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(54) Title: METHODS OF TREATMENT UTILIZING BINDING PROTEINS OF THE INTERLEUKIN-21 RECEPTOR

(57) **Abrégé/Abstract:**

The present invention provides binding proteins and antigen-binding fragments thereof, including human antibodies, that specifically bind to the human interleukin-21 receptor (IL-21R), and methods of using them. The binding proteins can act as, e.g., antagonists of IL-21R activity, thereby modulating immune responses in general, and those mediated by IL-21R in particular. The disclosed compositions and methods may be used, e.g., in diagnosing, treating, and/or preventing IL-21R- associated disorders, e.g., inflammatory disorders, autoimmune diseases, allergies, transplant rejection, and other immune system disorders.



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(54) Title: METHODS OF TREATMENT UTILIZING BINDING PROTEINS OF THE INTERLEUKIN-21 RECEPTOR

(57) Abstract: The present invention provides binding proteins and antigen-binding fragments thereof, including human antibodies, that specifically bind to the human interleukin-21 receptor (IL-21R), and methods of using them. The binding proteins can act as, e.g., antagonists of IL-21R activity, thereby modulating immune responses in general, and those mediated by IL-21R in particular. The disclosed compositions and methods may be used, e.g., in diagnosing, treating, and/or preventing IL-21R-associated disorders, e.g., inflammatory disorders, autoimmune diseases, allergies, transplant rejection, and other immune system disorders.



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METHODS OF TREATMENT UTILIZING BINDING PROTEINS OF THE INTERLEUKIN-21 RECEPTOR

Related Applications

[0001] This application claims the benefit of priority from U.S. Provisional Patent Application No. 61/055,543, filed May 23, 2008, and U.S. Provisional Patent Application No. 61/099,476, filed September 23, 2008, the contents of which are hereby incorporated by reference herein in their entireties.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to binding proteins and antigen-binding fragments thereof that bind interleukin-21 receptor (IL-21R), in particular, human IL-21R, and their use in regulating IL-21R-associated activities, e.g., IL-21 effects on the levels of expression of IL-21 responsive genes. The binding proteins disclosed herein are useful in treating and/or diagnosing IL-21R-associated disorders, e.g., inflammatory disorders, autoimmune diseases, allergies, transplant rejection, hyperproliferative disorders of the blood, and other immune system disorders. The invention further provides methods for determining pharmacodynamic and pharmacokinetic properties of the antibodies of the invention.

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Related Background Art

[0003] Antigens initiate immune responses and activate the two largest populations of lymphocytes: T cells and B cells. After encountering antigen, T cells proliferate and differentiate into effector cells, while B cells proliferate and differentiate into antibody-secreting plasma cells. These effector cells secrete and/or respond to cytokines, which are small proteins (less than about 30 kDa) secreted by lymphocytes and other cell types.

[0004] Human IL-21 is a cytokine that shows sequence homology to IL-2, IL-4 and IL-15 (Parrish-Novak et al. (2000) *Nature* 408:57-63). Despite low sequence homology among interleukin cytokines, cytokines share a common fold into a “four-helix-bundle” structure that is representative of the family. Most cytokines bind either class I or class II cytokine receptors. Class II cytokine receptors include the receptors for IL-10 and the interferons, whereas class I cytokine receptors include the receptors for IL-2 through IL-7, IL-9, IL-11, IL-12, IL-13, and IL-15, as well as hematopoietic growth factors, leptin, and growth hormone (Cosman (1993) *Cytokine* 5:95-106).

[0005] Human IL-21R is a class I cytokine receptor. The nucleotide and amino acid sequences encoding human IL-21 and its receptor (IL-21R) are described in International Application Publication Nos. WO 00/053761 and WO 01/085792; Parrish-Novak et al. (2000) *supra*; and Ozaki et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:11439-44. IL-21R has the highest sequence homology to the IL-2 receptor β chain and the IL-4 receptor α chain (Ozaki et al. (2000) *supra*). Upon ligand binding, IL-21R associates with the common gamma cytokine receptor chain (γ_c) that is shared by receptor complexes for IL-2, IL-3, IL-4, IL-7, IL-9, IL-13 and IL-15 (Ozaki et al. (2000) *supra*; Asao et al. (2001) *J. Immunol.* 167:1-5).

[0006] IL-21R is expressed in lymphoid tissues, particularly on T cells, B cells, natural killer (NK) cells, dendritic cells (DC) and macrophages (Parrish-Novak et al. (2000) *supra*), which allows these cells to respond to IL-21 (Leonard and Spolski (2005) *Nat. Rev. Immunol.* 5:688-98). The widespread lymphoid distribution of

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IL-21R indicates that IL-21 plays an important role in immune regulation. *In vitro* studies have shown that IL-21 significantly modulates the function of B cells, CD4⁺ and CD8⁺ T cells, and NK cells (Parrish-Novak et al. (2000) *supra*; Kasaian et al. (2002) *Immunity* 16:559-69). Recent evidence suggests that IL-21-mediated signaling can have antitumor activity (Sivakumar et al. (2004) *Immunology* 112:177-82), and that IL-21 can prevent antigen-induced asthma in mice (Shang et al. (2006) *Cell. Immunol.* 241:66-74).

[0007] In autoimmunity, disruption of the IL-21 gene and injection of recombinant IL-21 have been shown to modulate the progression of experimental autoimmune myasthenia gravis (EAMG) and experimental autoimmune encephalomyelitis (EAE), respectively (King et al. (2004) *Cell* 117:265-77; Ozaki et al. (2004) *J. Immunol.* 173:5361-71; Vollmer et al. (2005) *J. Immunol.* 174:2696-2701; Liu et al. (2006) *J. Immunol.* 176:5247-54). In these experimental systems, it has been suggested that the manipulation of IL-21-mediated signaling directly altered the function of CD8⁺ cells, B cells, T helper cells, and NK cells.

[0008] Thus, the present invention provides novel therapeutic agents for treating, e.g., autoimmune diseases that act by blocking the IL-21 signaling pathway, i.e., anti-IL-21R antibodies. In order for a therapeutic agent, such as an anti-IL-21R antibody, to be effective *in vivo*, a minimum serum concentration of the anti-IL-21R antibody necessary to modulate IL-21 responses should be determined; thus a method that allows accurate determination of such minimum serum concentration is required.

SUMMARY OF THE INVENTION

[0009] The present invention describes the isolation and characterization of binding proteins, for example, human antibodies and fragments thereof, that specifically bind to the human and murine IL-21R. The binding proteins described herein are derived from antibody 18A5, which is disclosed in U.S. Patent No. 7,495,085, the entirety of which is hereby incorporated by reference herein. The binding proteins of the

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present invention have a much greater degree of affinity to human and/or murine IL-21R than does the parental 18A5 antibody.

[0010] The invention provides, at least in part, IL-21R binding agents (such as binding proteins and antigen-binding fragments thereof) that bind to IL-21R, in particular, human IL-21R, with high affinity and specificity. The binding proteins, and antigen-binding fragments thereof, of the present invention are also referred to herein as “anti-IL-21R binding proteins” and “fragments thereof,” respectively. In one embodiment, the binding protein or fragment thereof reduces, inhibits, or antagonizes IL-21R activity. Such binding proteins can be used to regulate immune responses or IL-21R-associated disorders by antagonizing IL-21R activity. In other embodiments, the anti-IL-21R binding protein can be used diagnostically, or as a targeting binding protein to deliver a therapeutic or cytotoxic agent to an IL-21R-expressing cell. Thus, the anti-IL-21R binding proteins of the invention are useful in diagnosing and treating IL-21R-associated disorders, e.g., inflammatory disorders, autoimmune diseases, allergies, transplant rejection, hyperproliferative disorders of the blood, and other immune system disorders, as described more fully herein.

[0011] Accordingly, in one aspect, the binding proteins of the invention feature an isolated binding protein (e.g., an isolated antibody) or antigen-binding fragment thereof that binds to IL-21R, in particular, human IL-21R. In certain embodiments, the anti-IL-21R binding protein (e.g., antibody) can have one or more of the following characteristics: (1) it is a monoclonal or single specificity binding protein; (2) it is a human binding protein; (3) it is an *in vitro*-generated binding protein; (4) it is an *in vivo*-generated (for example, a transgenic mouse system) binding protein; (5) it inhibits the binding of IL-21 to IL-21R; (6) it is an IgG1; (7) it binds to human IL-21R with an association constant of at least about $10^5 \text{ M}^{-1}\text{s}^{-1}$; (8) it binds to murine IL-21R with an association constant of at least about $5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$; (9) it binds to human IL-21R with a dissociation constant of about 10^{-3} s^{-1} or less; (10) it binds to murine IL-21R with a dissociation constant of about 10^{-2} s^{-1} or less; (11) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing BaF3 cells with

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an IC_{50} of about 1.75 nM or less; (12) it inhibits murine IL-21R-mediated proliferation of murine IL-21R-expressing BaF3 cells with an IC_{50} of about 0.5 nM or less; (13) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing TF1 cells with an IC_{50} of about 14.0 nM or less; (14) it inhibits IL-21-mediated proliferation of human primary B cells with an IC_{50} of about 1.9 nM or less; (15) it inhibits IL-21-mediated proliferation of human primary $CD4^+$ T cells with an IC_{50} of about 1.5 nM or less; (16) it inhibits IL-21-mediated proliferation of murine primary $CD4^+$ T cells with an IC_{50} of about 5.0 nM or less; (17) it has a mean total body clearance of about 0.1-7.5 ml/hr/kg following, e.g., intravenous (i.v.) administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (18) it has a mean elimination half-life of about 20-700 hr following, e.g., i.v., subcutaneous (s.c.), or intraperitoneal (i.p.) administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (19) it has a mean steady-state volume of distribution of about 40-1500 ml/kg in animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (20) it has a bioavailability of about 35-100% following, e.g., s.c. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (21) it has a mean dose-normalized AUC of about 200-10,000 $\mu\text{g}\cdot\text{hr}/\text{ml}$ (per 1 mg/kg dosage) following, e.g., i.v., s.c., or i.p. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (22) it has a mean dose-normalized C_{max} (maximum serum concentration) of about 0.5-30 $\mu\text{g}/\text{ml}$ following, e.g., i.v., s.c., or i.p. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; and (23) it modulates expression of IL-21 responsive cytokines or IL-21 responsive genes.

[0012] Nonlimiting illustrative embodiments of the binding proteins of the invention (the term “binding proteins” also includes and refers to antigen-binding fragments thereof, as appropriate) are referred to herein as AbA-AbZ, and correlation of these terms with terms used in U.S. Provisional Patent Application No. 61/055,543 is presented in **Table 2A**. Other illustrative embodiments of the binding proteins of the

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present invention, i.e., scFv, are referred to herein as H3-H6, L1-L6, L8-L21, and L23-L25, as detailed in **Table 2B**.

[0013] In one embodiment, the binding proteins of the invention are antibodies. In further embodiments, the antibodies are polyclonal, monoclonal, monospecific, polyspecific, nonspecific, humanized, human, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, grafted, *in vitro*-generated and/or multispecific (e.g., bispecific antibodies formed from at least two intact antibodies).

[0014] One embodiment of the invention is a method of treating or preventing an IL-21R-associated disorder in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

[0015] In embodiments of the invention, the binding protein or antigen-binding fragment can be, e.g., an antibody, an scFv, a V_H, a V_L, and/or a CDR.

[0016] Another embodiment of the invention is a method of treating or preventing an IL-21R-associated disorder in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21,

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23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

[0017] In one embodiment of the invention, the binding protein or antigen-binding fragment comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

[0018] Another embodiment of the invention is a method of treating or preventing an IL-21R-associated disorder in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248, and wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22,

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24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

[0019] Another embodiment of the invention is a method of treating or preventing an IL-21R-associated disorder in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, and wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, and 11, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

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[0020] In one embodiment of the invention, the binding protein or antigen-binding fragment comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248, wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

[0021] A further embodiment of the invention is a method of treating or preventing an IL-21R-associated disorder in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the heavy chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 68, 70, 72, 88, 90, 92, 94, 213, 218, 219, 240, and 242. In one embodiment, the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and the V_H domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, and 20.

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[0022] Another embodiment of the invention is a method of treating or preventing an IL-21R-associated disorder in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the light chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 74, 76, 78, 80, 82, 84, 86, 96, 98, 100, 102, 104, 106, 108, 214-217, 220-229, 244, 246, and 248. In one embodiment, the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and the V_L domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 215, 217, 221, 223, 225, 227, and 229.

[0023] In another embodiment of the methods of the invention, the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:88, 90, 92, 94, 213, 218, 219, 240, and 242, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:96, 98, 100, 102, 104, 106, 108, 214, 216, 220, 222, 224, 226, 228, 244, 246, and 248.

[0024] One embodiment of the invention is a method of treating or preventing an IL-21R-associated disorder in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to a human IL-21R epitope that is recognized by a binding protein selected from the group consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25, wherein the binding protein or antigen-binding fragment competitively inhibits the binding of a binding protein selected from the group consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25 to human IL-21R, in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder. In another embodiment, the binding protein or antigen-binding fragment thereof comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid

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sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248. In yet another embodiment, the binding protein or antigen-binding fragment thereof comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247. In still another embodiment, the binding protein specifically binds to an IL-21R epitope that is recognized by AbO, AbP, AbQ, AbR, AbS, AbT, AbU, AbV, and/or AbW, and the binding protein competitively inhibits the binding of AbO, AbP, AbQ, AbR, AbS, AbT, AbU, AbV, and/or AbW to human IL-21R.

[0025] In one embodiment of the methods of the invention, the IL-21R-associated disorder is selected from the group consisting of autoimmune disorders, inflammatory conditions, allergies, transplant rejections, and hyperproliferative disorders of the blood. In another embodiment, the IL-21R-associated disorder is selected from the group consisting of immune disorders, hyperproliferative disorders of the blood, transplant rejection, graft-versus-host disease, allergy (including atopic allergy), diabetes mellitus, arthritic disorders (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, ankylosing spondylitis), spondyloarthropathy, multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis, eczematous dermatitis), psoriasis, Sjögren's syndrome, IBD (including Crohn's disease, ulcerative colitis), asthma

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(including intrinsic asthma, allergic asthma), scleroderma and vasculitis. In a further embodiment, the IL-21R-associated disorder is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, psoriasis, transplant rejection, rheumatoid arthritis, and other arthritic disorders.

[0026] In one embodiment of the methods of the invention, the binding protein or antigen-binding fragment thereof has an association constant for human IL-21R of at least $10^5 \text{ M}^{-1} \text{ s}^{-1}$. In another embodiment, the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated BAF3 cell proliferation with an IC_{50} of about 1.75 nM or less, wherein the BAF3 cells comprise a human IL-21 receptor. In another embodiment, the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of TF1 cells with an IC_{50} of about 14 nM or less, wherein the TF1 cells comprise a human IL-21 receptor. In still another embodiment, the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of primary human B cells with an IC_{50} of about 1.9 nM or less, wherein the B cells comprise a human IL-21R. In yet another embodiment, the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of primary human CD4^+ cells with an IC_{50} of about 1.5 nM or less, wherein the CD4^+ cells comprise a human IL-21R. In further embodiments of the invention, other ranges and values for these parameters, and for other pharmacokinetic and pharmacodynamic parameters, are provided herein.

[0027] In one embodiment, the invention provides a method of determining whether an anti-IL-21R antibody is a therapeutic anti-IL-21R antibody comprising the steps of: contacting a first blood sample from a subject with an IL-21 ligand; determining a level of expression of at least one IL-21-responsive gene in the first blood sample contacted with the IL-21 ligand; contacting a second blood sample from the subject with the IL-21 ligand in the presence of an anti-IL-21R antibody; determining the level of expression of the at least one IL-21-responsive gene in the second blood sample contacted with the IL-21 ligand in the presence of the anti-IL-21R antibody; and comparing the levels of expression of the at least one IL-21-responsive gene

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determined above, wherein a change in the level of expression of the at least one IL-21-responsive gene indicates that the anti-IL-21R antibody is a therapeutic antibody. In another embodiment of the invention, the subject is a mammal, e.g., a monkey or a human. In another embodiment, the at least one IL-21-responsive gene is selected from the group consisting of TNF, IFN γ , IL-6, IL-8, IL-10, CD19, STAT3, TBX21, CSF1, GZMB, PRF1, IL-2R α , and IL-21R. In a further embodiment, the at least one IL-21-responsive gene is IL-2R α .

[0028] In one embodiment, the invention provides a method of determining the pharmacodynamic activity of an anti-IL-21R antibody comprising detecting a modulation in a level of expression of at least one IL-21-responsive gene in a blood sample of a subject. In a further embodiment, detecting the modulation in the level of expression of the at least one IL-21-responsive gene comprises the steps of: administering the anti-IL-21R antibody to the subject, wherein the subject is treated with the anti-IL-21R antibody; contacting a blood sample from the subject treated with the anti-IL-21R antibody with an IL-21 ligand; determining the level of expression of the at least one IL-21-responsive gene in the blood sample from the subject treated with the anti-IL-21R antibody and contacted with the IL-21 ligand; and comparing the level of expression of the at least one IL-21-responsive gene determined above with the level of expression of the at least one IL-21 responsive gene in a different blood sample contacted with the IL-21 ligand, wherein the different blood sample is from a subject not treated with the anti-IL-21R antibody. In another embodiment of the invention, the subject is a mammal, e.g., a monkey or a human. In another embodiment, the at least one IL-21-responsive gene is selected from the group consisting of TNF, IFN γ , IL-6, IL-8, IL-10, CD19, STAT3, TBX21, CSF1, GZMB, PRF1, IL-2R α , and IL-21R. In a further embodiment, the at least one IL-21-responsive gene is selected from the group consisting of CD19, GZMB, PRF1, IL-2R α , IFN γ , and IL-6. In another further embodiment, the at least one IL-21-responsive gene is IL-2R α .

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[0029] In one embodiment, the invention provides a method of decreasing, inhibiting, or reducing an acute phase response in a subject, comprising administering to the subject a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to decrease, inhibit or reduce the acute phase response in the subject, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248. In a further embodiment, the binding protein or antigen-binding fragment thereof is administered locally.

[0030] One embodiment of the invention is a method of increasing the efficacy of a vaccine formulation used to immunize a subject, comprising administering to the subject a therapeutically effective amount of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248. In some embodiments, the binding protein or antigen-binding fragment thereof is administered before, during and/or after immunization.

[0031] Another embodiment of the invention provides a method for detecting the presence of IL-21R in a sample *in vitro*, comprising (a) contacting a sample with a

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binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R, and (b) detecting formation of a complex between the binding protein or antigen-binding fragment thereof and the sample, wherein a significant difference in the formation of the complex in the sample relative to in a control or reference sample or level is indicative of the presence of IL-21R in the sample, and wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248. In a further embodiment, the sample is serum, plasma, or tissue.

[0032] Yet another embodiment of the invention provides a method for detecting the presence of IL-21R *in vivo*, comprising (a) administering a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R to a subject under conditions that allow for binding of the binding protein or antigen-binding fragment thereof to IL-21R, and (b) detecting formation of a complex between the binding protein or antigen-binding fragment thereof and IL-21R, wherein a significant difference in the formation of the complex in the subject relative to a control or reference sample or level of formation of the complex is indicative of the presence of IL-21R, and wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248. In some embodiments, the binding

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protein or antigen-binding fragment thereof is directly or indirectly labeled with a detectable substance to facilitate detection of bound or unbound antibody. In further embodiments, the detectable substance is an enzyme, a prosthetic group, a fluorescent material, a luminescent material, or a radioactive material.

[0033] In some embodiments, the invention provides methods further comprising administering to the subject another therapeutic agent chosen from the group consisting of a cytokine inhibitor, a growth factor inhibitor, an immunosuppressant, an anti-inflammatory agent, a metabolic inhibitor, an enzyme inhibitor, a cytotoxic agent, and a cytostatic agent. In further embodiments, the therapeutic agent is chosen from the group consisting of a TNF antagonist, an IL-12 antagonist, an IL-15 antagonist, an IL-17 antagonist, an IL-18 antagonist, an IL-19 antagonist, an IL-20 antagonist, an IL-21 antagonist, an IL-23 antagonist, a T cell-depleting agent, a B cell-depleting agent, methotrexate, leflunomide, sirolimus (rapamycin) or an analog thereof, a cox2 inhibitor, a cPLA2 inhibitor, an NSAID, and a p38 inhibitor. As in other methods of the invention, in some embodiments the subject is a mammal, e.g., a human.

[0034] Another embodiment of the invention provides a method for measuring, determining, and/or assessing the levels of production of anti-product antibodies, e.g., anti-product antibodies to anti-IL-21R binding proteins and antigen-binding fragments thereof.

[0035] Additional aspects of the disclosure will be set forth in part in the description, and in part will be obvious from the description, or may be learned by practicing the invention. The invention is set forth and particularly pointed out in the claims, and the disclosure should not be construed as limiting the scope of the claims. The following detailed description includes exemplary representations of various embodiments of the invention, which are not restrictive of the invention as claimed. The accompanying figures constitute a part of this specification and, together with the description, serve only to illustrate embodiments and not limit the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] **FIG. 1(a-c)** depicts the neutralization of proliferation of human IL-21R-BaF3 cells by scFv. Cells were mixed with the indicated scFv and then incubated with 100 pg/ml of human IL-21. Proliferation was measured by CELLTITER-GLO[®] (Promega Corporation, Madison, WI) after 48 hr.

[0037] **FIG. 2(a-c)** depicts the neutralization of proliferation of human IL-21R-TF1 cells by scFv. Cells were mixed with the indicated scFv and then incubated with 100 pg/ml of human IL-21. Proliferation was measured by CELLTITER-GLO[®] after 48 hr.

[0038] **FIG. 3(a-c)** depicts the neutralization of proliferation of murine IL-21R-BaF3 cells by scFv. Cells were mixed with the indicated scFv and then incubated with 400 pg/ml of murine IL-21. Proliferation was measured by CELLTITER-GLO[®] after 48 hr.

[0039] **FIG. 4(a-c)** depicts scFv competition with parental antibody 18A5 IgG for binding to murine IL-21R. The scFv were mixed with biotinylated-murine IL-21R-H/F, and the mixtures were added to antibody 18A5 immobilized on an ELISA plate. Capture of mIL-21R was detected with HRP-streptavidin, and competition for binding to mIL-21R was indicated by a reduction in the A450 signal.

[0040] **FIG. 5** depicts the neutralization of IL-21-dependent proliferation by 21 heavy chain / light chain pairs. Antibodies, as indicated in the figure, were added to cells. IL-21 was subsequently added, and proliferation measured with CELLTITER-GLO[®] after 48 hr. Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIGs. 5a-c**), human IL-21R-TF1 cells with 100 pg/ml of human IL-21 (**FIGs. 5d-f**), or murine IL-21R-BaF3 cells with 400 pg/ml of murine IL-21 (**FIGs. 5g-i**).

[0041] **FIG. 6** depicts the binding of 21 anti-IL-21R IgGs to CHO cells transiently expressing human IL-21R (**FIGs. 6a-c**), rat IL-21R (**FIGs. 6d-f**), cynomolgus monkey IL-21R (**FIGs. 6g-i**), and human gamma common chain (**FIGs. 6j-l**). CHO cells were transiently transfected with IL-21R or the control gamma common

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chain, and binding was detected with HRP-conjugated anti-human IgG in a cell-based ELISA.

[0042] **FIG. 7(a-c)** depicts the binding specificity of particular anti-IL-21R antibodies (**FIG. 7a**, AbS; **FIG. 7b**, AbQ, AbT, AbO; **FIG. 7c**, AbR, AbP, and AbU), measured by surface plasmon resonance. The anti-IL-21R antibodies were captured on anti-human IgG, and subsequent binding to either murine IL-21R-H/F, human IL-13-H/F, human IL-2R β , or human soluble IL-4R was measured in a BIACORETM instrument (GE Healthcare, Piscataway, NJ). **FIG. 7d** shows that human IL-2R β and human soluble IL-4R are captured by specific anti-IL-2R β and anti-IL-4R antibodies, respectively (control).

[0043] **FIG. 8(a-d)** depicts the binding of anti-IL-21R antibodies to human and murine IL-21R. The indicated human anti-IL-21R antibodies were captured on anti-human IgG immobilized on a BIACORETM chip. Varying concentrations of human IL-21R-His/FLAG (**FIGs. 8a-b**) and murine IL-21R-His/FLAG (**FIGs. 8c-d**) were allowed to flow over the chip, and binding and dissociation were monitored.

[0044] **FIG. 9** depicts the binding of anti-IL-21R antibodies to human and cynomolgus monkey IL-21R. Human anti-IL-21R antibodies AbS and AbT were captured on anti-human IgG immobilized on a BIACORETM chip. Varying concentrations of human and cynomolgus monkey IL-21R-His/FLAG were allowed to flow over the chip, and binding and dissociation were monitored. **FIG. 9a** shows cynomolgus monkey IL-21R-His/FLAG binding to AbS. **FIG. 9b** shows human IL-21R-His/FLAG binding to AbS. **FIG. 9c** shows cynomolgus monkey IL-21R-His/FLAG binding to AbT. **FIG. 9d** shows human IL-21R-His/FLAG binding to AbT.

[0045] **FIG. 10** depicts an epitope assessment of IL-21R antibodies. In the experiment depicted in **FIG. 10a** (see also illustration at left of Y-axis), murine IL-21R-H/F (His-Flag fusion protein) was captured by anti-IL-21R antibody AbS immobilized on a BIACORETM chip. Additional anti-IL-21R antibodies (AbS, AbT, D5 (D5-20, a neutralizing anti-murine IL-21R antibody), and 7C2 (a nonneutralizing

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anti-murine IL-21R control antibody)) were flowed over the chip and their binding to the captured IL-21R-H/F was monitored. In the experiment depicted in **FIG. 10b**, human IL-21R-H/F was captured by anti-IL-21R antibody AbS immobilized on a BIACORETM chip. Additional anti-IL-21R antibodies (AbS, AbT, and 9D2 (a nonneutralizing anti-human IL-21R control antibody)) were flowed over the chip and their binding to the captured IL-21R-H/F was monitored.

[0046] FIG. 11 depicts the neutralization of proliferation of human IL-21R-BaF3 cells and murine IL-21R-BaF3 cells by the indicated antibodies. Antibodies were added to cells. IL-21 was subsequently added and proliferation measured with CELLTITER-GLO[®] after 48 hr. Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIG. 11a**), murine IL-21R-BaF3 cells with 200 pg/ml of murine IL-21 (**FIG. 11b**), and human IL-21R-TF1 cells with 100 pg/ml of human IL-21 (**FIG. 11c**).

[0047] FIG. 12 depicts the neutralization of IL-21-dependent proliferation of human primary B cells. The indicated antibodies were added to primary human B cells along with anti-CD40 antibodies and human IL-21. Incorporation of ³H-thymidine was measured after three days. **FIG. 12a** depicts the comparison between AbQ, AbR, AbS, AbT, AbU, IL-13 triple-mutant, and 18A5 parental antibody; **FIG. 12b** depicts the comparison between AbT, AbV, AbW, AbU, and human IgG1 control (hIg1).

[0048] FIG. 13 depicts the neutralization of IL-21-dependent proliferation of human primary CD4⁺ T cells. The indicated antibodies were added to activated primary human CD4⁺ T cells along with human IL-21, and incorporation of ³H-thymidine was measured after three days.

[0049] FIG. 14 depicts the neutralization of IL-21-dependent proliferation of murine primary CD8⁺ T cells. The indicated antibodies were added to activated primary murine CD8⁺ T cells along with human IL-21, and incorporation of ³H-thymidine was measured after three days.

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[0050] FIG. 15 depicts the measurement of antibody-dependent cellular cytotoxicity (ADCC) induced by anti-IL-21R antibodies. PBMC-dependent killing of CFSE-labeled BJAB cells coated with the indicated anti-IL-21R antibodies was measured by incorporation of propidium iodide. The anti-CD20 antibody rituximab (RITUXAN[®], Genentech, Inc., South San Francisco, CA) was included as a positive control, and an anti-IL-13 antibody was included as a negative control.

[0051] FIG. 16 depicts complement C1q binding by anti-IL-21R antibodies. The indicated anti-IL-21R antibodies were immobilized on an ELISA plate and, following incubation with human serum, C1q binding was measured with chicken anti-human C1q and an HRP-conjugated anti-chicken IgY antibody. The anti-CD20 antibody rituximab (RITUXAN[®]) was included as a positive control, and an anti-IL-13 antibody was included as a negative control.

[0052] FIG. 17(a-c) depicts amino acid sequences for AbQ, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0053] FIG. 18(a-c) depicts amino acid sequences for AbR, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0054] FIG. 19(a-c) depicts amino acid sequences for AbW, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0055] FIG. 20(a-c) depicts amino acid sequences for AbS, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0056] FIG. 21(a-c) depicts amino acid sequences for AbT, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0057] FIG. 22(a-c) depicts amino acid sequences for AbO, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0058] FIG. 23(a-c) depicts amino acid sequences for AbP including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0059] FIG. 24(a-c) depicts amino acid sequences for AbU, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

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[0060] **FIG. 25(a-c)** depicts amino acid sequences for AbV, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0061] **FIGs. 26(a-g)** depict results generated from additional studies that were performed similarly to those performed to generate the results shown in **FIGs. 5, 11, 12, 13, and 14** (described above).

[0062] **FIG. 27a** depicts IL-21 cytokine competition with antibody AbT for binding to murine IL-21R. Vehicle, or increasing amounts of IL-21, was mixed with biotinylated murine IL-21R-His/FLAG, and the mixtures were added to AbT immobilized on an ELISA plate. Capture of mIL-21R was detected with HRP-streptavidin, and competition for binding to mIL-21R was indicated by a reduction in the A450 signal. **FIG. 27b** depicts the competition of AbS and IL-21 for binding to murine IL-21R. AbS and mIL-21 were mixed and applied to immobilized mIL-21R-Fc in ELISA plates, and the binding of AbS (filled diamonds), isotype control antibody (open triangles), or mIL-21 (squares) was monitored.

[0063] **FIG. 28** depicts IL-21-dependent ³H thymidine incorporation into rat CD3 T cells in the presence of anti-IL-21R antibodies AbS, AbU, AbV, or AbW, or an isotype control antibody, hIgG1-TM.

[0064] **FIG. 29** depicts binding of AbS to either of two isoforms of rabbit IL-21R-Fc presented by immobilized anti-mouse IgG antibodies in the absence (**FIG. 29a**) or presence (**FIG. 29b**) of 10% conditioned medium containing rabbit IL-21.

[0065] **FIG. 30** depicts NP-specific primary antibody response in animals treated with anti-IL-21R antibodies. **FIG. 30a** depicts NP-specific IgG response and **FIG. 30b** depicts NP-specific IgM response. **FIGs. 30c-f** depict the NP-specific IgG subclass responses in repeats of the experiment shown in **FIG. 30a**, with ELISA data shown for individual animals: total IgG (**FIG. 30c**), IgG1 (**FIG. 30d**), IgG2a (**FIG. 30e**), and IgG2c (**FIG. 30f**). **FIG. 30g** depicts NP-specific IgG response over a two-month period. **FIG. 30h** depicts NP-specific IgG antibody-secreting cells (ASC) found in bone marrow at the end of the two-month study period.

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[0066] FIG. 31 depicts anti-double-stranded DNA (anti-dsDNA) antibodies in MRL-*Fas^{lpr}* mice treated with anti-IL-21R antibodies. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated with either saline (control) or the indicated triple-mutant antibodies (10 mg/kg i.p., 3X / week); an anti-human IL-13 human IgG1 A234 A235 A237 triple-mutant antibody with no reactivity to murine IL-21 (IL-13 TM) was used as an isotype control. Serum collected biweekly was tested by ELISA for the presence of anti-dsDNA. **FIG. 31a** shows anti-dsDNA antibody titers following treatment. **FIG. 31b** shows prebleed anti-dsDNA antibody titers. **FIG. 31c** shows anti-dsDNA antibody titers after two weeks of dosing. **FIG. 31d** shows anti-dsDNA antibody titers after four weeks of dosing. **FIG. 31e** shows anti-dsDNA antibody titers after six weeks of dosing. **FIG. 31f** shows anti-dsDNA antibody titers after eight weeks of dosing. **FIG. 31g** shows anti-dsDNA antibody titers after ten weeks of dosing. In **FIGs. 31a** and **31c-g**, asterisks indicate a significant difference as compared to both the saline and IL-13 controls ($p<0.01$). In **FIG. 31b**, the asterisk indicates a significant difference as compared to the other three groups ($p<0.01$). The dashed line in **FIGs. 31b-g** indicates the level of detection.

[0067] FIG. 32 depicts IgG deposits in kidneys of MRL-*Fas^{lpr}* mice treated with anti-IL-21R antibodies. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated (10 mg/kg i.p., 3X / week) with either saline (control) or the indicated triple-mutant antibodies; anti-human IL-13 human IgG1 A234 A235 A237 triple-mutant antibody with no reactivity to murine IL-21 was used as an isotype control. Following 10 weeks of treatment, mice were sacrificed and IgG deposits in the kidneys were identified by immunocytochemistry (**FIG. 32b**; glomeruli are indicated by dashed circles; examples of IgG deposits are indicated by arrowheads). Staining intensity was scored on a scale of 1-5 (**FIG. 32a**).

[0068] FIG. 33 depicts IgM and complement C3 deposits in kidneys of MRL-*Fas^{lpr}* mice treated with anti-IL-21R antibodies. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated (10 mg/kg i.p., 3X / week) with either saline (control) or the indicated

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triple-mutant antibodies. Following ten weeks of treatment, mice were sacrificed and IgM (**FIG. 33a**) and complement C3 (**FIG. 33b**) deposits in the kidneys were identified by immunocytochemistry. Staining intensity was scored on a scale of 1-5.

[0069] **FIG. 34** depicts IgG, IgM and complement C3 deposits in brains of MRL-*Fas^{lpr}* mice treated (10 mg/kg i.p., 3X / week) with anti-IL-21R antibodies. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated with either saline (control) or the indicated triple-mutant antibodies. Following ten weeks of treatment, mice were sacrificed and IgG (**FIG. 34a**), IgM (**FIG. 34b**), and complement C3 (**FIG. 34c**) deposits in the brain were identified by immunocytochemistry. Staining intensity was scored on a scale of 1-5.

[0070] **FIG. 35** depicts lymphocytic infiltrates in kidneys of MRL-*Fas^{lpr}* mice treated with anti-IL-21R antibodies. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated (10 mg/kg i.p., 3X / week) with either saline (control) or the indicated triple-mutant antibodies. Following ten weeks of treatment, mice were sacrificed and hematoxylin/eosin (H/E)-stained kidney sections were examined for lymphocyte infiltration in three zones, cortex interstitium (a support structure for the glomeruli) (**FIG. 35a**), cortex perivascular (**FIG. 35b**), and peripelvic (near the origin of the ureter) (**FIG. 35c**). Lymphocyte numbers were scored on a scale of 1-5.

[0071] **FIG. 36** depicts lymphocytic infiltrates in lungs of MRL-*Fas^{lpr}* mice treated with anti-IL-21R antibodies. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated (10 mg/kg i.p., 3X / week) with either saline (control) or the indicated triple-mutant antibodies. Following ten weeks of treatment, mice were sacrificed and H/E-stained lung sections were examined for lymphocyte infiltration. Lymphocyte numbers were scored on a scale of 1-5.

[0072] **FIGs. 37a-b** depict the mouse anti-human antibody (MAHA) response in MRL-*Fas^{lpr}* mice treated with anti-IL-21R antibodies. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated (10 mg/kg i.p., 3X / week) with the indicated triple-mutant antibodies. Serum, collected biweekly, was tested by ELISA for the presence of murine antibodies capable of binding to the same human antibodies with which

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the mice were treated (**FIG. 37a**), or for the presence of murine antibodies capable of binding to the other anti-IL-21 human antibodies (**FIG. 37b**). Asterisks in **FIG. 37a** indicate a significant difference as compared to the anti-IL-13-treated group ($p<0.05$).

[0073] **FIG. 38** depicts development of anti-dsDNA antibodies in MRL-*Fas^{lpr}* mice treated with anti-IL-21R antibodies. **FIG. 38a** depicts anti-dsDNA antibody titer for mice treated with AbS, and **FIG. 38b** depicts anti-dsDNA antibody titer for mice treated with AbT. **FIGs. 38c-d** represent kidney pathology and renal inflammatory foci in MRL-*Fas^{lpr}* mice treated with AbS and AbT.

[0074] **FIG. 39** depicts cellular infiltration into the dorsal air pouch in mice or rats treated with anti-IL-21R antibodies. **FIGs. 39a-e**: three days after injection of 3 ml of air under the dorsal skin of BALB/C mice to create an air pouch, pouches were reinflated. Two days later, either saline (control) or the indicated triple-mutant antibodies were injected i.p. One hundred ng of murine IL-21 was injected into the air pouch 24 hr after antibody injection. Six hours later, the pouches were washed out with 3 ml of PBS, and total cell counts (**FIG. 39a**), monocytes (**FIG. 39b**), lymphocytes (**FIG. 39c**), and neutrophils (**FIG. 39d**) were determined. **FIG. 39e** depicts total cell counts in a replicate of the experiment shown in **FIGs. 39a-d**. **FIGs. 39f-j** depict total cell infiltration into air pouches in rats following treatment with mouse IL-21 and anti-IL-21R antibodies. **FIG. 39f** depicts cellular infiltration following administration of either 20 μ g mL-21 for 6 hr or 1 μ g mL-21 for 20 hr, in the presence or absence of AbS or control antibody. **FIGs. 39g-h** depict cellular infiltration into rat air pouches in replicate experiments in which 20 μ g mL-21 was administered for 6 hr in the presence of 1, 3, or 10 μ g/kg AbS or a control antibody. In the experiment shown in **FIG. 39i**, rats were treated for 6 hr with 20 μ g mL-21 and 1 mg/kg antibody (either AbS or isotype control), singly or in combination, as well as a combination of 20 μ g mL-21 and 10 mg/kg antibody. In **FIG. 39j**, 10, 20, or 40 μ g mL-21 were tested in combination with either 1 or 10 mg/kg AbS.

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[0075] **FIG. 40** depicts the concentration-time profiles of AbS in CD-1 mice after a single intravenous or subcutaneous administration. Concentrations below the limit of quantitation were treated as zero for the calculation of mean and standard deviation. N = 4-8 for each data point.

[0076] **FIG. 41** depicts the concentration-time profiles of AbS in DBA and MRL-*Fas^{lpr}* mice after a single intraperitoneal administration. Concentrations below the limit of quantitation were treated as zero for the calculation of mean and standard deviation. N = 4-8 for each data point.

[0077] **FIGs. 42a and b** depict the observed and predicted AbS concentrations in MRL-*Fas^{lpr}* mice after multiple i.p. administrations at 10, 5, and 2.5 mg/kg doses, 3X / week, for ten weeks. Concentrations below the limit of quantitation were treated as zero for the calculation of mean and SD. N = 7-8 per time point.

[0078] **FIGs. 43a and b** depict the concentration-time profiles of AbS in cynomolgus monkeys after administrations as shown. Concentrations below the limit of quantitation (<30 ng/ml) were treated as zero for the calculation of mean and standard deviation. N = 3 for each group. SAN in **FIG. 43a** is the study animal number.

[0079] **FIG. 44** depicts the concentration-time profiles of AbS and AbT after a single administration to mice. **FIG. 44a** shows a profile after a 10 mg/kg i.v. administration in CD-1 mice. **FIG. 44b** shows a profile after a 10 mg/kg i.p. administration in MRL-*Fas^{lpr}* mice. **FIG. 44c** shows a profile after an 8 mg/kg i.p. administration in DBA mice. Concentrations below the limit of quantitation were treated as zero for the calculation of mean and standard deviation. N = 4-8 for each data point.

[0080] **FIG. 45** depicts the observed and predicted AbT concentrations in MRL-*Fas^{lpr}* mice after multiple i.p. administrations at 20 mg/kg doses, 3X / week, for ten weeks. Concentrations below the limit of quantitation were treated as zero for the calculation of mean and standard deviation. N = 6-8 per time point.

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[0081] **FIGs. 46a and b** depict the concentration-time profiles of AbT and AbS in cynomolgus monkeys after administrations as shown. Concentrations below the limit of quantitation (<30 ng/ml) were treated as zero for the calculation of mean and standard deviation. N = 3 for each group.

[0082] **FIG. 47** depicts the concentration-time profiles of the anti-IL-21R antibodies AbS, AbT, and ^{125}I -D5 (8 mg/kg, i.p.) in male DBA mice. Human antibodies AbS and AbT were quantitated by anti-human IgG ELISA, and the ^{125}I -labeled murine antibody D5 was quantitated by monitoring the radiolabel.

[0083] **FIG. 48a** depicts mean serum concentrations (Y-axis; ng/ml) of AbS, AbT, and an isotype control in Sprague-Dawley (S-D) rats after a single 10 mg/kg i.v. dose. **FIG. 48b** depicts anti-IL-21R antibodies AbS-AbW after a single 10 mg/kg i.v. dose in S-D rats. Individual data points <LOQ (45 ng/mL) were treated as zero for calculations of mean and SD. If mean value was below LOQ (e.g., when all animals had value <LOQ), data points are not shown.

[0084] **FIG. 49** depicts means serum concentrations (Y-axis; ng/ml) of AbS in S-D rats after a single i.v., i.p., or s.c. dose.

[0085] **FIG. 50** depicts the biodistribution of ^{125}I -AbS in IL-21R knockout and wild-type C57BL/6 (control) mice after a single 2.5 mg/kg intravenous dose. **FIG. 50a** shows the concentration of ^{125}I -AbS in tissues in control C57BL/6 mice. **FIG. 50b** shows the concentration of ^{125}I -AbS in IL-21R knockout mice. **FIG. 50c** shows the serum concentration of ^{125}I -AbS in control C57BL/6 and IL-21R knockout mice. **FIG. 50d** shows cumulative urine counts of ^{125}I -AbS in control C57BL/6 and IL-21R knockout mice. Concentrations below LOQ were treated as zero for the calculation of mean and SD. N = 9-10 for each serum or tissue data point; N = 5-10 for each urine data point.

[0086] **FIG. 51** depicts the biodistribution of ^{125}I -AbS in MRL-*Fas^{lpr}* mice after a single 2.5 mg/kg i.p. dose. **FIG. 51a** shows the concentration of ^{125}I -AbS in tissues in MRL-*Fas^{lpr}* mice. **FIG. 51b** shows urine counts of ^{125}I -AbS in MRL-*Fas^{lpr}* mice.

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[0087] **FIG. 52** depicts the serum concentration of ^{125}I -AbS in MRL-*Fas*^{lpr} mice after single 2.5 and 10 mg/kg i.p doses. Concentrations below LOQ were treated as zero for the calculation of mean and SD. N = 4-8 for each data point.

[0088] **FIG. 53a** depicts percent inhibition (Y-axis) of IL-21-induced IL-2R α (IL2RA), PRF1, IL-6, and CD19 expression upon increasing concentrations of AbS (X-axis) in human whole blood samples, calculated from fold-change; **FIG. 53b** depicts inhibition of IL-21-induced relative expression level (RQ; Y-axis) of IL-6 in response to increasing AbS concentrations (X-axis; concentration Ab (nM)) in human whole blood samples.

[0089] **FIG. 54** depicts the genes included on custom TLDA (Taqman® Low Density Array) for assay; Endogenous (Endo) controls are indicated.

[0090] **FIG. 55** demonstrates relative quantification (RQ; Y-axis) of gene expression of six examined genes (CD19, GZMB, IFN γ (IFNG), IL-2R α (IL2RA), IL-6, and PRF1) at different concentrations of IL-21 at either 2, 4, 6, or 24 hr time points (X-axis).

[0091] **FIG. 56a** demonstrates relative quantification (RQ; Y-axis) of gene expression of six examined genes (CD19, GZMB, IFN γ , IL-2R α , IL-6, and PRF1) after incubation with IL-21 and different concentrations of either AbS or IgG1 TM control (X-axis). **FIGs. 56b-c** depict percent inhibition (Y-axes) of IL-21 response of the same genes after treatment with different concentrations of either AbS or control IgG1 TM (X-axes).

[0092] **FIG. 57** depicts distribution of Relative Quantification (RQ) values for IL-2R α gene expression in whole blood of male cynomolgus monkeys following *ex vivo* stimulation with recombinant human IL-21. **FIG. 57a** demonstrates the histogram analysis (Y-axis) for the RQ values (X-axis), and **FIG. 57b** demonstrates histogram analysis (Y-axis) for the log₂-transformed RQ values (X-axis), which were obtained using a statistical software package. The solid line represents Gaussian distribution. The dotted line represents cutoff RQ for the enrollment into the *in vivo* study, defined using the formula: $\log \{RQ_{\text{cutoff}}\} = \text{mean of the log-transformed RQ}$

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values – SD of the log-transformed RQ values. **FIG. 57c** compares individual animal and median (solid lines) RQ values (Y-axis) of whole blood aliquots stimulated with IL-21 and AbT (triangles) or IL-21 and control IgG (squares) as compared to those with IL-21 stimulation alone (circles).

[0093] **FIG. 58** depicts serum concentrations (Y-axis) following single 10 mg/kg i.v. administration of anti-IL-21R antibody AbS (“A”) or AbT (“B”) to male cynomolgus monkeys, collected up to day 148 for AbS and up to day 36 for AbT (X-axis). Data points with serum concentrations below the lower LOQ (30 ng/mL) are not shown.

[0094] **FIG. 59** shows correlation of antibody serum concentrations (second Y-axis), pharmacodynamic (PD) activity (“RQ”; first Y-axis), and anti-product antibody response (indicated by “A”), including neutralizing anti-product antibody response (indicated by “AN”), following single 10 mg/kg i.v. administration of anti-IL-21R antibody AbS to male cynomolgus monkeys, as measured at various times pre- and post-dose administration (X-axis; Time(days)). **FIGs. 59a-c** depict results for Animals 1-3, respectively.

[0095] **FIG. 60** shows correlations similar to those in **FIG. 59** for AbT (single 10 mg/kg i.v. administration). **FIGs. 60a-c** depict results for Animals 4-6, respectively.

[0096] **FIG. 61** depicts the concentration-time profiles of AbS-AbW in cynomolgus monkeys after a single i.v. administration. Concentrations below the limit of quantitation (<30 ng/ml) were treated as zero for calculations. For AbS (filled circles, Study 1; open diamonds, Study 2), AbT (open circles), AbV (filled diamonds), and AbU (open triangles), n=3; 10 mg/kg dose. For AbW (open squares), n=2; 1 mg/kg dose.

[0097] **FIG. 62** depicts individual concentrations (ng/ml) of AbS after three weekly i.v. administrations (arrows) of 2 (**FIG. 62a**) or 10 mg/kg (**FIG. 62b**) to tetanus-toxoid challenged male and female cynomolgus monkeys.

[0098] **FIG. 63** depicts allometric scaling of AbS PK parameters after i.v. administration. Body weight (W); maximum span potential (in years; MLP).

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FIG. 63a represents CL•MLP (Y-axis) plotted against body weight (X-axis);

FIG. 63b represents volume of distribution (Y-axis) plotted against body weight (X-axis). Solid lines represent fitted curves based on a linear regression using data from mice, rats, and cynomolgus monkeys. Dotted lines represent 95% confidence intervals.

[0099] **FIG. 64** represents allometric scaling of AbT PK parameters after i.v. administration. Body weight (W); brain weight ("BW"). **FIG. 64a** represents CL•BW (Y-axis) plotted against body weight (X-axis); **FIG. 64b** represents volume of distribution (Y-axis) plotted against body weight (X-axis).

[0100] **FIG. 65(a-b)** depicts the effects of AbS on the development of proteinuria (**FIG. 65a**) and anti-dsDNA IgG serum antibody titers (**FIG. 65b**) in NZBWF1/J mice. Female 26-week old NZBWF1/J mice were administered 400 µg of either saline, anti-E.tenella antibody, mCTLA-4Ig, hIgGTm antibody or AbS antibody 3X / week for 10 weeks. Urine protein levels and anti-dsDNA IgG serum antibody titers were measured at the beginning of the study, and every two weeks thereafter for 10 weeks. Animals treated with CTLA4-Ig showed significant reductions in anti-dsDNA titers (asterisks; $p < .05$) at weeks 4-10.

[0101] **FIG. 66** depicts the effects of anti-IL-21R neutralization on disease outcome in a semi-therapeutic CIA mouse model of rheumatoid arthritis. Female DBA/1 mice were immunized and boosted with bovine collagen type II; when 10% of the animals in the study exhibited paw swelling, mice were dosed 3X / week for 30 days with 8 mg/kg of either murine IgG2a isotype control antibody, anti-mouse IL-21R antibody D5 (murine IgG2a antibody), mTNFRII-Fc (positive control, murine IgG2a isotype) (**FIG. 66a**); or anti-IL-13TM antibody (human IgG1 isotype control antibody), AbT, or AbS (**FIG. 66b**). Individual animal scores on day 30 of the study are depicted in **FIG. 66c**.

[0102] **FIG. 67** depicts the development of amnestic tetanus-specific IgM and IgG serum antibody responses to tetanus toxoid in cynomolgus monkeys in the presence of AbS. Nine male and nine female cynomolgus monkeys were immunized with

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tetanus toxoid and rested for 43 days. After 43 days, male and female monkeys were randomly assigned into groups of three, and treated with either saline (vehicle), 2 mg/kg AbS, or 10 mg/kg AbS 1X / week for 3 weeks. Twenty-four hr after the first dose of either vehicle or AbS (arrows), monkeys were immunized a second time with tetanus toxoid. Monkeys were routinely bled throughout the course of the study, and examined for tetanus-specific IgM (FIG. 67a) and IgG (FIG. 67b) serum antibody titers.

DETAILED DESCRIPTION OF THE INVENTION

[0103] The binding proteins of the present invention were initially derived from parental antibody 18A5, but differ from 18A5 in the amino acid sequences of portions of the heavy chain and/or light chain complementarity determining region 3 (CDR3). Additionally, the present binding proteins show improved potency in binding to and neutralizing both human and murine IL-21R as compared to 18A5 in the equivalent format (e.g., scFv or IgG). High-potency neutralization of IL-21R from both species (human and mouse) by a single binding protein has not previously been reported. The present binding proteins having a greater neutralization potency than their parental antibody may translate into higher efficacy as compared to agents previously described. In addition, the amino acid sequence of the V_H and V_L framework regions has been altered to match sequences encoded by human genomic sequence, thereby reducing the potential for human anti-human antibody responses in patients treated with the present binding proteins.

Definitions

[0104] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description and elsewhere in the specification.

[0105] The term “binding protein” as used herein includes any naturally occurring, recombinant, synthetic, or genetically engineered protein, or a combination thereof, that binds an antigen, target protein, or peptide, or a fragment(s) thereof. Binding

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proteins of the invention can include antibodies, or be derived from at least one antibody fragment. The binding proteins can include naturally occurring proteins and/or proteins that are synthetically engineered. Binding proteins of the invention can bind to an antigen or a fragment thereof to form a complex and elicit a biological response (e.g., agonize or antagonize a particular biological activity). Binding proteins can include isolated antibody fragments, "Fv" fragments consisting of the variable regions of the heavy and light chains of an antibody, recombinant single-chain polypeptide molecules in which light and heavy chain variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region. Binding protein fragments can also include functional fragments of an antibody, such as, for example, Fab, Fab', F(ab')₂, Fc, Fd, Fd', Fv, and a single variable domain of an antibody (dAb). The binding proteins can be double or single chain, and can comprise a single binding domain or multiple binding domains.

[0106] Binding proteins can also include binding domain-immunoglobulin fusion proteins, including a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE (see, e.g., Ledbetter et al., U.S. Patent Publication 2005/0136049, for a more complete description). The binding domain-immunoglobulin fusion protein can further include a region that includes a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide, and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide (or CH4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE).

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Typically, such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody-dependent cell-mediated cytotoxicity, complement fixation, and/or binding to a target, for example, a target antigen. The binding proteins of the invention can be derived from any species including, but not limited to mouse, rat, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine.

[0107] The term “antibody” as used herein refers to an immunoglobulin that is reactive to a designated protein or peptide or fragment thereof. Suitable antibodies include, but are not limited to, human antibodies, primatized antibodies, chimeric antibodies, monoclonal antibodies, monospecific antibodies, polyclonal antibodies, polyspecific antibodies, nonspecific antibodies, bispecific antibodies, multispecific antibodies, humanized antibodies, synthetic antibodies, recombinant antibodies, hybrid antibodies, mutated antibodies, grafted conjugated antibodies (i.e., antibodies conjugated or fused to other proteins, radiolabels, cytotoxins), and *in vitro*-generated antibodies. The antibody can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (e.g., IgG1, IgG2, IgG3, and IgG4) of antibodies. The antibody can have a heavy chain constant region chosen from, e.g., IgG1, IgG2, IgG3, or IgG4. The antibody can also have a light chain chosen from, e.g., kappa (κ) or lambda (λ). The antibodies of the invention can be derived from any species including, but not limited to mouse, rat, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine. Constant regions of the antibodies can be altered, e.g., mutated, to modify the properties of the antibody (e.g., to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function). Typically, the antibody specifically binds to a predetermined antigen, e.g., an antigen associated with a disorder, e.g., an inflammatory, immune, autoimmune, neurodegenerative, metabolic, and/or malignant disorder.

[0108] The term “single domain binding protein” as used herein includes any single domain binding scaffold that binds to an antigen, protein, or polypeptide. Single

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domain binding proteins can include any natural, recombinant, synthetic, or genetically engineered protein scaffold, or a combination thereof, that binds an antigen or fragment thereof to form a complex and elicit a biological response (e.g., agonize or antagonize a particular biological activity). Single domain binding proteins may be derived from naturally occurring proteins or antibodies, or they can be synthetically engineered or produced by recombinant technology. Single domain binding proteins may be any in the art or any future single domain binding proteins, and may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine. In some embodiments of the invention, a single domain binding protein scaffold can be derived from a variable region of the immunoglobulin found in fish, such as, for example, that which is derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain binding scaffolds derived from a variable region of NAR ("IgNARs") are described in International Application Publication No. WO 03/014161 and Streltsov (2005) *Protein Sci.* 14(11):2901-09.

[0109] In other embodiments, a single domain binding protein is a naturally occurring single domain binding protein, which has been described in the art as a heavy chain antibody devoid of light chains. Such single domain binding proteins are disclosed in, e.g., International Application Publication No. WO 94/004678. For clarity reasons, a variable domain binding protein that is derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH or "nanobody" to distinguish it from the conventional VH of four-chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in *Camelidae* species, for example in camel, llama, dromedary, alpaca, and guanaco. Other families besides *Camelidae* may also be used to produce heavy chain binding proteins naturally devoid of light chains. VHH molecules are approximately ten times smaller than traditional IgG molecules. They are single polypeptides and are very stable, resisting extreme pH and temperature conditions. Moreover, they are resistant to the action of

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proteases, which is not the case for conventional antibodies. Furthermore, *in vitro* expression of VHHs can produce high-yield, properly folded functional VHHs. In addition, binding proteins generated in Camelids can recognize epitopes other than those recognized by antibodies generated *in vitro* via antibody libraries or via immunization of mammals other than Camelids (see, e.g., International Application Publication Nos. WO 97/049805 and WO 94/004678, both hereby incorporated by reference herein).

[0110] The terms “antigen-binding domain” and “antigen-binding fragment” refer to a part of a binding protein that comprises amino acids responsible for the specific binding between the binding protein and an antigen. The part of the antigen that is specifically recognized and bound by the binding protein is referred to as the “epitope.” An antigen-binding domain may comprise a light chain variable region (V_L) and a heavy chain variable region (V_H) of an antibody; however, it does not have to comprise both. Fd fragments, for example, have two V_H regions and often retain antigen-binding function of the intact antigen-binding domain. Examples of antigen-binding fragments of a binding protein include, but are not limited to: (1) a Fab fragment, a monovalent fragment having V_L , V_H , C_L and C_{H1} domains; (2) a $F(ab')_2$ fragment, a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; (3) an Fd fragment, having two V_H and one C_{H1} domains; (4) an Fv fragment, having the V_L and V_H domains of a single arm of an antibody; (5) a dAb fragment (see, e.g., Ward et al. (1989) *Nature* 341:544-46), having a V_H domain; (6) an isolated CDR; and (7) a single chain variable fragment (scFv).

Although the two domains of an Fv fragment, V_L and V_H are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as scFv) (see, e.g., Bird et al. (1988) *Science* 242:423-26; Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-83). These binding domain fragments can be obtained using conventional techniques

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known to those with skill in the art, and the fragments are evaluated for function in the same manner as are intact binding proteins such as, for example, antibodies.

[0111] The term “neutralizing” refers to a binding protein or antigen-binding fragment thereof (for example, an antibody) that reduces or blocks the activity of a signaling pathway or an antigen, e.g., IL-21/IL-21R signaling pathway or IL-21R antigen. “An anti-product antibody,” as used herein, refers to an antibody formed in response to exogenous protein, e.g., an anti-IL-21R antibody. “A neutralizing anti-product antibody,” as used herein, refers to an anti-product antibody that blocks the *in vivo* activity of the exogenously introduced protein, e.g., an anti-IL-21R antibody. In some embodiments of the invention, a neutralizing anti-product antibody diminishes *in vivo* activity of an IL-21R antibody, e.g., *in vivo* pharmacodynamic (PD) activity of an IL-21R antibody (such as the ability of an anti-IL-21R antibody to modulate expression of IL-21-responsive cytokines or genes).

[0112] The term “effective amount” refers to a dosage or amount that is sufficient to regulate IL-21R activity to ameliorate or lessen the severity of clinical symptoms or achieve a desired biological outcome, e.g., decreased T cell and/or B cell activity, suppression of autoimmunity, suppression of transplant rejection.

[0113] The term “human binding protein” includes binding proteins having variable and constant regions corresponding substantially to human germline immunoglobulin sequences known in the art, including, for example, those described by Kabat et al. (5th ed. 1991) *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example, in the CDRs, and in particular, CDR3. The human antibody can have at least one, two, three, four, five, or more positions replaced with an amino acid residue that is not encoded by the human germline immunoglobulin sequence.

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[0114] The phrases “inhibit,” “antagonize,” “block,” or “neutralize” IL-21R activity and its cognates refer to a reduction, inhibition, or otherwise diminution of at least one activity of IL-21R due to binding an anti-IL-21R antibody, wherein the reduction is relative to the activity of IL-21R in the absence of the same antibody. The IL-21R activity can be measured using any technique known in the art. Inhibition or antagonism does not necessarily indicate a total elimination of the IL-21R biological activity. A reduction in activity may be about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more.

[0115] The terms “interleukin-21 receptor” or “IL-21R” or the like refer to a class I cytokine family receptor, also known as MU-1 (see, e.g., U.S. Patent Application No. 09/569,384 and U.S. Application Publication Nos. 2004/0265960; 2006/0159655; 2006/0024268; and 2008/0241098), NLR or zalpa11 (see, e.g., International Application Publication No. WO 01/085792; Parrish-Novak et al. (2000) *supra*; Ozaki et al. (2000) *supra*), that binds to an IL-21 ligand. IL-21R is homologous to the shared β chain of the IL-2 and IL-15 receptors, and IL-4 α (Ozaki et al. (2000) *supra*). Upon ligand binding, IL-21R is capable of interacting with a common gamma cytokine receptor chain (γ_c) and inducing the phosphorylation of STAT1 and STAT3 (Asao et al. (2001) *supra*) or STAT5 (Ozaki et al. (2000) *supra*). IL-21R shows widespread lymphoid tissue distribution. The terms “interleukin-21 receptor” or “IL-21R” or the like also refer to a polypeptide (preferably of mammalian origin, e.g., murine or human IL-21R) or, as context requires, a polynucleotide encoding such a polypeptide, that is capable of interacting with IL-21 (preferably IL-21 of mammalian origin, e.g., murine or human IL-21) and has at least one of the following features: (1) an amino acid sequence of a naturally occurring mammalian IL-21R polypeptide or a fragment thereof, e.g., an amino acid sequence set forth in SEQ ID NO:2 (human - corresponding to GENBANK[®] (U.S. Dept. of Health and Human Services, Bethesda, MD) Accession No. NP_068570) or SEQ ID NO:4 (murine - corresponding to GENBANK[®] Acc. No. NP_068687), or a fragment thereof; (2) an amino acid sequence substantially homologous to, e.g., at least 85%, 90%, 95%,

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98%, or 99% homologous to, an amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4, or a fragment thereof; (3) an amino acid sequence that is encoded by a naturally occurring mammalian IL-21R nucleotide sequence or fragment thereof (e.g., SEQ ID NO:1 (human - corresponding to GENBANK[®] Accession No. NM_021798) or SEQ ID NO:3 (murine - corresponding to GENBANK[®] Acc. No. NM_021887), or a fragment thereof); (4) an amino acid sequence encoded by a nucleotide sequence that is substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, a nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 or a fragment thereof; (5) an amino acid sequence encoded by a nucleotide sequence degenerate to a naturally occurring IL-21R nucleotide sequence or a fragment thereof, e.g., SEQ ID NO:1 or SEQ ID NO:3, or a fragment thereof; or (6) a nucleotide sequence that hybridizes to one of the foregoing nucleotide sequences under stringent conditions, e.g., highly stringent conditions. In addition, other nonhuman and nonmammalian IL-21Rs are contemplated as useful in the disclosed methods.

[0116] The term “interleukin-21” or “IL-21” refers to a cytokine that shows sequence homology to IL-2, IL-4 and IL-15 (Parrish-Novak et al. (2000) *supra*), and binds to an IL-21R. Such cytokines share a common fold into a “four-helix-bundle” structure that is representative of the family. IL-21 is expressed primarily in activated CD4⁺ T cells, and has been reported to have effects on NK, B and T cells (Parrish-Novak et al. (2000) *supra*; Kasaian et al. (2002) *supra*). Upon IL-21 binding to IL-21R, activation of IL-21R leads to, e.g., STAT5 or STAT3 signaling (Ozaki et al. (2000) *supra*). The term “interleukin-21” or “IL-21” also refers to a polypeptide (preferably of mammalian origin, e.g., murine or human IL-21), or as context requires, a polynucleotide encoding such a polypeptide, that is capable of interacting with IL-21R (preferably of mammalian origin, e.g., murine or human IL-21R) and has at least one of the following features: (1) an amino acid sequence of a naturally occurring mammalian IL-21 or a fragment thereof, e.g., an amino acid sequence set forth in SEQ ID NO:212 (human), or a fragment thereof; (2) an amino

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acid sequence substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, an amino acid sequence set forth in SEQ ID NO:212, or a fragment thereof; (3) an amino acid sequence that is encoded by a naturally occurring mammalian IL-21 nucleotide sequence or a fragment thereof (e.g., SEQ ID NO:211 (human), or a fragment thereof); (4) an amino acid sequence encoded by a nucleotide sequence that is substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, a nucleotide sequence set forth in SEQ ID NO:211 or a fragment thereof; (5) an amino acid sequence encoded by a nucleotide sequence degenerate to a naturally occurring IL-21 nucleotide sequence or a fragment thereof; or (6) a nucleotide sequence that hybridizes to one of the foregoing nucleotide sequences under stringent conditions, e.g., highly stringent conditions.

[0117] The terms “IL-21R activity” and the like (e.g., “activity of IL-21R,” “IL-21/IL-21R activity”) refer to at least one cellular process initiated or interrupted as a result of IL-21R binding. IL-21R activities include, but are not limited to: (1) interacting with, e.g., binding to, a ligand, e.g., an IL-21 polypeptide; (2) associating with or activating signal transduction (also called “signaling,” which refers to the intracellular cascade occurring in response to a particular stimuli) and signal transduction molecules (e.g., gamma chain (γ c) and JAK1), and/or stimulating the phosphorylation and/or activation of STAT proteins, e.g., STAT5 and/or STAT3; (3) modulating the proliferation, differentiation, effector cell function, cytolytic activity, cytokine secretion, and/or survival of immune cells, e.g., T cells, NK cells, B cells, macrophages, regulatory T cells (Tregs) and megakaryocytes; and (4) modulating expression of IL-21-responsive genes or cytokines, e.g., modulating IL-21 effects on the level of expression of, e.g., TNF, IFN γ , IL-6, IL-8, IL-10, CD19, STAT3, ICAM-1, TBX21, CSF1, GZMB, PRF1, IL-2R α , IL-21R, etc.

[0118] As used herein, “*in vitro*-generated antibody” refers to an antibody where all or part of the variable region (e.g., at least one CDR) is generated in a nonimmune cell selection (e.g., an *in vitro* phage display, protein chip, or any other method in which candidate sequences can be tested for their ability to bind to an antigen).

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[0119] The term “isolated” refers to a molecule that is substantially free of its natural environment. For instance, an isolated protein is substantially free of cellular material or other proteins from the cell or tissue source from which it was derived. The term also refers to preparations where the isolated protein is sufficiently pure for pharmaceutical compositions, or is at least 70-80% (w/w) pure, at least 80-90% (w/w) pure, at least 90-95% (w/w) pure, or at least 95%, 96%, 97%, 98%, 99%, or 100% (w/w) pure.

[0120] The phrase “percent identical” or “percent identity” refers to the similarity between at least two different sequences. This percent identity can be determined by standard alignment algorithms, for example, the Basic Local Alignment Search Tool (BLAST) described by Altshul et al. ((1990) *J. Mol. Biol.* 215:403-10); the algorithm of Needleman et al. ((1970) *J. Mol. Biol.* 48:444-53); or the algorithm of Meyers et al. ((1988) *Comput. Appl. Biosci.* 4:11-17). A set of parameters may be the Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide sequences can also be determined using the algorithm of Meyers and Miller ((1989) *CABIOS* 4:11-17), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4. The percent identity is usually calculated by comparing sequences of similar length.

[0121] The term “repertoire” refers to at least one nucleotide sequence derived wholly or partially from at least one sequence encoding at least one immunoglobulin. The sequence(s) may be generated by rearrangement *in vivo* of the V, D, and J segments of heavy chains, and the V and J segments of light chains. Alternatively, the sequence(s) can be generated from a cell in response to which rearrangement occurs, e.g., *in vitro* stimulation. Alternatively, part or all of the sequence(s) may be obtained by DNA splicing, nucleotide synthesis, mutagenesis, or other methods (see, e.g., U.S. Patent No. 5,565,332). A repertoire may include only one sequence or may include a plurality of sequences, including ones in a genetically diverse collection.

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[0122] The terms “specific binding,” “specifically binds,” and the like refer to two molecules forming a complex that is relatively stable under physiologic conditions. Specific binding is characterized by a high affinity and a low-to-moderate capacity as distinguished from nonspecific binding, which usually has a low affinity with a moderate-to-high capacity. Typically, binding is considered specific when the association constant K_a is higher than about $10^6 \text{ M}^{-1} \text{ s}^{-1}$. If necessary, nonspecific binding can be reduced without substantially affecting specific binding by varying the binding conditions. The appropriate binding conditions, such as concentration of binding protein, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent (e.g., serum albumin or milk casein), etc., can be improved by a skilled artisan using routine techniques. Illustrative conditions are set forth herein, but other conditions known to the person of ordinary skill in the art fall within the scope of this invention.

[0123] As used herein, the terms “stringent,” “stringency,” and the like describe conditions for hybridization and washing. The isolated polynucleotides of the present invention can be used as hybridization probes and primers to identify and isolate nucleic acids having sequences identical to or similar to those encoding the disclosed polynucleotides. Therefore, polynucleotides isolated in this fashion may be used to produce binding proteins against IL-21R or to identify cells expressing such binding proteins. Hybridization methods for identifying and isolating nucleic acids include polymerase chain reaction (PCR), Southern hybridizations, *in situ* hybridization and Northern hybridization, and are well known to those skilled in the art.

[0124] Hybridization reactions can be performed under conditions of different stringencies. The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another and the conditions under which they will remain hybridized. Preferably, each hybridizing polynucleotide hybridizes to its corresponding polynucleotide under reduced stringency conditions, more preferably stringent conditions, and most preferably

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highly stringent conditions. Stringent conditions are known to those skilled in the art and can be found in, e.g., *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989) 6.3.1-6.3.6. Both aqueous and nonaqueous methods are described in this reference, and either can be used. One example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by at least one wash in 0.2X SSC / 0.1% SDS at 50°C. Stringent hybridization conditions are also accomplished with wash(es) in, e.g., 0.2X SSC / 0.1% SDS at 55°C, 60°C, or 65°C. Highly stringent conditions include, e.g., hybridization in 0.5M sodium phosphate / 7% SDS at 65°C, followed by at least one wash at 0.2X SSC / 1 % SDS at 65°C. Further examples of stringency conditions are shown in **Table 1** below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Table 1: Hybridization Conditions

Condition	Hybrid	Hybrid Length (bp) ¹	Hybridization Temperature and Buffer ²	Wash Temperature and Buffer ²
A	DNA:DNA	> 50	65°C; 1X SSC -or- 42°C; 1X SSC, 50% formamide	65°C; 0.3X SSC
B	DNA:DNA	< 50	T _B *; 1X SSC	T _B *; 1X SSC
C	DNA:RNA	> 50	67°C; 1X SSC -or- 45°C; 1X SSC, 50% formamide	67°C; 0.3X SSC
D	DNA:RNA	< 50	T _D *; 1X SSC	T _D *; 1X SSC
E	RNA:RNA	> 50	70°C; 1X SSC -or- 50°C; 1X SSC, 50% formamide	70°C; 0.3X SSC
F	RNA:RNA	< 50	T _F *; 1X SSC	T _F *; 1X SSC
G	DNA:DNA	> 50	65°C; 4X SSC -or- 42°C; 4X SSC, 50% formamide	65°C; 1X SSC
H	DNA:DNA	< 50	T _H *; 4X SSC	T _H *; 4X SSC
I	DNA:RNA	> 50	67°C; 4X SSC -or- 45°C; 4X SSC, 50% formamide	67°C; 1X SSC
J	DNA:RNA	< 50	T _J *; 4X SSC	T _J *; 4X SSC

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Condition	Hybrid	Hybrid Length (bp) ¹	Hybridization Temperature and Buffer ²	Wash Temperature and Buffer ²
K	RNA:RNA	> 50	70°C; 4X SSC -or- 50°C; 4X SSC, 50% formamide	67°C; 1X SSC
L	RNA:RNA	< 50	T _L *; 2X SSC	T _L *; 2X SSC
M	DNA:DNA	>50	50°C; 4X SSC -or- 40°C; 6X SSC, 50% formamide	50°C; 2X SSC
N	DNA:DNA	< 50	T _N *; 6X SSC	T _N *; 6X SSC
O	DNA:RNA	> 50	55°C; 4X SSC -or- 42°C; 6X SSC, 50% formamide	55°C; 2X SSC
P	DNA:RNA	< 50	T _P *; 6X SSC	T _P *; 6X SSC
Q	RNA:RNA	> 50	60°C; 4X SSC -or- 45°C; 6X SSC, 50% formamide	60°C; 2X SSC
R	RNA:RNA	< 50	T _R *; 4X SSC	T _R *; 4X SSC

¹ The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

² SSPE (1xSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 min after hybridization is complete.

T_B* - T_R*: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C) = 81.5 + 16.6(log₁₀Na⁺) + 0.41(%G + C) - (600/N), where N is the number of bases in the hybrid, and Na⁺ is the concentration of sodium ions in the hybridization buffer (Na⁺ for 1X SSC = 0.165 M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook et al. (1989) *Molecular Cloning: A Laboratory Manual*, Chs. 9 & 11, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, and Ausubel et al. eds. (1995) *Current Protocols in Molecular Biology* Sects. 2.10 & 6.3-6.4, John Wiley & Sons, Inc., herein incorporated by reference.

[0125] The isolated polynucleotides of the present invention may be used as hybridization probes and primers to identify and isolate DNAs having sequences encoding allelic variants of the disclosed polynucleotides. Allelic variants are naturally occurring alternative forms of the disclosed polynucleotides that encode

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polypeptides that are identical to or have significant similarity to the polypeptides encoded by the disclosed polynucleotides. Preferably, allelic variants have at least about 90% sequence identity (more preferably, at least about 95% identity; most preferably, at least about 99% identity) with the disclosed polynucleotides. The isolated polynucleotides of the present invention may also be used as hybridization probes and primers to identify and isolate DNAs having sequences encoding polypeptides homologous to the disclosed polynucleotides. These homologs are polynucleotides and polypeptides isolated from a different species than that of the disclosed polypeptides and polynucleotides, or within the same species, but with significant sequence similarity to the disclosed polynucleotides and polypeptides. Preferably, polynucleotide homologs have at least about 50% sequence identity (more preferably, at least about 75% identity; most preferably, at least about 90% identity) with the disclosed polynucleotides, whereas polypeptide homologs have at least about 30% sequence identity (more preferably, at least about 45% identity; most preferably, at least about 60% identity) with the disclosed binding proteins / polypeptides. Preferably, homologs of the disclosed polynucleotides and polypeptides are those isolated from mammalian species. The isolated polynucleotides of the present invention may additionally be used as hybridization probes and primers to identify cells and tissues that express the binding proteins of the present invention and the conditions under which they are expressed.

[0126] The phrases “substantially as set out,” “substantially identical,” and “substantially homologous” mean that the relevant amino acid or nucleotide sequence (e.g., CDR(s), V_H, or V_L domain(s)) will be identical to or have insubstantial differences (e.g., through conserved amino acid substitutions) in comparison to the sequences which are set out. Insubstantial differences include minor amino acid changes, such as one or two substitutions in a five amino acid sequence of a specified region. In the case of antibodies, the second antibody has the same specificity and has at least about 50% of the affinity of the first antibody.

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[0127] Sequences substantially identical or homologous to the sequences disclosed herein are also part of this application. In some embodiments, the sequence identity can be about 85%, 90%, 95%, 96%, 97%, 98%, 99%, or higher. Alternatively, substantial identity or homology exists when the nucleic acid segments will hybridize under selective hybridization conditions (e.g., highly stringent hybridization conditions), to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

[0128] The term “therapeutic agent” or the like is a substance that treats or assists in treating a medical disorder or symptoms thereof. Therapeutic agents may include, but are not limited to, substances that modulate immune cells or immune responses in a manner that complements the use of anti-IL-21R binding proteins. In one embodiment of the invention, a therapeutic agent is a therapeutic antibody, e.g., an anti-IL-21R antibody. In another embodiment of the invention, a therapeutic agent is a therapeutic binding protein, e.g., an anti-IL-21R nanobody. Nonlimiting examples and uses of therapeutic agents are described herein.

[0129] As used herein, a “therapeutically effective amount” of an anti-IL-21R binding protein (e.g., an antibody) refers to an amount of the binding protein that is effective, upon single or multiple dose administration to a subject (such as a human patient) for treating, preventing, curing, delaying, reducing the severity of, and/or ameliorating at least one symptom of a disorder or a recurring disorder, or prolonging the survival of the subject beyond that expected in the absence of such treatment. In one embodiment, a therapeutically effective amount may be an amount of an anti-IL-21R binding protein that is sufficient to modulate expression of at least one IL-21-responsive cytokine or gene.

[0130] The term “treatment” refers to a therapeutic or preventative measure. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay, reduce the severity of, and/or ameliorate one or more symptoms of a disorder or a recurring

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disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

Anti-IL-21R Binding Proteins

[0131] The disclosure of the present application provides novel anti-IL-21R binding proteins that comprise novel antigen-binding fragments. Numerous methods known to those skilled in the art are available for obtaining binding proteins or antigen-binding fragments thereof. For example, anti-IL-21R binding proteins that comprise antibodies can be produced using recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). Monoclonal antibodies may also be produced by generation of hybridomas in accordance with known methods (see, e.g., Kohler and Milstein (1975) *Nature* 256:495-99). Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assays (ELISA) and surface plasmon resonance (BIAcore™) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a particular antigen. Any form of the specified antigen may be used as the immunogen, e.g., recombinant antigen, naturally occurring forms, any variants or fragments thereof, and antigenic peptides thereof.

[0132] One exemplary method of making binding proteins that comprise antibodies includes screening protein expression libraries, e.g., phage or ribosome display libraries. Phage display is described, for example, in U.S. Patent No. 5,223,409; Smith (1985) *Science* 228:1315-17; Clackson et al. (1991) *Nature* 352:624-28; Marks et al. (1991) *J. Mol. Biol.* 222:581-97; and International Application Publication Nos. WO 92/018619; WO 91/017271; WO 92/020791; WO 92/015679; WO 93/001288; WO 92/001047; WO 92/009690; and WO 90/002809.

[0133] In addition to the use of display libraries, the specified antigen can be used to immunize a nonhuman animal, e.g., a cynomolgus monkey, a chicken, or a rodent (e.g., a mouse, hamster, or rat). In one embodiment, the nonhuman animal includes at least a part of a human immunoglobulin gene. For example, it is possible to

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engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci. Using the hybridoma technology, antigen-specific monoclonal binding proteins, such as antibodies, derived from the genes with the desired specificity may be produced and selected (see, e.g., XENOMOUSE™ (Amgen, Inc., Thousand Oaks, CA); Green et al. (1994) *Nat. Genet.* 7:13-21; U.S. Patent No. 7,064,244; and International Application Publication Nos. WO 96/034096 and WO 96/033735).

[0134] In one embodiment of the invention, the binding proteins is a monoclonal antibody that is obtained from a nonhuman animal, and then modified (e.g., humanized, deimmunized, or chimeric) using recombinant DNA techniques known in the art. A variety of approaches for making chimeric antibodies have been described (see, e.g., Morrison et al. (1985) *Proc. Natl. Acad. Sci. USA* 81(21):6851-55; Takeda et al. (1985) *Nature* 314(6010):452-54; U.S. Patent Nos. 4,816,567 and 4,816,397; European Application Publication Nos. EP 0 171 496 and EP 0 173 494; and United Kingdom Patent No. GB 2 177 096). Humanized binding proteins may also be produced, for example, using transgenic mice that express human heavy and light chain genes, but are incapable of expressing the endogenous mouse immunoglobulin heavy and light chain genes. Winter (U.S. Patent No. 5,225,539) describes an exemplary CDR-grafting method that may be used to prepare the humanized binding proteins described herein. All of the CDRs of a particular human binding protein may be replaced with at least a portion of a nonhuman CDR, or only some of the CDRs may be replaced with nonhuman CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized binding protein to a predetermined antigen.

[0135] Humanized binding proteins or fragments thereof can be generated by replacing sequences of the Fv variable domain that are not directly involved in antigen binding with equivalent sequences from human Fv variable domains. Exemplary methods for generating humanized binding proteins or fragments thereof are provided by, e.g., Morrison (1985) *Science* 229:1202-07; Oi et al. (1986)

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BioTechniques 4:214; and U.S. Patent Nos. 5,585,089; 5,693,761; 5,693,762; 5,859,205; and 6,407,213. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable domains from at least one of a heavy or light chain. Such nucleic acids may be obtained from a hybridoma producing an antibody against a predetermined target, as described above, as well as from other sources. The recombinant DNA encoding the humanized antibody molecule can then be cloned into an appropriate expression vector.

[0136] In certain embodiments, a humanized binding protein is improved by the introduction of conservative substitutions, consensus sequence substitutions, germline substitutions and/or backmutations. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, (see, e.g., Teng et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:7308-73; Kozbor et al. (1983) *Immunol. Today* 4:7279; Olsson et al. (1982) *Meth. Enzymol.* 92:3-16); International Application Publication No. WO 92/006193; and European Patent No. EP 0 239 400).

[0137] A binding protein or fragment thereof may also be modified by specific deletion of human T cell epitopes or “deimmunization” by the methods disclosed in, e.g., International Application Publication Nos. WO 98/052976 and WO 00/034317. Briefly, the heavy and light chain variable domains of a binding protein (such as, for example, a binding protein derived from an antibody) can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T cell epitopes (as defined in, e.g., International Application Publication Nos. WO 98/052976 and WO 00/034317). For detection of potential T cell epitopes, a computer modeling approach termed “peptide threading” can be applied and, in addition, a database of human MHC class II binding peptides can be searched for motifs present in the V_H and V_L sequences, as described in International Application Publication Nos. WO 98/052976 and WO 00/034317. These motifs bind to any of the 18 major MHC Class II DR allotypes and thus, constitute potential T cell epitopes. Potential T cell

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epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable domains or by single amino acid substitutions. Typically, conservative substitutions are made. Often, but not exclusively, an amino acid common to a position in human germline antibody sequences may be used. Human germline sequences are disclosed in, e.g., Tomlinson et al. (1992) *J. Mol. Biol.* 227:776-98; Cook et al. (1995) *Immunol. Today* 16(5):237-42; Chothia et al. (1992) *J. Mol. Biol.* 227:799-817; and Tomlinson et al. (1995) *EMBO J.* 14:4628-38. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson et al., MRC Centre for Protein Engineering, Cambridge, UK). These sequences can be used as a source of human sequence, e.g., for framework regions and CDRs. Consensus human framework regions can also be used, as described in, e.g., U.S. Patent No. 6,300,064.

[0138] In certain embodiments, a binding protein can contain an altered immunoglobulin constant or Fc region. For example, binding proteins produced in accordance with the teachings herein may bind more strongly or with more specificity to effector molecules such as complement and/or Fc receptors, which can control several immune functions of the binding protein such as effector cell activity, lysis, complement-mediated activity, binding protein clearance, and binding protein half-life. Typical Fc receptors that bind to an Fc region of a binding protein (e.g., an IgG antibody) include, but are not limited to, receptors of the Fc γ RI, Fc γ RII, and FcRn subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc receptors are reviewed in, e.g., Ravetch and Kinet (1991) *Annu. Rev. Immunol.* 9:457-92; Capel et al. (1994) *Immunomethods* 4:25-34; and de Haas et al. (1995) *J. Lab. Clin. Med.* 126:330-41. For additional binding protein / antibody production techniques, see, e.g., *Antibodies: A Laboratory Manual* (1988) Harlow et al. eds., Cold Spring Harbor Laboratory. The present invention is not necessarily limited to any particular source, method of production, or other special characteristic of a binding protein or an antibody.

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[0139] Binding proteins comprising antibodies (immunoglobulins) are typically tetrameric glycosylated proteins composed of two light (L) chains of approximately 25 kDa each and two heavy (H) chains of approximately 50 kDa each. Two types of light chains, termed lambda (λ) and kappa (κ), may be found in antibodies. Depending on the amino acid sequence of the constant domain of heavy chains, immunoglobulins can be assigned to five major classes: A, D, E, G, and M, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. Each light chain includes an N-terminal variable (V) domain (V_L) and a constant (C) domain (C_L). Each heavy chain includes an N-terminal V domain (V_H), three or four C domains (C_{HS}), and a hinge region. The C_H domain most proximal to V_H is designated as C_{H1} . The V_H and V_L domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4) that form a scaffold for three regions of hypervariable sequences, called CDRs. The CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen. CDRs are referred to as CDR1, CDR2, and CDR3. CDR constituents on the heavy chain are referred to as H1, H2, and H3 (also referred to herein as CDR H1, CDR H2, and CDR H3, respectively), while CDR constituents on the light chain are referred to as L1, L2, and L3 (also referred to herein as CDR L1, CDR L2, and CDR L3, respectively).

[0140] CDR3 is typically the greatest source of molecular diversity within the antigen-binding site. CDR H3, for example, can be as short as two amino acid residues or greater than 26 amino acids. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in the art. For a review of antibody structure, see, e.g., Harlow et al. (1988) *supra*. One of skill in the art will recognize that each subunit structure, e.g., a C_H , V_H , C_L , V_L , CDR, and/or FR structure, comprises active fragments, e.g., the portion of the V_H , V_L , or CDR subunit that binds to the antigen, i.e., the antigen-binding fragment, or, e.g., the portion of the C_H subunit that binds to and/or activates, e.g., an Fc receptor and/or complement. The CDRs typically refer to the Kabat CDRs (as

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described in Kabat et al. (1991) *supra*). Another standard for characterizing the antigen-binding site is to refer to the hypervariable loops as described in, e.g., Chothia et al. (1992) *supra* and Tomlinson et al. (1995) *supra*. Still another standard is the “AbM” definition used by Oxford Molecular’s AbM antibody modeling software (see, generally, e.g., *Protein Sequence and Structure Analysis of Antibody Variable Domains* in: *Antibody Engineering* (2001) Duebel and Kontermann eds., Springer-Verlag, Heidelberg). Embodiments described with respect to Kabat CDRs can alternatively be implemented using similar described relationships with respect to Chothia hypervariable loops or to the AbM-defined loops.

[0141] The Fab fragment consists of V_H - C_H1 and V_L - C_L domains covalently linked by a disulfide bond between the constant regions. The F_v fragment is smaller and consists of V_H and V_L domains noncovalently linked. To overcome the tendency of noncovalently linked domains to dissociate, an scFv can be constructed. The scFv contains a flexible polypeptide that links (1) the C-terminus of V_H to the N-terminus of V_L , or (2) the C-terminus of V_L to the N-terminus of V_H . A 15-mer $(Gly_4Ser)_3$ peptide, for example, may be used as a linker, but other linkers are known in the art.

[0142] The sequence of antibody genes after assembly and somatic mutation is highly varied, and these varied genes are estimated to encode 10^{10} different antibody molecules (*Immunoglobulin Genes* (2nd ed. 1995) Jonio et al. eds., Academic Press, San Diego, CA).

[0143] In certain embodiments of the invention, the binding protein is a single domain binding protein. Single domain binding proteins include binding proteins wherein the CDRs are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain binding proteins, binding proteins that are naturally devoid of light chains, single domain binding proteins derived from conventional four-chain antibodies, engineered binding proteins, and single domain protein scaffolds other than those derived from antibodies. Single domain binding proteins include any known in the art, as well as any future-determined or -learned single domain binding proteins.

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[0144] Single domain binding proteins may be derived from any species including, but not limited to, mouse, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine. In one aspect of the invention, the single domain binding protein can be derived from a variable region of the immunoglobulin found in fish, such as, for example, that which is derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain binding proteins derived from a variable region of NAR (IgNARs) are described in, e.g., International Application Publication No. WO 03/014161 and Streltsov (2005) *Protein Sci.* 14:2901-09. Single domain binding proteins also include naturally occurring single domain binding proteins known in the art as heavy chain antibodies devoid of light chains. This variable domain derived from a heavy chain antibody naturally devoid of a light chain is known herein as a VHH, or a nanobody, to distinguish it from the conventional V_H of four-chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in *Camelidae* species, for example, in camel, llama, dromedary, alpaca, and guanaco, and is sometimes called a camelid or camelized variable domain (see, e.g., Muyldermans (2001) *J. Biotechnol.* 74(4):277-302, incorporated herein by reference). Other species besides those in the family *Camelidae* may also produce heavy chain binding proteins naturally devoid of light chains. VHH molecules are about ten times smaller than IgG molecules. They are single polypeptides and are very stable, resisting extreme pH and temperature conditions. Moreover, they are resistant to the actions of proteases, which is not the case for conventional antibodies. Furthermore, *in vitro* expression of VHHs can produce high-yield, properly folded functional VHHs. In addition, binding proteins generated in camelids will recognize epitopes other than those recognized by antibodies generated *in vitro* via antibody libraries or via immunization of mammals other than camelids (see, e.g., International Application Publication Nos. WO 97/049805 and WO 94/004678, which are incorporated herein by reference).

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[0145] A “bispecific” or “bifunctional” binding protein is an artificial hybrid binding protein having two different heavy/light chain pairs and two different binding sites. Bispecific binding proteins can be produced by a variety of methods including fusion of hybridomas or linking of Fab’ fragments (see, e.g., Songsivilai and Lachmann (1990) *Clin. Exp. Immunol.* 79:315-21; Kostelny et al. (1992) *J. Immunol.* 148:1547-53). In one embodiment, the bispecific binding protein comprises a first binding domain polypeptide, such as a Fab’ fragment, linked via an immunoglobulin constant region to a second binding domain polypeptide.

[0146] Another binding protein according to the invention can comprise, for example, a binding domain-immunoglobulin fusion protein that includes a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain, other than C_H1, for example, the C_H2 and C_H3 regions of IgG and IgA1 or the C_H3 and C_H4 regions of IgE (see, e.g., U.S. Application Publication No. 2005/0136049, which is incorporated by reference herein, for a more complete description). The binding domain-immunoglobulin fusion protein can further include a region that includes a native or engineered immunoglobulin heavy chain C_H2 constant region polypeptide (or C_H3 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide and a native or engineered immunoglobulin heavy chain C_H3 constant region polypeptide (or C_H4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the C_H2 constant region polypeptide (or C_H3 in the case of a construct derived in whole or in part from IgE). Typically, such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody-dependent cell-mediated cytotoxicity (ADCC), complement fixation, and/or binding to a target, for example, a target antigen, such as human IL-21R.

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[0147] Binding proteins of the invention can also comprise peptide mimetics. Peptide mimetics are peptide-containing molecules that mimic elements of protein secondary structure (see, for example, Johnson et al. *Peptide Turn Mimetics* in *Biotechnology and Pharmacy* (1993), Pezzuto et al. eds., Chapman and Hall, New York, incorporated by reference herein in its entirety). The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those between antibody and antigen. A peptide mimetic is expected to permit molecular interactions similar to the natural molecule. These principles may be used to engineer second generation molecules having many of the natural properties of the targeting peptides disclosed herein, but with altered and potentially improved characteristics.

[0148] Other embodiments of binding proteins useful for practicing the invention include fusion proteins. These molecules generally have all or a substantial portion of a targeting peptide, for example, IL-21R or an anti IL-21R antibody, linked at the N- or C-terminus, to all or a portion of a second polypeptide or protein. For example, fusion proteins may employ leader (or signal) sequences from other species to permit the recombinant expression of a protein in a heterologous host. For example, amino acid sequences, or nucleic acid sequences encoding amino acid sequences, of the binding proteins and antigen-binding fragments thereof of the present invention comprising leader (or signal) sequence may be selected from SEQ ID NOs:87-109 and 239-248. Another useful fusion includes the addition of an immunologically active domain, such as a binding protein epitope, to facilitate purification of the fusion protein. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other useful fusions include the linking of functional domains, such as active sites from enzymes, glycosylation domains, cellular targeting signals, or transmembrane regions. Examples of proteins or peptides that may be incorporated into a fusion protein include, but are not limited to, cytostatic proteins, cytotoxic proteins, pro-

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apoptosis agents, anti-angiogenic agents, hormones, cytokines, growth factors, peptide drugs, antibodies, Fab fragments of antibodies, antigens, receptor proteins, enzymes, lectins, MHC proteins, cell adhesion proteins, and binding proteins. Methods of generating fusion proteins are well known to those of skill in the art. Such proteins can be produced, for example, by chemical attachment using bifunctional cross-linking reagents, by de novo synthesis of the complete fusion protein, or by attachment of a DNA sequence encoding the targeting peptide to a DNA sequence encoding the second peptide or protein, followed by expression of the intact fusion protein.

[0149] In one embodiment, the targeting peptide, for example, IL-21R, is fused with an immunoglobulin heavy chain constant region, such as an Fc fragment, which contains two constant region domains and a hinge region, but lacks the variable region (see, e.g., U.S. Patent Nos. 6,018,026 and 5,750,375, incorporated by reference herein). The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, e.g., therapeutic qualities, circulation time, reduced aggregation. Peptides and proteins fused to an Fc region typically exhibit a greater half-life *in vivo* than the unfused counterpart does. In addition, a fusion to an Fc region permits dimerization / multimerization of the fusion polypeptide.

[0150] One aspect of the present invention comprises binding proteins and antigen-binding fragments thereof that bind IL-21R. The disclosure provides novel CDRs that have been derived from human immunoglobulin gene libraries. The protein structure that is generally used to carry a CDR is an antibody heavy or light chain or a portion thereof, wherein the CDR is localized to a region associated with a naturally occurring CDR. The structures and locations of variable domains may be determined as described in Kabat et al. ((1991) *supra*).

[0151] Illustrative embodiments of the binding proteins (and antigen-binding fragments thereof) of the invention are identified as AbA-AbU, H3-H6, L1-L6, L8-L21, and L23-L25. DNA and amino acid sequences of the nonlimiting illustrative embodiments of the anti-IL-21R binding proteins of the invention are set

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forth in SEQ ID NOs:5-195, 213-229, and 239-248. DNA and amino acid sequences of some illustrative embodiments of the anti-IL-21R binding proteins of the invention, including their scFv fragments, V_H and V_L domains, and CDRs, as well as their present codes and previous designations, are set forth in **FIGs. 17-25**, and **Tables 2A** and **2B**.

Table 2A: Correlation of Present Antibody Codes and Previous Designations

Present Code	Previous Designation
AbA	VHP/VL2
AbB	VHP/VL3
AbC	VHP/VL11
AbD	VHP/VL13
AbE	VHP/VL14
AbF	VHP/VL17
AbG	VHP/VL18
AbH	VHP/VL19
AbI	VHP/VL24
AbJ	VH3/VLP
AbK	VH3/VL3
AbL	VH3/VL13
AbM	VH6/VL13
AbN	VH6/VL24
AbO	VHP/VL16; VHPTM/VL16
AbP	VHP/VL20; VHPTM/VL20
AbQ	VH3/VL2; VH3DM/VL2
AbR	VH3/VL18; VH3DM/VL18
AbS	VHP/VL6; VHPTM/VL6; VL6
AbT	VHP/VL9; VHPTM/VL9; VL9
AbU	VHP/VL25; VHPTM/VL25
AbV	VH3TM/VL2
AbW	VH3TM/VL18
AbX	VHPDM/VL9
AbY	VHPg4/VL9
AbZ	VHPWT/VL9

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Table 2B: Amino Acid and Nucleotide Sequences of V_H and V_L Domains, scFv, and CDRs of Illustrative Binding Proteins of the Invention

REGION	TYPE	H3 SEQ ID	H4 SEQ ID	H5 SEQ ID	H6 SEQ ID	L1 SEQ ID
V _H	AA	NO:14	NO:16	NO:18	NO:20	NO:6
V _L	AA	NO:10	NO:10	NO:10	NO:10	NO:22
scFv	AA	NO:110	NO:112	NO:114	NO:116	NO:118
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:165	NO:166	NO:167	NO:168	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:170	NO:170	NO:170	NO:170	NO:171
V _H	DNA	NO:13	NO:15	NO:17	NO:19	NO:5
V _L	DNA	NO:9	NO:9	NO:9	NO:9	NO:21
scFv	DNA	NO:109	NO:111	NO:113	NO:115	NO:117

Table 2B (continued)

REGION	TYPE	L2 SEQ ID	L3 SEQ ID	L4 SEQ ID	L5 SEQ ID	L6 SEQ ID
V _H	AA	NO:6	NO:6	NO:6	NO:6	NO:6
V _L	AA	NO:24	NO:26	NO:28	NO:30	NO:32
scFv	AA	NO:120	NO:122	NO:124	NO:126	NO:128
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:172	NO:173	NO:174	NO:175	NO:176
V _H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V _L	DNA	NO:23	NO:25	NO:27	NO:29	NO:31
scFv	DNA	NO:119	NO:121	NO:123	NO:125	NO:127

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Table 2B (continued)

REGION	TYPE	L8 SEQ ID	L9 SEQ ID	L10 SEQ ID	L11 SEQ ID	L12 SEQ ID
V _H	AA	NO:6	NO:6	NO:6	NO:6	NO:6
V _L	AA	NO:34	NO:36	NO:38	NO:40	NO:42
scFv	AA	NO:130	NO:132	NO:134	NO:136	NO:138
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:177	NO:178	NO:179	NO:180	NO:181
V _H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V _L	DNA	NO:33	NO:35	NO:37	NO:39	NO:41
scFv	DNA	NO:129	NO:131	NO:133	NO:135	NO:137

Table 2B (continued)

REGION	TYPE	L13 SEQ ID	L14 SEQ ID	L15 SEQ ID	L16 SEQ ID	L17 SEQ ID
V _H	AA	NO:6	NO:6	NO:6	NO:6	NO:6
V _L	AA	NO:44	NO:46	NO:48	NO:50	NO:52
scFv	AA	NO:140	NO:142	NO:144	NO:146	NO:148
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:182	NO:183	NO:184	NO:185	NO:186
V _H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V _L	DNA	NO:43	NO:45	NO:47	NO:49	NO:51
scFv	DNA	NO:139	NO:141	NO:143	NO:145	NO:147

Table 2B (continued)

REGION	TYPE	L18 SEQ ID	L19 SEQ ID	L20 SEQ ID	L21 SEQ ID	L23 SEQ ID
V _H	AA	NO:6	NO:6	NO:6	NO:6	NO:6
V _L	AA	NO:54	NO:56	NO:58	NO:60	NO:62
scFv	AA	NO:150	NO:152	NO:154	NO:156	NO:158
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:187	NO:188	NO:189	NO:190	NO:191
V _H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V _L	DNA	NO:53	NO:55	NO:57	NO:59	NO:61
scFv	DNA	NO:149	NO:151	NO:153	NO:155	NO:157

Table 2B (continued)

REGION	TYPE	L24 SEQ ID	L25 SEQ ID
V _H	AA	NO:6	NO:6
V _L	AA	NO:64	NO:66
scFv	AA	NO:160	NO:162
CDR H1	AA	NO:163	NO:163
CDR H2	AA	NO:164	NO:164
CDR H3	AA	NO:169	NO:169
CDR L1	AA	NO:194	NO:194
CDR L2	AA	NO:195	NO:195
CDR L3	AA	NO:192	NO:193
V _H	DNA	NO:5	NO:5
V _L	DNA	NO:63	NO:65
scFv	DNA	NO:159	NO:161

[0152] Anti-IL-21R binding proteins of the present invention may comprise antibody constant regions or parts thereof. For example, a V_L domain may be attached at its C-terminal end to a light chain constant domain like C_κ or C_λ. Similarly, a V_H domain, or portion thereof, may be attached to all or part of a heavy chain like IgA, IgD, IgE, IgG, and IgM, and any isotype subclass. Constant regions are known in the art (see, e.g., Kabat et al. (1991) *supra*). Therefore, binding

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proteins within the scope of this invention include V_H and V_L domains, or portions thereof, combined with constant regions known in the art.

[0153] Certain embodiments comprise a V_H domain, a V_L domain, or a combination thereof, of the Fv fragment from AbA-AbZ, H3-H6, L1-L6, L8-L21, and/or L23-L25. Further embodiments comprise one, two, three, four, five or six CDRs from the V_H and V_L domains. Binding proteins whose CDR sequence(s) are the same as, or similar to (i.e., differ insubstantially from), one or more CDR sequence(s) present within the sequences set forth in SEQ ID NOs:5-195, 213-229, and 239-248 are encompassed within the scope of the invention.

[0154] In certain embodiments, the V_H and/or V_L domains may be germlined, i.e., the FR of these domains are mutated using conventional molecular biology techniques to match those produced by the germline cells. In other embodiments, the FR sequences remain diverged from the consensus germline sequences.

[0155] In one embodiment, mutagenesis is used to make a binding protein more similar to one or more germline sequences. This may be desirable when mutations are introduced into the FR of a binding protein (e.g., an antibody) through somatic mutagenesis or through error prone PCR. Germline sequences for the V_H and V_L domains can be identified by performing amino acid and nucleic acid sequence alignments against the VBASE database (MRC Center for Protein Engineering, UK). VBASE is a comprehensive directory of all human germline variable region sequences compiled from over a thousand published sequences, including those in the current releases of the GENBANK[®] and EMBL data libraries. In some embodiments, the FRs of scFvs are mutated in conformity with the closest matches in the VBASE database and the CDR portions are kept intact.

[0156] In certain embodiments, binding proteins of the invention specifically react with an epitope that is the same as the epitope recognized by AbA-AbZ, H3-H6, L1-L6, L8-L21, or L23-L25, such that they competitively inhibit the binding of AbA-AbZ, H3-H6, L1-L6, L8-L21, or L23-L25 to human IL-21R. Such binding proteins can be determined in competitive binding assays. In one embodiment, the

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association constant (K_A) of these binding proteins for human IL-21R is at least $10^5 \text{ M}^{-1} \text{ s}^{-1}$. The binding affinity may be determined using techniques known in the art, such as ELISA, biosensor technology (such as biospecific interaction analysis) or other techniques, including those described in this application.

[0157] It is contemplated that binding proteins of the invention may bind other proteins, such as, for example, recombinant proteins comprising all or a portion of IL-21R.

[0158] One of ordinary skill in the art will recognize that the disclosed binding proteins may be used to detect, measure, and/or inhibit proteins that differ somewhat from IL-21R. For example, these proteins may be homologs of IL-21R. Anti-IL-21R binding proteins are expected to bind proteins that comprise a sequence that is at least about 60%, 70%, 80%, 90%, 95%, or more identical to any sequence of at least 100, 80, 60, 40, or 20 contiguous amino acids in the sequence set forth SEQ ID NOs:2 or 4.

[0159] In addition to sequence homology analyses, epitope mapping (see, e.g., *Epitope Mapping Protocols* (1996) Morris ed., Humana Press), and secondary and tertiary structure analyses can be carried out to identify specific 3D structures assumed by the presently disclosed binding proteins and their complexes with antigens. Such methods include, but are not limited to, x-ray crystallography (Engstrom (1974) *Biochem. Exp. Biol.* 11:7-13) and computer modeling of virtual representations of the present binding proteins (Fletterick et al. (1986) *Computer Graphics and Molecular Modeling, in Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0160] The disclosure provides a method for obtaining anti-IL-21R binding proteins. The method comprises creating binding proteins with V_H and/or V_L sequence(s) that are altered from those sequences disclosed herein. Such binding proteins may be derived by a skilled artisan using techniques known in the art. For example, amino acid substitutions, deletions, or additions can be introduced in FR and/or CDR regions. FR changes are usually designed to improve the stability and

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immunogenicity of the binding protein, while CDR changes are typically designed to increase a binding protein's affinity for its antigen. The changes that increase affinity may be tested by altering one or more CDR sequences and measuring the affinity of the binding protein for its target (see, e.g., *Antibody Engineering* (2nd ed. 1995) Borrebaeck ed., Oxford University Press).

[0161] Binding proteins whose CDR sequences differ insubstantially from those set forth in or included within the sequences of SEQ ID NOs:5-195, 213-229, and 239-248 are encompassed within the scope of this invention. Typically, such an insubstantial difference(s) involves substitution of an amino acid with an amino acid having similar charge, hydrophobicity, or stereochemical characteristics. More drastic substitutions in FR regions, in contrast to CDR regions, may also be made as long as they do not adversely affect (e.g., reduce affinity by more than 50% as compared to the unsubstituted binding protein) the binding properties of the binding protein. Substitutions may also be made to germline the binding protein or stabilize its antigen-binding site.

[0162] Conservative modifications will produce molecules having functional and chemical characteristics similar to those of the molecule from which such modifications are made. In contrast, substantial modifications in the functional and/or chemical characteristics of the molecules may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (1) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (2) the charge or hydrophobicity of the molecule at the target site, and/or (3) the size of the molecule.

[0163] For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position (see, e.g., MacLennan et al. (1998) *Acta Physiol. Scand. Suppl.* 643:55-67; Sasaki et al. (1998) *Adv. Biophys.* 35:1-24).

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[0164] Desired amino acid substitutions (whether conservative or nonconservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the molecule sequence, or to increase or decrease the affinity of the molecules described herein. Exemplary amino acid substitutions include, but are not limited to, those set forth in **Table 3**.

Table 3: Exemplary Amino Acid Substitutions

Original Residues	Exemplary Substitutions	More Conservative Substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4-diamino-butyric acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala, Gly	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0165] In certain embodiments, conservative amino acid substitutions also encompass nonnaturally occurring amino acid residues that are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

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[0166] In one embodiment, the method for making a variant V_H domain comprises adding, deleting, or substituting at least one amino acid in the disclosed V_H domains, or combining the disclosed V_H domains with at least one V_L domain, and testing the variant V_H domain for IL-21R binding or modulation of IL-21R/IL-21 activity.

[0167] An analogous method for making a variant V_L domain comprises adding, deleting, or substituting at least one amino acid in the disclosed V_L domains, or combining the disclosed V_L domains with at least one V_H domain, and testing the variant V_L domain for IL-21R binding or modulation of IL-21R activity.

[0168] In some alternative embodiments, the anti-IL-21R binding proteins can be linked to a protein (e.g., albumin) by chemical cross-linking or recombinant methods. The disclosed binding proteins may also be linked to a variety of nonproteinaceous polymers (e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes) in manners set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; and 4,179,337. The binding proteins can be chemically modified by covalent conjugation to a polymer, for example, to increase their half-life in blood circulation. Exemplary polymers and attachment methods are shown in U.S. Patent Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546.

[0169] The disclosed binding proteins can be modified to alter their glycosylation; that is, at least one carbohydrate moiety can be deleted or added to the binding protein. Deletion or addition of glycosylation sites can be accomplished by changing amino acid sequence to delete or create glycosylation consensus sites, which are well known in the art. Another means of adding carbohydrate moieties is the chemical or enzymatic coupling of glycosides to amino acid residues of the binding protein, e.g., antibody (see, e.g., International Application Publication No. WO 87/05330 and Aplin et al. (1981) *CRC Crit. Rev. Biochem.* 22:259-306). Removal of carbohydrate moieties can also be accomplished chemically or enzymatically (see, e.g., Hakimuddin et al. (1987) *Arch. Biochem. Biophys.* 259:52; Edge et al. (1981) *Anal. Biochem.* 118:131; and Thotakura et al. (1987) *Meth. Enzymol.* 138:350). Modification of carbohydrate structures may be preferable as amino acid changes in

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the Fc domain may enhance immunogenicity of a pharmaceutical composition (see, e.g., International Application Publication No. WO 2008/052030). For immunoglobulin molecules, it has been demonstrated that attachment of N-linked carbohydrate to Asn-297 of the CH2 domain is critical for ADCC activity. Its removal enzymatically or through mutation of the N-linked consensus site results in little to no ADCC activity. In glycoproteins, carbohydrates may attach to the amide nitrogen atom in the side chain of an asparagine in a tripeptide motif Asn-X-Thr/Ser. This type of glycosylation, termed N-linked glycosylation, commences in the endoplasmic reticulum (ER) with the addition of multiple monosaccharides to a dolichol phosphate to form a 14-residue branched carbohydrate complex. This carbohydrate complex is then transferred to the protein by the oligosaccharyltransferase (OST) complex. Before the glycoprotein leaves the lumen of the ER, three glucose molecules are removed from the 14-residue oligosaccharide. The enzymes ER glucosidase I, ER glucosidase II and ER mannosidase are involved in ER processing. Subsequently, the polypeptides are transported to the Golgi complex, where the N-linked sugar chains are modified in many different ways. In the cis and medial compartments of the Golgi complex, the original 14-saccharide N-linked complex may be trimmed through removal of mannose (Man) residues and elongated through addition of N-acetylglucosamine (GlcNac) and/or fucose (Fuc) residues. The various forms of N-linked carbohydrates generally have in common a pentasaccharide core consisting of three mannose and two N-acetylglucosamine residues. Finally, in the trans Golgi, other GlcNac residues can be added, followed by galactose (Gal) and a terminal sialic acid (Sial). Carbohydrate processing in the Golgi complex is called “terminal glycosylation” to distinguish it from “core glycosylation,” which takes place in the ER. The final complex carbohydrate units can take on many forms and structures, some of which have two, three or four branches (termed biantennary, triantennary or tetraantennary). A number of enzymes are involved in Golgi processing, including Golgi mannosidases IA, IB and IC,

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GlcNAc-transferase I, Golgi mannosidase II, GlcNAc-transferase II, galactosyl transferase and sialyl transferase.

[0170] Methods for altering the constant region of a binding protein (such as, for example, the constant region of an antibody) are known in the art. Binding proteins with altered function (e.g., altered affinity for an effector ligand such as FcR on a cell or the C1 component of complement) can be produced by replacing at least one amino acid residue in the constant portion with a different residue (see, e.g., European Application Publication No. EP 0 388 151 and U.S. Patent Nos. 5,624,821 and 5,648,260). Similar types of alterations could be described that, if applied to a murine or other species of binding protein, would reduce or eliminate similar functions.

[0171] For example, it is possible to alter the affinity of an Fc region of a binding protein (e.g., an IgG, such as a human IgG) for FcR (e.g., Fc gamma R1) or C1q. The affinity may be altered by replacing at least one specified residue with at least one residue having an appropriate functionality on its side chain, or by introducing a charged functional group, such as glutamate or aspartate, or perhaps an aromatic nonpolar residue such as phenylalanine, tyrosine, tryptophan or alanine (see, e.g., U.S. Patent No. 5,624,821).

[0172] For example, replacing residue 297 (asparagine) with alanine in the IgG constant region significantly inhibits recruitment of effector cells, while only slightly reducing (about three-fold weaker) affinity for C1q (see, e.g., U.S. Patent No. 5,624,821). The numbering of the residues in the heavy chain is that of the EU index (see Kabat et al. (1991) *supra*). This alteration destroys the glycosylation site, and it is believed that the presence of carbohydrate is required for Fc receptor binding. Any other substitution at this site that destroys the glycosylation site is believed to cause a similar decrease in lytic activity. Other amino acid substitutions, e.g., changing any one of residues 318 (Glu), 320 (Lys) and 322 (Lys), to Ala, are also known to abolish C1q binding to the Fc region of IgG antibodies (see, e.g., U.S. Patent No. 5,624,821).

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[0173] Modified binding proteins can be produced which have a reduced interaction with an Fc receptor. For example, it has been shown that in human IgG₃, which binds to the human Fc gamma R1 receptor, changing Leu 235 to Glu destroys its interaction with the receptor. Mutations on adjacent or close sites in the hinge link region of a binding protein (e.g., replacing residues 234, 235 and 237 with Ala) can also be used to affect binding protein affinity for the Fc gamma R1 receptor. The numbering of the residues in the heavy chain is based on the EU index (see Kabat et al. (1991) *supra*). Thus, in some embodiments of the invention, the Fc region of the binding proteins of the invention contains at least one constant region mutation, such as, for example, changing Leu to Ala at position 234 (L234A), changing Leu to Ala at position 235 (L235A), and/or changing Gly to Ala at position 237 (G237A). In one embodiment, the Fc region of the binding protein contains two constant region mutations, L234A and G237A (i.e., “double-mutant” or “DM”). In another embodiment, the Fc region of the binding protein contains three constant region mutations, L234A, L235A, and G237A (i.e., “triple-mutant” or “TM”). For example, a human IgG constant region triple-mutant is set forth in SEQ ID NO:196.

[0174] Additional methods for altering the lytic activity of a binding protein, for example, by altering at least one amino acid in the N-terminal region of the CH2 domain, are described in International Application Publication No. WO 94/029351 and U.S. Patent No. 5,624,821.

[0175] The binding proteins of the invention can be tagged with a detectable or functional label. These labels include radiolabels (e.g., ¹³¹I and ⁹⁹Tc), enzymatic labels (e.g., horseradish peroxidase and alkaline phosphatase), and other chemical moieties (e.g., biotin).

[0176] The invention may also feature an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, in particular, human IL-21R. In certain embodiments, the anti-IL-21R binding protein may have at least one of the following characteristics: (1) it is a monoclonal or single specificity binding protein; (2) it is a human binding protein; (3) it is an *in vitro* generated binding protein; (4) it is an

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in vivo generated binding protein (e.g., transgenic mouse system); (5) it inhibits the binding of IL-21 to IL-21R; (6) it is an IgG1; (7) it binds to human IL-21R with an association constant of at least about $10^5 \text{ M}^{-1}\text{s}^{-1}$; (8) it binds to murine IL-21R with an association constant of at least about $5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$; (9) it binds to human IL-21R with a dissociation constant of about 10^{-3} (1/s) or less; (10) it binds to murine IL-21R with a dissociation constant of about 10^{-2} (1/s) or less; (11) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing BaF3 cells with an IC_{50} of about 1.75 nM or less; (12) it inhibits murine IL-21R-mediated proliferation of murine IL-21R-expressing BaF3 cells with an IC_{50} of about 0.5 nM or less; (13) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing TF1 cells with an IC_{50} of about 14.0 nM or less; (14) it inhibits IL-21-mediated proliferation of human primary B cells with an IC_{50} of about 1.9 nM or less; (15) it inhibits IL-21-mediated proliferation of human primary CD4^+ T cells with an IC_{50} of about 1.5 nM or less; (16) it inhibits IL-21-mediated proliferation of murine primary CD4^+ T cells with an IC_{50} of about 5.0 nM or less; (17) it has a mean total body clearance of about 0.1-7.5 ml/hr/kg following, e.g., i.v. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (18) it has a mean elimination half-life of about 20-700 hr following, e.g., i.v., s.c., or i.p. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (19) it has a mean steady-state volume of distribution of about 40-1500 ml/kg in animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (20) it has a bioavailability of about 35-100% following, e.g., s.c. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (21) it has a mean dose-normalized AUC of about 200-10,000 $\mu\text{g} \cdot \text{hr}/\text{mL}$ (per 1 mg/kg dosage) following, e.g., i.v., s.c., or i.p. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; (22) it has a mean dose-normalized C_{max} (maximum serum concentration) of about 0.5-30 $\mu\text{g}/\text{ml}$ following, e.g., i.v., s.c., or i.p. administration to animals, e.g., mammals, e.g., humans, nonhuman primates, rodents; and (23) it modulates expression of IL-21 responsive cytokines or IL-21 responsive genes.

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[0177] One of skill in the art will appreciate that the modifications described above are not exhaustive, and that many other modifications will be obvious to a skilled artisan in light of the teachings of the present disclosure.

Nucleic Acids, Cloning and Expression Systems

[0178] The disclosure provides isolated nucleic acids encoding the disclosed binding proteins. The nucleic acids may comprise DNA or RNA, and they may be synthetic (completely or partially) or recombinant (completely or partially). Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses an RNA molecule with the specified sequence in which U is substituted for T.

[0179] Also contemplated are nucleic acids that comprise a coding sequence for one, two, or three CDRs, a V_H domain, a V_L domain, or combinations thereof, as disclosed herein, or a sequence substantially identical thereto (e.g., a sequence at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identical thereto, or which is capable of hybridizing under stringent conditions to the sequences).

[0180] In one embodiment, the isolated nucleic acids have nucleotide sequences encoding heavy chain and light chain variable regions of an anti-IL-21R binding protein comprising at least one CDR chosen from the amino acid sequences of SEQ ID NOs:163-195, or a sequence encoding a CDR which differs by one or two or three or four amino acids from the sequences described herein.

[0181] The nucleic acid can encode only the light chain or the heavy chain variable region, or can encode a binding protein light or heavy chain constant region, operatively linked to the corresponding variable region. In one embodiment, the light chain variable region is linked to a constant region chosen from a kappa or a lambda constant region. The light chain constant region may also be a human kappa or lambda type. In another embodiment, the heavy chain variable region is linked to a heavy chain constant region of a binding protein isotype chosen from IgG (e.g.,

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IgG1, IgG2, IgG3, and IgG4), IgM, IgA1, IgA2, IgD, and IgE. The heavy chain constant region may be an IgG (e.g., an IgG1) isotype.

[0182] The nucleic acid compositions of the present invention, while often in the native sequence (of cDNA or genomic DNA or mixtures thereof), except for modified restriction sites and the like, can be mutated in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid sequences as desired. In particular, nucleotide sequences substantially identical to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where “derived” indicates that a sequence is identical to or modified from another sequence).

[0183] In one embodiment, the nucleic acid differs (e.g., differs by substitution, insertion, or deletion) from that of the sequences provided (e.g., by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid). If necessary for this analysis, the sequences should be aligned for maximum homology. “Looped” out sequences from deletions or insertions, or mismatches, are considered differences. The difference may be at a nucleotide(s) encoding a nonessential residue(s), or the difference may be a conservative substitution(s).

[0184] The disclosure also provides nucleic acid constructs in the form of plasmids, vectors, and transcription or expression cassettes, which comprise at least one nucleic acid as described herein.

[0185] The disclosure further provides a host cell that comprises at least one nucleic acid construct described herein.

[0186] Also provided is a method of making an encoded protein(s) from a nucleic acid(s) comprising the sequence(s) described herein. The method comprises culturing host cells under appropriate conditions so they express the protein from the nucleic acid. Following expression and production, the V_H or V_L domain, or specific binding member, may be isolated and/or purified using any suitable technique, and then used as appropriate. The method can also include the steps of fusing a nucleic

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acid encoding an scFv with nucleic acids encoding an Fc portion of a binding protein, and expressing the fused nucleic acid in a cell. The method can also include a step of germlining.

[0187] Antigen-binding fragments, V_H and/or V_L domains, and encoding nucleic acid molecules and vectors may be isolated and/or purified from their natural environment, in substantially pure or homogenous form, or, in the case of nucleic acids, free or substantially free of nucleic acids or genes of origin other than the sequence encoding a polypeptide with the require function.

[0188] Systems for cloning and expressing polypeptides in a variety of host cells are known in the art. Cells suitable for producing binding proteins are described in, for example, Fernandez et al. (1999) *Gene Expression Systems*, Academic Press. In brief, suitable host cells include mammalian cells, insect cells, plant cells, yeast cells, or prokaryotic cells, e.g., *E. coli*. Mammalian cells available in the art for heterologous polypeptide expression include lymphocytic cell lines (e.g., NSO), HEK293 cells, Chinese hamster ovary (CHO) cells, COS cells, HeLa cells, baby hamster kidney cells, oocyte cells, and cells from a transgenic animal, e.g., mammary epithelial cells. In other embodiments, the nucleic acids encoding the binding proteins of the invention are placed under the control of a tissue-specific promoter (e.g., a mammary-specific promoter) and the binding proteins are produced in transgenic animals. For example, the binding proteins are secreted into the milk of the transgenic animal, such as a transgenic cow, pig, horse, sheep, goat, or rodent.

[0189] Suitable vectors may be chosen or constructed to contain appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes, and other sequences. The vectors may also contain a plasmid or viral backbone. For details, see, e.g., Sambrook et al. *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989), Cold Spring Harbor Laboratory Press. Many established techniques used with vectors, including the manipulation, preparation, mutagenesis, sequencing, and transfection of

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DNA, are described, e.g., in *Current Protocols in Molecular Biology* (2nd ed. 1992) Ausubel et al. eds., John Wiley & Sons.

[0190] A further aspect of the disclosure provides a method of introducing the nucleic acid into a host cell. For eukaryotic cells, suitable transfection techniques may include calcium phosphate, DEAE-Dextran, electroporation, liposome-mediated transfection, and transduction using a retrovirus or other virus(es), e.g., vaccinia or baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation, and transfection using bacteriophage. DNA introduction may be followed by a selection method (e.g., drug resistance) to select cells that contain the nucleic acid.

Uses of Anti-IL-21R Binding Proteins

[0191] Anti-IL-21R binding proteins that act as antagonists to IL-21R (e.g., binding proteins, or antigen-binding fragments thereof, of the present invention) can be used to regulate at least one IL-21R-mediated immune response, such as one or more of cell proliferation, cytokine expression or secretion, chemokine secretion, and cytolytic activity, of T cells, B cells, NK cells, macrophages, or synovial cells. Accordingly, the binding proteins of the invention can be used to inhibit the activity (e.g., proliferation, differentiation, and/or survival) of an immune or hematopoietic cell (e.g., a cell of myeloid, lymphoid, or erythroid lineage, or precursor cells thereof), and, thus, can be used to treat a variety of immune disorders and hyperproliferative disorders of the blood. Examples of IL-21R-associated disorders / immune disorders that can be treated include, but are not limited to, transplant rejection, graft-versus-host disease (GVHD), allergies (for example, atopic allergy), and autoimmune diseases. Autoimmune diseases include, but are not limited to, diabetes mellitus, arthritic disorders (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), spondyloarthropathy, multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus (SLE), cutaneous lupus erythematosus, autoimmune

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thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's syndrome, IBD (including Crohn's disease and ulcerative colitis), asthma (including intrinsic asthma and allergic asthma), scleroderma and vasculitis. The binding proteins, or antigen-binding fragments thereof, of the present invention can be used in methods to treat or prevent IL-21R-associated disorders / immune disorders in subjects, e.g., in humans.

[0192] Multiple sclerosis is a central nervous system disease that is characterized by inflammation and loss of myelin sheaths (the fatty material that insulates nerves and is needed for proper nerve function). Inflammation that results from an immune response that is dependent on IL-21/IL-21R can be treated with the binding proteins and compositions of this invention. In the experimental autoimmune encephalitis (EAE) mouse model for multiple sclerosis (see, e.g., Tuohy et al. (1988) *J. Immunol.* 141:1126-30; Sobel et al. (1984) *J. Immunol.* 132:2393-401; and Traugott (1989) *Cell Immunol.* 119:114-29), treatment of mice with injections of an IL-21R antibody prior to (and continuously after) induction of EAE profoundly delayed the onset of the disease. The binding proteins, or antigen-binding fragments thereof, of the present invention can be used in methods to treat or prevent multiple sclerosis in subjects, e.g., in humans.

[0193] Arthritis is a disease characterized by inflammation in the joints. Rheumatoid arthritis (RA) is the most frequent form of arthritis, involving inflammation of connective tissue and the synovial membrane, a membrane that lines the joint. The inflamed synovial membrane often infiltrates the joint and damages joint cartilage and bone. Studies show that treatment of synovial cells and macrophages with IL-21 induces these cells to secrete cytokines and chemokines associated with inflammation. In the collagen-induced arthritis (CIA) mouse model for rheumatoid arthritis (see, e.g., Courtenay et al. (1980) *Nature* 283:666-28; and Williams et al. (1995) *Immunol.* 84:433-39), treatment of mice with IL-21 subsequent to CIA induction (and continuously) exacerbates the disease. Increased secretion of inflammatory cytokines and chemokines, and more importantly,

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increased levels of disease resulting from immune responses that are dependent on IL-21, may be treated with the binding proteins of the invention. Similarly, the binding proteins, or antigen-binding fragments thereof, of the present invention can be used in methods to treat or prevent RA or other arthritic diseases in subjects, e.g., in humans.

[0194] Transplant rejection is the immunological phenomenon where tissues from a donor are specifically “attacked” by immune cells of the host. The principle “attacking” cells are T cells, whose T cell receptors recognize the donor’s MHC molecules as “foreign.” This recognition activates the T cell, which proliferates and secretes a variety of cytokines and cytolytic proteins that ultimately destroy the transplant. T cells in a mixed lymphocyte reaction (MLR), an *in vitro* assay of transplant rejection, proliferate more strongly when supplemented with IL-21. MLR and transplantation models have been described in *Current Protocols in Immunology* (2nd ed. 1994) Coligan et al. eds., John Wiley & Sons (see also Kasaian et al. (2002) *supra*; Fulmer et al. (1963) *Am. J. Anat.* 113:273-85; and Lenschow et al. (1992) *Science* 257:789-92). The binding proteins, or antigen-binding fragments thereof, of the present invention can be used in methods to reduce or prevent the MLR and/or to treat or prevent transplant rejection and related diseases (e.g., GVHD) that are dependent on IL-21 in subjects, e.g., in humans.

[0195] Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the presence of autoantibodies, including antibodies to DNA, nuclear antigens, and ribonucleoproteins. These autoantibodies are associated with tissue and organ damage. The cause of SLE is unknown, but the occurrence of autoantibodies suggests inadequate inhibition of autoreactive T cells or B cells. The binding proteins, or antigen-binding fragments thereof, of the present invention can be used in methods to inhibit the IL-21-mediated activities of autoreactive T cells and B cells and/or to treat or prevent SLE or related diseases in subjects, e.g., in humans or in MRL-*Fas^{lpr}* mice (a mouse model for SLE) (*Immunologic Defects in Laboratory Animals* (1981) Gershwin et al. eds., Plenum Press).

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[0196] The binding proteins, or antigen-binding fragments thereof, of the present invention also can be used in methods to treat or prevent hyperproliferative disorders of the blood that are associated with aberrant activity of IL-21-responsive cells and IL-21R-responsive cells in subjects, e.g., in humans. Examples of such cells include neoplastic cells of hematopoietic origin, e.g., cells arising from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. Examples of such neoplastic disorders include leukemic cancers and tumors of the blood, bone marrow (e.g., myeloma), and lymph tissue (e.g., lymphomas). In certain embodiments, the present invention is directed to the treatment of various leukemic cancers including, but not limited to, acute promyeloid leukemia (APML), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) (reviewed in Vaickus (1991) *Crit. Rev. Oncol./Hematol.* 11:267-97). Examples of lymphoid malignancies that may be treated by the present methods include, but are not limited to, acute lymphoblastic leukemia (ALL, which includes B-lineage ALL and T-lineage ALL), chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), hairy cell leukemia (HCL), and Waldenstrom's macroglobulinemia (WM). Additional forms of malignant lymphomas that can be treated by the present invention include, but are not limited to, non-Hodgkin's lymphoma, peripheral T cell lymphomas, adult T cell leukemia/lymphoma (ATL), cutaneous T cell lymphoma (CTCL), large granular lymphocytic leukemia (LGL), Hodgkin's lymphoma, and variants thereof.

[0197] In another aspect, the invention features a method of decreasing, inhibiting or reducing an acute phase response in a subject. An acute phase response is a response to inflammation, including the modulation of levels of acute phase proteins (e.g., C-reactive protein and serum albumin). The method includes administering to the subject an anti-IL-21R binding protein or antigen-binding fragment thereof as described herein, in an amount sufficient to decrease, inhibit or reduce the acute phase response in the subject. In one embodiment, the subject is a mammal, e.g., a human suffering from an IL-21R-associated disorder as described herein, including, e.g., respiratory disorders, inflammatory disorders and autoimmune disorders.

Combination Therapy

[0198] In one embodiment, a pharmaceutical composition comprising at least one anti-IL-21R binding protein or antigen-binding fragment thereof and at least one therapeutic agent is administered in combination therapy. The therapy is useful for treating pathological conditions or disorders, such as immune and inflammatory disorders. The term “in combination” in this context means that the binding protein composition and the therapeutic agent are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound, the first of the two compounds may still be detectable at effective concentrations at the site of treatment.

[0199] For example, the combination therapy can include at least one anti-IL-21R binding protein, such as, for example, an anti-IL-21R antibody, coformulated with, and/or coadministered with, at least one additional therapeutic agent. The additional agents may include at least one cytokine inhibitor, growth factor inhibitor, immunosuppressant, anti-inflammatory agent, metabolic inhibitor, enzyme inhibitor, cytotoxic agent, and/or cytostatic agent, as described in more detail below. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. Moreover, the therapeutic agents disclosed herein act on pathways that differ from the IL-21/IL-21R pathway, and thus are expected to enhance and/or synergize with the effects of the anti-IL-21R binding proteins.

[0200] Therapeutic agents used in combination with anti-IL-21R binding proteins may be those agents that interfere at different stages in the autoimmune and subsequent inflammatory response. In one embodiment, at least one anti-IL-21R binding protein described herein may be coformulated with, and/or coadministered with, at least one cytokine and/or growth factor antagonist. The antagonists may include soluble receptors, peptide inhibitors, small molecules, ligand fusions, antibodies (that bind cytokines or growth factors, or their receptors or other cell surface molecules), and “anti-inflammatory cytokines” and agonists thereof.

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[0201] Examples of the agents that can be used in combination with the anti-IL-21R binding proteins described herein, include, but are not limited to, antagonists of at least one interleukin (e.g., IL-1, IL-2, IL-6, IL-7, IL-8, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, and IL-22), cytokine (e.g., TNF- α , LT, EMAP-II, and GM-CSF), or growth factor (e.g., FGF and PDGF). The agents may also include, but are not limited to, antagonists of at least one receptor for an interleukin, cytokine, or growth factor. Anti-IL-21R binding proteins can also be combined with inhibitors (e.g., antibodies) to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, or their ligands (e.g., CD154 (gp39, CD40L)), or LFA-1/ICAM-1 or VLA-4/VCAM-1 (see Yusuf-Makagiansar et al. (2002) *Med. Res. Rev.* 22(2):146-67)). Antagonists that can be used in combination with anti-IL-21R binding proteins described herein may include antagonists of IL-1, IL-12, TNF- α , IL-15, IL-17, IL-18, IL-22, and their receptors.

[0202] Examples of IL-12 antagonists include antibodies that bind IL-12 (see, e.g., International Application Publication No. WO 00/056772); IL-12 receptor inhibitors (e.g., antibodies to the IL-12 receptor), and soluble IL-12 receptor and fragments thereof. Examples of IL-15 antagonists include antibodies against IL-15 or its receptor, soluble fragments of the IL-15 receptor, and IL-15-binding proteins. Examples of IL-18 antagonists include antibodies to IL-18, soluble fragments of the IL-18 receptor, and IL-18 binding proteins (IL-18BP, Mallet et al. (2001) *Circ. Res.* 28). Examples of IL-1 antagonists include Interleukin-1-Converting Enzyme (ICE) inhibitors (such as Vx740), IL-1 antagonists (e.g., IL-1RA (anakinra, Amgen)), sIL-1RII (Immunex), and anti-IL-1 receptor antibodies.

[0203] Examples of TNF antagonists include antibodies to TNF (e.g., human TNF- α), such as D2E7 (human anti-TNF- α antibody, U.S. Patent No. 6,258,562, HUMIRATM, BASF, Parsippany, NJ), CDP-571/CDP-870/BAY-10-3356 (humanized anti-TNF- α antibodies, Celltech/Pharmacia), cA2 (chimeric anti-TNF- α antibody, REMICADETM, Centocor), and anti-TNF antibody fragments (e.g., CPD870). Other examples include soluble TNF receptor (e.g., human p55 or p75)

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fragments and derivatives, such as p55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein, LENERCEPTTM) and 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein, ENBRELTM, Immunex; see, e.g., *Arthritis Rheumatism* (1994) 37:S295 and *J. Invest. Med.* (1996) 44:235A). Further examples include enzyme antagonists (e.g., TNF- α converting enzyme inhibitors (TACE) such as alpha-sulfonyl hydroxamic acid derivative (WO 01/055112) or N-hydroxyformamide inhibitor (GW 3333, -005, or -022)) and TNF-bp/s-TNFR (soluble TNF binding protein; see, e.g., *Arthritis Rheumatism* (1996) 39(9):S284 and *Am. J. Physiol. Heart Circ. Physiol.* (1995) 268:37-42).

[0204] In other embodiments, the anti-IL-21R binding proteins described herein can be administered in combination with at least one of the following: IL-13 antagonists, such as soluble IL-13 receptors and/or anti-IL-13 antibodies; and IL-2 antagonists, such as IL-2 fusion proteins (e.g., DAB 486-IL-2 and/or DAB 389-IL-2, Seragen (see, e.g., *Arthritis Rheumatism* (1993) 36:1223)) and anti-IL-2R antibodies (e.g., anti-Tac (humanized antibody, Protein Design Labs (see *Cancer Res.* (1990) 50(5):1495-502))). Another combination includes anti-IL-21R binding proteins in combination with nondepleting anti-CD4 inhibitors such as IDEC-CE9.1/SB 210396 (anti-CD4 antibody, IDEC/SmithKline). Yet other combinations include anti-IL-21R binding proteins with CD80 (B7.1) and CD86 (B7.2) costimulatory pathway antagonists (such as antibodies, soluble receptors, or antagonistic ligands), P-selectin glycoprotein ligand (PSGL), and/or anti-inflammatory cytokines and agonists thereof (e.g., antibodies). The anti-inflammatory cytokines may include IL-4 (DNAX/Schering, Palo Alto, CA), IL-10 (SCH 52000, recombinant IL-10, DNAX/Schering), IL-13, and TGF.

[0205] In other embodiments, at least one anti-IL-21R binding protein can be coformulated with, and/or coadministered with, at least one anti-inflammatory drug, immunosuppressant, metabolic inhibitor, and enzymatic inhibitor. Nonlimiting examples of the drugs or inhibitors that can be used in combination with the IL-21R binding proteins described herein, include, but are not limited to, at least one of:

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nonsteroidal anti-inflammatory drugs (NSAID) (such as ibuprofen, tenidap (see, e.g., *Arthritis Rheumatism* (1996) 39(9):S280), naproxen (see, e.g., *NeuroReport* (1996) 7:1209-13), meloxicam, piroxicam, diclofenac, and indomethacin); sulfasalazine (see, e.g., *Arthritis Rheumatism* (1996) 39(9):S281)); corticosteroids (such as prednisolone); cytokine suppressive anti-inflammatory drugs (CSAID); and inhibitors of nucleotide biosynthesis (such as an inhibitor of purine biosynthesis (e.g., a folate antagonist such as methotrexate) and an inhibitor of pyrimidine biosynthesis (e.g., a dihydroorotate dehydrogenase (DHODH) inhibitor such as leflunomide (see, e.g., *Arthritis Rheumatism* (1996) 39(9):S131 and *Inflammation Research* (1996) 45:103-7)).

[0206] Examples of additional inhibitors include at least one of: corticosteroids (oral, inhaled and local injection); immunosuppressants (such as cyclosporin and tacrolimus (FK-506)); mTOR inhibitors (such as sirolimus (rapamycin) or a rapamycin derivative (e.g., an ester rapamycin derivative such as CCI-779 (see Elit (2002) *Current Opinion Investig. Drugs* 3(8):1249-53 and Huang et al. (2002) *Current Opinion Investig. Drugs* 3(2):295-304))); agents which interfere with the signaling of proinflammatory cytokines such as TNF- α and IL-1 (e.g., IRAK, NIK, IKK, p38, or a MAP kinase inhibitor); cox2 inhibitors (e.g., celecoxib and variants thereof (MK-966); see, e.g., *Arthritis Rheumatism* (1996) 39(9):S81); phosphodiesterase inhibitors (such as R973401; see, e.g., *Arthritis Rheumatism* (1996) 39(9):S282); phospholipase inhibitors (e.g., an inhibitor of cytosolic phospholipase 2 (cPLA2) such as trifluoromethyl ketone analogs; see U.S. Patent No. 6,350,892); inhibitors of vascular endothelial cell growth factor (VEGF); inhibitors of the VEGF receptor; and inhibitors of angiogenesis.

[0207] The anti-IL-21R binding proteins disclosed herein can be used in combination with other therapeutic agents to treat specific immune disorders as discussed in further detail below.

[0208] Nonlimiting examples of agents for treating arthritic disorders (e.g., rheumatoid arthritis, inflammatory arthritis, rheumatoid arthritis, juvenile rheumatoid

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arthritis, osteoarthritis and psoriatic arthritis), with which an anti-IL-21R binding protein can be combined include at least one of the following: TNF antagonists (such as anti-TNF antibodies); soluble fragments of TNF receptors (e.g., human p55 and p75) and derivatives thereof (such as p55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein, LENERCEPTTM) and 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein, ENBRELTM)); TNF enzyme antagonists (such as TACE inhibitors); antagonists of IL-12, IL-15, IL-17, IL-18, and IL-22; T cell- and B cell-depleting agents (such as anti-CD4 or anti-CD22 antibodies); small molecule inhibitors (such as methotrexate and leflunomide); sirolimus (rapamycin) and analogs thereof (e.g., CCI-779); cox2 and cPLA2 inhibitors; NSAIDs; p38, TPL-2, Mk-2, and NFκB inhibitors; RAGE or soluble RAGE; P-selectin or PSGL-1 inhibitors (such as antibodies thereto and small molecule inhibitors); estrogen receptor beta (ERB) agonists; and ERB-NFκB antagonists. Therapeutic agents that can be coadministered and/or coformulated with at least one IL-21/IL-21R antagonist may include at least one of: a soluble fragment of a TNF receptor (e.g., human p55 or p75) such as 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein, ENBRELTM); methotrexate; leflunomide; and sirolimus (rapamycin) or an analog thereof (e.g., CCI-779).

[0209] Nonlimiting examples of agents for treating multiple sclerosis with which an anti-IL-21R binding protein can be combined include interferon-β (for example, IFNβ-1a and IFNβ-1b), copaxone, corticosteroids, IL-1 inhibitors, TNF inhibitors, antibodies to CD40 ligand, antibodies to CD80, and IL-12 antagonists.

[0210] Nonlimiting examples of agents for treating inflammatory bowel disease or Crohn's disease with which an anti-IL-21R binding protein can be combined include at least one of the following: budenoside; epidermal growth factor; corticosteroids; cyclosporine; sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 monoclonal antibodies; anti-IL-6 monoclonal antibodies; growth factors; elastase

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inhibitors; pyridinyl-imidazole compounds; TNF antagonists as described herein; IL-4, IL-10, IL-13, and/or TGF β or agonists thereof (e.g., agonistic antibodies); IL-11; glucuronide- or dextran-conjugated prodrugs of prednisolone, dexamethasone or budesonide; ICAM-1 antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TP10; T Cell Sciences, Inc.); slow-release mesalazine; methotrexate; antagonists of Platelet Activating Factor (PAF); ciprofloxacin; and lignocaine.

[0211] In other embodiments, an anti-IL-21R binding protein can be used in combination with at least one antibody directed at other targets involved in regulating immune responses, e.g., transplant rejection or GVHD. Nonlimiting examples of agents for treating immune responses with which an IL-21/IL-21R antagonist can be combined include the following antibodies against cell surface molecules (e.g., CD25 (IL-2 receptor α), CD11a (LFA-1), CD54 (ICAM-1), CD4, CD45, CD28/CTLA4, CD80 (B7-1), CD86 (B7-2), or combinations thereof). In another embodiment, an anti-IL-21R binding protein is used in combination with at least one general immunosuppressive agent, such as cyclosporin A or FK506.

[0212] Another aspect of the present invention relates to kits for carrying out the combined administration of anti-IL-21R binding proteins with other therapeutic agents. In one embodiment, the kit comprises at least one anti-IL-21R binding protein formulated in a pharmaceutical carrier, and at least one therapeutic agent, formulated as appropriate in one or more separate pharmaceutical preparations.

Diagnostic Uses

[0213] The binding proteins of the invention may also be used to detect the presence of IL-21R in biological samples. By correlating the presence or level of these proteins with a medical condition, one of skill in the art can diagnose the associated medical condition. For example, stimulated T cells increase their expression of IL-21R, and an unusually high concentration of IL-21R expressing T cells in joints may indicate joint inflammation and possible arthritis. Illustrative medical

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conditions that may be diagnosed by the binding proteins of this invention include, but are not limited to, multiple sclerosis, rheumatoid arthritis, and transplant rejection.

[0214] Binding protein-based detection methods, such as those commonly used for antibodies, are well known in the art, and include ELISA, radioimmunoassays, immunoblots, Western blots, flow cytometry, immunofluorescence, immunoprecipitation, and other related techniques. The binding proteins may be provided in a diagnostic kit that incorporates at least one of these procedures to detect IL-21R. The kit may contain other components, packaging, instructions, reagents, and/or other material to aid the detection of the protein and use of the kit.

[0215] Binding proteins may be modified with detectable markers, including ligand groups (e.g., biotin), fluorophores, chromophores, radioisotopes, electron-dense reagents, or enzymes. Enzymes are detected by their activity. For example, horseradish peroxidase is detected by its ability to convert tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. Other suitable binding partners include biotin and avidin, IgG and protein A, and other receptor-ligand pairs known in the art.

[0216] Binding proteins can also be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association, or otherwise) to at least one other molecular entity, such as another binding protein (e.g., a bispecific or a multispecific binding protein), toxins, radioisotopes, cytotoxic or cytostatic agents, among others. Other permutations and possibilities are apparent to those of ordinary skill in the art, and they are considered equivalents within the scope of this invention.

Pharmaceutical Compositions and Methods of Administration

[0217] Certain embodiments of the invention include compositions comprising the disclosed binding proteins. The compositions may be suitable for pharmaceutical use and administration to patients. The compositions comprise a binding protein of the present invention and a pharmaceutical excipient. As used herein, “pharmaceutical

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excipient” includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, etc., that are compatible with pharmaceutical administration. Use of these agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions. The pharmaceutical compositions may also be included in a container, pack, or dispenser, together with instructions for administration.

[0218] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known to those of ordinary skill in the art. Pharmaceutical compositions may be topically or orally administered, or capable of transmission across mucous membranes. Examples of administration of a pharmaceutical composition include oral ingestion or inhalation. Administration may also be intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, cutaneous, or transdermal.

[0219] Solutions or suspensions used for intradermal or subcutaneous application typically include at least one of the following components: a sterile diluent such as water, saline solution, fixed oils, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetate, citrate, or phosphate; and tonicity agents such as sodium chloride or dextrose. The pH can be adjusted with acids or bases by methods known in the art. Such preparations may be enclosed in ampoules, disposable syringes, or multiple dose vials.

[0220] Solutions or suspensions used for intravenous administration include a carrier such as physiological saline, bacteriostatic water, CREMOPHOR EL[®] (BASF Corp., Ludwigshafen, Germany), ethanol, or polyol. In all cases, the composition must be sterile and fluid for easy syringability. Proper fluidity can often be obtained using lecithin or surfactants. The composition must also be stable under the

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conditions of manufacture and storage. Prevention of microorganisms can be achieved with antibacterial and antifungal agents, e.g., parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, etc. In many cases, isotonic agents (sugar), polyalcohols (e.g., mannitol and sorbitol), or sodium chloride may be included in the composition. Prolonged absorption of the composition can be accomplished by adding an agent that delays absorption, e.g., aluminum monostearate or gelatin.

[0221] Oral compositions include an inert diluent or edible carrier. For the purpose of oral administration, the binding proteins can be incorporated with excipients and placed, e.g., in tablets, troches, capsules, or gelatin. Pharmaceutically compatible binding agents or adjuvant materials can be included in the composition. The compositions may contain (1) a binder such as microcrystalline cellulose, gum tragacanth or gelatin; (2) an excipient such as starch or lactose, (3) a disintegrating agent such as alginic acid, Primogel, or corn starch; (4) a lubricant such as magnesium stearate; (5) a glidant such as colloidal silicon dioxide; and/or (6) a sweetening or flavoring agent.

[0222] The composition may also be administered by a transmucosal or transdermal route. For example, binding proteins that comprise an Fc portion (for example, an antibody) may be capable of crossing mucous membranes in the intestine, mouth, or lungs (via Fc receptors). Transmucosal administration can be accomplished by lozenges, nasal sprays, inhalers, or suppositories. Transdermal administration can be accomplished with a composition containing ointments, salves, gels, or creams known in the art. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used. For administration by inhalation, the binding proteins may be delivered in an aerosol spray from a pressured container or dispenser, which contains a propellant (e.g., liquid or gas), or a nebulizer.

[0223] In certain embodiments, the binding proteins of this invention are prepared with carriers to protect the binding proteins against rapid elimination from the body. Biodegradable polymers (e.g., ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid) are often used. Methods for the

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preparation of such formulations are known by those skilled in the art. Liposomal suspensions can be used as pharmaceutically acceptable carriers also. The liposomes can be prepared according to established methods known in the art (see, e.g., U.S. Patent No. 4,522,811).

[0224] The binding proteins or binding protein compositions of the invention are administered in therapeutically effective amounts as described. Therapeutically effective amounts may vary with the subject's age, condition, sex, and severity of medical condition. Appropriate dosages can be determined by a physician based upon clinical indications. The binding proteins or compositions may be given as a bolus dose to maximize the circulating levels of binding proteins for the greatest length of time. Continuous infusion may also be used.

[0225] As used herein, the term "subject" is intended to include human and nonhuman animals. Subjects may include a human patient having a disorder characterized by cells that express IL-21R, e.g., a cancer cell or an immune cell. The term "nonhuman animals" of the invention includes all vertebrates, such as nonhuman primates, sheep, dogs, cows, chickens, amphibians, reptiles, etc.

[0226] Examples of dosage ranges that can be administered to a subject can be chosen from: 1 µg/kg to 20 mg/kg, 1 µg/kg to 10 mg/kg, 1 µg/kg to 1 mg/kg, 10 µg/kg to 1 mg/kg, 10 µg/kg to 100 µg/kg, 100 µg/kg to 1 mg/kg, 250 µg/kg to 2 mg/kg, 250 µg/kg to 1 mg/kg, 500 µg/kg to 2 mg/kg, 500 µg/kg to 1 mg/kg, 1 mg/kg to 2 mg/kg, 1 mg/kg to 5 mg/kg, 5 mg/kg to 10 mg/kg, 10 mg/kg to 20 mg/kg, 15 mg/kg to 20 mg/kg, 10 mg/kg to 25 mg/kg, 15 mg/kg to 25 mg/kg, 20 mg/kg to 25 mg/kg, and 20 mg/kg to 30 mg/kg (or higher). These dosages may be administered daily, weekly, biweekly, monthly, or less frequently, for example, biannually, depending on dosage, method of administration, disorder or symptom(s) to be treated, and individual subject characteristics.

[0227] In certain circumstances, it may be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited for the patient. Each

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dosage unit contains a predetermined quantity of binding protein calculated to produce a therapeutic effect in association with the carrier. The dosage unit depends on the characteristics of the binding protein and the particular therapeutic effect to be achieved.

[0228] Toxicity and therapeutic efficacy of the composition can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Binding proteins that exhibit large therapeutic indices may be less toxic and/or more therapeutically effective.

[0229] The data obtained from the cell culture assays and animal studies can be used to formulate a dosage range in humans. The dosage of these compounds may lie within the range of circulating binding protein concentrations in the blood, which includes an ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage composition form employed and the route of administration. For any binding protein used in the present invention, the therapeutically effective dose can be estimated initially using cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of binding protein that achieves a half-maximal inhibition of symptoms). The effects of any particular dosage can be monitored by a suitable bioassay. Examples of suitable bioassays include DNA replication assays, transcription-based assays, gene expression assays, IL-21/IL-21R binding assays, and other immunological assays.

[0230] In one embodiment of the invention, a dose may be formulated in an *ex vivo* whole blood cell assay. In such embodiment, a suitable bioassay for determining and monitoring a particular dosage includes a method of determining a minimum serum concentration of an anti-IL-21R binding protein necessary to inhibit or reduce IL-21R activity, such as modulation of expression of IL-21-responsive genes. In one

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embodiment of the invention, the IL-21-responsive gene is selected from the following nonlimiting list: TNF, IFN γ , IL-6, IL-8, IL-10, CD19, STAT3, TBX21, CSF1, GZMB, PRF1, IL-2R α , and IL-21R. In a preferred embodiment, the IL-21-responsive gene is selected from CD19, GZMB, IFN γ , IL-2R α , IL6, and PRF-1. In a most preferred embodiment, the IL-21-responsive gene is IL-2R α . Thus, the method of determining a minimum serum concentration of an anti-IL-21R antibody necessary to inhibit or reduce IL-21R activity may include determining a level of expression of more than one IL-21-responsive gene, e.g., two, three, four, five, or six IL-21-responsive genes.

[0231] The entire contents of all references, patent applications, and patents cited throughout this application are hereby incorporated by reference herein.

EXAMPLES

[0232] The invention will be further illustrated in the following nonlimiting examples. These Examples are set forth to aid in the understanding of the invention but are not intended to, and should not be construed to, limit its scope in any way. The Examples do not include detailed descriptions of conventional methods that would be well known to those of ordinary skill in the art.

Example 1: Generation of Binding Proteins by Phage Display

[0233] The scFv parental clone 18A5, described in U.S. Patent No. 7,495,085 (incorporated by reference herein), was obtained from the CS human scFv library by standard phage display methods, using BaF3 cells expressing human IL-21R as a target in rounds 1 and 3 and biotinylated IL-21R-Fc fusion protein as a target in round 2.

Example 2: Library Construction

[0234] Phage display libraries were based upon the parental 18A5 scFv, using a pCANTAB6 vector in which the scFv was fused at its 3' end to the intact gene III. Various CDR3 sequences were derived using techniques well known in the art.

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[0235] Two overlapping blocks of six consecutive codons were randomized in the CDR3 of the V_H and the V_L , producing a total of four libraries: H3B1, H3B2, L3B1, and L3B2. The following identify nucleotide and amino acid sequences, respectively: IL-21R: 18A5 V_H CDR3 [SEQ ID NOs:199 and 200]; H3B1 (library size 1.40×10^9) [SEQ ID NOs:201 and 202]; H3B2 (library size 1.00×10^9) [SEQ ID NOs:203 and 204]; IL-21R: 18A5 V_L CDR3 [SEQ ID NOs:205 and 206]; L3B1 (library size 9.00×10^9) [SEQ ID NOs:207 and 208]; L3B2 (library size 6.40×10^9) [SEQ ID NOs:209 and 210].

Example 3: Phage Selection

[0236] All derivatives of 18A5 were isolated from the scFv libraries above by selection of phage able to bind in solution phase to biotinylated human IL-21R extracellular domain His-Flag fusion proteins (“biotin-hIL-21R-H/F”) and biotinylated murine IL-21R extracellular domain His-Flag fusion proteins (“biotin-mIL-21R-H/F”); all procedures and techniques related to selection are well known to one of skill in the art. A total of twenty-seven anti-IL-21R scFv were isolated by phage selection procedures.

Example 4: Library Screening

[0237] Resulting binding proteins in scFv format were chosen based on their ability to compete with parental 18A5 in human IgG1 format for binding to biotin-hIL-21R-H/F and biotin-mIL-21R-H/F, to prevent the hIL-21-dependent proliferation of genetically engineered cell lines expressing human IL-21R and the mIL-21-dependent proliferation of genetically engineered cell lines expressing murine IL-21R.

Example 4.1: Preparation of Crude Periplasmic Material (“peri-preps”) for Use in Screening Assays

[0238] Depending on the growth conditions used, scFv can be expressed in solution in the bacterial periplasmic space. To induce release of scFv into the periplasm,

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96-deep-well plates containing 990 μ l 2xTY media with 0.1% glucose / 100 μ g/ml ampicillin were inoculated from thawed glycerol stocks (one clone per well) using the QPix2 Colony picker (Genetix, New Milton, England) and grown at 37°C (999 rpm) for about 4 hr. Cultures were induced with IPTG at a final concentration of 0.02 mM and grown overnight at 30°C (999 rpm). The contents of the bacterial periplasm (peri-preps) were released by osmotic shock. Briefly, plates were centrifuged and pellets were resuspended in 150 μ l TES periplasmic buffer (50 mM Tris / HCl (pH 8.0) / 1 mM EDTA (pH 8.0) / 20% Sucrose), followed by the addition of 150 μ l 1:5 TES:water, and incubated on ice for 30 min. Plates were centrifuged and the scFv-containing supernatant was harvested.

Example 4.2: Epitope Competition Assay for Library Screening

[0239] Those scFv able to compete with the parental 18A5 antibody for binding to human or murine IL-21R were identified from selected phage by a homogeneous time-resolved fluorescence (HTRF[®]) assay. Purified parental 18A5 antibody was covalently modified with cryptate, a derivative of europium, according to the instructions in an HTRF[®] Cryptate Labeling Kit (Cisbio, Bedford, MA). Peri-preps of scFv were prepared as described above and diluted to 0.25% in PBS / 0.4 M potassium fluoride / 0.1% BSA (HTRF[®] buffer); then 10 μ l of the mixture was transferred to the wells of black 384-shallow-well plates (Nunc, Rochester, NY). Five μ l of cryptate-conjugated 18A5 antibody was then added to each well, followed by 5 μ l of a mixture of a 1:800 dilution of streptavidin-XL665 conjugate (Cisbio) and either 4.8 nM biotin-hIL-21R-H/F or 40 nM biotin-mIL-21R-H/F. The mixture was incubated for 2 hr at RT, and time-resolved fluorescence measurements were made (340 nm excitation, 615 nm and 665 nm emission). Competition with 18A5 antibody was indicated by a reduction in the background-corrected ratio of emission at 665 nm to emission at 615 nm.

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[0240] A total of 8280 independently isolated scFv were screened in the HTRF[®] assay using human IL-21R-H/F, and 376 clones able to compete with the parental 18A5 antibody for binding to biotin-hIL-21R-H/F were chosen for further analysis.

Example 5: DNA Sequence Analysis of Library-derived scFv - PCR Amplification of scFv Regions for Sequencing Analysis

[0241] The sequences of 287 18A5-derived scFv variants with improved IL-21R binding over that of the parent 18A5 scFv molecule were determined, and the frequencies of amino acids found at each position were determined. Among the V_H clones, only two (1.7%) were derived from a library which mutated the last six amino acids of, e.g., SEQ ID NO:169 (at the C-terminus of V_H CDR3), while the remainder were derived from a library which mutated the first six amino acids of, e.g., SEQ ID NO:169. Among the V_L clones, only one clone (0.6%) was derived from a library in which the last six amino acids of, e.g., SEQ ID NO:170 (at the C-terminus of V_L CDR3) were mutated, while the majority were derived from alterations in the first six amino acids of, e.g., SEQ ID NO:170 (at the N-terminus of V_L CDR3).

[0242] PCR amplification of scFvs was carried out using VENT[®] DNA Polymerase (New England Biolabs, Ipswich, MA) in HN buffer (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's instructions. Five µl of a 1:10 dilution of a stationary phase bacterial culture was used as the template for a final reaction volume of 20 µl. The cycling conditions used were a 2-min hot start at 94°C, 30 cycles of denaturation at 94°C (1 min), primer annealing at 55°C (2 min) and extension at 72°C (1 min), followed by a final extension at 72°C (5 min). PCR products were verified by agarose gel electrophoresis and cleaned up with ExoI/SAP (shrimp alkaline phosphatase) prior to sequencing with the M13rev primer.

[0243] The SEQ ID NOs for the CDR3 sequences of twenty-seven scFv are listed in **Table 4**. These scFv were chosen for further analysis based on assays described in Example 6.

Table 4: CDR3 SEQ ID NOs of Improved 18A5-derived scFv

scFv	Heavy CDR3	Light CDR3
H3	165	170
H4	166	170
H5	167	170
H6	168	170
L1	169	171
L2	169	172
L3	169	173
L4	169	174
L5	169	175
L6	169	176
L8	169	177
L9	169	178
L10	169	179
L11	169	180
L12	169	181
L13	169	182
L14	169	183
L15	169	184
L16	169	185
L17	169	186
L18	169	187
L19	169	188
L20	169	189
L21	169	190
L23	169	191
L24	169	192
L25	169	193

*Example 6: Characterization of Library-derived scFv**Example 6.1: Preparation of Purified scFv for Quantitative Analysis*

[0244] Individual scFv clones were purified on a small scale by Ni-NTA purification on PHYTIP[®] columns (PhyNexus, Inc., San Jose, CA). Single colonies were grown in 20 ml 2xTY medium with 0.1% glucose / 100 µg/ml ampicillin in 50-ml conical tubes to mid-logarithmic phase at 37°C with shaking at 250 rpm. Expression of scFv was induced with IPTG at a final concentration of 0.02 mM, and cultures were grown overnight at 30°C. Cells were harvested by centrifugation and resuspended in 1 ml TES periplasmic buffer, followed by the addition of 1 ml 1:5 TES:water and incubation on ice for 30 min. Lysates were centrifuged at 3200 rpm for 10 min at

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4°C, and supernatants were brought to 2 mM MgCl₂. scFv were captured on Ni-NTA PHYTIPs[®] (PhyNexus) by repeated passage of the supernatant over the PHYTIPs[®] on a Perkin Elmer (Waltham, MA) MINITRAK[™] IX liquid handling robot, followed by washing in IMAC wash buffer and elution with 200 mM imidazole, 50 mM Tris, 300 mM NaCl (pH 8.0). The buffer was exchanged to PBS by three cycles of dilution 1:10 into PBS, followed by concentration on a 10,000 molecular weight cutoff filter plate (Millipore MULTISCREEN[®] ULTRACEL[™] 96-well ultrafiltration plate, Millipore, Billerica, MA). Samples were quantitated using a Micro BCA[™] kit (Thermo Fisher Scientific Inc., Rockford, IL) using the manufacturer's bovine serum albumin standard.

Example 6.2: Assays for IL-21-dependent Proliferation of Cells Overexpressing Human or Murine IL-21R

[0245] Inhibition assays were performed with 18A5-derived binding proteins (scFv and IgG) to measure their blockade of IL-21-dependent proliferation of cell lines transfected with human or murine IL-21R. BaF3 cells, a murine pre-B cell line, and TF1 cells, a human erythroid cell line, were retrovirally transduced with IL-21R and green fluorescent protein (GFP). Cells were routinely grown in RPMI 1640 with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.00036% β-mercaptoethanol. Human IL-21R-BaF3 cell cultures were supplemented with 50 ng/ml of human IL-21; murine IL-21R-BaF3 cell cultures were supplemented with 10 U/ml of IL-3; TF1 cell cultures were supplemented with 50 ng/ml of GM-CSF. Prior to assay, cells were washed 3X in assay medium lacking supplemental growth factors, resuspended in assay medium, and incubated at 37°C / 5% CO₂ for 6 hr. To prepare assay plates, 5000 cells were added to the central 60 wells of a 96-well flat-bottomed white tissue culture plate (Thermo Scientific, Waltham, MA) in a volume of 55 µl/well. Test scFv or IgG samples were prepared by diluting the stock sample in assay medium and diluting serially three-fold. Twenty-five µl of the binding protein samples were added to the cells and incubated for 30 min at 37°C / 5% CO₂. Twenty µl of assay medium containing 100-400 pg/ml

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of human or murine IL-21 was added to each well, and the cells were incubated for an additional 48 hr. Proliferation was measured by bringing plates to RT, adding 15 μ l/well CELLTITER-GLO[®], incubating for 10 min at RT, and measuring luminescence with a Perkin Elmer ENVISION[™] plate reader. After purification with PhyNexus IMAC tips, 108 scFv were tested for neutralization of IL-21-dependent proliferation of all three cell lines. All showed neutralization of human IL-21R-BaF3 cells, with IC₅₀s lower than or equal to that of the parental 18A5 scFv. A subset showed strong neutralization of proliferation of murine IL-21R-BaF3 cells and human IL-21R-TF1 cells. Data from the 27 most potent clones are shown in **FIGs. 1-3**, and are summarized in **Table 5**.

[0246] **FIGs. 1-3** show the neutralization of proliferation by scFv of human IL-21R-BaF3 cells (**FIGs. 1a-c**); human IL-21R-TF1 cells (**FIGs. 2a-c**); and murine IL-21R-BaF3 cells (**FIGs. 3a-c**). Cells were mixed with the indicated scFv and incubated with 100 pg/ml (**FIGs. 1-2**) or 400 pg/ml (**FIG. 3**) of human IL-21.

Example 6.3: Quantitative Epitope Competition Assay

[0247] Purified scFv were analyzed quantitatively for their ability to compete with the parental 18A5 antibody for binding to murine IL-21R in an enzyme-linked immunosorbent assay (ELISA). Parental 18A5 antibody was coated overnight at 4°C on 96-well Nunc MAXISORP[®] plates at a concentration of 0.75 μ g/ml in PBS. Plates were washed 3X using PBS, and then blocked for 3 hr at RT in PBS / 1% BSA / 0.05% Tween-20. scFv were mixed with 36 nM biotinylated mIL-21R-H/F and incubated for 10 min at RT. Blocked plates were washed 3X with PBS, and 50 μ l/well of scFv / IL-21R mixtures were transferred to the appropriate plates and incubated for 1 hr at RT. Plates were washed 5X with PBS prior to the addition of a 1:6000 dilution of horseradish peroxidase-conjugated streptavidin (Southern Biotech, Birmingham, AL) secondary antibody to detect bound biotinylated mIL-21R-H/F. Plates were then incubated for 1 hr at RT and washed 7X with PBS. Signal was developed using 3,3',5,5'-tetramethylbenzidine (TMB), the

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reaction stopped with H₂SO₄, and the absorbance read at 450 nm on an ENVISION™ plate reader (Perkin Elmer). 108 scFv purified by PhyNexus IMAC tips were tested in this assay, and most competed with the parental 18A5 antibody for binding to biotinylated murine IL-21R-H/F with IC₅₀s lower than that of the parental 18A5 scFv. Epitope competition data for the 27 clones with the highest potencies in cell-based neutralization assays are shown in **FIGs. 4a-c** and summarized in **Table 5**.

Table 5: Neutralization of Human and Murine IL-21R in Cell-based Assays and Competition with 18A5 Antibody for Murine IL-21R Binding

	IC ₅₀ (nM) in Human IL-21R-BaF3 Neutralization Assay	IC ₅₀ (nM) in Human IL-21R-TF1 Neutralization Assay	IC ₅₀ (nM) in Murine IL-21R-BaF3 Neutralization Assay	IC ₅₀ (nM) in Murine IL-21R Epitope Competition ELISA
H3	7.7	98.1	25.68	14
H4	3.8	9.3	nd	nd
H5	7.9	178.5	53.66	17
H6	13.8	150 (estimated)	nd	13
L1	3.7	55 (estimated)	28.77	7
L2	3.1	37.5	2.41	5
L3	27.6	7 (estimated)	13.78	100
L4	2.1	60 (estimated)	nd	8
L5	2.1	20 (estimated)	38.52	7
L6	5.9	150 (estimated)	0.29	4
L8	4.1	51.3	715.27	7
L9	2.8	27.7	3.61	7
L10	15.1	7	nd	40
L11	4.2	38.3	10.03	6
L12	2.6	54.9	87.77	8
L13	11.0	257.4	1.25	7
L14	3.2	33.5	6.49	6
L15	3.3	30.3	53.49	14
L16	3.7	67.4	4.71	6
L17	1.6	60.3	2.66	12
L18	3.7	54.4	8.34	8
L19	4.5	35.3	13.59	15
L20	3.1	57.5	15.39	5
L21	9.4	100 (estimated)	162.27	28
L23	1.5	15.3	nd	12
L24	2.4	18.7	3.73	6
L25	3.7	33.1	15.55	9

Example 7: Conversion of Parental 18A5 IgG to Germline Sequence

[0248] The following fifteen scFv with modified V_L regions, along with the germlined parental 18A5 V_L (see below), were chosen for conversion to full-length

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human IgG lambda: L2, L3, L6, L9, L11, L13, L14, L16, L17, L18, L19, L20, L23, L24, and L25. Four scFv with modified V_H regions, H3, H4, H5, and H6, along with the germlined parental 18A5 V_H (see below) were chosen for conversion to full-length human IgG1.

[0249] The V_H and V_L amino sequences of the parental 18A5 antibody were modified so that the sequences outside the CDR regions matched the closest human germline sequences: DP67/VH4B+ (VBASE_AA:WAP00CEAZ_1) and JH1/JH4/JH5 in the case of the V_H, and DPL16/VL3.1 (VBASE_AA:WAP00CEMI_1) in the case of the V_L. Modifications were done by a combination of gene synthesis at GENEART (Regensburg, Germany) and site-directed changes introduced by PCR. In addition, the sequences were codon-optimized for expression in mammalian cells by GENEART using their proprietary methods. An alignment of the parental 18A5 sequences and the germline-corrected 18A5 sequences is shown below:

18A5 Heavy Chain Comparison

Parental 18A5 V_H

```
CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGACTTCGGAGACCCTGTCCCTCACCTGCGCT
GTCTCTGGTTACTCCATCAGCAGTGGTTACTACTGGGGCTGGATCCGGCAGCCCCCAGGGAAGGGTTG
GAGTGGATTGGGAGTATCTCTCATACTGGGAACACCTACTACAACCCGCCCTCAAGAGTCGCGTCACC
ATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAACTGAGCTCTGTGACCGCCGCAGACACGGCC
GTGTATTACTGTGCGCGAGGTGGGGGAATTAGCAGGCCGGAGTACTGGGGCAAAGGCACCCTGGTCACC
GTCTCGAGT (SEQ ID NO:5)
```

Germlined 18A5 V_H

```
CAGGTGCAGCTGCAGGAGTCTGGCCCTGGCCTGGTGAAGCCTTCCGAGACCCTGTCTCTGACCTGTGCC
GTGTCCGGCTACTCCATCTCCTCCGGCTACTACTGGGGCTGGATCAGACAGCCTCCTGGCAAGGGCCTG
GAGTGGATCGGCTCCATCTCTCACACCGGCAACACCTACTACAACCCCCCTCTGAAGTCCAGAGTGACC
ATCTCCGTGGACACCTCCAAGAACCAGTTCTCCCTGAAGCTGTCTCTGTGACCGCTGCCGATACCGCC
GTGTACTACTGTGCCAGAGGCGGCGGAATCTCCAGACCTGAGTACTGGGGCCAGGGCACCCCTGGTGACC
GTGTCCTCT (SEQ ID NO:7)
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Germlined 18A5 V_H x Parental 18A5 V_H

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      .       .       .       .       .
1  CAGGTGCAGCTGCAGGAGTCTGGCCCTGGCCTGGTGAAGCCTTCCGAGAC  50
   ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1  CAGGTGCAGCTGCAGGAGTCGGGCCAGGACTGGTGAAGACTTCGGAGAC  50
      .       .       .       .       .

51  CCTGTCTCTGACCTGTGCCGTGTCCGGCTACTCCATCTCCTCCGGCTACT  100
   ||||| || ||||| || || || || ||||| ||||| || |||||
51  CCTGTCCCTCACcTGCGCTGTCTCTGGTTACTCCATCAGCAGTGGTTACT  100
      .       .       .       .       .

101 ACTGGGGCTGGATCAGACAGCCTCCTGGCAAGGGCCTGGAGTGGATCGGC  150
     ||||| ||||| || ||||| || || ||||| ||||| ||||| ||
101 ACTGGGGCTGGATCCGGCAGCCCCAGGGAAGGGGTTGGAGTGGATTGGG  150
      .       .       .       .       .

151 TCCATCTCTCACACCGGCAACACCTACTACAACCCCCCTCTGAAGTCCAG  200
     ||||| || || ||||| ||||| ||||| || || || || ||
151 AGTATCTCTCATACTGGGAACACCTACTACAACCCGCCCTCAAGAGTCG  200
      .       .       .       .       .

201 AGTGACCATCTCCGTGGACACCTCCAAGAACCAGTTCTCCCTGAAGCTGT  250
     || ||||| || || ||||| ||||| ||||| ||||| ||||| ||
201 CGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTGA  250
      .       .       .       .       .

251 CCTCTGTGACCGCTGCCGATAACGCCGTGTACTACTGTGCCAGAGGCGGC  300
     ||||| ||||| || || || ||||| ||||| ||||| || ||
251 GCTCTGTGACCGCCGCAGACACGGCCGTGTATTACTGTGCGCGAGGTGGG  300
      .       .       .       .       .

301 GGAATCTCCAGACCTGAGTACTGGGGCCAGGGCACCCCTGGTGACCGTGTC  350
     ||||| || || ||||| ||||| || ||||| ||||| || ||
301 GGAATTAGCAGGCCGGAGTACTGGGGCAAAGGCACCCTGGTCACCGTCTC  350

351 CTCT 354 (SEQ ID NO:7)
     |
351 GAGT 354 (SEQ ID NO:5)

```

18A5 Light Chain ComparisonParental 18A5 V_L

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TCTTCTGAGCTGACTCAGGACCCTCCTGTGTCTGTGGCCTTGGGACAGACAGTCACGCTCACATGCCAA
GGAGACAGCCTCAGAACCTATTATGCAAGCTGGTACCAGCAGAAGTCAGGACAGGCCCTATACTTCTC
CTCTATGGTAAACACAAACGGCCCTCAGGGATCCCAGACCGCTTCTCTGGCTCCACCTCAGGAGACACA
GCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGACGAGGCTGACTATTACTGTAACCTCCCGGGACTCC
AGTGGCAACCCCCATGTTCTGTTCGGCGGAGGGACCCAGCTCACCGTTTTA (SEQ ID NO:9)

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Germlined 18A5 V_L

TCCTCTGAGCTGACCCAGGATCCTGCTGTGTCTGTGGCCCTGGGCCAGACCGTCAGGATCACCTGCCAG
 GGCGATAGCCTGAGAACCTACTACGCCTCCTGGTATCAGCAGAAGCCTGGACAGGCCCTGTGCTGGTG
 ATCTACGGCAAGCACAAAGAGGCCATCCGGCATCCCTGACAGATTCTCCGGCTCCTCCTCTGGCAATACC
 GCCTCCCTGACCATCACCGGCGCTCAGGCCGAGGACGAGGCCGACTACTACTGTAACTCCCGGGACTCT
 TCCGGCAACCCTCACGTGCTGTTTGGCGGCGGAACCCAGCTGACCGTGCTA (SEQ ID NO:11)

Germlined 18A5 V_L x Parental 18A5 V_L

1	TCCTCTGAGCTGACCCAGGATCCTGCTGTGTCTGTGGCCCTGGGCCAGAC	50
1	TCTTCTGAGCTGACTCAGGACCCTCCTGTGTCTGTGGCCTTGGGACAGAC	50
		
51	CGTCAGGATCACCTGCCAGGGCGATAGCCTGAGAACCTACTACGCCTCCT	100
51	AGTCACGCTCACATGCCAAGGAGACAGCCTCAGAACCTATTATGCAAGCT	100
		
101	GGTATCAGCAGAAGCCTGGACAGGCCCTGTGCTGGTGATCTACGGCAAG	150
101	GGTACCAGCAGAAGTCAGGACAGGCCCTATACTTCTCCTCTATGGTAAA	150
		
151	CACAAGAGGCCATCCGGCATCCCTGACAGATTCTCCGGCTCCTCCTCTGG	200
151	CACAAACGGCCCTCAGGGATCCCAGACCGCTTCTCTGGCTCCACCTCAGG	200
		
201	CAATACCGCCTCCCTGACCATCACCGGCGCTCAGGCCGAGGACGAGGCCG	250
201	AGACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGACGAGGCTG	250
		
251	ACTACTACTGTAACTCCCGGGACTCTTCCGGCAACCCTCACGTGCTGTTT	300
251	ACTATTACTGTAACTCCCGGGACTCCAGTGGCAACCCCATGTTCTGTTC	300
		
301	GGCGGCGGAACCCAGCTGACCGTGCTA	327 (SEQ ID NO:11)
301	GGCGGAGGGACCCAGCTCACCGTTT	327 (SEQ ID NO:9)

Germline-corrected V_H sequence (changes from parental sequence are bold and underlined):

Parental (SEQ ID NO:6) QVQLQESGPGLVKTSETLSLTCAVSGYSISSGYYWGWIRQPPGKG

Germlined (SEQ ID NO:8) QVQLQESGPGLVK**P**SETLSLTCAVSGYSISSGYYWGWIRQPPGKG

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Parental LEWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGGISRP

Germlined LEWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGGISRP

Parental EYWGKGTLVTVSS

Germlined EYWQGTLVTVSS

Germline-corrected V_L sequence (changes from parental sequence are bold and underlined):

Parental (SEQ ID NO:10) SSELTDPPVSVALGQTVTLTCQGDSLRTYYASWYQQKSGQAPIL

Germlined (SEQ ID NO:12) SSELTDPPAVSVAGQTVRITCQGDSLRTYYASWYQQKPGQAPVL

Parental LLYGKHKRPSGIPDRFSGTSGDTASLTITGAQAEDEADYYCNSRDSSGNPHVLFGGGTQ

Germlined VIYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNPHVLFGGGTQ

Parental LTVL

Germlines LTVL

Example 8: Conversion of Library-Derived scFv to IgG

[0250] The CDR3 regions of the V_L and V_H domains of improved 18A5 scFv derivatives were amplified by PCR and subcloned into the germline-corrected V_L and V_H frameworks of the parental 18A5 by the following method. A PCR fragment encompassing the 5' portion of the germlined 18A5 V_H gene was generated by amplification of the plasmid pSMED2_OP18A5G_huIgG1 with primers BssHII_II_V_H_F (5'-GCTTGGCGCGCACTCTCAGGTGCAGCTGCAGGAG-3') [SEQ ID NO:230] and GV_H_R_for_BssHII (5'-TCAGGGAGAACTGGTTCTTGG-3') [SEQ ID NO:231]. A PCR fragment encompassing the 3' portion of the V_H gene from the improved scFv clone VH3 was amplified with the following primers: G_V_H_F_for_Sall

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(5'-TCCAAGAACCAGTTCTCCCTG-3') [SEQ ID NO:232] and scFv_SalI_V_H_R (5'-GCGACGTCGACAGGACTCACCCTCGAGACGGTGACCAGGGTGCC-3') [SEQ ID NO:233]. Fragments were gel-purified, and then the two were mixed and amplified with the outside primer sets BssHII_G_V_H_F and SalI_V_H_R to generate a complete V_H gene fragment. This was digested with BssHII and SalI and ligated into a vector containing the constant regions of human IgG1 with a triple-mutant hinge region. The insert was reamplified with BssHII_II_V_H_F and a new primer (Sal_V_H_R_RJ (5'-GCGACGTCGACAGGACTCACCCTCGAGACGG-3')) [SEQ ID NO:234] in order to alter the coding sequence of the V_H J segment to conform to the JH1 germline sequence, and ligated into a human IgG1-triple-mutant constant region vector.

[0251] The V_L genes from improved scFv were subcloned by a similar method. A PCR fragment encompassing the 5' portion of the 18A5 V_L gene was generated by amplification of the plasmid pSMEN2_OP18A5G_hu Lambda with primers BssHII_II_V_L_F (5'-GCTTGGCGCGCACTCTTCCTCTGAGCTGACCCAG-3') [SEQ ID NO:235] and scFv_V_L_R_for_BssHII (5'-GCCTGAGCCCCAGTGATGGTCA-3') [SEQ ID NO:236]. PCR fragments encompassing the 3' portions of the V_L genes from improved scFv clones were amplified with the primers GV_L_F_for_XbaI (5'-ACCGCCTCCCTGACCATCAC-3') [SEQ ID NO:237] and scFv_XbaI_V_L_R (5'-GCGCCGTCTAGAGTTATTCTACTCACCTAAAACGGTGAGCTGGGTCCC TC-3') [SEQ ID NO:238]. Fragments were gel-purified, and then fragments corresponding to the 5' and 3' portions of each gene were mixed and amplified with the outside primer set BssHII_II_V_L_F and scFv_XbaI_V_L_R to generate complete V_L gene fragments. These were digested with BssHII and XbaI, and ligated into a vector containing the constant regions of the human lambda gene.

*Example 9: Characterization of Improved IgG In Vitro**Example 9.1: Transient Small-scale Expression of Binding Proteins*

[0252] Clones were tested for function in full IgG format following transient expression in cos-7 cells. Each light chain in the set of sixteen test sequences (germlined parental 18A5 V_L and L2, L3, L6, L9, L11, L13, L14, L16, L17, L18, L19, L20, L23, L24 and L25) was paired with each heavy chain in the set of five test sequences (H3, H4, H5, and H6, along with V_H-P, the germlined parental 18A5 V_H domain). Each plasmid in the pair (1.4 µg) was combined with the TRANSIT[®] transfection reagent (Mirus, Madison, WI) according to the manufacturer's instructions, and DNA:TRANSIT[®] reagent complexes were added to monolayers of cos-7 cells growing in Dulbecco's Modified Eagle's medium (DMEM) / 10% heat-inactivated fetal bovine serum / penicillin / streptomycin / 2 mM L-glutamine in 6-well tissue culture plates. After 24 hr, the medium was changed to a serum-free medium (R1CD1), and was then collected 48 hr later. Binding proteins, now comprising full-length antibodies, were quantitated by anti-human IgG ELISA.

Example 9.2: Activity of Anti-IL-21R IgG in Neutralization of Cell Proliferation

[0253] The 80 transiently expressed IgGs in serum-free conditioned medium were tested for activity in IL-21-dependent proliferation assays in three cell lines as described above: (1) human IL-21R-BaF3 cells, (2) murine IL-21R-BaF3 cells, and (3) human IL-21R-TF1 cells. All 80 pairs showed neutralization of proliferation of human IL-21R-expressing BaF3 cells, and all pairs except those involving VH4 showed neutralization of human IL-21R-expressing TF1 cells (data not shown). All 80 pairs also showed neutralization of proliferation of murine IL-21R-expressing BaF3 cells, with the strongest neutralization generally associated with light chains paired with the parental heavy chain and the weakest neutralization generally associated with the VH4 heavy chain (data not shown). Neutralization data from the most potent 21 IgG combinations (AbA-AbU) are shown in **FIG. 5**, and IC₅₀ data are summarized in **Table 6**.

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[0254] Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIGs. 5a-c**), human IL-21R-TF1 cells with 100 pg/ml of human IL-21 (**FIGs. 5d-f**), or murine IL-21R-BaF3 cells with 400 pg/ml of murine IL-21 (**FIGs. 5g-i**). IL-21 was added to the cells after the indicated antibodies; proliferation was measured with CELLTITER-GLO[®] after 48 hr. **FIGs. 26a-c** show additional studies demonstrating similar inhibition in the same three cell lines.

Example 9.3: Anti-IL-21R IgG Binding to Transiently Expressed Rat and Cynomolgus Monkey IL-21R

[0255] A subset of binding proteins was tested for binding to rat, cynomolgus monkey, human IL-21R, or human IL-2R- γ common subunit expressed transiently on the surfaces of CHO-PA-Dukx cells. Cells were transfected 48 hr prior to the assay. On the day of the assay, cells were washed gently 5X in PBS containing 0.9 mM CaCl₂ and 0.45 mM MgCl₂ (PBS / CaMg) on an automated plate washer (Titertek, Huntsville, AL), and blocked for 1 hr at RT in PBS / CaMg / 5% nonfat dry milk. Conditioned media from transiently expressed anti-IL-21R IgGs were serially diluted in blocking buffer and added to the cells in the blocked plates for 1 hr at RT. Cells were washed 5X with PBS / CaMg and then incubated with horseradish peroxidase-conjugated anti-human IgG for 1 hr at RT. Cells were then washed 10X in PBS / CaMg and all of the wash buffer was removed. Cells were incubated with 100 μ l TMB until the color reaction reached saturation, stopped with 100 μ l of 0.18 M H₂SO₄, and read at A450 on a Perkin Elmer ENVISION[™] plate reader.

[0256] All of the twenty-one IgGs bound to CHO cells transiently expressing human (**FIGs. 6a-c**), rat (**FIGs. 6d-f**), or cynomolgus monkey (**FIGs. 6g-i**) IL-21R. Most showed no binding above background to a control protein (human gamma (γ) common chain) transiently expressed on CHO cells, but a subset of IgGs (AbD, AbE, AbF, AbH, AbL, and AbM) bound above background at 13 nM or greater (**FIGs. 6j-l**). Data are summarized in **Table 6**.

Table 6: Summary of Neutralization of Human and Murine IL-21R Activity in Cell-proliferation Assays and Binding to Human, Rat, and Cynomolgus Monkey IL-21R Expressed on CHO Cells

Binding Protein	Human IL-21R-BaF3 Proliferation IC ₅₀ (nM)	Human IL-21R-TF1 Proliferation IC ₅₀ (nM)	Murine IL-21R-BaF3 Proliferation IC ₅₀ (nM)	Human IL-21R Binding (13 nM Ab in Cell ELISA; A450)	Rat IL-21R Binding (13 nM Ab in Cell ELISA; A450)	Monkey IL-21R Binding (13 nM Ab in Cell ELISA; A450)	Human γ -Common Binding (13 nM Ab in Cell ELISA; A450)
AbA	0.97	3.80	0.08	1.196	1.124	1.352	0.111
AbB	1.14	3.34	0.421	1.147	1.09	1.333	0.107
AbC	0.82	3.36	0.03	1.218	0.999	1.277	0.137
AbD	0.91	2.67	0.01	1.247	0.874	1.375	0.197
AbE	0.56	2.28	0.04	1.257	1.111	1.423	0.223
AbF	0.54	2.41	0.304	1.347	1.001	1.458	0.433
AbG	0.77	3.84	0.07	1.35	1.112	1.304	0.108
AbH	0.94	3.64	0.327	1.35	1.097	1.324	0.152
AbI	1.00	3.80	0.224	1.237	1.088	1.209	0.107
AbJ	0.65	4.60	0.4	1.217	1.261	1.273	0.126
AbK	0.98	4.00	0.079	1.364	1.175	1.338	0.108
AbL	0.68	4.25	0.227	1.454	1.257	1.514	0.219
AbM	1.08	4.22	0.125	1.197	0.78	1.45	0.224
AbN	0.50	1.59	0.435	1.214	0.702	1.497	0.136
AbO	0.52	2.91	0.065	1.107	1.101	1.358	0.108
AbP	0.75	3.48	0.03	1.308	1.03	1.313	0.112
AbQ	0.68	4.62	0.153	1.255	1.161	1.31	0.125
AbR	0.87	3.94	0.302	1.334	1.108	1.35	0.109
AbS	1.53	5.00	0.04	1.017	1.166	1.224	0.118
AbT	0.67	3.26	0.093	1.078	0.994	1.219	0.102
AbU	0.73	3.13	0.184	1.289	0.927	1.314	0.104

Example 9.4: BIACORE™ Analysis of Selectivity of Anti-IL-21R IgG Binding to Human IL-21R

[0257] The specificity of binding of a subset of transiently expressed anti-IL-21R binding proteins (here antibodies) was tested on a BIACORE™ 2000 surface plasmon resonance instrument. Anti-human-IgG, anti-murine immunoglobulin antibodies, and murine IL-21R-H/F were immobilized onto a research-grade carboxymethyl-dextran chip (CM5) using standard amine coupling. The sensor chip surface was activated with EDC/NHS for 7 min at a flow rate of 20 μ l/min. The first flow cell was used as reference surface to correct for bulk refractive index, matrix effects, and nonspecific binding. Capture antibodies (7,150 resonance units (RU) of anti-human-Fc antibody (Invitrogen Corporation, Carlsbad, CA) on flow cell 2 and

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7,500 RU of anti-murine-Fc antibody on flow cell 3) were diluted to 10 µg/ml in sodium acetate buffer (pH 5.0) and injected over the activated surface. Remaining activated groups were blocked with 1.0 M ethanolamine (pH 8.0). The molecular weights of the anti-human IgG and the anti-murine IgG were both 150 kD, and the molecular weight of the IL-21R monomer was 27 kD.

[0258] Conditioned media containing anti-IL-21R antibodies and antibody controls (murine anti-human IL-2Rβ and murine anti-human IL-4R (R&D Systems, Minneapolis, MN); human anti-human IL-13 (Wyeth, Cambridge, MA)) were diluted in HBS / EP buffer supplemented with 0.2% bovine serum and injected onto all four flow cells of the BIACORE™ chip, capturing 500-700 (RU) of antibody on the species-appropriate capture antibody. Following a 5 sec washing period, 50 nM solutions of a positive control protein (murine IL-21R-H/F), two human proteins related to IL-21R (human IL-2Rβ and human sIL-4R (R&D Systems)), or an unrelated His/FLAG-tagged protein (human IL-13-H/F) were injected over the captured antibodies on the chip. The association and dissociation phases were monitored for 120 and 180 sec, respectively, followed by two 5 µl injections of glycine (pH 1.5) to regenerate a fully active capturing surface. All binding experiments were done at 25°C in HBS / EP buffer. Blank and buffer effects were subtracted for each sensorgram using double referencing.

[0259] All of the anti-IL-21R antibodies tested (18A5 antibody and AbA-AbU) showed clear binding to murine IL-21R, but no binding to the IL-21R-related proteins human IL-2Rβ and human soluble IL-4R, or to the unrelated His/FLAG-tagged protein human IL-13-His/FLAG (**FIGs. 7a-c**). Controls indicated that IL-2Rβ and human soluble IL-4R could be captured by specific anti-IL-2Rβ and anti-IL-4R antibodies (**FIG. 7d**).

Example 9.5: Purification of Transiently Expressed Antibodies

[0260] Seven antibodies (human IgG1 triple-mutant versions: AbS, AbT, AbO, AbP, and AbU; and double-mutant versions: AbQ and AbR) were transiently expressed in

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cos-7 cells and purified for further analysis. In addition, three versions of AbT with human IgG tails expected to have different levels of Fc receptor binding (wild-type IgG1, IgG4, and IgG1 double-mutants) were also prepared. The *TRANSIT*[®] protocol described above was followed, except that 25 µg of each plasmid was used to transfect cells in each of eight T-175 flasks. Following the first harvest of conditioned medium, fresh R1CD1 was added and then collected after an additional 72 hr. Conditioned media were pooled and filtered on a 0.22 µm filter. Antibodies were loaded onto protein A resin, eluted with 20 mM citric acid / 150 mM sodium chloride (pH 2.5), neutralized with Tris (pH 8.5), and dialyzed into PBS.

Example 9.6: BIACORE[™] Analysis of Antibody Binding to Human and Murine IL-21R

[0261] The kinetics of binding of anti-IL-21R antibodies to human and murine IL-21R-H/F was tested on a BIACORE[™] surface plasmon resonance instrument. Anti-human IgG antibodies (Invitrogen Corporation) were immobilized onto a research-grade carboxy-methyl-dextran chip (CM5) using standard amine coupling. The surface was activated with EDC/NHS for 7 min at a flow of 20 µl/min. The first flow cell was used as a reference surface to correct for bulk refractive index, matrix effects, and nonspecific binding. The anti-human-Fc antibody was diluted to 20 µg/ml in 10 mM sodium acetate buffer (pH 5.0), and 2950-3405 resonance units (RU) were captured on each of the four flow cells. Remaining activated groups were blocked with 1.0 M ethanolamine-HCl (pH 8.5).

[0262] Anti-IL-21R antibodies were diluted to 0.1-0.2 µg/ml in HBS / EP buffer supplemented with 0.2% bovine serum albumin and loaded onto the BIACORE[™] chip. Following a brief washing period, solutions of 0-100 nM human IL-21R-H/F or 10-500 nM murine IL-21R-H/F were injected over the chip at a flow rate of 50 µl/min. The association phase was run for 3 min for human and murine IL-21R kinetics, and the dissociation phase was monitored for 15 min for hIL-21R and for 5 min for mIL-21R, followed by two 10 µl injections and one 30 µl injection of glycine (pH 1.5), to regenerate a fully active capturing surface. All binding

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experiments were done at 25°C in HBS / EP buffer, and the sample rack was kept at 15°C. Blank and buffer effects were subtracted for each sensorgram using double referencing. Sensorgrams are shown in **FIGs. 8a-b** (human IL-21R-His/FLAG) and **FIGs. 8c-d** (murine IL-21R-His/FLAG). Binding kinetic parameters are shown in **Table 7A**, and additional kinetic data from a replicate experiment are shown in **Table 7B**.

[0263] In addition, AbS and AbT were tested for binding kinetics to cynomolgus monkey IL-21R-His/FLAG by the above-described protocol. Binding profiles to human and cynomolgus monkey IL-21R-H/F were similar for both AbS and AbT (**FIG. 9**). **FIG. 9** shows cynomolgus monkey IL-21R-His/FLAG binding to AbS (**9a**); and to AbT (**9c**); and human IL-21R-His/FLAG binding to AbS (**9b**); and AbT (**9d**).

Table 7A: Kinetic Parameters of Anti-IL-21R Antibody Binding Human and Murine IL-21R-His/FLAG

Antibody	Human IL-21R			Murine IL-21R		
	ka (1/Ms)	kd (1/s)	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)
18A5	2.43E+05	1.08E-03	4.43E-09	2.12E+05	1.53E-02	7.20E-08
AbO	2.41E+05	1.14E-04	4.75E-10	1.12E+05	5.49E-03	4.92E-08
AbP	1.94E+05	1.19E-04	6.15E-10	9.99E+04	5.08E-03	5.08E-08
AbQ	4.39E+05	9.34E-05	2.13E-10	3.01E+05	2.07E-02	6.88E-08
AbR	1.70E+05	9.61E-05	5.67E-10	7.65E+04	4.93E-03	6.45E-08
AbS	1.44E+05	2.91E-04	2.02E-09	1.99E+05	3.32E-03	1.67E-08
AbT	1.79E+05	6.78E-05	3.79E-10	2.11E+05	3.31E-03	1.57E-08
AbU	1.86E+05	8.18E-05	4.40E-10	9.81E+04	4.34E-03	4.42E-08

Table 7B: Kinetic Parameters of Anti-IL-21R Antibody Binding Human IL-21R-His/FLAG

	Human IL-21R		
Antibody	ka (1/Ms)	kd (1/s)	KD (M)
18A5	3.04E+05	1.34E-03	4.40E-09
AbP	2.33E+05	1.02E-04	4.36E-10
AbQ	4.39E+05	9.34E-05	2.13E-10
AbR	2.48E+05	9.76E-05	3.94E-10
AbS	2.02E+05	3.05E-04	1.51E-09
AbT	2.73E+05	7.42E-05	2.72E-10
AbU	2.38E+05	7.83E-05	3.29E-10

Example 9.7: BIACORE™ Epitope Competition Assay

[0264] Antibodies AbS and AbT and the parental antibody 18A5 were immobilized directly onto a CM5 BIACORE™ chip. Murine IL-21R-H/F (100 nM) was allowed to flow over the chip for 300 sec, followed by a wash (100 sec), and then a 5 µg/ml solution of either AbS, AbT, D5, or a nonneutralizing anti-mIL-21R antibody (7C2) was allowed to flow over the surface. No additional binding was observed with AbS, AbT, and D5, indicating that their binding site on mIL-21R-H/F was blocked by concurrent binding to AbS, AbT, or 18A5 antibody (**FIG. 10a**). In contrast, the nonneutralizing control anti-IL-21R antibody 7C2 was able to bind to mIL-21R-H/F captured on AbS, AbT, or 18A5 antibody, indicating that this control antibody bound at a different epitope from the one bound by the capture antibodies.

[0265] Similarly, AbS and AbT did not bind to human IL-21R-H/F captured by AbS or AbT immobilized on a CM5 BIACORE™ chip, while the control anti-human IL-21R antibody (9D2) was able to bind human IL-21R-H/F captured by AbS or AbT (**FIG. 10b**). This observation suggested that the binding site for AbS is blocked by concurrent binding by AbT, and vice versa.

Example 9.8: Cell-based Proliferation Assays

[0266] Purified IgGs were tested for activity in IL-21-dependent proliferation assays in three cell lines as described above: human IL-21R-BaF3 cells, murine IL-21R-BaF3 cells, and human IL-21R-TF-1 cells. All showed strong inhibition of

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both human and murine IL-21R-dependent proliferation with greater potency than that of the parental 18A5 IgG (**FIG. 11, Table 8**). Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIG. 11a**), murine IL-21R-BaF3 cells with 200 pg/ml of murine IL-21 (**FIG. 11b**), and human IL-21R-TF-1 cells with 100 pg/ml of human IL-21 (**FIG. 11c**). **FIG. 26d** depicts the results of an additional study of the effects of these antibodies on human IL-21R-BaF3 cells.

Table 8: Neutralization of Proliferation of Human IL-21R-BaF3 Cells, Murine IL-21R-BaF3 Cells, and Human IL-21R-TF-1 Cells

Antibody	Human IL-21R-BaF3 Neutralization IC₅₀ (nM)	Murine IL-21R-BaF3 Neutralization IC₅₀ (nM)	Human IL-21R-TF1 Neutralization IC₅₀ (nM)
18A5 antibody	1.71	177.23	13.99
AbR	0.56	0.34	1.63
AbS	0.68	0.04	6.67
AbT	0.30	0.05	2.32
AbX	0.54	nd	nd
IL21R-Fc	0.20 (human IL-21R-Fc)	0.04 (mouse IL-21R-Fc)	7.22 (human IL-21R-Fc)

Example 9.9: Primary Human B cell Proliferation Assays

[0267] Anti-IL-21R antibodies were tested for their ability to inhibit IL-21-dependent proliferation of primary human B cells. Buffy coat cells from healthy human donors were obtained from Massachusetts General Hospital (Boston, MA). The cells were incubated with a ROSETTESEP™ B cell enrichment cocktail (StemCell Technologies, Vancouver, Canada), and B cells isolated according to the manufacturer's instructions. The resulting population (60-80% CD19⁺ B cells) were cultured in RPMI containing 10% FBS, 50 U/ml penicillin, 50 µg/ml streptomycin, and 2 mM L-glutamine at 1x10⁵/well in 96-well flat-bottom plates. B cells were pretreated with serially diluted anti-human IL-21R antibodies in a 37°C incubator adjusted to 5% CO₂ for 30 min. The treated B cells were then stimulated with 0.5 µg/ml anti-CD40 mAb (BD Biosciences, San Jose, CA) and 10 ng/ml IL-21

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cytokine for 3 days in a 37°C incubator adjusted to 5% CO₂. On day 3, cultures were pulsed with 0.5 µCi/well ³H-thymidine (Perkin Elmer (NEN)) and harvested 5 hr later onto glass fiber filter mats. ³H-thymidine incorporation was determined by liquid scintillation counting. All of the improved antibodies neutralized IL-21-dependent proliferation with greater potency than the parental 18A5 antibody (FIGs. 12a-b, Table 9; also see FIG. 26e).

Table 9: Neutralization of Human Primary B cell Proliferation

Antibody	Neutralization of B cell proliferation IC₅₀ (nM)
AbQ	0.16
AbR	0.22
AbS	0.44
AbT	0.14
AbU	0.13
18A5 antibody	1.86

Example 9.10: Primary Human T cell Proliferation Assays

[0268] Anti-IL-21R antibodies were tested for their ability to inhibit IL-21-dependent proliferation of primary human CD4⁺ T cells. Buffy coat cells from healthy human donors were obtained from Massachusetts General Hospital. CD4⁺ T cells were isolated by negative selection using ROSETTESEP™ CD4⁺ T cell enrichment cocktail (StemCell Technologies), according to the manufacturer's instructions. The resulting population was ~80-90% CD4⁺/CD3⁺ T cells. Enriched human CD4⁺ T cells were activated for 3 days with anti-CD3/anti-CD28-coated microspheres in RPMI containing 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, and HEPES in a 37°C incubator adjusted to 5% CO₂. After activation, the microspheres were removed and the cells were washed and rested overnight at approximately 1x10⁶ cells/ml in culture medium. The rested cells were then washed again before addition to the assay plates. Serial dilutions of anti-human IL-21 receptor antibodies were made in culture medium in flat-bottomed

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96-well plates, followed by the sequential addition of human IL-21 (20 ng/ml final concentration) and the activated and rested CD4⁺ T cells (10⁵ cells/well). The plates were then incubated for an additional 3 days and pulsed with 1 μ Ci/well ³H-thymidine (Perkin Elmer (NEN)) during the final 6 hr of the assay. Cells were harvested onto glass fiber filter mats and ³H-thymidine incorporation was determined by liquid scintillation counting. All of the improved antibodies neutralized IL-21-dependent proliferation with greater potency than the parental 18A5 antibody (FIG. 13, Table 10A; also see FIG. 26f).

Table 10A: Neutralization of Human Primary T cell Proliferation

Antibody	Neutralization of T cell Proliferation IC ₅₀ (nM)
AbO	0.06
AbP	0.02
AbQ	0.08
AbR	0.04
AbS	0.06
AbT	0.03
AbU	0.03
18A5 antibody	1.42

Example 9.11: Primary Murine T cell Proliferation Assays

[0269] Anti-IL-21R antibodies were tested for their ability to inhibit IL-21-dependent proliferation of primary murine CD8⁺ T cells. Popliteal, axillary, brachial, and inguinal lymph nodes and spleens from 12-week-old female BALB/C mice were collected. A single-cell suspension of the spleen cells was depleted of red blood cells using 0.16 M NH₄Cl in 0.017 M Tris (pH 7.4). The spleen and lymph node cells were pooled and enriched for CD8⁺ cells using a murine T cell CD8 Subset Column Kit (R&D Systems). Murine CD8⁺ cells (3x10⁴; suspended in DMEM containing 10% fetal calf serum and supplemented with 0.05 mM β -mercaptoethanol, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μ g/ml streptomycin and 50 μ g/ml gentamicin) were plated in 96-well,

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anti-mCD3 activation plates (BD Biosciences); mIL-21 (50 ng/ml) was added to all the wells. The test antibodies were titrated in triplicate beginning at 20 μ g/ml. Cells were grown for 3 days in a 37°C / 10% CO₂ incubator. During the last 5 hr of culture, cells were labeled with 0.5 μ Ci methyl-³H-thymidine/well (GE Healthcare). The cells were harvested using a Mach III cell harvester (TomTec, Hamden, CT) and counted using a Trilux microbeta counter (Perkin Elmer). Aside from AbP, all of the improved antibodies neutralized IL-21-dependent proliferation with greater potency than the parental 18A5 antibody (**FIG. 14, Table 10B**; also see **FIG. 26g**).

Table 10B: Neutralization of Murine Primary T cell Proliferation

Antibody	Neutralization of T cell Proliferation IC₅₀ (nM)
AbO	4.92
AbP	no inhibition
AbQ	0.85
AbR	0.13
AbS	0.02
AbT	0.61
AbU	1.79
18A5 antibody	>85

Example 9.12: ADCC Assay

[0270] Anti-IL-21R antibodies were tested for their ability to induce antibody-dependent cellular cytotoxicity (ADCC) when bound to target cells. The day before the experiment, PBMC were isolated from buffy coat by diluting the buffy coat 1:1 in PBS, layering it over FICOLL[®] (GE Healthcare) and centrifuging at 1200 g for 20 min. PBMCs were removed from the top of the FICOLL[®] layer, washed, and stimulated overnight with 10 ng/ml IL-2 and 10 ng/ml IL-12 (R&D Systems). The day of the experiment, stimulated PBMCs were collected by centrifugation and resuspended in media at 1x10⁸ cells/ml. BJAB cells were labeled with 0.5 μ M CFSE (MOLECULAR PROBES[®], Invitrogen Corporation) for 10 min at 37°C, and then washed with fetal bovine serum once and PBS twice. Cells were then plated into a

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96-well flat-bottom plate at 2×10^5 cells/well in 100 μ l media. Fifty μ l of the 4x antibodies were added to the BJAB cells, followed by 5×10^6 PBMC in 50 μ l, giving a final 1:25 target:effector cell ratio. Cells were incubated at 37°C for 6 hr and stained with propidium iodide (PI) to label dead and dying cells. Killing of target cells (CFSE⁺) was assessed by measuring PI staining in a FACSCALIBUR™ flow cytometer (BD Biosciences). Only one anti-IL-21R antibody, AbZ, which has a wild-type human IgG1 constant region, showed ADCC above the background level displayed by a control anti-IL-13 antibody that did not bind to the target cells. All antibodies with the same variable domains as AbZ, including forms with human IgG4 (AbY), and those with double-mutant (AbX) and triple-mutant (AbT) forms of human IgG1, showed only background levels of ADCC (**FIG. 15**). All other anti-IL-21R antibodies tested contained the triple-mutant form of human IgG1 and showed background ADCC. A positive control antibody, rituximab (RITUXAN®), induced ADCC in all experiments.

Example 9.13: C1q ELISA

[0271] In order to determine whether cell-surface binding by anti-IL-21R antibodies is likely to lead to complement-dependent cytotoxicity (CDC), the antibodies were tested for their ability to bind to the complement component C1q in an ELISA. IL-21R antibodies and rituximab (RITUXAN®) were diluted in PBS to 5 μ g/ml. Diluted antibodies (100 μ l) were coated onto a COSTAR® high-binding ELISA plate (Corning Life Sciences, Lowell, MA) overnight at 4°C. Plates were washed 3X with PBS / Tween-20 and blocked with 200 μ l of blocking buffer (0.1 M NaPO₄, 0.1 M NaCl, 0.1% gelatin, 0.01% Tween) for 1 hr at RT. Human serum previously determined to contain C1q (Quidel, San Diego, CA) was diluted 1:50 in PBS. After 1 hr of blocking, plates were washed and 100 μ l of diluted serum was added to each well and incubated for 2 hr at RT on a shaker. Following three washes, 100 μ l of 0.1 μ g/ml chicken polyclonal anti-human C1q antibody (AbCam, Cambridge, MA) was added to each well and incubated for 1 hr at RT. Plates were again washed and

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incubated with 100 μ l of a rabbit polyclonal antibody to chicken Ig-Y-HRP diluted 1:4000 (AbCam) for 1 hr at RT. Plates were washed and developed with TMB for 5 min, followed by 50 μ l of 1 M H_2SO_4 to stop the reaction, and then read at 450 nm. Only one anti-IL-21R antibody, AbZ, which has a wild-type human IgG1 constant region, showed C1q binding above the background level displayed by a control antibody with a triple-mutant human IgG1 constant region that had previously been shown to lack C1q binding. All antibodies with the same variable domains as AbZ, including forms with human IgG4 (AbY), and those with double-mutant (AbX) and triple-mutant (AbT) forms of human IgG1, showed only background levels of C1q binding (**FIG. 16**). All other anti-IL-21R antibodies tested contained the triple-mutant form of human IgG1 and showed background C1q binding.

Example 9.14: Cytokine competition assay

[0272] In order to demonstrate that antibody AbT binds to the murine IL-21R in a manner that competes with the IL-21 cytokine, a cytokine competition assay was performed. Antibody AbT was coated at 1 μ g/ml onto ELISA plates, which were then blocked with 1% BSA in PBS/.05% Tween. Biotinylated murine IL-21R-His/FLAG (1.5 ng/ml) was added to the wells, either alone or in the presence of increasing concentrations of murine IL-21, and the binding of the receptor to the immobilized antibody was detected with HRP-conjugated streptavidin and subsequent incubation with TMB detection reagent. Mouse IL-21 was able to block the binding of mIL-21R to AbT nearly completely above 4 ng/ml, indicating that the antibody and the cytokine compete for binding to murine IL-21R (**FIG. 27a**).

[0273] A second assay was performed to demonstrate that antibody AbS binds to the murine IL-21R in a manner that competes with the IL-21 cytokine. Murine IL-21R-Fc was captured on ELISA plates coated with an anti-mouse IgG2a antibody. Plates were blocked with 1% BSA in PBS and washed, and varying concentrations of AbS were added to the plate in the presence of 10 μ g/ml mIL-21. The binding of mIL-21 to the receptor was detected by an HRP-conjugated anti-His₆ antibody, and

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the binding of AbS to the receptor was detected by an anti-human Ig antibody. Concentrations of AbS above approximately 2 µg/ml completely prevented binding of mIL-21 to mIL-21R-Fc, indicating that the antibody and the cytokine compete for binding to murine IL-21R (**FIG. 27b**).

Example 9.15: Inhibition of Rat T cell Proliferation by Anti-IL-21R Antibodies

[0274] Lewis female rat splenic T cells were purified to 95% CD3⁺ using Rat T cell Enrichment Columns (RTCC-25; R&D Systems) according to the manufacturer's instructions. Serial dilutions of the anti-human IL-21R antibodies and isotype control protein were made in culture medium (Dulbecco's Modified Eagle Medium containing 10% FCS, L-glutamine, beta-mercaptoethanol, nonessential amino acids, sodium pyruvate, penicillin, streptomycin, and gentamycin) in flat-bottomed 96-well tissue culture plates which had been precoated with 1 µg of anti-rat CD3 antibody (BD Pharmingen Cat# 554829), followed by the addition of 5 ng/ml rat IL-21 and 20,000 CD3 T cells per well. The cells were grown for 3 days in a 10% CO₂, 37°C, humidified incubator. For the last 5 hr of culture, cells were labeled with 0.5 µCi of ³H-thymidine (GE Amersham Cat# TRA-120). The plates were harvested onto glass fiber filter mats by a Tomtec Mach III plate harvester and were counted on a Perkin Elmer 1450 Microbeta Counter.

[0275] The response of the rat T cells to 5 ng/ml rat IL-21 was 6-fold above the background response to 1 µg of anti-CD3 alone. Antibodies AbS, AbU, AbV, and AbW were able to inhibit the ³H thymidine incorporation stimulated by 5 ng/ml rat IL-21 (57,000 cpm in the absence of antibody treatment; **FIG. 28**). IC₅₀ values for neutralization in two independent experiments are shown in **Table 11**.

Table 11: Blockade of IL-21-dependent Rat T cell Proliferation by Anti-IL-21R Antibodies.

Antibody	IC50 (nM) experiment 1	IC50 (nM) experiment 2
AbS	35.98	27.07
AbU	172.79	105.46

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AbV	70.55	59.23
AbW	159.06	94.74

Example 9.16: Binding of AbS to Rabbit IL-21R

[0276] In order to demonstrate that antibody AbS binds to rabbit IL-21R, the extracellular domains of two isoforms of rabbit IL-21R were subcloned as Fc fusions and transiently expressed in HEK293 cells. Rabbit IL-21R-Fc (either isoform 1 or isoform 2) was captured from conditioned medium onto ELISA plates coated with anti-mouse IgG2a. Varying concentrations of AbS were added, the plates were washed, and antibody binding was detected with an HRP-conjugated anti-human IgG antibody. AbS showed clear binding to both isoforms of rabbit IL-21R Fc (**FIG. 29a**). When binding of AbS to rabbit IL-21R-Fc was carried out in the presence of 10% conditioned medium containing rabbit IL-21, binding of AbS to either receptor isoform was reduced by approximately 10-fold, indicating that AbS competes with rabbit IL-21 for binding to rabbit IL-21R (**FIG. 29b**).

Example 10: Characterization of Improved IgG In Vivo

Example 10.1: Neutralization of IL-21R with the Anti-IL-21R Antibodies, AbS and AbT, Inhibits the Generation of IgG Antibody Responses to a T cell-dependent Antigen In Vivo

[0277] IL-21 is important for B cell isotype switching to certain subclasses of IgG and differentiation to plasma cells. Thus, to determine the efficacy of the anti-IL-21R antibodies, AbS and AbT, *in vivo*, the ability of these antibodies to inhibit IgM and IgG antibody responses to the T cell-dependent antigen, NP-chicken gamma globulin (NP-CGG) were tested in C57BL/6 mice. Mice were treated 3X / week with 10 mg/kg anti-IL-21R antibody or isotype control beginning one day prior to immunization with NP-CGG. NP-specific IgG and IgM were detected by ELISA. NP-specific IgM and IgG antibodies were readily detected in serum of isotype control-treated animals within 7 days following immunization, and these responses increased in magnitude to day 14 (**FIG. 30b**). Treatment with either AbS or AbT did

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not affect the magnitude of IgM responses in this study. NP-specific IgG antibody responses were delayed in AbS- or AbT-treated cells, as demonstrated by a decreased response compared to isotype control on day 7. NP-specific IgG antibody responses were similar in isotype control-, AbS- and AbT-treated mice at day 14 (**FIG. 30a**). These data show that neutralization of IL-21R *in vivo* using either AbS or AbT can transiently inhibit the induction of early IgG antibody responses to a T cell-dependent antigen.

[0278] A second study testing AbS and AbT also yielded similar results (**FIGs. 30c-f**). NP-specific IgG responses were transiently reduced at day 7 of immunization in mice treated with either AbS or AbT, but were similar to isotype control-treated mice at later time points. Isotype-specific ELISAs indicated that IgG2b and IgG2c were significantly reduced by AbS and AbT compared to controls at day 7 (**FIGs. 30e-f**). These data show that neutralization of IL-21R *in vivo* using either AbS or AbT can transiently inhibit the induction of early IgG antibody responses to a T cell-dependent antigen.

[0279] To test the effects of IL-21R neutralization on the maintenance of established long-term humoral immunity, C57BL/6 mice were immunized with NP-CGG and rested for at least one month to allow them to generate memory B cells and long-lived plasma cells, which give rise to long-term IgG serum antibody titers.

Approximately one month after immunization, mice were treated 3X / week i.p. with saline, or 10 mg/kg of either AbS or AbT, or isotype control antibody for two months. NP-specific IgG serum antibody titers were monitored every two weeks by ELISA. C57BL/6 mice had high titers of NP-specific IgG serum antibodies one month after immunization when compared to naïve C57BL/6 mice, consistent with the formation of long-term humoral immunity to NP. NP-specific IgG serum antibody titers remained stable over the course of the study in both mice treated with the isotype control antibody, and treatment with either AbS or AbT did not affect these antibody titers (**FIG. 30g**). Following immunization, B cells generate plasma cells (in bone marrow), which give rise to long-term serum antibody titers. At the

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end of the study, the number of NP-specific IgG plasma cells in the bone marrow of the mice was measured, and the cell numbers were unaffected by treatment with AbS or AbT (**FIG. 30h**). These data indicate that neutralization of IL-21R with AbS and AbT in this treatment regimen does not affect the maintenance of established long-term serum antibody titers.

Example 10.2: Anti-IL-21R Antibody Function in a Model of SLE

[0280] The anti-IL-21R antibodies AbS and AbT were tested for their ability to ameliorate disease in the MRL-*Fas*^{lpr} murine model of systemic lupus erythematosus (SLE). MRL-*Fas*^{lpr} mice spontaneously develop symptoms resembling those observed in human lupus, including high titers of anti-double-stranded DNA (anti-dsDNA) autoantibodies in circulation, immunoglobulin and complement C3 deposits in the glomeruli, presence of lymphocytic infiltrates in the kidney and lung, and, in severe disease, proteinuria, lymphadenopathy, and skin lesions. Male MRL-*Fas*^{lpr} mice were obtained from Jackson Laboratory (Bar Harbor, ME) and, starting at 12 weeks of age, were given AbS, AbT, saline, or a control anti-human IL-13 antibody with the same triple-mutant human IgG1 constant region at a dosage of 400 µg/mouse (10 mg/kg) 3X / week over 10 weeks via i.p. injection. Serum samples were taken biweekly and examined for anti-dsDNA antibodies by ELISA. Urine was collected biweekly and tested for protein (using protein test strips); neither control animals nor treated animals developed significant proteinuria. Animals were also monitored for enlarged lymph nodes and skin lesions; neither control animals nor treated animals showed abnormalities. After 10 weeks of treatment, animals were sacrificed, and kidney and brain sections were examined for Ig and C3 deposits by immunohistochemistry. Cellular infiltrations into kidney and lungs were measured by examination of H/E-stained tissue sections.

[0281] Treatment of MRL-*Fas*^{lpr} mice with the IL-21R blocking antibody AbS significantly reduced anti-dsDNA IgG serum antibody levels as compared to animals treated with saline or a control anti-human IL-13 antibody, beginning at 2 weeks post-treatment and continuing until 10 weeks post-treatment (**FIG. 31**). Anti-dsDNA

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antibodies were reduced to undetectable levels by AbS treatment in 8/10 mice at week 4 (**FIG. 31d**), 5/10 at week 6 (**FIG. 31e**), and 7/10 at week 8 (**FIG. 31f**), as compared to saline and IL-13 control antibody-treated animals (all control mice had detectable anti-dsDNA antibody titers). Treatment with the IL-21R blocking antibody AbT did not significantly affect anti-dsDNA IgG serum antibody levels.

[0282] **FIG. 31a** shows anti-dsDNA antibody titers following treatment (AbS-treated group is significantly different from both saline- and anti-IL-13-treated groups ($p<0.01$)). **FIG. 31b** shows prebleed anti-dsDNA antibody titers (saline-treated group is significantly different from AbS-, AbT-, and anti-IL-13-treated groups ($p<0.01$)). The AbS-treated group is significantly different from both saline- and anti-IL-13-treated groups ($p<0.01$) after 2 weeks of dosing (**FIG. 31c**); 4 weeks (**FIG. 31d**); 6 weeks (**FIG. 31e**); 8 weeks (**FIG. 31f**); and 10 weeks (**FIG. 31g**).

[0283] Treatment with AbS also significantly reduced Ig and C3 immune complex deposition and kidney pathology as compared to anti-IL-13 antibody-treated controls ($p<0.01$) (**FIGs. 32, 33**). Twelve-week-old male MRL-*Fas*^{lpr} mice were treated (10 mg/kg i.p., 3X / week) with either saline (control) or the indicated triple-mutant antibodies (an anti-human IL-13 human IgG1 A234 A235 A237 mutant antibody with no reactivity to murine IL-21 was used as an isotype control). Following 10 weeks of treatment, mice were sacrificed and Ig and C3 deposits in the kidneys were identified by immunocytochemistry. **FIG. 32** depicts IgG deposits in kidneys of MRL-*Fas*^{lpr} mice treated as indicated (in **FIG. 32b**, glomeruli are indicated by dashed circles, and examples of diffuse stain, indicating IgG deposits, are indicated with white arrowheads). Staining intensity was scored on a scale of 1-5 (**FIG. 32a**). Kidney IgG deposits in animals treated with AbS were significantly lower ($p<0.01$) than those in IL-13 triple-mutant-treated controls. **FIG. 33** depicts IgM (**FIG. 33a**) and complement C3 (**FIG. 33b**) deposits in kidneys of MRL-*Fas*^{lpr} mice treated as indicated. Staining intensity was scored on a scale of 1-5. IgM and complement C3 deposition in the kidneys were significantly reduced ($p<0.05$) by AbS compared to the anti-IL-13 control antibody (**FIG. 33**). **FIG. 34** depicts deposition of IgG

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(**FIG. 34a**), IgM (**FIG. 34b**), and C3 (**FIG. 34c**) in the brains of treated mice. IgG ($p<0.05$; **FIG. 34a**) but not IgM (**FIG. 34b**) or C3 (**FIG. 34c**) deposits were reduced in the brains of AbS-treated mice as compared to anti-IL-13 antibody-treated controls. Staining intensity was scored on a scale of 1-5. Treatment with AbT did not reduce immune complex deposition in the kidneys or brains of MRL-*Fas^{lpr}* mice (**FIGs. 32-34**).

[0284] Infiltration of lymphocytes into the kidneys and lungs of MRL-*Fas^{lpr}* mice was also examined histologically. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated (10 mg/kg i.p., 3X / week) with either saline (control) or the indicated triple-mutant antibodies. Following 10 weeks of treatment, mice were sacrificed and H/E-stained kidney and lung sections were examined for lymphocyte infiltration. Lymphocyte numbers were scored on a scale of 1-5. In the kidney, AbS but not AbT significantly reduced lymphocyte infiltration in three zones: cortex-interstitium (a support structure for the glomeruli) (**FIG. 35a**), cortex-perivascular region (**FIG. 35b**), and peripelvic region (near the origin of the ureter) (**FIG. 35c**) ($p<0.01$). Both AbS and AbT treatment significantly reduced the number of lymphocytes measured in the lungs of MRL-*Fas^{lpr}* mice as compared to saline-treated and anti-IL-13 antibody-treated controls ($p<0.01$ and $p<0.05$, respectively; **FIG. 36**).

[0285] Taken together, these data show that treatment with AbS ameliorates lupus-like disease in MRL-*Fas^{lpr}* mice, whereas treatment with AbT seemed to be less efficacious in this model of SLE. To examine the discrepancy in efficacy of AbS and AbT treatment of MRL-*Fas^{lpr}* mice, sera from these mice were examined for anti-product antibody responses by ELISA. Twelve-week-old male MRL-*Fas^{lpr}* mice were treated (10 mg/kg i.p.; 3X / week) with the indicated triple-mutant antibodies. Serum collected biweekly was tested by ELISA for the presence of murine antibodies capable of binding to the same human antibodies with which the mice were treated. MRL-*Fas^{lpr}* mice generated anti-human antibody (MAHA) responses to all three tested antibodies as early as 2 weeks after treatment (**FIG. 37a**). However, anti-product IgG antibody responses were more than 10-fold greater against AbT or the

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control antibody than against AbS ($p < 0.05$ for AbS vs. IL-13 control). It is unlikely that the AbS CDR sequence is more immunogenic than that of AbT, as for both AbS and AbT the majority of anti-product antibodies recognized epitopes common to both molecules (**FIG. 37b**)

[0286] To examine the dose-dependency of IL-21R neutralization on disease development in the MRL-*Fas^{lpr}* model, eight-week-old female mice were administered either AbS (10, 5 or 2.5 mg/kg doses), AbT (20 mg/kg doses) or isotype control antibody (20, 10, 5, or 2.5 mg/kg doses) i.p. 3X / week for 10 weeks. Mice were tested for anti-dsDNA serum antibodies, and examined for proteinuria, lymphadenopathy, and skin lesions every two weeks. After 10 weeks of dosing, animals were sacrificed and kidney sections were examined for immunoglobulin deposits by immunohistochemistry, and immune cell infiltrates were measured by examination of H&E-stained kidney sections.

[0287] MRL-*Fas^{lpr}* mice had detectable levels of anti-dsDNA IgG antibodies at the onset of the study, and these antibodies increased in titer over the course of the study in all treatment groups. However, treatment with AbS at all doses tested (10, 5 and 2.5 mg/kg) significantly reduced antibody titers in MRL-*Fas^{lpr}* mice in a dose-dependant fashion when compared to isotype control-treated mouse serum (**FIG. 38a**). AbT (20 mg/kg) also reduced anti-dsDNA antibody titers in these mice, but only at one time point (week 2 of dosing) (**FIG. 38b**).

[0288] To assess further the impact of IL-21R neutralization on disease progression in MRL-*Fas^{lpr}* mice, the mice were examined for clinical signs of disease. Very few of the MRL-*Fas^{lpr}* mice used in this study developed skin lesions or clinically significant proteinuria, so the effects of AbS and AbT treatment on these aspects of disease could not be assessed. Treatment with AbS and AbT at all of the doses tested did not affect the development of lymphadenopathy in the study. However, IgG deposits and immune cell infiltrates were readily observed in kidneys from isotype control-treated mice, consistent with the development of lupus nephritis in these mice. Treatment with 10 mg/kg AbS significantly reduced the mean number of

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immune cell infiltrates and IgG deposits in the kidney, whereas treatment with 5 and 2.5 mg/kg AbS had no effect on these parameters in this study (**FIG. 38c-d**).

Treatment with AbT at the 20 mg/kg dose increased mean number of immune cell infiltrates and did not affect IgG deposits in the kidney of MRL-*Fas^{lpr}* mice in this study (**FIG. 38c-d**).

Example 10.3: IL-21R Antibodies Block Cellular Infiltration in a Murine Air Pouch Assay

[0289] The ability of AbS and AbT to neutralize IL-21R function *in vivo* was tested in a short-term murine air pouch assay. Air pouches were created by injecting 8-10 week old BALB/C mice with 3 ml of air under the dorsal skin. Three days later, pouches were reinflated. Two days after reinflation, either saline (control) or the indicated antibodies were injected i.p. at the indicated dose. Murine IL-21 (100 ng) was injected into the air pouch 24 hr after antibody injection. Six hr later, the pouches were washed out with 3 ml PBS, and total cell counts (**FIG. 39a**), monocytes (**FIG. 39b**), lymphocytes (**FIG. 39c**), and neutrophils (**FIG. 39d**) were determined with a CELL-DYN® (Abbott, Abbott Park, IL).

[0290] Injection of murine IL-21 into the air pouch led to a significant increase in total cells ($p=0.0002$), monocytes ($p=0.0137$), lymphocytes ($p=0.0035$), and neutrophils ($p=0.0004$) in the air pouch 6 hr later as compared to saline (**FIG. 39a**). Treatment with either AbS or AbT led to significant reductions in cellular infiltration into the air pouch as compared to treatment with the control IgG1 (anti-IL-13). Total cellular infiltration was reduced significantly compared to the control IgG by both AbS (10 mg/kg, $p=0.0031$; 1 mg/kg, $p=0.00426$) and AbT (10 mg/kg, $p=0.0198$) (**FIG. 39a**). Monocyte infiltration was significantly decreased: AbS (5 mg/kg, $p=0.0031$; 2 mg/kg $p=0.0239$; 1 mg/kg, $p=0.0008$) and AbT (10 mg/kg, $p=0.0002$; 5 mg/kg $p=0.0066$; 1 mg/kg, $p=0.0009$) (**FIG. 39b**). Lymphocyte infiltration was also significantly decreased: AbS (1 mg/kg, $p=0.0049$) and AbT (10 mg/kg, $p=0.0222$) (**FIG. 39c**). Neutrophil infiltration was significantly reduced by AbS (10 mg/kg, $p=0.0032$), but not AbT.

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[0291] Similar results were obtained in a second study conducted to examine the ability of AbS and AbT in the murine air pouch model. Total cell infiltration into the air pouch was significantly reduced by AbS when administered at 10 ($p=0.0113$), 5 ($p=0.0027$), and 2 mg/kg ($p=0.029$), and by AbT at 10 mg/kg ($p=0.0007$) (**FIG. 39e**).

[0292] The effect of AbS on cell infiltration in response to IL-21 in the rat air pouch model was also examined to determine if these antibodies could neutralize IL-21R in the rat. Pouches were created by injecting 8-week-old female S-D rats with 20 ml of air into the subcutaneous tissue of the back. Three days later, pouches were reinflated by injecting an additional 10 mL of air into the pouch. Two days later, the rats were injected with the designated amount of isotype control or AbS antibody. The next day, either saline or 1-20 μ g of murine IL-21 was injected into the pouch, and six hr later the pouch contents were washed out and total cells counted using a Cell Dyne machine.

[0293] Injection of 1 μ g of murine IL-21 into the air pouch did not result in significant cell infiltration into the pouch. However, administration of 20 μ g of murine IL-21 led to an increase in total cells in the air pouch 6 hr later as compared to saline, and treatment with 10 mg/kg AbS significantly reduced IL-21 mediated cell infiltration into the pouch under these conditions ($p<0.05$) (**FIG. 39f**). To determine the minimal dose of AbS that could inhibit cell infiltration into the air pouch in this rat model, additional studies were performed in which rats were administered 20 μ g of murine IL-21 and either 10 mg/kg isotype control or 10, 3 or 1 mg/kg of AbS. Administration of 10 mg/kg AbS significantly reduced cell infiltration into the rat air pouch ($p<0.05$). A nonsignificant increase in cell infiltration into the air pouch was observed in rats treated with 3 mg/kg of AbS. Treatment with 1 mg/kg AbS significantly increased cell infiltration into the pouch compared to isotype control treated rats ($p<0.05$) (**FIG. 39g**). These observations were repeated in a second study, in which treatment with 10 mg/kg significantly reduced cell infiltration into the rat air pouch induced by 20 μ g murine IL-21 ($p<0.05$); in this study, treatment

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with both 3 and 1 mg/kg AbS significantly increased cell infiltration into the pouch ($p<0.05$) (**FIG. 39h**).

[0294] Treatment with AbS significantly increased cell infiltration into the pouch in response to IL-21 when AbS was administered at low doses (e.g., 1 mg/kg). To determine if administration of antibody alone into the pouch at low concentrations (1 mg/kg) elicited cell migration into the pouch, an additional study was performed in which rats were treated with 1 mg/kg AbS or isotype control antibody in the absence of IL-21 treatment. As observed in previous studies, administration of AbS at 10 mg/kg significantly reduced IL-21-driven cell infiltration into the pouch ($p=0.0075$), whereas administration of AbS at 1 mg/kg significantly increased IL-21-induced cell infiltration into the pouch ($p=0.0257$). Treatment with either AbS or isotype control antibody at 1 mg/kg in the absence of IL-21 treatment did not increase cell infiltration into the pouch, indicating that the increased cell infiltration into the pouch elicited by treatment with AbS at 1 mg/kg was dependent on administration of both murine IL-21 and anti-IL-21R antibody in this model (**FIG. 39i**).

[0295] To determine whether the increase in cells in the rat air pouch model was dependent on administration of the dose of murine IL-21 (20 μ g), an experiment was done in which rats were treated with either 10, 20 or 40 μ g murine IL-21 and either 1 or 10 mg/kg AbS. A significant inhibition of cell infiltration into the pouch was observed with 10 mg/kg AbS in response to both 10 ($p=0.003$) and 20 μ g ($p=0.003$) murine IL-21. Treatment with 10 mg/kg AbS did not affect cell infiltration in response to 40 μ g murine IL-21 in this study. Treatment with 1 mg/kg AbS significantly increased cell infiltration into the air pouch in response to 20 μ g murine IL-21 ($p=0.0454$), as previously observed. However, treatment with 1 mg/kg AbS did not affect cell infiltration into rat air pouch in response to either 10 or 40 μ g murine IL-21, indicating that increased cell infiltration into the rat air pouch is very specific to the dose of 1 mg/kg Abs and 20 μ g murine IL-21 in this model (**FIG. 39j**). These data indicate that AbS can affect cell infiltration into the rat air pouch in

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response to murine IL-21, with inhibition of this response achieved with a dose of 10 mg/kg of AbS.

Example 10.4: Pharmacokinetics in CD-1 Mice After Intravenous or Subcutaneous Administration of AbS

[0296] The serum concentrations of human anti-IL-21R antibodies were determined by qualified ELISAs as described in **Table 13**. The anti-IL-21R ELISA used a monomeric His-tagged IL-21R as a capture reagent and an anti-human-Fc (conjugated to horseradish peroxidase (HRP)) as a detector reagent. The enzyme substrate, 3,3',5,5'-tetramethylbenzidine (TMB), was used to produce a colored endproduct to visualize the bound test article. Optical density (OD) was measured colorimetrically at a wavelength of 405 or 450 nm. Sample concentrations were determined by interpolation from a standard curve that was fit using a four-parameter equation.

[0297] Pharmacokinetic parameters were calculated based on mean concentrations. Individual concentration values below the LOQ were treated as zero for calculation of the mean and SD. The PK parameters were determined using a noncompartmental analysis module (Model 200 for i.p. and s.c. dosing and Module 201 for i.v. dosing) of the PK software package WinNonlin (version 4.1; Pharsight, Mountain View, CA). The program applies a model-independent approach and the standard methods described by Gibaldi and Perrier (Pharmacokinetics (2nd ed. 1982) Marcel-Dekker, Inc., New York). The area under the serum concentration vs. time curve (AUC) was calculated using the linear trapezoidal method. The slope of the apparent terminal phase was estimated by log-linear regression using at least three data points and the terminal rate constant (λ) was derived from the slope. $AUC_{0-\infty}$ was estimated as the sum of the AUC_{0-t} (where t is the time of the last measurable concentration) and C_t/λ . The apparent elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda$. Predictions of concentrations after a multiple dose regimens, were conducted by nonparametric superposition using WinNonlin software.

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[0298] After intravenous administration of 10 mg/kg of AbS to male CD-1 mice, the exposure ($AUC_{0-\infty}$) of AbS was 7272 $\mu\text{g}\cdot\text{hr}/\text{ml}$. The mean concentration at the first sampling time point after intravenous administration ($C_{5\text{min}}$) was 113 $\mu\text{g}/\text{ml}$. The elimination of AbS in CD-1 mice was relatively slow, as evidenced by the low total body clearance (CL) of ~ 1.4 ml/hr/kg and long elimination half-life ($t_{1/2}$) of 162 hr (~ 6.8 days). The steady-state volume of distribution ($V_{d\text{ss}}$) was 306 ml/kg, suggesting that AbS was mainly confined to the vascular system (**FIG. 40; Table 14**).

[0299] Pharmacokinetics of AbS in CD-1 mice appeared linear in the 10-100 mg/kg dose range. After intravenous administration of 100 mg/kg of AbS to male CD-1 mice, $AUC_{0-\infty}$ was 75792 $\mu\text{g}\cdot\text{hr}/\text{ml}$, $C_{5\text{min}}$ was 1160 $\mu\text{g}/\text{ml}$, CL was ~ 1.3 ml/hr/kg, $V_{d\text{ss}}$ was 473 ml/kg, and elimination half-life was ~ 391 hr (~ 16.2 days) (**FIG. 40; Table 14**).

[0300] After 10 mg/kg s.c. administration of AbS to CD-1 mice, the absorption of AbS was slow (T_{max} of 48 hr) and the subcutaneous bioavailability was 81%. The mean $t_{1/2}$ value after subcutaneous administration was ~ 195 hr (~ 8.1 days) and similar to that observed after 10 mg/kg intravenous administration.

[0301] The presence of anti-AbS antibodies was evaluated using a qualified immunoassay at 576 hr (24 days) and 672 hr (28 days) following 10 mg/kg i.v. or s.c. administration of AbS to CD-1 mice ($n=8$ per time point per group). An electrochemiluminescent, paramagnetic bead assay was used to detect anti-AbS antibodies. In this assay, samples were coincubated with biotinylated AbS overnight. Streptavidin-coated paramagnetic beads were incubated with the mixture. After incubation with the beads, the plate was placed in the BioVeris M-Series 384 Analyzer. A magnet was applied to capture the paramagnetic beads onto a surface electrode, and unbound reactants were washed away. The ruthenylated AbS captured on the beads was electrically excited by a voltage application, resulting in the production of light. The light was measured by photodetectors with the read-out in response units (RU).

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[0302] Positive and negative controls were also used to determine the cutpoint RU, which was defined as twice the mean RU of the negative control. Samples were initially tested in a screening format at dilutions of 1:25 and 1:75. Samples generating an RU greater than or equal to the cutpoint RU were considered positive and reanalyzed in a full-dilution series to confirm the positive result and determine the titer (the reciprocal dilution that would generate an RU equal to the cutpoint RU). For positive samples, the log of titer is reported. The minimum required dilution was 1:25, and the limit of detection was 1.40 (the log of 25). Therefore, negative samples were designated as <1.40.

[0303] In both i.v. and s.c. treatment groups, 5 or 6 (of 8) mice per time point per group tested positive for anti-AbS antibodies (**Table 15**). The majority of the mice with detectable anti-AbS antibodies had lower AbS concentrations than those observed in animals without anti-AbS antibodies; of some note, the high levels of AbS in some samples could have interfered with the detection of anti-AbS antibodies. The summary of the onset of anti-AbS antibody response across various times, and across animal species and strains/models, is shown in **Table 23**.

[0304] The presence of anti-AbS antibodies was also tested at 648 hr (27 days), 984 hr (41 days), and 1320 hr (55 days) following 100 mg/kg i.v. of AbS to CD-1 mice (n=3 per time point). None of the mice tested were positive for anti-AbS antibodies at these time points.

Example 10.5: Pharmacokinetics in DBA and MRL-Fas^{lpr} Mice After Intraperitoneal Administration of AbS

[0305] The serum concentrations of human anti-IL-21R antibodies were determined by qualified ELISAs as described in **Table 13**. The anti-IL-21R ELISA used anti-human-Fc as a capture reagent and biotinylated anti-human-Fc as a detector reagent. Avidin-HRP, and the enzyme substrate TMB or 2,2'-azino di (3-ethyl-benzthiazoline-6-sulfonate) (ABTS), were used to produce a colored endproduct. OD was measured colorimetrically at a wavelength of 405 or 450 nm. Sample concentrations were determined by interpolation from a standard curve that was fit

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using a four-parameter equation. PK parameters were calculated as noted in Example 10.4. Predictions of concentrations after a multiple dose regimens, were conducted by nonparametric superposition using WinNonlin software and assuming linear kinetics in MRL-*Fas^{lpr}* and DBA mice in the 2.5–10 mg/kg dose range (single dose).

[0306] Following a single i.p. dose of 8 mg/kg of AbS to male DBA mice, the maximum serum concentration ($C_{\max}$) and exposure ($AUC_{0-\infty}$) of AbS were 62 $\mu\text{g/ml}$ and 7229 $\mu\text{g}\cdot\text{hr/ml}$, respectively. The $T_{\max}$ and the elimination half-life ($t_{1/2}$) of AbS were 6 hr and 140 hr (~5.8 days), respectively (**FIG. 41**). Out of eight animals tested, three developed anti-AbS antibody response at 672 hr, determined using the paramagnetic bead assay described in Example 10.4 (**Table 12**). The summary of the onset of anti-AbS antibody across various times and species/strains is shown in **Table 23**.

Table 12: Formation of Anti-AbS Antibodies After a Single 8 mg/kg i.p. Dose to DBA Mice

Onset (hr)	408 hr	504 hr	576 hr	672 hr
Positive	0 of 8	0 of 8	0 of 8	3 of 8

[0307] Following a single intraperitoneal dose of 10 mg/kg to female, 12-week-old MRL-*Fas^{lpr}* mice, the maximum serum concentration ($C_{\max}$) and exposure ($AUC_{0-\infty}$) of AbS were 51 $\mu\text{g/ml}$ and 2798 $\mu\text{g}\cdot\text{hr/ml}$, respectively. The $T_{\max}$ and the elimination half-life ($t_{1/2}$) of AbS were 3 hr and 46 hr (~1.9 days), respectively (**FIG. 41**).

[0308] Compared to DBA and CD-1 mice, dose-normalized exposure of AbS appeared to be lower in MRL-*Fas^{lpr}* mice (**Table 14**). This was not entirely unexpected, as fast elimination of normal IgG in MRL-*Fas^{lpr}* mice (especially with disease symptoms) and in SLE patients has been reported (see Zhou et al. (2005) *Lupus* 4(6):458-66; Newkirk et al. (1996) *Clin. Exp. Immunol.* 106(2):259-64; Wochner (1970) *J. Clin. Invest.* 49(3):454-64). The hypercatabolism of mouse IgG

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in MRL-*Fas*^{lpr} mice has been proposed to be due to the disease-induced impairment of the function of the receptor FcRn, which regulates the homeostasis of IgG (Zhou et al. (2005) *supra*). As AbS is a human IgG, differences in elimination of AbS at the terminal phase among the different strains of mice also may be explained, at least in part, by a differential MAHA response.

[0309] When AbS was administered to MRL-*Fas*^{lpr} mice i.p. at 2.5, 5, or 10 mg/kg per dose 3X / week for 10 weeks (Example 10.2), all dose groups had significant reduction in titers of anti-dsDNA antibodies compared to the isotype control (anti-human IL-13 human IgG1 triple-mutant). For the 2.5 and 10 mg/kg dose groups, steady-state trough levels of AbS were assayed by ELISA. At 2- and 4-week time points, almost all samples tested from the 2.5 mg/kg group had undetectable levels (<34 ng/ml) of AbS in the serum; one sample had very low levels (57 ng/ml). For the 10 mg/kg group, there was high interanimal variability in the AbS serum concentrations; however, median steady-state trough levels were ~3-10 fold higher, compared to those predicted by simulations using PK data from the single dose study in MRL-*Fas*^{lpr} mice (**FIG. 42**). When single-dose i.p. data obtained in the DBA mouse strain were used for predictions, there appeared to be an improved correlation between the observed and predicted AbS concentrations in the 10 mg/kg/dose group (**FIG. 42**). Without intending to be bound by theory, a possible explanation for the undetectable levels of AbS in the low-dose group is marked MAHA production triggered by multiple administration of AbS at relatively low dose levels, as week-2 serum samples from the 2.5 mg/kg/dose group were assayed for anti-AbS antibodies and all samples had very high titers above the upper limit of quantitation of 4.74 log titer units. However, in lieu of expected pharmacological action of AbS to delay and/or reduce IgG responses, it is possible that higher dosage of AbS under the multiple dose regimens would reduce MAHA response against itself, resulting in higher observed serum AbS concentrations, compared to those expected based solely on the single-dose PK data. In fact, in MRL-*Fas*^{lpr} mice, MAHA response to AbS

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was ~10-fold lower, compared to that to an isotype control human IgG administered via the same multiple dose regimen of 10 mg/kg 3X / week for 10 weeks (**FIG. 37a**).

Example 10.6: PK of AbS in Female Cynomolgus Monkeys

[0310] PK of AbS in female monkeys were determined following a single 10 mg/kg i.v. or s.c. administration. Individual animal and mean concentration-time profiles from this study are shown in **FIGs. 43a** and **43b**, respectively, and mean PK parameters are summarized in **Table 14**.

[0311] The serum concentrations of human anti-IL-21R antibodies were determined by qualified ELISAs as described in **Table 12**. The anti-IL-21R ELISA used a monomeric His-tagged IL-21R as a capture reagent and an anti-human-Fc conjugated to HRP as a detector reagent, as described for CD-1 mice in Example 10.4. The serum concentration of the isotype control antibody in monkeys (anti-IL-13 antibody) was also measured by ELISA. In this assay, the recombinant human IL-13 ligand, which contains a FLAG octapeptide fusion tag, was captured by an anti-FLAG monoclonal antibody. The serum samples containing anti-IL-13 antibody were detected with an anti-human-Fc-HRP. The enzyme substrate ABTS was used to produce a colored endproduct to visualize the bound test article. PK parameters were calculated for each individual animal using noncompartmental methods.

[0312] All six female monkeys that were administered AbS at 10 mg/kg (n=3 for both i.v. and s.c. routes of administration) had a sharp drop in AbS levels in the terminal phase (at ~408 hr post-dose), suggesting the possible formation of anti-product antibodies. The elimination half-life values in the 10 mg/kg groups were calculated with and without the time points at which a sharp concentration drop was observed.

[0313] After a single 10 mg/kg i.v. administration of AbS to female cynomolgus monkeys, the mean serum clearance (CL) was 1.03 ml/hr/kg and the mean elimination half-life ($t_{1/2}$) was 74 hr (~3 days). When time points at which a sharp concentration drop was observed were excluded from calculations, the mean $t_{1/2}$

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estimate was ~7.9 days. The mean steady-state volume of distribution (V_{dss}) was low (~125 ml/kg), suggesting that AbS was mainly confined to the vascular system. The mean exposure ($AUC_{0-\infty}$) of AbS was 9728 $\mu\text{g}\cdot\text{hr}/\text{ml}$.

[0314] After a single 100 mg/kg i.v. administration of AbS to cynomolgus monkeys, CL was ~1.08 ml/hr/kg, $t_{1/2}$ was 279 hr (~11.6 days), $AUC_{0-\infty}$ was ~92867 $\mu\text{g}\cdot\text{hr}/\text{ml}$, and V_{dss} was ~211 ml/kg. Thus, in general, the PK of AbS were approximately linear in the 10-100 mg/kg dose range.

[0315] After 10 mg/kg s.c. administration of AbS to female monkeys, the absorption of AbS was slow (mean T_{max} of ~34 hr) and the mean s.c. bioavailability was ~43%. The mean $t_{1/2}$ value after 10 mg/kg s.c. administration to monkeys was 52 hr (~2.2 days), and similar to that observed after 10 mg/kg i.v. administration. When time points at which a sharp concentration drop was observed were excluded from calculations, the mean $t_{1/2}$ was 258 hr (~10.8 days).

[0316] All six female monkeys that were administered AbS at 10 mg/kg had detectable anti-AbS antibodies starting at 504 hr (3 weeks) post-dose (**Table 16**), determined using the paramagnetic bead assay described in Example 10.4. These data are consistent with the observed sharp drop in AbS serum levels. The anti-AbS response persisted at all subsequent time points (up to 27 weeks). Thus, the anti-AbS antibody response appears to affect the PK of AbS in monkeys after a single 10 mg/kg i.v. or s.c. administration. For the 100 mg/kg i.v. dose group, 1 of 3 monkeys was positive for anti-AbS antibodies at weeks 6 and 8, with log titers of 1.8 and 4.6, respectively.

Table 13: ELISA Summary for AbS in Mouse and Female Monkey Serum

Species strain	Assay Capture	Assay Detector	Range of quantitation (ng/mL in 100% serum)	Minimum Required Dilution	Standard curve (ng/ml in 5% serum)	Serum used for assay diluent
Mouse DBA	anti-human IgG	anti-human IgG-biotin	32-500	20	0.78-100	Sprague-Dawley rat
Mouse MRL ^{lpr}	anti-human IgG	anti-human IgG-biotin	33.4-630	20	0.514-102	C57BL/6 mouse
Mouse CD-1	Monomeric His-tagged IL-21R	anti-human IgG-HRP	45-768	20	1-38.4	CD-1 mouse
Monkey (cyno)	Monomeric His-tagged IL-21R	anti-human IgG-HRP	30-512	20	0.44-38.4	Pooled monkey

Table 14: Mean PK Parameters of AbS in Mice and Female Monkeys

Species strain	Dose (mg/kg), Route, Protocol	C _{5min} or C _{max} ^a (µg/ml)	T _{max} (hr)	AUC _{0-∞} (µg*hr/ml)	CL (ml/hr/kg)	Vd _{ss} (ml/kg)	t _{1/2} (hr)	F (%)
Mouse DBA	8, ip	62	6	7229	NA	NA	140	ND
Mouse MRL ^{lpr}	10, ip	51	3	2798	NA	NA	46	ND
	2.5, ip	12	3	823	NA	NA	12 (54) ^b	ND
Mouse CD-1	10, iv	113	NA	7272	1.38	306	162	NA
	10, sc	17	48	5913	NA	NA	195	81
	100, iv	1160	NA	75792	1.32	473	391	NA
Mouse C57BL/6	2.5, iv	29	NA	1927	1.30	208	120	NA
Mouse IL-21R knockout	2.5, iv	32	NA	3415	0.73	273	256	NA
Monkey (cyno)	10, iv	177±10	NA	9728±621	1.03±0.07	125±16	74±46 (190±38) ^b	NA
	10, sc	15±3	34±34	4188±542	NA	NA	52±20 (258±35) ^b	43±6
	100, iv	2030±95	NA	92867±9768	1.08±0.11	211±11	279±28	NA

^a C_{5min} was determined for i.v. route, or C_{max} was determined for i.p. and s.c. routes.

^b Terminal half-life was calculated excluding the data points with the sharp concentration drop.

NA = not applicable

ND = not determined

Table 15: Formation of Anti-AbS Antibodies (Log Titer) in Male CD-1 Mice After a Single IV or SC Dose of 10 mg/kg**Intravenous Group**

Time (hr)

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SAN	576	672
26	2.65	
27	2.54	
28	<1.40 ^a	
29	2.16	
30	<1.40 ^a	
40	2.81	
41	2.34	
42	2.32	
43		2.79
44		<1.40 ^a
45		3.25
46		1.79
47		3.28
48		<1.40 ^a
49		3.21
50		3.18

Subcutaneous Group

Time (hr)		
SAN	576	672
76	2.30	
77	2.55	
78	<1.40 ^a	
79	2.71	
80	1.87	
90	<1.40 ^a	
91	3.75	
92	2.35	
93		<1.40 ^a
94		2.75
95		<1.40 ^a
96		3.27
97		3.53
98		3.73
99		<1.40 ^a
100		3.65

^a <1.40 signifies a negative result.

SAN = study animal number

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Table 16: Formation of Anti-AbS Antibodies (Log Titer) in Female Cynomolgus Monkeys After a Single IV or SC Dose of 10 mg/kg**Intravenous Group**

Time (hr)	SAN 1	SAN 2	SAN 3
336	<1.40 ^a	<1.40	<1.40
504	2.35	2.33	2.26
672	3.61	3.22	2.47
804	3.78	3.61	3.31
1008	3.80	3.72	4.18
1176	3.72	3.76	4.09
2016	3.70	4.44	4.14
2856	3.60	4.67	4.44
3696	3.60	4.71	4.24
4536	3.53	4.64	4.31

Subcutaneous Group

Time (hr)	SAN 4	SAN 5	SAN 6
336	<1.40 ^a	<1.40	<1.40
504	3.00	2.68	2.39
672	3.52	3.64	2.59
804	4.10	3.71	2.47
1008	3.91	3.73	2.89
1176	4.11	3.59	3.27
2016	4.29	3.77	3.65
2856	4.25	3.61	3.50
3696	4.26	3.49	3.45
4536	4.39	3.56	3.58

^a <1.40 signifies a negative result.

SAN = study animal number

Example 10.7: PK of AbT in Mice

[0317] The serum concentrations of human anti-IL-21R antibodies were determined by qualified ELISAs as described in **Table 17**. The anti-IL-21R ELISA used a monomeric His-tagged IL-21R as a capture reagent and an anti-human-Fc (conjugated to horseradish peroxidase (HRP)) as a detector reagent for CD-1 mice, and anti-human Fc as a capture reagent and biotinylated anti-human Fc as a detector

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reagent for DBA and MRL-*Fas*^{lpr} mice. PK parameters were calculated as noted in Example 10.4. Predictions of concentrations after a multiple dose regimens were conducted by nonparametric superposition using WinNonlin software and assuming linear kinetics in MRL-*Fas*^{lpr} and DBA mice in the 2.5-10 mg/kg dose range (single dose) for AbS and in the 8-20 mg/kg dose range (single dose) for AbT.

[0318] After i.v. administration of 10 mg/kg of AbT to male CD-1 mice, the exposure ($AUC_{0-\infty}$) of AbT was 4551 $\mu\text{g}\cdot\text{hr}/\text{ml}$ (**FIG. 44a; Table 18**). The mean concentration at the first sampling time point after i.v. administration ($C_{5\text{min}}$) was ~ 70 $\mu\text{g}/\text{ml}$. The elimination of AbT in CD-1 mice was relatively slow, as evidenced by the low total body clearance (CL) of ~ 2.2 $\text{ml}/\text{hr}/\text{kg}$ and long elimination half-life ($t_{1/2}$) of 210 hr (~ 8.8 days). The steady-state volume of distribution ($V_{d\text{ss}}$) was 572 ml/kg . Following 10 mg/kg i.v. administration to mice, AbT appeared to have higher CL ($\sim 60\%$ increase), lower $AUC_{0-\infty}$ ($\sim 37\%$ decrease) and larger $V_{d\text{ss}}$ ($\sim 87\%$ increase), compared to the corresponding values for AbS.

[0319] Following a single i.p. dose of 8 mg/kg to male DBA mice, the maximum serum concentration (C_{max}) and exposure ($AUC_{0-\infty}$) of AbT were 21 $\mu\text{g}/\text{ml}$ and 3320 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively (**FIG. 44c; Table 18**). The T_{max} and the elimination half-life ($t_{1/2}$) of AbT were 1 hr and 80 hr (~ 3.3 days), respectively. After i.p. administration to DBA mice, AbT exposure and half-life were reduced $\sim 54\%$ and $\sim 43\%$, respectively, compared to the corresponding PK parameters of AbS.

[0320] Following a single i.p. dose of 10 mg/kg to female 12-week-old MRL-*Fas*^{lpr} mice, the maximum serum concentration (C_{max}) and exposure ($AUC_{0-\infty}$) of AbT were 23 $\mu\text{g}/\text{ml}$ and 957 $\mu\text{g}\cdot\text{hr}/\text{ml}$, respectively (**FIG. 44b; Table 18**). The T_{max} and the elimination half-life ($t_{1/2}$) of AbT were 6 hr and 21 hr, respectively. After i.p. administration to MRL-*Fas*^{lpr} mice, AbT exposure and half-life was reduced $\sim 66\%$ and $\sim 54\%$, respectively, compared to the corresponding PK parameters of AbS. At 2 weeks, the only time point for AbT at which a reduction in anti-ds DNA titers was observed, median AbT levels were 4-5-fold higher compared to those predicted by simulations using PK data from the single-dose study in MRL-*Fas*^{lpr} mice and

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similar compared to those predicted by simulation with single-dose data obtained from DBA mice (**FIG. 45**). This observation is in line with that for the AbS 10 mg/kg group. Without intending to be bound by theory, a possible explanation for relatively lower levels of AbT at the 2-week time point (compared to those of AbS) and undetectable levels of AbT at later time points is marked MAHA production triggered by multiple administrations of AbT and the inability of AbT to suppress the MAHA response against itself.

[0321] In line with results described for AbT, dose-normalized exposure of AbT appeared to be lower in MRL-*Fas*^{lpr} mice, compared to those in DBA and CD-1 mice (**Table 18**).

[0322] Unlike AbS, when AbT was administered to MRL-*Fas*^{lpr} mice i.p. at 10 mg/kg or 20 mg/kg per dose 3X / week for 10 weeks, only limited effects on the development of disease in MRL-*Fas*^{lpr} mice were observed (Example 10.2). Administration of AbT (10 mg/kg) did not result in significant reduction of titers of anti-dsDNA at all time points evaluated. Twenty mg/kg administration of AbT resulted in transient reduction in titers of anti-dsDNA at 2 weeks; however anti-dsDNA titers were not different from control at later time points (4, 6, 8, and 10 weeks) (**Table 19**). For the 20 mg/kg AbT group, steady-state trough levels of AbT were assayed by ELISA. There was high interanimal variability in the AbT serum concentrations; however, in general, median steady-state trough levels of AbT appeared to have an inverse correlation with the effect on median anti-dsDNA titers (**Table 19**). At 2 weeks, median AbT levels were ~8-fold higher than those at 4 weeks (but still lower than median AbS levels at 2 weeks in the 10 mg/kg group). At later time points (4, 6, 8, and 10 weeks), median AbT trough levels were less than the limit of detection (~66 ng/ml). At 2 weeks, the only time point for AbT at which a reduction in anti-dsDNA titers was observed, median AbT levels were 4-5-fold higher compared to those predicted by simulations using PK data from the single-dose study in MRL-*Fas*^{lpr} mice and similar compared to those predicted by simulation with single-dose data obtained from DBA mice (**FIG. 45**). This

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observation is in line with that for the AbS 10 mg/kg group. Without intending to be bound by theory, a possible explanation for relatively lower levels of AbT at the 2-week time point (compared to those of AbS) and undetectable levels of AbT at later time points, is marked MAHA production triggered by multiple administrations of AbT and the inability of AbT to suppress the MAHA response against itself.

Example 10.8: PK of AbT in Cynomolgus Monkeys

[0323] PK of AbT in female monkeys were determined following a single i.v. (10 mg/kg or 100 mg/kg) or s.c. (10 mg/kg) administration. Mean concentration-time profiles from this study were compared to those for AbS (**FIGs. 46a-b**) and mean PK parameters are summarized in **Table 18**.

[0324] The serum concentrations of human anti-IL-21R antibodies were determined by qualified ELISAs as described in **Table 17**. The anti-IL-21R ELISA used a monomeric His-tagged IL-21R as a capture reagent and an anti-human-Fc conjugated to HRP as a detector reagent, as described in Example 10.6. TMB was used to produce a colored endproduct to visualize the bound test article. The serum concentration of the isotype control antibody in monkeys (anti-IL-13 antibody) was also measured by ELISA, as described in Example 10.6. PK parameters were calculated as noted in Example 10.6.

[0325] After a single 10 mg/kg intravenous administration of AbT to female cynomolgus monkeys, the mean serum clearance (CL) was 7.01 ml/hr/kg and the mean elimination half-life ($t_{1/2}$) was ~2.6 days. The mean steady-state volume of distribution (V_{dss}) was ~202 ml/kg and the mean exposure ($AUC_{0-\infty}$) of AbT was 1476 $\mu\text{g}\cdot\text{hr}/\text{ml}$ (**Table 18**).

[0326] After a single 100 mg/kg i.v. administration of AbT to cynomolgus monkeys, CL was ~4.87 ml/hr/kg, $t_{1/2}$ was ~3.7 days, $AUC_{0-\infty}$ was ~20955 $\mu\text{g}\cdot\text{hr}/\text{ml}$, and V_{dss} was ~146 ml/kg (**Table 18**). There was no significant difference between the mean PK parameters of AbT in monkeys after a 10 vs. 100 mg/kg single i.v. dose; thus the PK of AbT were approximately linear in the 10-100 mg/kg dose range.

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[0327] After a 10 mg/kg s.c. administration of AbT to monkeys, the mean $T_{\max}$ was ~6 hr and the mean subcutaneous bioavailability was ~43%. The mean $t_{1/2}$ value after a 10 mg/kg s.c. administration to monkeys was 63 hr (~2.6 days), and similar to that observed after a 10 or 100 mg/kg i.v. administration (Table 18).

Table 17: ELISA Summary for AbT in Mouse and Female Monkey Serum

Species strain	Assay Capture	Assay Detector	Range of quantitation (ng/ml in 100% serum)	Minimum Required Dilution	Standard curve (ng/ml)	Serum used for assay diluent
Mouse DBA	anti-human IgG	anti-human IgG-biotin	32-500	20	0.78-100 (in 5% serum)	S-D rat
Mouse MRL ^{lpr}	anti-human IgG	anti-human IgG-biotin	66.8-1260	40	0.514-102 (in 2.5% serum)	C57BL/6 mouse
Mouse CD-1	Monomeric His-tagged IL-21R	anti-human IgG-HRP	45-768	20	1-38.4 (in 5% serum)	CD-1 mouse
Monkey (cyno)	Monomeric His-tagged IL-21R	anti-human IgG-HRP	30-512	20	0.44-38.4 (in 5% serum)	Pooled cyno

Table 18: Mean PK Parameters of AbT in Mice and Female Monkeys

Species strain	Dose (mg/kg), Route	$C_{5\min}$ or $C_{\max}^a$ ($\mu\text{g/ml}$)	$T_{\max}$ (hr)	$\text{AUC}_{0-\infty}$ ($\mu\text{g}\cdot\text{hr/ml}$)	CL (ml/hr/kg)	Vd_{ss} (ml/kg)	$t_{1/2}$ (hr)	F (%)
Mouse DBA	8, i.p.	21	1	3320	NA	NA	80	ND
Mouse MRL ^{lpr}	10, i.p.	23	6	957	NA	NA	21	ND
Mouse CD-1	10, i.v.	70	NA	4551	2.194	572	210	NA
Monkey cyno	10, i.v.	113±14	NA	1476± 312	7.01 ± 1.68	202 ± 44	63 ± 24	NA

	10, s.c.	11±3	6±0	638 ± 56	NA	NA	63 ± 10	43 ± 4
	100, i.v.	1850±415	NA	20955 ± 3702	4.87 ± 0.79	146 ± 24	88 ± 34	NA

Table 19: Median AbS and AbT Concentrations After Multiple Dosing to MRL^{lpr} Mice

Antibody (Dose)	2 wk	4 wk	6 wk	8 wk	10 wk
AbS (2.5 mg/kg)	0*	0*	ND*	ND	ND
AbS (10 mg/kg)	137940*	196887*	198707*	68363	49563
AbT (20 mg/kg)	94456*	12765	0	0	0

Example 10.9: Pharmacokinetics of ¹²⁵I-D5 in DBA Mice

[0328] The ¹²⁵I-anti-murine IL-21R antibody D5-20 (“D5”) was injected intraperitoneally into nonfasted male DBA mice in a single 8 mg/kg dose. Serum samples were taken at time points from 1-576 hr, and D5 levels were quantified by measuring trichloroacetic acid (TCA)-precipitable radioactivity. The PK profile of D5 antibody after i.p. dosage to DBA mice was, in general, similar to that of the human anti-IL-21R antibodies (**Table 20, FIG. 47**; see also **Tables 14 and 18**).

Table 20: Pharmacokinetic Parameters of ¹²⁵I-D5 in Male DBA Mice

	t _{1/2} (hr)	T _{max} (hr)	C _{max} (µg/mL)	AUC _{0-∞} (µg*hr/ml)
¹²⁵ I-D5	143.9	6	36	5422

Example 10.10: Pharmacokinetics in Sprague-Dawley Rats After Intravenous, Subcutaneous, or Intraperitoneal Administration of Anti-IL-21R Antibodies

[0329] PK of AbS, AbT, AbV, AbU, and AbW, as well as an isotype control antibody, were examined after a single 10 mg/kg i.v. dose to S-D rats. Bioanalytical assay for quantitation of test article serum concentrations, an assay for detection of anti-AbS antibodies in rat serum, and pharmacokinetic calculations were performed as described for AbS and AbT for monkeys (Examples 10.6 and 10.8), with the following modifications: S-D serum was used as an assay diluent and LOQ for test

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article serum concentration assay was 45 ng/mL. For the ELISA method employed for an isotype control, an anti-human Fc antibody was used as both the capture and detector.

[0330] Following a single 10 mg/kg i.v. dose to S-D rats, antibody AbS was eliminated slowly (CL ~1.6 mL/hr/kg); albeit significantly faster ($p < 0.05$) than that for an isotype control IgG (~0.4 mL/hr/kg). (**Table 21** and **FIG. 48a**). All rats ($n=6$) had a sharp decline in AbS serum concentration, such that there was no detectable AbS at and after day 15 post-dose. This decline in AbS serum concentration was likely related to the presence of anti-AbS antibodies detected at day 15 and all subsequent time points in all six animals. It should be noted that differences in concentration-time profiles in rats between the AbS and the control IgG are not likely to be explained entirely by anti-product responses, as mean serum concentrations started to diverge as early as 24 hr post-dose and differed by more than 4-fold at the one-week time point. The concentration-time profile and PK parameters of AbV closely resembled those of AbS in rats.

[0331] After a single 10 mg/kg i.v. dose to rats, AbT elimination was faster (CL ~2.1 mL/hr/kg) and the resulting $AUC_{0-\infty}$ was ~2-fold lower, compared to AbS. All of six AbT-dosed rats also had a sharp decline in the serum concentrations in the terminal phase. The concentration-time profiles of AbU and AbW and PK parameters (such as $AUC_{0-\infty}$, $AUC_{0-240hr}$, and CL) in rats were intermediate between those of AbS and AbT (**FIG. 48b**).

Table 21: Pharmacokinetic Properties of Anti-IL-21R Antibodies in S-D Rats
After 10 mg/kg i.v. Administration

Compound (n)	C_{5min} (µg/ml)	AUC_{0-∞} (µg*hr/ml)	AUC_{0-240h}^b (µg*hr/ml)	CL (ml/hr/kg)	Vd_{ss} (ml/kg)
AbS (n=6)	191±38.5	6361±184	5266 ± 625	1.60 ± 0.210	191 ± 33.0
AbT (n=5)	149±51.1	3233 ± 356	3036 ± 167	3.12 ± 0.312	182 ± 46.0
AbU (n=7)	161±15.0	4371±552	4116 ± 392	2.32 ± 0.328	160 ± 24.2
AbV (n=7)	153±14.4	5541±691	4907 ± 374	1.83 ± 0.211	161 ± 20.3
AbW (n=5)	144±7.78	4002 ± 737	3613 ± 591	2.58 ± 0.522	200 ± 36.7

CL=serum clearance. Vd_{ss}=Volume of distribution at steady-state. AUC=Area under the curve.

- Concentration at 5 min, the first sampling time point after IV administration.
- AbS levels were <LOQ after 240 hr time point in all rats.
- All animals in all dose groups had sharp drop in test article levels during the 168-504 hr period. Thus, the terminal phase was not well defined and the resulting apparent t_{1/2} values were driven by the differences in the onset of this concentration drop. Therefore, t_{1/2} values were not used for comparison of PK across constructs and are not shown.

[0332] For AbS, PK in rats was also examined after 10 mg/kg s.c. and i.p. administrations, as well as at two dose levels (1 and 10 mg/mg) after i.v. administration.

[0333] At 168-504 hr after a single i.v., s.c., or i.p. administration to rats, there was a sharp decline in AbS levels in all animals (**FIG. 49**). In the i.v. and s.c. groups, AbS levels were below LOQ for the assay (45.0 ng/mL) at 360 hr through the end of the study (840 hr). In the i.p. group, there was no detectable AbS starting at 576 hr through the end of the study. Using the paramagnetic bead assay described in Examples 10.4 and 11, anti-AbS antibodies were detected in all animals for all dose groups as early as 360 hr post-dose (the first sampling time-point taken for anti-AbS antibody evaluation) and persisted through the end of the study (840 hr). Log titers for the anti-AbS response reached 4.01 to >4.74 units by the end of the study for all samples tested. Thus, the sharp decline in AbS was likely related to the formation of anti-AbS antibodies.

[0334] After a single 1 or 10 mg/kg i.v. dose of AbS in rats, the average exposures (AUC_{0-∞}) were 470 ± 45 or 6361 ± 845 µg•hr/mL, respectively (**Table 22**). The average steady-state volume of distribution (Vd_{ss}) after a single 1 or 10 mg/kg

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i.v. dose of AbS was low (101 ± 24 or 191 ± 33 mL/kg, respectively), suggesting that AbS was mainly distributed throughout the vascular space and into limited extracellular fluids after i.v. administration. The average clearance (CL) of AbS was 2.14 ± 0.21 and 1.60 ± 0.21 mL/hr/kg for the 1 mg/kg and 10 mg/kg i.v. doses, respectively. The average elimination half-life ($t_{1/2}$) was 40 ± 10 and 113 ± 24 hr for the 1 mg/kg and 10 mg/kg i.v. doses, respectively. AbS PK parameters were not dose-proportional in the 1-10 mg/kg dose range after i.v. administration to rats, as the average dose-normalized exposures ($AUC_{0-\infty}/\text{dose}$), CL, and $t_{1/2}$ were significantly different ($p < 0.01$, unpaired t-test) between the 1 and 10 mg/kg dose groups.

[0335] After a single 10 mg/kg i.p. dose of AbS to rats, the average serum $AUC_{0-\infty}$ was 3929 ± 979 $\mu\text{g}\cdot\text{hr}/\text{mL}$ and the average estimated bioavailability (BA) was $62 \pm 15\%$ (with the $AUC_{0-\infty}$ data from the 10 mg/kg, i.v. group used for the BA calculation) (**Table 22**). The average serum C_{max} was 31 ± 14 $\mu\text{g}/\text{mL}$ and was reached at T_{max} of 20 ± 9 hr. After a 10 mg/kg i.p. dose to rats, there was significant interanimal variability in the apparent terminal $t_{1/2}$ with the average value of 78 ± 70 hr.

[0336] After a single 10 mg/kg s.c. dose of AbS to rats, the average serum $AUC_{0-\infty}$ was 1595 ± 456 $\mu\text{g}\cdot\text{hr}/\text{mL}$ and the average estimated BA was relatively low, $25 \pm 7\%$ (with the $AUC_{0-\infty}$ data from the 10 mg/kg i.v. group used for the BA calculation) (**Table 22**). The average serum C_{max} was 8 ± 1 $\mu\text{g}/\text{mL}$ and was reached at T_{max} of 77 ± 26 hr. After 10 mg/kg s.c. dose to rats, there was also significant interanimal variability in the apparent terminal $t_{1/2}$ with the average value of 88 ± 78 hr.

Table 22: Mean ($\pm$ SD) Pharmacokinetic Parameters of AbS in S-D Rats After a Single i.v., i.p., or s.c. Dosage

Group	N	Route, Dose (mg/kg)	C _{5min} or C _{max} (μ g/mL) ^a	T _{max} (hr)	AUC _{0-∞} (μ g•hr/mL)	AUC _{0-∞} /Dose (μ g•hr/mL)/(mg/kg)	CL (mL/hr/kg)	t _{1/2} (hr)	Vd _{ss} (mL/kg)	BA ^b (%)
1	6	IV, 10	191 $\pm$ 38	NA	6361 $\pm$ 845	636 $\pm$ 85	1.60 $\pm$ 0.21	113 $\pm$ 24	191 $\pm$ 33	NA
2	6 ^c	IV, 1	19 $\pm$ 3	NA	470 $\pm$ 45	470 $\pm$ 45 ^e	2.14 $\pm$ 0.2 ^e	40 $\pm$ 10 ^e	101 $\pm$ 24	NA
3	4	IP, 10	31 $\pm$ 14	20 $\pm$ 9	3929 $\pm$ 979	393 $\pm$ 98	NA	78 $\pm$ 70	NA	62 $\pm$ 15
4	4 ^c	SC, 10	8 $\pm$ 1	77 $\pm$ 26	1595 $\pm$ 456	160 $\pm$ 46	NA	88 $\pm$ 78	NA	25 $\pm$ 7

NA=Not applicable.

Note: PK parameters were calculated for each individual animal.

- C_{5min}, concentration at the first sampling time point after i.v. administration is shown for Groups 1 and 2. C_{max} is shown for Groups 3 and 4.
- BA, bioavailability after i.p. or s.c. administration, was calculated using AUC data for Group 1 (10 mg/kg, IV).
- Statistically significant difference from Group 1 (p<0.01).

[0337] The summary of the onset anti-AbS antibody response in all animals, including S-D rats, is summarized in **Table 23**.

Table 23: Onset of Anti-AbS Antibody Response Following a Single Dose of AbS to Mice, Rats, and Monkeys

Species, strain	Dose (mg/kg) Route	Onset (hr) ^a	% positive at onset
Mouse, DBA	8, ip	672	37%
Mouse, MRL-fas ^{lpr}	10, ip	336	100%
	2.5 ip	312	100%
Mouse, CD-1	10, iv	576	75%
Rat, Sprague-Dawley	10, iv	360	100%
Monkey, Cynomolgus	10, iv	504	100%
	10, sc	504	100%

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Example 11: Pharmacokinetics and Biodistribution of ^{125}I -AbS in Wild-Type Control (C57BL/6), IL-21R Knockout, and MRL-Fas^{lpr} Mice

Example 11.1: ^{125}I Labeling

[0338] Iodination was performed using the IODO-BEADS method (Pierce, Rockford, IL) according to the manufacturer's instructions, and purified by filtration. Briefly, ~100-200 μg of test article were incubated for 25 min with 1-2 mCi of ^{125}I (PerkinElmer), three IODO-BEADS, and ~100-200 μl of PBS. The reaction mixture was separated from the IODO-BEADS and transferred to a Centricon filtration device (10 kD cut-off, Millipore). The purification was performed by adding ~5-10 ml of PBS (in aliquots of 1-2 mL) and spinning at 2,000X g until the volume in the upper chamber of the filtration device was down to ~200-500 μl .

[0339] The dosing solution was prepared by combining the stock solution of unlabeled test article, the formulation buffer, and the ^{125}I tracer. The purity of the dosing solution was analyzed using reducing and nonreducing SDS-PAGE. The dosing solution was prepared once, one day prior to dosing of the first cohort of animals.

Example 11.2: Determination of Radioactive Equivalent Concentrations in Serum

[0340] Total radioactivity (in counts per min (cpm)) in 50-100 μl of serum samples (in duplicate) was determined by gamma-counting (1480 WIZARDTM, Wallac Inc., Gaithersburg, MD). An equal volume of 20% TCA (trichloroacetic acid) was added to each aliquot, and samples were spun at ~12,000 rpm for 10 min. TCA-soluble radioactivity in the supernatant (with the volume equal to the sample volume used for total radioactivity count) was determined by gamma-counting. TCA-precipitable radioactivity in a given sample [total cpm – 2 x TCA - soluble cpm], the specific activity of the dosing solution (cpm/ng), as well as the dates of the sample (t_s (day)) and the dosing solution (t_D (day)) measurements, were used to calculate the radioactive equivalent concentration (ng eq/ml) in a given sample using the formula:

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[average TCA – precipitable cpm / $\text{EXP}(-0.693 / 60.2 \times (t_s - t_D))$] / [specific activity x sample volume].

Example 11.3: Determination of the Cumulative Total Count (as Percentage of Dose Count) and Free ^{125}I Iodine Fraction in Urine

[0341] Total counts were used to calculate urinary excretion (excreted cpm as % dose) for each collection period, using the formula: $[100\% \times \text{urine cpm} / \text{EXP}(-0.693 / 60.2 \times (t_s - t_D))]$ / [specific activity x dose]. Cumulative radioactivity in the urine (cumulative excreted cpm as % dose) was defined as the sum of urinary excretions (cpm, as % dose) from time 0 up to an indicated time point.

[0342] To determine the fraction of TCA-soluble radioactivity, 50 μl urine aliquots were mixed with 50 μl of normal mouse serum (resulting in 100 μl samples) and analyzed by gamma-counting. A 100 μl aliquot of 20% TCA was added to each 100 μl sample, and the 200 μl samples were spun at $\sim 12,000$ rpm for 10 min. TCA-soluble radioactivity in the 100 μl of supernatant was determined by gamma-counting. The fraction of free iodine was obtained using the formula: $[2 \times 100\% \times \text{TCA-soluble cpm in } 100 \mu\text{l of supernatant} / \text{total cpm in } 50 \mu\text{l of urine}]$.

Example 11.4: Biodistribution Analysis

[0343] Tissue samples were placed into preweighed tubes, weighed to determine tissue weights in grams, and counted for total radioactivity (cpm). The quantitation of radioactive equivalent tissue concentration (ng eq/g) was based on the total radioactivity in tissues and the specific activity of the dosing solution (cpm/ng) after a correction for half-life of ^{125}I using the formula: $[\text{sample cpm} / \text{EXP}(-0.693 / 60.2 \times (t_s - t_D))]$ / [specific activity x sample weight].

[0344] Tissue to serum concentration ratios (T/S) for a given tissue at a given time point were calculated using the ratio of radioactive equivalent concentration in tissue ($\mu\text{g eq/g}$) to that in serum ($\mu\text{g eq/ml}$). Total tissue counts as % dose were calculated

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using the formula: $[100\% \times \text{sample cpm} / \text{EXP}(-0.693 / 60.2 \times (t_s - t_D))] / [\text{specific activity} \times \text{dose}]$.

Example 11.5: Pharmacokinetics and Biodistribution in Control and IL-21R

Knockout Mice After a 2.5 mg/kg Intravenous Dose

[0345] In wild-type C57BL/6 (control) mice, there was a decline in mean AbS serum levels relative to those in IL-21R knockout mice, starting at 10-14 days after a single 2.5 mg/kg i.v. dose of ^{125}I -AbS (**FIG. 50c**). At day 14 (336 hr), 60% of controls (n=10) and 0% of IL-21R knockout mice (n=10) had undetectable or very low levels of AbS. After day 20 (480 hr), there were no detectable AbS levels in the serum of all control mice. In IL-21R knockout mice, AbS was detected in serum as late as day 36 (864 hr) post-dose, the last sampling time point. The differences in concentration-time profile of ^{125}I -AbS in control vs. IL-21R knockout appears to be due to possible differences in anti-AbS responses in these two mouse strains. At the five-week time point, about 55% (five out of nine) wild-type serum samples and none of IL-21R knock out serum samples tested positive in the anti-AbS antibody assay.

[0346] In control mice, the total body clearance (CL) was ~1.3 ml/hr/kg, and the elimination half-life ($t_{1/2}$) was 120 hr (~5.0 days). In IL-21R knockout mice, CL was ~0.7 ml/hr/kg, and $t_{1/2}$ was 256 hr (10.7 days) (**Table 14**). Accordingly, after a 2.5 mg/kg i.v. dose, IL-21R knockout mice had a higher serum exposure ($\text{AUC}_{0-\infty}$) as compared to controls (**Table 14**).

[0347] In general, for both controls and IL-21R knockout mice, the highest concentrations of ^{125}I -AbS were found in serum as compared to other tissues examined (**FIGs. 50a and 50b; Tables 24 and 25**). However, at the last tissue sampling time point (864 hr), detectable ^{125}I -AbS levels were found in tissues, but not in serum, of control mice.

[0348] In accordance with differences in serum levels of AbS between control and IL-21R knockout mice, radioactive equivalent concentrations of AbS in tissues were

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lower in controls as compared to IL-21R knockouts, starting at ~2 weeks post-dose (**FIG. 50**). Likewise, tissue elimination half-lives ($t_{1/2}$) were shorter in control mice (~90-165 hr) as compared to those in tissues of IL-21R knockout mice (~230-270 hr). Accordingly, the tissue exposures ($AUC_{0-\infty}$) were lower in control mice compared to IL-21R knockout mice (**Table 26**). Thus, after a 2.5 mg/kg i.v. dose of ^{125}I to IL-21R knockout mice, exposure in both serum and tissues was higher as compared to controls.

Table 24: Mean ($\pm$ SD) Tissue to Serum (T/S) Radioactive Equivalent Concentration Ratio After a Single Intravenous Dose of 2.5 mg/kg of ^{125}I -AbS to C57BL/6 Mice

	1 hr	48 hr	168 hr	336 hr
fat	0.0099 $\pm$ 0.0062	0.0849 $\pm$ 0.0289	0.0619 $\pm$ 0.0144	0.2316 $\pm$ 0.2619
femurs	0.0360 $\pm$ 0.0126	0.1203 $\pm$ 0.0124	0.1108 $\pm$ 0.0140	0.6979 $\pm$ 1.0550
heart	0.0776 $\pm$ 0.0109	0.1588 $\pm$ 0.0143	0.1122 $\pm$ 0.0123	0.3837 $\pm$ 0.4993
kidney	0.1293 $\pm$ 0.0311	0.2497 $\pm$ 0.2105	0.0957 $\pm$ 0.0215	0.6350 $\pm$ 0.8669
large intestine	0.0122 $\pm$ 0.0056	0.0760 $\pm$ 0.0127	0.0466 $\pm$ 0.0078	0.2102 $\pm$ 0.2544
liver	0.1600 $\pm$ 0.0770	0.1342 $\pm$ 0.0679	0.1206 $\pm$ 0.0717	1.1834 $\pm$ 1.4455
lungs	0.1968 $\pm$ 0.0863	0.2522 $\pm$ 0.0714	0.1462 $\pm$ 0.0776	1.3408 $\pm$ 2.4852
lymph node	0.0314 $\pm$ 0.0163	0.1554 $\pm$ 0.0333	0.1504 $\pm$ 0.0542	3.7491 $\pm$ 5.7164
skeletal muscle	0.0099 $\pm$ 0.0048	0.0789 $\pm$ 0.0108	0.0724 $\pm$ 0.0064	0.2461 $\pm$ 0.3171
skin	0.0243 $\pm$ 0.0195	0.3125 $\pm$ 0.0327	0.3145 $\pm$ 0.1155	1.4147 $\pm$ 1.6123
spleen	0.2450 $\pm$ 0.0289	0.2043 $\pm$ 0.0403	0.1470 $\pm$ 0.0232	2.2414 $\pm$ 3.4892
stomach	0.0471 $\pm$ 0.0530	0.0791 $\pm$ 0.0249	0.0674 $\pm$ 0.014	0.4292 $\pm$ 0.5801
thymus	0.0215 $\pm$ 0.0117	0.0842 $\pm$ 0.0174	0.0590 $\pm$ 0.0125	0.2794 $\pm$ 0.3901

Individual values below the limit of quantitation (LOQ, defined as 3X the background cpm) were treated as "0" for calculations of the mean and the standard deviation (SD).

T/S were not calculated for time points after 336 hr (480 and 864 hr), as serum concentrations were below the limit of detection.

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Table 25: Mean ($\pm$ SD) Tissue to Serum (T/S) Radioactive Equivalent Concentration Ratio After a Single Intravenous Dose of 2.5 mg/kg of ^{125}I -AbS to IL-21R Knockout Mice

	1 hr	48 hr	168 hr	336 hr
fat	0.0063 $\pm$ 0.0022	0.0718 $\pm$ 0.0353	0.0856 $\pm$ 0.0475	0.0885 $\pm$ 0.0381
femurs	0.0327 $\pm$ 0.0069	0.1283 $\pm$ 0.0555	0.1169 $\pm$ 0.0104	0.1238 $\pm$ 0.0217
heart	0.0733 $\pm$ 0.0152	0.1569 $\pm$ 0.0172	0.1526 $\pm$ 0.0213	0.1681 $\pm$ 0.0315
kidney	0.1491 $\pm$ 0.0417	0.1570 $\pm$ 0.0218	0.1588 $\pm$ 0.0347	0.1687 $\pm$ 0.0417
large intestine	0.0112 $\pm$ 0.0034	0.0666 $\pm$ 0.0133	0.0612 $\pm$ 0.0111	0.0568 $\pm$ 0.0122
liver	0.1550 $\pm$ 0.0466	0.1434 $\pm$ 0.0706	0.1190 $\pm$ 0.0549	0.0784 $\pm$ 0.0282
lungs	0.3495 $\pm$ 0.1671	0.2722 $\pm$ 0.0529	0.1178 $\pm$ 0.0707	0.2510 $\pm$ 0.1016
lymph node	0.0224 $\pm$ 0.0096	0.1337 $\pm$ 0.0369	0.1215 $\pm$ 0.0345	0.1118 $\pm$ 0.0384
skeletal muscle	0.0068 $\pm$ 0.0016	0.0663 $\pm$ 0.0077	0.0761 $\pm$ 0.0115	0.1227 $\pm$ 0.1479
skin	0.0185 $\pm$ 0.0029	0.2716 $\pm$ 0.0315	0.3244 $\pm$ 0.0607	0.3593 $\pm$ 0.1247
spleen	0.2682 $\pm$ 0.0425	0.1943 $\pm$ 0.0304	0.1809 $\pm$ 0.0322	0.1846 $\pm$ 0.0483
stomach	0.0318 $\pm$ 0.0151	0.0617 $\pm$ 0.0165	0.0689 $\pm$ 0.0237	0.0724 $\pm$ 0.0174
thymus	0.0206 $\pm$ 0.0099	0.0929 $\pm$ 0.0178	0.0979 $\pm$ 0.0127	0.1049 $\pm$ 0.0233

Individual values below the limit of quantitation (LOQ, defined as 3X the background cpm) were treated as "0" for calculations of the mean and the standard deviation (SD).

T/S were not calculated for time points after 336 hr (480 and 864 hr), as serum concentrations were below the limit of detection.

Table 26: Exposure of ^{125}I -AbS in Tissue of C57BL/6 and IL-21R Knockout Mice Following a Single 2.5 mg/kg Intravenous Dose

C57BL/6				IL-21R KO		
	Cmax ^a	Tmax	AUC _{0-∞}	Cmax ^a	Tmax	AUC _{0-∞}
	μg eq/ml	hr	hr* μg eq/ml	μg eq/ml	hr	hr* μg eq/ml
fat	0.66	48	120	0.64	48	236
femurs	0.93	48	213	1.17	48	370
heart	1.79	1	258	1.97	1	501
kidney	3.06	1	352	4.07	1	573
large intestine	0.59	48	105	0.61	48	181
liver	3.77	1	317	4.24	1	419
lymph node	1.21	48	303	9.88	1	882
lung	4.84	1	447	1.21	48	357
skeletal muscle	0.61	48	128	0.60	48	238
skin	2.44	48	567	2.48	48	939

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spleen	5.77	1	454	7.20	1	706
stomach	0.78	1	163	0.81	1	222
thymus	0.65	48	141	0.85	48	320
serum	29	NA	1927	32	NA	3415

^a C_{5min} concentration at the first sampling time point is shown for serum. Tissue sampling time points were: 1, 48, 168, 336, 480, and 864 hr.

Example 11.6: Pharmacokinetics and Biodistribution in MRL-Fas^{lpr} Mice After a 2.5 mg/kg Intraperitoneal Dose

[0349] Following a single i.p. dose of 2.5 mg/kg of ¹²⁵I to female MRL-Fas^{lpr} mice (~10-12 weeks old), the serum concentration-time profile was biphasic, showing a relatively slower decline in AbS levels during the first week post-dose (t_{1/2} ~54 hr) and a faster decline after 5 days post-dose (t_{1/2} ~12 hr), suggesting the formation of anti-AbS antibodies (**FIGs. 51a** and **52**). In fact, 4 of 4 mice at the 312 hr time point, and 4 of 4 mice at the 408 hr time point, tested positive for anti-product antibodies (with relative media titers of ~4.01 and ~4.43 log titer units, respectively). After a 2.5 mg/kg i.p. dose of ¹²⁵I, the maximum serum concentration (C_{max}) and exposure (AUC_{0-∞}) of AbS were 12 µg eq/ml and 823 µg eq•hr/ml, respectively. Thus, after a single i.p. dose to MRL-Fas^{lpr} mice, PK were approximately linear in the 2.5-10 mg/kg dose range (**FIG. 52; Table 14**) (see also Example 10.4). Urine counts of ¹²⁵I-AbS in MRL-Fas^{lpr} mice were elevated as of the 10 day time point (**FIG. 51b**).

[0350] During the first week following a single 2.5 mg/kg i.p. dose (likely prior to formation of anti-product antibodies), the highest concentrations of AbS were found in serum for all tissues examined. After the first week, serum concentration declined more rapidly as compared to the decline of AbS in tissue, so that T/S concentration ratios were significantly higher than 1 (**Table 27**), and AUC_{0-∞} in tissues was ~90-300 µg eq•hr/ml (**Table 28**). In tissue, the apparent elimination half-life (calculated based on the 24-312 hr data set) was ~40-55 hr. Relatively low (but still detectable) levels of radioactivity persisted in tissues until the end of the study (seven weeks post-dose) (**FIG. 51a**).

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Table 27: Mean ($\pm$ SD) Tissue to Serum (T/S) Radioactive Equivalent Concentration Ratio After a Single IP Dose of 2.5 mg/kg of ^{125}I -AbS to MRL-*Fas*^{lpr} Mice

	24 hr	72 hr	168 hr	240 hr
fat	0.321 $\pm$ 0.073	0.233 $\pm$ 0.070	41.125 $\pm$ 69.735	71.500 $\pm$ 131.496
kidney	0.195 $\pm$ 0.026	0.202 $\pm$ 0.036	9.831 $\pm$ 6.787	56.205 $\pm$ 25.079
liver	0.104 $\pm$ 0.028	0.091 $\pm$ 0.032	8.067 $\pm$ 5.587	16.820 $\pm$ 6.892
lungs	0.177 $\pm$ 0.074	0.159 $\pm$ 0.070	7.621 $\pm$ 6.512	20.127 $\pm$ 12.783
lymph node	0.197 $\pm$ 0.055	0.141 $\pm$ 0.020	5.490 $\pm$ 3.707	22.497 $\pm$ 16.812
skin	0.318 $\pm$ 0.023	0.305 $\pm$ 0.056	12.237 $\pm$ 8.734	50.869 $\pm$ 59.413
spleen	0.166 $\pm$ 0.022	0.105 $\pm$ 0.020	9.648 $\pm$ 6.834	50.418 $\pm$ 33.424

Individual values below the limit of quantitation (LOQ, defined as 3X the background cpm) were treated as “0” for calculations of the mean and the standard deviation (SD).

T/S were not calculated for time points after 240 hr, as serum concentrations were below the limit of detection in most of the mice.

Table 28: Exposure of ^{125}I -AbS in Tissue of MRL-*Fas*^{lpr} Mice Following a Single 2.5 mg/kg IP Dose

	Cmax $\mu\text{g eq/ml}$	Tmax hr	AUC _{0-∞} hr* $\mu\text{g eq/ml}$
fat	2.9	24	255
kidney	1.8	24	197
liver	1.0	24	95
lymph node	1.8	24	133
lung	1.6	24	150
skin	2.9	24	285
spleen	1.5	24	161
serum	12	3	823

Tissue sampling time points were: 24, 72, 168, 240, 312, 528, 864, and 1176 hr.

Example 12: Inhibitory Properties of Anti-IL-21R Antibodies in Human Blood

Example 12.1: Agonistic Response of Human Whole Blood to IL-21 is Neutralized by Ex Vivo Treatment of Anti-IL-21R Antibody

[0351] Human whole blood was drawn by the Human Blood Donor Program in Cambridge, MA. All human blood samples were collected in BD Vacutainer™

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CPT™ cell preparation tubes. Collection tubes contained sodium heparin. Samples were maintained at ambient temperature and processed immediately. Blood was divided into 1 to 2 mL aliquots in cryovials, treated with IL-21, AbS, or control proteins. When samples were treated with both antibody and IL-21, the antibody was added immediately prior to IL-21. Samples were then incubated at 37°C in a Forma Scientific Reach-In Incubator Model # 3956 for four hr while mixed continuously at 15 RPM using the Appropriate Technical Resources Inc (ATR) Rotamix (Cat. # RKVS) rotating mixer (serial #0995-52 and #0695-36), or using the Labquake® Tube Shaker/Rotator (Cat. # 400110) during the incubation. Aliquots (0.5 mL) were removed using a Gilson P1000 pipette with ART 1000E tips (Cat. # 72830-042) and added to 2.0 mL microtubes (Axygen Scientific, Cat. # 10011-744) containing 1.3 mL of RNA*later*® supplied with the Human RiboPure™-Blood Kit (Ambion, Austin, TX; Cat. # AM1928) and mixed thoroughly by five complete inversions. Samples were stored at ambient temperature overnight and then frozen at –80°C pending RNA purification.

[0352] RNA was isolated using the Human RiboPure™-Blood Protocol (Ambion, Cat. # AM1928). The Human RiboPure™ RNA isolation procedure consists of cell lysis in a guanidinium-based solution and initial purification of the RNA by phenol/chloroform extraction, and final RNA purification by solid-phase extraction on a glass-fiber filter. The residual genomic DNA was removed according to the manufacturer's instructions for DNase treatment using the DNA-*free*™ reagents provided in the kit. For all samples, RNA quantity was determined by absorbance at 260 nm with a NanoDrop 1000 (NanoDrop, Wilmington, DE). RNA quality was spot-checked using a 2100 Bioanalyzer (Agilent, Palo Alto, CA). Samples were stored at –80°C until cDNA synthesis was performed.

[0353] According to the manufacturer's instructions, cDNA was reverse transcribed from total RNA using a High Capacity cDNA Reverse Transcription Kit (ABI, Cat. # 4368814) with additional RNase inhibitor at 50 U / sample (ABI, Cat. # N808-0119). cDNA samples were stored at –20°C until RT-PCR (real-time

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PCR) was performed. The amount of cDNA loaded on a Taqman® Low Density Array card was determined using the lowest RNA yield obtained within an experiment. cDNA samples were assayed on an ABI PRISM 7900 Sequence detector (Sequence Detector Software v2.2.2, Applied Biosystems) using universal thermal cycling conditions of 50 °C for 2 min, 95 °C for 10 min, then 40 cycles of 95 °C for 15 sec and 60 °C for 1 min.

[0354] To check for *ex vivo* effects of IL-21, experiments were conducted to test whether human whole blood responded to IL-21 with detectable changes in gene expression levels. Whole blood from human donors was incubated in the presence and absence of IL-21, and RNA levels were determined using TLDA cards. Two different TLDAs were used to measure RNA expression levels. The first, Human Immune TLDA (ABI, Catalog #4370573), tested 96 genes, of which 91 were detectable in stimulated human blood. PBMCs stimulated with LPS or PHA from human donor whole blood was used as a positive control. To test the upregulation of IL-21R in response to IL-21 stimulation, results were obtained using a custom designed TLDA that contained the IL-21R gene.

[0355] Data for the seven most consistent IL-21-dependent gene expression changes observed in whole blood samples of the four humans tested under the selected assay conditions are shown in **Table 29**. Significant responses to IL-21 were observed for IL6, IFN γ , CD19, PRF1, IL10, IL2R α , and GNLY. Interestingly, IL-21 treatment at concentrations of 30 ng/mL, 100 ng/mL and 500 ng/mL produced similar results (**Table 29**).

Table 29: IL-21-dependent gene expression changes in human whole blood samples

Gene	IL21 (30ng/mL) Average Fold Change (2 Humans)	IL21 (100ng/mL) Average Fold Change (4 Humans)	IL21 (500ng/mL) Average Fold Change (2 Humans)	Composite Average Fold Change	Composite Standard Deviation	Paired T-test (P-Value)
IL6	20.17	13.24	32.14	19.70	22.51	1.35E-01
IFNG	7.65	6.51	7.23	6.97	2.34	5.86E-02
CD19	2.72	2.78	2.78	2.76	0.94	6.09E-02
PRF1	2.43	2.87	2.72	2.72	1.08	5.21E-02
IL10	3.17	2.49	2.66	2.70	1.26	3.78E-02
IL2RA	2.82	2.07	2.15	2.28	0.86	5.35E-02
GNLY	2.12	2.07	2.66	2.23	0.38	1.76E-03

Fold change was calculated by dividing RQ of treated group by RQ of control group. Paired T-test values were calculated using the RQ values and pairing control and treated values from each human donor.

[0356] To determine if AbS had the desired blocking activity of IL-21/IL-21R-dependent activation of human cells, the ability of this antibody to block IL-21-dependent activation in whole blood was tested on one of the human donors.

FIGs. 53a-b show the effective inhibition (compared to the Hu IgG1 control) of IL-21-dependent activation of IL-21-responsive genes by the antibodies.

[0357] These results define methods for measuring the response to IL-21 in human whole blood samples; thus, this assay can be used to detect the inhibition of IL-21-dependent activation by anti-IL-21R antibodies in experimental or clinical settings, because the assay does not require more blood than can be routinely collected and involves minimal *ex vivo* manipulation.

Example 12.2: An Assay Measuring PD Activity of AbS in Whole Human Blood

[0358] A total of five healthy human donors were used in these studies. All human whole blood samples were collected in BD Vacutainer™ CPT™ cell preparation tubes containing sodium heparin (Catalog #362753). Human whole blood was drawn by the Human Blood Donor Program in Cambridge, MA. Samples were

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maintained at ambient temperature and, except where otherwise stated, were processed within an hour of collection. When samples were treated with immunoglobulin reagents and IL-21, the immunoglobulin reagent was added prior to addition of IL-21. Samples were incubated at 37°C in a Forma Scientific Reach-In Incubator Model #3956 for the duration noted while mixed continuously at about 7 RPM using the Appropriate Technical Resources Inc (ATR) Rotamix (Catalog #RKVS) rotating mixer (serial #0995-52 and #0695-36), or using the Labquake® Tube Shaker/Rotator (Catalog #400110) during the incubation. Subsequently, 0.5 mL of each sample was removed and added to 2.0 mL microtubes (Axygen Scientific, Catalog #10011-744,) containing 1.3 ml of RNAlater® which is provided with the Human RiboPure™-Blood Kit (Ambion, Catalog #AM1928), and mixed thoroughly by five complete inversions. Following procedure described in Example 12.1, samples were processed to isolate and quantify RNA yield, and synthesize cDNA; and 200 ng of cDNA was loaded onto a custom TLDA plate, comprising 24 genes (19 test genes whose RNA expression levels had been observed to change in whole human blood upon stimulation with IL-21, and 5 endogenous control genes), shown in **FIG. 54**.

[0359] For quantification of RNA expression levels, average Real-Time PCR threshold cycle (Ct) values from several endogenous controls (GAPDH, GUSB, ZNF592, and PGK1 in **FIG. 54**) were used as “normalizers” because the expression of these genes did not change with treatment and the genes yielded the most consistent Ct values across all samples. Average Ct values of experimental controls (no AbS and no IL-21 added) were used as “calibrators.” Expression of a gene in a given sample was calculated as Ct of gene – Ct of average of endogenous controls for that sample (ΔCt of sample). The gene expression value ($\Delta\Delta\text{Ct}$) was calculated as ΔCt of sample – ΔCt of “calibrator.” Relative quantification (RQ) or fold change was calculated as $2^{-\Delta\Delta\text{Ct}}$.

[0360] In order to determine optimal time and dose of IL-21 treatment for generation of maximal signal, whole blood samples from five healthy donors were incubated in

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the presence of 3.3, 10 or 30 ng/ml of IL-21 for 2, 4, 6 or 24 hr. RNA was isolated and gene expression levels measured. Significant and robust IL-21 dependent signals were obtained for six genes: IL6, IFN γ , IL2R α , GZMB, PRF1, CD19. The optimal signal for all but CD19 was obtained at 2 hr (**FIG. 55**). There was little difference in the response obtained at 3.3, 10 or 30 ng/ml IL-21. Response to *ex vivo* IL-21 treatment was consistent between all five donors (data not shown). Based on the results obtained with these five donors, the assay conditions chosen to titrate the inhibitory effect of AbS on the *ex vivo* response to IL-21 were: two hr stimulation with 10 ng/ml of IL-21. The most reliable IL-21-responsive genes were GZMB, IFN γ , IL-21RA, IL-6, and PRF1.

[0361] To determine the dose of AbS to optimally block the effect of IL-21, samples from four individual donors were preincubated for 2 hr at the indicated concentrations of AbS and IgG₁TM, both diluted in PBS, before the addition of 10 ng/ml of IL-21. Following the addition of IL-21, samples were incubated for an additional two hr.

[0362] Addition of 0.1 μ g/mL AbS resulted in full inhibition, so 0.003 μ g/mL of AbS was used for subsequent experiments. AbS, but not IgG₁TM, inhibited the response of all six genes tested in all four donors, as demonstrated in **FIGs. 56a-c**. The IC₅₀ for all six genes is presented in **Table 30**.

Table 30: Inhibitory Activity of AbS on IL-21 Response Signal in Whole Blood

	CD19	GZMB	IFNG	IL2R α	IL6	PRF1
IC ₅₀ (μ g/mL)	0.007	<0.003	0.015	0.002	0.005	0.007

[0363] As demonstrated in **FIG. 56**, AbS at concentration as low as 0.03 μ g/mL (200 pM or 6×10^4 molecules/cell) successfully blocked the effects of 10 ng/mL of IL-21 on gene expression. This inhibition was reduced when the concentration of AbS was lowered to ≤ 0.003 μ g/mL (20 pM). GZMB, IFN γ , IL-2R α , IL-6, and PRF1 were reliable blood biomarkers of IL-21R/IL-21 and antibody activity at 2 hr;

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however all six genes (GZMB, IFN γ , IL-2R α , IL-6, PRF1, and CD19) were identified as useful human blood biomarkers for IL-21R/IL-21 activity. Moreover, 10 ng/mL of IL-21 was shown to decrease gene expression of TBX-21 gene in the same assay, and this decrease was completely reversed by AbS at 0.03 μ g/mL (data not shown).

Example 13: PK and PD of AbS and AbT in Male Cynomolgus Monkeys

Example 13.1: Animal Selection for AbS and AbT Study

[0364] Animals used in the study were protein-naïve and selected for inclusion based on results obtained with recombinant human IL-21 stimulation in the whole blood *ex vivo* assay prior to dosing.

[0365] Specifically, for male monkeys, whole blood samples (0.5-1.5 mL) were placed in sterile, nuclease-free, 2 mL micro-centrifuge tubes (Axygen, cat. #1011-744) and treated with vehicle (10 mM L-histidine, 5% sucrose), 50 ng/mL recombinant human IL-21 (rhuIL-21), 50 ng/mL rhuIL-21 with 30 nM IgG control antibody, or 50 ng/mL rhuIL-21 with 30 nM anti-IL-21R antibody for 4 hrs at 37°C on a platform shaker. For female monkeys, whole blood samples (0.5 mL) were treated with either vehicle or 20 ng/mL rhuIL-21. Peripheral blood mononuclear cells in the blood samples were isolated by Ficoll method according to manufacturer's instructions (GE Healthcare, Ficoll-PaqueTM plus) and washed once in PBS.

[0366] RNA isolation was performed using the RiboPureTM-Blood Kit (Ambion, Cat#AM1928; males) or RNeasy kit (Qiagen, females) according to manufacturer's instructions. RNA yield was determined using a NanoDrop 1000A spectrophotometer (NanoDrop, Wilmington, DE) and RNA quality was assessed using a 2100 Bioanalyzer (Agilent, Santa Clara, CA). RNA concentration was adjusted to 28 ng/mL (males) or 20 ng/ μ L (females).

[0367] For male monkeys, synthesis of cDNA was performed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, cat. #4368814) according to manufacturer's instructions with 700 ng of RNA and gene

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expression analysis was performed using a Wyeth custom TLDA card (Applied Biosystems, part # 4342249) designed for detection of cynomolgus monkey genes. Each cDNA synthesis reaction was mixed with TaqMan[®] 2x PCR Master Mix (Applied Biosystems, cat. # 4304437), and 100 μ L was loaded onto a TLDA card. TLDA cards were processed according to manufacturer instructions and amplification was performed using an ABI Prism[®] 7900HT Sequence Detection System. Cycling parameters used for each run were as follows: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 sec followed by 60°C for 1 min. Cycle thresholds (C_T) were calculated using Sequence Detection Software (version 2.3, Applied Biosystems).

[0368] For female monkeys, TaqMan quantitative RT-PCR for IL-2R α only was performed using prequalified primers and probes to IL-2R α (Applied Biosystems; the same IL-2R α primers and probes as those in custom TLDA for male monkeys).

[0369] For both males and females, the relative quantification (RQ) of gene expression was then calculated using the delta delta C_t ($\Delta\Delta C_t$) method where $RQ = 2^{-\Delta\Delta C_t}$. Zinc finger protein 592 (ZNF592, males) or protein kinase G-1 (PKG1, females) was used as the endogenous control and the vehicle control sample was used as the calibrator for RQ calculations. Samples with RQ values greater or equal to 1.5 were considered to have gene expression higher than the corresponding vehicle control sample.

[0370] Male monkeys whose blood showed higher expression of several immune function-related genes when stimulated *ex vivo* with IL-21 compared with vehicle ($RQ \geq 1.5$) were selected for inclusion in this study. The RQ values of five genes (IL-2R α , IL-21R, PRF1, GZMB, and IL-6) for nine monkeys selected for further studies described below, represented by animal number (Animal #), are shown in **Table 31**. The group number (A-C) represents whether the animals selected were treated with AbS (Group A), AbT (Group B), or IgG control (Group C) in subsequent experiments. Animals 10-13 were not selected for further studies.

Table 31: Relative Quantification (RQ) of Gene Expression Induced by Ex Vivo Addition of IL-21 to Whole Blood Obtained from Male Cynomolgus Monkeys

Group; Animal #	IL-2R α	IL-21R	PRF1	GZMB	IL-6
A; 1	2.8	2.5	2.8	2.0	4.2
A; 2	3.4	2.3	1.2	1.1	1.7
A; 3	5.5	2.9	2.5	1.4	3.6
B; 4	4.1	2.1	2.5	1.1	4.9
B; 5	5.3	3.2	2.1	2.1	4.1
B; 6	2.8	1.8	1.8	2.0	0.5
C; 7	6.3	1.9	2.4	1.9	2.6
C; 8	2.2	1.8	1.6	1.6	6.7
C; 9	3.8	2.0	1.9	2.2	1.2
10	2.9	1.8	0.7	1.0	0.9
11	7.7	3.4	2.6	2.2	2.8
12	4.5	3.6	1.4	1.5	1.1
13	2.1	1.5	1.1	1.2	1.4

[0371] IL-2R α was determined to have the largest magnitude (highest RQ) and most consistent change (highest percentage of animals that had RQ>1.5) in IL-21-induced gene expression of the genes evaluated, and was therefore considered the best single gene for assessing pharmacodynamic (PD) activity of the anti-IL-21R antibodies.

Whole blood samples from all monkeys included in the *in vivo* study had IL-2R α RQ values greater than 1.5 following *ex vivo* stimulation with IL-21. However, significant interanimal variability in IL-2R α RQ values was observed, with values ranging from 2.8 to 6.3.

[0372] To obtain a larger number of samples for characterization of the distribution of the IL-2R α response to rhuIL-21 in the *ex vivo* assay, blood samples were obtained from 24 additional female cynomolgus monkeys, stimulated *ex vivo* with rhuIL-21, and analyzed for IL-2R α gene expression by using a quantitative RT-PCR method (IL-21R, PRF1, GZMB, and IL-6 expression was not analyzed for these monkeys). There were no noticeable differences in IL-2R α RQ distribution between male and

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female monkeys (with the median $\pm$ SD RQ values of 3.8 ± 1.7 and 3.0 ± 1.9 , respectively) and subsequent analysis of IL-2R α RQ distribution was performed using a combined data set with n=37. All cynomolgus monkeys tested had IL-2R α RQ values greater or equal to 1.5 following *ex vivo* stimulation with rhuIL-21. The median IL-2R α RQ value (n=37) was 3.2 and the range was 1.5-8.1.

[0373] RQ values for IL-2R α expression obtained at baseline from 37 monkeys were log-transformed and the distribution of the RQ and log {RQ} values was tested in the Shapiro-Wilk and D'Agostino & Pearson normality tests (GraphPad Prism 5, GraphPad Software Inc). The normality hypothesis was rejected for the RQ distribution ($p < 0.05$) but not for log {RQ} distribution ($p = 0.16$ for the Shapiro-Wilk test; and $p = 0.48$ for the D'Agostino & Pearson test). The log-transformed RQ values were fitted into normal distribution ($R^2 = 0.69$) using GraphPad Prism 5 software. The distribution of the IL-2R α RQ values and log transformation ($\log_2$) of the RQ values obtained in the *ex vivo* assay for all 37 monkeys (n=13 males; n=24 females) are shown in **FIGs. 57a** and **57b**, respectively. The distribution of IL-2R α RQ values appeared approximately log-normal and passed normality tests. The inclusion criterion for future pharmacodynamic (PD) studies with anti-IL-21R antibodies in cynomolgus monkeys was defined as 2.3, based on the formula: $\log \{RQ_{\text{cutpoint}}\} = \text{mean of the log-transformed RQ values} - \text{standard deviation of the log-transformed RQ values}$. Thus, animals with distribution of the IL-2R α RQ values greater than 2.3 were considered to be good responders to IL-21 stimulation. Animals with RQ values of greater than 2.3 (~81%; 30 of 37) were defined as good responders and were considered to satisfy the inclusion criterion for the PD study of anti-IL-21R antibodies.

[0374] To confirm that IL-21-induced gene expression was dependent on engagement of cynomolgus monkey IL-21R, monkey whole blood samples were incubated simultaneously with IL-21 and an anti-IL-21R antibody (AbT; 30 nM) prior to RNA isolation and gene expression analysis. As expected, *ex vivo* addition

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of AbT simultaneously with IL-21 strongly inhibited IL-21-induced gene expression changes in the whole blood assay (i.e., RQ value <1.5; **FIG. 57c**).

Example 13.2: Study Design for In Vivo Experiments

[0375] Nine male protein-naive cynomolgus monkeys that were identified as responders to IL-21 stimulation in the *ex vivo* pharmacodynamic (PD) whole blood assay (described in Example 12.1), were dosed with 10 mg/kg of AbS (Group A), AbT (Group B), or IgG control antibody (Group C), with three animals per group. The dose was administered i.v. (infusion rate of ~4 mL/min) into the saphenous vein with a dose volume of 2.5 mL/kg.

[0376] Blood samples (~7.0 mL) for the determination of PD activity (all three groups) were collected into tubes containing sodium citrate as the anticoagulant. Blood samples (~3.0 mL) for the determination of serum AbS or AbT concentrations and for the evaluation of anti-product antibodies were collected into tubes without anticoagulant, allowed to clot at room temperature for approximately 15 min, and processed for serum collection by centrifugation. The sample collection schedule is shown in **Table 32**. After day 50, additional sampling time points were added for animals 1 and 3 in the AbS group (Group A) to demonstrate reversibility of PD activity. Day 1, also referred to as “dose administration,” is the day on which antibodies were administered to the monkeys. “Pre-dose” refers to sample collection time prior to dose administration, and “post-dose” refers to sample collection time after dose administration.

Table 32: *In Vivo* Study Design and Sample Collection in Male Cynomolgus Monkeys

Group (Dose) Animal #	Time (days)	Sample collection ^a
A; AbS (10 mg/kg, IV) Animals 1-3	-13, 1(pre- ^b and 5 min post-dose), 2, 8, 15, 22, 36, 50	Animals 1-3
	71, 92 ^c	Animal 3
	92, 106, 113, 134, 148 ^c	Animal 1
B; AbT (10 mg/kg, IV) Animals 4-6	-13, 1(pre- and 5 min post-dose), 2, 8, 15, 22, 36	Animals 4-6
C; IgG control (10 mg/kg, IV) Animals 7-9	-13, 1(pre- and 5 min post-dose), 2, 8, 15, 22, 36	Animals 7-9

- For Groups A and B, serum was collected to assay for test article concentrations and anti-product antibodies, and whole blood was collected for *ex vivo* PD assay. For Group C, only whole blood samples were collected.
- For animal 1, pre-dose day 1 samples were not collected.
- Following PD analysis at day 50, additional sampling time points were included to demonstrate reversibility of PD activity.

Example 13.3: AbS and AbT Serum Concentrations

[0377] To determine AbS and AbT serum concentrations, ELISAs described in Example 10 were used. PK studies in cynomolgus monkeys described in Example 10 indicated that following single i.v. administration, AbS was cleared markedly faster compared to AbT. In this study, extensive serum sampling required for determination of a complete set of PK parameters were not performed because of the relatively large sample volume required for the PD assay and limitations on blood volumes that could be collected from each individual cynomolgus monkey. Samples for determination of anti-IL-21R serum concentrations were taken only at those time points at which PD activity was assessed to enable correlation between the serum concentrations and PD activity for each individual animal. Thus, only elimination half-life ($t_{1/2}$) was estimated based on the terminal phases of serum concentration-time profiles. The apparent $t_{1/2}$ was determined as described in Example 10.

[0378] Following a single 10 mg/kg i.v. dose, AbS was eliminated slowly from male cynomolgus monkeys, with a mean apparent terminal half-life ($t_{1/2}$) of $\sim 10.6 \pm 3.92$

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days (**Table 33**). Up until day 22, AbS serum concentrations were very similar between all three AbS-dosed animals (**FIG. 58**). However, at day 36 and later time points, AbS serum concentrations in animal 2 declined rapidly (to ~0.6 µg/mL) compared to those for animals 1 and 3 (to ~2 µg/mL). At day 50, animal 2 had no detectable AbS in the serum (less than LOQ of 30 ng/mL), while animals 1 and 3 had AbS serum concentrations of ~0.9-1 µg/mL. Thus, the estimated $t_{1/2}$ of AbS was shorter for animal 2 (~6.2 days), compared to that for animals 1 and 3 (~12 and 14 days, respectively).

[0379] As expected based on the initial PK studies, after a single 10 mg/kg i.v. dose administration, the serum concentration of AbT declined markedly faster, compared to that of AbS (**FIG. 58**). All three AbS-dosed monkeys had similar concentration time-profiles and apparent $t_{1/2}$ values (**Table 33**), with serum concentrations declining to relatively low levels at day 15 (<0.4 µg/mL) and to less than the LOQ at day 22. The estimated mean $t_{1/2}$ of AbT was 2.3 ± 0.16 days. AbS and AbT concentrations started to diverge as early as 24 hrs post-dose and differed by more than ten-fold at the one-week time point. These data confirmed observations from the earlier PK studies in which AbT had ~5-7 fold faster total body clearance (CL), compared to AbS (see, e.g., Example 10).

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Table 33: Peak and Last Detectable Concentrations and Elimination Half-Life After 10 mg/kg IV Administration of AbS and AbT to Male Cynomolgus Monkeys

Group	Animal	C _{peak} (µg/mL)	t _{1/2} (days)	C _{last} (µg/mL)	T _{last} (days)
A (AbS)	1	200	12	0.91	50
	2	139	6.2	0.56	36
	3	153	14	0.37	71
	Mean	164	11	0.61	52
	SD	32	3.9	0.27	18
B (AbT)	4	145	2.5	0.36	15
	5	155	2.3	0.26	15
	6	201	2.1	0.25	15
	Mean	167	2.3	0.29	15
	SD	30	0.16	0.06	0

C_{peak} Concentration at 5 min (also referred to as C_{5min}), the first sampling time point after i.v. administration

t_{1/2} Elimination half-life

T_{last} Last time point at which the test article concentration was above the lower limit of quantitation (LOQ=30.0 ng/mL)

C_{last} Concentration at T_{last}

Example 13.4: AbS and AbT PD Response in Male Cynomolgus Monkeys

[0380] *Ex vivo* whole blood assay for detection of inhibition of IL-21-induced expression by anti-IL-21R antibodies was described in Example 12.1. For all nine monkeys enrolled into this study, each pre-dose and post-dose whole blood sample was divided into four 1.5 mL aliquots. First and second aliquots were treated with either recombinant human IL-21 or vehicle (a calibrator for RQ calculations), and were used to assess whether the circulating test article affected *ex vivo* IL-21-induced IL-2Rα gene expression (i.e., PD activity). The third aliquot was treated with IL-21

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and an anti-IL-21R antibody (30 nM), and the fourth aliquot was treated with IL-21 and an IgG control antibody (negative control for the anti-IL-21R antibody). The third and fourth aliquots were used to assess whether inhibition of IL-21-induced IL-2R α gene expression by the circulating test article in a given post-dose sample was complete (for time points at which PD activity was observed), whether the return of IL-21-induced gene expression was mediated through the IL-21R (for time points at which PD activity was later lost), and to monitor for the presence of neutralizing anti-product antibodies.

[0381] For AbS, full inhibition of IL-21-induced IL-2R α gene expression (IL-2R α RQ <1.5) was observed immediately after dose administration and persisted until at least day 22 for animal 2 and at least day 50 for animals 1 and 3, when serum AbS concentrations were at or above 6 nM (0.9 μ g/mL) for all three monkeys (**FIGs. 59a-c** and **Table 33**). *Ex vivo* IL-21-induced IL-2R α expression returned to pre-dose values (i.e., PD activity was lost) at day 92 for animals 1 and 3, coincident with the time points at which serum concentrations were <LOQ (**FIGs. 59a** and **c**). For animal 2, PD activity was lost at day 36, when serum AbS concentration declined to a relatively low level of ~4 nM (0.6 μ g/mL). For all time points examined in this study, PD activity of AbS appeared all or none, such that there was typically either complete inhibition of IL-21-induced IL-2R α gene expression (RQ <1.5), or a lack of inhibition (RQ similar to that in the corresponding pre-dose sample). A partial PD response was difficult to differentiate because of the intra-animal variability observed in IL-2R α RQ values. It is possible that data points with partial PD responses for AbS would have been observed if additional sampling time points were collected at the terminal phase. The minimum concentration that was needed to maintain minimum PD activity of AbS ($C_{\min}$) could not be precisely estimated, but is likely to be ~4-6 nM.

[0382] For AbT, PD activity was also observed immediately after dose administration and persisted until at least day 8 (RQ <1.5), when serum AbT concentrations were at or above 1.3 μ g/mL (**FIGs. 60a-c** and **Table 33**). PD activity

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was lost ($RQ > 1.5$) at day 15 for all three monkeys. In blood samples obtained at day 15 from animals 5 and 6, IL-2R α RQ values appeared similar to the corresponding pre-dose values (i.e., complete loss of PD activity) and serum AbT concentrations were less than 1.8 nM. There was a partial PD response in blood samples obtained at day 15 from animal 4, as the observed IL-2R α RQ value of 2.7 was less than that at pre-dose ($RQ=4.8$) and at the subsequent day 22 time-point ($RQ=5.3$; **FIG. 60a**). Animal 4 also had a slightly longer estimated $t_{1/2}$ and a somewhat higher AbT serum concentration at day 15 (~ 2.5 nM), compared to animals 5 and 6 (**FIGs. 60a-c**). These data suggest that the C_{min} of AbT needed to maintain PD activity was approximately 2.5 nM.

[0383] For the isotype control group, *ex vivo*-added recombinant human IL-21 induced IL-2R α gene expression in whole blood samples from all three monkeys at all time points, with noticeable intra-animal variability in the IL-2R α RQ values (data not shown).

[0384] In agreement with the earlier PK studies (see Example 10), AbT had faster elimination in monkeys compared with AbS, with a mean apparent $t_{1/2}$ of 10.6 and 2.3 days for AbS and AbT, respectively. At the day 15 time point, PD activity was completely or partially lost in all three AbT-dosed monkeys, while all three monkeys in the AbS dose group had relatively high serum AbS concentrations (~ 6.0 - 7.4 $\mu\text{g/mL}$) and full PD activity. Thus, AbS had a longer duration of PD activity and a longer $t_{1/2}$ in cynomolgus monkeys.

Example 13.5: Anti-product Antibody Response

[0385] At the first time point where loss of PD activity was observed, *ex vivo* addition of an anti-IL-21R antibody simultaneous with rhuIL-21 inhibited the induction of IL-2R α gene expression ($RQ < 1.5$) in all AbS- and AbT-dosed monkeys, indicating that the return of rhuIL-21-induced gene expression was mediated through the IL-21R and that neutralizing anti-IL-21R antibodies were not present (**FIGs. 59 and 60**). *Ex vivo* addition of AbS continued to demonstrate inhibitory activity at

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subsequent time points collected from animals 1 and 3. However, AbS had no *ex vivo* inhibitory activity at the day 50 time point from animal 2 (**FIG. 59**). Similarly, AbT had no *ex vivo* inhibitory activity at days 22 and/or 36 collected from all animals in AbT-dosed groups (**FIG. 60**). These data suggested that animal 2 in the AbS group, and all three animals (4-6) in the AbT group, had developed neutralizing anti-product antibodies.

[0386] The presence of neutralizing anti-AbT antibodies in AbT-dosed animals was confirmed using an orthogonal flow cytometric (FACS)-based assay. TF-1 and TF-1/rhuIL-21R (TF-1 cells transfected with rhuIL-21R) were grown in RPMI media containing 25 ng/ml huGMCSF (R&D Systems). Confluent cell cultures were centrifuged at 300 g for 10 min, resuspended in OptiMEM serum-free medium (Invitrogen Corporation) at 10^6 cells/mL, and incubated at 37°C for 2 hr. The cells were then washed in cold PBS/0.5% BSA, resuspended in ice-cold PBS buffer, and kept on ice until staining. To determine the EC₅₀ for AbS-biotin and AbT-biotin binding to TF-1/rhuIL-21R cells, both the parental TF-1 and the TF-1/rhuIL-21R cells (10^5 cells per test) were incubated with either AbT-biotin, or IgG-biotin control using serial 3-fold dilutions (range=16 - 0.0002µg/mL) on ice for 30 min, washed in PBS/0.5% BSA, and then incubated with streptavidin-allophycocyanin (APC; Invitrogen Corporation). Geometric mean fluorescent intensities (“GMFI”) of the APC channel peaks was collected on an LSRII flow cytometer (BD Biosciences) and analyzed using Flowjo 8.3.3 software (Treestar). Linear regression analysis of the plots was performed using Prism 4 for Macintosh v4.0b (GraphPad Software, Inc.).

[0387] The minimum required dilution (MRD) for testing serum samples in this assay was determined to be 1:6 in PBS/0.5% BSA. To test for inhibition of AbT-biotin to TF-1/rhuIL-21R cells (i.e. for the presence of neutralizing activity), TF-1/rhuIL-21R cells were preincubated with sera from anti-IL-21R-dosed monkeys (using a 3-fold dilution series starting at the MRD), stained with an anti-IL-21R-biotin (at the estimated EC₅₀ concentration), washed in PBS/0.5% BSA, stained with streptavidin-APC, and analyzed for GMFI as described above. Each serum sample

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was run in duplicate in two individual experiments, and the average GMFI value for the four replicates was obtained for each dilution point. The relative GMFI value for each serum sample for each dilution point was calculated using the formula $[100\% \times \text{average GMFI} / \text{average GMFI pre-dose}]$. A sample was considered positive if the relative GMFI value was less than or equal to 80% at the MRD. For positive samples, the log titer was calculated as the log [reciprocal dilution that would generate relative GMFI >80%]. Based on the MRD, log titers for negative samples were reported as <0.78 (log 6).

[0388] All three AbT-dosed animals tested positive in the FACS-based neutralizing antibody assay at days 22 and 36, with log titers ranging from 2.2 to 4.1 (**Table 34**).

Table 34: Formation of Neutralizing anti-AbT Antibodies (log Titer) After 10 mg/kg i.v. Administration of AbT to Male Cynomolgus Monkeys

TIME (DAYS)	ANIMAL 4	ANIMAL 5	ANIMAL 6
Pre-dose	Negative	Negative	Negative
15	Negative	Negative	Negative
22	4.12	2.7	2.2
36	2.2	2.7	2.7

[0389] As only one of the AbS-dosed animals showed evidence of neutralizing anti-AbS antibodies in the *ex vivo* IL-2R α gene expression assay, serum samples from AbS-dosed monkeys were tested in an electrochemiluminescent paramagnetic bead-based assay described in Example 11.5 that detected both neutralizing and nonneutralizing anti-AbS antibodies. In this assay, serum samples were coincubated with biotinylated-AbS and ruthenylated-AbS, streptavidin-coated paramagnetic beads were added to the mixture, and the emitted light was detected using BioVeris technology. All three AbS-dosed monkeys were positive for anti-AbS antibodies in this assay, with log titers ranging from 1.86 to 3.43 (**Table 35**). There was significant interanimal variability in the apparent onset of anti-AbS generation. The first serum sample that was positive for anti-AbS antibodies in the BioVeris-based

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assay was obtained at days 134, 36, and 92 for animals 1, 2, and 3, respectively. Thus, among the three AbS-dosed animals, animal 2 had the shortest $t_{1/2}$ and the fastest onset and highest titer of anti-AbS antibody response. Animal 2 was also the only AbS-dosed monkey that showed evidence of neutralizing anti-AbS antibody response in the *ex vivo* IL-2R α gene expression assay, similar to all three AbS-dosed monkeys.

Table 35: Formation of Anti AbS Antibodies (log Titer) After 10 mg/kg i.v. Administration of AbS to Male Cynomolgus Monkeys

TIME (DAYS)	ANIMAL 1	ANIMAL 2	ANIMAL 3
Pre-dose	Negative	Negative	Negative
15	Negative	Negative	Negative
22	Negative	Negative	Negative
36	Negative	2.13	Negative
50	Negative	3.43	Negative
71	ND	ND	Negative
92	Negative	ND	2.27
106	ND	ND	2.79
113	Negative	ND	ND
134	1.86	ND	ND
148	2.4	ND	ND

“ND”=Not determined

Example 14: PK and PD of Additional Anti-IL-21R Antibodies in Cynomolgus Monkeys

[0390] Pharmacokinetics of AbV, AbU, and AbW were examined after a single i.v. dose (10, 10, and 1 mg/kg, respectively) to protein-naïve cynomolgus monkeys, and PK parameters were compared to earlier PK data obtained with AbS and AbT (Table 36 and FIG. 61). Bioanalytical assays and PK calculations were performed as described for AbS and AbT in Example 13.

[0391] For all compounds, there was significant interanimal variability in the terminal phase, likely related to different onset of formation of anti-product antibodies. Following a single 10 mg/kg i.v. dose to monkeys, AbV and AbS

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appeared to have similar mean serum concentrations and PK parameters. After a single i.v. dose to monkeys, AbV and AbS appeared to have the slowest mean CL, longest mean $t_{1/2}$, and highest (dose-normalized) mean serum concentrations among all human anti-IL-21R antibodies tested. AbT had the fastest mean CL, shortest mean $t_{1/2}$ and lowest (dose-normalized) mean serum concentrations among all human anti-IL-21R antibodies tested. Comparison of mean concentration profiles and PK parameters after a single i.v. dose to cynomolgus monkeys suggested that ranking of human anti-IL-21R Ab antibodies based on PK profiles was similar between the S-D rats and cynomolgus monkeys (see also Example 10.10).

Table 36: PK Parameters After a Single i.v. Administration of Human Anti-IL-21R Antibodies to Cynomolgus Monkeys

Com- pound	Sex		Dose (mg/kg)	C_{5min}^a ($\mu g \cdot hr/ml$)	$AUC_{0-\infty}$ ($\mu g \cdot hr/ml$)	$AUC_{0-\infty}/$ Dose ($\mu g \cdot hr/ml$)/ (mg/kg)	CL (ml/hr/kg)	Vd_{ss} (ml/kg)	$t_{1/2}$ (hr)
AbS	F	MEAN	2	33.3	1575	788	1.28	122	78.5
		SD		3.84	184	91.9	0.150	25.0	16.4
	F	MEAN	10	177	9728	973	1.03	125	74
		SD		10	621	62.1	0.07	16	46
	M	MEAN	10^b	164	ND	ND	ND	ND	255
		SD		32	ND	ND	ND	ND	94
	M	MEAN	100	2030	92867	929	1.08	211	279
		SD		95	9768	97.7	0.108	8.00	27.6
AbT	F	MEAN	10	113	1476	148	7.01	202	63
		SD		14	312	31.2	1.68	44	24
	M	MEAN	10^b	167	ND	ND	ND	55	
		SD		30	ND	ND	ND	4	
	M	MEAN	100	1850	20955	210	4.87	146	87.8
		SD		415	3702	37.0	0.790	24.4	34.0
AbW	F	MEAN	1	10.0	430	430	2.33	165	73.2
		SD	NA	NA	NA	NA	NA	NA	NA
AbV	F	MEAN	10	152	9895	989	1.00	228	201
		SD		46.0	3181	318	0.367	70.4	113

	F	MEAN	10	197	6807	1947	1.55	140	89.6
AbU		SD		35.5	681	195	0.447	25.6	35.2

All studies were run in protein-naïve monkeys, n=3 per dose group, unless otherwise noted
a. C_{5min} =concentration at 5 min, the first time point after IV administration
b. PK data from a separate PK-PD study
NA=Not applicable (n=2)
ND=Not determined; insufficient sampling time points

[0392] In the same study, PD activity of AbU-AbW (AbU, AbV, and AbW) in monkeys was examined using *ex vivo* rhuIL-21-induced gene expression assay (described in Example 13).

[0393] At five min, the first sampling time point, AbU-AbW had PD activity in all monkeys, i.e., displayed complete inhibition of rhuIL-21-induced gene expression in the *ex vivo* whole blood assay, similar to data obtained for AbS and AbT. PD activity was lost with the washout of AbU-AbW from serum.

Example 15: Pharmacokinetics of AbS After Intravenous Administration to Tetanus-Toxoid-Challenged Male and Female Cynomolgus Monkeys

[0394] PK of AbS was examined after a single three weekly i.v. administration of 2 or 10 mg/kg of AbS to tetanus-toxoid-challenged cynomolgus monkeys. AbS serum concentrations and anti-AbS antibodies were monitored by specific ELISA, and PK parameters were calculated by noncompartmental analysis, as described, e.g., in Example 13.

[0395] Following three weekly i.v. administrations of 2 or 10 mg/kg to monkeys, AbS concentration-time profiles and PK parameters were generally similar between the male and female monkeys in the same dose group (n=3 per sex per group). The mean concentration at 5 min after the 1st dose (C_{5min}) and the 3rd dose (C_{day14, 5min}), as well as the mean exposure after the 3rd dose (AUC_{day14-day21}) increased with the dose level. In the 2 mg/kg group, the mean C_{5min} was 28.4 ± 2.50 µg/mL, the mean C_{day14, 5min} was 35.7 ± 11.0 µg/mL, and the mean AUC_{day14-day21} was 1279 ± 592 µg•hr/mL. In the 10 mg/kg group, the mean C_{5min} was 120 ± 59.9, the mean C_{day14, 5min} was 152 ± 21.9 µg/mL, and the mean AUC_{day14-day21} was

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$7700 \pm 782 \mu\text{g}\cdot\text{hr/mL}$. Elimination half-life ($t_{1/2}$) after the third IV administration of AbS was 25.6 ± 22.7 and 168 ± 56.5 hr in the 2 and 10 mg/kg groups, respectively ($p=0.0002$) (**FIG. 62**).

[0396] In the 2 mg/kg group, AbS concentrations declined rapidly after the third i.v. administration, and all six monkeys tested positive for anti-AbS antibodies at day 28 (two weeks after the third dose) through the end of the study, in line with the relatively short $t_{1/2}$ (**FIG. 62a**). In the 10 mg/kg group, two of the six monkeys (SAN 7 and SAN 8) tested positive for anti-AbS antibodies (at day 42 or 49), and these monkeys had the shortest $t_{1/2}$ values in the dose group, suggesting that formation of anti-AbS antibodies correlated with the $t_{1/2}$ of AbS (**FIG. 62b**).

Example 16: Prediction of Pharmacokinetics of AbS and AbT in Humans After Single Administration

[0397] To predict the PK of AbS and AbT in humans, two approaches were used. For the first approach, it was assumed that the PK parameters (such as CL and volume of distribution) of an anti-IL-21R Ab in humans would be similar to those in cynomolgus monkeys. For the second approach, allometric scaling was used to estimate human PK parameters based on the animal data obtained from mice, rats, and cynomolgus monkeys following single i.v. administration of an anti-IL-21R antibody. Allometric scaling of CL was performed using adjustment for maximal life-span potential (MLP) for AbS and brain weight adjustments for AbT, according to the methods previously described in Mahmood (1996) *Eur. J. Drug Metab. Pharmacokinet.* 21:275-78; Mahmood and Balian (1996) *Xenobiotica* 26:887-95; Sacher, "Relation of lifespan to brain weight and body weight in mammals" in CIBA Foundation Symposium – The Lifespan of Animals (Colloquia on Aging), 115-33 (Wolstenholme GEW, O'Connor M, eds, 1959); and Hahn ME, Haber SB. (1978) *Behav Genet.*, 8:251-260. The allometric scaling data for AbS and AbT is demonstrated in **FIGs. 63a-b** and **64a-b**, respectively.

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[0398] Collectively, these two approaches suggested a low clearance (CL) and a small steady-state volume of distribution (V_{dss}) of AbS in humans. The predicted CL of AbS would be ~0.72-1.3 mL/hr/kg and the estimated V_{dss} would be ~92.4-125 mL/kg. Following an i.v. dose of 1 mg/kg of AbS to a 60 kg human subject, the estimate of exposure ($AUC_{0-\infty}$) is 1393 $\mu\text{g}\cdot\text{hr/mL}$, based on the CL value obtained by the allometric scaling method. For AbT, these two approaches suggested a higher CL of ~ 5-8.5 mL/hr/kg and V_{dss} of ~115-202 mL/hr/kg.

Example 17: Treatment with AbS Does Not Affect Disease in the NZBWF1/J Murine Lupus Nephritis Model

[0399] The anti-IL-21R antibody AbS was tested for its ability to reduce disease in a murine model of lupus, using NZBWF1/J mice. Female NZBWF1/J mice spontaneously develop symptoms resembling those observed in human lupus nephritis, including high titers of circulating IgG anti-nuclear and anti-double-stranded DNA autoantibodies, IgG deposits in the glomeruli, and proteinuria. The onset of kidney disease, as measured by the presence of protein in the urine, occurs at approximately 26 weeks of age in female NZBWF1/J mice. To examine the effects of IL-21R blockade with AbS, 26 week old female NZBWF1/J mice were administered either saline (vehicle control), CTLA-4Ig (murine IgG2a, positive control), anti-E.tenella antibody (murine IgG2a isotype control), AbS, or a human antibody isotype control (human IgG1 antibody with triple mutation) at a dosage of 400 $\mu\text{g}/\text{mouse}$ 3X / week over 10 weeks via i.p. injection. Serum samples were taken every two weeks and assayed for IgG anti-dsDNA antibodies by ELISA. Urine was collected and examined every two weeks for protein levels using Albustix (Bayer HealthCare, Tarrytown, NY). All groups of animals had similar levels of proteinuria at the onset of the study, and the degree of proteinuria escalated in the saline, anti-E.tenella, and hIgG1TM control over the course of the study (**FIG. 65a**). Treatment with CTLA-4Ig protein, which has previously been shown to ameliorate disease in this model, prevented the development of increased proteinuria in these

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mice. In contrast, treatment with AbS did not affect the development of proteinuria in NZBWF1/J mice when compared to control mice (**FIG. 65a**). Similarly, treatment with CTLA-4 Ig significantly reduced anti-dsDNA IgG serum antibody titers in NZBWF1/J mice, where treatment with AbS did not affect the development of anti-dsDNA antibodies in these mice (**FIG. 65b**).

Example 18: Effects of Treatment with Anti-IL-21R Antibodies in a Semi-therapeutic Collagen-Induced Arthritis (CIA) Mouse Model

[0400] Antibodies AbS and AbT were tested for their ability to reduce disease in a murine collagen-induced arthritis model of rheumatoid arthritis. Female DBA/1 mice were immunized intradermally in the tail with 100 mg bovine collagen type II emulsified in complete Freund's adjuvant, and then boosted with 100 mg bovine collagen type II emulsified in incomplete Freund's adjuvant 21 days later at the same site. After the second immunization with bovine collagen type II, paws were examined for swelling and scored for severity on a scale of 0-16, with 0 representing no swelling and 16 indicating severe disease. Once 10% of animals in the study showed signs of disease, animals were either left untreated, or dosed 3X / week for 30 days with 8 mg/kg either murine IgG2a isotype control antibody, anti-mouse IL-21R antibody D5 (murine IgG2a antibody), mTNFRII-Fc (positive control, murine IgG2a isotype), anti-IL-13TM antibody (human IgG1 isotype control antibody), AbT, or AbS. Overall disease severity in this study was not observed to be as severe as normally observed in this model. Mean disease severity in the untreated and isotype control treated mice on the final day of the study was less than 4, on a scale of 0-16 (**FIG. 66c**). Additionally, although treatment with the mTNFRII-Fc positive control reduced disease in this study, statistically significant differences in disease severity were only observed between this treatment group and isotype control treated group disease on day 22 after dosing. Treatment with anti-mouse IL-21R antibody (D5) or anti-human IL-21R antibodies (AbS and AbT) also did not affect disease severity in this study (**FIGs. 66a-b**).

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Example 19: Testing Antibody Effects on the Formation of Amnestic Antibody Response to Tetanus Immunization in Cynomolgus Monkeys

[0401] The anti-IL-21R antibody AbS was examined for its effects on the development of amnestic antibody responses to tetanus immunization in cynomolgus monkeys. Nine female and nine male cynomolgus monkeys were tested for serum antibody titers to tetanus toxoid to identify tetanus-naïve animals. These animals were then administered 0.5 ml of tetanus toxoid in two equally divided (0.25 ml) doses intramuscularly, and blood was collected every 7 days for 35 days and examined for anti-tetanus IgM and IgG serum antibodies by ELISA. Forty-three days after primary immunization, groups of 3 male and 3 female cynomolgus monkeys were randomly assigned and administered either saline (vehicle), 2 mg/kg AbS, or 10 mg/kg AbS as an i.v slow bolus into the brachial/cephalic or saphenous vein at a dose volume of 1 ml/kg (flushed with 2 ml vehicle) 1X / week for 3 weeks. Twenty-four hr after administering the first dose of AbS or vehicle, monkeys were immunized a second time with 0.5 ml of tetanus toxoid in two equally divided (0.25 ml) doses intramuscularly, and blood was collected routinely and examined for anti-tetanus IgM and IgG antibody titers by ELISA. IgM and IgG tetanus serum antibodies were detectable in both male and female cynomolgus monkeys within 14 days after the first immunization with tetanus toxoid (**FIG. 67a**). Tetanus specific serum IgM titers did not change following secondary immunization with tetanus toxoid in any of the treatment groups. Tetanus-specific IgG serum titers were approximately 10-20 fold greater, and generated more rapidly in saline treated animals following secondary immunization, consistent with the kinetics of amnestic antibody responses (**FIG. 67b**). Treatment with 2 mg/kg or 10 mg/kg AbS did not affect the development of tetanus-specific IgG serum antibody responses in cynomolgus monkeys following secondary immunization, indicating that treatment with AbS does not affect the formation of amnestic antibody responses to tetanus toxoid using this treatment protocol (**FIG. 67b**).

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Equivalents

[0402] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

WHAT IS CLAIMED IS:

1. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248, in the treatment or prevention of an IL-21R-associated disorder in a subject.
2. The use of claim 1, wherein the binding protein or antigen-binding fragment is an antibody.
3. The use of claim 1, wherein the binding protein or antigen-binding fragment is an scFv.
4. The use of claim 1, wherein the binding protein or antigen-binding fragment is a V_H.
5. The use of claim 1, wherein the binding protein or antigen-binding fragment is a V_L.
6. The use of claim 1, wherein the binding protein or antigen-binding fragment is a CDR.
7. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, in the treatment or prevention of an IL-21R-associated disorder in a subject.

8. The use of claim 1, wherein the binding protein or antigen-binding fragment comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

9. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248, and

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248, in the treatment or prevention of an IL-21R-associated disorder in a subject.

10. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide

sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, and

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, and 11, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, in the treatment or prevention of an IL-21R-associated disorder in a subject.

11. The use of claim 9, wherein the binding protein or antigen-binding fragment comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248,

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132,

134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

12. A use of a binding protein or antigen-binding fragment thereof that specifically binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the heavy chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 68, 70, 72, 88, 90, 92, 94, 213, 218, 219, 240, and 242, in the treatment or prevention of an IL-21R-associated disorder in a subject.

13. A use of a binding protein or antigen-binding fragment thereof that specifically binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the light chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 74, 76, 78, 80, 82, 84, 86, 96, 98, 100, 102, 104, 106, 108, 214-217, 220-229, 244, 246, and 248, in the treatment or prevention of an IL-21R-associated disorder in a subject.

14. The use of claim 12, wherein the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and wherein the V_H domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, and 20.

15. The use of claim 13, wherein the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and wherein the V_L domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 215, 217, 221, 223, 225, 227, and 229.

16. The use of claim 12 or 13, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:88, 90, 92, 94, 213, 218, 219, 240, and 242, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:96, 98, 100, 102, 104, 106, 108, 214, 216, 220, 222, 224, 226, 228, 244, 246, and 248.

17. A use of a binding protein or antigen-binding fragment thereof that specifically binds to a human IL-21R epitope that is recognized by a binding protein selected from the group

consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25, wherein the binding protein or antigen-binding fragment competitively inhibits the binding of a binding protein selected from the group consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25 to human IL-21R, in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, in the treatment or prevention of an IL-21R-associated disorder in a subject.

18. The use of claim 17, wherein the binding protein or antigen-binding fragment thereof comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

19. The use of claim 17, wherein the binding protein or antigen-binding fragment thereof comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

20. The use of claim 17, wherein the binding protein specifically binds to an IL-21R epitope that is recognized by AbO, AbP, AbQ, AbR, AbS, AbT, AbU, AbV, and/or AbW, and wherein the binding protein competitively inhibits the binding of AbO, AbP, AbQ, AbR, AbS, AbT, AbU, AbV, and/or AbW to human IL-21R.

21. The use of any one of claims 1, 9, 12, 13, and 17, wherein the IL-21R-associated disorder is selected from the group consisting of autoimmune disorders, inflammatory conditions, allergies, transplant rejections, and hyperproliferative disorders of the blood.

22. The use of claim 21, wherein the IL-21R-associated disorder is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, psoriasis, transplant rejection, rheumatoid arthritis, and other arthritic disorders.

23. The use of any one of claims 1, 9, 12, 13, and 17, wherein the binding protein or antigen-binding fragment thereof has an association constant for human IL-21R of at least $10^5 \text{ M}^{-1} \text{ s}^{-1}$.

24. The use of any one of claims 1, 9, 12, 13, and 17, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated BAF3 cell proliferation with an IC_{50} of about 1.75 nM or less, and wherein the BAF3 cells comprise a human IL-21 receptor.

25. The use of any one of claims 1, 9, 12, 13, and 17, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of TF1 cells with an IC_{50} of about 14 nM or less, and wherein the TF1 cells comprise a human IL-21 receptor.

26. The use of any one of claims 1, 9, 12, 13, and 17, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of primary human B cells with an IC_{50} of about 1.9 nM or less, and wherein the B cells comprise a human IL-21R.

27. The use of any one of claims 1, 9, 12, 13, and 17, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of primary human CD4^+ cells with an IC_{50} of about 1.5 nM or less, and wherein the CD4^+ cells comprise a human IL-21R.

28. A method of determining whether an anti-IL-21R antibody is a therapeutic anti-IL-21R antibody comprising the steps of:

- (a) contacting a first blood sample from a subject with an IL-21 ligand;
- (b) determining a level of expression of at least one IL-21-responsive gene in the first blood sample contacted with the IL-21 ligand;
- (c) contacting a second blood sample from the subject with the IL-21 ligand in the presence of an anti-IL-21R antibody;
- (d) determining the level of expression of the at least one IL-21-responsive gene in the second blood sample contacted with the IL-21 ligand in the presence of the anti-IL-21R antibody; and
- (e) comparing the levels of expression of the at least one IL-21-responsive gene determined in steps (b) and (d),

wherein a change in the level of expression of the at least one IL-21-responsive gene indicates that the anti-IL-21R antibody is a therapeutic antibody.

29. The method of claim 28, wherein the at least one IL-21-responsive gene is selected from the group consisting of TNF, IFN γ , IL-6, IL-8, IL-10, CD19, STAT3, TBX21, CSF1, GZMB, PRF1, IL-2R α , and IL-21R.

30. The method of claim 29, wherein the at least one IL-21-responsive gene is IL-2R α .

31. A method of determining the pharmacodynamic activity of an anti-IL-21R antibody comprising detecting a modulation in a level of expression of at least one IL-21-responsive gene in a blood sample of a subject.

32. The method of claim 31, wherein detecting the modulation in the level of expression of the at least one IL-21-responsive gene comprises the steps of:

(a) administering the anti-IL-21R antibody to the subject, wherein the subject is treated with the anti-IL-21R antibody;

(b) contacting a blood sample from the subject treated with the anti-IL-21R antibody with an IL-21 ligand;

(c) determining the level of expression of the at least one IL-21-responsive gene in the blood sample from the subject treated with the anti-IL-21R antibody and contacted with the IL-21 ligand; and

(d) comparing the level of expression of the at least one IL-21-responsive gene determined in step (c) with the level of expression of the at least one IL-21 responsive gene in a different blood sample contacted with the IL-21 ligand, wherein the different blood sample is from a subject not treated with the anti-IL-21R antibody.

33. The method of claim 32, wherein the at least one IL-21-responsive gene is selected from the group consisting of TNF, IFN γ , IL-6, IL-8, IL-10, CD19, STAT3, TBX21, CSF1, GZMB, PRF1, IL-2R α , and IL-21R.

34. The method of claim 33, wherein the at least one IL-21-responsive gene is selected from the group consisting of CD19, GZMB, PRF1, IL-2R α , IFN γ , and IL-6.

35. The method of claim 34, wherein the at least one IL-21-responsive gene is IL-2R α .

36. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-

binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248, in the manufacture of a medicament for use in the treatment or prevention of an IL-21R-associated disorder in a subject.

37. The use of claim 36, wherein the binding protein or antigen-binding fragment is an antibody.

38. The use of claim 36, wherein the binding protein or antigen-binding fragment is an scFv.

39. The use of claim 36, wherein the binding protein or antigen-binding fragment is a V_H .

40. The use of claim 36, wherein the binding protein or antigen-binding fragment is a V_L .

41. The use of claim 36, wherein the binding protein or antigen-binding fragment is a CDR.

42. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, in the manufacture of a medicament for use in the treatment or prevention of an IL-21R-associated disorder in a subject.

43. The use of claim 36, wherein the binding protein or antigen-binding fragment comprises at least one amino acid sequence selected from the group consisting of SEQ ID

NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

44. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248, and

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248, in the manufacture of a medicament for use in the treatment or prevention of an IL-21R-associated disorder in a subject.

45. A use of a binding protein or antigen-binding fragment thereof that specifically binds to human IL-21R in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37,

39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, and

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, and 11, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, in the manufacture of a medicament for use in the treatment or prevention of an IL-21R-associated disorder in a subject.

46. The use of claim 44, wherein the binding protein or antigen-binding fragment comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248,

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

47. A use of a binding protein or antigen-binding fragment thereof that specifically binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the heavy chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 68, 70, 72, 88, 90, 92, 94, 213, 218, 219, 240, and 242, in the manufacture of a medicament for use in the treatment or prevention of an IL-21R-associated disorder in a subject.

48. A use of a binding protein or antigen-binding fragment thereof that specifically binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the light chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 74, 76, 78, 80, 82, 84, 86, 96, 98, 100, 102, 104, 106, 108, 214-217, 220-229, 244, 246, and 248, in the manufacture of a medicament for use in the treatment or prevention of an IL-21R-associated disorder in a subject.

49. The use of claim 47, wherein the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and wherein the V_H domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, and 20.

50. The use of claim 48, wherein the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and wherein the V_L domain comprises at least one sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 215, 217, 221, 223, 225, 227, and 229.

51. The use of claim 47 or 48, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:88, 90, 92, 94, 213, 218, 219, 240, and 242, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:96, 98, 100, 102, 104, 106, 108, 214, 216, 220, 222, 224, 226, 228, 244, 246, and 248.

52. A use of a binding protein or antigen-binding fragment thereof that specifically binds to a human IL-21R epitope that is recognized by a binding protein selected from the group consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25, wherein the binding protein or antigen-binding fragment competitively inhibits the binding of a binding protein selected

from the group consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25 to human IL-21R, in an amount sufficient to inhibit or reduce immune cell activity in the subject thereby treating or preventing the disorder, in the manufacture of a medicament for use in the treatment or prevention of an IL-21R-associated disorder in a subject.

53. The use of claim 52, wherein the binding protein or antigen-binding fragment thereof comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

54. The use of claim 52, wherein the binding protein or antigen-binding fragment thereof comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

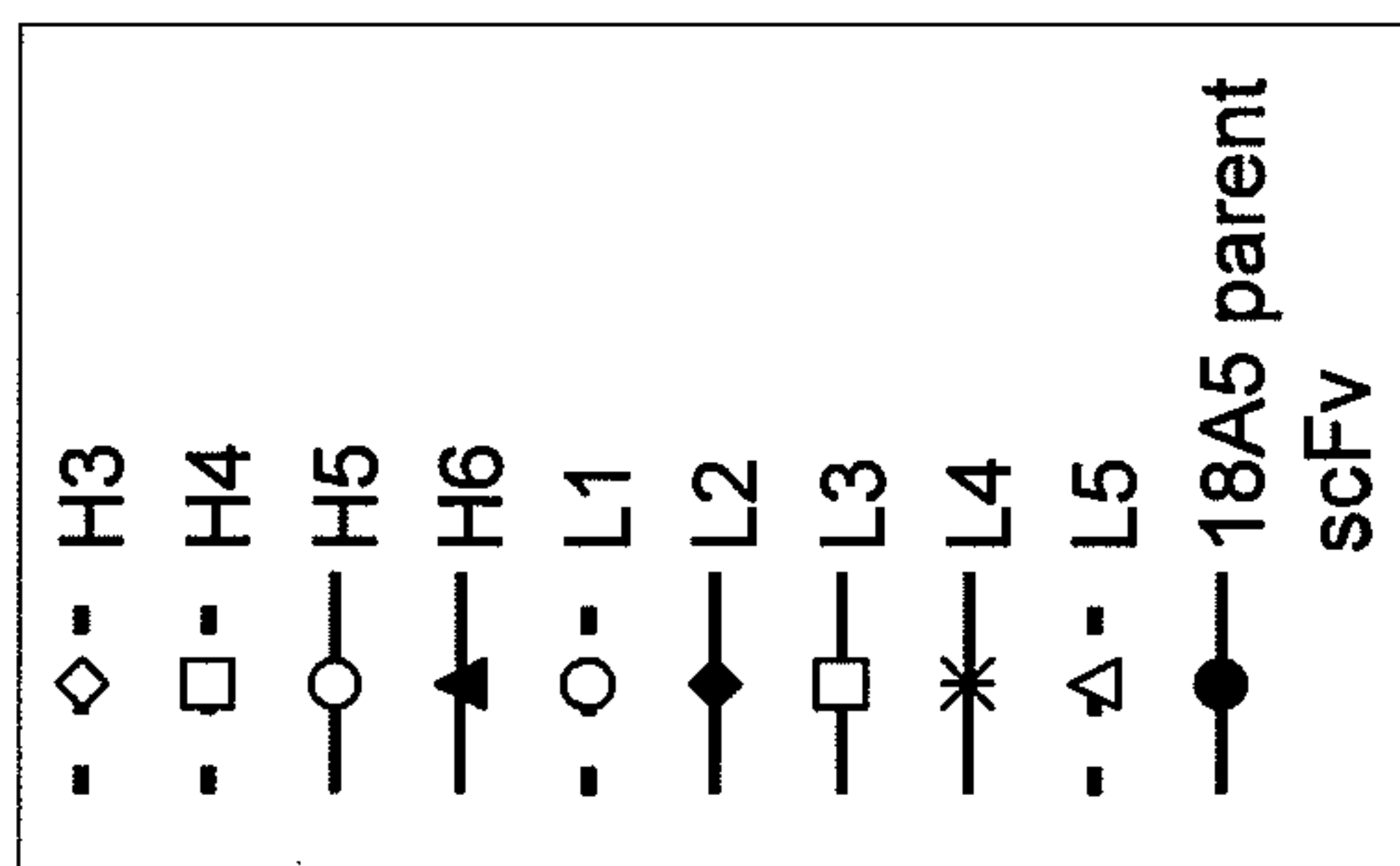
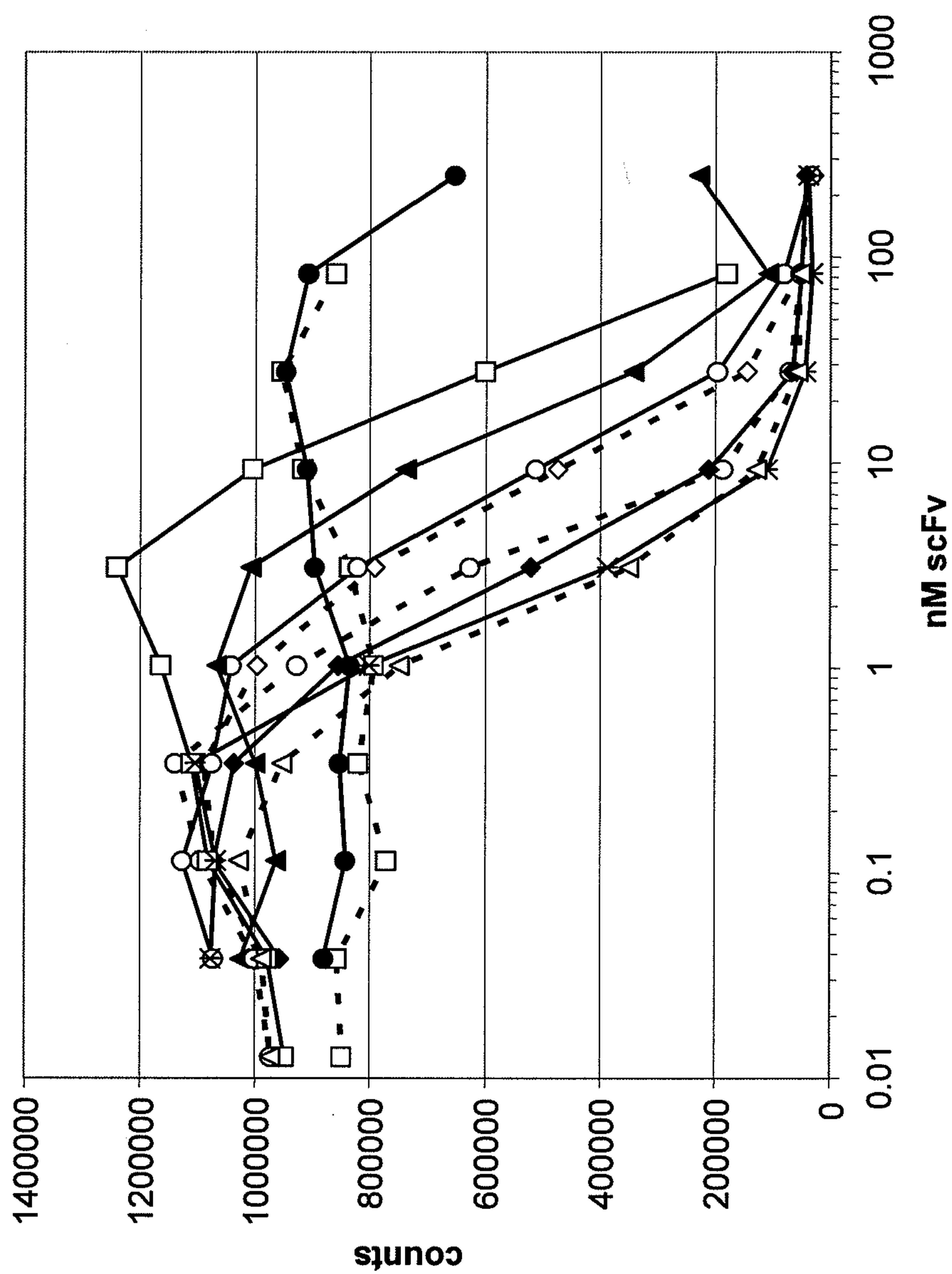
55. The use of claim 52, wherein the binding protein specifically binds to an IL-21R epitope that is recognized by AbO, AbP, AbQ, AbR, AbS, AbT, AbU, AbV, and/or AbW, and wherein the binding protein competitively inhibits the binding of AbO, AbP, AbQ, AbR, AbS, AbT, AbU, AbV, and/or AbW to human IL-21R.

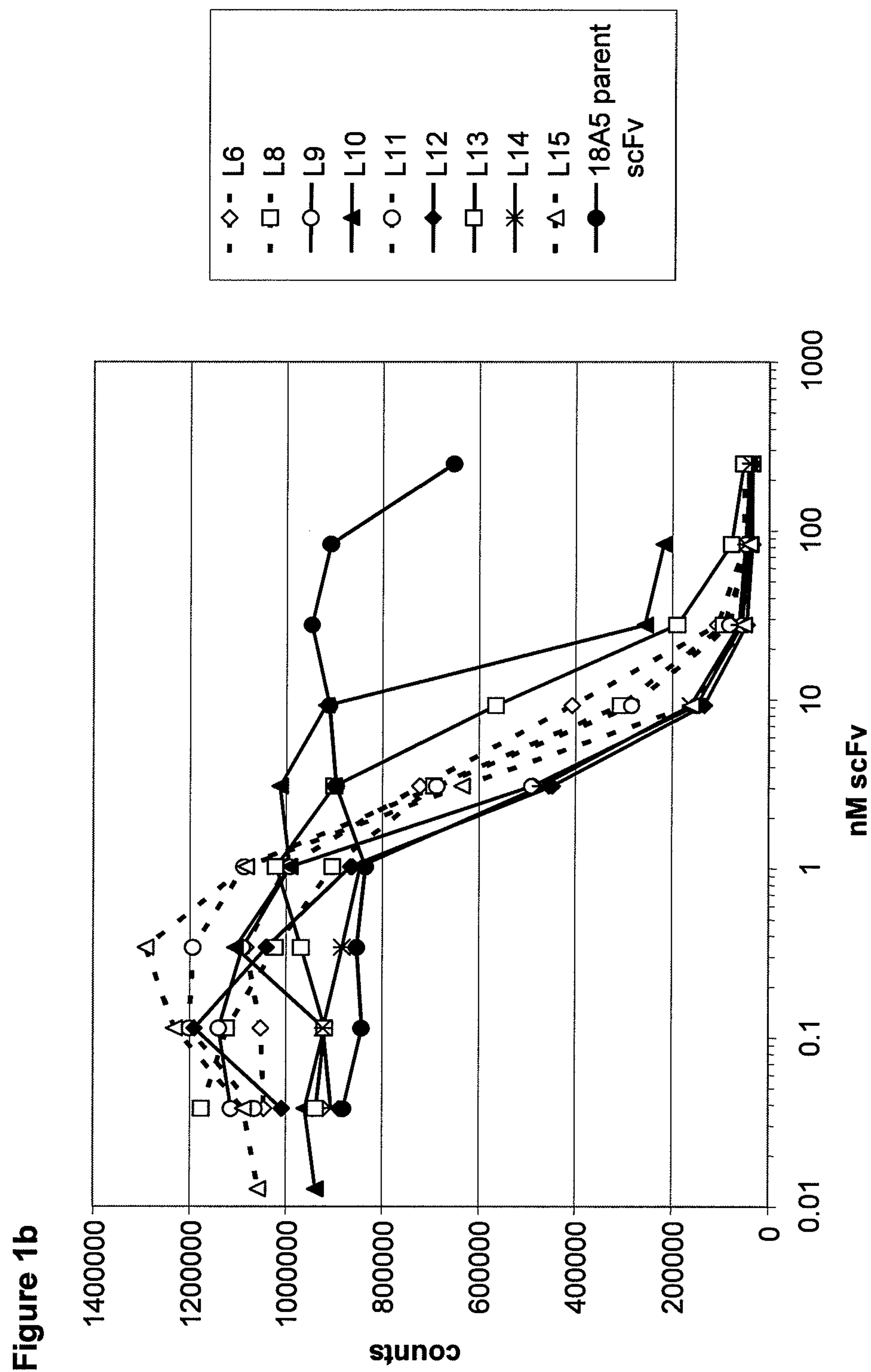
56. The use of any one of claims 36, 44, 47, 48, and 52, wherein the IL-21R-associated disorder is selected from the group consisting of autoimmune disorders, inflammatory conditions, allergies, transplant rejections, and hyperproliferative disorders of the blood.

57. The use of claim 56, wherein the IL-21R-associated disorder is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus, psoriasis, transplant rejection, rheumatoid arthritis, and other arthritic disorders.

58. The use of any one of claims 36, 44, 47, 48, and 52, wherein the binding protein or antigen-binding fragment thereof has an association constant for human IL-21R of at least $10^5 \text{ M}^{-1} \text{ s}^{-1}$.
59. The use of any one of claims 36, 44, 47, 48, and 52, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated BAF3 cell proliferation with an IC_{50} of about 1.75 nM or less, and wherein the BAF3 cells comprise a human IL-21 receptor.
60. The use of any one of claims 36, 44, 47, 48, and 52, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of TF1 cells with an IC_{50} of about 14 nM or less, and wherein the TF1 cells comprise a human IL-21 receptor.
61. The use of any one of claims 36, 44, 47, 48, and 52, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of primary human B cells with an IC_{50} of about 1.9 nM or less, and wherein the B cells comprise a human IL-21R.
62. The use of any one of claims 36, 44, 47, 48, and 52, wherein the binding protein or antigen-binding fragment thereof inhibits IL-21-mediated proliferation of primary human CD4^+ cells with an IC_{50} of about 1.5 nM or less, and wherein the CD4^+ cells comprise a human IL-21R.

Figure 1a





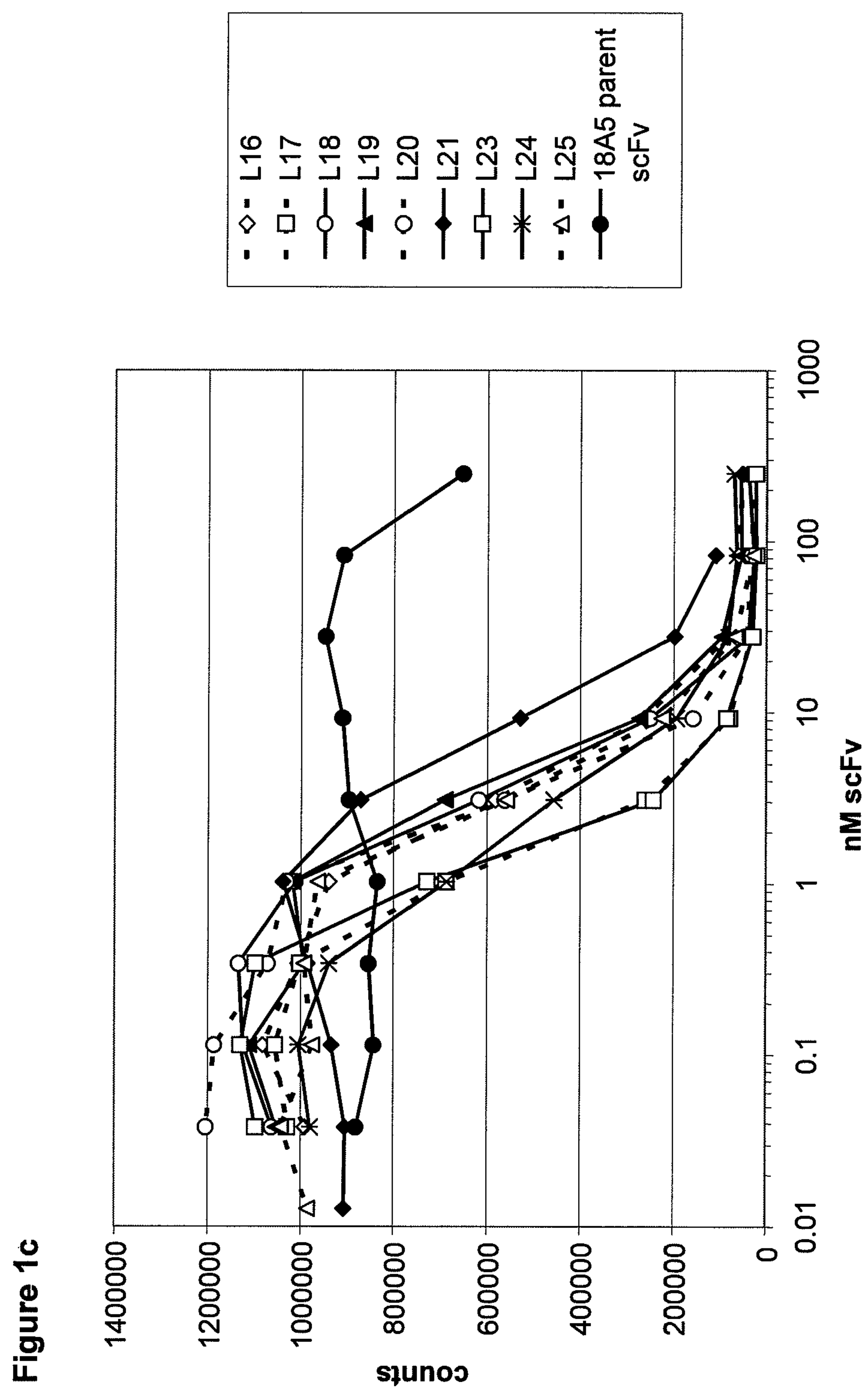


Figure 2a

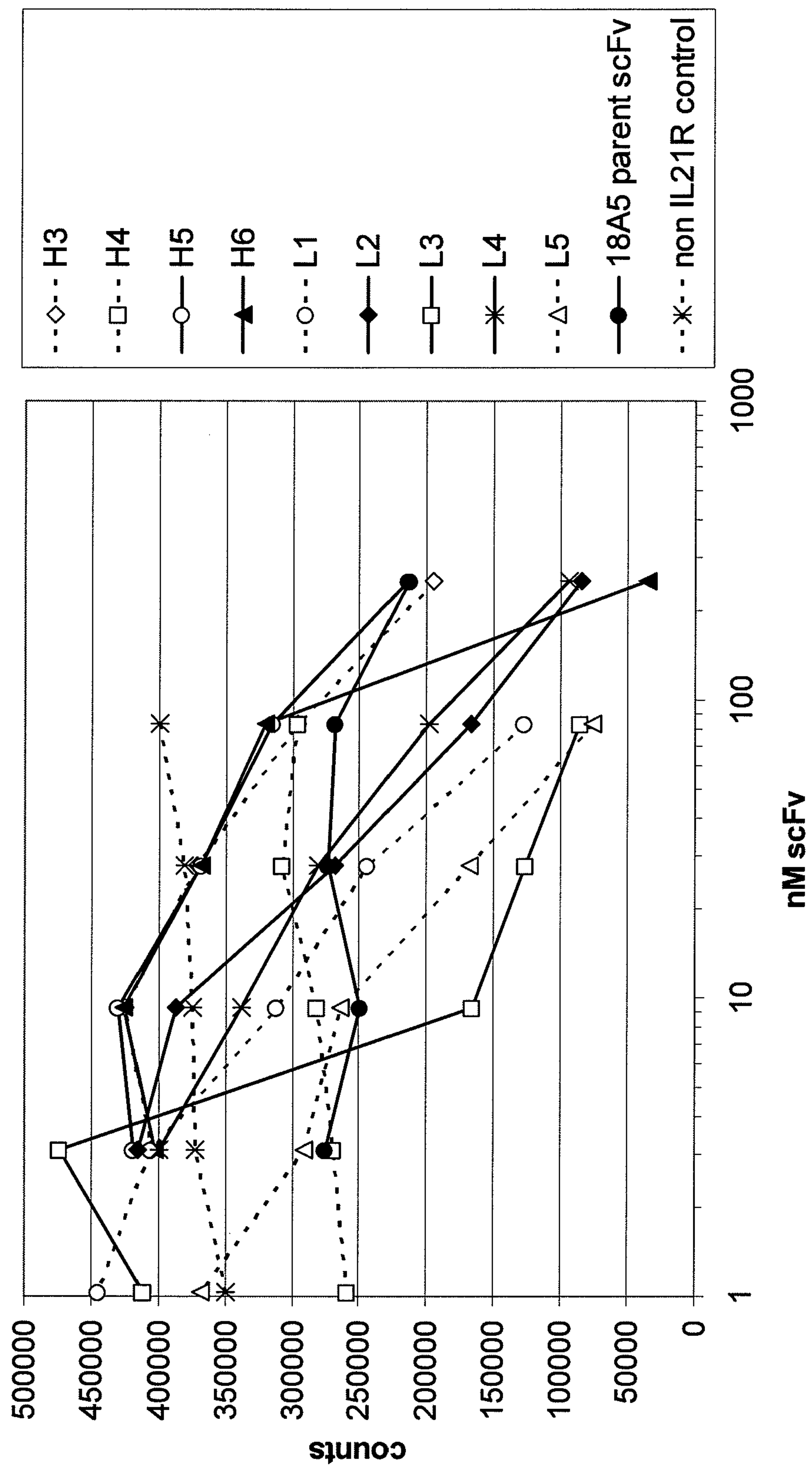


Figure 2b

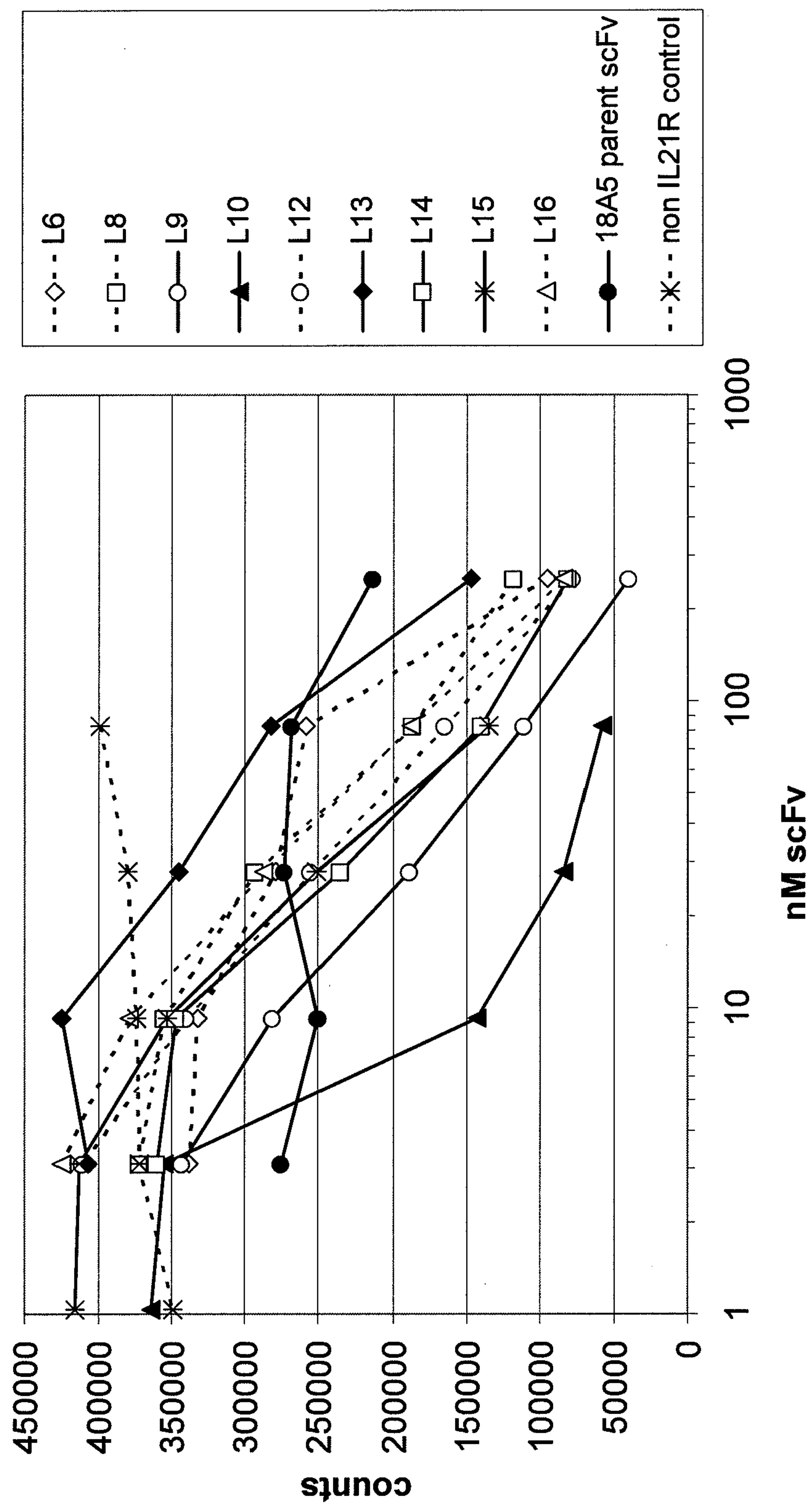


Figure 2c

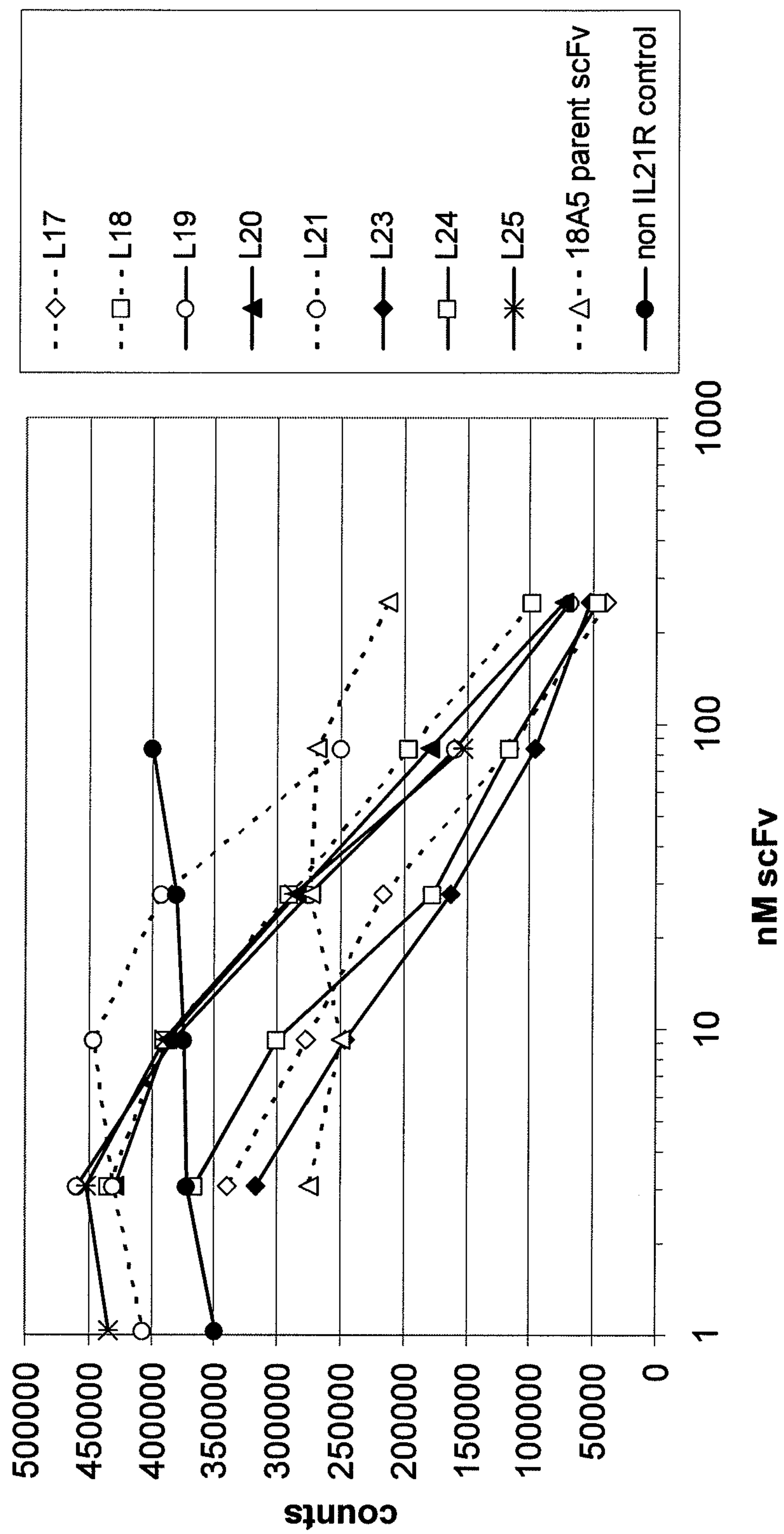


Figure 3a

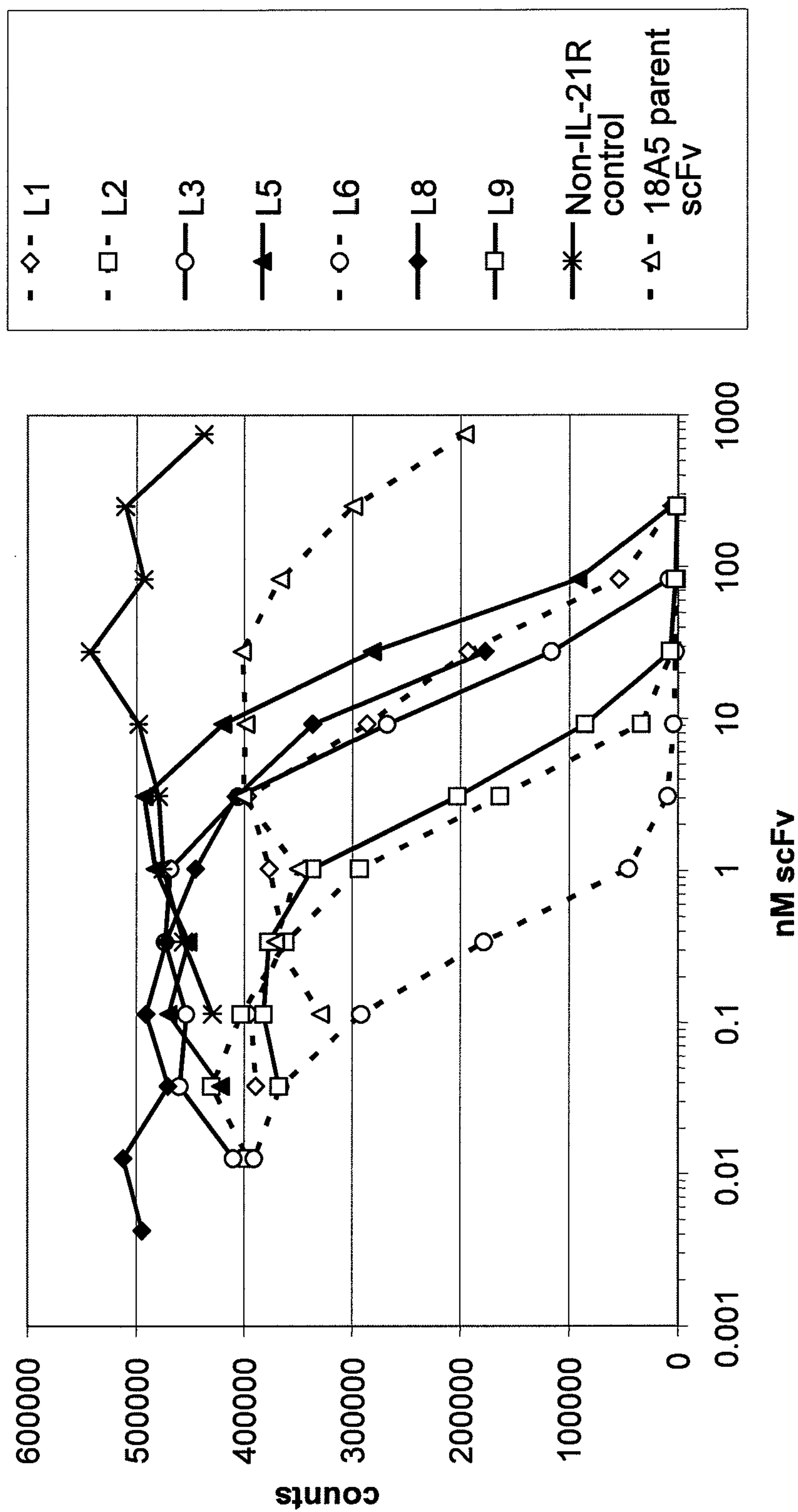


Figure 3b

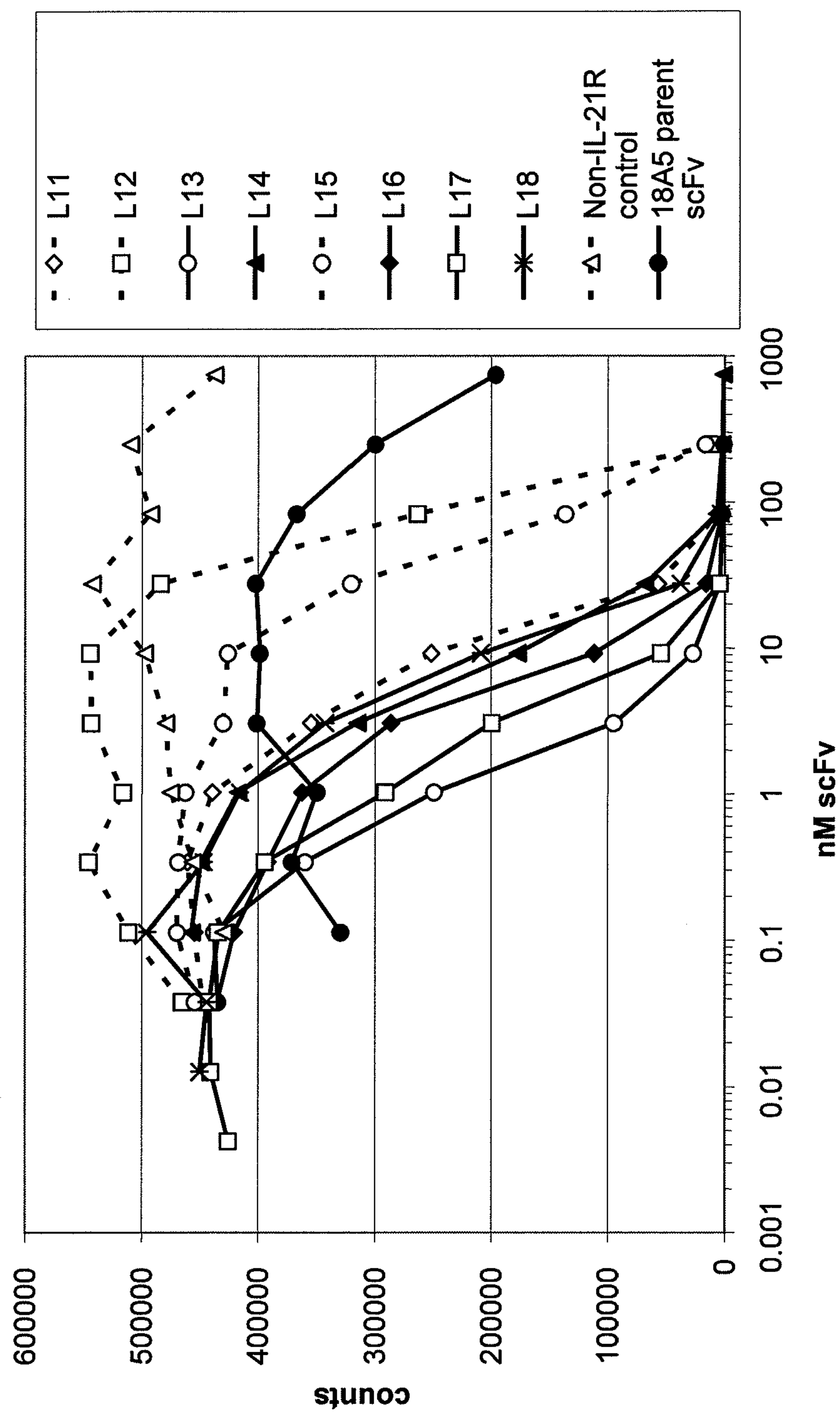
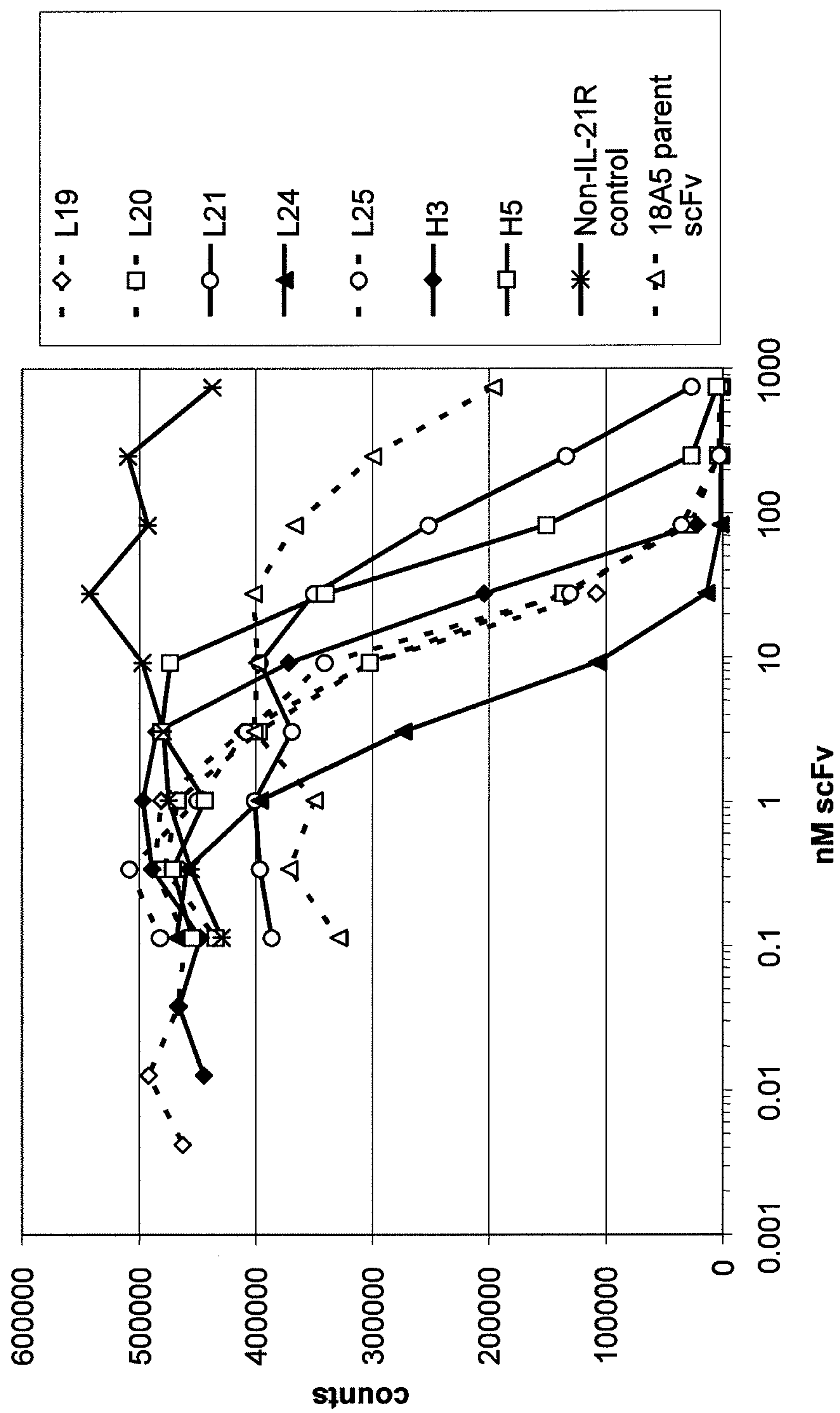


Figure 3c



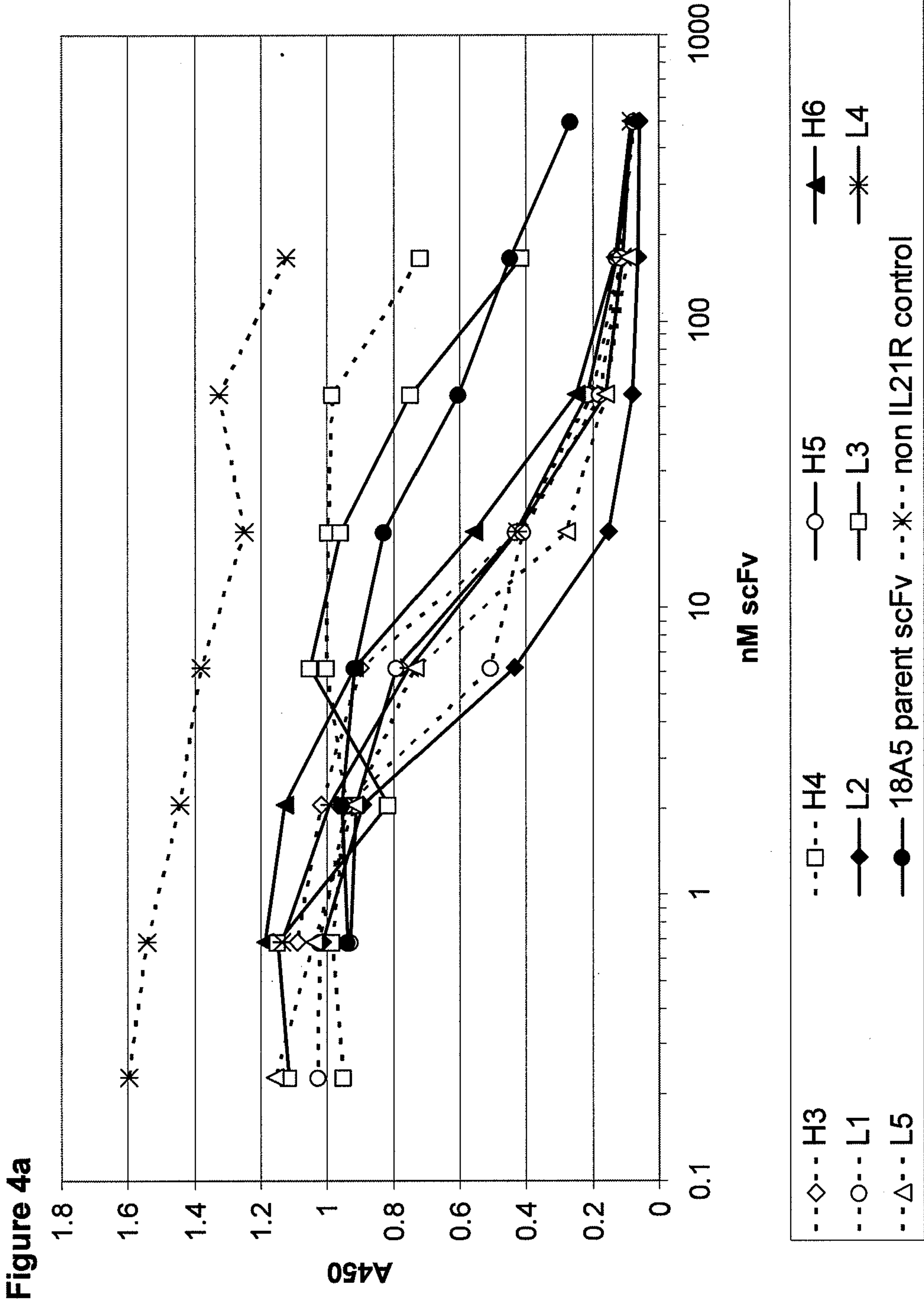


Figure 4b

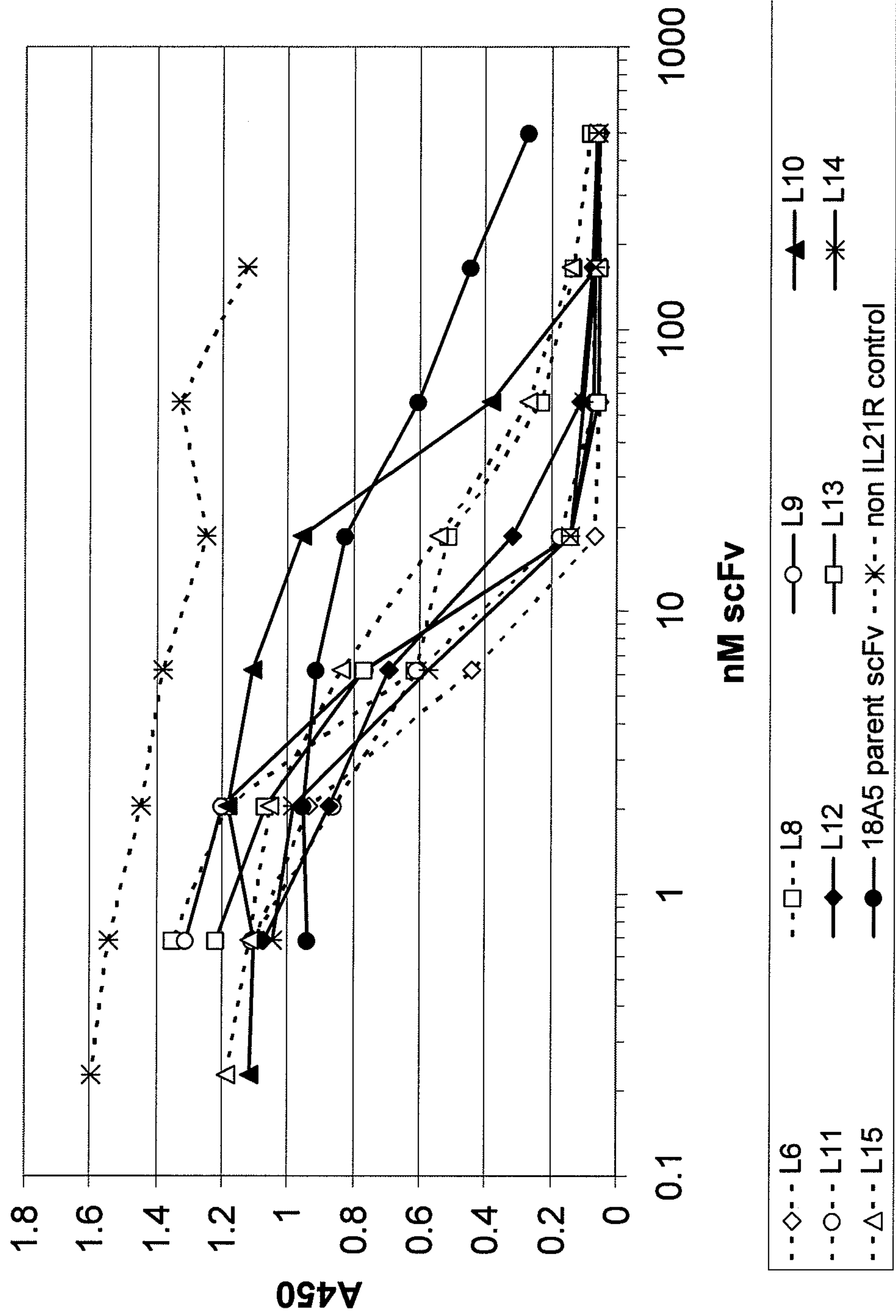
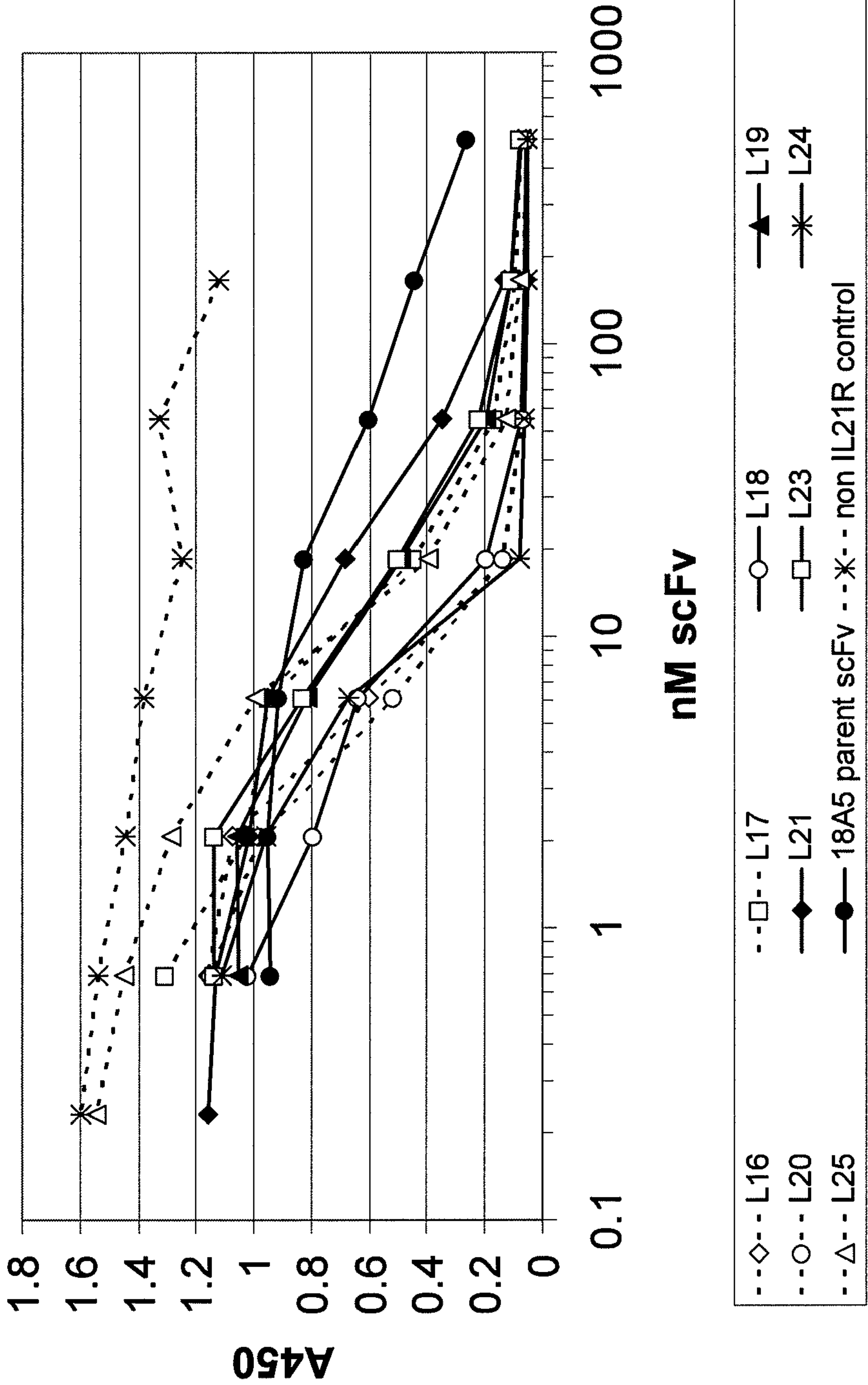


Figure 4c



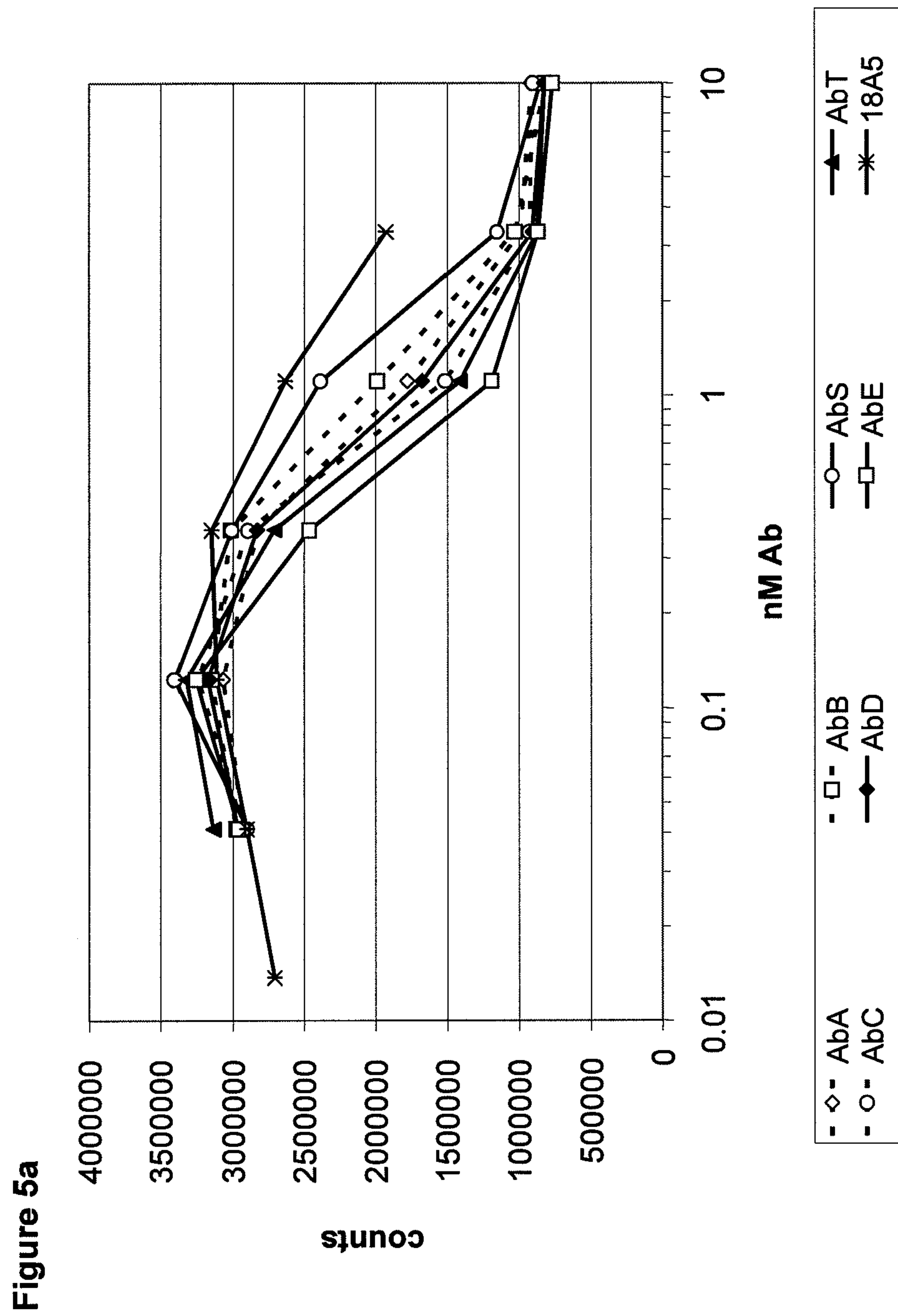
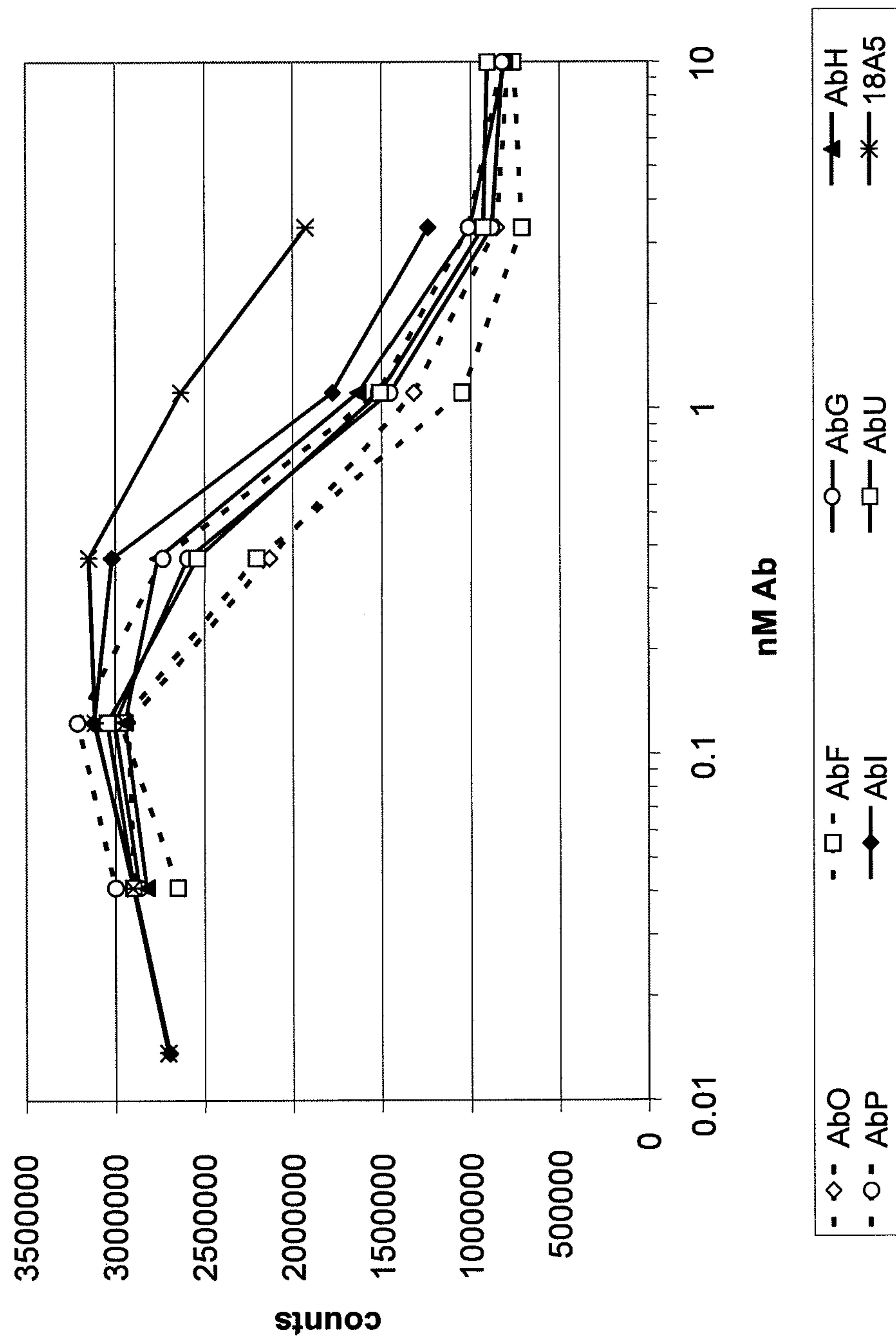
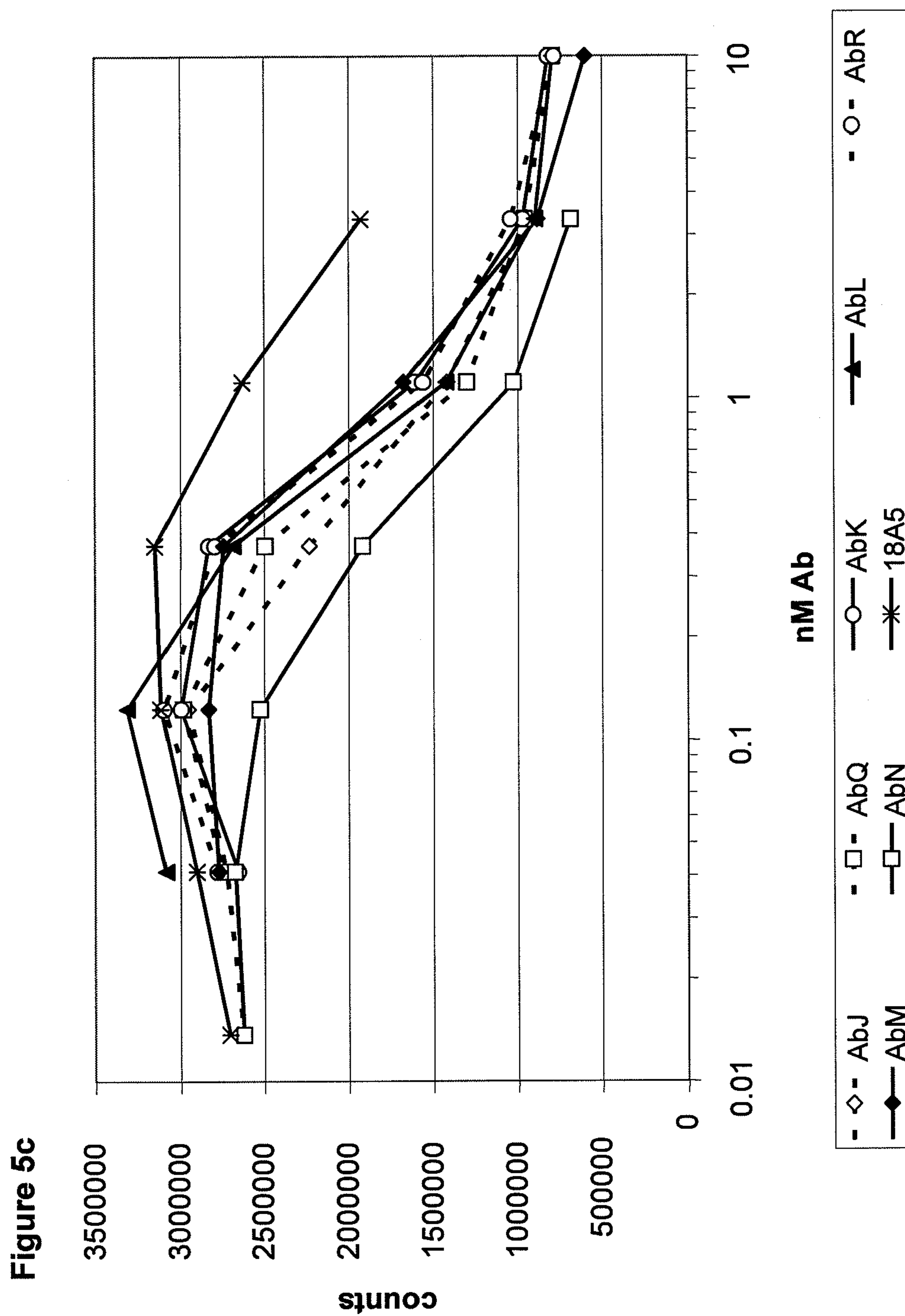
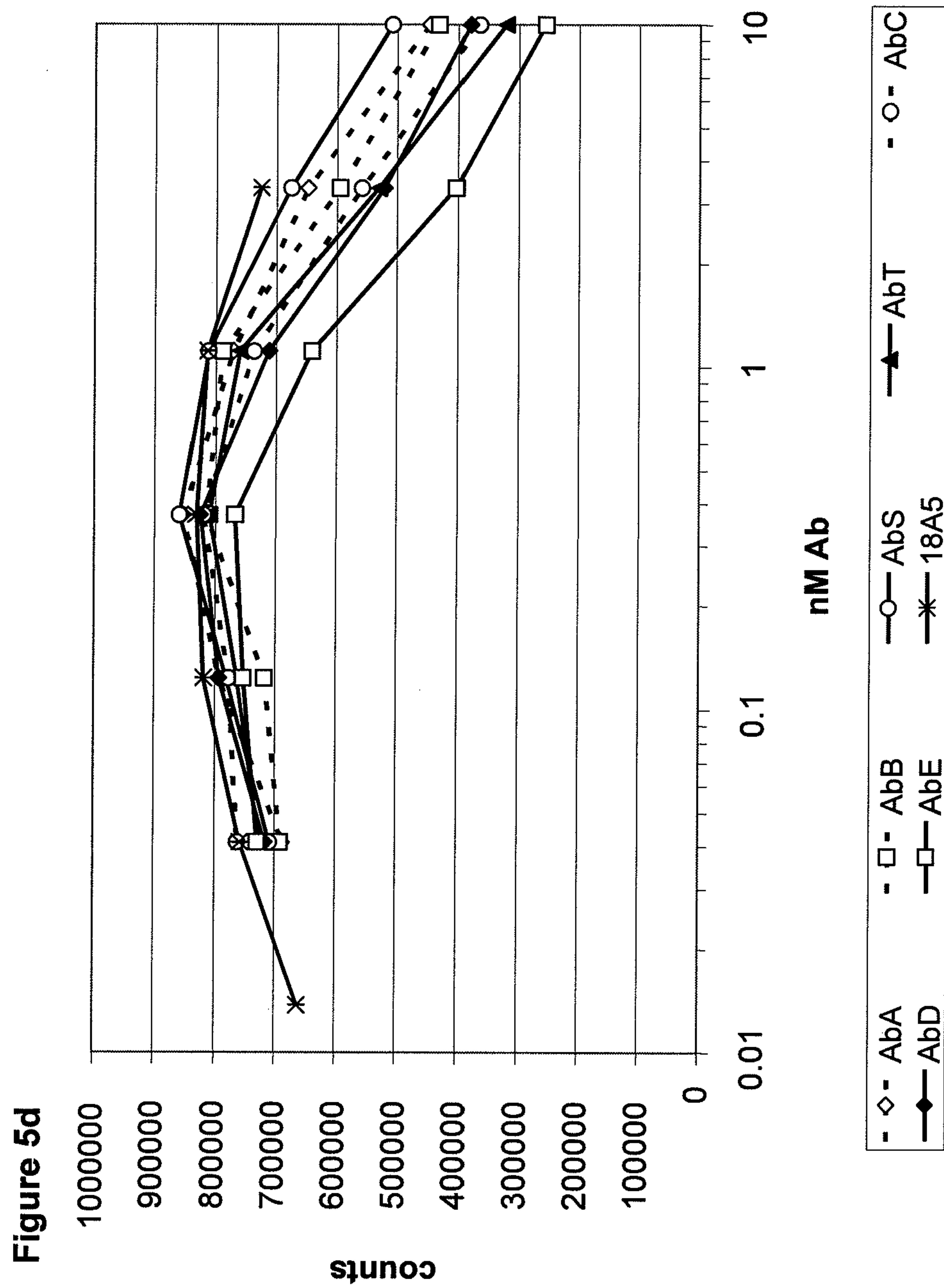


Figure 5b







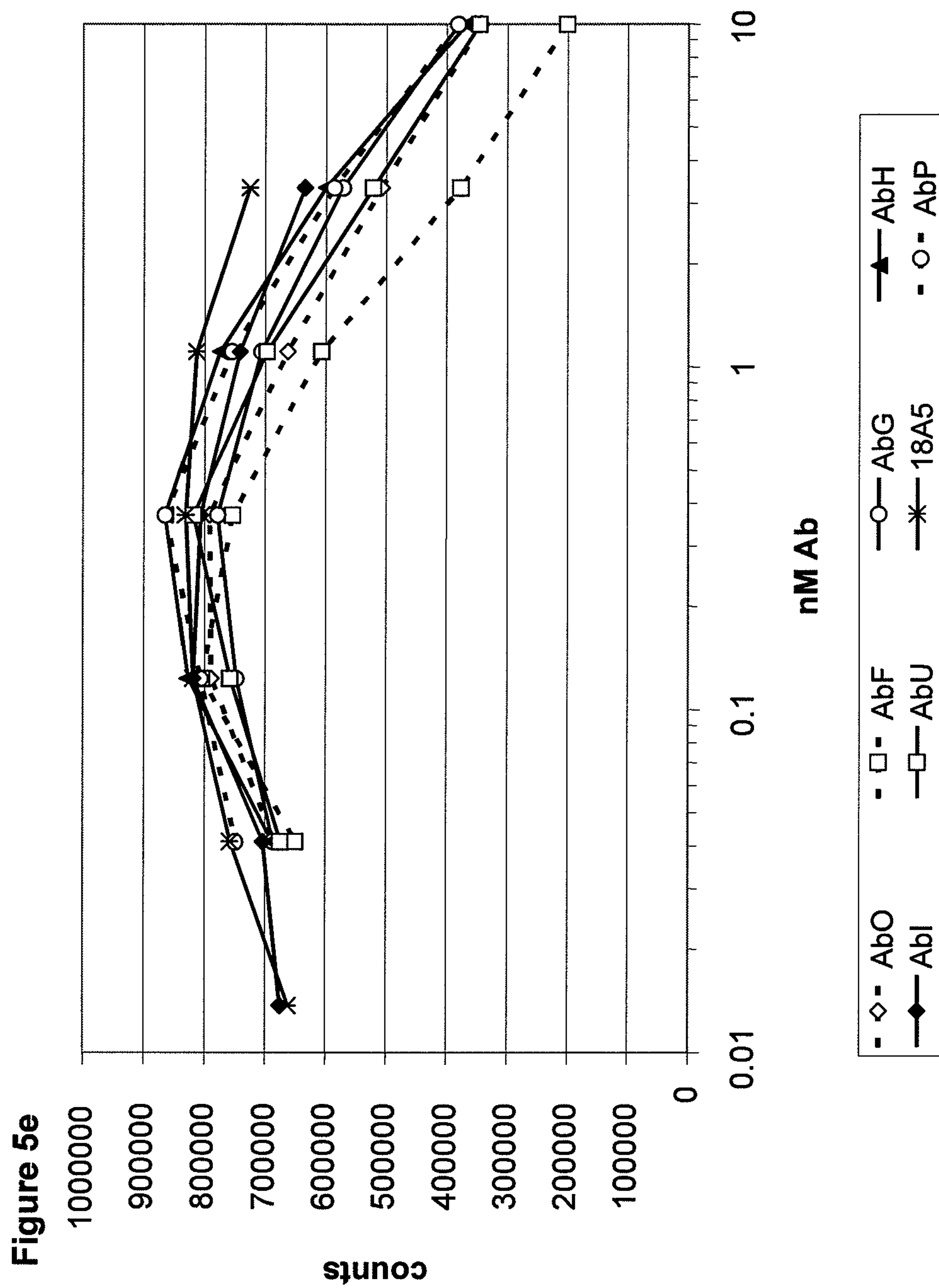
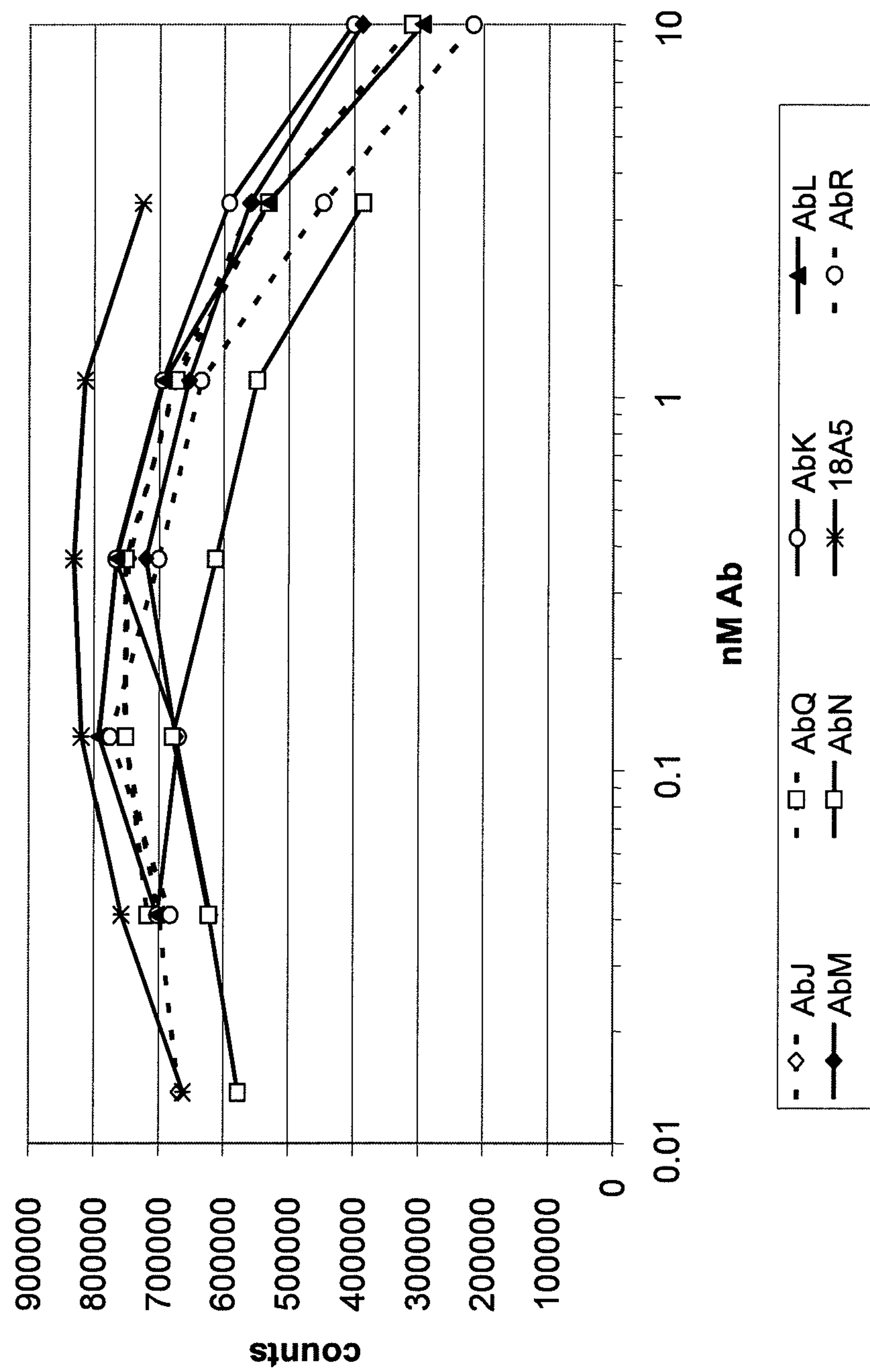
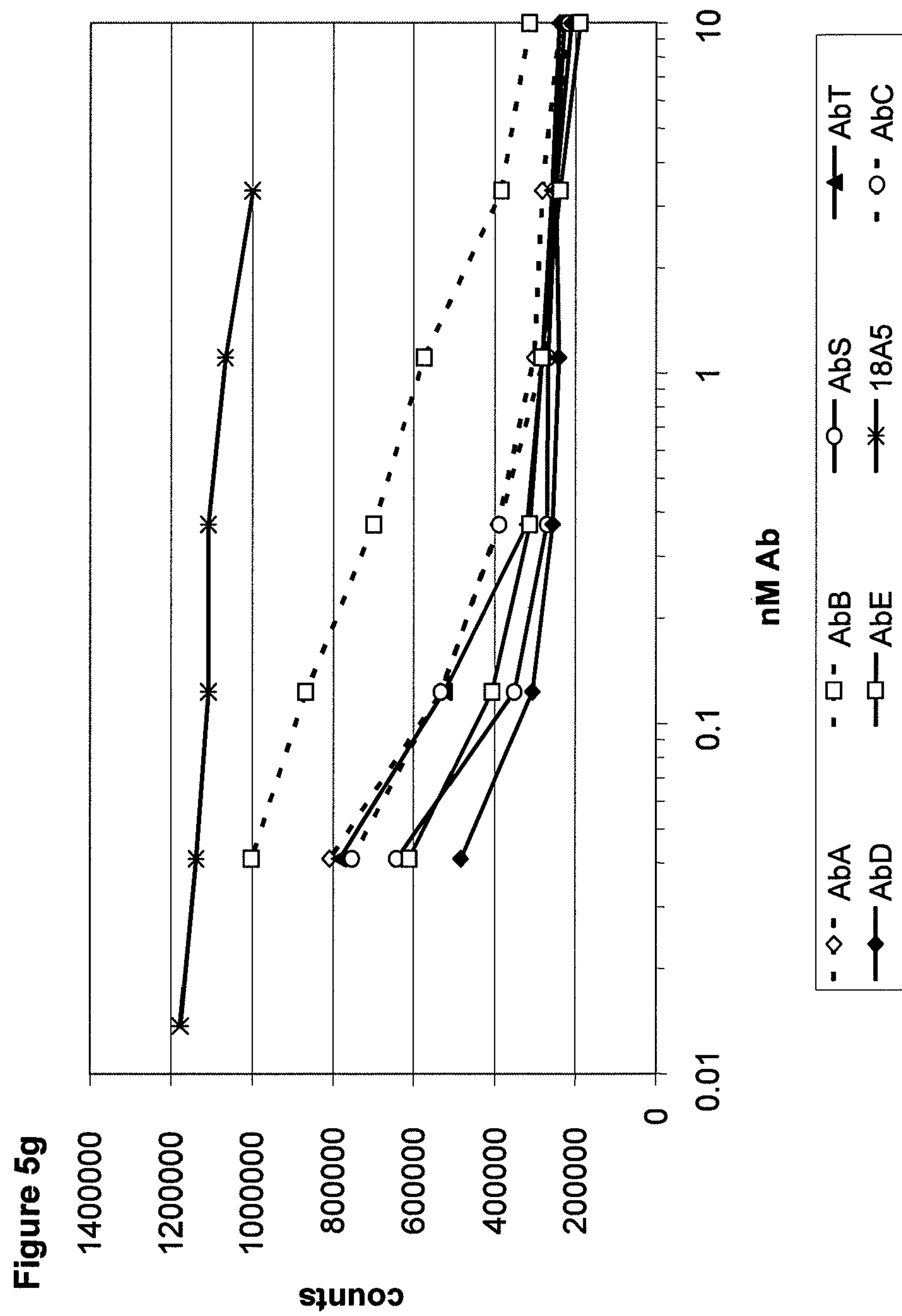
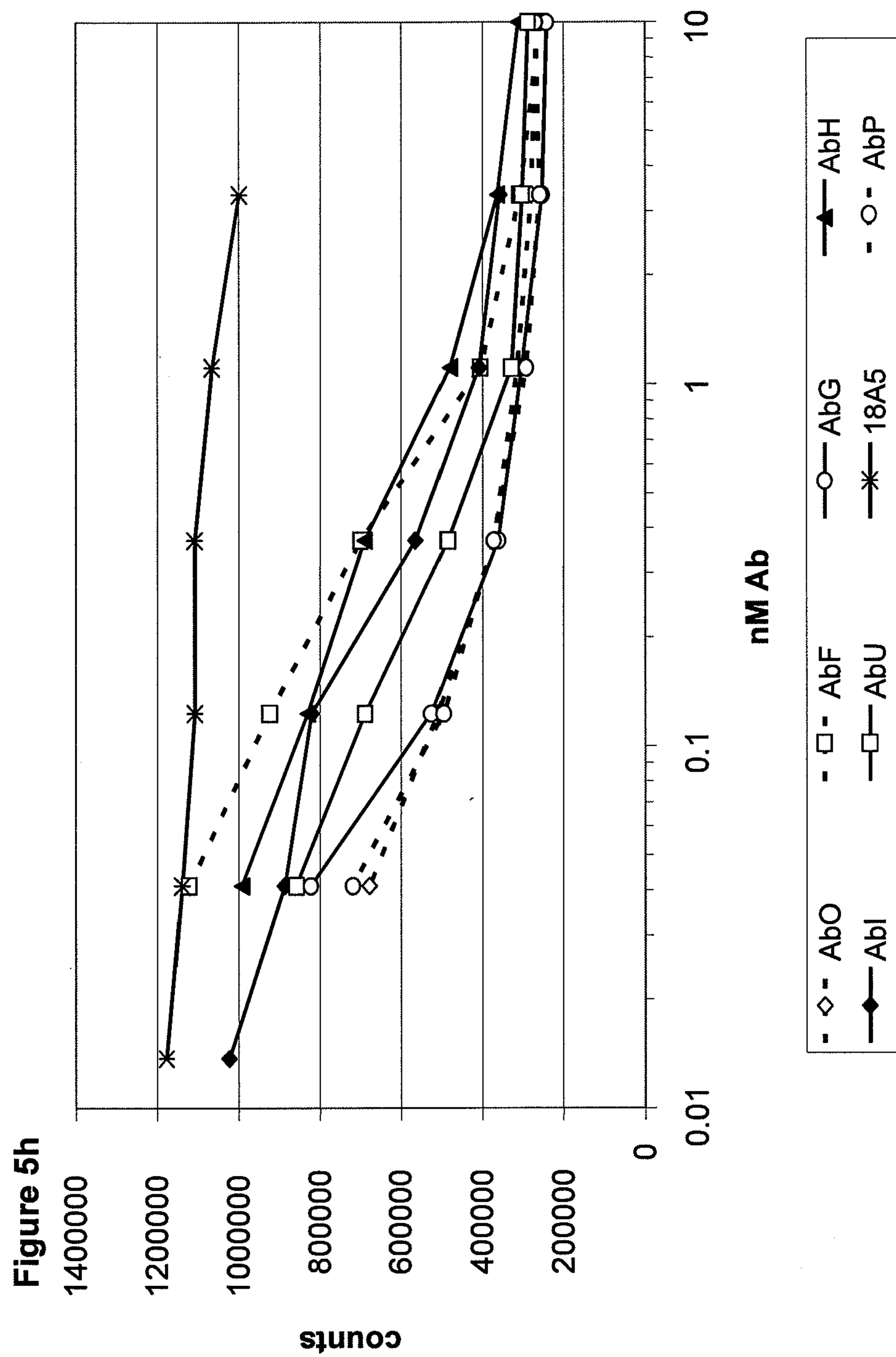


Figure 5f







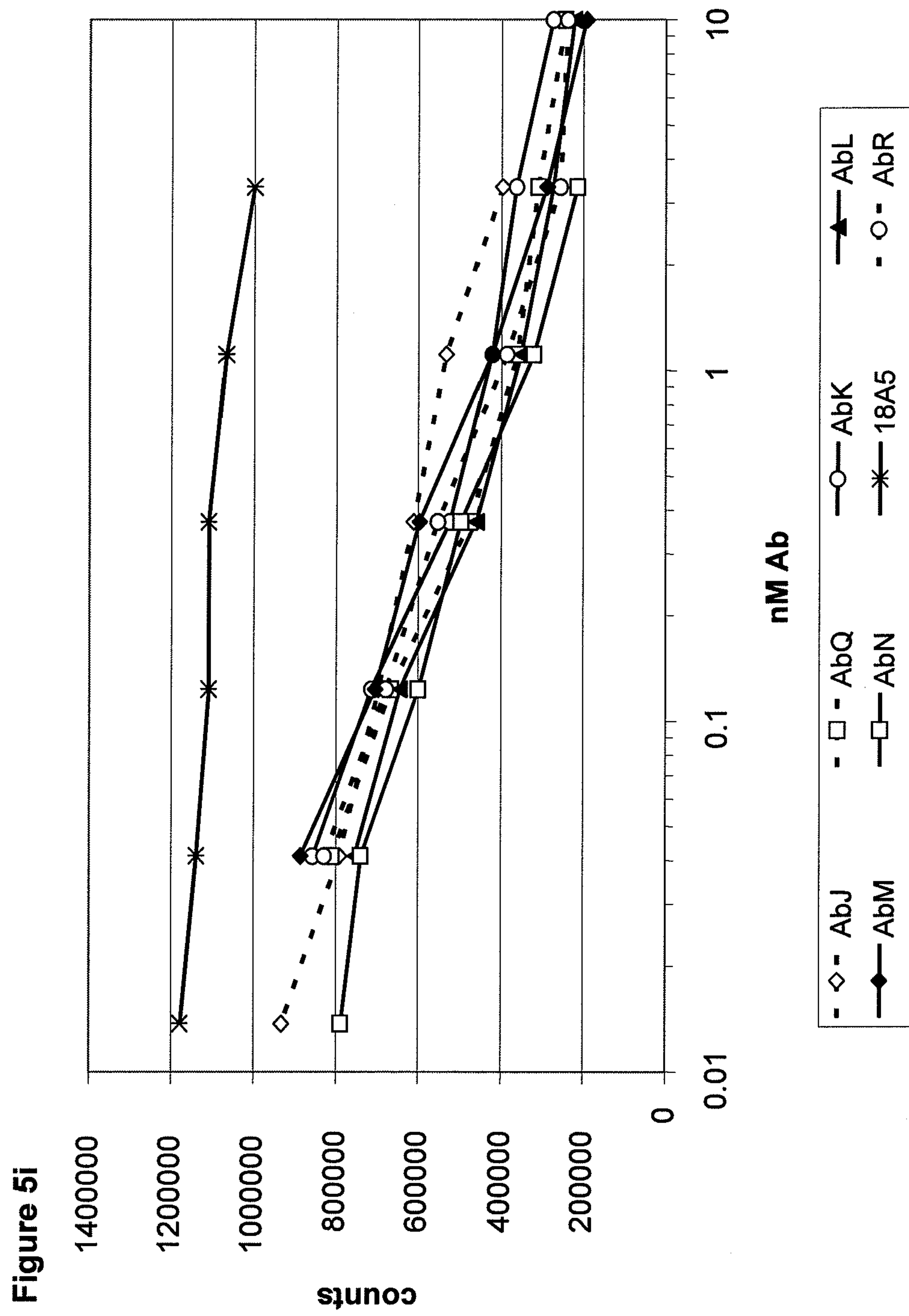
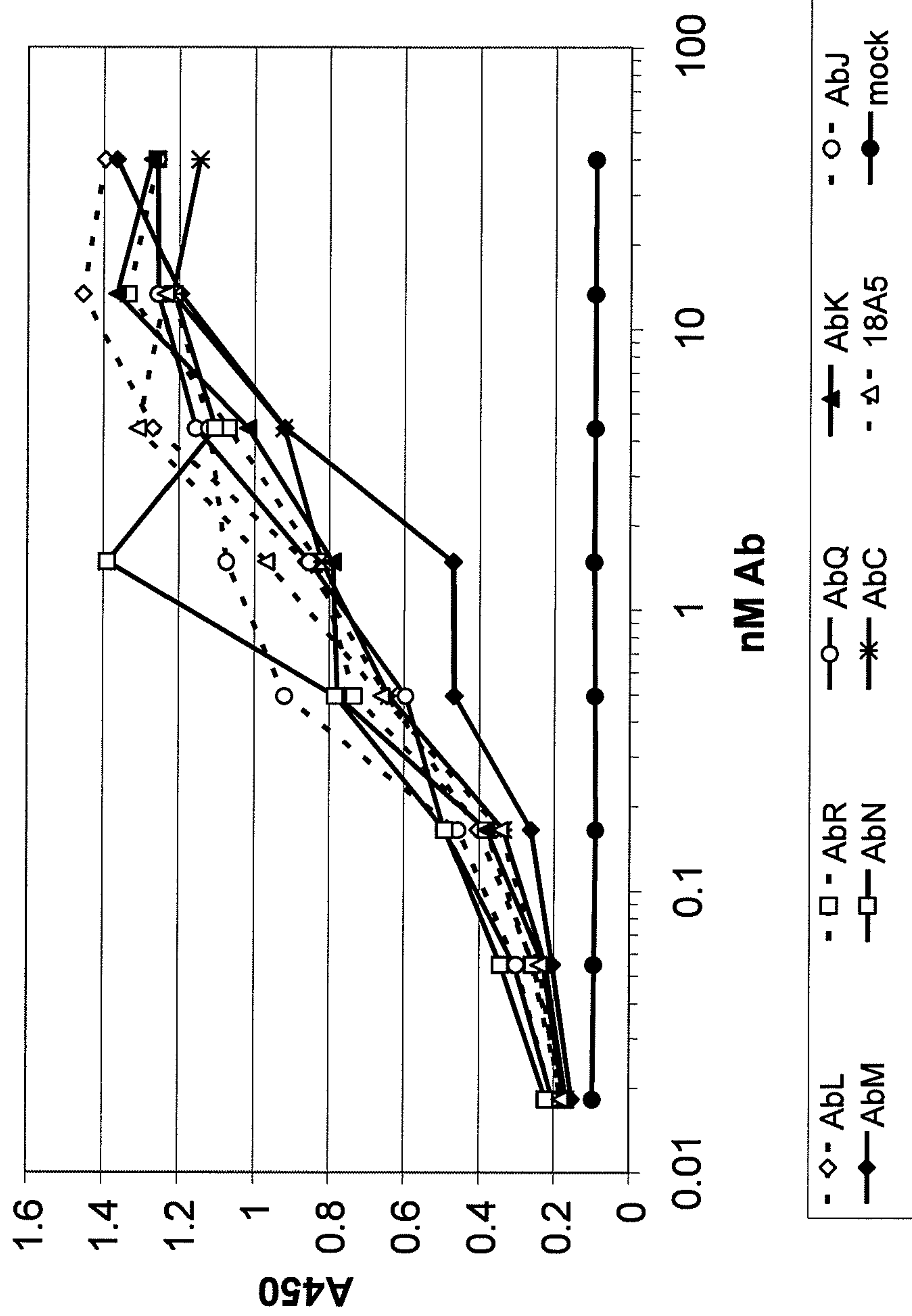


Figure 6a



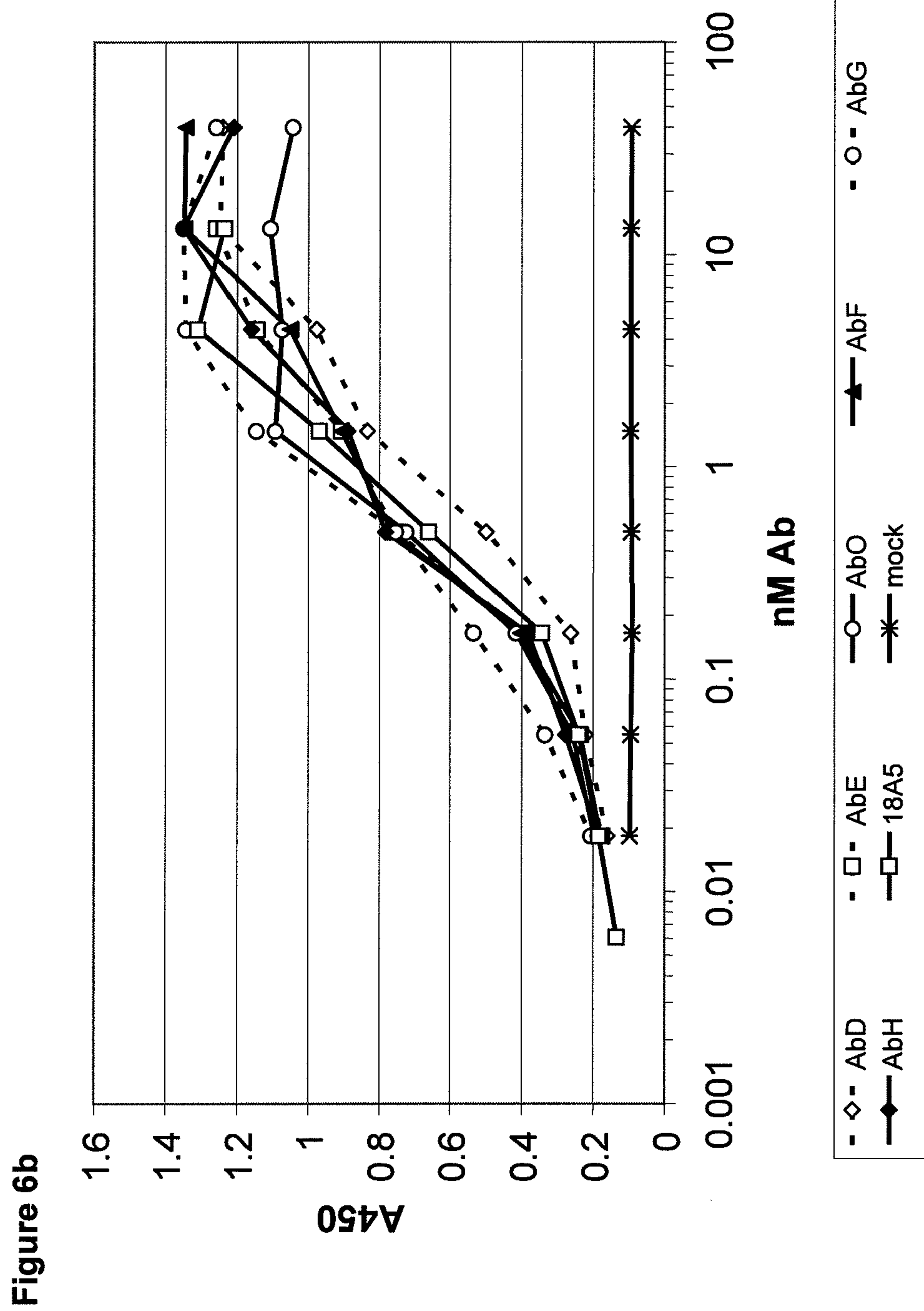


Figure 6c

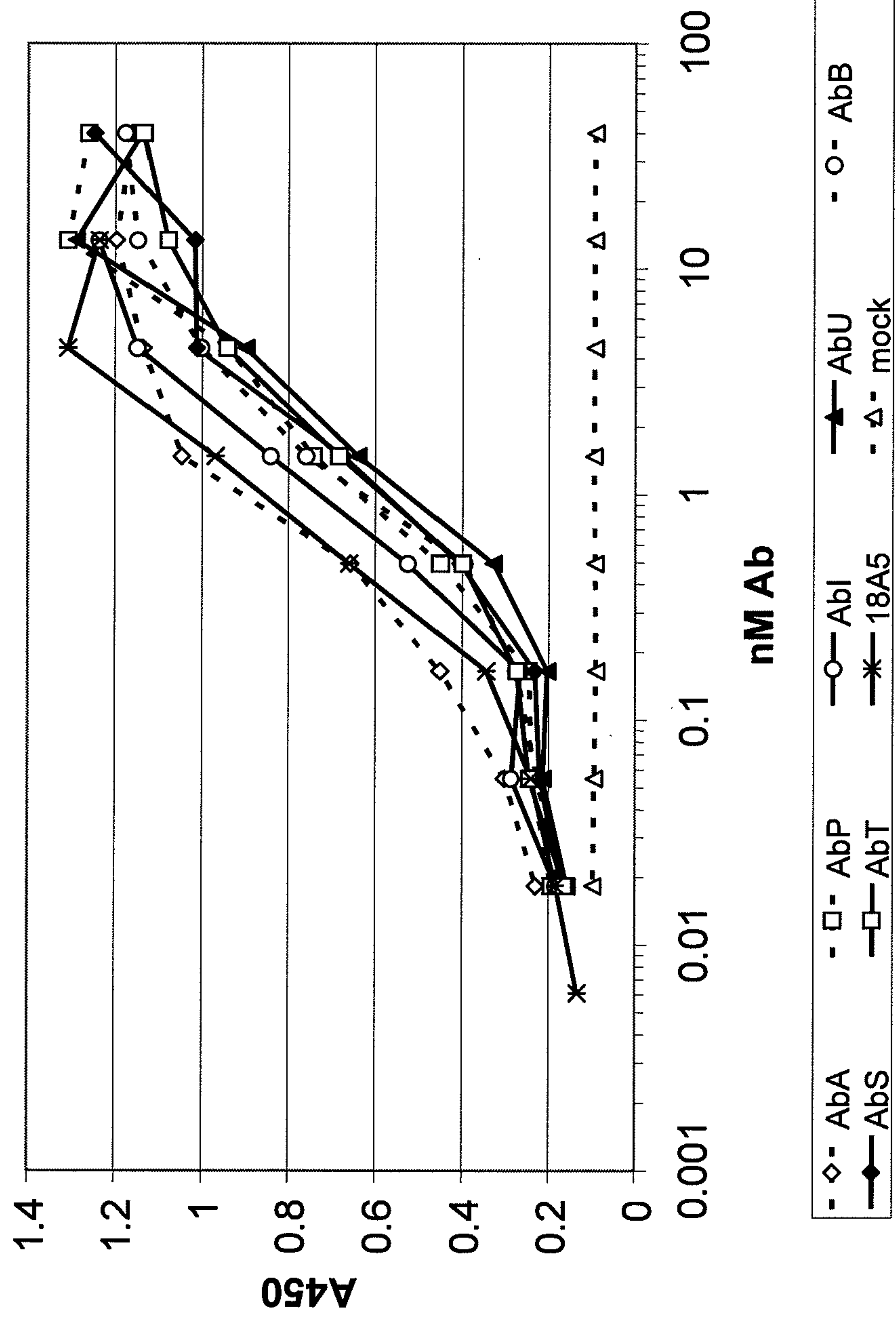


Figure 6d

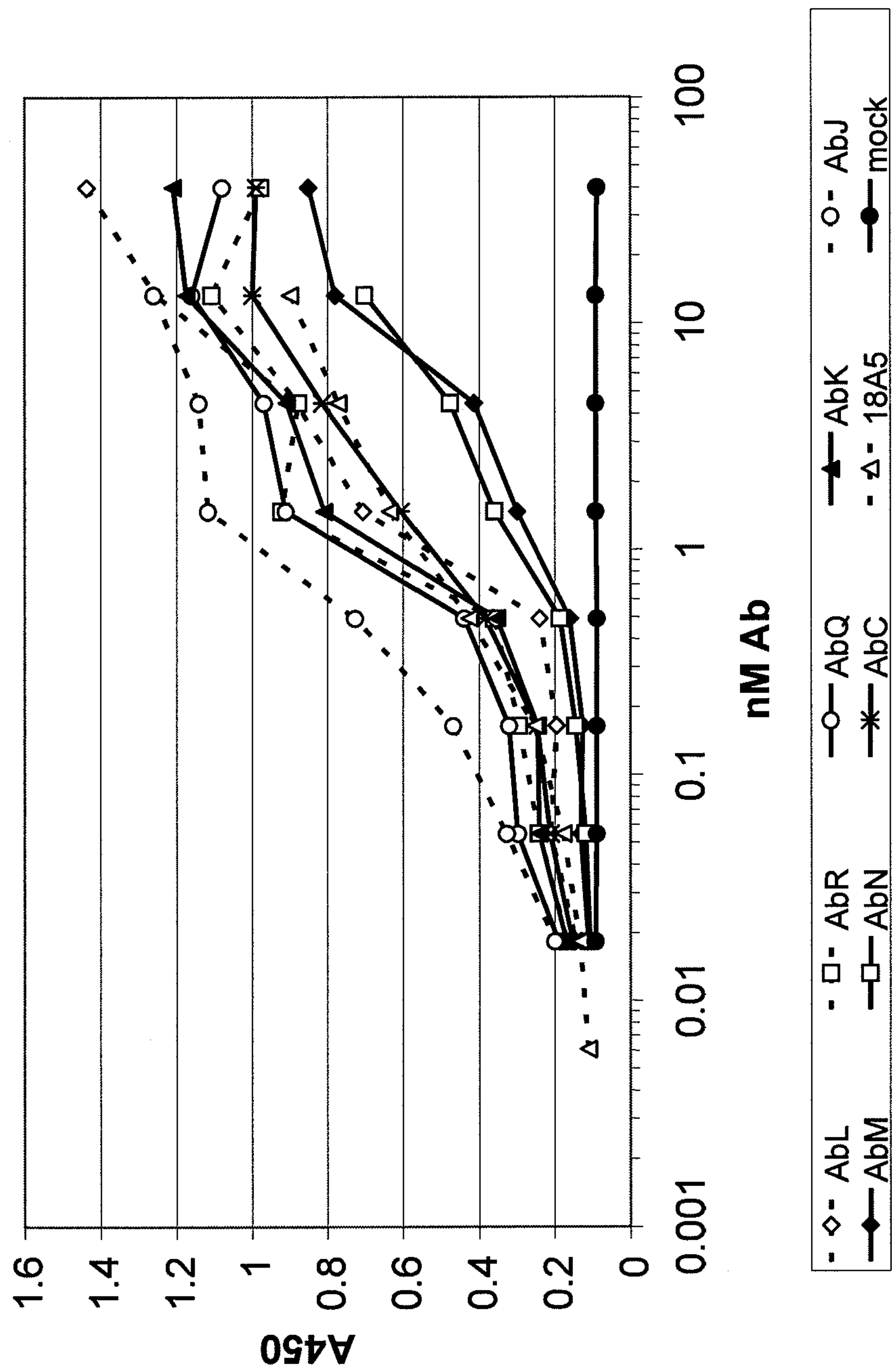


Figure 6e

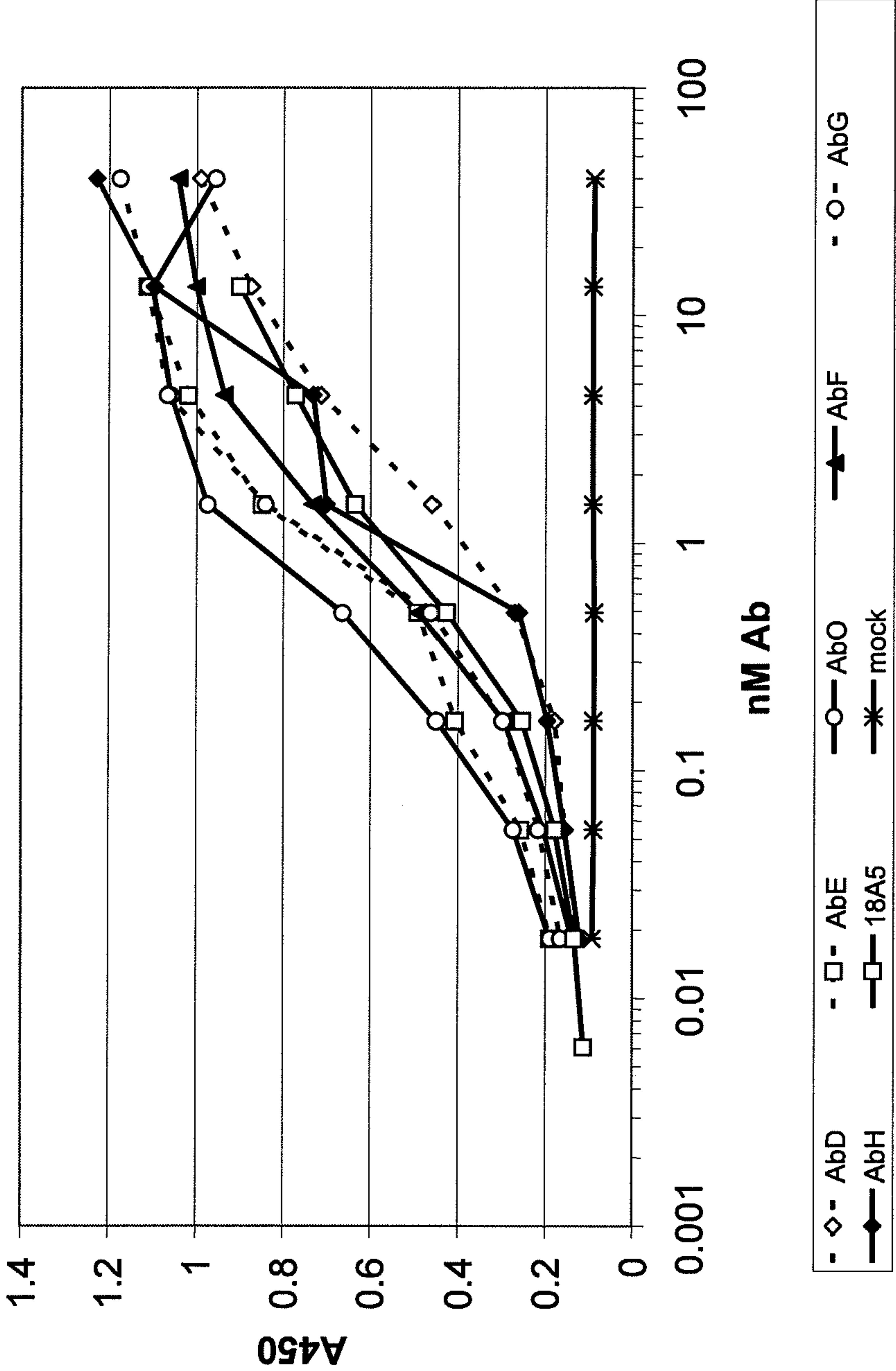


Figure 6f

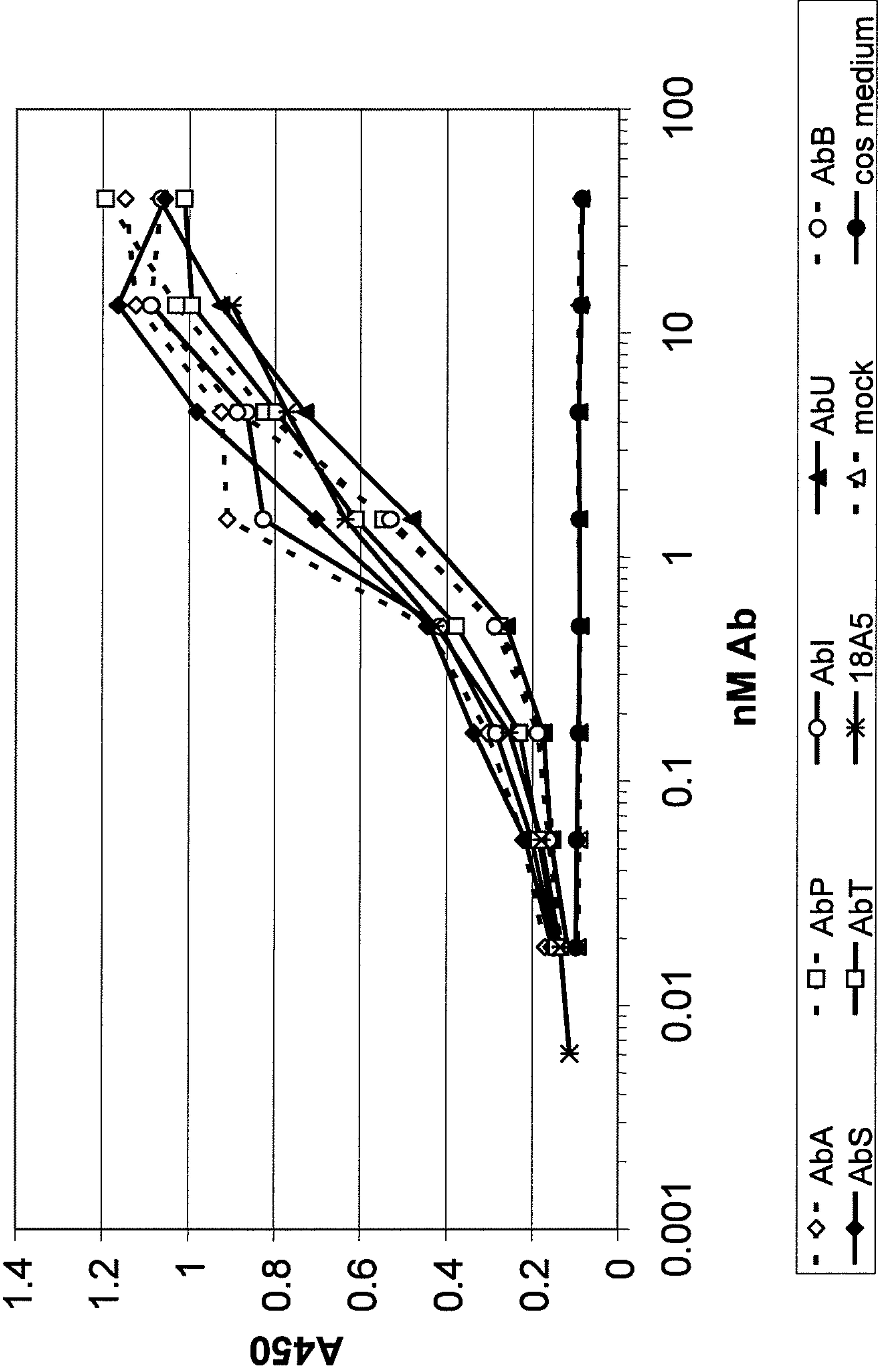


Figure 6g

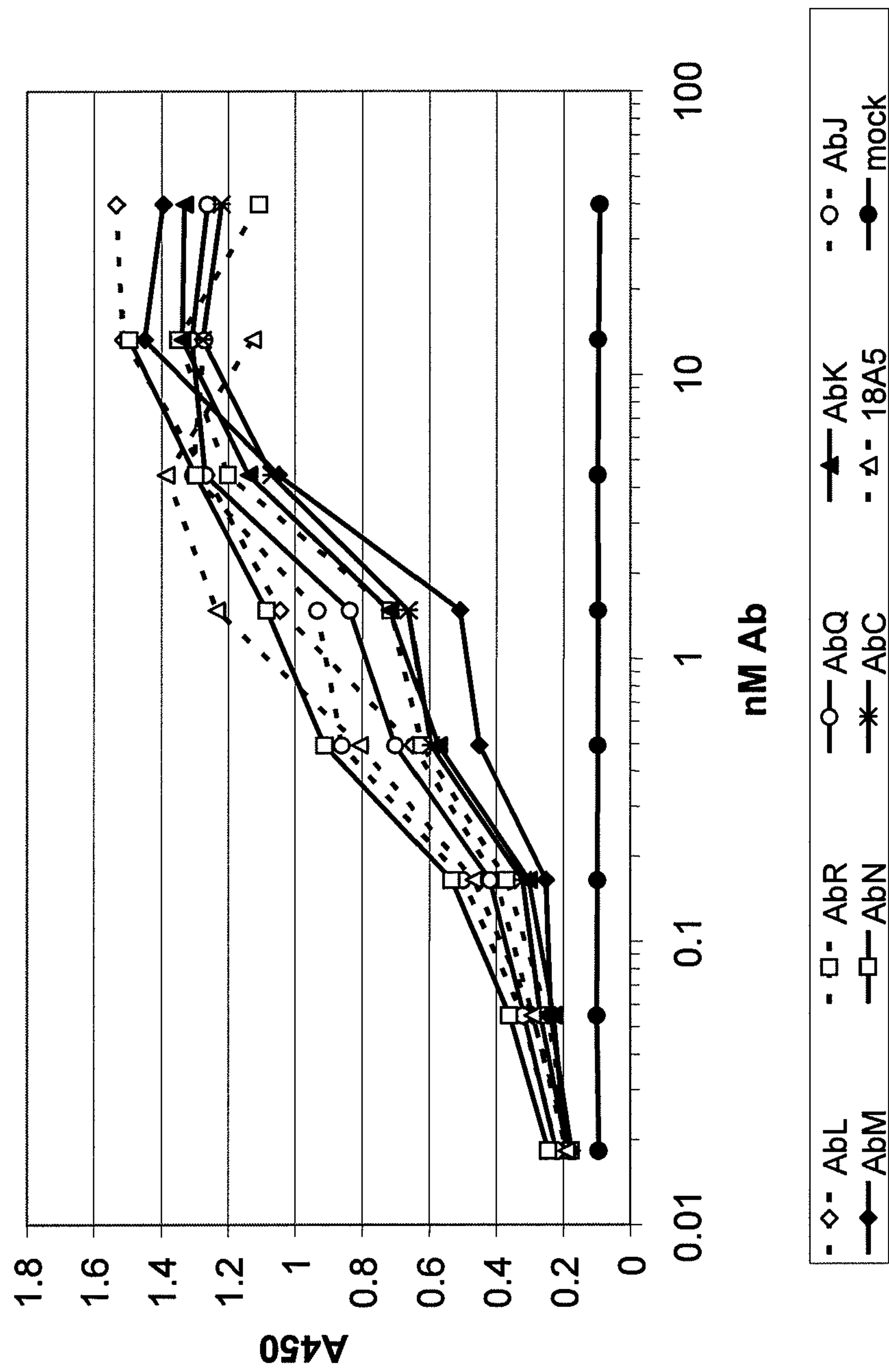


Figure 6h

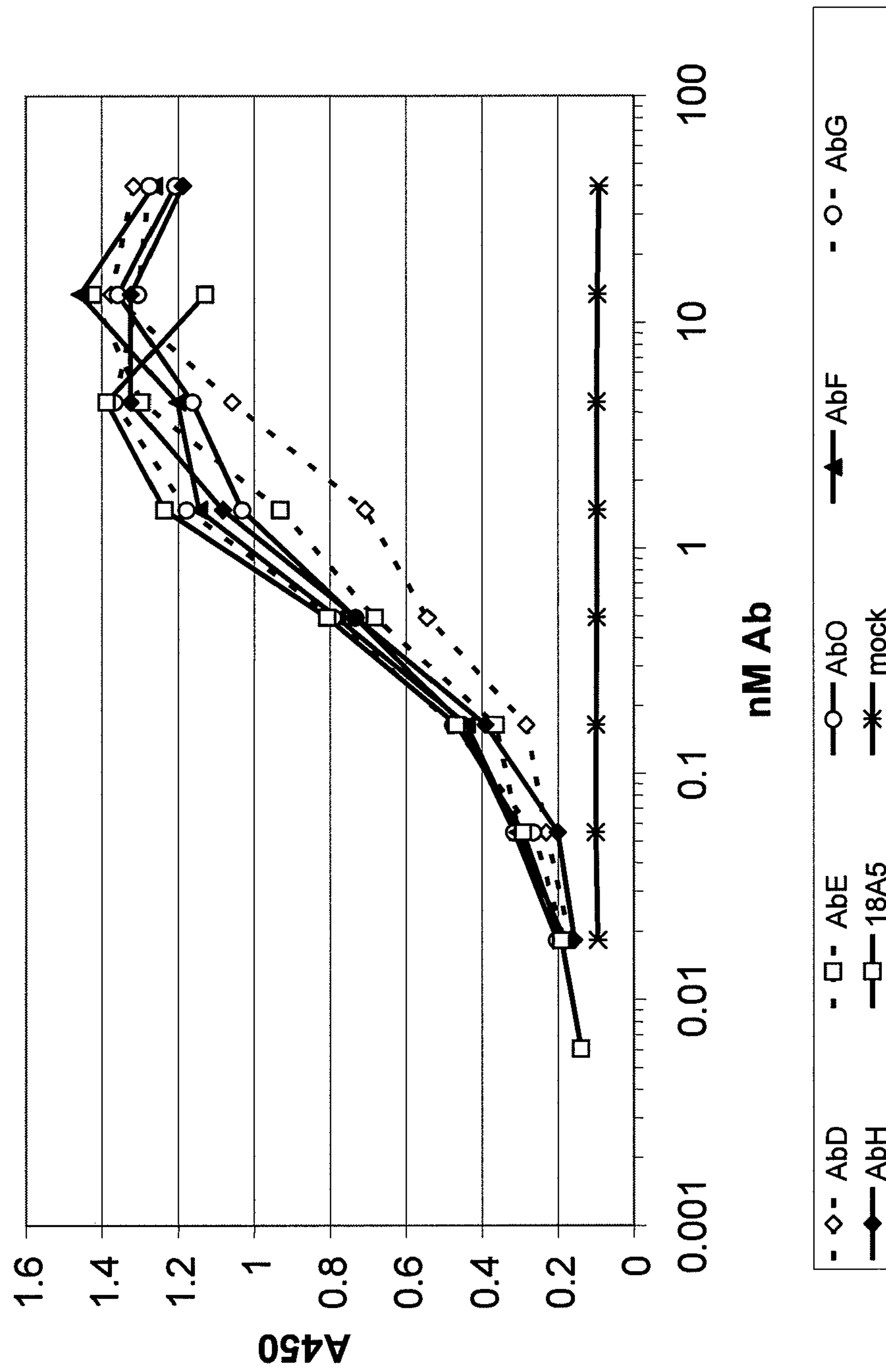


Figure 6i

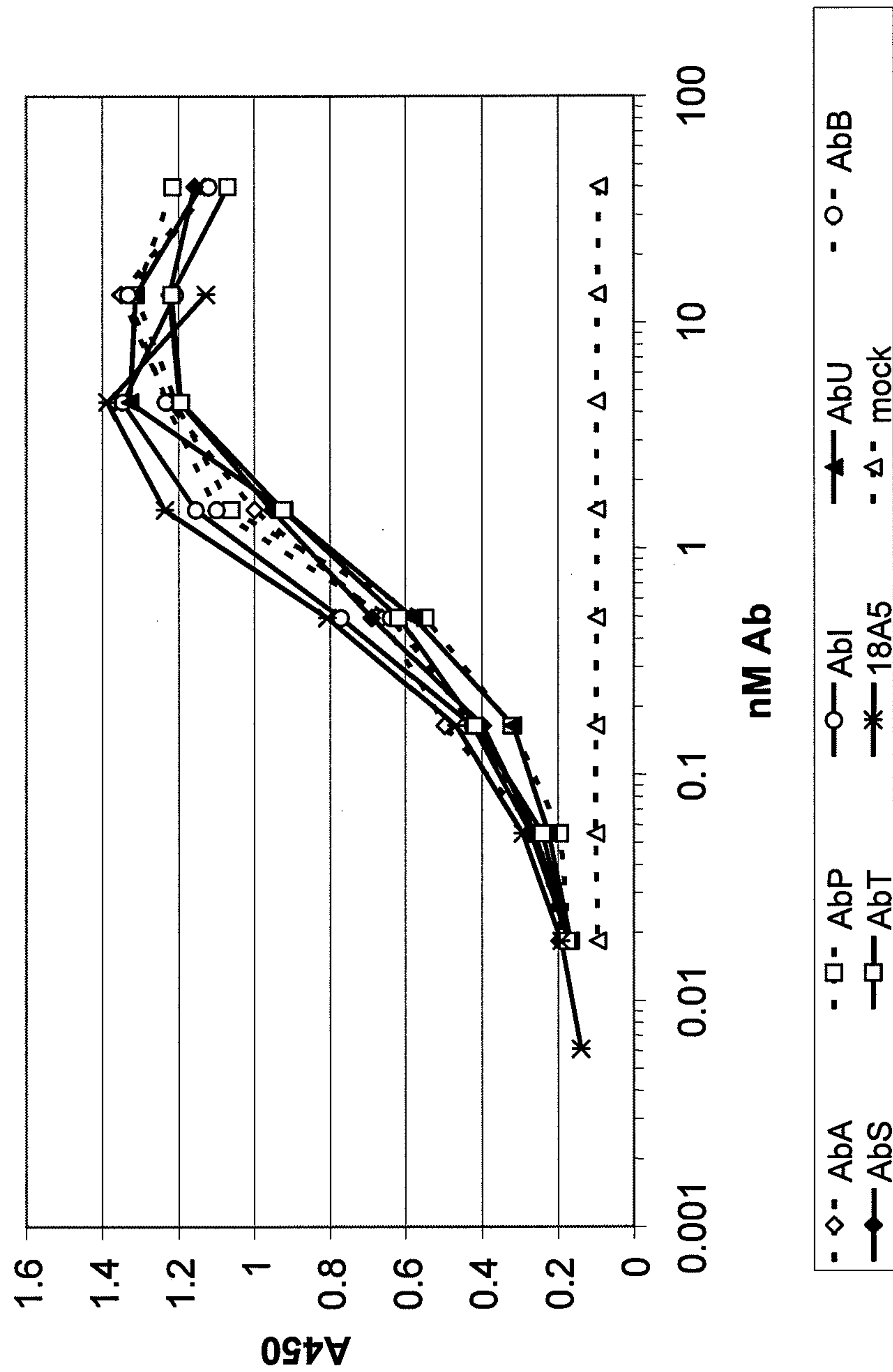


Figure 6j

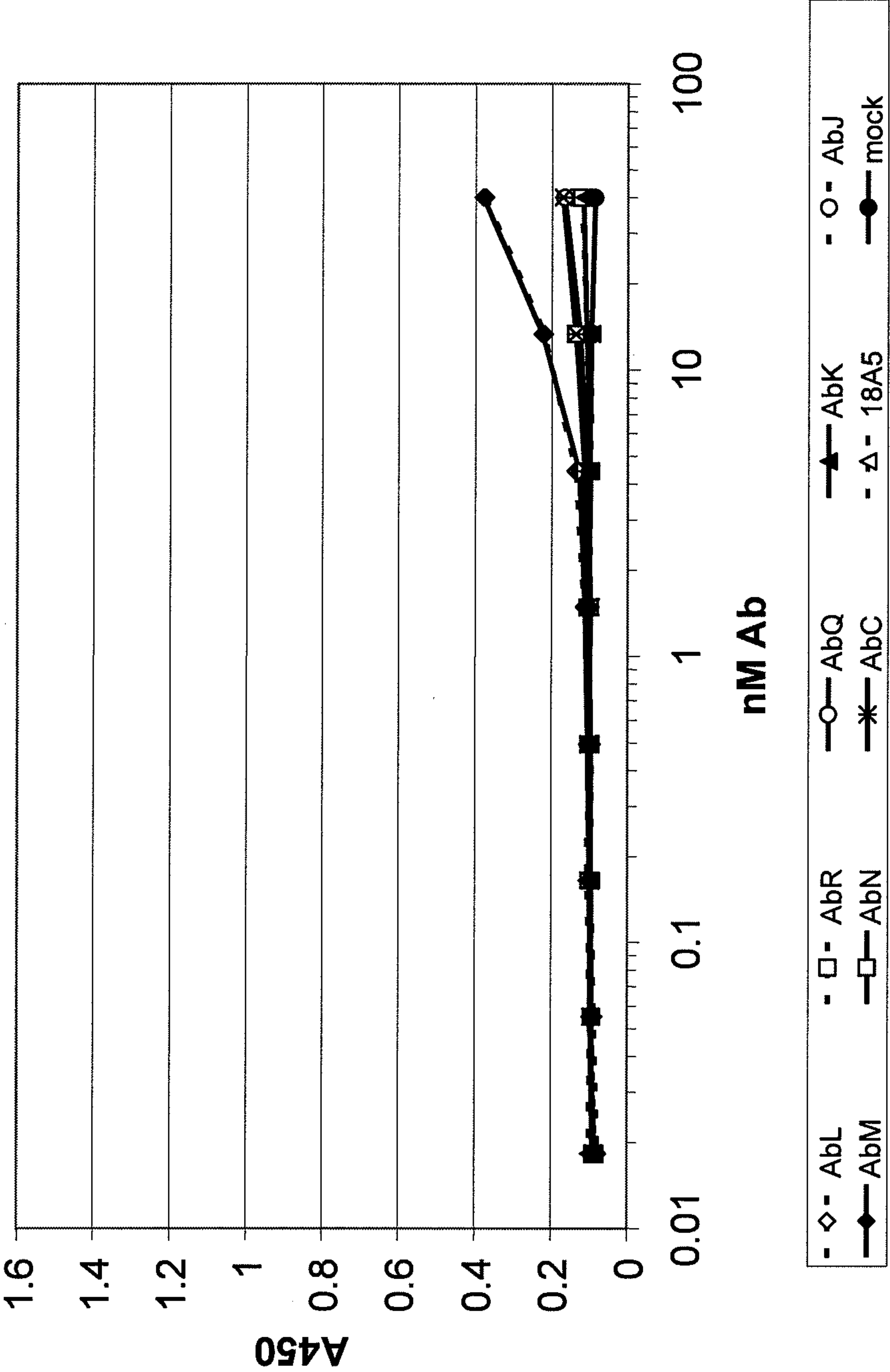


Figure 6k

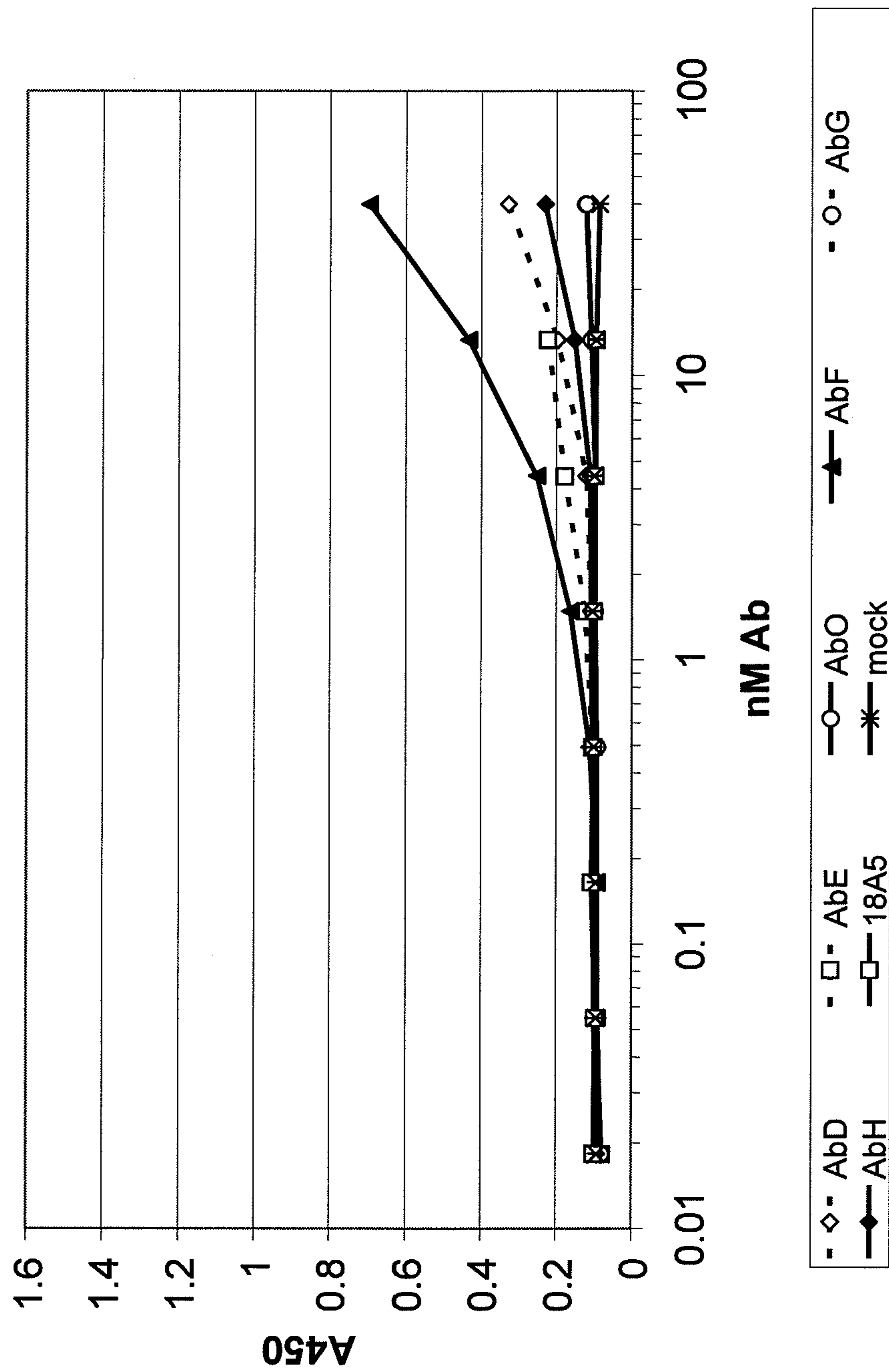
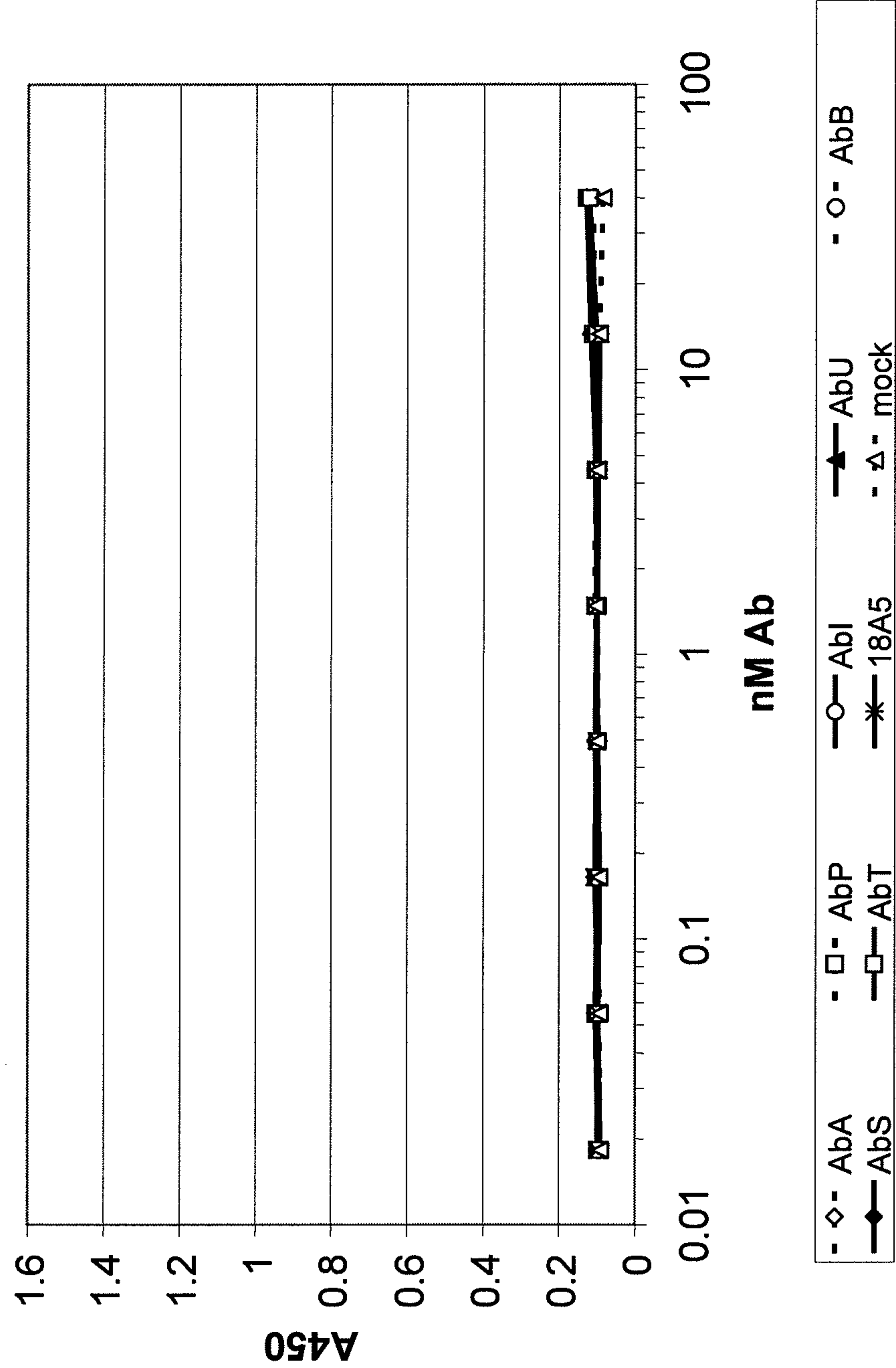


Figure 6I



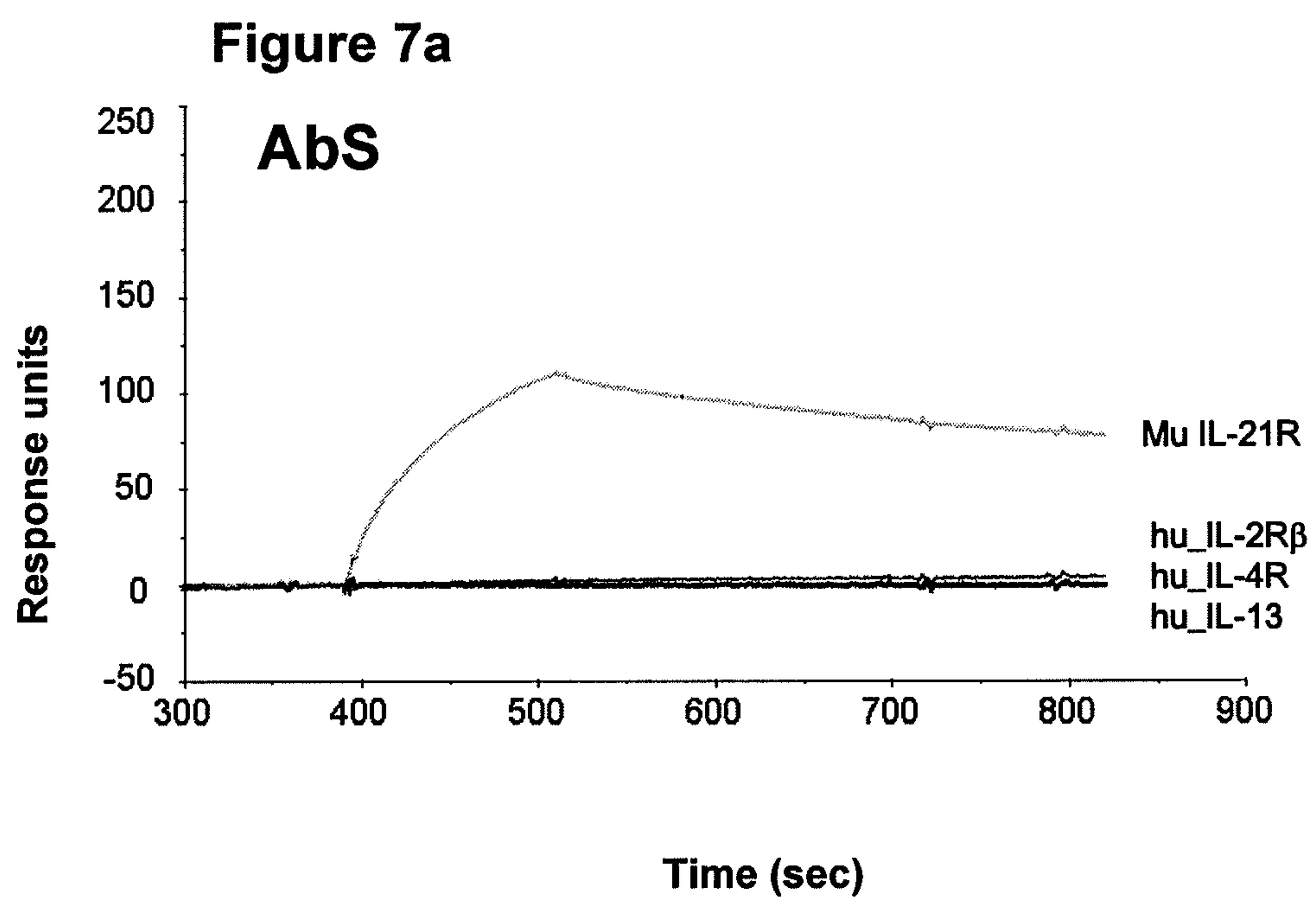


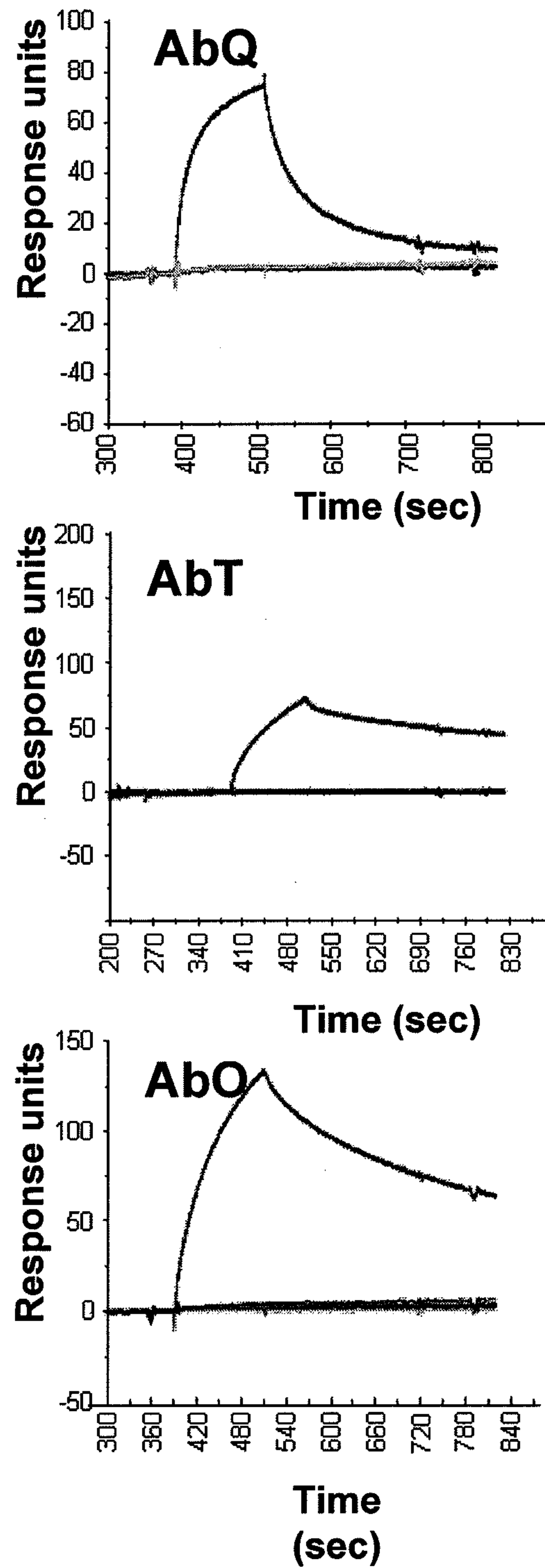
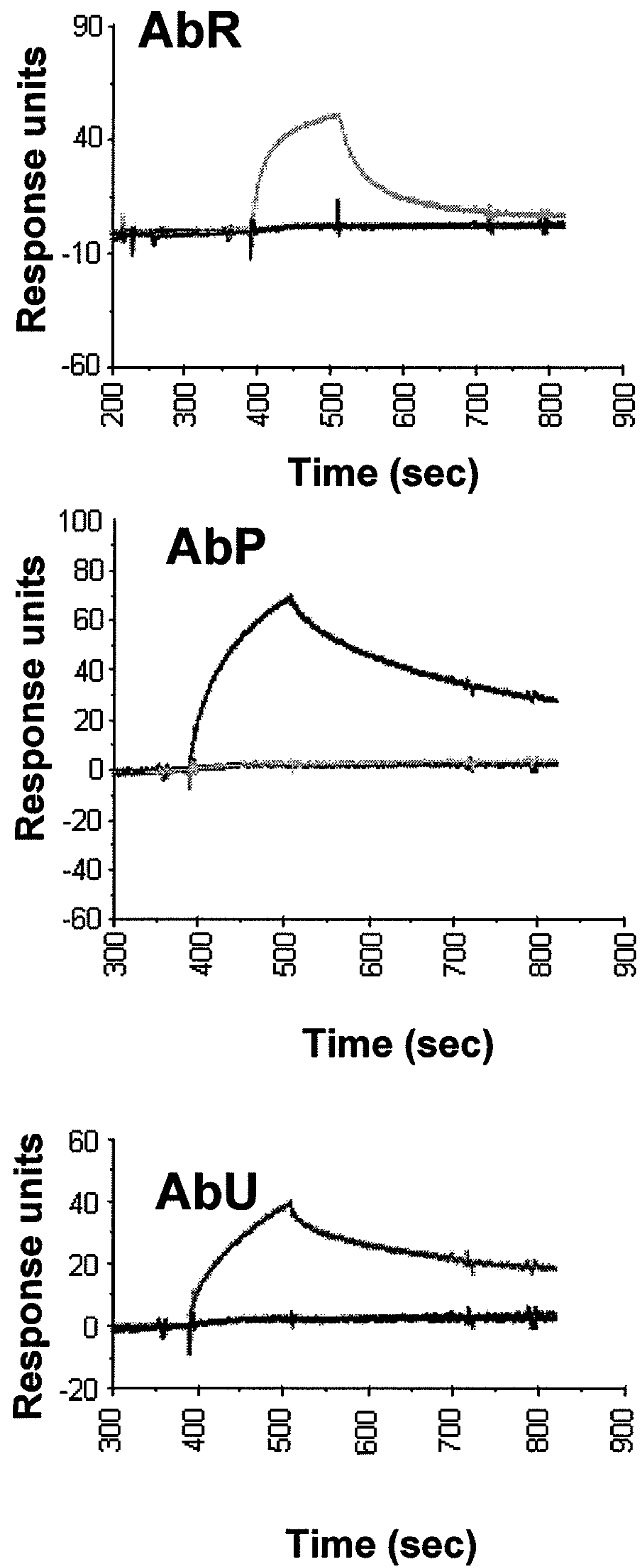
Figure 7b

Figure 7c

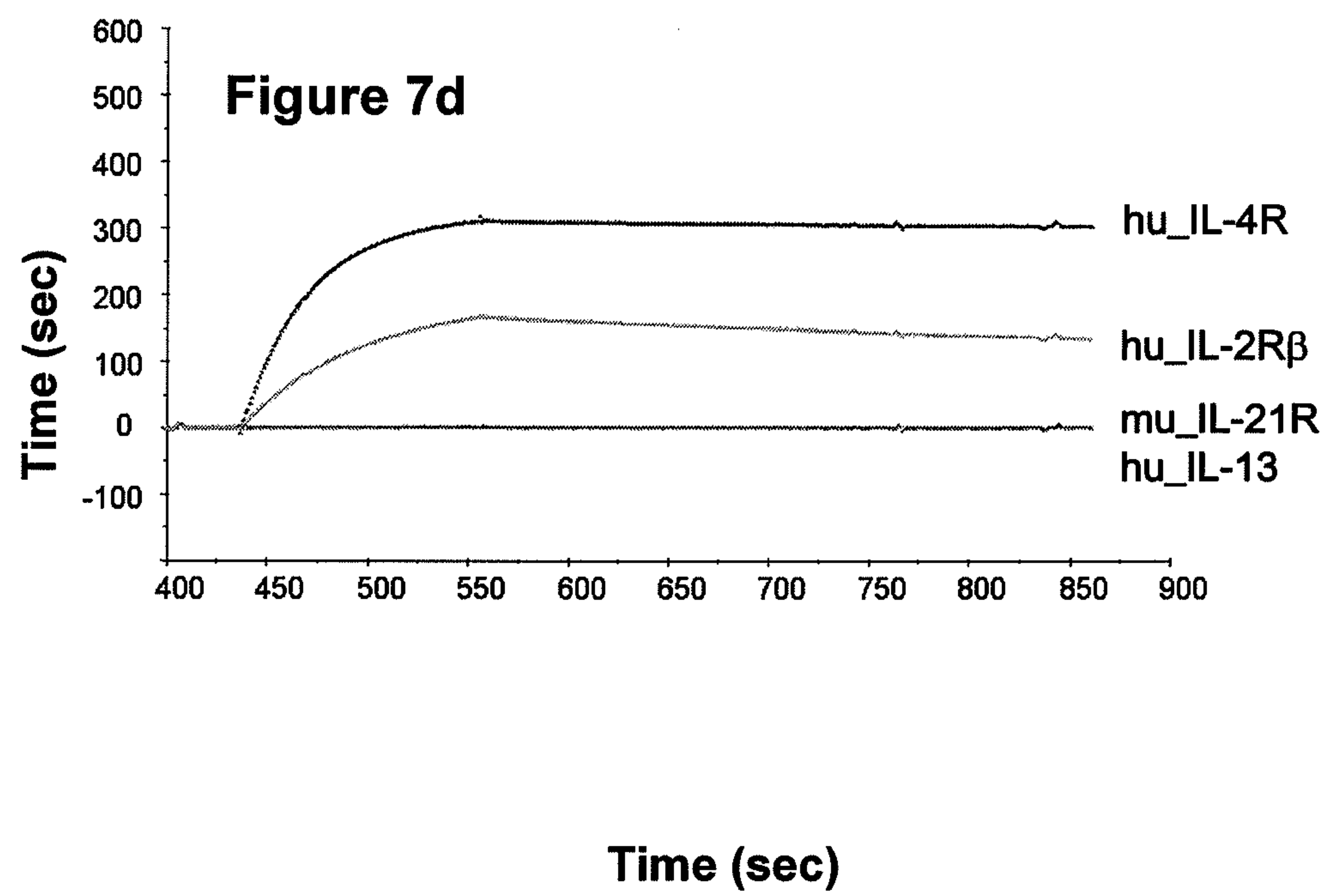


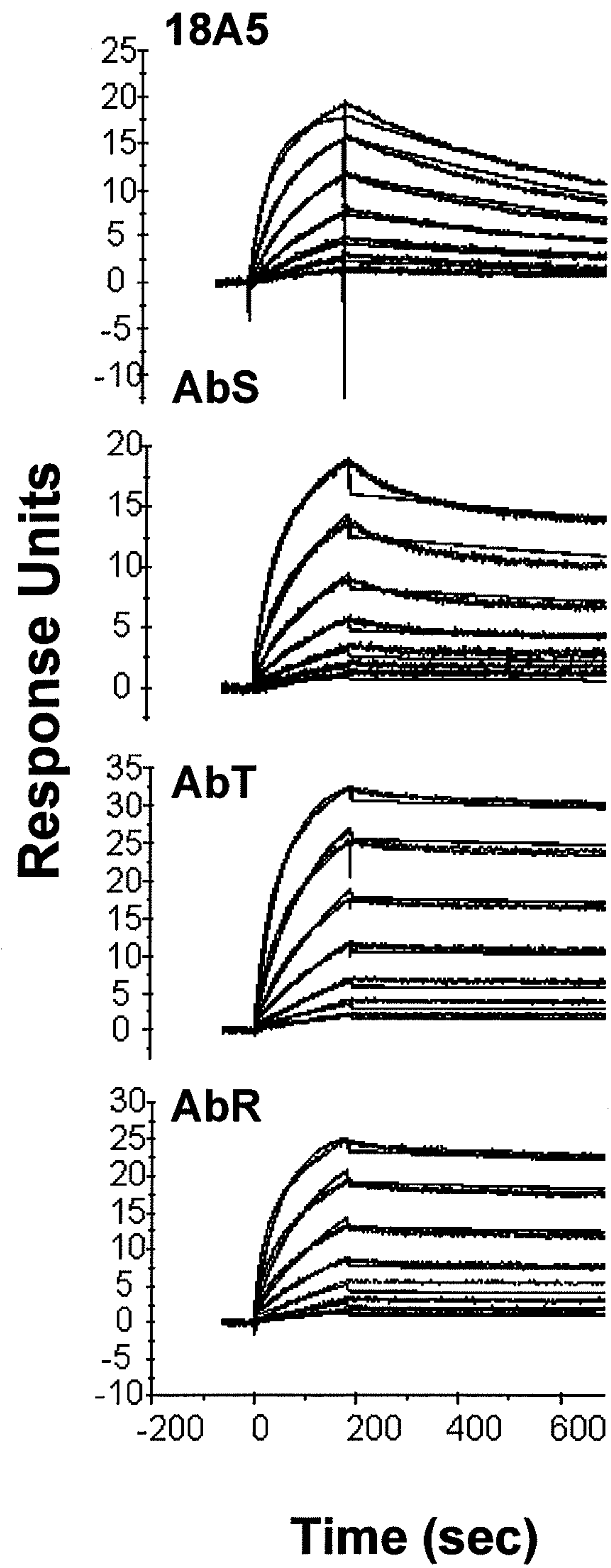
Figure 8a

Figure 8b

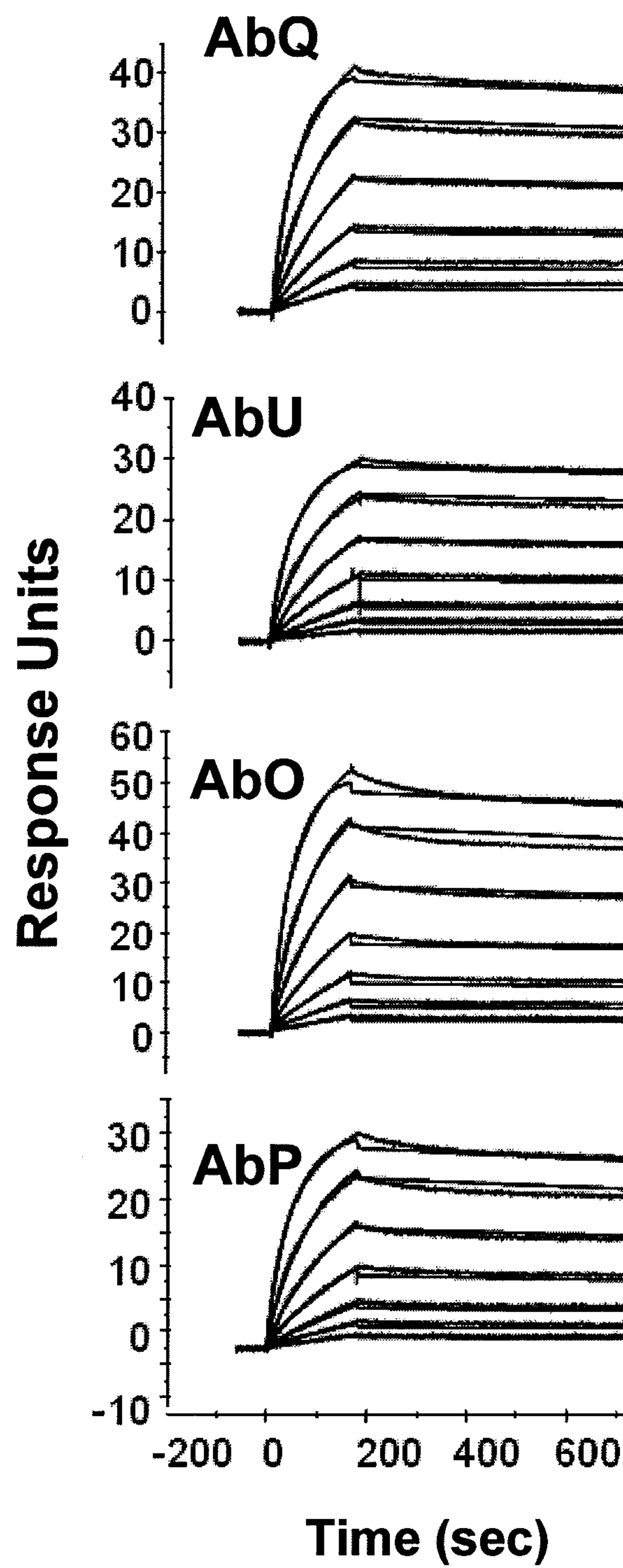


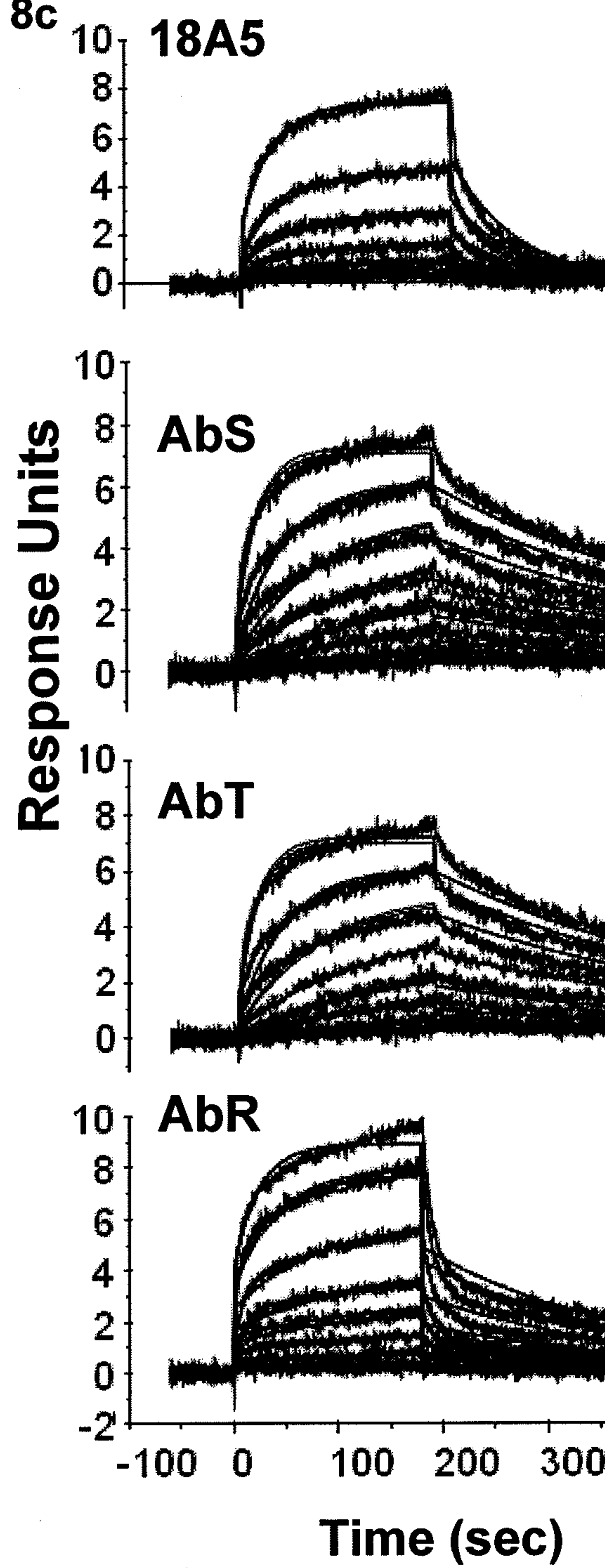
Figure 8c

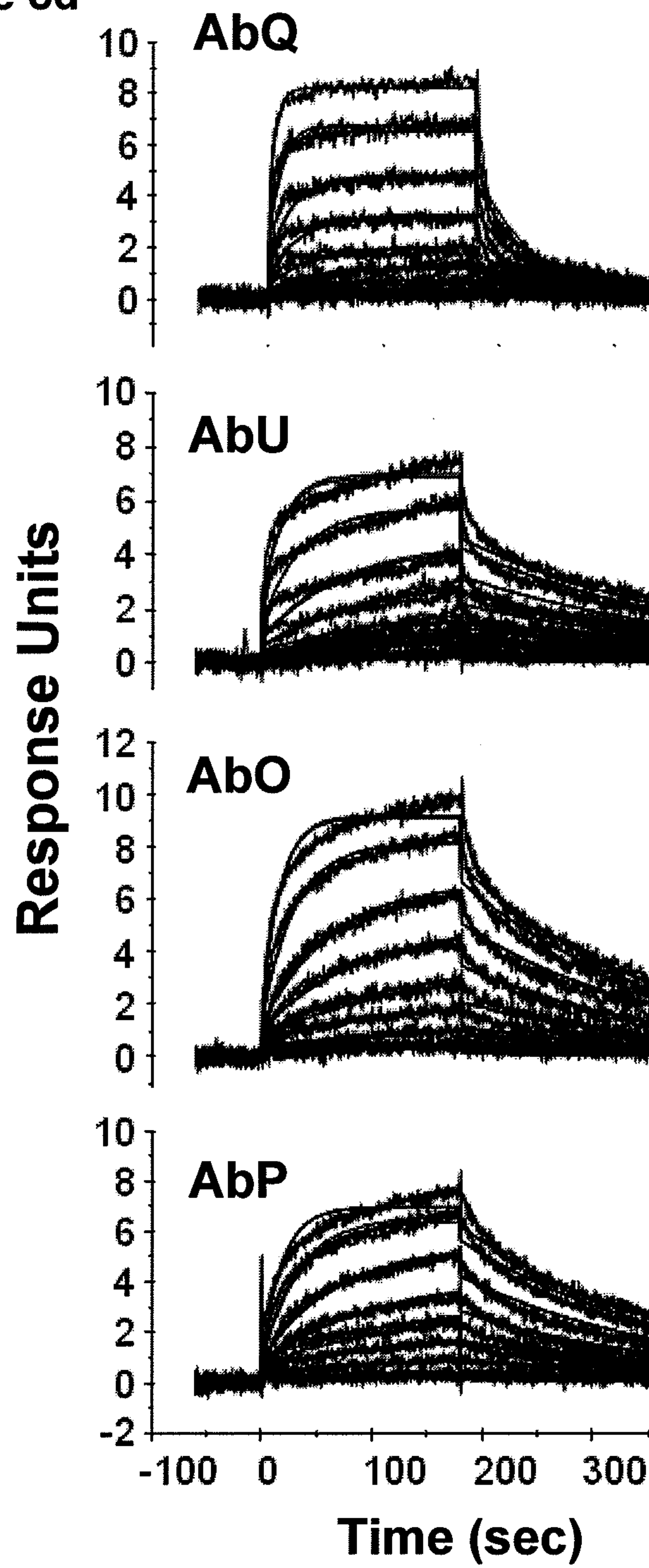
Figure 8d

Figure 9a

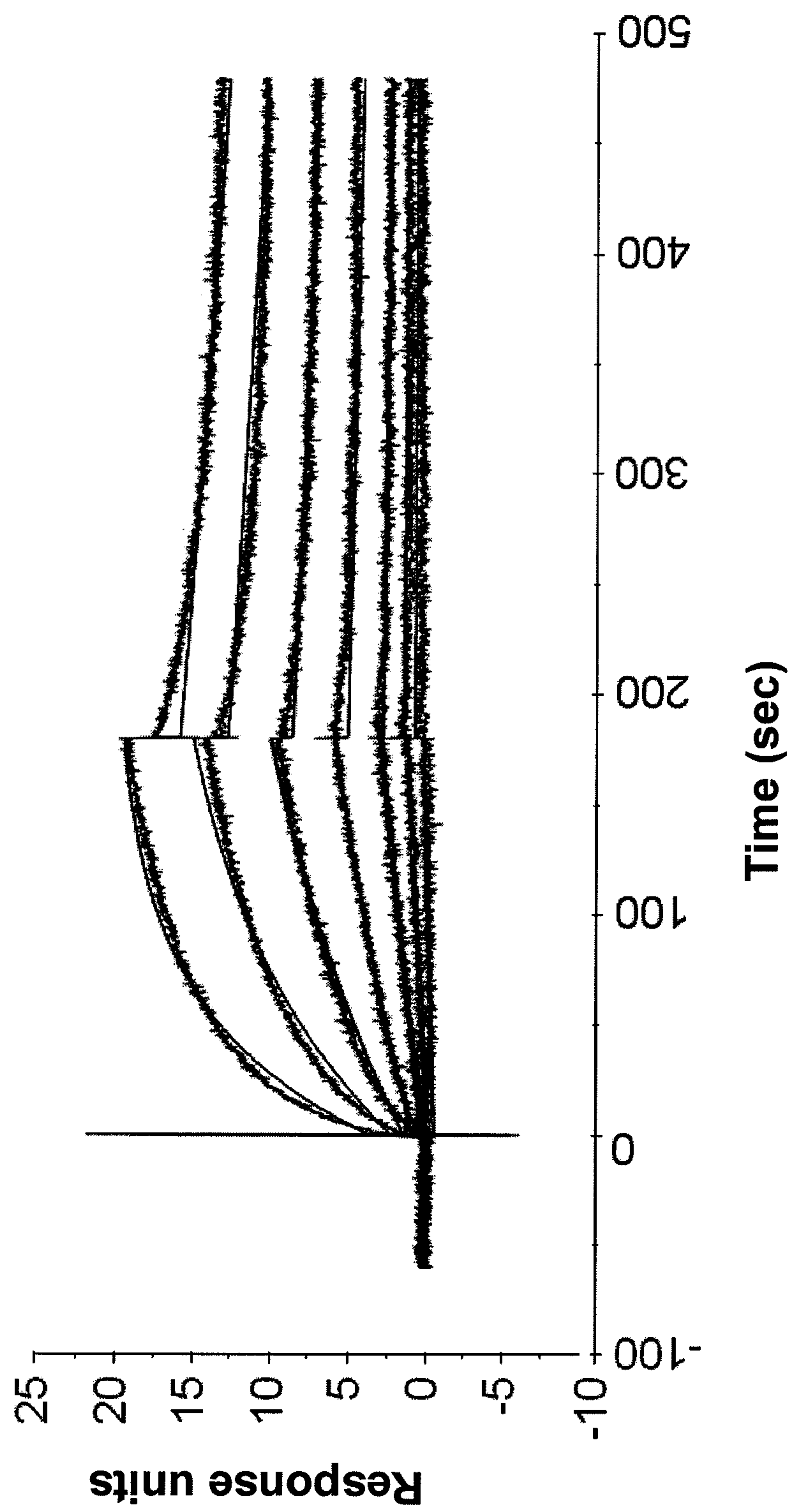


Figure 9b

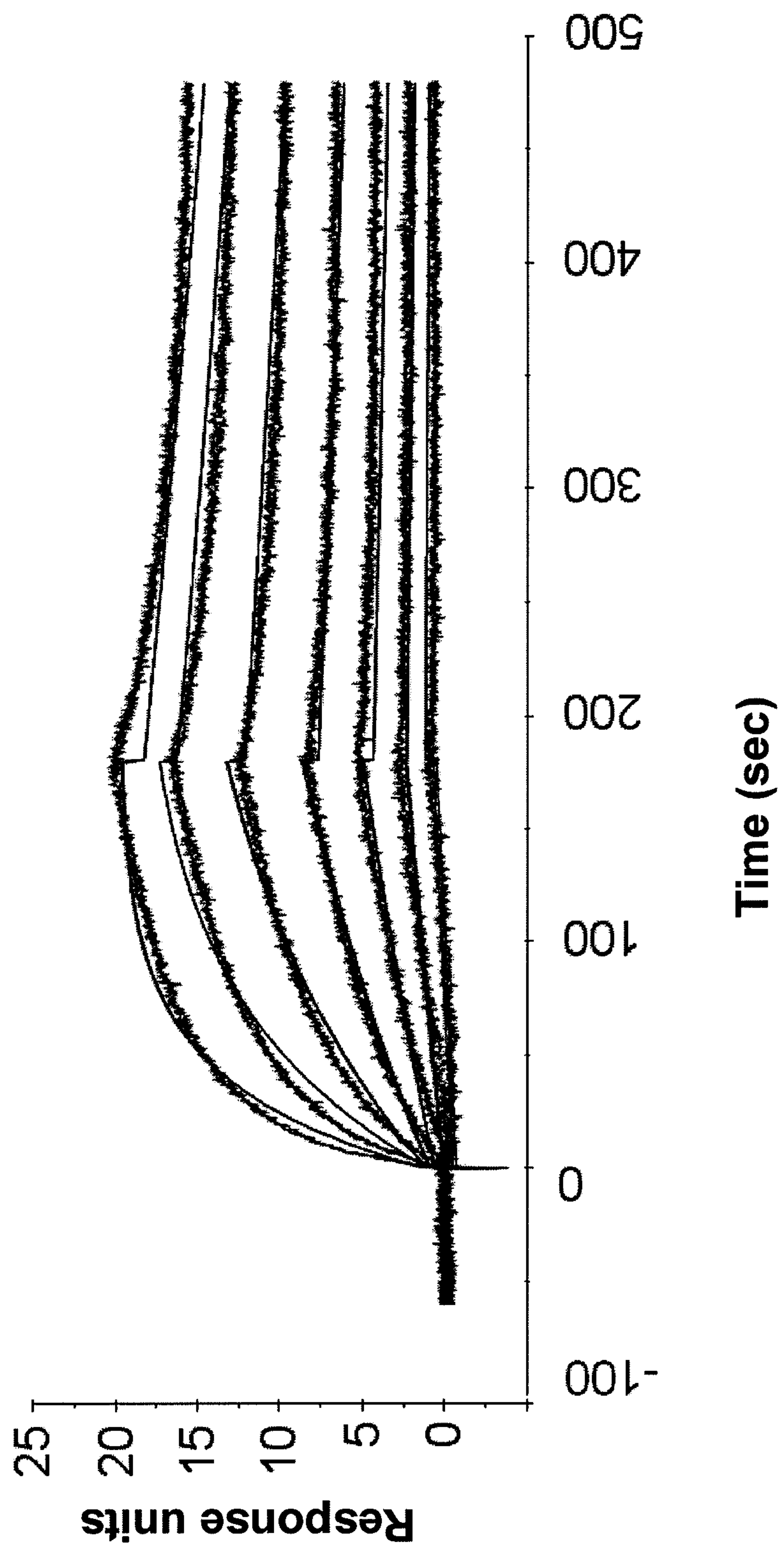


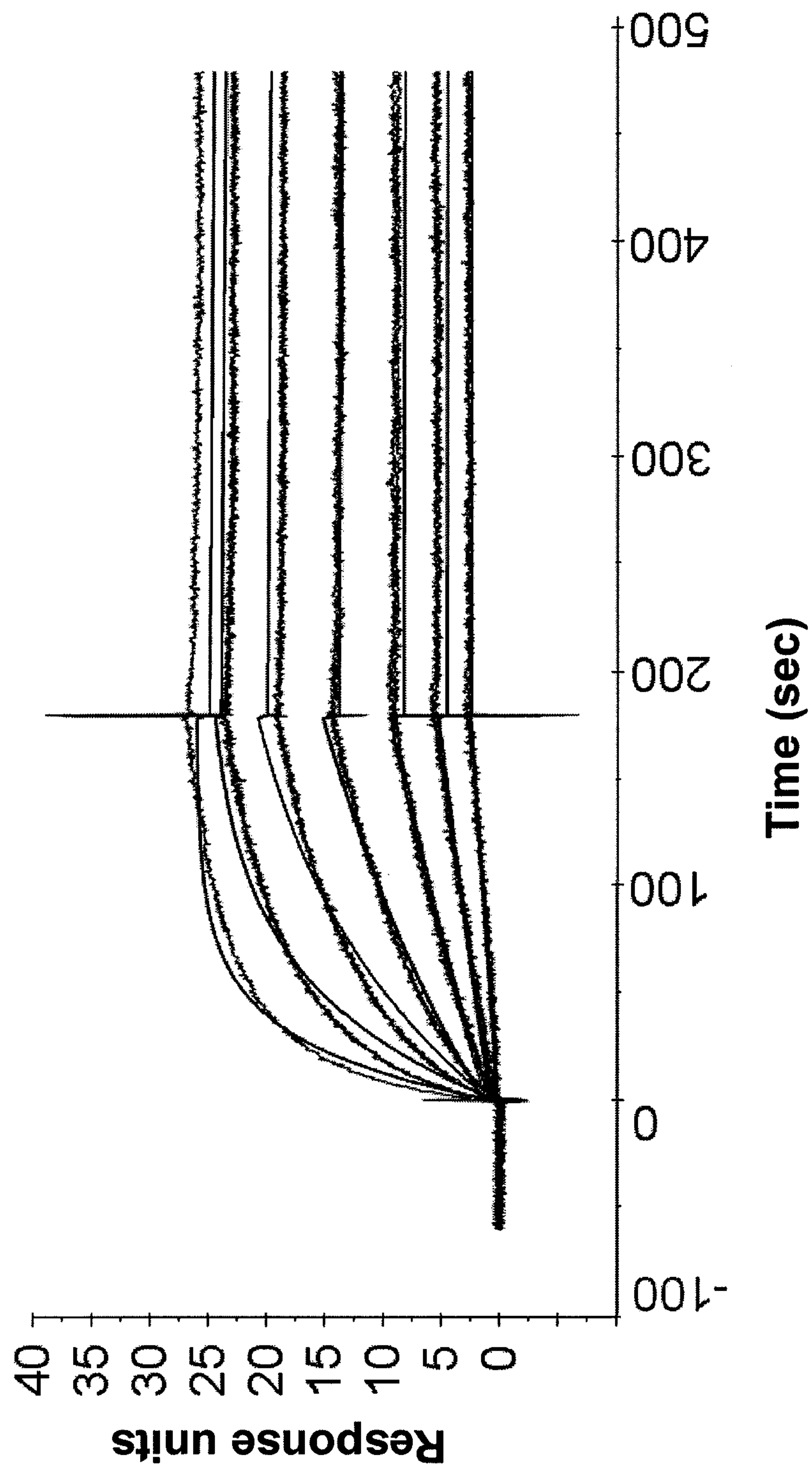
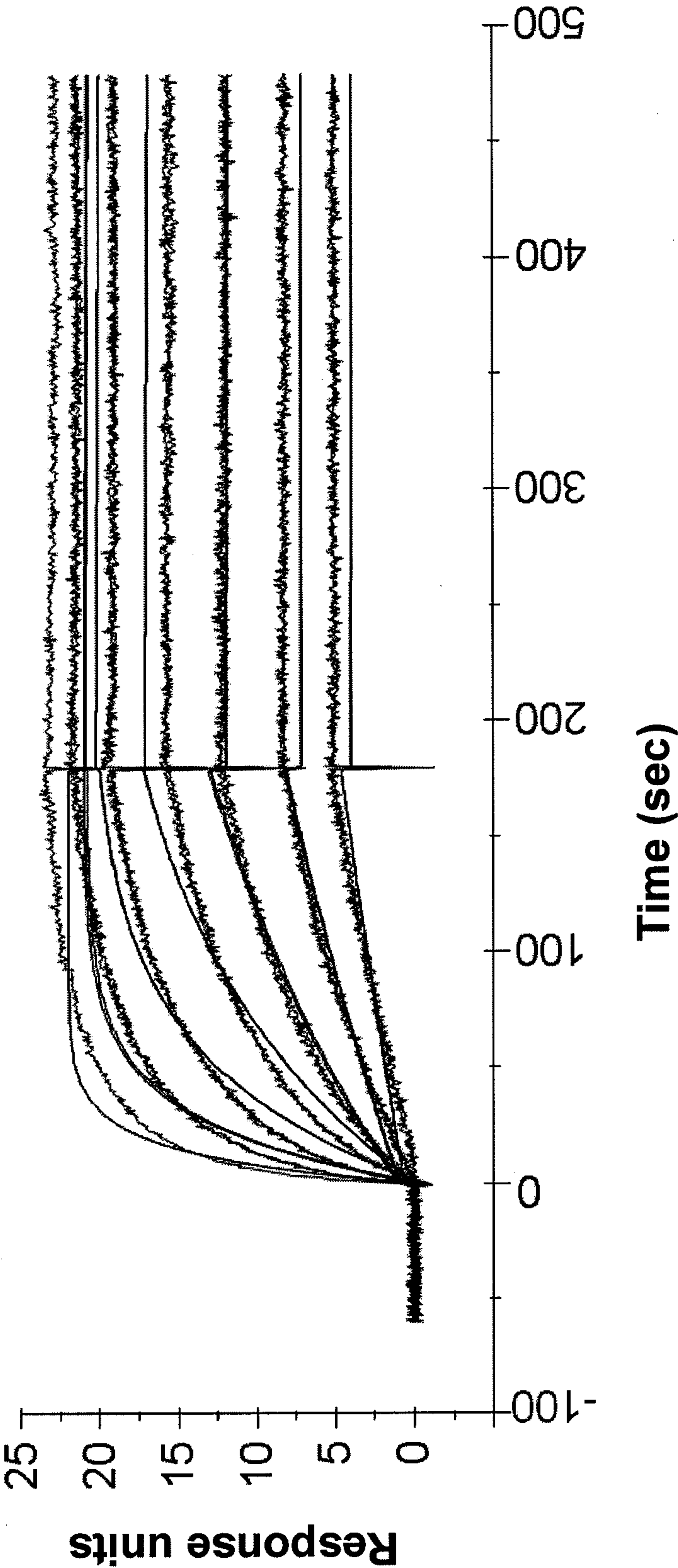
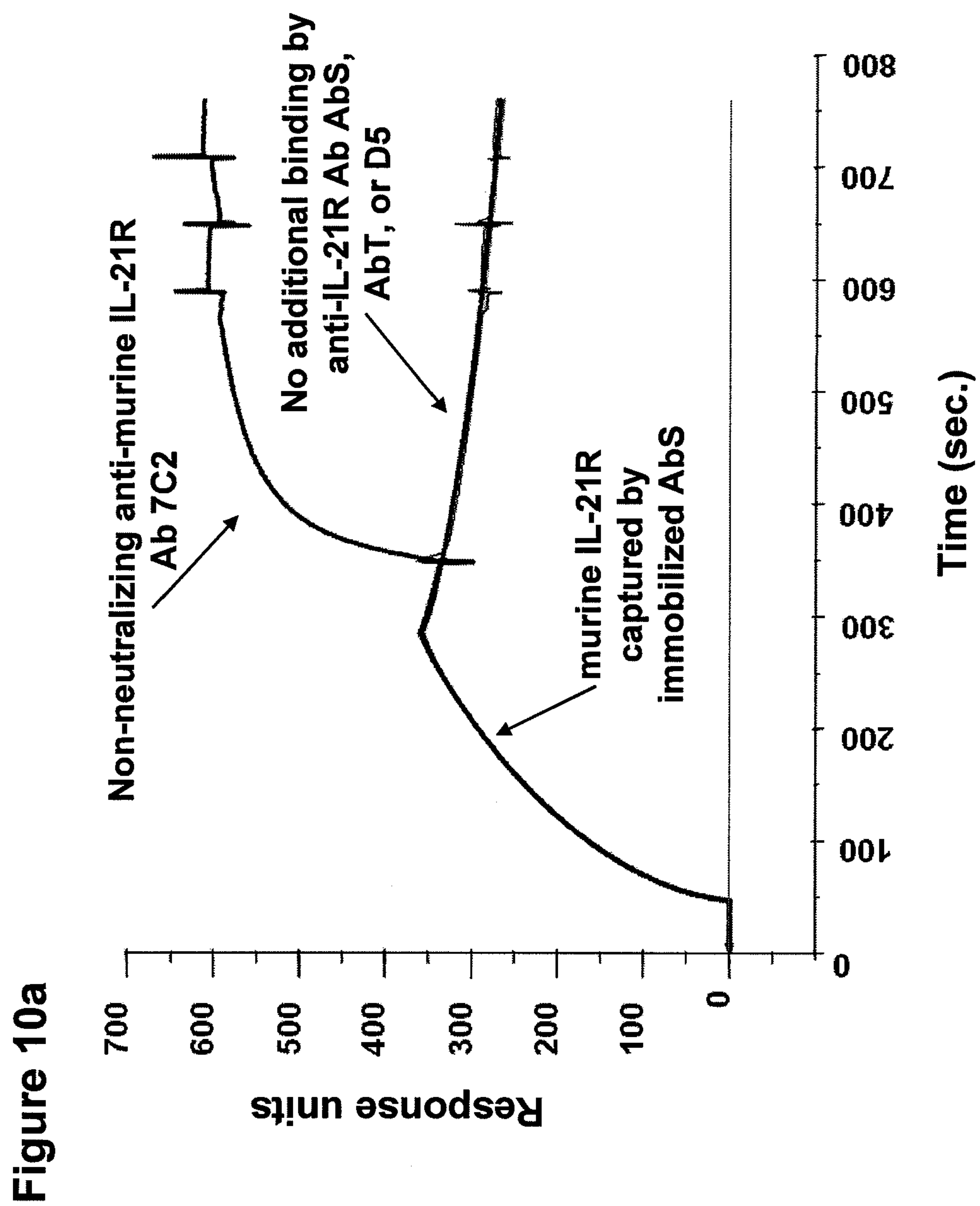
Figure 9c

Figure 9d





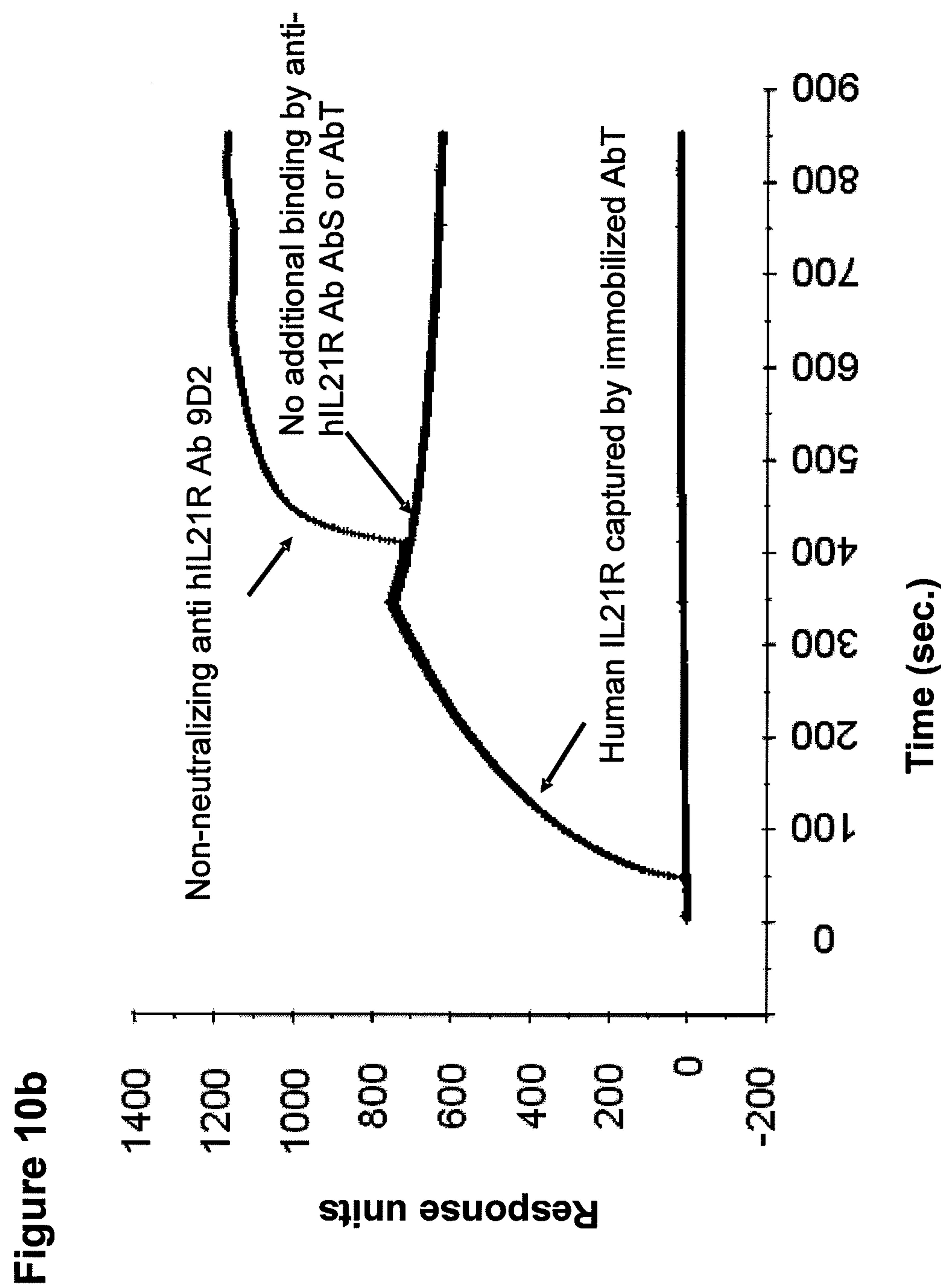
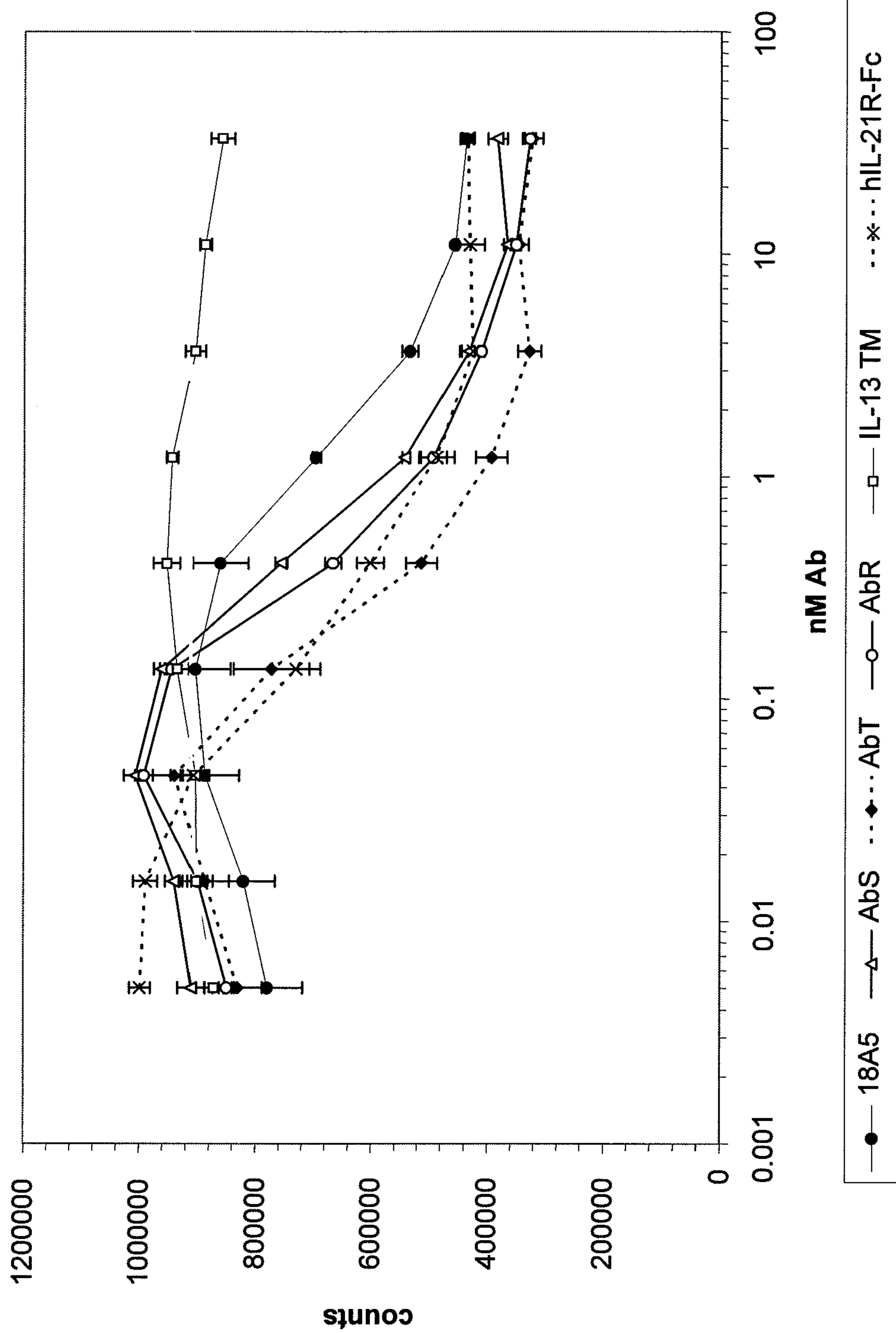


Figure 11a



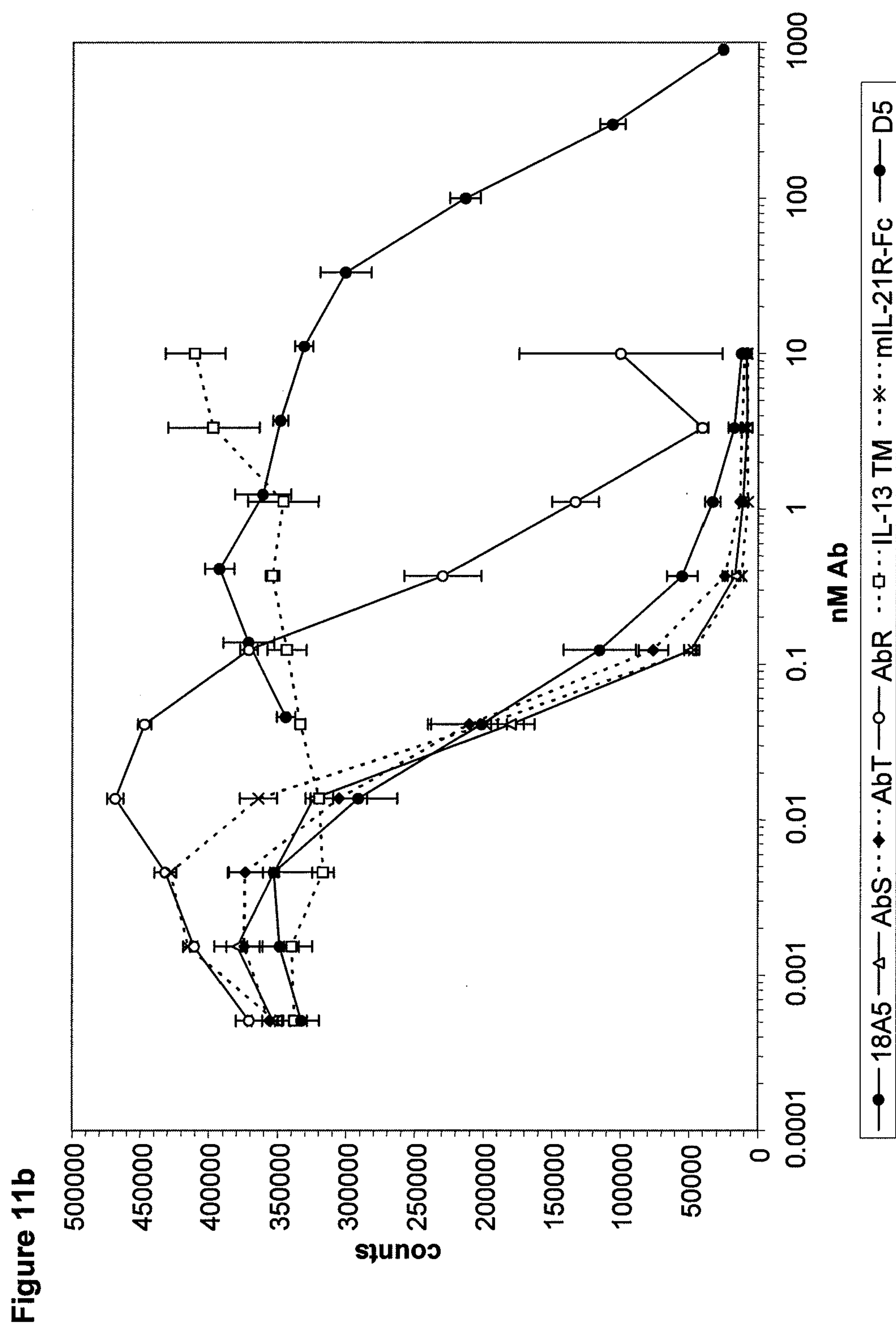


Figure 11c

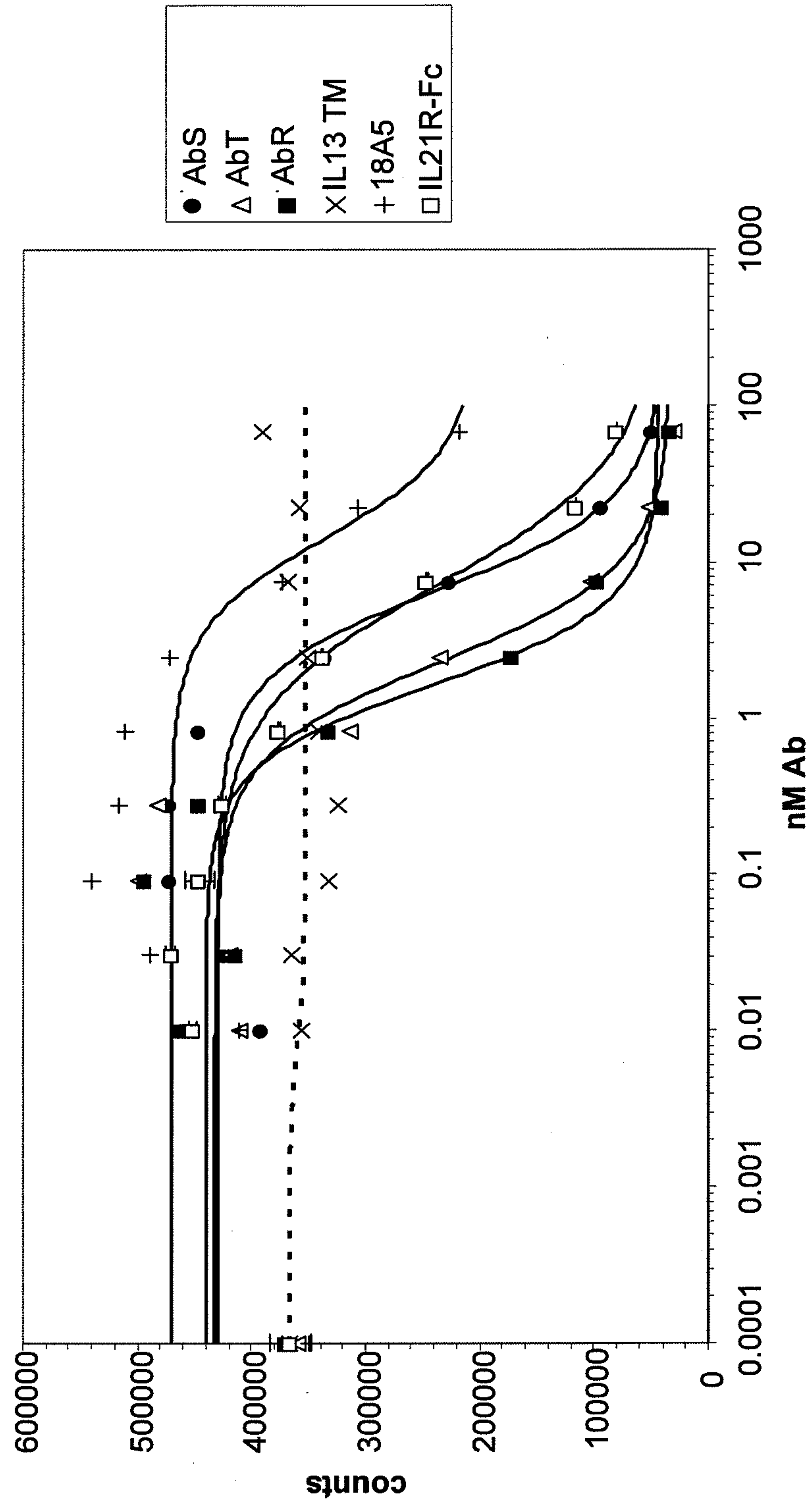


Figure 12a

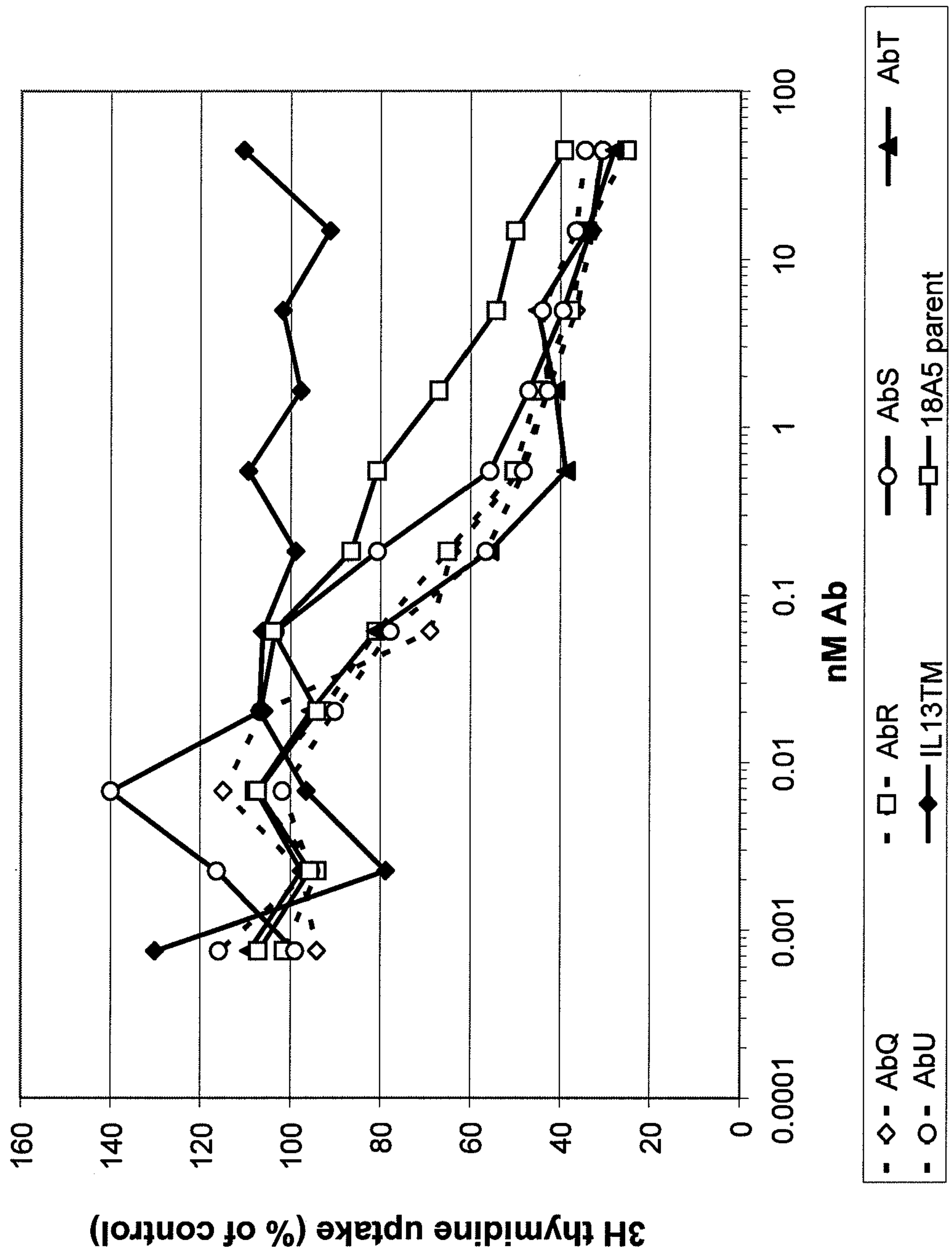


Figure 12b

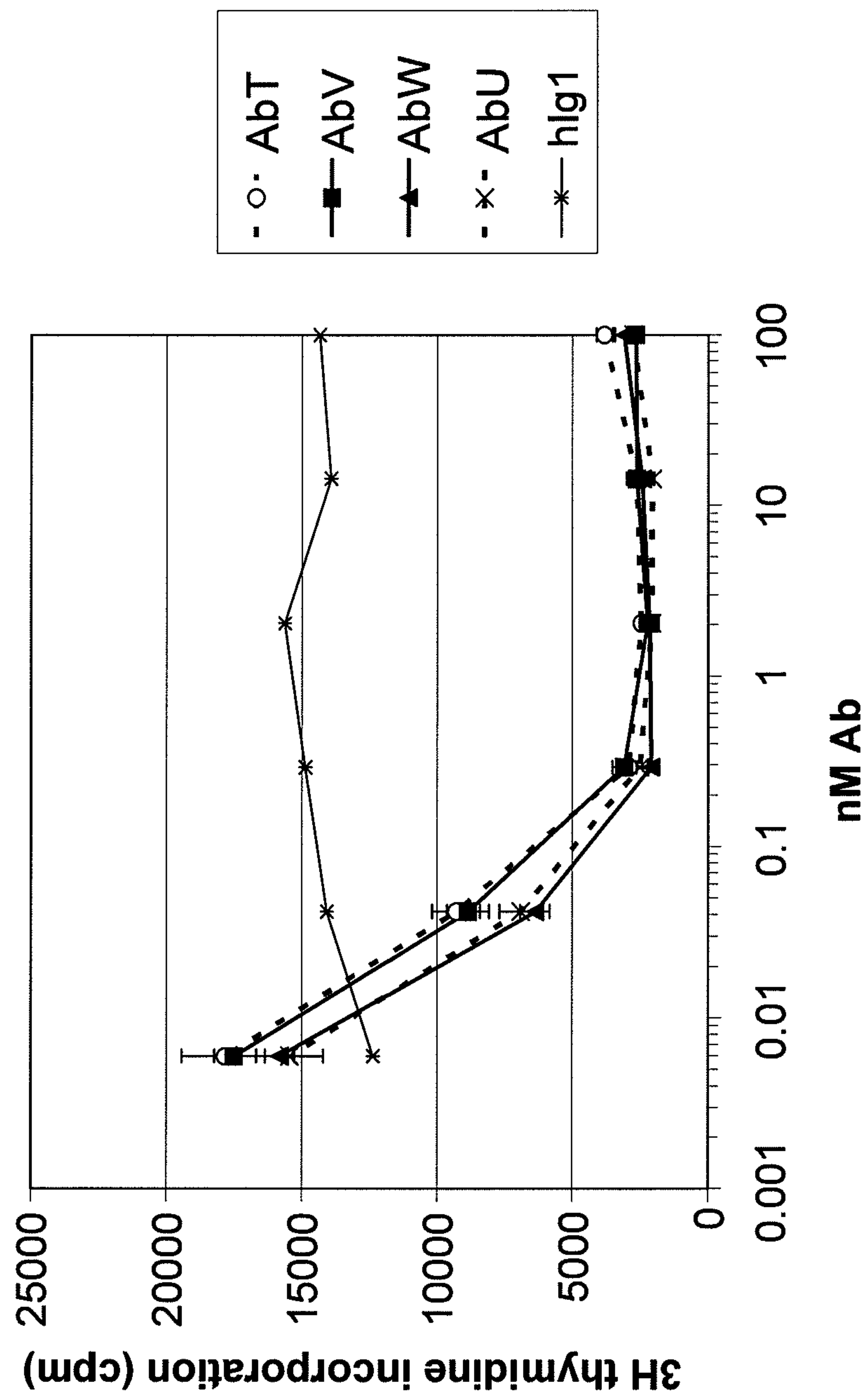


Figure 13

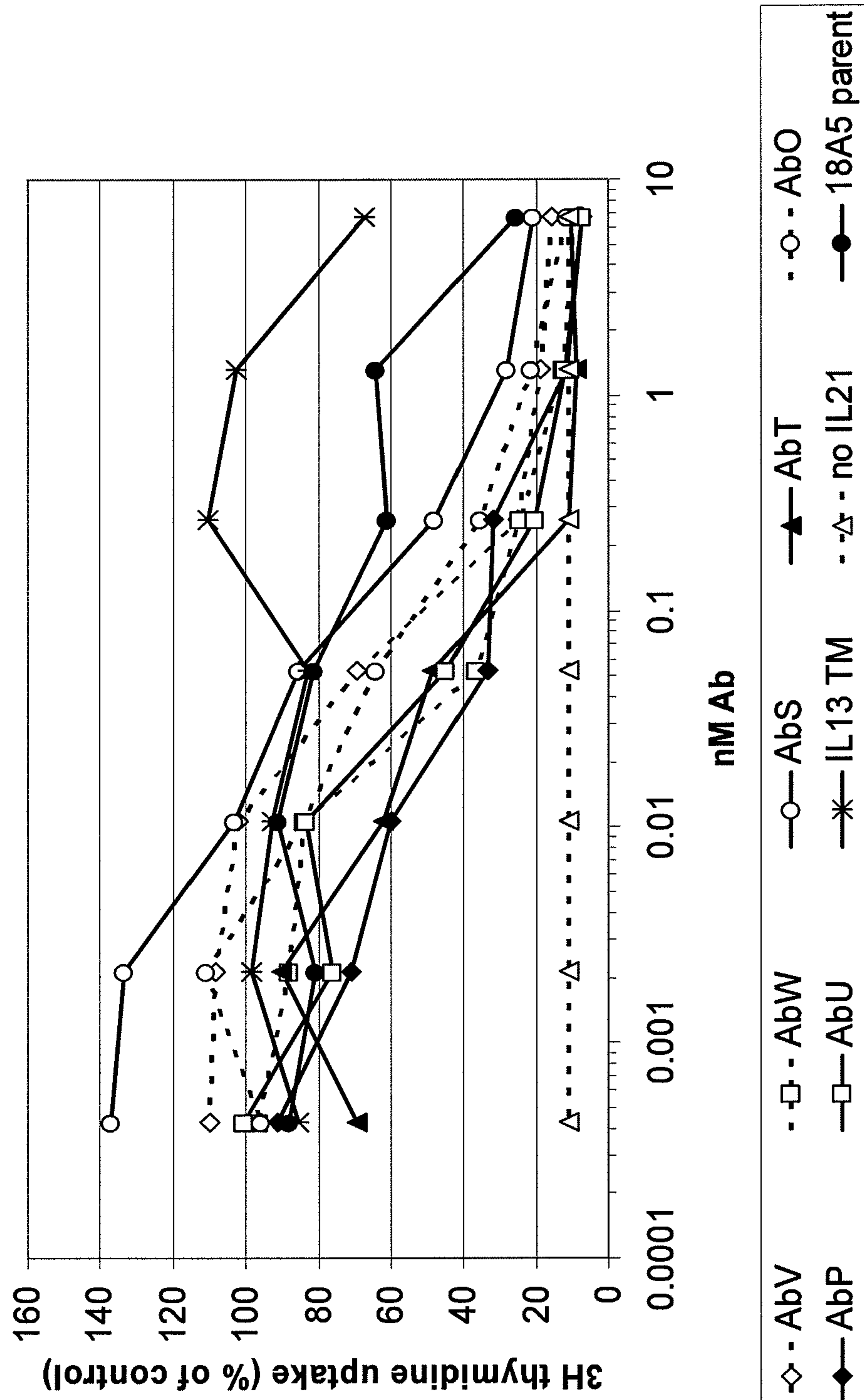
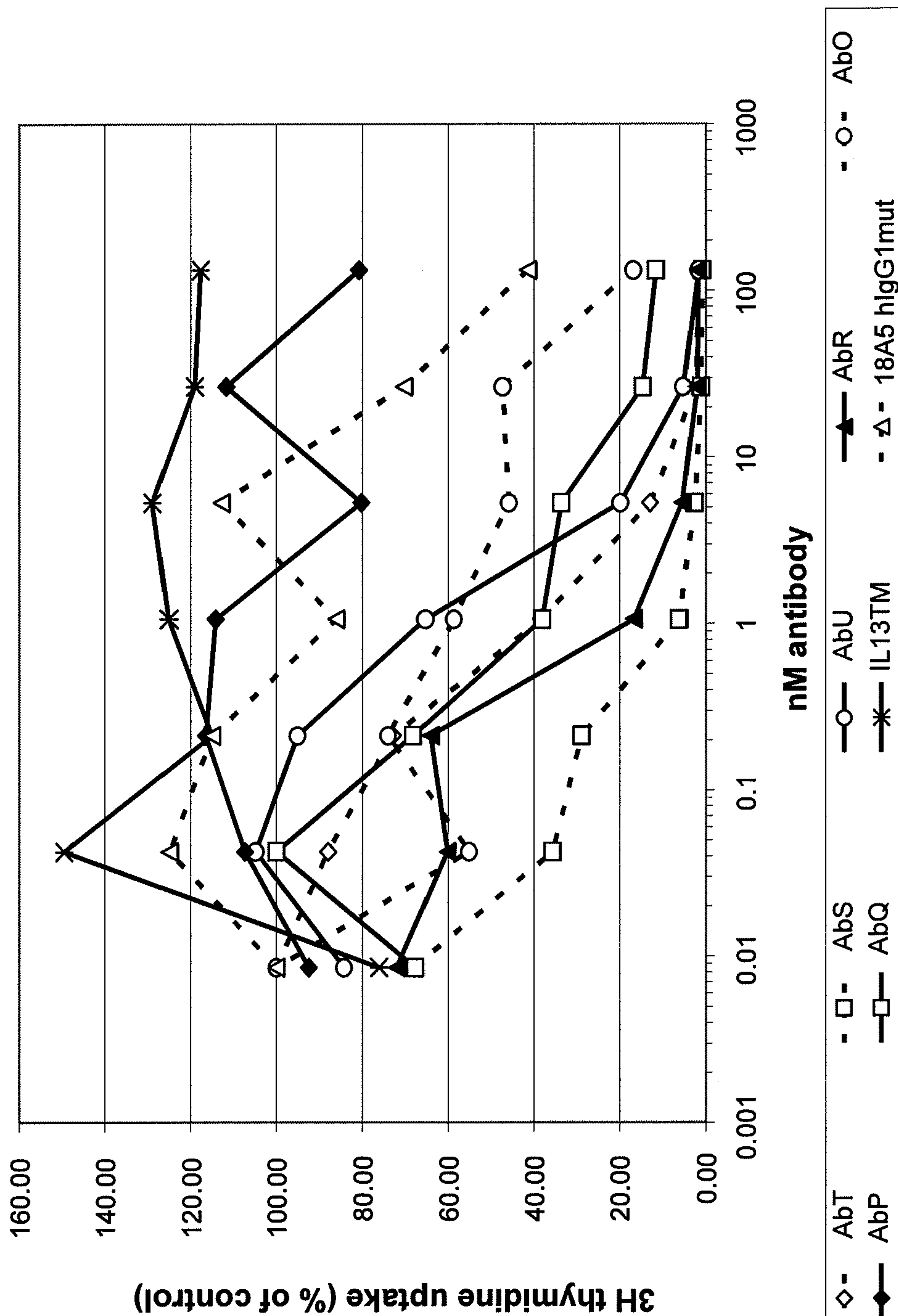
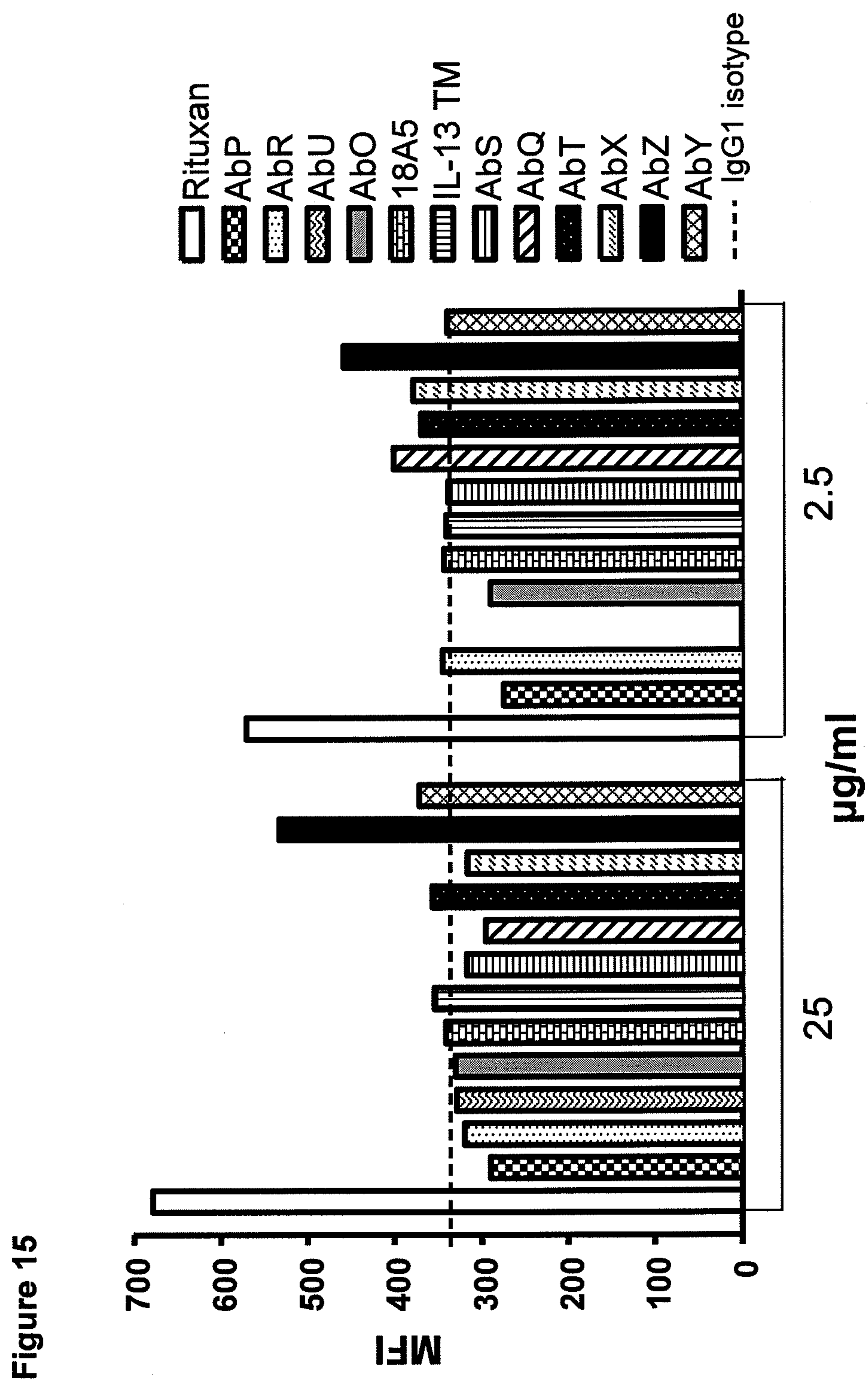


Figure 14





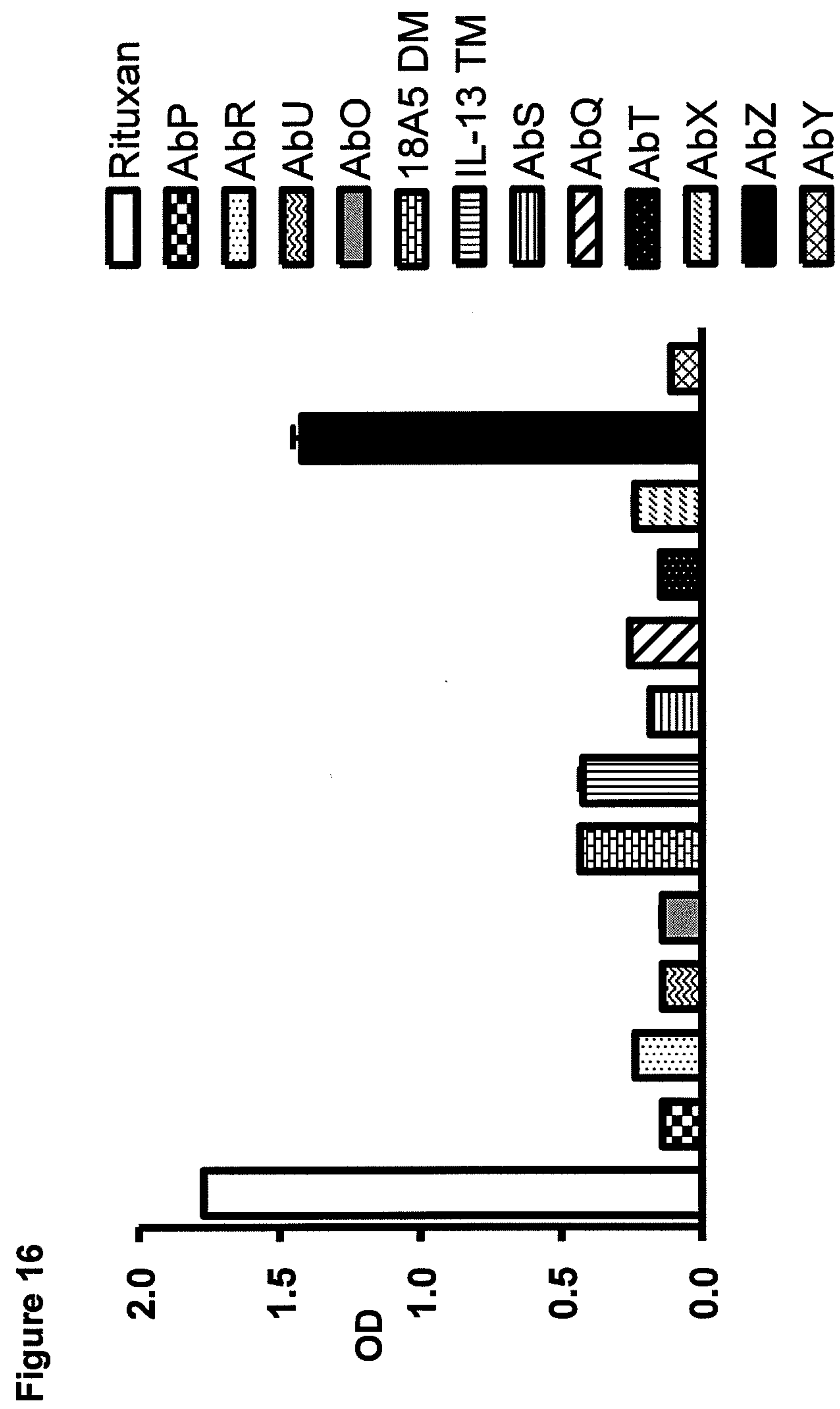


Figure 17a

AbQ:

Heavy chain for AbQ (SEQ ID NO:213), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 165, respectively). The heavy chain constant region is shown in lower case italics with the double mutation (L234A G237A) shown in bold lowercase underlined text (SEQ ID NO: 197).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWI RQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFG RPEYWGQGT LVT VSS astkgpsvfplapsskstsg
gtaalgclvkdyfpepvtvswnsgaltsgvhtfpavqlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papealgapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykc
kvsnk alpapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfypsdia vewesngqpennykttppvl dsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls l spgk

Light chain for AbQ (SEQ ID NO: 214), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 172, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGD SLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQA EDEADYYCVARSV
VGNPHVLFGGGTQLTVL gqpkaapsvtlfppsseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvthegstvektvaptecs

SEQ ID NO:213 is the full-length heavy chain for AbQ:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWI RQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFG RPEYWGQGT LVT VSS astkgpsvfplapsskstsg
gtaalgclvkdyfpepvtvswnsgaltsgvhtfpavqlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papealgapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykc
kvsnk alpapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfypsdia vewesngqpennykttppvl dsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls l spgk

Figure 17b

SEQ ID NO:14 is the V_H portion of the full-length heavy chain:

QVQLQESGPGGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGTLLVTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of the full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of the full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:165 is the CDR H3 portion of the V_H portion of the full-length heavy chain:

FMGFGRPEY

SEQ ID NO:197 is the heavy chain constant region with the double mutation (L234A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpapealgapsvfllfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltclvkgyfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:214 is the full-length light chain for AbQ:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVARSV
VGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassy lsl t
peqwkshrsyscqvt hegstvektvaptecs

Figure 17c

SEQ ID NO:215 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVARSV
VGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:172 is the CDR L3 portion of the V_L portion of the full-length light chain:

VARSVVGNPHVL

SEQ ID NO:198 is the lambda light chain constant region:

*gqpkaapsvtlfppsseelqankatlvcclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassy ls l t p e q w k s h r s y s c q v t h e
gstvektvaptecs*

Figure 18a

AbR:

Heavy chain for AbR (SEQ ID NO:213), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 165, respectively). The heavy chain constant region is shown in lower case italics with the double mutation (L234A G237A) shown in bold lowercase underlined text (SEQ ID NO: 197).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGT^LTVVSSastkgpsvfplapsskstsg
gtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
pape^alg^apsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltv^lhqdwlngkeykc
kvsnk^alpapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfy^psdiavewesngqpennykttppvldsdgsfflyskltvd
ksrwqqggnvfscsvmhealhnhytqksls^lspgk

Light chain for AbR (SEQ. ID NO:216), with CDRs L1, L2, and L3 underlined (SEQ. ID NOs:194, 195, and 187, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ. ID. NO:198).

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVTRSV
KGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvcl
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvthegstvektvaptecs

SEQ ID NO:213 is the full-length heavy chain for AbR:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGT^LTVVSSastkgpsvfplapsskstsg
gtaalgclvkdyfpepvtvswnsgaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papealgapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltv^lhqdwlngkeykc
kvsnk^alpapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfy^psdiavewesngqpennykttppvldsdgsfflyskltvd
ksrwqqggnvfscsvmhealhnhytqksls^lspgk

Figure 18b

SEQ ID NO:14 is the V_H portion of the full-length heavy chain:

QVQLQESGPGGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGTLLTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of the full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of the full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:165 is the CDR H3 portion of the V_H portion of the full-length heavy chain:

FMGFGRPEY

SEQ ID NO:197 is the heavy chain constant region with the double mutation (L234A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpape algapsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vsvltvlnhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltclvkgyfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:216 is the full-length light chain for **AbR**:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDYCYVTRSV
KGNPHVLFGGGTQLTVLgqpkaapsvtlfppsseelqankatlvcl
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassy slt
peqwkshrsyscqvt hegstvektvapt ecs

Figure 18c

SEQ ID NO:217 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDYVCVTRSV
KGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:187 is the CDR L3 portion of the V_L portion of the full-length light chain:

VTRSVKGNPHVL

SEQ ID NO:198 is the lambda light chain constant region

*gqpkaapsvtlfppsseelqankatlvcclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassylsltpeqwkshrsyscqvtthe
gstvektvaptecs*

Figure 19a

AbW:

Heavy chain for AbW (SEQ ID NO:218), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 165, respectively). The heavy chain constant region is shown in lower case italics with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text (SEQ ID NO:196).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIROPKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGTLVTVSSastkgpsvfp~~lapssk~~stsg
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
pape~~**aag**~~apsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykc
kvsnkalpapiektiskakgqprepqvvtlppsreemtknqvsltc
lvkgfypsdia~~vewesngqpennyk~~ttppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls~~l~~spgk

Light chain for AbW (SEQ ID NO:216), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 187, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVTLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVTRSV
KGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvc
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvthegstvektvaptecs

SEQ ID NO:218 is the full-length heavy chain for AbW:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIROPKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGTLVTVSSastkgpsvfp~~lapssk~~stsg
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlhqdwlngkeykc
kvsnkalpapiektiskakgqprepqvvtlppsreemtknqvsltc
lvkgfypsdia~~vewesngqpennyk~~ttppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls~~l~~spgk

Figure 19b

SEQ ID NO:14 is the V_H portion of the full-length heavy chain:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGGTLVTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of the full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of the full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:165 is the CDR H3 portion of the V_H portion of the full-length heavy chain:

FMGFGRPEY

SEQ ID NO:196 is the heavy chain constant region with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswnsgal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpapeaagapsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vvsvltvlnhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltcclvkgyfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhcalhnhytqk
slslspgk*

SEQ ID NO:216 is the full-length light chain for AbW:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVTRSV
KGNPHVLFGGGTQLTVLgqpkaapsvtlfppsseelqankatlvcl
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvthegstvektvaptacs

Figure 19c

SEQ ID NO:217 is V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDYCYCVTRSV
KGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:187 is the CDR L3 portion of the V_L portion of the full-length light chain:

VTRSVKGNPHVL

SEQ ID NO:198 is the lambda light chain constant region

*gqpkaapsvtlfppsseelqankatlvclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassylsltpeqwkshrsyscqvtthe
gstvektvaptecs*

Figure 20a

AbS:

Heavy chain for AbS (SEQ ID NO:219), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 169, respectively). The heavy chain constant region is shown in lower case italics with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text (SEQ ID NO:196).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGLVTVSSastkgpsvfplapsskstsg
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlgssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papeaagapsvfllfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlgdwlngkeykc
kvsnkaklpapiektiskakgqprepqvvtlppsreemtknqvsltc
lvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqkslsispkg

Light chain for AbS (SEQ ID NO:220), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 176, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQEDEADYYCMSRSI
WGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvc
lisdypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvtthegstvektvaptecs

SEQ ID NO:219 is the full-length heavy chain for AbS:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGLVTVSSastkgpsvfplapsskstsg
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlgssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papeaagapsvfllfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvlgdwlngkeykc
kvsnkaklpapiektiskakgqprepqvvtlppsreemtknqvsltc
lvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqkslsispkg

Figure 20b

SEQ ID NO:6 is the V_H portion of the full-length heavy chain:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGTLLVTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of the full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of the full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:169 is the CDR H3 portion of the V_H portion of the full-length heavy chain:

GGGISRPEY

SEQ ID NO:196 is the heavy chain constant region with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpape **aag**apsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vvsvlthqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltcclvkgyfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:220 is the full-length light chain for AbS:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCMSRSI
WGNPHVLFGGGTQLTVLgqpkaapsvtlfppsseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvtthegstvektvaptecs

Figure 20c

SEQ ID NO:221 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCMSRSI
WGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:176 is the CDR L3 portion of the V_L portion of the full-length light chain:

MSRSIWGNPHVL

SEQ ID NO:198 is the lambda light chain constant region:

*gqpkaapsvtlfppsseelqankatlvclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassylsltpewkshrsyscqvtthe
gstvektvaptecs*

Figure 21a

AbT:

Heavy chain for AbT (SEQ ID NO:219), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 169, respectively). The heavy chain constant region is shown in lower case italics with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text (SEQ ID NO:196).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGT^LTVVSSastk^gpsvfplapssk^sts^g
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
pape^{aag}apsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltv^lhqdwl^{ng}keykc
kvsnk^{al}papiektiskakgqprepqv^{yt}lppsreemtknqvsltc
lvkgfypsdia^vewesngqpennyk^{tt}ppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqkslslspgk

Light chain for AbT (SEQ ID NO:222), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 178, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYC^{VAR}SN
KGNPHVLFGGGTQLTVLgqpkaapsvtlfp^{ps}seelqankatl^vcl
isdfypgavtvawkadsspvkagvetttpskqsnⁿkyaa^{ss}ylslt
peqwkshrsyscqvthegstvektvaptecs

SEQ ID NO:219 is the full-length heavy chain for AbT:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGT^LTVVSSastk^gpsvfplapssk^sts^g
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltv^lhqdwl^{ng}keykc
kvsnk^{al}papiektiskakgqprepqv^{yt}lppsreemtknqvsltc
lvkgfypsdia^vewesngqpennyk^{tt}ppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqkslslspgk

Figure 21b

SEQ ID NO:6 is the V_H portion of full-length heavy chain:

QVQLQESGPGGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGTLLTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:169 is the CDR H3 portion of the V_H portion of full-length heavy chain:

GGGISRPEY

SEQ ID NO:196 is the heavy chain constant region with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpape aagapsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltclvkgyfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:222 is the full-length light chain for AbT:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVARSN
KGNPHVLFGGGTQLTVLgqpkaapsvtlfppsseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvtthegstvektvaptecs

Figure 21c

SEQ ID NO:223 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDYCYCVARSN
KGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:178 is the CDR L3 portion of the V_L portion of the full-length light chain:

VARSNKGNPHVL

SEQ ID NO:198 is the lambda light chain constant region:

*gqpkaapsvtlfppsseelqankatlvclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassylsltpeqwkshrsyscqvtthe
gstvektvaptecs*

Figure 22a

AbO:

Heavy chain for **AbO** (SEQ ID NO:219), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 169, respectively). The heavy chain constant region is shown in lower case italics with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text (SEQ ID NO:196).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSSGYYWGWIRQPPGKGL
 EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
 VYYCARGGGISRPEYWGQGTLLVTVSSastkgpsvfplapssksts
 gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
 svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
 papeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
 nwyvdgvevhnaktkpreeqynstyrvvsvltvhlhqdwlngkeykc
 kvsnkaklpapiektiskakgqprepqvytlppsreemtknqvsltc
 lvkgfyfypsdiavewesngqpennykttppvldsdgsfflyskltvd
 ksrwqqgnvfscsvmhealhnhytqkslsispkg

Light chain for **AbO** (SEQ ID NO:224), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 185, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGD~~SL~~RTYYASWYQQKPGQAPVLR
 IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAED~~EA~~DYYCVTRSA
 KGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvc
 lisdffypgavtvawkadsspvkagvetttpskqsnnkyaassylsl
 tpeqwkshrsyscqvtthegstvektvaptecs

SEQ ID NO:219 is the full-length heavy chain for **AbO**:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSSGYYWGWIRQPPGKGL
 EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
 VYYCARGGGISRPEYWGQGTLLVTVSSastkgpsvfplapssksts
 gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
 svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
 papeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
 nwyvdgvevhnaktkpreeqynstyrvvsvltvhlhqdwlngkeykc
 kvsnkaklpapiektiskakgqprepqvytlppsreemtknqvsltc
 lvkgfyfypsdiavewesngqpennykttppvldsdgsfflyskltvd
 ksrwqqgnvfscsvmhealhnhytqkslsispkg

Figure 22b

SEQ ID NO:6 is the V_H portion of full-length heavy chain:

QVQLQESGPGGLVKPSETLSLTCAVSGYSISSGYYWGWI RQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGLVTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:169 is the CDR H3 portion of the V_H portion of full-length heavy chain:

GGGISRPEY

SEQ ID NO:196 is the heavy chain constant region with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpape aagapsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vsvltvltlhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltclvkgfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:224 is the full-length light chain for AbO:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVTRSA
KGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvtthegstvektvaptecs

Figure 22c

SEQ ID NO:225 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDYCYVTRSA
KGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:185 is the CDR L3 portion of the V_L portion of the full-length light chain:

VTRSAKGNPHVL

SEQ ID NO:198 is the lambda light chain constant region:

*gqpkaapsvtlfppsseelqankatlvclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassy ls l tpeqwkshrsyscqvt he
gstvektvapt e cs*

Figure 23a

AbP:

Heavy chain for AbP (SEQ ID NO:219), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 169, respectively). The heavy chain constant region is shown in lower case italics with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text (SEQ ID NO:196).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSSGYWGWIRQPPGKGL
 EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
 VYYCARGGGISRPEYWGQGT~~LVTVSS~~*astkgpsvfplapsskstsg*
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
*pape***aagap***svflfppkpkdtlmisrtpevtcvvvdvshedpevkf*
nwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykc
kvsnkalspapiektiskakgqprepqv tylppsreemtknqvsltc
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqkslsispkg

Light chain for AbP (SEQ ID NO:226), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 189, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
 IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVSRSA
 KGNPHVLFGGGTQLTVL*gqpkaapsvtlfpssseelqankatlvc*
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylsl
peqwkshrsyscqvt hegstvektvaptecs

SEQ ID NO:219 is the full-length heavy chain for AbP:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSSGYWGWIRQPPGKGL
 EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
 VYYCARGGGISRPEYWGQGT~~LVTVSS~~*astkgpsvfplapsskstsg*
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
*pape***aagap***svflfppkpkdtlmisrtpevtcvvvdvshedpevkf*
nwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykc
kvsnkalspapiektiskakgqprepqv tylppsreemtknqvsltc
lvkgfypsdiavewesngqpennykttpvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqkslsispkg

Figure 23b

SEQ ID NO:6 is the V_H portion of full-length heavy chain:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGLVTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:169 is the CDR H3 portion of the V_H portion of full-length heavy chain:

GGGISRPEY

SEQ ID NO:196 is the heavy chain constant region with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpape aagapsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltclvkgyfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdkstrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:226 is the full-length light chain for AbP:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVSRSA
KGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvt hegstvektvaptecs

Figure 23c

SEQ ID NO:227 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVSRSA
KGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:189 is the CDR L3 portion of the V_L portion of the full-length light chain:

VSRSAKGNPHVL

SEQ ID NO:198 is the lambda light chain constant region:

*gqpkaapsvtlfppsseelqankatlvcclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassylsltpeqwkshrsyscqvtthe
gstvektvaptecs*

Figure 24a

AbU:

Heavy chain for AbU (SEQ ID NO:219), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 169, respectively). The heavy chain constant region is shown in lower case italics with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text (SEQ ID NO:196).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGT~~LV~~TVSSastk~~g~~psvfplapsskstsg
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvhlhqdwlngkeykc
kvsnkaklpapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfyfypsdiavewesngqpennykttppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls~~l~~spgk

Light chain for AbU (SEQ ID NO:228), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 193, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGD~~SL~~R~~TY~~YASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAED~~EAD~~YYCTTRSN
KGNPHVLFGGGTQLTVLgqpkaapsvtlfpssseelqankatlvc
lsdfypgavtvawkadsspvkagvetttpskqsnnkyaassylsl
peqwkshrsyscqvthegstvektvaptecs

SEQ ID NO:219 is the full-length heavy chain for AbU:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGT~~LV~~TVSSastk~~g~~psvfplapsskstsg
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
papeaagapsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf
nwyvdgvevhnaktkpreeqynstyrvvsvltvhlhqdwlngkeykc
kvsnkaklpapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfyfypsdiavewesngqpennykttppvldsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls~~l~~spgk

Figure 24b

SEQ ID NO:6 is the V_H portion of full-length heavy chain:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARGGGISRPEYWGQGLVTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:169 is the CDR H3 portion of the V_H portion of full-length heavy chain:

GGGISRPEY

SEQ ID NO:196 is the heavy chain constant region with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpape aagapsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vvsvltvlnhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltclvkgfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:228 is the full-length light chain for AbU:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCTTRSN
KGNPHVLFGGGTQLTVLgqpkaapsvtlfppsseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvt hegstvektvaptecs

Figure 24c

SEQ ID NO:229 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCTTRSN
KGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

GKHKRPS

SEQ ID NO:193 is the CDR L3 portion of the V_L portion of the full-length light chain:

TTRSNKGNPHVL

SEQ ID NO:198 is the lambda light chain constant region:

*gqpkaapsvtlfppsseelqankatlvcclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassyisltpeqwkshrsyscqvtthe
gstvektvaptecs*

Figure 25a

AbV:

Heavy chain for AbV (SEQ ID NO:218), with CDRs H1, H2, and H3 underlined (SEQ ID NOs:163, 164, and 165, respectively). The heavy chain constant region is shown in lower case italics with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text (SEQ ID NO:196).

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIROPKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGT¹⁶³LVTVSS¹⁶⁴*astkgpsvfplapsskstsg*
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
*pape***aag***apsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf*
nwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykc
kvsnkalspapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfypsdiavewesngqpennykttppvl¹⁶⁵dsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls¹⁹⁶spgkk

Light chain for AbV (SEQ ID NO:214), with CDRs L1, L2, and L3 underlined (SEQ ID NOs:194, 195, and 172, respectively). The constant lambda light chain sequence is indicated by lower-case italics (SEQ ID NO:198).

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLR
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDADYYCVARSV
VGNPHVLFGGGTQLTVL*gqpkaapsvtlfpssseelqankatlvcl*
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvthegstvektvaptecs

SEQ ID NO:218 is the full-length heavy chain for AbV:

QVQLQESGPGLVKPSETLSLTCAVSGYSISSGYYWGWIROPKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGT¹⁶³LVTVSS¹⁶⁴*astkgpsvfplapsskstsg*
gtaalgclvkdyfpepvtvswngaltsgvhtfpavlqssglysls
svvtvpssslgtqtyicnvnhkpsntkvdkkvepkscdkthtcppc
*pape***aag***apsvflfppkpkdtlmisrtpevtcvvvdvshedpevkf*
nwyvdgvevhnaktkpreeqynstyrvsvltvlhqdwlngkeykc
kvsnkalspapiektiskakgqprepqvytlppsreemtknqvsltc
lvkgfypsdiavewesngqpennykttppvl¹⁶⁵dsdgsfflyskltvd
ksrwqqgnvfscsvmhealhnhytqksls¹⁹⁶spgk

Figure 25b

SEQ ID NO:14 is the V_H portion of the full-length heavy chain:

QVQLQESGPGGLVKPSETLSLTCAVSGYSISSGYYWGWIRQPPGKGL
EWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTA
VYYCARFMGFGRPEYWGQGTLLTVSS

SEQ ID NO:163 is the CDR H1 portion of the V_H portion of the full-length heavy chain:

SGYYWG

SEQ ID NO:164 is the CDR H2 portion of the V_H portion of the full-length heavy chain:

SISHTGNTYYNPPLKS

SEQ ID NO:165 is the CDR H3 portion of the V_H portion of the full-length heavy chain:

FMGFGRPEY

SEQ ID NO:196 is the heavy chain constant region with the triple mutation (L234A L235A G237A) shown in bold lowercase underlined text:

*astkgpsvfplapsskstsggtaalgclvkdyfpepvtvswns gal
tsgvhtfpavlqssglyslssvvtvpssslgtqtyicnvnhkpsnt
kvdkkvepkscdkthtcppcpape **aag**apsvflfppkpkdtlmisr
tpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyr
vsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepq
vytlppsreemtknqvsltclvkgyfypsdiavewesngqpennykt
tppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqk
slslspgk*

SEQ ID NO:214 is the full-length light chain for AbV:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVARSV
VGNPHVLFGGGTQLTVLgqpkaapsvtlfppsseelqankatlvc l
isdfypgavtvawkadsspvkagvetttpskqsnnkyaassylslt
peqwkshrsyscqvt hegstvektvaptecs

Figure 25c

SEQ ID NO:215 is the V_L portion of the full-length light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRTYYASWYQQKPGQAPVLV
IYGKHKRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCVARSV
VGNPHVLFGGGTQLTVL

SEQ ID NO:194 is the CDR L1 portion of the V_L portion of the full-length light chain:

QGDSLRTYYAS

SEQ ID NO:195 is the CDR L2 portion of the V_L portion of the full-length light chain:

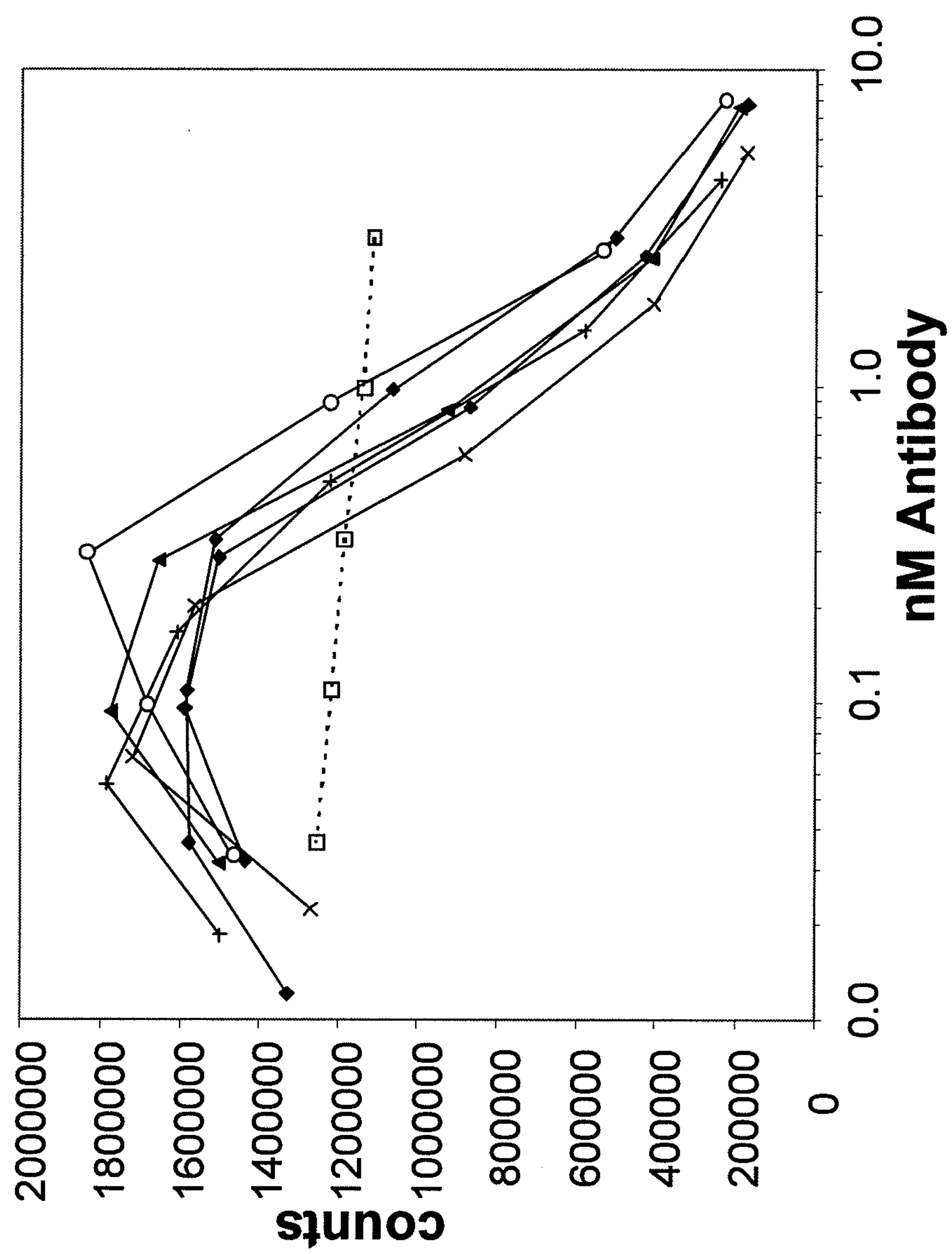
GKHKRPS

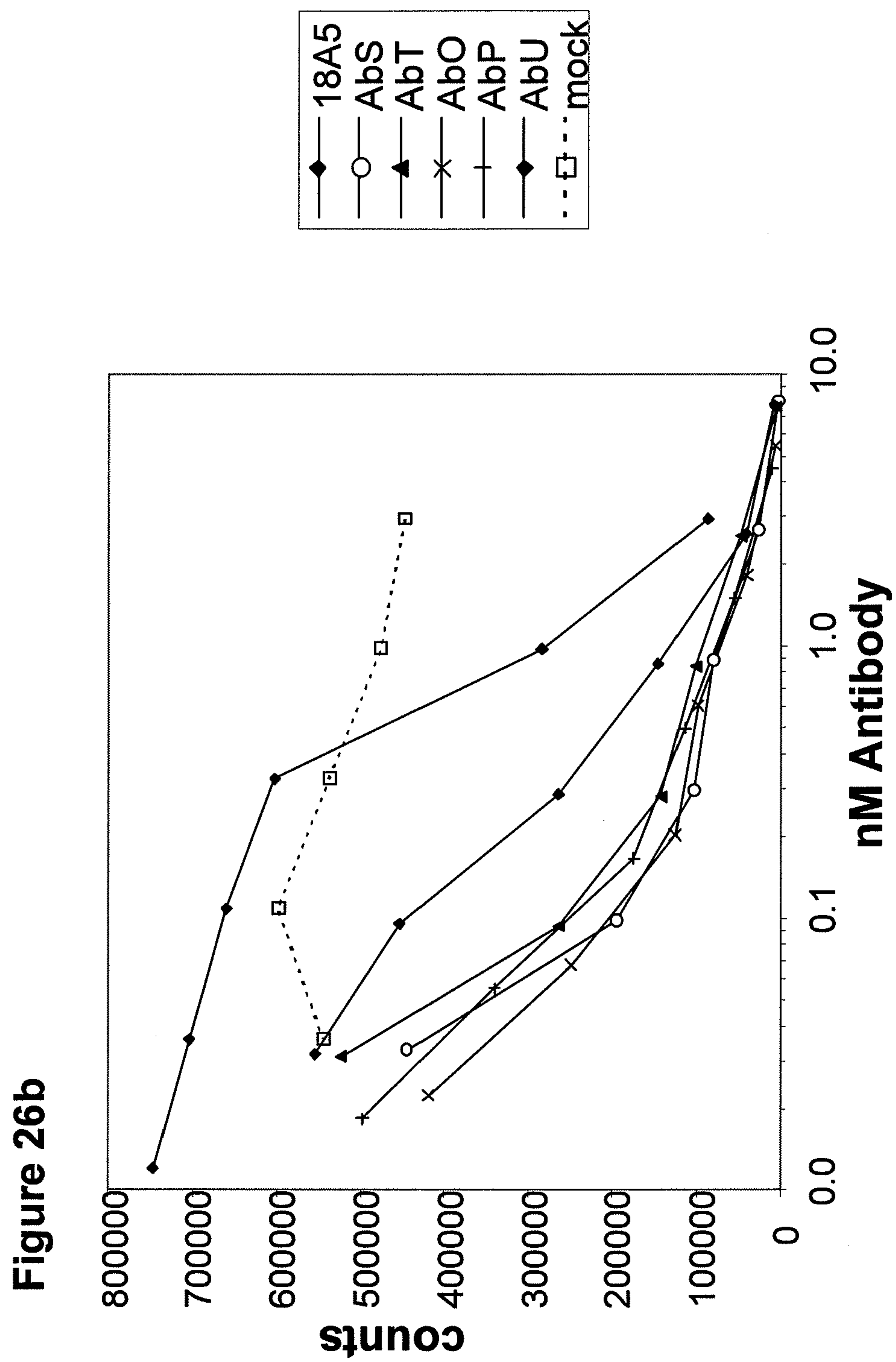
SEQ ID NO:172 is the CDR L3 portion of the V_L portion of the full-length light chain:

VARSVVGNPHVL

SEQ ID NO:198 is the lambda light chain constant region:

*gqpkaapsvtlfppsseelqankatlvclisdfypgavtvawkads
spvkagvetttpskqsnnkyaassy ls l tpeqwks hrsyscqvt he
gstvektvaptecs*

Figure 26a



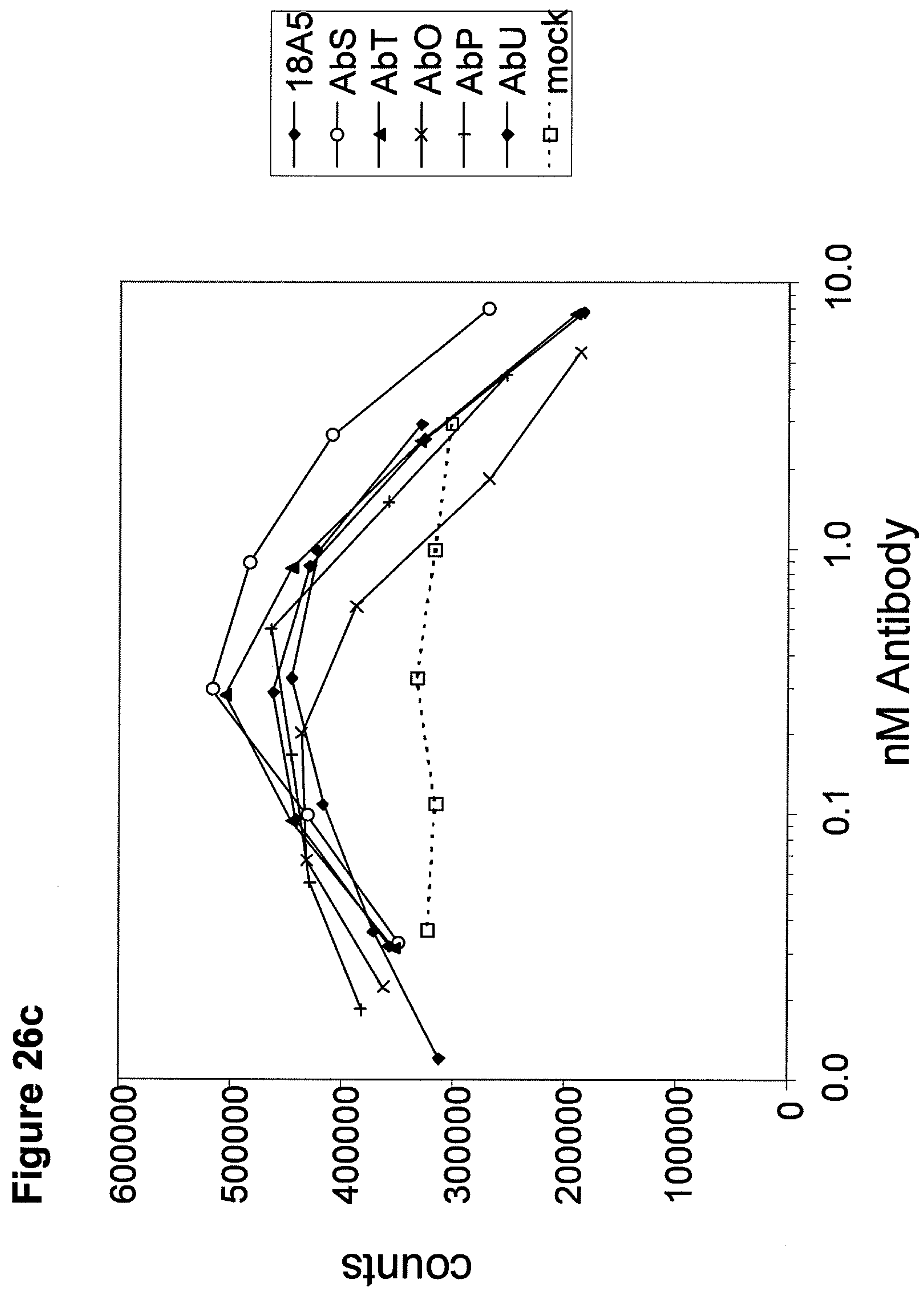


Figure 26d

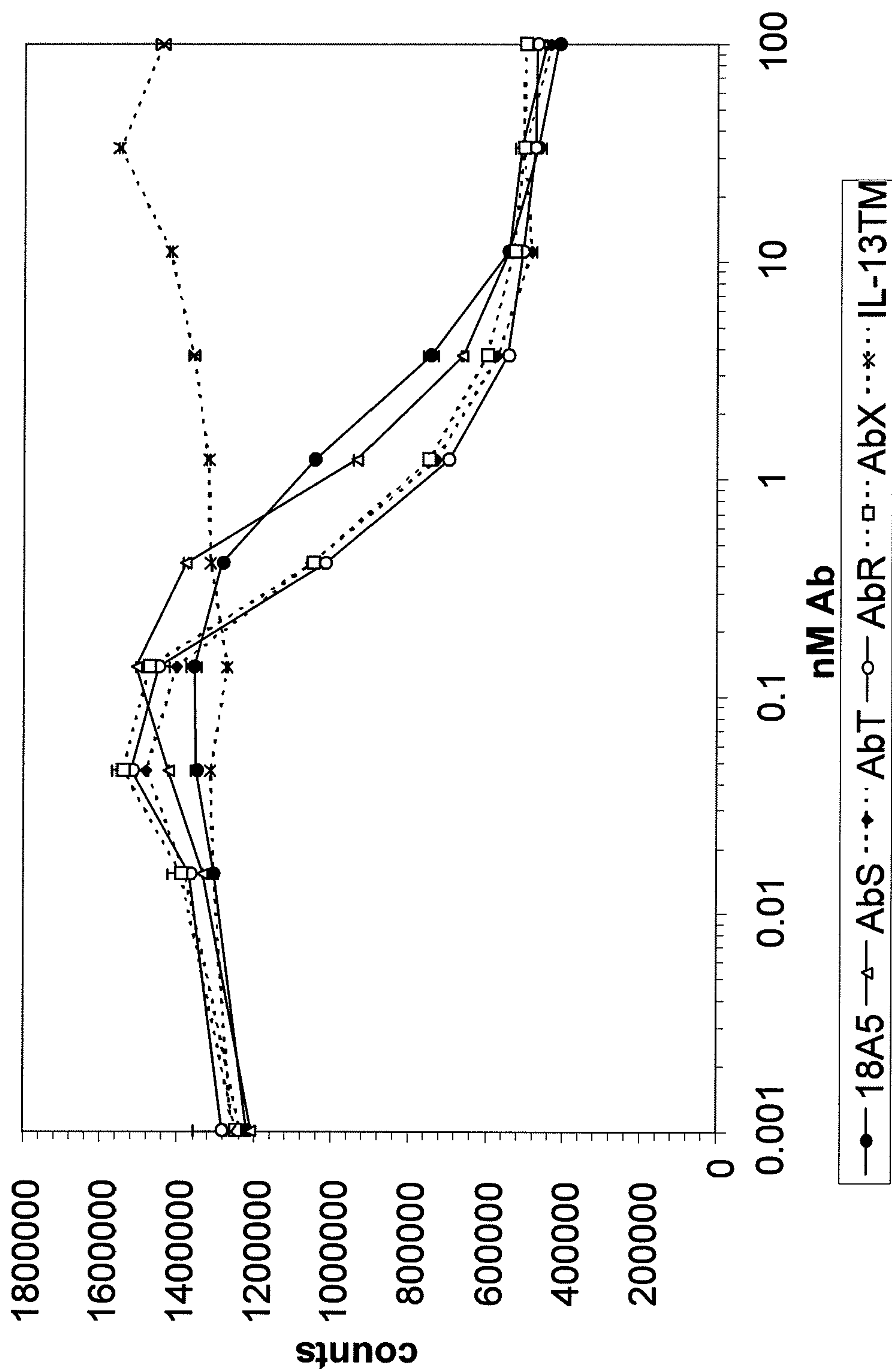
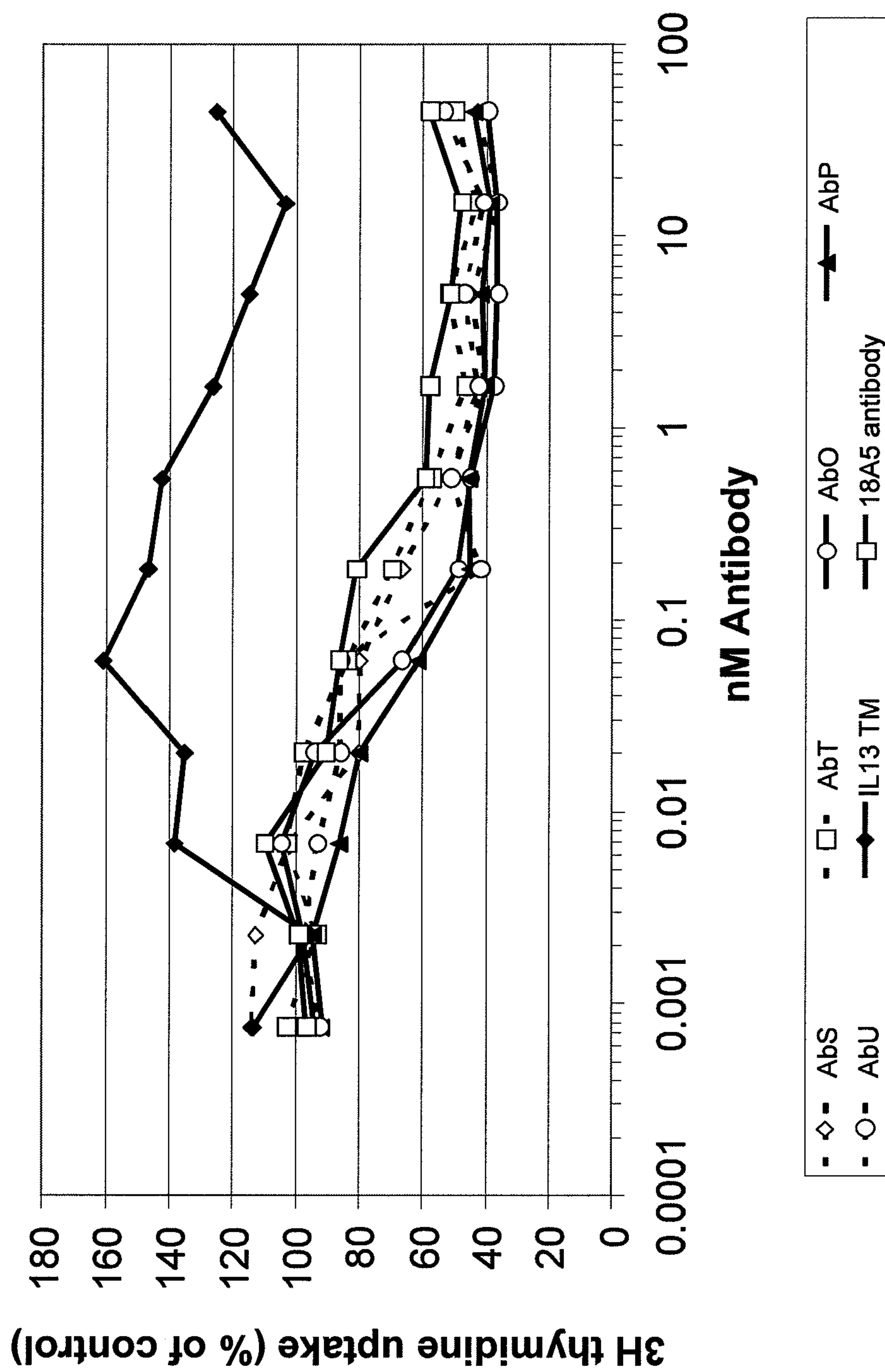


Figure 26e



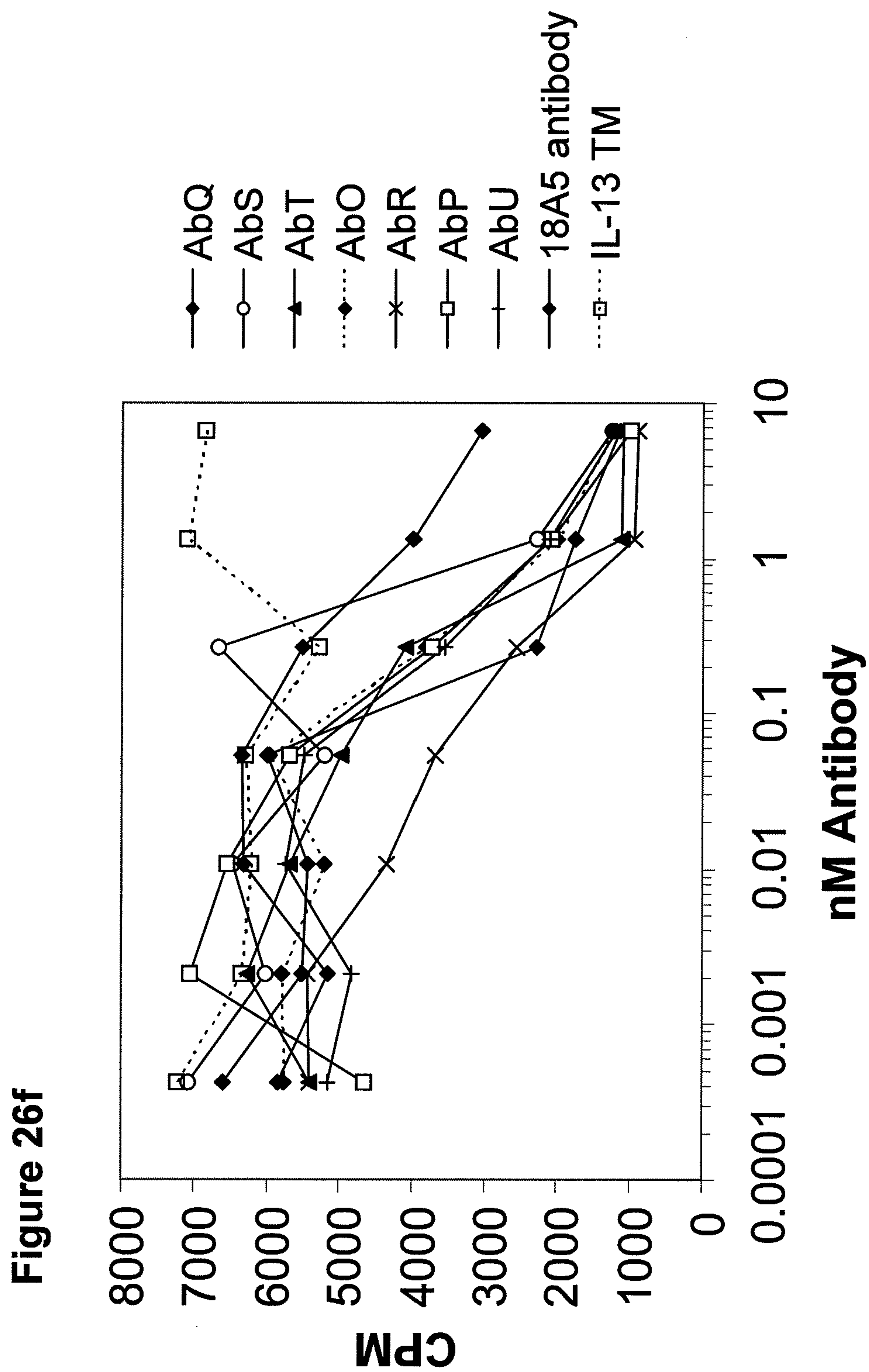


Figure 26g

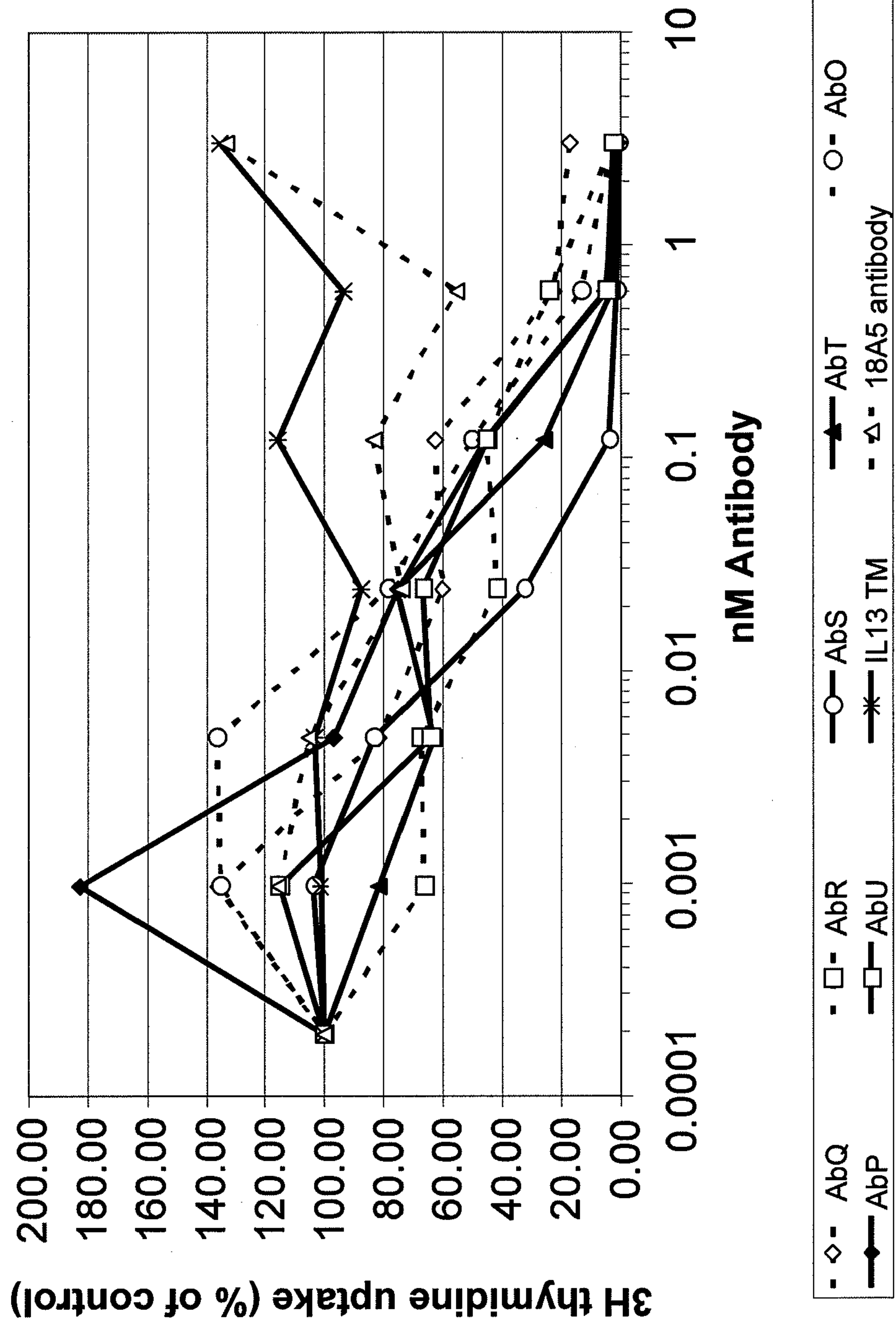


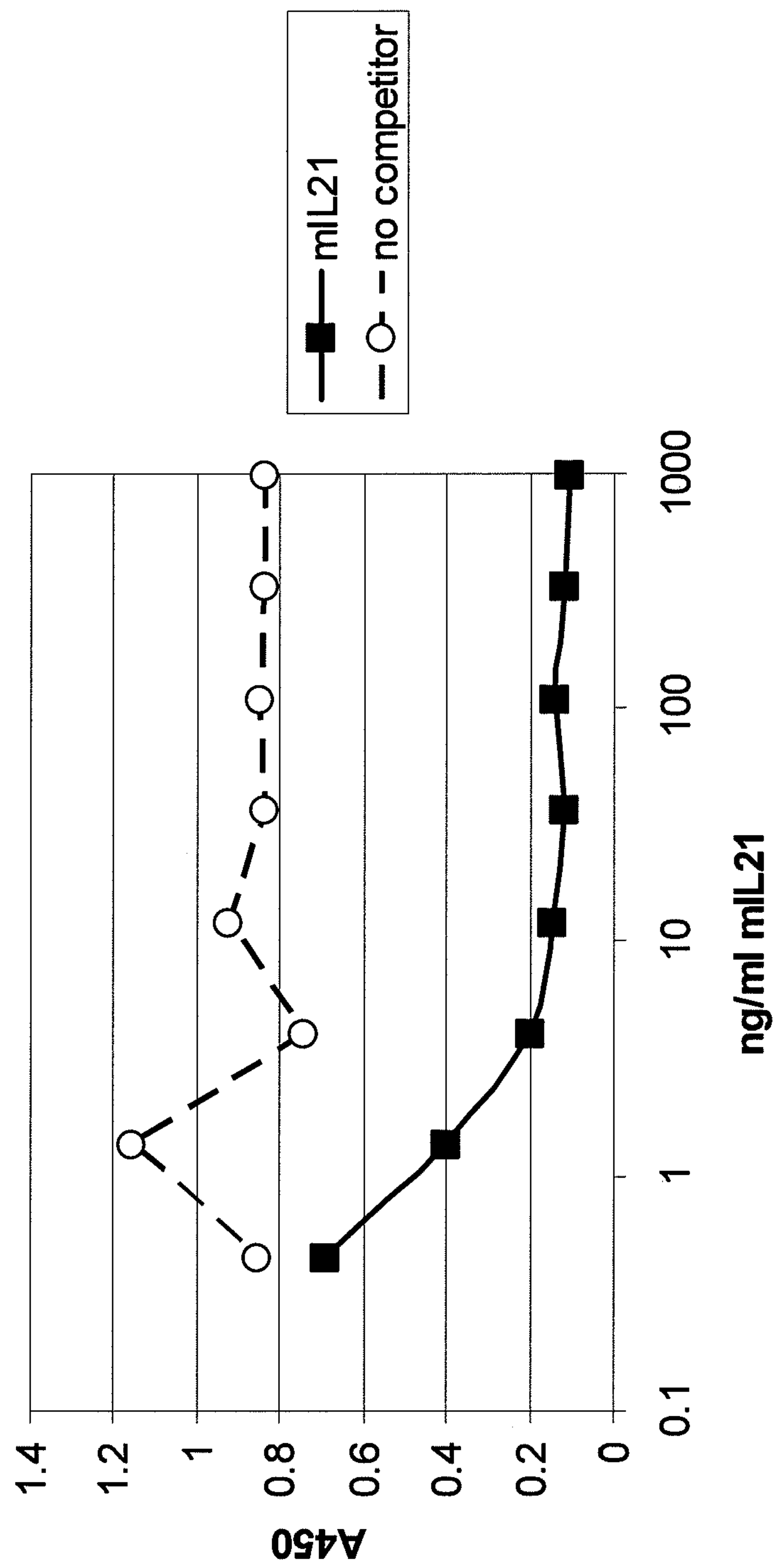
Figure 27a

Figure 27b

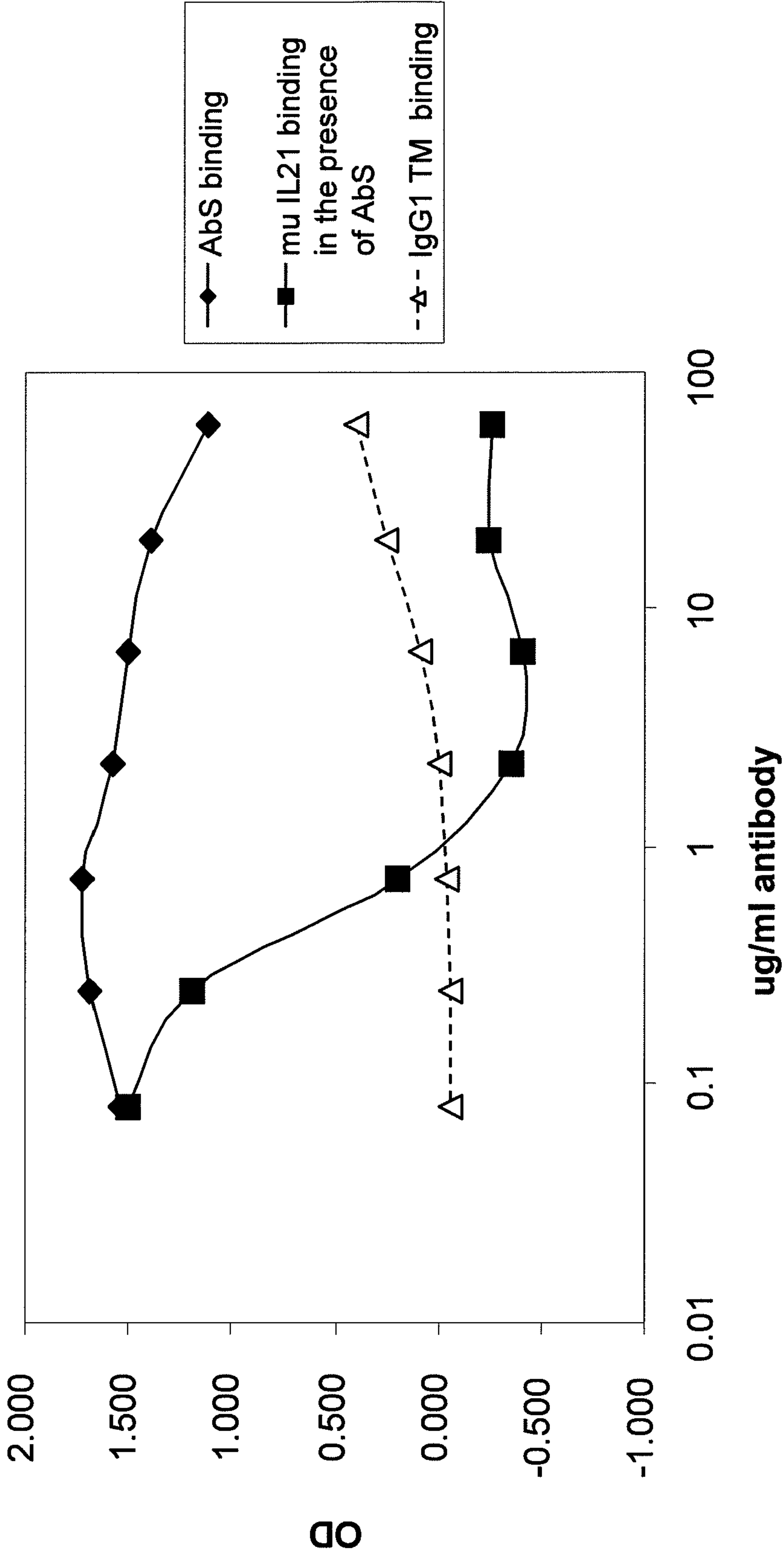


Figure 28

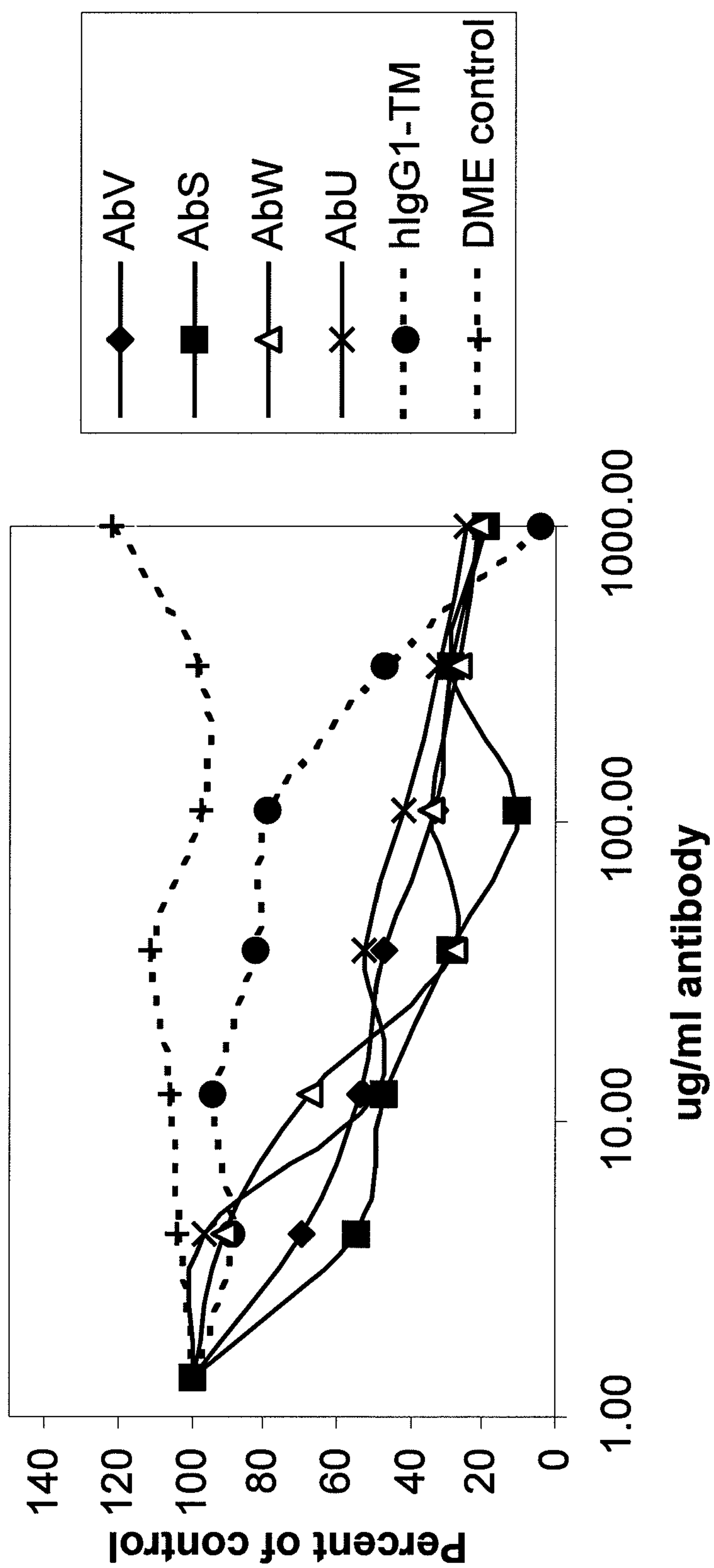


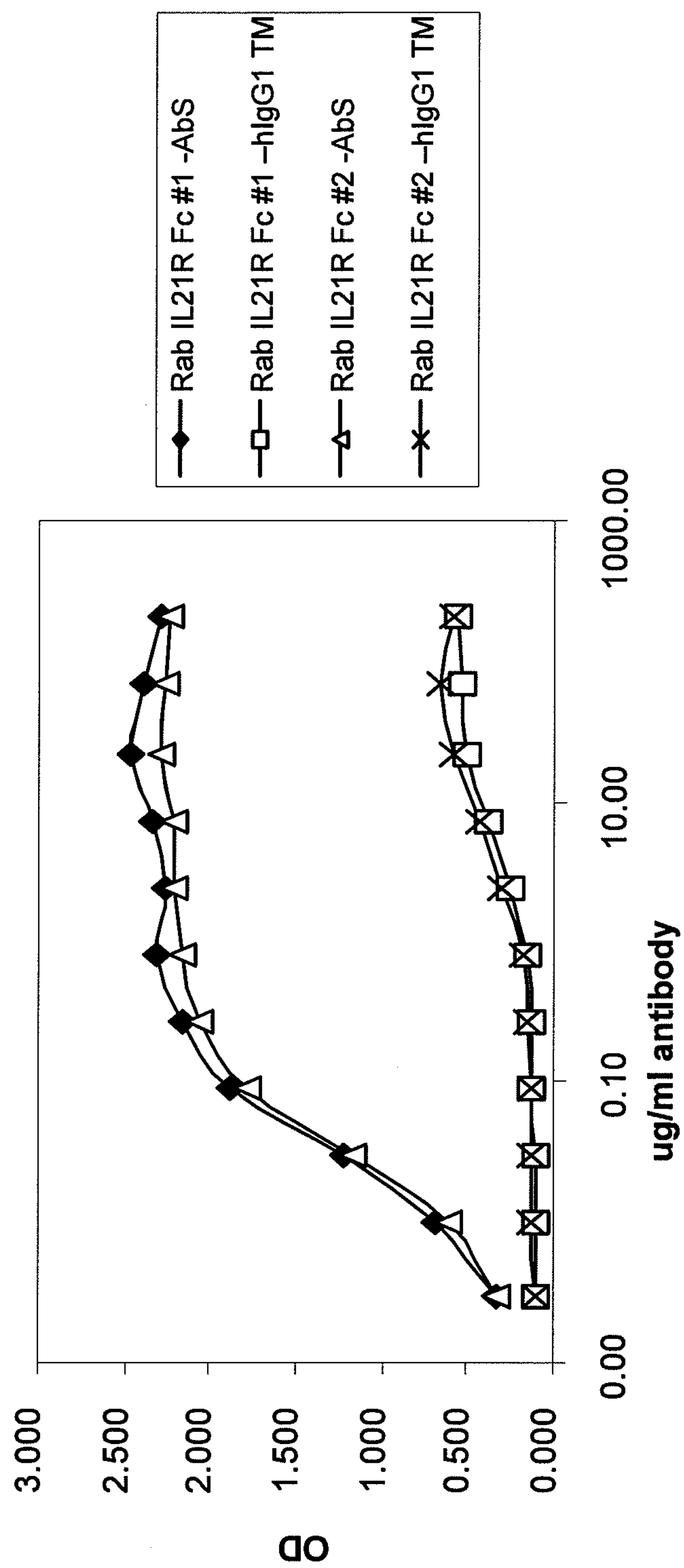
Figure 29a

Figure 29b

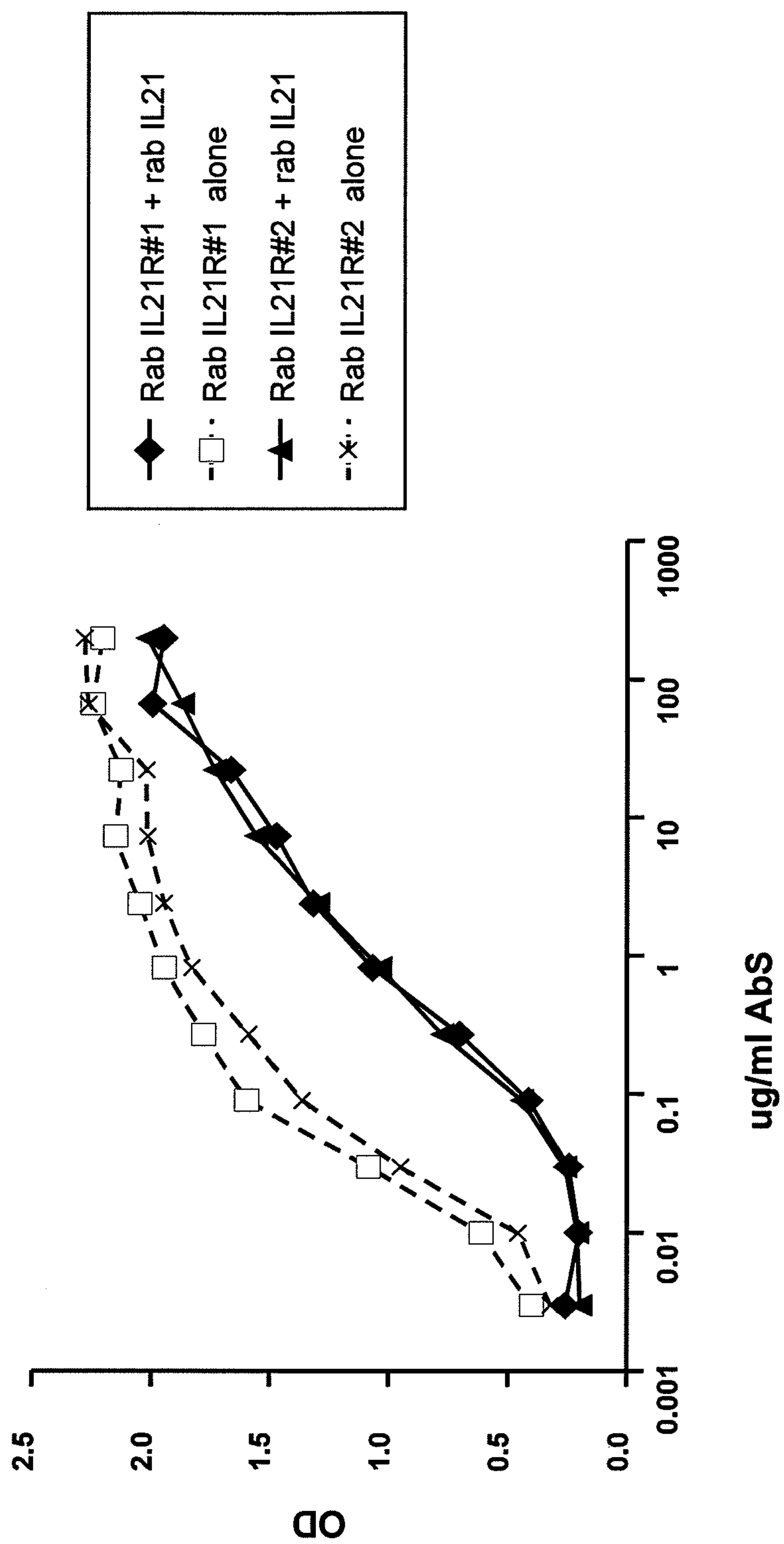


Figure 30a

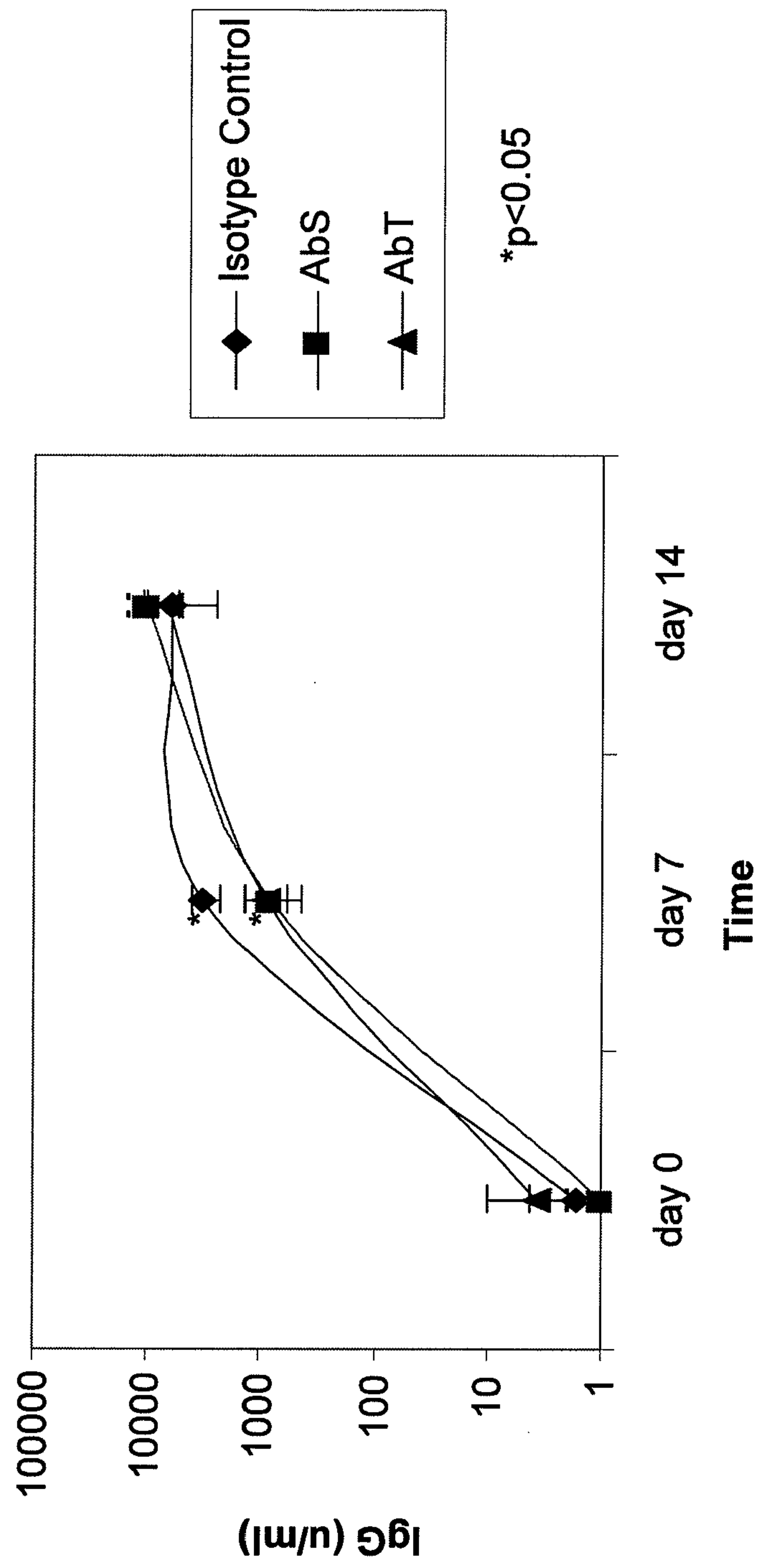


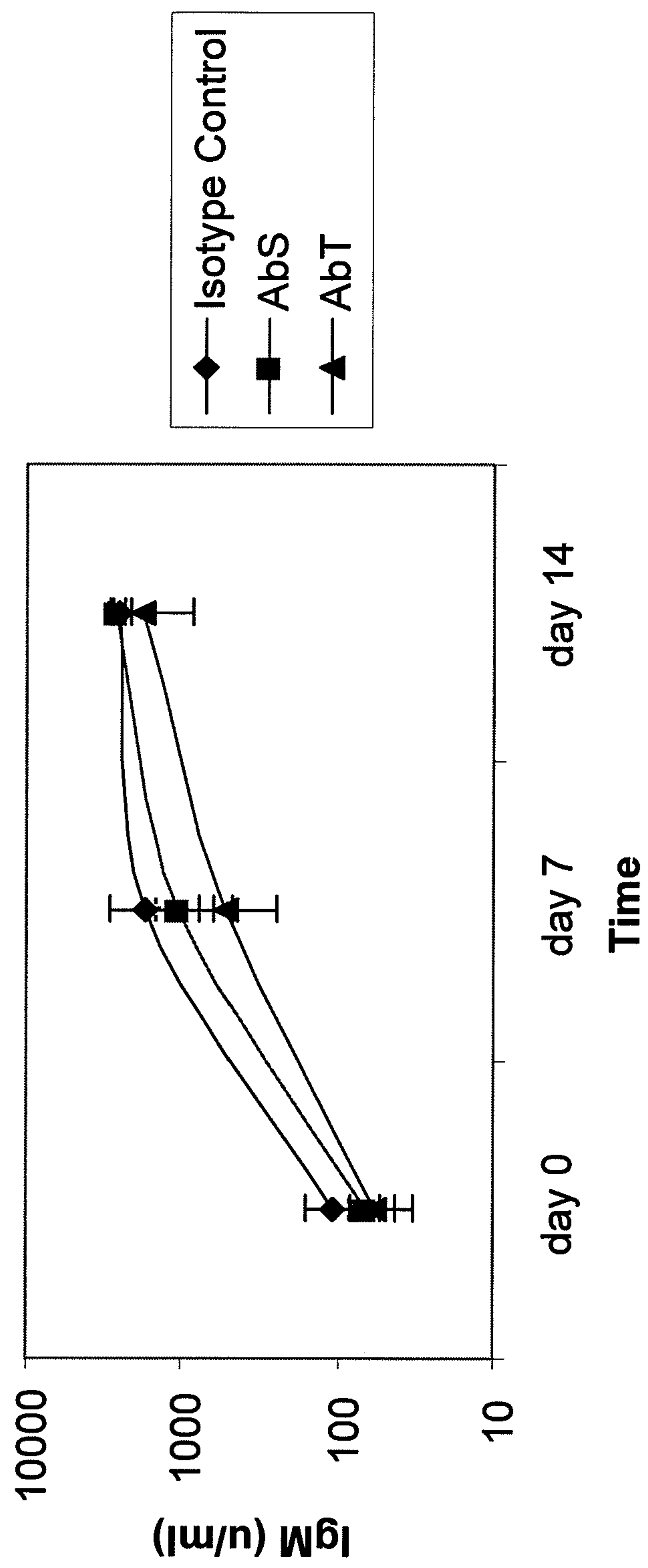
Figure 30b

Figure 30c

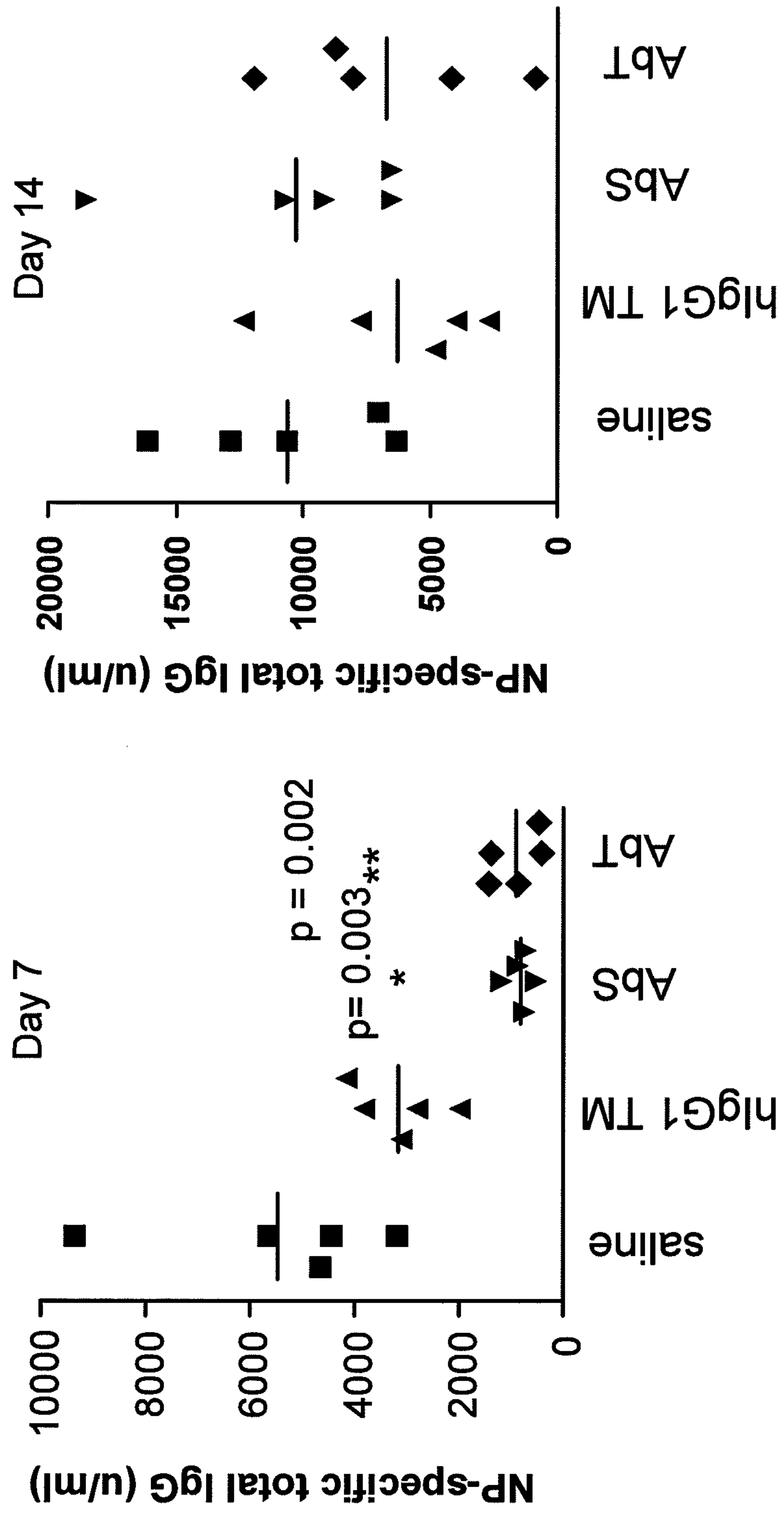


Figure 30d

Figure 30e

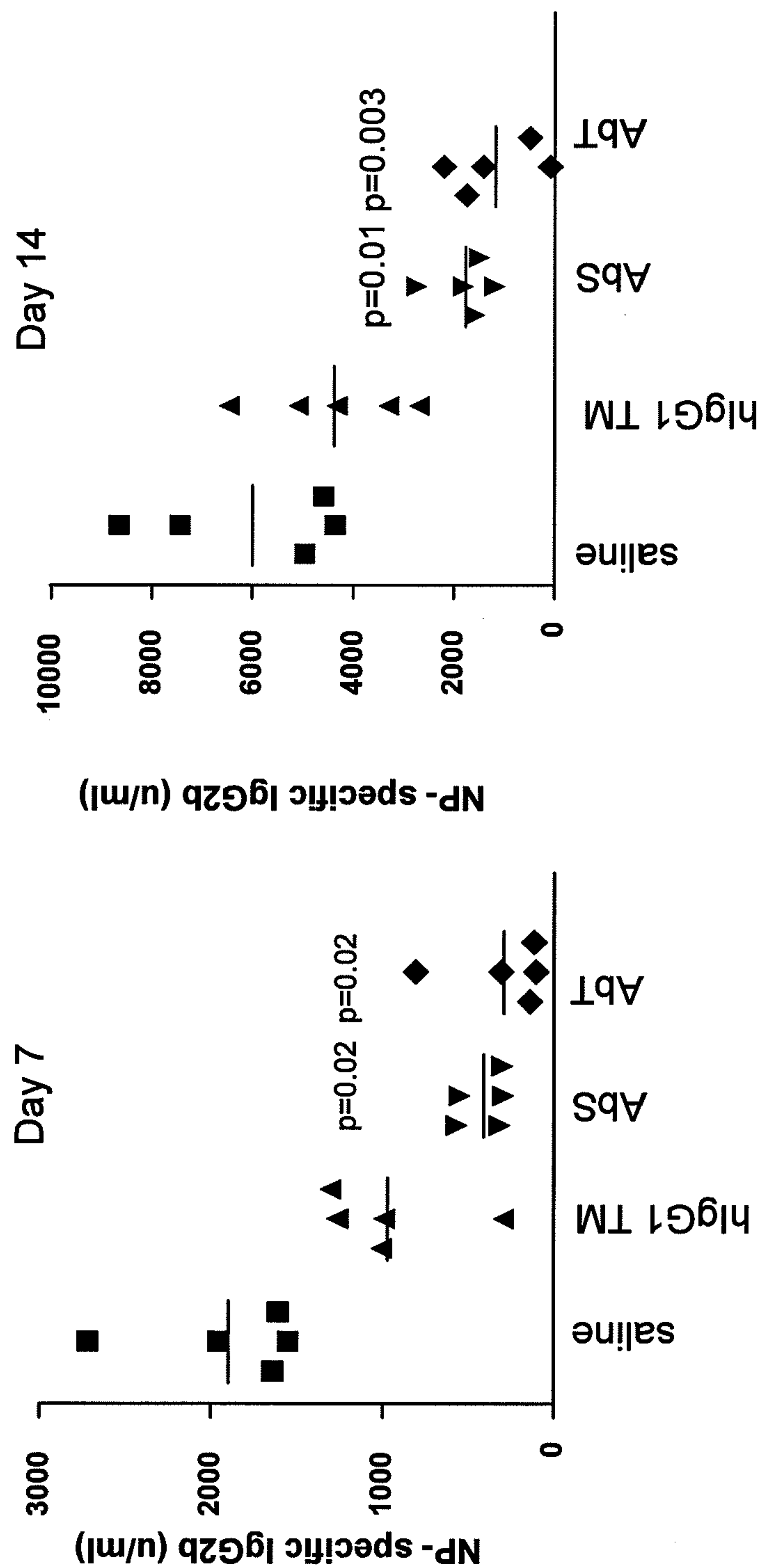


Figure 30f

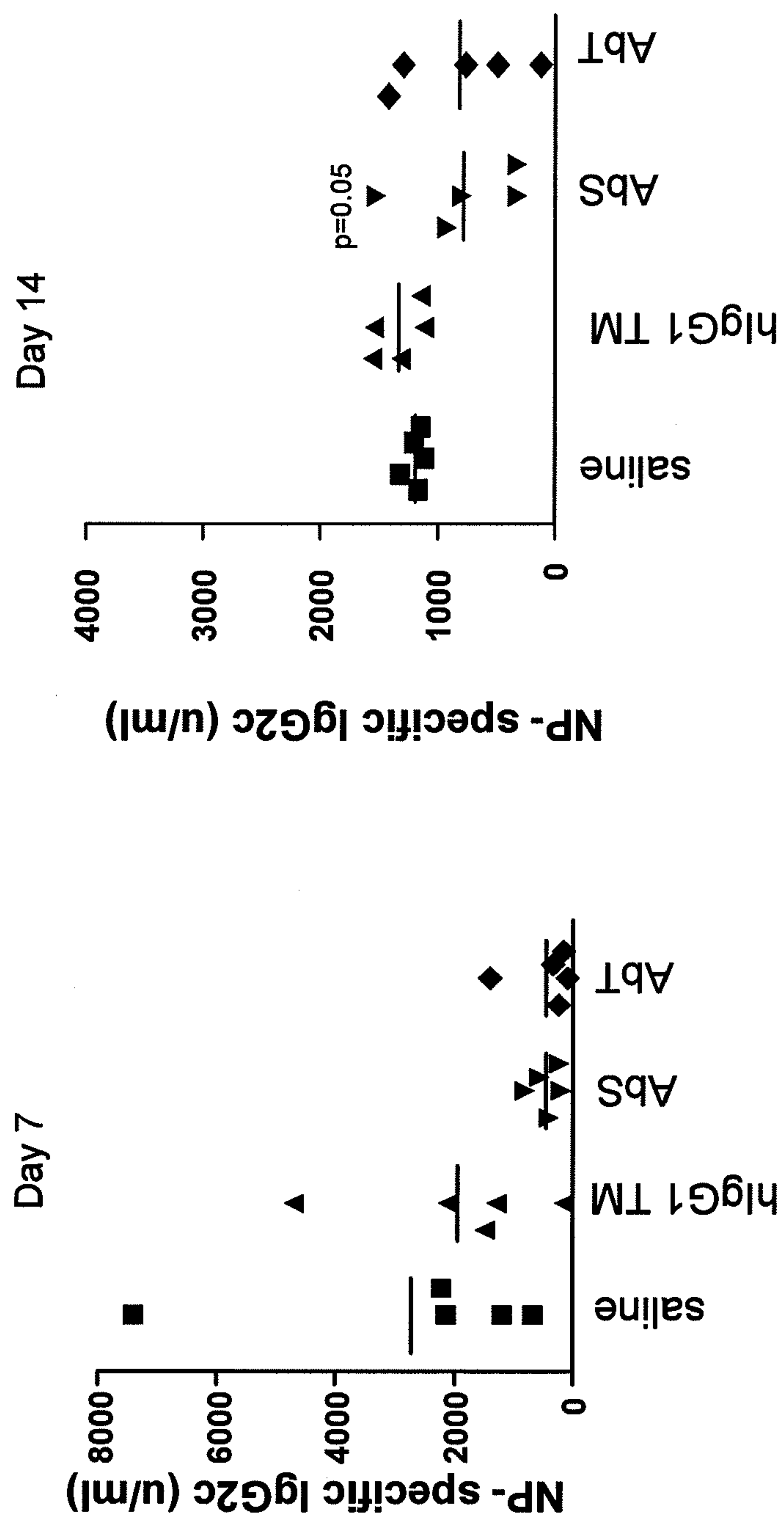
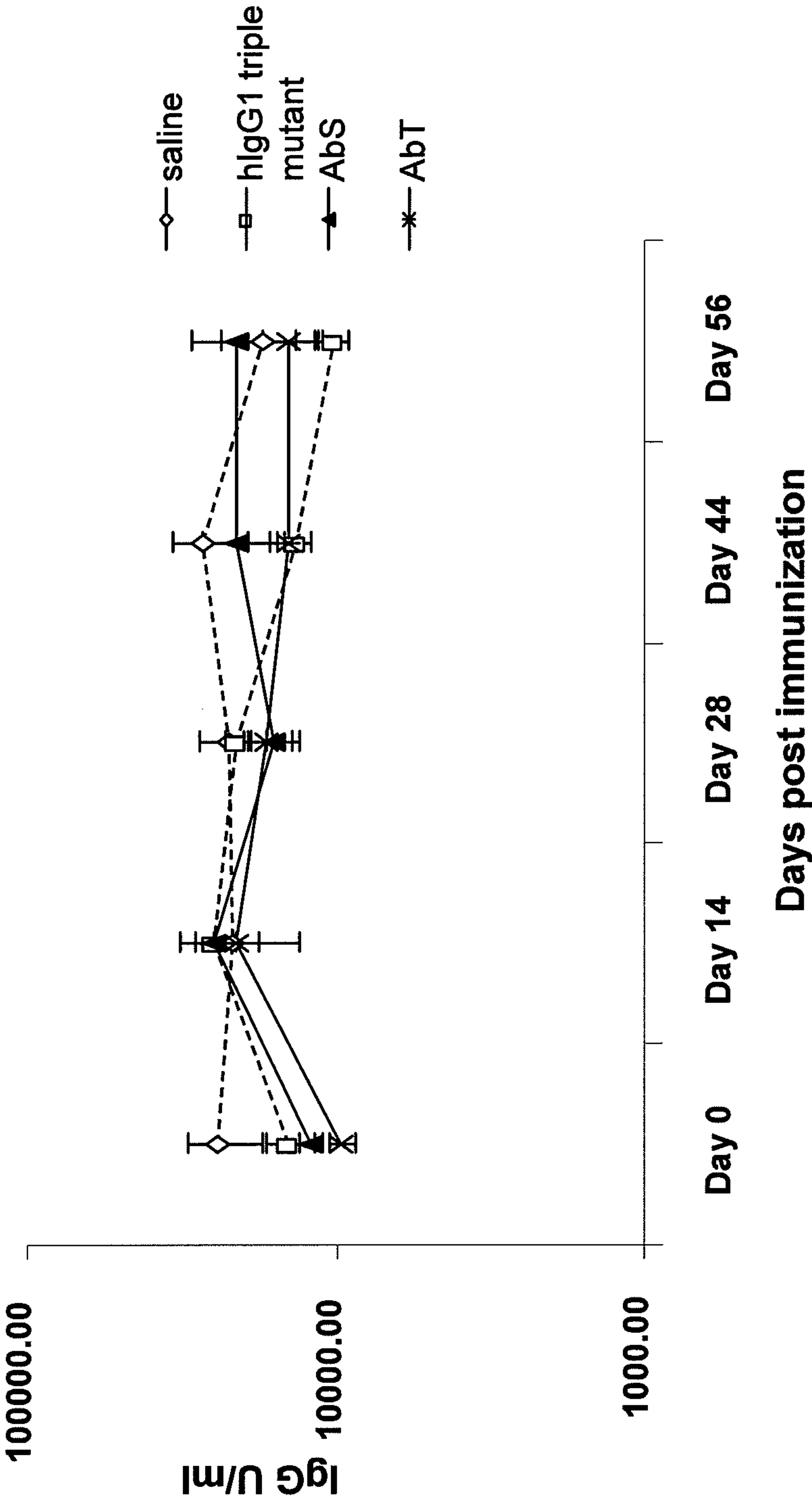


Figure 30g



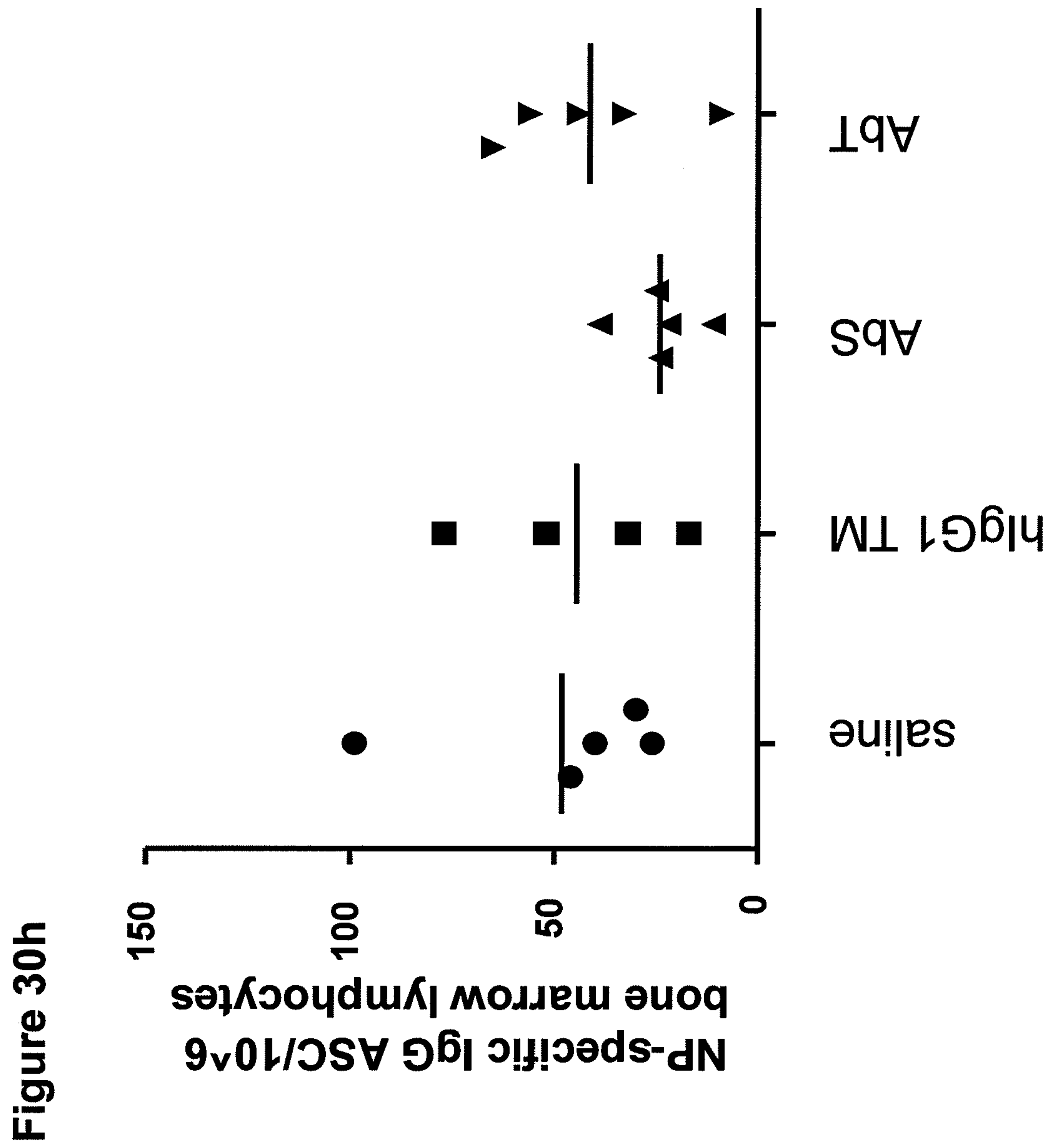


Figure 31a

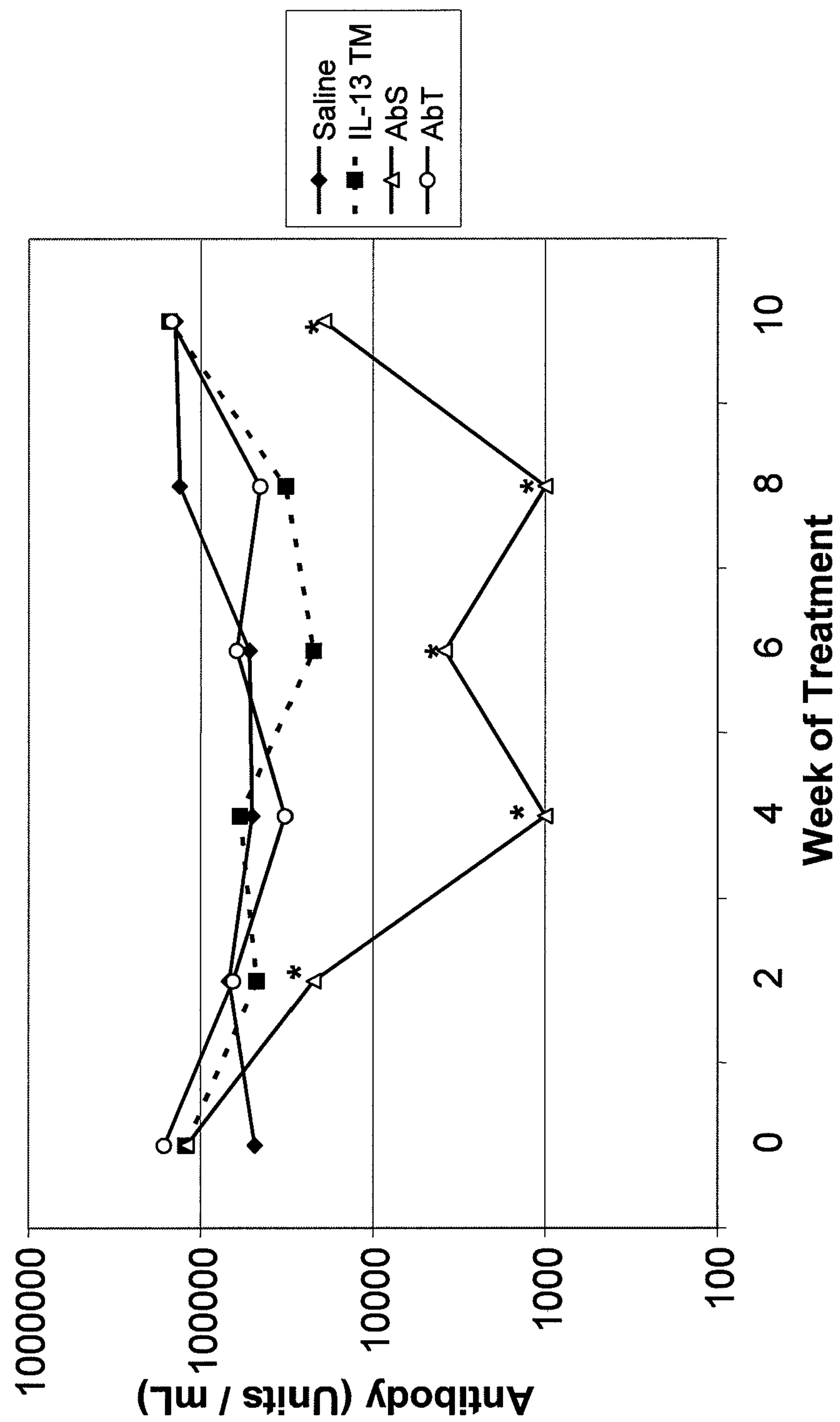


Figure 31b

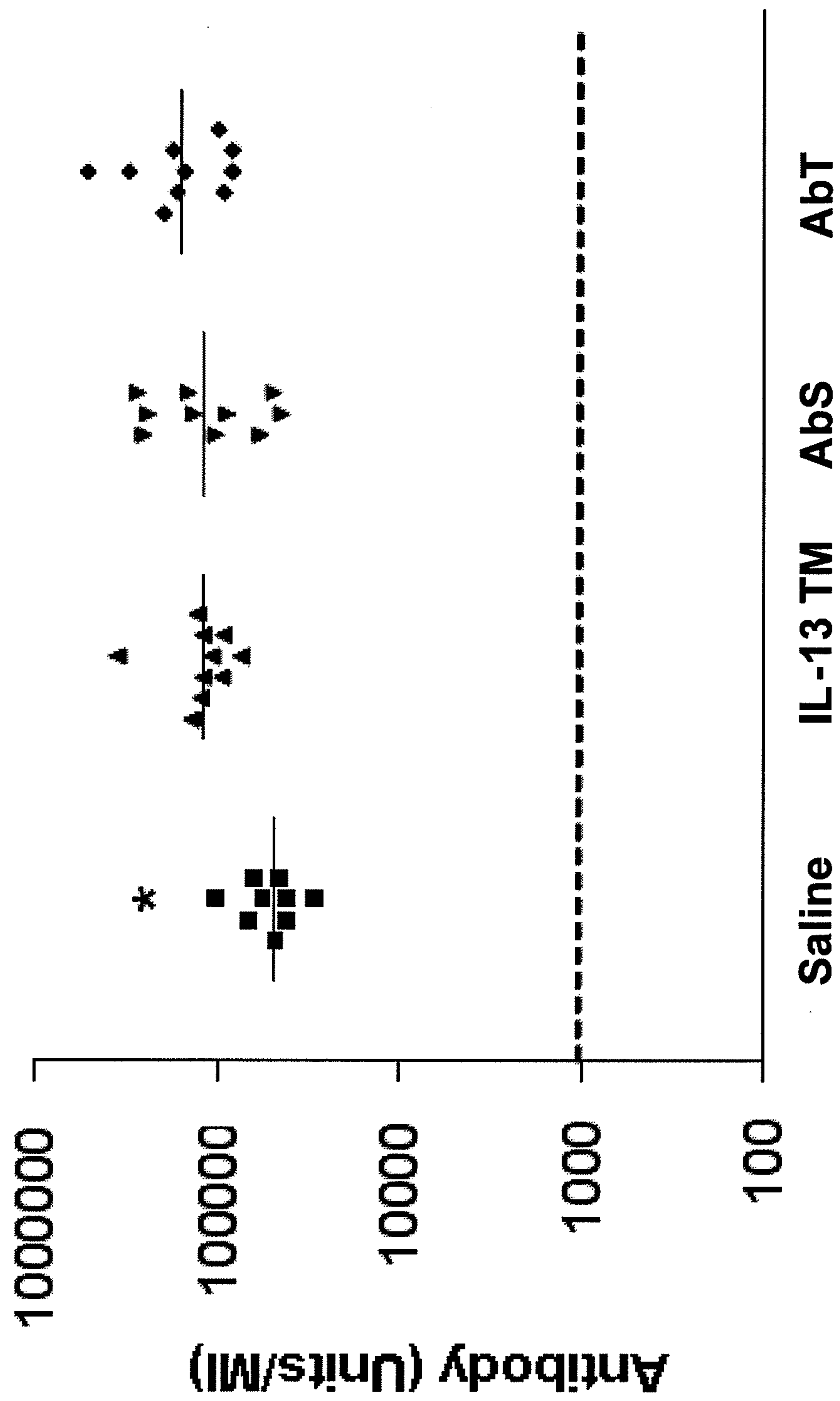
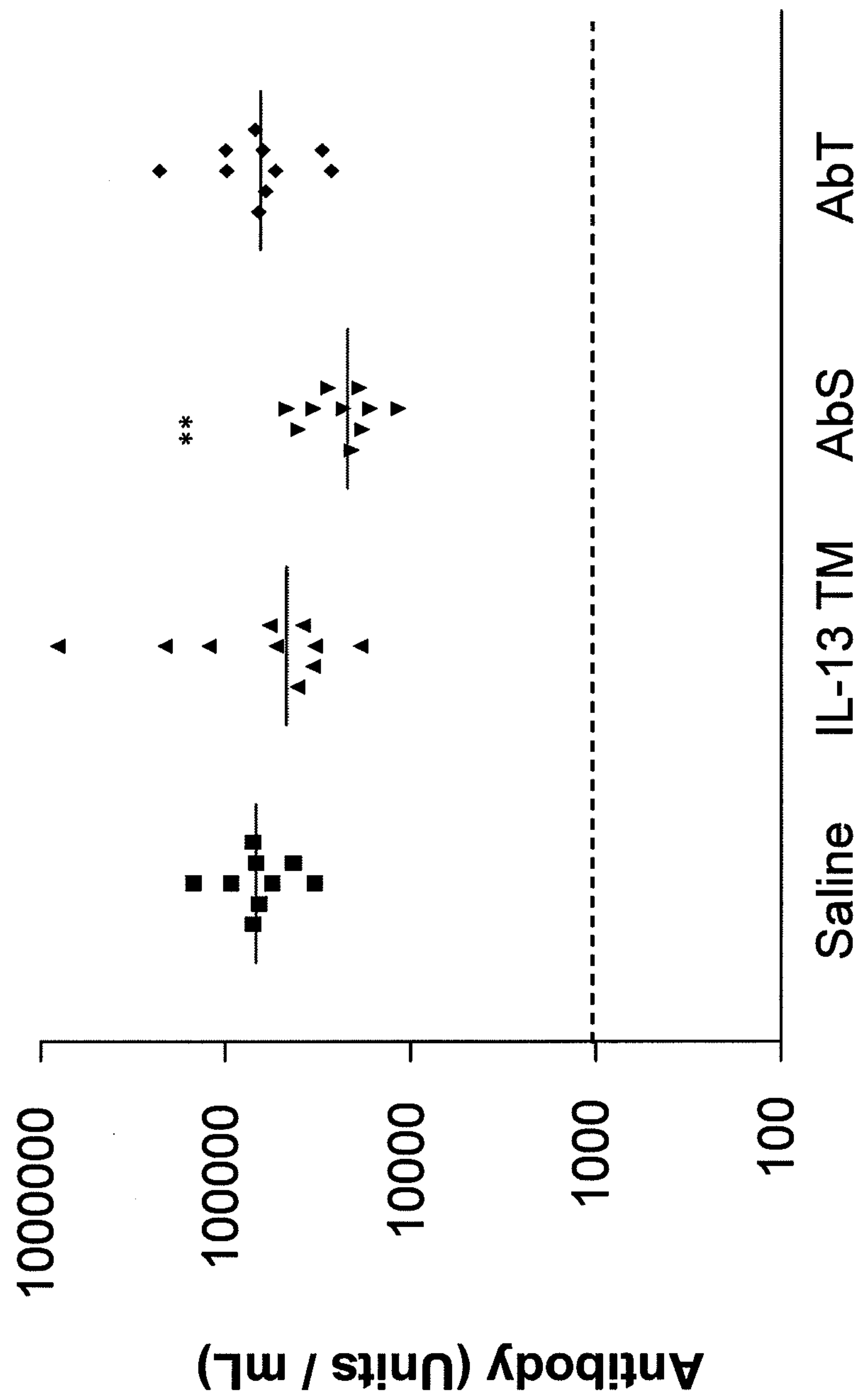


Figure 31c



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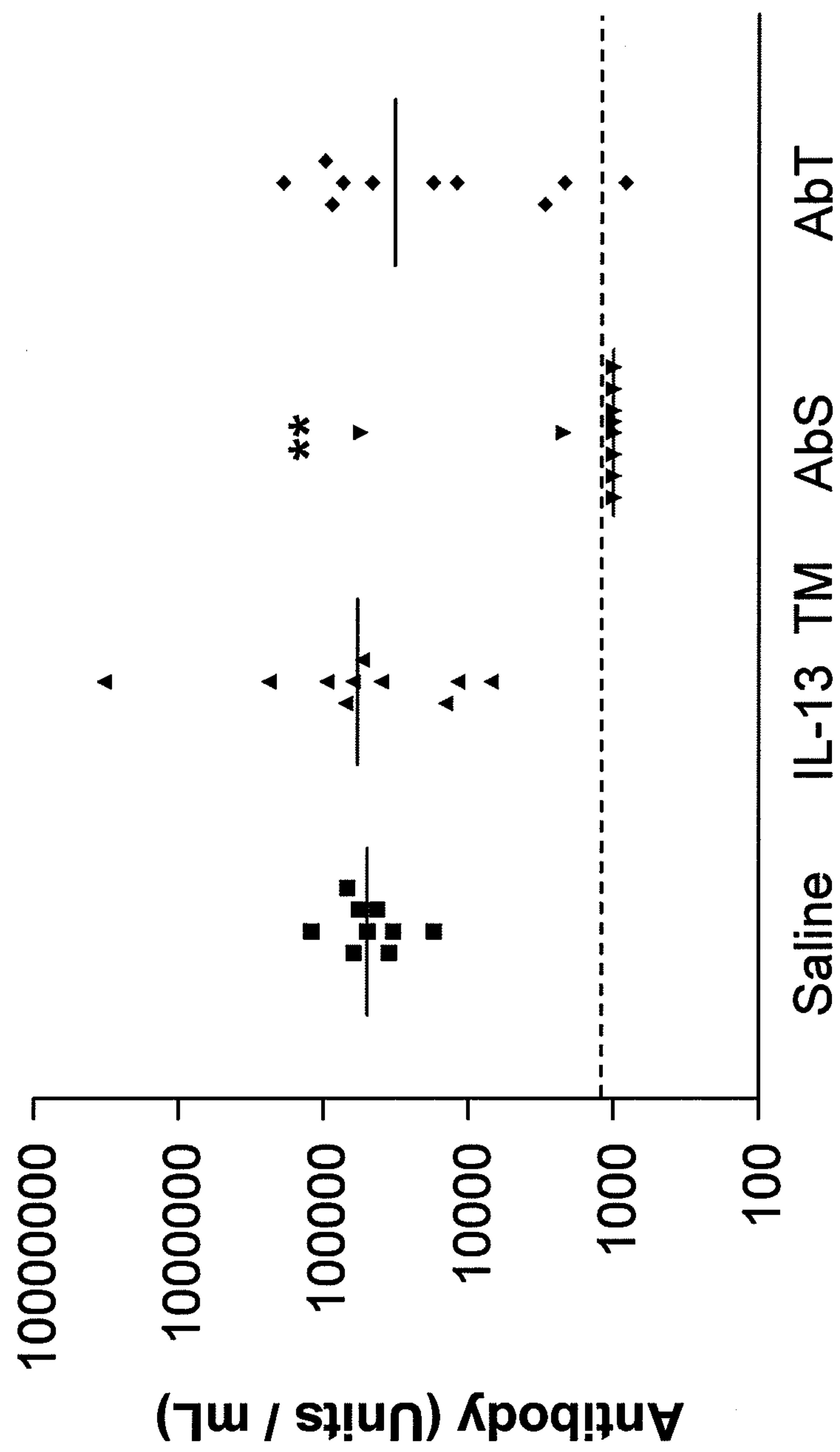
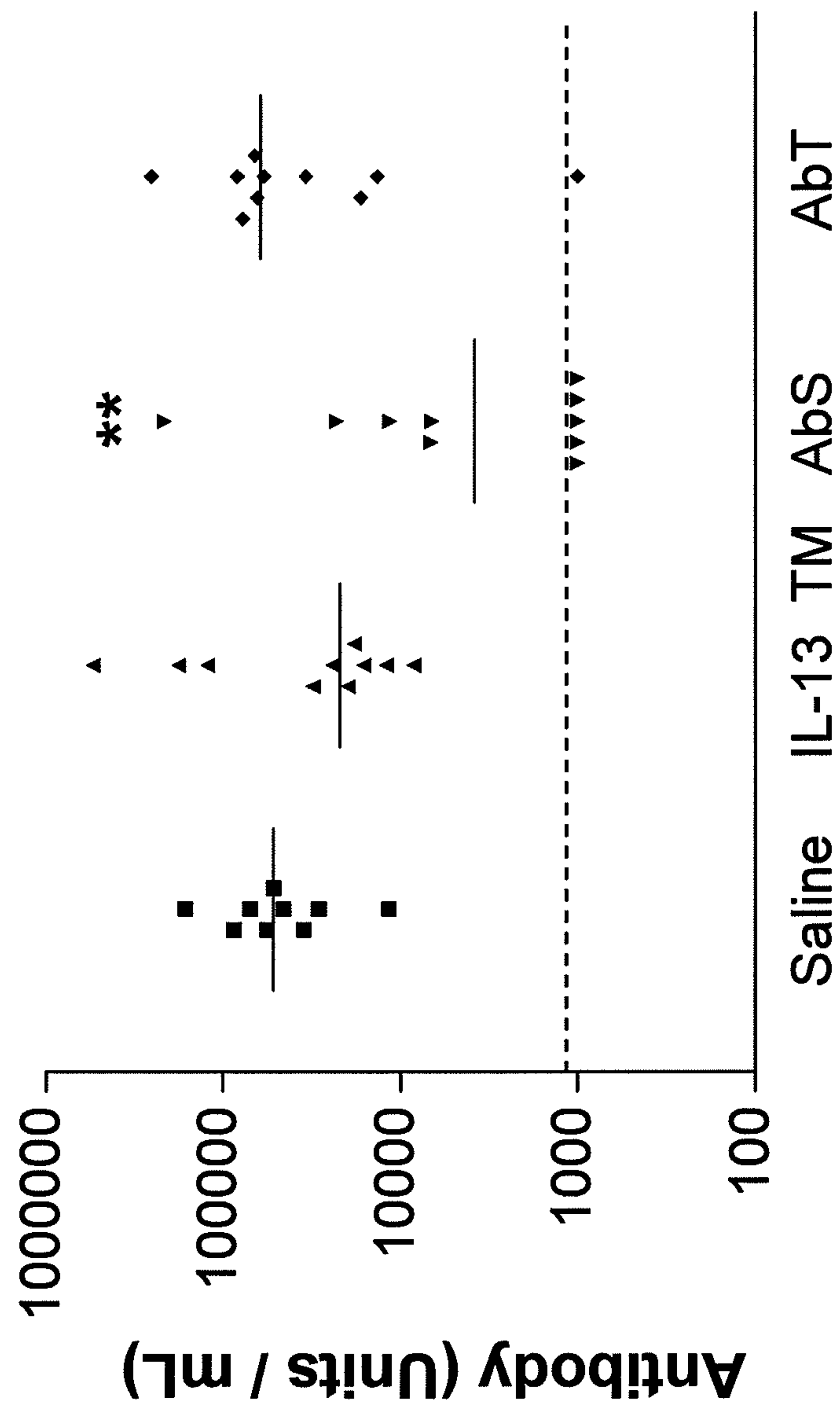
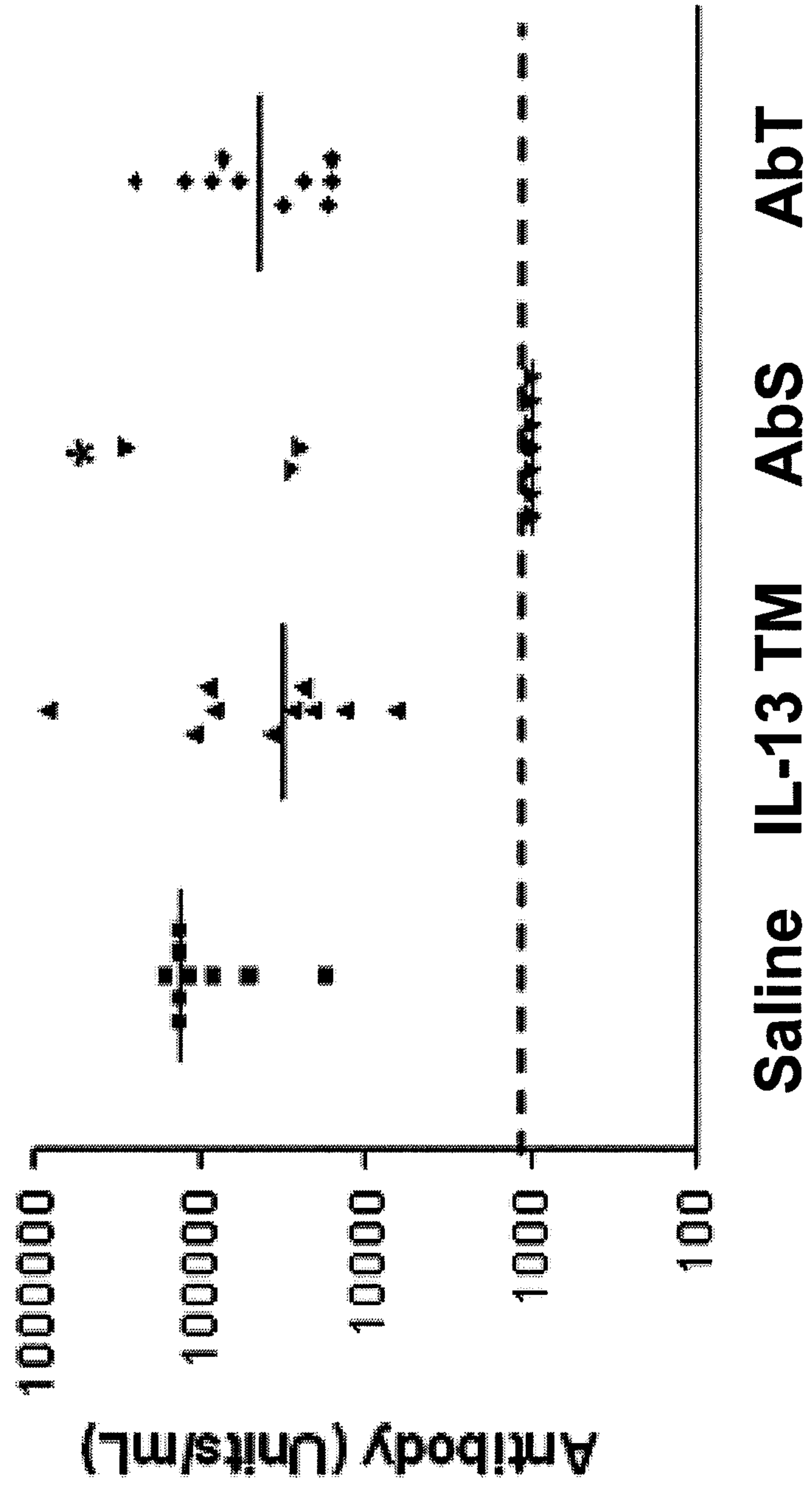


Figure 31e



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Antibody (Units / mL)

Saline IL-13 TM AbS AbT

10000000
1000000
100000
10000
1000
100

**
*

Figure 32a

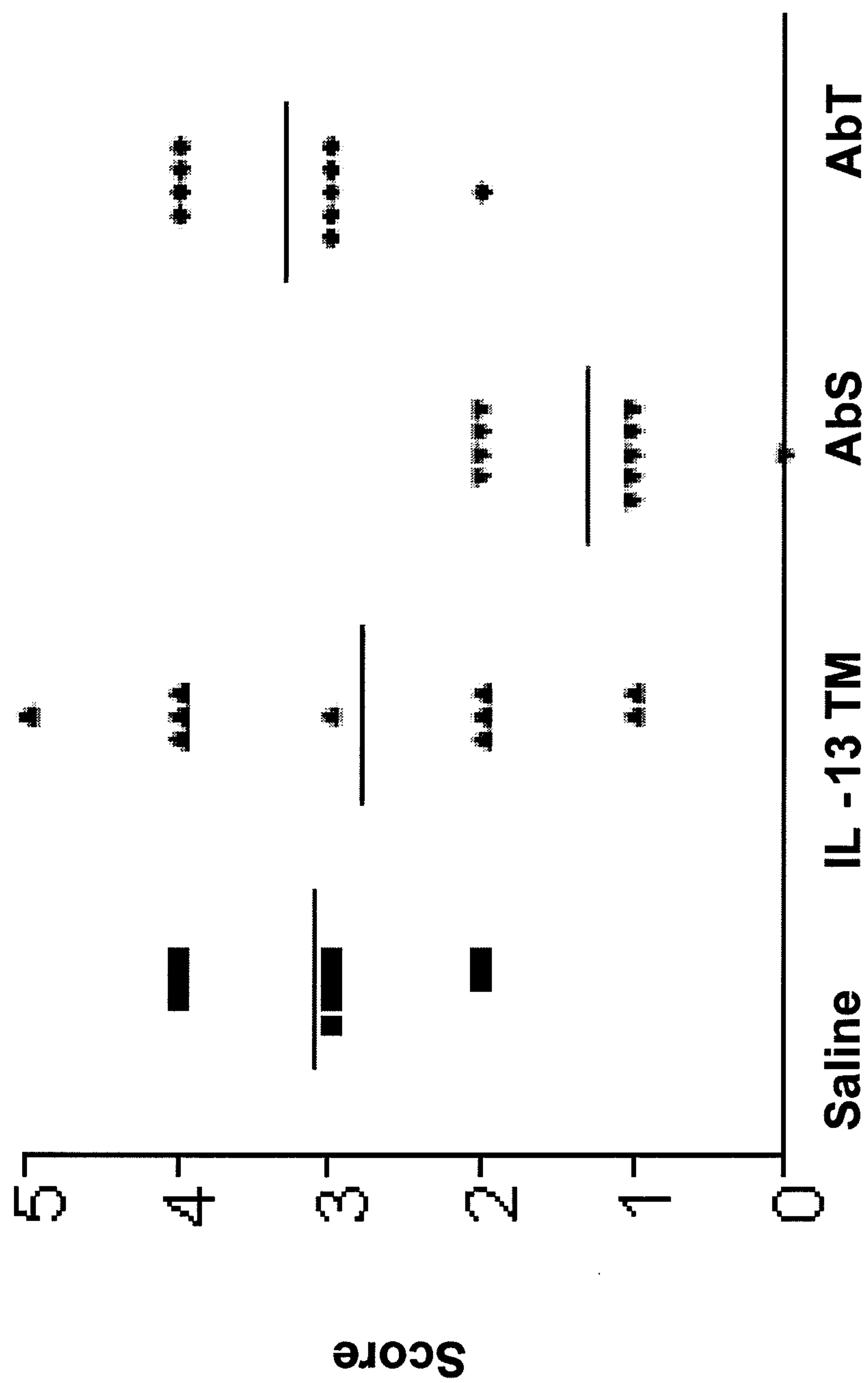


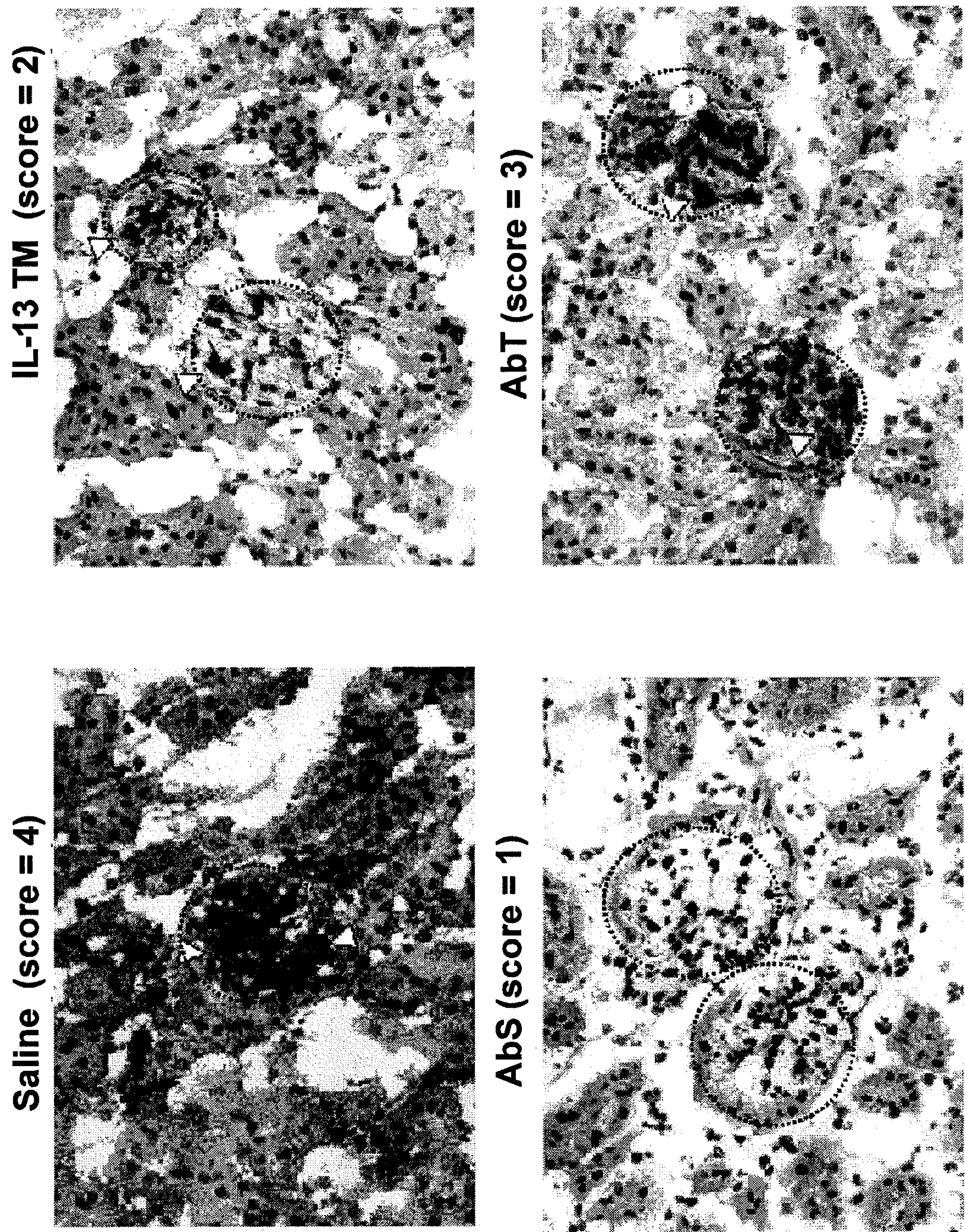
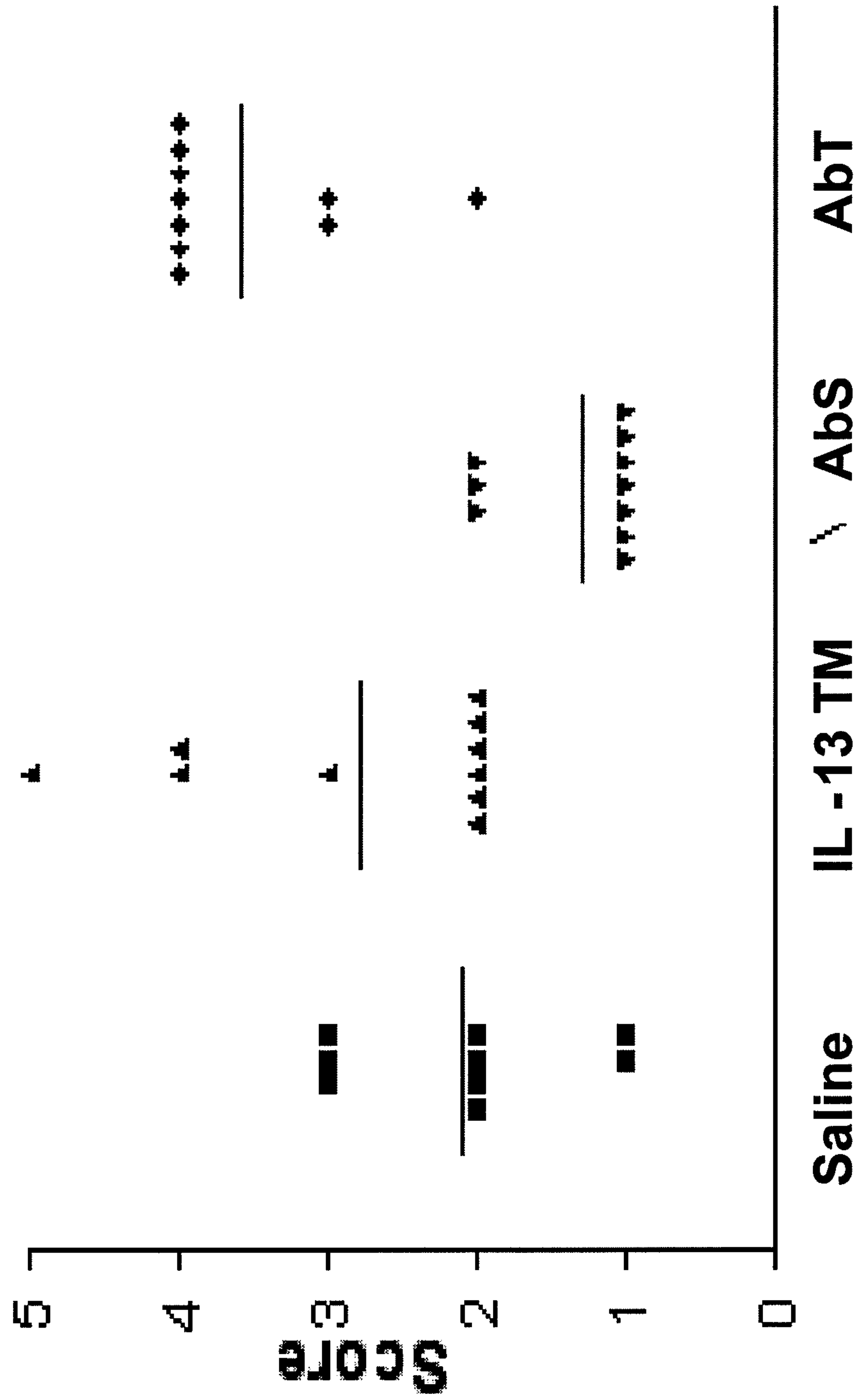
Figure 32b

Figure 2 is a scatter plot showing the effect of IL-13 treatment on the score of allergic rhinitis in mice. The y-axis represents the 'Score' (0 to 5), and the x-axis shows four groups: Saline, IL-13 TM, AbS, and AbT. The Saline and IL-13 TM groups show significantly higher scores (around 1.5-2.5) compared to the AbS and AbT groups (around 0.5-1.0). Statistical significance is indicated by asterisks and horizontal bars.

Group	Score (Mean ± SEM)	Significance
Saline	1.5 ± 0.2	
IL-13 TM	2.5 ± 0.2	
AbS	0.5 ± 0.1	***
AbT	1.0 ± 0.1	***



[illegible]

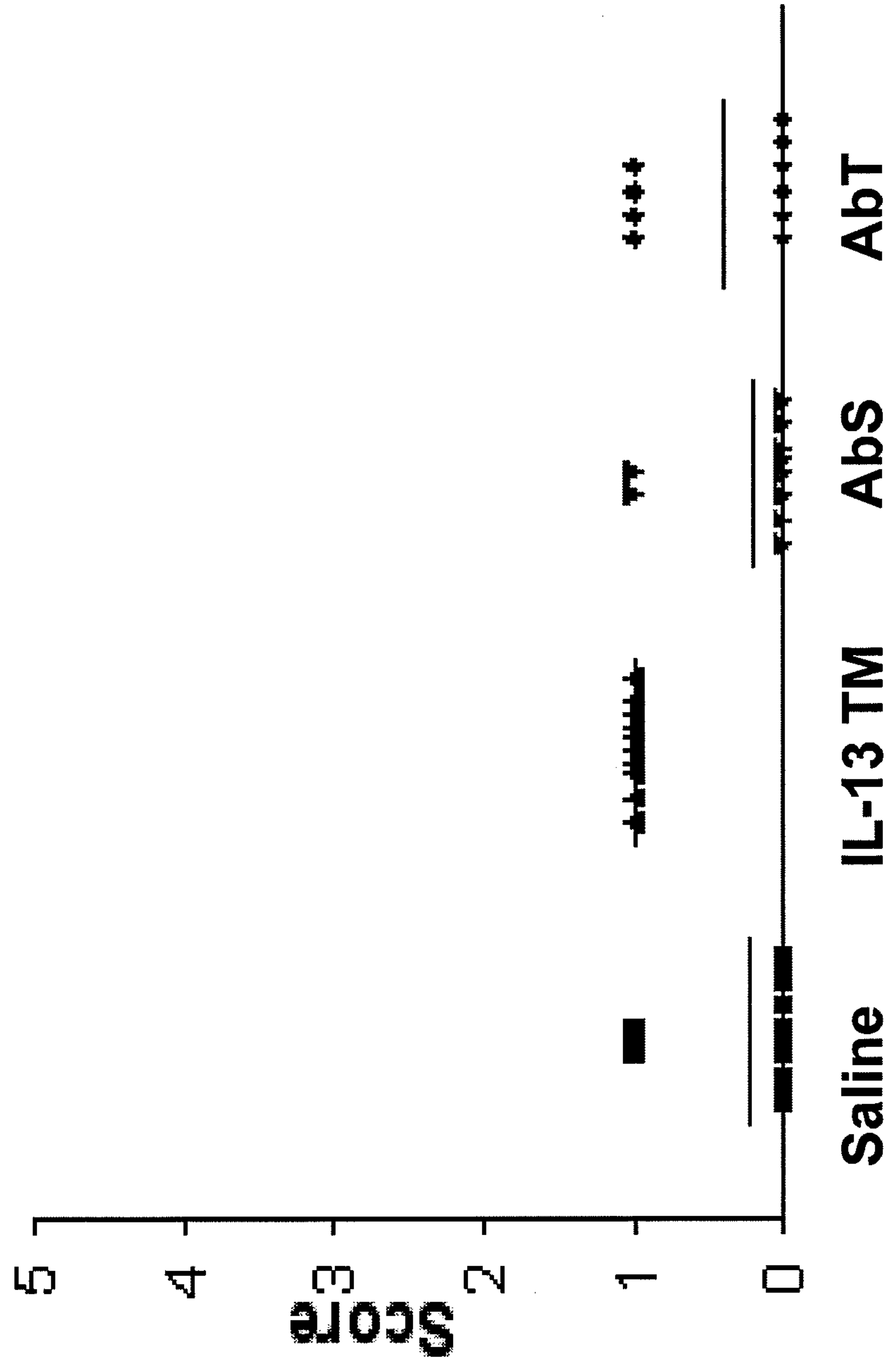
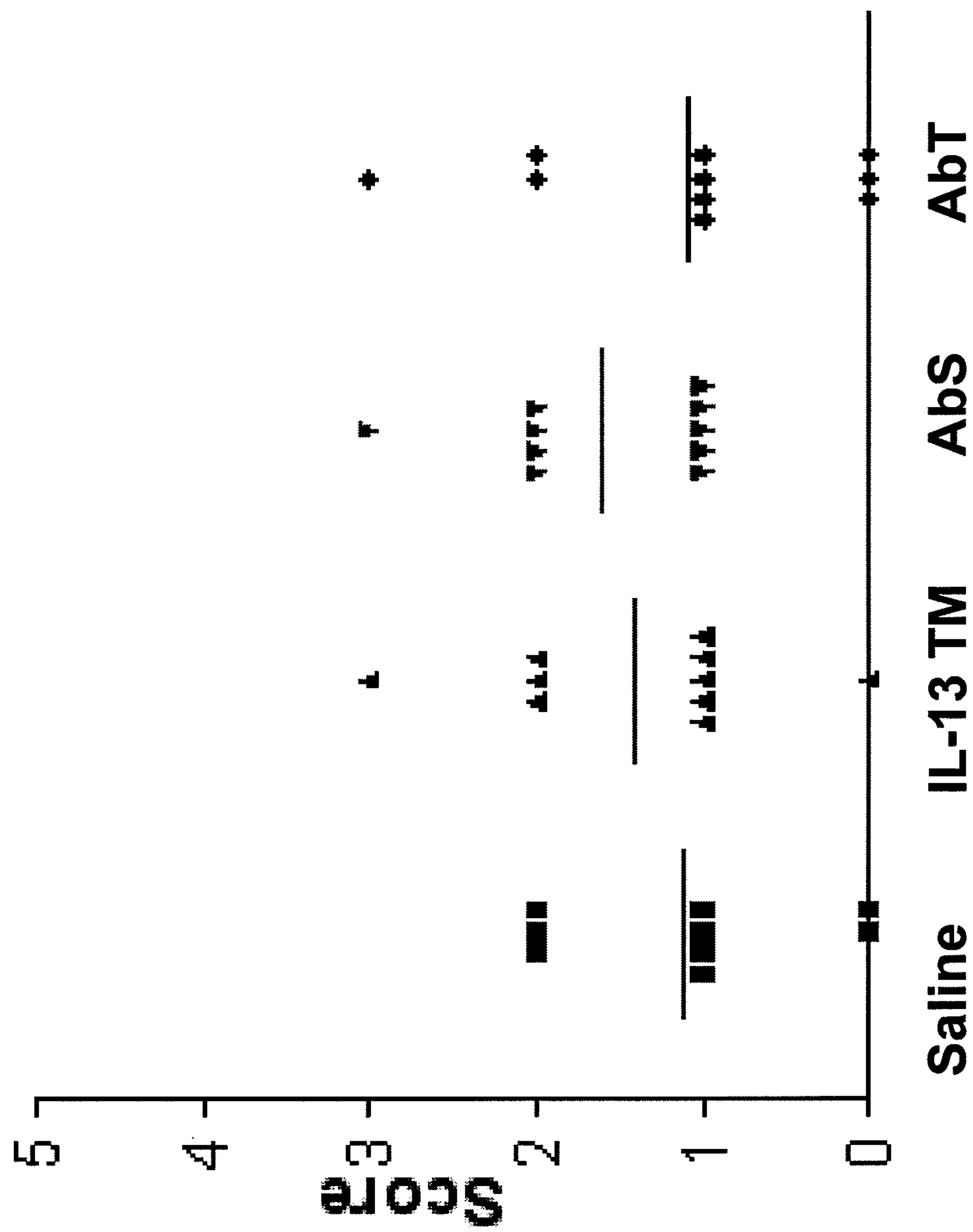
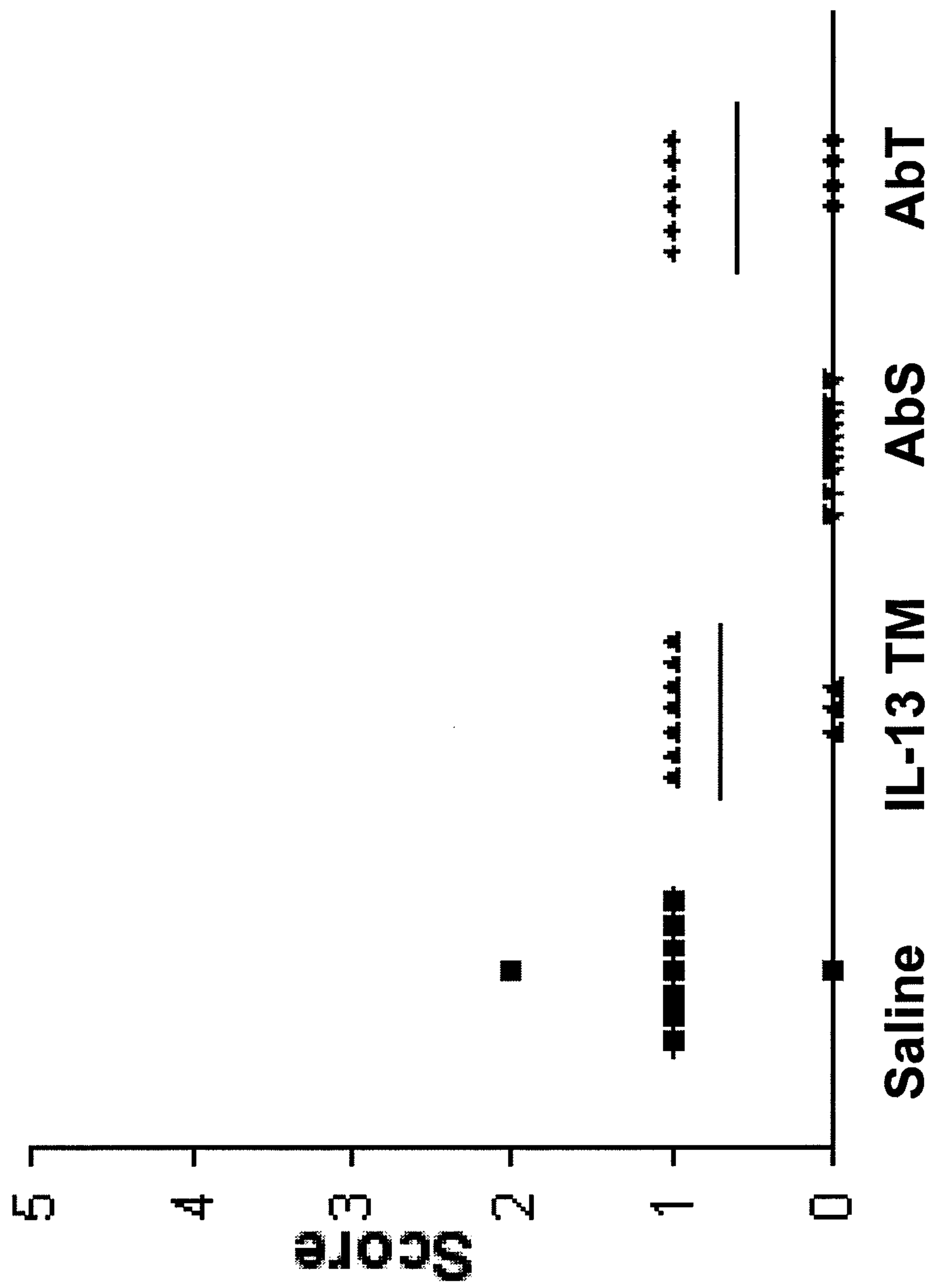
Figure 34b

Figure 34c





Group	Score 0	Score 1	Score 2	Score 3	Score 4	Score 5
Saline	0	0	1	10	1	0
IL-13 TM	0	0	1	10	0	0
AbT	0	10	1	0	0	0

Group	Score
Saline	2.8
IL-13 TM	2.2
AbT	1.5

Figure 36

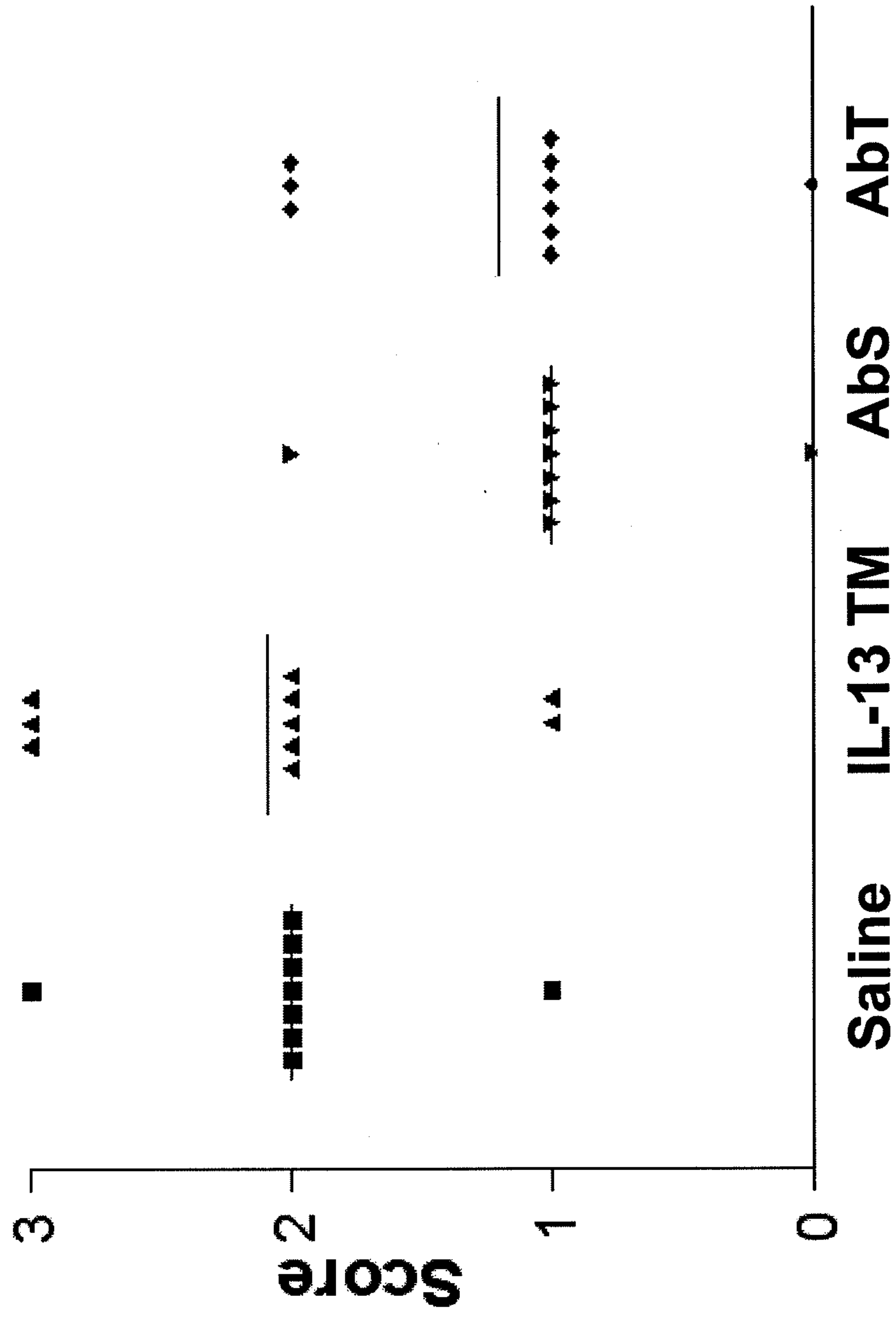


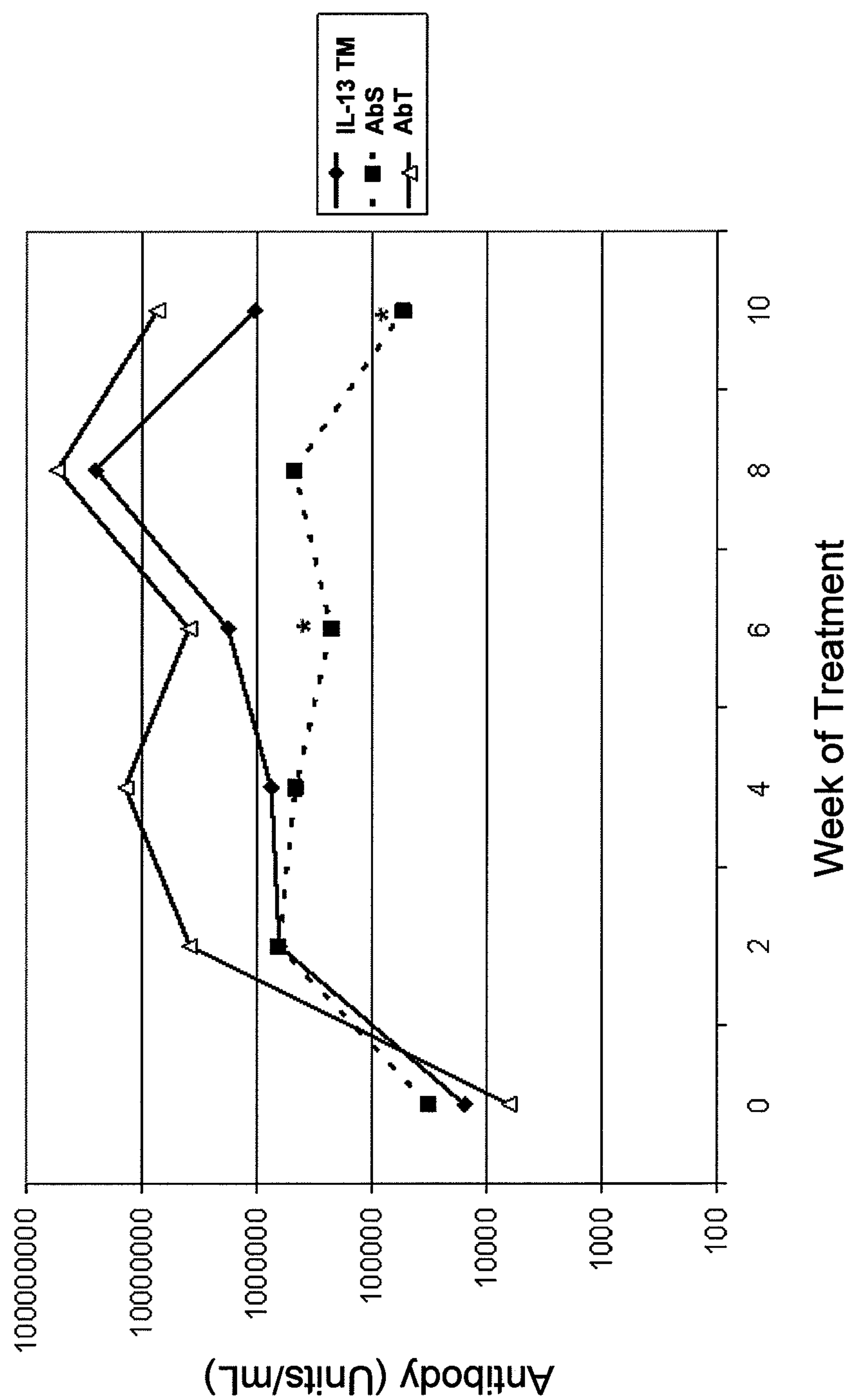
Figure 37a

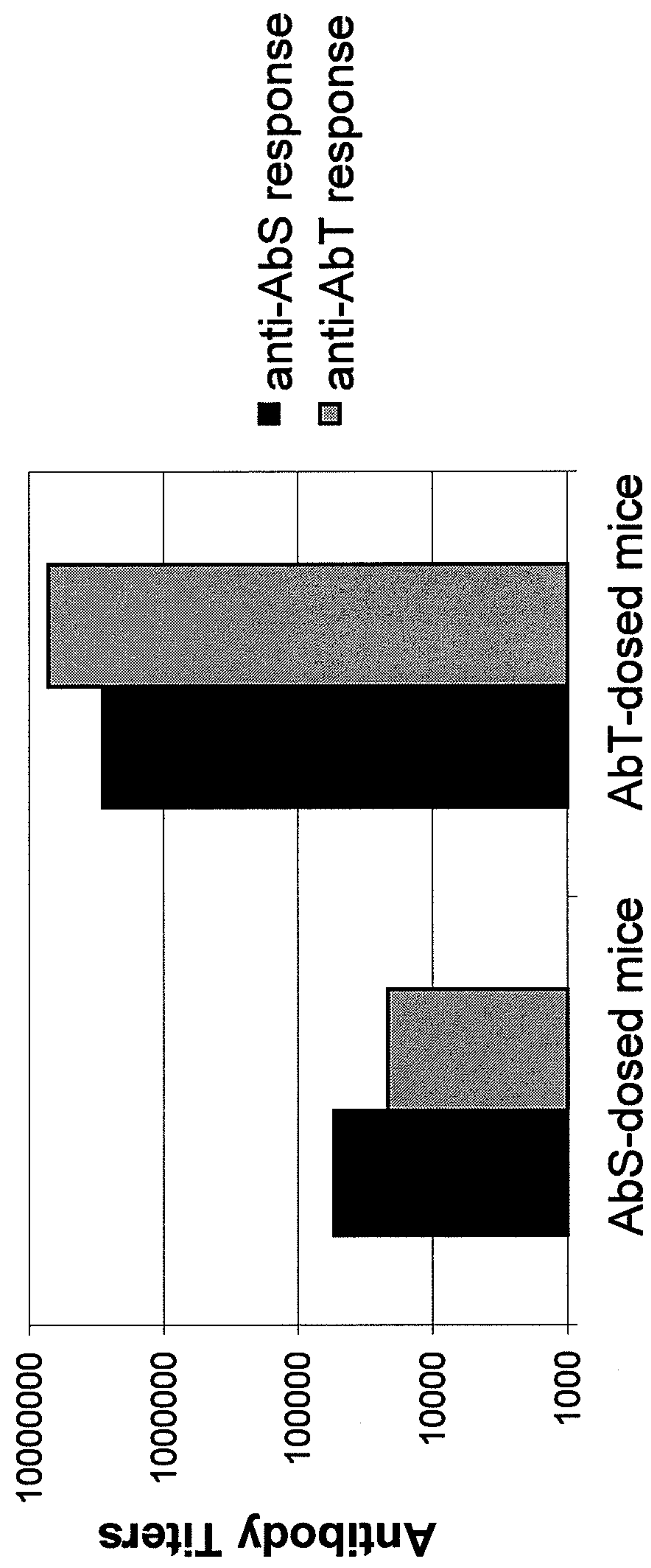
Figure 37b

Figure 38a

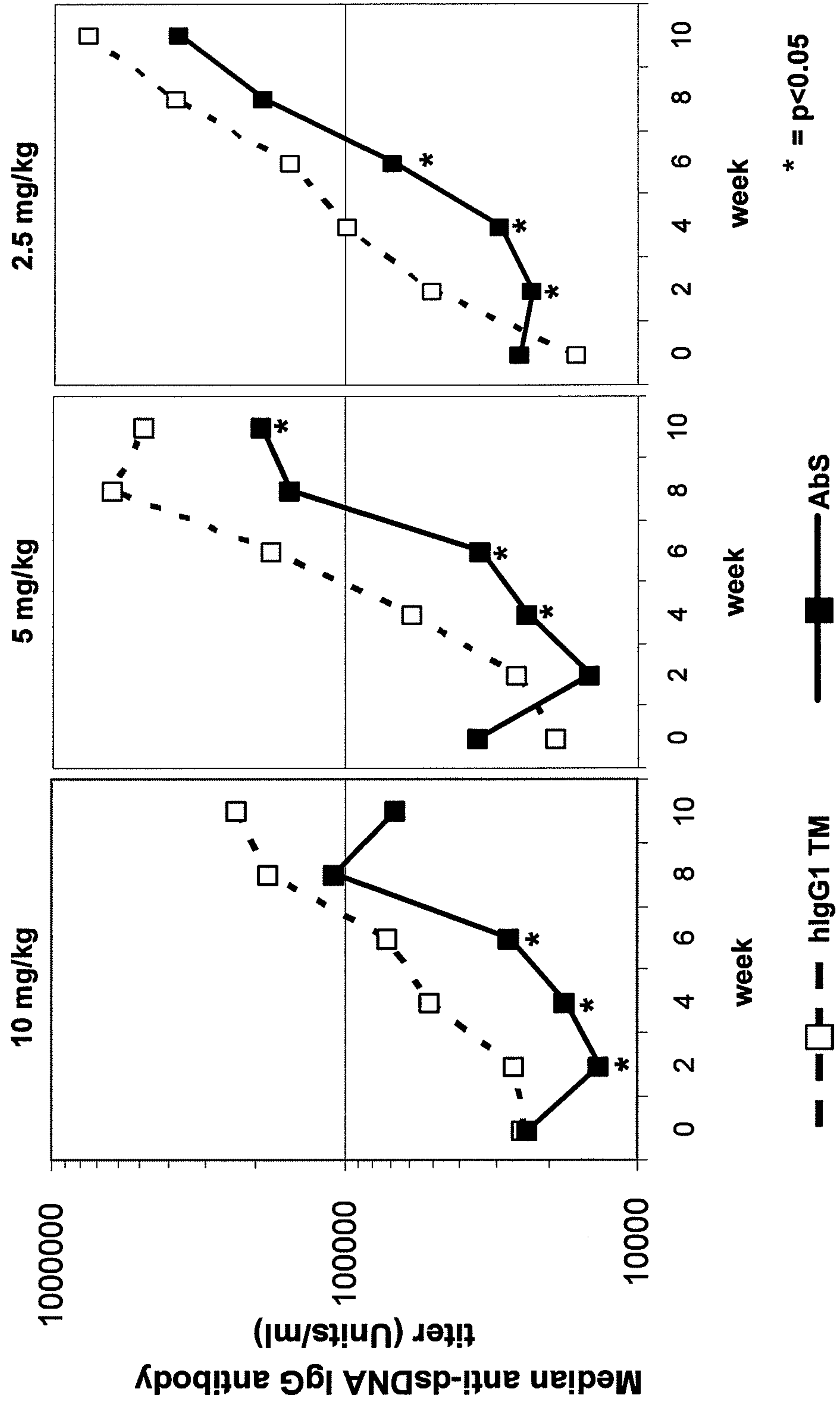


Figure 38b

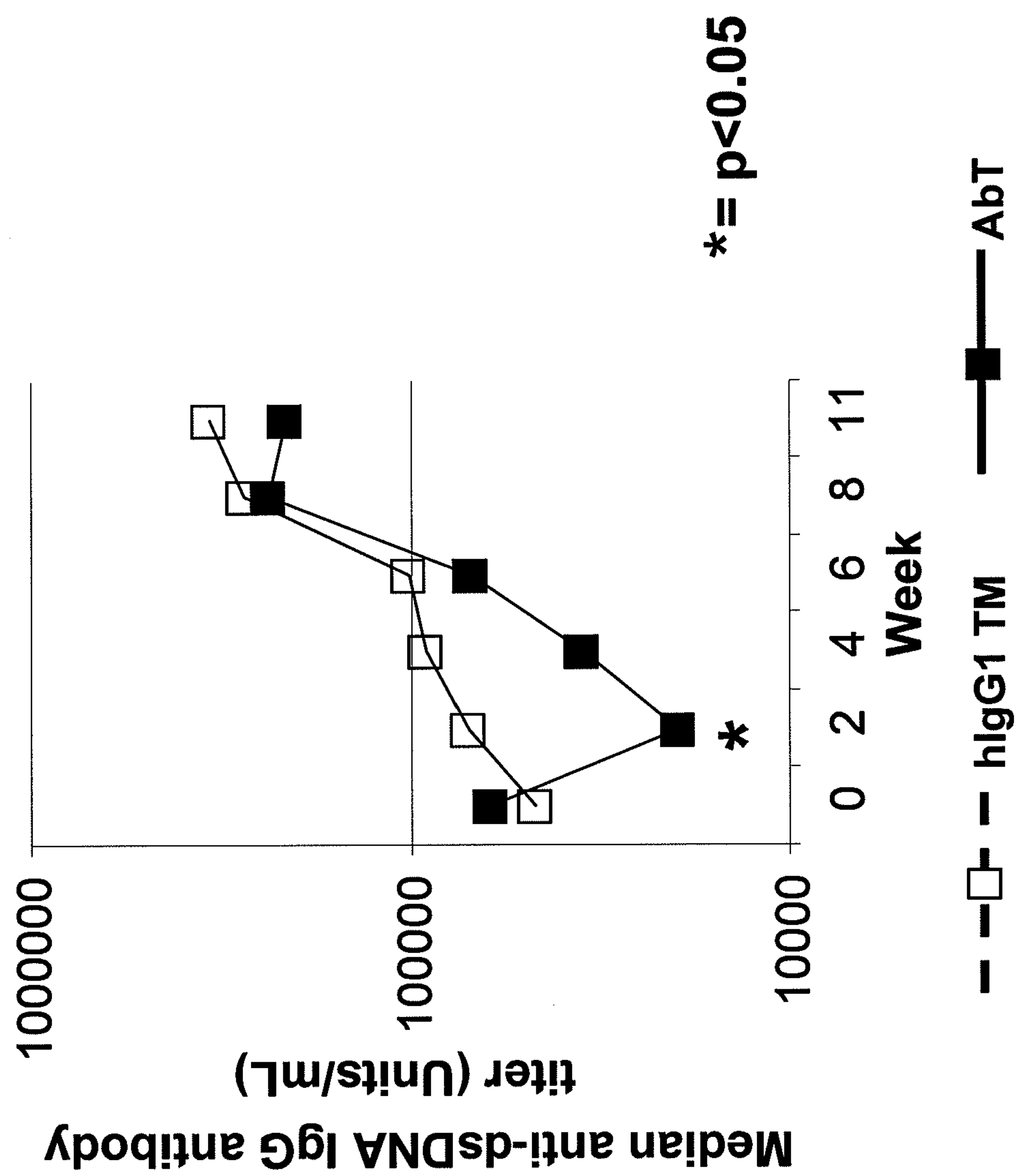


Figure 38c

Renal IgG Deposits

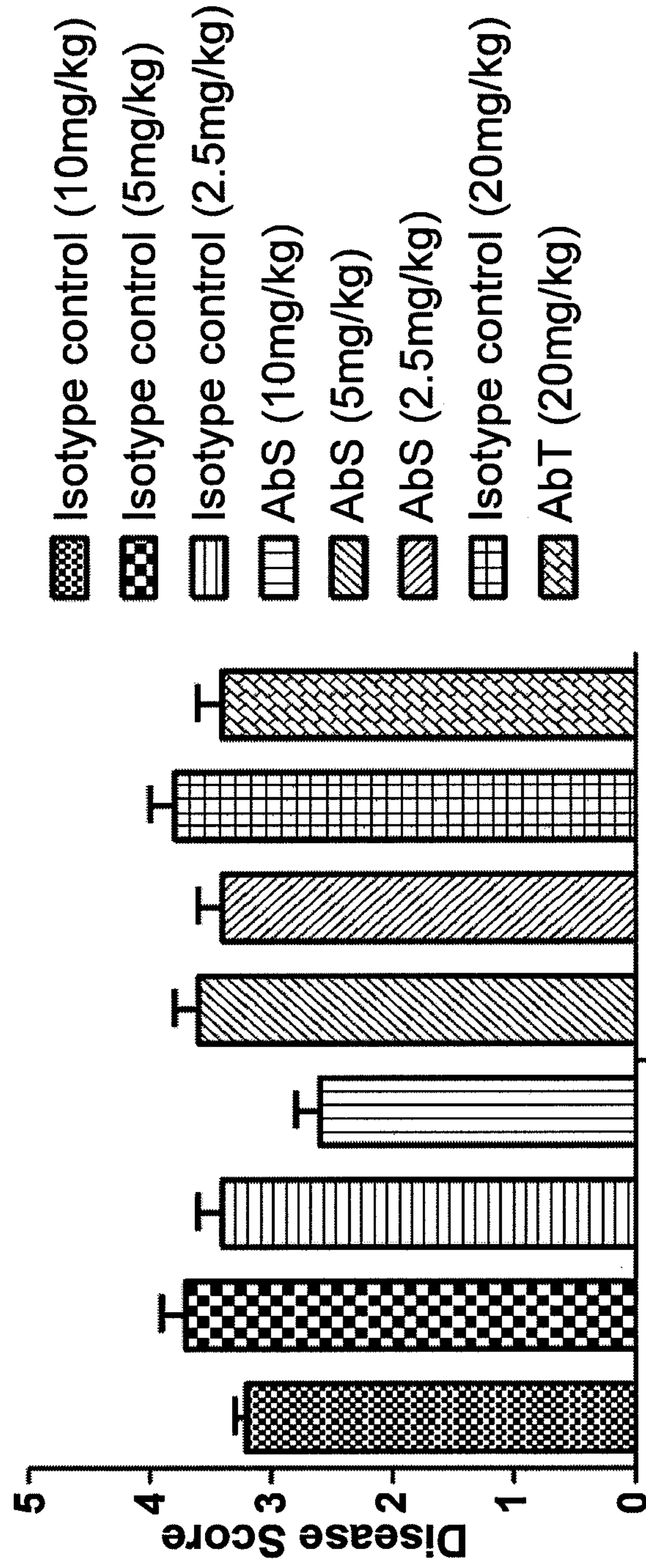


Figure 38d

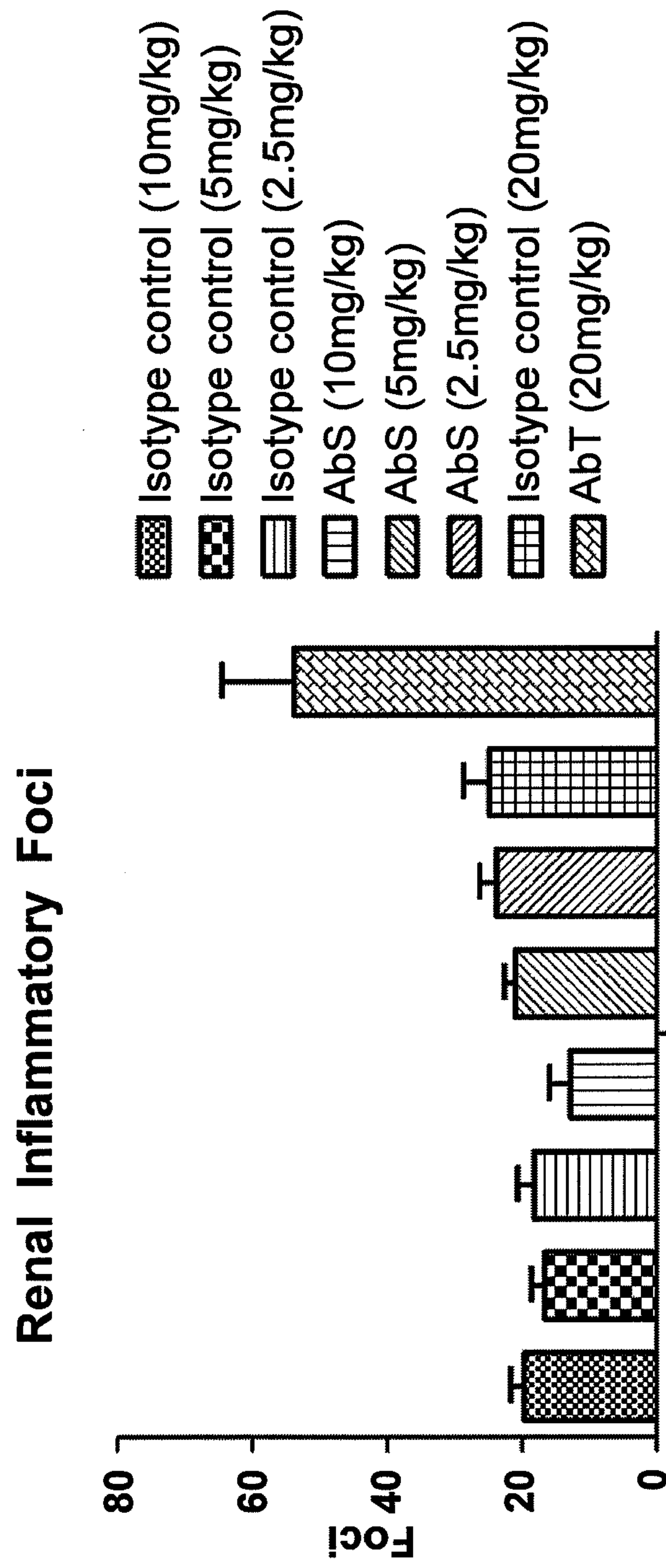


Figure 39a

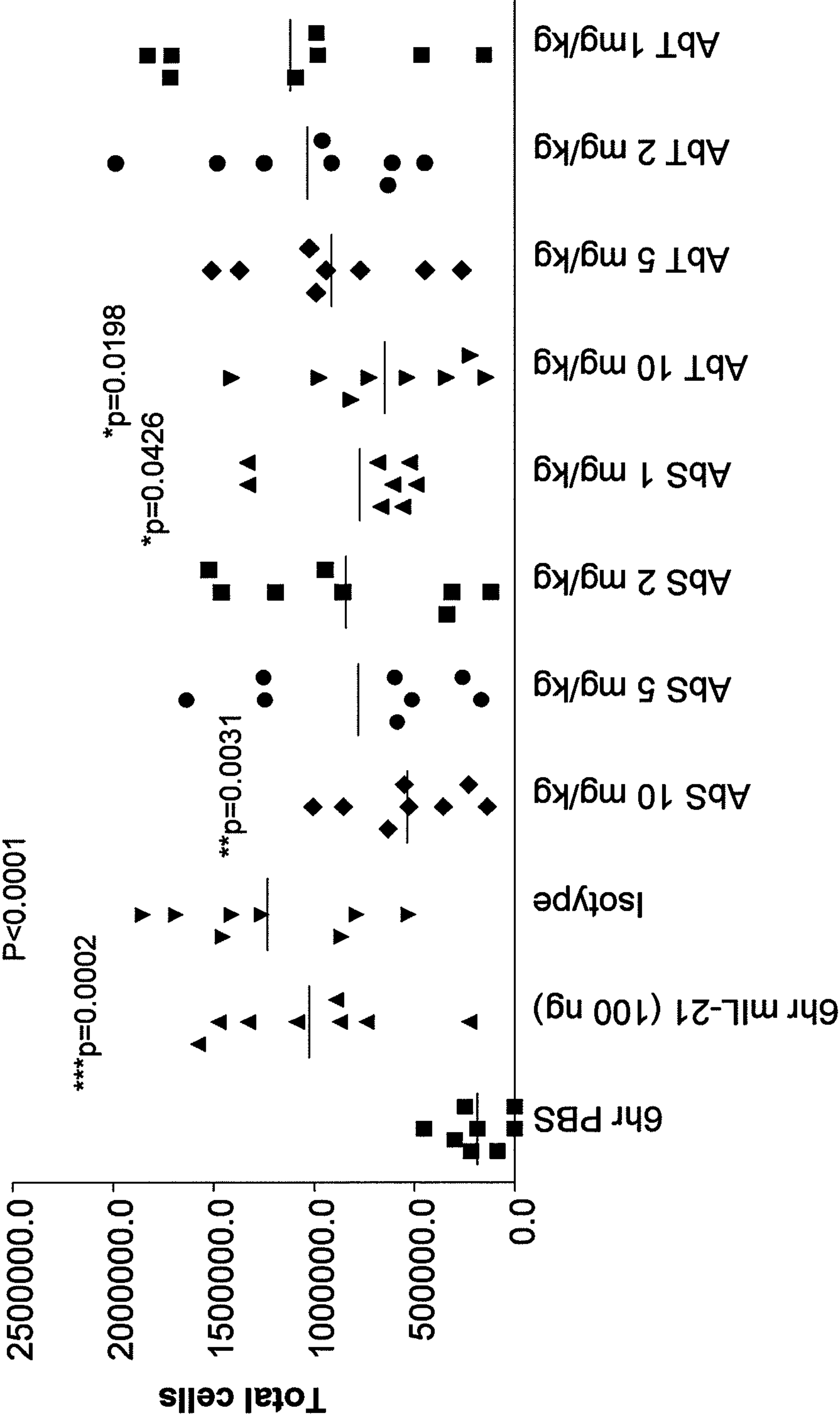


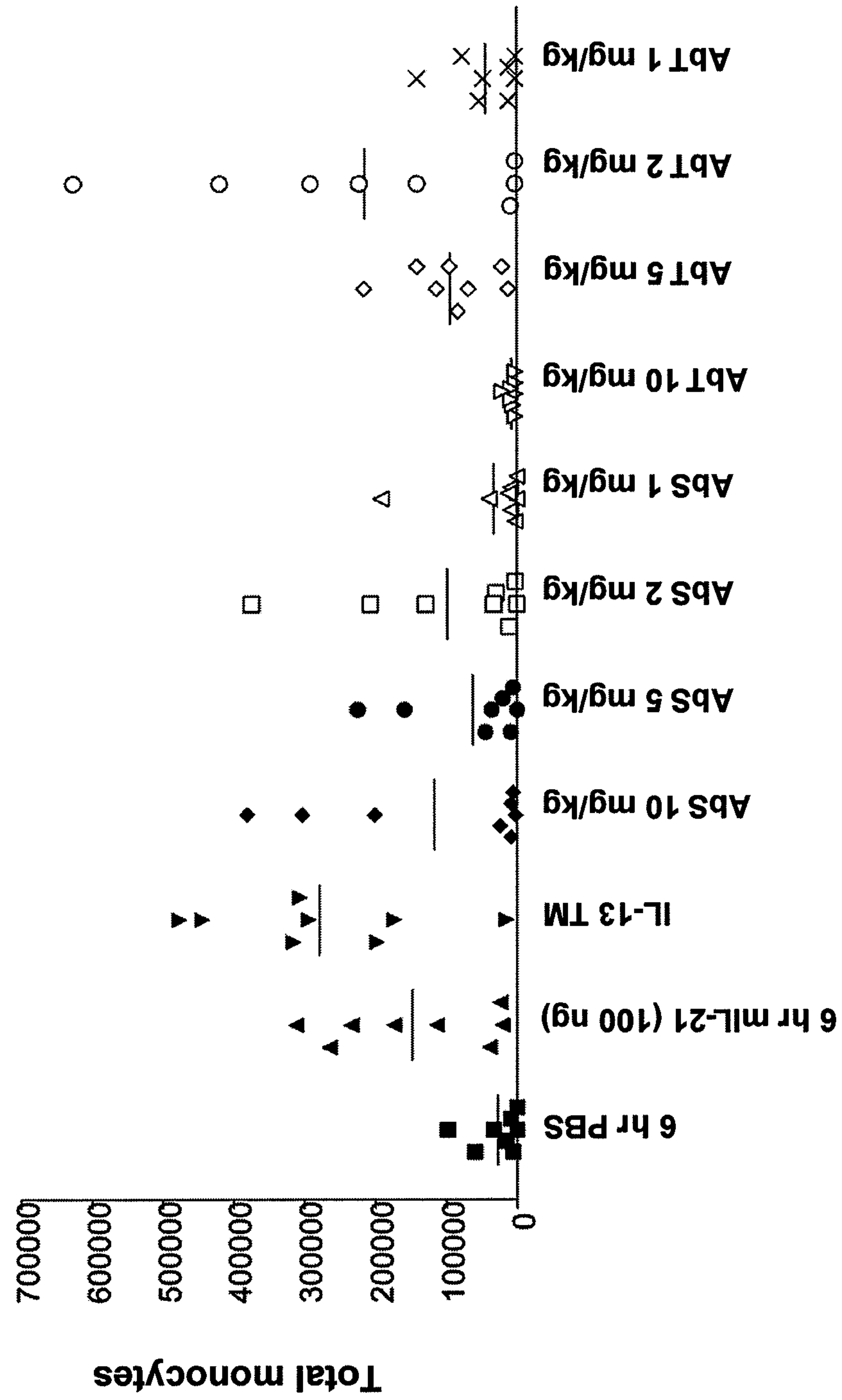
Figure 39b

Figure 39c

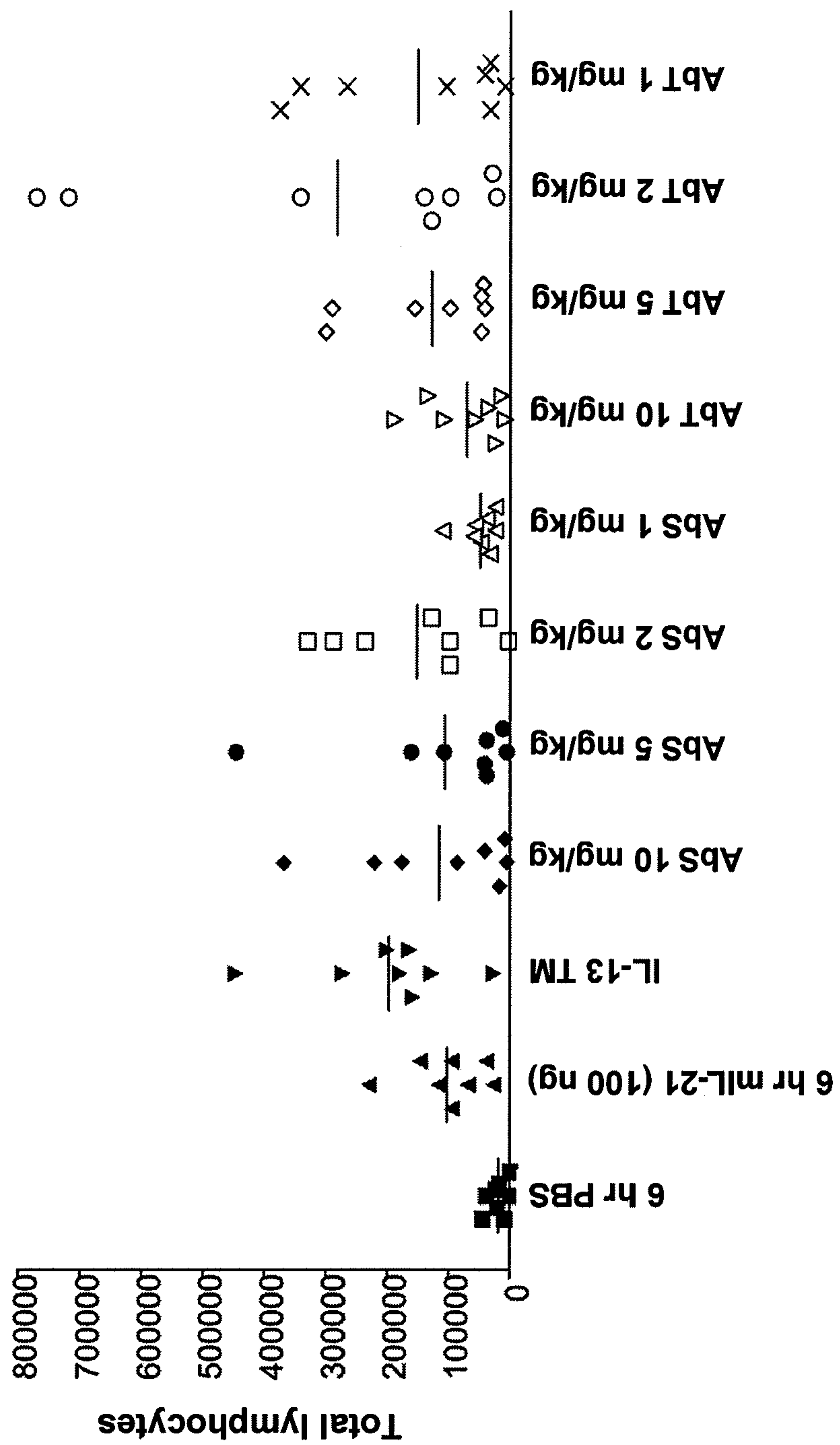


Figure 39d

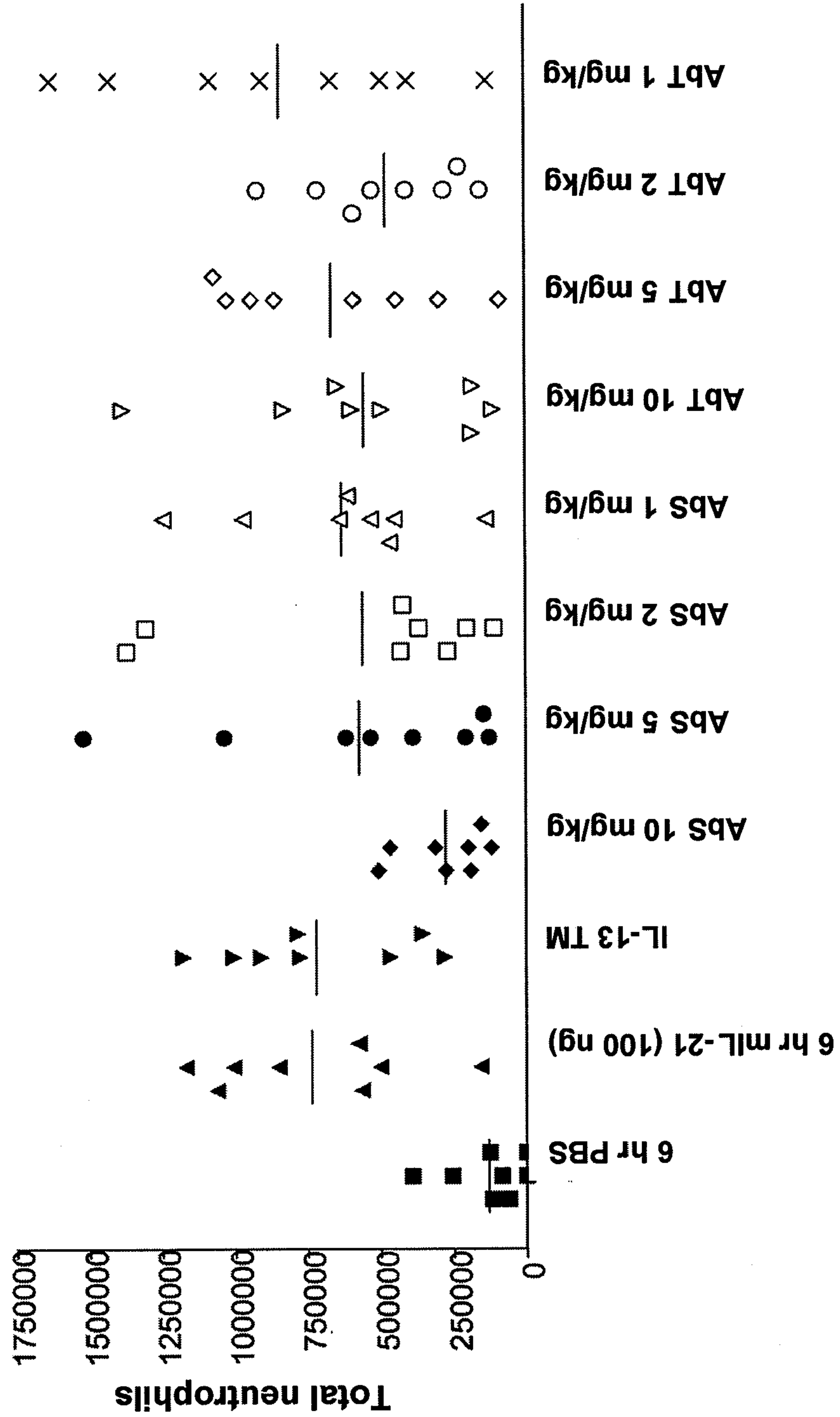
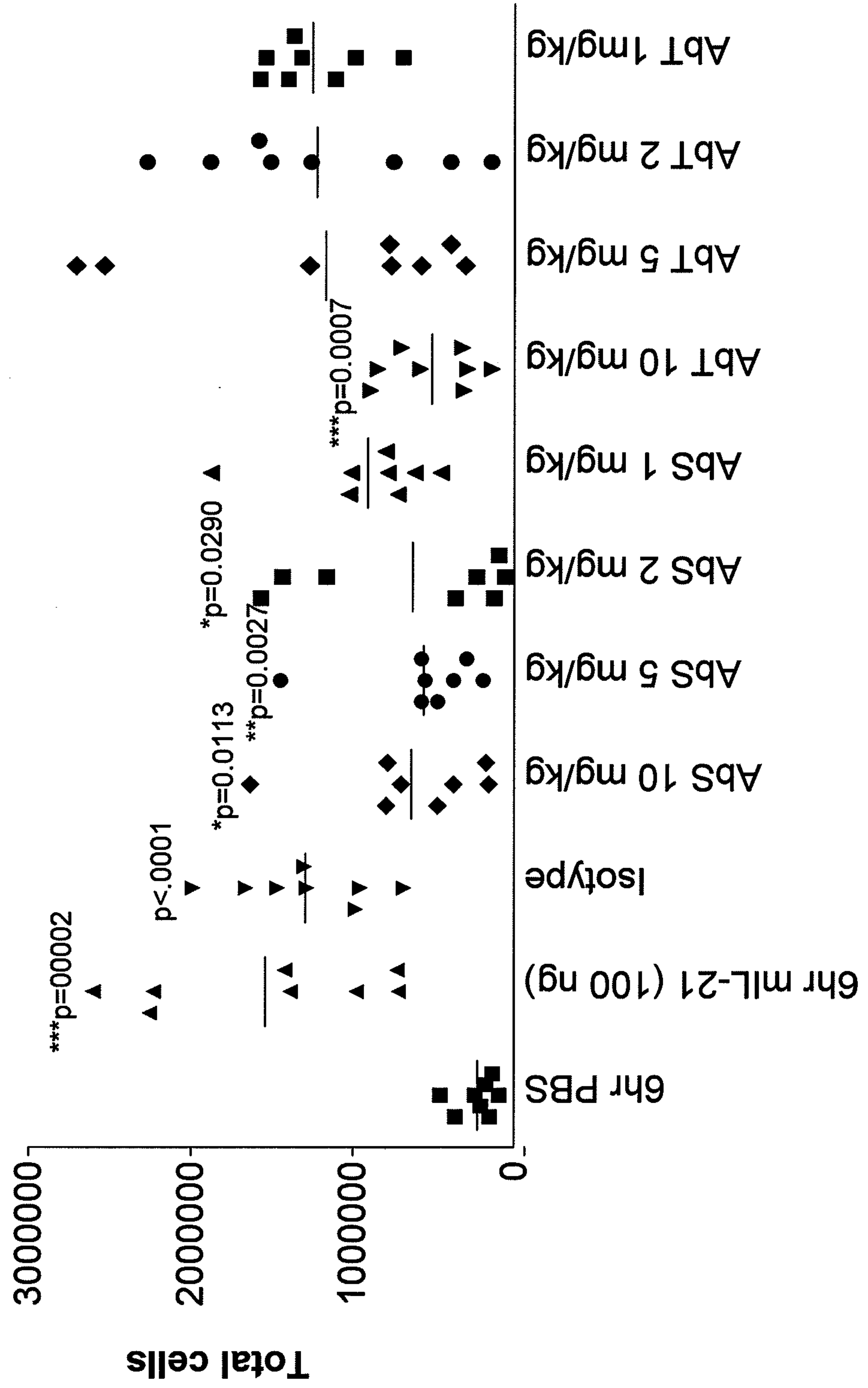


Figure 39e

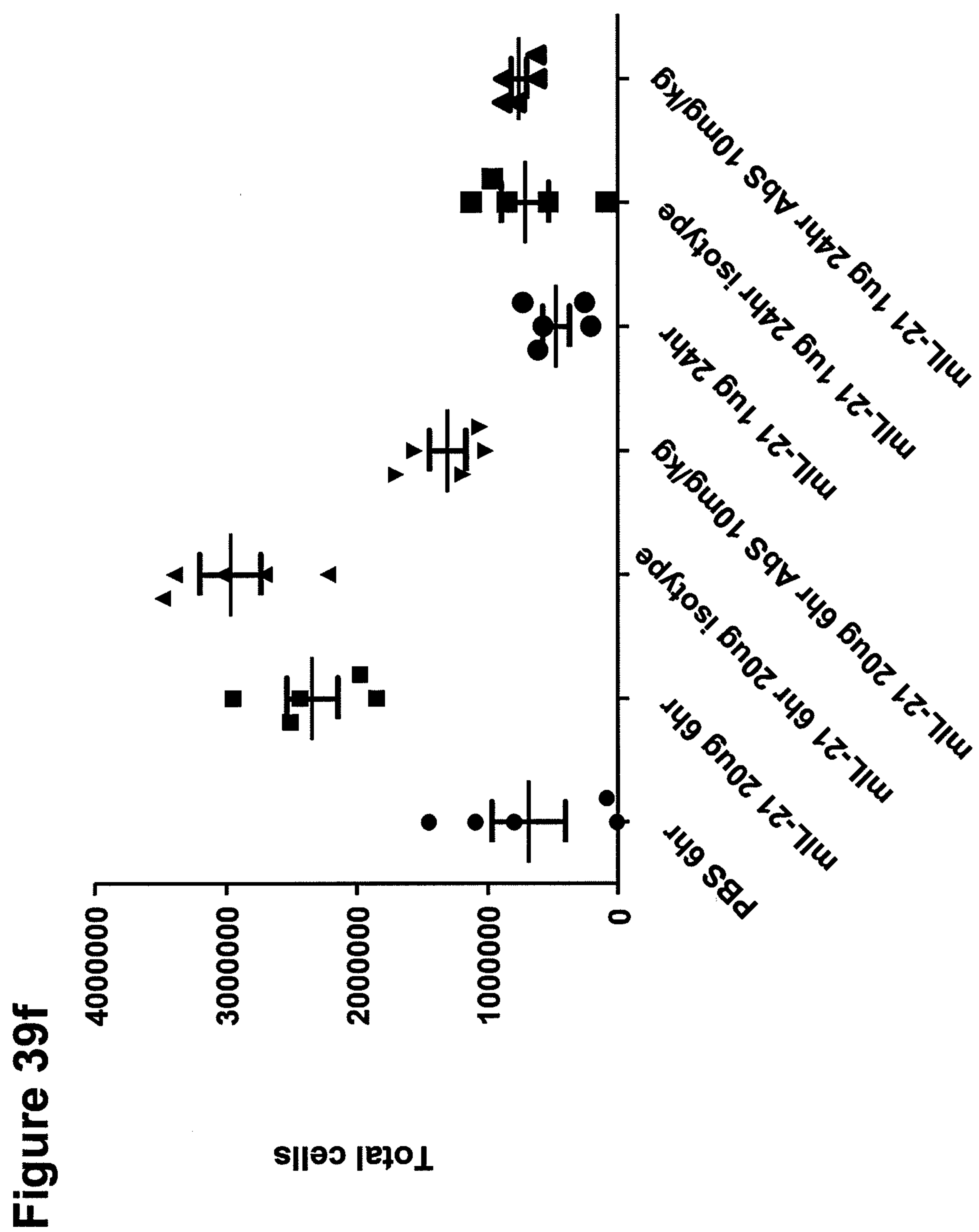


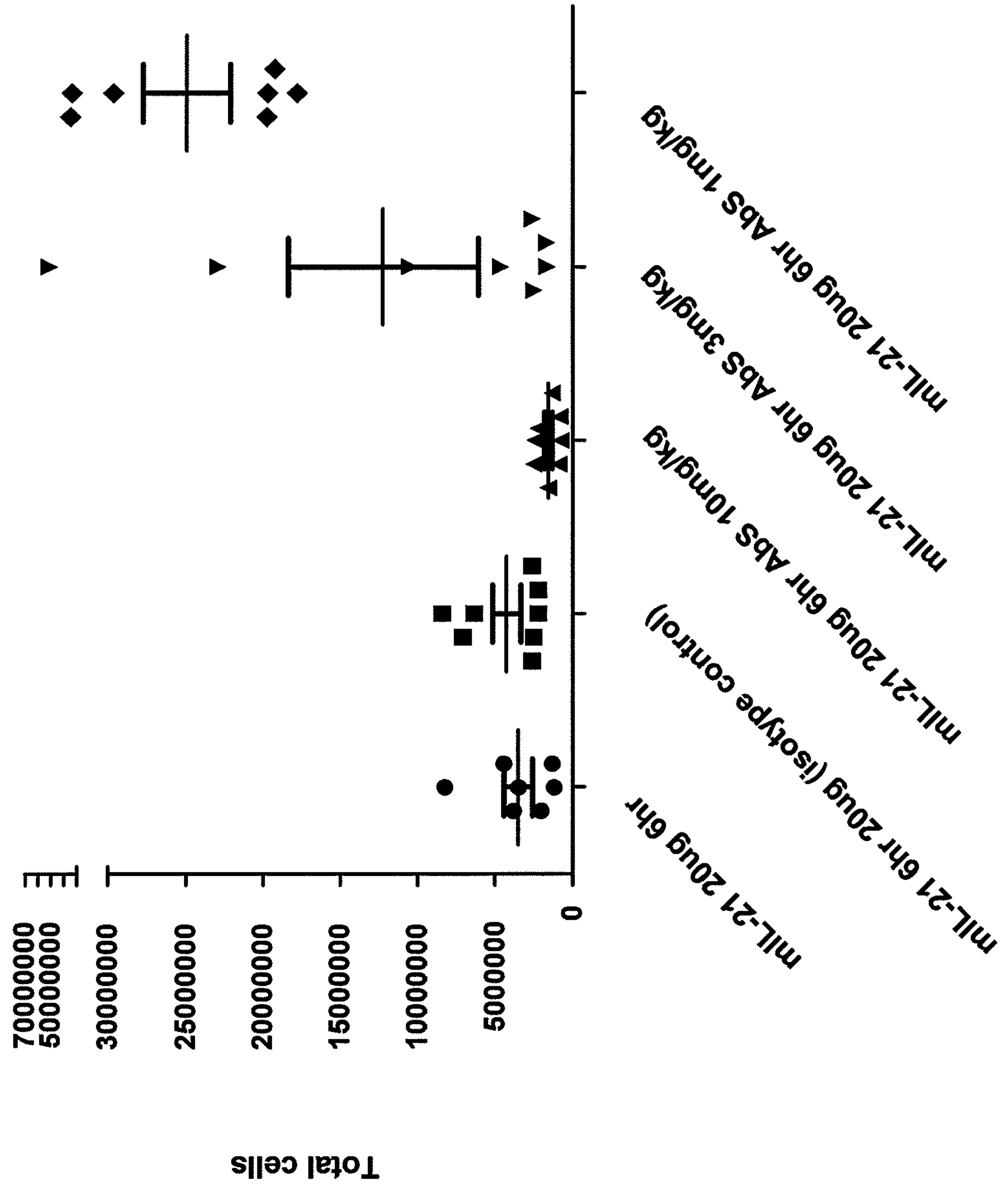
Figure 39g

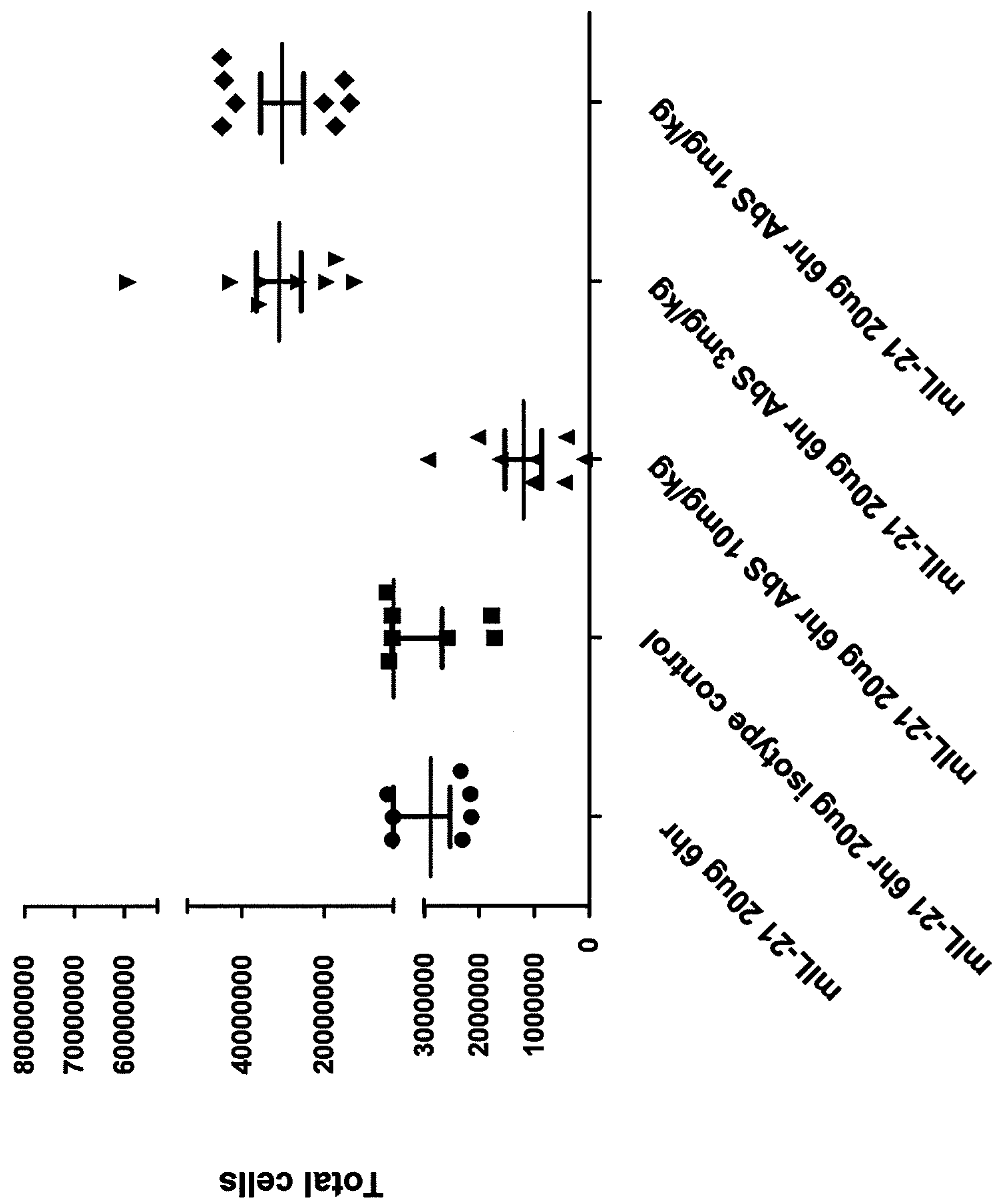
Figure 39h

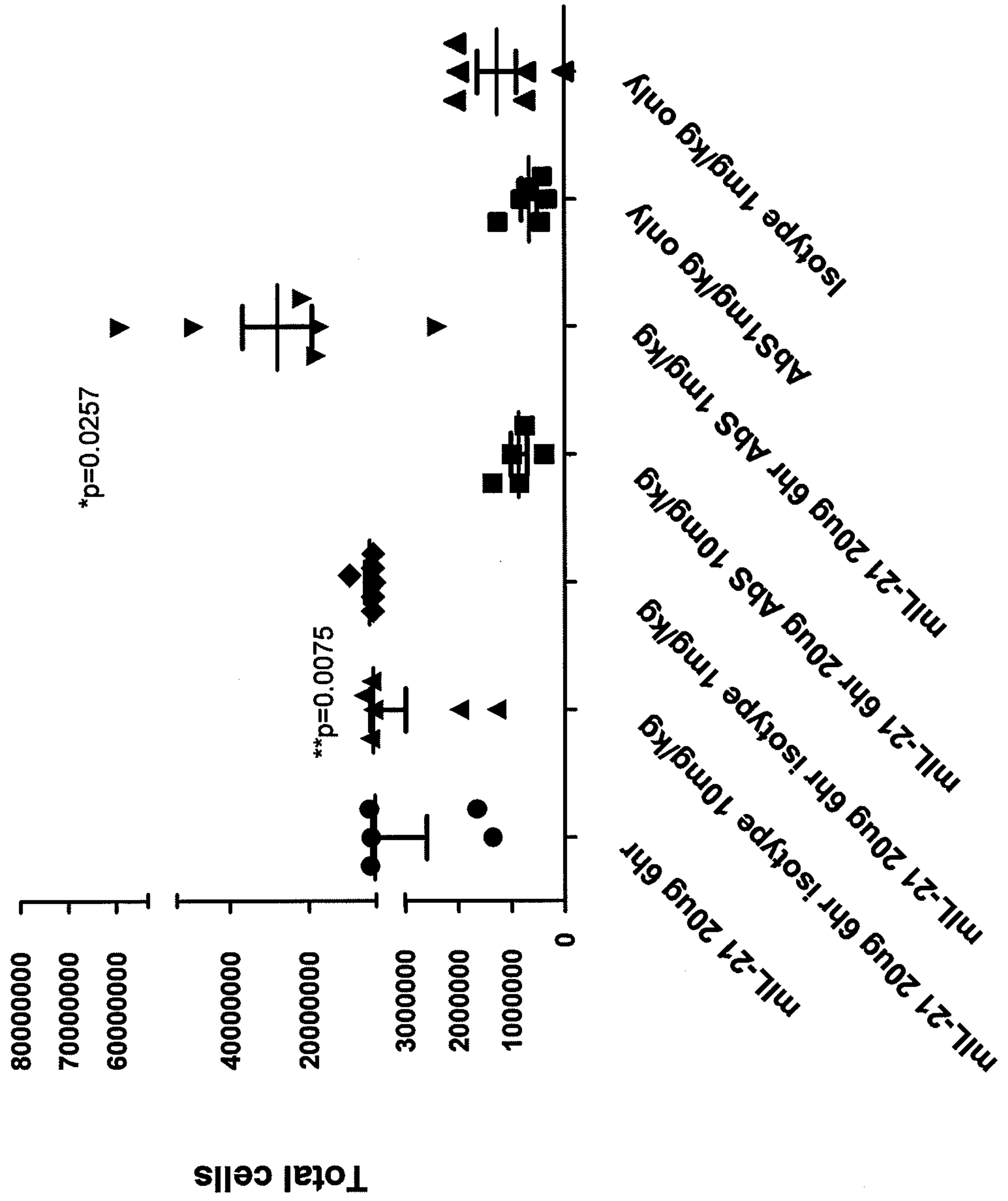
Figure 39i

Figure 39j

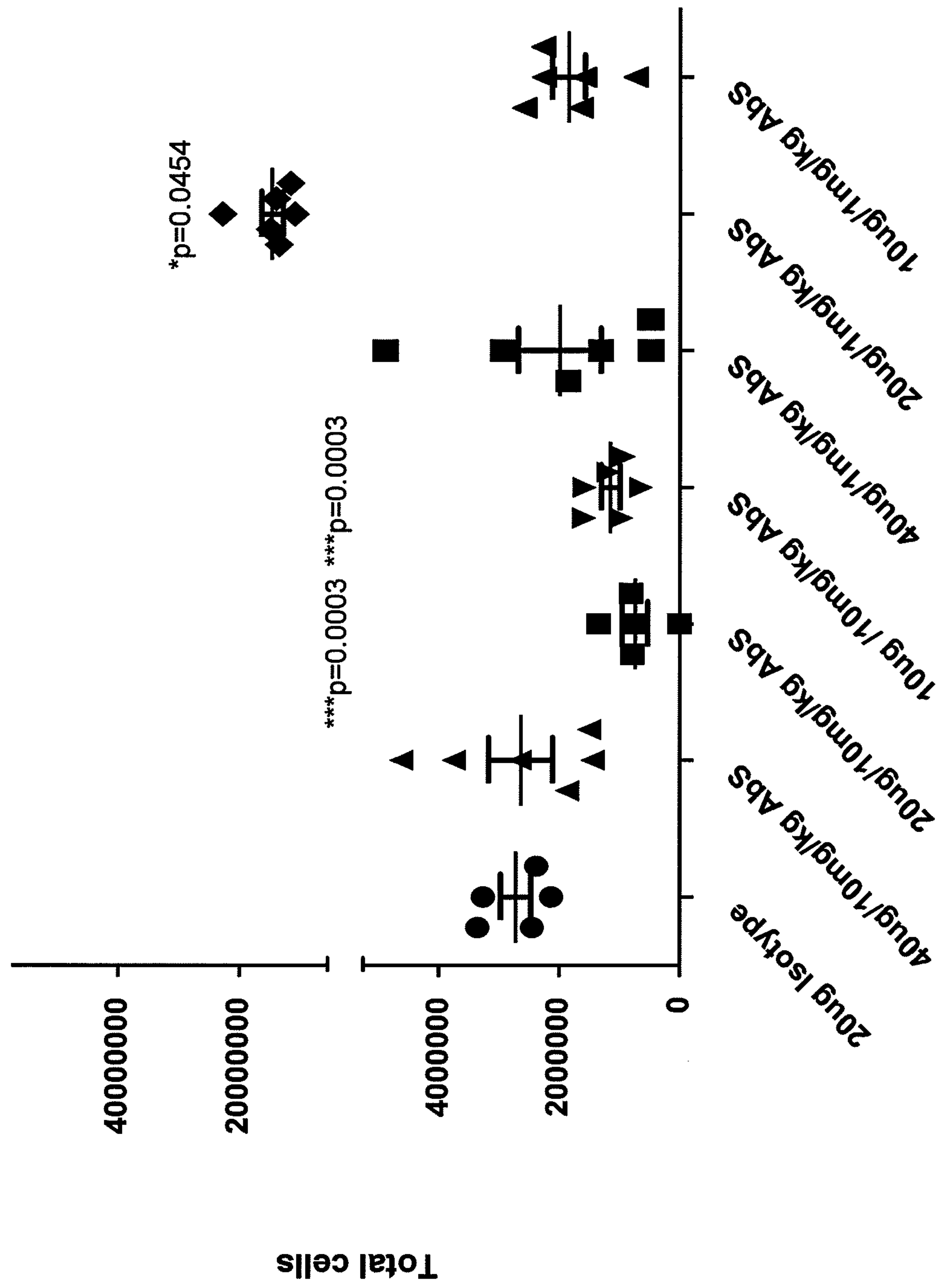


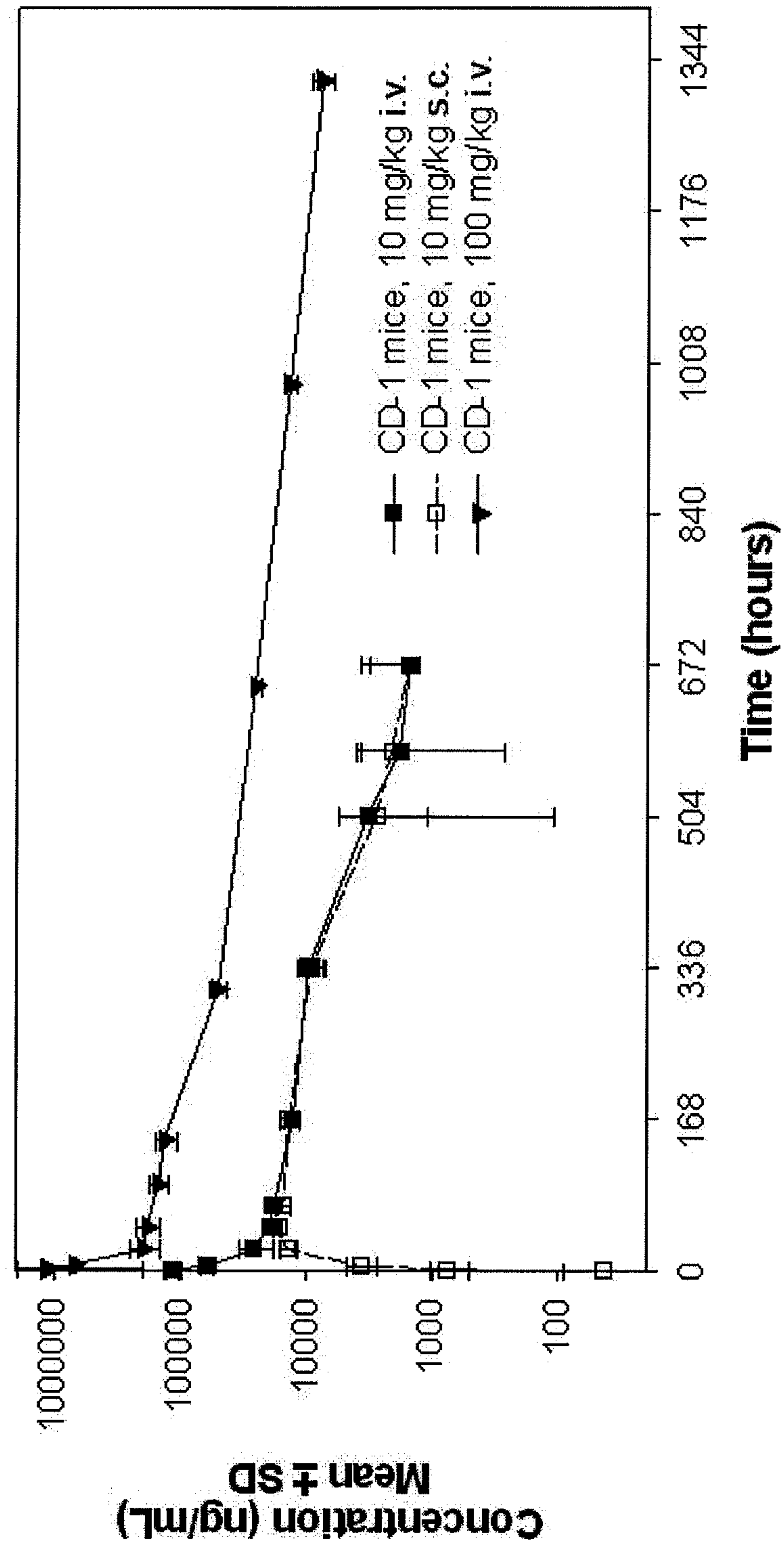
Figure 40

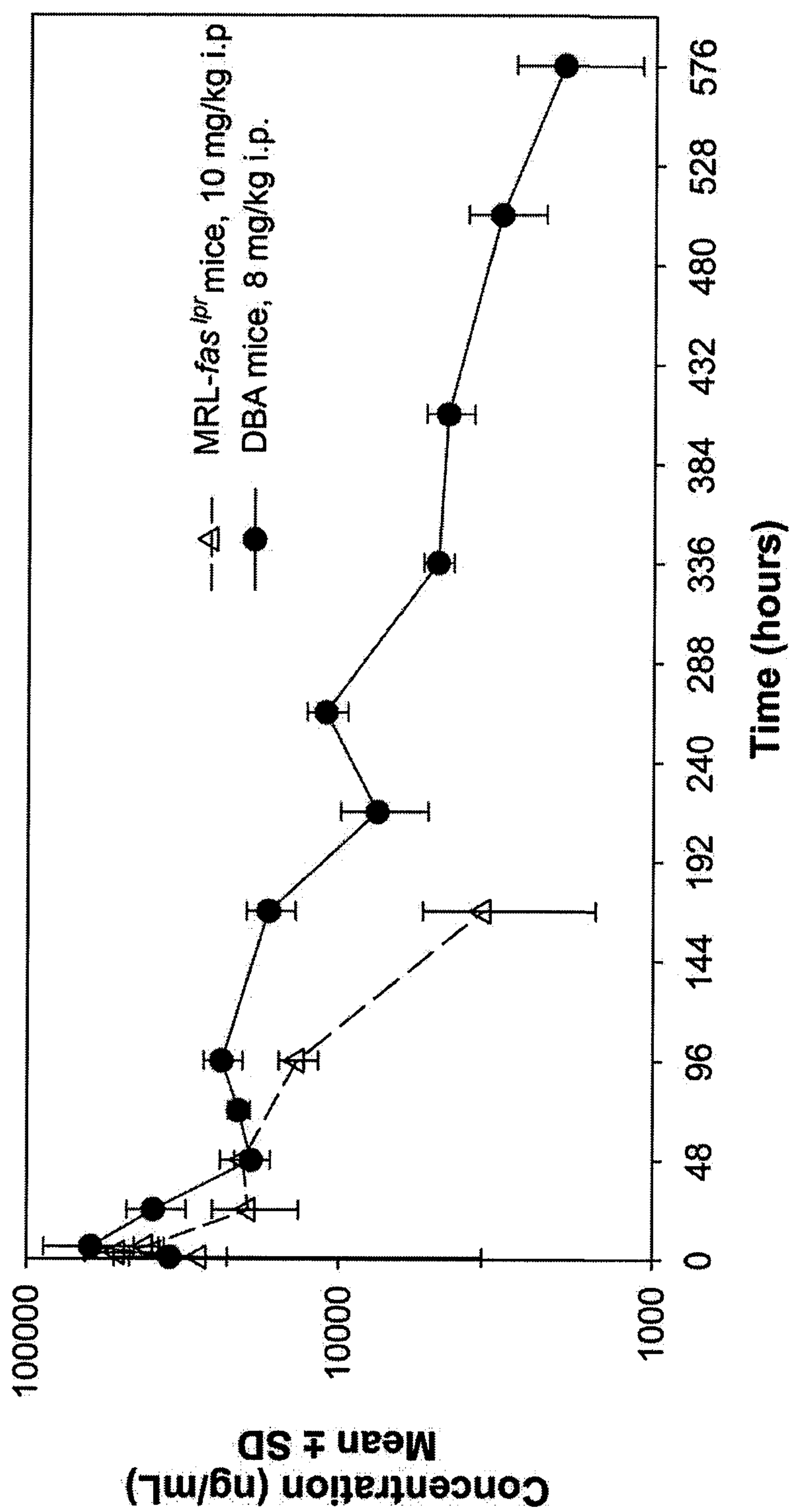
Figure 41

Figure 42a

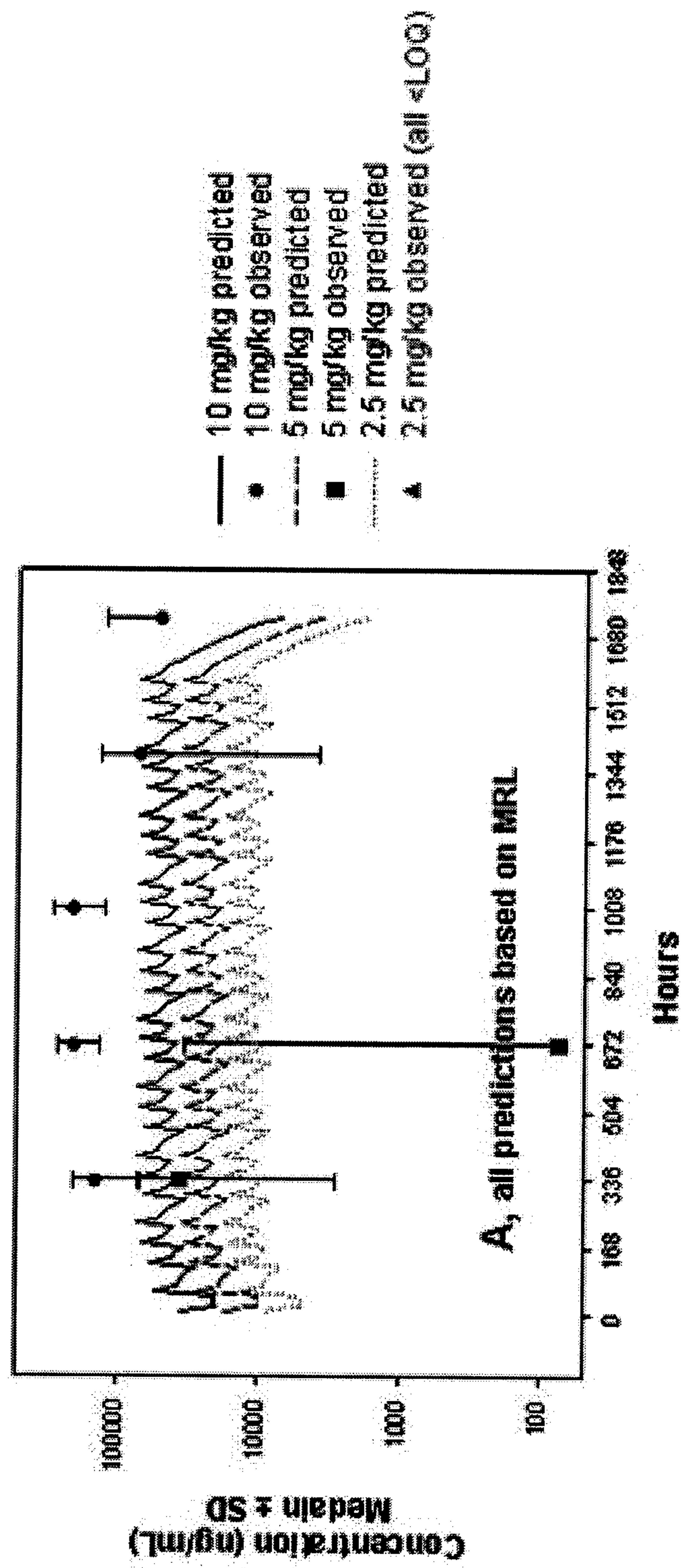


Figure 42b

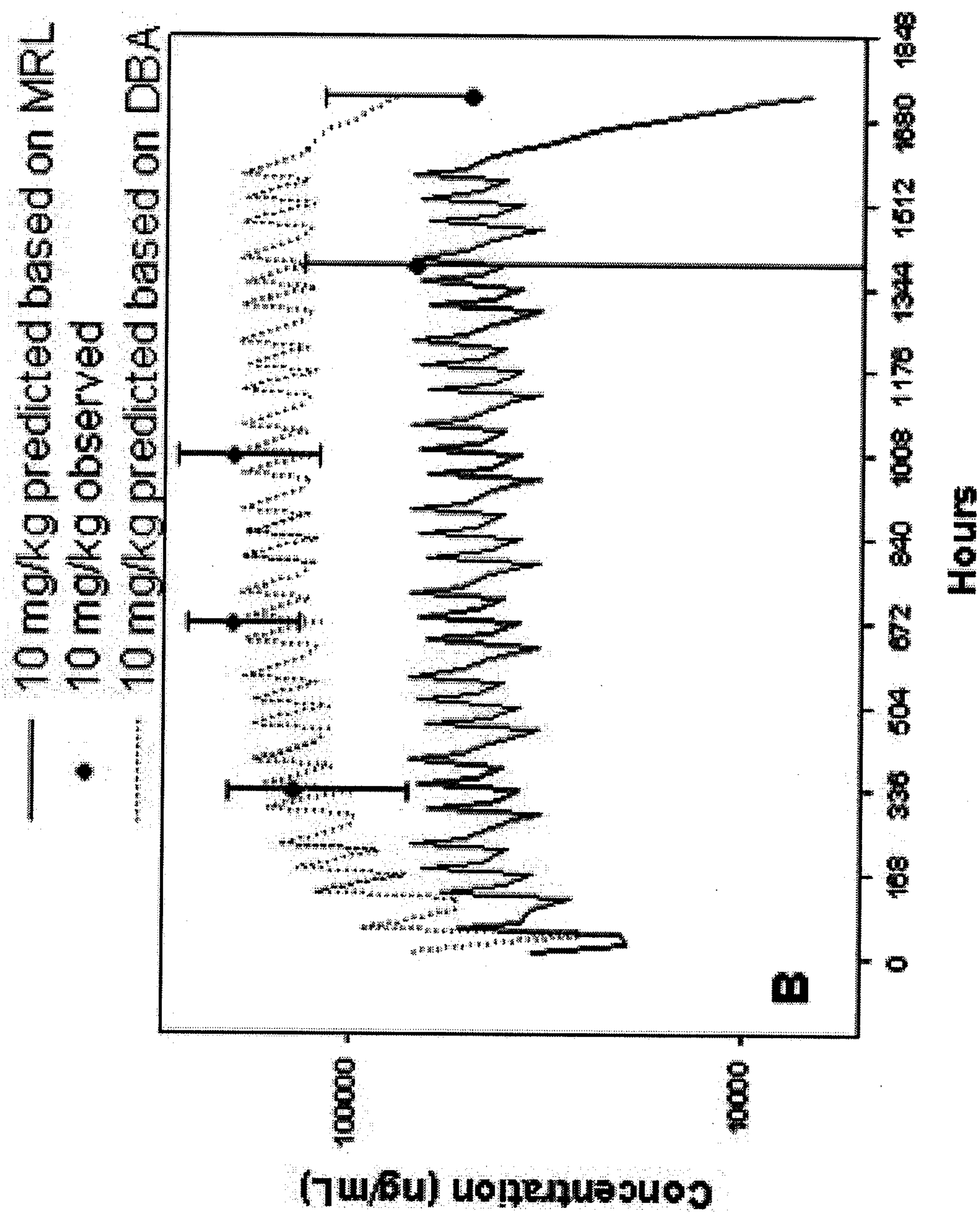


Figure 43a

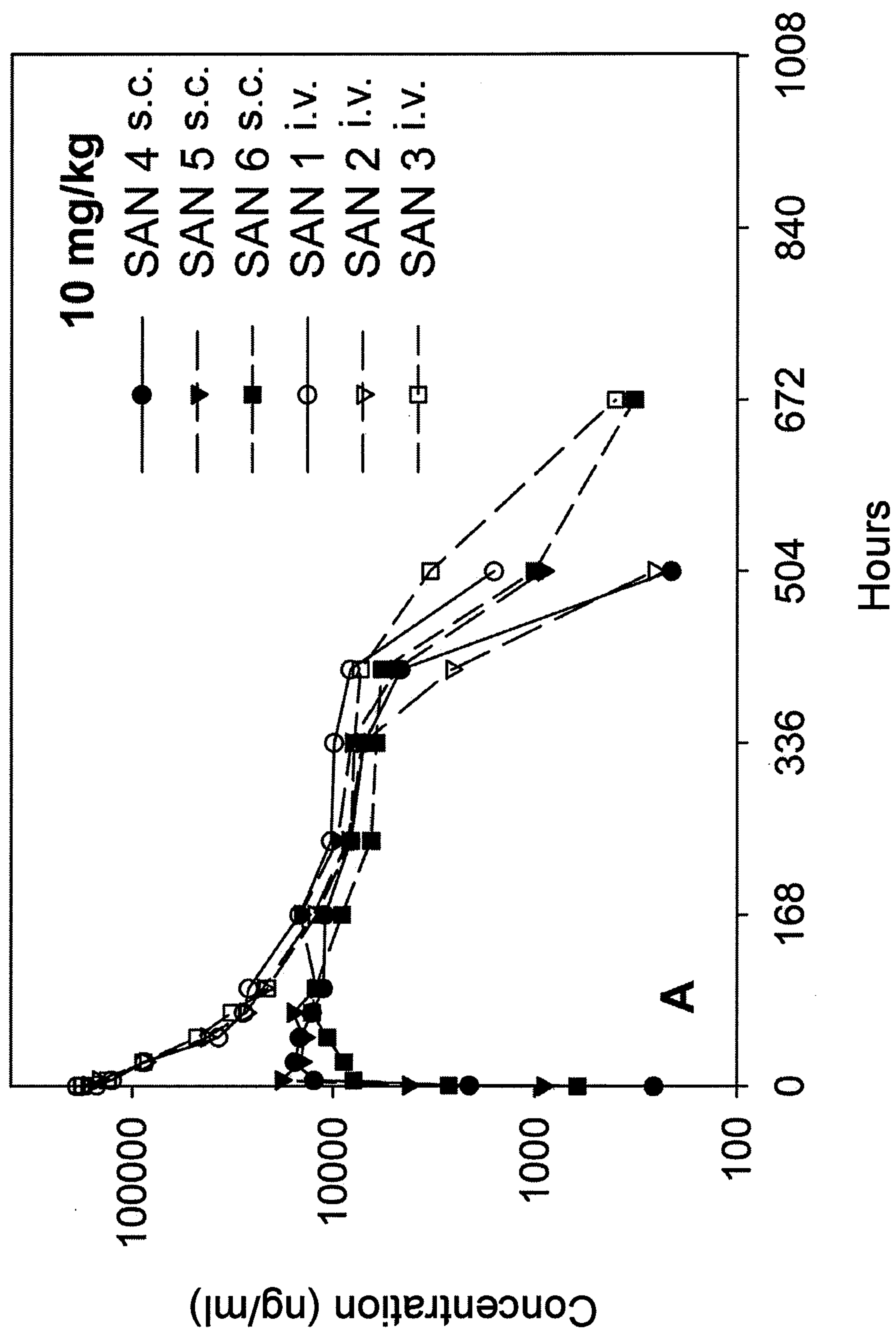
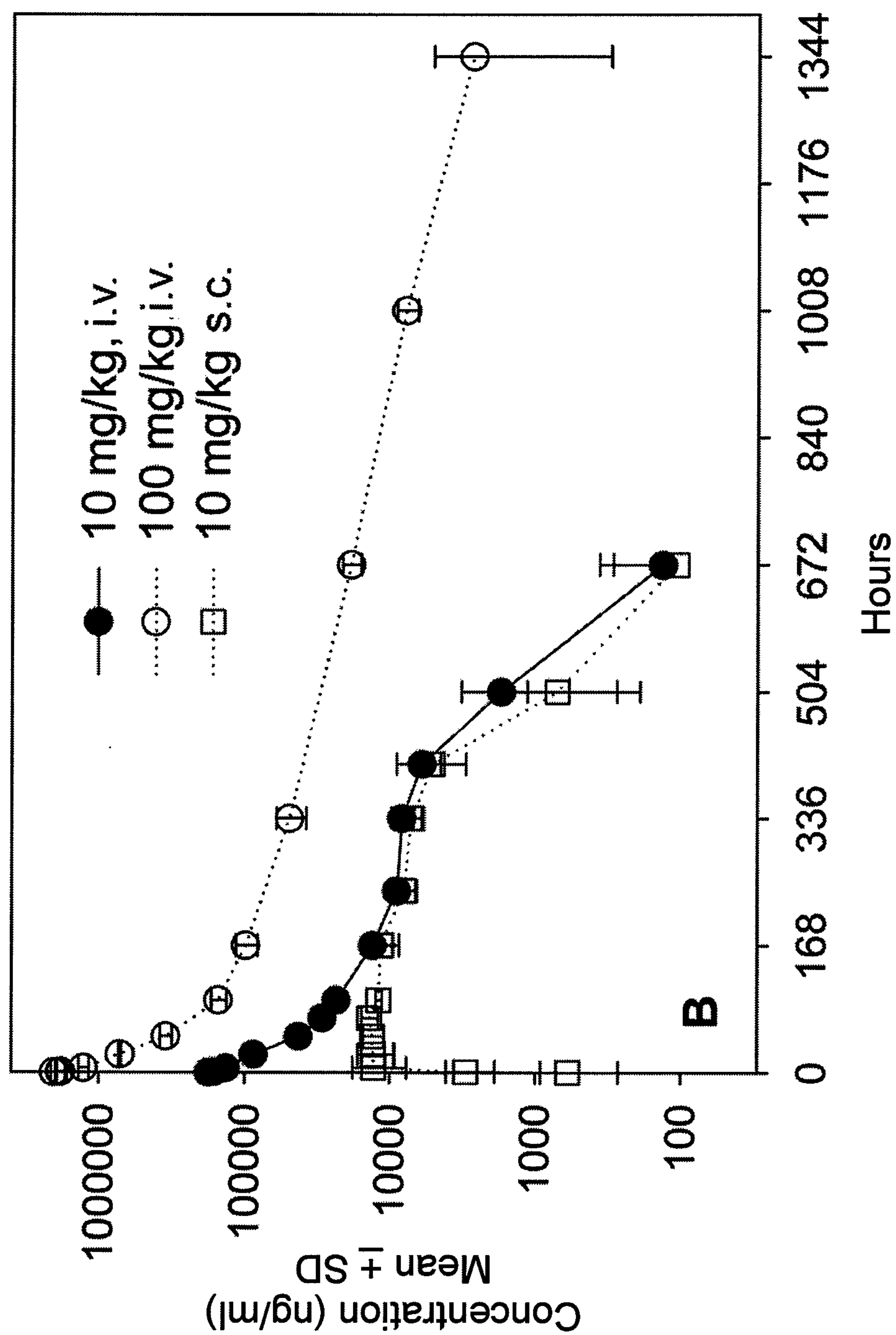


Figure 43b



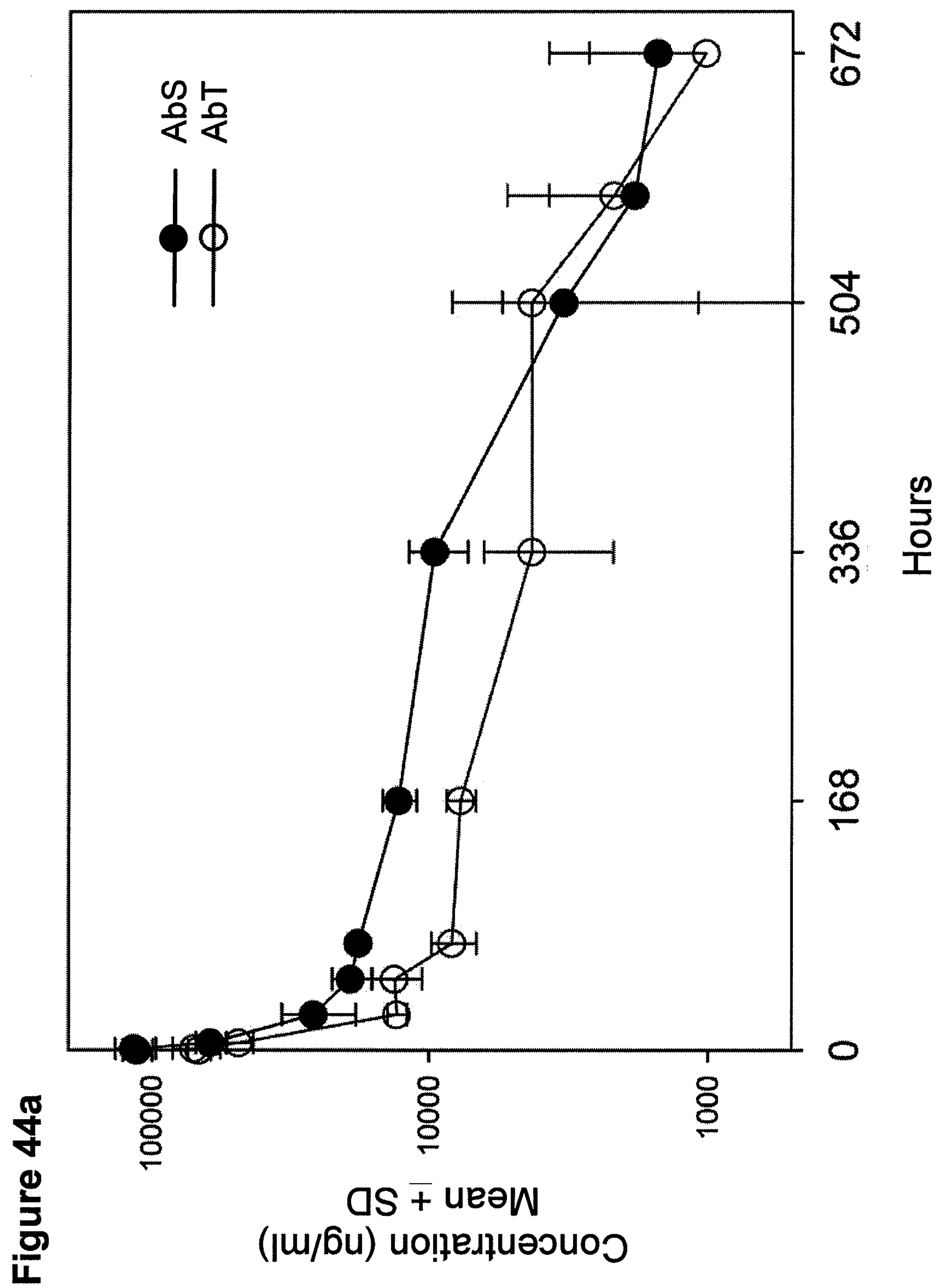


Figure 44b

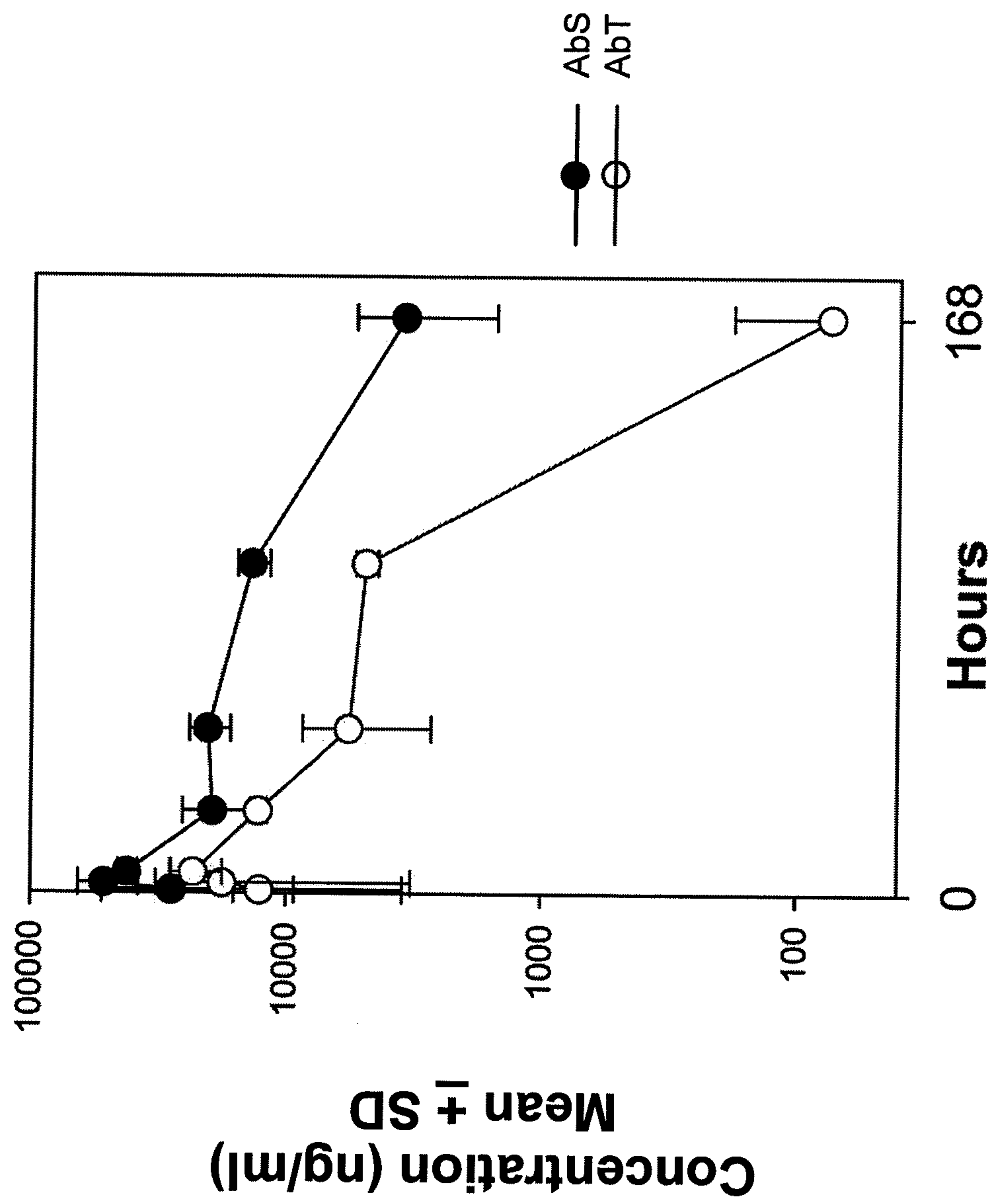


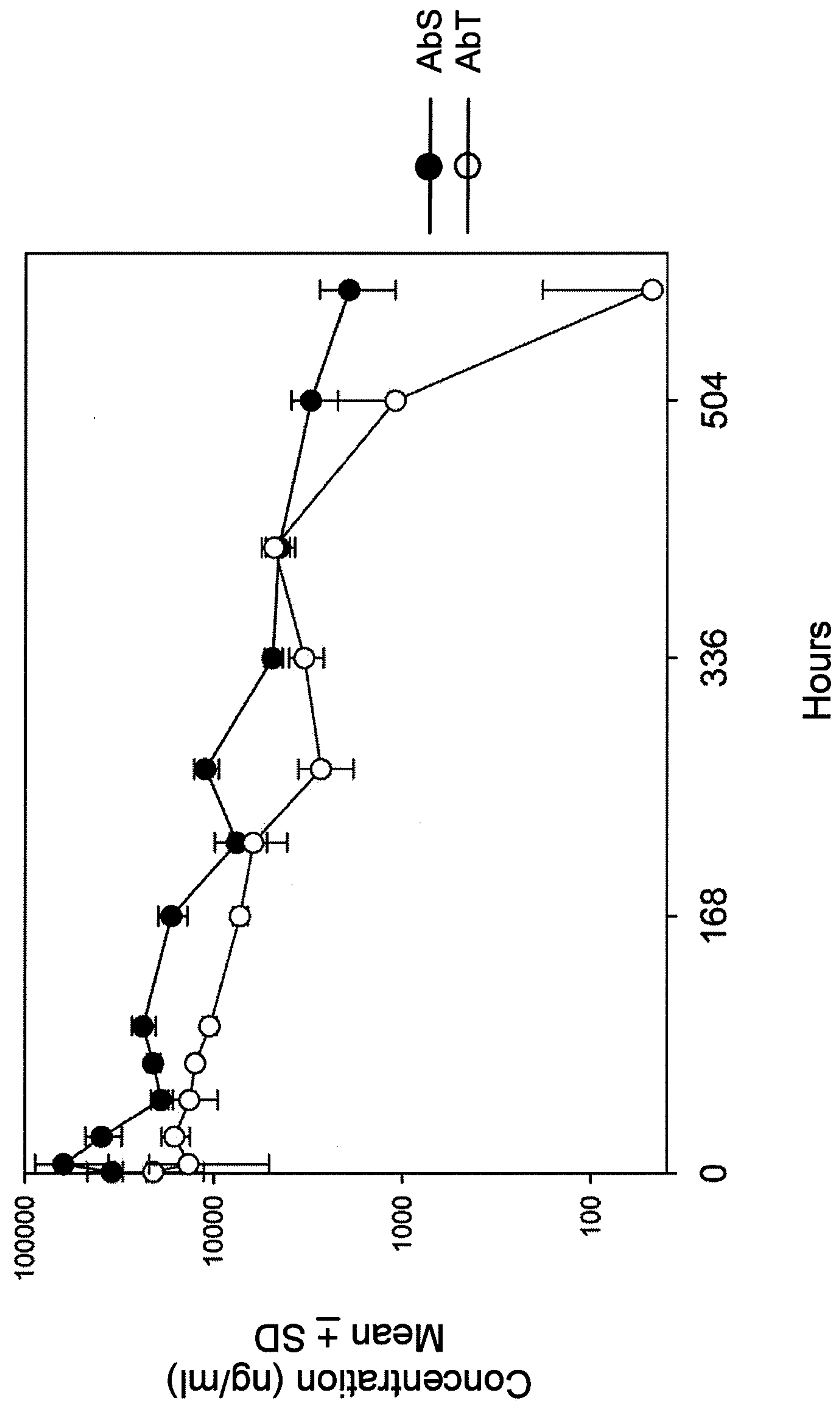
Figure 44c

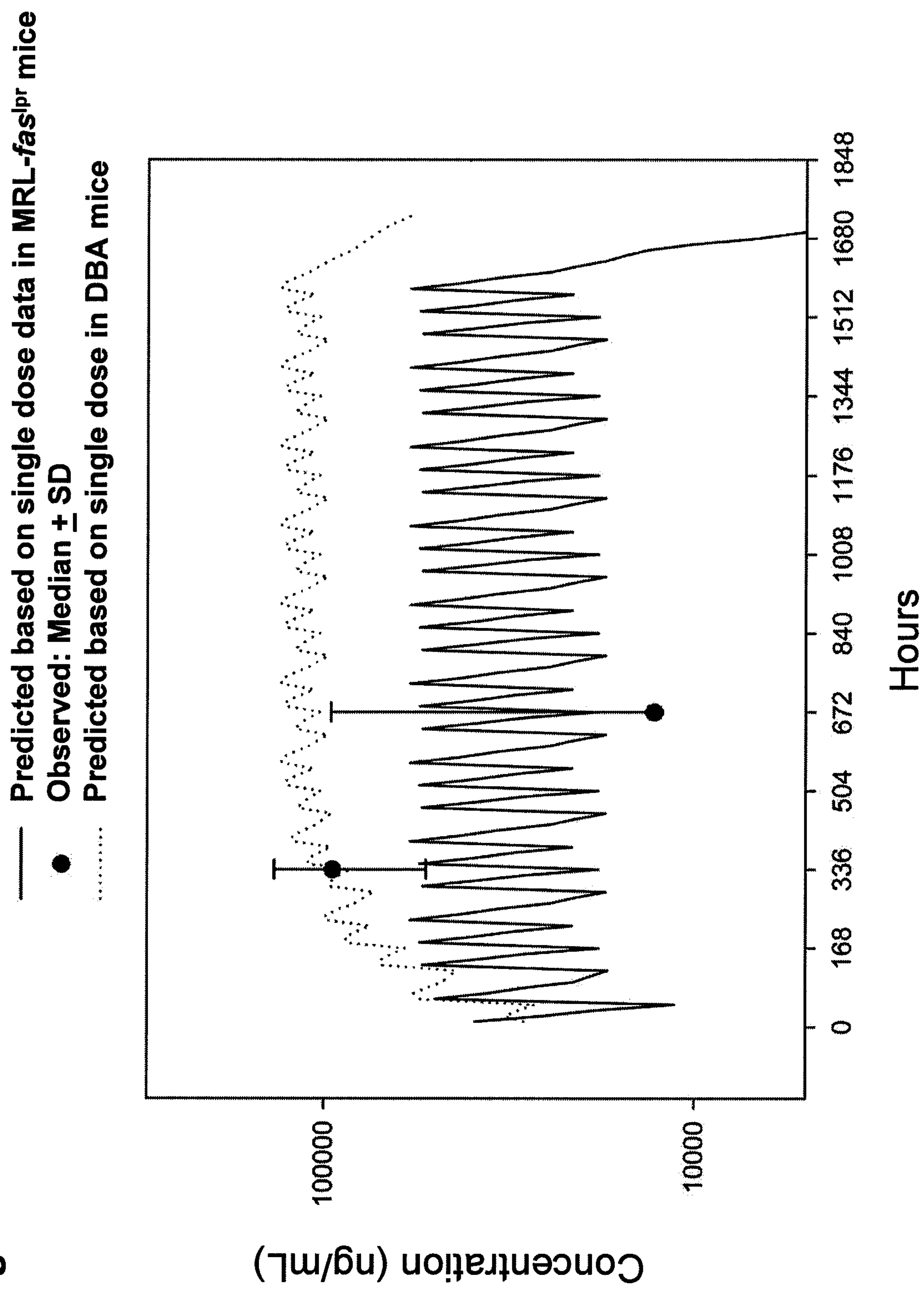
Figure 45

Figure 46a

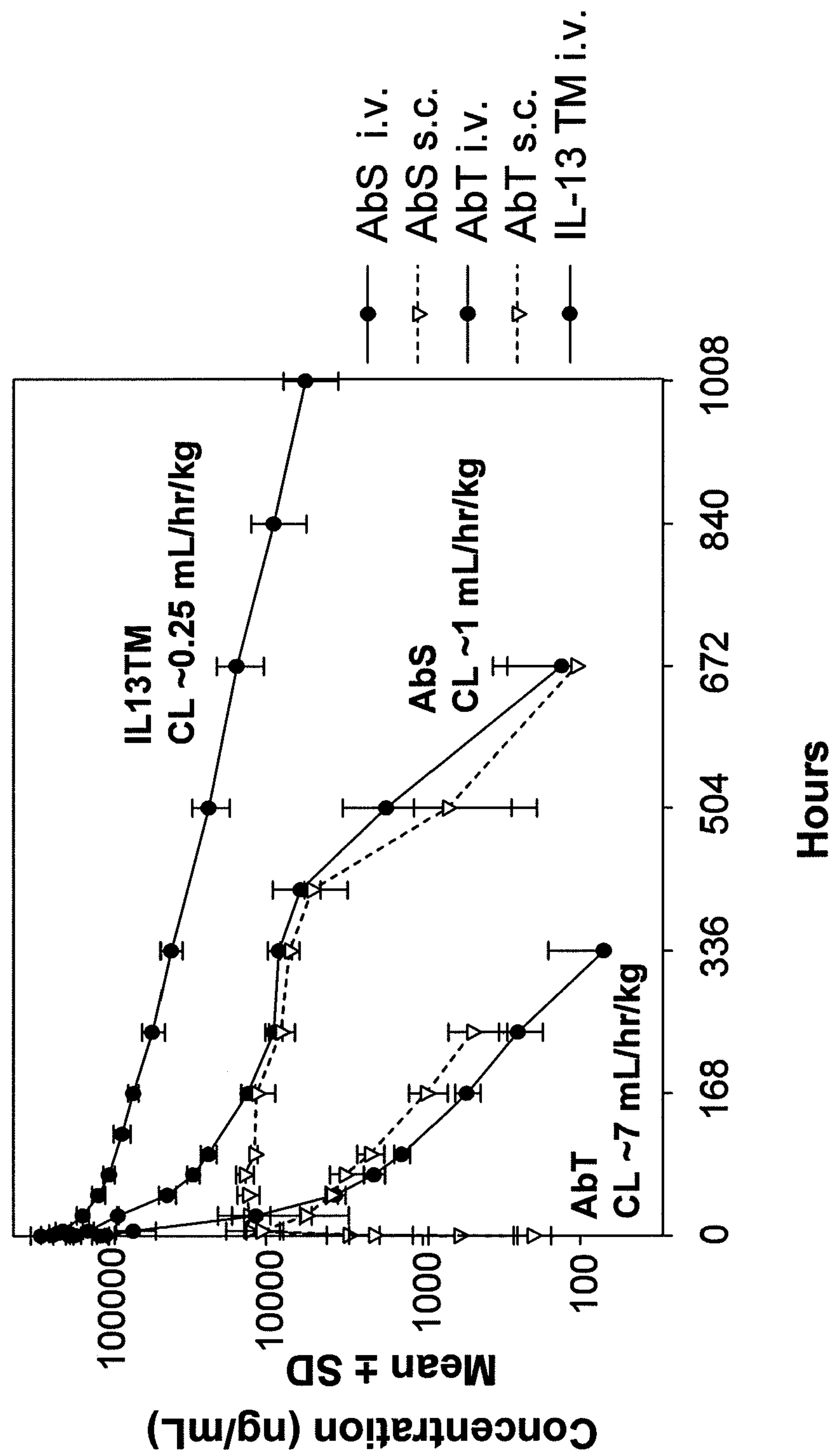


Figure 46b

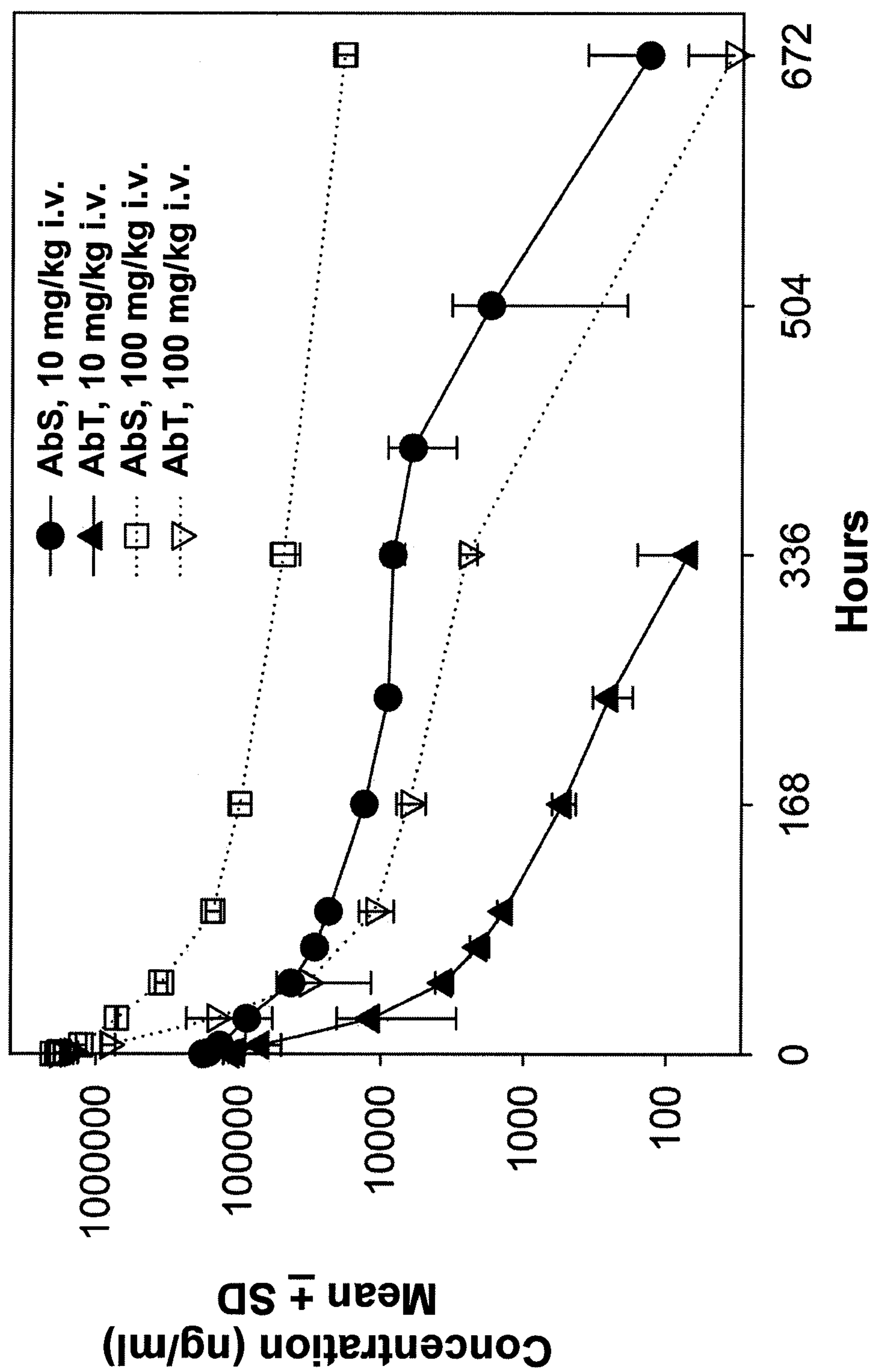


Figure 47

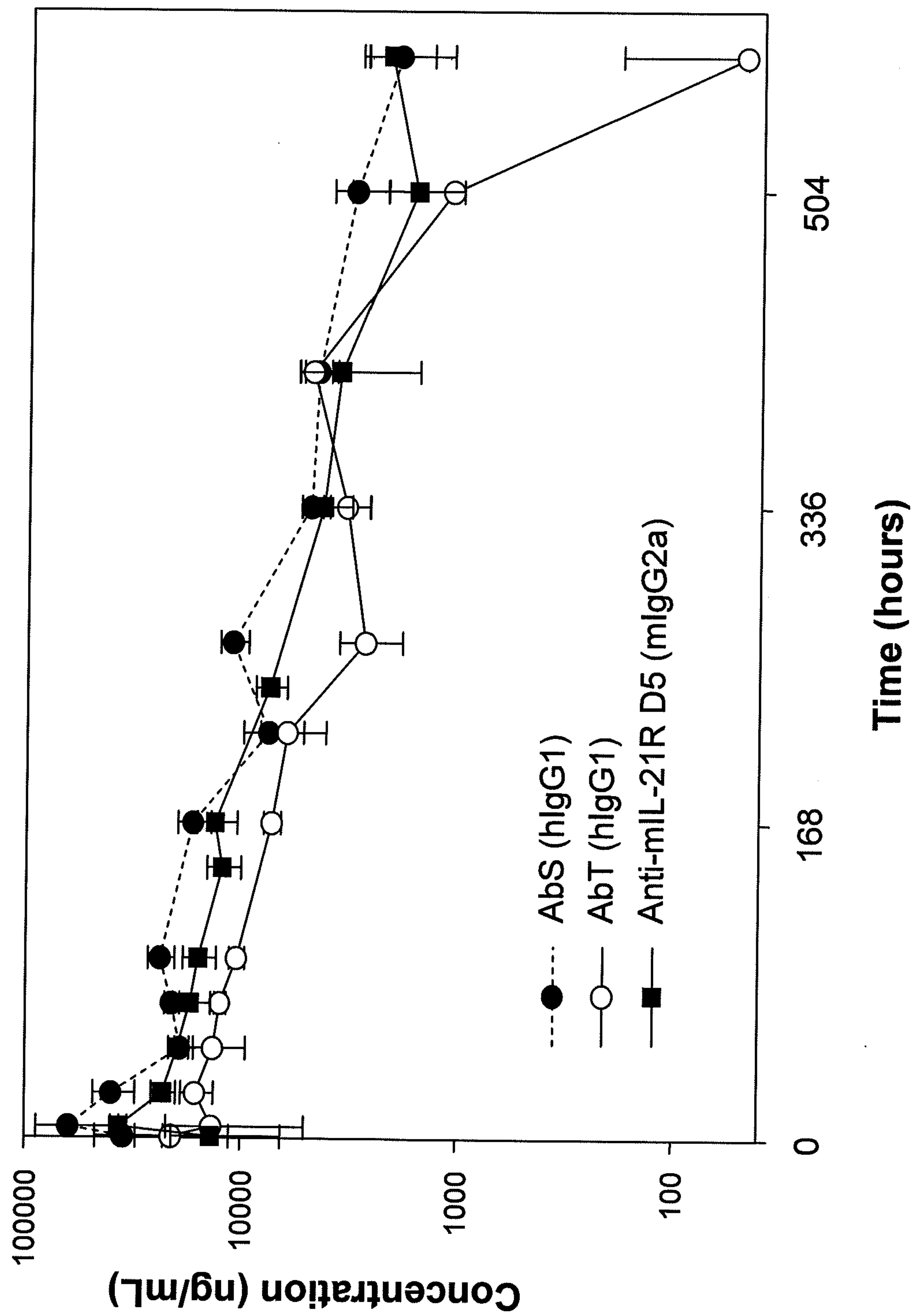


Figure 48a

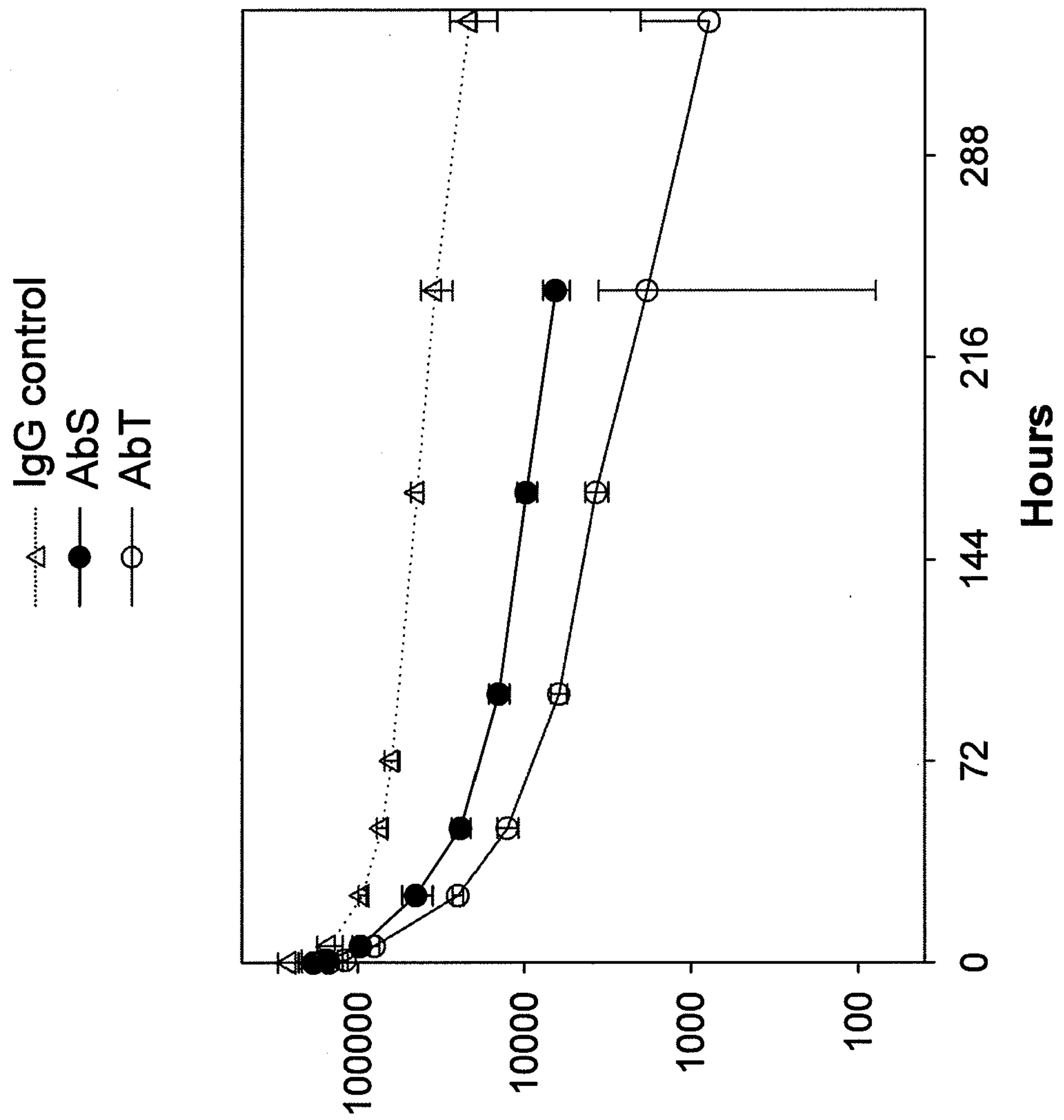


Figure 48b

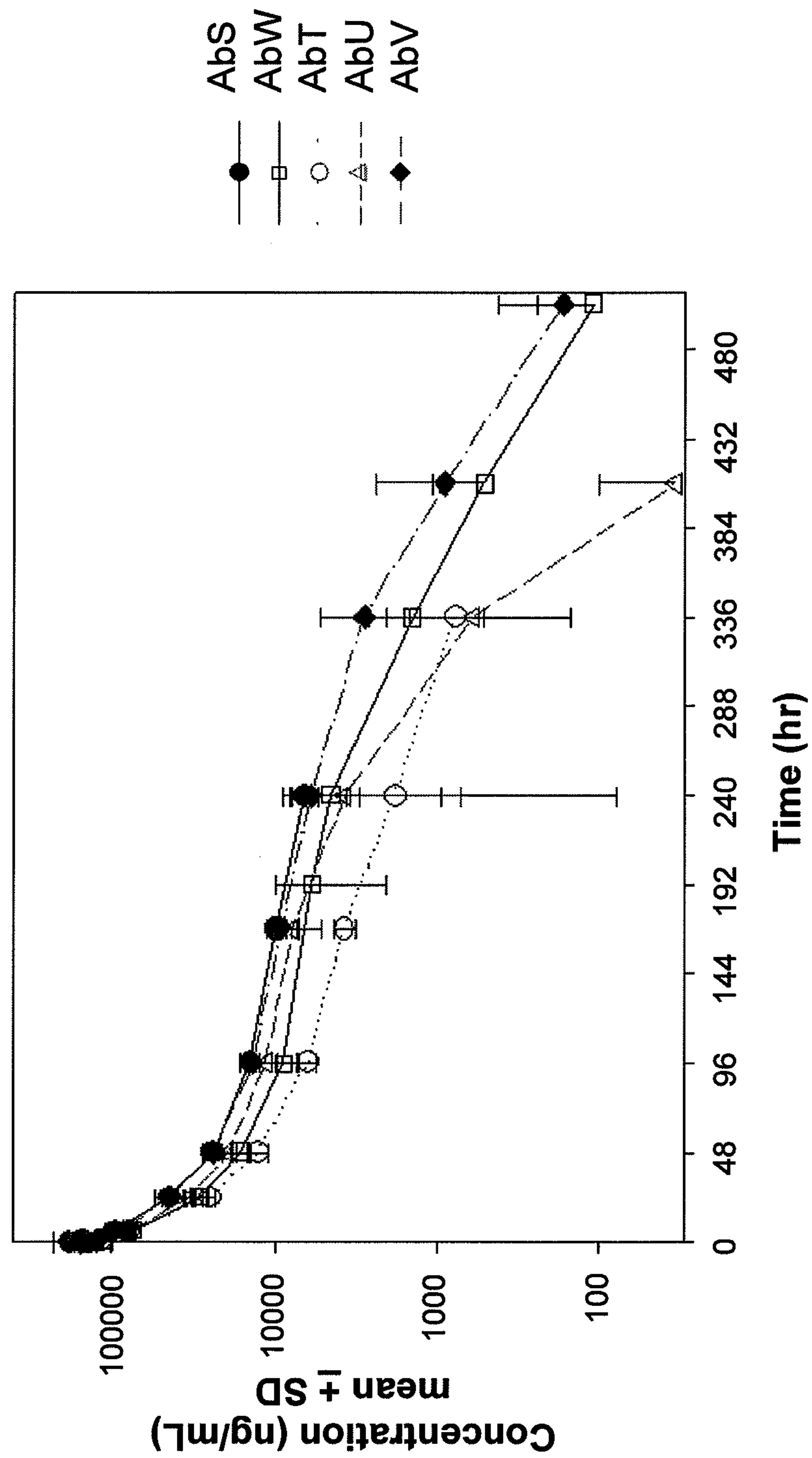


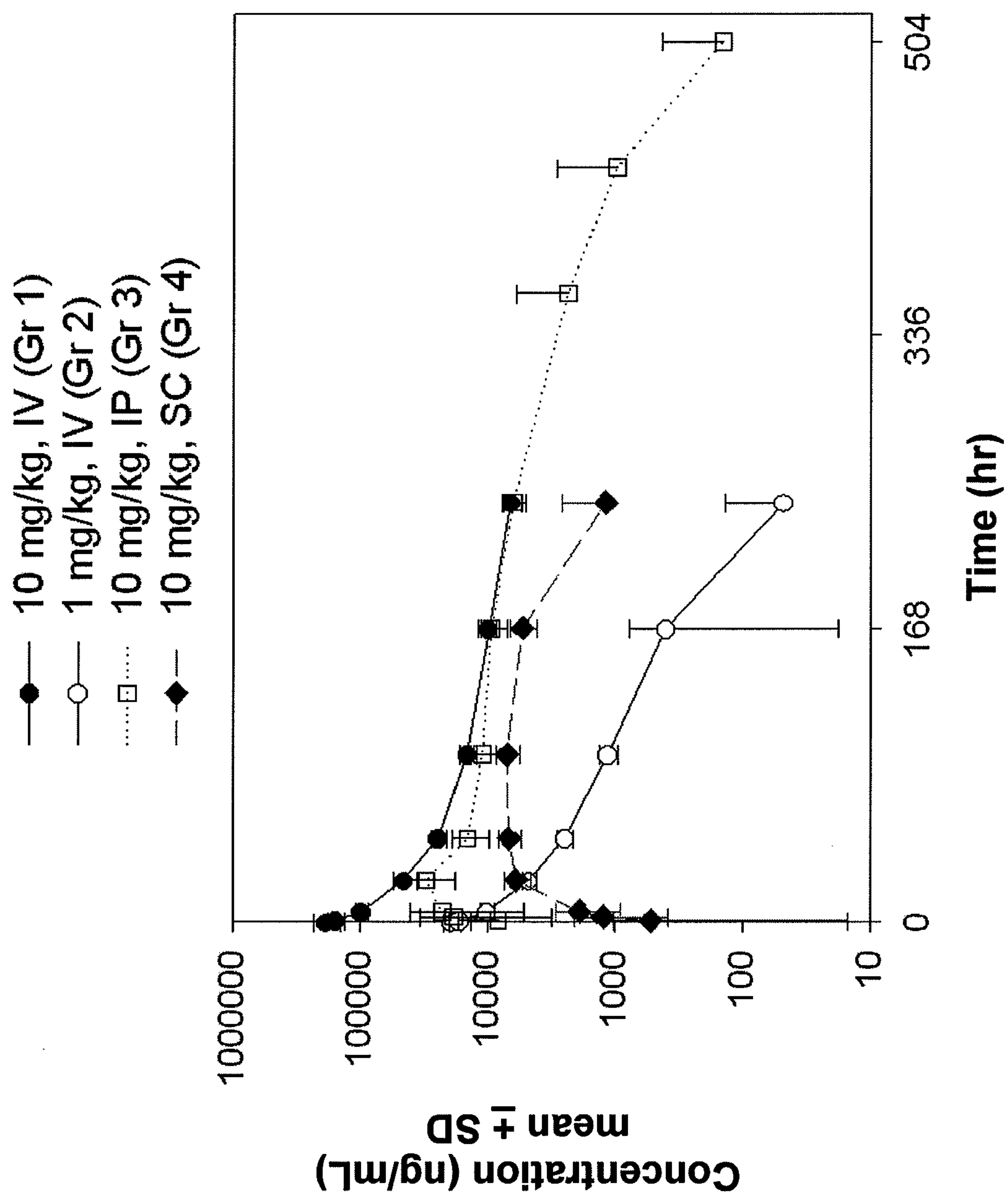
Figure 49

Figure 50a

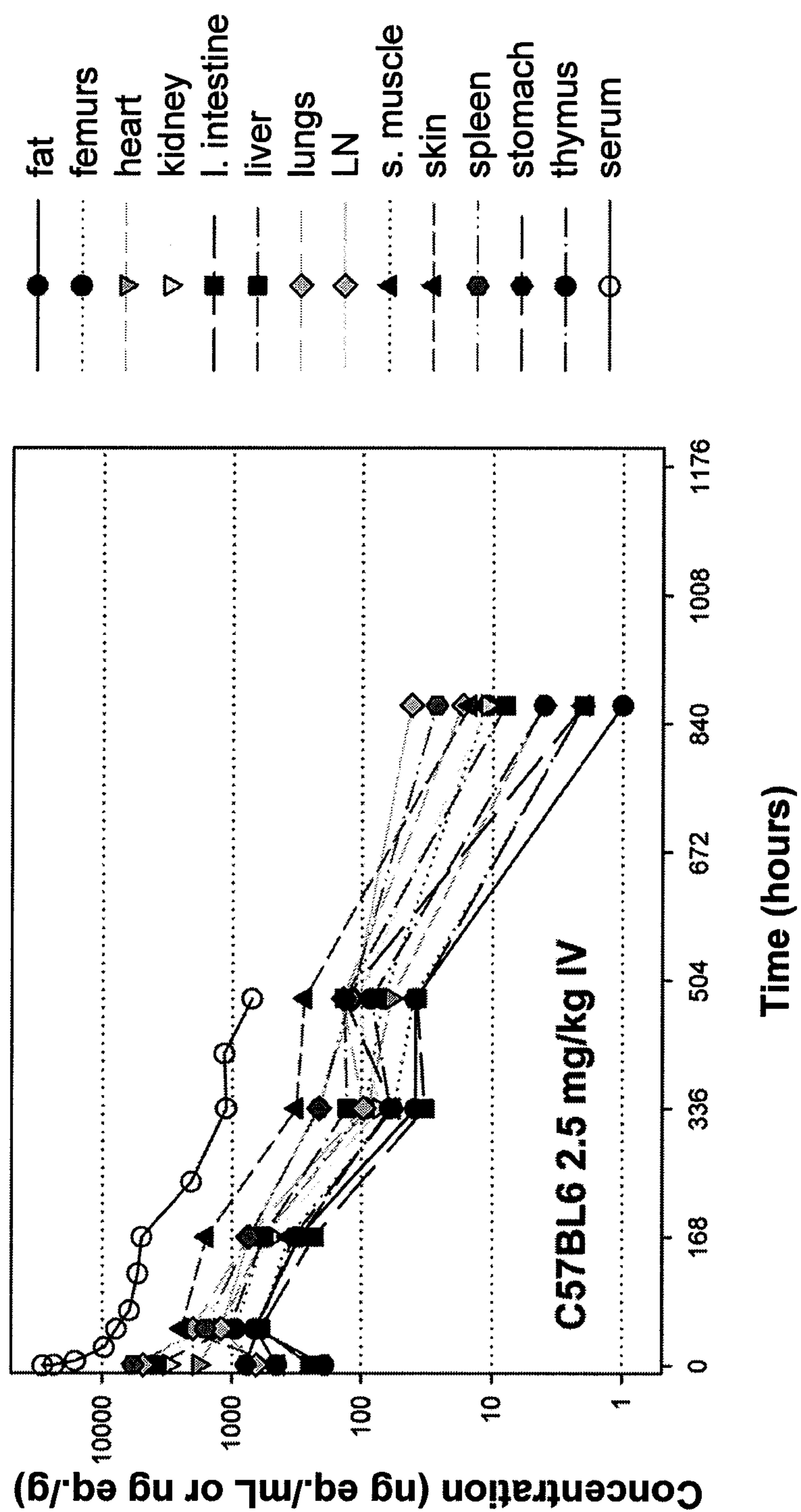
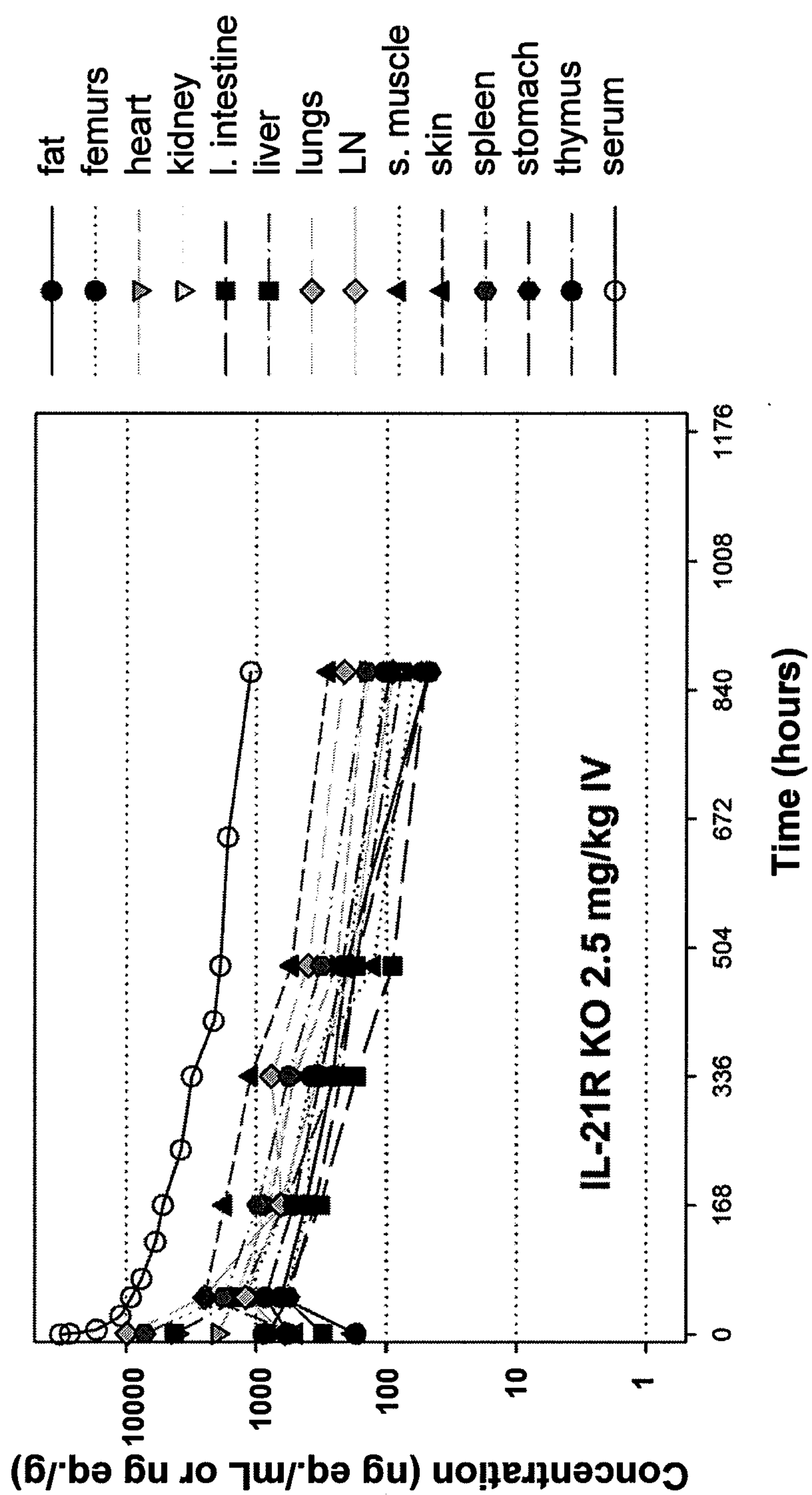


Figure 50b



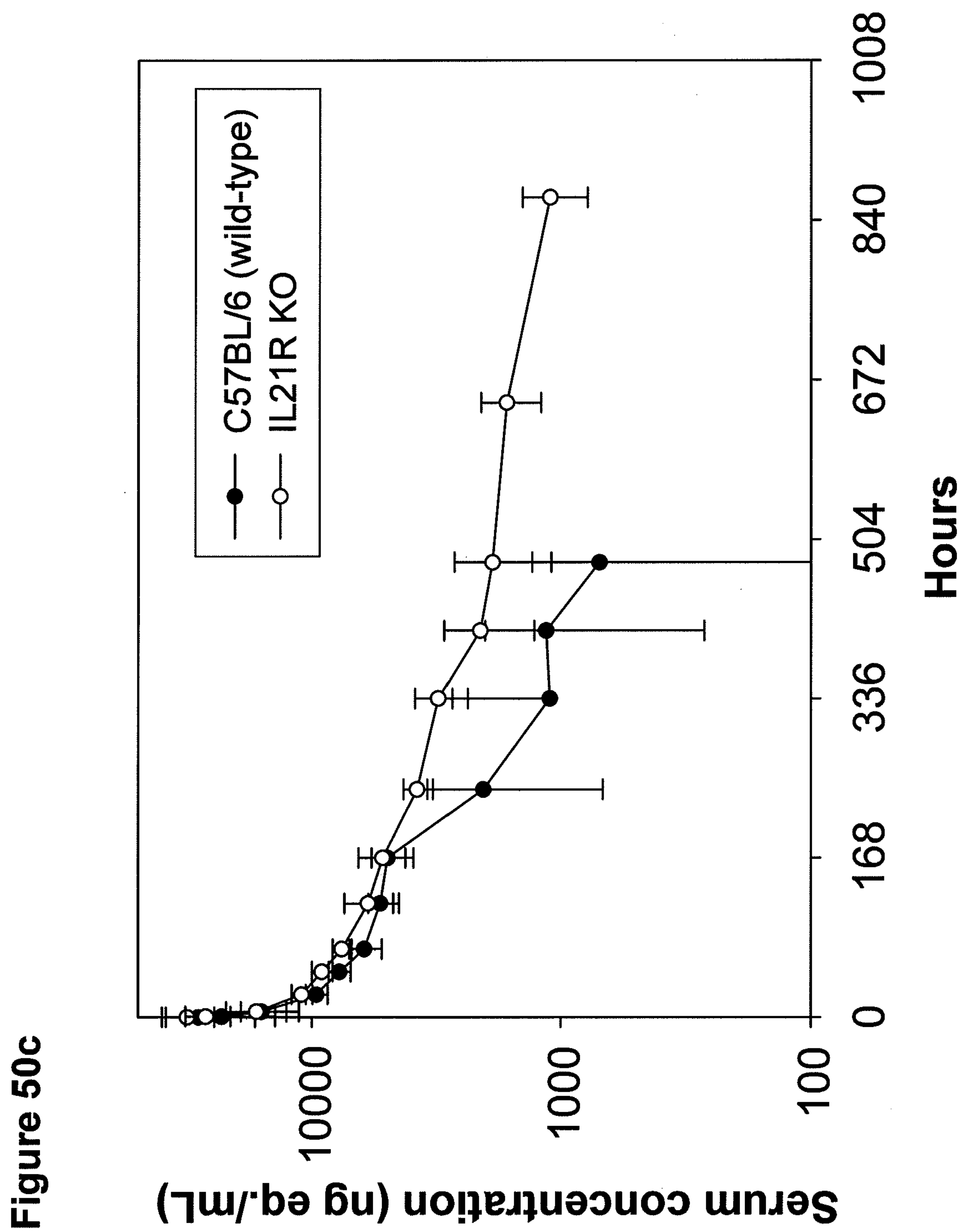


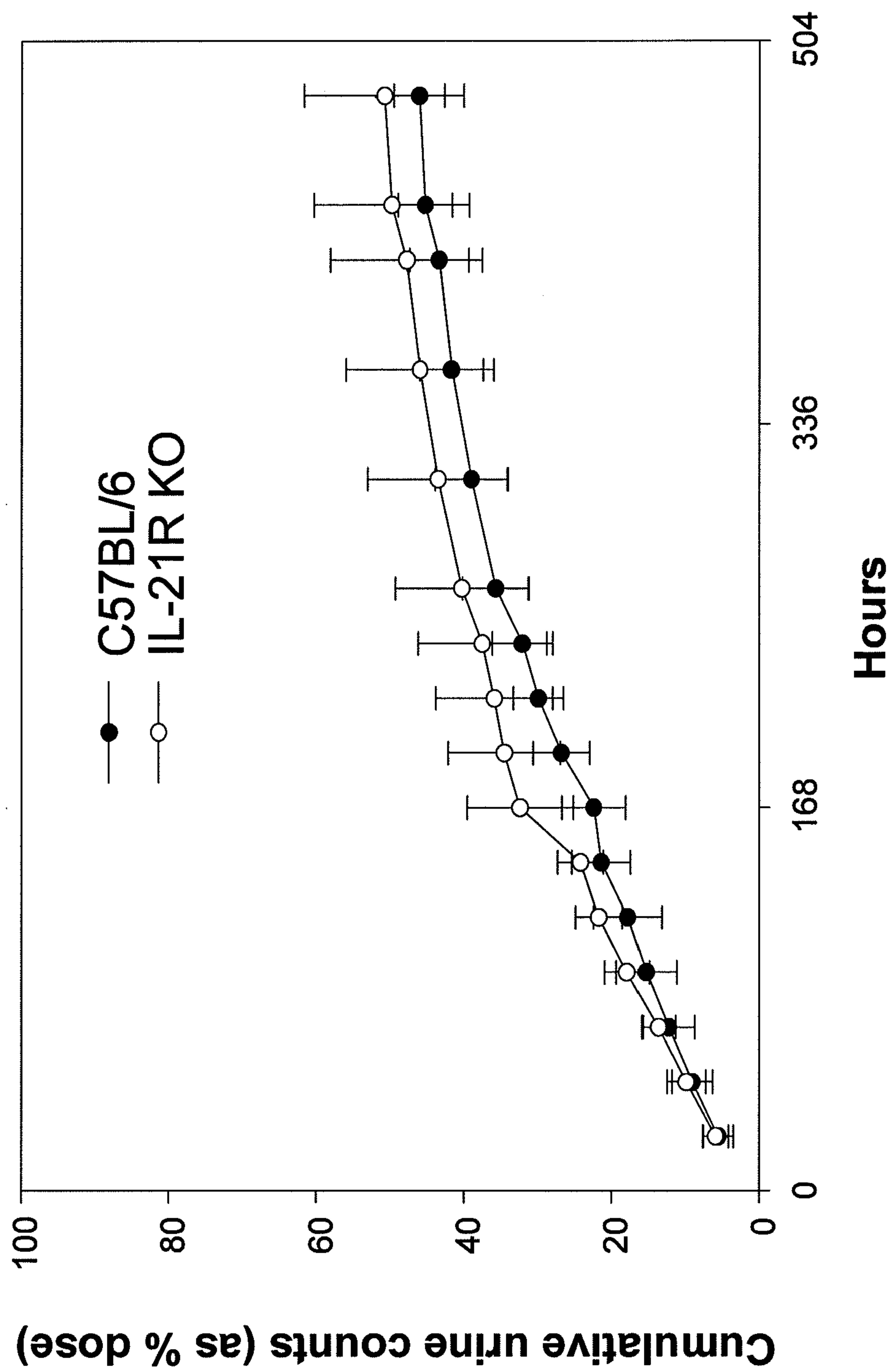
Figure 50d

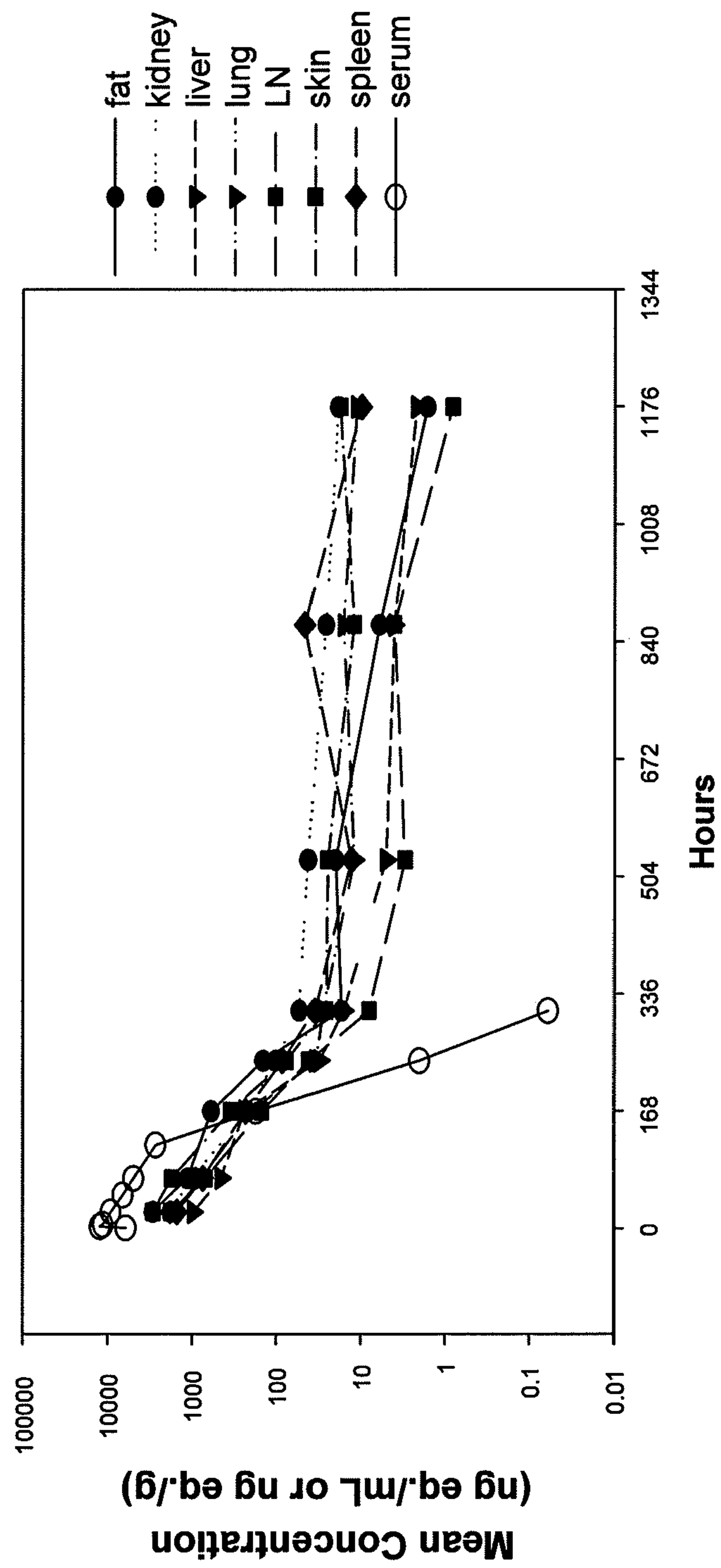
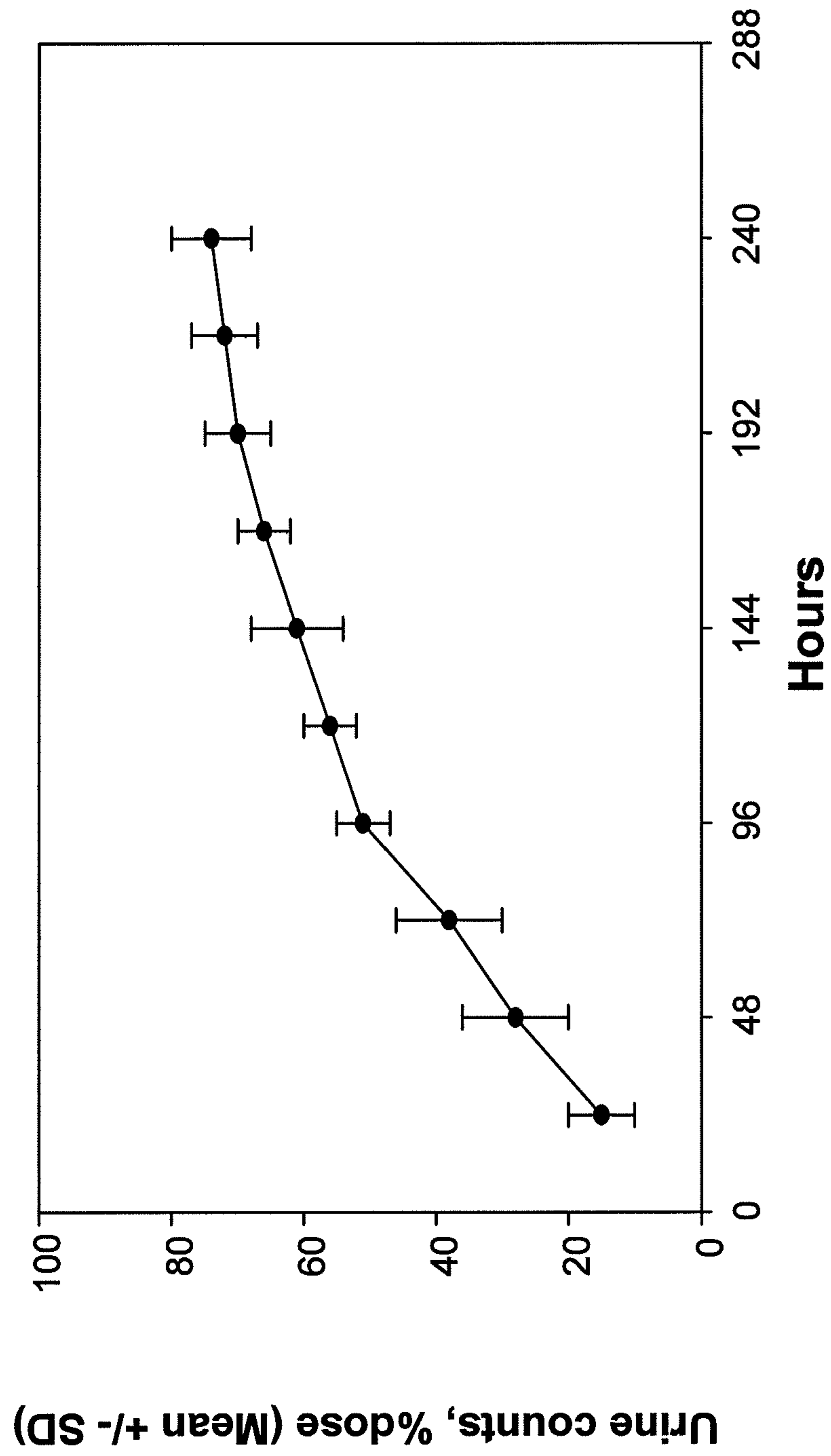
Figure 51a

Figure 51b

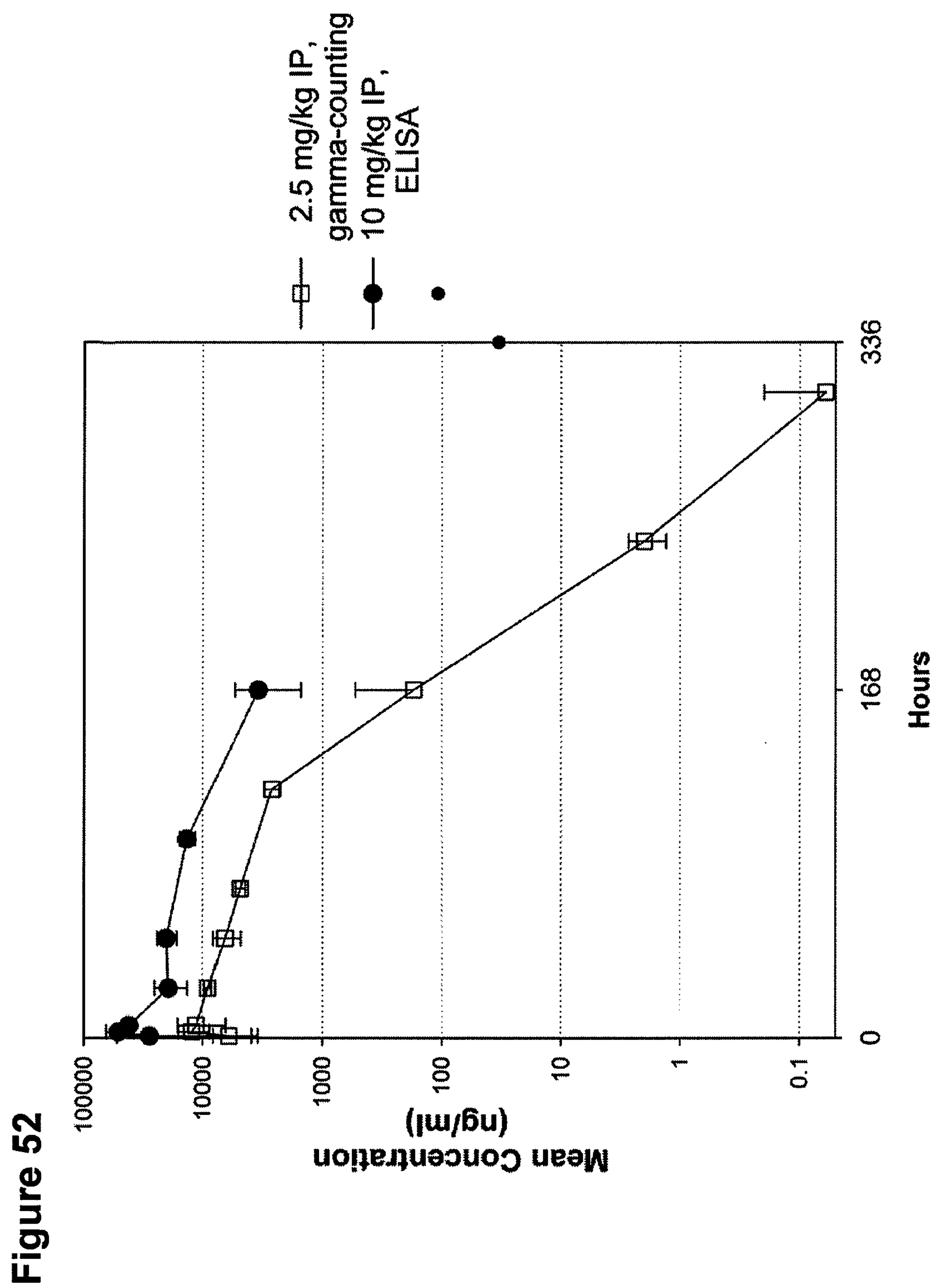


Figure 53a

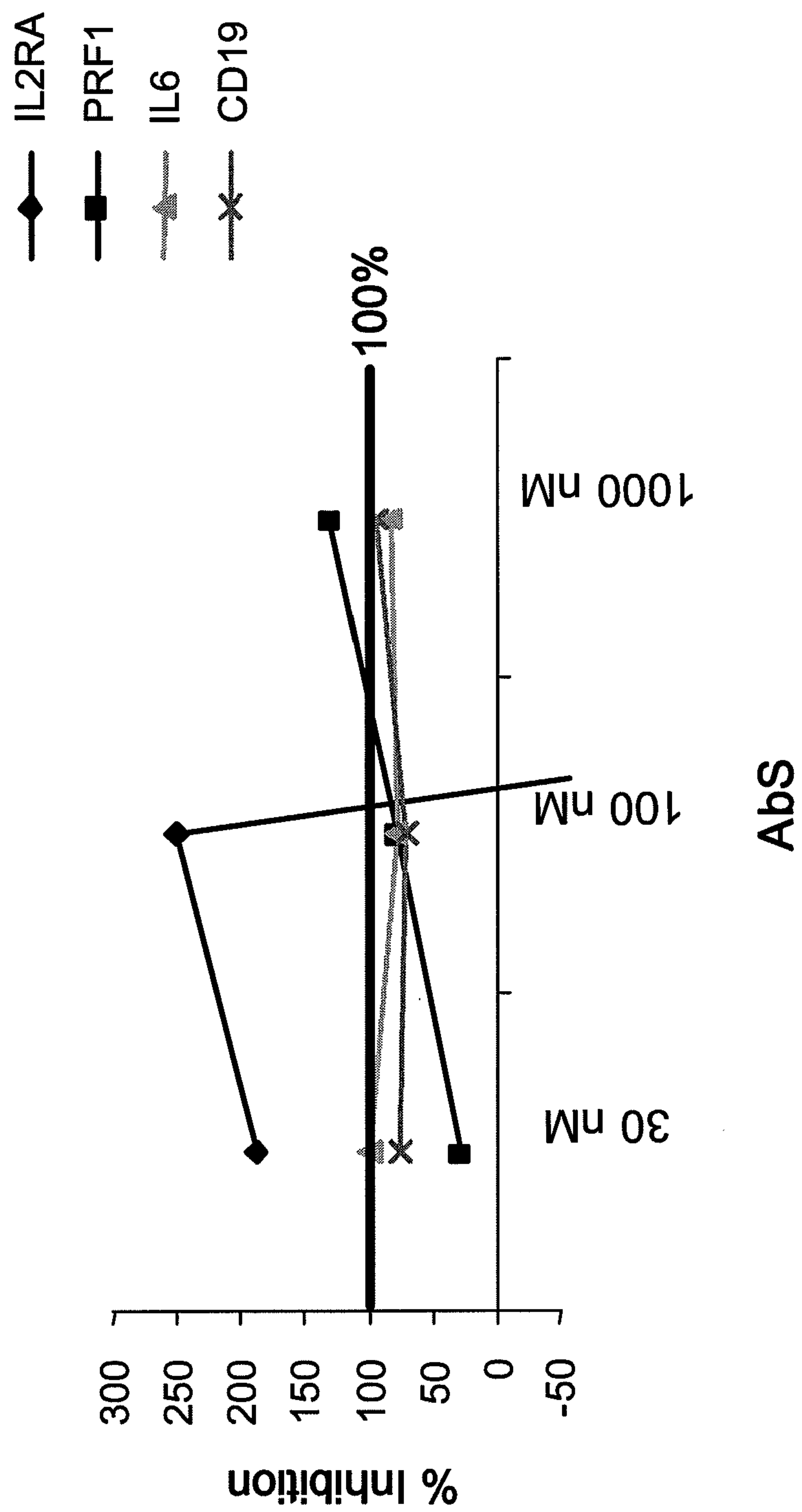


Figure 53b

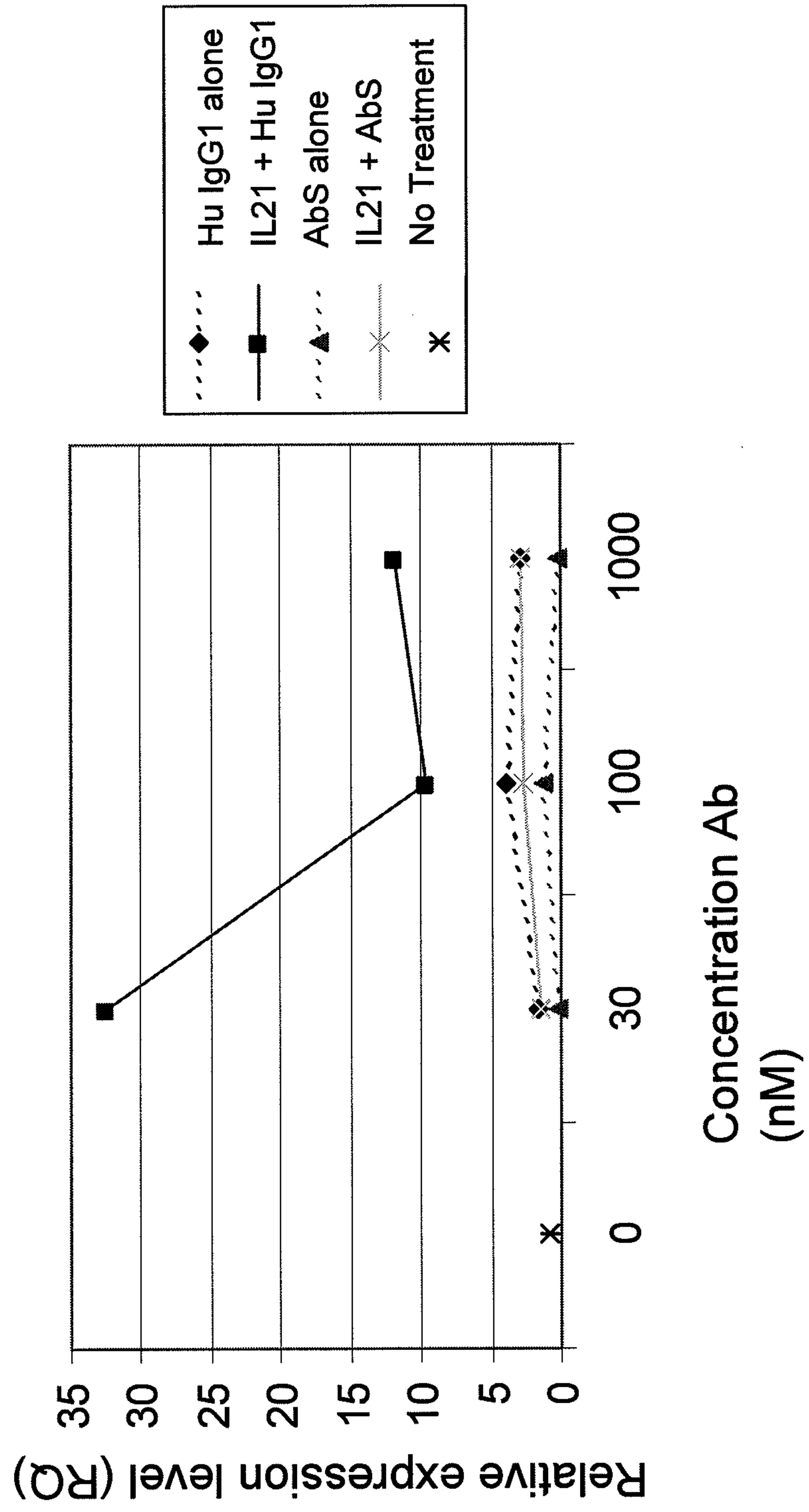


Figure 54

18S	IFNG
GAPDH	IL10
GUSB	IL12A
PGK1	IL1B
ZNF592	IL21R
CCL3	IL2RA
CD19	IL6
CXCL10	IL8
CXCL11	PRF1
GNLY	STAT3
GZMB	TBX21
ICAM1	TNF

Endo Controls

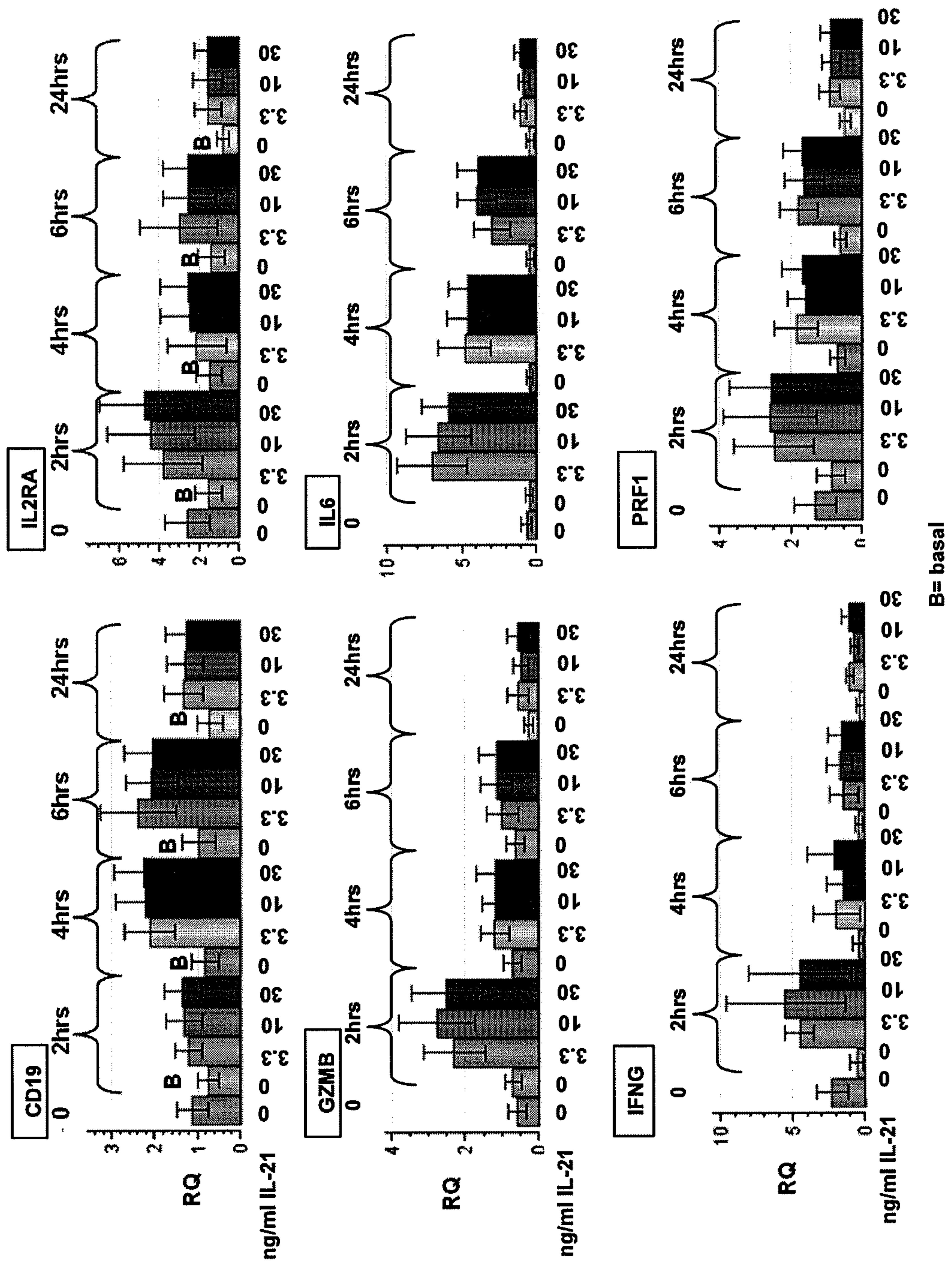
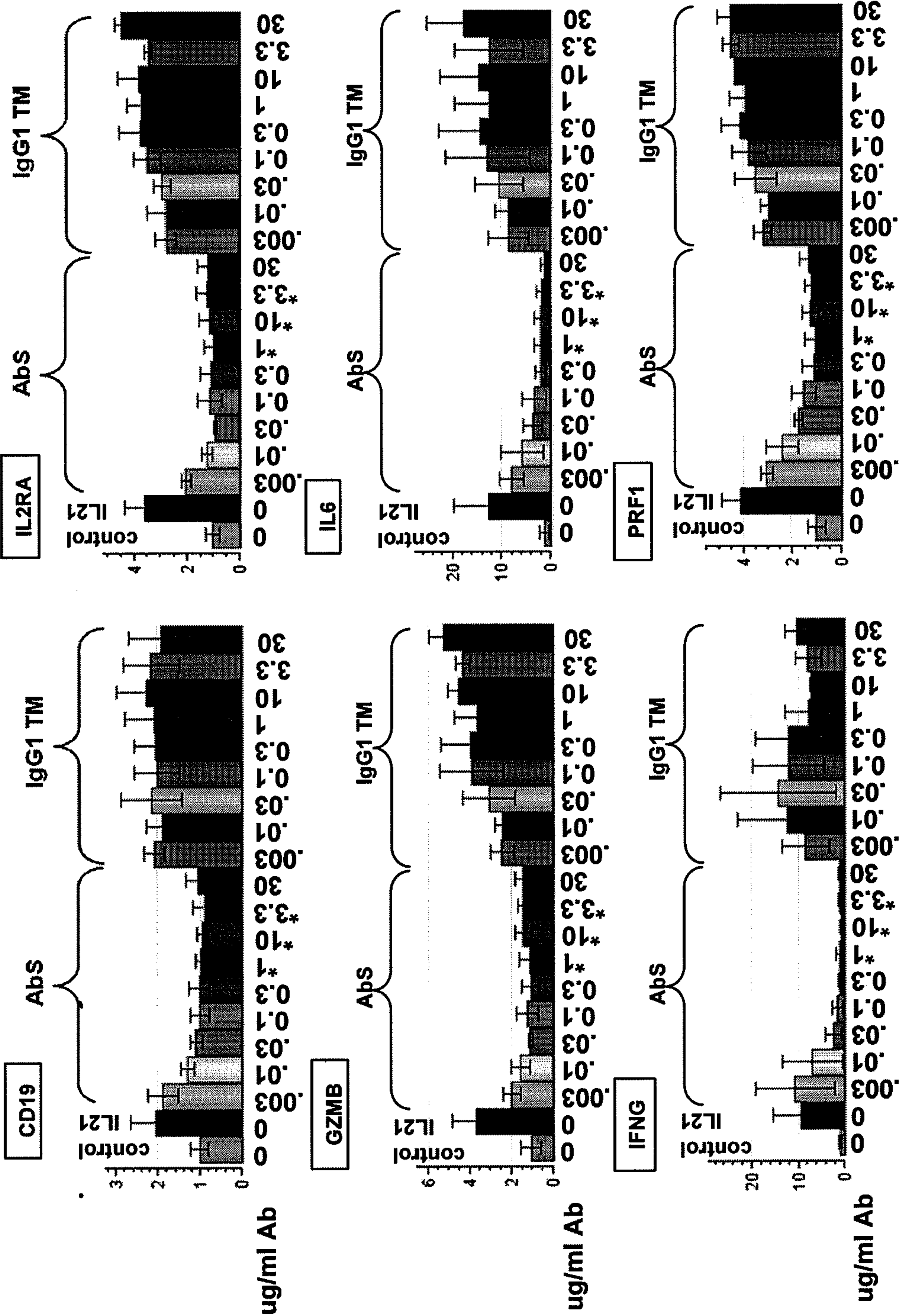
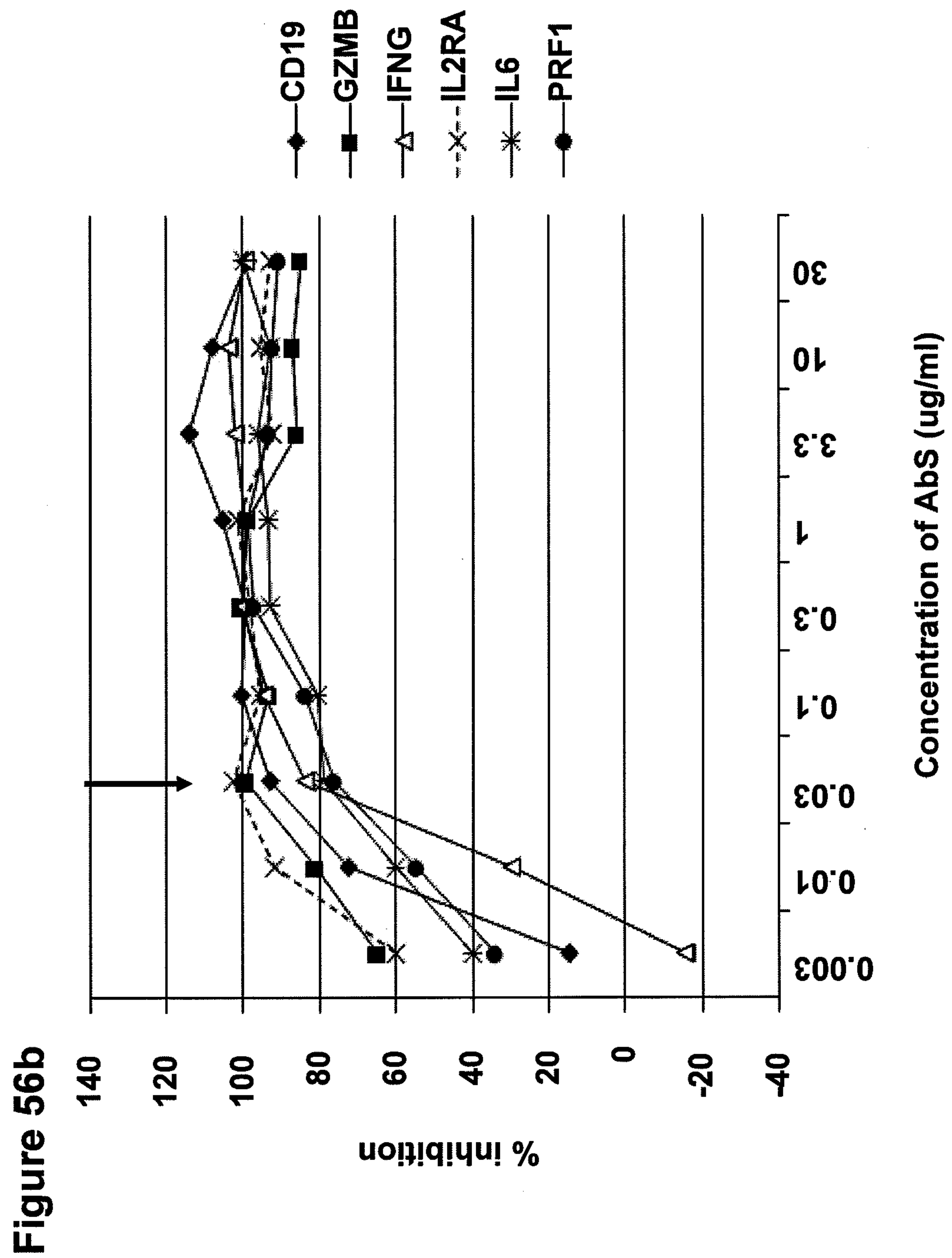
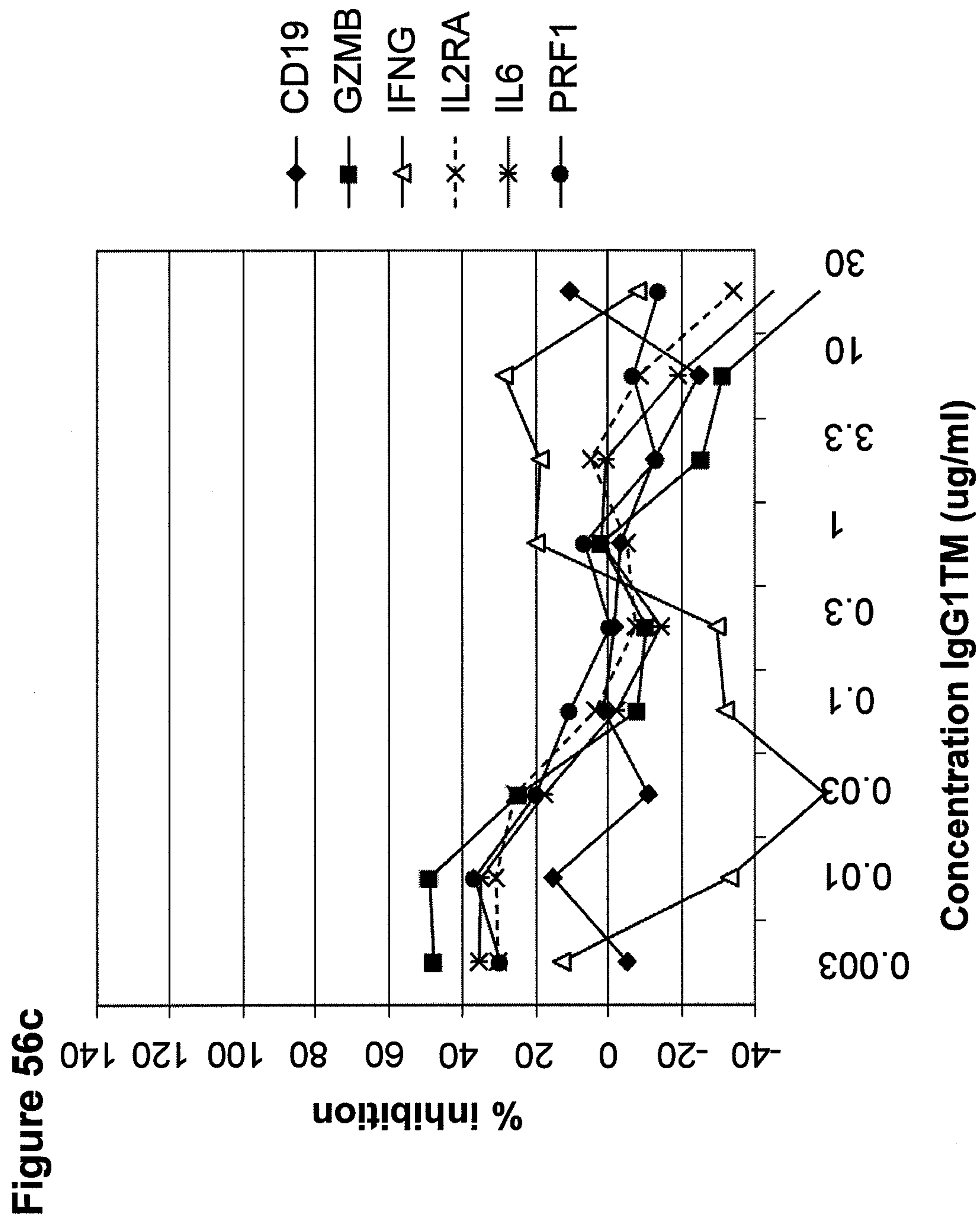
Figure 55

Figure 56a



* n=8; for other samples, n=4





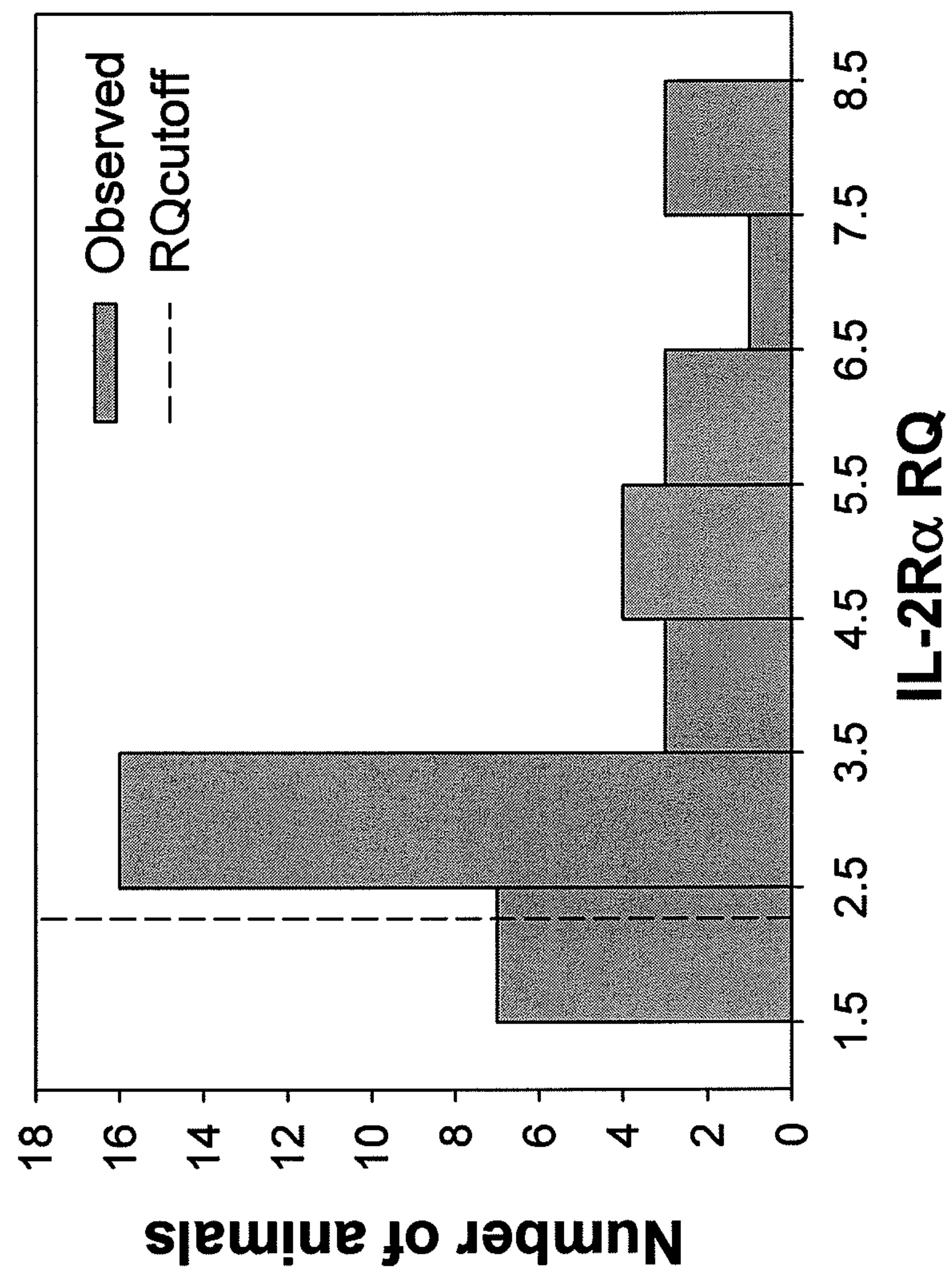
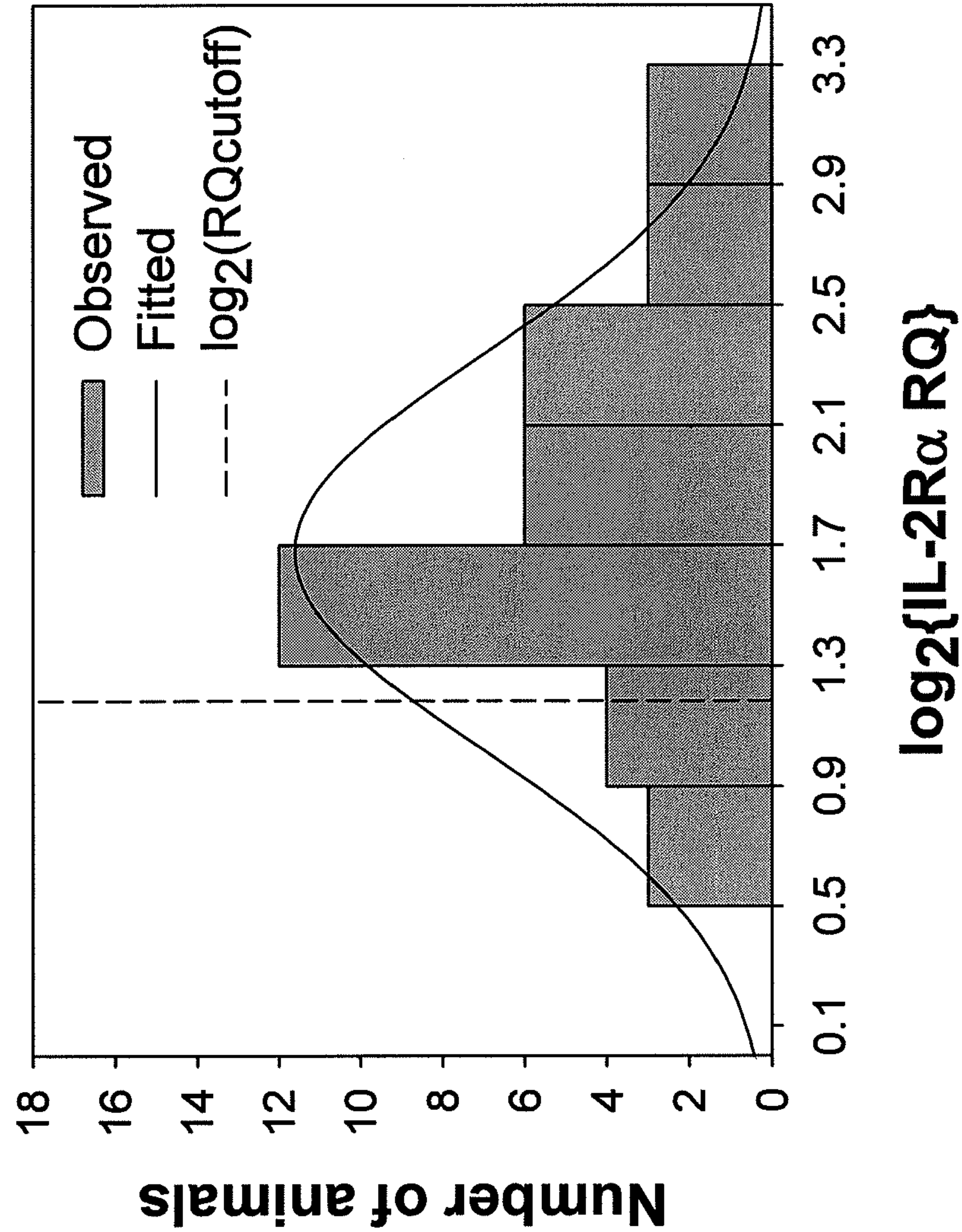
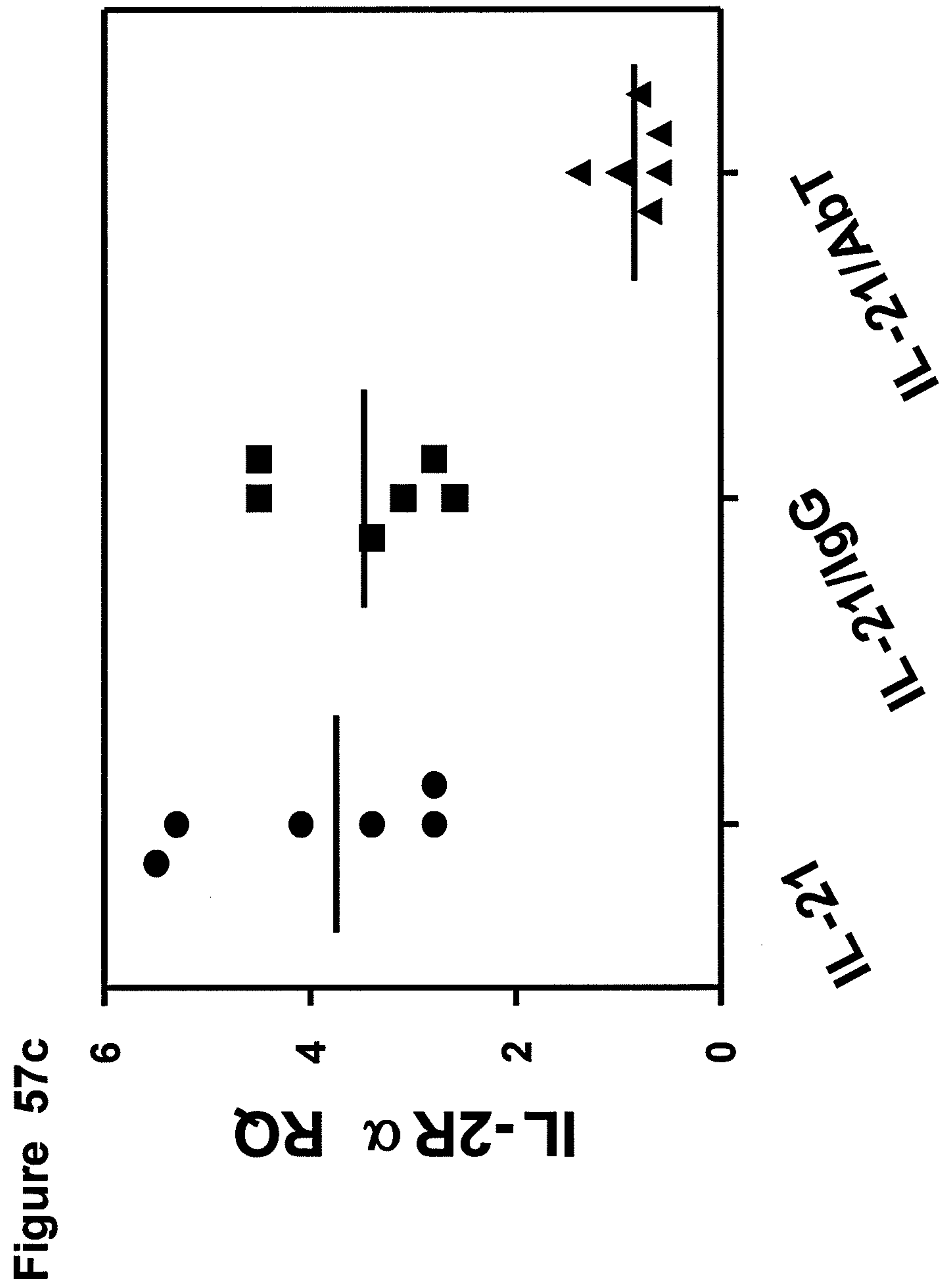
**Figure 57a**

Figure 57b





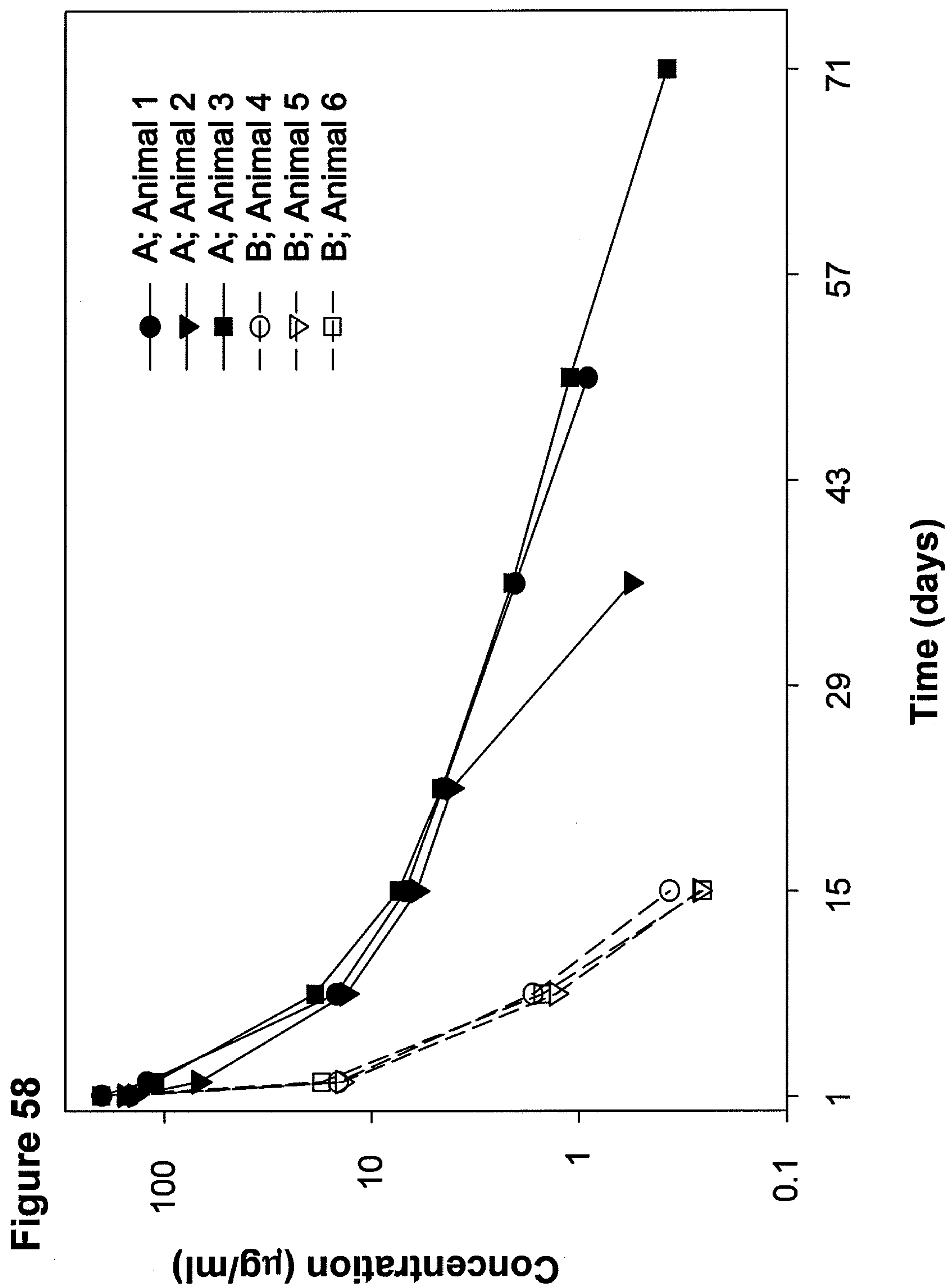


Figure 59a

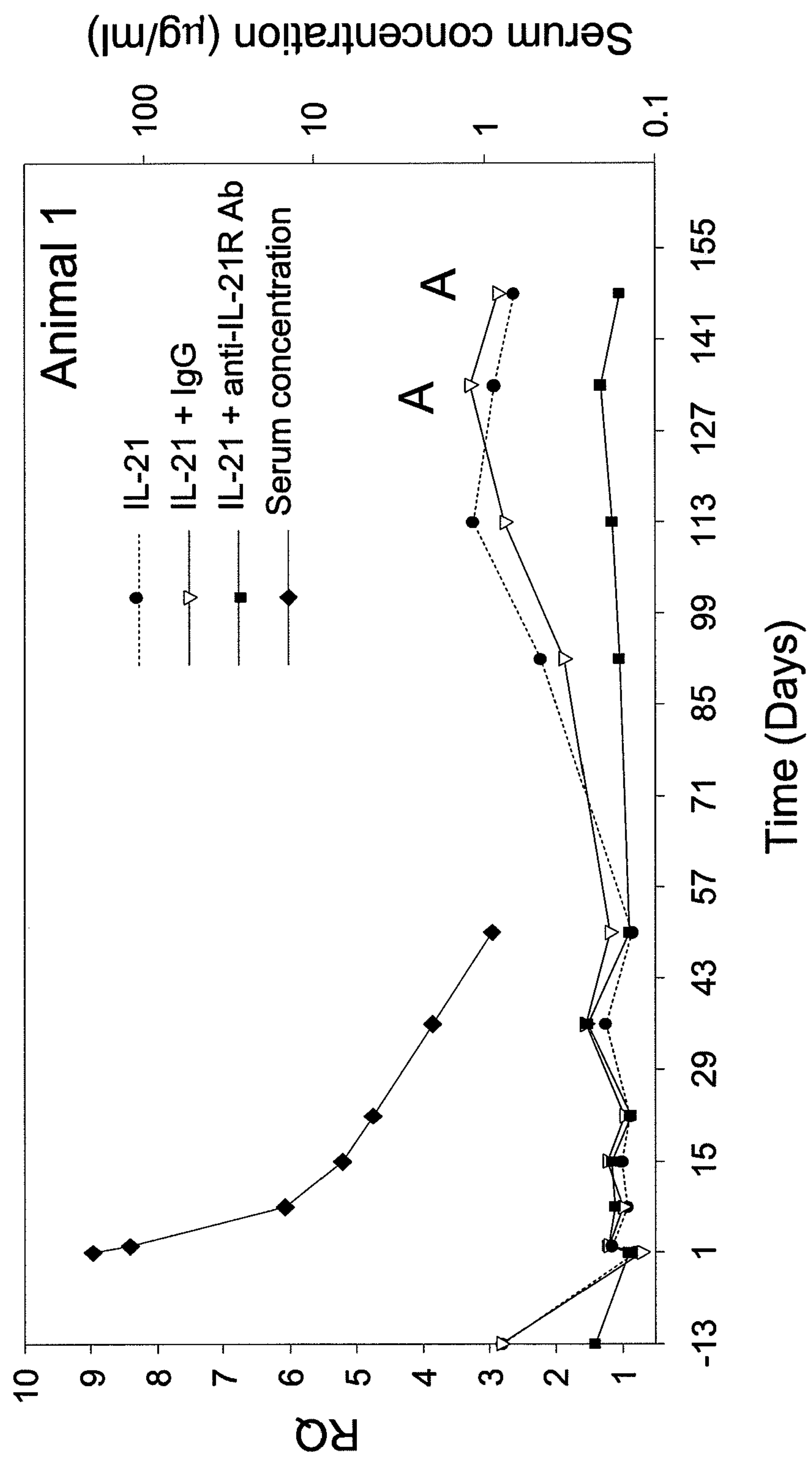


Figure 59b

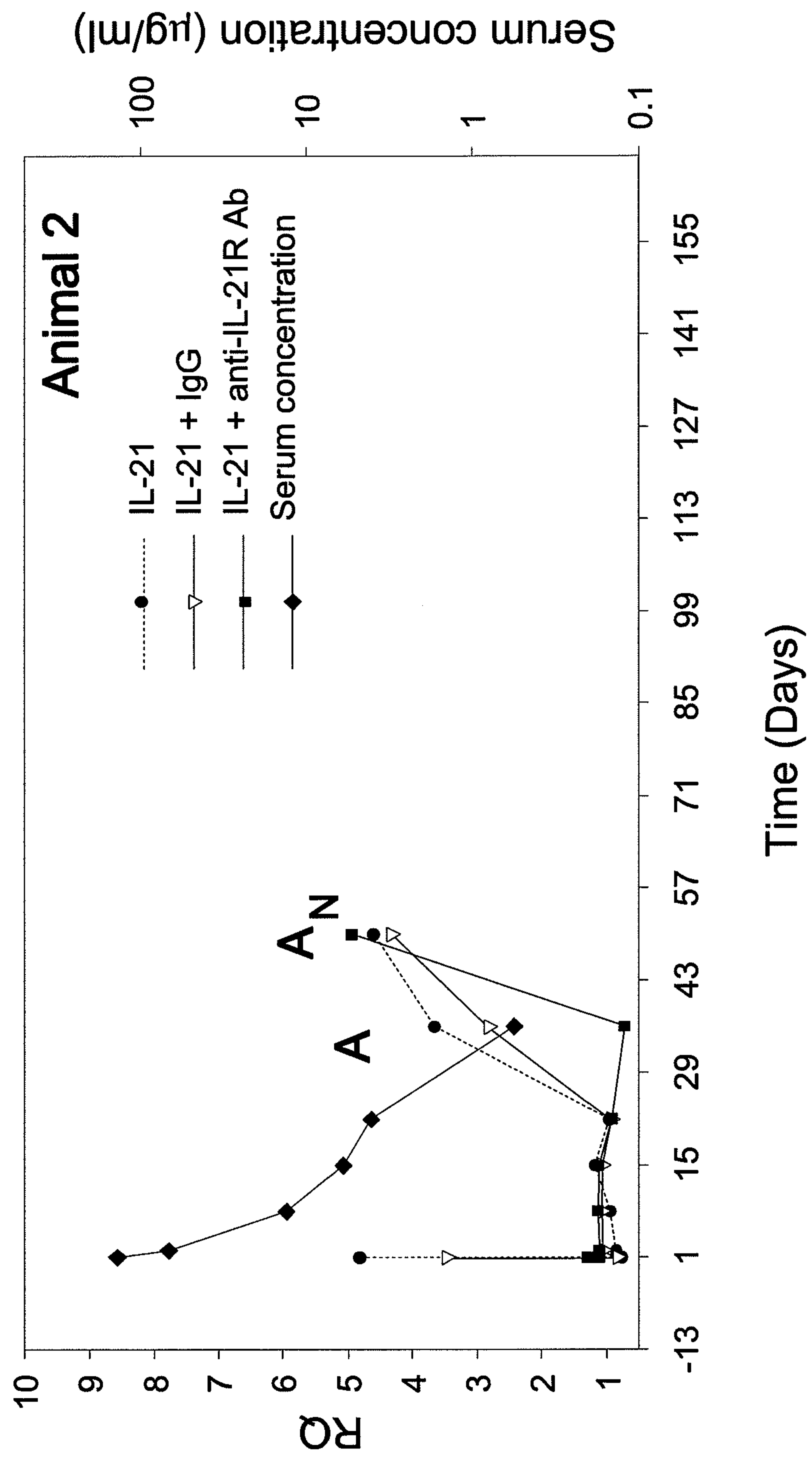


Figure 59c

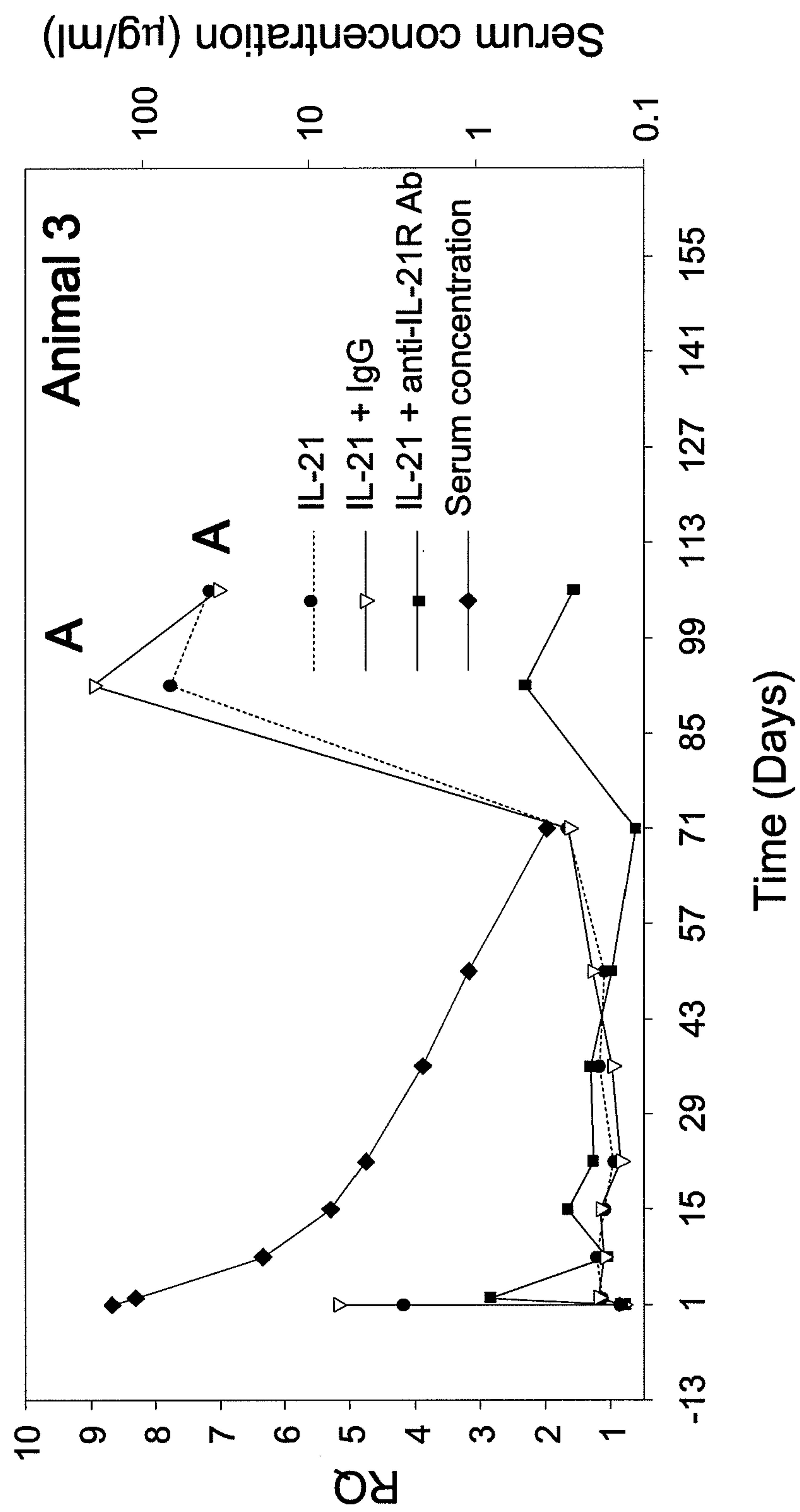


Figure 60a

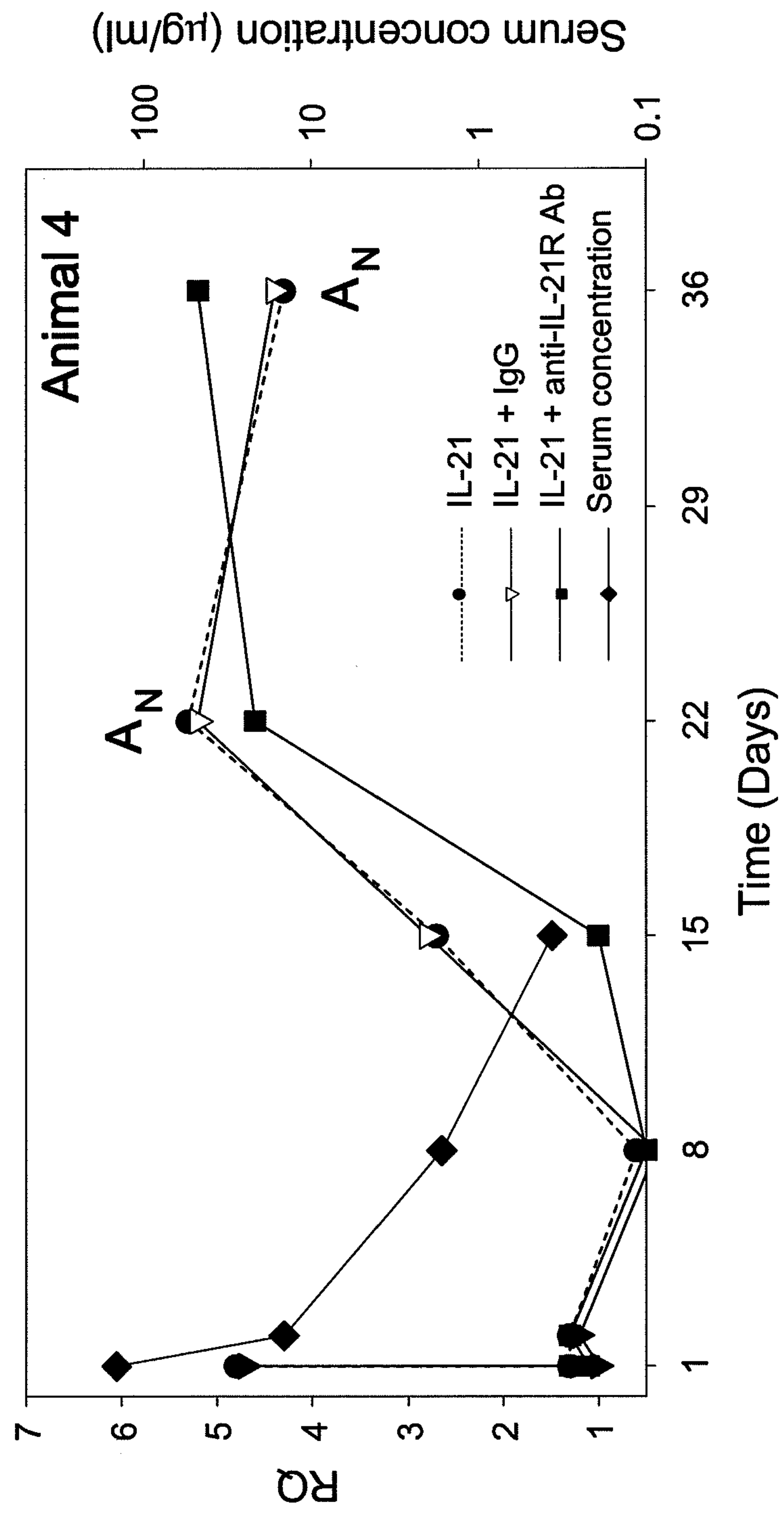


Figure 60b

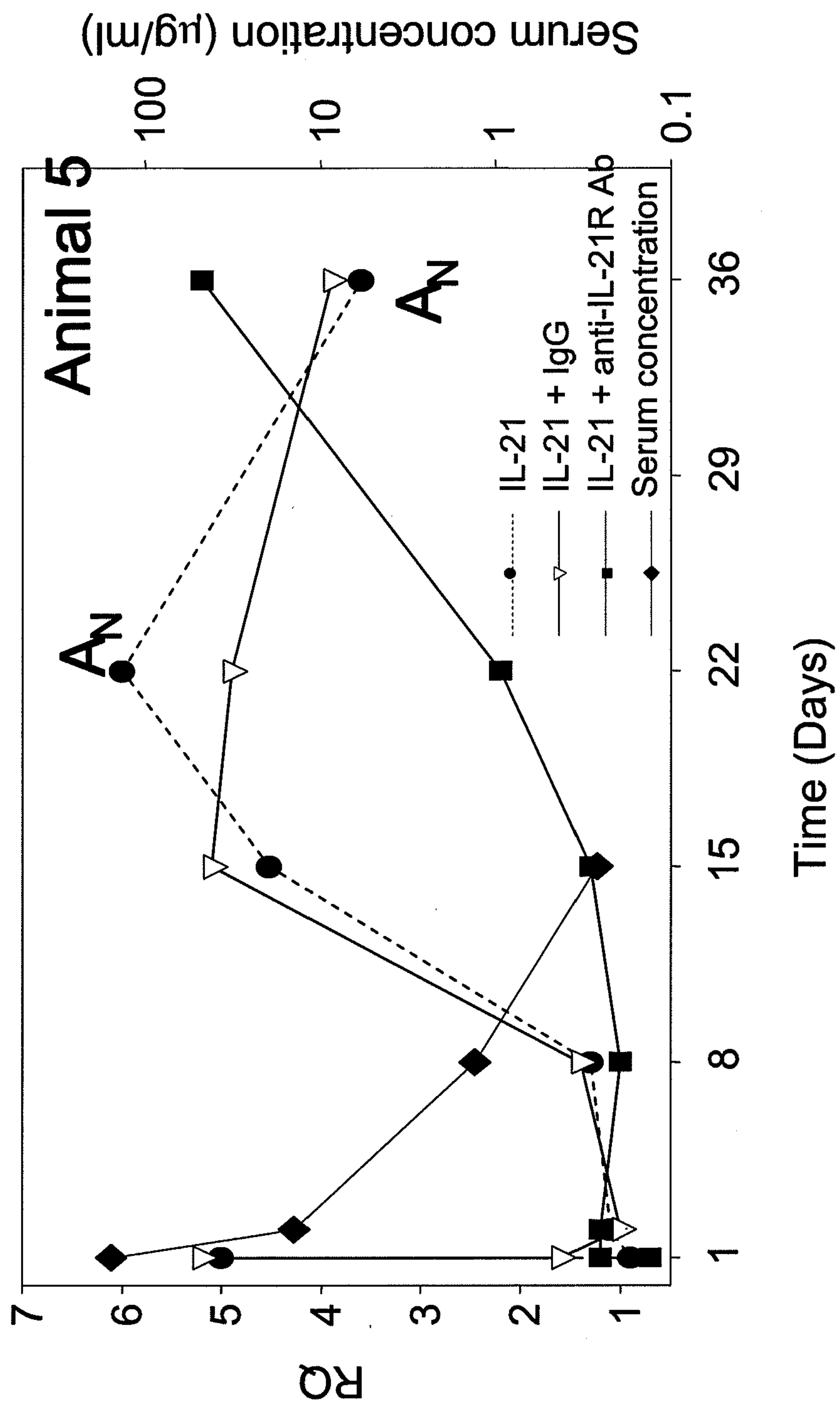
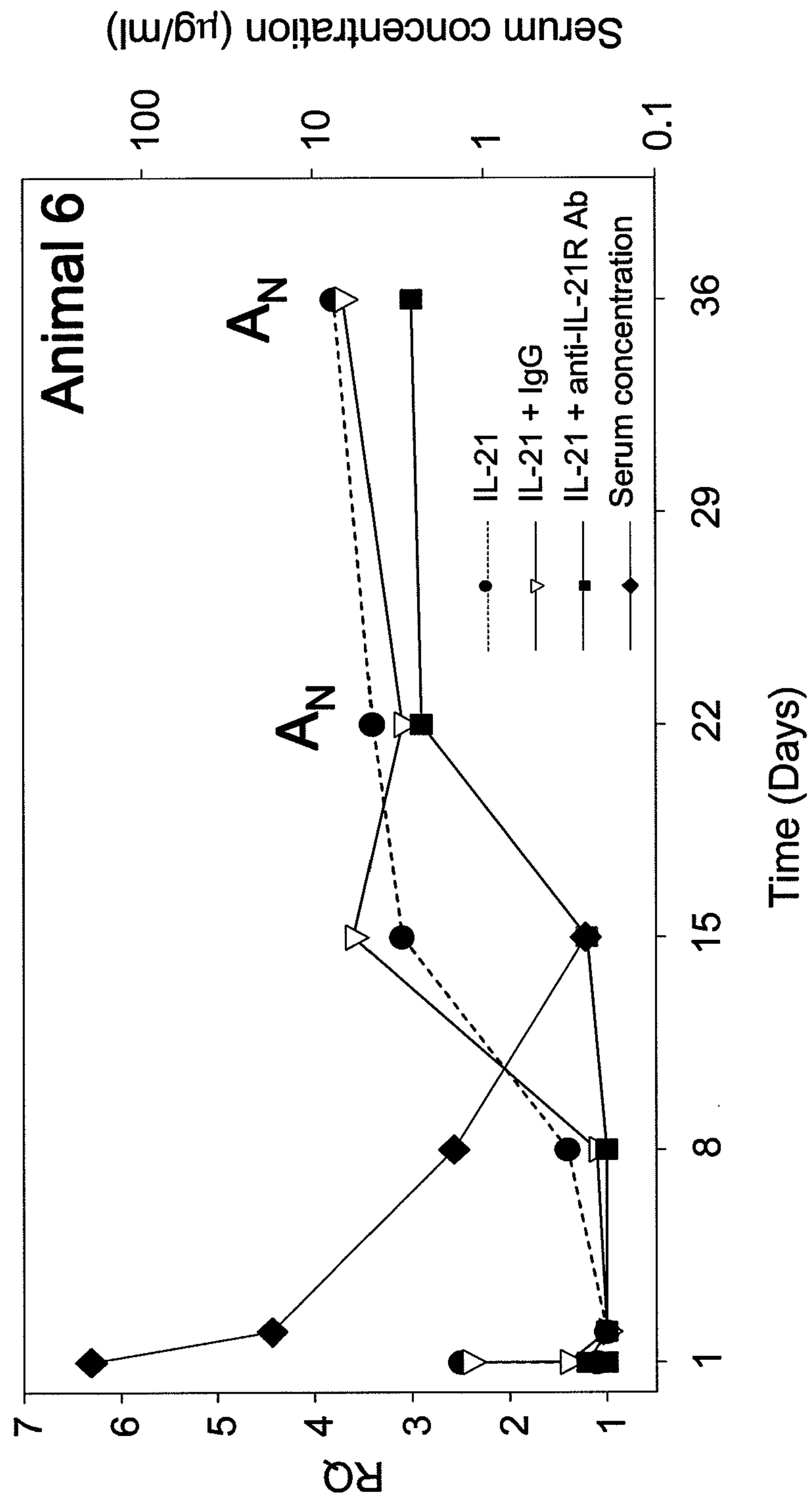


Figure 60c



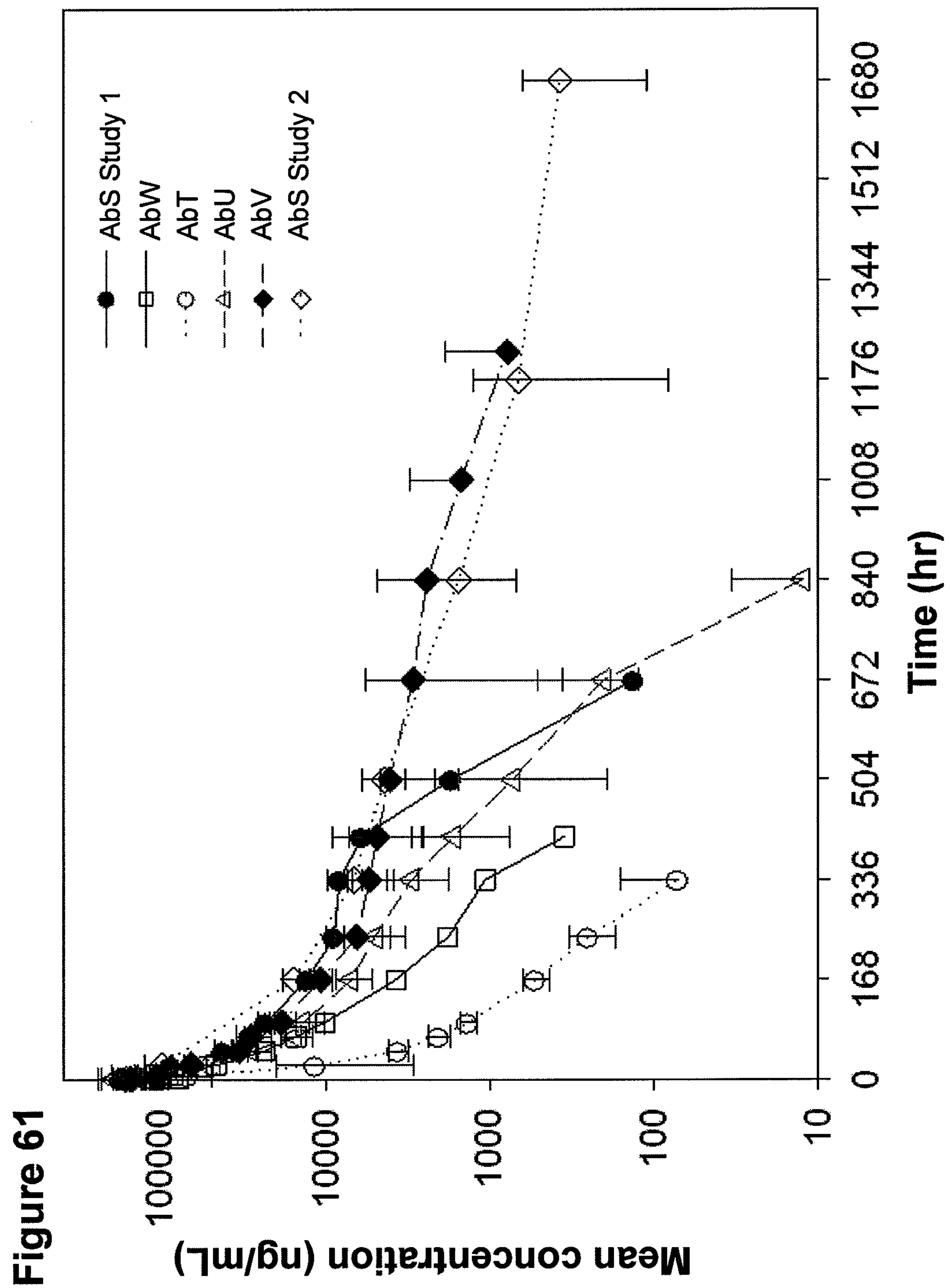


Figure 62a

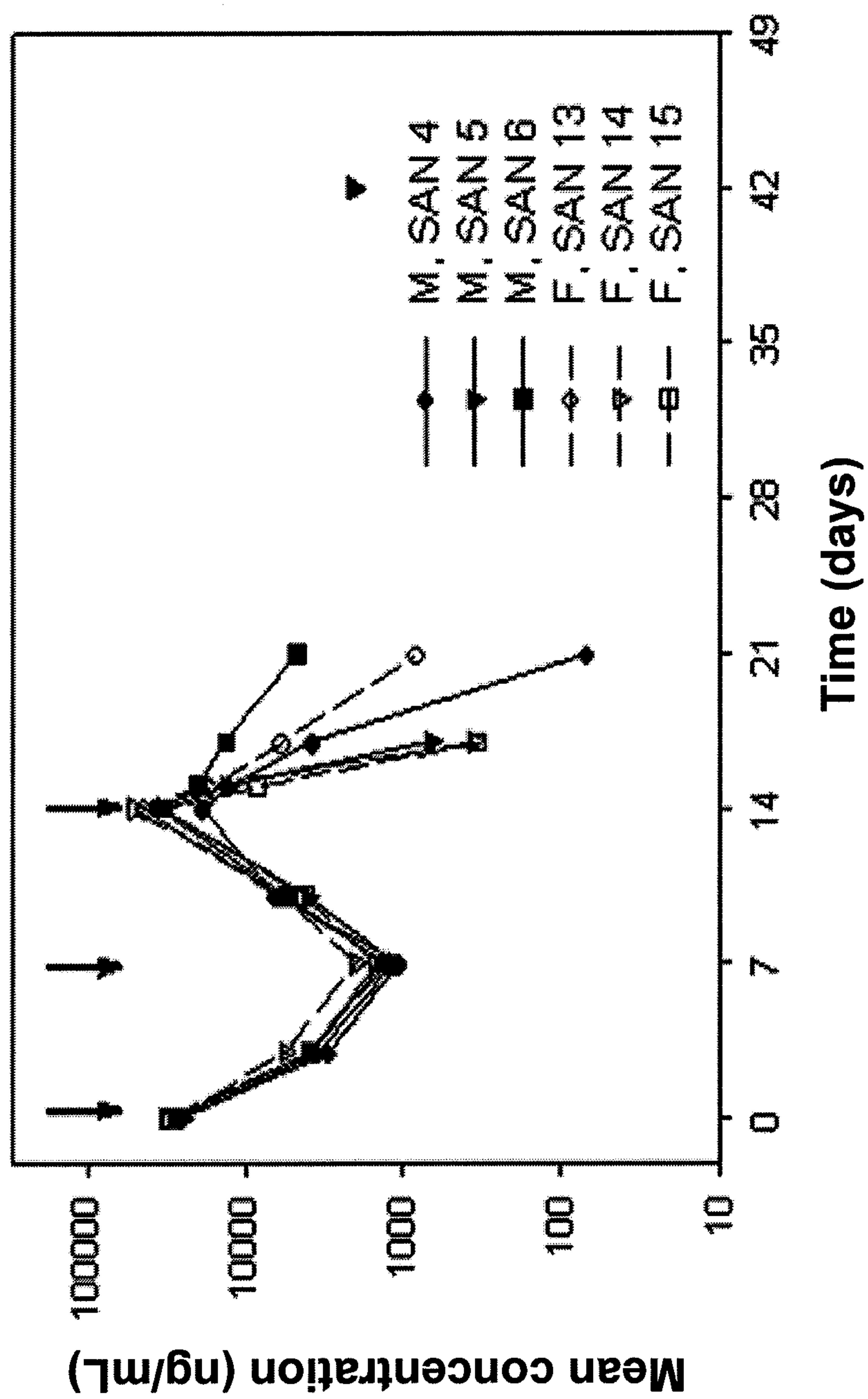


Figure 62b

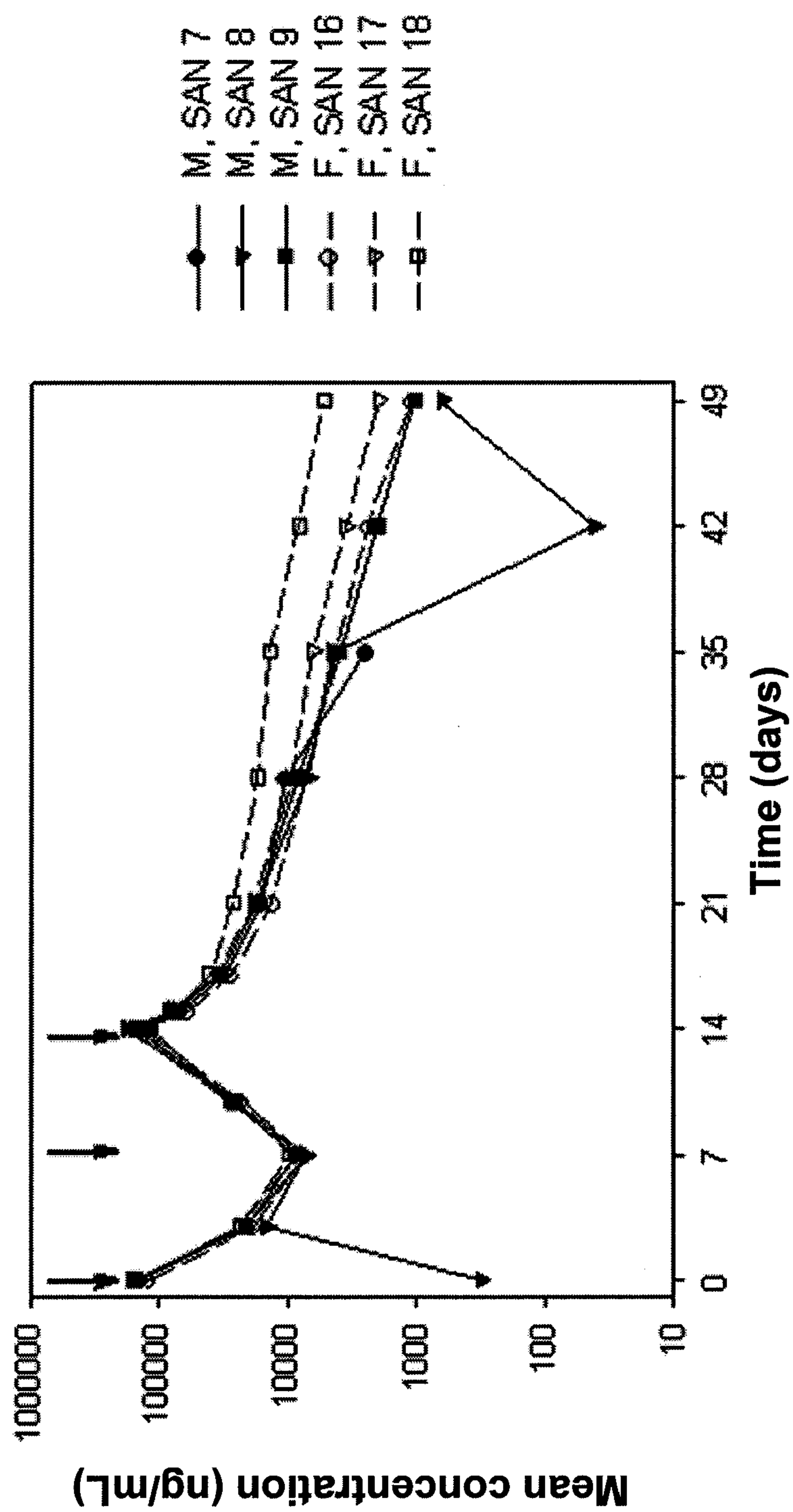


Figure 63a

$CL \text{ (mL/hr)} \cdot MLP = 14.2 \cdot W^{1.36} \quad (r^2 = 0.994)$

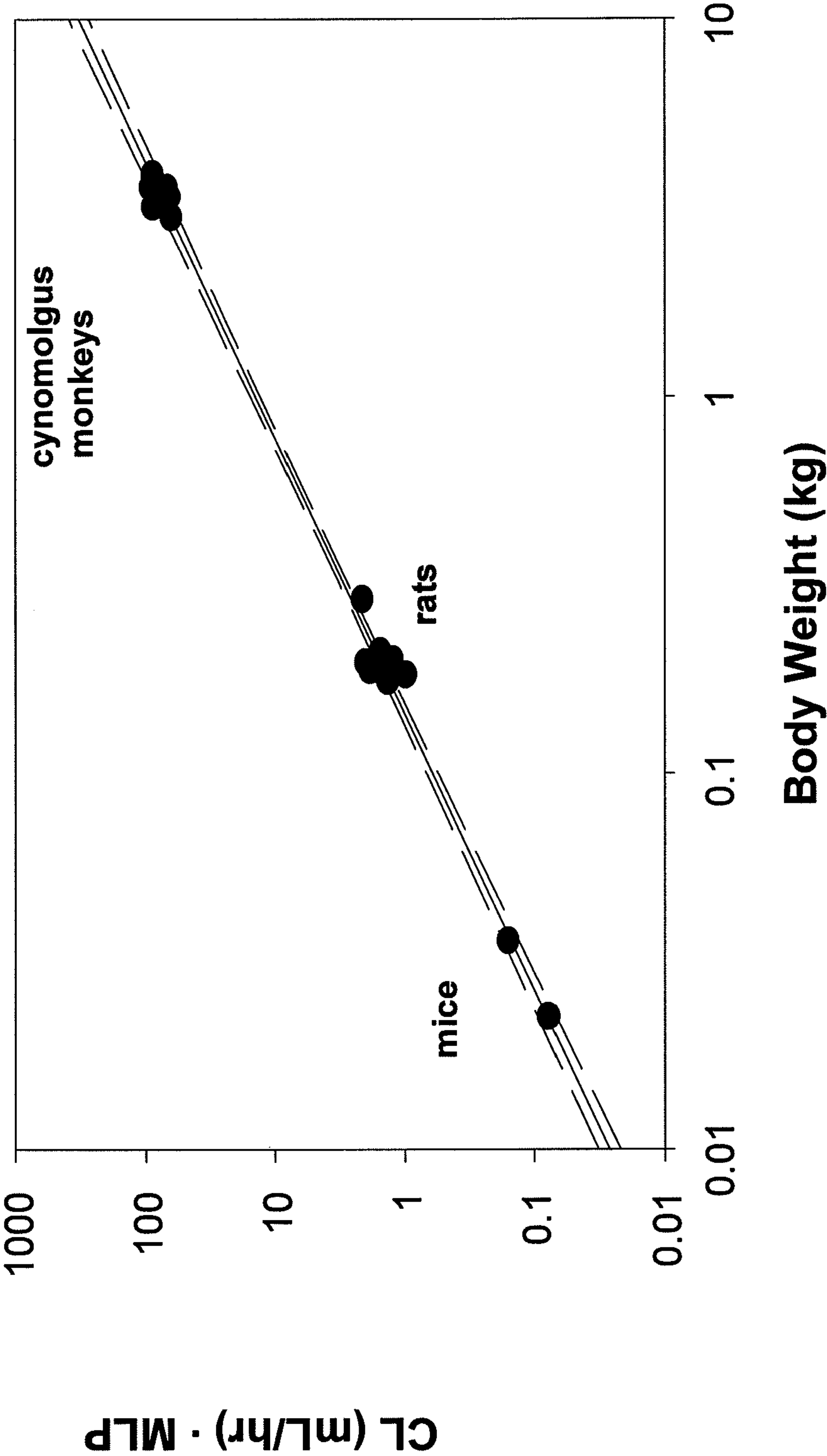


Figure 63b

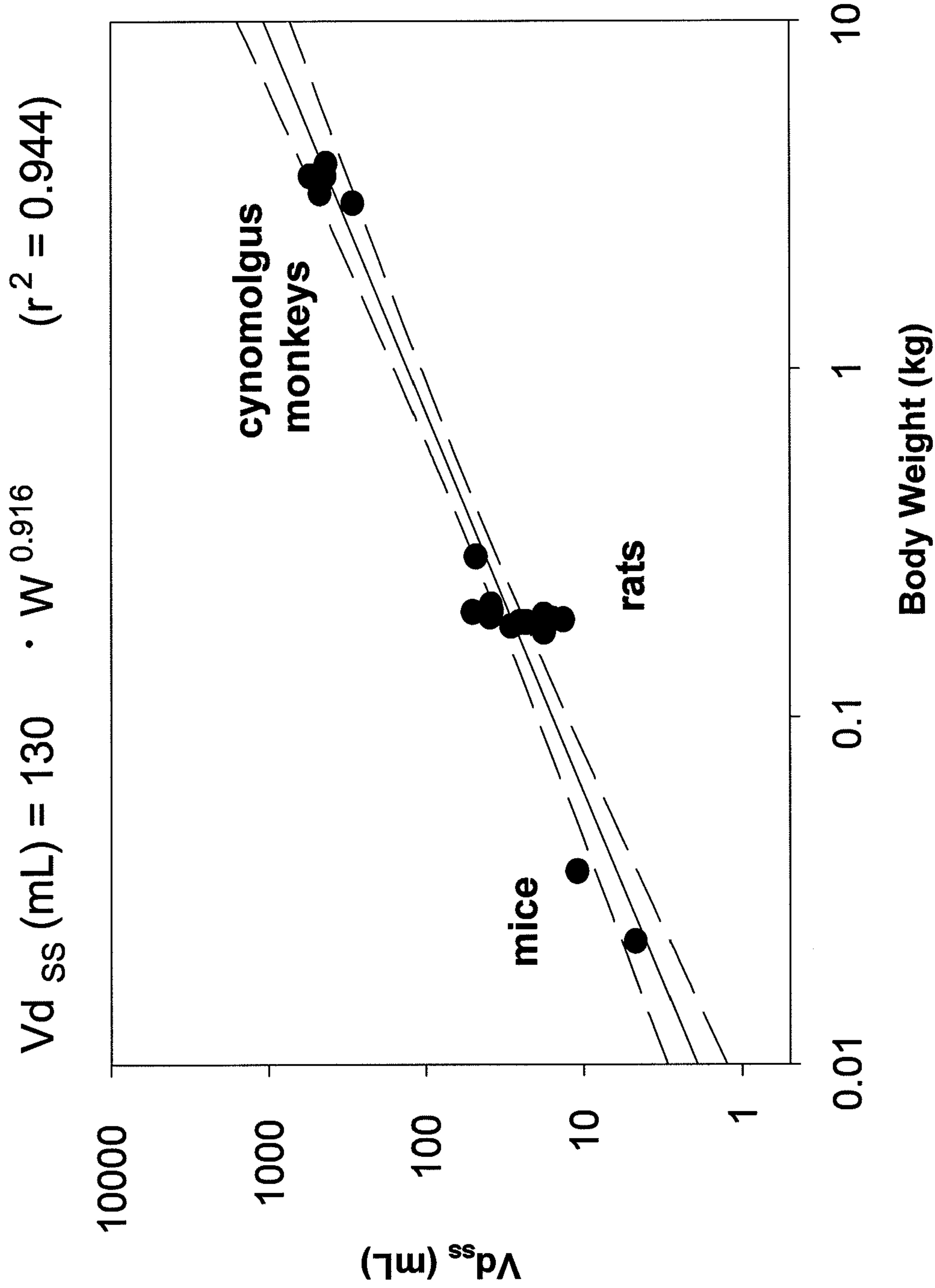


Figure 64a

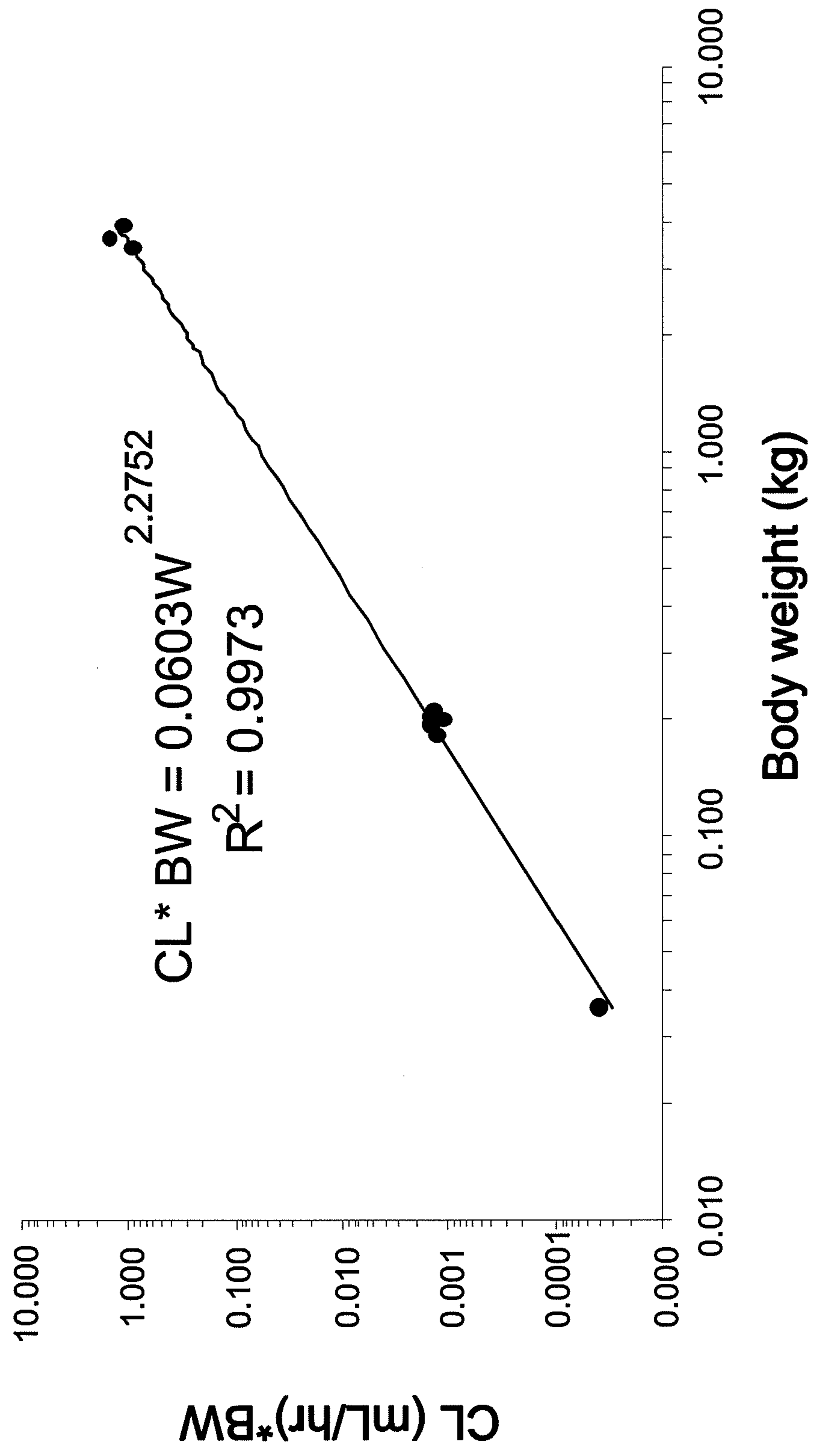


Figure 64b

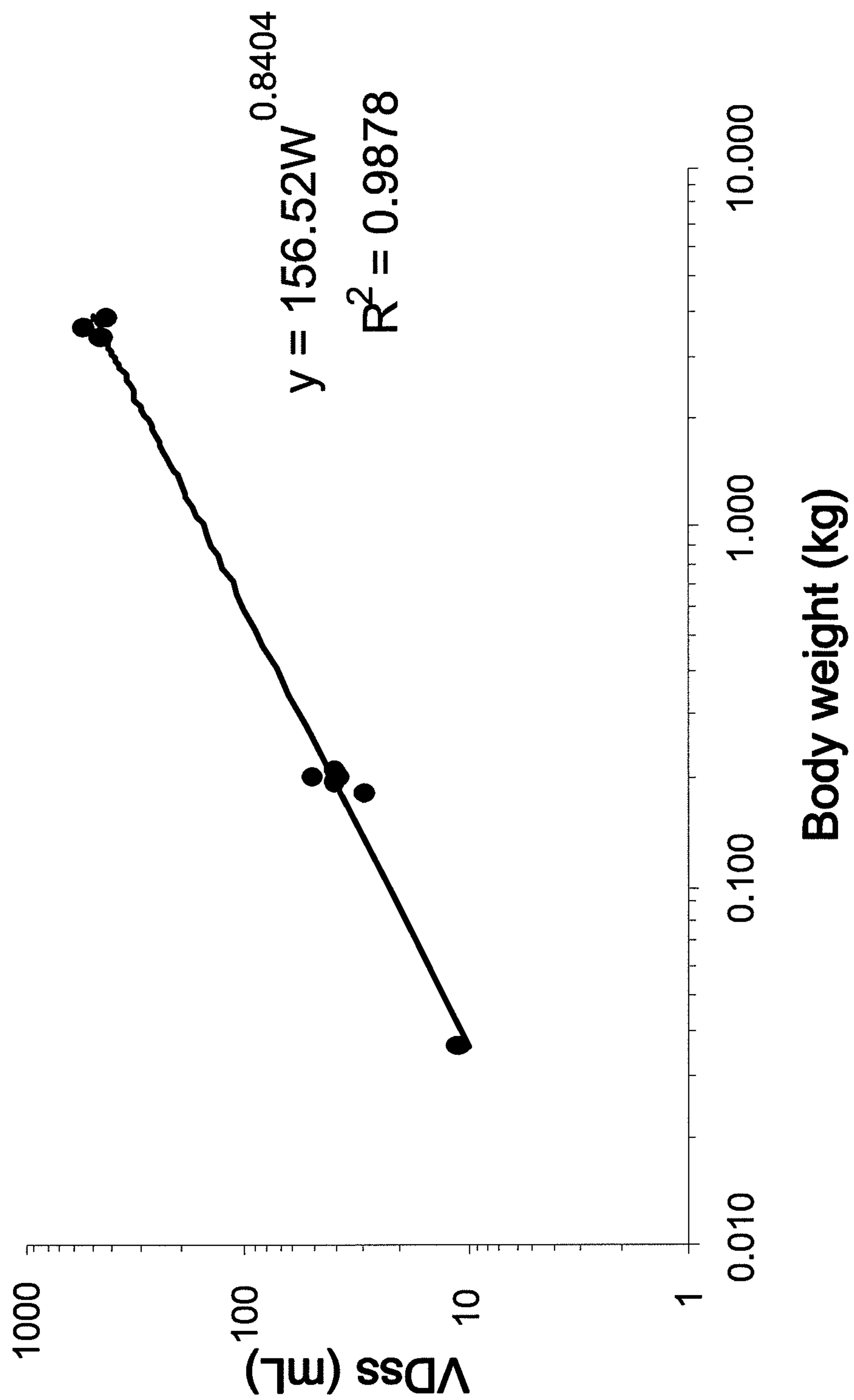


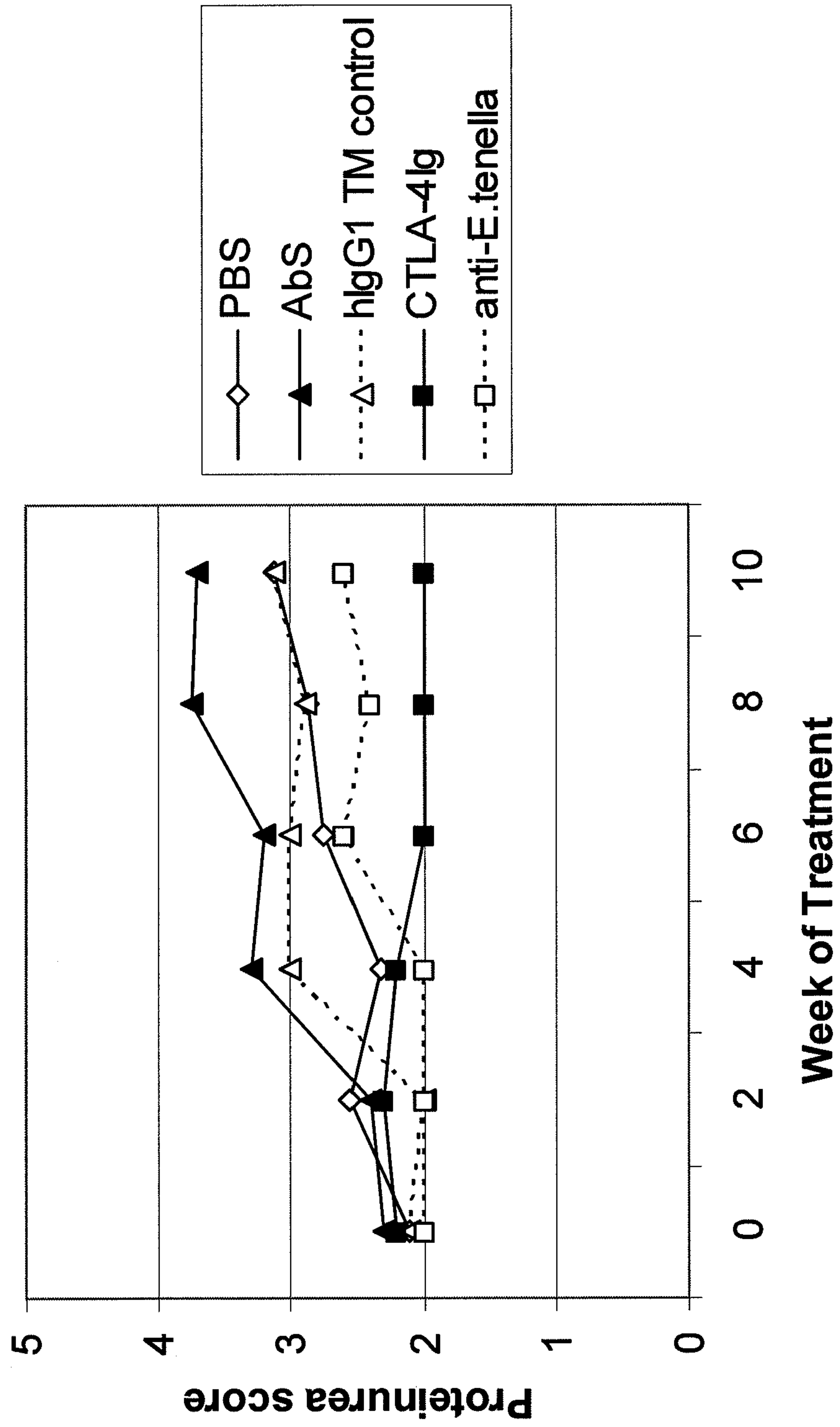
Figure 65a

Figure 65b

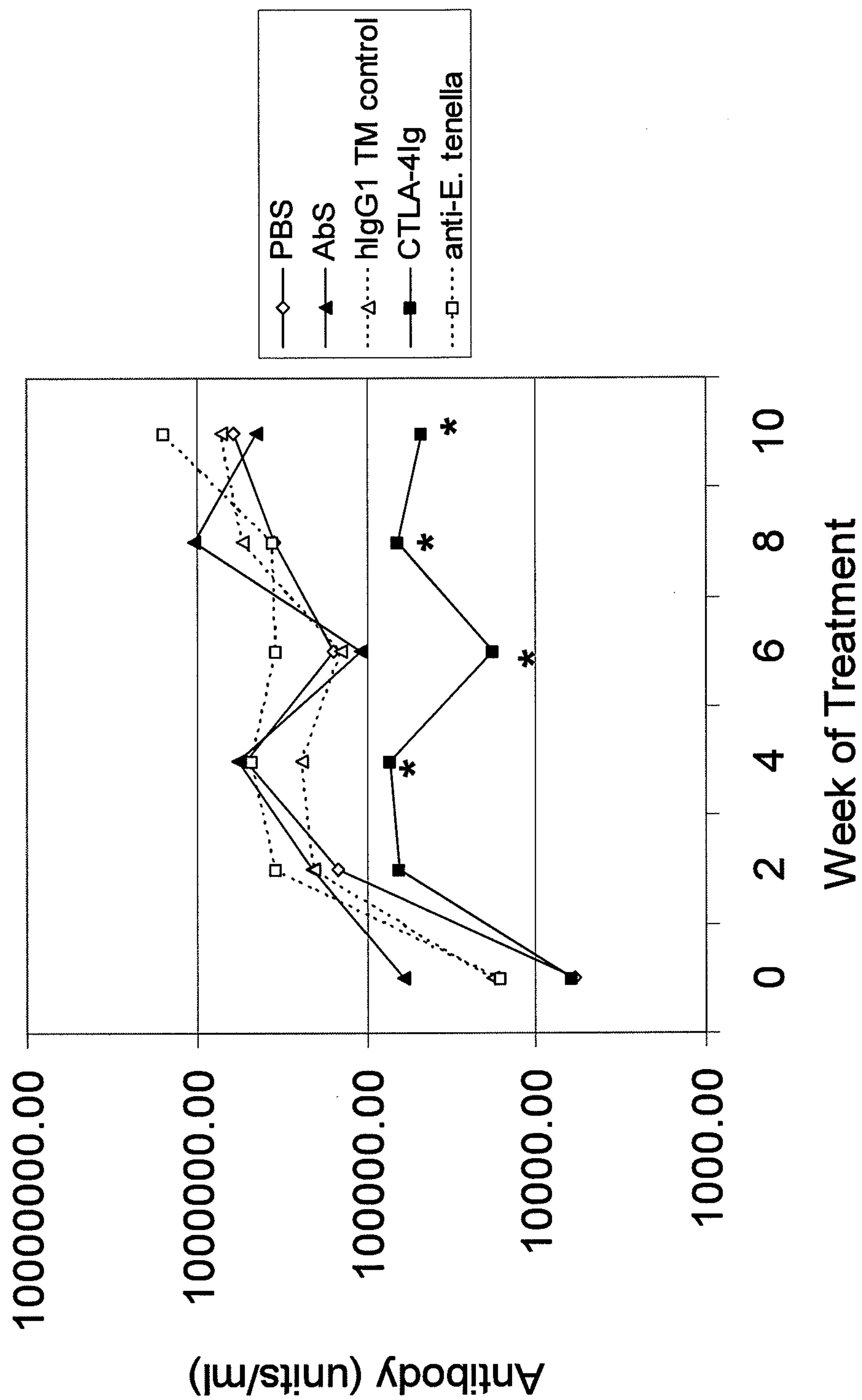
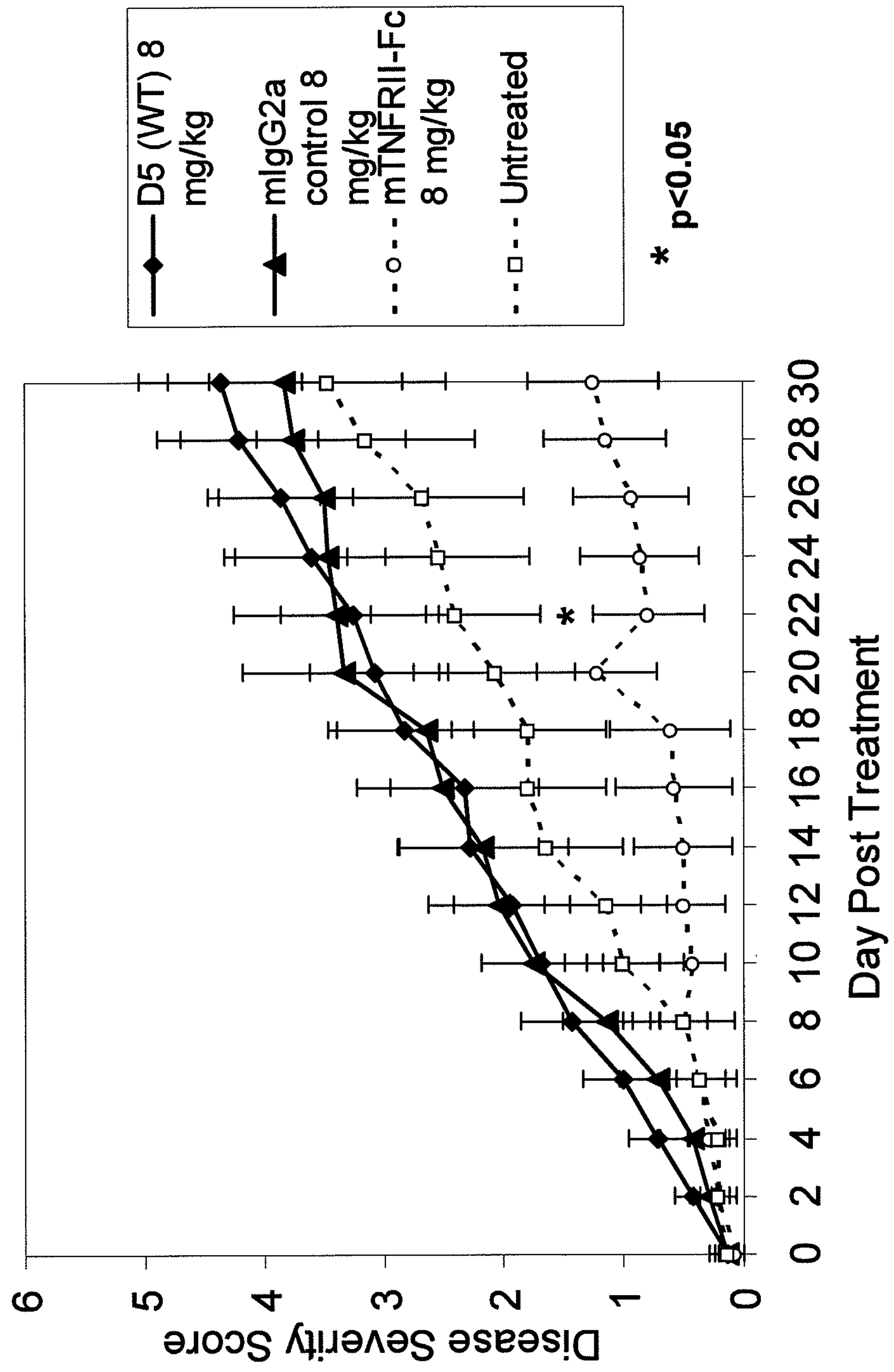
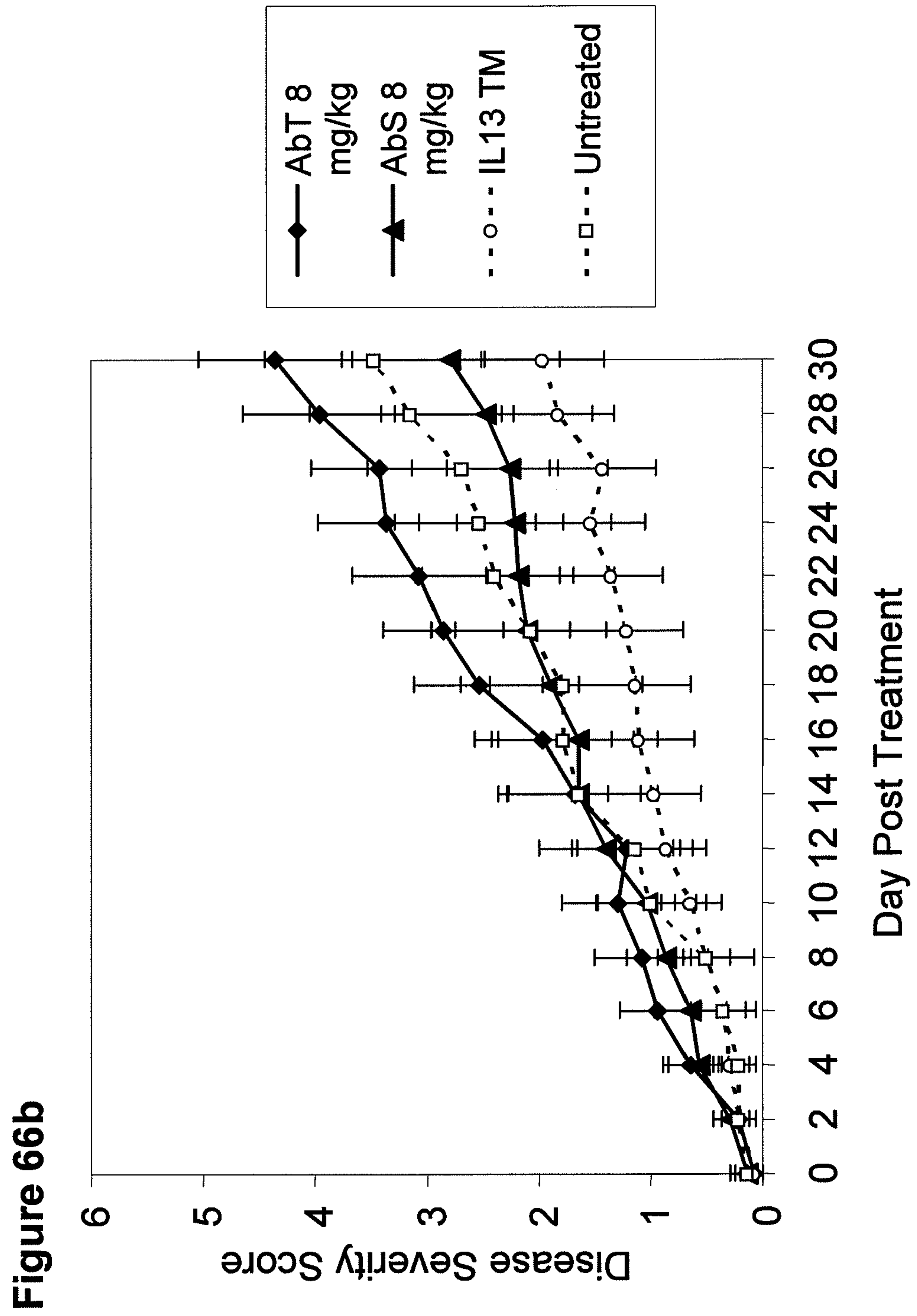


Figure 66a





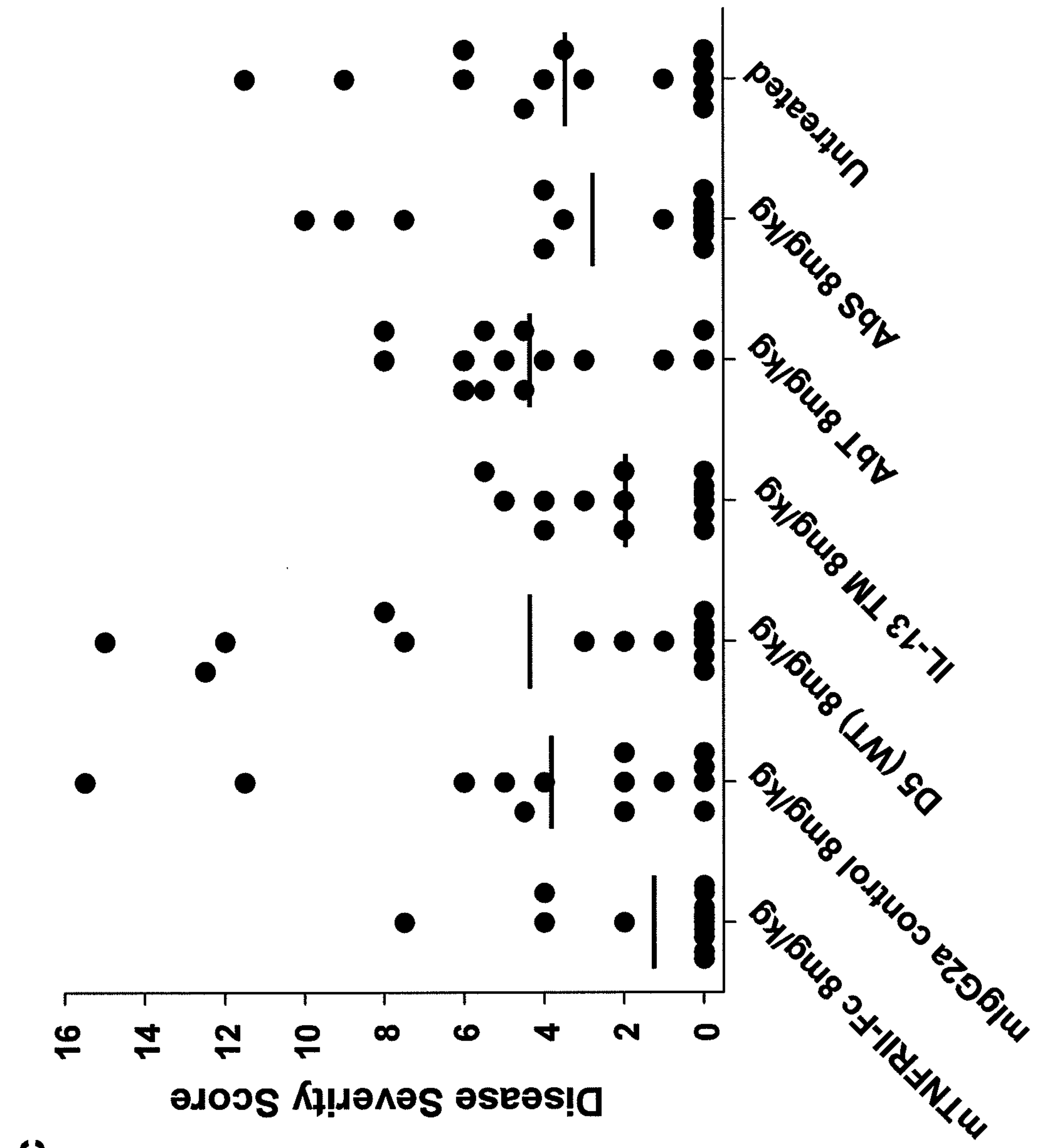


Figure 67a