEVQLVESGG* GLVKPGGSLR LSCAASGFSL
 SSYDMSWVRQ APGKLEWIS IYGGSGSTW YASWAKGRFT MSKDSAKNSV
 YLQMNSLRAE DTAVYYCARG RDAGAGGSRN GYSLWGQGLT VTVSSASTKG

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PSVFPLAPSS KSTSGGTAAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA
VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSC

4856 gH20 Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:79

atgaagaaga ctgctatagc aattgcagtg gcgctagctg gtttcgccac
cgtaggcgcaa gctgaggttc agctggtcga gtctggaggc gggcttgtca
aacctggagg gagcctgcgt ctctcttgtg cagcaagcgg cttcagcctg
tcctcttacg atatgtcctg ggtgcgccag gcacctggga agggcctgga
gtggatttct attatttatg gcggcagcgg cagcacatgg tacgcgagct
gggcgaaggg ccgtttcacc atgtccaaag acagcgcaaa gaatagcgtg
tacctccaga tgaactctct ccgcgcagag gacacagcag tctattactg
tgcgcggggc cgcgatgcag gcgcggggcg cagccgcaat gggatatagcc
tgtggggaca ggggaccctt gtgacagtct cgagcgcttc tacaaagggc
ccatcgggtct tccccctggc accctcctcc aagagcacct ctgggggcac
agcggccctg ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg
tgtcgtggaa ctcaggcgcc ctgaccagcg gcgtgcacac cttcccggct
gtcctacagt cctcaggact ctactccctc agcagcgtgg tgaccgtgcc
ctccagcagc ttgggcaccc agacctacat ctgcaacgtg aatcacaagc
ccagcaacac caaggtcgac aagaaagttg agcccaaata ttgt

FIGURE 1G**4856gH23 V-region SEQ ID NO: 80**

EVQLVESGGG LVKPGGSLRL SCAASGFSL S YDMSWVRQA PGKGLEWIGI
 IYGGSGSTWY ASWAKGRFTI SRDSAKNSVY LQMNSLRAED TAVYYCARGR
 DAGAGGSRNA YSLWGQGTLV TVSS

4856gH23 V-region (mammalian expression) SEQ ID NO: 81

gaagtgcaac tgggtggaag cggaggaggt ttggtgaaac cgggagggtc
 actgcggctc tcctgtgccg cctccggatt cagcctctcg tcctacgaca
 tgagctgggt cagacaggcc cccgggaagg gcctggagtg gattgggtatc
 atctacggcg gctccggctc gacttgggtac gcttcgtggg ccaagggacg
 gttcaccatc tcccgcgatt ccgcgaagaa cagcgtgtat ctgcagatga
 actctctgcg ggccgaggac accgcagtgt actactgcgc gagggggcgc
 gacgccggcg ccgggggata acgcaacgcct actcccttt ggggacaggg
 aaccctggtc actgtctcca gc

4856gH23 V-region (*E. coli* expression) SEQ ID NO: 82

gaggttcagc tggtcgagtc tggaggcggg cttgtcaaac ctggaggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gattggcatt
 atttatggcg gcagcggcag cacatgggtac gcgagctggg cgaagggccg
 tttcaccatc tcccgggaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcaacgcg tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gc

4856gH23 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 83

MKWVTFISLL *FLFSSAYSEV* QLVESGGGLV KPGGSLRLSC AASGFSLSSY
 DMSWVRQAPG KGLEWIGIIY GSGGSTWYAS WAKGRFTISR DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRNAYS LWGQGTLVTV SS

4856gH23 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 84

atgaagtggg *tcaccttcat* *ctccctgctg* *tttctgttct* *ccagcgccta*
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctcgctctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggattgg
 tatcatctac ggcggctccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catctcccgc gattccgcga agaacagcgt gtatctgcag
 atgaactctc tgcggggccga ggacaccgca gtgtactact gcgcgagggg

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gcgcgacgcc ggcgccgggg gatcacgcaa cgcctactcc ctttggggac
agggaaccct ggtcactgtc tccagc

4856gH23 V-region with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:85

*MKKT**AI**AI**AV* *ALAGFATVAQ* *AEVQLVESGG* *GLVKPGGSLR* *LSCAASGFSL*
SSYDMSWVRQ *APGKGLEWIG* *IIYGGSGSTW* *YASWAKGRFT* *ISRDSAKNSV*
YLQMNSLRAE *DTAVYYCARG* *RDAGAGGSRN* *AYSLWGQGT**L* *VT**VSS*

4856gH23 V-region with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:86

atgaagaaga *ctgctatagc* *aattgcagtg* *gcgctagctg* *gtttcgccac*
cgtggcgcaa *gctgaggttc* *agctggtcga* *gtctggaggc* *gggcttgtca*
aacctggagg *gagcctgcgt* *ctctcttgtg* *cagcaagcgg* *cttcagcctg*
tcctcttacg *atatgtcctg* *ggtgcgccag* *gcacctggga* *agggcctgga*
gtggattggc *attatttatg* *gcggcagcgg* *cagcacatgg* *tacgcgagct*
gggcgaaggg *ccgtttcacc* *atctcccggg* *acagcgcaaa* *gaatagcgtg*
tacctccaga *tgaactctct* *ccgcgcagag* *gacacagcag* *tctattactg*
tgcgcggggc *cgcgatgcag* *gcgcggggcg* *cagccgcaac* *gcgtatagcc*
tgtggggaca *ggggaccctt* *gtgacagtct* *cgagc*

4856 gH23 Fab heavy chain (V + human gamma-1 CH1)

SEQ ID NO:87

EVQLVESGGG *LVKPGGSLRL* *SCAASGFSL**S* *SYDMSWVRQA* *PGKGLEWIGI*
IYGGSGSTWY *ASWAKGRFTI* *SRDSAKNSVY* *LQMNSLRAED* *TAVYYCARGR*
DAGAGGSRNA *YSLWGQGT**LV* *TVSSASTKGP* *SVFPLAPSSK* *STSGGTAALG*
CLVKDYFPEP *VTVSWNSGAL* *TSGVHTFPAV* *LQSSGLYSLS* *SVVTVPSSSL*
GTQTYICNVN *HKPSNTKV**DK* *KVEPKSC*

4856 gH23 Fab heavy chain (V + human gamma-1 CH1) (mammalian expression)

SEQ ID NO:88

*gaagtgc**aac* *tgg**tgg**aaag* *cgg**agg**aggt* *ttg**gtg**aaac* *cggg**agg**ggtc*
*actgc**gggtc* *tcct**gtg**ccg* *cctcc**ggatt* *cagc**ctctcg* *tcct**acgaca*
*tgag**ctgggt* *cag**acaggcc* *ccc**gggaagg* *gcct**ggagt**g* *gatt**gg**tatc*
*atct**acggcg* *gctcc**ggctc* *gact**tgg**tac* *gcttc**gtggg* *cca**agg**gacg*
*gttc**accatc* *tccc**gcgatt* *ccgc**gaagaa* *cagc**gtgtat* *ctgc**agatga*
actctctgcg *ggcc**gaggac* *accgc**agtgt* *actactgcgc* *gagggg**gcgc*
*gacgc**ggcg* *ccggg**ggatc* *acgca**acgcc* *tactcc**cttt* *gggg**acaggg*
*aacct**ggtc* *actgtctcca* *gcg**cttctac* *aaagg**gccca* *tcgg**tcttcc*
*ccct**ggcacc* *ctcctccaag* *agcac**ctctg* *gggg**cacagc* *ggcc**ctgggc*
*tgct**gg**tca* *agg**actactt* *cccc**gaaccg* *gtg**acgg**tg**t* *cgt**gga**actc*
*aggc**gc**ccctg* *acc**agcggcg* *tgc**acac**ctt* *ccc**ggctgtc* *ctac**ag**tcct*
*cagg**actcta* *ctcc**ctcagc* *agc**gtgg**tga* *ccgt**gccctc* *cagc**ag**cttg*
*ggc**acccaga* *cct**acatctg* *caac**gtgaat* *caca**ag**ccca* *gca**acac**caa*
*ggt**ggacaag* *aaag**ttgagc* *ccaa**atcttg* *t*

4856 gH23 Fab heavy chain (V + human gamma-1 CH1) (*E. coli* expression)

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SEQ ID NO:89

gaggttcagc tggtcgagtc tggagggcggg cttgtcaaac ctggagggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gattggcatt
 atttatggcg gcagcggcag cacatggtac gcgagctggg cgaagggccg
 tttcaccatc tcccgggaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcaacgcg tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gcgcttctac aaagggccca tcggtcttcc
 ccctggcacc ctccccaag agcacctctg ggggcacagc ggccctgggc
 tgccctggta aggactactt ccccgaaccg gtgacgggtg cgtggaactc
 aggcgccctg accagcggcg tgcacacctt cccggctgtc ctacagtcct
 caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcagcttg
 ggcaccaga cctacatctg caacgtgaat cacaagccca gcaacaccaa
 ggtcgacaag aaagttgagc ccaaattctg t

4856 gH23 Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:90

MKWVTFISLL *FLFSSAYSEV* QLVESGGGLV KPGGSLRLSC AASGFSLSY
 DMSWVRQAPG KGLEWIGIIY GSGSTWYAS WAKGRFTISR DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRNAYS LWGQGLTVTV SSASTKGPSV
 FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ
 SSGLYSLSSV VTPVSSSLGT QTYICNVN HK PSNTKVDKKV EPKSC

4856 gH23 Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:91

atgaagtggg *tcaccttcat* *ctccctgctg* *tttctgttct* *ccagcgccta*
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctcgtcctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggattgg
 tatcatctac ggcggctccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catctcccgc gattccgcga agaacagcgt gtatctgcag
 atgaactctc tgcggggccga ggacaccgca gtgtactact gcgcgagggg
 gcgcgacgcc ggcgccgggg gatcacgcaa cgcctactcc ctttggggac
 agggaaaccct ggtcactgtc tccagcgctt ctacaaaggg cccatcggtc
 ttccccctgg caccctcctc caagagcacc tctgggggca cagcggccct
 gggctgcctg gtcaaggact acttccccga accggtgacg gtgtcgtgga
 actcaggcgc cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag
 tcctcaggac tctactccct cagcagcgtg gtgaccgtgc cctccagcag
 cttgggcacc cagacctaca tctgcaacgt gaatcacaag cccagcaaca
 ccaaggtgga caagaaagtt gagcccaaat cttgt

4856 gH23 Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:92

*MKKTALAI**AV* *ALAGFATVAQ* *AEVQLVESGG* GLVKPGGSLR LSCAASGFSL
 SSYDMSWVRQ APGKGLEWIG IYGGSGSTW YASWAKGRFT ISRDSAKNSV
 YLQMNSLRAE DTAVYYCARG RDAGAGGSRN AYSLWGQGLT VTVSSASTKG

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PSVFPLAPSS KSTSGGTAAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA
VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSC

4856 gH23 Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:93

atgaagaaga ctgctatagc aattgcagtg gcgctagctg gtttcgccac
cgtggcgcaa gctgagggttc agctgggtcga gtctggaggc gggcttgtca
aacctggagg gagcctgcgt ctctcttgtg cagcaagcgg cttcagcctg
tcctcttacg atatgtcctg ggtgcgccag gcacctggga agggcctgga
gtggattggc attatttatg gcggcagcgg cagcacatgg tacgcgagct
gggcgaaggg ccgtttcacc atctcccggg acagcgcaaa gaatagcgtg
tacctccaga tgaactctct ccgcgcagag gacacagcag tctattactg
tgcgcggggc cgcgatgcag gcgcggggcg cagccgcaac gcgtatagcc
tgtggggaca ggggaccctt gtgacagtct cgagcgcttc tacaaagggc
ccatcgggtct tccccctggc accctcctcc aagagcacct ctgggggcac
agcggccctg ggctgcctgg tcaaggacta ctccccgaa ccggtgacgg
tgtcgtggaa ctcaggcgcc ctgaccagcg gcgtgcacac cttcccggct
gtcctacagt cctcaggact ctactccctc agcagcgtgg tgaccgtgcc
ctccagcagc ttgggcaccc agacctacat ctgcaacgtg aatcacaagc
ccagcaacac caaggtcgac aagaaagttg agcccaaata ttgt

FIGURE 1H

4856gH29 V-region SEQ ID NO: 94

EVQLVESGGG LVKPGGSLRL SCAASGFSL S YDMSWVRQA PGKGLEWIGI
 IYGGSGSTWY ASWAKGRFTI SRDSAKNSVY LQMNSLRAED TAVYYCARGR
 DAGAGGSRDG YSLWGQGTLV TVSS

4856gH29 V-region (mammalian expression) SEQ ID NO: 95

gaagtgcaac tgggtggaag cggaggaggt ttggtgaaac cgggagggtc
 actgcggctc tcctgtgccg cctccgatt cagcctctcg tcctacgaca
 tgagctgggt cagacaggcc cccgggaagg gcctggagtg gattgggtatc
 atctacggcg gctccggctc gacttgggtac gcttcgtggg ccaagggacg
 gtccaccatc tcccgcgatt ccgcgaagaa cagcgtgtat ctgcagatga
 actctctgcg ggccgaggac accgcagtgt actactgcgc gagggggcgc
 gacgccggcg ccgggggatc acgcgacggg tactcccttt ggggacaggg
 aaccctggtc actgtctcca gc

4856gH29 V-region (*E. coli* expression) SEQ ID NO: 96

gaggttcagc tgggtcgagtc tggaggcggg cttgtcaaac ctggaggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gattggcatt
 atttatggcg gcagcggcag cacatgggtac gcgagctggg cgaagggccg
 ttccaccatc tcccgggaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcgatggg tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gc

4856gH29 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 97

MKWVTFISLL *FLFSSAYSEV* QLVESGGGLV KPGGSLRLSC AASGFSLSSY
 DMSWVRQAPG KGLEWIGIY GSGSTWYAS WAKGRFTISR DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRDGYS LWGQGTLVTV SS

4856gH29 V-region with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO: 98

atgaagtggg *tcaccttcat* *ctccctgctg* *tttctgttct* *ccagcgccta*
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctctgcctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggattgg
 tatcatctac ggcggctccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catctcccgc gattccgcga agaacagcgt gtatctgcag
 atgaactctc tgcggggccga ggacaccgca gtgtactact gcgcgagggg

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gcgcgacgcc ggcgccgggg gatcacgcga cggttactcc ctttggggac
agggaaccct ggtcactgtc tccagc

4856gH29 V-region with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:99

*MKKT**AI**AI**AV* *ALAGFATVAQ* AEVQLVESGG GLVKPGGSLR LSCAASGFSL
SSYDMSWVRQ APGKGLEWIG IIYGGSGSTW YASWAKGRFT ISRDSAKNSV
YLQMNSLRAE DTAVYYCARG RDAGAGGSRD GYSLWGQGTL VTVSS

4856gH29 V-region with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:100

atgaagaaga *ctgctatagc* *aattgcagtg* *gcgctagctg* *gtttcgccac*
cgtggcgcaa *gctgaggttc* agctggtcga gtctggaggc gggcttgtca
aacctggagg gagcctgcgt ctctcttgtg cagcaagcgg cttcagcctg
tcctcttacg atatgtcctg ggtgcgccag gcacctggga agggcctgga
gtggattggc attatttatg gcggcagcgg cagcacatgg tacgcgagct
gggcgaaggg ccgtttcacc atctcccggg acagcgcaaa gaatagcgtg
tacctccaga tgaactctct ccgcgcagag gacacagcag tctattactg
tgcgcggggc cgcatgcag gcgcgggcgg cagccgcgat gggatatagcc
tgtggggaca ggggaccctt gtgacagtct cgagc

4856 gH29 Fab heavy chain (V + human gamma-1 CH1)

SEQ ID NO:101

EVQLVESGGG LVKPGGSLRL SCAASGFSL S YDMSWVRQA PGKGLEWIGI
IYGGSGSTWY ASWAKGRFTI SRDSAKNSVY LQMNSLRAED TAVYYCARGR
DAGAGGSRDG YSLWGQGTLV TVSSASTKGP SVFPLAPSSK STSGGTAALG
CLVKDYFPEP VTVSWNSGAL TSGVHTFPAV LQSSGLYSLS SVVTVPSSSL
GTQTYICNVN HKPSNTKVDK KVEPKSC

4856 gH29 Fab heavy chain (V + human gamma-1 CH1) (mammalian expression)

SEQ ID NO:102

gaagtgcaac tgggtgaaag cggaggaggt ttggtgaaac cgggagggtc
actgcggctc tcctgtgccg cctccgatt cagcctctcg tcctacgaca
tgagctgggt cagacaggcc cccgggaagg gcctggagtg gattgggtatc
atctacggcg gctccggctc gacttggtac gcttcgtggg ccaagggacg
gttcaccatc tcccgcgatt ccgcgaagaa cagcgtgtat ctgcagatga
actctctgcg ggccgaggac accgcagtgt actactgcgc gagggggcgc
gacgccggcg ccgggggatc acgcgacggg tactcccttt ggggacaggg
aacctgggtc actgtctcca gcgcttctac aaagggccca tcggtcttcc
ccctggcacc ctctccaag agcacctctg ggggcacagc ggccctgggc
tgcttggtca aggactactt ccccgaaccg gtgacgggtg cgtggaactc
aggcgccctg accagcggcg tgcacacctt cccggctgtc ctacagtcct
caggactcta ctccctcagc agcgtgggtga ccgtgccctc cagcagcttg
ggcaccacaga cctacatctg caacgtgaat cacaagccca gcaacaccaa
ggtggacaag aaagttgagc ccaaattcttg t

4856 gH29 Fab heavy chain (V + human gamma-1 CH1) (*E. coli* expression)

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SEQ ID NO:103

gaggttcagc tggtcgagtc tggagggcggg cttgtcaaac ctggagggag
 cctgcgtctc tcttgtgcag caagcggctt cagcctgtcc tcttacgata
 tgtcctgggt gcgccaggca cctgggaagg gcctggagtg gattggcatt
 atttatggcg gcagcggcag cacatggtac gcgagctggg cgaagggccg
 tttcaccatc tcccgggaca gcgcaaagaa tagcgtgtac ctccagatga
 actctctccg cgcagaggac acagcagtct attactgtgc gcggggccgc
 gatgcaggcg cgggcggcag ccgcgatggg tatagcctgt ggggacaggg
 gacccttgtg acagtctcga gcgcttctac aaagggccca tcggtcttcc
 ccctggcacc ctccccaag agcacctctg ggggcacagc ggccctgggc
 tgccctggta aggactactt ccccgaaccg gtgacgggtg cgtggaactc
 aggcgccctg accagcggcg tgcacacctt cccggctgtc ctacagtcct
 caggactcta ctccctcagc agcgtggtga ccgtgccctc cagcagcttg
 ggcaccacga cctacatctg caacgtgaat cacaagccca gcaacaccaa
 ggtcgacaag aaagttgagc ccaaattctg t

4856 gH29 Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:104

MKWVTFISLL FLFSSAYSEV QLVESGGGLV KPGGSLRLSC AASGFSLSY
 DMSWVRQAPG KGLEWIGIIY GSGSTWYAS WAKGRFTISR DSAKNSVYLQ
 MNSLRAEDTA VYYCARGRDA GAGGSRDGYS LWGQGLTVTV SSASTKGPSV
 FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ
 SSGLYSLSSV VTPVSSSLGT QTYICNVN HK PSNTKVDKKV EPKSC

4856 gH29 Fab heavy chain with signal sequence underlined and italicized (mammalian expression)

SEQ ID NO:105

atgaagtggg tcaccttcat ctccctgctg tttctgttct ccagcgccta
ctccgaagtg caactggtgg aaagcggagg aggtttggtg aaaccgggag
 ggtcactgcg gctctcctgt gccgcctccg gattcagcct ctcgtcctac
 gacatgagct gggtcagaca ggcccccggg aagggcctgg agtggattgg
 tatcatctac ggcgggtccg gctcgacttg gtacgcttcg tgggccaagg
 gacggttcac catctcccgc gattccgcga agaacagcgt gtatctgcag
 atgaactctc tgcggggccga ggacaccgca gtgtactact gcgcgagggg
 gcgcgacgcc ggcgccgggg gatcacgcga cggttactcc ctttggggac
 agggaaccct ggtcactgtc tccagcgtt ctacaaaggg cccatcggtc
 ttccccctgg caccctcctc caagagcacc tctgggggca cagcggccct
 gggctgcctg gtcaaggact acttccccga accggtgacg gtgtcgtgga
 actcaggcgc cctgaccagc ggcgtgcaca ccttcccggc tgtcctacag
 tcctcaggac tctactccct cagcagcgtg gtgaccgtgc cctccagcag
 cttgggcacc cagacctaca tctgcaacgt gaatcacaag cccagcaaca
 ccaagggtga caagaaagtt gagcccaaat cttgt

4856 gH29 Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:106

MKKTALAIIV ALAGFATVAQ AEVQLVESGG GLVKPGGSLR LSCAASGFSL
 SSYDMSWVRQ APGKGLEWIG IYGGSGSTW YASWAKGRFT ISRDSAKNSV
 YLQMNSLRAE DTAVYYCARG RDAGAGGSRD GYSLWGQGLT VTVSSASTKG

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PSVFPLAPSS KSTSGGTAAAL GCLVKDYFPE PVTVSWNSGA LTSGVHTFPA
VLQSSGLYSL SSVVTVPSSS LGTQTYICNV NHKPSNTKVD KKVEPKSC

4856 gH29 Fab heavy chain with signal sequence underlined and italicized (*E. coli* expression)

SEQ ID NO:107

atgaagaaga ctgctatagc aattgcagtg gcgctagctg gtttcgccac
cgtaggcgcaa gctgaggttc agctggtcga gtctggaggc gggcttgtca
aacctggagg gagcctgcgt ctctcttgtg cagcaagcgg cttcagcctg
tcctcttacg atatgtcctg ggtgcgccag gcacctggga agggcctgga
gtggattggc attatttatg gcggcagcgg cagcacatgg tacgcgagct
gggcgaaggg ccgtttcacc atctcccggg acagcgcaaa gaatagcgtg
tacctccaga tgaactctct ccgcgcagag gacacagcag tctattactg
tgcgcggggc cgcgatgcag gcgcggggcg cagccgcgat gggatatagcc
tgtggggaca ggggaccctt gtgacagtct cgagcgcttc tacaaagggc
ccatcgggtct tccccctggc accctcctcc aagagcacct ctgggggcac
agcggccctg ggctgcctgg tcaaggacta cttccccgaa ccggtgacgg
tgtcgtggaa ctcaggcgcc ctgaccagcg gcgtgcacac cttcccggct
gtcctacagt cctcaggact ctactccctc agcagcgtgg tgaccgtgcc
ctccagcagc ttgggcaccc agacctacat ctgcaacgtg aatcacaagc
ccagcaacac caaggtcgac aagaaagttg agcccaaate ttgt

4856 scFv

SEQ ID NO:108

EVQLVESGGG LVKPGGSLRL SCAASGFSLs SYDMSWVRQA PGKCLEWIGI
IYGSGSTWY ASWAKGRFTI SRDSAKNSVY LQMNSLRAED TAVYYCARGR
DAGAGGSRNG YSLWGQGTIV TVSSGGGGSG GGGSGGGSG GGSAYDMTQ
SPSTLSASVG DRVTITCQAS ESIYSGLAWY QQKPGKAPKL LIYAASDLAS
GVPSRFSGSG SGTEYTLTIS SLQPDFFATY YCQQTWTDGG IDNPFSGCGTK
VEIKRTENLY FQ

FIGURE 11

Human IGKV1-5 JK4 acceptor framework SEQ ID NO:109

DIQMTQSPST LSASVGDRVT ITCRASQSIG SWLAWYQQKP GKAPKLLIYD
ASSLESGVPS RFSGSGSGTE FTLTISSLQP DDFATYYCQQ YNSYSLTFGG
GTKVEIK

Human IGKV1-5 JK4 acceptor framework SEQ ID NO:110

gacatccaga tgacccagtc tccttccacc ctgtctgcat ctgtaggaga
cagagtcacc atcacttgcc gggccagtc gagtattagt agctgggttg
cctgggtatca gcagaaacca gggaaagccc ctaagctcct gatctatgat
gcctccagtt tggaaagtgg ggtcccatca aggttcagcg gcagtggatc
tgggacagaa ttcactctca ccatcagcag cctgcagcct gatgattttg
caacttatta ctgccaacag tataatagtt attctctcac tttcggcgga
gggaccaagg tggagatcaa a

Human IGHV3-21 JH5 acceptor framework SEQ ID NO:111

EVQLVESGGG LVKPGGSLRL SCAASGFTFS SYSMNWVRQA PGKGLEWVSS
ISSSSSYIYY ADSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARNW
FDSWGQGLV TVSS

Human IGHV3-21 JH5 acceptor framework SEQ ID NO:112

gaggtgcagc tgggtggagtc tgggggaggc ctggtcaagc ctgggggggtc
cctgagactc tcctgtgcag cctctggatt caccttcagt agctatagca
tgaactgggt ccgccaggct ccagggaagg ggctggagtg ggtctcatcc
attagtagta gtagtagtta catatactac gcagactcag tgaagggccg
attcaccatc tccagagaca acgccaagaa ctactgtat ctgcaaataga
acagcctgag agccgaggac acggctgtgt attactgtgc gagaaactgg
ttcgactcct ggggccaagg aaccctggtc accgtctcct ca

Figure 2A

LIGHT CHAIN Graft 4856

Light 4856	1	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95abc	100	105		
	AYD	MTQ	TPAS	VEVA	GGTV	TIKCC	<u>QASESIYSGLA</u>	WYQQ	TPGQ	RPKLLIY	<u>AASDLAS</u>	GVPS	SRFK	SGSG	TEYTL	TI	ISGVE	CADA	ATYYC	<u>QQTWT</u>	<u>DGGIDNP</u>	FGG	TEVVVK	
IGKV1-5	DIQ	MTQ	SPST	LSAS	VGDR	VTIT	TC	<u>RASQSISSWLA</u>	WYQQ	KPGK	APKLLIY	<u>DASSLES</u>	GVPS	SRFS	SGSG	TEFT	LT	ISSLP	DD	FATYYC	<u>QQYN---</u>	<u>SYSLT</u>	FGG	GTKVEIK
4856gL3	AYD	MTQ	SPST	LSAS	VGDR	VTIT	TC	<u>QASESIYSGLA</u>	WYQQ	KPGK	APKLLIY	<u>AASDLAS</u>	GVPS	SRFS	SGSG	TEY	TL	TISSLP	DD	FATYYC	<u>QQTWT</u>	<u>DGGIDNP</u>	FGG	GTKVEIK
4856mL1.1	AYD	MNQS	PSSL	SASL	GDTIT	ITIT	TC	<u>QASESIYSGLA</u>	WYQQ	KPGN	IPKLLIY	<u>AASDLAS</u>	GVPS	SRFK	SGSG	TEYTL	TI	SSLP	ED	IATYYC	<u>QQTWT</u>	<u>DGGIDNP</u>	FGG	GTKLEIK

Legend

Light 4856 = Rabbit light chain variable region sequence (SEQ ID NO: 10)

IGKV1-5 = human germline acceptor framework VK1 sequence IGKV1-5 with JK4 (SEQ ID NO:108)

4856gL3 = Humanized graft of 4856 light chain variable region using IGKV1-5 human germline as the acceptor framework. (SEQ ID NO:38)

4856mL1.1 = Murinized graft of 4856 light chain variable region using a murine germline as the acceptor framework (SEQ ID NO:26)

CDRs are shown in bold/underlined

Donor residues are shown in bold/italic and are highlighted: A1, Y2, D3 and Y71

FIGURE 3A**HUMAN Latency-associated Peptide and Transforming growth factor beta-1 SEQ ID NO:113**

LSTCKTIDMELVKKRIEAI RQILSKLRLASPPSQGEVPPGPLPEAVLALYNSTRDRVAGESAEPEPEPEADYYAK
 EVTRVLMVETHNEIYDKFKQSTHSIYMFFNTSELREAVPEPVLLSRAELRLRLKLVKVEQHVELYQKYSNNSWRYS
 NRLLAPSDSPEWLSFDVTGVVRQWLSRGGEIEGFRLSAHCSCDSRDNTLQVDINGFTTGRRGDLATIHGMNRPFLLL
 MATPLERAQHLQSSRRHRALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYS
 KVLALYNQHNP GASAAPCCVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

FIGURE 3B**Mature HUMAN Transforming growth factor beta-1 SEQ ID NO:114**

ALDTNYCFSSTEKNCCVRQLYIDFRKDLGWKWIHEPKGYHANFCLGPCPYIWSLDTQYSKVLALYNQHNP GASAAPC
 CVPQALEPLPIVYYVGRKPKVEQLSNMIVRSCKCS

FIGURE 3C**HUMAN Latency-associated Peptide and Transforming growth factor beta-2 SEQ ID NO:115**

LSTCSTLDMQFMRKRIEAI RQILSKLKLTSPPEDYPEPEEVPPEVISIYNSTRDLLQEKASRRAAACERERSDEE
 YYAKEVYKIDMPPFFPSENAIPPTFYRPFYFRIVRFDVSAMEKNASNLVKAEFRVFERLQNP KARVPEQRIELYQILKS
 KDLTSTPTQRYIDSKVVKTRAEGEWLSFDVTDVHEWLHHKDRNLGFKISLHCPCTFVPSNNYIIPNKSEELEARFA
 GIDGTSTYTSGDQKTIKSTRKKNSGKTPHLLLMLLPSYRLESQQTNRRKKRALDAAYCFRNVQDNCCLRPLYIDFKR
 DLGWKWIHEPKGYNANFCAGACPYLWSSDTQHSRVLSLYNTINPEASASPCCVSQDLEPLTILYYIGKTPKIEQLSN
 MIVKSKCS

FIGURE 3D**Mature HUMAN Transforming growth factor beta-2 SEQ ID NO:116**

ALDAAYCFRNVQDNCCLRPLYIDFKRDLGWKWIHEPKGYNANFCAGACPYLWSSDTQH
 SRVLSLYNTINPEASASPCCVSQDLEPLTILYYIGKTPKIEQLSNMIVKSKCS

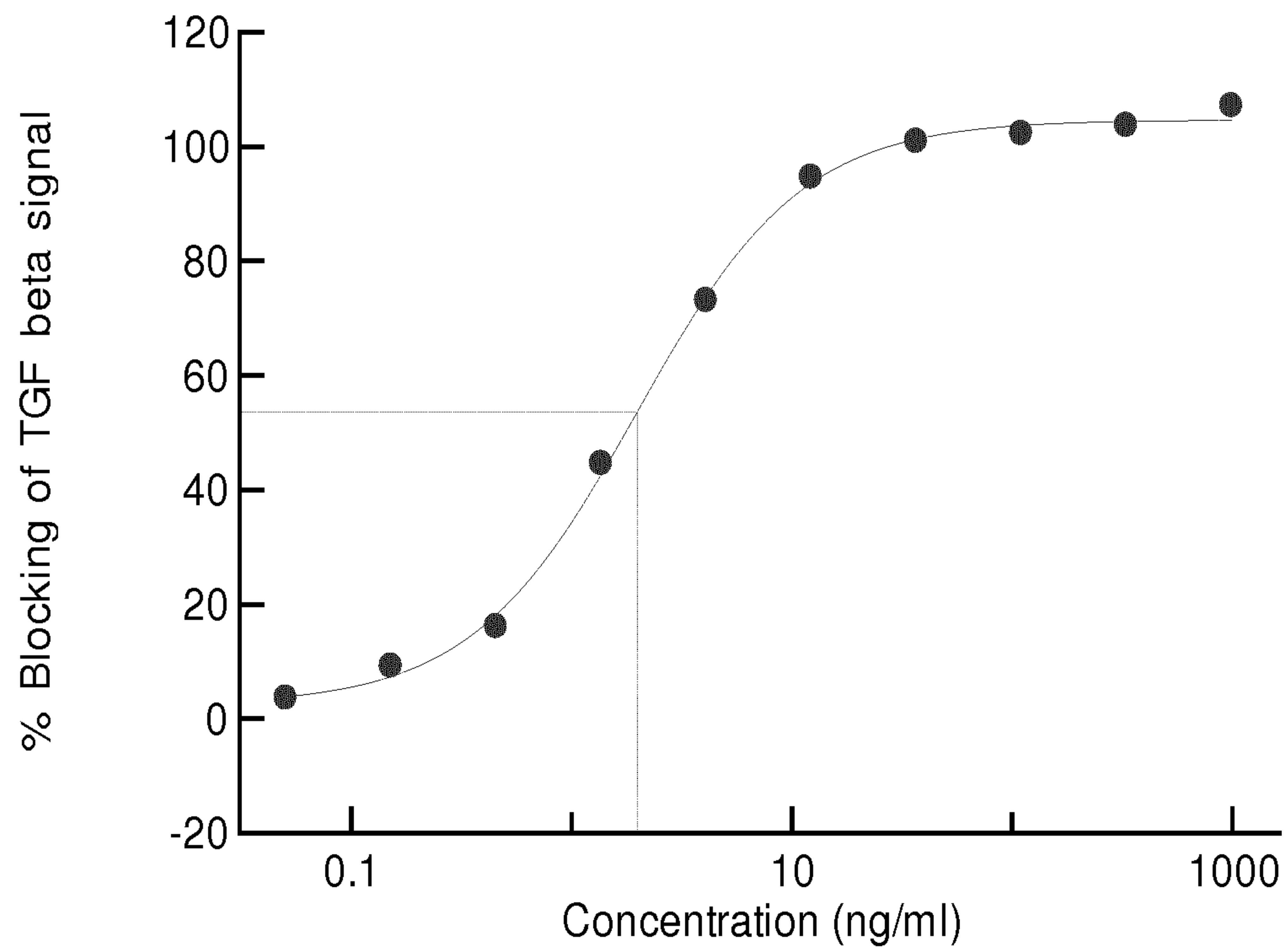
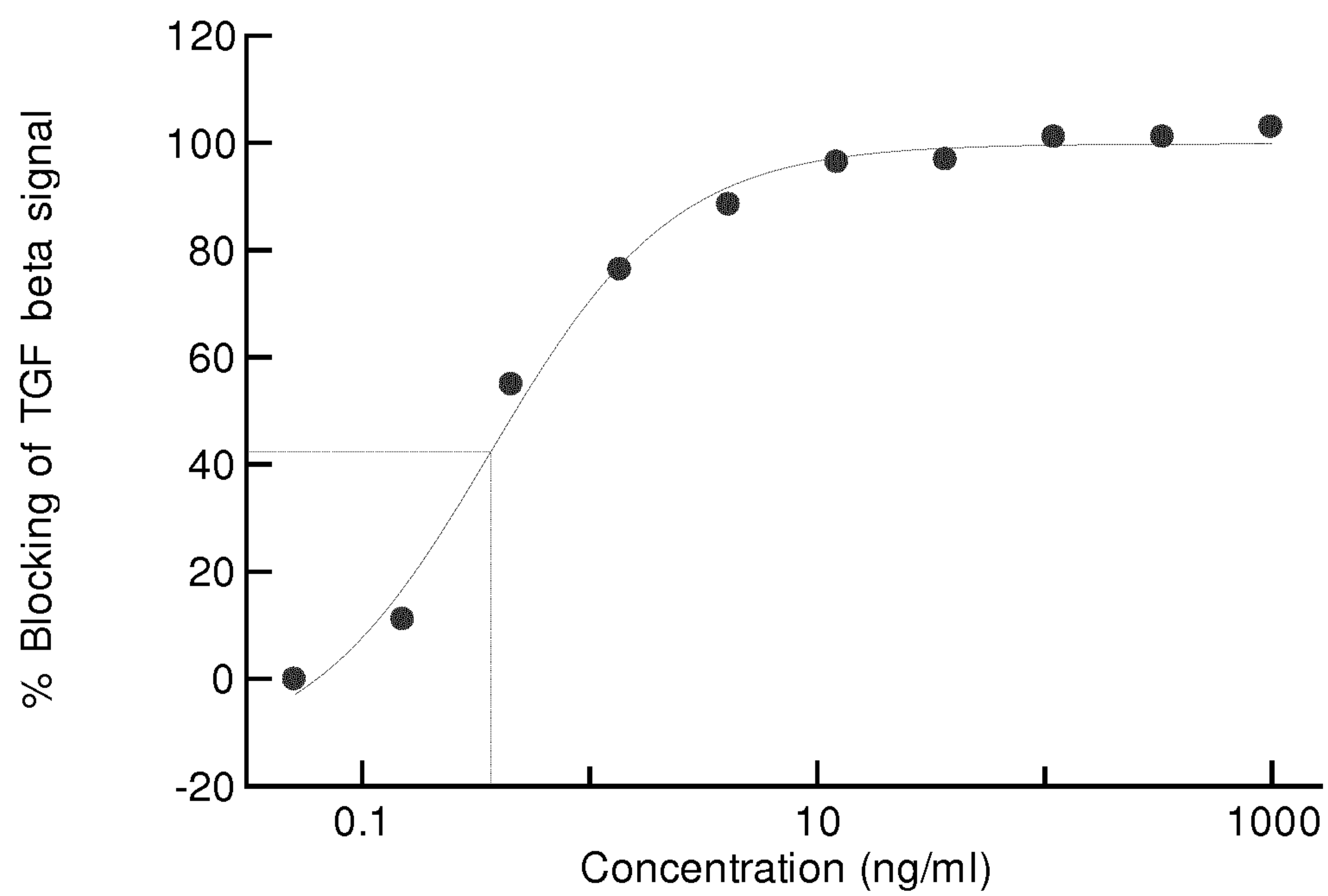
FIGURE 3E**HUMAN Latency-associated Peptide and Transforming growth factor beta-3 SEQ ID NO:117**

LSTCTTLDFGHIKKRVEAI RQILSKLRLTSPPEPTVMTHVPYQVLALYNSTRELLEEMHGEREEGCTQENTESEY
 YAKEIHKFDMIQGLAEHNELAVCPKGITSKVFRFNVSSVEKNRTNLFRAEFRVLRVPNPSSKRNEQRIELFQILRPD
 EHI AKQRYIGGKNLPTRGTAEWLSFDVTDTVREWLLRRESNLGLEISIHCPCHTFQPNGDILENIHEVMEIKFKGVD
 NEDDHGRGDLGRLKKQKDHHNP HLI LMMIPPHRLDNPGQGGQRKKRALDTNYCFRNLEENCCVRPLYIDFRQDLGWK
 WVHEPKGYANFCSGPCPYLRSADTTHSTVLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRTPKVEQLSNMVVKS
 CKCS

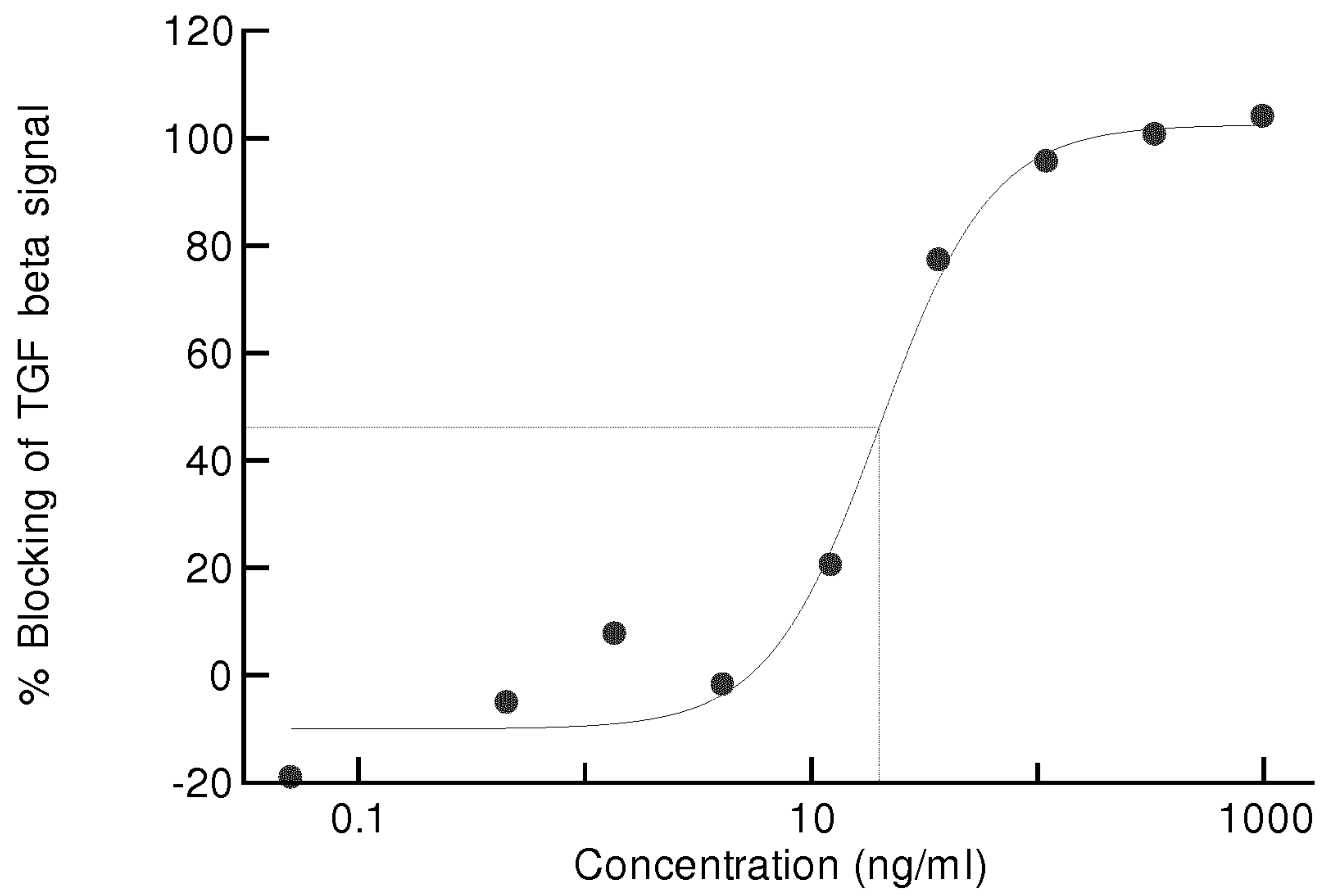
FIGURE 3F**Mature HUMAN Transforming growth factor beta-3 SEQ ID NO:118**

ALDTNYCFRNLEENCCVRPLYIDFRQDLGWK WVHEPKGYANFCSGPCPYLRSADTTHST
 VLGLYNTLNPEASASPCCVPQDLEPLTILYYVGRTPKVEQLSNMVVKSCKCS

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Figure 4A**Figure 4B**

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Figure 4C

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Figure 5

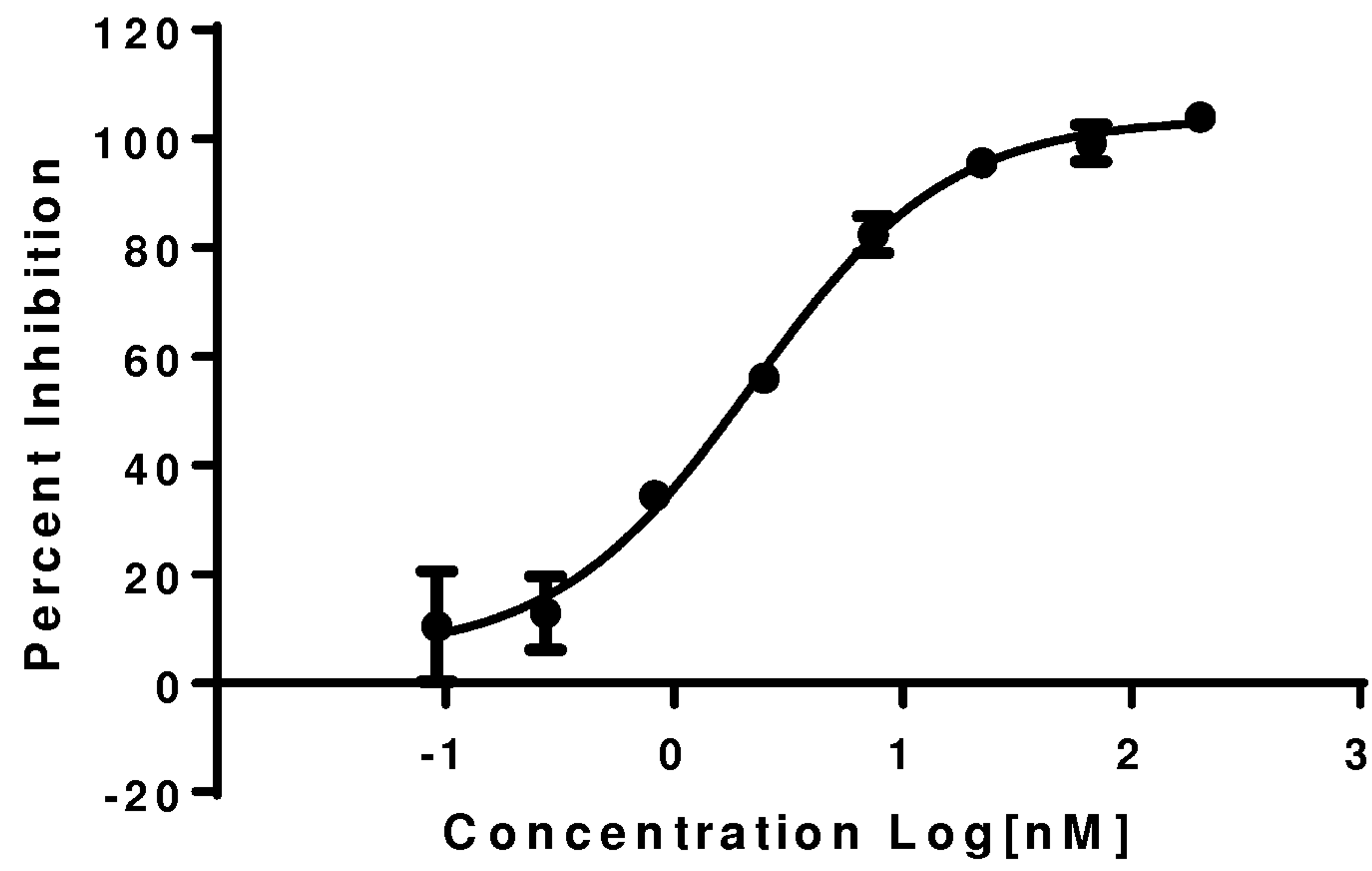


Figure 6

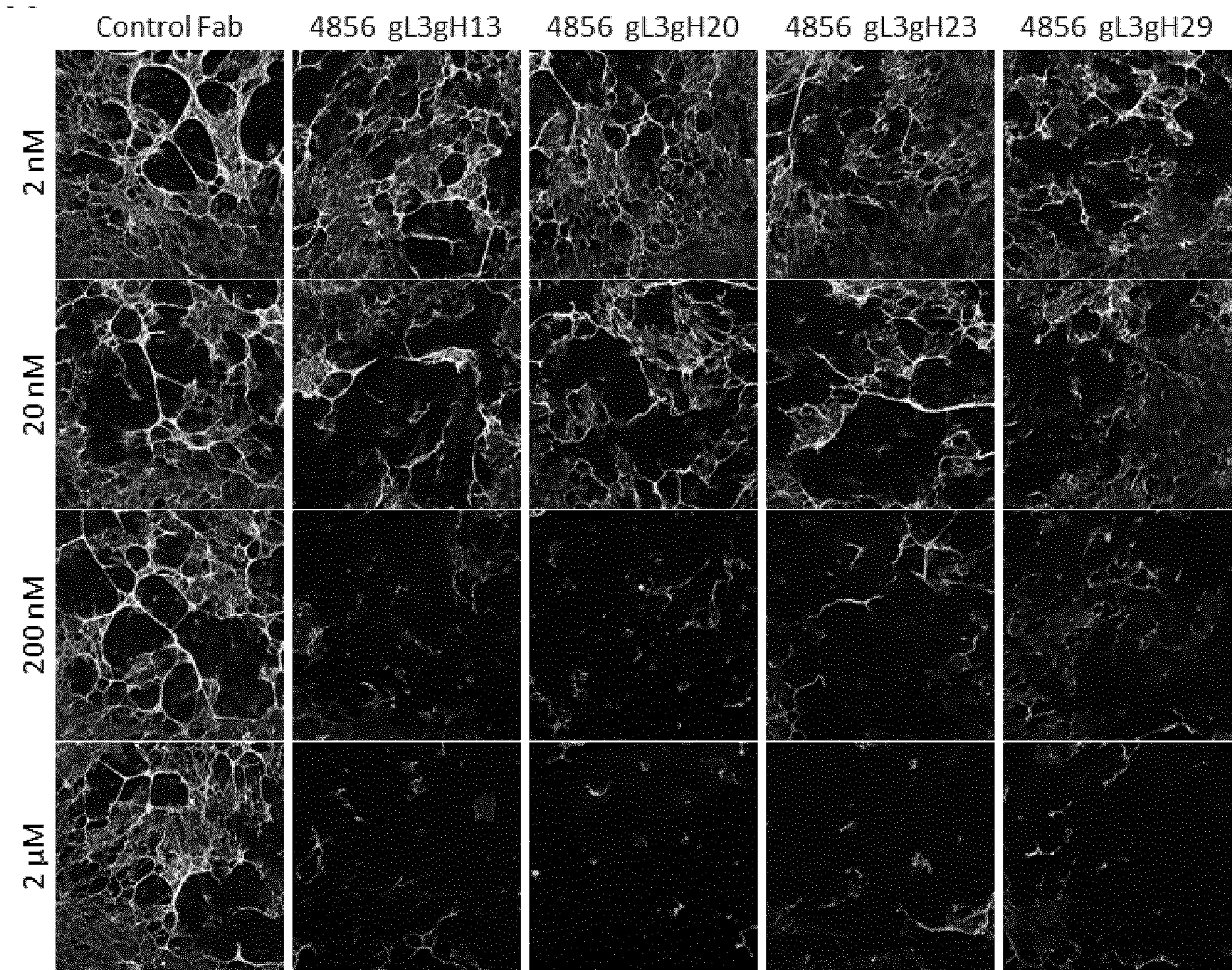


Figure 7A

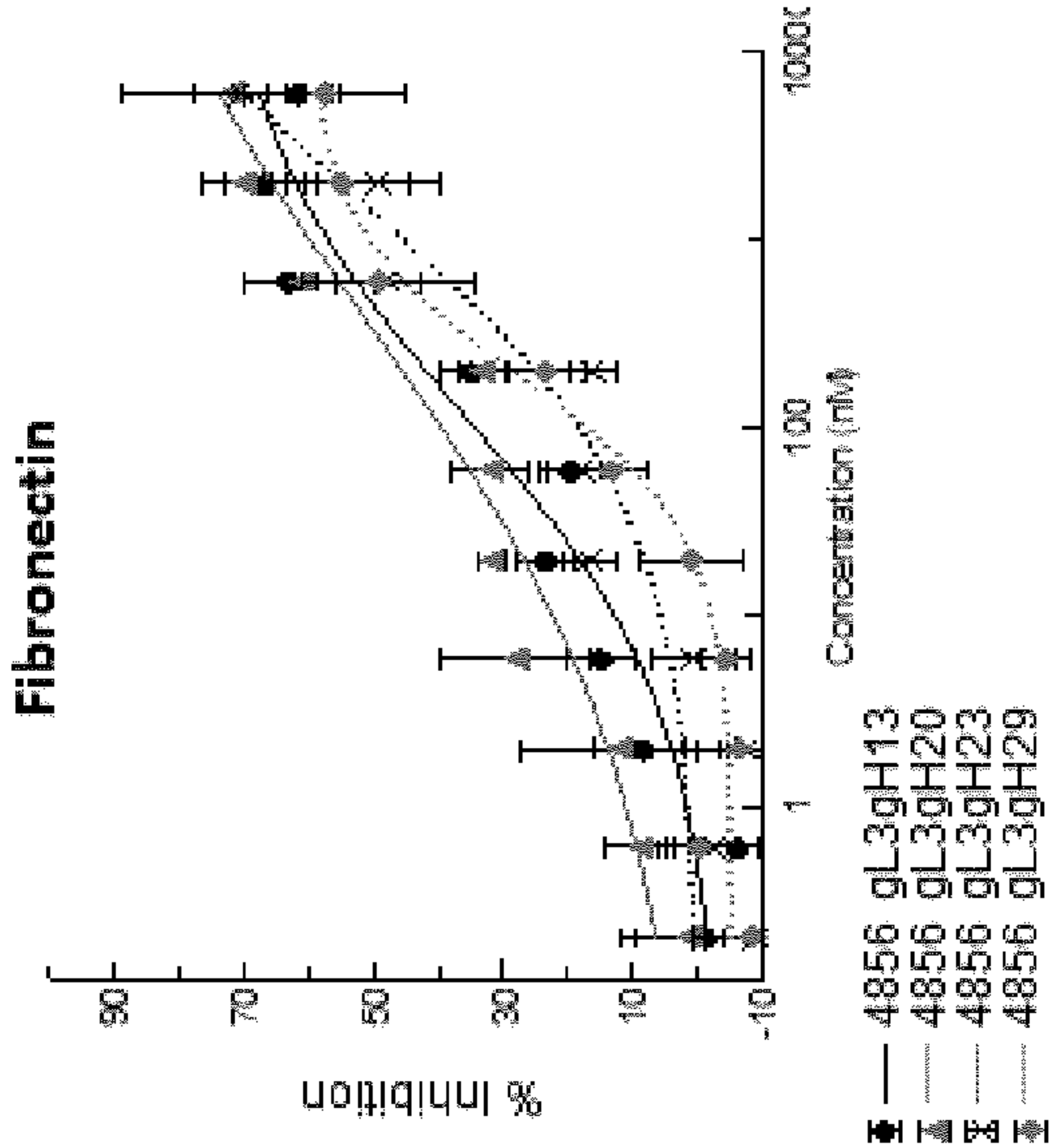


Figure 7B

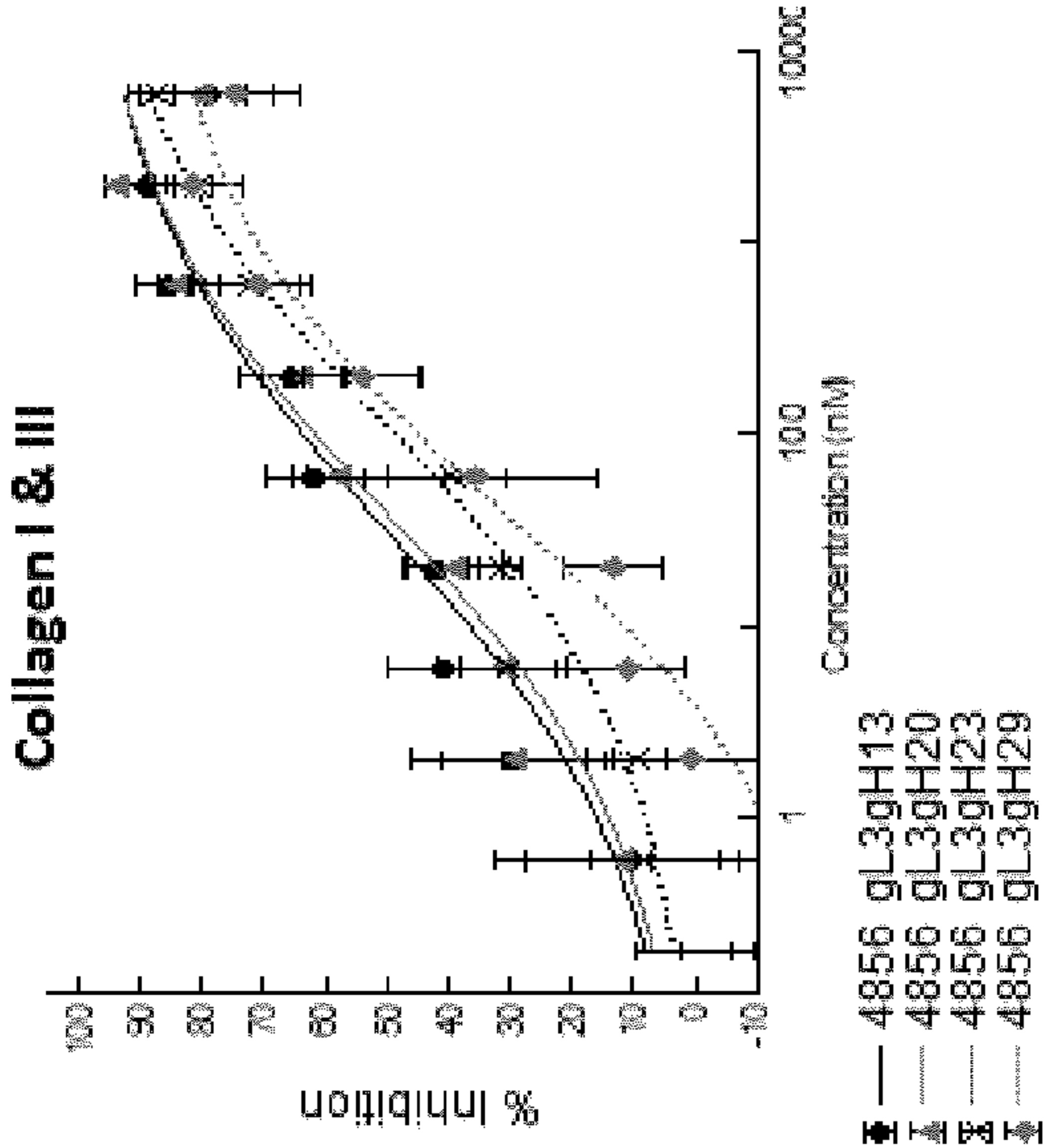
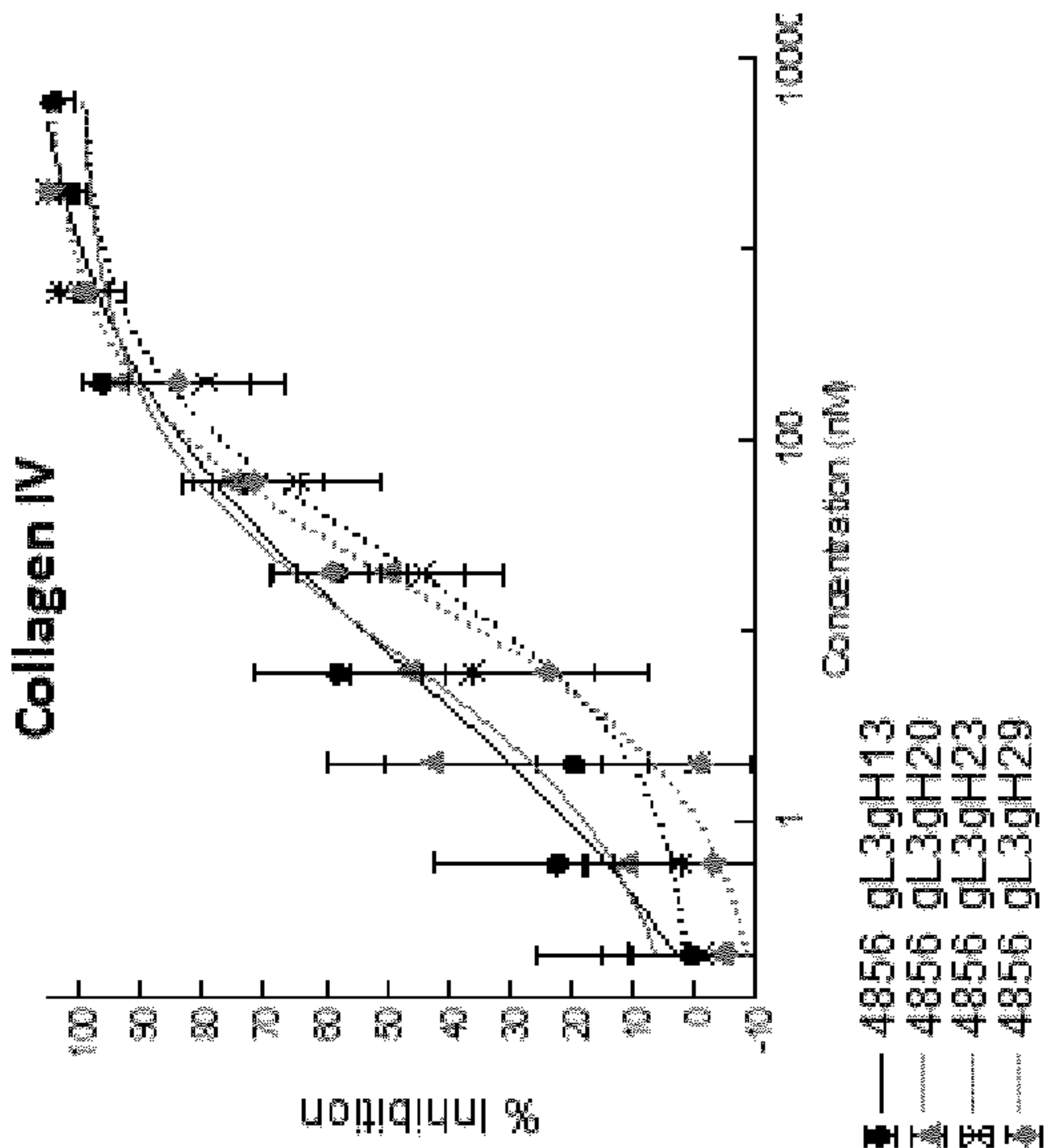


Figure 7C



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Figure 8

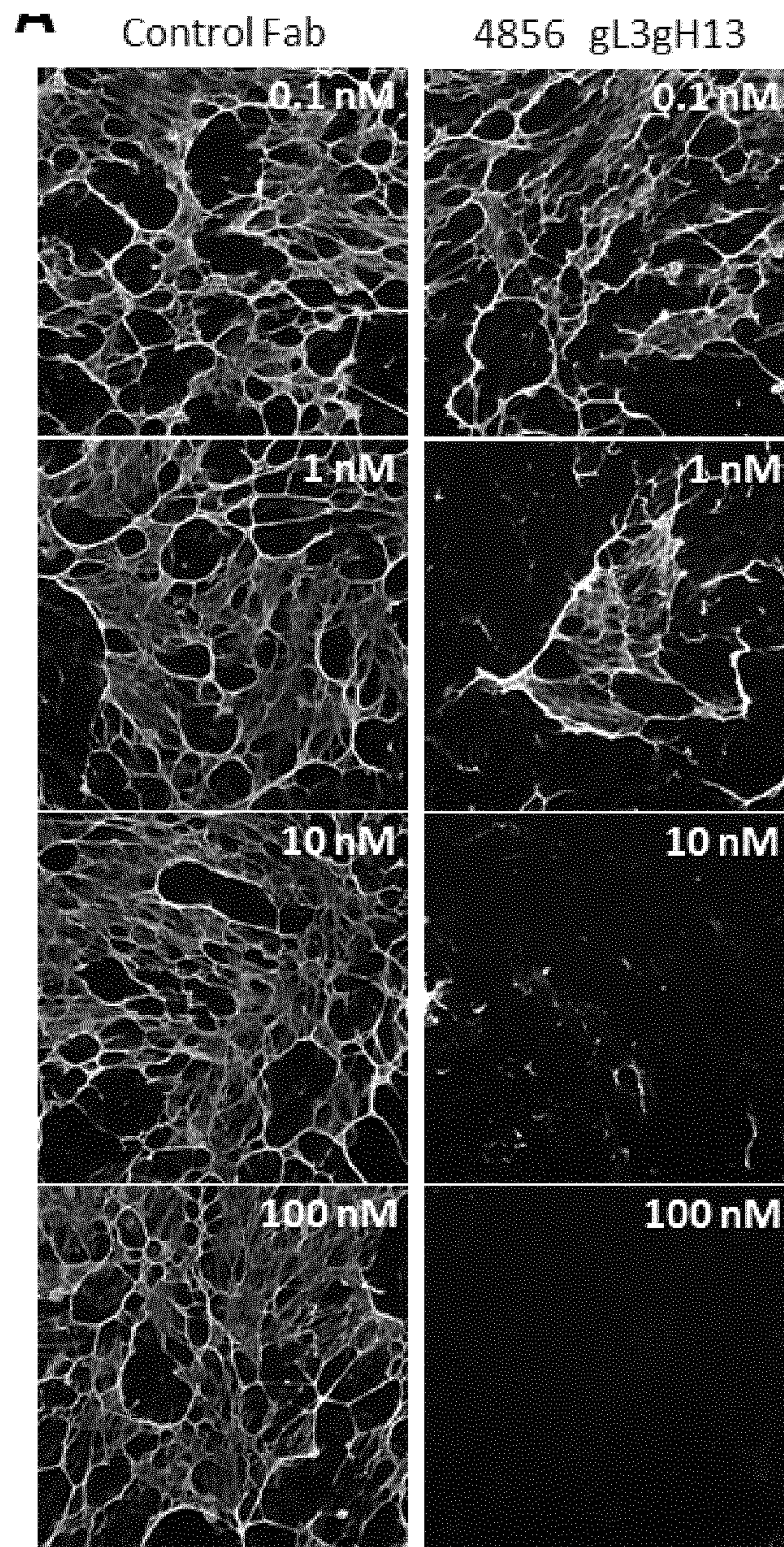


Figure 9A

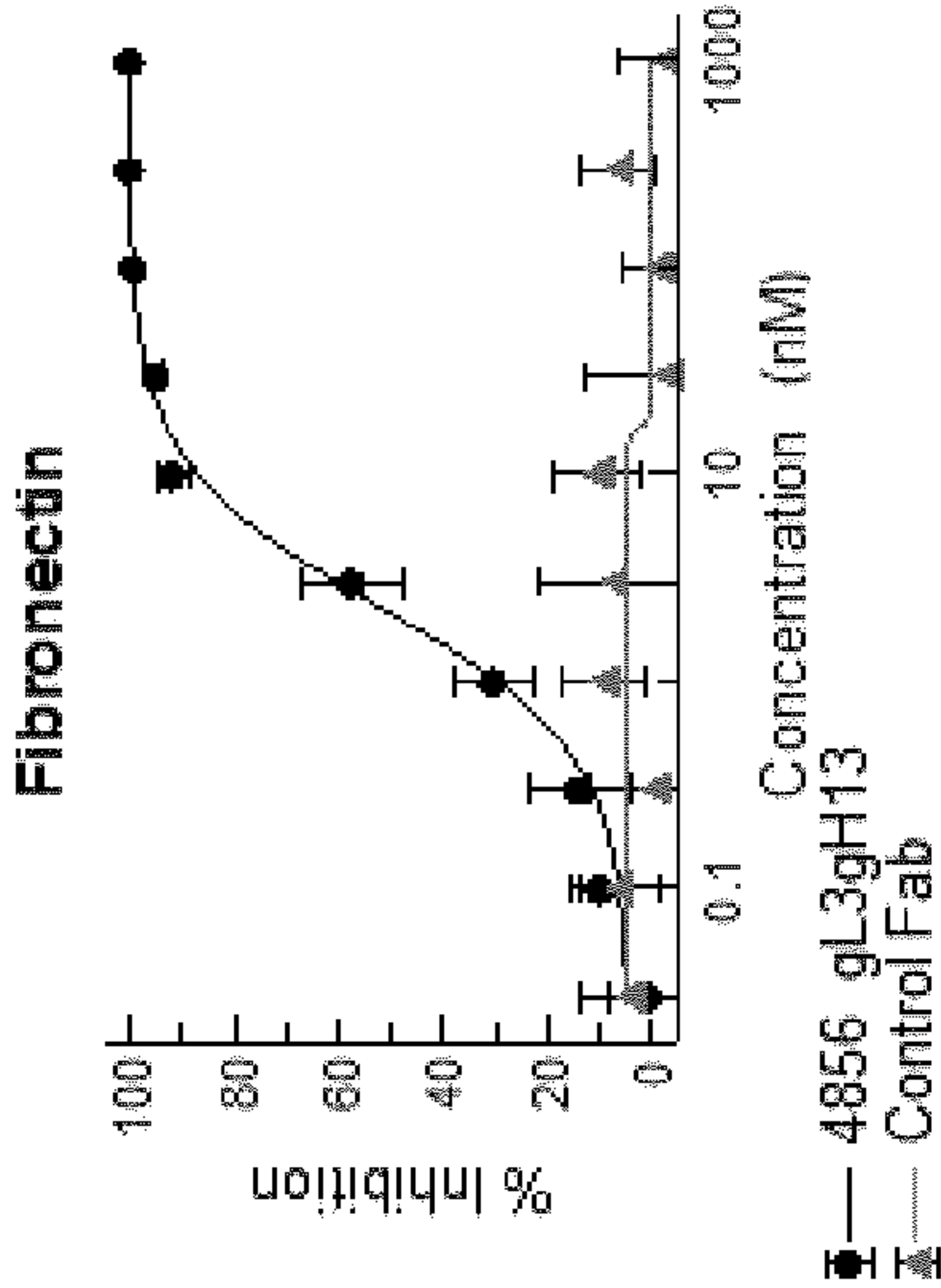


Figure 9B

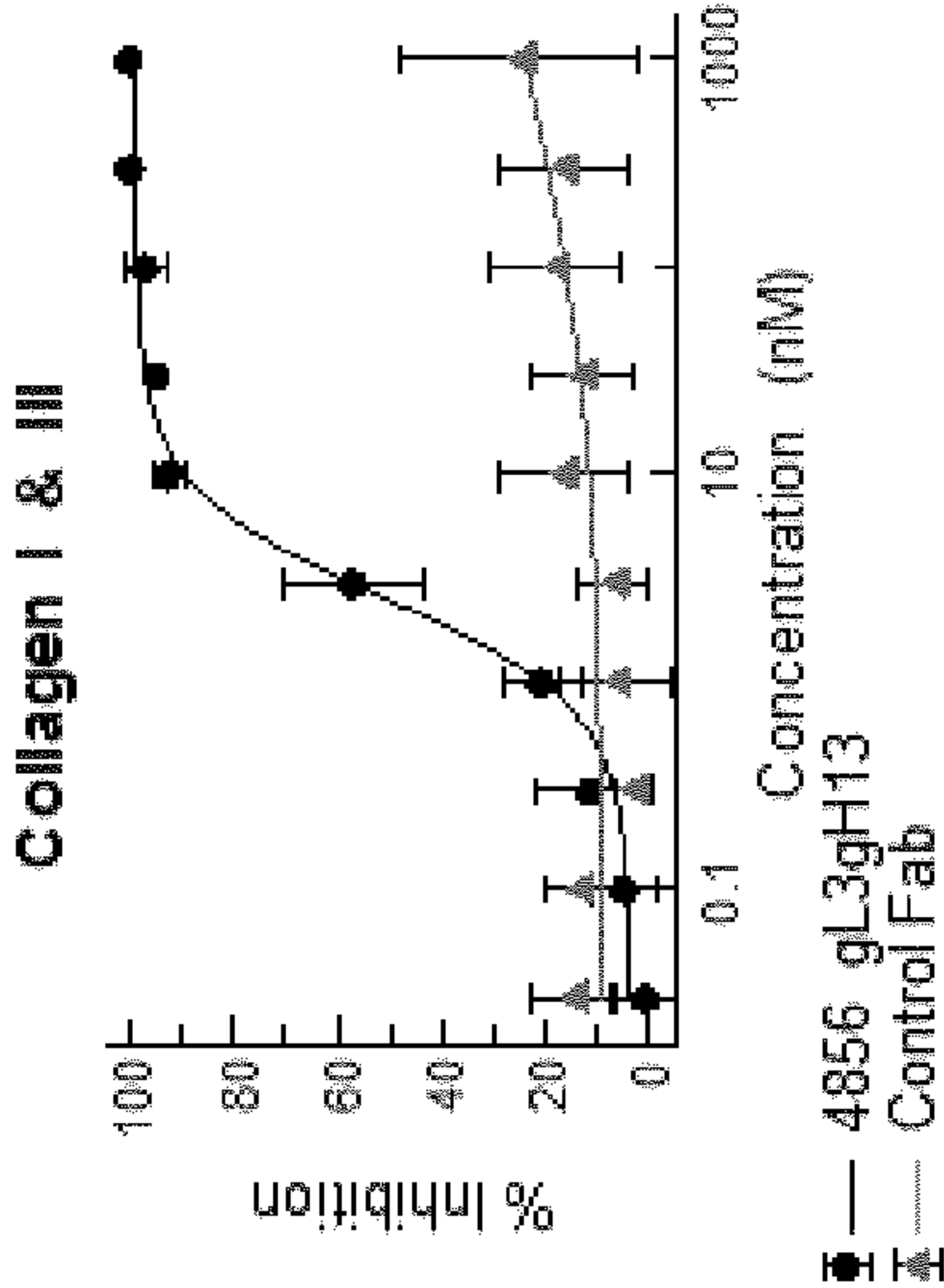
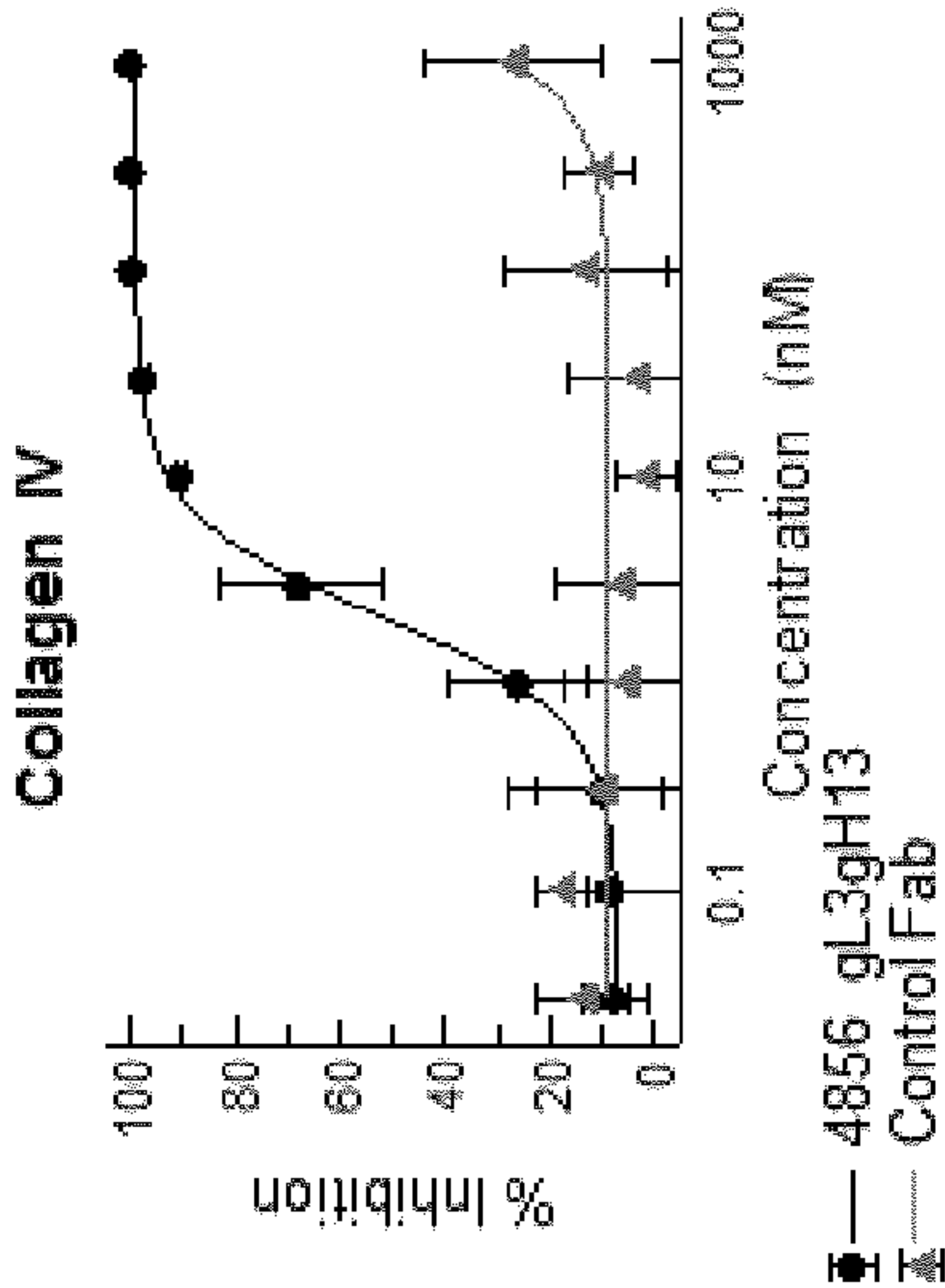


Figure 9C



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Figure 10A

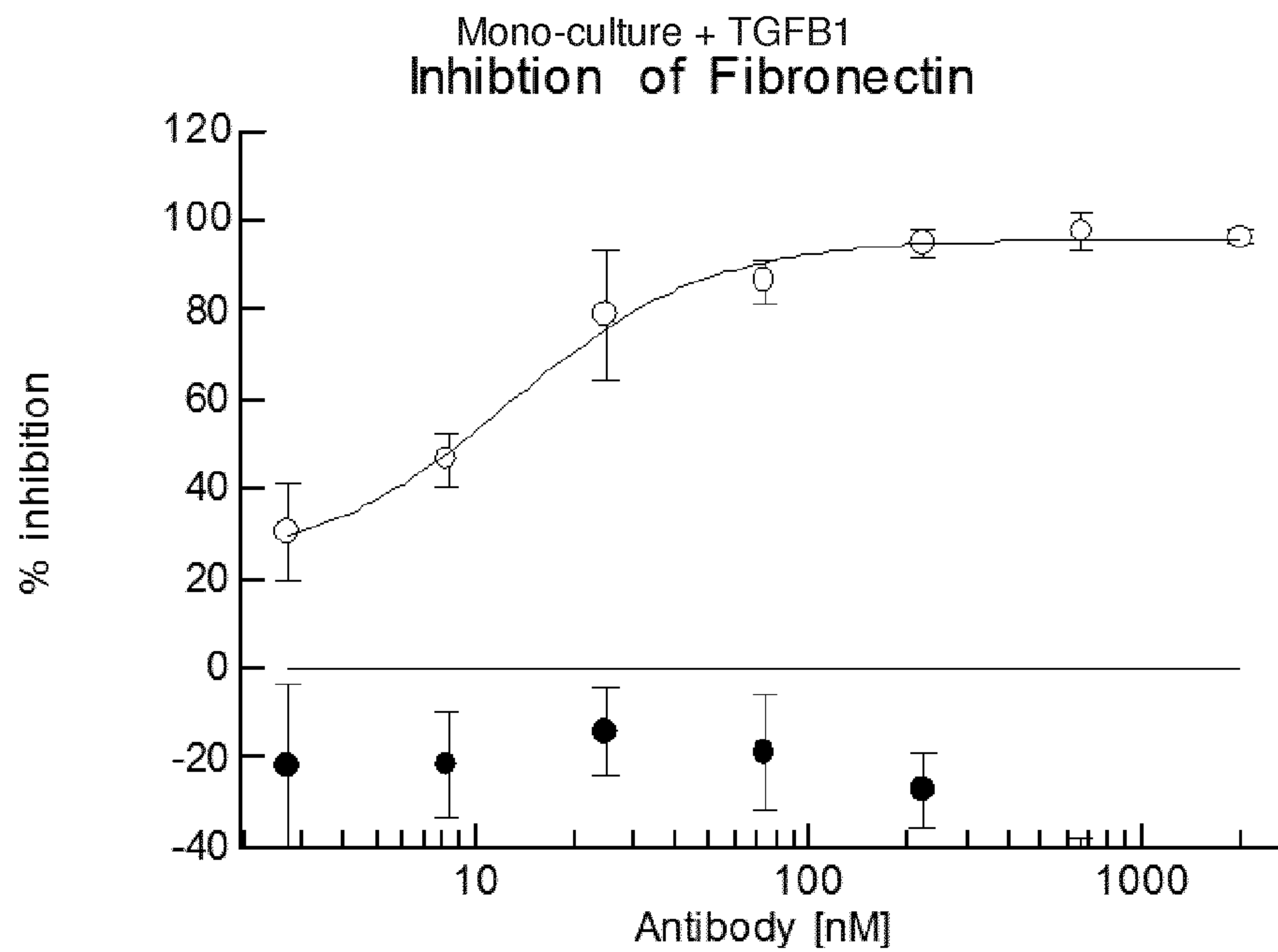
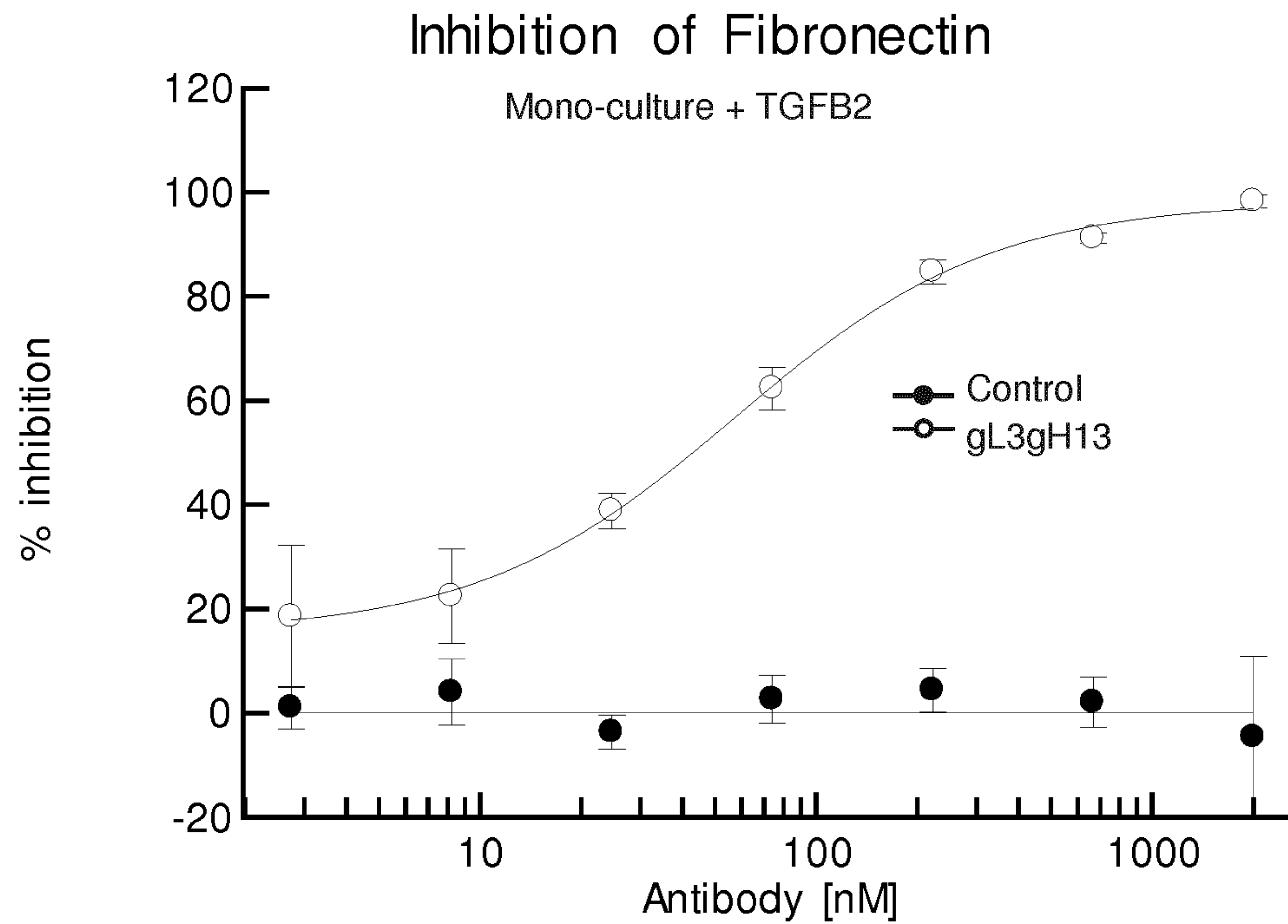


Figure 10B



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Figure 10C

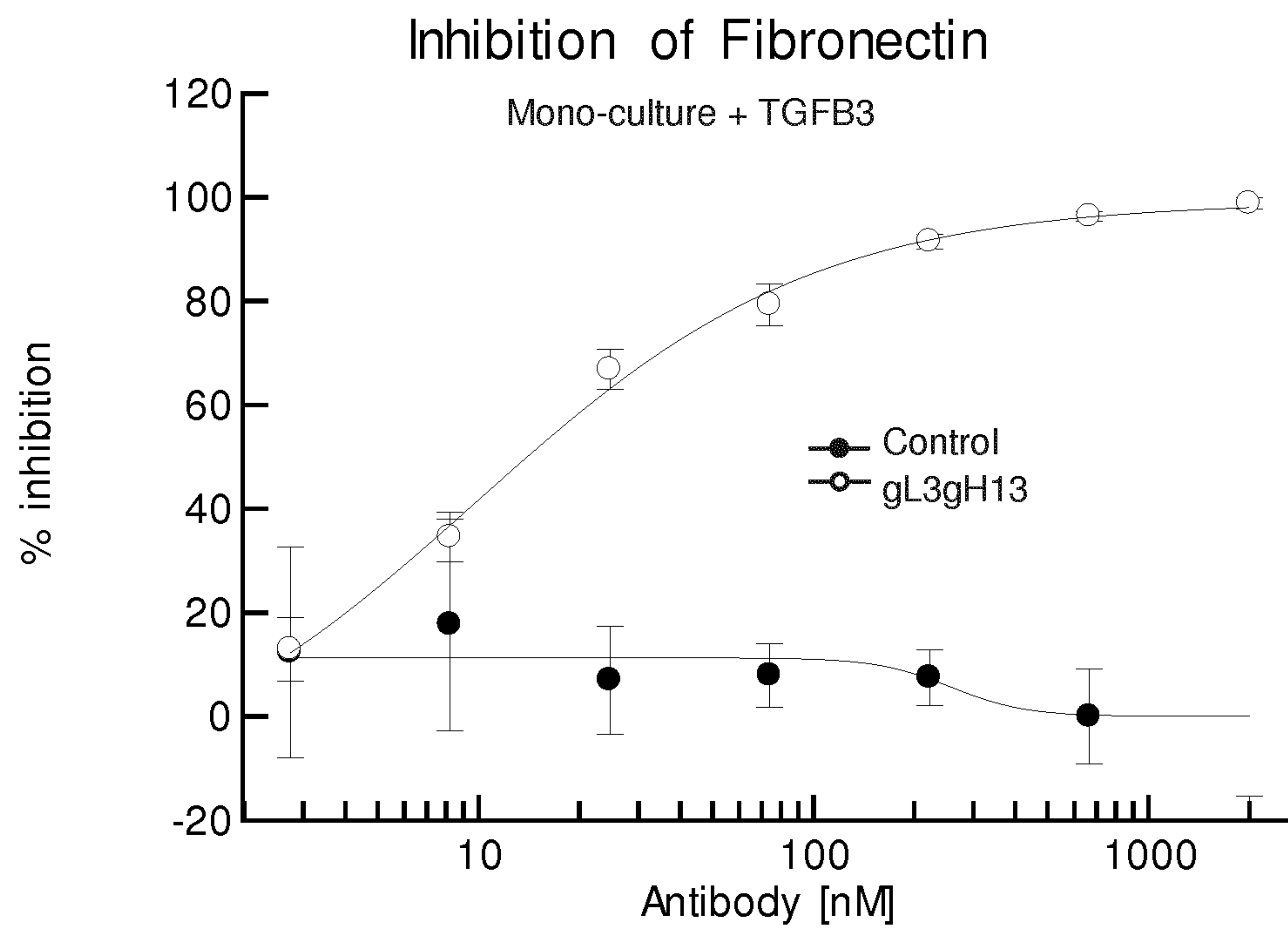
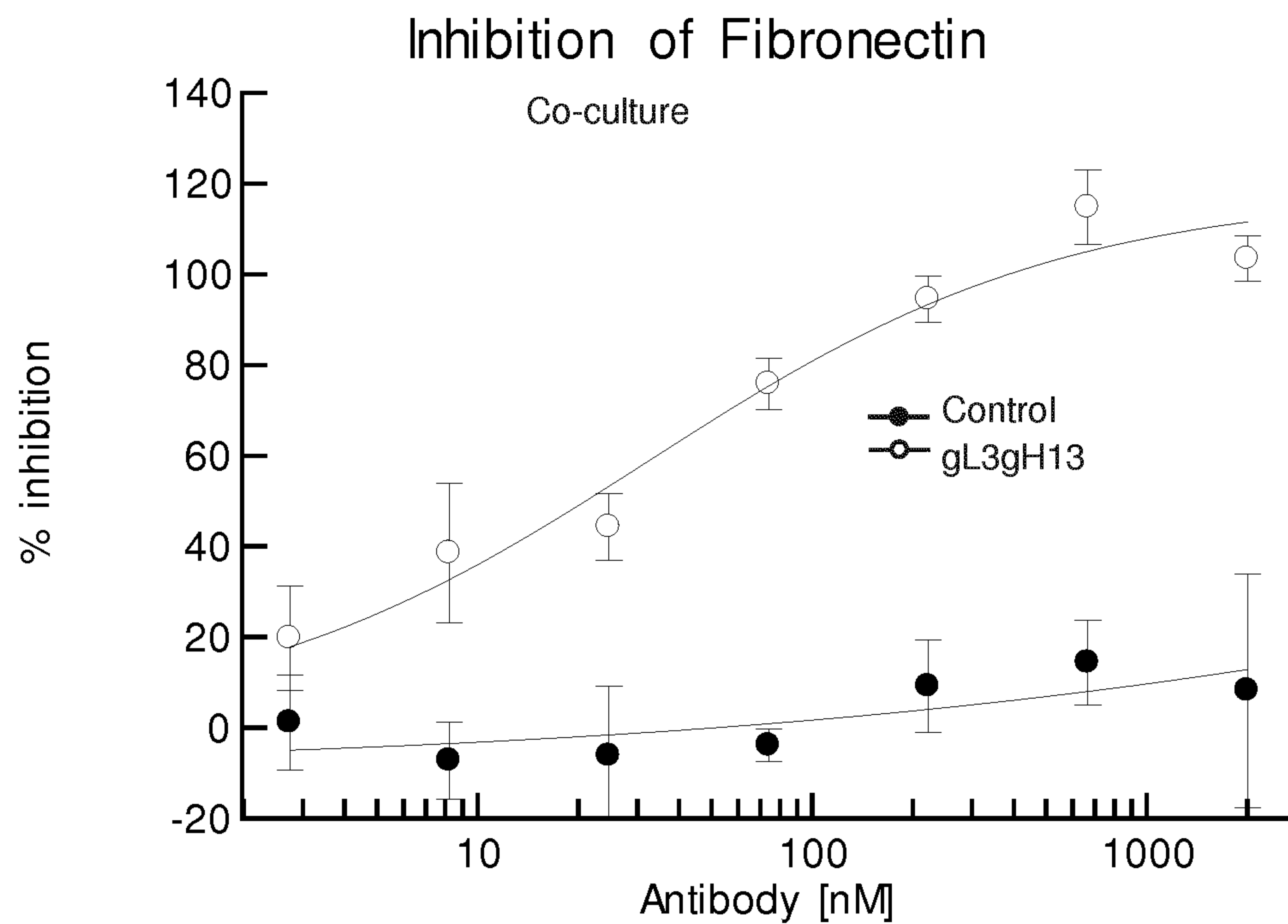


Figure 10D



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Figure 11A

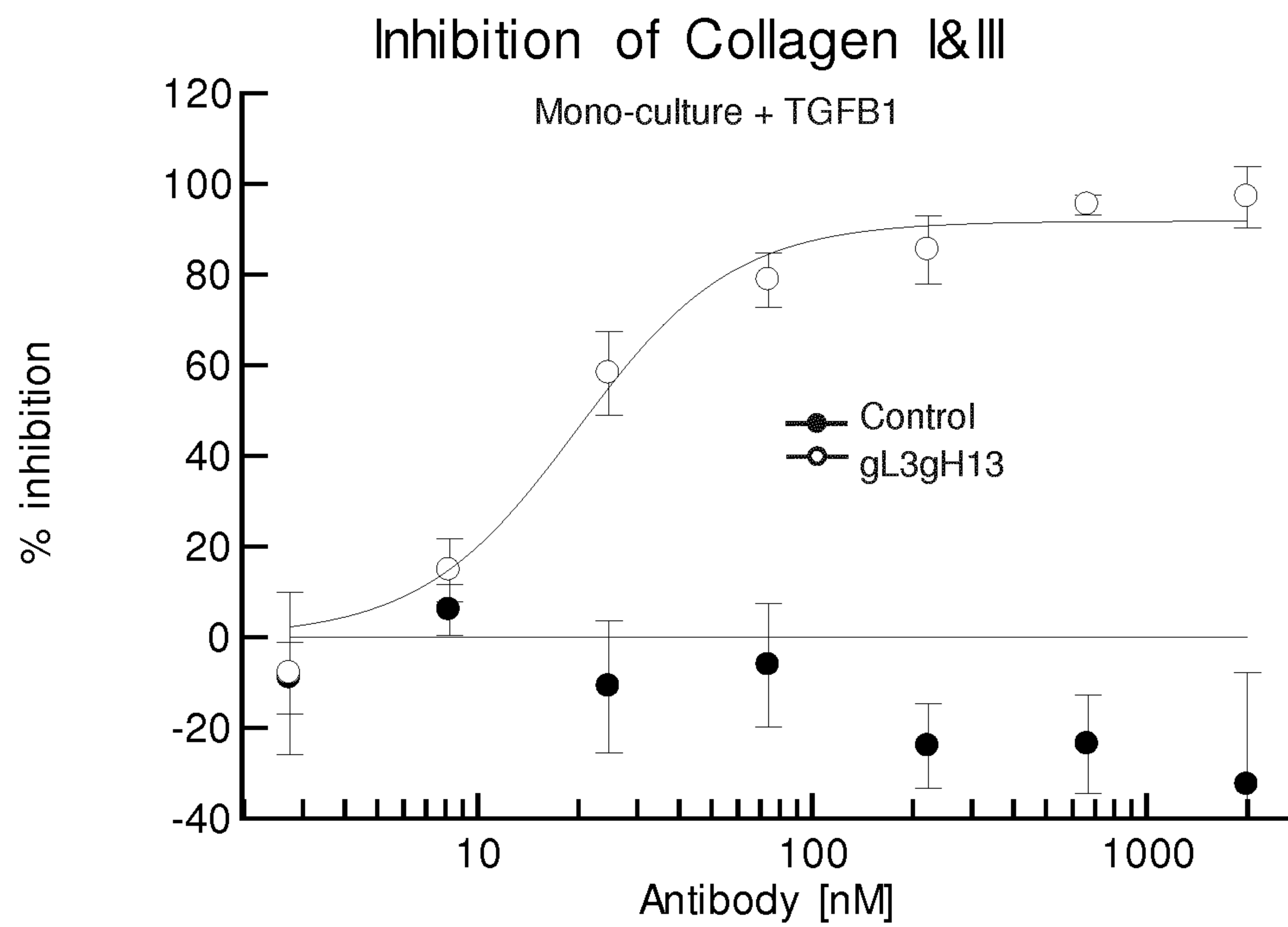
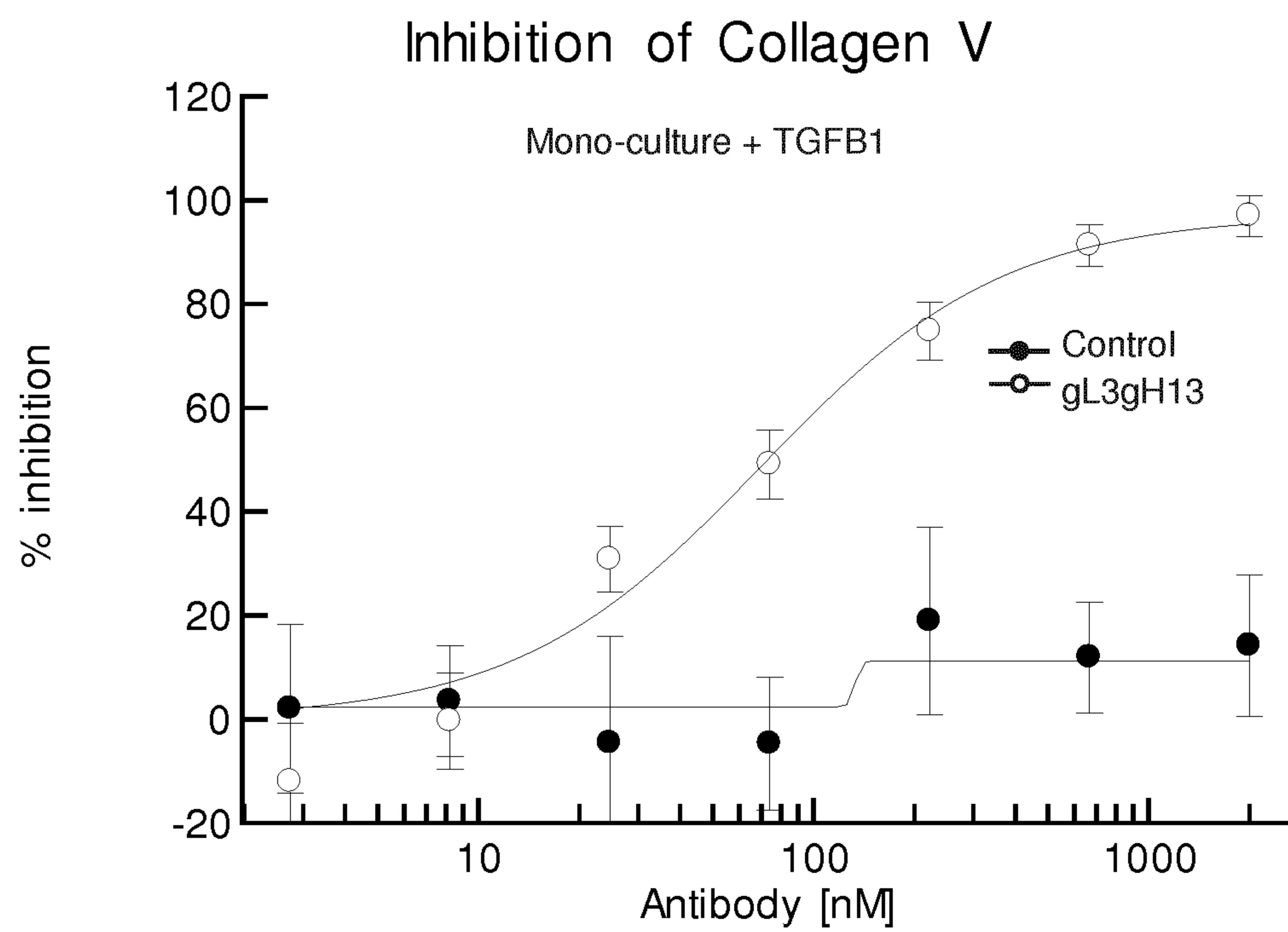


Figure 11B



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Figure 12

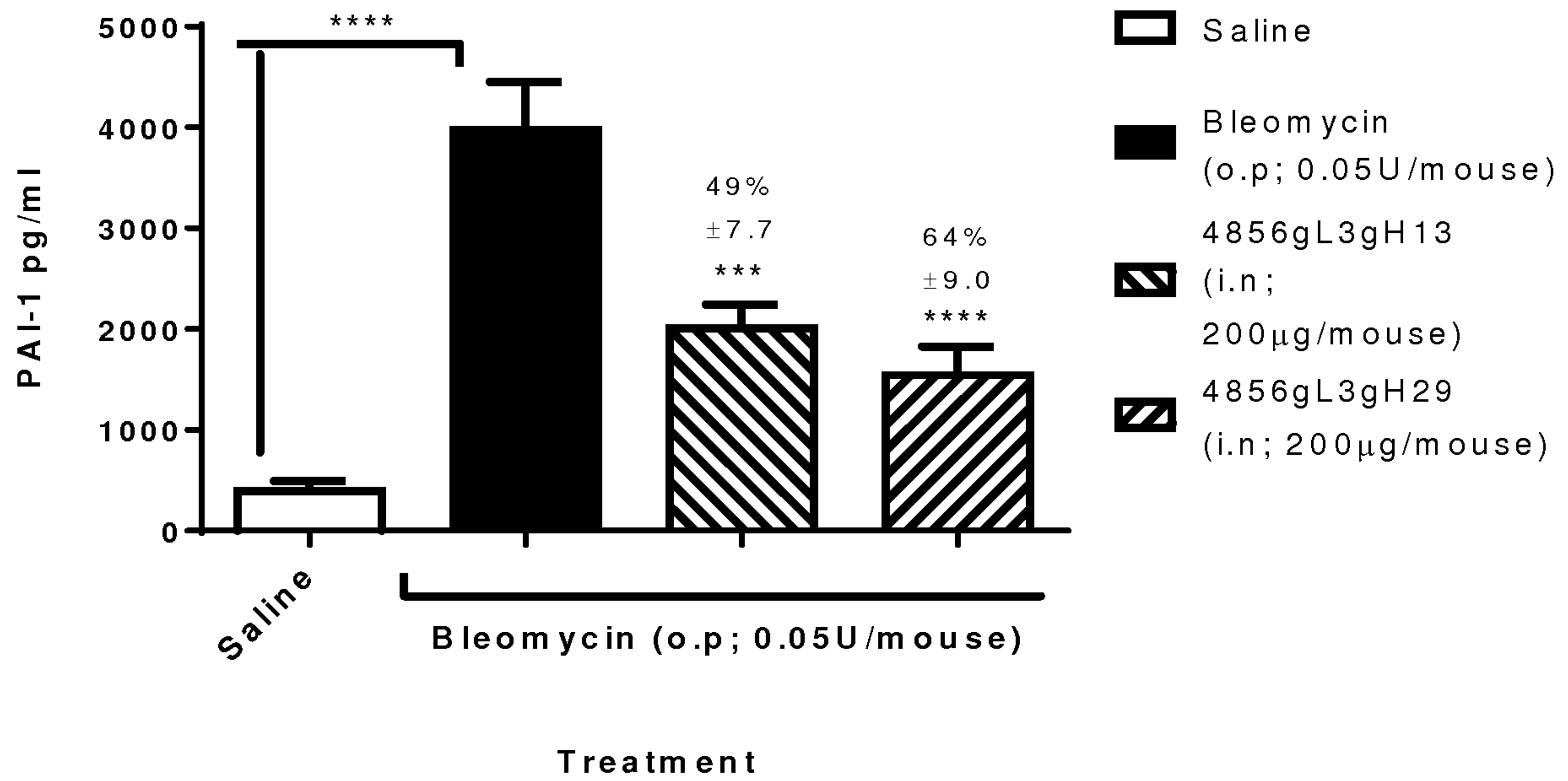
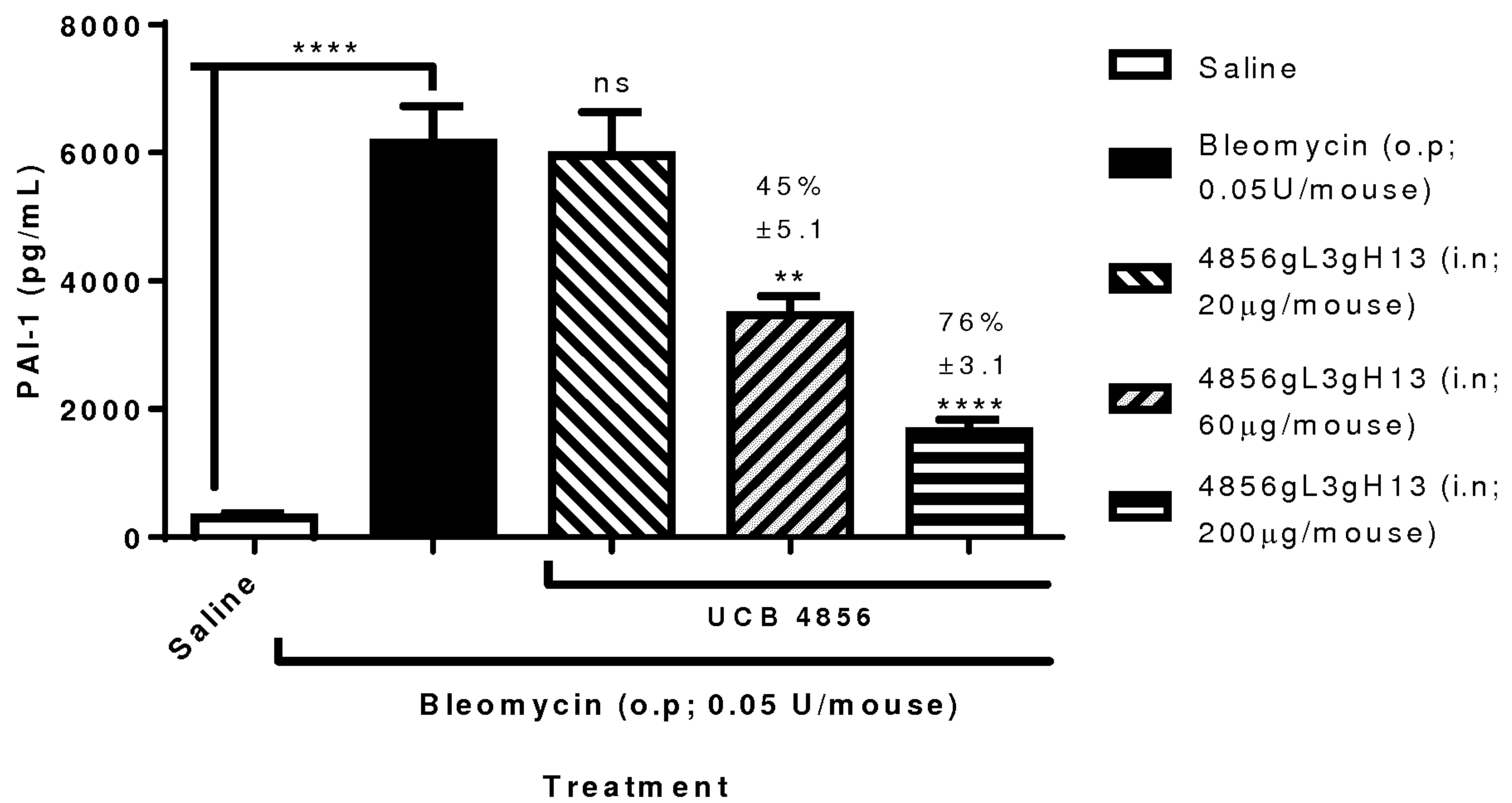


Figure 13



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Figure 14

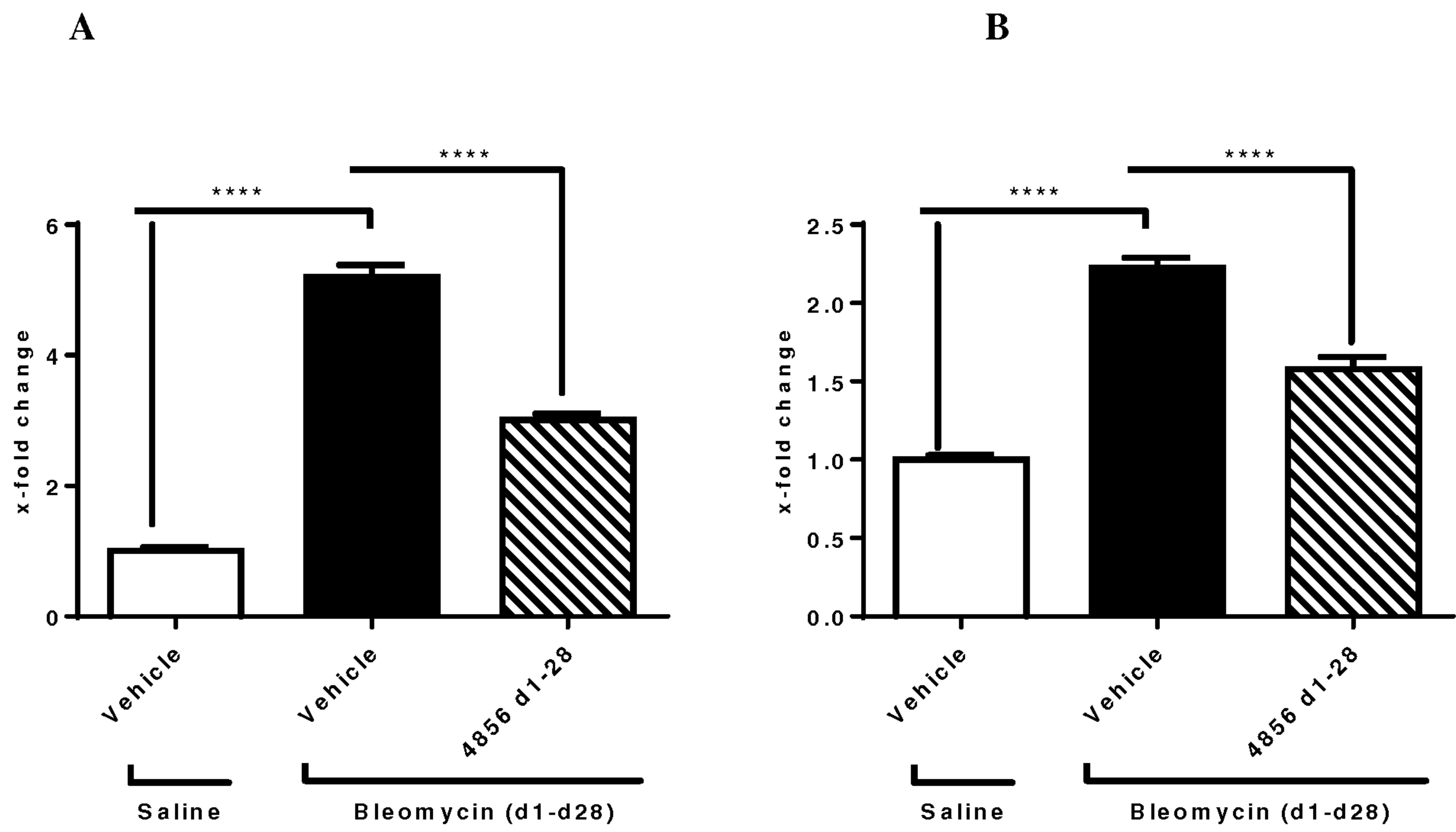
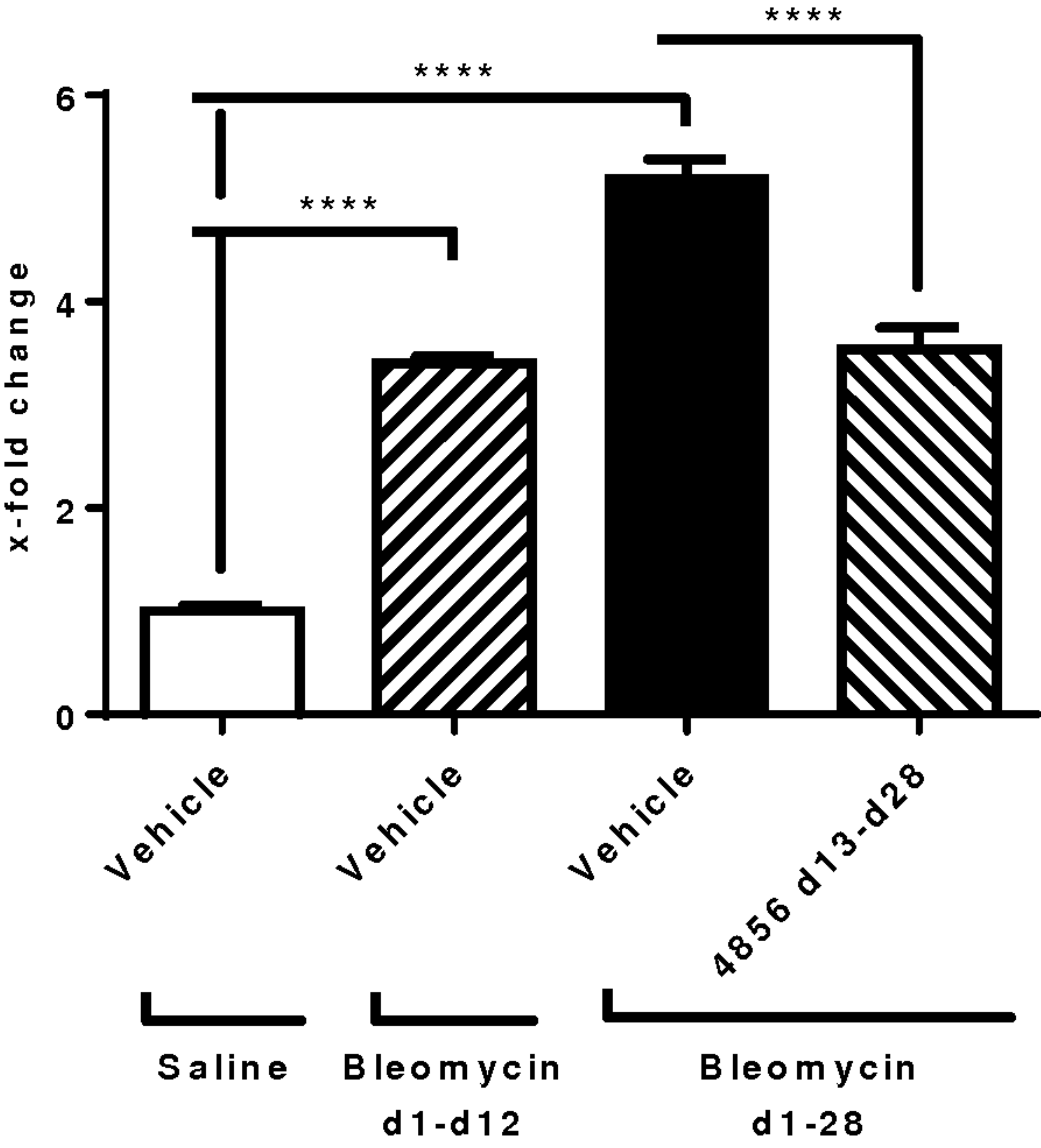
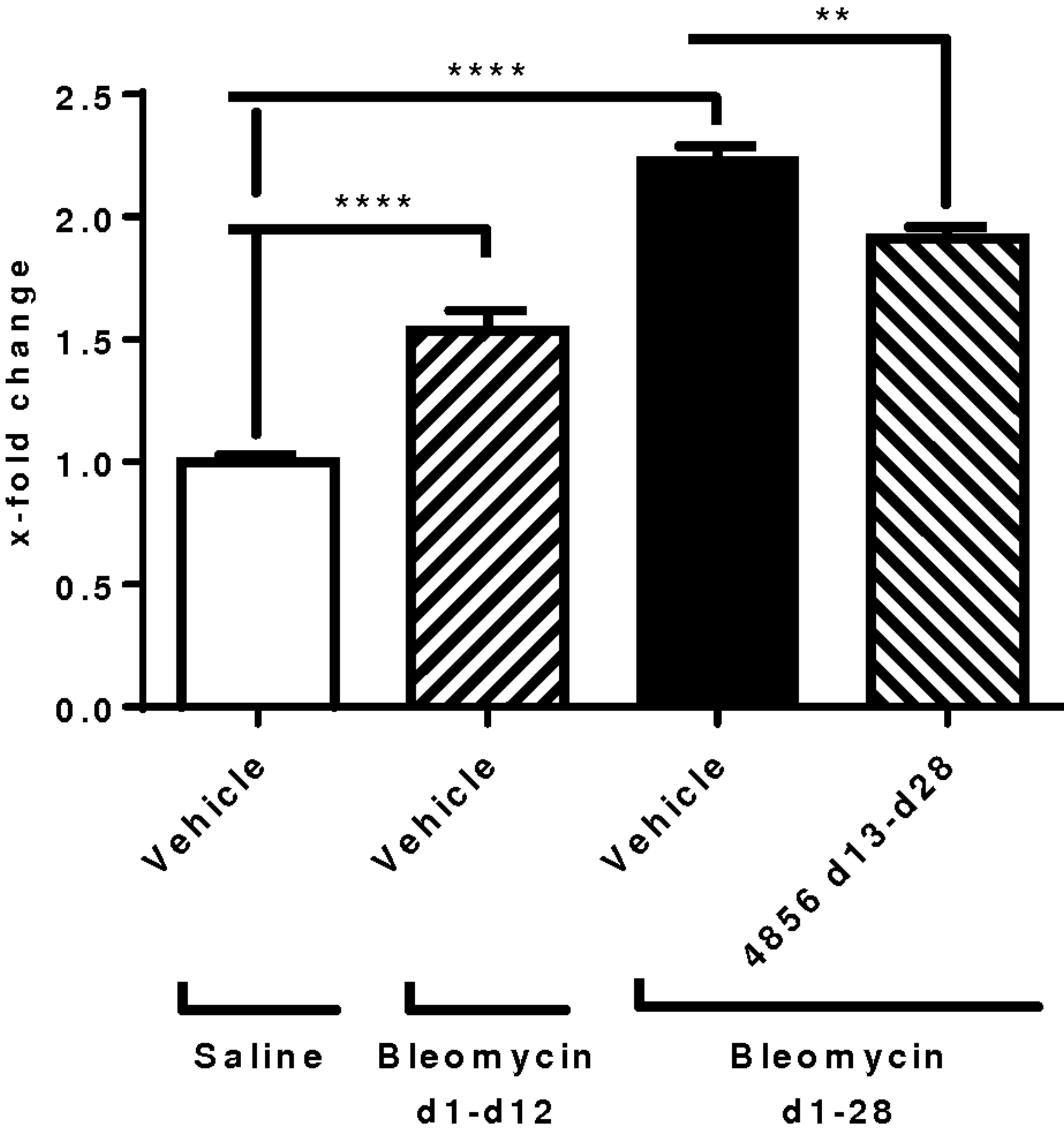


Figure 15

A



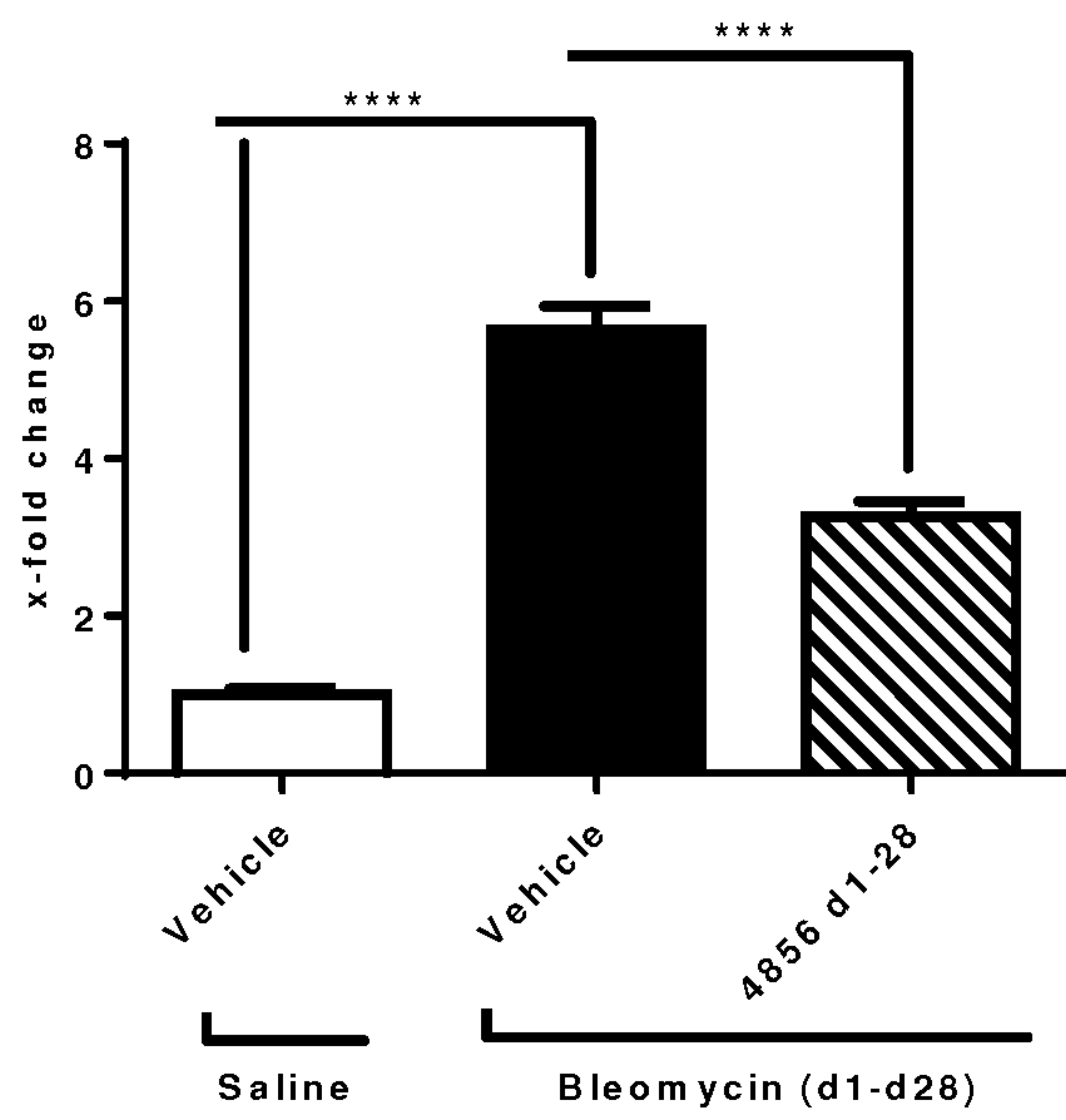
B



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Figure 16

A



B

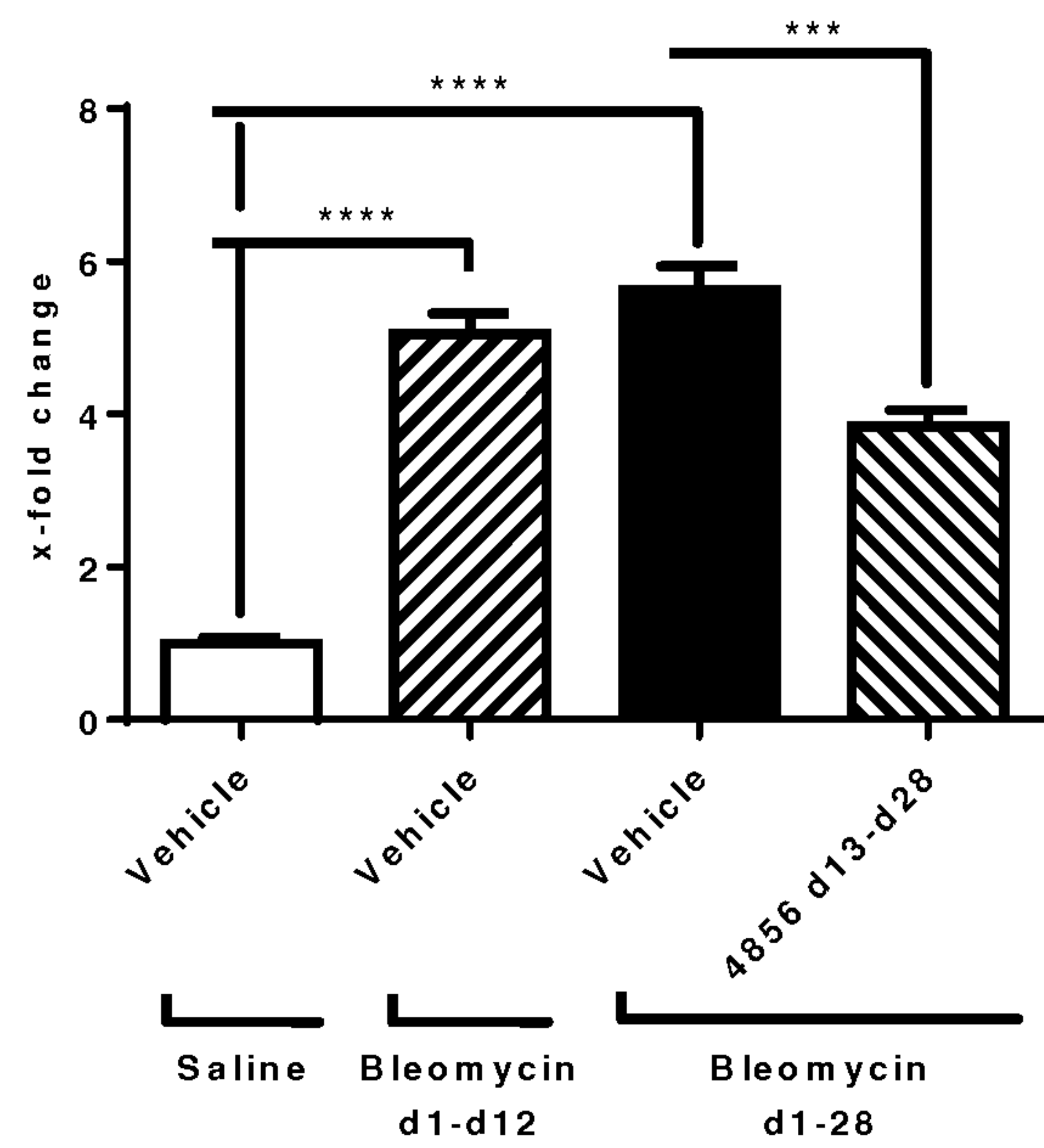


Figure 17

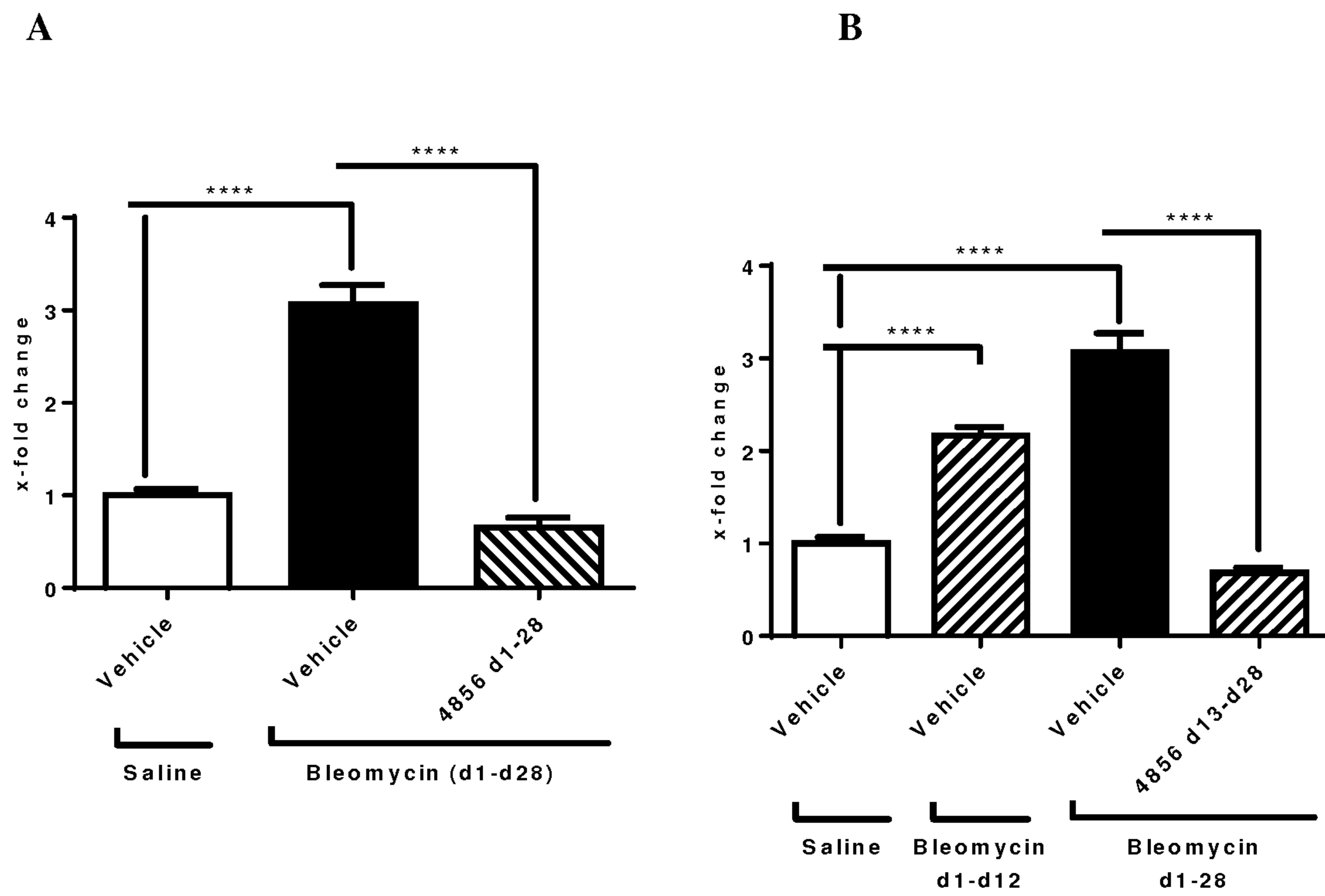


FIGURE 18A

Mature human TGF-beta 1 (SEQ ID NO:114) showing residues involved in interaction with 4856 Fab gL3gH13 (underlined) and residues critical for interaction with TbetaRI and TbetaRII (**bold**). Contacting residues within 4Å.

1
ALDTNYCFSSTEKNCCVRQLYIDF**R**KDLGWKWIHEPKGY
40
HANFCLGPCPYIWSLDTQYS**K**VLALYNQHNP GASAAPCCV
80
PQALEPLPIV**YYVGRKP****K**VEQLSNMIVRSCKCS

4856: R25, K26, D27, L28, G29, W30, K31, W32, I33, H34, K60, L64, H68, Y90, V92, G93, R94, K97, E99, L101

FIGURE 18B

Mature human TGF-beta 1 (SEQ ID NO:114) showing residues involved in interaction with 4856 Fab gL3gH13 (underlined) and residues critical for interaction with TbetaRI and TbetaRII (**bold**). Contacting residues within 5Å.

1
ALDTNYCFSSTEKNCCVRQLYIDF**R**KDLGWKWIHEPKGY
40
HANFCLGPCPYIWSLDTQYS**K**VLALYNQHNP GASAAPCCV
80
PQALEPLPIV**YYVGRKP****K**VEQLSNMIVRSCKCS

4856: R25, K26, D27, L28, G29, W30, K31, W32, H34, I33, Q57, K60, L64, H68, I88, Y90, Y91, V92, G93, R94, K95, K97, E99, L101

FIGURE 18C

Mature human TGF-beta 2 (SEQ ID NO:116) showing residues involved in interaction with scFv 4856 (underlined). Contacting residues within 4Å.

ALDAAYCFRN VQDNCCLRPL YIDF**K**RDLGW **K**WIHEPKGYN ANFCAGACP**Y** **L**WSSDTQH**S**R
VLS**L**YNT**I**NP EASASPCCVS QDLEPLTIL**Y** **Y**IGKTPKIE**Q** **L**SNMIVKSCK CS