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(54) **Titre : ANTICORPS ANTI-NGF ET LEURS UTILISATIONS**
(54) **Title: ANTI-NGF ANTIBODIES AND USES THEREOF**

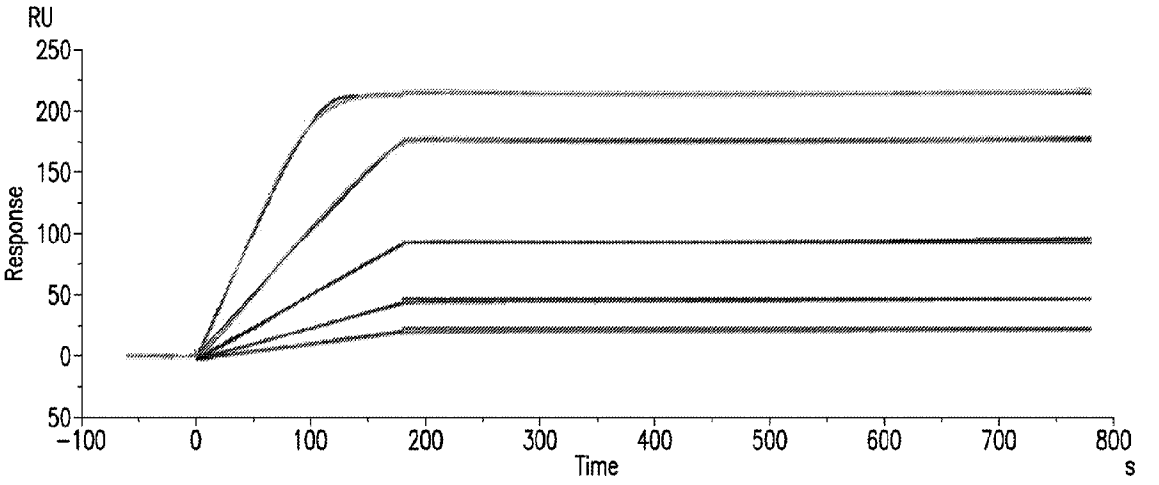

FIG. 5

(57) **Abrégé/Abstract:**
The invention provides novel anti-NGF proteins, antibodies, and NGF-binding fragments thereof which inhibit association of NGF with TrkA and/or p75 and are suitable for administration to a canine or feline subject. The invention also provides novel compositions and methods of treating pain or eliciting an analgesic effect in a canine or feline subject, comprising administering an effective amount of an anti-NGF protein, antibody or fragment thereof. The methods and compositions are used to treat or prevent NGF-related disorders.

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Abstract:

The invention provides novel anti-NGF proteins, antibodies, and NGF-binding fragments thereof which inhibit association of NGF with TrkA and/or p75 and are suitable for administration to a canine or feline subject. The invention also provides novel compositions and methods of treating pain or eliciting an analgesic effect in a canine or feline subject, comprising administering an effective amount of an anti-NGF protein, antibody or fragment thereof. The methods and compositions are used to treat or prevent NGF-related disorders.

ANTI-NGF ANTIBODIES AND USES THEREOF

RELATED APPLICATIONS AND INCORPORATION BY REFERENCE

[0001] This application claims priority to US provisional application Serial No. 63/282,590, filed November 23, 2021, and US provisional application Serial No. 63/383,173, filed November 10, 2022, each incorporated by reference herein in its entirety.

[0002] All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which is being submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 22, 2022, is named G9432-99003.xml and is 292,984 bytes in size.

FIELD OF THE INVENTION

[0004] The invention provides novel anti-NGF proteins, antibodies, and NGF-binding fragments thereof which inhibit association of NGF with TrkA and/or p75 and are suitable for administration to a canine or feline subject. The invention also provides novel compositions and methods of treating pain or eliciting an analgesic effect in a canine or feline subject, comprising administering an effective amount of an anti-NGF protein, antibody or fragment thereof. The methods and compositions are used to treat or prevent NGF-related disorders.

BACKGROUND OF THE INVENTION

[0005] Nerve growth factor (NGF) is critical in the development and maintenance of peripheral sympathetic and embryonic sensory neurons and of basal forebrain cholinergic neurons. NGF upregulates expression of neuropeptides in sensory neurons and its activity is mediated through two different membrane-bound receptors. Several neurotropins (NTs) including NGF bind

to a low-affinity receptor identified as p75. NGF selectively binds to, and displays a high affinity for the high affinity neurotrophin receptor TrkA.

[0006] Upon neurotrophin binding, TrkA undergoes autophosphorylation as well as phosphorylates members of the MAPK pathway. The presence of this kinase leads to cell differentiation and may play a role in specifying sensory neuron subtypes.

[0007] NGF plays a role in several diseases and disorders, including but not limited to pain associated with a broad range of diseases and disorders, such as pain associated with cancers, neuropathic pain, and neurogenic pain. Due to the involvement of NGF in a wide range of pain-related diseases and disorders, there is a need in the art for compositions and methods useful for preventing or treating diseases and disorders associated with NGF, particularly those associated with pain, including in canines, felines and other animals. Particularly preferred anti-NGF compositions are those having minimal or minimized adverse reactions, such as inflammation when administered to a subject.

[0008] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

[0009] The invention provides novel anti-NGF binding protein for treatment or amelioration of NGF-related disorders, particularly adapted for use in dogs and cats but not limited thereby.

[0010] The invention provides binding proteins that specifically binds to NGF. In certain embodiments, the binding proteins are optimized for administration to a canine. In certain embodiments, the binding proteins are optimized for administration to a feline.

[0011] In an aspect, the invention provides binding proteins designed or adapted to bind NGF in the manner of an antibody, i.e. by one or more complementarity determining regions (CDRs). CDRs can be identified by the international ImMunoGeneTics (IMGT) information system. Accordingly, in certain embodiments, the anti-NGF binding protein comprises an antigen binding portion that comprises one or more of (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO:146), wherein X_1 comprises A, G, or N, X_2 comprises L or M, X_3 comprises A, D, E, or S, X_4 comprises F, I, L, M, or V, X_5 comprises N or T, X_6 comprises E, S, or T, X_7 comprises G, H, N, S, or Q, and X_8 comprises A or S; (b) a heavy chain complementarity determining region 2 (VH-CDR2)

comprising the amino acid sequence $X_1X_2SNX_5GT$ (SEQ ID NO:147), wherein X_1 comprises I or L, X_2 comprises W or Y, and X_5 comprises G or R; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $AX_2IX_4X_5YX_7X_8X_9YLX_{12}X_{13}YX_{15}X_{16}X_{17}$ (SEQ ID NO:148), wherein X_2 comprises D, E, K, N, Q, S, or T, X_4 comprises W or Y, X_5 comprises F, H, W, or Y, X_7 comprises D or E, X_8 comprises A or S, X_9 comprises D or Y, X_{12} comprises H or Y, X_{13} comprises F or W, X_{15} comprises F, I, L, W, or Y, X_{16} comprises D or Q, and X_{17} comprises F, I, L, M, W, or Y; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1X_2IX_4X_5X_6$ (SEQ ID NO:149), wherein X_1 comprises D, E, or K, X_2 comprises A, G, or N, X_4 comprises G, N, Q or S, X_5 comprises N or S, X_6 comprises A, G, N, S or T; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence AX_2X_3 (SEQ ID NO:150), wherein X_2 comprises A, S, or T, X_3 comprises A, D, E, N, Q, S, or T; and (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QX_2GX_4X_5X_6PX_8T$ (SEQ ID NO:151), wherein X_2 comprises H or Q, X_4 comprises F, H, W, or Y, X_5 comprises K or Q, X_6 comprises F or W, and X_8 comprises L or M.

[0012] In certain embodiments, the anti-NGF binding protein comprises an antigen binding portion which comprises (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO:152), wherein X_1 comprises A or G, X_2 comprises L or M, X_3 comprises E or S, X_4 comprises F or L, X_5 comprises N or T, X_6 comprises E, S, or T, X_7 comprises H, N, or S, and X_8 comprises A or S; (b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence X_1WSNX_5GT (SEQ ID NO:153), wherein X_1 comprises I or L, X_5 comprises G or R; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $AX_2IYYYX_7ADYLHX_{13}YX_{15}DX_{17}$ (SEQ ID NO:154), wherein X_2 comprises N, Q, S, or T, X_7 comprises D or E, X_{13} comprises F or W, X_{15} comprises F, I, L, W, or Y, and X_{17} comprises F, I, L, or M; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1GIX_4X_5X_6$ (SEQ ID NO:155), wherein X_1 comprises D or E, X_4 comprises N, Q, or S, X_5 comprises N or S, X_6 comprises G, N, S or T; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence ATX_3 (SEQ ID NO:156), wherein X_3 comprises D, E, N, Q, or S; and (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QQGX_4X_5X_6PX_8T$ (SEQ ID NO:157), wherein

X_4 comprises F, H, W, or Y, X_5 comprises K or Q, X_6 comprises F or W, and X_8 comprises L or M.

[0013] In certain embodiments, the anti-NGF binding protein comprises a heavy chain CDR1 set forth in FIG. 1. In certain embodiments, the anti-NGF binding protein comprises a heavy chain CDR2 set forth in FIG. 1. In certain embodiments, the anti-NGF binding protein comprises a heavy chain CDR3 set forth in FIG. 1. In certain embodiments, the anti-NGF binding protein comprises a light chain CDR1 set forth in FIG. 2. In certain embodiments, the anti-NGF binding protein comprises a light chain CDR2 set forth in FIG. 2. In certain embodiments, the anti-NGF binding protein comprises a light chain CDR3 set forth in FIG. 2.

[0014] In certain embodiments, the anti-NGF binding protein comprises heavy chain CDRs of a heavy chain variable domain set forth in FIG. 1.

[0015] In certain embodiments, the anti-NGF binding protein comprises a heavy chain variable domain (V_H) at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 98%, or identical to a V_H domain set forth in FIG. 1.

[0016] In certain embodiments, the anti-NGF binding protein comprises light chain CDRs of a light chain variable domain set forth in FIG. 2.

[0017] In certain embodiments, the anti-NGF binding protein comprises a light chain variable domain (V_L) at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 98%, or identical to a light chain variable domain set forth in FIG. 2.

[0018] In certain embodiments, the anti-NGF binding protein comprises V_H and V_L from an Fv set forth in FIG. 1 and FIG. 2.

[0019] In FIG. 1 and FIG. 2, CDRs are identified by the IMGT system. Alternatively, CDRs can be identified according to the Kabat numbering system or the Chothia numbering system. Accordingly, in certain embodiments, the anti-NGF binding protein comprises an antigen binding portion that comprises one or more of VH-CDR1, VH-CDR2, VH-CDR3, VL-CDR1, VL-CDR2, and VL-CDR3 according to the Kabat or Chothia numbering system as further set forth herein.

[0020] In paring of V_H and V_L domains described herein, any V_H domain can be used with any V_L domain. Similarly, the CDRs of any V_H domain can be used with the CDRs of any V_L domain. In an embodiment, an antibody of the invention comprises V_H CDRs of SEQ ID NO:137 (SC-42_101) and V_L CDRs of SEQ ID NO:3 (SC-42_006). In an embodiment which comprises the

amino acid arginine at position 55 in VH-CDR2, an antibody of the invention comprises V_H CDRs of SEQ ID NO:207 (SC-42_101R) and V_L CDRs of SEQ ID NO:3 (SC-42_006).

[0021] In certain embodiments, an antibody of the invention incorporate V_H and V_L domains that were selected together, i.e. identified in the same clone. V_H and V_L clones selected together are identified as having the same clone name in FIG. 1 as in FIG. 2. Similarly, the CDRs of a V_H domain identified by clone name in FIG. 1 can be used with the CDRs of the V_L domain identified by the same clone name in FIG. 2. The paired V_H and V_L domains can further comprise conservative substitutions, such as but not limited to conservative variation observed at specific positions of V_H CDRs and V_L CDRs, positions adjacent to V_H and V_L CDRs and positions of the V_H domains and V_L domains set forth herein.

[0022] In certain embodiments, an antibody of the invention comprises V_H and V_L CDRs of clone 2166, SC-42_006, SC-42_007, SC-42_008, SC-42_010, SC-42_011, SC-42_023, SC-42_032, SC-42_045, SC-42_047, SC-42_048, SC-42_052, SC-42_070, SC-42_073, SC-42_077, SC-42_082, SC-42_090, or SC-42_101 (FIG. 1 and FIG. 2).

[0023] In certain embodiments, an antibody of the invention comprises V_H and V_L domains at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 98%, or identical to those of clone 2166, SC-42_006, SC-42_007, SC-42_008, SC-42_010, SC-42_011, SC-42_023, SC-42_032, SC-42_045, SC-42_047, SC-42_048, SC-42_052, SC-42_070, SC-42_073, SC-42_077, SC-42_082, SC-42_090, or SC-42_101.

[0024] In certain embodiments, a felinized anti-NGF binding protein comprises (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1LX_3X_4X_5X_6X_7X_8MX_{10}$ (SEQ ID NO:208), wherein X_1 comprises A, G, L, N, or Q, X_3 comprises A, D, E, G, H, I, M, S, T, or Y, X_4 comprises L, M, or V, X_5 comprises A, M, N, R, S, T, or V, X_6 comprises A, E, G, H, K, R, S, or T, X_7 comprises A, D, H, I, N, Q, S, T, or Y, X_8 comprises A or S, and X_{10} comprises S or V; (b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $X_1X_2X_3X_4X_5GTX_8YX_{10}DX_{12}VX_{14}$ (SEQ ID NO:209), wherein X_1 comprises I or L, X_2 comprises W or Y; X_3 comprises A, P, or S, X_4 comprises D, E, N, Q, R, or S, X_5 comprises G, R, or Y, X_8 comprises D or Y, X_{10} comprises D, E, H, S, or T, X_{12} comprises D or S, and X_{14} comprises D, E, or K; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}LX_{12}X_{13}X_{14}FX_{16}X_{17}$ (SEQ ID NO:210), wherein X_1 comprises A, D,

E, K, N, Q, S, or T, X_2 comprises A, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, or Y, X_3 comprises I, L, W, or Y, X_4 comprises F, T, W, or Y, X_5 comprises F, H, or Y, X_6 comprises H or Y, X_7 comprises D or E, X_8 comprises A, S, or V, X_9 comprises D, E, H, K, N, Q, or Y, X_{10} comprises F, H, or Y, X_{12} comprises H or Y, X_{13} comprises F or W, X_{14} comprises D, I, L, W, or Y, X_{16} comprises D or Q, and X_{17} comprises E, F, H, I, L, M, N, P, W, or Y; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1ASX_4X_5X_6X_7X_8X_9LX_{11}$ (SEQ ID NO:211), wherein X_1 comprises F or R, X_4 comprises E, K, or N, X_5 comprises A, or G, X_6 comprises I, L, or V, X_7 comprises A, D, G, L, P, Q, S, V, or Y, X_8 comprises K, Q, N, S, or Y, X_9 comprises A, D, E, F, G, H, K, L, N, Q, R, S or T, and X_{11} comprises A, G, or S; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence $AX_2X_3X_4X_5X_6X_7$ (SEQ ID NO:212), wherein X_2 comprises A, D, L, Q, S, T, V, or Y, X_3 comprises D, E, K, N, Q, or S, X_4 comprises H, I, K, L, M, N, or V; X_5 comprises H or L, X_6 comprises H, I, L, or M, and X_7 comprises D, E, N, S, or T; and (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QQX_3X_4X_5X_6X_7X_8T$ (SEQ ID NO:213), wherein X_3 comprises G or Y, X_4 comprises D, F, G, H, K, L, R, S, T, V, W, or Y, X_5 comprises E, K, Q, R, or S, X_6 comprises I, F, T, or W, X_7 comprises E or P, and X_8 comprises L, M, or W.

[0025] In certain embodiments, the anti-NGF binding protein comprises a heavy chain CDR1 set forth in FIG. 17A. In certain embodiments, the anti-NGF binding protein comprises a heavy chain CDR2 set forth in FIG. 17A. In certain embodiments, the anti-NGF binding protein comprises a heavy chain CDR3 set forth in FIG. 17A. In certain embodiments, the anti-NGF binding protein comprises a light chain CDR1 set forth in FIG. 17B. In certain embodiments, the anti-NGF binding protein comprises a light chain CDR2 set forth in FIG. 17B. In certain embodiments, the anti-NGF binding protein comprises a light chain CDR3 set forth in FIG. 17B.

[0026] In certain embodiments, the anti-NGF binding protein comprises heavy chain CDRs of a heavy chain variable domain set forth in FIG. 17A.

[0027] In certain embodiments, the anti-NGF binding protein comprises a heavy chain variable domain (V_H) at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 98%, or identical to a V_H domain set forth in FIG. 17A.

[0028] In certain embodiments, the anti-NGF binding protein comprises light chain CDRs of a light chain variable domain set forth in FIG. 17B.

[0029] In certain embodiments, the anti-NGF binding protein comprises a light chain variable domain (V_L) at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 98%, or identical to a light chain variable domain set forth in FIG. 17B.

[0030] The V_H CDRs set forth in FIG. 17A can be used with the V_L CDRs set forth in FIG. 17B in any combination. The V_H domains set forth in FIG. 17A can be used with the V_L domains set forth in FIG. 17B in any combination. Table 11 and Table 12 provide exemplary combinations.

[0031] In certain embodiments, the anti-NGF binding protein comprises V_H and V_L from an Fv whose V_H is set forth in FIG. 17A and V_L set forth in FIG. 17B.

[0032] In FIG. 17, CDRs are identified by the IMGT system. Alternatively, CDRs can be identified according to the Kabat numbering system or the Chothia numbering system. Accordingly, in certain embodiments, the anti-NGF binding protein comprises an antigen binding portion that comprises one or more of VH-CDR1, VH-CDR2, VH-CDR3, VL-CDR1, VL-CDR2, and VL-CDR3 according to the Kabat or Chothia or IMGT numbering system as further set forth herein.

[0033] In certain embodiments, the anti-NGF binding protein comprises CDRs as described above, with the further limitation that each of the CDRs comprises no more than one or two amino acid differences as compared to specific antibody heavy and light chains described herein, for example, CDRs of the heavy and light chains whose sequences are set forth in FIG. 1, FIG. 2, FIG. 17A, and FIG. 17B, which are of similar sequence and bind to NGF with high affinity. In certain embodiments, the anti-NGF binding protein comprise CDRs of a V_H and V_L disclosed herein.

[0034] In certain embodiments, the anti-NGF protein comprises no more than one or two amino acid differences per CDR as compared to specificcaninized antibody heavy and light chains described herein, for example, CDRs of the heavy and light chains set forth in FIG. 1 and FIG. 2 which are of similar sequence and bind to NGF with the highest affinity. Such antibodies include antibodies which comprise no more than two (2) changes per VH-CDR, i.e. 2, 1 or no changes per CDR as compared to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, SEQ ID NO:137, or SEQ ID NO:141 and no more than two (2) changes per VL-CDR, i.e. 2, 1 or no changes per CDR as compared to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ

ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

[0035] In certain embodiments, the anti-NGF protein comprises no more than one or two amino acid differences per CDR as compared to specificfelinized antibody heavy and light chains described herein, for example, CDRs of the heavy and light chains set forth in FIG. 17A and FIG. 17B which are of similar sequence and bind to NGF with the highest affinity. Such antibodies include antibodies which comprise no more than two (2) changes per VH-CDR, i.e. 2, 1 or no changes per CDR as compared to SEQ ID NO:141, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:205, or SEQ ID NO:206 and no more than two (2) changes per VL-CDR, i.e. 2, 1 or no changes per CDR as compared to SEQ ID NO:142, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, or SEQ ID NO:203.

[0036] Mutations and combinations thereof, within CDRs and among CDRs, including allowed mutations and advantageous mutations are evident from the sequence datasets shown in FIG. 1 and FIG. 2, and FIG. 17A and FIG. 17B. For example, by comparing sequence variability or lack thereof at various CDR positions in the datasets as a whole, one can observe CDR locations at which particular amino acids are beneficial for binding. Similarly, by comparing sequence variability among V_H chains or among V_L chains from the same germline, one can observe CDR locations at which amino acid changes may be cooperative. The dataset further allows on to identify CDR positions that are likely to be critical for binding.

[0037] Certain antibodies disclosed herein were selected from canine or feline libraries on the basis of CDR sequence similarity to other anti-NGF antibodies. Accordingly both CDRs and FRs are canine-like or feline-like and there will be observed some degree of uniformity among antibody heavy and light chains resulting from the same germline sequence. It is understood that such uniformity is not a necessity but a consequence of the caninization and felinization methods employed. It is also understood that a substantial degree of sequence variability is allowed or can be introduced into FRs that is not detrimental to antigen binding. In certain embodiments, the anti-NGF protein comprises a heavy chain framework (FR1H+FR2H+FR3HH) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to a heavy chain set forth in FIG. 1. In certain embodiments, the anti-NGF protein comprises a heavy chain

framework (FR1H+FR2H+FR3H+FR4H) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, SEQ ID NO:137, or SEQ ID NO:141.

[0038] In certain embodiments, the anti-NGF protein comprises a heavy chain framework (FR1H+FR2H+FR3HH) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to a heavy chain set forth in FIG. 17A. In certain embodiments, the anti-NGF protein comprises a heavy chain framework (FR1H+FR2H+FR3H+FR4H) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:141, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:205, or SEQ ID NO:206.

[0039] In certain embodiments, the anti-NGF protein comprises a light chain framework (FR1+FR2+FR3+FR4) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to a light chain set forth in FIG. 2. In certain embodiments, the anti-NGF protein comprises a light chain framework (FR1L+FR2L+FR3L+FR4L) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

[0040] In certain embodiments, the anti-NGF protein comprises a light chain framework (FR1+FR2+FR3+FR4) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to a light chain set forth in FIG. 17B. In certain embodiments, the anti-NGF protein comprises a light chain framework (FR1L+FR2L+FR3L+FR4L) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:142, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, or SEQ ID NO:203.

[0041] For a discussion of naturally conserved networks of amino acids that support antibody V(H) and V(L) function, see, e.g., Wang et al., Conserved amino acid networks involved in antibody variable domain interactions. Proteins 2009 Jul;76(1):99-114. Wang identifies conserved and non-conserved amino acid pairs in antibody V_H and V_L domains, the V_H-C_H1 variable-constant domain interface, as well as in camelid V_{HH} domains, which have evolved to lack interactions with V_L and C_H1. In certain embodiments, mutations are introduces to optimize biopharmaceutical and biophysical properties, such as efficacy, safety, and manufacturability, and stability of therapeutic antibodies. See, e.g. Douillard et al., Optimization of an Antibody Light Chain Framework Enhances Expression, Biophysical Properties and Pharmacokinetics. Antibodies (Basel) 2019 Sep 6;8(3):46.

[0042] In certain embodiments, the invention provides an isolated, recombinant NGF-binding protein wherein the variable heavy chain comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a heavy chain variable domain set forth in FIG. 1. In certain embodiments, the heavy chain variable domain comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, or SEQ ID NO:137.

[0043] In certain embodiments, the invention provides an isolated, recombinant NGF-binding protein wherein the variable light chain comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a light chain variable domain set forth in FIG. 2. In certain embodiments, the light chain variable domain comprises an amino acid sequence having at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, or SEQ ID NO:138.

[0044] In another aspect, the invention provides a nucleic acid that encodes an anti-NGF protein of the invention. In another aspect, the invention provides a vector which comprises a nucleic acid that encodes an anti-NGF protein of the invention.

[0045] In another aspect, the invention provides a cell which comprises a nucleic acid of vector or the invention or expresses an anti-NGF protein of the invention.

[0046] The anti-NGF binding proteins, such as but not limited to antibodies and antibody fragments, specifically bind NGF which inhibits the association of NGF with TrkA and further inhibits the association of NGF with p75. In certain embodiments, these novel anti-NGF binding proteins are suitable for detecting NGF, and for treating pain and pain-associated disorders and conditions, e.g., pain associated with inflammation, cancer, specific pain and inflammation associated disorders, especially pain-associated disorders associated with elevated NGF levels, and may be administered alone or in association with another active agent, such as but not limited to another biologic, an NSAID or opioid analgesic.

[0047] Accordingly, it is an object of the invention not to encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product. It may be advantageous in the practice of the invention to be in compliance with Art. 53(c) EPC and Rule 28(b) and (c) EPC. All rights to explicitly disclaim any embodiments that are the subject of any granted patent(s) of applicant in the lineage of this application or in any other lineage or in any prior filed application of any third party is explicitly reserved. Nothing herein is to be construed as a promise.

[0048] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0049] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] FIG. 1A-1B shows an alignment of amino acid sequences of exemplary V_H heavy chain variable domains of the invention. The aligned variable domains are divided in two parts: part (A) shows the N-terminal end and part (B) shows the C-terminal end. For alignment purposes frameworks (FRs) and complementarity determining regions (CDRs) are identified according to the IMGT system. CDRs and FRs may be mapped according to other systems disclosed herein.

[0051] FIG. 2A-2B shows an alignment of amino acid sequences of exemplary V_κ light chain variable domains of the invention. The aligned variable domains are divided in two parts: part (A) shows the N-terminal end and part (B) shows the C-terminal end. For alignment purposes frameworks (FRs) and complementarity determining regions (CDRs) are identified according to the IMGT system. CDRs and FRs may be mapped according to other systems disclosed herein. Each of the V_L domains is suitable for paring with any one of the V_H domains depicted in FIG. 1. Together with FIG. 1, the Clone Names indicate a selection of exemplary V_H-V_L pairs that were tested for binding.

[0052] FIG. 3 depicts inhibition of proliferation of TF-1 cells.

[0053] FIG. 4 depicts heavy chain (SEQ ID NO:144) and light chain (SEQ ID NO:145) amino acid sequences of chimeric 2166 antibody. Two residue changes ("AA," underlined and in bold font) were made in the Fc to eliminate effector activity. The changes are analogous to the "LALA" mutation described for human IgG1 Fc. The chimeric 2166 antibody comprises a canine IgGB heavy chain constant region and kappa light chain constant region.

[0054] FIG. 5 is a sensorgram for canine 2166 chimeric antibody binding to canine NGF. The NGF concentrations were 0.78, 1.56, 3.12, 6.25, and 12.5 nM.

[0055] FIG. 6 is a sensorgram for canine NGF only binding to canine p75-Fc. The NGF concentrations were 0.78, 1.56, 3.12, 6.25, 12.5, 25, and 50 nM.

[0056] FIG. 7 is a sensorgram for canine NGF only binding to canine TrkA-Fc. The NGF concentrations were 0.78, 1.56, 3.12, 6.25, 12.5, 25, and 50 nM.

[0057] FIG. 8 is a sensorgram for canine 2166 chimeric antibody-NGF mixture binding to canine p75-Fc. The NGF concentrations were 12.5, 25, and 50 nM.

[0058] FIG. 9 is a sensorgram for canine 2166 chimeric antibody-NGF mixture binding to canine TrkA-Fc. The NGF concentrations were 12.5, 25, and 50 nM.

[0059] FIG. 10 shows sensorgrams of 70 caninized clones binding to canine NGF. The NGF concentrations were 0.23, 0.69, 2.06, 6.17, and 18.52 nM

[0060] FIG. 11 is a sensorgram for canine NGF only binding to canine p75-Fc. The NGF concentrations 0.78, 1.56, 3.12, 6.25, 12.5, 25, and 50 nM.

[0061] FIG. 12 is a sensorgram for canine NGF only binding to canine TrkA-Fc. The NGF concentrations 0.78, 1.56, 3.12, 6.25, 12.5, 25, and 50 nM.

[0062] FIG. 13 is a sensorgram for caninized SC42_101 antibody-NGF mixture binding to canine p75-Fc. The NGF concentrations were 12.5, 25, and 50 nM.

[0063] FIG. 14 is a sensorgram for caninized SC42_101 antibody-NGF mixture binding to canine TrkA-Fc. The NGF concentrations were 12.5, 25, and 50 nM.

[0064] FIG. 15 depicts the V_H (SEQ ID NO:141) and V_L (SEQ ID NO:142) amino acid sequence of a felinized anti-NGF antibody.

[0065] FIG. 16 is a sensorgram for felinized clone 101 binding to NGF. The NGF concentrations were 1.23, 3.7, 11, 33, and 100 nM.

[0066] FIG. 17A-17B shows alignments of amino acid sequences of exemplary felinized and affinity matured felinized V_H heavy chain variable domains (A) and V_K light chain variable domains (B) of the invention. For alignment purposes frameworks (FRs) and complementarity determining regions (CDRs) are identified according to the IMGT system. CDRs and FRs may be mapped according to other systems disclosed herein. See, e.g., Table 4 and CDRs defined using a combination of Kabat and IMGT methodology.

[0067] FIG. 18A-18H shows sensorgrams for affinity-matured feline antibodies: (A) clone 101; (B) AHF17602; (C) SC-184_76; (D) SC-184_76-Arg; (E) SC-102; (F) SC-184_102-Arg; (G) SC-110; (H) SC-184_110-Arg. The NGF concentrations were 50, 25, 12.5, 6.25, 3.125, and 1.56 nM.

[0068] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0069] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings.

DETAILED DESCRIPTION OF THE INVENTION

[0070] According to certain exemplary embodiments of the present invention, the NGF binding protein is an anti-NGF antibody or antigen-binding fragment thereof. The term "antibody," as used herein, includes immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (e.g., IgM). In a typical antibody, each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, C_H1, C_H2 and C_H3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the anti-NGF antibody (or antigen-binding portion thereof) may be identical to the canine germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0071] Antibody residues that have a substantial impact on affinity and specificity of binding to target antigen are primarily located in CDRs. Kabat et al. compiled and aligned immunoglobulin heavy and light chain sequences and were the first to propose a standardized numbering scheme for the variable regions of immunoglobulins identifying conserved and hypervariable regions and residues. (Kabat EA et al., 1979, Sequences of Immunoglobulin Chains: Tabulation and Analysis of Amino Acid Sequences of Precursors, V-regions, C-regions, J-Chain and BP-Microglobulins, Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health). While the Kabat system is a widely adopted standard for numbering antibody residues, the hypervariable regions defined by Kabat do not exactly match with the structural aspects of antigen-binding loops. Chothia and Lesk developed a structure-based numbering scheme by aligning crystal structures of antibody variable regions and classified CDR loops in a small number of "canonical" classes (Chothia C, et al., 1987, Canonical structures for the hypervariable regions of immunoglobulins. J. Mol. Biol. 196:901–17. doi: 10.1016/0022-2836(87)90412-8). An advantage of the Chothia numbering scheme is that topologically aligned residues from different

antibodies are localized at the same position number and the Chothia CDR definition corresponds in most antibody sequences to the structural antigen-binding loop. Lefranc introduced a new system based on germ-line sequences intended to standardize numbering for all proteins of the immunoglobulin superfamily, including T cell receptor chains. (Giudicelli V et al., 1997, IMGT, the international ImMunoGeneTics database. Nucleic Acids Res. 25:206–11), which was then extended to entire variable domains (Lefranc M-P et al., 2003, IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev Comp Immunol. 27:55–77. doi: 10.1016/S0145-305X(02)00039-3). Additional numbering systems have been proposed to align unconventional frameworks (Abhinandan KR et al., 2008, Analysis and improvements to Kabat and structurally correct numbering of antibody variable domains. Mol Immunol. 45:3832–9. doi: 10.1016/j.molimm.2008.05.022) and to subdivide variable chain sequences into multiple fragments including structurally invariant "cores" (Gelfand et al., 1998, Algorithmic determination of core positions in the V_L and V_H domains of immunoglobulin molecules. J Comput Biol. (1998) 5:467–77). In certain embodiments of the invention, CDR residues are identified according to such a standard system as set forth above. In certain embodiments, antibodies of the invention are identified by all or a subset of Kabat CDR residues of the antibody sequences set forth herein. In certain embodiments, antibodies of the invention are identified by all or a subset of Chothia CDR residues of the antibody sequences set forth herein. In certain embodiments, antibodies of the invention are identified by all or a subset of IMGT CDR residues of the antibody sequences set forth herein. In certain embodiments, antibodies of the invention are identified by CDR residues defined by two or more systems, comprising e.g., but not limited to, all or a subset of residues of VH-CDR1 according to Kabat, all or a subset of residues of VH-CDR2 according to Chothia, all or a subset of residues of VH-CDR3 according to Kabat, all or a subset of residues of VL-CDR1 according to Kabat, all or a subset of residues of VL-CDR2 according to IMGT, and all or a subset of residues of VL-CDR3 according to Chothia. Table 1 shows the correspondence of FRs and CDRs for the antibody sequences shown in FIGS. 1 and 2.

Table 1 - CDR amino acids

	VH-FR1	VH-CDR1	VH-FR2	VH-CDR2	VH-FR3	VH-CDR3	VH-FR4
IMGT	1-25	26-33	34-50	51-57	58-95	96-112	113-123
Kabat	1-30	31-35	36-49	50-65	66-97	98-112	113-123

Chothia	1-25	26-32	33-51	52-56	57-97	98-112	113-123
	VL-FR1	VL-CDR1	VL-FR2	VL-CDR2	VL-FR3	VL-CDR3	VL-FR4
IMGT	1-26	27-32	33-49	50-52	53-88	89-97	98-107
Kabat	1-23	24-34	35-49	50-56	57-88	89-97	98-107
Chothia	1-25	26-32	33-49	50-52	53-90	91-96	97-107

[0072] Identifying CDRs according to Kabat, in certain embodiments, a caninized anti-NGF binding protein comprises an antigen binding portion that comprises one or more of (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4X_5$ (SEQ ID NO:158), wherein X_1 comprises E, S, or T, X_2 comprises G, H, N, S, or Q, X_3 comprises A or S; and X_4 comprises I, M, or V, and X_5 comprises D or S; (b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $X_1X_2X_3SNX_6GTX_9YX_{11}X_{12}AX_{14}X_{15}X_{16}$ (SEQ ID NO:159), wherein X_1 comprises V, L, M, or T, X_2 comprises I or L, X_3 comprises W or Y, X_6 comprises G or R, X_9 comprises D, Q, or S, X_{11} comprises A, N, or T, X_{12} comprises D or S, X_{14} comprises I or V, X_{15} comprises E or K, and X_{16} comprises G or S; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $IX_2X_3YX_5X_6X_7YLX_{10}X_{11}YX_{13}X_{14}X_{15}$, (SEQ ID NO:160), wherein X_2 comprises W or Y, X_3 comprises F, H, W, or Y, X_5 comprises D or E, X_6 comprises A or S, X_7 comprises D or Y, X_{10} comprises H or Y, X_{11} comprises F or W, X_{13} comprises F, I, L, W, or Y, X_{14} comprises D or Q, and X_{15} comprises F, I, L, M, W, or Y; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1ASX_4X_5IX_7X_8X_9X_{10}X_{11}$ (SEQ ID NO:161), wherein X_1 comprises L or R, X_4 comprises D, E, or K, X_5 comprises A, G, or N, X_7 comprises G, N, Q or S, X_8 comprises N or S, X_9 comprises A, G, N, S or T, X_{10} comprises L or V, and X_{11} comprises A or N; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence $AX_2X_3X_4X_5X_6X_7$ (SEQ ID NO:162), wherein X_2 comprises A, S, or T, X_3 comprises A, D, E, N, Q, S, or T, X_4 comprises A, E, K, L, N, Q, S, or T, X_5 comprises L, M, or N, X_6 comprises A or Q, and X_7 comprises G, D, R, S, or T; and (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $X_1X_2GX_4X_5X_6PX_8T$ (SEQ ID NO:163), wherein X_1 comprises H, M Q, or R, X_2 comprises H, N, Q, or S, X_4 comprises F, H, W, or Y, X_5 comprises K or Q, X_6 comprises F or W, and X_8 comprises L or M.

[0073] In certain embodiments, the caninized anti-NGF binding protein comprises an antigen binding portion which comprises (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4S$ (SEQ ID NO:164), wherein X_1 comprises E, S or T, X_2 comprises H or N, X_3 comprises A or S, X_4 comprises I or M, (b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $TIWSNX_6GTDYX_{11}X_{12}AVKG$ (SEQ ID NO:165), wherein X_6 comprises G or R, X_{11} comprises A or T, and X_{12} comprises D or S; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $IYYYX_5ADYLX_{10}X_{11}YX_{13}DX_{15}$ (SEQ ID NO:166), wherein X_5 comprises D or E, X_{10} comprises H or Y, X_{11} comprises F or W, X_{13} comprises F, I, L, W, or Y, and X_{15} comprises F, I, L, or M; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $RASEGIX_7X_8X_9X_{10}A$ (SEQ ID NO:167), wherein X_7 comprises N, Q, or S, X_8 comprises N or S, X_9 comprises G, N, S or T, and X_{10} comprises L or V; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence $ATX_3X_4LX_6X_7$ (SEQ ID NO:168), wherein X_3 comprises A, D, E, N, Q, or S, X_4 comprises E, K, Q, or S, X_6 comprises A or Q, and X_7 comprises R or T; and (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QQGX_4X_5X_6PLT$ (SEQ ID NO:169), wherein X_4 comprises F, H, W, or Y, X_5 comprises K or Q, and X_6 comprises F or W.

[0074] Identifying CDRs according to Chothia, in certain embodiments, the anti-NGF binding protein comprises an antigen binding portion that comprises one or more of (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7$ (SEQ ID NO:170), wherein X_1 comprises A, G, or N, X_2 comprises L or M, X_3 comprises A, D, E, or S, X_4 comprises F, I, L, M, or V, X_5 comprises N or T, X_6 comprises E, S, or T, and X_7 comprises G, H, N, S, or Q; (b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence X_1SNX_4G (SEQ ID NO:171), wherein X_1 comprises W or Y and X_4 comprises G orR; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $IX_2X_3YX_5X_6X_7YLX_{10}X_{11}YX_{13}X_{14}X_{15}$, (SEQ ID NO:172), wherein X_2 comprises W or Y, X_3 comprises F, H, W, or Y, X_5 comprises D or E, X_6 comprises A or S, X_7 comprises D or Y, X_{10} comprises H or Y, X_{11} comprises F or W, X_{13} comprises F, I, L, W, or Y, X_{14} comprises D or Q, and X_{15} comprises F, I, L, M, W, or Y; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid

sequence $SX_2X_3IX_5X_6X_7$ (SEQ ID NO:173), wherein X_2 comprises D, E, or K, X_3 comprises A, G, or N, X_5 comprises G, N, Q or S, X_6 comprises N or S, X_7 comprises A, G, N, S or T; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence AX_2X_3 (SEQ ID NO:174), wherein X_2 comprises A, S, or T, and X_3 comprises A, D, E, N, Q, S, or T; and (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $GX_2X_3X_4PX_6$ (SEQ ID NO:175), wherein X_2 comprises F, H, W, or Y, X_3 comprises K or Q, X_4 comprises F or W, and X_6 comprises L or M.

[0075] In certain embodiments, the anti-NGF binding protein comprises an antigen binding portion which comprises (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7$ (SEQ ID NO:176), wherein X_1 comprises A, G, or N, X_2 comprises L or M, X_3 comprises A, E, or S, X_4 comprises F or L, X_5 comprises N or T, X_6 comprises E, S or T, and X_7 comprises H, N, or S; (b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $WSNX_4G$ (SEQ ID NO:177), wherein X_4 comprises G or R; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $IYX_3YX_5ADYLX_{10}X_{11}YX_{13}DX_{15}$ (SEQ ID NO:178), wherein X_3 comprises F or Y, X_5 comprises D or E, X_{10} comprises H or Y, X_{11} comprises F or W, X_{13} comprises F, I, L, W, or Y, and X_{15} comprises F, I, L, M, W, or Y; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $SX_2GIX_5X_6X_7$ (SEQ ID NO:179), wherein X_2 comprises D or E, X_5 comprises N, Q, or S, X_6 comprises N or S, and X_7 comprises G, N, S, or T; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence ATX_3 (SEQ ID NO:180), wherein X_3 comprises D, E, N, Q, or S; and (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $GX_2X_3X_4PX_6$ (SEQ ID NO:181), wherein X_2 comprises F, H, W, or Y, X_3 comprises K or Q, X_4 comprises F or W, and X_6 comprises L or M.

[0076] In another aspect, the invention provides a binding protein suitable for use in a mammal, for example, but without limitation, a feline. In certain embodiments, a felinized anti-NGF binding protein comprises (a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1LX_3X_4X_5X_6X_7X_8MX_{10}$ (SEQ ID NO:208), wherein X_1 comprises A, G, L, N, or Q, X_3 comprises A, D, E, G, H, I, M, S, T, or Y, X_4 comprises L, M, or V, X_5 comprises A, M, N, R, S, T, or V, X_6 comprises A, E, G, H, K, R, S, or T, X_7 comprises A, D, H, I, N, Q, S, T, or Y, X_8 comprises A or S, and X_{10} comprises S or V; (b) a heavy chain

complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $X_1X_2X_3X_4X_5GTX_8YX_{10}DX_{12}VX_{14}$ (SEQ ID NO:209), wherein X_1 comprises I or L, X_2 comprises W or Y; X_3 comprises A, P, or S, X_4 comprises D, E, N, Q, R, or S, X_5 comprises G, R, or Y, X_8 comprises D or Y, X_{10} comprises D, E, H, S, or T, X_{12} comprises D or S, and X_{14} comprises D, E, or K; (c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}LX_{12}X_{13}X_{14}FX_{16}X_{17}$ (SEQ ID NO:210), wherein X_1 comprises A, D, E, K, N, Q, S, or T, X_2 comprises A, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, or Y, X_3 comprises I, L, W, or Y, X_4 comprises F, T, W, or Y, X_5 comprises F, H, or Y, X_6 comprises H or Y, X_7 comprises D or E, X_8 comprises A, S, or V, X_9 comprises D, E, H, K, N, Q, or Y, X_{10} comprises F, H, or Y, X_{12} comprises H or Y, X_{13} comprises F or W, X_{14} comprises D, I, L, W, or Y, X_{16} comprises D or Q, and X_{17} comprises E, F, H, I, L, M, N, P, W, or Y; (d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1ASX_4X_5X_6X_7X_8X_9LX_{11}$ (SEQ ID NO:211), wherein X_1 comprises F or R, X_4 comprises E, K, or N, X_5 comprises A, or G, X_6 comprises I, L, or V, X_7 comprises A, D, G, L, P, Q, S, V, or Y, X_8 comprises K, Q, N, S, or Y, X_9 comprises A, D, E, F, G, H, K, L, N, Q, R, S or T, and X_{11} comprises A, G, or S; (e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence $AX_2X_3X_4X_5X_6X_7$ (SEQ ID NO:212), wherein X_2 comprises A, D, L, Q, S, T, V, or Y, X_3 comprises D, E, K, N, Q, or S, X_4 comprises H, I, K, L, M, N, or V; X_5 comprises H or L, X_6 comprises H, I, L, or M, and X_7 comprises D, E, N, S, or T; (f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QQX_3X_4X_5X_6X_7X_8T$ (SEQ ID NO:213), wherein X_3 comprises G or Y, X_4 comprises D, F, G, H, K, L, R, S, T, V, W, or Y, X_5 comprises E, K, Q, R, or S, X_6 comprises I, F, T, or W, X_7 comprises E or P, and X_8 comprises L, M, or W.

[0077] In certain embodiments, the anti-NGF binding protein comprises: VH-CDR1 comprises $GLSLTSX_7SMX_{10}$ (SEQ ID NO:214), wherein X_7 comprises A, D, or N, and X_{10} comprises S or V; VH-CDR2 comprises $X_1X_2SNX_5GT$ (SEQ ID NO:215), wherein X_1 comprises I or L, X_2 comprises W or Y, and X_5 comprises G or R; VH-CDR3 comprises $ASIYYYX_7AX_9YLHWYFDX_{12}$ (SEQ ID NO:216), wherein X_7 comprises D or E, X_9 comprises D or E, and X_{12} comprises E or F; VL-CDR1 comprises $RASX_4GIX_7X_8NLS$ (SEQ ID NO:217), wherein X_4 comprises E or K, X_7 comprises A, Q, or S, X_8 comprises K or N; VL-CDR2 comprises $AX_2X_3X_4LHS$ (SEQ ID NO:218), wherein X_2 comprises Q or T, X_3 comprises D or S, and X_4

comprises I, N, or V; and VL-CDR3 comprises QQGX$_4$KWPLT (SEQ ID NO:219), wherein X$_4$ comprises F, W, or Y.

[0078] In certain embodiments, the anti-NGF binding protein comprises one or more (i.e. one, two, three, four, five, or all six) CDRs of felinized antibody 101 disclosed herein. In certain embodiments, the anti-NGF binding protein comprises one or more (i.e. one, two, three, four, five, or all six) CDRs of an affinity matured felinized antibody disclosed herein. In certain embodiments, the anti-NGF binding protein comprises CDRs from one or more of felinized antibody 101 and the affinity matured variants provided herein. In certain embodiments, the anti-NGF binding protein comprises V_H CDRs set forth in Fig. 17A. In certain embodiments, the anti-NGF binding protein comprises V_L CDRs set forth in Fig. 17B. In certain embodiments, the anti-NGF binding protein comprises V_H CDRs of an antibody V_H domain set forth in Fig. 17A. In certain embodiments, the anti-NGF binding protein comprise V_L CDRs of an antibody V_L domain set forth in Fig. 17B.

[0079] According to the invention, in certain embodiments, the anti-NGF binding protein comprises an amino acid of a felinized antibody 101 variant disclosed herein, for example one or more of the following amino acids in V_H: S28H, T30N, T30R, S31H, S35V, Y52W, S53P, G55R, G55Y, Y58D, T60D, T60E, T60H, T60S, S62D, K64D, K64E, S97H, S97K, S97M, S97N, S97Q, S97T, Y99F, Y101H, D104E, D104K, D104N, D104Q, F112E, D112H, F112N, F112P; and/or in V_L: R24F, S30A, S30L, S30P, S30Q, S30V, S30Y, N31Q, S34A, S34G, N53H, N53I, N53K, N53L, N53M, N53V, L54H, H55I, H55L, H55M, S56D, S56E, S56N, S56T. In certain embodiments, the anti-NGF binding protein does not comprise one or more of the above-listed amino aicd variants. For example, in certain embodiments, the anti-NGF binding protein does not comprise arginine of G55R. Amino acid positions are indicated by residue and number in felinized antibody 101, e.g., S28H indicates H at the position corresponding to S28 of V_H or antibody 101. The aforementioned positions include CDR and framework amino acid residues.

[0080] In certain embodiments, the anti-NGF binding protein comprises one or more of the following amino acids in V_H: S35V, G55R, S97Q, F112E; and/or in V_L: S30A, S30Q, N53I, N53V. Pairings of VH and VL chains comprising the above-described sequence variation demonstrate compatibility of the VH and VL mutations and interchangeability of the VH and VL domains comprising the mutations.

[0081] In certain embodiments, the binding proteins comprise a canine or a caninized antibody. In certain embodiments, the binding proteins comprise a feline or a felinized antibody.

[0082] In certain embodiments, an amino acid residue is mutated into one that allows the properties of the amino acid side-chain to be conserved. Examples of the properties of amino acid side chains comprise: hydrophobic amino acids (A, I, L, M, F, P, W, Y, V), hydrophilic amino acids (R, D, N, C, E, Q, G, H, K, S, T), and amino acids comprising the following side chains: aliphatic side-chains (G, A, V, L, I, P); hydroxyl group-containing side-chains (S, T, Y); sulfur atom-containing side-chains (C, M); carboxylic acid- and amide-containing side-chains (D, N, E, Q); base-containing side-chains (R, K, H); and aromatic-containing side-chains (H, F, Y, W). The letters within parenthesis indicate the one-letter amino acid codes. Amino acid substitutions within each group are called conservative substitutions. It is well known that a polypeptide comprising a modified amino acid sequence in which one or more amino acid residues is deleted, added, and/or substituted can retain the original biological activity (Mark D. F. et al., Proc. Natl. Acad. Sci. U.S.A. 81:5662-5666 (1984); Zoller M. J. and Smith M., Nucleic Acids Res. 10: 6487-6500 (1982); Wang A. et al., Science 224: 1431-1433; Dalbadie-McFarland G. et al., Proc. Natl. Acad. Sci. U.S.A. 79: 6409-6413 (1982)). The number of mutated amino acids is not limited, but in general, the number falls within 40% of amino acids of each CDR, and preferably within 35%, and still more preferably within 30% (e.g., within 25%). The identity of amino acid sequences can be determined as described herein.

[0083] The invention provides recombinant antibodies designed or modified to minimize antigenicity in canines and felines. In certain embodiments, the antibodies are further modified to remove T cell epitopes.

[0084] As used herein, the term "canine" includes all domestic dogs, Canis lupus familiaris or Canis familiaris, unless otherwise indicated.

[0085] As used herein, the term "feline" refers to any member of the Felidae family. Domestic cats, pure-bred and/or mongrel companion cats, and wild or feral cats are all felines.

[0086] As used herein the term "canine framework" or "feline framework" refers to the amino acid sequence of the heavy chain and light chain of a canine antibody other than the hypervariable region residues defined herein as CDR residues. With regard to a caninized antibody, in certain embodiments, canine CDRs are identified in canine antibody heavy and light chains variable domain sequences that closely match CDRs of NGF-binding antibodies originating in other

species. In certain embodiments, native canine CDRs are replaced with the corresponding foreign CDRs (e.g ., those from a rat or a mouse antibody) in both chains. With regard to a felinized antibody, in certain embodiments, feline CDRs are identified in feline antibody heavy and light chains variable domain sequences that closely match CDRs of NGF-binding antibodies originating in other species. In certain embodiments, native feline CDRs are replaced with the corresponding foreign CDRs (e.g ., those from a rat or a mouse antibody) in both chains. Optionally the heavy and/or light chains of the caninized or felinized antibody may contain some mutated or foreign non-CDR residues, e.g., framework amino acid residues that vary among germline antibody sequence or mutations that preserve the conformation of the foreign CDRs within the antibody.

[0087] Five major isotypes (IgA, IgG, IgM, IgD, IgE) and two forms of light chain (κ and λ) are present in dogs. In the dog, there are four subtypes for IgG, which are IgGA, IgGB, IgGC, and IgGD (Bergeron etal al, 2014, *Comparative functional characterization of canine IgG subclasses*. Veterinary Immunology and Immunopathology. 157:31-41). For the cat, there are three subtypes of IgG which are IgG1a, IgG1b, and IgG2 (Streitzel et al. 2014, *In vitro functional characterization of feline IgGs*. Vet Immunol Immunopathol 158, 214–223, doi.org/10.1016/j.vetimm.2014.01.012).

[0088] The invention provides caninized and felinized antibodies engineered to modulate one or more effector functions or circulation half-life. Hinge and constant domains of an antibody engage host receptors or complement protein to mediate effector functions and regulate antibody circulation. In certain embodiments, one or more effector functions is enhanced. In certain embodiments, one or more effector funtions is reduced or eliminated. In certain embodiments, antibodies of the invention comprise modifications to modulate antibody-dependent cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). A non-limiting example involves engineering of canine IgGB constant region residues Met242 and/or Leu243 to reduce effector function. In certain embodiments, a IgGB constant region of the invention comprises M242A and L243A substitution. In certain embodiments, the second constant domain (CH2) and/or the third constant domain (CH3) comprises mutations and combinations of mutations from wild-type designed to modulate binding to FcRn (neonatal Fc) receptor. In canine constant regions, such mutations include, without limitation substitutions of Ala426, for example A426Y or A426H, substitutions of Thr286, for example T286L or T286Y, substitutions of Tyr436, for example Y436H, and combinations of such mutations including but not limited to A426Y + T286L, A426Y

+ Y436H, A426H + T286L, and A426H + T286Y. In certain embodiments a chimeric or caninized antibody of the invention comprises a substitution at amino acid Asn434, such as but not limited to N434H. In feline constant regions, such mutations include, without limitation substitutions of Ser428, including but not limited to S428Y or S428L, substitutions of Gln311, including but not limited to Q311V, substitutions of Leu309, including but not limited to L309V, substitutions of Thr286, including but not limited to T286E, substitutions of Glu380, including but not limited to E380T, and combinations of such mutations including but not limited to S428Y + Q311V, S428Y + L309V, S428Y + Q311V + T286E, S428Y + Q311V + E380T, and S428Y +L309V + E380T. In certain embodiments a chimeric or felinized antibody of the invention comprises a substitution at amino acid Ser428 and/or Ser434 including but not limited to S428L and/or S434H.

[0089] The term "antibody," as used herein, includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. As used herein, the term "specifically binds" or "binds specifically" means that an NGF binding protein of the invention reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with NGF than it does with alternative antigens. For example, NGF binding protein binds to NGF with materially greater affinity (e.g., at least 2-fold or 5-fold or 10-fold or 20-fold or 50-fold or 100-fold or 500-fold or 1000-fold or 10,000-fold or greater) than it does to other proteins or peptides. In certain embodiments, the NGF-binding proteins binds to NGF with an equilibrium dissociation constant K_D for the epitope or target to which it binds of, e.g., 10^{-4} M or smaller, e.g., 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. It will be recognized by one of skill that an antibody that specifically binds to a target (e.g., NGF) from one species may also specifically bind to orthologs of NGF.

[0090] Antigen-binding fragments of an antibody may be derived, e.g., from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, e.g., commercial sources, DNA libraries (including, e.g., phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular

biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0091] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')2 fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (e.g. monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0092] In certain embodiments, an antigen-binding fragment of an antibody comprises at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V_H domain associated with a V_L domain, the V_H and V_L domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V_H-V_H, V_H-V_L or V_L-V_L dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V_H or V_L domain.

[0093] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H-C_H1; (ii) V_H-C_H2; (iii) V_H-C_H3; (iv) V_H-C_H1-C_H2; (V) V_H-C_H1-C_H2-C_H3; (vi) V_H-C_H2-C_H3; (vii) V_H-C_L; (viii) V_L-C_H1; (ix) V_L-C_H2, (x) V_L-C_H3; (xi) V_L-C_H1-C_H2; (xii) V_L-C_H1-C_H2-C_H3; (xiii) V_L-C_H2-C_H3; and (xiv) V_L-C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (e.g., 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule.

Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (e.g., by disulfide bond(s)).

[0094] The term "diabody (Db)" refers to a bivalent antibody fragment constructed by gene fusion (for example, P. Holliger et al., Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993), EP 404,097, WO 93/11161). In general, a diabody is a dimer of two polypeptide chains. In the each of the polypeptide chains, a light chain variable region (V_L) and a heavy chain variable region (V_H) in an identical chain are connected via a short linker, for example, a linker of about five residues, so that they cannot bind together. Because the linker between the two is too short, the V_L and V_H in the same polypeptide chain cannot form a single chain V region fragment, but instead form a dimer. Thus, a diabody has two antigen-binding domains. When the V_L and V_H regions against the two types of antigens (a and b) are combined to form V_{La}-V_{Hb} and V_{Lb}-V_{Ha} via a linker of about five residues, and then co-expressed, they are secreted as bispecific Dbs. The antibodies of the present invention may be such Dbs.

[0095] A single-chain antibody (also referred to as "scFv") can be prepared by linking a heavy chain V region and a light chain V region of an antibody (for a review of scFv see Pluckthun "The Pharmacology of Monoclonal Antibodies" Vol. 113, eds. Rosenburg and Moore, Springer Verlag, N.Y., pp. 269-315 (1994)). Methods for preparing single-chain antibodies are known in the art (see, for example, U.S. Pat. Nos. 4,946,778; 5,260,203; 5,091,513; and 5,455,030). In such scFvs, the heavy chain V region and the light chain V region are linked together via a linker, preferably, a polypeptide linker (Huston, J. S. et al., Proc. Natl. Acad. Sci. U.S.A, 1988, 85, 5879-5883). The heavy chain V region and the light chain V region in a scFv may be derived from the same antibody, or from different antibodies. The peptide linker used to ligate the V regions may be any single-chain peptide consisting of 12 to 19 residues. A DNA encoding a scFv can be amplified by PCR using, as a template, either the entire DNA, or a partial DNA encoding a desired amino acid sequence, selected from a DNA encoding the heavy chain or the V region of the heavy chain of the above antibody, and a DNA encoding the light chain or the V region of the light chain of the above antibody; and using a primer pair that defines the two ends. Further amplification can be subsequently conducted using a combination of the DNA encoding the peptide linker portion, and the primer pair that defines both ends of the DNA to be ligated to the heavy and light chain

respectively. After constructing DNAs encoding scFvs, conventional methods can be used to obtain expression vectors comprising these DNAs, and hosts transformed by these expression vectors. Furthermore, scFvs can be obtained according to conventional methods using the resulting hosts. These antibody fragments can be produced in hosts by obtaining genes that encode the antibody fragments and expressing these as outlined above. Antibodies bound to various types of molecules, such as polyethylene glycols (PEGs), may be used as modified antibodies. Methods for modifying antibodies are already established in the art. The term "antibody" in the present invention also encompasses the above-described antibodies.

[0096] The term "Kd" as used herein, refers to the dissociation constant of an antibody-antigen interaction. The dissociation constant, Kd, and the association constant, Ka, are quantitative measures of affinity. At equilibrium, free antigen (Ag) and free antibody (Ab) are in equilibrium with antigen-antibody complex (Ag-Ab), and the rate constants, ka and kd, quantitate the rates of the individual reactions. At equilibrium, ka [Ab][Ag]=kd [Ag-Ab]. The dissociation constant, Kd, is given by: Kd=kd/ka=[Ag][Ab]/[Ag-Ab]. Kd has units of concentration, most typically M, mM, nM, pM, etc. When comparing antibody affinities expressed as Kd, having greater affinity for NGF is indicated by a lower value. The association constant, Ka, is given by: Ka=ka/kd=[Ag-Ab]/[Ag][Ab]. Ka has units of inverse concentration, most typically M^{-1}, mM^{-1}, nM^{-1}, pM^{-1}, etc. As used herein, the term "avidity" refers to the strength of the antigen-antibody binding taking valency into account.

[0097] The antibodies obtained can be purified to homogeneity. The antibodies can be isolated and purified by a method routinely used to isolate and purify proteins. The antibodies can be isolated and purified by the combined use of one or more methods appropriately selected from column chromatography, filtration, ultrafiltration, salting out, dialysis, preparative polyacrylamide gel electrophoresis, and isoelectro-focusing, for example (Strategies for Protein Purification and Characterization: A Laboratory Course Manual, Daniel R. Marshak et al. eds., Cold Spring Harbor Laboratory Press (1996); Antibodies: A Laboratory Manual. Ed Harlow and David Lane, Cold Spring Harbor Laboratory, 1988). Such methods are not limited to those listed above. Chromatographic methods include affinity chromatography, ion exchange chromatography, hydrophobic chromatography, gel filtration, reverse-phase chromatography, and adsorption chromatography. These chromatographic methods can be practiced using liquid phase chromatography, such as HPLC and FPLC. Columns to be used in affinity chromatography include

protein A columns and protein G columns. For example, protein A columns include Hyper D, POROS, and Sepharose F. F. (Pharmacia). Antibodies can also be purified by utilizing antigen binding, using carriers on which antigens have been immobilized.

[0098] As used herein, the term "therapeutic agent" refers to any agent or material that has a beneficial effect on the mammalian recipient. Thus, "therapeutic agent" embraces both therapeutic and prophylactic molecules having nucleic acid or protein components.

[0099] "Treating" as used herein refers to ameliorating at least one symptom of, curing and/or preventing the development of a given disease or condition.

[00100] The anti-NGF proteins described herein, including antibodies or fragments thereof, are useful for ameliorating, or reducing the symptoms of, or treating, or preventing, diseases and disorders associated with NGF. The anti-NGF proteins or fragments, as well as combinations with other agent, are to be administered in a therapeutically effective amount to subjects in need of treatment of diseases and disorders associated with NGF in the form of a pharmaceutical composition as described herein

[00101] In certain embodiments the method comprises ameliorating, or reducing the symptoms of, or treating, or preventing pain in a subject. In certain embodiments, the anti-NGF proteins, antibodies, or fragments thereof inhibit the association of NGF with TrkA and/or p75, for example administered alone or in conjunction with a second agent and are used to treat, ameliorate, reduce the symptoms of, or prevent inflammatory pain, post-operative incision pain, complex, cancer pain (including but not limited to primary or metastatic bone cancer pain), fracture pain, osteoporotic fracture pain, pain from osteoporosis, pain resulting from burn, and other nociceptic pain.

[00102] In certain embodiments the antibody compositions and methods are used for ameliorating, or reducing the symptoms of, or treating, or preventing pain of osteoarthritis (OA). OA is a slowly progressive degenerative joint disease characterized by whole-joint structural changes including articular cartilage, synovium, subchondral bone and periarticular components, leading to pain and loss of joint function. Chronic pain and OA are common in dogs and cats. 20-30% of dogs are affected clinically and have signs of OA. Up to 40% of all cats being affected clinically, with 90% of all cats over 12 years of age have signs of OA.

[00103] In dogs the most common site of OA is the hip, followed by stifle (knee), shoulder and carpus. In cats hip, stifle, carpus or spine are most commonly affected.

[00104] The anti-NGF proteins, antibodies or antibody fragments, are optionally administered in combination with one or more active agents including other analgesic agents. Such active agents include analgesic, anti-histamine, antipyretic, anti-inflammatory, antibiotic, antiviral, and anti-cytokine agents. Active agents include agonists, antagonists, and modulators of TNF-α, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-18, IFN-α, IFN-γ, BAFF, CXCL13, IP-10, VEGF, EPO, EGF, HRG, Hepatocyte Growth Factor (HGF), Hepcidin, including antibodies reactive against any of the foregoing, and antibodies reactive against any of their receptors. Active agents also include, without limitation, 2-arylpropionic acids, aceclofenac, acemetacin, acetylsalicylic acid (Aspirin), alclofenac, alminoprofen, amoxiprin, ampyrone, arylalkanoic acids, azapropazone, benorylate/benorilate, benoxaprofen, bromfenac, carprofen, celecoxib, choline magnesium salicylate, clofezone, COX-2 inhibitors, dexibuprofen, dexketoprofen, diclofenac, diflunisal, droxicam, ethenzamide, etodolac, etoricoxib, faislamine, fenamic acids, fenbufen, fenoprofen, flufenamic acid, flunoxaprofen, flurbiprofen, ibuprofen, ibuproxam, indometacin, indoprofen, kebuzone, ketoprofen, ketorolac, lomoxicam, loxoprofen, lumiracoxib, magnesium salicylate, meclofenamic acid, mefenamic acid, meloxicam, metamizole, methyl salicylate, mofebutazone, nabumetone, naproxen, n-arylanthranilic acids, nerve growth factor (NGF), oxametacin, oxaprozin, oxicams, oxyphenbutazone, parecoxib, phenazone, phenylbutazone, phenylbutazone, piroxicam, pirprofen, profens, proglumetacin, pyrazolidine derivatives, rofecoxib, salicyl salicylate, salicylamide, salicylates, sulfinpyrazone, sulindac, suprofen, tenoxicam, tiaprofenic acid, tolfenamic acid, tolmetin, and valdecoxib.

[00105] An anti-histamine can be any compound that opposes the action of histamine or its release from cells (e.g., mast cells). Anti-histamines include but are not limited to acrivastine, astemizole, azatadine, azelastine, betatastine, brompheniramine, buclizine, cetirizine, cetirizine analogues, chlorpheniramine, clemastine, CS 560, cyproheptadine, desloratadine, dexchlorpheniramine, ebastine, epinastine, fexofenadine, HSR 609, hydroxyzine, levocabastine, loratidine, methscopolamine, mizolastine, norastemizole, phenindamine, promethazine, pyrilamine, terfenadine, and tranilast.

[00106] Antibiotics include but are not limited to amikacin, aminoglycosides, amoxicillin, ampicillin, ansamycins, arsphenamine, azithromycin, azlocillin, aztreonam, bacitracin, carbacephem, carbapenems, carbenicillin, cefaclor, cefadroxil, cefalexin, cefalothin, cefalotin, cefamandole, cefazolin, cefdinir, cefditoren, cefepime, cefixime, cefoperazone, cefotaxime,

cefoxitin, cefpodoxime, cefprozil, ceftazidime, ceftibuten, ceftizoxime, ceftobiprole, ceftriaxone, cefuroxime, cephalosporins, chloramphenicol, cilastatin, ciprofloxacin, clarithromycin, clindamycin, cloxacillin, colistin, co-trimoxazole, dalfopristin, demeclocycline, dicloxacillin, dirithromycin, doripenem, doxycycline, enoxacin, ertapenem, erythromycin, ethambutol, flucloxacillin, fosfomycin, furazolidone, fusidic acid, gatifloxacin, geldanamycin, gentamicin, glycopeptides, herbimycin, imipenem, isoniazid, kanamycin, levofloxacin, lincomycin, linezolid, lomefloxacin, loracarbef, macrolides, mafenide, meropenem, meticillin, metronidazole, mezlocillin, minocycline, monobactams, moxifloxacin, mupirocin, nafcillin, neomycin, netilmicin, nitrofurantoin, norfloxacin, ofloxacin, oxacillin, oxytetracycline, paromomycin, penicillin, penicillins, piperacillin, platensimycin, polymyxin B, polypeptides, prontosil, pyrazinamide, quinolones, quinupristin, rifampicin, rifampin, roxithromycin, spectinomycin, streptomycin, sulfacetamide, sulfamethizole, sulfanilimide, sulfasalazine, sulfisoxazole, sulfonamides, teicoplanin, telithromycin, tetracycline, tetracyclines, ticarcillin, tinidazole, tobramycin, trimethoprim, trimethoprim-sulfamethoxazole, troleandomycin, trovafloxacin, and vancomycin.

[00107] Active agents also include aldosterone, beclometasone, betamethasone, corticosteroids, cortisol, cortisone acetate, deoxycorticosterone acetate, dexamethasone, fludrocortisone acetate, glucocorticoids, hydrocortisone, methylprednisolone, prednisolone, prednisone, steroids, and triamcinolone. Any suitable combination of these active agents is also contemplated.

[00108] The most common form of current treatment for OA and pain related to OA is NSAIDs (which are also anti-pain medications). NSAIDs are not always sufficiently effective, typically need to be administered daily and none are approved for long-term use in cats in the US. Additionally, there are safety and tolerability concerns with the use of NSAIDS in both dogs and cats, especially with long-term treatment. NSAIDs are not recommended to be co-administered with anti-NGF mAbs for long periods.

[00109] In certain embodiments, treatment comprises coadministration of dietary supplements containing Omega-3 fatty acids, microlactin, and/or glucosamine/chondroitin as an aid to joint health. Adequan (polysulfated glycosaminoglycan) is an FDA-approved disease modifying drug that inhibits cartilage loss and may also be co-administered.

[00110] Formulations and Methods of Administration

[00111] For *in vivo* use, a therapeutic agent as described herein is generally incorporated into a pharmaceutical composition prior to administration. Within such compositions, one or more therapeutic compounds as described herein are present as active ingredient(s) (i.e., are present at levels sufficient to provide a statistically significant effect on the symptoms of cystic fibrosis, as measured using a representative assay). A pharmaceutical composition comprises one or more such compounds in combination with any pharmaceutically acceptable carrier(s) known to those skilled in the art to be suitable for the particular mode of administration. In addition, other pharmaceutically active ingredients (including other therapeutic agents) may, but need not, be present within the composition.

[00112] The antibodies of the present invention can be formulated according to standard methods (see, for example, Remington's Pharmaceutical Science, latest edition, Mark Publishing Company, Easton, U.S.A), and may comprise pharmaceutically acceptable carriers and/or additives. The present invention relates to compositions (including reagents and pharmaceuticals) comprising the antibodies of the invention, and pharmaceutically acceptable carriers and/or additives. Exemplary carriers include surfactants (for example, PEG and Tween), excipients, antioxidants (for example, ascorbic acid), coloring agents, flavoring agents, preservatives, stabilizers, buffering agents (for example, phosphoric acid, citric acid, and other organic acids), chelating agents (for example, EDTA), suspending agents, isotonizing agents, binders, disintegrators, lubricants, fluidity promoters, and corrigents. However, the carriers that may be employed in the present invention are not limited to this list. In fact, other commonly used carriers can be appropriately employed: light anhydrous silicic acid, lactose, crystalline cellulose, mannitol, starch, carmelose calcium, carmelose sodium, hydroxypropylcellulose, hydroxypropylmethyl cellulose, polyvinylacetaldiethylaminoacetate, polyvinylpyrrolidone, gelatin, medium chain fatty acid triglyceride, polyoxyethylene hydrogenated castor oil 60, sucrose, carboxymethylcellulose, corn starch, inorganic salt, and so on. The composition may also comprise other low-molecular-weight polypeptides, proteins such as serum albumin, gelatin, and immunoglobulin, and amino acids such as glycine, glutamine, asparagine, arginine, and lysine. When the composition is prepared as an aqueous solution for injection, it can comprise an isotonic solution comprising, for example, physiological saline, dextrose, and other adjuvants, including, for example, D-sorbitol, D-mannose, D-mannitol, and sodium chloride, which can also contain an

appropriate solubilizing agent, for example, alcohol (for example, ethanol), polyalcohol (for example, propylene glycol and PEG), and non-ionic detergent (polysorbate 80 and HCO-50).

[00113] If necessary, antibodies of the present invention may be encapsulated in microcapsules (microcapsules made of hydroxycellulose, gelatin, polymethylmethacrylate, and the like), and made into components of colloidal drug delivery systems (liposomes, albumin microspheres, microemulsions, nano-particles, and nano-capsules) (for example, see "Remington's Pharmaceutical Science 16th edition", Oslo Ed. (1980)). Moreover, methods for making sustained-release drugs are known, and these can be applied for the antibodies of the present invention (Langer et al., J. Biomed. Mater. Res. 15: 167-277 (1981); Langer, Chem. Tech. 12: 98-105 (1982); U.S. Pat. No. 3,773,919; EP Patent Application No. 58,481; Sidman et al., Biopolymers 22: 547-556 (1983); EP: 133,988).

[00114] A preferred route of administration in both canines and felines is by subcutaneous injection usually into the skin at the base of the neck. In certain embodiments, the anti-NGF protein is packaged in an integrated delivery system such as a pen or prefilled syringe for subcutaneous administration. Ghil et al. describes administration of the adalimumab biosimilar, SB5, via prefilled syringe (PFS) and autoinjector (AI) pen based on injection site pain, patient preference, and safety in rheumatoid arthritis (RA) (See Ghil et al., Usability and safety of SB5 (an adalimumab biosimilar) prefilled syringe and autoinjector in patients with rheumatoid arthritis. Curr Med Res Opin 2019 Mar;35(3):497-502.) Compositions of the invention are similarly administered to canines, felines, and other mammals.

[00115] The term "therapeutically effective amount," in reference to treating a disease state/condition, refers to an amount of a compound either alone or as contained in a pharmaceutical composition that is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease state/condition when administered as a single dose or in multiple doses. Such effect need not be absolute to be beneficial.

[00116] The terms "treat," "treating" and "treatment" as used herein include administering a compound prior to the onset of clinical symptoms of a disease state/condition so as to prevent any symptom, as well as administering a compound after the onset of clinical symptoms of a disease state/condition so as to reduce or eliminate any symptom, aspect or characteristic of the disease state/condition. Such treating need not be absolute to be useful.

[00117] In certain embodiments, the present therapeutic agent may be systemically administered, *e.g.*, orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of active compound in such therapeutically useful compositions is such that an effective dosage level will be obtained.

[00118] The tablets, troches, pills, capsules, and the like may also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring may be added. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the active compound, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and devices.

[00119] The active compound may also be administered intravenously or intraperitoneally by infusion or injection. Solutions of the active compound or its salts may be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[00120] The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient that are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00121] Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

[00122] Useful dosages of the compounds of the present invention can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. In certain embodiments, a useful dose is from about 0.1 mg/kg to about 5 mg/kg or from about 0.5 mg/kg to about 2 mg/kg. Methods for the extrapolation of effective dosages in humans and animals of different sizes are known to the art; for example, see U.S. Pat. No. 4,938,949.

[00123] The amount of the compound, or an active salt or derivative thereof, required for use in treatment will vary not only with the particular salt selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician.

[00124] In general, however, a suitable dose will be in the range of from about 0.5 to about 100 mg/kg, *e.g.*, from about 10 to about 75 mg/kg of body weight per day, such as 3 to about 50 mg per kilogram body weight of the recipient per day, preferably in the range of 6 to 90 mg/kg/day, most preferably in the range of 15 to 60 mg/kg/day.

[00125] The compound is conveniently administered in unit dosage form; for example, containing 5 to 1000 mg, conveniently 10 to 750 mg, most conveniently, 50 to 500 mg of active ingredient per unit dosage form.

[00126] Ideally, the active ingredient should be administered to achieve peak plasma concentrations of the active compound of from about 0.5 to about 75 μM, preferably, about 1 to 50 μM, most preferably, about 2 to about 30 μM. This may be achieved, for example, by the intravenous injection of a 0.05 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1-100 mg of the active ingredient. Desirable blood levels may be maintained by continuous infusion to provide about 0.01-5.0 mg/kg/hr or by intermittent infusions containing about 0.4-15 mg/kg of the active ingredient(s).

[00127] The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations

[00128] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

[00129] The present invention will be further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

Examples

Example 1

[00130] Generation and characterization of rat antibody 2166 that binds to canine NGF.

[00131] Lewis rats were immunized with human NGF (R&D Systems, 256-GF-100/CF) on a weekly basis for eight weeks. The titers were measured in a flow cytometry assay using human NGF-coated beads. Beads were conjugated with human NGF (R&D Systems, 256-GF-100/CF) and incubated with different dilutions of serum (1:100, 1:500, 1:2500) for 30 minutes. Beads were

washed and binding was detected by using a fluorescently labeled anti-rat IgG secondary antibody. Fluorescence was measured using the Intellicyt iQue Screener Plus. Titers were measured at a 1:2500 dilution for all three rats and they were ~100-fold greater than the values of normal Lewis rat serum.

[00132] Lymph nodes (brachial, axillary, inguinal, popliteal and sciatic) and bone marrow from femur, tibia and pelvis were collected from rats with significant NGF titers. Cells from both tissues were isolated and enriched for plasma cells using flow cytometry. Enriched plasma cell suspension was injected into AbCellera's microfluidic screening devices with either 91,000 or 153,000 individual nanoliter-volume reaction chambers. Single cells secreting NGF-specific antibodies were identified and isolated using a bead-based assay. Beads coated with anti-rat IgG antibody were flowed onto microfluidic screening devices and incubated with single antibody-secreting cells. The IgG secreted by plasma cells were captured on beads using the constant region. Binding to secreted IgG immobilized onto beads was subsequently assessed using fluorescently labeled human NGF antigen. Positive hits were identified using machine vision and recovered using automated robotics-based protocols. Approximately 269,000 individual B cells were screened in the NGF binding assay and 592 cells expressed antibodies recognized NGF. From these positive cells, 190 unique antibody sequences were identified. Eighty-eight antibodies were selected from the 190 antibodies based on the diversity of the clonotypes.

[00133] Single cell polymerase chain reaction and custom molecular biology protocols generated NGS sequencing libraries (MiSeq, Illumina) using automated workstations (Bravo, Agilent). Sequencing data were analyzed using a custom bioinformatics pipeline to yield paired heavy and light chain sequences for each recovered antibody-secreting cell (Jones et al., 2020, bioRxiv 2020.09.30.318972. doi: 10.1101/2020.09.30.318972). The amino acid sequences of the heavy and light variable domains of rat antibody 2166 are shown in FIG. 1 and FIG. 2 respectively and the CDRs are indicated. The variable (V(D)J) region of each antibody chain was synthesized and inserted into mammalian expression plasmids using a custom, automated high-throughput cloning pipeline.

[00134] The expression vectors were transfected into Expi293-F cells (Gibco, ThermoFisher Scientific) in 24 deep well plates using the manufacturer's recommended protocol. Four days post-transfection, the conditioned medium was purified with protein A beads and the antibody was

eluted by the addition of 100 mM glycine, pH 2.0 and neutralized to pH 7.0 by the addition of 1 M Tris-HCL, pH 8.0. The neutralized antibodies were buffer exchanged into PBS, pH 7.2.

[00135] The analytics for the purified antibodies included CE-SDS (denaturing capillary sodium dodecyl sulfate gel electrophoresis) and DSF (differential scanning fluorimetry). The CE-SDS was used to determine the purity of the purified antibodies and was completed by using the LabChip GXII Touch instrument (Perkin Elmer). Two microliters of antibody solution at a concentration of 350 μg/mL in PBS was mixed with a non-reducing denaturing buffer solution (Perkin Elmer) and incubated at 70°C for 10 minutes. Separation and detection were performed using the HT Antibody Analysis 200 assay setting on the LabChip instrument (Perkin Elmer). The fluorescence data was analyzed using the LabChip GX Reviewer Software (Perkin Elmer), with percent purity. The percent purity of the rat monoclonal antibody 2166 was 96%.

[00136] The melting point (Tm) of antibodies was assessed by differential scanning fluorimetry (DSF) using the SYPRO™ Orange fluorescence probe (5000X concentrated solution, Thermo Fisher Scientific). 6 μL of mAb solution at 350 μg /mL in PBS was mixed with 6 μL of a 19X concentrated SYPRO™ Orange solution diluted in PBS. Thermal unfolding as assessed by a change in fluorescence was measured on a Bio-Rad C1000 Touch Thermal Cycler instrument (Bio-Rad Laboratories) using a CFX96 Real-Time System reader head (Bio-Rad Laboratories). The wavelengths for excitation and emission were 450-490 nm and 560-580 nm, respectively. The fluorescence signal was measured at a starting temperature of 25°C and increased to 95°C in 0.5°C/min increments. Data was analyzed and melting curves integrated using the Bio-Rad CFX Maestro software (v1.1). The Tm was defined as the local minimum taken from the derivative of the melting curve. The Tm of the rat antibody 2166 was 66.5°C.

[00137] A binding assay was completed to confirm binding of the antibodies to NGF (R&D Systems, 256-GF-100/CF). In addition, the specificity of the antibodies was determined by testing the binding of the antibodies to NT-3 and BDNF which are closely related proteins. Unique antibody sequences were confirmed to bind the screening target using a multiplexed bead assay on a high throughput flow cytometer. Different optically encoded beads were conjugated to either human NGF (R&D Systems, 256-GF-100/CF), NT-3 (R&D Systems, 267-N3-025/CF) or BDNF (R&D Systems, 248-BDB-050/CF). Purified antibodies were incubated with the multiplexed beads at different antibody concentrations for 30 minutes at room temperature. Beads were washed and

binding was detected by using a fluorescently labelled secondary antibody. Fluorescence was measured using high throughput plate-based flow cytometry on an Intellicyt® iQue Screener Plus.

[00138] Median fluorescence intensity of each antibody was normalized over the median fluorescence intensity of the appropriate isotype control for individual bead types. Antibody values greater than 10-fold over isotype were considered as binders.

[00139] Antibody 2166 bound to NGF greater than 59-fold higher than background levels and the binding of this antibody to NT-3 and BDNF was at background levels.

[00140] A functional assay with TF-1 cells was used to determine if the binding of the 2166 antibody to canine NGF blocks the ability of canine NGF to induce signaling with human TrkA which is the high affinity receptor for NGF (Chevalier *et al.*, 1994. Blood, 83:1479). For these studies, canine NGF (Genbank NP_001181879.1) was used for the NGF source. Canine NGF with a strep-tag (Trp-Ser-His-Pro-Gln-Phe-Glu-Lys) at the C-terminus was stably expressed in Dmel-2 cells and purified using StrepTactinXT chromatography followed by a polishing step with Superdex 200 16/600 chromatography. The proliferation of TF-1 cells can be stimulated by different growth factors such as GM-CSF and NGF. TF-1 cells (ATCC-CRL2003) were cultured in RPMI-1640 media containing 10% fetal bovine serum, 100 U/mL Penicillin, 100 µg/ml Streptomycin and 2 ng/mL recombinant human GM-CSF. Cells were maintained between 3 X 10^4 and 5 X 10^5 viable cells/mL and passaged every 48 hours. Each condition was run in triplicate wells. Cells were collected and counted. Cells were resuspended in media without GM-CSF at $1.75x10^5$ cells/ml and incubated in a flask in a humidified 37°C, 5% CO2 incubator for 4 hours. During the incubation, NGF/antibody mixtures were prepared in media as 2x media solutions in full media without GM-CSF and with 10 ng/mL canine NGF. Antibodies were added to the appropriate 2x media solutions and the NGF/antibody solutions were incubated for at least 1 hour at room temperature before being added to the cells. Cells were then collected and resuspended in appropriate media volume to achieve a 0.5 x 10^6 cells/ml suspension in media without GM-CSF. 50 µl of the cell suspension was added per well in a 96-well plate, to which 50 µl of the 2x NGF/antibody media was added per well to the cell plate. Cells were incubated in a humidified 37°C, 5% CO2 incubator for 48 hours, then 20 µl of Aqueous One solution Reagent (Promega) was added per well. Cells were incubated for further 4 hours in a humidified 37°C, 5% CO2 incubator and then absorbance was read at 490 nm on a BioTek Synergy/neO2. Data was analyzed by subtracting the blank well from all measured values. Percent inhibition was calculated using

the following formula: % inhibition = 100 x [1 - (X - MIN)/(MAX - MIN)], where X = signal at a given concentration, MAX = 0% inhibition = Canine NGF only and MIN = 100% inhibition = No NGF control. The average of the triplicates for each condition was calculated. The proliferation data for rat antibody 2166 and the isotype rat antibody control are shown in FIG. 3. The data demonstrates that the rat antibody 2166 effectively blocks NGF from binding to TrkA.

[00141] The VH domain of antibody 2166 was fused with the canine IgGB constant domains (Tang *et al.* 2001. Vet. Immunol. Immunopathol. 80:259) and the VL domain of antibody 2166 was fused to the canine kappa constant domain to generate a canine chimeric antibody (FIG. 4). Two residue changes (AA) were made in the Fc (underlined and in bold font) to eliminate effector activity and these changes are analogous to the "LALA" mutation described for human IgG1 Fc (Tamm & Schmidt, 1997. Int. Rev. Immunol. 16:57). These two constructs were subcloned into pcDNA3.4 (ThermoFisher Scientific) and co-transfected with the Expi293 system (ThermoFisher Scientific) and purified with HiTrap Protein A HP chromatography. The purity of the antibody as measured by SDS/PAGE was >95% and by SEC (size exclusion chromatography) the antibody was 98% monomeric.

[00142] The affinity of the canine 2166 chimeric antibody for canine NGF was measured by SPR (surface plasmon resonance). For these studies, canine NGF (Genbank NP_001181879.1) was generated by fusing the C-terminus with the Flag tag (DYKDDDDK), expressing the canine NGF construct with baculovirus technology and then purifying the NGF with Anti-DYKDDDDK G1 affinity chromatography. The binding kinetics of canine 2166 chimeric antibody to canine NGF was measured with a Biacore T200 instrument. The format of the assay was to capture the Fc of 2166 antibody onto a protein A sensor chip and use canine NGF as the analyte. The running buffer was HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% tween 20) and the instrument temperature was set at 25° C. The flow rate was 40 µl/min and the five analyte concentrations tested in duplicate ranged from 0.78 nM to 12.5 nM. The binding signals were corrected for the blank and the resulting sensorgram (FIG. 5) was used to determine the rate constants (k_a and k_d) and binding affinity (K_D) using a one-to-one binding model with the BIAEVAL software. Under these binding conditions, the binding kinetics for canine 2166 chimeric antibody were k_a (1/Ms)= 1.2E+7, k_d (1/s)= 3.5E-6, and K_D = 3E-13. The measured affinity (K_D) of this antibody exceeds the sensitivity of the Biacore instrument but from these data it is estimated to be at least 50 pM.

[00143] The ability of canine 2166 chimeric antibody to block canine NGF from binding to the canine NGF receptors (TrkA and p75) was measured in an SPR assay on a Biacore T200. The format of the assay was to capture the NGF receptor on a sensor chip and flow over either canine NGF only, NGF mixed with canine 2166 chimeric antibody, or canine 2166 chimeric antibody only.

[00144] The NGF receptors used in the assay consist of the extracellular domains of canine p75 (XP_038340439.1) and canine TrkA (XP_038398906.1) fused to the human IgG1 Fc (UniProtKB P01857) with a 2X Gly-Gly-Gly-Ser linker between the receptor and the Fc. The fusion proteins were expressed in CHO cells and purified with protein A chromatography. For this assay, the proteins p75-Fc and Trk-Fc were captured onto a human anti-Fc sensor chip.

[00145] NGF only, canine 2166 chimeric antibody only and canine 2166 chimeric antibody-NGF mixture (at a 2:1 molar ratio) were the analytes. The seven concentrations of NGF in NGF only condition ranged from 0.78 nM to 50 nM. The concentrations of canine NGF in the canine 2166 chimeric antibody-NGF mixture was 50 nM, 25 nM and 12.5 nM. Lastly, the four concentrations of canine 2166 chimeric antibody alone condition ranged from 12.5 nM to 100 nM. The instrument temperature and flow rate were set at 25°C and 40 µL/min, respectively.

[00146] The binding signals were corrected for the reference, and the resulting sensorgrams were used to determine the rate constants (k_a and k_d) and binding affinity (K_D) using a one-to-one binding model with the BIAEVAL software. The sensorgrams (FIGS. 5, 6, 7, 8) represent the NGF only condition and the canine 2166 chimeric antibody-NGF mixture for both the p75-Fc and TrkA-Fc receptors. Under these binding conditions, the binding kinetics for canine NGF only to canine p75 in a dimer format were k_a (1/Ms)= 1.2E+10, k_d (1/s)= 45, and K_D = 3.7E-9 (FIG. 6). The binding kinetics for canine NGF only to canine TrkA in a dimer format were k_a (1/Ms)= 1.3E+7, k_d (1/s)= 1.7E-4, and K_D = 1.3E-11 (FIG. 7). No binding to the canine NGF receptors was observed with the canine 2166 chimeric antibody only condition (data not shown). As evidenced by the sensorgrams (FIGS. 8 and 9), canine 2166 chimeric antibody effectively blocks canine NGF binding to canine TrkA and p75.

Example 2

[00147] Caninization of rat 2166 antibody

[00148] A canine antibody database was generated by performing NGS (next generation sequencing) on canine PBMCs (peripheral blood mononuclear cells). This database contains the sequences from 5.0 x 10^6 VH domains, 3.7 x 10^6 VK domains and 2.6 x 10^6 VL domains. The HCDR 1, 2 and LCDR 1, 2, 3 sequences from the 2166 parental antibody were used in an algorithm to identify the closest canine CDR sequences and their linked framework sequences in the canine antibody database. These linked framework sequences were included in the scFv phage display library along with the closest framework germline sequences and the linked framework sequences with 1 to 3 residues reverted back to the closest germline. A proprietary algorithm was used to identify a set of CDR sequences that are similar to the original 2166 CDRs and closer in identity to the germline and expressed CDR sequences. These CDRs and framework sequences were used to generate a scFv antibody phage display library with a theoretical complexity of 3 x 10^{12}. Antibody phage selections were completed with canine NGF for four rounds and with each round the stringency was increased by reducing the antigen concentration and increasing the number of washes. Specifically, 96-multi well plates were coated with 200 pmol of NGF for the first round, 100 pmol for the second round and 50 pmol for the third and fourth rounds. The number of washes with PBS- tween 20 (0.01%) after the selection were six for the first round, seven for the second round, eight for the third round and nine for the fourth round. The output scFv clones from the third and fourth rounds were sequenced and unique clones were reformatted into IgGs and screened for binding to canine NGF by SPR. The sequences and the binding kinetics to canine NGF of the top 69 caninized clones along with parental clone 2166 are shown in FIGS. 1 and 2 and Table 2.

Table 2 - Binding to canine NGF

Clone	ka	kd	KD
2166	2.38E+06	1.00E-05	4.20E-12
SC-42_006	2.22E+06	1.75E-04	7.88E-11
SC-42_007	1.32E+06	1.38E-04	1.05E-10
SC-42_008	1.96E+06	9.40E-05	4.79E-11
SC-42_010	2.06E+06	8.55E-05	4.16E-11
SC-42_011	1.27E+06	7.28E-05	5.75E-11
SC-42_023	4.19E+06	4.66E-04	1.11E-10
SC-42_024	3.63E+06	5.22E-04	1.44E-10
SC-42_025	3.90E+06	5.88E-04	1.51E-10
SC-42_026	4.26E+06	9.20E-04	2.16E-10
SC-42_027	3.43E+06	6.51E-04	1.90E-10

SC-42_028	3.19E+06	4.96E-04	1.56E-10
SC-42_029	4.09E+06	5.44E-04	1.33E-10
SC-42_030	4.10E+06	6.34E-04	1.55E-10
SC-42_031	4.23E+06	6.46E-04	1.53E-10
SC-42_032	4.61E+06	2.64E-04	5.73E-11
SC-42_033	3.73E+06	5.81E-04	1.56E-10
SC-42_034	2.81E+06	1.51E-03	5.39E-10
SC-42_035	3.02E+06	6.24E-04	2.06E-10
SC-42_036	3.40E+06	8.77E-04	2.58E-10
SC-42_037	3.25E+06	4.82E-04	1.49E-10
SC-42_038	3.21E+06	5.30E-04	1.65E-10
SC-42_040	4.82E+06	7.09E-04	1.47E-10
SC-42_041	3.42E+06	5.55E-04	1.62E-10
SC-42_042	3.51E+06	9.76E-04	2.78E-10
SC-42_043	2.79E+06	7.13E-04	2.55E-10
SC-42_044	3.12E+06	4.53E-04	1.45E-10
SC-42_045	3.34E+06	4.12E-04	1.23E-10
SC-42_046	3.45E+06	4.89E-04	1.42E-10
SC-42_047	3.55E+06	4.41E-04	1.24E-10
SC-42_048	4.25E+06	4.89E-04	1.15E-10
SC-42_049	3.80E+06	5.49E-04	1.44E-10
SC-42_050	3.49E+06	5.02E-04	1.44E-10
SC-42_051	3.27E+06	6.09E-04	1.86E-10
SC-42_052	3.35E+06	4.13E-04	1.23E-10
SC-42_053	3.41E+06	4.71E-04	1.38E-10
SC-42_054	3.32E+06	5.91E-04	1.78E-10
SC-42_055	4.23E+06	7.73E-04	1.83E-10
SC-42_057	3.73E+06	5.53E-04	1.48E-10
SC-42_058	3.31E+06	5.88E-04	1.78E-10
SC-42_059	3.20E+06	6.38E-04	2.00E-10
SC-42_060	3.87E+06	5.85E-04	1.51E-10
SC-42_061	3.16E+06	4.94E-04	1.56E-10
SC-42_062	3.27E+06	6.36E-04	1.95E-10
SC-42_063	3.57E+06	8.52E-04	2.39E-10
SC-42_064	2.43E+06	6.51E-04	2.68E-10
SC-42_065	3.47E+06	5.95E-04	1.71E-10
SC-42_066	3.16E+06	5.21E-04	1.65E-10
SC-42_067	3.57E+06	7.02E-04	1.97E-10
SC-42_068	3.59E+06	4.87E-04	1.36E-10

SC-42_069	2.39E+06	4.67E-04	1.96E-10
SC-42_070	4.70E+06	5.43E-04	1.15E-10
SC-42_071	1.45E+06	6.89E-04	4.74E-10
SC-42_072	3.98E+06	7.86E-04	1.98E-10
SC-42_073	5.83E+06	5.46E-04	9.37E-11
SC-42_075	4.54E+06	1.55E-03	3.42E-10
SC-42_077	3.35E+06	2.91E-04	8.71E-11
SC-42_079	3.53E+06	8.49E-04	2.40E-10
SC-42_080	3.31E+06	4.46E-04	1.35E-10
SC-42_081	3.41E+06	2.17E-03	6.35E-10
SC-42_082	3.45E+06	2.52E-04	7.29E-11
SC-42_083	4.17E+06	6.23E-04	1.49E-10
SC-42_084	3.60E+06	4.73E-04	1.32E-10
SC-42_085	4.28E+06	8.34E-04	1.95E-10
SC-42_088	3.39E+06	1.06E-03	3.11E-10
SC-42_089	4.26E+06	7.74E-04	1.82E-10
SC-42_090	1.91E+06	1.52E-04	7.99E-11
SC-42_091	1.00E+04	1.00E-04	1.00E-08
SC-42_101	3.41E+06	3.80E-04	1.11E-10
SC-42_102	2.91E+06	1.07E-03	3.67E-10

[00149] Sensorgrams for all 69 clones are in FIG. 10. The SPR was completed by amine coupling the antibody (~5 μg/ml) to the HC30M sensor chip by EDC/NHS activation, followed by ethanolamine HCL quenching. The canine NGF (Genbank NP_001181879.1) used as the analyte for the SPR analyses was the same preparation described in Example 1 for the canine NGF tagged at the C-terminus with the Flag tag (DYKDDDDK).

[00150] The ability of caninized SC42_101 antibody to block canine NGF from binding to the canine NGF receptors (TrkA and p75) was measured in an SPR assay on a Biacore T200. The format of the assay was to capture the NGF receptor on a sensor chip and flow over either canine NGF only, NGF mixed with caninized SC42_101 antibody, or caninized SC42_101 antibody only. The receptor blocking methods are identical to those described for canine 2166 chimeric antibody in Example 1. The sensorgrams (FIGS. 11, 12, 13, 14) represent the NGF only condition and the caninized SC42_101 antibody-NGF mixture for both the p75-Fc and TrkA-Fc receptors. Under these binding conditions, the binding kinetics for canine NGF only to canine p75 in a dimer format were k_a (1/Ms)= 3.8E+7, k_d (1/s)= 0.1, and K_D = 2.7E-9 (FIG. 11). The binding kinetics for canine

NGF only to canine TrkA in a dimer format were k_a (1/Ms)= 2.4E+7, k_d (1/s)= 1.6E-4, and K_D = 6.6E-12 (FIG. 12). No binding to the canine NGF receptors was observed with the caninized SC42_101 antibody only condition (data not shown). As evidenced by the sensorgrams (FIGS. 13 and 14), caninized SC42_101 antibody effectively blocks canine NGF binding to canine TrkA and p75.

Example 3

[00151] Felinization of rat 2166 antibody

[00152] A feline antibody database was generated by performing NGS (next generation sequencing) on feline PBMCs (peripheral blood mononuclear cells). This database contains the sequences from 7.5 x 10^6 VH domains, 1.3 x 10^6 VK domains and 3.8 x 10^6 VL domains. The HCDR 1, 2 and LCDR 1, 2, 3 sequences from the 2166 parental antibody were used in an algorithm to identify the closest feline CDR sequences and their linked framework sequences in the feline antibody database. These linked framework sequences were included in the scFv phage display library along with the closest framework germline sequences and the linked framework sequences with 1 to 3 residues reverted back to the closest germline. A proprietary algorithm was used to identify a set of CDR sequences that are similar to the original 2166 CDRs and closer in identity to the germline and expressed CDR sequences. These CDRs and framework sequences were used to generate a scFv antibody phage display library with a theoretical complexity of 3 x 10^{12}. The processed form of feline NGF (XP_004001166.1) is identical to the processed form of canine NGF (NP_001181879.1) so for the felininization studies, canine NGF tagged at the C-terminus with the Flag tag (DYKDDDDK) described in Example 1 was used.

[00153] Antibody phage selections were completed with NGF for four rounds and with each round the stringency was increased by reducing the antigen concentration and increasing the number of washes. Specifically, 96-multi well plates were coated with 200 pmol of NGF for the first round, 100 pmol for the second round and 50 pmol for the third and fourth rounds. The number of washes with PBS-tween 20 (0.01%) after the selection were six for the first round, seven for the second round, eight for the third round and nine for the fourth round. The output scFv clones from the third and fourth rounds were sequenced and unique clones were reformatted into IgGs and screened for binding to NGF by SPR. The variable domains of clone 101 are shown in FIG. 15 with the CDR regions underlined. The affinity of felinized clone 101 for NGF was determined by

SPR. The format of this assay was to immobilize goat anti-cat IgG (30 μg/ml) on a series S CM5 biosensor using EDC/NHS and quenching the remaining sites with ethanolamine. Captured felinized clone 101 (1 μg/ml) onto the goat anti-cat IgG sensor chip. Using single cycle kinetics, five concentrations of NGF were captured. The binding kinetics for felinized clone 101 for NGF were k_a (1/Ms) = 3.8E+5, k_d (1/s) = 3E-3, and K_D = 7.8E-9.

Example 4

[00154] Affinity maturation of felinized clone 101 using site-specific mutagenesis of the CDRs.

[00155] In the first affinity maturation approach, the heavy variable domain and CH1 domain of feline clone 101 (Table 3) were subcloned in the GenScript FASEBA plasmid. The construct included at the C-terminus of the heavy chain (VH-CH1) a single-domain antibody against serum albumin (SASA) tag (see, e.g. US 2013/0129727A1) which has low pM affinity for albumin, and further downstream a His-tag for purification. The light chain variable domain was subcloned with feline Cκ (Table 3) into a proprietary *E. coli* expression vector. Both the heavy chain and light chain had the PelB (pectate lyase B) signal peptide at the N-terminus to facilitate secretion of the Fab when expressed in TG1 *E. coli*. The expression of the variable domains was regulated by the Lac promoter.

Table 3 - Sequences

clone 101 VH-CH1 (IgG1)	DVQLVESGGD LVKPGGSLRL TCVASGLSLT SSSMSWVRQA PGKGLQWVST IYSNGGTYYT DSVKGRFTIS KDNAENTLYL QMNNLKTEDT ATYYCASIYY YDADYLHWYF DFWGQGALVT VSSASTTAPS VFPLAPSCGT TSGATVALAC LVLGYFPEPV TVSWNSGALT SGVHTFPAVL QASGLYSLSS MVTVPSSRWL SDTFTCNVAH PPSNTKVDKT V
clone 1 VL-Cκ1	EIQMTQSPTS LSASVGDRVT ITCRASEGIS NNLSWYQQTP GKAPKLLIYA TSNLHSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ GYKWPLTFGG GTKLEITRSD AQPSVFLFQP SLDELHTGSA SIVCILNDFY PKEVNVKWKV DGVVQNKGIQ ESTTEQNSKD STYSLSSTLT MSSTEYQSHE KFSCEVTHKS LASTLVKSFN RSECQRE

[00156] A variant library was generated for each CDR position in the heavy and light chains using the GenScript proprietary Precision Mutant Library (PML) which utilizes semiconductor-based oligonucleotide synthesis technology. In generating the mutants, the CDRs were defined using a combination of Kabat and IMGT methodology and the residues selected for each CDR are shown below in Table 4. The residue numbers for the CDRs are shown in parentheses.

Table 4 - CDR sequences and numbering			
	VH		VL
CDR1	GLSLTSSSMS (26-35)	CDR1	RASEGISNNLS (24-34)
CDR2	TIYSNGGTYYTDSVKG (50-65)	CDR2	ATSNLHS (50-56)
CDR3	ASIYYYDADYLHWYFDF (96-112)	CDR3	QQGYKWPLT (89-97)

[00157] The quality of the libraries was verified using NGS (Next Generation Sequencing). Forty-four PML clones were selected from each library for expression in *E. coli* in 96 deep-well plates by inoculating into 2YT medium and inducing with 0.2 mM IPTG overnight at room temperature. The Fab secreted in the medium was analyzed for binding activity by completing an ELISA. In this ELISA, plates were coated with 10 μg/ml of BSA overnight at 4°C, washed 3X with 0.1% tween 20 in PBS, pH 7.4 (PBST), blocked non-specific interactions with 3% non-fat dry milk in PBS (phosphate-buffered saline, pH 7.4) at 37°C for 1 hour, washed 3X with PBST, added crude Fab supernatant (diluted 1:1 with PBST) incubated at 37°C for 1 hour, washed 3X with PBST, added 0.15 μg/ml of NGF incubated at 37°C for 1 hour, washed 3X with PBST, added horseradish peroxidase (HRP) conjugated anti-Flag tag antibody (Flag tag present on NGF) incubated at room temperature for 45 minutes, washed 3X with PBST and detected the HRP conjugate by incubating with TMB substrate for 10 minutes at room temperature and measured absorbance at 450 nm. The top 100 clones with an apparent increase in affinity as measured by ELISA were sequenced to detect the variant in the CDR and 57 unique clones were identified. Mutations from clone 101 for each of the 57 clones are tallied in Table 5.

Table 5 - Individual mutations at each position compared to clone 101																
	CDR1H				CDR2H							CDR3H				
	28	30	31	35	52	53	55	58	60	62	64	97	99	101	104	112
101	S	T	S	S	Y	S	G	Y	T	S	K	S	Y	Y	D	F
	H	N	H	V	W	P	R	D	H	D	E	Q	F	H	K	E
		R					Y		D		D	M			E	P
									S			N			N	H
									E			K			Q	N
												H				
												T				
	CDR1L				CDR2L											

	24	30	31	34	53	54	55	56
101	R	S	N	S	N	L	H	S
	F	A	Q	G	I	H	M	T
		Q		A	V		I	D
		V			K		L	E
		L			H			N
		P			M			
		Y			L			

[00158] Binding of the 57 unique clones were confirmed by an off-rate screening assay in an SPR assay performed on a Biacore T200. For the SPR analyses, bovine serum albumin (BSA) was immobilized to CM5 sensor chip. The sensor chip surface was activated with 50 mmol/L H-Hydroxysuccinimide and 200 mmol/L 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride for 420 s. Afterwards, BSA diluted in 10 mM sodium acetate, pH 4.5 was injected. After the amine coupling reaction, the remaining active coupling sites on the chip surface were blocked with 1 mM ethanolamine hydrochloride. The selected Fab-SASA variants in conditioned medium were captured on the BSA-coated chips. The running buffer was HBS-EP (10 mM HEPES 500 mM NaCl, 3 mM EDTA, 0.05% Tween 20, pH 7.4). After equilibration, antigen was injected for 120 seconds (association phase) followed by the injection of running buffer for 420 sec (dissociation phase). The off-rates of the Fab-SASA clones were obtained from fitting the experimental data locally to a 1:1 interaction model using the Biacore T200 evaluation software. The Fab variants were ranked by their dissociation rate constants (off-rates, kd) shown in Table 6.

Table 6 - Rank ordering of the Fab variants based on dissociation constants

Sequence Analysis	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)	Chi² (RU^2)	Ratio WT-kd / clone-kd
G55R	1.20E+05	1.47E-04	1.23E-09	19.7	0.00634	6.41
S30A	2.77E+05	2.92E-04	1.05E-09	152.2	0.618	3.23
H55M	3.33E+05	3.12E-04	9.35E-10	137.7	0.712	3.02
S30Q	2.59E+05	3.24E-04	1.25E-09	132.5	0.462	2.91
D104K	6.09E+04	3.32E-04	5.45E-09	64.6	0.0316	2.84
S35V	3.09E+05	3.60E-04	1.17E-09	106.1	0.414	2.62

N53I	2.76E+05	3.89E-04	1.41E-09	118	0.347	2.42
F112E	2.84E+05	3.99E-04	1.41E-09	147.2	0.488	2.36
S97Q	2.97E+05	4.03E-04	1.36E-09	79.5	0.287	2.34
N53V	2.88E+05	4.07E-04	1.42E-09	108.7	0.375	2.32
H55L	2.64E+05	4.18E-04	1.58E-09	91.8	0.494	2.26
G55Y	2.29E+05	4.19E-04	1.83E-09	17.7	0.0131	2.25
D104E	2.37E+05	4.26E-04	1.80E-09	66.4	0.126	2.21
H55I	3.34E+05	4.36E-04	1.31E-09	100.7	0.67	2.16
D104N	2.39E+05	4.46E-04	1.87E-09	52.8	0.0774	2.11
S97M	2.66E+05	4.66E-04	1.75E-09	64.3	0.149	2.02
Y99F	3.21E+05	4.69E-04	1.46E-09	100.8	0.439	2.01
K64E	1.96E+05	4.76E-04	2.42E-09	50.2	0.164	1.98
D104Q	2.20E+05	4.78E-04	2.17E-09	49.6	0.056	1.97
N31Q	3.27E+05	4.80E-04	1.47E-09	80.2	0.343	1.96
S97N	3.73E+05	4.81E-04	1.29E-09	69.6	0.317	1.96
S30V	2.87E+05	4.83E-04	1.68E-09	59.3	0.22	1.95
Y101H	3.07E+05	4.96E-04	1.62E-09	61.8	0.194	1.90
S30L	2.70E+05	5.07E-04	1.87E-09	92.6	0.914	1.86
S97K	3.31E+05	5.11E-04	1.54E-09	62.3	0.226	1.85
S34G	4.56E+05	5.14E-04	1.13E-09	65.8	0.571	1.83
S97H	2.97E+05	5.33E-04	1.79E-09	72	0.198	1.77
F112P	3.98E+05	5.52E-04	1.39E-09	48	0.164	1.71
K64D	3.40E+05	5.54E-04	1.63E-09	48.1	0.189	1.70
S62D	3.01E+05	5.60E-04	1.86E-09	103.3	0.405	1.68
L54H	3.40E+05	5.60E-04	1.65E-09	75.6	0.357	1.68
T60H	3.12E+05	5.61E-04	1.80E-09	77.5	0.316	1.68
Y58D	3.81E+05	5.62E-04	1.47E-09	108.4	0.571	1.68
T60D	2.94E+05	5.67E-04	1.93E-09	90.6	0.299	1.66
T60S	3.25E+05	5.73E-04	1.76E-09	80.6	0.33	1.65
N53K	2.92E+05	5.96E-04	2.04E-09	60.5	0.172	1.58
S31H	3.10E+05	6.12E-04	1.98E-09	66.6	0.291	1.54

N53H	3.22E+05	6.27E-04	1.95E-09	69.4	0.177	1.50
F112H	3.28E+05	6.39E-04	1.95E-09	81.8	0.407	1.48
F112N	3.47E+05	6.43E-04	1.85E-09	72	0.316	1.47
N53M	3.46E+05	6.48E-04	1.87E-09	53.7	0.312	1.46
Y52W	4.34E+05	6.51E-04	1.50E-09	41.3	0.258	1.45
R24F	2.87E+05	6.55E-04	2.28E-09	89.3	0.273	1.44
N53L	3.21E+05	6.67E-04	2.08E-09	60	0.188	1.41
S30P	2.62E+05	6.70E-04	2.56E-09	75.4	0.211	1.41
T60E	2.87E+05	6.83E-04	2.38E-09	77.7	0.289	1.38
S53P	3.23E+05	6.95E-04	2.15E-09	56.6	0.183	1.36
T30N	3.33E+05	7.04E-04	2.12E-09	71.2	0.287	1.34
F112E	2.94E+05	7.08E-04	2.41E-09	64.9	0.221	1.33
S56T	3.58E+05	7.14E-04	1.99E-09	65.5	0.414	1.32
S97T	3.80E+05	7.16E-04	1.88E-09	51.1	0.207	1.32
T30R	3.57E+05	7.30E-04	2.05E-09	61.2	0.261	1.29
S56D	3.72E+05	7.55E-04	2.03E-09	49.1	0.224	1.25
S30Y	3.55E+05	7.82E-04	2.20E-09	60.5	0.4	1.21
S34A	4.50E+05	7.83E-04	1.74E-09	50.4	0.361	1.20
S28H	3.15E+05	8.02E-04	2.55E-09	79.8	0.244	1.18
S56E	3.70E+05	8.09E-04	2.19E-09	54.6	0.249	1.17
S56N	3.41E+05	9.03E-04	2.64E-09	50.6	0.178	1.04
Wild-Type	3.59E+05	9.39E-04	2.62E-09	59.1	0.241	1.00
Wild-Type	3.55E+05	9.43E-04	2.655E-09			1.00
Wild-Type	3.51E+05	9.47E-04	2.69E-09	60.1	0.235	1.00

[00159] Fab variants G55R, S30A, S30Q, S35V, N53I, F112E, S97Q, and N53V were selected for combinatorial library construction (Table 7).

Table 7 - Selected Fab variants for combinatorial library						
	VH				VL	
Position	35	55	97	112	30	53

Wild-Type	S	G	S	F	S	N
Variant	V	R	Q	E	A	I
					Q	V

[00160] The combinatorial library was constructed in the same Fab-SASA vector described above. The theoretical diversity of the combinatorial library is 2 x 2 x 2 x 2 x 3 x 3 = 144 and the size of the constructed library was 5.6 x 10^7 CFU (colony forming units). The library in-frame rate and diversity were evaluated by DNA sequencing and the results are shown in the tables below.

Table 8 - Percentage of in-frame Fab-SASA construct in combinatorial library				
	Clones for sequencing	Sequences with stop codons	In-frame rate	Unique clones
VH	47	3	44/47 = 93.6%	12
VL	47	2	45/47 = 95.7%	9

Table 9 - Diversity of heavy chain variants in combinatorial library					
Chain	Number of clones	S35V	G55R	S97Q	F112E
VH	44	S(30), V(14)	G(10), R(34)	S(36), Q(8)	F(33), E(11)

Table 10 - Diversity of light chain variants in combinatorial library			
Chain	Number of clones	S30A/Q	N53I/V
VL	43	S(16), A(21), Q(8)	N(13), I(17), V(15)

[00161] From the combinatorial library, 184 clones were randomly selected and the binding by the NGF ELISA was completed. The NGF ELISA was the same method as described above. The top 20 clones in ELISA binding were sequenced and tested in the SPR off-rate assay. Binding results are shown by amino acid combination in Table 11. Table 12 indicates variable domain sequence IDs for the top 20 clones. The pairings of VH and VL indicate substantial compatibility of the VH and VL mutations and interchangeability of the VH and VL domains.

Table 11 - Affinity matured Fab variants from the combinatorial library												
Antibody ID	**VH**				**VL**		**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**Ratio (kd)**	**Ratio (KD)**	**Rmax (RU)**
	35	**55**	**97**	**112**	**30**	**53**						

	S	G	S	F	S	N				WT / clones	WT / clones	
AHF17591	V	R	S	E	A	I	2.68E+05	1.00E-06	3.73E-12	1685.00	925.94	19.3
AHF17598	V	R	S	E	A	I	2.94E+05	5.25E-05	1.79E-10	32.10	19.30	25.9
AHF17592	V	R	S	F	S	V	1.92E+05	1.46E-05	7.61E-11	115.41	45.40	28.5
AHF17600	V	R	S	F	S	V	1.77E+05	5.81E-05	3.29E-10	29.00	10.50	26.7
AHF17593	V	R	Q	F	A	V	1.63E+05	1.00E-06	6.13E-12	1685.00	563.17	24.4
AHF17594	V	G	S	F	A	V	3.53E+05	3.28E-04	9.31E-10	5.14	3.71	86.1
AHF17595	V	R	S	E	Q	N	1.78E+05	1.00E-06	5.62E-12	1685.00	614.99	41.6
AHF17596	V	R	S	F	A	V	2.27E+05	1.82E-05	8.02E-11	92.58	43.08	31.5
AHF17608	V	R	S	F	A	V	2.28E+05	1.00E-06	4.39E-12	1685.00	787.74	28.8
AHF17599	V	R	S	F	A	V	2.12E+05	1.00E-06	4.72E-12	1685.00	732.46	34.9
AHF17597	V	R	S	F	Q	I	1.99E+05	1.08E-05	5.44E-11	156.02	63.51	37.6
AHF17601	V	R	Q	E	A	N	2.91E+05	1.00E-06	3.44E-12	1685.00	1005.41	42.6
AHF17602	V	R	S	F	A	N	2.10E+05	1.00E-06	4.76E-12	1685.00	725.55	27.9
AHF17606	V	R	S	F	A	N	1.87E+05	2.01E-05	1.07E-10	83.83	32.29	46.3
AHF17610	V	R	S	F	A	N	1.82E+05	1.00E-06	5.49E-12	1685.00	628.81	35
AHF17610	V	R	S	F	A	N	1.82E+05	1.00E-06	5.49E-12	1685.00	628.81	35
AHF17603	V	G	S	E	A	I	3.68E105	2.45E-04	6.64E-10	6.88	5.20	108.7
AHF17604	V	R	Q	F	Q	I	1.45E+05	4.66E-05	3.21E-10	36.16	10.76	27.9
AHF17605	V	G	Q	F	A	I	2.53E+05	2.07E-04	8.20E-10	8.14	4.21	94.2
AHF17607	V	R	S	E	S	I	2.26E+05	1.00E-06	4.42E-12	1685.00	780.83	36.9
AHF17609	V	R	S	F	A	I	2.28E+05	1.00E-06	4.39E-12	1685.00	787.74	35.9
Blank							1.92E+04	NA	NA	NA	NA	NA
Blank							1.45E+04	NA	NA	NA	NA	NA
Wild-type							5.69E+05	1.79E-03	3.15E-09	0.94	0.00	28.7
Wild-type							4.19E+05	1.58E-03	3.78E-09	1.07	0.00	30.2
Wild-type							4.94E+05	1.69E-03	3.47E-09	1.00	0.00	29.45

Table 12 - VH and VL variable domain sequences of affinity matured Fab variants

Antibody ID	VH					VL		
	SEQ ID NO:	35	55	97	112	SEQ ID NO:	30	53

		S	G	S	F		S	N
AHF17591	184	V	R	S	E	198	A	I
AHF17598	184	V	R	S	E	198	A	I
AHF17592	188	V	R	S	F	192	S	V
AHF17600	188	V	R	S	F	192	S	V
AHF17593	185	V	R	Q	F	193	A	V
AHF17594	186	V	G	S	F	193	A	V
AHF17595	184	V	R	S	E	194	Q	N
AHF17596	188	V	R	S	F	193	A	V
AHF17608	188	V	R	S	F	193	A	V
AHF17599	188	V	R	S	F	193	A	V
AHF17597	188	V	R	S	F	195	Q	I
AHF17601	187	V	R	Q	E	196	A	N
AHF17602	188	V	R	S	F	196	A	N
AHF17606	188	V	R	S	F	196	A	N
AHF17610	188	V	R	S	F	196	A	N
AHF17610	188	V	R	S	F	196	A	N
AHF17603	189	V	G	S	E	198	A	I
AHF17604	187	V	R	Q	F	195	Q	I
AHF17605	190	V	G	Q	F	198	A	I
AHF17607	184	V	R	S	E	197	S	I
AHF17609	188	V	R	S	F	198	A	I

Example 5

[00162] Affinity maturation of felinized clone 101 by scFv phage display

[00163] In the second affinity maturation approach, a scFv phage display library was constructed containing the frameworks of felinized clone 101 and the following sequences for the heavy and light CDR sequences shown in Table 13.

Table 13 - CDRs of felinized clone 101 and variants in scFv phage display combinatorial library

HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
GLSLTSSS ------H-	IYSNGGT -W-----	ASIYYYDADYLHWYFDF -N----------------	EGISNN Q-----	ATS --E	QQGYKWPLT ---W-----

-----TN-	---Q---	-E----------	N-----	-S-	---H-----
--E---N-	--A----	-Q----------	K-----	--N	---F-----
A-------	---E---	-D----------	-A----	--D	----R----
L-------	---R---	-K----------	--L---	--Q	----E----
N-------	---S---	--L---------	--V---	--K	----S----
--E-----	LW-----	---T--------	---G--	-A-	----Q----
--G-----	-W-S---	---W--------	---D--	-Q-	-----F---
--A-----	-W-E---	---F--------	----S-	-D-	-----I---
--D-----	-W-D---	----H-------	----K-	-Y-	------E--
--T-----		----F-------	----Y-	-V-	-------M-
---M----		------E------	-----S	-L-	---S-----
---V----		-------S------	-----G		---T-----
----A---		-------V------	-----Q		---H-----
----S---		--------E-----	-----E		---G-----
----V---		---------H----	-----K		---L-----
----N---		-A----------	-----D		---V-----
----M---		-Y----------	-----T		---R-----
-----TH-		-T----------	-----L		---D-----
-----H--		-V----------	-----A		---K-----
-----G--		-L----------	-----H		--Y-ST-W-
-----E--		-P----------	-----F		
-----R--		-H----------	-----R		
-----K--		-R----------			
------I-		-I----------			
------T-		-G----------			
------D-		--Y---------			
------N-		S----------			
------Q-		T----------			
------A-		D----------			
Q-------		N----------			
------Y-		E----------			
-------A		Q----------			
-----A--		K----------			
--Y-----		--Y---------M			
--M-----		--W---------			
		-D-F--------			
		-N-W--------			
		-Q--F-------			
		------E-E--------			
		-------SY--------			
		--------Y--------			
		----------Y-----			
		----------F----			
		---------F-------			
		----------L---			
		----------W---			
		----------I---			

		----------D--- ----------Q- ----------YF---- ----------Y ----------L ----------M ----------I ----------W			

[00164] The library diversity for the heavy chain was 37 (HCDR1) x 11 (HCDR2) x 57 (HCDR3) = 23,199 and for the light chain was 24 (LCDR1) x 13 (LCDR2) x 22 (LCDR3) = 6, 864. The library containing the combined heavy and light chains has a diversity of 1.59×10^8. Antibody phage selections were completed with NGF for five rounds and with each round the stringency was increased by reducing the antigen concentration and increasing the number of washes. Specifically, 96-multi-well plates were coated with 200 pmol of NGF for the first round, 50 pmol of NGF for the second and third rounds, 25 pmol for the fourth round and 10 pmol for the fifth round. The number of washes with PBS, pH 7.4-Tween 20 (0.01%) after the selection step was three after the first round, four after the second round, five after the third round, six after the fourth round and seven after the fifth round. Isolated 760 clonal phage from each of the outputs of the third, fourth and fifth rounds that were screened in an NGF-binding ELISA. The positive clones were sequenced and 140 unique positive clones were reformatted into feline IgG1a, expressed in CHO cells and purified with protein A. The SPR was completed by amine coupling the antibody (~5 µg/ml) to the HC30M sensor chip by EDC/NHS activation, followed by ethanolamine HCL quenching. NGF was the analyte diluted in HEPES-buffered saline with 0.01% tween 20 and 0.5 mg/ml BSA. The NGF was run at concentrations of 500 nM, 166 nM, 55 nM, 18 nM, 6.2 nM, 2.0 nM, 0.68 nM, and 0.23 nM. The affinities of the top three affinity-matured clones are shown below in Table 14. The sequences of variable domains of the top three clones (SC-184_76; SC-184_102; SC-184_110) are shown in FIG. 17

Table 14 -SPR data of top three affinity-matured feline antibodies

Clone	ka (1/Ms)	kd (1/s)	KD (M)
101	1.10E+05	2.20E-04	2.10E-09
SC-184_76	2.70E+05	6.90E-05	2.50E-10
SC-184_102	2.10E+05	6.30E-05	6.30E-10

SC-184_110	1.60E+05	3.60E-05	3.60E-10

Example 6

[00165] SPR and NGF receptor blocking data of affinity-matured feline antibodies directed against feline NGF

[00166] Affinity-matured antibodies AHF17602, SC-184_76, SC-184_102, and SC-184_110 along with the latter three clones containing the G55R mutation (SC-184_76-Arg, SC-184_102-Arg, and SC-184_110-Arg) described in the first affinity maturation approach were evaluated for their affinity to NGF using SPR with a Biacore T200 instrument. The variable domain sequences of AHF17602, SC-184_76-Arg, SC-184_102-Arg, and SC-184_110-Arg are shown in FIG. 17. In addition, clone 101 was also evaluated. Antibodies were captured using an anti-feline coupled CM5 chip. NGF binding was then assessed at multiple concentrations starting at 50 nM using PBSP+ running buffer (Cytiva) with a flow rate of 30 µL/min. The length of the association time was 120s and the dissociation time was run for 600s. The chip surface was regenerated with 10 mM glycine. Reference-subtracted sensorgrams were fitted to a 1:1 binding model using Biacore T100 Evaluation software. The data is shown in Table 15 below and the sensorgrams in FIG. 18.

Table 15 - Affinity of feline NGF antibodies

Clone	ka (1/Ms)	kd (1/s)	KD	Rmax (RU)
101	7.84E+05	1.38E-03	1.75E-09	26.3
AHF17602	3.94E+05	6.32E-05	1.61E-10	61.4
SC-184_76	8.38E+05	3.96E-04	4.72E-10	61.9
SC-184_76-Arg	5.85E+05	9.01E-05	1.54E-10	73.4
SC-184_102	4.71E+05	1.98E-04	4.20E-10	82.1
SC-184_102-Arg	3.43E+05	4.43E-05	1.29E-10	87.8
SC-184_110	4.92E+05	3.80E-04	7.73E-10	43.2
SC-184_110-Arg	3.87E+05	5.48E-05	1.42E-10	53.2

[00167] For the NGF receptor blocking experiments the feline TrkA and p75 NGF receptors were generated and used in an SPR experiment with a Biacore T200. The extracellular domain of

feline TrkA (XP_023103311) was cloned with an AviTag (GLNDIFEAQKIEWHE) and 8X His tag at the C-terminus and expressed in HEK293 cells. The recombinant feline TrkA protein was purified from the conditioned medium using nickel chromatography. The extracellular domain of feline p75 (XP_023099534) was cloned with an AviTag (GLNDIFEAQKIEWHE) and 8X His tag at the C-terminus and expressed in HEK293 cells. The recombinant feline p75 protein was purified from the conditioned medium using nickel chromatography. Both receptors were biotinylated at the AviTag site using the BirA biotin protein ligase reaction kit (Avidity). Biotinylated receptors were captured on a Series S CAP chip and Biotin CAPture reagent (Cytiva). Antibodies were titrated in running buffer (1 X PBSP+, Cytiva) and pre-incubated with 10 nM NGF (TrkA assay) or 50 nM NGF (p75 assay) at the indicated ratios. Binding was assessed by injecting these samples over the captured receptor for 180s. The Rmax was used to calculate the inhibition percent by dividing the Rmax of the pre-mixed samples by an average of the NGF-only Rmax samples that were collected throughout the assay. The ability of each antibody to block binding of NGF to feline TrkA and p75 are shown in Table 16.

Table 16 - Antibody blocking NGF ability to bind to the NGF receptor

	TrkA		p75	
Clone	Ab to NGF ratio	% Block	Ab to NGF ratio	% Block
101	10 to 1	15	1 to 1	42
101	50 to 1	76	5 to 1	87
SC-184_76-Arg	10 to 1	99	1 to 1	100
SC-184_76-Arg	50 to 1	100	5 to 1	100
SC-184_110-Arg	10 to 1	100	1 to 1	100
SC-184_110-Arg	50 to 1	100	5 to 1	100
AHF17602	10 to 1	99	1 to 1	100
AHF17602	50 to 1	100	5 to 1	100

Example 7

[00168] Testing the affinity maturation mutation G55R in the canine clone SC-42_101_006.

[00169] The feline clone 101, has significant CDR similarity as the canine clone SC-42_101_006 (V_H domain of SC-42_101; V_L domain of SC-42_006). Affinity-matured feline clone AHF17602 removes a potential NG deamidation site by mutation of the G55R and this potential

deamidation site exists in the canine clones as well. Clone SC-42_101_006 was mutated to R55 and both the parental and the R55 variant were transiently expressed in CHO cells and purified by Protein A. The variable domain sequences of are shown in FIG. 1 and FIG. 2. Affinity to NGF was assessed using SPR with a Biacore T200 instrument. Antibodies were captured using a Protein A Series S chip. NGF binding was then assessed at multiple concentrations starting at 50 nM using PBSP+ running buffer (Cytiva) with a flow rate of 30 µL/min. The length of the association time was 120s and the dissociation time was run for 600s. The chip surface was regenerated with 10 mM glycine. Reference-subtracted sensorgrams were fitted to a 1:1 binding model using Biacore T200 Evaluation software. The data are shown in Table 17 below.

Table 17 - Affinity of canine NGF antibodies

Clone	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
SC-42_101_006	1.51E+06	1.74E-04	1.15E-10	46.7
SC-42_101_006-Arg	1.38E+06	1.89E-04	1.39E-10	39

[00170] The invention is further described by the following numbered paragraphs:

[00171] 1. A isolated protein that specifically binds to canine NGF, which comprises an antigen binding portion that comprises:

(a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO:146), wherein X_1 comprises A, G, or N, X_2 comprises L or M, X_3 comprises A, D, E, or S, X_4 comprises F, I, L, M, or V, X_5 comprises N or T, X_6 comprises E, S, or T, X_7 comprises G, H, N, S, or Q, and X_8 comprises A or S;

(b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence X_1X_2SNGGT (SEQ ID NO:147), wherein X_1 comprises I or L, X_2 comprises W or Y;

(c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $AX_2IX_4X_5YX_7X_8X_9YLX_{12}X_{13}YX_{15}X_{16}X_{17}$ (SEQ ID NO:148), wherein X_2 comprises D, E, K, N, Q, S, or T, X_4 comprises W or Y, X_5 comprises F, H, W, or Y, X_7 comprises D or E, X_8 comprises A or S, X_9 comprises D or Y, X_{12} comprises H or Y, X_{13} comprises F or W, X_{15} comprises F, I, L, W, or Y, X_{16} comprises D or Q, and X_{17} comprises F, I, L, M, W, or Y;

(d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1X_2IX_4X_5X_6$ (SEQ ID NO:149), wherein X_1 comprises D, E, or K, X_2 comprises A, G, or N, X_4 comprises G, N, Q or S, X_5 comprises N or S, X_6 comprises A, G, N, S or T;

(e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence AX_2X_3 (SEQ ID NO:150), wherein X_2 comprises A, S, or T, X_3 comprises A, D, E, N, Q, S, or T; and

(f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QX_2GX_4X_5X_6PX_8T$ (SEQ ID NO:151), wherein X_2 comprises H or Q, X_4 comprises F, H, W, or Y, X_5 comprises K or Q, X_6 comprises F or W, and X_8 comprises L or M.

[00172] 2. The protein of paragraph 1, which comprises an antigen binding portion that comprises:

(a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4TX_6X_7S$ (SEQ ID NO:152), wherein X_1 comprises A or G, X_2 comprises L or M, X_3 comprises E or S, X_4 comprises F or L, X_6 comprises S or T, and X_7 comprises H, N, or S;

(b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence IWSNGGT (SEQ ID NO:153);

(c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $AX_2IYYYX_7ADYLHX_{13}YX_{15}DX_{17}$ (SEQ ID NO:154) comprises, wherein X_2 comprises N, Q, or S, X_7 comprises D or E, X_{13} comprises F or W, X_{15} comprises F, I, L, W, or Y, and X_{17} comprises F, I, L, or M;

(d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1GIX_4NX_6$ (SEQ ID NO:155), wherein X_1 comprises D or E, X_4 comprises Q or S, X_6 comprises G, N, S or T;

(e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence ATX_3 (SEQ ID NO:156), wherein X_3 comprises D, E, N, Q, or S; and

(f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence QQG$X_4X_5X_6$PLT (SEQ ID NO:157), wherein X_4 comprises F, H, or Y, X_5 comprises K or Q, and X_6 comprises F or W.

[00173] 3. The protein of paragraph 1 or 2, which comprises no more than two (2) changes per VH-CDR as compared to SEQ ID NO:137 and no more than two (2) changes per VL-CDR as compared to SEQ ID NO:138.

[00174] 4. The protein of paragraph 1 or 2, which comprises no more than one (1) changes per VH-CDR as compared to SEQ ID NO:137 and no more than one (1) change per VL-CDR as compared to SEQ ID NO:138.

[00175] 5. The protein of any one of paragraphs 1 to 4, which comprises a heavy chain framework (FR1H+FR2H+FR3H+FR4H) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, SEQ ID NO:137, or SEQ ID NO:141.

[00176] 6. The protein of any one of paragraphs 1 to 5, which comprises a light chain framework (FR1L+FR2L+FR3L+FR4L) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

[00177] 7. The protein of any one of paragraphs 1 to 6, which comprises a V_H domain comprising SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, SEQ ID NO:137, or SEQ ID NO:141.

[00178] 8. The protein of any one of paragraphs 1 to 7, which comprises a V_L domain comprising SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

[00179] 9. An isolated nucleic acid sequence encoding an anti-NGF antibody or antibody fragment of any one of paragraphs 1 to 8.

[00180] 10. A vector that comprises the nucleic acid of paragraph 9.

[00181] 11. A recombinant cell which comprises the nucleic acid of any one of paragraphs 9 or 10.

[00182] 12. A cell that expresses the protein of any one of paragraphs 1 therapeutically effective amount of the anti-NGF protein of any one of paragraphs 1 to 8 or the nucleic acid of paragraphs 9 or 10.

[00183] 13. A method of producing the anti-NGF protein of any one of paragraphs 1 to 8, which comprises culturing the host cell of paragraph 11 under conditions that result in production of the anti-NGF protein.

[00184] 14. A pharmaceutical composition comprising a therapeutically effective amount of the anti-NGF protein of any one of paragraphs 1 to 8.

[00185] 15. A method of treating pain in a subject which comprises administering to the subject a therapeutically effective amount of the anti-NGF protein of any one of paragraphs 1 to 8.

[00186] 16. The method of paragraph 15, wherein the pain comprises inflammatory pain, post-operative incision pain, cancer pain, primary or metastatic bone cancer pain, fracture pain, osteoporotic fracture pain, pain resulting from burn, pain from trauma, musculoskeletal pain, rheumatic pain, or osteoporosis pain.

[00187] 17. The method of paragraph 16, wherein the subject comprises a canine.

[00188] 18. The method of paragraph 16, wherein the subject comprises a feline.

[00189] 19. The method of paragraph 16, wherein the subject comprises a human.

[00190] 20. A method of detecting NGF in a sample comprising incubating a sample comprising NGF in the presence of an anti-NGF protein of any one paragraphs 1 to 8 and detecting the anti-NGF protein bound to NGF in the sample.

* * *

[00191] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

WHAT IS CLAIMED IS:

1. An antigen binding protein that specifically binds to nerve growth factor (NGF), which comprises:

(a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8$ (SEQ ID NO:146), wherein X_1 comprises A, G, or N, X_2 comprises L or M, X_3 comprises A, D, E, or S, X_4 comprises F, I, L, M, or V, X_5 comprises N or T, X_6 comprises E, S, or T, X_7 comprises G, H, N, S, or Q, and X_8 comprises A or S;

(b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $X_1X_2SNX_5GT$ (SEQ ID NO:147), wherein X_1 comprises I or L, X_2 comprises W or Y, and X_5 comprises G or R;

(c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $AX_2IX_4X_5YX_7X_8X_9YLX_{12}X_{13}YX_{15}X_{16}X_{17}$ (SEQ ID NO:148), wherein X_2 comprises D, E, K, N, Q, S, or T, X_4 comprises W or Y, X_5 comprises F, H, W, or Y, X_7 comprises D or E, X_8 comprises A or S, X_9 comprises D or Y, X_{12} comprises H or Y, X_{13} comprises F or W, X_{15} comprises F, I, L, W, or Y, X_{16} comprises D or Q, and X_{17} comprises F, I, L, M, W, or Y;

(d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1X_2IX_4X_5X_6$ (SEQ ID NO:149), wherein X_1 comprises D, E, or K, X_2 comprises A, G, or N, X_4 comprises G, N, Q or S, X_5 comprises N or S, X_6 comprises A, G, N, S or T;

(e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence AX_2X_3 (SEQ ID NO:150), wherein X_2 comprises A, S, or T, X_3 comprises A, D, E, N, Q, S, or T; and

(f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QX_2GX_4X_5X_6PX_8T$ (SEQ ID NO:151), wherein X_2 comprises H or Q, X_4 comprises F, H, W, or Y, X_5 comprises K or Q, X_6 comprises F or W, and X_8 comprises L or M.

2. The antigen binding protein of claim 1, which comprises:

(a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1X_2X_3X_4TX_6X_7S$ (SEQ ID NO:152), wherein X_1 comprises A or G, X_2 comprises L or M, X_3 comprises E or S, X_4 comprises F or L, X_6 comprises S or T, and X_7 comprises H, N, or S;

(b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $IWSNX_5GT$ (SEQ ID NO:153), wherein X_5 comprises G or R;

(c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $AX_2IYYYX_7ADYLHX_{13}YX_{15}DX_{17}$ (SEQ ID NO:154) comprises, wherein X_2 comprises N, Q, or S, X_7 comprises D or E, X_{13} comprises F or W, X_{15} comprises F, I, L, W, or Y, and X_{17} comprises F, I, L, or M;

(d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1GIX_4NX_6$ (SEQ ID NO:155), wherein X_1 comprises D or E, X_4 comprises Q or S, X_6 comprises G, N, S or T;

(e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence ATX_3 (SEQ ID NO:156), wherein X_3 comprises D, E, N, Q, or S; and

(f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QQGX_4X_5X_6PLT$ (SEQ ID NO:157), wherein X_4 comprises F, H, or Y, X_5 comprises K or Q, and X_6 comprises F or W.

3. The antigen binding protein of claim 1 or 2, which comprises no more than two (2) substitutions per VH-CDR as compared to SEQ ID NO:137 and no more than two (2) substitutions per VL-CDR as compared to SEQ ID NO:138.

4. The antigen binding protein of claim 1 or 2, which comprises no more than one (1) substitution per VH-CDR as compared to SEQ ID NO:137 and no more than one (1) substitution per VL-CDR as compared to SEQ ID NO:138.

5. The antigen binding protein of claim 1, which comprises one or more VH-CDRs of any one of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID

NO:121, SEQ ID NO:133, SEQ ID NO:137, SEQ ID NO:141, or SEQ ID NO:207 and one or more VL-CDRs of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

6. The antigen binding protein of claim 1, which comprises the VH-CDRs of any one of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, SEQ ID NO:137, SEQ ID NO:141, or SEQ ID NO:207 and the VL-CDRs of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

7. The antigen binding protein of claim 1, which comprises the VH-CDRs and VL-CDRs of 2166, SC-42_006, SC-42_007, SC-42_008, SC-42_010, SC-42_011, SC-42_023, SC-42_032, SC-42_045, SC-42_047, SC-42_048, SC-42_052, SC-42_070, SC-42_073, SC-42_077, SC-42_082, SC-42_090, or SC-42_101.

8. The antigen binding protein of any one of claims claims 1 to 7, wherein the CDRs are according to the IMGT system..

9. The antigen binding protein of any one of claims claims 1 to 7, wherein the CDRs are according to Kabat.

10. The antigen binding protein of any one of claims claims 1 to 7, wherein the CDRs are according to Chothia.

11. The antigen binding protein of any one of claims 1 to 7, which comprises a heavy chain framework (FR1H+FR2H+FR3H+FR4H) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID

NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, SEQ ID NO:137, SEQ ID NO:141, or SEQ ID NO:207.

12. The antigen binding protein of any one of claims 1 to 11, which comprises a light chain framework (FR1L+FR2L+FR3L+FR4L) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

13. The antigen binding protein of any one of claims 1 to 12, which comprises a V_H domain at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:31, SEQ ID NO:55, SEQ ID NO:61, SEQ ID NO:69, SEQ ID NO:77, SEQ ID NO:103, SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:121, SEQ ID NO:133, SEQ ID NO:137, SEQ ID NO:141, or SEQ ID NO:207.

14. The antigen binding protein of any one of claims 1 to 13, which comprises a V_L domain at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:32, SEQ ID NO:56, SEQ ID NO:62, SEQ ID NO:70, SEQ ID NO:78, SEQ ID NO:104, SEQ ID NO:110, SEQ ID NO:114, SEQ ID NO:122, SEQ ID NO:134, SEQ ID NO:138, or SEQ ID NO:142.

15. An antigen binding protein that specifically binds to NGF, which comprises:

(a) a heavy chain complementarity determining region 1 (VH-CDR1) comprising the amino acid sequence $X_1LX_3X_4X_5X_6X_7X_8MX_{10}$, wherein X_1 comprises A, G, L, N, or Q, X_3 comprises A, D, E, G, H, I, M, S, T, or Y, X_4 comprises L, M, or V, X_5 comprises A, M, N, R, S, T, or V, X_6 comprises A, E, G, H, K, R, S, or T, X_7

comprises A, D, H, I, N, Q, S, T, or Y, X_8 comprises A or S, and X_{10} comprises S or V;

(b) a heavy chain complementarity determining region 2 (VH-CDR2) comprising the amino acid sequence $X_1X_2X_3X_4X_5GTX_8YX_{10}DX_{12}VX_{14}$, wherein X_1 comprises I or L, X_2 comprises W or Y; X_3 comprises A, P, or S, X_4 comprises D, E, N, Q, R, or S, X_5 comprises G, R, or Y, X_8 comprises D or Y, X_{10} comprises D, E, H, S, or T, X_{12} comprises D or S, and X_{14} comprises D, E, or K;

(c) a heavy chain complementarity determining region 3 (VH-CDR3) comprising the amino acid sequence $X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}LX_{12}X_{13}X_{14}FX_{16}X_{17}$, wherein X_1 comprises A, D, E, K, N, Q, S, or T, X_2 comprises A, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, or Y, X_3 comprises I, L, W, or Y, X_4 comprises F, T, W, or Y, X_5 comprises F, H, or Y, X_6 comprises H or Y, X_7 comprises D or E, X_8 comprises A, S, or V, X_9 comprises D, E, H, K, N, Q, or Y, X_{10} comprises F, H, or Y, X_{12} comprises H or Y, X_{13} comprises F or W, X_{14} comprises D, I, L, W, or Y, X_{16} comprises D or Q, and X_{17} comprises E, F, H, I, L, M, N, P, W, or Y;

(d) a light chain complementarity determining region 1 (VL-CDR1) comprising the amino acid sequence $X_1ASX_4X_5X_6X_7X_8X_9LX_{11}$, wherein X_1 comprises F or R, X_4 comprises E, K, or N, X_5 comprises A, or G, X_6 comprises I, L, or V, X_7 comprises A, D, G, L, P, Q, S, V, or Y, X_8 comprises K, Q, N, S, or Y, X_9 comprises A, D, E, F, G, H, K, L, N, Q, R, S or T, and X_{11} comprises A, G, or S;

(e) a light chain complementarity determining region 2 (VL-CDR2) comprising the amino acid sequence $AX_2X_3X_4X_5X_6X_7$, wherein X_2 comprises A, D, L, Q, S, T, V, or Y, X_3 comprises D, E, K, N, Q, or S, X_4 comprises H, I, K, L, M, N, or V; X_5 comprises H or L, X_6 comprises H, I, L, or M, and X_7 comprises D, E, N, S, or T;

(f) a light chain complementarity determining region 3 (VL-CDR3) comprising the amino acid sequence $QQX_3X_4X_5X_6X_7X_8T$, wherein X_3 comprises G or Y, X_4 comprises D, F, G, H, K, L, R, S, T, V, W, or Y, X_5 comprises E, K, Q, R, or S, X_6 comprises I, F, T, or W, X_7 comprises E or P, and X_8 comprises L, M, or W.

16. The antigen binding protein of claim 15, wherein:

VH-CDR1 comprises GLSLTSX$_7$SMX$_{10}$, wherein X$_7$ comprises A, D, or N, and X$_{10}$ comprises S or V;

VH-CDR2 comprises X$_1$X$_2$SNX$_5$GT, wherein X$_1$ comprises I or L, X$_2$ comprises W or Y, and X$_5$ comprises G or R;

VH-CDR3 comprises ASIYYYX$_7$AX$_9$YLHWYFDX$_{12}$, wherein X$_7$ comprises D or E, X$_9$ comprises D or E, and X$_{12}$ comprises E or F;

VL-CDR1 comprises RASX$_4$GIX$_7$X$_8$NLS, wherein X$_4$ comprises E or K, X$_7$ comprises A, Q, or S, X$_8$ comprises K or N;

VL-CDR2 comprises AX$_2$X$_3$X$_4$LHS, wherein X$_2$ comprises Q or T, X$_3$ comprises D or S, and X$_4$ comprises I, N, or V; and

VL-CDR3 comprises QQGX$_4$KWPLT, wherein X$_4$ comprises F, W, or Y.

17. The antigen binding protein of claim 15 or 16, which comprises no more than two (2) substitutions per VH-CDR as compared to SEQ ID NO:204 and no more than two (2) substitutions per VL-CDR as compared to SEQ ID NO:199.

18. The antigen binding protein of claim 15 or 16, which comprises no more than one (1) substitution per VH-CDR as compared to SEQ ID NO:204 and no more than one (1) substitution per VL-CDR as compared to SEQ ID NO:199.

19. The antigen binding protein of claim 15, which comprises one or more VH-CDRs of any one of SEQ ID NO:141, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:205, or SEQ ID NO:206 and one or more VL-CDRs of any one of SEQ ID NO:142, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, or SEQ ID NO:203.

20. The antigen binding protein of claim 15, which comprises the VH-CDRs of any one of SEQ ID NO:141, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:205, or SEQ ID NO:206 and the VL-CDRs of any one of SEQ ID NO:142, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194,

SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, or SEQ ID NO:203.

21. The antigen binding protein of claim 15, which comprises the VH-CDRs and VL-CDRs of clone AHF17591, AHF17592, AHF17593, AHF17595, AHF17597, AHF17602, AHF17607, SC-184_76, SC-184_102, SC-184_110, SC-184_76-Arg, SC-184_102-Arg, or SC-184_110-Arg.

22. The antigen binding protein of any one of claims 15 to 21, which comprises a heavy chain framework (FR1H+FR2H+FR3H+FR4H) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:141, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:205, or SEQ ID NO:206.

23. The protein of any one of claims 15 to 22, which comprises a light chain framework (FR1L+FR2L+FR3L+FR4L) at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:142, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, or SEQ ID NO:203.

24. The antigen binding protein of any one of claims 15 to 23, which comprises a V_H domain at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:141, SEQ ID NO:184, SEQ ID NO:185, SEQ ID NO:186, SEQ ID NO:187, SEQ ID NO:188, SEQ ID NO:189, SEQ ID NO:190, SEQ ID NO:198, SEQ ID NO:200, SEQ ID NO:202, SEQ ID NO:204, SEQ ID NO:205, or SEQ ID NO:206.

25. The antigen binding protein of any one of claims 15 to 24, which comprises a V_H domain at least 80%, or at least 85%, or at least 90%, or at least 93%, or at least 95% identical to SEQ ID NO:142, SEQ ID NO:191, SEQ ID NO:192, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:195, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:199, SEQ ID NO:201, or SEQ ID NO:203.

26. An isolated nucleic acid sequence encoding an anti-NGF antibody or antibody fragment of any one of claims 1 to 25.

27. A vector that comprises the nucleic acid of claim 26.

28. A recombinant cell which comprises the nucleic acid of any one of claims 26 or 27.

29. A cell that expresses the antigen binding protein of any one of claims 1 to 25 or the nucleic acid of claims 26 or 27.

30. A method of producing the antigen binding protein of any one of claims 1 to 25, which comprises culturing the cell of claim 29 under conditions that result in production of the antigen binding protein.

31. A pharmaceutical composition comprising a therapeutically effective amount of the anti-NGF protein of any one of claims 1 to 25.

32. A method of treating or reducing pain in a subject which comprises administering to the subject a therapeutically effective amount of the anti-NGF protein of any one of claims 1 to 25.

33. The method of claim 32, wherein the pain comprises inflammatory pain, post-operative incision pain, cancer pain, primary or metastatic bone cancer pain, fracture pain, osteoporotic fracture pain, pain resulting from burn, pain from trauma, musculoskeletal pain, rheumatic pain, or osteoporosis pain.

34. The method of claim 33, wherein the subject comprises a canine.

35. The method of claim 33, wherein the subject comprises a feline.

36. The method of claim 33, wherein the subject comprises a human.

37. A method of detecting NGF in a sample comprising incubating a sample comprising NGF in the presence of an anti-NGF protein of any one claims 1 to 25 and detecting the anti-NGF protein bound to NGF in the sample.

SEQ ID NO:	Clone Name	FR1H	CDR1H	FR2H	CDR2H
1	2166	QVQLKESGPGLVQPSQTLSLTCTVS	GLSLTSNS	VSWIRQPPGKGLEWMGV	IWSNGGT
3	SC-42_006	EVQLVESGGDLVQPAGSLRLSCVAS	GLSLNSNS	MSWVRQAPEKGLQLVAT	IWSNGGT
5	SC-42_007	EVQLVESGGDLVAPSQSLSITCTVS	GLSLTSNA	ISWVRQPPGRGLEWLGT	IWSNGGT
7	SC-42_008	EVQLVESGGDLVKPEGSLRLSCVVS	GLELTSNS	MSWVRQAPGKGLQWVGV	LWSNGGT
9	SC-42_010	EVQLVESGGDLVKPEGSLSLSCVVS	GLSLTSNA	MSWVRQAPGKGLQWVAT	IWSNGGT
11	SC-42_011	EVQLVESGGDLVAPSQSLSITCTVS	GLSLTENS	ISWVRQPPGRGLEWLGV	IWSNGGT
13	SC-42_023	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
15	SC-42_024	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
17	SC-42_025	EVQLVESGGELVKPGGSLRLSCVAS	GLSLNSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
19	SC-42_026	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
21	SC-42_027	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
23	SC-42_028	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
25	SC-42_029	EVQLVESGGELVKPGGSLRLSCVAS	GLELTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
27	SC-42_030	EVQLVESGGELVKPGGSLRLSCVAS	NLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
29	SC-42_031	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
31	SC-42_032	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTTNS	MSWIRQAPGKGLQWVAT	IWSNGGT
33	SC-42_033	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
35	SC-42_034	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
37	SC-42_035	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
39	SC-42_036	EVQLVESGGELVKPGGSLRLSCVAS	NLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
41	SC-42_037	EVQLVESGGELVKPGGSLRLSCVAS	GLSLNSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
43	SC-42_038	EVQLVESGGELVKPGGSLRLSCVAS	GLELTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
45	SC-42_040	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
47	SC-42_041	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTTNS	MSWIRQAPGKGLQWVAT	IWSNGGT

FIG. 1A

SEQ ID NO:	Clone Name	FR1H	CDR1H	FR2H	CDR2H
49	SC-42_042	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
51	SC-42_043	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
53	SC-42_044	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
55	SC-42_045	EVQLVESGGELVKPGGSLRLSCVAS	GLELTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
57	SC-42_046	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
59	SC-42_047	EVQLVESGGELVKPGGSLRLSCVAS	NLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
61	SC-42_048	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
63	SC-42_049	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
65	SC-42_050	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTTNS	MSWIRQAPGKGLQWVAT	IWSNGGT
67	SC-42_051	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
69	SC-42_052	EVQLVESGGELVKPGGSLRLSCVAS	GLELTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
71	SC-42_053	EVQLVESGGELVKPGGSLRLSCVAS	GLSLNSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
73	SC-42_054	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTTNS	MSWIRQAPGKGLQWVAT	IWSNGGT
75	SC-42_055	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
77	SC-42_057	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
79	SC-42_058	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
81	SC-42_059	EVQLVESGGELVKPGGSLRLSCVAS	NLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
83	SC-42_060	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
85	SC-42_061	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
87	SC-42_062	EVQLVESGGELVKPGGSLRLSCVAS	GLALTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
89	SC-42_063	EVQLVESGGELVKPGGSLRLSCVAS	GLSLNSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
91	SC-42_064	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
93	SC-42_065	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
95	SC-42_066	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT

FIG. 1A Cont'd

SEQ ID NO:	Clone Name	FR1H	CDR1H	FR2H	CDR2H
97	SC-42_067	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
99	SC-42_068	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSHS	MSWIRQAPGKGLQWVAT	IWSNGGT
101	SC-42_069	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTTNS	MSWIRQAPGKGLQWVAT	IWSNGGT
103	SC-42_070	EVQLVESGGELVKPGGSLRLSCVAS	GLSLTSNS	MSWIRQAPGKGLQWVAT	IWSNGGT
105	SC-42_071	EVQLVESGGDLVKPEGSLRLSCVVS	GLSLTSGS	MSWVRQAPGKGLQWVGV	IYSNGGT
107	SC-42_072	EVQLVESGGDLVQPGGSLRLSCVVS	GLDLTSNS	MSWVRQAPGKGLQWVTV	IWSNGGS
109	SC-42_073	EVQLVESGGDLVKPAGSLRLSCVAS	ALSLTSNS	MSWVRQAPGKGLQLVAT	IWSNGGT
111	SC-42_075	EVQLVESGGDLVQPAGSLRLSCVAS	GLSLTSQS	MSWVRQAPGKGLQLVAI	IWSNGGT
113	SC-42_077	EVQLVESGGDLVQPGGSLRLSCVVS	GLSLTTNS	MSWVRQAPGKGLQWVTT	IWSNGGT
115	SC-42_079	EVQLVESGGDLVKPGGSLSLSCVAS	GLSVTSNS	MDWVRQAPGKGLQWLST	IWSNGGT
117	SC-42_080	EVQLVESGGDLVKPEGSLSLSCVVS	GLSLTSSS	MSWVRQAPGKGLEWVAT	IWSNGGT
119	SC-42_081	EVQLVESGGDLVKPEGSLRLTCVVS	GLSMTSNS	MSWVRQAPGKGLQWVAT	IWSNGGI
121	SC-42_082	EVQLVESGGDLVAPSQSLSITCTVS	GLSLTSHS	ISWVRQAPGKGLQWVAT	IWSNGGT
123	SC-42_083	EVQLVESGGDLVKPEGSLRLSCVVS	GLSLTSNG	MSWVRQAPGKGLEWVAT	IWSNGGT
125	SC-42_084	EVQLVESGGDLVKPEGSLSLSCVVS	GLSLTSHS	MSWVRQAPGKGLQWVAT	IWSNGGT
127	SC-42_085	EVQLVESGGDLVKPEGSLRLSCVVS	GLELTSNS	MSWVRQAPGKGLQWVGT	IWSNGGT
129	SC-42_088	EVQLVESGGDLVKPGGSLRLSCVAS	GLSLTSNS	MSWVRQAPEKGLQLVAT	IWSNGGT
131	SC-42_089	EVQLVESGGDLVKPAGSLRLSCVAS	GLSLTSHS	MSWVRQAPGKGLQLVAT	IWSNGGT
133	SC-42_090	EVQLVESGGDLVKPAGSLRLSCVAS	GLSFTSNS	MSWVRQAPGKGLQLVAT	IWSNGGT
135	SC-42_091	EVQLVESGGDLVKPGGSLSLSCVAS	GLSLTSHS	MDWVRQAPGKGLQWLST	IWSNGGT
137	SC-42_101	EVQLVESGGDLVAPSQSLSITCTVS	GMSLTSNS	ISWVRQPPGRGLEWLGT	IWSNGGT
207	SC-42_101R	EVQLVESGGDLVAPSQSLSITCTVS	GMSLTSNS	ISWVRQPPGRGLEWLGT	IWSNRGT
139	SC-42_102	EVQLVESGGDLVKPAGSLRLSCVAS	GLGLTSNS	MSWVRQAPEKGLQLVAV	IWSNGGT

FIG. 1A Cont'd

SEQ ID NO:	Clone Name	FR3H	CDR3H	FR4
1	2166	DYNSAIESRLSINRDTSKSQVFLKMNSLQPEDTAMYFC	ASIYYYDADYLHWYFDF	WGPGTMVTVSS
3	SC-42_006	QYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	ATIYYYDADYLHWYFDF	WGQGTLVTVSS
5	SC-42_007	SYTDAVKGRFTISRDNAKNTLYLQMNSLRTEDTARYYC	ASIYYYDADYLHWYFDM	WGQGTLVTVSS
7	SC-42_008	DYTDAVKGRFTISRDNAKNTLYLQMNSLRTEDTARYYC	ASIYYYDADYLHWYFDY	WGQGTLVTVSS
9	SC-42_010	DYTDAVKGRFTISRDNAKNTLYLQMNSLRTEDTARYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
11	SC-42_011	SYNSAVKGRFTISRDNAKNTLYLQMNSLRTEDTAVYYC	ASIYYYDADYLHWYLDF	WGPGTLVTISS
13	SC-42_023	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDL	WGQGTLVTVSS
15	SC-42_024	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHFYFDF	WGQGTLVTVSS
17	SC-42_025	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYEYDADYLHWYFDF	WGQGTLVTVSS
19	SC-42_026	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYFYDADYLHWYFDF	WGQGTLVTVSS
21	SC-42_027	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
23	SC-42_028	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ADIYYYDADYLHWYFDF	WGQGTLVTVSS
25	SC-42_029	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
27	SC-42_030	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYLHWYFDF	WGQGTLVTVSS
29	SC-42_031	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
31	SC-42_032	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDM	WGQGTLVTVSS
33	SC-42_033	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYLHWYFDF	WGQGTLVTVSS
35	SC-42_034	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYILHWYFDF	WGQGTLVTVSS
37	SC-42_035	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDI	WGQGTLVTVSS
39	SC-42_036	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	AEIYYYDADYLHWYFDF	WGQGTLVTVSS
41	SC-42_037	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYWDF	WGQGTLVTVSS
43	SC-42_038	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	AQIYYYDADYLHWYFDF	WGQGTLVTVSS
45	SC-42_040	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDW	WGQGTLVTVSS
47	SC-42_041	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	AQIYYYDADYLHWYFDF	WGQGTLVTVSS

FIG. 1B

SEQ ID NO:	Clone Name	FR3H	CDR3H	FR4
49	SC-42_042	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
51	SC-42_043	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ADIYYYDADYLHWYFDF	WGQGTLVTVSS
53	SC-42_044	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYLHWYFDF	WGQGTLVTVSS
54	SC-42_045	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYWDF	WGQGTLVTVSS
55	SC-42_046	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHFYFDF	WGQGTLVTVSS
59	SC-42_047	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYEADYLHWYFDF	WGQGTLVTVSS
61	SC-42_048	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYLHWYFDF	WGQGTLVTVSS
63	SC-42_049	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDI	WGQGTLVTVSS
65	SC-42_050	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYEADYLHWYFDF	WGQGTLVTVSS
67	SC-42_051	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHFYFDF	WGQGTLVTVSS
69	SC-42_052	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	AQIYYYDADYLHWYFDF	WGQGTLVTVSS
71	SC-42_053	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDL	WGQGTLVTVSS
73	SC-42_054	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
75	SC-42_055	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDL	WGQGTLVTVSS
77	SC-42_057	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
79	SC-42_058	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHFYFDF	WGQGTLVTVSS
81	SC-42_059	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYLHWYFDF	WGQGTLVTVSS
83	SC-42_060	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDI	WGQGTLVTVSS
85	SC-42_061	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
87	SC-42_062	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ADIYYYDADYLHWYFDF	WGQGTLVTVSS
89	SC-42_063	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYLHWYFDF	WGQGTLVTVSS
91	SC-42_064	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	AKIYYYDADYLHWYFDF	WGQGTLVTVSS
93	SC-42_065	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYYDF	WGQGTLVTVSS
95	SC-42_066	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYEADYLHWYFDF	WGQGTLVTVSS

FIG. 1B Cont'd

SEQ ID NO:	Clone Name	FR3H	CDR3H	FR4
97	SC-42_067	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYEYYDADYLHWYFDF	WGQGTLVTVSS
99	SC-42_068	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ANIYYYDADYLHWYFDF	WGQGTLVTVSS
101	SC-42_069	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHWYFDL	WGQGTLVTVSS
103	SC-42_070	DYTDAVKGRFTISRDNVKNSLYLQMNSLRAEDTAVYYC	ASIYYYDADYLHFYFDF	WGQGTLVTVSS
105	SC-42_071	DYTDAVKGRFTISRDNAKNTVYLQMNSLRTEDTAVYYC	ASIYYYDAYYLHWYYDF	WGQGTLVTVSS
107	SC-42_072	DYADAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	ASIYYYDADYLHWYTQF	WGQGTLVTVSS
109	SC-42_073	DYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	ASIYYYDADYLHWYWDF	WGQGTLVTVSS
111	SC-42_075	DYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	ASIYYYDADYLHWYFDM	WGQGTLVTVSS
113	SC-42_077	DYADAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	AQIYYYDADYLHWYFDF	WGQGTLVTVSS
115	SC-42_079	DYADAVKGRFTISRDNAKNTLYLQMNSLRTEDTAVYYC	AKIYYYDADYLHWYFDF	WGQGTLVTVSS
117	SC-42_080	DYNDAVKGRFTISRDNAKNTLYLKMNSLRTEDTAVYYC	ASIYYYDADYLYWYFDF	WGQGTLVTVSS
119	SC-42_081	DYTDAVKGRFTISRDNAKNTLYLQMNSLRTEDTARYYC	ASIYYYDSDYLHWYFDF	WGQGTLVTVSS
121	SC-42_082	DYTSAVKGRFTISRDNAKNTVYLQMNSLRTEDTAVYYC	ASIYYYDADYLHWYWDF	WGQGTLVTVSS
123	SC-42_083	DYTDAVKGRFTISRDNAKNTLYLQMNSLRTEDTAVYYC	ASIYYYEADYLHWYYDF	WGQGTLVTVSS
125	SC-42_084	DYTDAVKGRFTISKDNSKSQVFLKMNSLQTDDTARYYC	ASIYYYDADYLHWYIDF	WGQGTLVTVSS
127	SC-42_085	DYTDAVKGRFTISRDNAKNTLYLQMNSLQTEDTAVYYC	ASIYYYDADYLHWYWDF	WGQGTLVTVSS
129	SC-42_088	DYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTGMYYC	ASIYYYDADYLHWYFDL	WGQGTLVTVSS
131	SC-42_089	DYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	ASIYYYDADYLHWYWDF	WGQGTLVTVSS
133	SC-42_090	DYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	ASIYYYDADYLHWYLDF	WGQGTLVTVSS
135	SC-42_091	QYADAVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYC	ASIWYYDADYLHWYFDF	WGQGTLVTVSS
137	SC-42_101	DYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	AQIYYYDADYLHWYFDF	WGQGTLVTVSS
207	SC-42_101R	DYTDAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	AQIYYYDADYLHWYFDF	WGQGTLVTVSS
139	SC-42_102	QYADAVKGRFTISRDNAKNTVYLQMNSLRAEDTAMYYC	AKIYYYDADYLHWYFDF	WGQGTLVTVSS

FIG. 1B Cont'd

SEQ ID NO:	Clone Name	FR1L	CDR1L	FR2L	CDR2L
2	2166	DIQMTQSPASLSASLGETVSIECLAS	EGISNS	LAWYQLKPGKSPQFLIY	ATS
4	SC-42_006	EIVMTQSPASLSLSQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATN
6	SC-42_007	EIVMTQSPASLSLSVEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
8	SC-42_008	EIVMTQSPASLSLSQEEKVTITCRAS	EGISNN	VAWYQQKPGQAPKLLIY	ATS
10	SC-42_010	EIVMTQSPASLSLSQEDKVTITCRAS	EGISSS	LAWYQQKPGQAPKLLIY	ATS
12	SC-42_011	EIVMTQSPASLSLSQEDKVTITCRAS	EGINNS	LAWYQQKPGQAPKLLIY	ATQ
14	SC-42_023	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
16	SC-42_024	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
18	SC-42_025	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
20	SC-42_026	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
22	SC-42_027	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
24	SC-42_028	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
26	SC-42_029	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
28	SC-42_030	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
30	SC-42_031	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
32	SC-42_032	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
34	SC-42_033	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
36	SC-42_034	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
38	SC-42_035	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
40	SC-42_036	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
42	SC-42_037	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
44	SC-42_038	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
46	SC-42_040	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
48	SC-42_041	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS

FIG. 2A

SEQ ID NO:	Clone Name	FR1L	CDR1L	FR2L	CDR2L
50	SC-42_042	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
52	SC-42_043	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
54	SC-42_044	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
56	SC-42_045	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
58	SC-42_046	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
60	SC-42_047	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
62	SC-42_048	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
64	SC-42_049	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
66	SC-42_050	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
68	SC-42_051	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
70	SC-42_052	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
72	SC-42_053	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
74	SC-42_054	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
76	SC-42_055	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
78	SC-42_057	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
80	SC-42_058	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
82	SC-42_059	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
84	SC-42_060	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
86	SC-42_061	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
88	SC-42_062	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
90	SC-42_063	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
92	SC-42_064	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
94	SC-42_065	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATD
96	SC-42_066	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS

FIG. 2A Cont'd

SEQ ID NO:	Clone Name	FR1L	CDR1L	FR2L	CDR2L
98	SC-42_067	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
100	SC-42_068	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
102	SC-42_069	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
104	SC-42_070	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
106	SC-42_071	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
108	SC-42_072	EIVMTQSPASLSLSQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	AAS
110	SC-42_073	EIVMTQSPASLSASQEEKVTITCRAS	EGIQNS	LAWYQQKPGQAPKLLIY	ATN
112	SC-42_075	EIVMTQSPASLSLSQEEKVTITCRAS	EGISNA	LAWYQQKPGKAPKLLIY	ATE
114	SC-42_077	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATE
116	SC-42_079	EIVMTQSPASLSLSQEDKVTITCRAS	EGIGNS	LAWYQQKPGQAPKLLIY	ATS
118	SC-42_080	EIVMTQSPASLSLSQEEKVTITCRAS	DGISNS	LAWYQQKPGQAPKLLIY	ATQ
120	SC-42_081	EIVMTQSPASLSASQEEKVTITCRAS	EAISNS	LAWYQQKPGQAPKLLIY	ASS
122	SC-42_082	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNG	LAWYQQKPGQAPKLLIY	ATS
124	SC-42_083	EIVMTQSPASLSLSQGEKVTITCRAS	EGISSS	LAWYQQKPGQAPKLLIY	ATA
126	SC-42_084	EIVMTQSPASLSLSQEDKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS
128	SC-42_085	EIVMTQSPASLSLSQGEKVTITCRAS	DGISNS	LAWYQQKPGQAPKLLIY	ATS
130	SC-42_088	EIVMTQSPASLSLSQGEKVTITCRAS	EAISNS	LAWYQQKPGQAPKLLIY	ATT
132	SC-42_089	EIVMTQSPASLSLSQGEKVTITCRAS	KGISNS	LAWYQQKPGQAPKLLIY	ATS
134	SC-42_090	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNT	LAWYQQKPGQAPKLLIY	ATE
136	SC-42_091	EIVMTQSPASLSLSQGEKVTITCRAS	ENISNS	LAWYQQKPGQAPKLLIY	ATS
138	SC-42_101	EIVMTQSPASLSASQEEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIH	ATS
140	SC-42_102	EIVMTQSPASLSLSQGEKVTITCRAS	EGISNN	LAWYQQKPGQAPKLLIY	ATS

FIG. 2A Cont'd

SEQ ID NO:	Clone Name	FR3L	CDR3L	FR4L
2	2166	SLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGVYYC	QQGYKFPLT	FGSGTKLKIK
4	SC-42_006	SLATGVPSRFSGSGSGTDFSLTISSLEPEDVAVYYC	QQGYKFPLT	FGQGTKVEIK
6	SC-42_007	SLATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGWKFPLT	FGQGTKVEIK
8	SC-42_008	ELATGVPSRFSGSGSGTDFSLTISSLEPEDVAVYYC	QQGYKFPMT	FGQGTKVEIK
10	SC-42_010	QLATGVPSRFSGSGSGTDFSFTISSLEPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
12	SC-42_011	SLATGVPSRFSGSGSGTDFSLTISSLEPEDVAVYYC	QQGFKFPLT	FGQGTKVEIK
14	SC-42_023	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
16	SC-42_024	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
18	SC-42_025	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
20	SC-42_026	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
22	SC-42_027	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
24	SC-42_028	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
26	SC-42_029	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
28	SC-42_030	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
30	SC-42_031	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
32	SC-42_032	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
34	SC-42_033	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
36	SC-42_034	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
38	SC-42_035	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
40	SC-42_036	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
42	SC-42_037	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
44	SC-42_038	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
46	SC-42_040	QLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
48	SC-42_041	QLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK

FIG. 2B

SEQ ID NO:	Clone Name	FR3L	CDR3L	FR4L
50	SC-42_042	QLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
52	SC-42_043	QLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
54	SC-42_044	QLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
56	SC-42_045	QLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
58	SC-42_046	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
60	SC-42_047	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
62	SC-42_048	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
64	SC-42_049	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
66	SC-42_050	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
68	SC-42_051	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
70	SC-42_052	ELATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
72	SC-42_053	ELATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
74	SC-42_054	ELATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
76	SC-42_055	ELATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
78	SC-42_057	ELATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
80	SC-42_058	ELATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
82	SC-42_059	ELATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
84	SC-42_060	SLATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
86	SC-42_061	SLATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
88	SC-42_062	SLATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
90	SC-42_063	SLATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
92	SC-42_064	SLATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
94	SC-42_065	SLATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
96	SC-42_066	ELATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGQGTKVEIK

FIG. 2B Cont'd

SEQ ID NO:	Clone Name	FR3L	CDR3L	FR4L
98	SC-42_067	ELATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGQGTKVEIK
100	SC-42_068	ELATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGQGTKVEIK
102	SC-42_069	ELATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGQGTKVEIK
104	SC-42_070	ELATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGQGTKVEIK
106	SC-42_071	SMATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGAGTKVELK
108	SC-42_072	SLQTGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QHGYKFPLT	FGAGTKVELK
110	SC-42_073	SLATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGHKFPLT	FGAGTKVELK
112	SC-42_075	SLATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGFKFPLT	FGAGTKVELK
114	SC-42_077	SLATGVPSRFSGSGSGTDFSFTISSLQPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
116	SC-42_079	QLATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGHKFPLT	FGQGTKVEIK
118	SC-42_080	SLARGVPSRFSGSGSGTDFSLTISSLEPEDVAVYYC	QQGYKWPLT	FGAGTKVELK
120	SC-42_081	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGWKFPLT	FGAGTKVELK
122	SC-42_082	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVAVYYC	QQGYKWPLT	FGQGTKVEIK
124	SC-42_083	SLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
126	SC-42_084	KLATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKWPLT	FGQGTKVEIK
128	SC-42_085	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVATYYC	QQGYKFPLT	FGQGTKVEIK
130	SC-42_088	SLATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGQGTKVEIK
132	SC-42_089	ELATGVPSRFSGSGSGTDFSFTISSLEPEDVAVYYC	QQGYKFPLT	FGQGTKVEIK
134	SC-42_090	SLATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGFKFPLT	FGQGTKVEIK
136	SC-42_091	TLATGVPSRFSGSGSGTDFSLTISSLEPEDVATYYC	QQGFKFPLT	FGQGTKVEIK
138	SC-42_101	SLQTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYQFPLT	FGQGTKVEIK
140	SC-42_102	ALATGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKFPLT	FGQGTKVEIK

FIG. 2B Cont'd

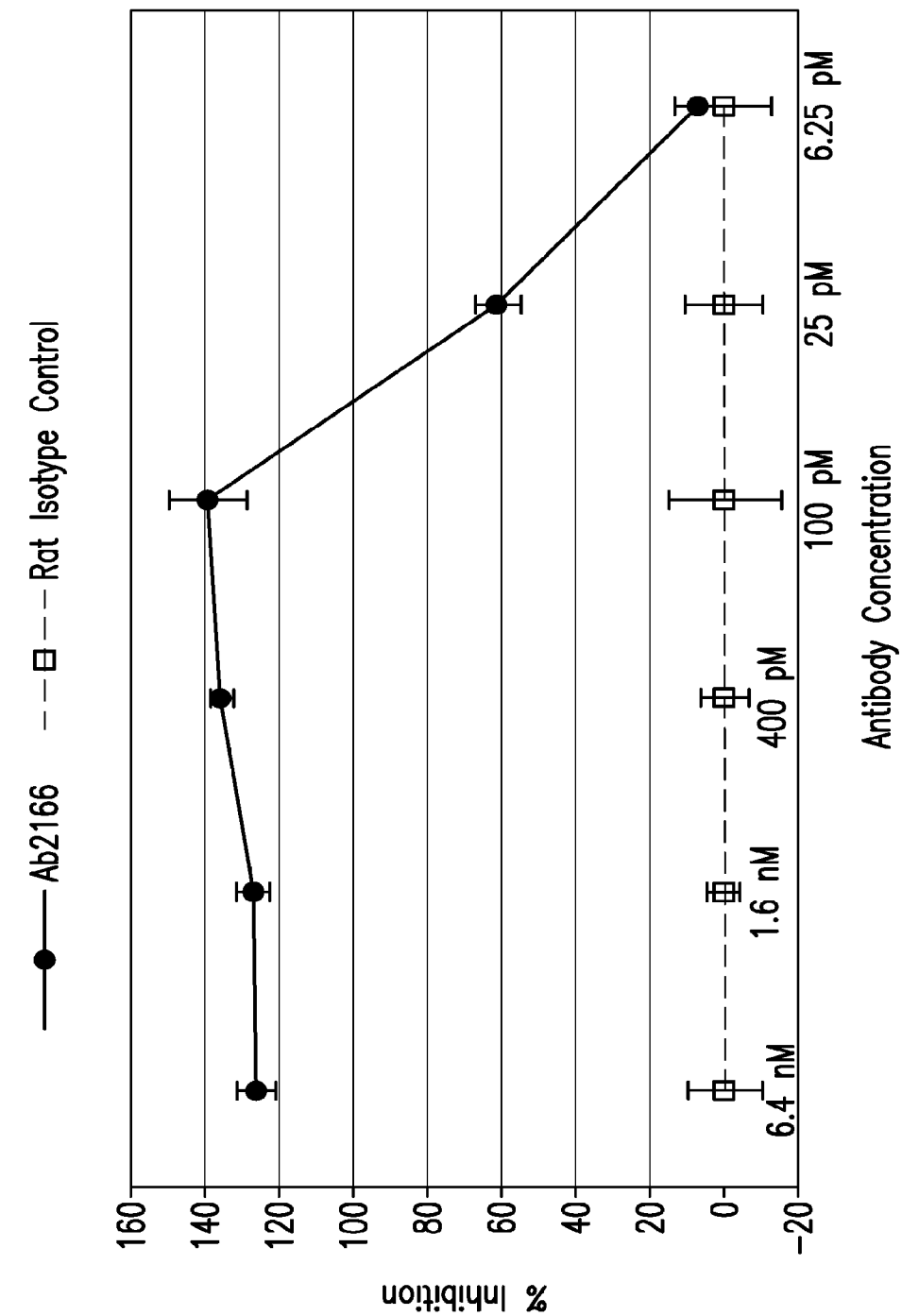

Ab2166
Rat Isotype Control
% Inhibition
160
140
120
100
80
60
40
20
0
-20
6.4 nM
1.6 nM
400 pM
100 pM
25 pM
6.25 pM
Antibody Concentration

FIG. 3

Heavy chain
QVQLKESGPGLVQPSQTLSLTCTVSGLSLTSNSVSWIRQPPGKGLEWMGVIWSNGGTDYNSAIESRLSINRDTSKSQ
VFLKMNSLQPEDTAMYFCASIYYYDADYLHWYFDFWGPGTMVTVSSASTTAPSVFPLAPSCGSTSGSTVALACLVSG
YFPEPVTVSWNSGSLTSGVHTFPSVLQSSGLYSLSSMVTVPSSRWPSETFTCNVAHPASKTKVDKPVPKRENGRVPR
PPDCPKCPAPE**AA**GGPSVFIFPPKPKDTLLIARTPEVTCVVVDLDPEDPEVQISWFVDGKQMQTAKTQPREEQFNGT
YRVVSVLPIGHQDWLKGKQFTCKVNNKALPSPIERTISKARGQAHQPSVYVLPPSREELSKNTVSLTCLIKDFFPPD
IDVEWQSNGQQEPESKYRTTPPQLDEDGSYFLYSKLSVDKSRWQRGDTFICAVMHEALHNHYTQESLSHSPG

Light chain
DIQMTQSPASLSASLGETVSIECLASEGISNSLAWYQLKPGKSPQFLIYATSSLQDGVPSRFSGSGSGTQYSLKISG
MQPEDEGVYYCQQGYKFPLTFGSGTKLKIKRNDAQPAVYLFQPSPDQLHTGSASVVCLLNSFYPKDINVKWKVDGV
IQDTGIQESVTEQDKDSTYSLSSTLTMSSTEYLSHELYSCEITHKSLPSTLIKSFQRSECQRVD

FIG. 4

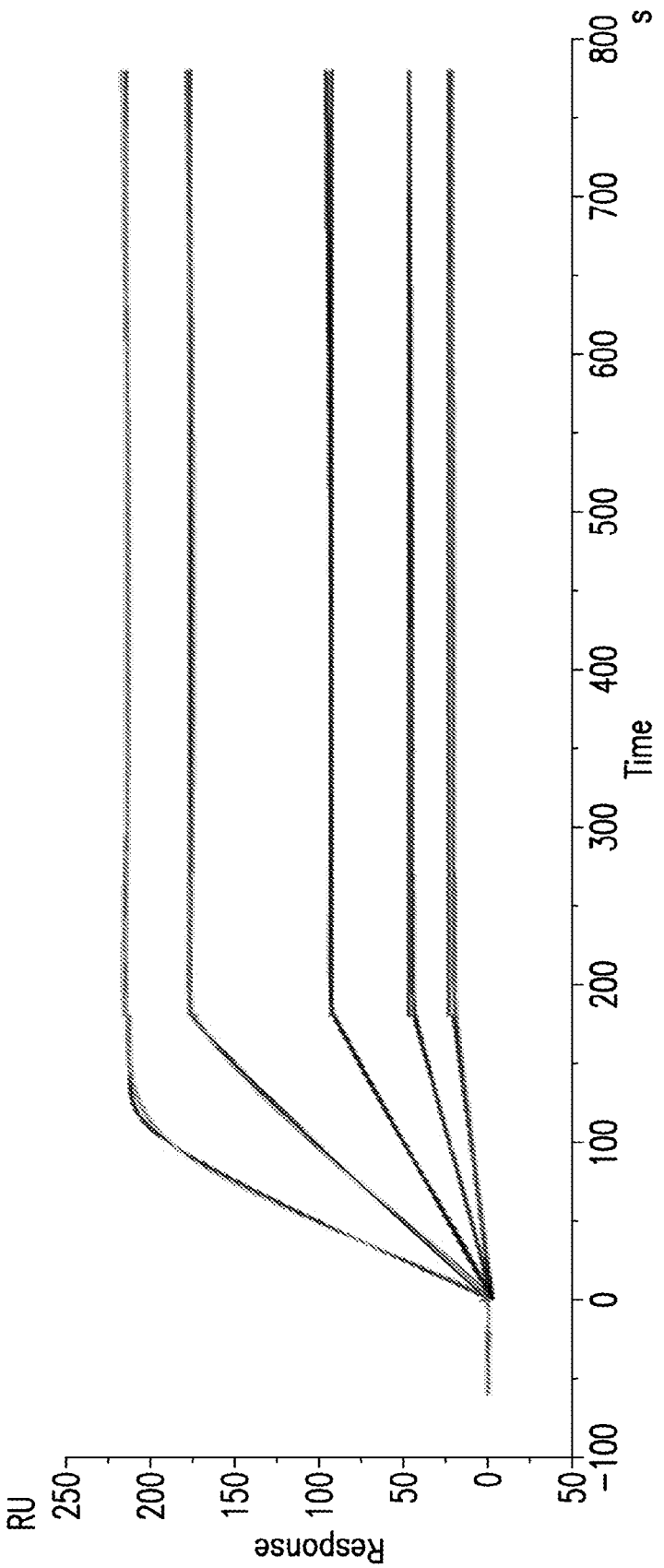

RU
250
200
150
100
50
0
50
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 5

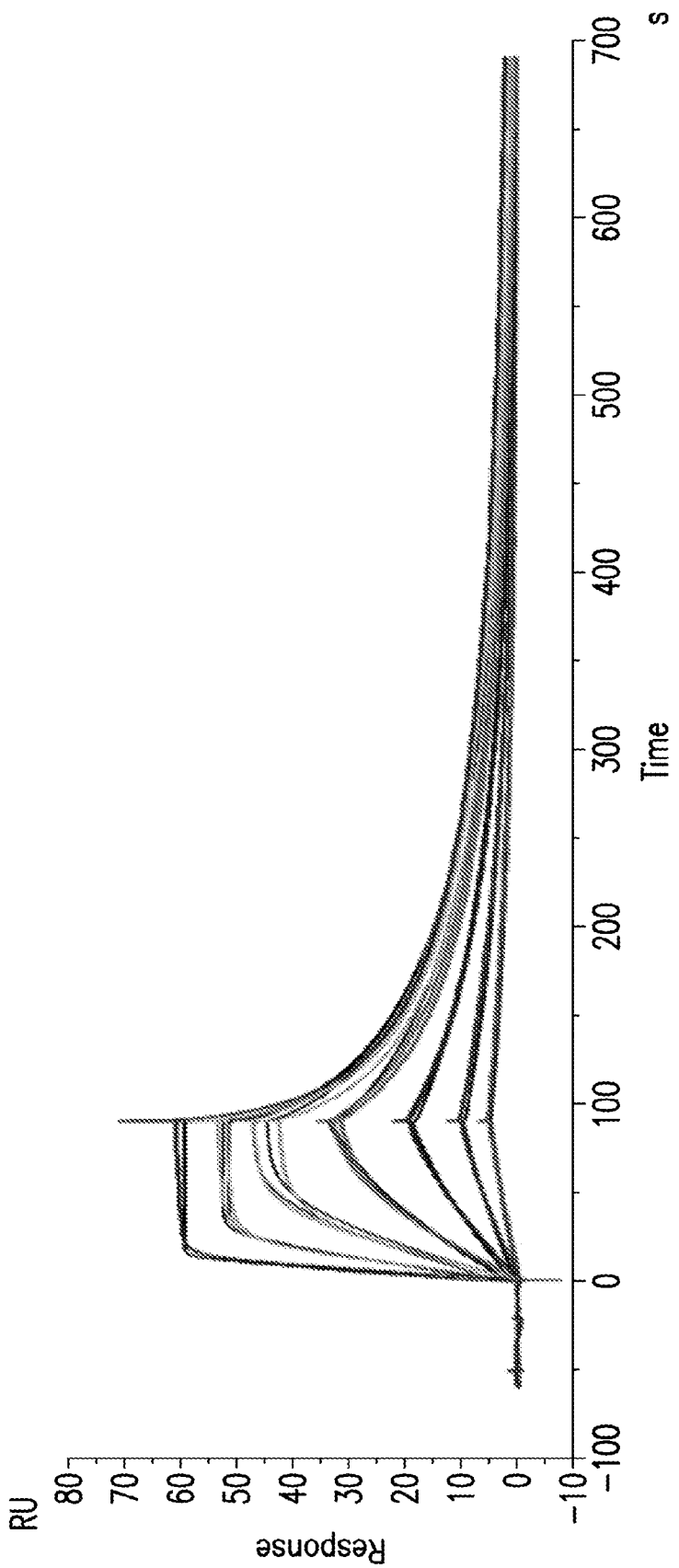

RU
80
70
60
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
s
Time

FIG. 6

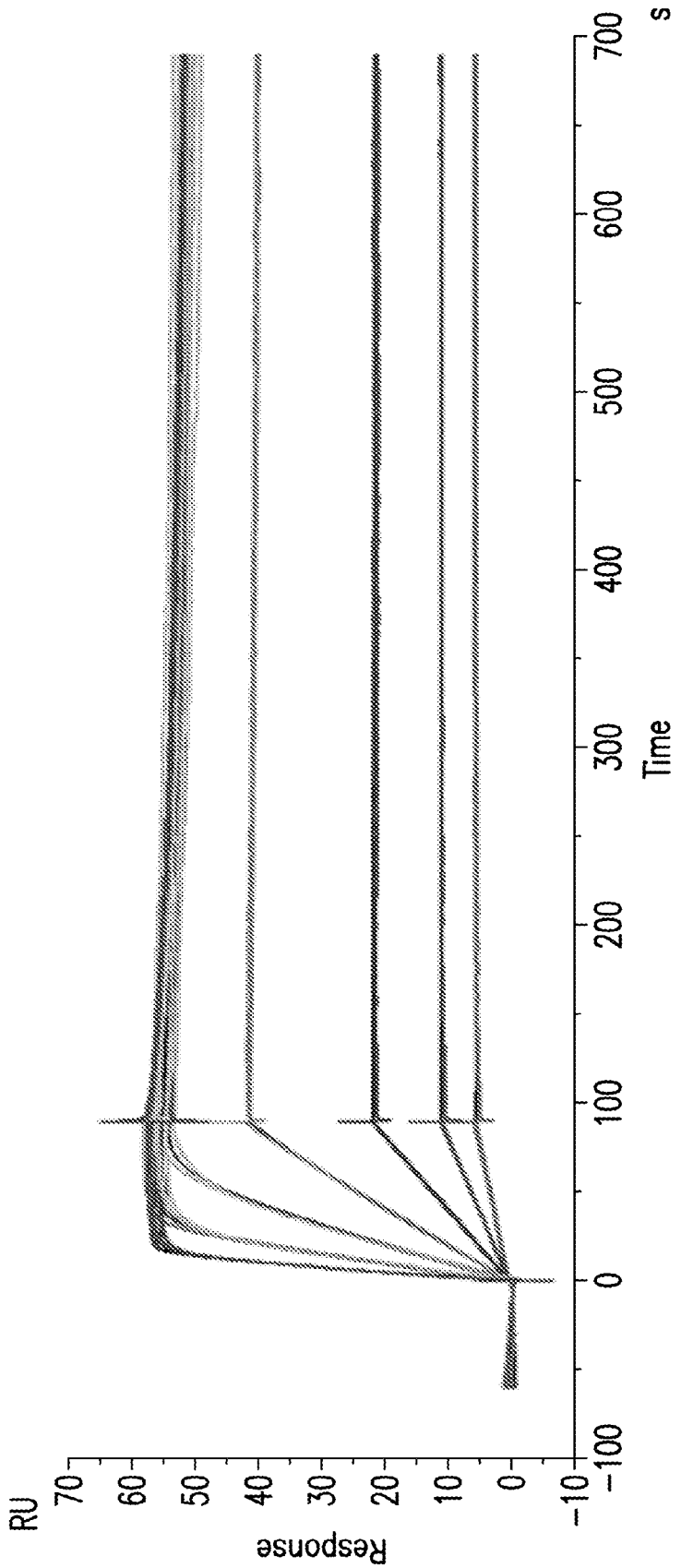

Response
RU
70
60
50
40
30
20
10
0
−10
−100
0
100
200
300
400
500
600
700
Time
s

FIG. 7

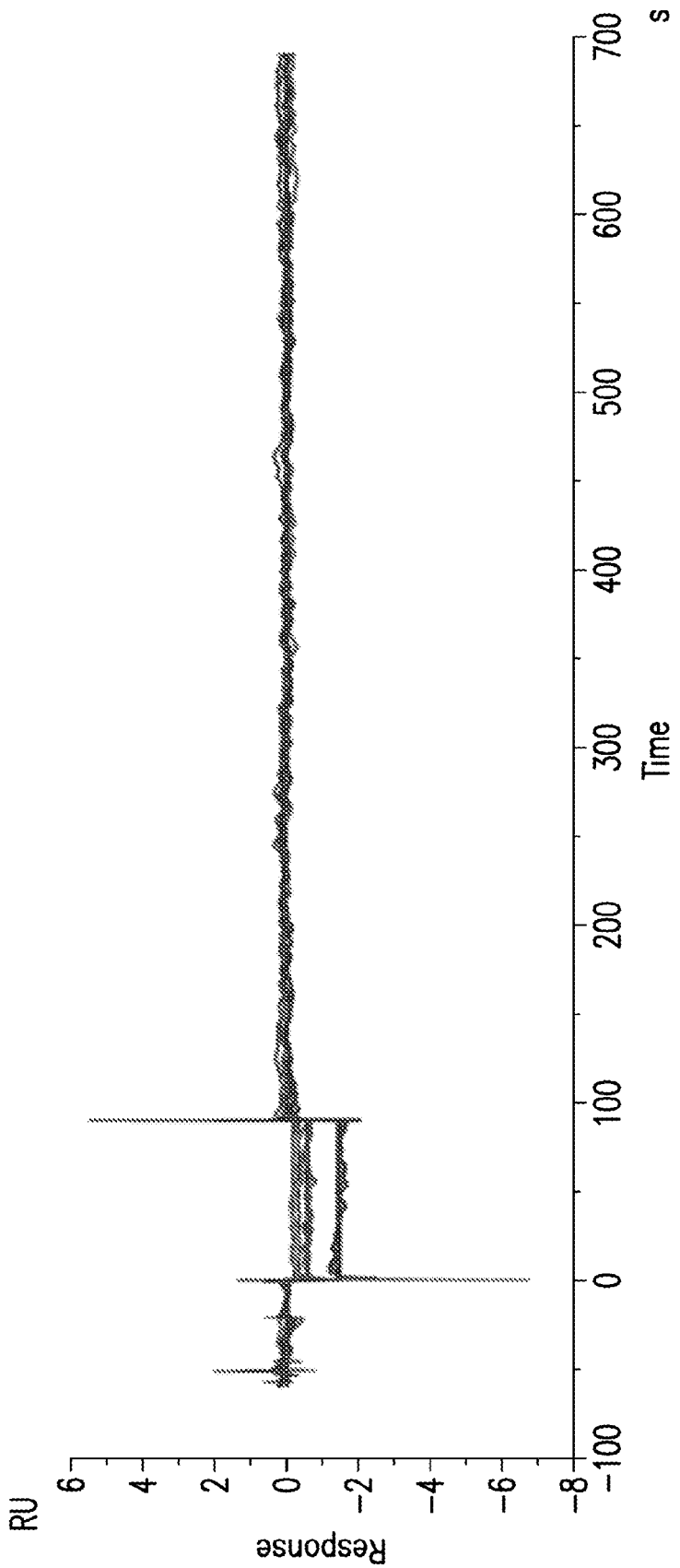


FIG. 8

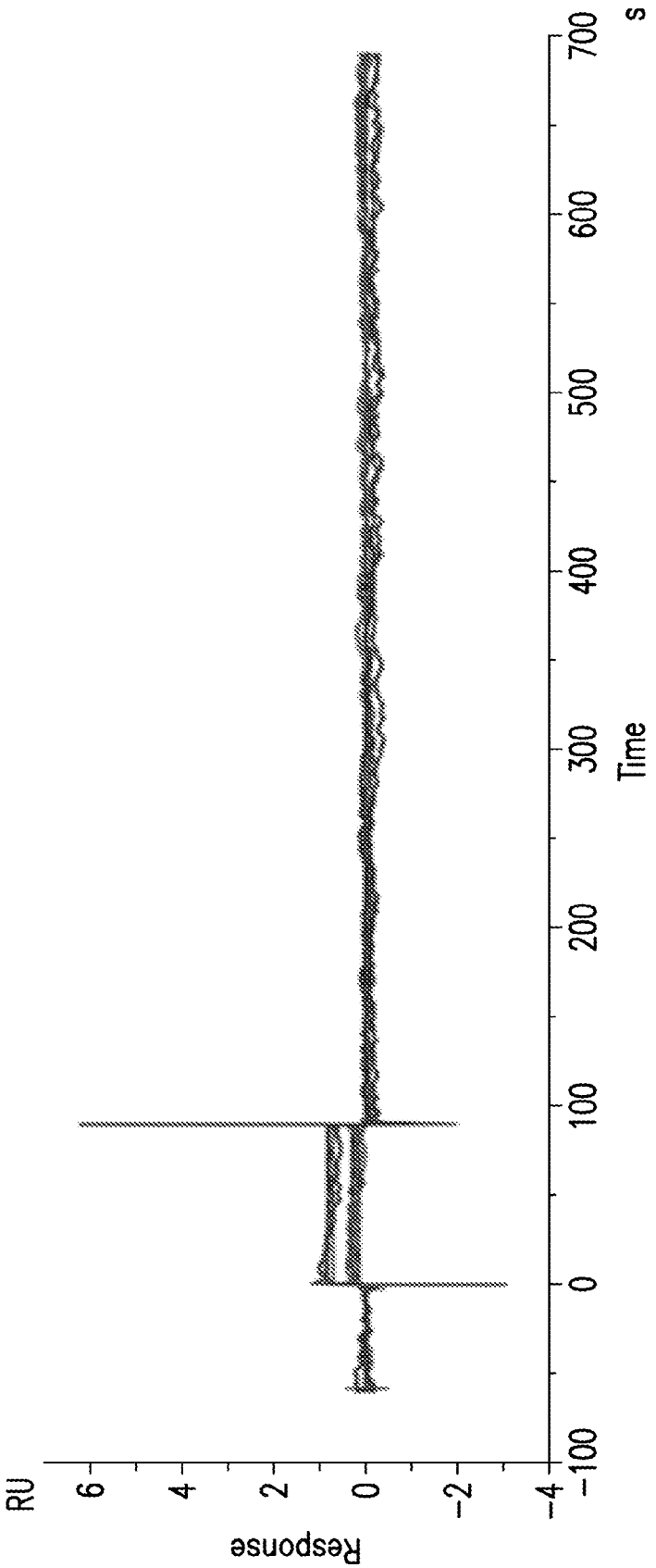


FIG. 9

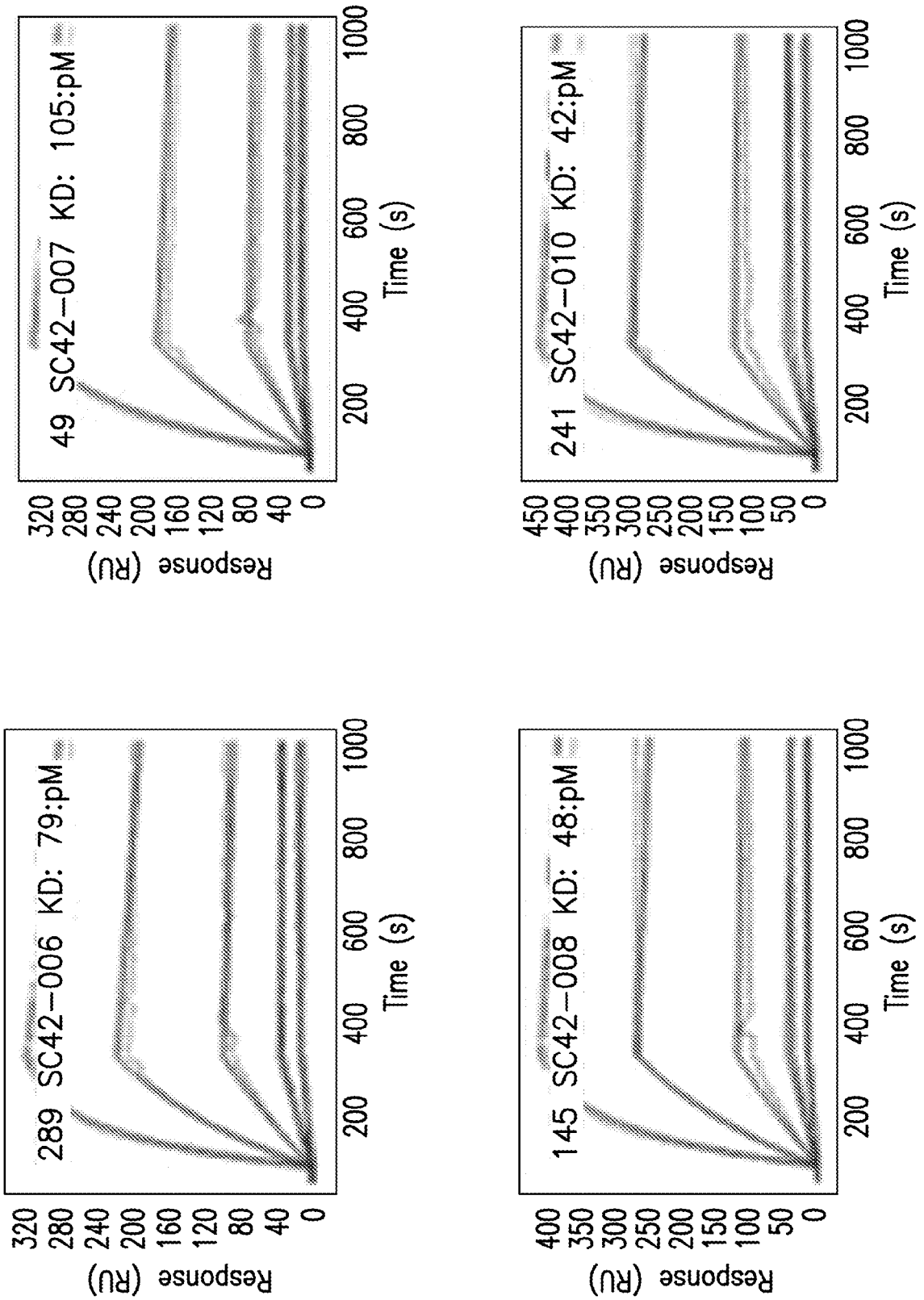

49 SC42-007 KD: 105:pM
241 SC42-010 KD: 42:pM
289 SC42-006 KD: 79:pM
145 SC42-008 KD: 48:pM
Response (RU)
Time (s)
0
40
80
120
160
200
240
280
320
50
100
150
200
250
300
350
400
450
200
400
600
800
1000

FIG. 10

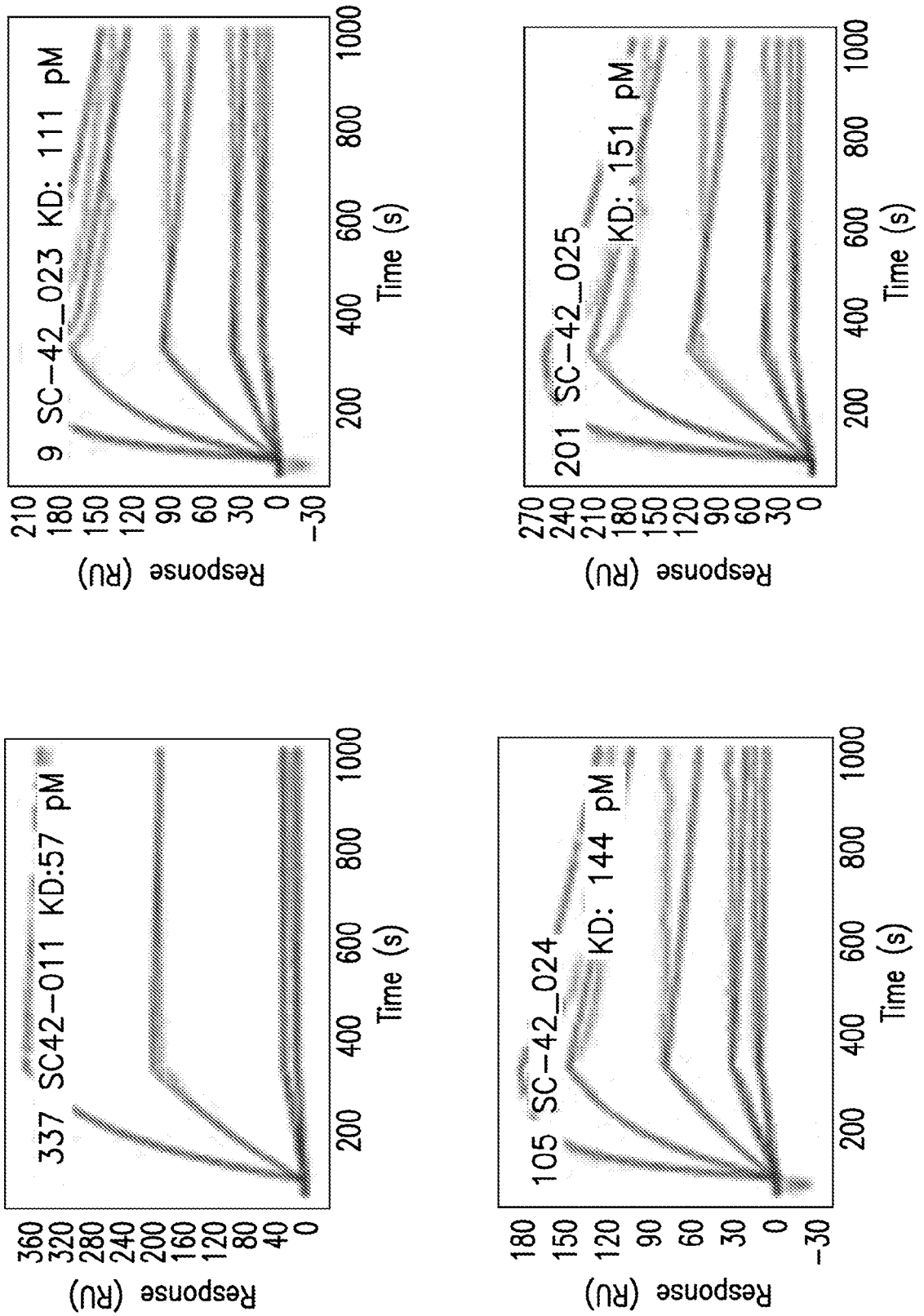

9 SC-42_023 KD: 111 pM
201 SC-42_025 KD: 151 pM
337 SC42-011 KD:57 pM
105 SC-42_024 KD: 144 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

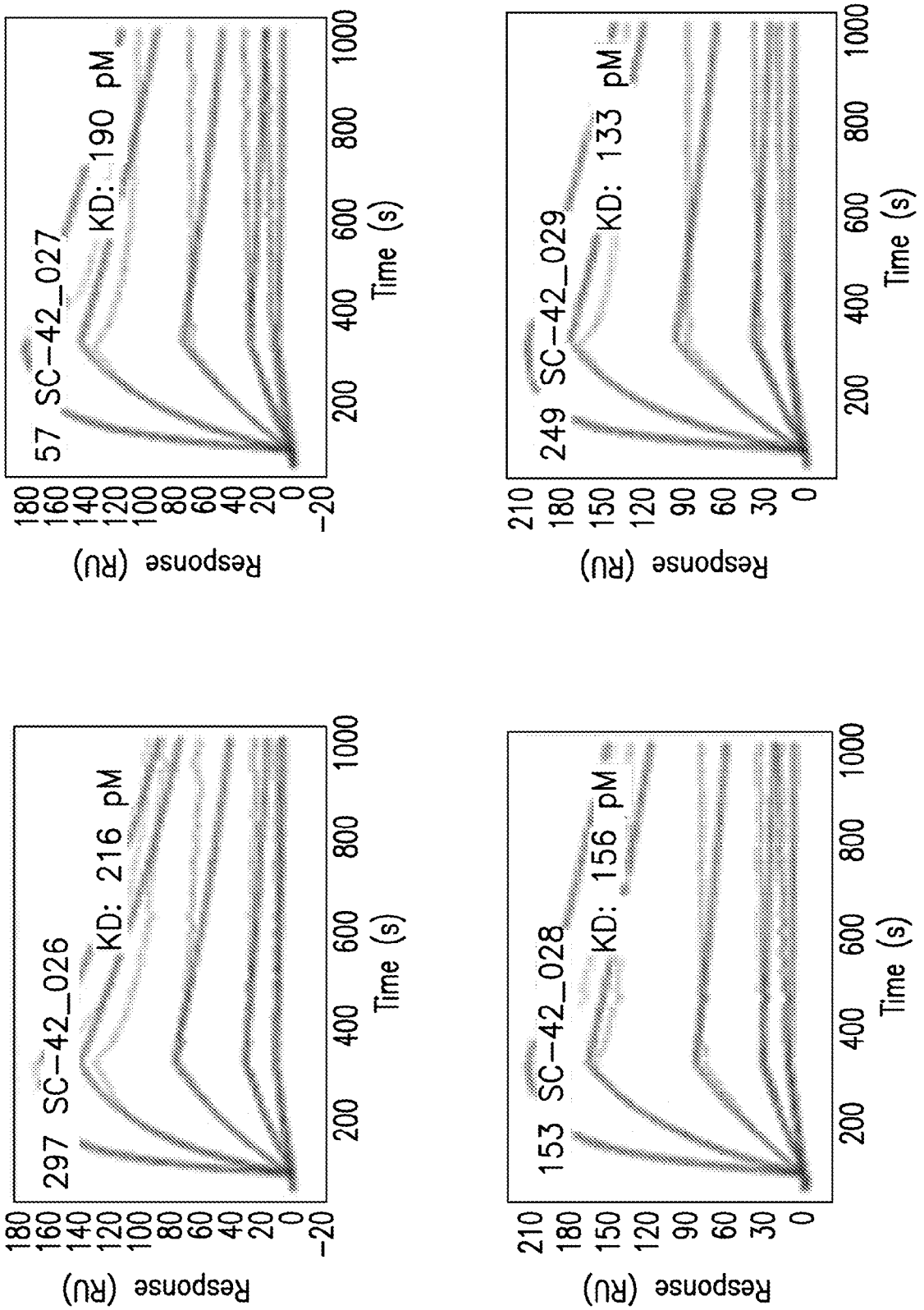

57 SC-42_027
KD: 190 pM
249 SC-42_029
KD: 133 pM
297 SC-42_026
KD: 216 pM
153 SC-42_028
KD: 156 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

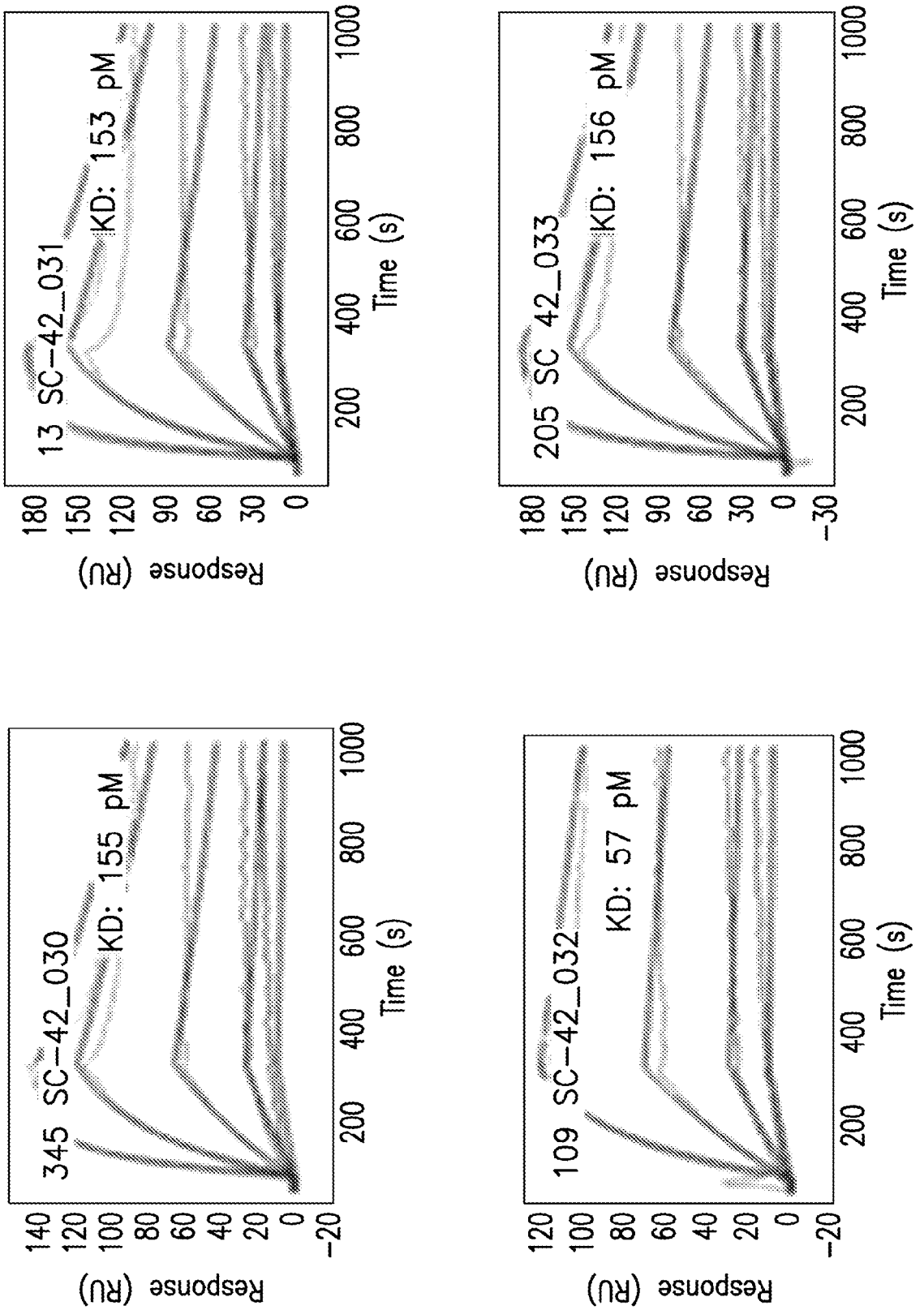

13 SC-42_031
KD: 153 pM
205 SC 42_033
KD: 156 pM
345 SC-42_030
KD: 155 pM
109 SC-42_032
KD: 57 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

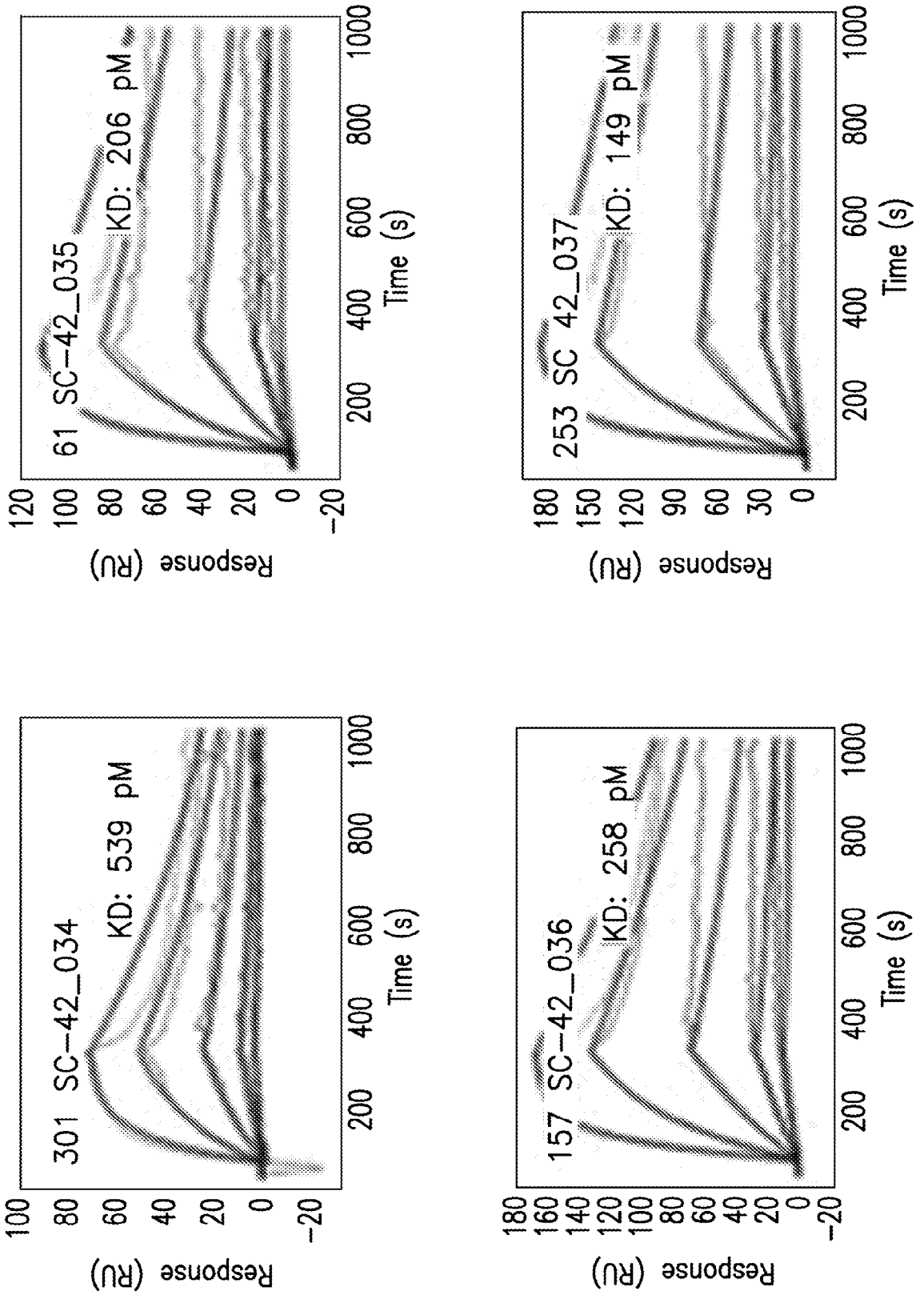

61 SC-42_035
KD: 206 pM
253 SC 42_037
KD: 149 pM
301 SC-42_034
KD: 539 pM
157 SC-42_036
KD: 258 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

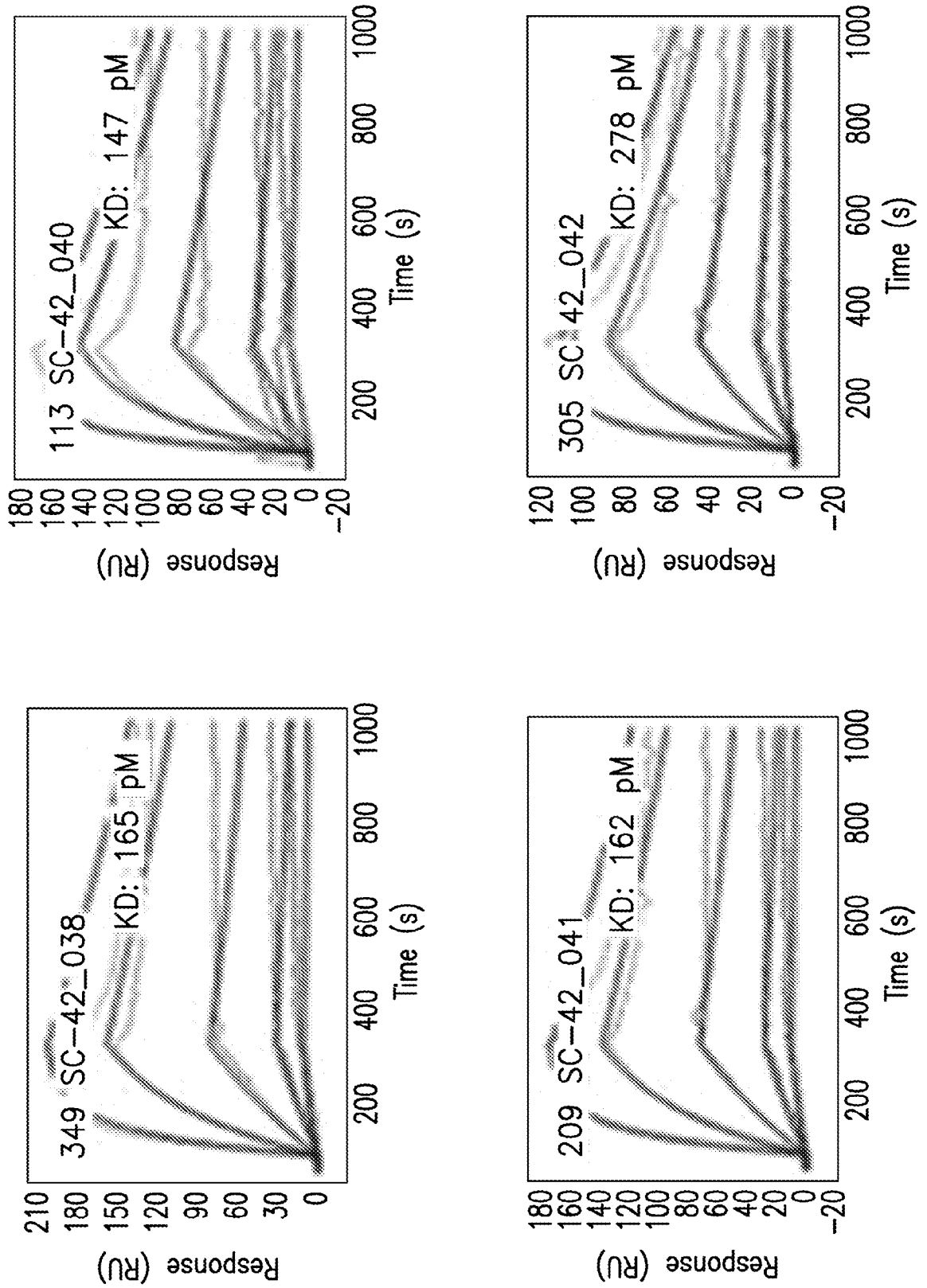

113 SC-42_040
KD: 147 pM
305 SC-42_042
KD: 278 pM
349 SC-42_038
KD: 165 pM
209 SC-42_041
KD: 162 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

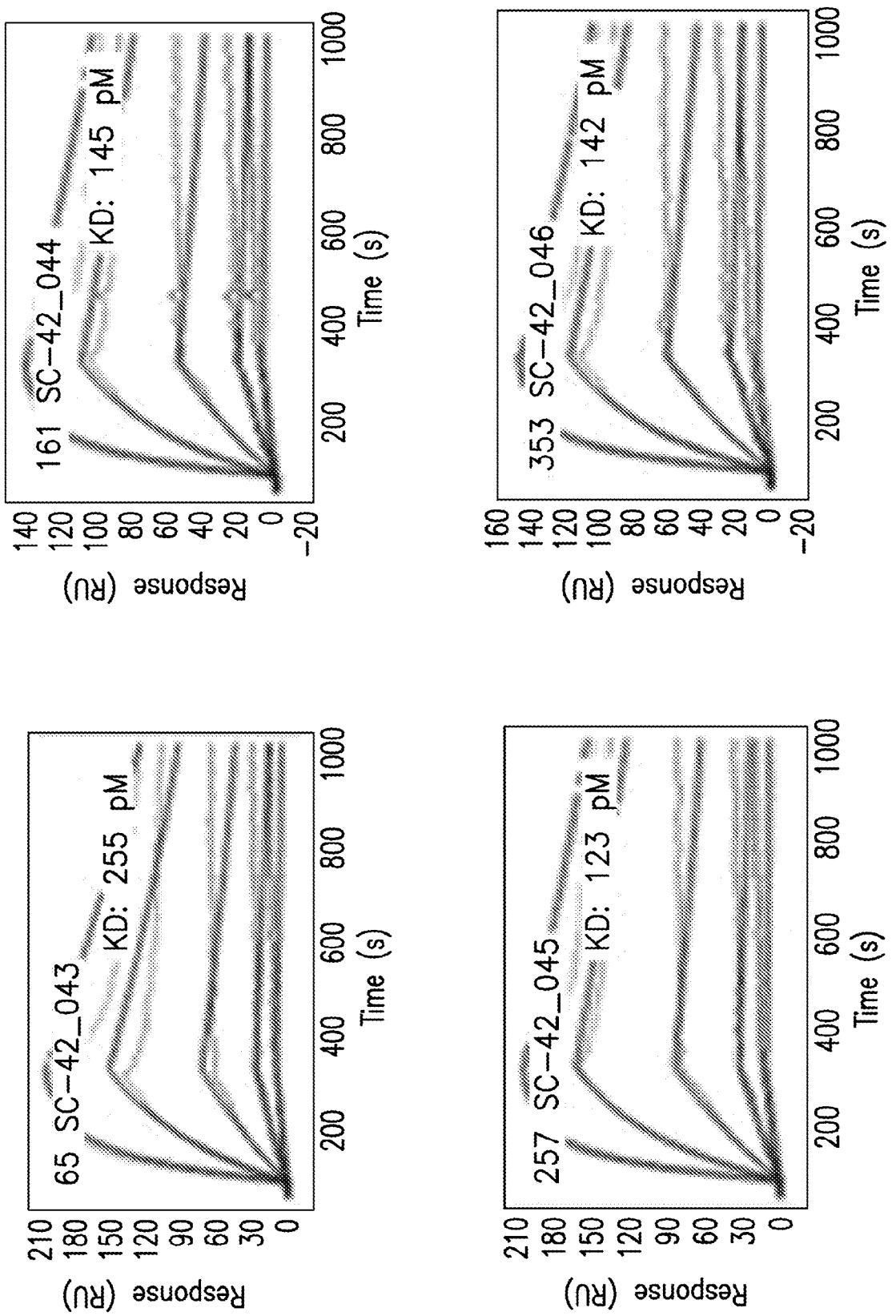

161 SC-42_044
KD: 145 pM
353 SC-42_046
KD: 142 pM
65 SC-42_043
KD: 255 pM
257 SC-42_045
KD: 123 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

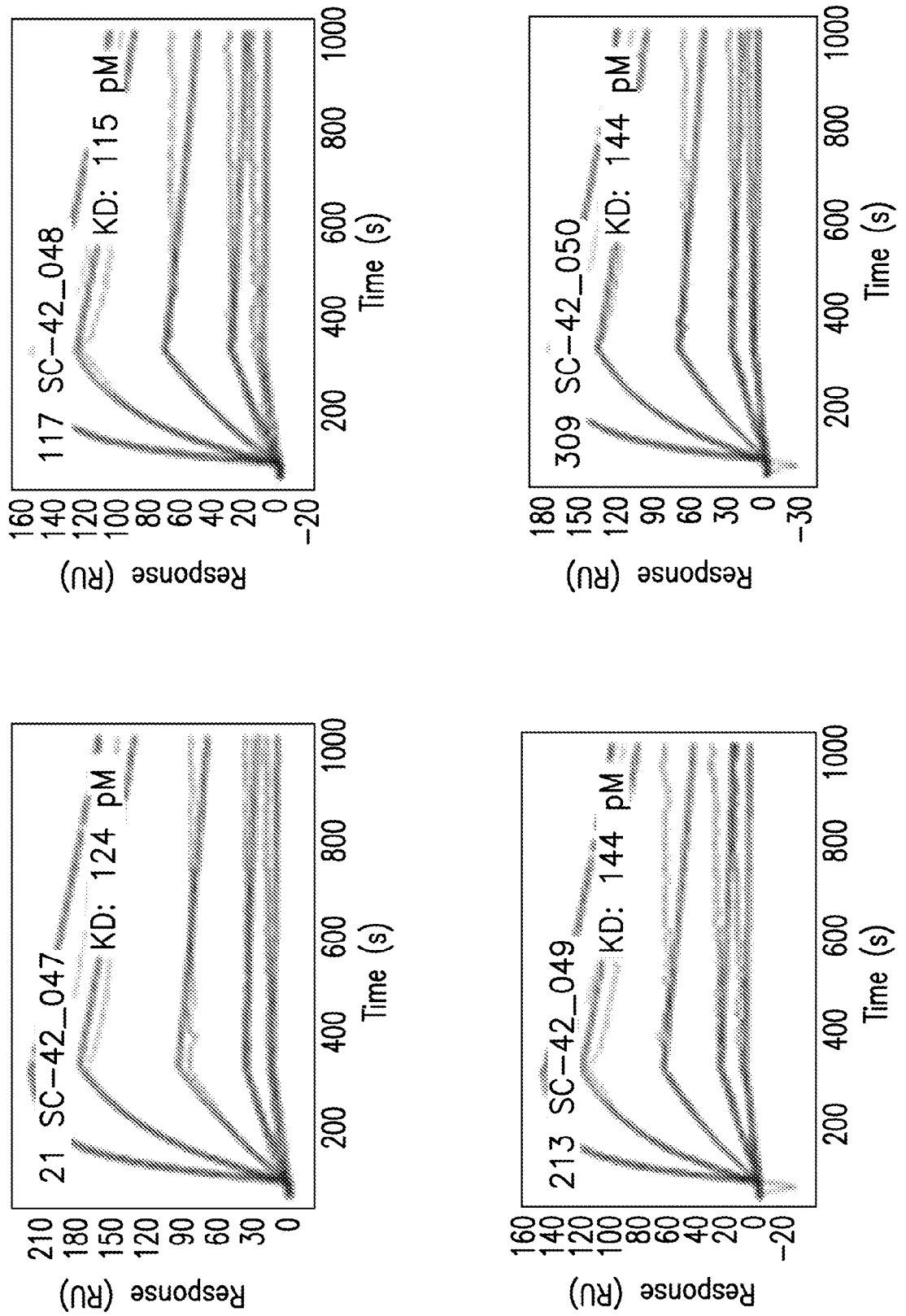

117 SC-42_048
KD: 115 pM
309 SC-42_050
KD: 144 pM
21 SC-42_047
KD: 124 pM
213 SC-42_049
KD: 144 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

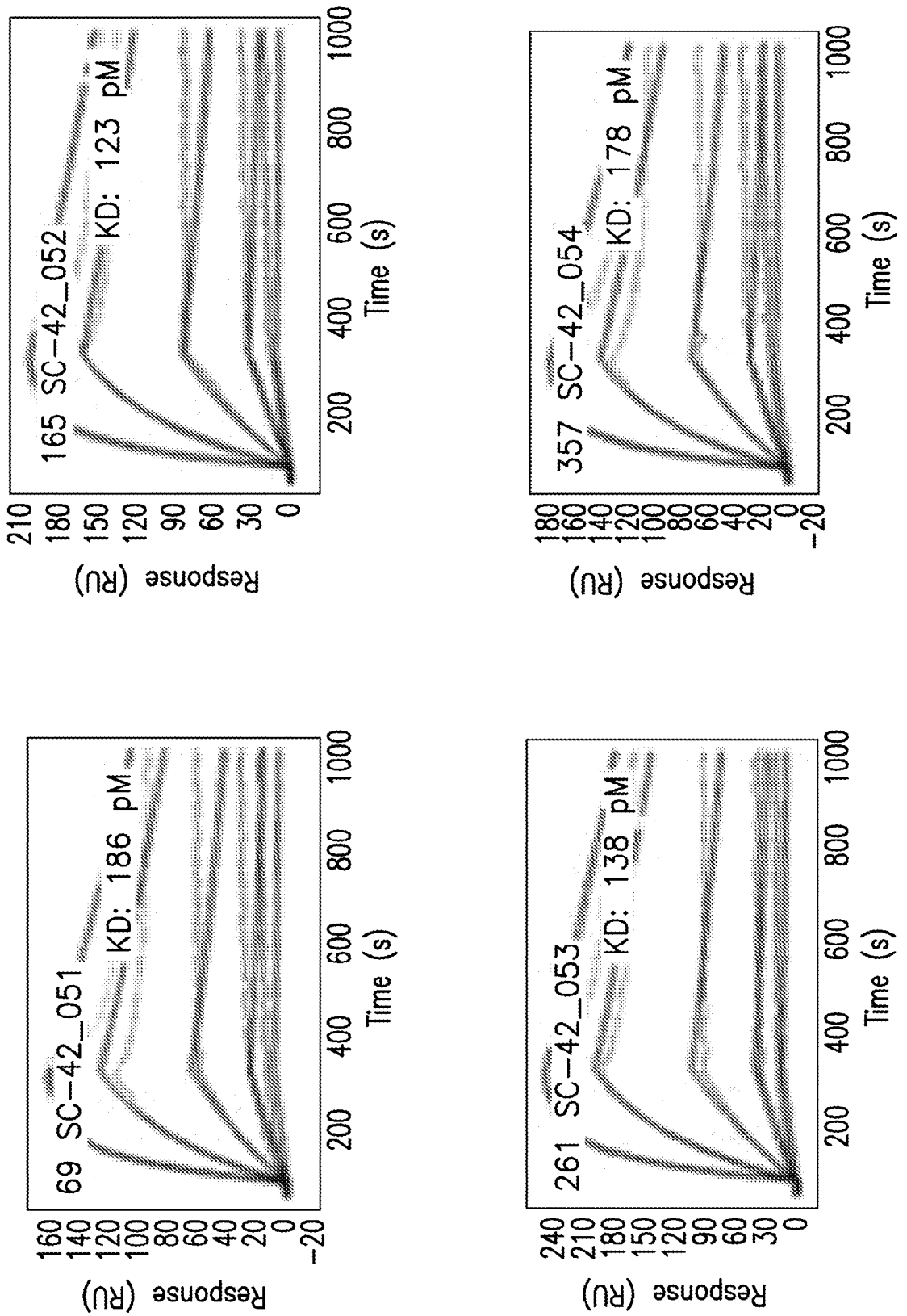

165 SC-42_052
KD: 123 pM
357 SC-42_054
KD: 178 pM
69 SC-42_051
KD: 186 pM
261 SC-42_053
KD: 138 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

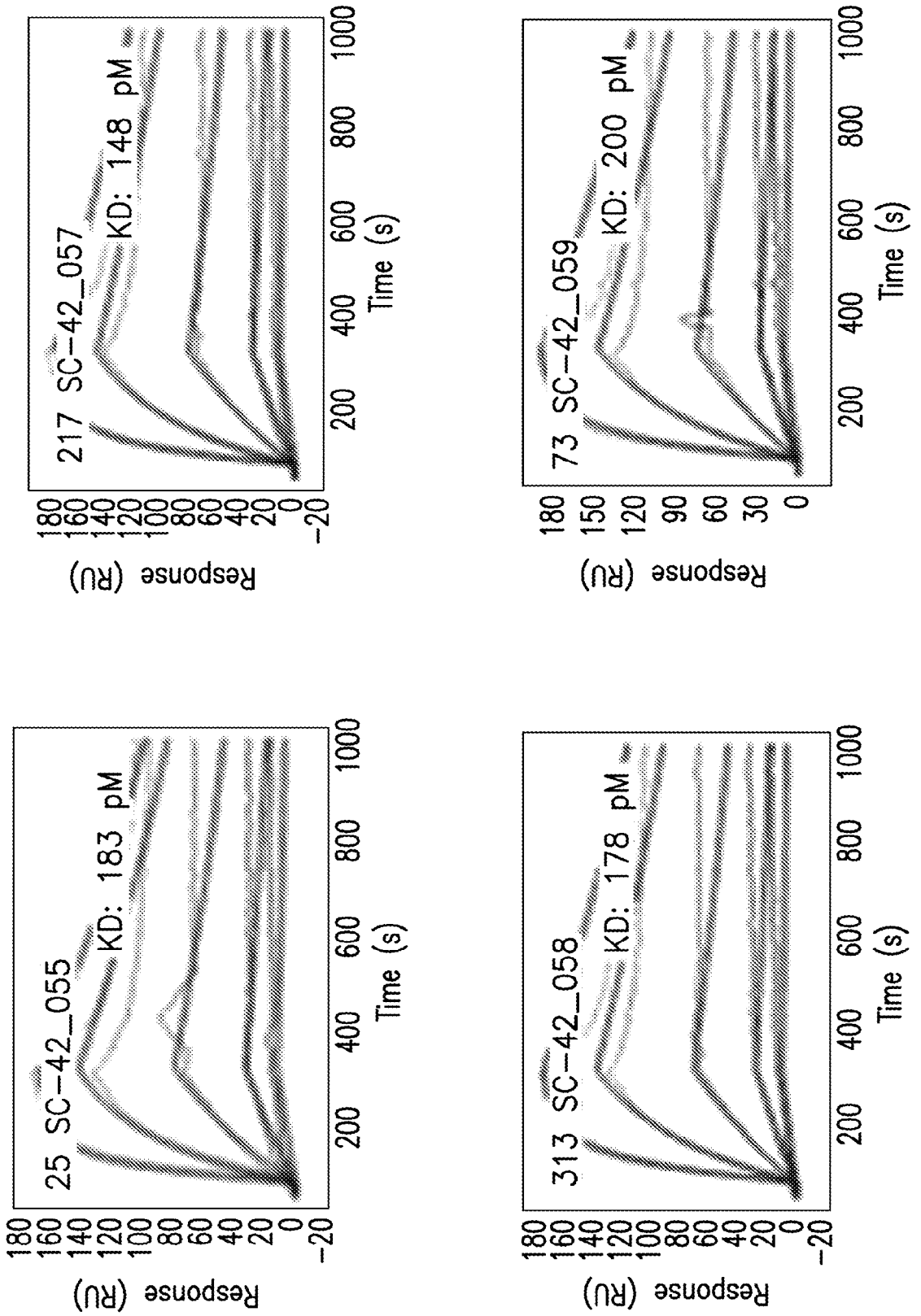

217 SC-42_057
KD: 148 pM
73 SC-42_059
KD: 200 pM
25 SC-42_055
KD: 183 pM
313 SC-42_058
KD: 178 pM
Response (RU)
Time (s)
180
160
140
120
100
80
60
40
20
0
-20
150
90
30
200
400
600
800
1000

FIG. 10 Cont'd

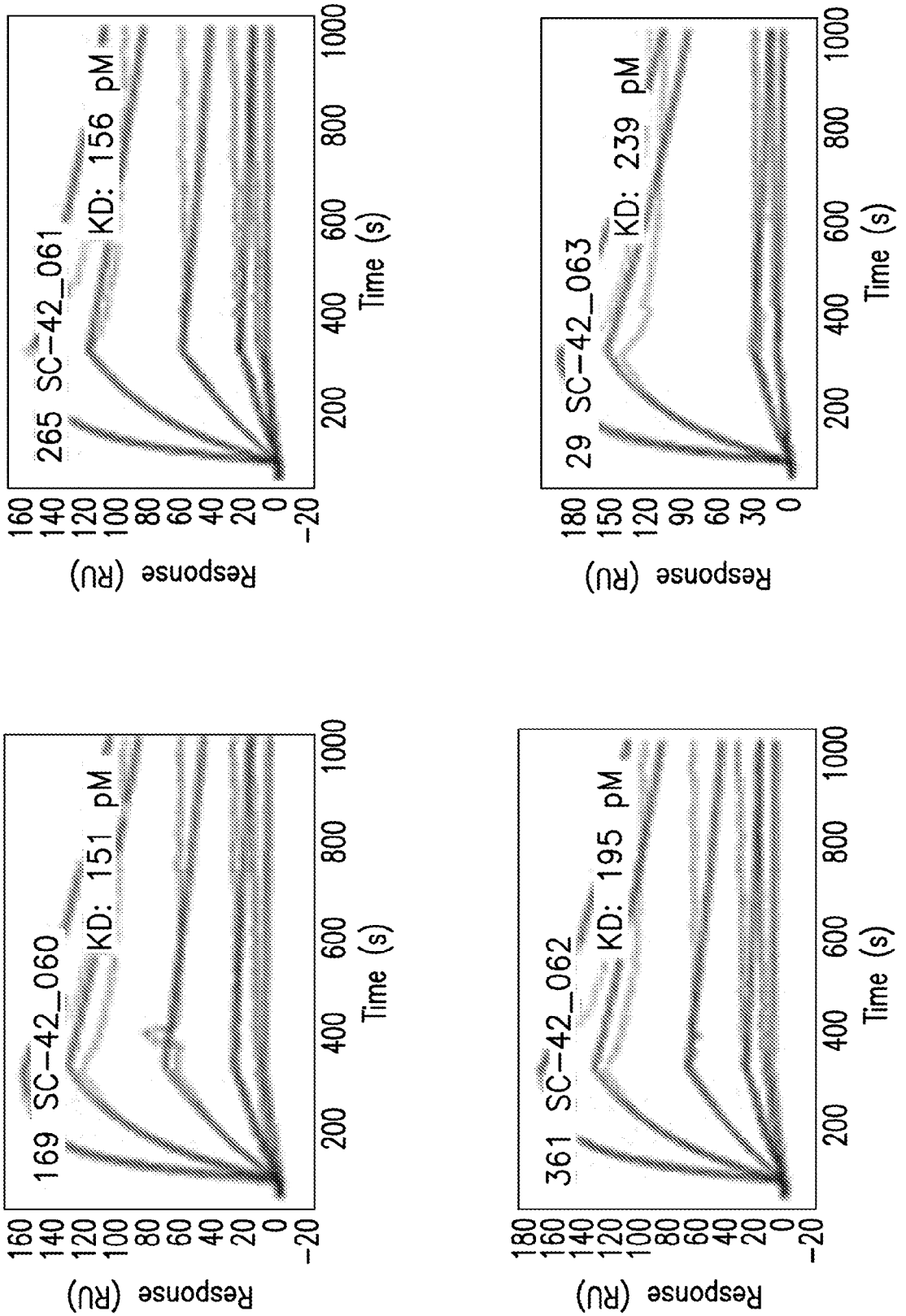

265 SC-42_061
KD: 156 pM
29 SC-42_063
KD: 239 pM
169 SC-42_060
KD: 151 pM
361 SC-42_062
KD: 195 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

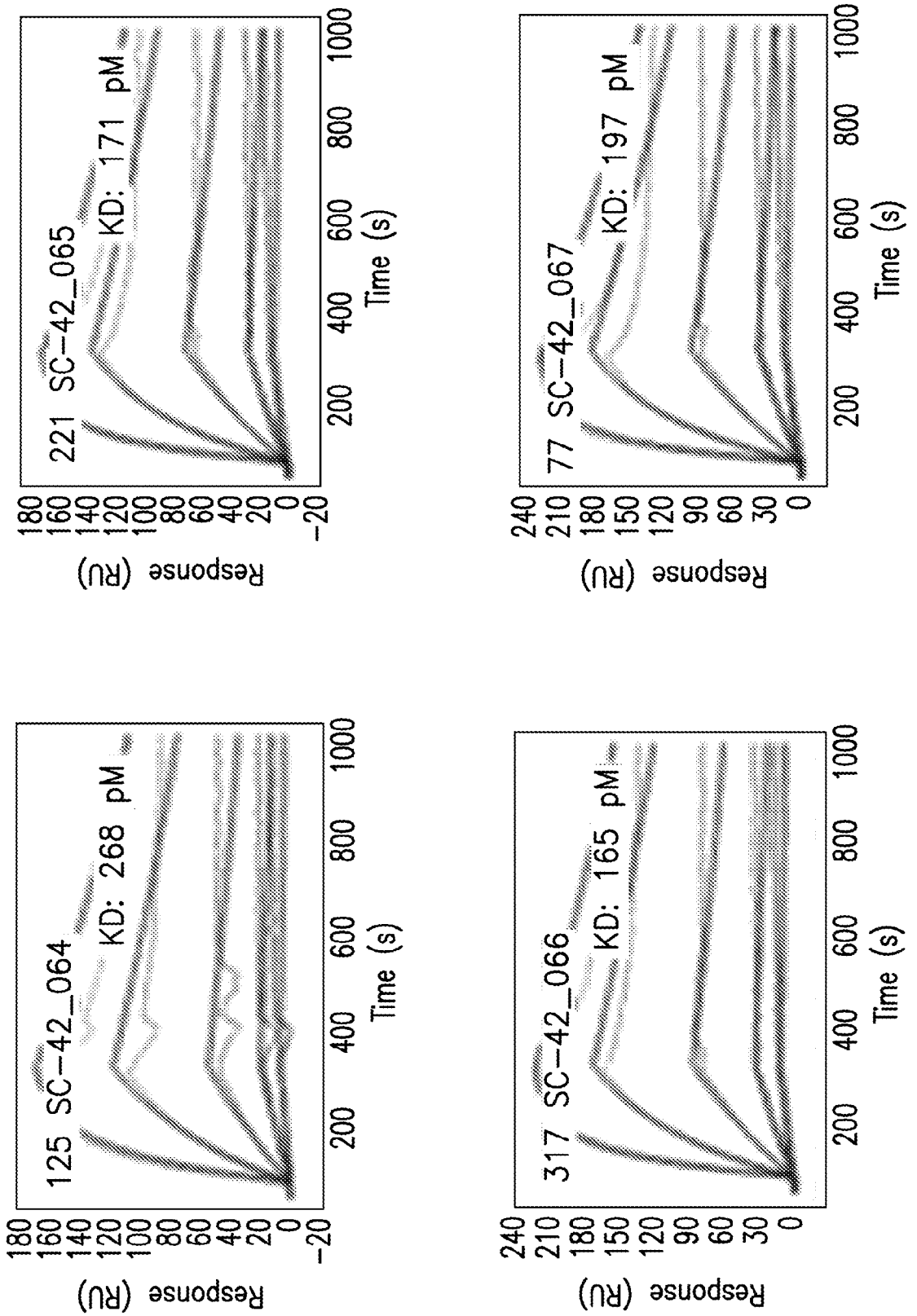

221 SC-42_065
KD: 171 pM
77 SC-42_067
KD: 197 pM
125 SC-42_064
KD: 268 pM
317 SC-42_066
KD: 165 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

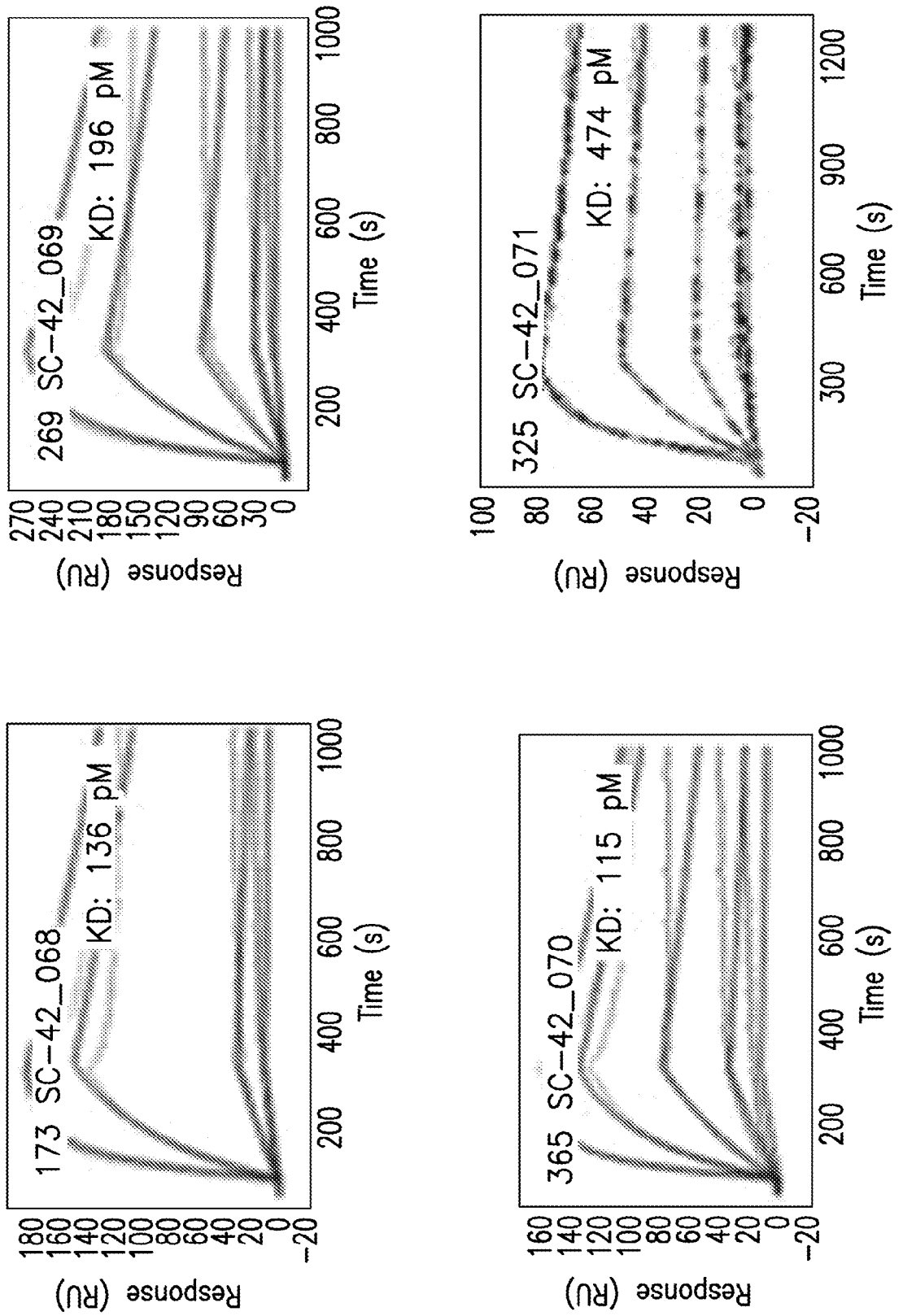

269 SC-42_069
KD: 196 pM
325 SC-42_071
KD: 474 pM
173 SC-42_068
KD: 136 pM
365 SC-42_070
KD: 115 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

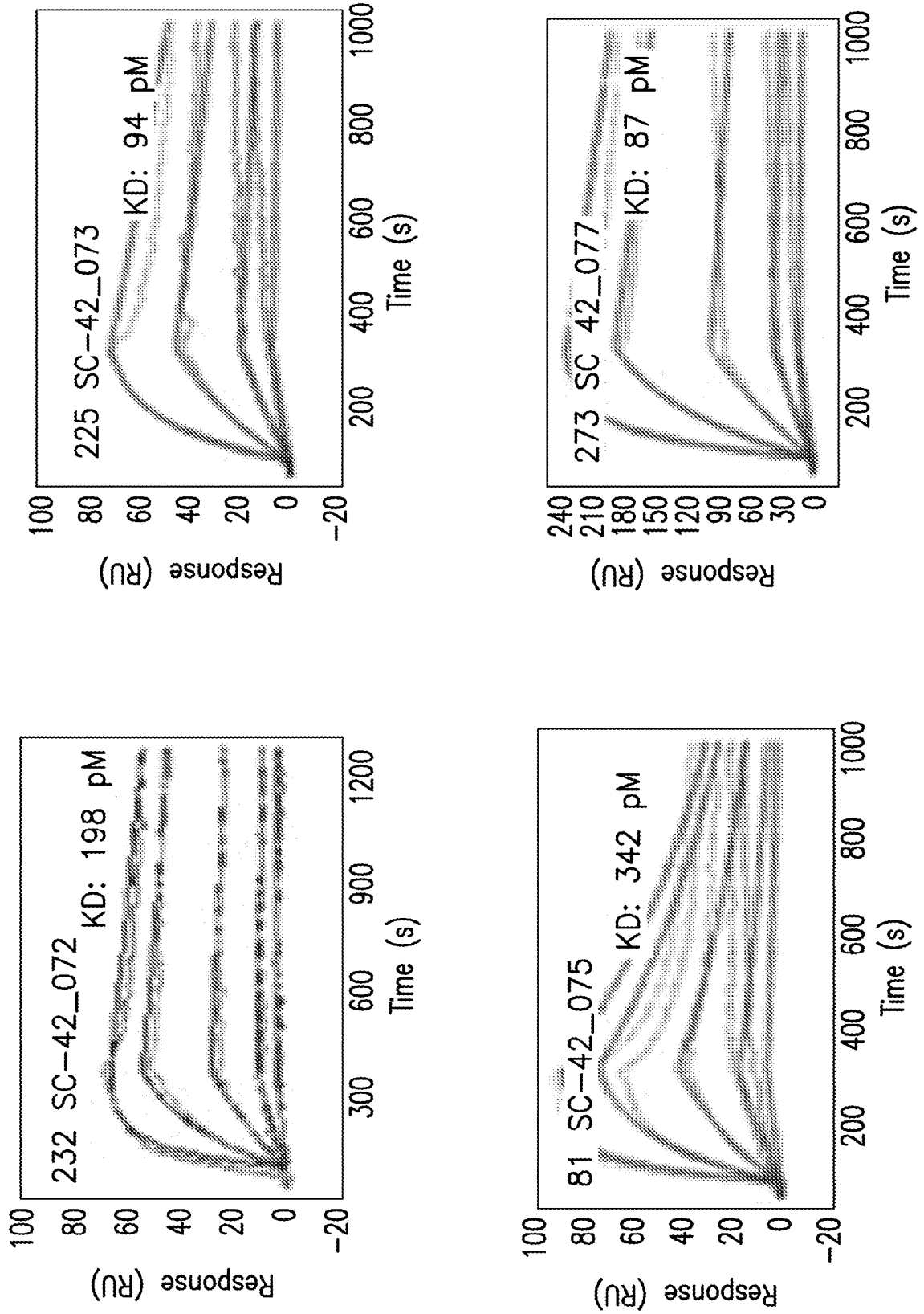

225 SC-42_073
KD: 94 pM
273 SC 42_077
KD: 87 pM
232 SC-42_072
KD: 198 pM
81 SC-42_075
KD: 342 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

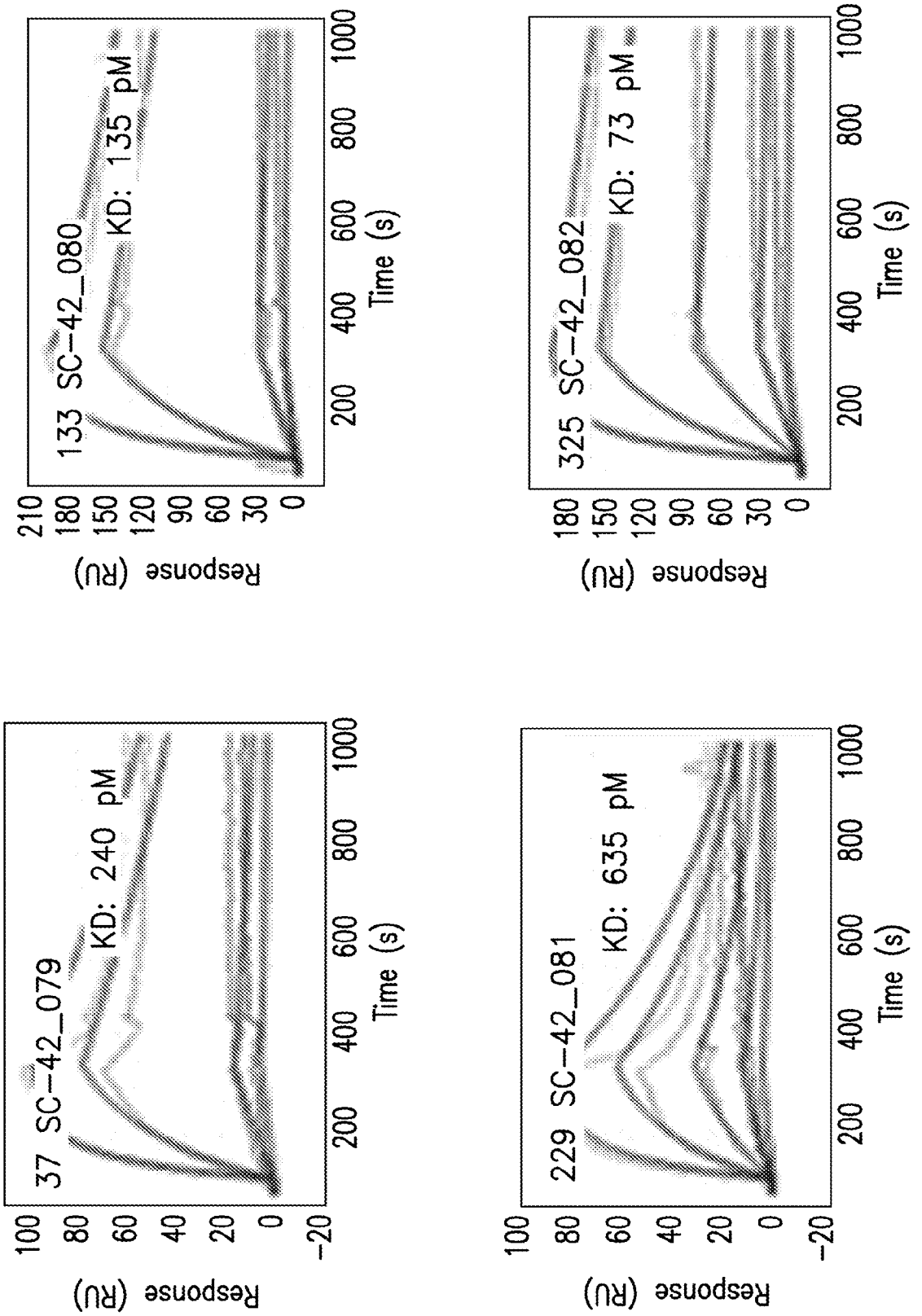

133 SC-42_080
KD: 135 pM
325 SC-42_082
KD: 73 pM
37 SC-42_079
KD: 240 pM
229 SC-42_081
KD: 635 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

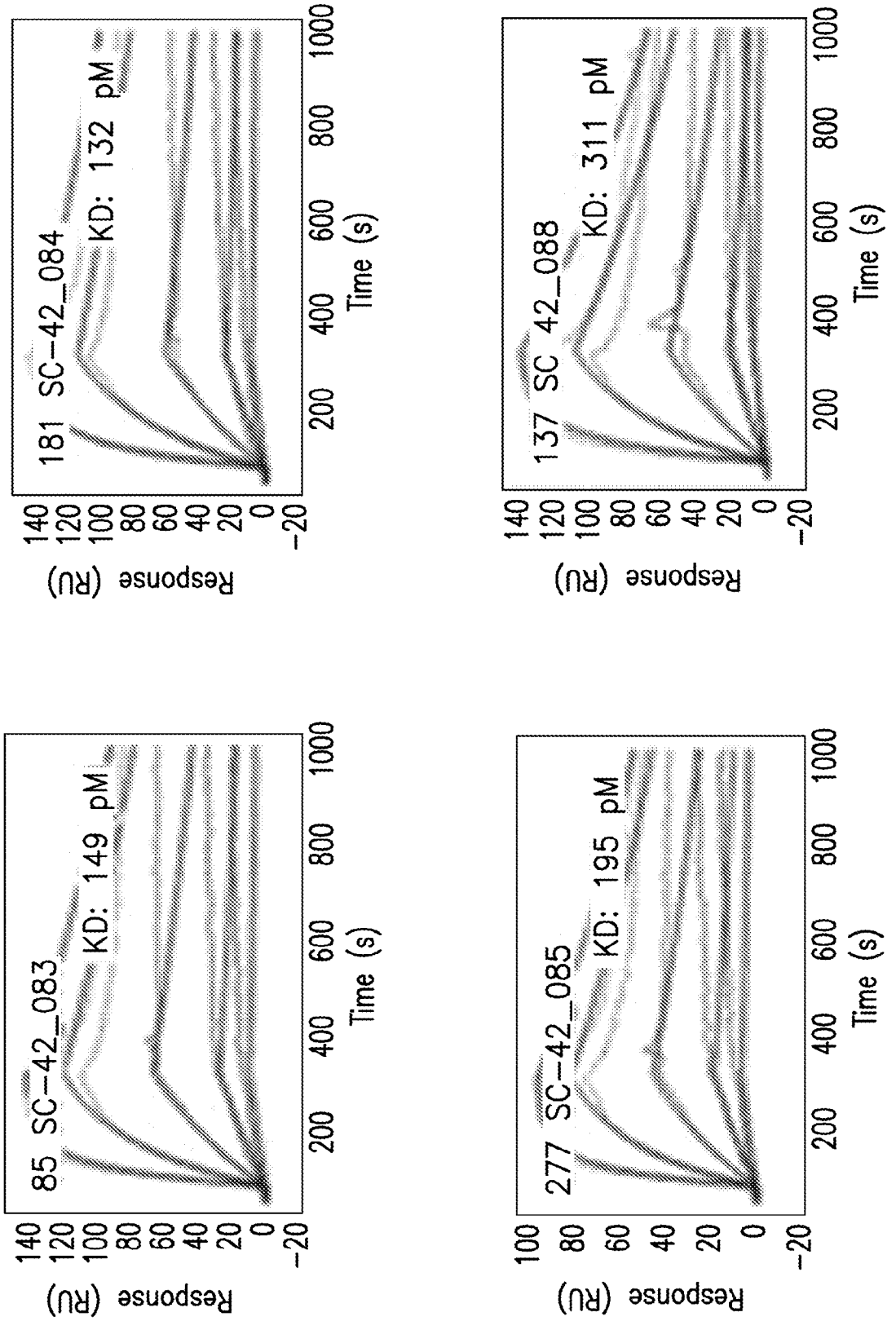

181 SC-42_084
KD: 132 pM
137 SC 42_088
KD: 311 pM
85 SC-42_083
KD: 149 pM
277 SC-42_085
KD: 195 pM
Response (RU)
Time (s)

FIG. 10 Cont'd

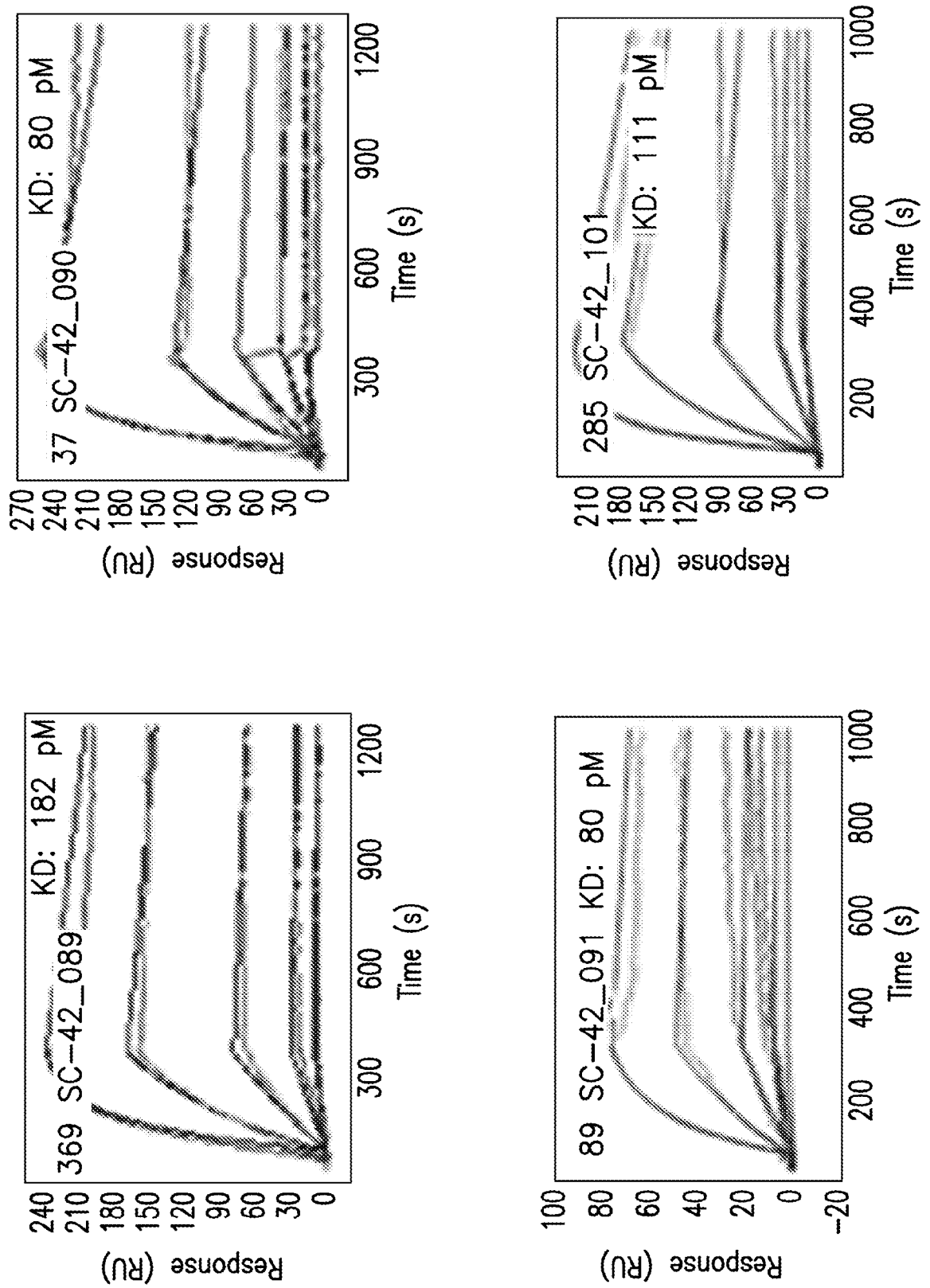
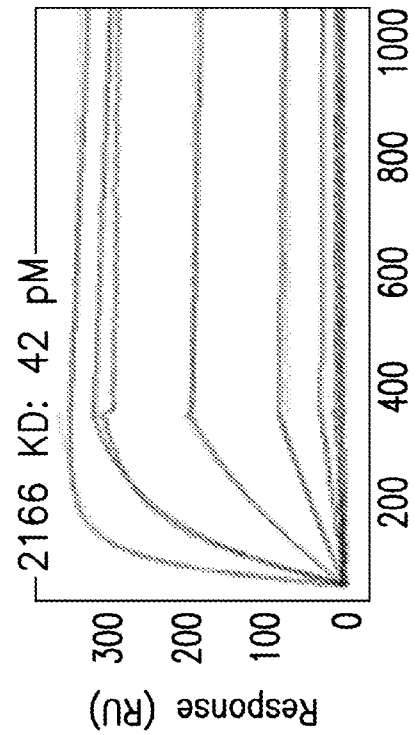

37 SC-42_090 KD: 80 pM
285 SC-42_101 KD: 111 pM
369 SC-42_089 KD: 182 pM
89 SC-42_091 KD: 80 pM
Response (RU)
Time (s)

FIG. 10 Cont'd


2166 KD: 42 pM
Response (RU)
300
200
100
0
200
400
600
800
1000
Time (s)

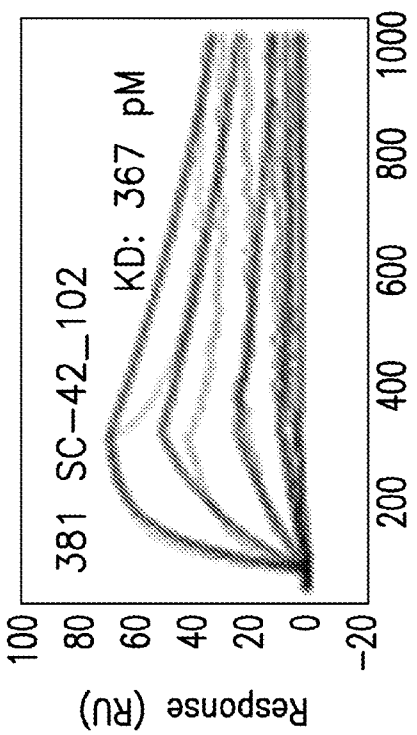

381 SC-42_102
KD: 367 pM
Response (RU)
100
80
60
40
20
0
-20
200
400
600
800
1000
Time (s)

FIG. 10 Cont'd

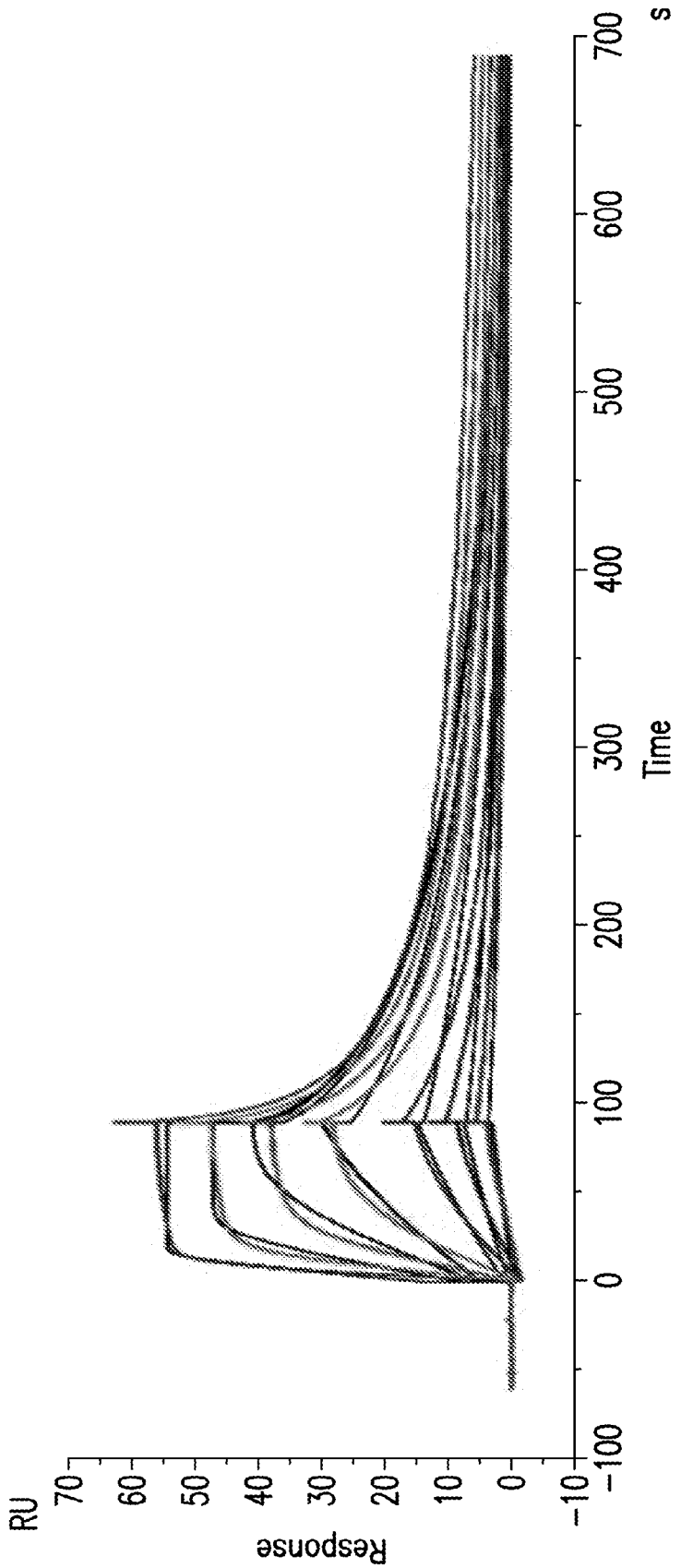

RU
70
60
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
s
Time

FIG. 11

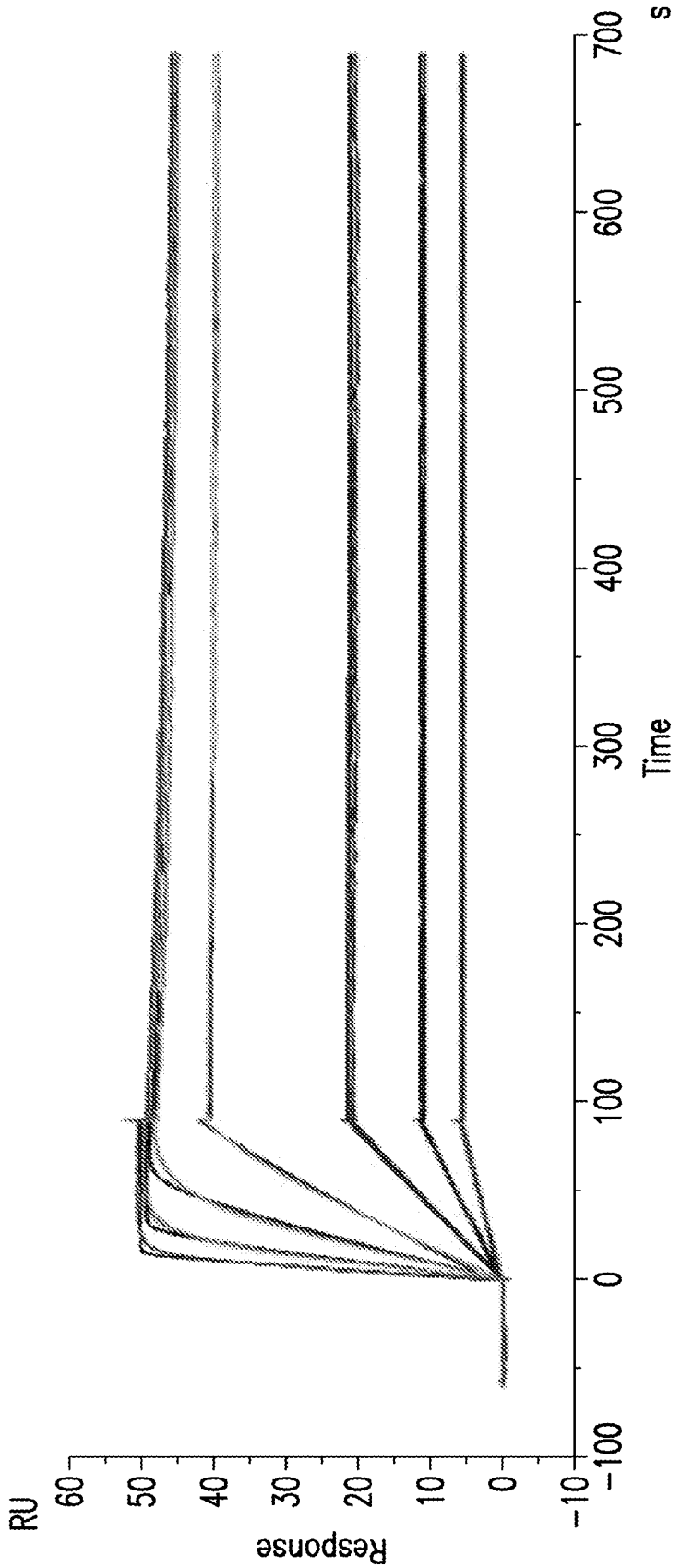

RU
60
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
s
Time

FIG. 12

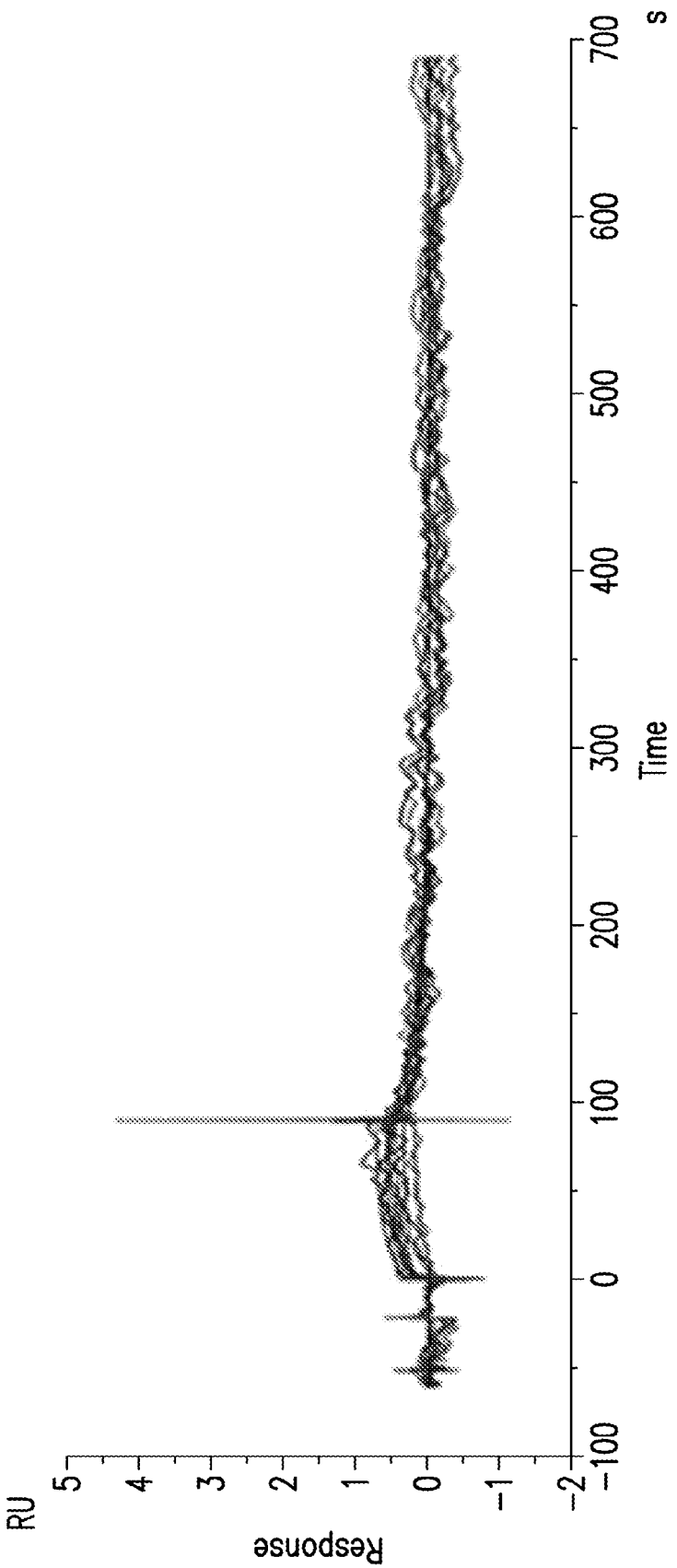


FIG. 13

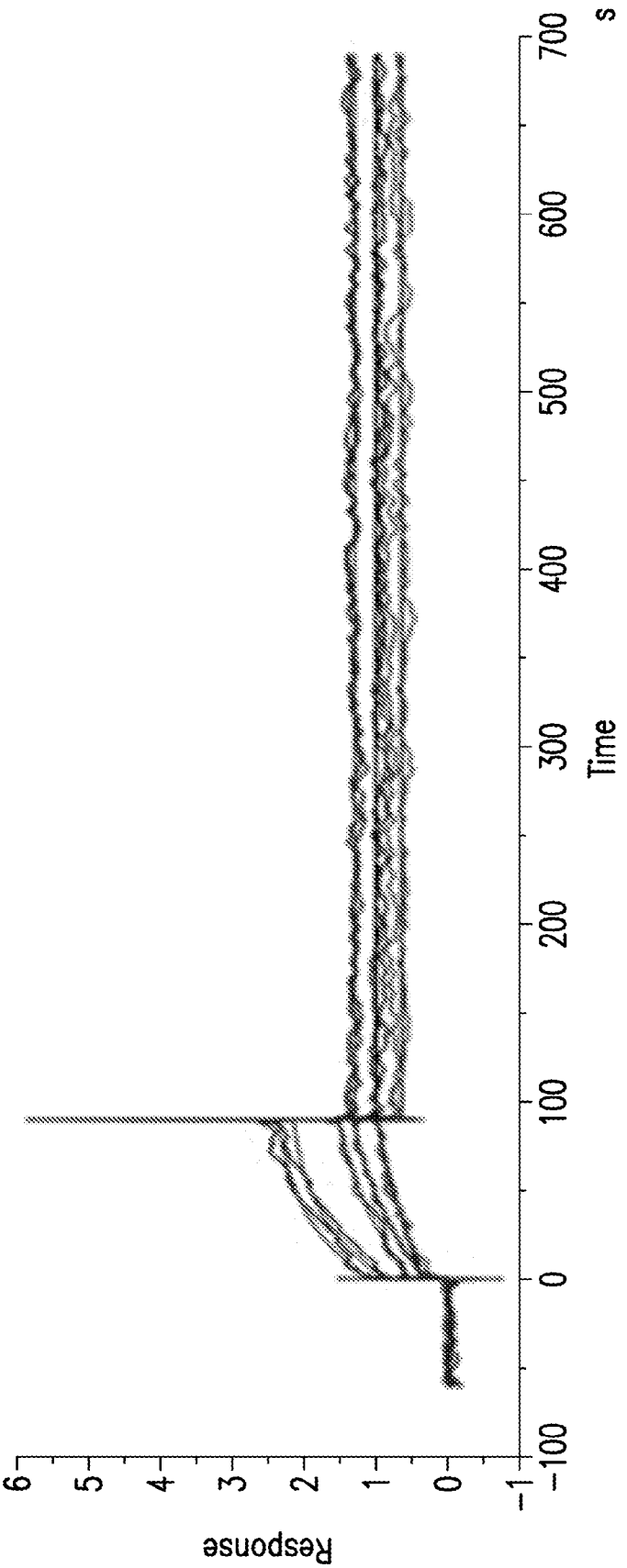


FIG. 14

Heavy Chain Variable Domain
DVQLVESGGDLVKPGGSLRLTCVASGLSLTSSSMSWVRQAPGKGLQWVSTIYSNGGTYYTDSVKGRFTISKDN
AENTLYLQMNNLKTEDTATYYCASIYYYDADYLHWYFDFWGQGALVTVSS

Light Chain Variable Domain
EIQMTQSPTSLSASVGDRVTITCRASEGISNNLSWYQQTPGKAPKLLIYATSNLHSGVPSRFSGSGSGTDFTL
TISSLQPEDFATYYCQQGYKWPLTFGGGTKLEIT

FIG. 15

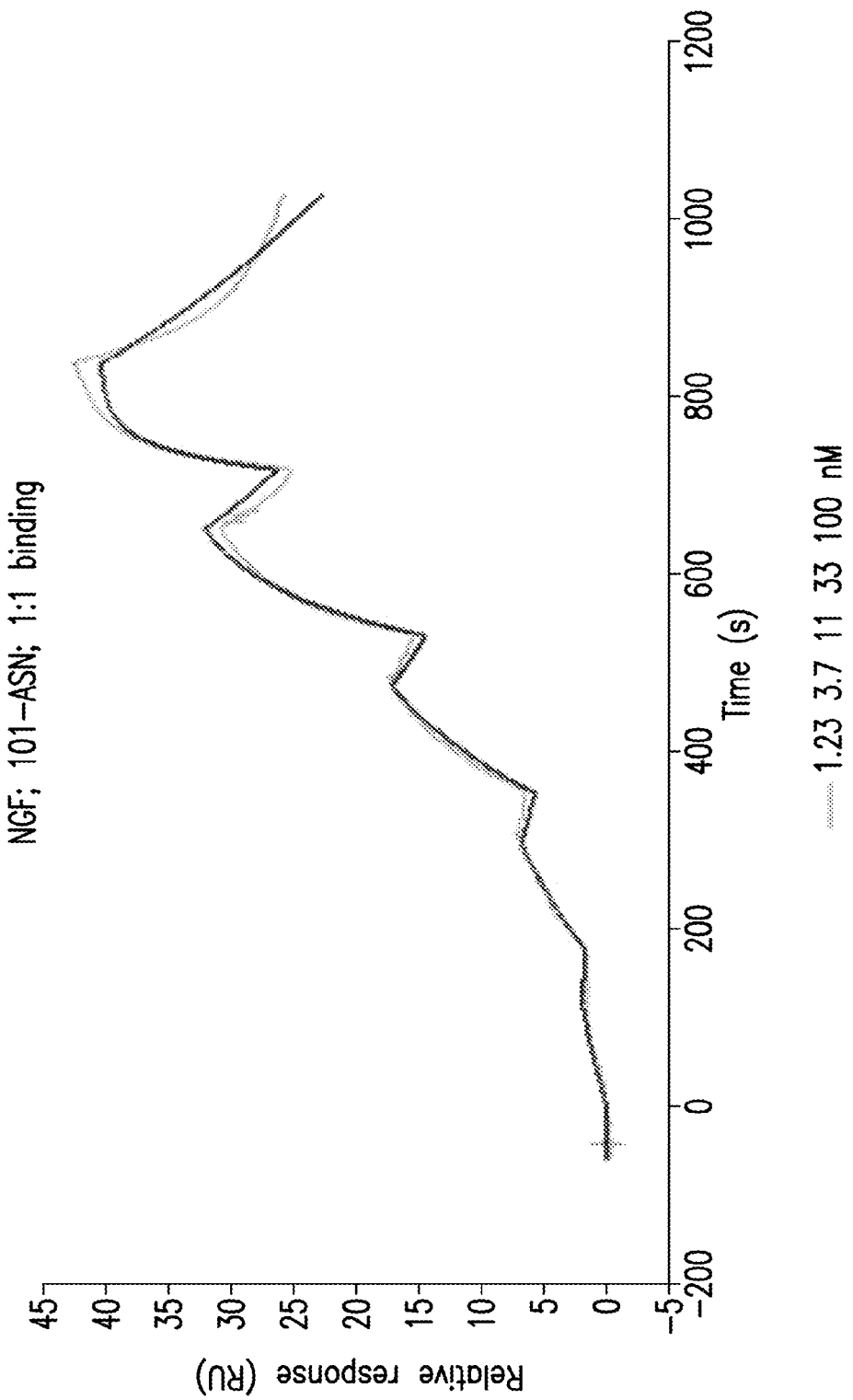

NGF; 101-ASN; 1:1 binding
Relative response (RU)
45
40
35
30
25
20
15
10
5
0
-5
-200
0
200
400
600
800
1000
1200
Time (s)
1.23 3.7 11 33 100 nM

FIG. 16

SEQ ID NO:	Clone Name	FR1H	CDR1H	FR2H	CDR2H
141	101	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MSWVRQAPGKGLQWVST	IYSNGGT
184	AHF17591	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MVWVRQAPGKGLQWVST	IYSNRGT
185	AHF17593	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MVWVRQAPGKGLQWVST	IYSNRGT
186	AHF17594	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MVWVRQAPGKGLQWVST	IYSNGGT
187	AHF17601	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MVWVRQAPGKGLQWVST	IYSNRGT
188	AHF17602	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MVWVRQAPGKGLQWVST	IYSNRGT
189	AHF17603	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MVWVRQAPGKGLQWVST	IYSNGGT
190	AHF17605	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSSS	MVWVRQAPGKGLQWVST	IYSNGGT
198	SC-184_76	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSDS	MSWVRQAPGKGLQWVST	LWSNGGT
200	SC-184_102	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSNS	MSWVRQAPGKGLQWVST	IWSNGGT
202	SC-184_110	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSAS	MSWVRQAPGKGLQWVST	IYSNGGT
204	SC-184_76-Arg	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSDS	MSWVRQAPGKGLQWVST	LWSNRGT
205	SC-184_102-Arg	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSNS	MSWVRQAPGKGLQWVST	IWSNRGT
206	SC-184_110-Arg	DVQLVESGGDLVKPGGSLRLTCVAS	GLSLTSAS	MSWVRQAPGKGLQWVST	IYSNRGT

FIG. 17A

SEQ ID NO:	Clone Name	FR3H	CDR3H	FR4
141	101	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYDADYLHWYFDF	WGQGALVTVSS
184	AHF17591	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYDADYLHWYFDE	WGQGALVTVSS
185	AHF17593	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	AQIYYYDADYLHWYFDF	WGQGALVTVSS
186	AHF17594	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYDADYLHWYFDF	WGQGALVTVSS
187	AHF17601	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	AQIYYYDADYLHWYFDE	WGQGALVTVSS
188	AHF17602	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYDADYLHWYFDF	WGQGALVTVSS
189	AHF17603	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYDADYLHWYFDE	WGQGALVTVSS
190	AHF17605	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	AQIYYYDADYLHWYFDF	WGQGALVTVSS
198	SC-184_76	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYEADYLHWYFDF	WGQGALVTVSS
200	SC-184_102	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYEAEYLHWYFDF	WGQGALVTVSS
202	SC-184_110	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYEAEYLHWYFDF	WGQGALVTVSS
204	SC-184_76-Arg	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYEADYLHWYFDF	WGQGALVTVSS
205	SC-184_102-Arg	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYEAEYLHWYFDF	WGQGALVTVSS
206	SC-184_110-Arg	YYTDSVKGRFTISKDNAENTLYLQMNNLKTEDTATYYC	ASIYYYEAEYLHWYFDF	WGQGALVTVSS

FIG. 17A Cont'd

SEQ ID NO:	Clone Name	FR1L	CDR1L	FR2L	CDR2L
142	101	EIQMTQSPTSLSASVGDRVTITCRAS	EGISNN	LSWYQQTPGKAPKLLIY	ATS
191	AHF17591	EIQMTQSPTSLSASVGDRVTITCRAS	EGIANN	LSWYQQTPGKAPKLLIY	ATS
192	AHF17592	EIQMTQSPTSLSASVGDRVTITCRAS	EGISNN	LSWYQQTPGKAPKLLIY	ATS
193	AHF17593	EIQMTQSPTSLSASVGDRVTITCRAS	EGIANN	LSWYQQTPGKAPKLLIY	ATS
194	AHF17595	EIQMTQSPTSLSASVGDRVTITCRAS	EGIQNN	LSWYQQTPGKAPKLLIY	ATS
195	AHF17597	EIQMTQSPTSLSASVGDRVTITCRAS	EGIQNN	LSWYQQTPGKAPKLLIY	ATS
196	AHF17602	EIQMTQSPTSLSASVGDRVTITCRAS	EGIANN	LSWYQQTPGKAPKLLIY	ATS
197	AHF17607	EIQMTQSPTSLSASVGDRVTITCRAS	EGISNN	LSWYQQTPGKAPKLLIY	ATS
199	SC-184_76	EIQMTQSPTSLSASVGDRVTITCRAS	EGIANN	LSWYQQTPGKAPKLLIY	ATS
201	SC-184_102	EIQMTQSPTSLSASVGDRVTITCRAS	KGISNN	LSWYQQTPGKAPKLLIY	AQS
203	SC-184_110	EIQMTQSPTSLSASVGDRVTITCRAS	EGISKN	LSWYQQTPGKAPKLLIY	ATD
199	SC-184_76-Arg	EIQMTQSPTSLSASVGDRVTITCRAS	EGIANN	LSWYQQTPGKAPKLLIY	ATS
201	SC-184_102-Arg	EIQMTQSPTSLSASVGDRVTITCRAS	KGISNN	LSWYQQTPGKAPKLLIY	AQS
203	SC-184_110-Arg	EIQMTQSPTSLSASVGDRVTITCRAS	EGISKN	LSWYQQTPGKAPKLLIY	ATD

FIG. 17B

SEQ ID NO:	Clone Name	FR3L	CDR3L	FR4L
142	101	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
191	AHF17591	ILHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
192	AHF17592	VLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
193	AHF17593	VLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
194	AHF17595	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
195	AHF17597	ILHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
196	AHF17602	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
197	AHF17607	ILHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
199	SC-184_76	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGFKWPLT	FGGGTKLEIT
201	SC-184_102	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
203	SC-184_110	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGWKWPLT	FGGGTKLEIT
199	SC-184_76-Arg	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGFKWPLT	FGGGTKLEIT
201	SC-184_102-Arg	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGYKWPLT	FGGGTKLEIT
203	SC-184_110-Arg	NLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QQGWKWPLT	FGGGTKLEIT

FIG. 17B Cont'd

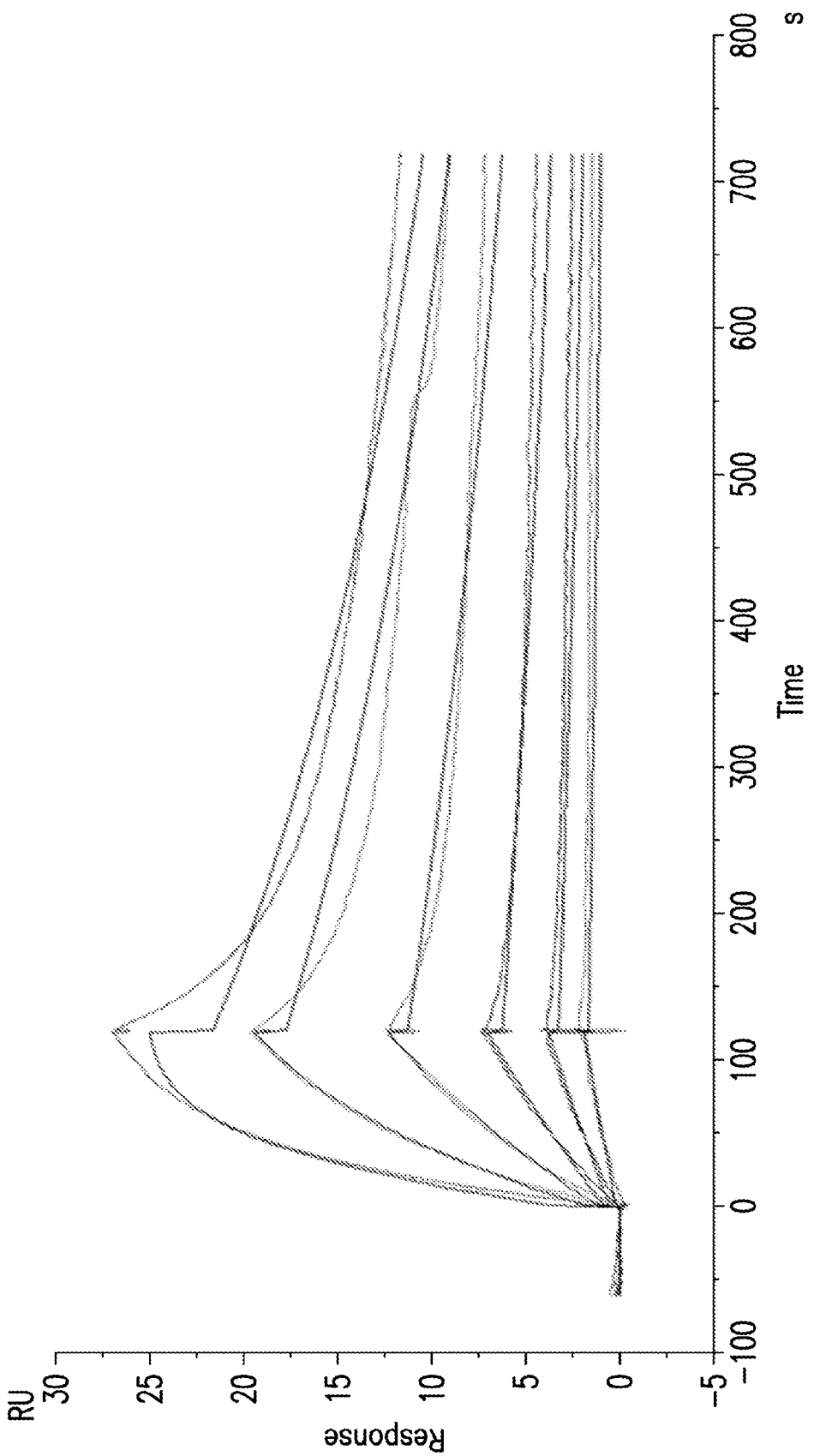

RU
30
25
20
15
10
5
0
−5
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 18A

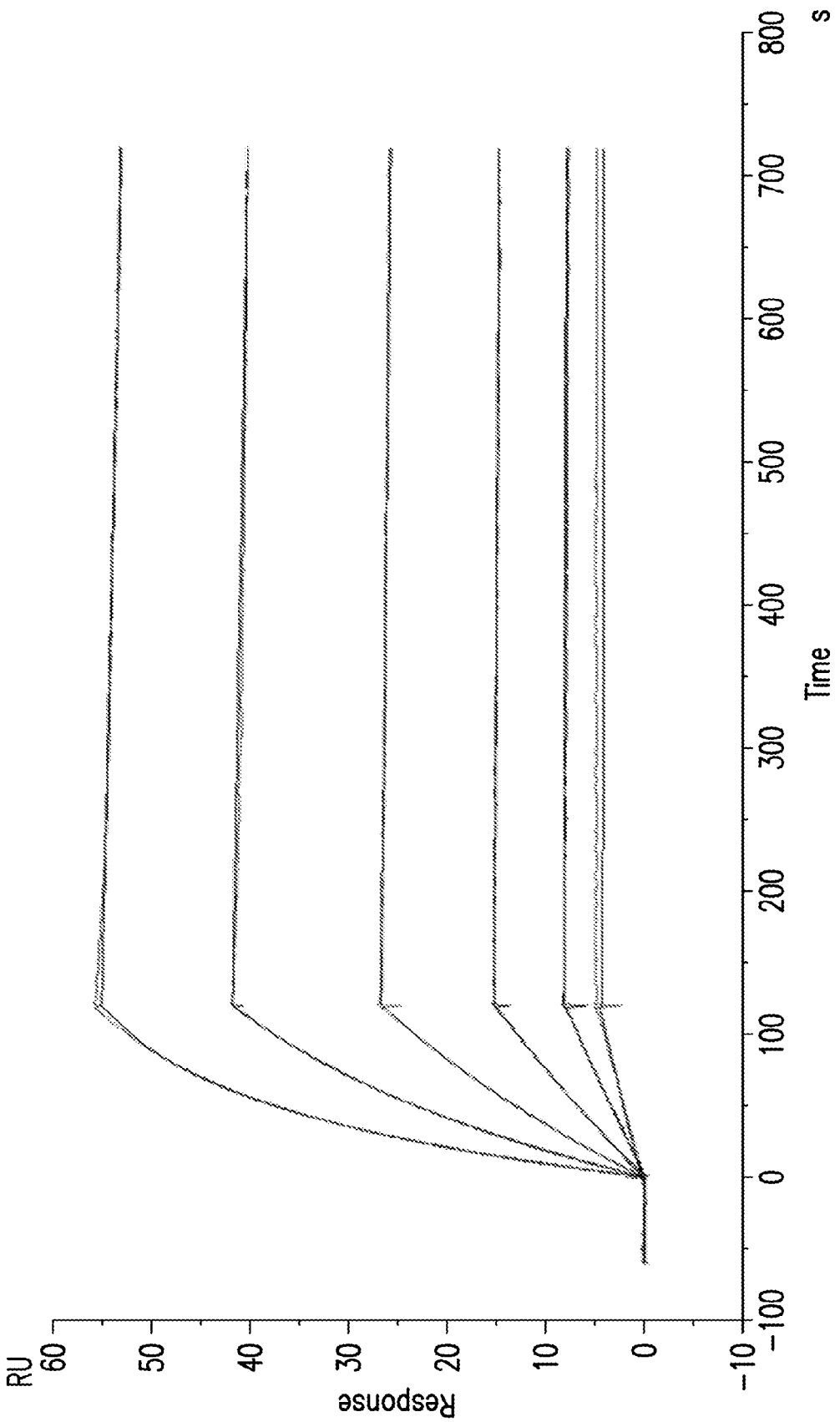

RU
60
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 18B

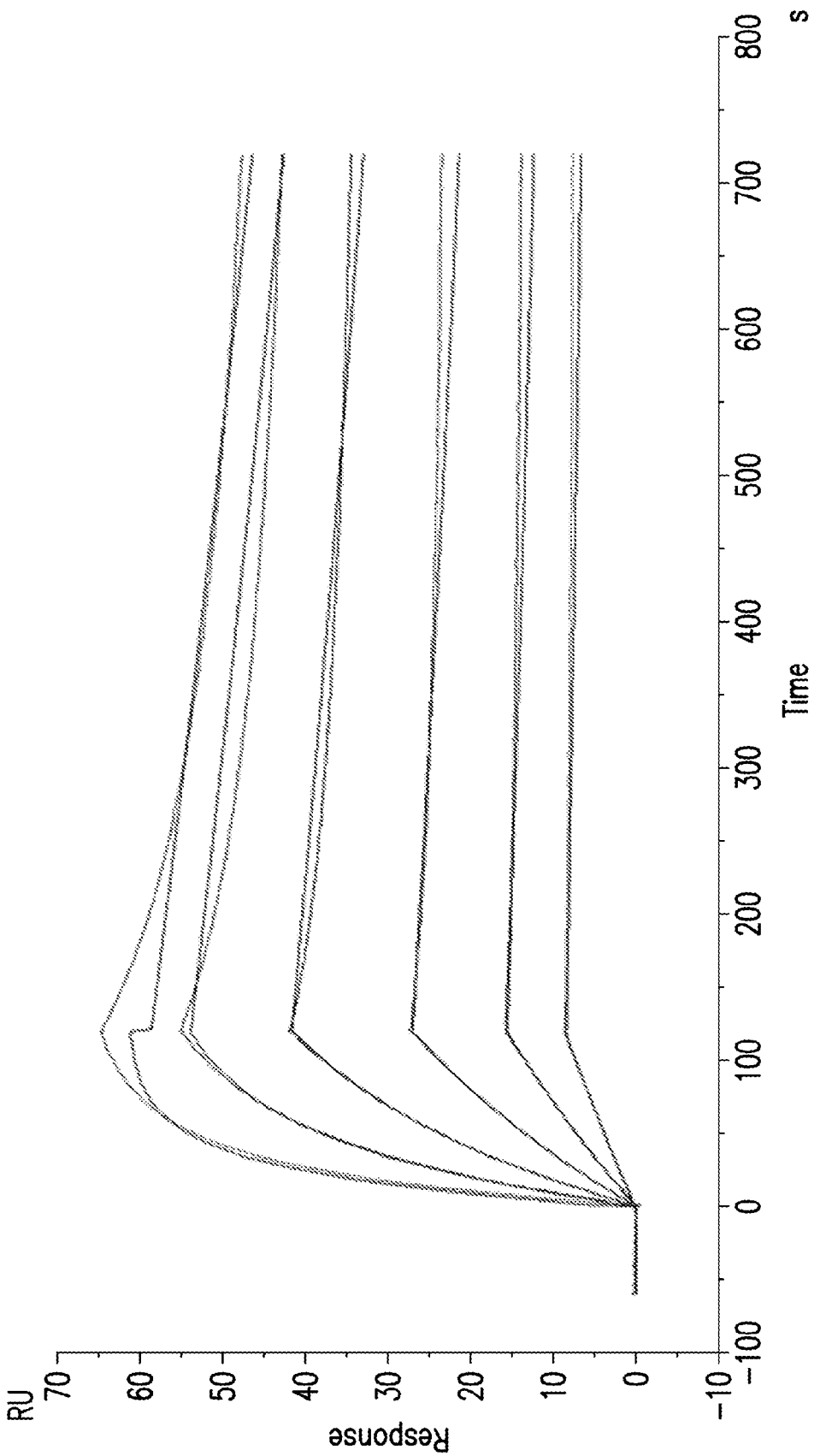

RU
70
60
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 18C

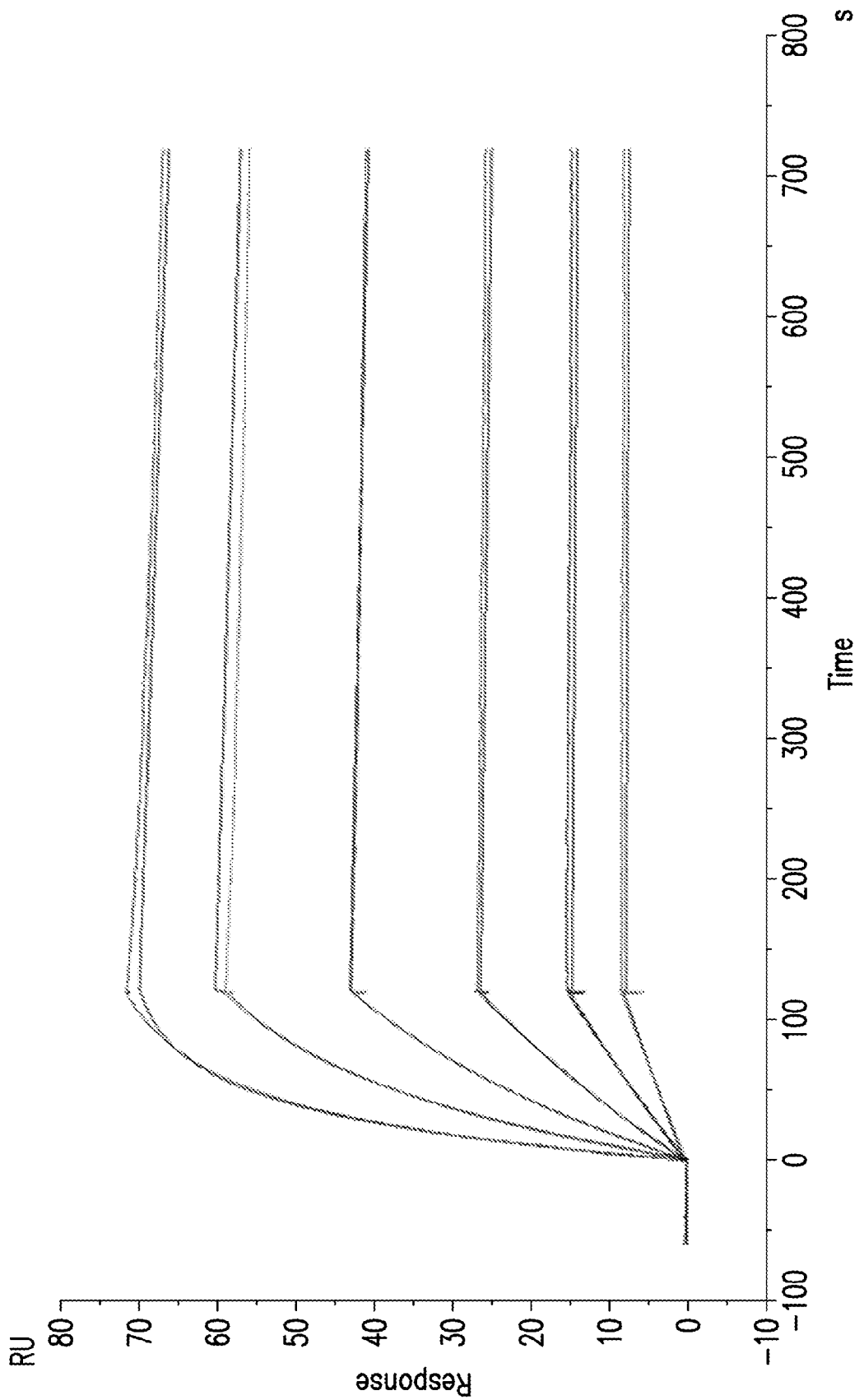

RU
Response
80
70
60
50
40
30
20
10
0
−10
−100
0
100
200
300
400
500
600
700
800
Time
s

FIG. 18D

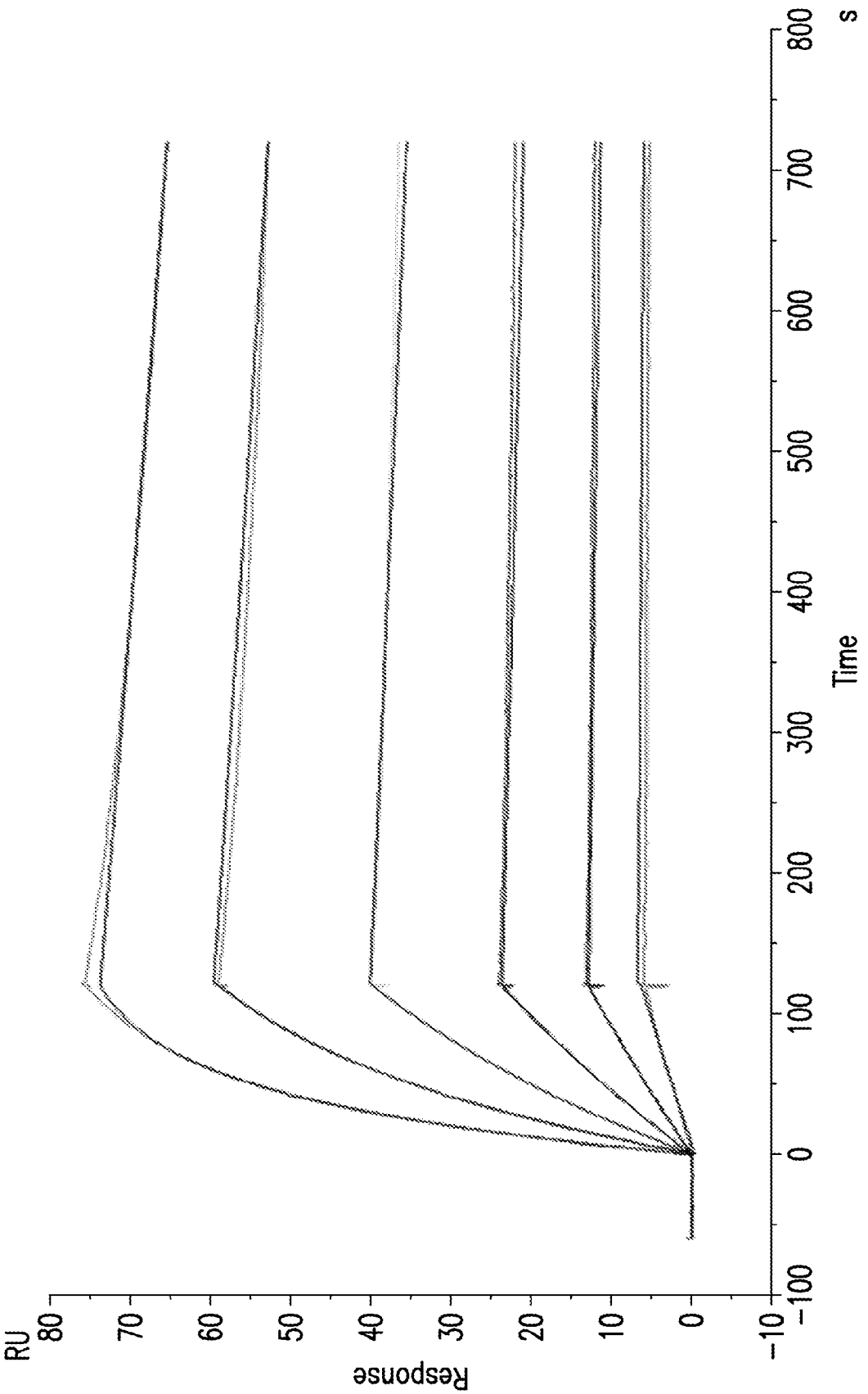

RU
80
70
60
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 18E

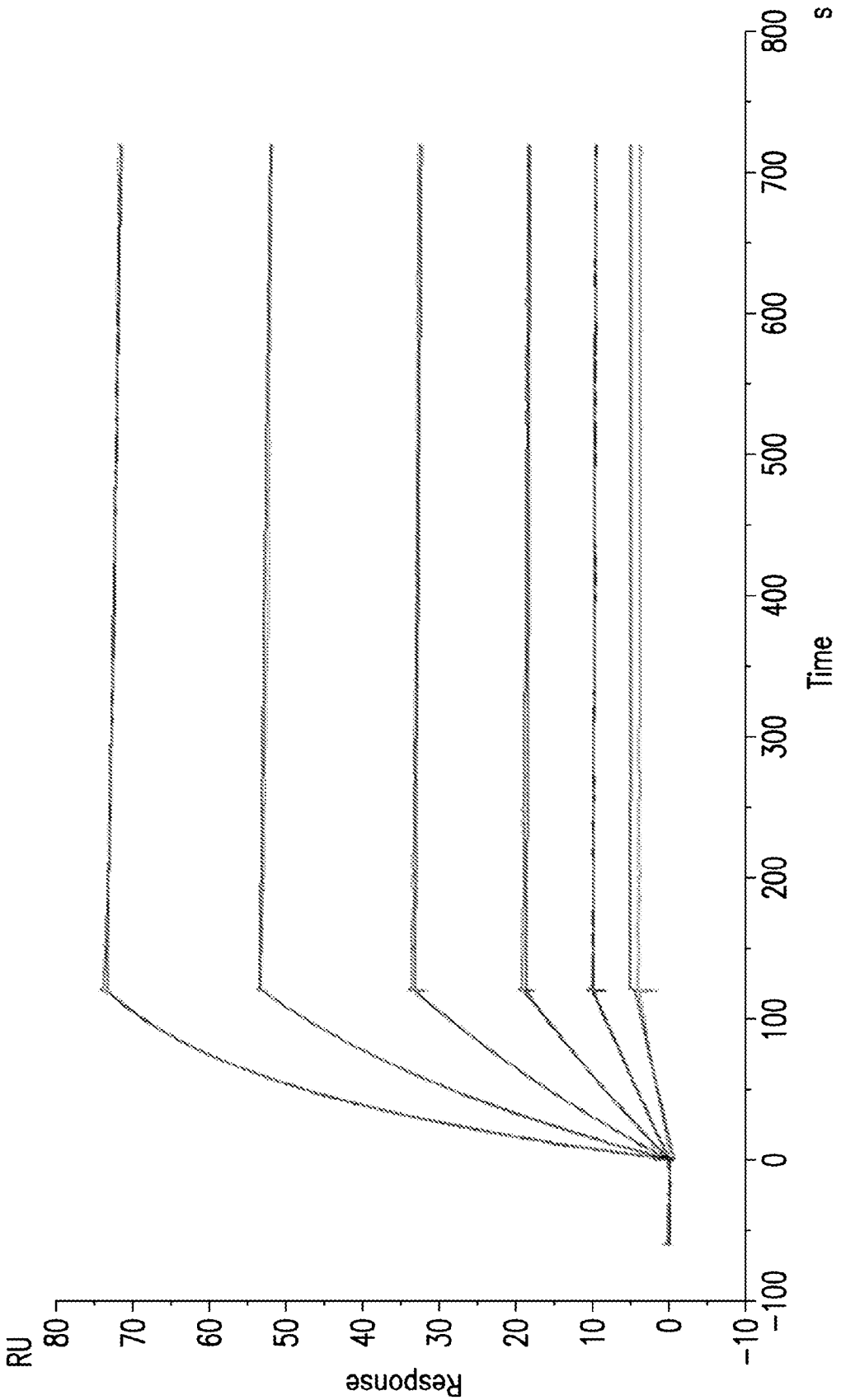

RU
80
70
60
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 18F

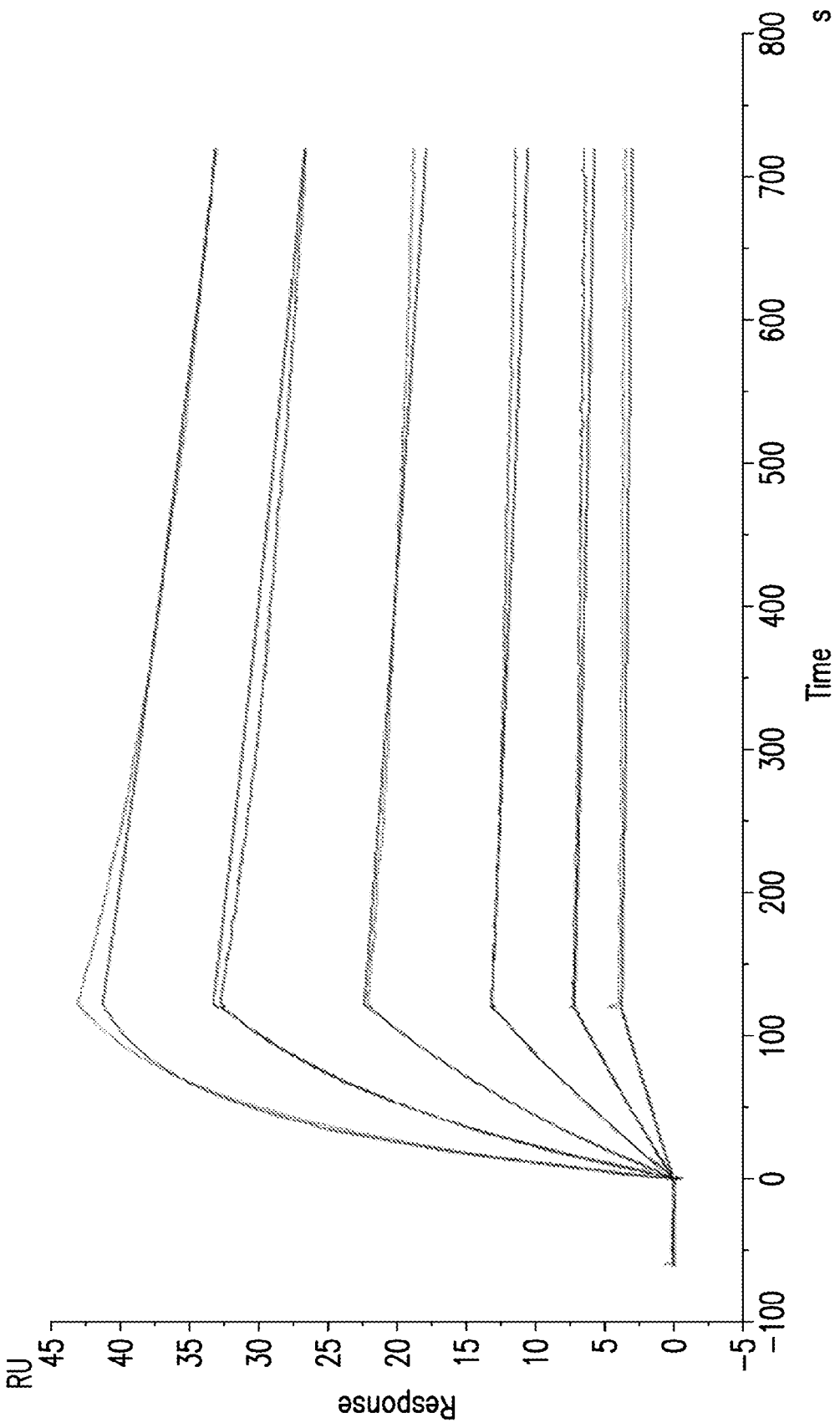

RU
45
40
35
30
25
20
15
10
5
0
−5
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 18G

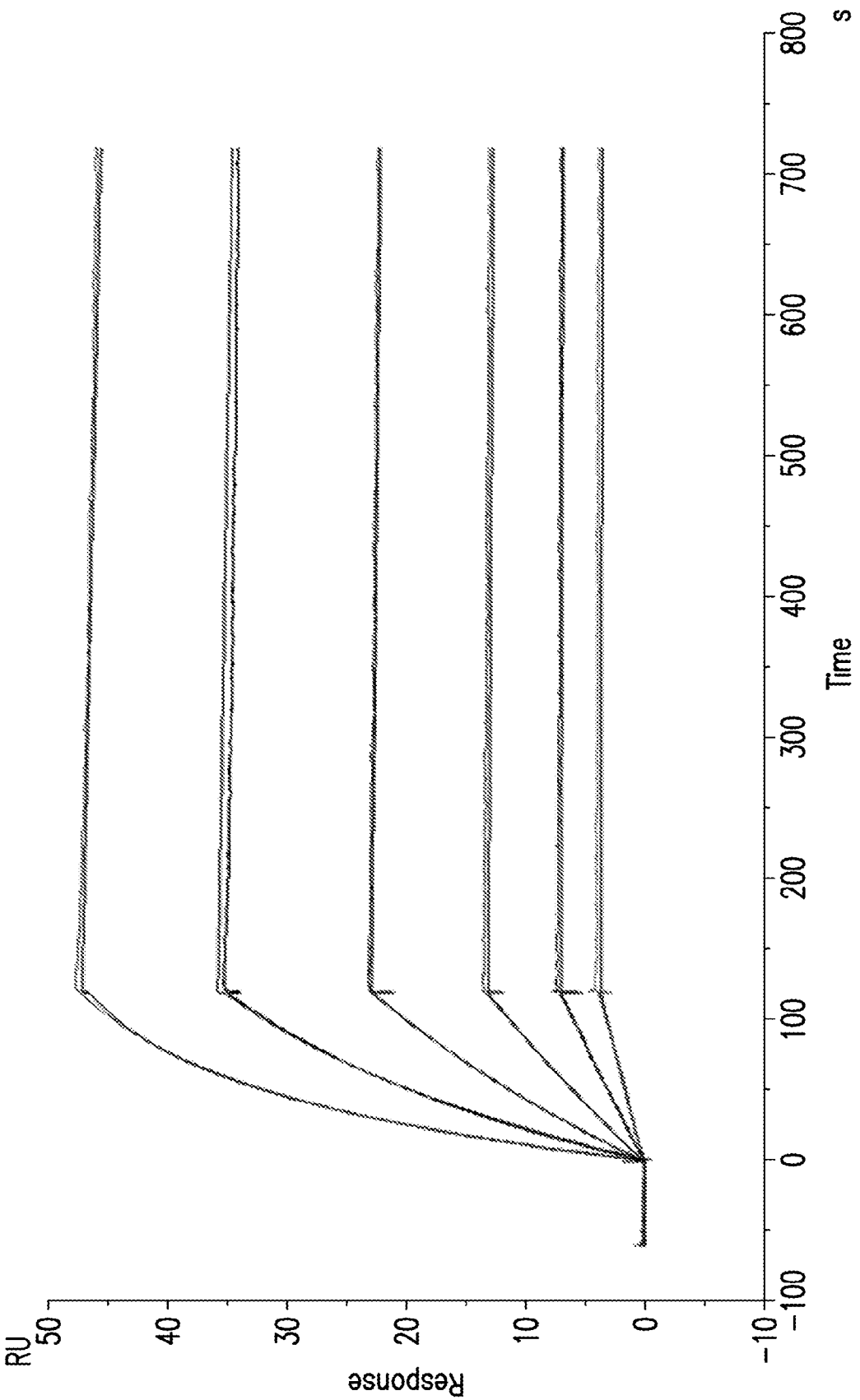

RU
50
40
30
20
10
0
−10
Response
−100
0
100
200
300
400
500
600
700
800
s
Time

FIG. 18H

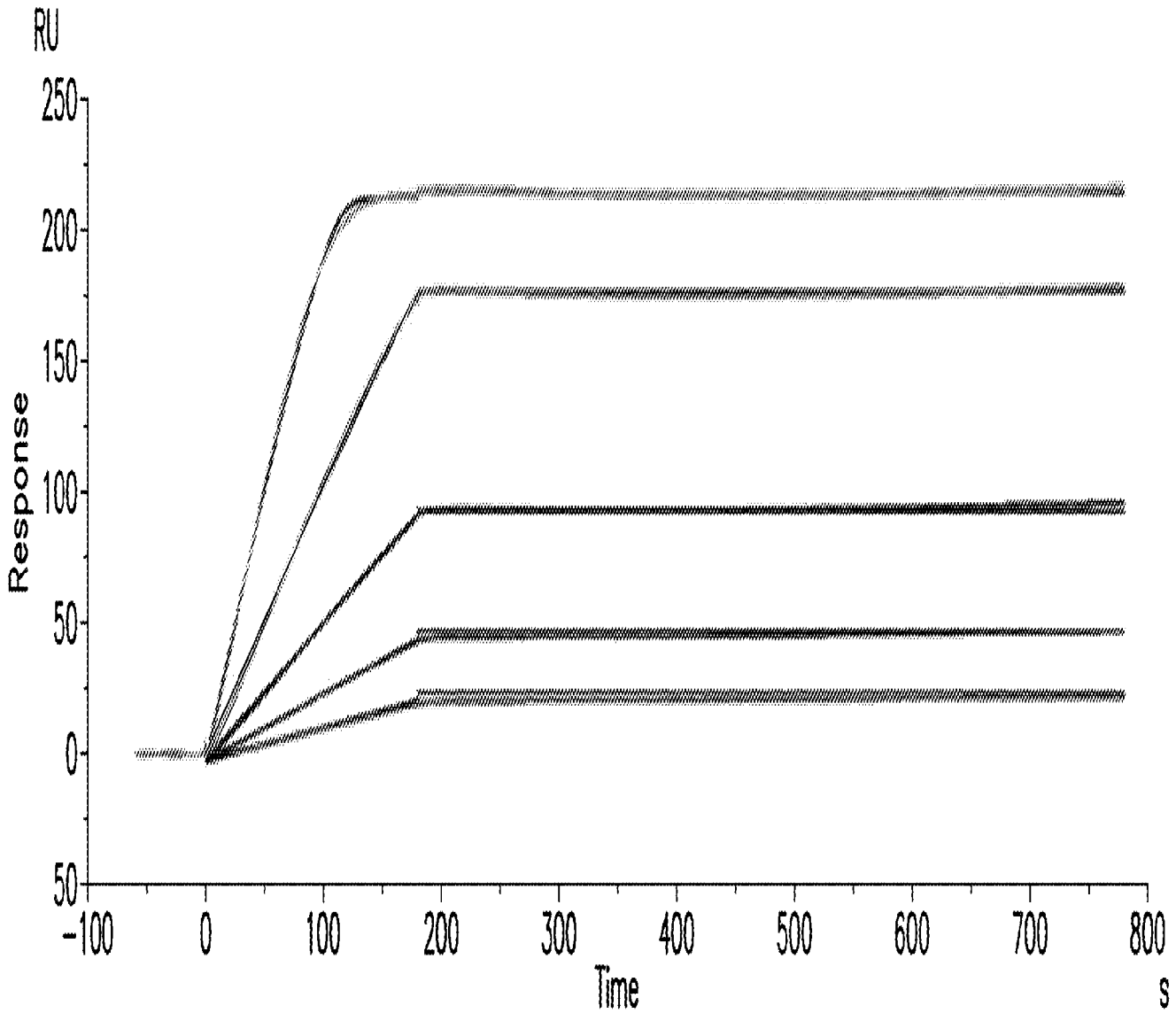

RU
250
200
150
100
50
0
50
Response
-100
0
100
200
300
400
500
600
700
800
Time
s

FIG. 5