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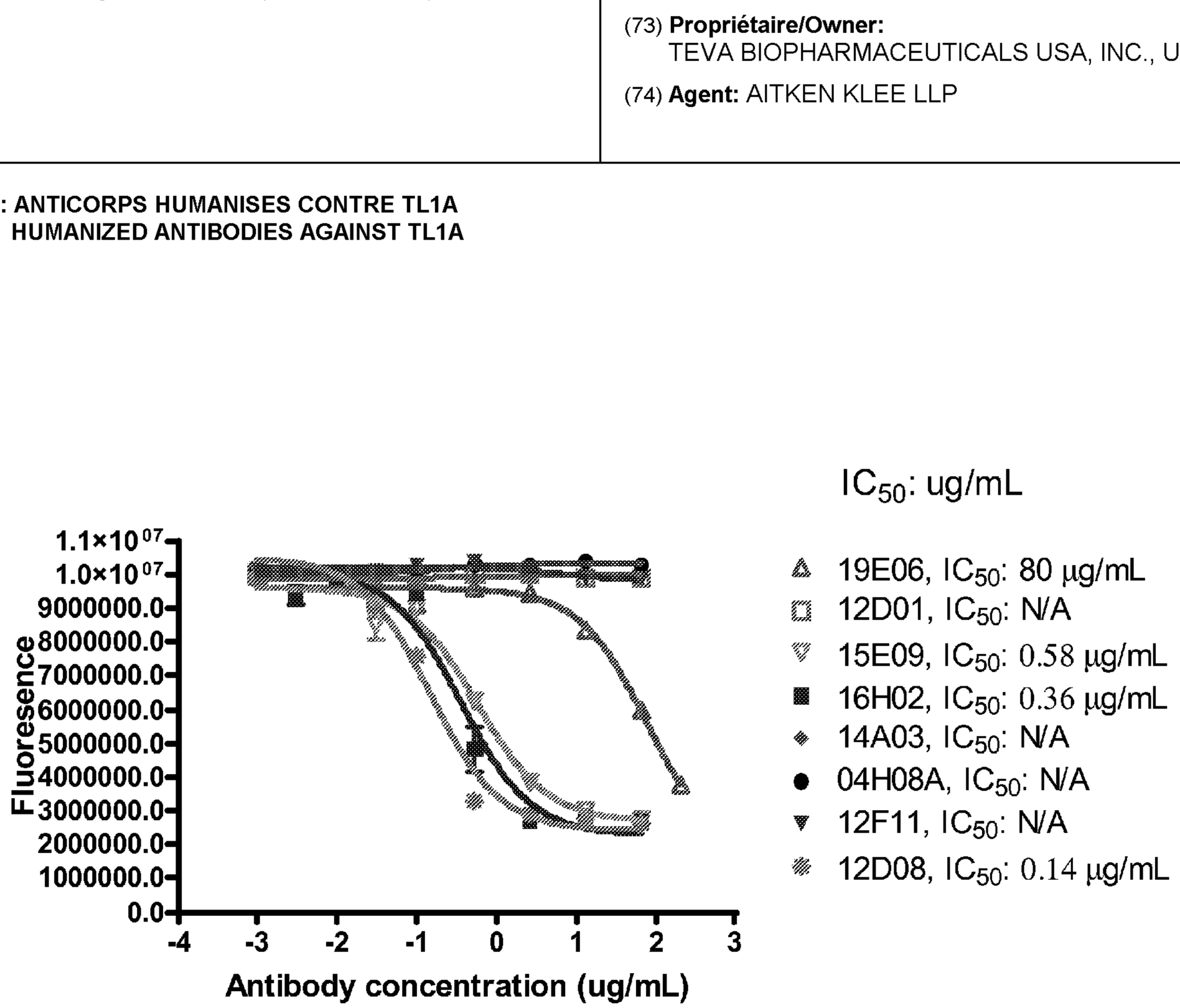
(54) **Titre : ANTICORPS HUMANISES CONTRE TL1A**
(54) **Title: HUMANIZED ANTIBODIES AGAINST TL1A**



(57) **Abrégé/Abstract:**
Disclosed are humanized antibodies that bind specifically to TNF superfamily member 15 (TNFSF 15), also known as TL1A. Methods of making and using the anti-TL1 A antibodies are also described. The humanized antibodies may be antagonists and may used to treat or diagnose conditions associated with TL1A function.

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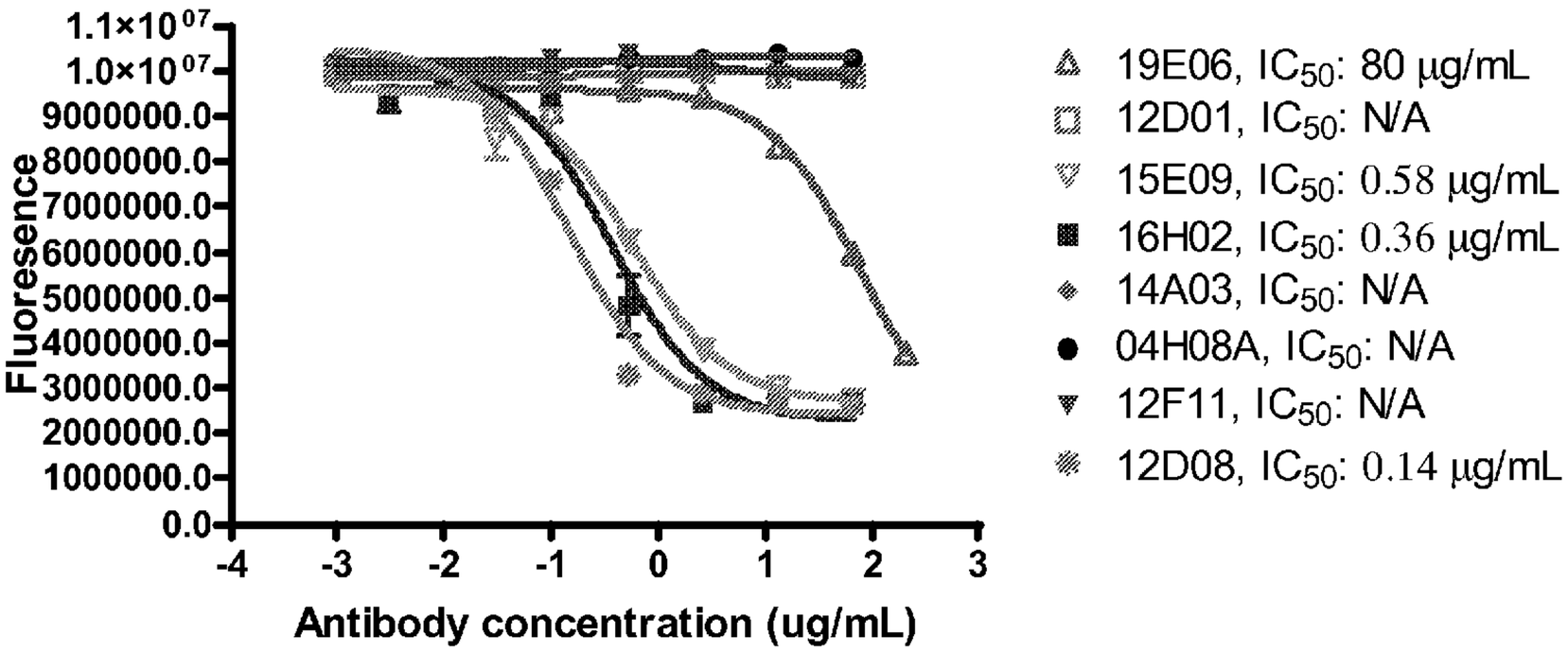

FIGURE 1

(57) Abstract: Disclosed are humanized antibodies that bind specifically to TNF superfamily member 15 (TNFSF 15), also known as TLlA. Methods of making and using the anti-TLl A antibodies are also described. The humanized antibodies may be antagonists and may used to treat or diagnose conditions associated with TLlA function.

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HUMANIZED ANTIBODIES AGAINST TL1A

SUMMARY

[0001] The present invention is directed to antibodies against TL1A, and methods of making and using such antibodies. The antibodies are expected to be particularly useful in treating inflammatory conditions such as Crohn's disease.

BACKGROUND

[0002] Proteins that are structurally related to tumor necrosis factor (TNF) are collectively referred to as the TNF superfamily. TL1A, a TNF superfamily member, is a TNF-like cytokine that binds to the death-domain receptor (DR)3 and provides costimulatory signals to activated lymphocytes. Through this interaction, TL1A induces secretion of IFN-gamma and may, therefore, participate in the development of T helper-1-type effector responses.

[0003] TL1A is a type II transmembrane protein and has been designated TNF superfamily member 15 (TNFSF15). TL1A is expressed predominantly by endothelial cells and monocytes, and its expression is inducible by TNF-a and IL-1a. Migone et al., *Immunity*, 16:479-92 (2002). TL1a is upregulated by the proinflammatory cytokines TNF and IL-1 and also by immune complexes (IC). Hsu et al., *Exp. Cell Res.*, 292:241-51 (2004).

[0004] TL1A mediates signaling via its cognate receptor DR3, a death receptor whose activation was known to induce both death and survival factors. TL1A, like TNF, is also presumed to circulate as a homotrimeric soluble form. Kim et al., *J. Immunol. Methods*, 298(1-2):1-8 (Mar. 2005).

[0005] TL1A binds with high affinity to death receptor 3 (DR3) which is a member of the death-domain containing TNF receptor family, and is also termed Wsl-1, Apo-3, TRAMP, and LARD, and now designated TNF receptor superfamily member 25 (TNFRSF25). Depending on the cell context, ligation of DR3 by TL1A can trigger one of two signaling pathways, activation of the transcription factor NF-kB or activation of caspases and apoptosis. TL1 functions in T cell costimulation and Th1 polarization. On activated T cells, TL1A functions specifically via its surface-bound receptor DR3 to

promote cell survival and secretion of proinflammatory cytokines. The secreted decoy receptor 3 (DcR3), a soluble protein of the tumor necrosis factor receptor (TNFR) superfamily, blocks the action of TL1A. Kim et al., "Identification of naturally secreted soluble form of TL1A, a TNF-like cytokine," *J Immunol Methods*, 298:1-8 (2005).

Potential Therapeutic Targets

Allergy and Asthma

[0006] Th2 polarization of CD4 T cells with elevated IgE levels and production of IL-13 by NKT cells are major cause of lung inflammation in Allergy and asthma. TL1A plays a major role in allergic lung inflammation (Fang et al J. Exp. Med. 2008). TL1A co-stimulates IL-4 and IL-13 production in NKT cells. Blocking TL1A and DR3 interaction by TL1A antibody or dominant negative TL1A mutant abolishes lung inflammation.

Lung and Colon Carcinomas

[0007] Members in the TNF and its receptor superfamilies regulate immune responses and induce apoptosis. DR3 is preferentially expressed by T lymphocytes and upregulated during T cell activation. The ligand for DR3 is TL1A. TL1A also binds decoy receptor DcR3/TR6, which is expressed in several lung and colon carcinomas and in some normal tissues. TL1A is upregulated by proinflammatory cytokines TNF and IL-1. TL1A is a longer variant of TL1 (also called VEGI).

Atherosclerosis

[0008] In addition, TL1A has also been reported to be angiostatic and to induce metalloproteinase and IL-8 gene expression (Su et al., *Exp. Cell Res.*, 312:266–277 (2006); Kang et al., *Cytokine*, 29:229–235 (2005)). Indeed, TL1A and DR3 may be involved in the pathogenesis of atherosclerosis by increasing the production of proinflammatory cytokines and chemokines and decreasing plaque stability by inducing extracellular matrix-degrading enzymes (Kang et al., *Cytokine*, 29:229–235 (2005)).

Rheumatoid arthritis

[0009] There is also evidence to suggest that TL1A/DR3 is involved in the etiology of rheumatoid arthritis (Bossen et al., J. Biol. Chem., 281(20):13964-13971 (May 19, 2006).

Inflammatory bowel disease

[0010] Researchers have found an association of the expression of TL1A and inflammatory bowel disease (Prehn et al., *Clin. Immunol.*, 112:66–77 (2004); Bamias et al., *J. Immunol.*, 171:4868–4874 (2003)).

Th1-mediated intestinal diseases, such as Crohn's Disease

[0011] Crohn's disease is a severe inflammatory bowel disorder that strikes young adults (ages 20–30). The condition is thought to originate from predisposing genetic and environmental factors that cause an imbalance of effector (proinflammatory) and regulatory T cell responses, resulting in inflammation of the gastrointestinal mucosa and disease.

[0012] The TL1A/DR3 pathway plays an important role in Th1-mediated intestinal diseases, such as Crohn's disease. Konstantinos et al., *The Journal of Immunology*, 2005, 174: 4985-4990 (2005); Bamias et al., *J. Immunol.*, 171:4868-74 (2003). Blockade of the TL1A/DR3 pathway may, therefore, offer therapeutic opportunities in Crohn's disease.

[0013] TL1A augments IFN-gamma production by anti-CD3 plus anti-CD28 and IL-12/IL-18-stimulated peripheral blood (PB) T cells. Activation of DR3 by TL1A induced the formation of a signaling complex containing TRADD, TRAF2, and RIP and activated the NF-kB and the ERK, JNK, and p38 mitogen-activated protein kinase pathways. Kang et al., *Cytokine*, 29:229-35 (2005). TL1A can be released to circulate as a homotrimeric soluble form. Wen et al., "TL1A-induced NF-kappaB activation and c-IAP2 production prevent DR3-mediated apoptosis in TF-1 cells," *J. Biol. Chem.*, 278:39251-8 (2003).

[0014] Death receptors and their ligands play a key role in the maintenance of tissue homeostasis and the physiological regulation of programmed cell death. Binding of a death ligand induces oligomerization of the receptor, recruitment of an adapter protein via a conserved cytoplasmic signaling element termed the death domain, activation of caspases, and induction of apoptosis. Young et al., *Proc Natl. Acad. Sci. U S A.*, 103(22): 8303--8304 (May 30, 2006).

[0015] Although death receptors such as Fas/Apo-1/CD95, TNF-R1, TRAIL-R1, TRAIL-R2, or DR3 were initially characterized as inducers of apoptosis, there is growing evidence that these receptors also have nonapoptotic functions, including

regulation of the adaptive immune response. Bamias et al., *Proc. Natl. Acad. Sci. USA*, 103:8441–8446 (2006), report that TL1A is expressed by lamina propia dendritic cells and that it functions by increasing the proliferation of memory cells, but not naïve $CD4^+$ T cells, and synergizes with IL-12 and/or low-dose stimulation of the T cell receptor to strongly enhance IFN-γ gene expression. IFN-γ expression in the gut has been considered a marker of inflammation, and many strategies for treating Crohn's disease rely on broad attempts to suppress the immune-activated state. However, such approaches (steroid treatment and immunosuppressive drugs) do not focus on the gut specifically and thus have their own complications. Targeted therapies based on the use of antagonists of TNF-α were introduced with success in the 1990s, and the results reported in ref. 1 suggest that therapy directed specifically against TL1A or its receptor may provide an alternative targeted therapy for this debilitating disorder.

[0016] As reported in Bamias et al., *Proc. Natl. Acad. Sci. USA.*, 103:8441–8446 (2006), TL1A seems to have a most profound effect when expressed in the gut during inflammation. TL1A synergizes in the induction of IFN-γ expression in human T cells when combined with IL-12/18, although increased expression can also be observed in natural killer cells (Migone et al., Immunity., 16:479–492 (2002); Papadakis et al., *J. Immunol.*, 174:4985–4990 (2005); Papadakis et al., *J. Immunol.*, 172:7002–7007 (2004)). Bamias et al., Proc. Natl. Acad. Sci. USA., 103:8441–8446 (2006), is the first report of a similar observation in mouse models of Crohn's disease and extends earlier data by showing that the synergy occurs when the T cell receptor is weakly stimulated or T cells are treated with IL-12. Although in Bamias et al. no synergy is observed when TL1A treatment is combined with IL-18, this result may not be surprising because both IL-18 and TL1A signal through NF-κB. Whereas the initial report by Migone et al. on TL1A demonstrated that it was a T cell costimulatory signal, Bamias et al. demonstrate that it is the memory T cell that most strongly responds, consistent with the increased capacity of this T cell population to express IFN-γ. Because this population does proliferate, it also expresses higher levels of the TL1A receptor, thus further enhancing the ability of the cells to proliferate and express IFN-γ. This finding might be considered somewhat surprising given that the only known receptor of TL1A is DR3, a death domain-containing receptor, and it might have been hypothesized that triggering this receptor would lead to cell death. (TL1A signals through DR3, its only known cell surface

receptor. TL1A also binds to the soluble decoy receptor (DcR3)). However NF-κB-dependent antiapoptotic genes, such as inhibitor of apoptosis 2, have been shown to be induced by TL1A (Wen et al., J. Biol. Chem., 278:39251–39258 (2003)), and therefore triggering of apoptosis vs. proliferation may be cell-type dependent.

[0017] Current treatment options for Crohn's disease include the monoclonal antibody against TNF-α, infliximab (Remicade; Centocor, Inc., Horsham, PA), the monoclonal antibody Adalimumab (brand name Humira; Abbott), as well as antiinflammatories (*e.g.*, sulfasalazine), cortisone or steroids (*e.g.*, prednisone), immune system suppressors (e.g., 6-mercaptopurine), and antibiotics. However, infliximab is the only treatment option having a high degree of specificity; the remaining treatment options have a low specificity. *Proc Natl Acad Sci U.S.A.*, 103(22): 8303–8304 (May 30, 2006). This means that the treatment is not targeted to the disease area. While infliximab has a high specificity and is generally well tolerated, infliximab can cause recrudescence of tuberculosis infection and worsening of heart failure, demyelinating disease, and an increased incidence of lymphoma.

[0018] Therefore, there remains a need in the art for compositions that can be used in the treatment and diagnosis of diverse inflammatory and immune diseases and disorders, such as allergy/asthma, rheumatoid arthritis, multiple sclerosis, Crohn's disease, inflammatory bowel disease, chronic obstructive pulmonary disease, psoriasis, type 1 diabetes and transplant rejection. The present invention, directed to monoclonal antibodies against TL1A, satisfies this need.

SUMMARY

[0019] Disclosed are antigen-binding polypeptide molecules that bind specifically to the TNF-like cytokine TL1A (*see* GenBank accession no. AF520785). The polypeptides include a humanized heavy chain variable region and a humanized light chain variable region. For example, the polypeptides may include the framework (FR) regions of the light and heavy chain variable regions of a human antibody, while retaining substantially the antigen-binding specificity of a parental monoclonal antibody. The humanized heavy chain variable region and/or the humanized light chain variable region are at least about 87% humanized, at least about 90% humanized, at least about 95% humanized, at least about 98% humanized, or at least about 100% humanized, excluding the complementary-

determining regions (CDRs). The antigen-binding polypeptides molecules may be derived from monoclonal antibody donors (*e.g.*, mouse monoclonal antibody donors) and may include CDRs from the monoclonal antibodies (*e.g.*, mouse monoclonal CDRs). The polypeptides may function as antagonists for the TL1A receptor.

[0020] Also encompassed by the invention are pharmaceutical compositions comprising the polypeptides of the invention, methods of making such polypeptides and compositions, and methods of treating subjects in need with the compositions of the invention. Exemplary conditions that may be treated with the compositions of the invention include, but are not limited to autoimmune disease (*e.g.*, lupus), inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), arthritis (*e.g.*, rheumatoid arthritis), multiple sclerosis, transplant rejection, central nervous system injury, Th1-mediated intestinal diseases such as Crohn's disease, psoriasis, leukemia or lymphoma (*e.g.*, chronic lymphocytic leukemia (CLL)), atherosclerosis, and lung and colon carcinomas.

[0021] In some embodiments, the antigen-binding polypeptide binds specifically to TL1A, and includes: (a) a humanized antibody heavy chain variable region comprising: (1) a CDR-H1 comprising an amino acid sequence of ({L,S,N}Y{G,A}MN) (SEQ ID NO: 1); (2) a CDR-H2 comprising an amino acid sequence of (WINT{Y,N}TG{E,N}PTYA{D,Q}{D,G}F{K,T}G) (SEQ ID NO: 2); and (3) a CDR-H3 comprising an amino acid sequence of (D{T,Y}{A,G}{M,K}{D,Y}{Y,G}{A,D}{M,Y}{A,Y}{Y,A}MDY) (SEQ ID NO: 3); and (b) a humanized antibody light chain variable region comprising: (1) a CDR-L1 comprising an amino acid sequence of ({K,R}SSQ{N,S}{I,L}V{H,Y}S{D,N}GNTYL{E,N,D}) (SEQ ID NO: 4); (2) a CDR-L2 comprising an amino acid sequence of (KVSNR{F,D}S) (SEQ ID NO: 5); and (3) a CDR-L3 comprising an amino acid sequence of ({F,M}QG{S,T}H{V,-}{P,-}{L,-}{T,-}) (SEQ ID NO: 6).

[0022] In certain embodiments the antigen-binding polypeptide binds specifically to TL1A and includes: a humanized antibody heavy chain variable region comprising (1) the CDR-H1 comprising, consisting essentially of or consisting of the amino acid sequence of TSNMGVV (SEQ ID NO: 7); (2) the CDR-H2 comprising, consisting

essentially of or consisting of the amino acid sequence of HILWDDREYSNPALKS (SEQ ID NO: 8); and (3) the CDR-H3 comprising, consisting essentially of or consisting of the amino acid sequence of MSRNYYGSSYVMDY (SEQ ID NO: 9).

[0023] In some embodiments, the antigen-binding polypeptide comprises a humanized antibody heavy chain variable region comprising, consisting essentially of or consisting of the amino acid sequence of:

QVTLKESGPALVKPTQTLTLTCTFSGFSLSTSNMGVVWIRQPPGKALEWLAHIL WDDREYSNPALKSRLTISKDTSKNQVVLTMTNMDPVDTATYYCARMSRNYYG SSYVMDYWGQGTLVTVSS (SEQ ID NO: 10).

[0024] In some embodiments of the polypeptides, (1) the CDR-H1 consists of the amino acid sequence of (LYGMN) (SEQ ID NO: 11) or (NYGMN) (SEQ ID NO: 12); (2) the CDR-H2 consists of the amino acid sequence of (WINTYTGEPTYADDFKG) (SEQ ID NO: 13); (3) the CDR-H3 consists of the amino acid sequence of (DTAMDYAMAY) (SEQ ID NO: 14) or DYGKYGDYYAMDY (SEQ ID NO: 15); (4) the CDR-L1 consists of the amino acid sequence of (KSSQNIVHSDGNTYLE) (SEQ ID NO: 16) or (RSSQSIVHSNGNTYLD) (SEQ ID NO: 17); (5) the CDR-L2 consists of the amino acid sequence of (KVSNRFS) SEQ ID NO: 18); and (6) the CDR-L3 consists of the amino acid sequence of (FQGSHVPLT) (SEQ ID NO: 19).

[0025] In some embodiments, the polypeptide comprises a humanized antibody heavy chain variable region of (Q{V,I}QLVQSG{S,P}ELKKPG{A,E}{S,T}VK{V,I}SCKASGYTFT{L,S,N}Y{G,A}MNWV{R,K}QAPG{Q,K}GL{E,K}WMGWINT{Y,N}TG{E,N}PTYA{D,Q}{D,G}F{K,T}GRF{V,A}FSL{D,E}TS{V,A}STAYLQI{S,N}{S,T}LK{A,N}ED{T,M}A{V,T}Y{Y,F}CARD{T,Y}{A,G}{M,K}{D,Y}{Y,G}{A,D}{M,Y}{A,Y}{Y,A}MDY)WGQGT{L,S}VTVSS) (SEQ ID NO: 20). For example, the polypeptide may comprise a humanized antibody heavy chain variable region of (QVQLVQSGSELKKPGASVKVSCKASGYTFTLYGMNWVRQAPGQGLEWMGWINTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARDTAMDYAMAYWGQGTLVTVSS) (SEQ ID NO: 21) or

(QVQLVQSGSELKKPGASVKVSCKASGYTFTLYGMNWVKQAPGKGLKWMGWI
NTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARDTAMDYA
MAYWGQGTLVTVSS) (SEQ ID NO: 22).

[0026] Alternatively, the polypeptide may comprise a humanized antibody heavy chain variable region of
(QVQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWI
NTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARDYGKYGD
YYAMDYWGQGTLVTVSS) (SEQ ID NO: 23) or
(QVQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGKGLKWMGWI
NTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARDYGKYGD
YYAMDYWGQGTLVTVSS) (SEQ ID NO: 24).

[0027] In some embodiments, the polypeptide comprises a humanized antibody light chain variable region of
(DVVMTQ{T,S}PLSLPV{T,S}{P,L}G{E,D,Q}{P,Q}ASISC{K,R}SSQ{N,S}{I,L}V{
H,Y}SDGNTYL{E,N}W{Y,F}{L,Q}Q{K,R}PGQSP{Q,K,R}{L,V,R}LIYKVSNR{F,
D}SGVPDRFSGSGSGTDFTLKI{S,N}RVEAED{L,V}GVY{Y,F}C{F,M}QG{S,T}H{
V,-}{P,-}{L,-}{T,-}{F,W}G{G,S,Q}GTK{V,L}EIKR) (SEQ ID NO: 25). For example, the polypeptide may comprise a humanized antibody light chain variable region of
(DVVMTQTPLSLPVTPGEPASISCKSSQNIVHSDGNTYLEWYLQKPGQSPQLLIYK
VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGGGTKVE
IKR) (SEQ ID NO: 26) or
(DVVMTQSPLSLPVTLGQPASISCKSSQNIVHSDGNTYLEWFQQRPGQSPRRLIYK
VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGGGTKVE
IKR) (SEQ ID NO: 27). In another embodiment, the polypeptide may comprise a humanized antibody light chain variable region of
(DVVMTQSPLSLPVTLGQPASISCKSSQNIVHSDGNTYLEWFQQRPGQSPRRLIYK
VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGQGTKVE
IKR) (SEQ ID NO: 28).

[0028] Also disclosed are humanized antibody heavy chain variable regions. The humanized antibody heavy chain region may comprise: (1) a CDR-H1 comprising an

amino acid sequence of ({L,S,N}Y{G,A}MN) (SEQ ID NO: 29); (2) a CDR-H2 comprising an amino acid sequence of (WINT{Y,N}TG{E,N}PTYA{D,Q}{D,G}F{K,T}G) (SEQ ID NO: 2); and (3) a CDR-H3 comprising an amino acid sequence of (DTAMDYAMAY) (SEQ ID NO: 14). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence of

(QVQLVQSGSELKKPGASVKVSCKASGYTFTLYGMNWVRQAPGQGLEWMGWI NTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARDTAMDYA MAYWGQGTLVTVSS) (SEQ ID NO: 21). Alternatively, the polypeptide may comprise a humanized antibody heavy chain variable region of (QVQLVQSGSELKKPGASVKVSCKASGYTFTLYGMNWVKQAPGKGLKWMGWI NTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARDTAMDYA MAYWGQGTLVTVSS) (SEQ ID NO: 22).

[0029] In another example, a humanized antibody heavy chain variable region comprises: (1)a CDR-H1 comprising an amino acid sequence of (NYGMN) (SEQ ID NO: 12); (2) a CDR-H2 comprising an amino acid sequence of (WINTYTGEPTYADDFKG) (SEQ ID NO: 13); and (3) a CDR-H3 comprising an amino acid sequence of (DYGKYGDYYAMDY) (SEQ ID NO: 15). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence of

(QVQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVRQAPGQGLEWMGWI NTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARDYGKYGD YYAMDYWGQGTLVTVSS) (SEQ ID NO: 23). Alternatively, the polypeptide may comprise a humanized antibody heavy chain variable region of (QVQLVQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGKGLKWMGWI NTYTGEPTYADDFKGRFVFSLDTSVSTAYLQISSLKAEDTAVYFCARDYGKYGD YYAMDYWGQGTLVTVSS) (SEQ ID NO: 30).

[0030] In another example, a humanized antibody heavy chain variable region comprises: (1) a CDR-H1 comprising an amino acid sequence of (NYAMS) (SEQ ID NO: 31); (2) a CDR-H2 comprising an amino acid sequence of (TIYSGGGYTFYLDSLKG) (SEQ ID NO: 32); and (3) a CDR-H3 comprising an amino

acid sequence of (HSYPMTTVITYAPYYFYY) (SEQ ID NO: 33). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence of
(QVQLVQSGSELKKPGASVKVSCKASGYTFTNYAMSWVKQAPGKGLKWMGTI YSGGGYTFYLDSLKGRFVFSLDTSVSTAYLQSSLKAEDTAVYFCARHSYPMTTV ITYAPYYFYYWGQGTLVTVSS) (SEQ ID NO: 34).

[0031] Also disclosed are humanized antibody light chain variable regions. The humanized antibody light chain variable region may comprise: (1) a CDR-L1 comprising an amino acid sequence of ({K,R}SSQ{N,S}{I,L}V{H,Y}S{D,N}GNTYL{E,N,D}) (SEQ ID NO: 4); (2) a CDR-L2 comprising an amino acid sequence of (KVSNR{F,D}S) (SEQ ID NO: 5); and (3) a CDR-L3 comprising an amino acid sequence of ({F,M}QG{S,T}H{V,-}{P,-}{L,-}{T,-}) (SEQ ID NO: 6).

[0032] In other emobodiments the antigen-binding polypeptide binds specifically to TL1A and includes: a humanized antibody light chain variable region comprising: (1) a CDR-L1 comprising, consisting essentially of, or consisting of an amino acid sequence of SASSSVNYMH (SEQ ID NO: 35); (2) a CDR-L2 comprising, consisting essentially of, or consisting of an amino acid sequence of STSNLAS (SEQ ID NO: 36); and (3) a CDR-L3 comprising, consisting essentially of, or consisting of an amino acid sequence of HQWNNYGT (SEQ ID NO: 37).

[0033] In some embodiments, the antigen-binding polypeptide comprises a humanized antibody light chain variable region comprising, consisting essentially of or consisting of the amino acid sequence of:
DIQLTQSPSFLSASVGDRVTITCSASSSVNYMHWYQQKPGKAPKLLIYSTSNLAS GVPSRFSGSGSGTEFTLTISSLQPEDFATYYCHQWNNYGTFGQGTKVEIKR (SEQ ID NO: 38).

[0034] For example, the humanized antibody light chain variable region may comprise an amino acid sequence of
DVVMTQSPLSLPVTLGQPASISCKSSQNIVHSDGNTYLEWFQQRPGQSPRRLIYK VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGGGTKVE IKR) (SEQ ID NO: 27). In another embodiment, the polypeptide may comprise a

humanized antibody light chain variable region of (DVVMTQSPLSLPVTLGQPASISCKSSQNIVHSDGNTYLEWFQQRPGQSPRRLIYK VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGQGTKVE IKR) (SEQ ID NO: 28). Alternatively, the polypeptide may comprise a humanized antibody light chain variable region of DVVMTQTPLSLPVTPGEPASISCKSSQNIVHSDGNTYLEWYLQKPGQSPQLLIYK VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGGGTKVE IKR (SEQ ID NO: 39) or DVVMTQTPLSLPVSLGDQASISCKSSQNIVHSDGNTYLEWYLQKPGQSPKVLIYK VSNRFSGVPDRFSGSGSGTDFTLKINRVEAEDVGVYFCFQGSHVPLTFGGGTKLE IKR (SEQ ID NO: 40).

[0035] The humanized antibody light chain region may also comprise: (1) a CDR-L1 comprising an amino acid sequence of (RSSQSIVHSNGNTYLD) (SEQ ID NO: 17); (2) a CDR-L2 comprising an amino acid sequence of (KVSNRFS) (SEQ ID NO: 18); and (3) a CDR-L3 comprising an amino acid sequence of (FQGSHVPLT) (SEQ ID NO: 19). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTYLDWFQQRPGQSPRRLIYK VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGGGTKVE IKR (SEQ ID NO: 41). Alternatively, the polypeptide may comprise a humanized antibody light chain variable region of (DVVMTQSPLSLPVTLGQPASISCRSSQSIVHSNGNTYLDWFQQRPGQSPRRLIYK VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPLTFGQGTKVE IKR)) (SEQ ID NO: 42).

[0036] In another example, a humanized antibody light chain variable region comprises: (1) a CDR-L1 comprising an amino acid sequence of (RSSQSIVHSNGNTYLD) (SEQ ID NO: 17); (2) a CDR-L2 comprising an amino acid sequence of (KVSNRFS) (SEQ ID NO: 18); and (3) a CDR-L3 comprising an amino acid sequence of (FQGSHVPLT) (SEQ ID NO: 19). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (DVVMTQTPLSLPVTPGEPASISCRSSQSIVHSNGNTYLDWYLQKPGQSPQLLIYK

VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGGGTKVE IKR) (SEQ ID NO: 43)) or (DVVMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLDWYLQKPGQSPKVLIY KVSNRFSGVPDRFSGSGSGTDFTLKINRVEAEDLGVYFCFQGSHVPLTFGGGTKL EIKR) (SEQ ID NO: 44)).

[0037] The aforementioned humanized heavy chains and humanized light chains may be present in the antigen binding polypeptides that binds specifically to TL1A.

[0038] The antigen-binding polypeptide may be selected from the group consisting of an antibody molecule, a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, and an scFv molecule. In some embodiments, the polypeptide is an antibody molecule. Antibody molecules may include chimeric antibodies that include a human heavy chain constant region and a human light chain constant region. For example, the antibody molecule may be an IgG molecule (*e.g.*, a IgG1 or an IgG4 molecule), where the polypeptide includes the heavy chain and light chain constant domains of an IgG molecule. The polypeptide may be an scFv molecule. For example, the scFv may have a formula selected from the group consisting of NH_2-L-VH-X-VK-COOH and NH_2-L-VK-X-VH-COOH; wherein L is a leader sequence; VH is the humanized antibody heavy chain variable region; X is a linking polypeptide; and VK is the humanized antibody light chain variable region. The polypeptide may be an Fab HSA fusion molecule. For example, the Fab HSA fusion has a formula selected from the group consisting of NH_2-VH-CH1-HSA-COOH combined with NH_2-VK-CK-COOH; wherein the VH-CH1-HSA is the humanized antibody heavy chain variable region (VH) and human constant heavy chain domain 1 (CH1) produced as a fusion protein with human serum albumin (HSA) that then folds with its cognate humanized antibody light chain variable region (VK) and human constant kappa domain (CK) to form the Fab HSA fusion protein.

[0039] The antigen-binding polypeptide further may be conjugated or fused to a therapeutic or diagnostic agent. For example, therapeutic agents may be selected from the group consisting of a cytotoxic agent, a radioactive label, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic agent or a

combination thereof. Examples of diagnostic agents may include a radioactive label, a photoactive diagnostic agent, an ultrasound-enhancing agent or a non-radioactive label.

[0040] The antigen-binding polypeptide may be an antagonist of TL1A. Typically, the polypeptide is not an agonist of TL1A.

[0041] The antigen-binding polypeptide binds to the TL1A receptor with specificity and high affinity. Typically, the polypeptide binds to TL1A with an affinity constant of at least about 10^6M^{-1} (preferably at least about 10^7M^{-1}, more preferably at least about 10^8M^{-1}, even more preferably at least about 10^9M^{-1}).

[0042] Also disclosed are pharmaceutical compositions comprising the aforementioned antigen-binding polypeptides and a carrier, such as a diluent or excipient. The pharmaceutical may further comprise an additional therapeutic or diagnostic agent as disclosed herein.

[0043] Also disclosed are methods of treating or diagnosing a disease or condition that comprise administering the disclosed pharmaceutical compositions to a patient in need thereof. For example, the pharmaceutical compositions may be administered to treat or diagnose an inflammatory, immune, and/or malignant disease or condition. Examples of diseases and conditions may include autoimmune disease (*e.g.*, lupus), inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), arthritis (*e.g.*, rheumatoid arthritis), multiple sclerosis, transplant rejection, central nervous system injury, Crohn's disease, psoriasis, type 1 diabetes, lung and colon carcinomas, and leukemia or lymphoma (*e.g.*, chronic lymphocytic leukemia (CLL)).

[0044] Also disclosed are polynucleotides that encode the aforementioned polypeptides. The polynucleotides may be operably linked to a promoter for expressing the encoded polypeptides in a suitable host cell. As such, methods of producing the polypeptide encoded by the recombinant polynucleotide may include: a) culturing a cell transformed with the recombinant polynucleotide to express the encoded polypeptide; and b) recovering the polypeptide so expressed.

[0045] Both the foregoing general description and the following brief description of the drawings and detailed description are exemplary and explanatory and are intended to

provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] **Figure 1** illustrates inhibition of human TL1A (huTL1A)-induced Caspase activity on TF-1 cells by mouse and hamster anti-TL1A antibodies. Ab#1 – 19E06; Ab#2 – 12D01; Ab#3 – 15E09; Ab#4 – 16H02; Ab#5 – 14A03; Ab#6 – 04H08A; Ab#7 – 12F11; Ab#8 – 12D08.

[0047] **Figure 2** illustrates an alignment of the VH Domain of mouse anti-TL1A 16H02 (SEQ ID NO: 70) with the closest human germline gene, IGHV7-4-1-02 (SEQ ID NO: 69). The alignment was used as a template to create 2 different versions of humanized 16H02 VH, identified in the figure as New Hum 16H02 VH#1 (SEQ ID NO: 21) and VH#2 (SEQ ID NO; 22). Figure 2 discloses the majority sequences as SEQ ID NO; 68.

[0048] **Figure 3** illustrates the number of mutations from mouse to human and percent humanization of two versions of a humanized anti-TL1A VH.

[0049] **Figure 4** illustrates an alignment of the VK domain of mouse anti-TL1A 16H02 (SEQ ID NO: 74) with the closest human germline gene, A17 (SEQ ID NO: 73). The alignment was used as a template to create 2 different versions of humanized 16H02 VH, identified in the figure as New Hum 16H02 VK#1 (SEQ ID NO: 72) and VK#2 (SEQ ID NO: 26). Figure 4 discloses the majority sequence as SEQ ID NO: 71.

[0050] **Figure 5** illustrates the number of mutations from mouse to human and percent humanization of two versions of humanized anti-TL1A 16H02 VK.

[0051] **Figure 6** illustrates results of a transient transfection of 293F cells with human 16H02 anti-TL1A VH#1 and VH#2 and human 16H02 VK#1 and VK#2 to produce a full length humanized antibody molecule.

[0052] **Figure 7** illustrates the activity of 16H02 TL1A monoclonal antibodies after one round of humanization by showing the inhibition of TL1A-induced caspase activity

in TF-1 cells by the humanized antibodies compared to mouse anti-TL1A antibody controls . 12D01= mouse anti-TL1a negative control; 16H02 = mouse anti-TL1A positive control; ; 19E06 = hamster anti-TL1A control; 3009 =humanized anti TL1A 16H02 VH#1+VK#1; 3010 =humanized anti TL1A 16H02 VH#2+VK#1;3011 =humanized anti TL1A 16H02 VH#1+VK#2; 3012 =humanized anti TL1A 16H02 VH#2+VK#2.

[0053] **Figure 8** illustrates the final mutations required for complete humanization of 16H02 VK framework. To create a fully humanized 16H02 light chain the New Hum16H02 VK#2 (SEQ ID NO: 26) (see Figure 4) starting sequence was aligned with the A17 germline sequence (SEQ ID NO: 76) and 3 disinct regions or blocks identified (solid circles) for further mutagenesis. Synthetic light chains were constructed that contained all possible combinations of either the non-mutated wild type VK#2 sequence (=W) or A17 human germline sequence (=M) within each of the 3 regions or blocks. Figure 8 discloses the majority sequence as SEQ ID NO: 75.

[0054] **Figure 9** illustrates the transient transfection ID and LDC # for final versions of the humanized 16H02 VK. Synthetic light chains were generated that contained all possible combinations of either wild type (W) or mutant (M) sequence in 3 distinct regions or blocks of New Hum 16H02 VK#2 from Figure 8. The synthetic light chains were then cotransfected with New Hum 16H02 VH#1 from Figure 2 and the resulting antibodies tested for inhibition of huTL1A-induced Caspase activity.

[0055] **Figure 10** illustrates inhibition of human TL1A (huTL1A)-induced Caspase activity on TF-1 cells by a panel of various humanized anti-TL1A antibodies from Figure 9. Activity of a fully humanized 16H02 TL1A antibody identifed as 3038 is compared to that of the original mouse anti-TL1A antibody 16H02.

[0056] **Figure 11** illustrates a sequence alignment of the VH Domain of five lead candidate mouse anti-TL1A antibodies: 1B4 (SEQ ID NO: 58), 25B9 (SEQ ID NO: 59), 11D8 (SEQ ID NO: 60), 27A8 (SEQ ID NO: 61), and 38D6 (SEQ ID NO: 62) .

[0057] **Figure 12** illustrates a sequence alignment of the VK Domain of five lead candidate mouse anti-TL1A antibodies: 1B4 (SEQ ID NO: 49), 25B9 (SEQ ID NO: 50), 11D8 (SEQ ID NO: 52), 27A8 (SEQ ID NO: 51), and 38D6 (SEQ ID NO: 53).

[0058] **Figure 13** illustrates a sequence alignment of the humanized 1B4 VH domain (hum 1B4 VH AA) (SEQ ID NO: 77) with the original mouse VH domain (1B4 VH AA) (SEQ ID NO: 58) and the closest matching human germline VH domain (VH2-70-10) (SEQ ID NO: 78.

[0059] **Figure 14** illustrates a sequence alignment of the humanized 1B4 VK domain (hum 1B4 VK AA) (SEQ ID NO: 67) with the original mouse VK domain (1B4 VK AA) (SEQ ID NO: 49) and the closest matching human germline VK domain (VK-L8) (SEQ ID NO: 78).

[0060] **Figure 15** illustrates the inhibition of mammalian derived TL1A-induced caspase activity in TF-1 cells by humanized TL1A antibodies (1B4, 11D8, 25B9) compared to the mouse 11D8 antibody.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

[0061] An antibody, as described herein, refers to a full-length (*i.e.*, naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes) immunoglobulin molecule (*e.g.*, an IgG antibody) or an immunologically active (*i.e.*, specifically binding) portion of an immunoglobulin molecule, like an antibody fragment.

[0062] An antibody fragment is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" includes aptamers, speigelmers, and diabodies. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. For example, antibody fragments include isolated fragments consisting of the variable regions, such as the "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain

polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), Fab HSA fusion polypeptides in which the VH-CH1 are produced as a fusion to HSA, which then folds with its cognate VK- CK light chain to form a Fab, and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

[0063] A humanized antibody is a recombinant protein in which the CDRs from an antibody from one species, *e.g.*, a rodent antibody, are transferred from the heavy and light variable chains of the rodent antibody into human heavy and light variable domains or heavy and light variable domains that have been mutagenized to include at least a portion of the amino acid sequence of the human heavy and light variable domains (as represented by "percent humanization"). The constant domains of the antibody molecule may be derived from those of a human antibody.

[0064] As used herein, "percent humanization" is calculated by determining the number of framework amino acid differences (*i.e.*, non-CDR difference) between the humanized domain and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

[0065] As used herein, "CDR" means a "complementarity determining region" that is present in a variable domain of an antibody heavy chain (VH) or a variable domain of an antibody light chain (VL or VK). Each variable domain includes three CDRs which are designated CDR-H1, CDR-H2, and CDR-H3, for those present in the heavy chain variable domain, and CDR-L1, CDR-L2, and CDR-L3 for those present in the light chain variable domain. The Kabat numbering system is used herein. As such, CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue);

includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (*i.e.*, following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

Conjugation to a Therapeutic or Diagnostic Agent

[0066] The antigen-binding polypeptides disclosed herein may be conjugated or fused to a therapeutic agent, which may include radioactive labels, an immunomodulator, a hormone, a photoactive therapeutic agent, a cytotoxic agent, which may be a drug or a toxin, and a combination thereof. Drugs may include those drugs that possess the pharmaceutical property selected from the group consisting of antimitotic, antikinase, alkylating, antimetabolite, antibiotic, alkaloid, antiangiogenic, apoptotic agents and combinations thereof. More specifically, these drugs are selected from the group consisting of nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, COX-2 inhibitors, pyrimidine analogs, purine analogs, antibiotics, enzymes, epipodophyllotoxins, platinum coordination complexes, vinca alkaloids, substituted ureas, methyl hydrazine derivatives, adrenocortical suppressants, antagonists, endostatin, taxols, camptothecins, anthracyclines, taxanes, and their analogs, and a combination thereof. The toxins encompassed by the present invention may be selected from the group consisting of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), *e.g.*, onconase, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

[0067] Immunomodulators may be selected from the group consisting of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interferon (IFN), erythropoietin, thrombopoietin and a combination thereof. Specifically useful are lymphotoxins such as tumor necrosis factor (TNF), hematopoietic factors, such as interleukin (IL), colony stimulating factor, such as granulocyte-colony stimulating factor (G-CSF) or granulocyte macrophage-colony stimulating factor (GM-CSF)), interferon, such as interferons-alpha, -beta, or -gamma, and stem cell growth factor, such as designated "S1 factor". More specifically,

immunomodulators may include IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21 interferon-gamma, TNF-alpha or a combination thereof.

[0068] The antigen-binding polypeptides disclosed herein may be conjugated or fused to a diagnostic agent. Diagnostic agents may include photoactive diagnostic agents or radiolabels having an energy between 60 and 4,000 keV, or a non-radioactive label. The radioactive label is preferably a gamma-, beta-, and positron-emitting isotope and is selected from the group consisting of ^{125}I, ^{131}I, ^{123}I, ^{124}I, ^{86}Y, ^{186}Re, ^{188}Re, ^{62}Cu, ^{64}Cu, ^{111}In, ^{67}Ga, ^{68}Ga, ^{99m}Tc, ^{94m}Tc, ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{76}Br and combinations thereof. Diagnostic agents may include contrast agents, for example, such as manganese, iron or gadolinium.

Exemplary method of making anti-TL1A antibodies using hybridoma technology

[0069] BALB/c mice can be immunized with recombinant TL1A protein (extracellular domain). In a typical procedure 10 mg of protein in 50 ml of complete Freund's adjuvant (Sigma) is injected subcutaneously. Two to four additional injections in incomplete Freund's adjuvant can be given at 2 week intervals followed by a final boost in PBS. Alternatively, injections can be given in the foot pads. Three days later mice can be sacrificed, their spleens or poplietal lymph nodes can be harvested and lymphocytes can be isolated for fusion. Lymphocytes can be fused with P3X63Ag8.653 plasmacytoma cells at 5:1 ratio using PEG/DMSO (Sigma) as a fusion agent. After fusion cells can be resuspended in selective HAT media and seeded at 10^6 cells per well in 96 well plates. The supernatants from hybridomas that survived HAT selection can be screened by direct binding ELISA for the presence of TL1A binding antibodies. Hybridomas secreting TL1A binding antibodies can be identified and their supernatants can be further screened by inhibition of binding ELISA for antibodies inhibiting binding of TL1A to its receptor DR3. The hybridomas identified as positives for inhibition of TL1A binding can then be screened for inhibition of TL1A induced caspase activity in TF-1 cells to identify TL1A antagonistic clones.

Exemplary Antibody Humanization Strategy

[0070] One goal in humanizing the anti-TL1A antibodies is to obtain 70-100% humanized VH and VK domains that retain 90-100% of original binding affinity and specificity. Site-directed mutagenesis of individual high risk positions in VH and VK can be used to further humanize the antibodies while maintaining binding affinity and specificity.

[0071] Humanization can be performed by CDR grafting and structure based analysis and variable region resurfacing. (*See* Jones *et al.*, NATURE (1986) May 29-Jun. 4;321(6069):522-5; Roguska *et al.*, PROTEIN ENGINEERING, 1996, 9(10):895-904; and Studnicka *et al.*, Humanizing Mouse Antibody Frameworks While Preserving 3–D Structure. PROTEIN ENGINEERING, 1994, Vol.7, pg 805). The primary antibody sequence and 3-D structure data can be utilized to identify key framework residues required to maintain the binding affinity and specificity. The "Blast for Ig sequences" website sponsored by the NCBI can be used to identify the closest match to the mouse VH and VK region used in the study. Human germline VH and VK genes can be chosen as the best matches to the mouse sequence VH and VK sequences. Alternatively, sequences from the naturally expressed human antibody repertoire can be used as a template for humanization either alone or in combination with the closest matching human germline gene.

[0072] After aligning mouse anti-TL1A VH and VK to the nearest human germline or expressed repertoire of genes, the amino acid at every position can be evaluated for potential influence on binding and immunogenicity. This information can be used to assign a low, moderate, or high risk value for mutation at each position. In one embodiment, only the low and moderate risk positions are mutated while avoiding the high risk positions. If necessary, an affinity maturation strategy can be performed by incorporating tyrosines pair wise at each position in the CDR's of VH, VK or both .

Exemplary Cloning and Sequencing of Murine anti-TL1A VH and VK domains from Hybridoma Cell Lines

[0073] Hybridoma cells can be pelleted, washed 3X with PBS and RNA extracted using Trizol reagent (Invitrogen, Cat. No. 15596-026) following the manufacturers protocol. Total RNA can be converted to cDNA using a 5' RACE kit (Rapid

Amplification of cDNA Ends, Invitrogen, Cat. No. 18374-058) following the manufacturers protocol. Briefly, RNA can be ligated to random hexamer primer, Random N6, and 1st strand cDNA can be generated using superscript II RNAase H negative reverse transcriptase. The cDNA can be purified using a GlassMax spin cartridge provided with the kit and then reacted with TdT (terminal deoxynucleotidyl transferase) in the presence of dCTP to append the cDNA with C basepairs at the 5'end. The dC-tailed cDNA can be PCR amplified using an anchor primer specific for the dC tail and a gene specific primer that hybridizes to highly conserved DNA sequence in the mouse constant heavy 1 (CH1) for VH and constant kappa (CK) for VK. The resulting PCR product can be analyzed by gel electrophoresis for correct size corresponding to intact VH or VK domain then purified and ligated into a TOPO TA vector (Invitrogen Cat. No. K4575-01) following the manufacturers protocol. After transformation into bacteria DNA can be prepared from clones containing the correct size insert and the DNA sequence can be determined using a Big Dye terminator sequencing reaction mix (Applied Biosystems, Part No. 4336699) and a 3700 ABI/Prism DNA analyzer following manufacturers protocol.

Exemplary Humanizing Murine anti-TL1A Antibodies

[0074] Murine anti-TL1A antibodies can be identified based on binding data and sequence data generated as described above. The amino acid sequence of the VH and VK domains from these antibodies can be aligned to human germline VH and VK domains using currently available public databases (*i.e.*, Blast for IgG at the NCBI and V-base at the MRC). At those positions in the framework where the mouse sequence differed from the human germline, an iterative process can be used to convert or mutate the mouse framework so it matches the corresponding human germline framework. In addition, or alternatively, certain CDR amino acid residues for both the VH and VK can be mutated by replacement with tyrosine (*i.e.*, affinity matured) to potentially help compensate for any losses in affinity due to the framework residues changes. The affinity matured and humanized mouse VH and VK domains can be generated by a polymerase chain reaction process using a panel of overlapping synthetic DNA oligonucleotides. As part of the synthetic gene design process a codon optimization strategy can be used, that is to say the triplet code for each amino acid that is

preferentially utilized by mammalian cells for gene expression can be incorporated at each position. The synthetic VH and VK domains can be cloned into specialized mammalian expression vectors that allow the corresponding domains to be expressed in the context of a fully human IgG1, G4 or Kappa antibody backbone. Small-scale production of the humanized antibodies can be achieved by co-tranfection of an IgG1 or G4 construct with the Kappa construct into 293F cells with lipofectamine (Invitrogen) following manufactures protocol. Supernatants from the transient transfections can be passed through Protein A or G resin and the IgG can be purified to homogeneity for testing in cell based assays.

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples.

Example 1

[0075] Amino Acid Sequences of VH and VK Domains of Mouse and Hamster anti-TL1A monoclonal antibodies prepared as described herein are shown below. The CDR regions of the variable domains are underlined.

12D08 VK (SEQ ID NO: 45):

DVLMTQTPLS LPVSLGDQAS ISCRSSQSIV HSNGNTYLDW YLQKPGQSPN LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP LTFGAGTKLE LKR

16H02 VK (SEQ ID NO: 46):

DVLMTQTPLS LPVSLGDQAS ISCKSSQNIV HSDGNTYLEW YLQKPGQSPK LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YYCFQGSHVP LTFGSGTKLE IKR

15E09 VK (SEQ ID NO: 47):

ETTVTQSPAS LSMAIGEKVT IRCITSTDID DDMNWYQQKP GEPPKLLISE GNTLRPGVPS RFSSSGYGTD FVFTIENMLS EDVADYYCLQ SDNLPLTFGA GTKLELKR

19E06 VK (SEQ ID NO: 48):

DIVMTQSPSS LAVSTGGTVT LTCLSSQSLF SSDTNKNYLN WYLQKPGQSP KLLVYHASTR LTGVPDRFIG SGSGTDFTLT INSVQAEDLG DYYCQQHFRP PFTFGRGTKL EIKR

IB4 VK AA (SEQ ID NO: 49)

QIVLTQSPAIMSASLGAEITLTC**SASSSVNYMH**WYQQRSGTSPKLLIY**STSNLAS** GVPSRFSGSGSGTFYSLTISSVEAEDAADYYC**HQWNNYGT**FGGGTKLEIKR

25B9 VK AA (SEQ ID NO: 50)

ENVLTQSPAILAASLGQKVTMTC**SASSSVSSGYLH**WYQQKSGASPKPLIH**RTSN**
LASGVPPRFSGSGSGTSYSLSISSVEAEDDATYYC**QQWSGFPFT**FGSGTKLEIKR

27A8 VK AA (SEQ ID NO: 51)

DIVLTQSPASLTVSLGQRATISC**RASQNVSTSSYSHMH**WSQQKPGQPPKLLIK**YA**
SNLDSGVPARFSGSGSGTDFTLNIHPVEEEDIATYYC**QHSWEIPYT**FGGGTKLEI
KR

11D8 VK AA (SEQ ID NO: 52)

DIVMTQSPASLTVSLGQRATISC**RASQSVSTSSYSHMH**WYQQKPGQPPKLLIR**Y**
ASNLESGVPARFSGSGSGTDFTLNIHPVEEEDTAIYYC**QHSWELPYT**FGGGTKL
EIKR

38D6 VK AA (SEQ ID NO: 53)

DIVLTQFPASLPVSLGQRATISC**RASQSVSTSSYSHMH**WYQQKPGQPPKLLIT**YA**
SNLDSGVPARISGSGSGTDFTLNIHPVEEEDTATYYC**HHSWELPYT**FGGGTKLEI
KR

12D08 VH (SEQ ID NO: 54):

QIQLVQSGPE LKKPGETVKI SCKASGYTFT NYGMNWVKQA PGKGLKWMGW
INTYTGEPTY ADDFKGRFAF SLETSASTAY LQINNLKNED MATYFCAKDY
GKYGDYYAMD YWGQGTSVTV SS

16H02 VH (SEQ ID NO: 55):

QIQLVQSGPE LKKPGETVKI SCKASGYTFT LYGMNWVKQA PGKGLKWMGW
INTYTGEPTY ADDFKGRFAF SLETSASTAY LQINTLKNED MATYFCARDT
AMDYAMAYWG QGTSVTVSS

15E09 VH (SEQ ID NO: 56):

EVKLVDSGGG LVQPGDSLRL SCATSGFTFS DFYMEWVRQP PGKRLEWIAA SGNKANDYTT EYSASVKGRF IVSRDTSQSI LYLQMNDLRA EDTAIYYCVR DAGYGYWYFD VWGAGTTVTV SS

19E06 VH (SEQ ID NO: 57):

QIQLQESGPS LVKPSQSLSL TCSVTGYSIT SDSYWNWIRQ FPGKNLVWMG YISYRGSTNY NPSLKSRISI TRDTSRNQFF LQLNSVITED TATYYCARYS GYSFWYFDFW GQGTQVTVSS

1B4 VH (SEQ ID NO: 58)

a. QVTLKESGPGILQPSQTLSLTCSFSGFSLT**TSNMGVV**WIRQPSGKGLEWL L**HILWDDREYSNPALKS**RLTISKDPFNNQVFLKIANVDTADTATYYCAR**MSRN YYGSSYVMDY**WGQGTSVTVSS

25B9 VH (SEQ ID NO: 59)

EVQLQQSGPELVKPGASVKMSCKASGYTFT**SYVMH**WVKQKTGQGLEWIG**YIN SNNDGTKYNEKFKG**KATLTSDKSSSTAYMELSSLTSEDSAVYYCAT**GDYYGG TSYWYFDV**WGAGTTVTVSS

11D8 VH (SEQ ID NO: 60)

EVQLQQSGPELEKPGASVKISCKASGYSFT**GYNMN**WVKQSNGKSLEWIG**NIDP YFGDTNYNQNFKG**RATLTVDKSSNTAYMQLMSLTSEDSAVYYCAR**EGAARA KNYFDY**WGQGTTLTVSS

27A8 VH (SEQ ID NO: 61)

EVQLQQSGPELETPGASVKISCKASGYSFT**GYNMN**WVKQTNGKSLEWIG**NIDP YFGDANYNRKFKG**KATLTVDKSSSTAYMQLRSLTSEDSAVYYCAK**EGAARAK NYFDY**WGQGTTLTVSS

38D6 VH AA (SEQ ID NO: 62)

EVQLQQSGPELEKPGASVKISCKASGYSFTGYNMNWVRQTNGKSLEWIGHIDP

YYGDATYRQKFKGKATLTVDKSSNTAYMQLKSLTSEDSAVYFCAREGAARA

RNYFDYWGQGTTLTVSS

Example 2

[0076] This example describes an assay protocol to measure inhibition of TL1A-induced caspase activity on TF-1 cells.

[0077] To determine neutralizing activity of anti-TL1A antibodies, their effects on TL1A-induced caspase activity in TF-1 cells were determined. *See* Fig. 1. TF-1 cells were seeded at 75,000 cells/well in a black 96-well plate with clear bottom in RPMI medium containing 1% fetal bovine serum. Cells were treated with 10 μg/mL cyclohexamide and 100 ng/mL TL1A in the absence or presence of various concentrations of mouse or hamster parental TL1A antibodies for 6 hr at 37°C. Caspase activity was measured by Apo-One homogeneous caspase-3/7assay kit (Promega). Equal volume of Apo-One homogeneous caspase-3/7assay buffer containing caspase substrate, Z-DEVD-Rhodamine (SEQ ID NO: 63) was added to each well containing cells. After overnight incubation, fluorescence was measured by a Wallac Victor 2 fluorescence plate reader with excitation filter 485 nm and emission filter 535 nm.

[0078] The results, shown in Fig. 1, shows that the level of fluoresence, which correlates with caspase activity, decreases with increasing concentration of four (4) different anti-TL1A antibodies: Ab#1 - 19E06; Ab#3 - 15E09; Ab#4 - 16H02; and Ab#8 - 12D08.

* * * *

[0079] It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present invention without departing from the spirit or scope of the invention.

CLAIMS:

1. An isolated antigen-binding polypeptide that binds specifically to TL1A, comprising:

(A) a humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of TSNMGVV;

(2) a CDR-H2 comprising an amino acid sequence of HILWDDREYSNPALKS;

(3) a CDR-H3 comprising an amino acid sequence of MSRNYYGSSYVMDY; and

(B) a humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of SASSSVNYMH;

(2) a CDR-L2 comprising an amino acid sequence of STSNLAS;

(3) a CDR-L3 comprising an amino acid sequence of HQWNNYGT.

2. The polypeptide of claim 1, wherein the humanized antibody heavy chain variable region comprises an amino acid sequence of QVTLKESGPALVKPTQTLTLTCTFSGFSLSTSNMGVVWIRQPPGKALEWLAHILWDDREYSNPALKSRLTISKDTSKNQVVLTMTNMDPVDTATYYCARMSRNYYGSSYVMDYWGQGTLVTVSS.

3. The polypeptide of claim 1, wherein the humanized antibody light chain variable region comprises an amino acid sequence of DIQLTQSPSFLSASVGDRVTITCSASSSVNYMHWYQQKPGKAPKLLIYSTSNLASGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCHQWNNYGTFGQGTKVEIKR.

4. An antigen binding polypeptide that binds specifically to TL1A, comprising a humanized heavy chain comprising an amino acid sequence of QVTLKESGPALVKPTQTLTLTCTFSGFSLSTSNMGVVWIRQPPGKALEWLAHILWDDREYSNPALKSRLTISKDTSKNQVVLTMTNMDPVDTATYYCARMSRNYYGSSYVMDYWGQGTLVTVSS and a humanized light chain comprising an amino acid sequence of

DIQLTQSPSFLSASVGDRVTITCSASSSVNYMHWYQQKPGKAPKLLIYSTSNLASG VPSRFSGSGSGTEFTLTISSLQPEDFATYYCHQWNNYGTFGQGTKVEIKR.

5. The polypeptide of any one of claims 1-4, wherein the polypeptide is selected from the group consisting of an antibody molecule, a Fab fragment, a Fab' fragment, a F(ab')2 fragment, and an scFv molecule.

6. The polypeptide of claim 5, wherein the polypeptide is an antibody.

7. The polypeptide of claim 1, wherein the polypeptide is a chimeric antibody comprising a human heavy chain constant region and a human light chain constant region.

8. The polypeptide of claim 6, wherein the antibody is an IgG molecule.

9. The polypeptide of claim 1, wherein the polypeptide is an scFv molecule.

10. The polypeptide of claim 9, wherein the scFv has a formula selected from the group consisting of NH2-L-VH-X-VK-COOH and NH2-L-VK-X-VH-COOH; wherein L is a leader sequence; VH is the humanized antibody heavy chain variable region; X is a linking polypeptide; and VK is the humanized antibody light chain variable region.

11. The polypeptide of claim 1, wherein the polypeptide is a Fab HSA fusion molecule.

12. The polypeptide of claim 11, wherein the Fab HSA fusion has a formula selected from the group consisting of NH2-VH-CH1-HSA-COOH and NH2-HSA-CH1-VH-COOH combined with NH2-VK-CK-COOH; wherein the VH-CH1-HSA or HSA-CH1-VH is the humanized antibody heavy chain variable region (VH) and human constant heavy chain domain 1 (CH1) produced as a fusion protein with human serum albumin (HSA) that then folds with its cognate humanized antibody light chain variable region

(VK) and human constant kappa domain (CK) to form the Fab-HSA or HSA-Fab fusion protein.

13. The polypeptide of any one of claims 1-12, conjugated to a therapeutic or diagnostic agent.

14. The polypeptide of claim 13, wherein the therapeutic agent is selected from the group consisting of a cytotoxic agent, a radioactive label, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic agent, and a combination thereof.

15. The polypeptide of claim 13, wherein the diagnostic agent is selected from the group consisting of a radioactive label, a photoactive diagnostic agent, an ultrasound-enhancing agent, and a non-radioactive label.

16. The polypeptide of any one of claims 1-15, wherein the polypeptide is an antagonist of TL1A.

17. The polypeptide of any one of claims 1-16, wherein the polypeptide is not an agonist of TL1A.

18. The polypeptide of any one of claims 1-17, wherein the polypeptide binds to TL1A with an affinity constant of at least about $10^6 M^{-1}$.

19. A pharmaceutical composition comprising any one of the polypeptides of claims 1-18 and a carrier.

20. The pharmaceutical composition of claim 19, further comprising an additional therapeutic or diagnostic agent.

21. A polynucleotide encoding any one of the polypeptides of claims 1-18.

22. A recombinant polynucleotide comprising a promoter sequence operably linked to any one of the polynucleotides of claim 21.

23. An isolated cell transformed with the recombinant polynucleotide of claim 22.

24. A method of producing the polypeptide encoded by the recombinant polynucleotide of claim 22, the method comprising:

a) culturing a cell transformed with the recombinant polynucleotide to express the encoded polypeptide; and

b) recovering the polypeptide so expressed.

25. Use of a therapeutically effective amount of the composition of claim 19 or 20 to treat an inflammatory disease or condition.

26. Use of the polypeptide of any one of claims 1 to 18 in the manufacture of a medicament to treat an inflammatory disease or condition.

27. Use of a therapeutically effective amount of the composition of claim 19 or 20 to treat an immune disease or condition.

28. Use of the polypeptide of any one of claims 1 to 18 in the manufacture of a medicament to treat an immune disease or condition.

29. Use of a therapeutically effective amount of the composition of claim 19 or 20 to treat a malignant disease or condition.

30. Use of the polypeptide of any one of claims 1 to 18 in the manufacture of a medicament to treat a malignant disease or condition.

31. The use according to any one of claims 25 to 28, wherein the disease or condition is selected from the group consisting of autoimmune disease, inflammatory bowel disease

(IBD), chronic obstructive pulmonary disease (COPD), arthritis, multiple sclerosis, transplant rejection, central nervous system injury, Th1-mediated intestinal disease, psoriasis, and atherosclerosis.

32. The use according to claim 31, wherein the autoimmune disease is lupus.

33. The use according to claim 31, wherein the arthritis is rheumatoid arthritis.

34. The use according to claim 31, wherein the Th1-mediated intestinal disease is Crohn's disease.

35. The use according to claim 29 or 30, wherein the malignant disease or condition is leukemia, lymphoma, lung carcinoma or colon carcinoma.

36. The use according to claim 35, wherein the leukemia is chronic lymphocytic leukemia (CLL).

FIGURE 1

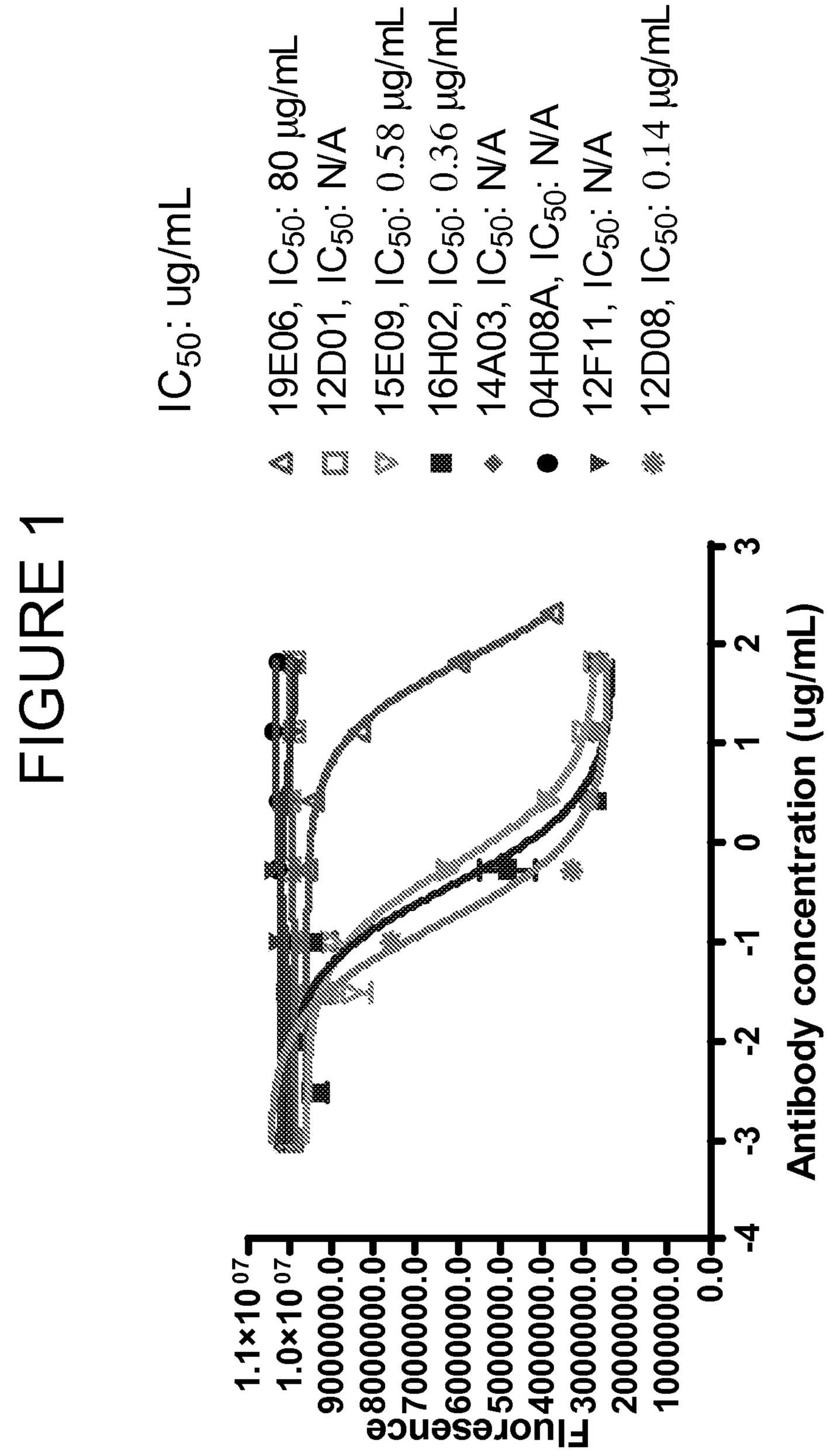
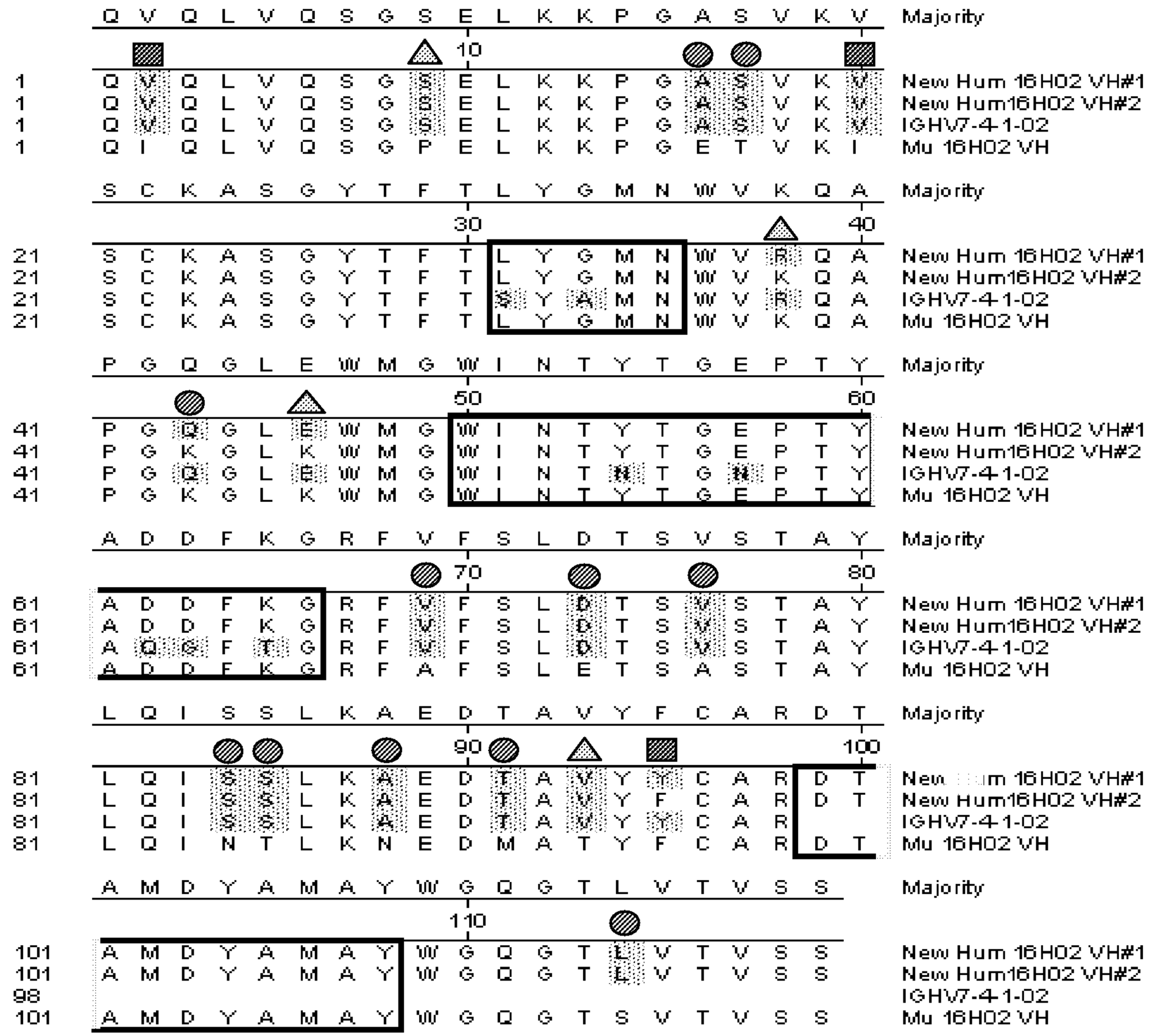

IC50: ug/mL
19E06, IC50: 80 µg/mL
12D01, IC50: N/A
15E09, IC50: 0.58 µg/mL
16H02, IC50: 0.36 µg/mL
14A03, IC50: N/A
04H08A, IC50: N/A
12F11, IC50: N/A
12D08, IC50: 0.14 µg/mL
Fluorescence
1.1×10^07
1.0×10^07
9000000.0
8000000.0
7000000.0
6000000.0
5000000.0
4000000.0
3000000.0
2000000.0
1000000.0
0.0
-4
-3
-2
-1
0
1
2
3
Antibody concentration (ug/mL)

FIGURE 2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10
1 Q V Q L V Q S G S E L K K P G A S V K V New Hum 16H02 VH#1
1 Q V Q L V Q S G S E L K K P G A S V K V New Hum16H02 VH#2
1 Q V Q L V Q S G S E L K K P G A S V K V IGHV7-4-1-02
1 Q I Q L V Q S G P E L K K P G E T V K I Mu 16H02 VH
S C K A S G Y T F T L Y G M N W V K Q A Majority
30
40
21 S C K A S G Y T F T L Y G M N W V R Q A New Hum 16H02 VH#1
21 S C K A S G Y T F T L Y G M N W V K Q A New Hum16H02 VH#2
21 S C K A S G Y T F T S Y A M N W V R Q A IGHV7-4-1-02
21 S C K A S G Y T F T L Y G M N W V K Q A Mu 16H02 VH
P G Q G L E W M G W I N T Y T G E P T Y Majority
50
60
41 P G Q G L E W M G W I N T Y T G E P T Y New Hum 16H02 VH#1
41 P G K G L K W M G W I N T Y T G E P T Y New Hum16H02 VH#2
41 P G Q G L E W M G W I N T N T G N P T Y IGHV7-4-1-02
41 P G K G L K W M G W I N T Y T G E P T Y Mu 16H02 VH
A D D F K G R F V F S L D T S V S T A Y Majority
70
80
61 A D D F K G R F V F S L D T S V S T A Y New Hum 16H02 VH#1
61 A D D F K G R F V F S L D T S V S T A Y New Hum16H02 VH#2
61 A Q G F T G R F V F S L D T S V S T A Y IGHV7-4-1-02
61 A D D F K G R F A F S L E T S A S T A Y Mu 16H02 VH
L Q I S S L K A E D T A V Y F C A R D T Majority
90
100
81 L Q I S S L K A E D T A V Y F C A R D T New Hum 16H02 VH#1
81 L Q I S S L K A E D T A V Y F C A R D T New Hum16H02 VH#2
81 L Q I S S L K A E D T A V Y Y C A R IGHV7-4-1-02
81 L Q I N T L K N E D M A T Y F C A R D T Mu 16H02 VH
A M D Y A M A Y W G Q G T L V T V S S Majority
110
101 A M D Y A M A Y W G Q G T L V T V S S New Hum 16H02 VH#1
101 A M D Y A M A Y W G Q G T L V T V S S New Hum16H02 VH#2
98 IGHV7-4-1-02
101 A M D Y A M A Y W G Q G T S V T V S S Mu 16H02 VH

FIGURE 3

Construct	# of Mutations	% Humanization
Hum VH #1	18	100
Hum VH #2	13	95

FIGURE 4

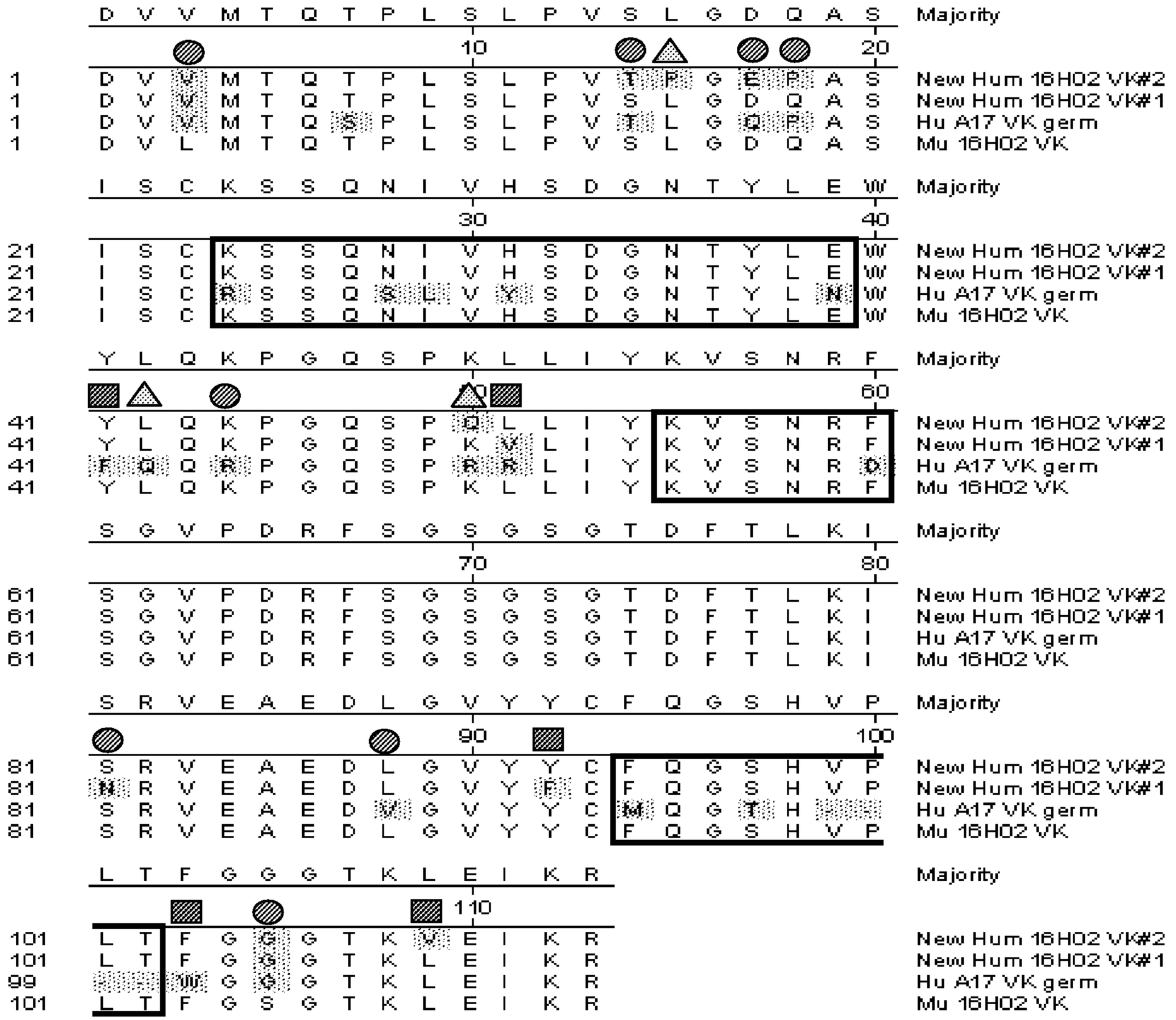

Majority
New Hum 16H02 VK#2
New Hum 16H02 VK#1
Hu A17 VK germ
Mu 16H02 VK
10
20
30
40
50
60
70
80
90
100
110

FIGURE 5

Construct	# of Mutations	% Humanization
Hum VK #1	8	87
Hum VK #2	5	87

FIGURE 6

Construct Names	Batch#/CID's	Protein / Conc.
Hum 16H02 Anti-TL1A VH#1+VK#1	LDC 3009	1.1 mgs (0.4 mg/ml)
Hum 16H02 Anti-TL1A VH#2+VK#1	LDC 3010	0.76 mgs (0.4 mg/ml)
Hum 16H02 Anti-TL1A VH#1+VK#2	LDC 3011	2.9 mgs (1.0 mg/ml)
Hum 16H02 Anti-TL1A VH#2+VK#2	LDC 3012	2.3 mgs (0.8 mg/ml)

FIGURE 7

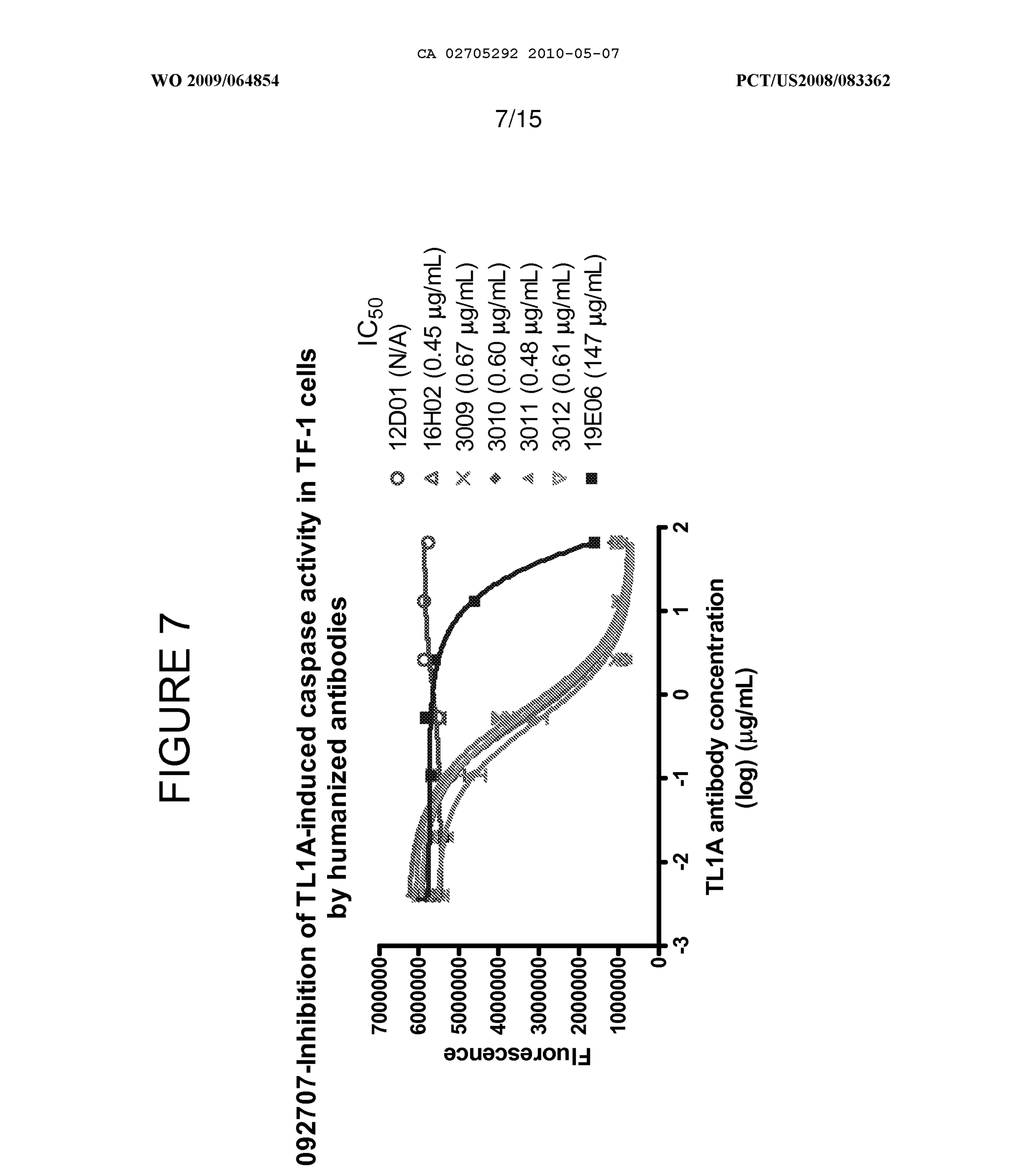

0927-Inhibition of TL1A-induced caspase activity in TF-1 cells by humanized antibodies
Fluorescence
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
-3
-2
-1
0
1
2
TL1A antibody concentration (log) (µg/mL)
IC50
12D01 (N/A)
16H02 (0.45 µg/mL)
3009 (0.67 µg/mL)
3010 (0.60 µg/mL)
3011 (0.48 µg/mL)
3012 (0.61 µg/mL)
19E06 (147 µg/mL)

FIGURE 8

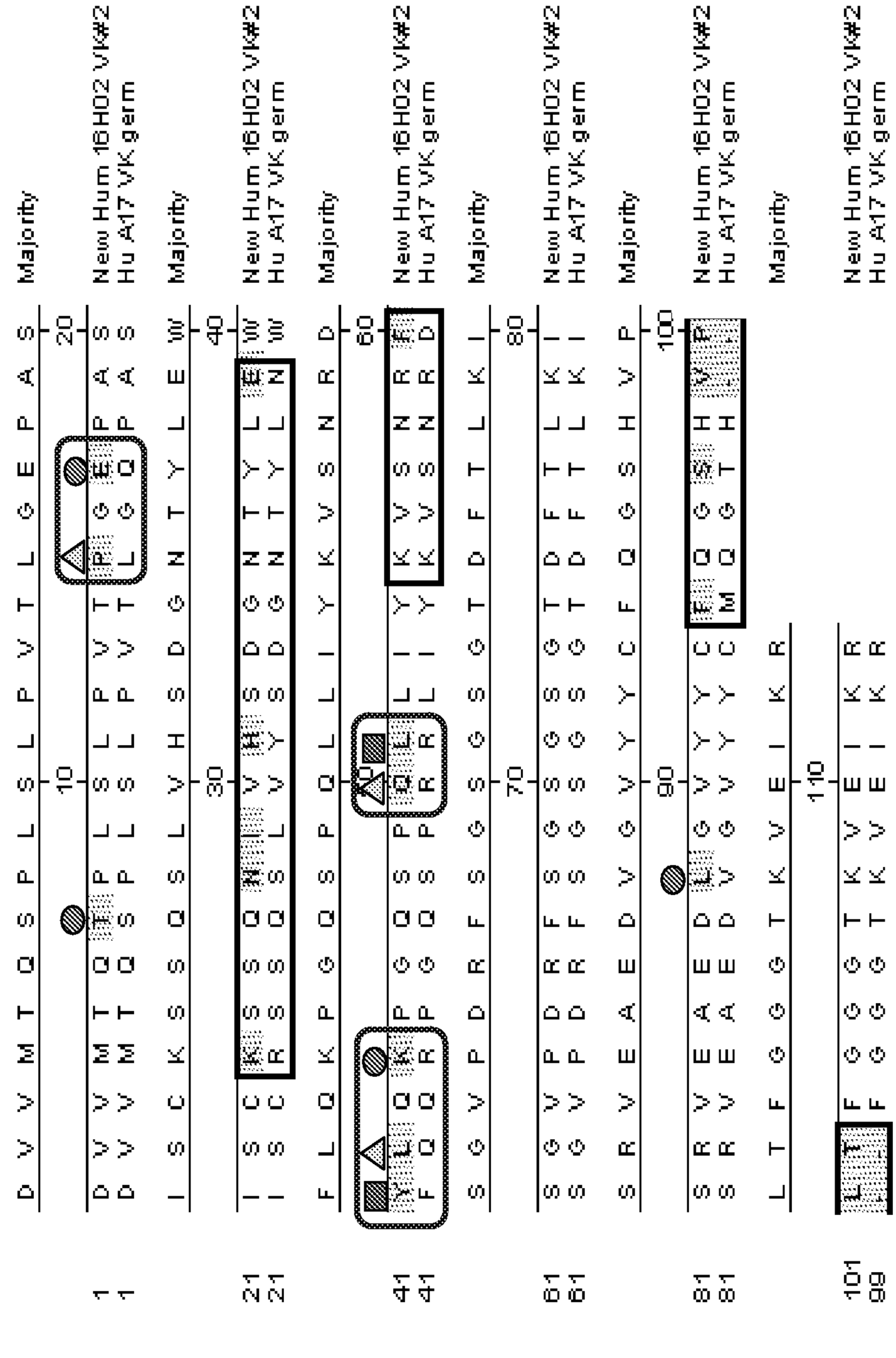

Majority
New Hum 1 VK#2 germ
Hu A17 VK#2 germ
20
40
60
80
100
10
30
70
90
110

FIGURE 9

Transfection ID	LDC #
W1	3030
W2	3031
M1	3032
M2	3033
M3	3034
M4	3035
M5	3036
M6	3037
M7	3038

16H02 VK
WWW Ver#2
New WWW
WWM
MWM
WMW-1 (QL)
WMW-2 (RL)
MWW
MMW
MMM

FIGURE 10

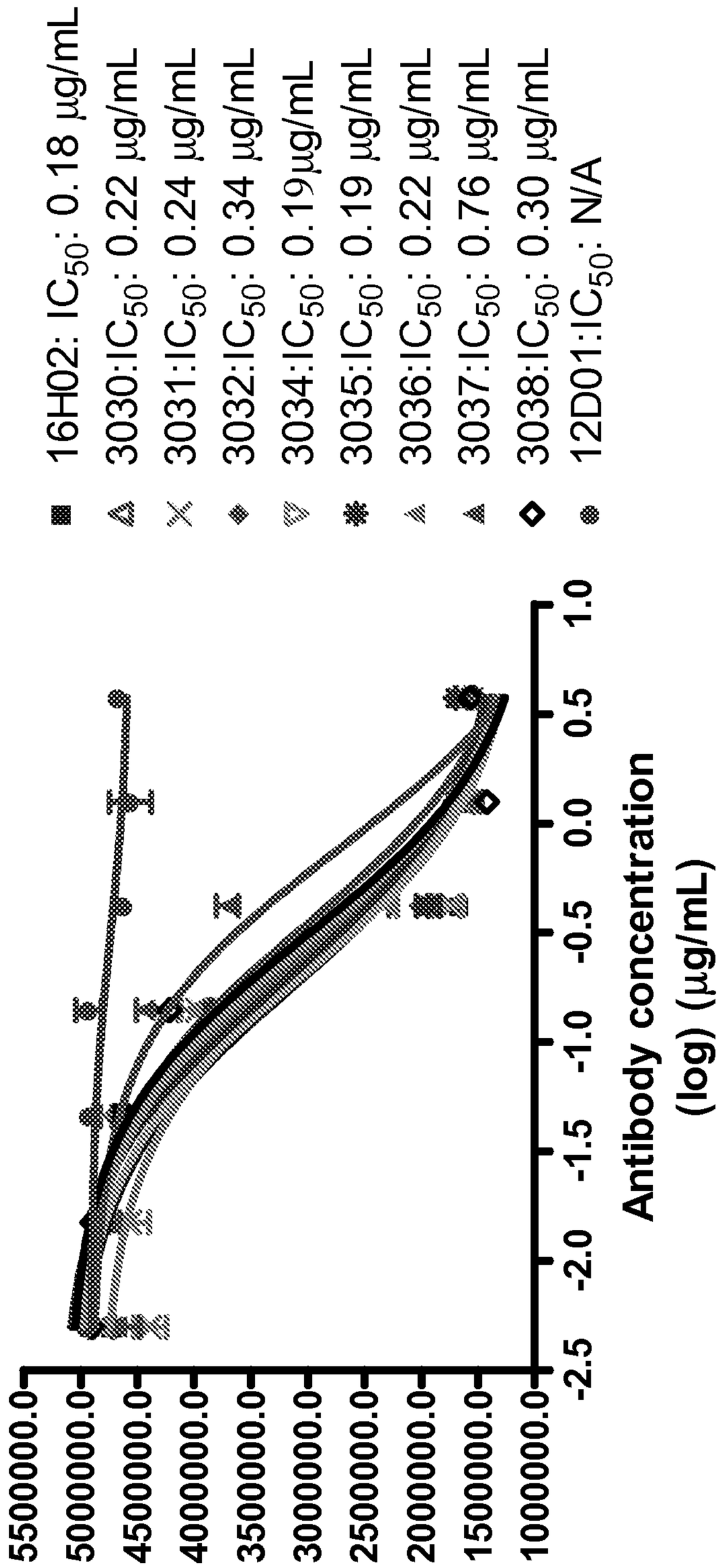
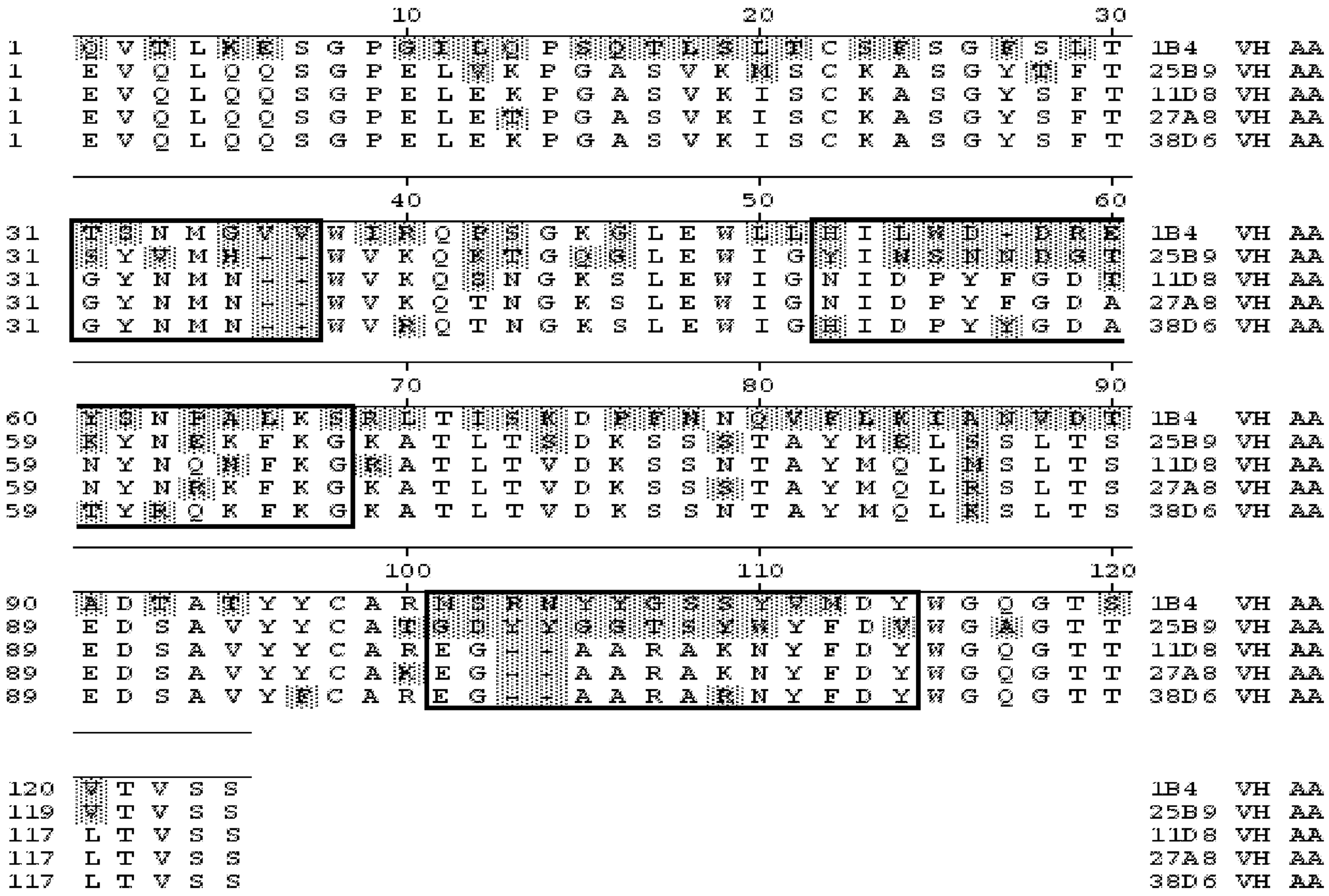

16H02: IC50: 0.18 µg/mL
3030:IC50: 0.22 µg/mL
3031:IC50: 0.24 µg/mL
3032:IC50: 0.34 µg/mL
3034:IC50: 0.19µg/mL
3035:IC50: 0.19 µg/mL
3036:IC50: 0.22 µg/mL
3037:IC50: 0.76 µg/mL
3038:IC50: 0.30 µg/mL
12D01:IC50: N/A
Antibody concentration (log) (µg/mL)
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
Caspase 3/7 activity (Fluorescence)
5500.0
5000.0
4500.0
4000.0
3500.0
3000.0
2500.0
2000.0
1500.0
1000.0

Figure 11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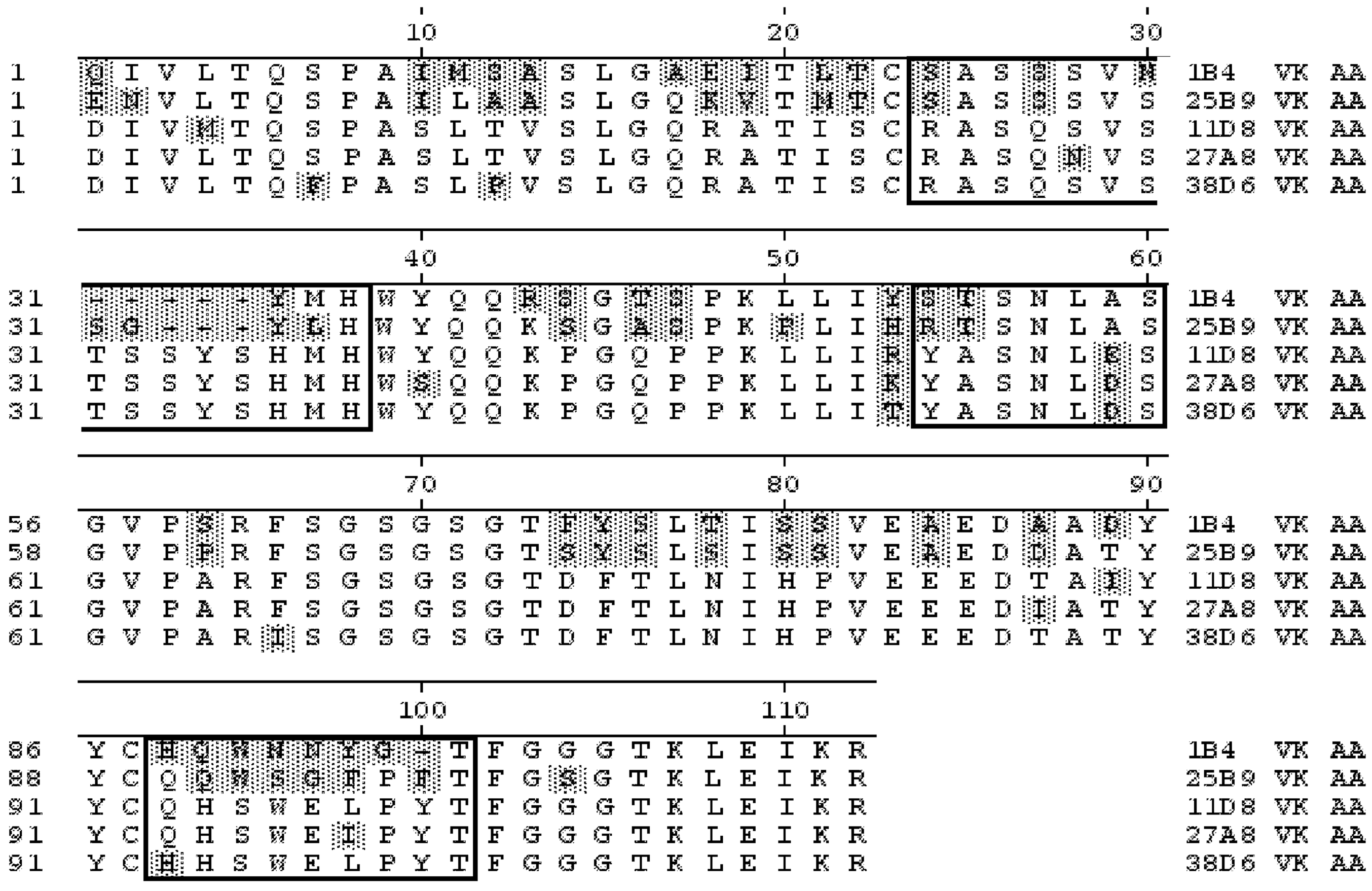
1 Q V T L K E S G P G I L Q P S Q T L S L T C S F S G F S L T 1B4 VH AA
1 E V Q L Q Q S G P E L V K P G A S V K M S C K A S G Y T F T 25B9 VH AA
1 E V Q L Q Q S G P E L E K P G A S V K I S C K A S G Y S F T 11D8 VH AA
1 E V Q L Q Q S G P E L E T P G A S V K I S C K A S G Y S F T 27A8 VH AA
1 E V Q L Q Q S G P E L E K P G A S V K I S C K A S G Y S F T 38D6 VH AA
40 50 60
31 T G N M G V W I R Q P S G K G L E W L L H I L W D - D K E 1B4 VH AA
31 S Y W M H - - W V K Q K T G Q G L E W I G Y I N S N N D G T 25B9 VH AA
31 G Y N M N - - W V K Q S N G K S L E W I G N I D P Y F G D T 11D8 VH AA
31 G Y N M N - - W V K Q T N G K S L E W I G N I D P Y F G D A 27A8 VH AA
31 G Y N M N - - W V R Q T N G K S L E W I G H I D P Y Y G D A 38D6 VH AA
70 80 90
60 Y G N F A L K S R L T I S K D F F N N Q V F L K I A N V D T 1B4 VH AA
59 K Y N E K F K G K A T L T S D K S S S T A Y M E L S S L T S 25B9 VH AA
59 N Y N Q N F K G K A T L T V D K S S N T A Y M Q L N S L T S 11D8 VH AA
59 N Y N K K F K G K A T L T V D K S S S T A Y M Q L R S L T S 27A8 VH AA
59 T Y R Q K F K G K A T L T V D K S S N T A Y M Q L K S L T S 38D6 VH AA
100 110 120
90 A D T A T Y Y C A R M S R M Y Y G G S Y V M D Y W G Q G T S 1B4 VH AA
89 E D S A V Y Y C A T S D Y Y G S T S Y W Y F D V W G A G T T 25B9 VH AA
89 E D S A V Y Y C A R E G - - A A R A K N Y F D Y W G Q G T T 11D8 VH AA
89 E D S A V Y Y C A K E G - - A A R A K N Y F D Y W G Q G T T 27A8 VH AA
89 E D S A V Y R C A R E G - - A A R A R N Y F D Y W G Q G T T 38D6 VH AA
120 V T V S S 1B4 VH AA
119 V T V S S 25B9 VH AA
117 L T V S S 11D8 VH AA
117 L T V S S 27A8 VH AA
117 L T V S S 38D6 VH AA

Figure 12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1 Q I V L T Q S P A I M S A S L G A E I T L T C S A S S S V M 1B4 VK AA
1 E N V L T Q S P A I L A A S L G Q K V T M T C S A S S S V S 25B9 VK AA
1 D I V M T Q S P A S L T V S L G Q R A T I S C R A S Q S V S 11D8 VK AA
1 D I V L T Q S P A S L T V S L G Q R A T I S C R A S Q N V S 27A8 VK AA
1 D I V L T Q F P A S L F V S L G Q R A T I S C R A S Q S V S 38D6 VK AA
40 50 60
31 - - - - - I M H W Y Q Q R S G T S P K L L I Y S T S N L A S 1B4 VK AA
31 S G - - - I L H W Y Q Q K S G A S P K F L I H R T S N L A S 25B9 VK AA
31 T S S Y S H M H W Y Q Q K P G Q P P K L L I F Y A S N L E S 11D8 VK AA
31 T S S Y S H M H W S Q Q K P G Q P P K L L I K Y A S N L D S 27A8 VK AA
31 T S S Y S H M H W Y Q Q K P G Q P P K L L I T Y A S N L D S 38D6 VK AA
70 80 90
56 G V P S R F S G S G S G T F S L T I S S V E A E D A A D Y 1B4 VK AA
58 G V P F R F S G S G S G T S Y S L S I S S V E A E D D A T Y 25B9 VK AA
61 G V P A R F S G S G S G T D F T L N I H P V E E E D T A I Y 11D8 VK AA
61 G V P A R F S G S G S G T D F T L N I H P V E E E D I A T Y 27A8 VK AA
61 G V P A R I S G S G S G T D F T L N I H P V E E E D T A T Y 38D6 VK AA
100 110
86 Y C H Q W N N Y P - T F G G G T K L E I K R 1B4 VK AA
88 Y C Q Q W S G F P F T F G S G T K L E I K R 25B9 VK AA
91 Y C Q H S W E L P Y T F G G G T K L E I K R 11D8 VK AA
91 Y C Q H S W E I P Y T F G G G T K L E I K R 27A8 VK AA
91 Y C H H S W E L P Y T F G G G T K L E I K R 38D6 VK AA

FIGURE 13

```
                    10                  20                  30
1   Q V T L K E S G P G L V Q P S Q T L S L T C S F S G F S L S  1B4  VH AA
1   Q V T L K E S G P A L V K P T Q T L T L T C T F S G F S L S  hum 1B4 VH AA
1   Q V T L K E S G P A L V K P T Q T L T L T C T F S G F S L S  VH2-70-10

                    40                  50                  60
31  T S N M G V Y W I R Q P S G K G L E W L A H I L W D D F K Y  1B4  VH AA
31  T S N M G V Y W I R Q P P G K A L E W L A H I L W D D F K Y  hum 1B4 VH AA
31  T S G M R V S W I R Q P P G K A L E W L A R I D W D D D K Y  VH2-70-10

                    70                  80                  90
61  S N P A L K S R L T I S K D P F N N Q V F L K I A N V D T A  1B4  VH AA
61  S N P A L K S R L T I S K D T S K N Q V V L T M T N M D P V  hum 1B4 VH AA
61  Y S T S L K T R L T I S K D T S K N Q V V L T M T N M D P V  VH2-70-10

                   100                 110                 120
91  D T A T Y Y C A R M S R N Y Y G S S Y V M D Y W G Q G T S V  1B4  VH AA
91  D T A T Y Y C A R M S R N Y Y G S S Y V M D Y W G Q G T L V  hum 1B4 VH AA
91  D T A T Y Y C A R                                          VH2-70-10

121 T V S S                                                    1B4  VH AA
121 T V S S                                                    hum 1B4 VH AA
99                                                             VH2-70-10
```

FIGURE 14

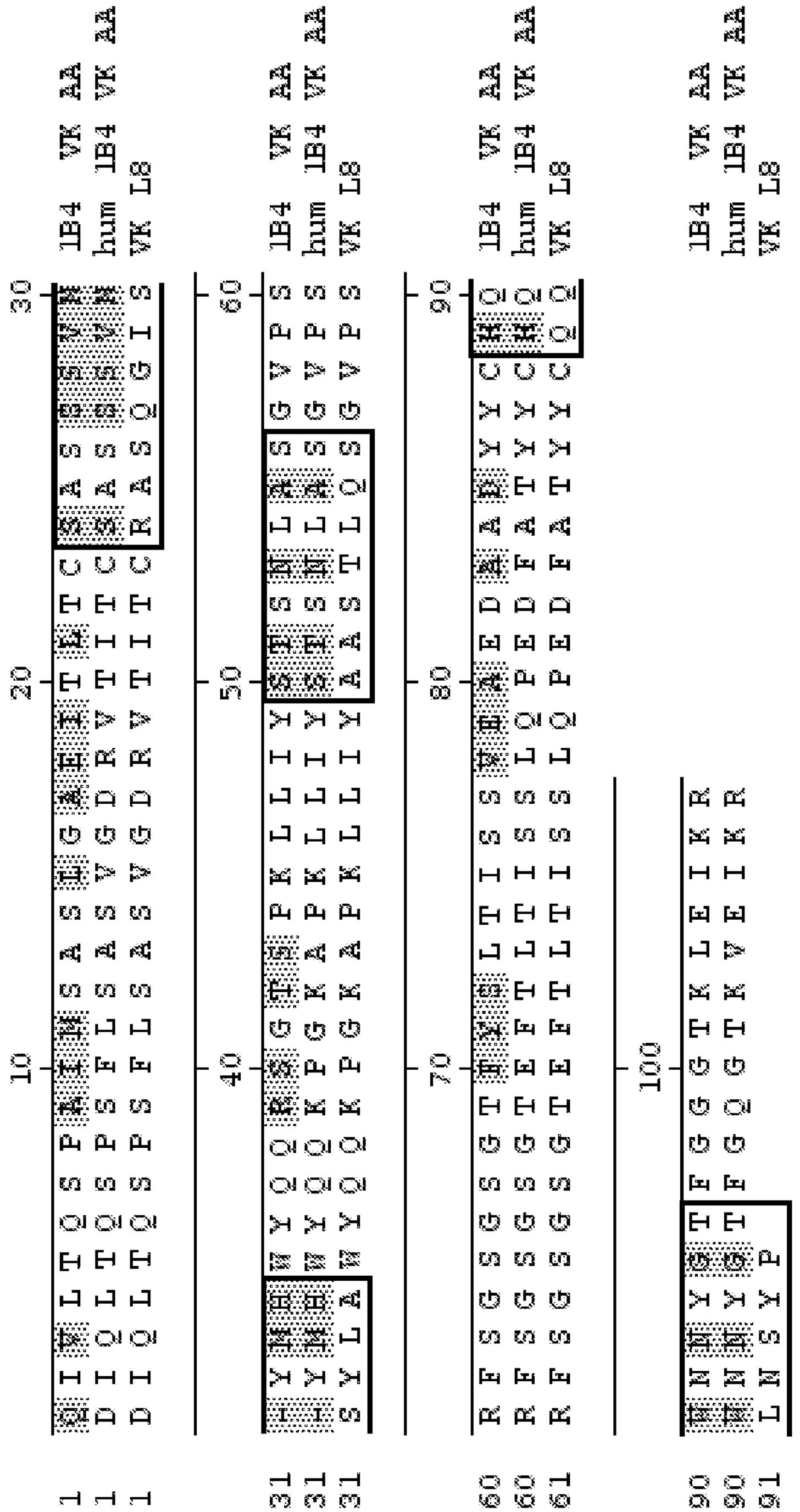
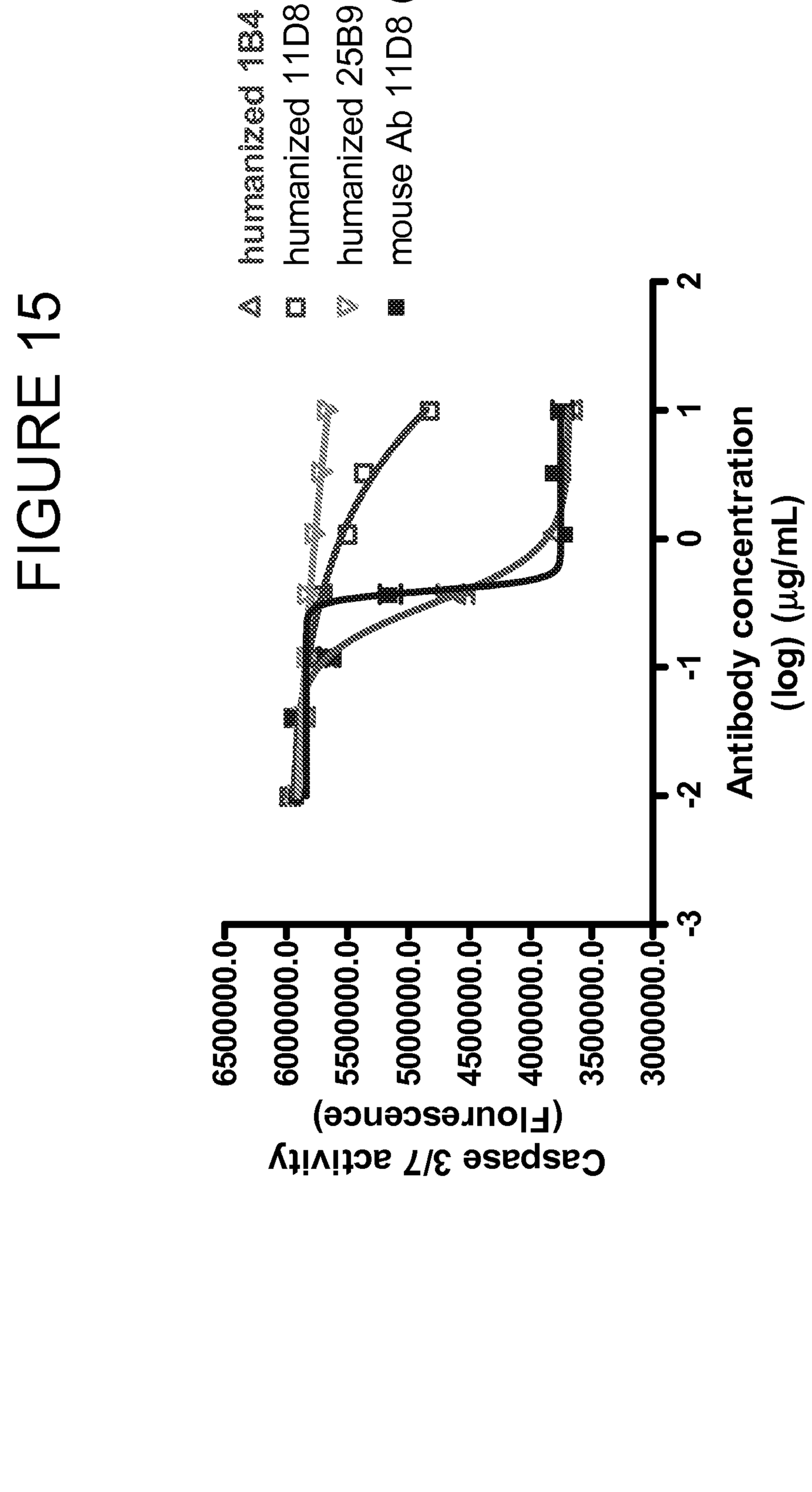
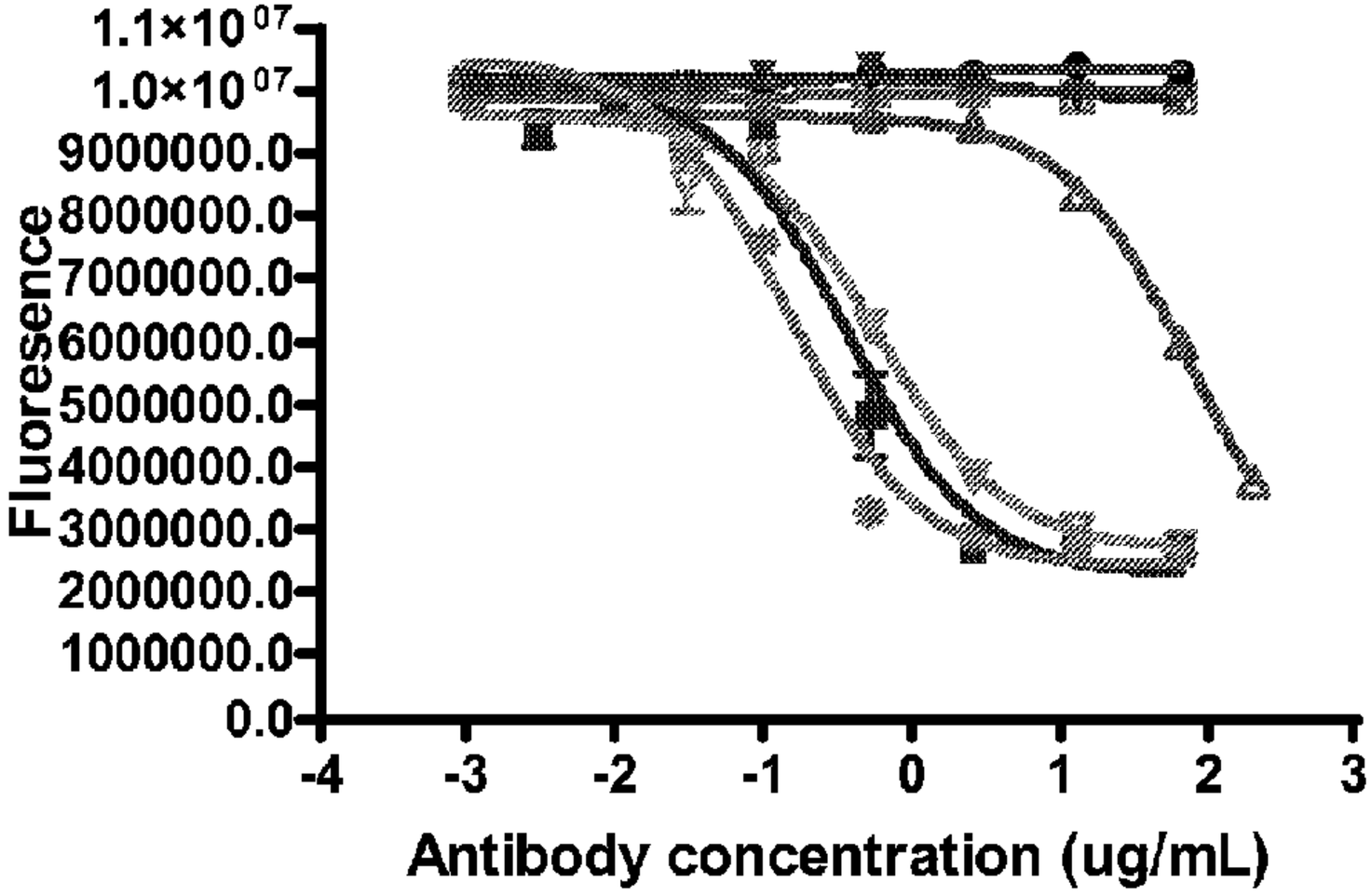


FIGURE 15


humanized 1B4 (3287): 0.32 µg/mL
humanized 11D8 (3288): N/A
humanized 25B9 (3289): 53.8 µg/mL
mouse Ab 11D8 (3196): 0.39 µg/mL
Caspase 3/7 activity (Flourescence)
65000.0
60000.0
55000.0
50000.0
45000.0
40000.0
35000.0
30000.0
-3
-2
-1
0
1
2
Antibody concentration (log) (µg/mL)



IC_{50}: ug/mL

- 19E06, IC_{50}: 80 µg/mL
- 12D01, IC_{50}: N/A
- 15E09, IC_{50}: 0.58 µg/mL
- 16H02, IC_{50}: 0.36 µg/mL
- 14A03, IC_{50}: N/A
- 04H08A, IC_{50}: N/A
- 12F11, IC_{50}: N/A
- 12D08, IC_{50}: 0.14 µg/mL