(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 January 2012 (05.01.2012)

PCT

(10) International Publication Number
WO 2012/003472 Al

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61P 35/00* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/US201 1/042843

(22) International Filing Date:
1 July 201 1 (01 .07.201 1)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/361,3 12 2 July 2010 (02.07.2010) US
61/497,289 15 June 201 1 (15.06.201 1) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ,

[Continued on next page]

(54) Title: ANTI-NOTCH 1 ANTIBODIES

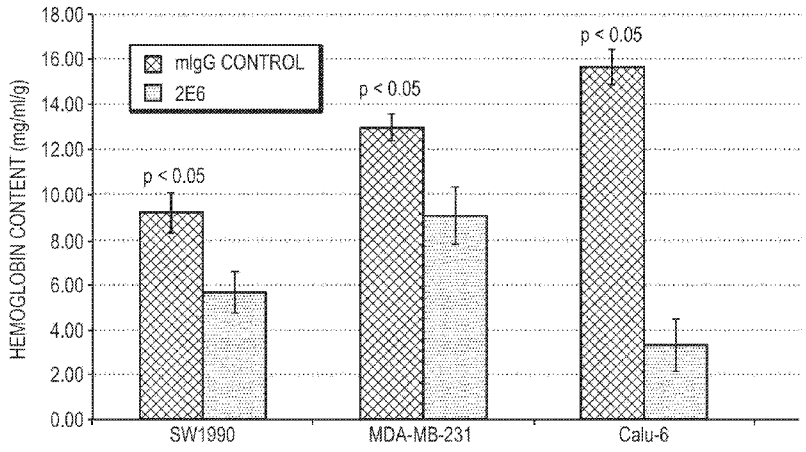

FIG. 11

(57) Abstract: Monoclonal antibodies that bind and inhibit activation of human Notch1 are disclosed. The antibodies can be used to treat cell proliferative diseases and disorders, including certain forms of cancer, associated with activation of Notch1.

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TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

ANTI-NOTCH1 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. provisional patent application serial numbers 61/361,312, filed July 2, 2010, and 61/497,289, filed June 15, 2011; the entire contents of each of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The field of the invention is molecular biology, immunology and oncology. More particularly, the field is antibodies that bind human Notch 1.

BACKGROUND

[0003] Notch pathway signaling is involved in numerous cellular processes, including cell fate determination, differentiation, proliferation, apoptosis, migration and angiogenesis. In mammals, there are four Notch proteins (sometimes called "Notch receptors"), designated Notch 1-Notch4. All four Notch proteins have a similar domain structure, which includes an extracellular domain, a negative regulatory (NRR) domain, a single-pass transmembrane domain, and an intracellular domain. The extracellular domain contains a series of EGF-like repeats that are involved in ligand binding. During maturation, the Notch polypeptide is cleaved by a furin-like protease. This cleavage divides the Notch protein into two subunits that are held together by the NRR. In the absence of ligand binding, the NRR domain functions to keep the Notch protein in a protease-resistant conformation. The intracellular domain is a transcription factor called Notch intracellular domain (NICD), which is released upon proteolytic cleavage by gamma secretase, in response to binding of the Notch protein by a ligand. In mammals, the Notch ligands are Delta-like and Jagged. When the NICD is released, it travels to the nucleus, where it activates transcription of the Notch-responsive genes, HES1, HES5, NRARP, Deltexl and c-MYC. For reviews of Notch-related biology, see, *e.g.,* Bray, 2006, **NATURE REVIEWS** 7:678-689; Kopan *et al.,* 2009, **CELL** 137:216-233.

[0004] While Notch proteins play crucial roles in normal development, dysregulation of the Notch proteins is associated with various types of cancer, including T-cell acute lymphatic

leukemia/lymphoma (T-All), breast cancer, colon cancer, ovarian cancer and lung cancer. See, *e.g.*, Miele *et al*, 2006, **CURRENT CANCER DRUG TARGETS** 6:313-323. Accordingly, one therapeutic approach for the treatment of cancer is inhibition of Notch pathway signaling. Inhibition of Notch pathway signaling has been achieved using monoclonal antibodies (Wu *et al*, 2010, **NATURE** 464:1052-1057; Aste-Amezaga *et al*, 2010, **PLOS ONE** 5:1-13 e9094).

[0005] Naturally-occurring antibodies are multimeric proteins that contain four polypeptide chains **(FIG. 1).** Two of the polypeptide chains are called immunoglobulin heavy chains (H chains), and two of the polypeptide chains are called immunoglobulin light chains (L chains). The immunoglobulin heavy and light chains are connected by an interchain disulfide bond. The immunoglobulin heavy chains are connected by interchain disulfide bonds. A light chain consists of one variable region (**V_L** in **FIG. 1)** and one constant region (**C_L** in **FIG. 1).** The heavy chain consists of one variable region (**V_H** in **FIG. 1)** and at least three constant regions (CHi, CH_2 and CH_3 in **FIG. 1).** The variable regions determine the specificity of the antibody.

[0006] Each variable region contains three hypervariable regions known as complementarity determining regions (CDRs) flanked by four relatively conserved regions known as framework regions (FRs). The three CDRs, referred to as CDR_1, CDR_2, and CDR_3, contribute to the antibody binding specificity. Naturally occurring antibodies have been used as starting material for engineered antibodies, such as chimeric antibodies and humanized antibodies.

[0007] There is a need for improved antibodies that neutralize the biological activity of human Notch 1 and that can be used as therapeutic agents to treat human patients.

SUMMARY OF THE INVENTION

[0008] The invention is based on the discovery of a family of antibodies that specifically bind human Notch 1. Antibodies disclosed herein contain Notch 1 binding sites based on the CDRs of the anti-Notch 1 antibodies described herein. The disclosed antibodies prevent or inhibit activation of human Notch 1. They do so by inhibiting Notch 1 from binding to Notch ligands, *i.e.*, Jagl, Jag2, DLLl, and DLL4. The disclosed antibodies can be used to inhibit the proliferation of tumor cells *in vitro* and/or *in vivo.* When administered to a human cancer patient, the antibodies inhibit or reduce tumor growth in the human patient.

[0009] These and other aspects and advantages of the invention are illustrated by the following figures, detailed description and claims. As used herein, "including" means without limitation, and examples cited are non-limiting.

DESCRIPTION OF THE DRAWINGS

[0010] The invention can be more completely understood with reference to the following drawings.

[0011] **FIG. 1** (prior art) is a schematic representation of a typical naturally-occurring antibody.

[0012] **FIG. 2** is a sequence alignment showing the amino acid sequence of the complete immunoglobulin heavy chain variable region of the antibodies designated 2G10, 2E6, 2A11 and 2D11. The amino acid sequences for each antibody are aligned against one another, and Complementary Determining Sequences (CDR) (Kabat definition), CDR_1, CDR_2, and CDR_3, are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0013] **FIG. 3** is a sequence alignment showing the CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) for each of the variable region sequences shown in **FIG. 2.**

[0014] **FIG. 4** is a sequence alignment showing the amino acid sequence of the complete immunoglobulin light chain variable region of antibodies 2G10, 2E6, 2A11 and 2D11. The amino acid sequences for each antibody are aligned against one another, and CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0015] **FIG. 5** is a sequence alignment showing the CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) for each of the variable region sequences shown in **FIG. 4.**

[0016] **FIG. 6** is a histogram summarizing results of an experiment to determine specificity of antibody binding to human Notch1 on the surface of CHO-Flpln-Notch1 cells. Antibodies 2G10, 2E6 (also referred to herein as antibody 2E06), 2A11, and 2D11 are shown from left to right.

[0017] **FIG. 7A** is a histogram summarizing results from a Notch1-specific reporter assay showing that antibody 2E6 (also referred to herein as antibody 2E06) inhibits Notch1-dependent reporter gene expression in the presence of Jag1. The Notch1-specific (N1-specific) control inhibitor is an anti-Notch1 polyclonal antibody (AF1057, R&D Systems). The Notch2-

specific (N2-specific) control inhibitor is an anti-Notch2 polyclonal antibody (AF1190, R&D Systems). The Notch3-specific (N3-specific) control inhibitor is an anti-Notch3 polyclonal antibody (AF1559, R&D Systems).

[0018] **FIG. 7B** is a histogram summarizing results from a Notch2-specific reporter assay showing that antibody 2E6 does not inhibit Notch2-dependent reporter gene expression in the presence of Jagl. The Notch 1-specific (Nl -specific) control inhibitor is an anti-Notch 1 polyclonal antibody (AF1057, R&D Systems). The Notch2-specific (N2-specific) control inhibitor is an anti-Notch2 polyclonal antibody (AF1 190, R&D Systems). The Notch3-specific (N3-specific) control inhibitor is an anti-Notch3 polyclonal antibody (AF1559, R&D Systems).

[0019] **FIG. 7C** is a histogram summarizing results from a Notch3-specific reporter assay showing that antibody 2E6 does not inhibit Notch3-dependent reporter gene expression in the presence of Jagl. The Notch 1-specific (Nl -specific) control inhibitor is an anti-Notch 1 polyclonal antibody (AF1057, R&D Systems). The Notch2-specific (N2-specific) control inhibitor is an anti-Notch2 polyclonal antibody (AF1 190, R&D Systems). The Notch3-specific (N3-specific) control inhibitor is an anti-Notch3 polyclonal antibody (AF1559, R&D Systems).

[0020] **FIG. 8A** is a histogram summarizing results of Notch 1-specific reporter assays showing that antibody 2E6 inhibits Notch 1-dependent reporter gene expression induced by the ligands Jagl, Jag2, DLL1 and DLL4. Reporter activity in the absence of any activating ligand (Fc + mlgG) was defined as 100% inhibition, and activity in the presence of ligand and treated with mouse IgG (Ligand alone + mlgG) was defined as 0% inhibition.

[0021] **FIG. 8B** is a histogram summarizing results of Notch 1-specific reporter assays showing that antibody 2A1 1 inhibits Notch 1-dependent reporter gene expression induced by the ligands Jagl, Jag2, DLL1 and DLL4. Reporter activity in the absence of any activating ligand (Fc + mlgG) was defined as 100% inhibition, and activity in the presence of ligand and treated with mouse IgG (Ligand alone + mlgG) was defined as 0% inhibition.

[0022] **FIG. 8C** is a histogram summarizing results of Notch 1-specific reporter assays showing that antibody 2D1 1 inhibits Notch 1-dependent reporter gene expression induced by the ligands Jagl, Jag2, DLL1 and DLL4. Reporter activity in the absence of any activating ligand (Fc + mlgG) was defined as 100% inhibition, and activity in the presence of ligand and treated with mouse IgG (Ligand alone + mlgG) was defined as 0% inhibition.

[0023] **FIG. 9A** is a histogram showing the effect of DBZ (dibenzazipine; a gamma secretase inhibitor dosed at 10 μMoI/kg once daily) and antibody 2E6 (dosed at 40, 100, or 150 mg/kg (abbreviated "mpk") three times per week) on thymocyte population in mice.

[0024] **FIG. 9B** is a graph showing mouse body weight over time, for mice treated with DBZ or antibody 2E6. This shows that DBZ at 30 μmoï/kg **(A)** or 10 μmoï/kg (■) induces weight loss in mice in 4 days and 17 days, respectively. By contrast, 40 mg/kg (abbreviated as mpk) (*), 100 mg/kg (·), or 150 mg/kg (+) of antibody 2E6 does not induce weight loss in mice (vehicle, (♦) and murine IgG (x)).

[0025] **FIG. 10** is a histogram summarizing data from an experiment to assess the effects of antibody 2E6 on functional angiogenesis *in vivo* induced by bFGF, with hemoglobin content serving as a surrogate indicator of functional angiogenesis.

[0026] **FIG. 11** is a histogram summarizing data from an experiment to assess the effects of antibody 2E6 on functional angiogenesis *in vivo* induced by human cancer cell lines (pancreatic cancer (SW1990) cells, breast cancer (MDA-MB-231 cells), and human lung cancer (Calu-6) cells), with hemoglobin content serving as a surrogate indicator of functional angiogenesis.

[0027] **FIG. 12** is a schematic diagram showing the amino acid sequences of the complete immunoglobulin heavy chain variable region of 2E6 (SEQ ID NO: 12) and the complete humanized heavy chain variable regions denoted as Hu2E6_Hvl (SEQ ID NO: 103), Hu2E6_Hvl T57A (SEQ ID NO: 105), Hu2E6_Hv2 (SEQ ID NO: 107), and Hu2E6_Hv2 T57A (SEQ ID NO: 109). The amino acid sequences for each heavy chain variable region are aligned against one another, and Complementary Determining Sequences (CDR) (Kabat definition), CDR_1, CDR_2, and CDR_3, are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0028] **FIG. 13** is a schematic diagram showing the CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) for each of the variable region sequences shown in **FIG. 12.**

[0029] **FIG. 14** is a schematic diagram showing the amino acid sequences of the complete light chain variable region of 2E6 (SEQ ID NO: 14) and the complete humanized light chain variable regions denoted as Hu2E6_Kvl (SEQ ID NO: 111) and Hu2E6_Kv2 (SEQ ID NO: 113). The amino acid sequences for each light chain variable region are aligned against one

another, and CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0030] **FIG. 15** is a sequence alignment showing the CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) for each of the variable region sequences shown in **FIG. 14.**

[0031] **Fig. 16** is a graph summarizing results from a Notch-1 specific reporter assay showing inhibition of DLL4-induced Notch-1 dependent reporter gene expression by antibodies mu2E6 (▪), Hu2E6-62 **(A),** A2-NRR1 **(T),** and a murine IgG control (·). Reporter activity in the absence of any activating ligand (Fc + mlgG) was defined as 100% inhibition, and activity in the presence of ligand and treated with mouse IgG (Ligand alone + mlgG) was defined as 0% inhibition.

[0032] **Fig. *17A*** is a histogram summarizing results from a Notch-1 specific reporter assay showing that antibody Hu2E6-62 inhibits Notch-1 dependent reporter gene expression in the presence of DLL4. **Fig. 17B** are histograms demonstrating inhibition of endogenous Notch 1-target genes by the Hu2E6-62 antibody.

[0033] **Fig. 18** is a histogram showing the effect of the Hu2E6-62 antibody dosed at 20 mg/kg (abbreviated "mpk") three times per week on thymocyte population in mice.

[0034] **Fig. 19A** is a graph showing mouse body weight over time, for mice treated with the antibodies Hu2E6-62 (▪), A2-NRR1 **(A)** and a human IgG (4)control. **Fig. 19B** are photographs showing alcian blue staining of small intestine sections after treatment with antibodies Hu2E6-62, A2-NRR1 and a human IgG control.

[0035] **Fig. 20** is a histogram summarizing data from an experiment to assess the effects of antibody Hu2E6-62 on functional angiogenesis ***in vivo*** induced by bFGF, with hemoglobin content serving as a surrogate indicator for functional angiogenesis.

[0036] **Fig. 21** is a histogram summarizing data from an experiment to assess the effects of antibody Hu2E6-62 on functional angiogenesis ***in vivo*** induced by the human lung cancer cell line Calu-6, with hemoglobin content serving as a surrogate indicator of functional angiogenesis.

DETAILED DESCRIPTION

[0037] The antibodies of the invention are based on the antigen binding sites of certain monoclonal antibodies that have been selected on the basis of binding and neutralizing the

activity of human Notch 1. The antibodies contain immunoglobulin variable region CDR sequences that define a binding site for human Notch 1.

[0038] Because of the neutralizing activity of these antibodies, they are useful for inhibiting the growth and/or proliferation of certain cancer cells and tumors. The antibodies can be engineered to minimize or eliminate an immune response when administered to a human patient. Various features and aspects of the invention are discussed in more detail below.

[0039] As used herein, unless otherwise indicated, the term "antibody" means an intact antibody *(e.g.,* an intact monoclonal antibody) or antigen-binding fragment of an antibody *(e.g.,* an antigen-binding fragment of a monoclonal antibody), including an intact antibody or antigen-binding fragment that has been modified, engineered or chemically conjugated. Examples of antibodies that have been modified or engineered are chimeric antibodies, humanized antibodies, and multispecific antibodies *(e.g.,* bispecific antibodies). Examples of antigen-binding fragments include Fab, Fab', $F(ab')_2$, Fv, single chain antibodies *(e.g.,* scFv), minibodies, and diabodies. An antibody conjugated to a toxin moiety is an example of a chemically conjugated antibody.

I. Antibodies that Bind Human Notchl

[0040] As disclosed herein, the antibodies may comprise: (a) an immunoglobulin heavy chain variable region comprising the structure CDRHI-$CDRH_2$-CDRH3 and (b) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human Notchl.

[0041] In some embodiments, the antibody comprises: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H}**i**-CDR_{H2}-CDR_{H3} and (b) an immunoglobulin light chain variable region, wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human Notchl. A CDRHI comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5 **(2G10),** SEQ ID NO: 38 **(2G10),** SEQ ID NO: 15 **(2E6),** SEQ ID NO: 40 **(2E6),** SEQ ID NO: 25 **(2A11),** SEQ ID NO: 42 **(2A11),** SEQ ID NO: 32 **(2D11),** and SEQ ID NO: 44 **(2D11);** a CDR_{H2} comprises an amino acid sequence selected from the group consisting SEQ ID NO: 6 **(2G10),** SEQ ID NO: 16 **(2E6),** SEQ ID NO: 26 **(2A11),** SEQ ID NO: 33 **(2D11),** SEQ ID NO: 94 **(Hu2E6_Hvl T57A),** SEQ ID NO: 95 **(Hu2E6_Hv2),** and SEQ ID NO: 96 **(Hu2E6_Hv2**

T57A); and a $CDRH_3$ comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 7 **(2G10),** SEQ ID NO: 17 **(2E6),** SEQ ID NO: 27 **(2A11),** and SEQ ID NO: 34 **(2D11).** Throughout the specification a particular SEQ ID NO. is followed in parentheses by the antibody that was the origin of that sequence. For example, "SEQ ID NO: 5 **(2G10)"** means that SEQ ID NO: 5 comes from antibody 2G10.

[0042] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a $CDR_{H}i$ comprising the amino acid sequence of SEQ ID NO: 5 **(2G10)** or SEQ ID NO: 38 **(2G10),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6 **(2G10),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7 **(2G10).**

[0043] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a $CDR_{H}i$ comprising the amino acid sequence of SEQ ID NO: 15 **(2E6)** or SEQ ID NO: 40 **(2E6),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 16 **(2E6),** and a $CDRH_3$ comprising the amino acid sequence of SEQ ID NO: 17 **(2E6).**

[0044] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a $CDR_{H}i$ comprising the amino acid sequence of SEQ ID NO: 25 **(2A11)** or SEQ ID NO: 42 **(2A11),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 26 **(2A11),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 27 **(2A11).**

[0045] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a $CDR_{H}i$ comprising the amino acid sequence of SEQ ID NO: 32 **(2D11)** or SEQ ID NO: 44 **(2D11),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 33 **(2D11),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 34 **(2D11).**

[0046] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a $CDR_{H}i$ comprising the amino acid sequence of SEQ ID NO: 15 **(2E6),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 94 **(Hu2E6_Hvl T57A),** SEQ ID NO: 95 **(Hu2E6_Hv2),** or SEQ ID NO: 96 **(Hu2E6_Hv2 T57A),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 17 **(2E6).**

[0047] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a $CDR_{H}i$ comprising the amino acid sequence of SEQ ID NO: 15 **(2E6),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 94 **(Hu2E6_Hvl T57A),** and a $CDRH_3$ comprising the amino acid sequence of SEQ ID NO: 17 **(2E6).**

[0048] Preferably, the CDR_{H1}, CDR_{H2}, and CDR_{H3} sequences are interposed between human or humanized immunoglobulin FRs. The antibody can be an intact antibody or an antigen-binding antibody fragment.

[0049] In other embodiments, the antibody comprises (a) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, and (b) an immunoglobulin heavy chain variable region, wherein the IgG light chain variable region and the IgG heavy chain variable region together define a single binding site for binding human Notch 1. A CDR_{L1} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8 **(2G10),** SEQ ID NO: 18 **(2E6, 2A11),** SEQ ID NO: 35 **(2D11),** and SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2);** a CDR_{L2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 9 **(2G10),** SEQ ID NO: 19 **(2E6, 2A11),** SEQ ID NO: 36 **(2D11),** SEQ ID NO: 100 **(Hu2E6_Kvl),** and SEQ ID NO: 101 **(Hu2E6_Kv2);** and a CDR_{L3} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 10 **(2G10),** SEQ ID NO: 20 **(2E6, 2A11),** and SEQ ID NO: 37 **(2D11).**

[0050] In one embodiment, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 8 **(2G10);** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9 **(2G10);** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10 **(2G10).**

[0051] In one embodiment, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 18 **(2E6, 2A11);** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 19 **(2E6, 2A11);** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6, 2A11).**

[0052] In one embodiment, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 35 **(2D11);** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 36 **(2D11);** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 37 **(2D11).**

[0053] In one embodiment, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 100 **(Hu2E6_Kvl)** or SEQ ID NO: 101 **(Hu2E6_Kv2),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6).**

[0054] In one embodiment, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 100 **(Hu2E6_Kvl),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6).**

[0055] In one embodiment, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 101 **(Hu2E6_Kv2),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6).**

[0056] Preferably, the CDR_{L1}, CDR_{L2}, and CDR_{L3} sequences are interposed between human or humanized immunoglobulin FRs. The antibody can be an intact antibody or an antigen-binding antibody fragment.

[0057] In some embodiments, the antibody comprises: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H}-CDR_{H2}-CDR_{H3} and (b) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human Notch1. The CDR_{H} is an amino acid sequence selected from the group consisting of SEQ ID NO: 5 **(2G10),** SEQ ID NO: 38 **(2G10),** SEQ ID NO: 15 **(2E6),** SEQ ID NO: 40 **(2E6),** SEQ ID NO: 25 **(2A11),** SEQ ID NO: 42 **(2A11),** SEQ ID NO: 32 **(2D11),** and SEQ ID NO: 44 **(2D11);** the CDR_{H2} is an amino acid sequence selected from the group consisting SEQ ID NO: 6 **(2G10),** SEQ ID NO: 16 **(2E6),** SEQ ID NO: 26 **(2A11),** SEQ ID NO: 33 **(2D11),** SEQ ID NO: 94 **(Hu2E6_Hvl T57A),** SEQ ID NO: 95 **(Hu2E6_Hv2),** and SEQ ID NO: 96 **(Hu2E6_Hv2 T57A);** and the CDR_{H3} is an amino acid sequence selected from the group consisting of SEQ ID NO: 7 **(2G10),** SEQ ID NO: 17 **(2E6),** SEQ ID NO: 27 **(2A11),** and SEQ ID NO: 34 **(2D11).** The CDR_{L} is an amino acid sequence selected from the group consisting of SEQ ID NO: 8 **(2G10),** SEQ ID NO: 18 **(2E6, 2A11),** SEQ ID NO: 35 **(2D11),** and SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2);** the CDR_{L2} is an amino acid sequence selected from the group consisting of SEQ ID NO:9 **(2G10),** SEQ ID NO: 19 **(2E6, 2A11),** SEQ ID NO: 36 **(2D11),** SEQ ID NO: 100 **(Hu2E6_Kvl),** and SEQ ID NO: 101

(Hu2E6_Kv2); and the CDR_{L3} is an amino acid sequence selected from the group consisting of SEQ ID NO: 10 **(2G10),** SEQ ID NO: 20 **(2E6, 2A11),** and SEQ ID NO: 37 **(2D11).**

[0058] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region selected from the group consisting of SEQ ID NO: 2 **(2G10),** SEQ ID NO: 12 **(2E6),** SEQ ID NO: 22 **(2A11),** SEQ ID NO: 29 **(2D11),** SEQ ID NO: 103 **(Hu2E6_Hvl),** SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** SEQ ID NO: 107 **(Hu2E6_Hv2),** and SEQ ID NO: 109 **(Hu2E6_Hv2 T57A),** and an immunoglobulin light chain variable region selected from the group consisting of SEQ ID NO: 4 **(2G10),** SEQ ID NO: 14 **(2E6),** SEQ ID NO: 24 **(2A11),** SEQ ID NO: 31 **(2D11),** SEQ ID NO: 111 **(Hu2E6_Kvl),** and SEQ ID NO: 113 **(Hu2E6_Kv2).**

[0059] In another embodiment, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2 **(2G10),** and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 **(2G10).**

[0060] In another embodiment, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 12 **(2E6),** and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 14 **(2E6).**

[0061] In another embodiment, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 22 **(2A11),** and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 24 **(2A11).**

[0062] In another embodiment, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 **(2D11),** and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 31 **(2D11).**

[0063] In another embodiment, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 111 **(Hu2E6_Kvl).**

[0064] In another embodiment, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 113 **(Hu2E6_Kv2).**

[0065] In other embodiments, the antibody comprises (i) an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO: 69 **(2G10),** SEQ ID NO: 73 **(2E6),** SEQ ID NO: 77 **(2A11),** SEQ ID NO: 81 **(2D11),** SEQ ID NO: 120 **(2E6 Chimeric Heavy IgGl),** SEQ ID NO: 124 **(Hu2E6_Hvl IgGl),** SEQ ID NO: 126 **(Hu2E6_Hvl T57A IgGl),** SEQ ID NO: 128 **(Hu2E6_Hv2 IgGl),** and SEQ ID NO: 130 **(Hu2E6_Hv2 T57A IgGl),** and (ii) an immunoglobulin light chain selected from the group consisting of SEQ ID NO: 71 **(2G10),** SEQ ID NO: 75 **(2E6),** SEQ ID NO: 79 **(2A11),** SEQ ID NO: 83 **(2D11),** SEQ ID NO: 122 **(2E6 Chimeric Kappa),** SEQ ID NO: 132 **(Hu2E6_Kvl Kappa),** and SEQ ID NO: 134 **(Hu2E6_Kv2 Kappa).**

[0066] In another embodiment, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 69 **(2G10),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 71 **(2G10).**

[0067] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 73 **(2E6),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 75 **(2E6).**

[0068] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 77 **(2A11),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 79 **(2A11).**

[0069] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 81 **(2D11),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 83 **(2D11).**

[0070] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 126 **(Hu2E6_Hvl T57A IgGl),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 132 **(Hu2E6_Kvl Kappa).**

[0071] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 126 **(Hu2E6_Hvl T57A IgGl),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 134 **(Hu2E6_Kv2 Kappa).**

[0072] In certain embodiments, an isolated antibody comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the framework region sequence of SEQ ID NO: 2 **(2G10),** SEQ ID NO: 12 **(2E6),** SEQ ID NO: 22 **(2A11),** SEQ ID NO: 29 **(2D11),** SEQ ID NO: 103 **(Hu2E6_Hvl),** SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** SEQ ID NO: 107 **(Hu2E6_Hv2),** or SEQ ID NO: 109 **(Hu2E6_Hv2 T57A).**

[0073] In certain embodiments, an isolated antibody comprises an immunoglobulin light chain variable region comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the framework region sequence of SEQ ID NO: 4 **(2G10),** SEQ ID NO: 14 **(2E6),** SEQ ID NO: 24 **(2A11),** SEQ ID NO: 31 **(2D11),** SEQ ID NO: 111 **(Hu2E6_Kvl),** or SEQ ID NO: 113 **(Hu2E6_Kv2).**

[0074] Homology or identity may be determined in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al,* (1990) PROC. NATL. ACAD. SCI. USA 87, 2264-2268; Altschul, (1993) J. MOL. EVOL. 36, 290-300; Altschul *et al,* (1997) NUCLEIC ACIDS RES. 25, 3389-3402, incorporated by reference) are tailored for sequence similarity searching. The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases see Altschul *et al,* (1994) NATURE GENETICS 6, 119-129 which is fully incorporated by reference. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect *(i.e.,* the statistical significance threshold for reporting matches against database sequences), cutoff,

matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al,* (1992) **PROC. NATL. ACAD. SCI.** USA 89, 10915-10919, fully incorporated by reference). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=l (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings may be Q=9; R=2; wink=l; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (e.g.: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and -Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, *e.g.,* Blosum62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

[0075] In each of the foregoing embodiments, it is contemplated herein that immunoglobulin heavy chain variable region sequences and/or light chain variable region sequences that together bind human Notchl may contain amino acid alterations *(e.g.,* at least 1, 2, 3, 4, 5, or 10 amino acid substitutions, deletions, or additions) in the framework regions of the heavy and/or light chain variable regions.

[0076] In certain embodiments, an isolated antibody binds human Notchl with a K_D of 100 nM, 50 nM, 20 nM, 15 nM, 12 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, 1 nM or lower. Unless otherwise specified, K_D values are determined by surface plasmon resonance methods under the conditions described in Examples 3 and 14.

[0077] Antibody Hu2E6-62 binds human Notchl with a K_D of 10 nM, 9 nM, 8 nM, 7 nM, 5 nM, 4 nM, 2 nM, 1 nM or lower as measured by surface plasmon resonance methods under the

conditions described in Examples 3 and 14. In an exemplary embodiment, antibody Hu2E6-62 binds human Notch 1 with a K_D of 8 nM or lower as measured by surface plasmon resonance methods at 37°C under the conditions described in Examples 3 and 14.

[0078] Antibody Hu2E6-74 binds human Notchl with a K_D of 10 nM, 9 nM, 8 nM, 7 nM, 5 nM, 4 nM, 2 nM, 1 nM or lower as measured by surface plasmon resonance methods under the conditions described in Examples 3 and 14. In an exemplary embodiment, antibody Hu2E6-74 binds human Notchl with a K_D of 8 nM or lower as measured by surface plasmon resonance methods at 37°C under the conditions described in Examples 3 and 14.

II. Antibody Production

[0079] Methods for producing antibodies of the invention are known in the art. For example, DNA molecules encoding light chain variable regions and/or heavy chain variable regions can be chemically synthesized using the sequence information provided herein. Synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, *e.g.*, constant region coding sequences, and expression control sequences, to produce conventional gene expression constructs encoding the desired antibody. Production of defined gene constructs is within routine skill in the art. Alternatively, the sequences provided herein can be cloned out of hybridomas by conventional hybridization techniques or polymerase chain reaction (PCR) techniques, using synthetic nucleic acid probes whose sequences are based on sequence information provided herein, or prior art sequence information regarding genes encoding the heavy and light chains of murine antibodies in hybridoma cells.

[0080] Nucleic acids encoding desired antibodies can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells *(e.g.,* Hep G2), and myeloma cells that do not otherwise produce IgG protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light and/or heavy chain variable regions.

[0081] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli,* it is first cloned into an expression vector by positioning the engineered gene downstream from a

suitable bacterial promoter, *e.g.,* Trp or Tac, and a prokaryotic signal sequence. The expressed secreted protein accumulates in refractile or inclusion bodies, and can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the proteins refolded and cleaved by methods known in the art.

[0082] If the engineered gene is to be expressed in eukayotic host cells, *e.g.,* CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, IgG enhancers, and various introns. This expression vector optionally contains sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed. The gene construct can be introduced into eukaryotic host cells using conventional techniques. The host cells express V_L or V_H fragments, V_L-V_H heterodimers, V_H-V_L or V_L-V_H single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a moiety having another function *(e.g.,* cytotoxicity). In some embodiments, a host cell is transfected with a single vector expressing a polypeptide expressing an entire, or part of, a heavy chain *(e.g.,* a heavy chain variable region) or a light chain *(e.g.,* a light chain variable region). In other embodiments, a host cell is transfected with a single vector encoding (a) a polypeptide comprising a heavy chain variable region and a polypeptide comprising a light chain variable region, or (b) an entire immunoglobulin heavy chain and an entire immunoglobulin light chain. In still other embodiments, a host cell is co-transfected with more than one expression vector *(e.g.,* one expression vector encoding a polypeptide comprising an entire, or part of, a heavy chain or heavy chain variable region, and another expression vector encoding a polypeptide comprising an entire, or part of, a light chain or light chain variable region).

[0083] A polypeptide comprising an immunoglobulin heavy chain variable region or light chain variable region can be produced by growing a host cell transfected with an expression vector encoding such variable region, under conditions that permit expression of the polypeptide. Following expression, the polypeptide can be harvested and purified using techniques well known in the art, *e.g.,* affinity tags such as glutathione-S-transferase (GST) and histidine tags.

[0084] A monoclonal antibody that binds human Notch 1, or an antigen-binding fragment of the antibody, can be produced by growing a host cell transfected with: (a) an expression vector that encodes a complete or partial immunoglobulin heavy chain, and a separate expression

vector that encodes a complete or partial immunoglobulin light chain; or (b) a single expression vector that encodes both chains *(e.g.,* complete or partial heavy and light chains), under conditions that permit expression of both chains. The intact antibody (or antigen-binding fragment of the antibody) can be harvested and purified using techniques well known in the art, *e.g.,* Protein A, Protein G, affinity tags such as glutathione-S-transferase (GST) and histidine tags. It is within ordinary skill in the art to express the heavy chain and the light chain from a single expression vector or from two separate expression vectors.

III. Antibody Modifications

[0085] Methods for reducing or eliminating the antigenicity of antibodies and antibody fragments are known in the art. When the antibodies are to be administered to a human, the antibodies preferably are "humanized" to reduce or eliminate antigenicity in humans. Preferably, the humanized antibodies have the same, or substantially the same, affinity for the antigen as the non-humanized mouse antibody from which it was derived.

[0086] In one humanization approach, chimeric proteins are created in which mouse immunoglobulin constant regions are replaced with human immunoglobulin constant regions. See, *e.g.,* Morrison *et al,* 1984, PROC. NAT. ACAD. SCI. 81:6851-6855, Neuberger *et al,* 1984, NATURE 312:604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

[0087] In an approach known as CDR grafting, the CDRs of the light and heavy chain variable regions are grafted into frameworks from another species. For example, murine CDRs can be grafted into human FRs. In some embodiments of the invention, the CDRs of the light and heavy chain variable regions of an anti-Notch 1 antibody are grafted into human FRs or consensus human FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described in U.S. Patent Nos. 7,022,500 (Queen); 6,982,321 (Winter); 6,180,370 (Queen); 6,054,297 (Carter); 5,693,762 (Queen); 5,859,205 (Adair); 5,693,761 (Queen); 5,565,332 (Hoogenboom); 5,585,089 (Queen); 5,530,101 (Queen); Jones *et al.* (1986) NATURE 321:522-525; Riechmann *et al.* (1988) NATURE 332:323-327; Verhoeyen *et al.* (1988) SCIENCE 239:1534-1536; and Winter (1998) FEBS LETT 430:92-94.

[0088] In an approach called "SUPERHUMANIZATION™," human CDR sequences are chosen from human germline genes, based on the structural similarity of the human CDRs to

those of the mouse antibody to be humanized. See, *e.g.*, U.S. Patent No. 6,881,557 (Foote); and Tan *et al.*, 2002, J. IMMUNOL 169:1119-1125.

[0089] Other methods to reduce immunogenicity include "reshaping," "hyperchimerization," and "veneering/resurfacing." See, *e.g.*, Vaswami *et al.*, 1998, ANNALS OF ALLERGY, ASTHMA, & IMMUNOL. 81:105; Roguska *et al.*, 1996, PROT. ENGINEER 9:895-904; and U.S. Patent No. 6,072,035 (Hardman). In the veneering/resurfacing approach, the surface accessible amino acid residues in the murine antibody are replaced by amino acid residues more frequently found at the same positions in a human antibody. This type of antibody resurfacing is described, *e.g.*, in U.S. Patent No. 5,639,641 (Pedersen).

[0090] Another approach for converting a mouse antibody into a form suitable for medical use in humans is known as ACTIVMAB™ technology (Vaccinex, Inc., Rochester, NY), which involves a vaccinia virus-based vector to express antibodies in mammalian cells. High levels of combinatorial diversity of IgG heavy and light chains are said to be produced. See, *e.g.*, U.S. Patent Nos. 6,706,477 (Zauderer); 6,800,442 (Zauderer); and 6,872,518 (Zauderer).

[0091] Another approach for converting a mouse antibody into a form suitable for use in humans is technology practiced commercially by KaloBios Pharmaceuticals, Inc. (Palo Alto, CA). This technology involves the use of a proprietary human "acceptor" library to produce an "epitope focused" library for antibody selection.

[0092] Another approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERING™ technology, which is practiced commercially by XOMA (US) LLC. See, *e.g.*, PCT Publication No. WO 93/11794 and U.S. Patent Nos. 5,766,886 (Studnicka); 5,770,196 (Studnicka); 5,821,123 (Studnicka); and 5,869,619 (Studnicka).

[0093] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of an antibody of the invention.

[0094] The antibody can be conjugated to an effector moiety such as a small molecule toxin or a radionuclide using standard *in vitro* conjugation chemistries. If the effector moiety is a polypeptide, the antibody can be chemically conjugated to the effector or joined to the effector as a fusion protein. Construction of fusion proteins is within ordinary skill in the art.

IV. Use of Antibodies

[0095] Antibodies disclosed herein can be used to treat various forms of cancer, *e.g.,* breast, ovarian, prostate, cervical, colorectal, lung, pancreatic, gastric, and head and neck cancers. The cancer cells are exposed to a therapeutically effective amount of the antibody so as to inhibit or reduce proliferation of the cancer cells. In some embodiments, the antibodies inhibit cancer cell proliferation by at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99% or 100%.

[0096] In some embodiments, the disclosed antibodies *(e.g.,* 2E6, 2G10, 2A11, 2D11, Hu2E6) may inhibit or reduce proliferation of a tumor cell by inhibiting binding of human Notch1 to a ligand, *e.g.,* Jag1, Jag2, DLL1, and DLL4. The antibodies *(e.g.,* 2E6, 2G10, 2A11, 2D11, Hu2E6) can also be used in therapy. The disclosed antibodies *(e.g.,* 2E6, 2G10, 2A11, 2D11, Hu2E6) can be used in a method to inhibit tumor growth in a mammal *(e.g.,* a human patient). The method comprises administering to the mammal a therapeutically effective amount of the antibody.

[0097] In certain embodiments, antibody Hu2E6-62 is used in therapy. For example, antibody Hu2E6-62 can be used for inhibiting or reducing proliferation of a tumor cell. Antibody Hu2E6-62 can also be used for inhibiting or reducing tumor growth in a mammal.

[0098] In other embodiments, antibody Hu2E6-74 is used in therapy. For example, antibody Hu2E6-74 can be used for inhibiting or reducing proliferation of a tumor cell. Antibody Hu2E6-74 can also be used for inhibiting or reducing tumor growth in a mammal.

[0099] Cancers associated with Notch1 overexpression and/or activation include breast cancer, ovarian cancer, prostate cancer, cervical cancer, lung cancer, brain cancers *(e.g.,* glioblastoma, astrocytoma, neuroblastoma), melanomas, gastrointestinal cancers *(e.g.,* colorectal, pancreatic, and gastric), head and neck cancer, and hematopoietic cell cancers, *(e.g.,* multiple myeloma, leukemia, *e.g.,* precursor T acute lymphoblastic leukemia (T-ALL), precursor B acute lymphoblastic leukemia (B-ALL) and B-cell chronic lymphoblastic leukemia (B-CLL)).

[00100] As used herein, "treat", "treating" and "treatment" mean the treatment of a disease in a mammal, *e.g.,* in a human. This includes: (a) inhibiting the disease, *i.e.,* arresting its development; and (b) relieving the disease, *i.e.,* causing regression of the disease state; and (c) curing the disease.

[00101] Generally, a therapeutically effective amount of active component is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1 mg/kg to 10 mg/kg. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue-level. Alternatively, the initial dosage can be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment. Human dosage can be optimized, *e.g.*, in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. A preferred route of administration is parenteral, *e.g.*, intravenous infusion. Formulation of monoclonal antibody-based drugs is within ordinary skill in the art. In some embodiments, a monoclonal antibody is lyophilized and reconstituted in buffered saline at the time of administration.

[00102] For therapeutic use, an antibody preferably is combined with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" means buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The carrier(s) should be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[00103] Pharmaceutical compositions containing antibodies disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, and rectal administration. A preferred route of administration for monoclonal antibodies is IV infusion. Useful formulations can be prepared by methods well known in the pharmaceutical art. For example, see *Remington's Pharmaceutical Sciences*, 18th

ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[0100] For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol), and suitable mixtures thereof.

[0101] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished by any suitable method, *e.g.,* filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

EXAMPLES

[0102] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1: Production of Anti-hNotch1 Monoclonal Antibodies

[0103] Immunizations, fusions, and primary screens were conducted at Maine Biotechnology Services Inc. following the Repetitive Immunization Multiple Sites (RIMMS) protocol. Five AJ mice and five Balb/c mice were immunized with a concatemeric protein containing 4 repeats of amino acids 413-488 of human Notch1 linked by two non-Notch1 amino acids, *i.e.,* alanine and glycine (AG linker) or glutamine and phenylalanine (QF linker). Subsequent boosts alternated between concatemers containing the AG linker and the QF linker. Two AJ mice and 2 Balb/c mice having sera displaying high binding to immunogen by Enzyme Linked Immunosorbent Assay (ELISA) were chosen for subsequent fusion. Spleens and lymph nodes from the selected mice were harvested. B cells were harvested and fused with a myeloma line. Fusion products from AJ mice and Balb/c mice were serially diluted in forty 96-

well plates to near clonality. A total of 10,560 supernatants from the cell fusions were screened for binding to human Notch1 on the surface of CHO cells, using a Mesoscale electrochemiluminescence assay (MSD). Three hundred supernatants that bound human Notch1 in this assay were identified from each of the AJ and Balb/c fusions. These 600 fusion products were further characterized by *in vitro* biochemical and cell-based assays, as discussed below. A panel of hybridomas was selected, the hybridomas were subcloned, and monoclonal hybridomas were expanded. Hybridoma cell lines were transferred to BioXCell (West Lebanon, NH) for antibody expression and purification by affinity chromatography on Protein G resin under standard conditions.

Example 2: Antibody Sequence Analysis

[0104] The light chain isotype and heavy chain isotype of each monoclonal antibody in Example 1 was determined using the IsoStrip™ Mouse Monoclonal Antibody Isotyping Kit according to the kit vendor's instructions (Roche Applied Science, Indianapolis, IN). All antibodies were found to be kappa light chain and IgG1 or IgG2b heavy chain.

[0105] The heavy and light chain variable regions of the mouse monoclonal antibodies were sequenced using 5' RACE (Rapid Amplification of cDNA Ends). Total RNA was extracted from each monoclonal hybridoma cell line using the RNeasy® Miniprep kit according to the vendor's instructions (Qiagen, Valencia, CA). Full-length first strand cDNA containing 5' ends was generated using either the GeneRacer™ Kit (Invitrogen, Carlsbad, California) or SMARTer™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA) according to the kit vendor's instructions, using random primers for 5' RACE.

[0106] The variable regions of the kappa and heavy (IgG1or IgG2b) chains were amplified by PCR, using KOD Hot Start Polymerase (EMD Chemicals, Gibbstown, NJ), Expand High Fidelity PCR System (Roche Applied Science), or Advantage 2 Polymerase Mix (Clontech, Mountain View, CA) according to the vendor's instructions. For amplification of 5' cDNA ends in conjunction with the GeneRacer™ Kit, the GeneRacer™ 5' Primer, 5' cgactggagcacgaggacactga 3' (SEQ ID NO: 84) (Invitrogen) was used as a 5' primer. For amplification of 5' cDNA ends in conjunction with the SMARTer™ RACE cDNA Amplification Kit, the Universal Primer Mix A primer (Clontech), a mix of 5'CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT 3' (SEQ ID NO: 85) and 5' CTAATACGACTCACTATAGGGC 3' (SEQ ID NO: 86), was used as a 5'

primer. Heavy chain variable regions were amplified using the above 5' primers and a 3' IgGl constant region specific primer, either 5' TATGCAAGGCTTACAACCACA 3' (SEQ ID NO: 87) or 5' GCCAGTGGATAGACAGATGGGGGTGTCG 3' (SEQ ID NO: 88). IgG2b sequences were amplified with 5' GGCCAGTGGATAGACTGATGGGGGTGTTGT 3' (SEQ ID NO: 89). Kappa chain variable regions were amplified with the above 5' primers and a 3' kappa constant region specific primer, either 5' CTCATTCCTGTTGAAGCTCTTGACAAT 3' (SEQ ID NO: 90) or 5' CGACTGAGGCACCTCCAGATGTT 3' (SEQ ID NO: 91).

[0107] Individual PCR products were isolated by agarose gel electrophoresis and purified using the Qiaquick® Gel Purification kit, according to the kit vendor's instructions (Qiagen). The PCR products were subsequently cloned into the pCR®4Blunt plasmid or pCR2.1®TOPO plasmid using the Zero Blunt® TOPO® PCR Cloning Kit or the TOPO® TA Cloning Kit, respectively, according to the kit vendor's instructions (Invitrogen) and transformed into DH5-α bacteria (Invitrogen), using standard molecular biology techniques. Plasmid DNA isolated from transformed bacterial clones was sequenced using M13 Forward (5'GTAAAACGACGGCCAGT 3') (SEQ ID NO: 92) and M13 Reverse primers (5' CAGGAAACAGCTATGACC 3') (SEQ ID NO: 93) by Beckman Genomics (Danvers, MA), using standard dideoxy DNA sequencing methods to identify the sequence of the variable region sequences. The sequences were analyzed using Vector NTI software (Invitrogen) and the IMGT/V-Quest web server (imgt.cines.fr) to identify and confirm variable region sequences.

[0108] The nucleic acid sequences encoding and the protein sequences defining variable regions of the murine monoclonal antibodies are summarized below (amino terminal signal peptide sequences are not shown). CDR sequences (Kabat definition) are shown in bold/underlined in the amino acid sequences.

[0109] Nucleic Acid Sequence of Heavy Chain Variable Region of Antibody 2G10 (SEQ ID NO: 1)

```
  1 gatgtgcagc tggtggagtc tgggggagtc ttagtgcagc ctggagggtc ccggaaactc
 61 tcctgtactg cctctggatt cactttcagt agctttggaa tgcactgggt tcgtcaggct
121 ccagagaagg ggctggagtg ggtcgcatac attagtagtg gcagtaaaac catctactat
181 gcagacacaa tgaagggccg attcaccatc tccagagaca atcccaagaa caccctgttc
241 ctgcaaatga cgagtctaag gtctgaggac acggccatat attactgtgc aagatcctac
301 gggtacttcg atgtctgggg cgcagggacc acggtcaccg tctcctca
```

[0110] Protein Sequence of Heavy Chain Variable Region of Antibody 2G10 (SEQ ID NO: 2)

```
  1 dvqlvesggv lvqpggsrkl sctasgftfs sfgmhwvrqa pekglewvay_ issgsktiyy
 61 adtmkgrfti srdnpkntlf lqmtslrsed taiyycarsy_ gyfdvwgagt tvtvss
```

[0111] Nucleic Acid Sequence Encoding Kappa Chain Variable Region of Antibody 2G10 (SEQ ID NO: 3)

```
  1 gacattgtga tgacccagtc tcaaaaattc atgtccacat cagtaggaga cagggtcagc
 61 gtcacctgca aggccagtca gaatgtgggt actaatgtgg cctggtatca acagaaacca
121 ggacaatctc ctaaagtgct gatttactcg gcatcctacc ggtacagtgg agtccctgat
181 cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcgccaa tgtgcagtct
241 gaagacttgg cagagtattt ctgtcagcaa tacgacagct atcctcggac gttcggtgga
301 gtcaccaagc tggaaatcaa a
```

[0112] Protein Sequence of Kappa Chain Variable Region of Antibody 2G10 (SEQ ID NO: 4)

```
  1 divmtqsqkf mstsvgdrvs vtckasqnvg tnvawyqqkp gqspkvliys_ asyrysgvpd
 61 rftgsgsgtd ftltianvqs edlaeyfcqcj ydsyprtfgg vtkleik
```

[0113] Nucleic Acid Sequence Encoding Heavy Chain Variable Region of Antibody 2E6 (SEQ ID NO: 11)

```
  1 gaggttcagc tccagcagtc tggggctgag ctggcaagac ctggggcttc agtgaagatg
 61 tcctgcaagg cttctggcta cacctttacc agctactgga tgcactgggt aaaacagagg
121 cctggacagg gtctggaatg gattggcgct gtttatccta gaaacaatga tactacttac
181 aatcagaagt tcaagggcaa ggccaagctg actgctgtca catccgccag cactgcctac
241 atggcactca gcagcctaac aaatgaggac tctgcggtct attactgtct ttatttttaac
301 tacaactttg actactgggg ccaaggcacc actctcacag tctcctca
```

[0114] Protein Sequence of Heavy Chain Variable Region of Antibody 2E6 (SEQ ID NO: 12)

```
  1 evqlqqsgae larpgasvkm sckasgytft sywmhwvkqr pgqglewiga vyprnndtty
 61 nqkfkgkakl tavtsastay malssltned savyyclyfn ynfdywgqgt tltvss
```

[0115] Nucleic Acid Sequence Encoding Kappa Chain Variable Region of Antibody 2E6 (SEQ ID NO: 13)

```
  1 caaattgttc tcacccagtc tccagcaatc atgtctgctt ctccagggga gaaggtcacc
 61 atgacctgca gtgccagctc aagtgtaagt tacatgcact ggtaccagca gaagccagga
121 tcctccccca gactcctgat ttatgacaca tccaacctgg cttctggagt ccctgtgcac
181 ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcatccgaat ggaggctgaa
241 gatgctgcca cttattactg ccagcagtgg agtagttacc cgtacacgtt cggaggggg
301 accaagctgg aaataaaa
```

[0116] Protein Sequence of Kappa Chain Variable Region of Antibody 2E6 (SEQ ID NO: 14)

```
  1 qivltqspai msaspgekvt mtcsasssvs ymhwyqqkpq ssprlliydt snlasgvpvh
 61 fsgsgsgtsy sltiirmeae daatyycqqw ssypyt fgqgq tkleik
```

[0117] Nucleic Acid Sequence Encoding Heavy Chain Variable Region of Antibody 2A1 1 (SEQ ID NO: 21)

```
  1 caggttcagc tgcagcagtc tggacctgag ctggtgaagc ctggggcctc agtgaagatt
 61 tcctgcaagg cttctggcta tgcattcagt agctcctgga tgaactgggt gaagcagagg
121 cctggaaagg gtcttgagtg gattggacgg atttatcctg gagatggaga tactaactac
181 aatgggaaat tcaagggcaa ggccacactg actgcagaca aatcctccag cacagcctac
241 atgcaactca gcagcctgac atctgaggac tctgcggtct acttctgtgc aagatcgggc
301 tccatctact atggtaacca cggggactac tttgactact ggggccaagg caccactctc
361 acagtctcct ca
```

[0118] Protein Sequence Defining Heavy Chain Variable Region of Antibody 2A1 1 (SEQ ID NO: 22)

```
  1 qvqlqqsgpe lvkpgasvki sckasgyafs sswmnwvkqr pgkglewigr iypgdgdtny
 61 ngkfkgkatl tadkssstay mqlssltsed savyfcarsg siyygnhgdy fdywqqttl
121 tvss
```

[0119] Nucleic Acid Sequence Encoding Kappa Chain Variable Region of Antibody 2A1 1 (SEQ ID NO: 23)

```
  1 caaattgttc tcacccagtc tccagcaatc atgtctgctt ctccagggga gaaggtcacc
 61 atgacctgca gtgccagctc aagtgtaagt tacatgcact ggtaccagca gaagccagga
121 tcctccccca gactcctgat ttatgacaca tccaacctgg cttctggagt ccctgtgcac
181 ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcatccgaat ggaggctgaa
241 gatgctgcca cttattactg ccagcagtgg agtagttacc cgtacacgtt cggaggggg
301 accaagctgg aaataaaa
```

[0120] Protein Sequence of Kappa Chain Variable Region of Antibody 2A1 1 (SEQ ID NO: 24)

```
  1 qivltqspai msaspgekvt mtcsasssvs ymhwyqqkpq ssprlliydt snlasgvpvh
 61 fsgsgsgtsy sltiirmeae daatyycqqw ssypyt fggg tkleik
```

[0121] Nucleic Acid Sequence Encoding Heavy Chain Variable Region of Antibody 2D1 1 (SEQ ID NO: 28)

```
  1 gaggttcagc tccagcagtc tggggctgag ctggcaagac ctggggcttc agtgaagatg
 61 tcctgcaagg cttctggcta caccttacc aggtactgga tgcactgggt aaaacagagg
121 cctggacagg gtctggaatg gattggcgct atttatcctg gaaatagtga tactacctac
181 aatcagaagt tcaagggcaa ggccaaactg actgcagtca catccgccag cactgcctac
241 atggagctca gcagcctaac aaatgaggac tctgcggtct attactgtat ataccctat
301 gattaccttg actactgggg ccaaggcacc actctcacag tctcctca
```

[0122] Protein Sequence Defining Heavy Chain Variable Region of Antibody 2D1 1 (SEQ ID NO: 29)

```
  1 evqlqqsgae larpgasvkm sckasgytft rywmhwvkqr pgqglewiga iypgnsdtty
 61 nqkfkgkakl tavtsastay melssltned savyyciypy dyldywgqgt tltvss
```

[0123] Nucleic Acid Sequence Encoding Kappa Chain Variable Region of Antibody 2D1 1 (SEQ ID NO: 30)

```
  1 caaattgttc tcacccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcacc
 61 atgacctgca gtgccagctc aagtttaagt tacatgcact ggtaccagca gaagccaggc
121 acctccccca aaagatgggt ttatgacaca tccaaactgg cttctggagt ccctgctcgc
181 ttcagtggca gtgggtctgg gacctcttat tctctcacaa tcagcagcat ggaggctgaa
241 gatgctgcca cttattactg ccatcagcgg agtagttacc cgtacacgtt cggaggggg
301 accaagctgg aaataaaa
```

[0124] Protein Sequence Defining Kappa Chain Variable Region of Antibody 2D1 1 (SEQ ID NO: 31)

```
  1 qivltqspai msaspgekvt mtcsasssls ymhwyqqkpg tspkrwvydt sklasgvpar
 61 fsgsgsgtsy sltissmeae daatyychqr ssypyt fggg tkleik
```

[0125] The amino acid sequences defining the immunoglobulin heavy chain variable regions for the antibodies in Example 1 are aligned in **FIG. 2.** Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR_1, CDR_2, and CDR_3 (Kabat definition) are identified by boxes. **FIG. 3** shows an alignment of the separate CDR_1, CDR_2, and CDR_3 sequences for each antibody.

[0126] The amino acid sequences defining the immunoglobulin light chain variable regions for the antibodies in Example 1 are aligned in **FIG. 4.** Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR_1, CDR_2 and CDR_3 are identified by boxes. **FIG. 5** shows an alignment of the separate CDR_1, CDR_2, and CDR_3 sequences for each antibody.

[0127] Table 1 shows the SEQ ID NO. of each sequence discussed in this Example.

Table 1

SEQ. ID NO.	Nucleic Acid or Protein
1	2G10 Heavy Chain Variable Region—nucleic acid
2	2G10 Heavy Chain Variable Region—protein
3	2G10 Light (kappa) Chain Variable Region—nucleic acid
4	2G10 Light (kappa) Chain Variable Region—protein
5	2G10 Heavy Chain CDR_1
6	2G10 Heavy Chain CDR_2
7	2G10 Heavy Chain CDR_3
8	2G10 Light (kappa) Chain CDR_1
9	2G10 Light (kappa) Chain CDR_2
10	2G10 Light (kappa) Chain CDR_3
11	2E6 Heavy Chain Variable Region—nucleic acid
12	2E6 Heavy Chain Variable Region—protein
13	2E6 Light (kappa) Chain Variable Region—nucleic acid
14	2E6 Light (kappa) Chain Variable Region—protein
15	2E6 Heavy Chain CDR_1
16	2E6 Heavy Chain CDR_2
17	2E6 Heavy Chain CDR_3
18	2E6 Light (kappa) Chain CDR_1
19	2E6 Light (kappa) Chain CDR_2
20	2E6 Light (kappa) Chain CDR_3
21	2A11 Heavy Chain Variable Region—nucleic acid
22	2A11 Heavy Chain Variable Region—protein
23	2A11 Light (kappa) Chain Variable Region—nucleic acid
24	2A11 Light (kappa) Chain Variable Region—protein
25	2A11 Heavy Chain CDR_1
26	2A11 Heavy Chain CDR_2
27	2A11 Heavy Chain CDR_3
18	2A11 Light (kappa) Chain CDR_1
19	2A11 Light (kappa) Chain CDR_2
20	2A11 Light (kappa) Chain CDR_3
28	2D11 Heavy Chain Variable Region—nucleic acid
29	2D11 Heavy Chain Variable Region—protein
30	2D11 Light (kappa) Chain Variable Region—nucleic acid
31	2D11 Light (kappa) Chain Variable Region—protein
32	2D11 Heavy Chain CDR_1
33	2D11 Heavy Chain CDR_2
34	2D11 Heavy Chain CDR_3
35	2D11 Light (kappa) Chain CDR_1
36	2D11 Light (kappa) Chain CDR_2
37	2D11 Light (kappa) Chain CDR_3

[0128] Mouse monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 2.

Table 2

	Kabat		
	CDR1	CDR2	CDR3
2G10	SFGMH (SEQ ID NO: 5)	YISSGSKTIYYADTMKG (SEQ ID NO: 6)	SYGYFDV (SEQ ID NO: 7)
2E6	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFKG (SEQ ID NO: 16)	FNYNFDY (SEQ ID NO: 17)
2A11	SSWMN (SEQ ID NO: 25)	RIYPGDGDTNYNGKFKG (SEQ ID NO: 26)	SGSIYYGNHGDYFDY (SEQ ID NO: 27)
2D11	RYWMH (SEQ ID NO: 32)	AIYPGNSDTTYNQKFKG (SEQ ID NO: 33)	PYDYLDY (SEQ ID NO: 34)
	Chothia		
	CDR1	CDR2	CDR3
2G10	GFTFSSF (SEQ ID NO: 38)	SSGSKTI (SEQ ID NO: 39)	SYGYFDV (SEQ ID NO: 7)
2E6	GYTFTSY (SEQ ID NO: 40)	YPRNNDT (SEQ ID NO: 41)	FNYNFDY (SEQ ID NO: 17)
2A11	GYAFSSS (SEQ ID NO: 42)	YPGDGDT (SEQ ID NO: 43)	SGSIYYGNHGDYFDY (SEQ ID NO: 27)
2D11	GYTFTRY (SEQ ID NO: 44)	YPGNSDT (SEQ ID NO: 45)	PYDYLDY (SEQ ID NO: 34)
	IMGT		
	CDR1	CDR2	CDR3
2G10	GFTFSSFG (SEQ ID NO: 46)	ISSGSKTI (SEQ ID NO: 47)	ARSYGYFDV (SEQ ID NO: 48)
2E6	GYTFTSYW (SEQ ID NO: 49)	VYPRNNDT (SEQ ID NO: 50)	LYFNYNFDY (SEQ ID NO: 51)
2A11	GYAFSSSW (SEQ ID NO: 52)	IYPGDGDT (SEQ ID NO: 53)	ARSGSIYYGNHGDYFDY (SEQ ID NO: 54)
2D11	GYTFTRYW (SEQ ID NO: 55)	IYPGNSDT (SEQ ID NO: 56)	IYPYDYLDY (SEQ ID NO: 57)

[0129] Mouse monoclonal antibody Kappa light chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 3.

Table 3

	Kabat/Chothia		
	CDR1	CDR2	CDR3
2G10	KASQNVGTNVA (SEQ ID NO: 8)	SASYRYS (SEQ ID NO: 9)	QQYDSYPRT (SEQ ID NO: 10)
2E6	SASSSVSYMH (SEQ ID NO: 18)	DTSNLAS (SEQ ID NO: 19)	QQWSSYPYT (SEQ ID NO: 20)
2A11	SASSSVSYMH (SEQ ID NO: 18)	DTSNLAS (SEQ ID NO: 19)	QQWSSYPYT (SEQ ID NO: 20)
2D11	SASSSLSYMH (SEQ ID NO: 35)	DTSKLAS (SEQ ID NO: 36)	HQRSSYPYT (SEQ ID NO: 37)
	IMGT		
	CDR1	CDR2	CDR3
2G10	QNVGTN (SEQ ID NO: 58)	SAS	QQYDSYPRT (SEQ ID NO: 10)
2E6	SSVSY (SEQ ID NO: 59)	DTS	QQWSSYPYT (SEQ ID NO: 20)
2A11	SSVSY (SEQ ID NO: 60)	DTS	QQWSSYPYT (SEQ ID NO: 20)
2D11	SSLSY (SEQ ID NO: 61)	DTS	HQRSSYPYT (SEQ ID NO: 37)

[0130] To create the complete heavy or kappa chain antibody sequences, each variable sequence above is combined with its respective constant region. For example, a complete heavy chain comprises a heavy variable sequence followed by the murine IgGl or IgG2b heavy chain constant sequence, and a complete kappa chain comprises a kappa variable sequence followed by the murine kappa light chain constant sequence.

[0131] Nucleic Acid Sequence Encoding Murine IgGl Heavy Chain Constant Region (SEQ ID NO: 62)

```
   1 gccaaaacga cacccccatc tgtctatcca ctggcccctg gatctgctgc ccaaactaac
  61 tccatggtga ccctgggatg cctggtcaag ggctatttcc ctgagccagt gacagtgacc
 121 tggaactctg gatccctgtc cagcggtgtg cacaccttcc cagctgtcct gcagtctgac
 181 ctctacactc tgagcagctc agtgactgtc ccctccagca cctggcccag cgagaccgtc
 241 acctgcaacg ttgcccaccc ggccagcagc accaaggtgg acaagaaaat tgtgcccagg
 301 gattgtggtt gtaagccttg catatgtaca gtcccagaag tatcatctgt cttcatcttc
 361 cccccaaagc ccaaggatgt gctcaccatt actctgactc ctaaggtcac gtgtgttgtg
 421 gtagacatca gcaaggatga tcccgaggtc cagttcagct ggtttgtaga tgatgtggag
 481 gtgcacacag ctcagacgca accccgggag gagcagttca acagcacttt ccgctcagtc
 541 agtgaacttc ccatcatgca ccaggactgg ctcaatggca aggagttcaa atgcagggtc
 601 aacagtgcag ctttccctgc ccccatcgag aaaaccatct ccaaaaccaa aggcagaccg
 661 aaggctccac aggtgtacac cattccacct cccaaggagc agatggccaa ggataaagtc
 721 agtctgacct gcatgataac agacttcttc cctgaagaca ttactgtgga gtggcagtgg
 781 aatgggcagc cagcggagaa ctacaagaac actcagccca tcatggacac agatggctct
```

```
841 tacttcgtct acagcaagct caatgtgcag aagagcaact gggaggcagg aaatactttc
901 acctgctctg tgttacatga gggcctgcac aaccaccata ctgagaagag cctctcccac
961 tctcctggta aa
```

[0132] Protein Sequence of Murine IgGl Heavy Chain Constant Region (SEQ ID NO: 63)

```
  1 akttppsvyp lapgsaaqtn smvtlgclvk gyfpepvtvt wnsgslssgv htfpavlqsd
 61 lytlsssvtv psstwpsetv tcnvahpass tkvdkkivpr dcgckpcict vpevssvfif
121 ppkpkdvlti tltpkvtcvv vdiskddpev qfswfvddve vhtaqtqpre eqfnstfrsv
181 selpimhqdw lngkefkcrv nsaafpapie ktisktkgrp kapqvytipp pkeqmakdkv
241 sltcmitdff peditvewqw ngqpaenykn tqpimdtdgs yfvysklnvq ksnweagntf
301 tcsvlheglh nhhtekslsh spgk
```

[0133] Nucleic Acid Sequence Encoding Murine IgG2b Heavy Chain Constant Region (SEQ ID NO: 64)

```
  1 gccaaaacaa cacccccatc agtctatcca ctggcccctg ggtgtggaga tacaactggt
 61 tcctccgtga cctctgggtg cctggtcaag gggtacttcc ctgagccagt gactgtgact
121 tggaactctg gatccctgtc cagcagtgtg cacaccttcc cagctctcct gcagtctgga
181 ctctacacta tgagcagctc agtgactgtc ccctccagca cctggccaag tcagaccgtc
241 acctgcagcg ttgctcaccc agccagcagc accacggtgg acaaaaaact tgagcccagc
301 gggcccattt caacaatcaa cccctgtcct ccatgcaagg agtgtcacaa atgcccagct
361 cctaacctcg agggtggacc atccgtcttc atcttccctc caaatatcaa ggatgtactc
421 atgatctccc tgacacccaa ggtcacgtgt gtggtggtgg atgtgagcga ggatgaccca
481 gacgtccaga tcagctggtt tgtgaacaac gtggaagtac acacagctca gacacaaacc
541 catagagagg attacaacag tactatccgg gtggtcagca ccctccccat ccagcaccag
601 gactggatga gtggcaagga gttcaaatgc aaggtgaaca acaaagacct cccatcaccc
661 atcgagagaa ccatctcaaa aattaaaggg ctagtcagag ctccacaagt atacactttg
721 ccgccaccag cagagcagtt gtccaggaaa gatgtcagtc tcacttgcct ggtcgtgggc
781 ttcaaccctg gagacatcag tgtggagtgg accagcaatg ggcatacaga ggagaactac
841 aaggacaccg caccagttct tgactctgac ggttcttact tcatatatag caagctcaat
901 atgaaaacaa gcaagtggga gaaaacagat tccttctcat gcaacgtgag acacgagggt
961 ctgaaaaatt actacctgaa gaagaccatc tcccggtctc cgggtaaa
```

[0134] Protein Sequence of Murine IgG2b Heavy Chain Constant Region (SEQ ID NO: 65)

```
  1 akttppsvyp lapgcgdttg ssvtsgclvk gyfpepvtvt wnsgslsssv htfpallqsg
 61 lytmsssvtv psstwpsqtv tcsvahpass ttvdkkleps gpistinpcp pckechkcpa
121 pnleggpsvf ifppnikdvl misltpkvtc vvvdvseddp dvqiswfvnn vevhtaqtqt
181 hredynstir vvstlpiqhq dwmsgkefkc kvnnkdlpsp iertiskikg lvrapqvytl
241 pppaeqlsrk dvsltclvvg fnpgdisvew tsnghteeny kdtapvldsd gsyfiyskln
301 mktskwektd sfscnvrheg lknyylkkti srspgk
```

[0135] Nucleic Acid Sequence Encoding Murine Kappa Light Chain Constant Region (SEQ ID NO: 66)

```
  1 cgggctgatg ctgcaccaac tgtatccatc ttcccaccat ccagtgagca gttaacatct
 61 ggaggtgcct cagtcgtgtg cttcttgaac aacttctacc ccaaagacat caatgtcaag
121 tggaagattg atggcagtga acgacaaaat ggcgtcctga acagttggac tgatcaggac
181 agcaaagaca gcacctacag catgagcagc accctcacgt tgaccaagga cgagtatgaa
241 cgacataaca gctatacctg tgaggccact cacaagacat caacttcacc cattgtcaag
301 agcttcaaca ggaatgagtg t
```

[0136] Protein Sequence of Murine Kappa Light Chain Constant Region (SEQ ID NO: 67)

```
  1 radaaptvsi fppsseqlts ggasvvcfln nfypkdinvk wkidgserqn gvlnswtdqd
 61 skdstysmss tltltkdeye rhnsytceat hktstspivk sfnrnec
```

[0137] The following sequences represent the actual or contemplated full length heavy and light chain sequence *(i.e.,* containing both the variable and constant regions sequences) for each antibody described in this Example. Signal sequences for proper secretion of the antibodies *(e.g.,* signal sequences at the 5' end of the DNA sequences or the amino terminal end of the protein sequences) are not shown in the full length heavy and light chain sequences disclosed herein and are not included in the final secreted protein. Also not shown are stop codons for termination of translation required at the 3' end of the DNA sequences. It is within ordinary skill in the art to select a signal sequence and/or a stop codon for expression of the disclosed full length IgG heavy chain and light chain sequences. It is also contemplated that the variable region sequences can be ligated to other constant region sequences to produce active full length IgG heavy and light chains.

[0138] Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 2G10 (SEQ ID NO: 68)

```
   1 gatgtgcagc tggtggagtc tgggggagtc ttagtgcagc ctggagggtc ccggaaactc
  61 tcctgtactg cctctggatt cactttcagt agctttggaa tgcactgggt tcgtcaggct
 121 ccagagaagg ggctggagtg ggtcgcatac attagtagtg gcagtaaaac catctactat
 181 gcagacacaa tgaagggccg attcaccatc tccagagaca atcccaagaa caccctgttc
 241 ctgcaaatga cgagtctaag gtctgaggac acggccatat attactgtgc aagatcctac
 301 gggtacttcg atgtctgggg cgcagggacc acggtcaccg tctcctcagc caaaacgaca
 361 cccccatctg tctatccact ggcccctgga tctgctgccc aaactaactc catggtgacc
 421 ctgggatgcc tggtcaaggg ctatttccct gagccagtga cagtgacctg gaactctgga
 481 tccctgtcca gcggtgtgca caccttccca gctgtcctgc agtctgacct ctacactctg
 541 agcagctcag tgactgtccc ctccagcacc tggcccagcg agaccgtcac ctgcaacgtt
 601 gcccacccgg ccagcagcac caaggtggac aagaaaattg tgcccaggga ttgtggttgt
 661 aagccttgca tatgtacagt cccagaagta tcatctgtct tcatcttccc cccaaagccc
 721 aaggatgtgc tcaccattac tctgactcct aaggtcacgt gtgttgtggt agacatcagc
 781 aaggatgatc ccgaggtcca gttcagctgg tttgtagatg atgtggaggt gcacacagct
 841 cagacgcaac cccgggagga gcagttcaac agcactttcc gctcagtcag tgaacttccc
 901 atcatgcacc aggactggct caatggcaag gagttcaaat gcagggtcaa cagtgcagct
 961 ttccctgccc ccatcgagaa aaccatctcc aaaaccaaag gcagaccgaa ggctccacag
1021 gtgtacacca ttccacctcc caaggagcag atggccaagg ataaagtcag tctgacctgc
1081 atgataacag acttcttccc tgaagacatt actgtggagt ggcagtggaa tgggcagcca
1141 gcggagaact acaagaacac tcagcccatc atggacacag atggctctta cttcgtctac
1201 agcaagctca atgtgcagaa gagcaactgg gaggcaggaa atactttcac ctgctctgtg
1261 ttacatgagg gcctgcacaa ccaccatact gagaagagcc tctcccactc tcctggtaaa
```

[0139] Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 2G10 (SEQ ID NO: 69)

```
  1 dvqlvesggv lvqpggsrkl sctasgftfs sfgmhwvrqa pekglewvay issgsktiyy
 61 adtmkgrfti srdnpkntlf lqmtslrsed taiyycarsy gyfdvwgagt tvtvssaktt
121 ppsvyplapg saaqtnsmvt Igclvkgyfp epvtvtwnsg slssgvhtfp avlqsdlytl
181 sssvtvpsst wpsetvtcnv ahpasstkvd kkivprdcgc kpcictvpev ssvfifppkp
241 kdvltitltp kvtcvvvdis kddpevqfsw fvddvevhta qtqpreeqfn stfrsvselp
301 imhqdwlngk efkcrvnsaa fpapiektis ktkgrpkapq vytipppkeq makdkvsltc
361 mitdffpedi tvewqwngqp aenykntqpi mdtdgsyfvy sklnvqksnw eagntftcsv
421 lheglhnhht ekslshspgk
```

[0140] Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2G10 (SEQ ID NO: 70)

```
  1 gacattgtga tgacccagtc tcaaaaattc atgtccacat cagtaggaga cagggtcagc
 61 gtcacctgca aggccagtca gaatgtgggt actaatgtgg cctggtatca acagaaacca
121 ggacaatctc ctaaagtgct gatttactcg gcatcctacc ggtacagtgg agtccctgat
181 cgcttcacag gcagtggatc tgggacagat ttcactctca ccatcgccaa tgtgcagtct
241 gaagacttgg cagagtattt ctgtcagcaa tacgacagct atcctcggac gttcggtgga
301 gtcaccaagc tggaaatcaa acgggctgat gctgcaccaa ctgtatccat cttcccacca
361 tccagtgagc agttaacatc tggaggtgcc tcagtcgtgt gcttcttgaa caacttctac
421 cccaaagaca tcaatgtcaa gtggaagatt gatggcagtg aacgacaaaa tggcgtcctg
481 aacagttgga ctgatcagga cagcaaagac agcacctaca gcatgagcag caccctcacg
541 ttgaccaagg acgagtatga acgacataac agctatacct gtgaggccac tcacaagaca
601 tcaacttcac ccattgtcaa gagcttcaac aggaatgagt gt
```

[0141] Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2G10 (SEQ ID NO: 71)

```
  1 divmtqsqkf mstsvgdrvs vtckasqnvg tnvawyqqkp gqspkvliys asyrysgvpd
 61 rftgsgsgtd ftltianvqs edlaeyfcqq ydsyprtfgg vtkleikrad aaptvsifpp
121 sseqltsgga svvcflnnfy pkdinvkwki dgserqngvl nswtdqdskd stysmsstlt
181 ltkdeyerhn sytceathkt stspivksfn rnec
```

[0142] Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 2E6 (SEQ ID NO: 72)

```
  1 gaggttcagc tccagcagtc tggggctgag ctggcaagac ctggggcttc agtgaagatg
 61 tcctgcaagg cttctggcta cacctttacc agctactgga tgcactgggt aaaacagagg
121 cctggacagg gtctggaatg gattggcgct gtttatccta gaaacaatga tactacttac
181 aatcagaagt tcaagggcaa ggccaagctg actgctgtca catccgccag cactgcctac
241 atggcactca gcagcctaac aaatgaggac tctgcggtct attactgtct ttattttaac
301 tacaactttg actactgggg ccaaggcacc actctcacag tctcctcagc caaaacgaca
361 cccccatctg tctatccact ggcccctgga tctgctgccc aaactaactc catggtgacc
421 ctgggatgcc tggtcaaggg ctatttccct gagccagtga cagtgacctg gaactctgga
481 tccctgtcca gcggtgtgca caccttccca gctgtcctgc agtctgacct ctacactctg
541 agcagctcag tgactgtccc ctccagcacc tggcccagcg agaccgtcac ctgcaacgtt
601 gcccacccgg ccagcagcac caaggtggac aagaaaattg tgcccaggga ttgtggttgt
661 aagccttgca tatgtacagt cccagaagta tcatctgtct tcatcttccc cccaaagccc
721 aaggatgtgc tcaccattac tctgactcct aaggtcacgt gtgttgtggt agacatcagc
781 aaggatgatc ccgaggtcca gttcagctgg tttgtagatg atgtggaggt gcacacagct
```

```
 841 cagacgcaac cccgggagga gcagttcaac agcactttcc gctcagtcag tgaacttccc
 901 atcatgcacc aggactggct caatggcaag gagttcaaat gcagggtcaa cagtgcagct
 961 ttccctgccc ccatcgagaa aaccatctcc aaaaccaaag gcagaccgaa ggctccacag
1021 gtgtacacca ttccacctcc caaggagcag atggccaagg ataaagtcag tctgacctgc
1081 atgataacag acttcttccc tgaagacatt actgtggagt ggcagtggaa tgggcagcca
1141 gcggagaact acaagaacac tcagcccatc atggacacag atggctctta cttcgtctac
1201 agcaagctca atgtgcagaa gagcaactgg gaggcaggaa atactttcac ctgctctgtg
1261 ttacatgagg gcctgcacaa ccaccatact gagaagagcc tctcccactc tcctggtaaa
```

[0143] Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 2E6 (SEQ ID NO: 73)

```
   1 evqlqqsgae larpgasvkm sckasgytft sywmhwvkqr pgqglewiga vyprnndtty
  61 nqkfkgkakl tavtsastay malssltned savyyclyfn ynfdywgqgt tltvssaktt
 121 ppsvyplapg saaqtnsmvt Igclvkgyfp epvtvtwnsg slssgvhtfp avlqsdlytl
 181 sssvtvpsst wpsetvtcnv ahpasstkvd kkivprdcgc kpcictvpev ssvfifppkp
 241 kdvltitltp kvtcvvvdis kddpevqfsw fvddvevhta qtqpreeqfn stfrsvselp
 301 imhqdwlngk efkcrvnsaa fpapiektis ktkgrpkapq vytipppkeq makdkvsltc
 361 mitdffpedi tvewqwngqp aenykntqpi mdtdgsyfvy sklnvqksnw eagntftcsv
 421 lheglhnhht ekslshspgk
```

[0144] Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2E6 (SEQ ID NO: 74)

```
   1 caaattgttc tcacccagtc tccagcaatc atgtctgctt ctccagggga gaaggtcacc
  61 atgacctgca gtgccagctc aagtgtaagt tacatgcact ggtaccagca gaagccagga
 121 tcctccccca gactcctgat ttatgacaca tccaacctgg cttctggagt ccctgtgcac
 181 ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcatccgaat ggaggctgaa
 241 gatgctgcca cttattactg ccagcagtgg agtagttacc cgtacacgtt cggaggggg
 301 accaagctgg aaataaaacg ggctgatgct gcaccaactg tatccatctt cccaccatcc
 361 agtgagcagt taacatctgg aggtgcctca gtcgtgtgct tcttgaacaa cttctacccc
 421 aaagacatca atgtcaagtg gaagattgat ggcagtgaac gacaaaatgg cgtcctgaac
 481 agttggactg atcaggacag caaagacagc acctacagca tgagcagcac cctcacgttg
 541 accaaggacg agtatgaacg acataacagc tatacctgtg aggccactca caagacatca
 601 acttcaccca ttgtcaagag cttcaacagg aatgagtgt
```

[0145] Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2E6 (SEQ ID NO: 75)

```
   1 qivltqspai msaspgekvt mtcsasssvs ymhwyqqkpg ssprlliydt snlasgvpvh
  61 fsgsgsgtsy sltiirmeae daatyycqqw ssypytfggg tkleikrada aptvsifpps
 121 seqltsggas vvcflnnfyp kdinvkwkid gserqngvln swtdqdskds tysmsstltl
 181 tkdeyerhns ytceathkts tspivksfnr nec
```

[0146] Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgG2b Constant Region) of 2A1 1 (SEQ ID NO: 76)

```
   1 caggttcagc tgcagcagtc tggacctgag ctggtgaagc ctggggcctc agtgaagatt
  61 tcctgcaagg cttctggcta tgcattcagt agctcctgga tgaactgggt gaagcagagg
 121 cctggaaagg gtcttgagtg gattggacgg atttatcctg gagatggaga tactaactac
 181 aatgggaaat tcaagggcaa ggccacactg actgcagaca aatcctccag cacagcctac
 241 atgcaactca gcagcctgac atctgaggac tctgcggtct acttctgtgc aagatcgggc
```

```
 301 tccatctact atggtaacca cggggactac tttgactact ggggccaagg caccactctc
 361 acagtctcct cagccaaaac aacaccccca tcagtctatc cactggcccc tgggtgtgga
 421 gatacaactg gttcctccgt gacctctggg tgcctggtca aggggtactt ccctgagcca
 481 gtgactgtga cttggaactc tggatccctg tccagcagtg tgcacacctt cccagctctc
 541 ctgcagtctg gactctacac tatgagcagc tcagtgactg tcccctccag cacctggcca
 601 agtcagaccg tcacctgcag cgttgctcac ccagccagca gcaccacggt ggacaaaaaa
 661 cttgagccca gcgggcccat ttcaacaatc aacccctgtc ctccatgcaa ggagtgtcac
 721 aaatgcccag ctcctaacct cgagggtgga ccatccgtct tcatcttccc tccaaatatc
 781 aaggatgtac tcatgatctc cctgacaccc aaggtcacgt gtgtggtggt ggatgtgagc
 841 gaggatgacc cagacgtcca gatcagctgg tttgtgaaca acgtggaagt acacacagct
 901 cagacacaaa cccatagaga ggattacaac agtactatcc gggtggtcag caccctcccc
 961 atccagcacc aggactggat gagtggcaag gagttcaaat gcaaggtgaa caacaaagac
1021 ctcccatcac ccatcgagag aaccatctca aaaattaaag ggctagtcag agctccacaa
1081 gtatacactt tgccgccacc agcagagcag ttgtccagga aagatgtcag tctcacttgc
1141 ctggtcgtgg gcttcaaccc tggagacatc agtgtggagt ggaccagcaa tgggcataca
1201 gaggagaact acaaggacac cgcaccagtt cttgactctg acggttctta cttcatatat
1261 agcaagctca atatgaaaac aagcaagtgg gagaaaacag attccttctc atgcaacgtg
1321 agacacgagg gtctgaaaaa ttactacctg aagaagacca tctcccggtc tccgggtaaa
```

[0147] Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgG2b Constant Region) of 2A1 1 (SEQ ID NO: 77)

```
   1 qvqlqqsgpe lvkpgasvki sckasgyafs sswmnwvkqr pgkglewigr iypgdgdtny
  61 ngkfkgkatl tadkssstay mqlssltsed savyfcarsg siyygnhgdy fdywgqgttl
 121 tvssakttpp svyplapgcg dttgssvtsg clvkgyfpep vtvtwnsgsl sssvhtfpal
 181 lqsglytmss svtvpsstwp sqtvtcsvah passttvdkk lepsgpisti npcppckech
 241 kcpapnlegg psvfifppni kdvlmisltp kvtcvvvdvs eddpdvqisw fvnnvevhta
 301 qtqthredyn stirvvstlp iqhqdwmsgk efkckvnnkd lpspiertis kikglvrapq
 361 vytlpppaeq lsrkdvsltc lvvgfnpgdi svewtsnght eenykdtapv ldsdgsyfiy
 421 sklnmktskw ektdsfscnv rheglknyyl kktisrspgk
```

[0148] Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2A1 1 (SEQ ID NO: 78)

```
   1 caaattgttc tcacccagtc tccagcaatc atgtctgctt ctccagggga gaaggtcacc
  61 atgacctgca gtgccagctc aagtgtaagt tacatgcact ggtaccagca gaagccagga
 121 tcctccccca gactcctgat ttatgacaca tccaacctgg cttctggagt ccctgtgcac
 181 ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcatccgaat ggaggctgaa
 241 gatgctgcca cttattactg ccagcagtgg agtagttacc cgtacacgtt cggaggggag
 301 accaagctgg aaataaaacg ggctgatgct gcaccaactg tatccatctt cccaccatcc
 361 agtgagcagt taacatctgg aggtgcctca gtcgtgtgct tcttgaacaa cttctacccc
 421 aaagacatca atgtcaagtg gaagattgat ggcagtgaac gacaaaatgg cgtcctgaac
 481 agttggactg atcaggacag caaagacagc acctacagca tgagcagcac cctcacgttg
 541 accaaggacg agtatgaacg acataacagc tatacctgtg aggccactca caagacatca
 601 acttcaccca ttgtcaagag cttcaacagg aatgagtgt
```

[0149] Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2A1 1 (SEQ ID NO: 79)

```
   1 qivltqspai msaspgekvt mtcsasssvs ymhwyqqkpg ssprlliydt snlasgvpvh
  61 fsgsgsgtsy sltiirmeae daatyycqqw ssypytfggg tkleikrada aptvsifpps
 121 seqltsggas vvcflnnfyp kdinvkwkid gserqngvln swtdqdskds tysmsstltl
 181 tkdeyerhns ytceathkts tspivksfnr nec
```

[0150] Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 2D1 1 (SEQ ID NO: 80)

```
   1 gaggttcagc tccagcagtc tggggctgag ctggcaagac ctggggcttc agtgaagatg
  61 tcctgcaagg cttctggcta cacctttacc aggtactgga tgcactgggt aaaacagagg
 121 cctggacagg gtctggaatg gattggcgct atttatcctg gaaatagtga tactacctac
 181 aatcagaagt tcaagggcaa ggccaaactg actgcagtca catccgccag cactgcctac
 241 atggagctca gcagcctaac aaatgaggac tctgcggtct attactgtat atacccctat
 301 gattaccttg actactgggg ccaaggcacc actctcacag tctcctcagc caaaacgaca
 361 cccccatctg tctatccact ggcccctgga tctgctgccc aaactaactc catggtgacc
 421 ctgggatgcc tggtcaaggg ctatttccct gagccagtga cagtgacctg gaactctgga
 481 tccctgtcca gcggtgtgca caccttccca gctgtcctgc agtctgacct ctacactctg
 541 agcagctcag tgactgtccc ctccagcacc tggcccagcg agaccgtcac ctgcaacgtt
 601 gcccacccgg ccagcagcac caaggtggac aagaaaattg tgcccaggga ttgtggttgt
 661 aagccttgca tatgtacagt cccagaagta tcatctgtct tcatcttccc cccaaagccc
 721 aaggatgtgc tcaccattac tctgactcct aaggtcacgt gtgttgtggt agacatcagc
 781 aaggatgatc ccgaggtcca gttcagctgg tttgtagatg atgtggaggt gcacacagct
 841 cagacgcaac cccgggagga gcagttcaac agcactttcc gctcagtcag tgaacttccc
 901 atcatgcacc aggactggct caatggcaag gagttcaaat gcagggtcaa cagtgcagct
 961 ttccctgccc ccatcgagaa aaccatctcc aaaaccaaag gcagaccgaa ggctccacag
1021 gtgtacacca ttccacctcc caaggagcag atggccaagg ataaagtcag tctgacctgc
1081 atgataacag acttcttccc tgaagacatt actgtggagt ggcagtggaa tgggcagcca
1141 gcggagaact acaagaacac tcagcccatc atggacacag atggctctta cttcgtctac
1201 agcaagctca atgtgcagaa gagcaactgg gaggcaggaa atactttcac ctgctctgtg
1261 ttacatgagg gcctgcacaa ccaccatact gagaagagcc tctcccactc tcctggtaaa
```

[0151] Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgGl Constant Region) of 2D1 1 (SEQ ID NO: 81)

```
   1 evqlqqsgae larpgasvkm sckasgytft rywmhwvkqr pgqglewiga iypgnsdtty
  61 nqkfkgkakl tavtsastay melssltned savyyciypy dyldywgqgt tltvssaktt
 121 ppsvyplapg saaqtnsmvt Igclvkgyfp epvtvtwnsg slssgvhtfp avlqsdlytl
 181 sssvtvpsst wpsetvtcnv ahpasstkvd kkivprdcgc kpcictvpev ssvfifppkp
 241 kdvltitltp kvtcvvvdis kddpevqfsw fvddvevhta qtqpreeqfn stfrsvselp
 301 imhqdwlngk efkcrvnsaa fpapiektis ktkgrpkapq vytipppkeq makdkvsltc
 361 mitdffpedi tvewqwngqp aenykntqpi mdtdgsyfvy sklnvqksnw eagntftcsv
 421 lheglhnhht ekslshspgk
```

[0152] Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2D1 1 (SEQ ID NO: 82)

```
   1 caaattgttc tcacccagtc tccagcaatc atgtctgcat ctccagggga gaaggtcacc
  61 atgacctgca gtgccagctc aagtttaagt tacatgcact ggtaccagca gaagccaggc
 121 acctccccca aaagatgggt ttatgacaca tccaaactgg cttctggagt ccctgctcgc
 181 ttcagtggca gtgggtctgg gacctcttat tctctcacaa tcagcagcat ggaggctgaa
 241 gatgctgcca cttattactg ccatcagcgg agtagttacc cgtacacgtt cggaggggg
 301 accaagctgg aaataaaacg ggctgatgct gcaccaactg tatccatctt cccaccatcc
 361 agtgagcagt taacatctgg aggtgcctca gtcgtgtgct tcttgaacaa cttctacccc
 421 aaagacatca atgtcaagtg gaagattgat ggcagtgaac gacaaaatgg cgtcctgaac
 481 agttggactg atcaggacag caaagacagc acctacagca tgagcagcac cctcacgttg
 541 accaaggacg agtatgaacg acataacagc tatacctgtg aggccactca caagacatca
 601 acttcaccca ttgtcaagag cttcaacagg aatgagtgt
```

[0153] Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 2D1 1 (SEQ ID NO: 83)

```
  1 qivltqspai msaspgekvt mtcsasssls ymhwyqqkpg tspkrwvydt sklasgvpar
 61 fsgsgsgtsy sltissmeae daatyychqr ssypytfggg tkleikrada aptvsifpps
121 seqltsggas vvcflnnfyp kdinvkwkid gserqngvln swtdqdskds tysmsstltl
181 tkdeyerhns ytceathkts tspivksfnr nec
```

[0154] Table 4 shows the correspondence between the full length sequences of the antibodies discussed in this Example with those presented in the Sequence Listing.

Table 4

SEQ ID NO.	Nucleic Acid or Protein
68	2G10 Heavy Variable + IgG1 Constant—nucleic acid
69	2G10 Heavy Variable + IgG1 Constant—protein
70	2G10 Kappa Variable + Constant—nucleic acid
71	2G10 Kappa Variable + Constant—protein
72	2E6 Heavy Variable + IgG1 Constant—nucleic acid
73	2E6 Heavy Variable + IgG1 Constant—protein
74	2E6 Kappa Variable + Constant—nucleic acid
75	2E6 Kappa Variable + Constant—protein
76	2A11 Heavy Variable + IgG1 Constant—nucleic acid
77	2A11 Heavy Variable + IgG1 Constant—protein
78	2A11 Kappa Variable + Constant—nucleic acid
79	2A11 Kappa Variable + Constant—protein
80	2D11 Heavy Variable + IgG1 Constant—nucleic acid
81	2D11 Heavy Variable + IgG1 Constant—protein
82	2D11 Kappa Variable + Constant—nucleic acid
83	2D11 Kappa Variable + Constant—protein

Example 3: Binding Affinities

[0155] The binding affinities and kinetics of interaction of monoclonal antibodies 2G10, 2E6, 2A1 1, and 2D1 1 to recombinant human Notch 1/Fc fusion protein (rhNotchl-Fc) were measured by surface plasmon resonance using a Biacore® T100 instrument (GE Healthcare, Piscataway, NJ).

[0156] Rabbit anti-mouse IgGs (Biacore, Cat. No. BR- 1008-38) were immobilized on carboxymethylated dextran CM4 sensor chips by amine coupling (GE Healthcare) using a standard coupling protocol according to vendor's instructions. The analyses were performed at

25°C, using PBS (Invitrogen, Cat. No. 14040-133) containing 0.05% surfactant P20 (GE Healthcare) as running buffer.

[0157] The antibodies were captured in individual flow cells, at a flow rate of 10 μl/min. Injection time was varied for each antibody to yield an Rmax between 30 and 60 RU. Buffer or rhNotch1-Fc diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 300 seconds, at 60 μl/min. The dissociation phase was monitored for up to 3600 seconds. The surface was then regenerated with two 60-second injections of 10 mM Glycine-HCl, pH 1.7, at a flow rate of 60 μl/min. The rhNotch1-Fc concentration range tested was 6.25 nM to 100 nM. Kinetic parameters were determined using the kinetic function of the BIAevalutation software (GE Healthcare) with double reference subtraction. Kinetic values of the monoclonal antibodies on rhNotch1-Fc at 25°C are summarized in Table 5.

Table 5

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	n
2E6	4.23E+04	1.54E-04	3.89E-09	2
2A11	4.76E+04	1.63E-04	3.43E-09	2
2D11	3.16E+04	2.06E-04	6.54E-09	2

[0158] The results in Table 5 demonstrate that antibodies 2E6, 2A11, and 2D11 bind rhNotch1-Fc with a K_D of about 10 nM or less, 7.5 nM or less, 5 nM or less, 4 nM or less.

Example 4: Binding Specificity

[0159] Antibodies 2G10, 2E6, 2A11 and 2G11 were tested for binding to human Notch1, human Notch2, or human Notch3, protein. Binding measurements were made by bio-layer interferometry (BLI), using a ForteBio Octet® QK instrument (ForteBio, Menlo Park, CA). Anti-human-Fc sensors were soaked in PBS containing 1 mg/ml BSA for 5 minutes prior to binding of antibodies. Then the following proteins (400 nM, in PBS containing 1 mg/ml BSA) were allowed to bind to the sensors: rhNotch1-Fc (R&D Systems, Minneapolis, MN; Cat. No. 3647-TK-050), rhNotch2-Fc (R&D Cat. No. 3735-NT-050), rhNotch3-Fc (R&D Cat. No. 1559-NT-050), or rmNotch1-Fc (R&D Cat. No. 5267-TK-050). Notch protein bound sensors were immersed in antibody solution (50 μg/ml) to allow binding of antibody to the Notch

protein. Binding was detected by shifts in the interference pattern. These results demonstrated that the antibodies bind specifically to human Notch 1 protein, but do not bind to human Notch2 or human Notch3 protein.

[0160] To determine specificity of binding to cell surface Notch proteins, the antibodies were tested for binding to human Notch 1, human Notch2, human Notch3 and human Notch4 expressed on the surface of CHO cells using electrochemiluminescence (Meso Scale Discovery). CHO Flpln™ cells (Invitrogen, Cat. No. R758-07) expressing each of the four human Notch proteins were produced, according to the vendor's instructions. A CHO line lacking any human Notch protein was also produced for use as a negative control. Cells were grown under standard conditions (37°C, DMEM/F12 + 10% FBS). For binding studies, cells were washed in PBS containing calcium and magnesium, removed from the plate, and disaggregated by treatment with dissociation buffer (GIBCO Cat. No. 13151014) for 10 minutes at 37°C.

[0161] Cells were seeded at a density of 30,000 cells per well, in hybridoma media, in a standard 96-well binding plates (Meso Scale Discovery, Cat. No. L15XA-6). Cells were incubated for one hour at 37°C. Antibodies or control IgG were added at 5 μg/ml, in 50 μï hybridoma media, and incubated for 1 hour at 37°C. The plates were washed twice with PBS containing 3% BSA. Binding of the antibodies to cell surface was detected using 2 μg/ml of MSD anti-mouse IgG secondary antibody (Meso Scale Discovery, Cat. No. R32AC-1) for 1 hour at 4°C. Plates were washed twice with PBS containing 3% BSA, and 150 μï of read buffer (Meso Scale Discovery Cat. No. R92TC-1) was added. The plates were analyzed on a Sector Imager 2400 instrument (Meso Scale Discovery). This analysis showed that antibodies 2G10, 2E6, 2A1 1 and 2D1 1 bind to human Notchl displayed on the surfaces of cells, but do not bind to human Notch2, Notch3 **(FIG. 6)** or Notch4 (data not shown), displayed on the surfaces of cells. The antibodies also do not bind CHO-EV (empty vector) cells that express endogenous hamster Notch proteins. These results indicated that the antibodies bind specifically to human Notchl protein *in vitro,* and when the Notchl protein is displayed on a cell surface.

Examples 5: Inhibition of Notchl-Ligand Binding

[0162] Antibodies 2E6, 2A1 1 and 2D1 1 were tested for their ability to inhibit the binding of rhNotchl to human Jagl, Jag2, DLLl and DLL4. Binding measurements were made by bio-

layer interferometry (BLI), using a ForteBio Octet® QK instrument (ForteBio, Menlo Park, CA). The ligands tested were rhJagl-Fc (R&D Cat. No. 1277-JG-050), rhJag2-Fc (R&D Cat. No. 1726-JG-050), rhDLLl-Fc (R&D Cat. No. 5026-DL-050), and His tagged rhDLL4 (R&D Cat. No. 1506-D4-050).

[0163] To determine the degree of inhibition of Notch 1-ligand binding by each antibody, the Octet sensors were loaded with recombinant human Notch 1, and each antibody was allowed to bind, as described in Example 4 (above). Subsequently sensors were immersed in 500 μg/ml human IgG, to block non-specific binding. Ligands were prepared at 400 nM, in PBS containing 3% BSA, and were allowed to bind. The on-rate and off-rate for ligand binding were detected using the Octet® QK instrument and software. Antibodies 2E6, 2A1 1, and 2D1 1 blocked binding of all four ligands to rhNotchl-Fc.

Example 6: Inhibition of Notchl Activation

[0164] The effect of the antibodies on signaling by Notchl was tested as follows. Blocking of ligand-induced activation of Notchl and subsequent proteolytic cleavages that release the intracellular domain of Notchl, were analyzed by using immunoblots to detect activated Notchl -intracellular domain (Nl-ICD).

[0165] To activate Notchl signaling in cell lines, various Notchl -expressing cells were plated in 96-well plates on wells coated with Notch ligands. The wells were prepared by diluting oc-human Fc (Jackson ImmunoResearch, West Grove, PA) to 10 μg/ml in sterile-filtered carbonate -bicarbonate coating buffer, pH 9.4 (Pierce 28382). Then 1 μg of the diluted antibody was added to each well of a 96-well Maxisorp™ plate and incubated overnight at 4°C. The next day, wells were washed three times with PBS before adding 1 μg soluble ligand Fc fusion protein or human IgG Fc (Jackson Immunolabs) diluted in PBS/0.5%BSA. After incubating for two hours at room temperature on an orbital shaker, the wells were washed three times with PBS to remove unbound ligand. Karpas45 or Notchl 293 Flpln™ cells were counted and resuspended in fresh growth media at 0.75 x 10^6 cells/ml or 0.3 x 10^6 cells/ml, respectively. Cells were pre-incubated with 10 μg/ml blocking antibody for one hour at 37°C, before seeding 100 μï of the suspension into 96-well plates coated with ligand or hFc. Cells were incubated for four hours at 37°C before being dislodged from the well by pipetting and harvested. Wells were washed with PBS and pooled with harvested cells to ensure complete collection. Cells were sedimented in a refrigerated microcentrifuge, washed with 100 μï PBS,

and lysed by resuspending cell pellet in 30 µï of RIPA buffer containing protease inhibitors. Lysates were clarified by centrifugation in a refrigerated microcentrifuge. Supernatants were boiled with 5X SDS sample buffer before SDS PAGE and for Western blotting. Blots were probed with a **a**-Notchl antibody specific for the cleaved intracellular domain (Cell Signaling, #2421). Notchl activation by all four ligands was inhibited by Notchl antibodies 2G10, 2E6, 2A1 1 and 2D1 1, at concentrations ranging from 0.1 µg/ml tolO µg/ml.

Example 7: Inhibition of Notchl-Dependent Transcription

[0166] Reporter cell lines dependent upon Notchl, Notch2, or Notch3 were produced by lentiviral introduction of a RBP-**jK**-dependent luciferase reporter gene (SABiosciences, Frederick, MD) into 293-FlpIn™ Notchl cells, Karpas45 cells, and DU4475 cells. To activate Notchl -dependent signaling and transcription, cells were plated on ligand-coated wells prepared, as described in Example 6 (above). Cells were pre-incubated with a 3-fold dilution series of Notchl antibody concentrations ranging from 0-300 µg/ml, for one hour at 37°C, before seeding 100 µï of the suspension into 96-well plates coated with ligand or hFc. Cells were incubated in ligand-coated or human-Fc-coated wells for four or 24 hours at 37°C, in 5% $C0_2$. Next, 100 µï of Promega Bright Glo™ (Promega, Madison, WI) was added to each well. The reaction was allowed to proceed for five minutes in the dark, and then the entire 200 µï volume was transferred to white walled plates and read using a luminometer. Polyclonal antibodies against Notchl (AF1057, R&D Systems), Notch2 (AF1 190, R&D Systems) or Notch3 (AF1559, R&D Systems) were used as controls to confirm that ligand-stimulated reporter activity in each cell line was specifically dependent upon the introduced Notch receptor. The Notchl antibodies specifically inhibited Notchl -dependent transcription **(FIG. 7A),** and did not inhibit Notch2-dependent **(FIG. 7B)** or Notch3-dependent transcription **(FIG. 7C).**

[0167] Activation of Notchl -dependent transcription by each of the ligands Jagl, Jag2, DLLl and DLL4 was inhibited by the Notchl antibodies 2E6 **(FIG. 8A),** 2A1 1 **(FIG. 8B)** and 2D1 1 **(FIG. 8C).** The data in Table 6 show that antibody 2E6 inhibited activation of Notch 1-dependent transcription by Jagl, Jag2, DLLl and DLL4 in the Notchl -dependent reporter cell lines.

Table 6

Ligand	Jag1	Jag2	DLL1	DLL4
EC_{50}	0.4 nM	0.1 nM	0.1 nM	0.2 nM
Maximum Inhibition	95%	98%	95%	100%

[0168] To determine the effect of Notch 1 antibodies on transcription of endogenous Notch target genes, Notchl signaling was activated by Jagl, as described above. The effect on expression of endogenous Notch targets, as a result of treatment of the Jagl -stimulated Karpas45 cells with IgG control, Notchl antibodies, or DBZ (gamma secretase inhibitor) was assessed by quantitative RT-PCR. Karpas45 cells were seeded into 6-well plates, in 2 ml of media. Replicate wells of cells were treated with antibody 2E6, IgG control, 1 μM DBZ, or vehicle control (DMSO), immediately after seeding. Cells were incubated at 37°C, 5% $C0_2$ for 20 hours after treatment, collected, and rinsed with PBS. Cell pellets were frozen on dry ice and stored -80°C. RNA was prepared using Qiagen RNeasy™ miniprep columns (Qiagen GR8RNA). Quantitative RT-PCR was performed to analyze Notch target gene expression, using a commercial kit according to the kit vendor's instructions (Quantitect SYBR GREEN RT-PCR Kit; Qiagen). Results were analyzed using the comparative Ct method. Beta actin was used as an internal standard, and Stratagene Universal Human Reference RNA (Stratagene 740000) was used as an external standard for measurement of expression levels of the genes investigated. These results showed that antibody 2E6 inhibits transcription of endogenous Notch target genes, including Heyl.

Example 8: Inhibition of Human Cancer Cell Line Proliferation

[0169] Antibody 2E6 was tested for inhibition of ligand-dependent and ligand-independent proliferation of human cancer cells that express Notchl. The T-ALL cell line Karpas45 expresses elevated levels of Notchl. To screen for antagonistic Notchl antibodies, cells were grown in 96-well plates in wells coated with either human Fc or rhJagl-Fc. Growth was measured in the presence of various concentrations of antibodies (0 - 300 μg/ml in 100 μï final volume) by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays conducted two days after plating cells on ligand or human Fc control. These results showed that antibody 2E6 inhibits proliferation of Karpas45 cells. In addition, quantitative RT-PCR

analysis, as described in Example 7 (above) demonstrated that antibody 2E6 inhibited expression of the Notch target gene Heyl in Karpas45 cells.

Example 9: Vascular Branching Morphogenesis

[0170] Antibodies 2G10, 2E6, 2A1 1 and 2D1 1 were shown to bind to Notchl on the surface of human umbilical vein endothelial cells (HUVEC) (ATCC Cat. No. CRL-1730), as determined by FACS analysis. To determine whether inhibition of Notchl signaling affects angiogenesis, these four antibodies were tested for promotion of endothelial cell branching morphogenesis. Matrigel™ was prepared in 24-well plates by adding 250 µï of growth factor reduced Matrigel (GFR; BD Bioscience Cat. No. 356231) to each well, and incubated for one hour at 37°C. In parallel, HUVECs were washed in PBS, and resuspended in EGM-2 growth media (Lonza Cat. No. CC-3156) plus 2% FBS. Antibodies, human IgG, or positive controls were added to the media containing cells, and 40,000 cells per well were plated on polymerized GFR matrix. Branching morphogenesis was assessed at various time points, by image capture and analysis, using ImageJ public domain image processing software. All four antibodies promoted vascular branching morphogenesis resulting in increased branching of vessels and increased overall vascular area. In addition, quantitative RT-PCR analysis, as described in Example 7 (above), demonstrated that the antibodies inhibited Notchl signaling in endothelial cells as indicated by the down-regulated expression of Notch target genes, including Heyl and Hey2.

Example 10: Inhibition of T-cell Fate Specification *In Vivo*

[0171] Antibodies 2G10, 2E6, 2A1 1 and 2D1 1 did not bind with high affinity to mouse Notchl. Therefore, to determine the effect of these Notchl antibodies on Notchl function in mice *in vivo,* the mouse Notchl gene was engineered to express a Notchl protein containing the human amino acid sequence from amino acid 413 to 488. No phenotypic difference was observed in these "humanized" Notchl mice. Importantly, the number and distribution of the thymocyte population in these animals was indistinguishable from wild-type mice. This indicated that the engineered Notchl protein was fully functional in the humanized mice.

[0172] Inhibition of thymocyte development and T-cell fate specification can be used as an indication that an anti-Notch 1 antibody is actually inhibiting Notchl function *in vivo.* Therefore, antibody 2E6 was tested for inhibition of thymocyte development and T-cell fate specification in humanized Notchl mice. Mice (C57bl/6; 129Sv/Ev mixed background)

homozygous for humanized Notch 1 gene were treated with the antibodies or IgG control at 40 mg/kg twice weekly, or the gamma secretase inhibitor DBZ at 10 μM/kg once daily, and monitored daily. After 18 days, the mice were sacrificed, thymus glands were removed, thymocytes were dissociated, and the total number of thymocytes was counted. FACS profiling was performed using antibodies against CD4 and CD8 (Beckton-Dickinson Cat. Nos. 553730 and 553031, respectively). The effect of the antibodies (or controls) on thymocyte number, and the distribution of CD4/CD8 double positive, double negative or single positive cells, were determined. Antibody 2E6 reduced the total number of thymocytes, and decreased the percent of CD4/CD8 double positive cells, while increasing the percentage of CD4 single positive, CD8 single positive, and CD4/CD8 double negative cells. These effects of antibody 2E6 were comparable to the effects observed when animals were treated with 10 μM/kg of the gamma-secretase inhibitor DBZ. This indicated that antibody 2E6 was inhibiting Notch 1 *in vivo* function in thymocyte development.

Example 11: Loss of Hair Pigmentation

[0173] Loss of hair pigmentation can be used as an indication that an anti-Notch 1 antibody is inhibiting Notch 1 function *in vivo.* Therefore, mice (C57bl/6; 129Sv/Ev mixed background) homozygous for humanized Notch 1 gene were treated with antibody 2E6, IgG control, or DBZ, as described in Example 10. Daily monitoring revealed loss of hair pigmentation in the antibody-treated mice within 2 weeks. No such loss of pigmentation was observed in IgG control treated mice.

Example 12: Lack of Toxicity

[0174] Mice were treated with antibody 2E6, IgG control, or DBZ, as described in Example 10 **(FIG. 9A).** Over time, DBZ treated animals exhibited loss of body weight, while animals treated with up to 150 mg/kg Notch 1 antibody three times per week exhibited normal weight gain **(FIG. 9B).** After 18 days, animals were sacrificed, small intestines were collected, fixed and embedded in paraffin. To observe goblet cells in the small intestine, sections of small intestine from antibody-treated, IgG-treated, and DBZ-treated animals were stained with Alcian Blue (Diagnostic Biosystems, Cat. No. KT 003). Mice treated with 40, 100 or 150 mg/kg of Notch 1 antibody 2E6 showed no increase in goblet cell numbers compared to control animals. By contrast, small intestines from animals treated with DBZ (10 μM/kg) showed extensive

alcian blue staining. These results indicated that antibody 2E6 did not lead to goblet cell metaplasia, and had little or no intestinal toxicity in treated mice.

[0175] The results in **FIG. 9A** also demonstrate that antibody 2E6 at doses of 40, 100 or 150 mg/kg inhibit thymocyte development as effectively as 10 μmoï/kg of DBZ. Therefore, as shown in **FIGS. 9A-9B,** and by alcian blue staining of the small intestine, antibody 2E6 does not have toxic effects (as measured by body weight loss and goblet cell conversion) at doses significantly higher than the dose required to inhibit thymocyte development.

Example 13: Inhibition of Angiogenesis *In Vivo*

[0176] The effect of antibody 2E6 on functional angiogenesis was determined using an *in vivo* matrigel plug assay. In this test, 400 ng/ml bFGF (R&D Systems) was prepared in 0.5 ml matrigel (Becton Dickinson). Mice were anaesthetized, and one or two matrigel plugs per animal were injected subcutaneously on either side on the ventral midline. Angiogenesis was allowed to proceed for 7 days, and the mice were treated intraperitoneally with 2-40 mg/kg Notch 1 antibody, or IgG control on day 0, and every 3 days thereafter. No significant loss of body weight was observed during these experiments. This indicated a lack of toxicity associated with treatment of the animals with 2-40 mg/kg of 2E6. Animals were sacrificed after 7 days. At this time, the plugs were removed, minced in water, and incubated overnight at 4°C. The following day, a standard curve of hemoglobin concentration was prepared using 180 mg/ml, 120 mg/ml, 60 mg/ml, 30 mg/ml, 15 mg/ml, 7.5 mg/ml and 0 mg/ml of hemoglobin in a 1:1 mixture of water and Drabkin's reagent (Sigma-Aldrich, St. Louis, MO). Test samples were centrifuged to pellet matrigel and cells. A 225 μï sample of supernatant was removed, mixed with an equal volume of Drabkin's reagent, and incubated for 15 minutes at room temperature. Absorbance at 540 nm was read, and hemoglobin concentration was determined by comparison to a standard curve. Hemoglobin concentration was normalized for plug weight for each sample. The results of these experiments indicated that antibody 2E6 inhibited bFGF-induced angiogenesis in humanized ($Notch1^{hll2/hl\ 12}$ knock-in) mice **(FIG. 10).** Dose response studies indicated that inhibition of bFGF-induced angiogenesis was inhibited at concentrations as low as 2 mg/kg.

[0177] To investigate the effect of antibody 2E6 on angiogenesis induced by human cancer cell lines *in vivo,* 0.5 - 1 x 10^6 human cancer cells were prepared in 0.5 ml matrigel. Mice were anaesthetized, and one or two matrigel plugs per animal were injected subcutaneously on either

side on the ventral midline. Angiogenesis was allowed to proceed for 7-12 days, and animals were treated intraperitoneally with 2-40 mg/kg antibody 2E6, or IgG control on day 0, and every 3 days thereafter. No significant loss of body weight was observed during these experiments. This indicated a lack of toxicity associated with treatment of the animals with 2-40 mg/kg of 2E6. Animals were sacrificed after 10-12 days, depending on the cell line. Plugs were removed and processed (as described above), to determine hemoglobin concentration. The results of this experiment indicated that antibody 2E6 inhibited angiogenesis induced by human lung cancer (Calu-6), breast cancer (MDA-MB-231) or pancreatic cancer (SW1990) cell lines **(FIG. 11).**

[0178] The second matrigel plug from each mouse (as described in the preceding paragraph) was processed for histologic analysis, in parallel with the hemoglobin measurements. Plugs were removed, fixed over night in 10% buffered formalin at room temperature, embedded in paraffin, and 10-20 um sections were prepared for immunohistochemistry. To detect blood vessels present in the matrigel plug, thin sections were stained for CD31, using an anti-mouse-CD3 1 antibody (Biocare Medical, Cat. Nos. CM303 and RT517SK) according to the vendor's instructions. CD31 staining of the matrigel plugs demonstrated increased vessel branching and smaller vessels after treatment with antibody 2E6, by comparison to control IgG. These data demonstrated that antibody 2E6 promoted vascular branching in the treated mice. However, despite the increase in vascular branching, the decrease in blood content (as measured by hemoglobin content) suggests that antibody 2E6 decreased the function of the vessels that were present, and thus inhibited functional angiogenesis. This apparent decrease in functional angiogenesis caused by antibody 2E6 is consistent with increased branching and decreased vascular function resulting from genetic loss of function of the Notch pathway in endothelial tissue.

Example 14: Humanization of Anti-human Notchl Antibodies

A. Construction of Humanized and Chimeric Anti-Human Notchl Antibodies

[0179] This Example describes the humanization of the murine antibody designated 2E6, and the characterization of the resulting humanized antibodies. The humanized anti-Notch 1 antibodies were designed using methods well-known in the art. Two different humanized versions were made for each chain and a predicted N-linked glycosylation site in 2E6 heavy CDR2 was mutated to prevent any possible glycosylation. The designed amino acid sequences were converted to codon-optimized DNA sequences and synthesized by DNA2.0, Inc. to

include (in the following order): 5' HindIII restriction site, Kozak consensus sequence, amino terminal signal sequence, humanized variable region, human IgG1 or Kappa constant region, stop codon, and a 3' EcoRI restriction site.

[0180] The anti-Notch 1 humanized antibody chains are designated with the prefix "Hu2E6_Hv" or "Hu2E6_Kv", referring to humanized 2E6 heavy or kappa light, respectively, and the designations are then followed by a numeric suffix *(e.g.,* Hu2E6_Hv1, Hu2E6_Hv2, Hu2E6_Kv1, or Hu2E6_Kv2). In some cases, the designation is also followed by an amino acid substitution abbreviation *(e.g.,* Hu2E6_Hv1 T57A or Hu2E6_Hv2 T57A). Combinations of humanized heavy light chains are designated with the prefix "Hu2E6" and a numeric suffix.

[0181] Chimeric (murine variable region and human constant region) 2E6 heavy (human IgG1) and light (human Kappa) chains were also constructed. The murine variable regions were fused to the human constant region using overlap extension PCR, including (in the following order): 5' HindIII restriction site, Kozak consensus sequence, amino terminal signal sequence, mouse variable region, human IgG1 or Kappa constant region, stop codon, and 3' EcoRI restriction site.

[0182] The humanized and chimeric heavy chains were subcloned into pEE6.4 (Lonza, Basel, Switzerland) via HindIII and EcoRI sites using In-Fusion™ PCR cloning (Clontech, Mountain View, CA). The humanized and chimeric Kappa light chains were subcloned into pEE14.4 (Lonza) via HindIII and EcoRI sites using In-Fusion™ PCR cloning.

[0183] Humanized antibody chains or chimeric antibody chains were transiently transfected into 293T cells to produce antibody. Antibody was either purified or used in cell culture media supernatant for subsequent *in vitro* analysis. Binding of the chimeric and humanized antibodies to human Notch 1 was measured as described below. The results are summarized in Table 13.

[0184] Additionally, some humanized antibody heavy and light chain combinations were stably expressed in CHOK1SV cells using the GS System™ (Lonza Biologies) in order to produce large quantities of purified humanized antibody. A single expression vector was constructed by combining pEE6.4 and pEE14.4 based vectors. First, pEE6.4 containing full length humanized heavy chain cDNA was digested with NotI and SalI to isolate the hCMV-MIE promoter + full length humanized heavy chain cDNA + SV40 poly A fragment. This fragment was inserted into the pEE14.4 vector already containing full length humanized light

chain cDNA via Notl/Sall sites, thus creating an expression vector that simultaneously expresses heavy and light chains. The combined heavy and light chain vector was linearized and transfected into CHOK1SV cells. Stable clones were selected in the presence of methionine sulfoximine.

[0185] Each of the possible combinations of the humanized immunoglobulin heavy chain and immunoglobulin light chain variable regions are set forth below in Table 7.

Table 7

Light Chain Variable Region	Heavy Chain Variable Region
Hu2E6_Kvl (SEQ ID NO: 111)	Hu2E6_Hvl (SEQ ID NO: 103)
Hu2E6_Kvl (SEQ ID NO: 111)	Hu2E6_Hvl T57A (SEQ ID NO: 105)
Hu2E6_Kvl (SEQ ID NO: 111)	Hu2E6_Hv2 (SEQ ID NO: 107)
Hu2E6_Kvl (SEQ ID NO: 111)	Hu2E6_Hv2 T57A (SEQ ID NO: 109)
Hu2E6_Kv2 (SEQ ID NO: 113)	Hu2E6_Hvl (SEQ ID NO: 103)
Hu2E6_Kv2 (SEQ ID NO: 113)	Hu2E6_Hvl T57A (SEQ ID NO: 105)
Hu2E6_Kv2 (SEQ ID NO: 113)	Hu2E6_Hv2 (SEQ ID NO: 107)
Hu2E6_Kv2 (SEQ ID NO: 113)	Hu2E6_Hv2 T57A (SEQ ID NO: 109)

[0186] The nucleic acid sequences encoding and the protein sequences defining variable regions of the humanized 2E6 antibodies are summarized below (amino terminal signal peptide sequences are not shown). CDR sequences (Kabat definition) are shown in bold and are underlined in the amino acid sequences.

[0187] Nucleic Acid Sequence Encoding the Hu2E6 Hyl Heavy Chain Variable Region (SEQ ID NO: 102)

```
  1 gaagtgcagt tggtacaaag tggggccgaa gttgeaaage caggggcctc agtgaagatg
 61 tettgeaagg ettceggata cacattcact tcatattgga tgcactgggt gaagcaagct
121 cccggccagg gtctggagtg gateggegea gtctacccta gaaacaacga taccacctat
181 aaccagaaat tcaagggcaa ggccaccctc accgctgaca ctagcacatc cacagcatac
241 atggagctgc gctctcttcg gagegacgat acagccgtct attactgtct gtatttcaat
301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttcc
```

[0188] Protein Sequence Defining the Hu2E6 Hyl Heavy Chain Variable Region (SEQ ID NO: 103)

```
  1 evqlvqsgae vakpgasvkm sckasgytft sywmhwvkqa pgqglewiga vyprnndtty
 61 nqkfkgkatl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvss
```

[0189] Nucleic Acid Sequence Encoding the Hu2E6_Hyl_T57A Heavy Chain Variable Region (SEQ ID NO: 104)

```
   1 gaagtgcagt tggtacaaag tggggccgaa gttgcaaagc caggggcctc agtgaagatg
  61 tcttgcaagg cttccggata cacattcact tcatattgga tgcactgggt gaagcaagct
 121 cccggccagg gtctggagtg gatcggcgca gtctacccta gaaacaacga tgccacctat
 181 aaccagaaat tcaagggcaa ggccaccctc accgctgaca ctagcacatc cacagcatac
 241 atggagctgc gctctcttcg gagcgacgat acagccgtct attactgtct gtatttcaat
 301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttcc
```

[0190] Protein Sequence Defining the Hu2E6_Hyl_T57A Heavy Chain Variable Region (SEQ ID NO: 105)

```
   1 evqlvqsgae vakpgasvkm sckasgytft sywmhwvkqa pgqglewiga vyprnndaty
  61 nqkfkgkatl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvss
```

[0191] Nucleic Acid Sequence Encoding the Hu2E6_Hv2 Heavy Chain Variable Region (SEQ ID NO: 106)

```
   1 caggtgcagt tggtacaaag tggggccgaa gttaagaagc caggggcctc agtgaagatg
  61 tcttgcaagg cttccggata cacattcact tcatattgga tgcactgggt gaggcaagct
 121 cccggccagg gtctggagtg gatcggcgca gtctacccta gaaacaacga taccacctat
 181 aaccagaaat tccagggcag ggccaccctc accgctgaca ctagcacatc cacagcatac
 241 atggagctgc gctctcttcg gagcgacgat acagccgtct attactgtct gtatttcaat
 301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttcc
```

[0192] Protein Sequence Defining the Hu2E6_Hv2 Heavy Chain Variable Region (SEQ ID NO: 107)

```
   1 qvqlvqsgae vkkpgasvkm sckasgytft sywmhwvrqa pgqglewiga vyprnndtty
  61 nqkfqgratl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvss
```

[0193] Nucleic Acid Sequence Encoding the Hu2E6_Hv2 T57A Heavy Chain Variable Region (SEQ ID NO: 108)

```
   1 caggtgcagt tggtacaaag tggggccgaa gttaagaagc caggggcctc agtgaagatg
  61 tcttgcaagg cttccggata cacattcact tcatattgga tgcactgggt gaggcaagct
 121 cccggccagg gtctggagtg gatcggcgca gtctacccta gaaacaacga tgccacctat
 181 aaccagaaat tccagggcag ggccaccctc accgctgaca ctagcacatc cacagcatac
 241 atggagctgc gctctcttcg gagcgacgat acagccgtct attactgtct gtatttcaat
 301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttcc
```

[0194] Protein Sequence Defining the Hu2E6_Hv2 T57A Heavy Chain Variable Region (SEQ ID NO: 109)

```
   1 qvqlvqsgae vkkpgasvkm sckasgytft sywmhwvrqa pgqglewiga vyprnndaty
  61 nqkfqgratl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvss
```

[0195] Nucleic Acid Sequence Encoding the Hu2E6 Kyl Kappa Chain Variable Region (SEQ ID NO: 110)

```
  1 gaaattgtcc tgacacagtc acccgcaaca atgtctgcct ctccaggcga gagagtcacc
 61 atgtcttgca gggcttcctc ctctgtgagc tacatgcatt ggtaccagca aaagccaggt
121 cagtcccctc ggctgcttat ctatgacacc tccaaccgag cctctggagt tcccgcccac
181 ttcagcggca gcgggagtgg gacagattac actctgacca taagttcaat ggagcctgag
241 gactttgcaa cctattactg ccagcaatgg agcagttatc cctatacttt cggccaggga
301 accaaactcg aaatcaag
```

[0196] Protein Sequence Defining the Hu2E6 Kyl Kappa Chain Variable Region (SEQ ID NO: 111)

```
  1 eivltqspat msaspgervt mscrasssvs ymhwyqqkpg qsprlliydt snrasgvpah
 61 fsgsgsgtdy tltissmepe dfatyycqqw ssypyt fgqg tkleik
```

[0197] Nucleic Acid Sequence Encoding the Hu2E6 Kv2 Kappa Chain Variable Region (SEQ ID NO: 112)

```
  1 gaaattgtcc tgacacagtc acccgcaaca ttgtctgcct ctccaggcga gagagtcacc
 61 atgtcttgca gggcttcctc ctctgtgagc tacatgcatt ggtaccagca aaagccaggt
121 caggctcctc ggctgcttat ctatgacacc tccaaccgag ccactggagt tcccgccagg
181 ttcagcggca gcgggagtgg gacagattac actctgacca taagttcaat ggagcctgag
241 gactttgcaa cctattactg ccagcaatgg agcagttatc cctatacttt cggccaggga
301 accaaactcg aaatcaag
```

[0198] Protein Sequence Defining the Hu2E6 Kv2 Kappa Chain Variable Region (SEQ ID NO: 113)

```
  1 eivltqspat lsaspgervt mscrasssvs ymhwyqqkpg qaprlliydt snratgvpar
 61 fsgsgsgtdy tltissmepe dfatyycqqw ssypyt fgqg tkleik
```

[0199] The amino acid sequences defining the immunoglobulin heavy chain variable regions for the antibodies produced in Example 14 are aligned in FIG. 12. Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR_1, CDR_2, and CDR_3 (Kabat definition) are identified by boxes. FIG. 13 shows an alignment of the separate CDRi, CDR_2, and CDR_3 sequences for each of the variable region sequences shown in FIG. 12.

[0200] The amino acid sequences defining the immunoglobulin light chain variable regions for the antibodies in Example 14 are aligned in FIG. 14. Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR_1, CDR_2 and CDR_3 are identified by boxes. FIG. 15 shows an alignment of the separate CDR_1, CDR_2, and CDR_3 sequences for each of the variable region sequences shown in FIG. 14.

[0201] Table 8 is a concordance chart showing the SEQ ID NO. of each sequence discussed

in this Example.

Table 8

SEQ. ID NO.	Nucleic Acid or Protein
102	Hu2E6_Hv1 Heavy Chain Variable Region—nucleic acid
103	Hu2E6_Hv1 Heavy Chain Variable Region—protein
15	Hu2E6_Hv1 Heavy Chain CDR_1
16	Hu2E6_Hv1 Heavy Chain CDR_2
17	Hu2E6_Hv1 Heavy Chain CDR_3
104	Hu2E6_Hv1 T57A Heavy Chain Variable Region—nucleic acid
105	Hu2E6_Hv1 T57A Heavy Chain Variable Region—protein
15	Hu2E6_Hv1 T57A Heavy Chain CDR_1
94	Hu2E6_Hv1 T57A Heavy Chain CDR_2
17	Hu2E6_Hv1 T57A Heavy Chain CDR_3
106	Hu2E6_Hv2 Heavy Chain Variable Region—nucleic acid
107	Hu2E6_Hv2 Heavy Chain Variable Region—protein
15	Hu2E6_Hv2 Heavy Chain CDR_1
95	Hu2E6_Hv2 Heavy Chain CDR_2
17	Hu2E6_Hv2 Heavy Chain CDR_3
108	Hu2E6_Hv2 T57A Heavy Chain Variable Region—nucleic acid
109	Hu2E6_Hv2 T57A Heavy Chain Variable Region—protein
15	Hu2E6_Hv2 T57A Heavy Chain CDR_1
96	Hu2E6_Hv2 T57A Heavy Chain CDR_2
17	Hu2E6_Hv2 T57A Heavy Chain CDR_3
110	Hu2E6_Kv1 Light (kappa) Chain Variable Region—nucleic acid
111	Hu2E6_Kv1 Light (kappa) Chain Variable Region—protein
99	Hu2E6_Kv1 Light (kappa) Chain CDR_1
100	Hu2E6_Kv1 Light (kappa) Chain CDR_2
20	Hu2E6_Kv1 Light (kappa) Chain CDR_3
112	Hu2E6_Kv2 Light (kappa) Chain Variable Region—nucleic acid
113	Hu2E6_Kv2 Light (kappa) Chain Variable Region—protein
99	Hu2E6_Kv2 Light (kappa) Chain CDR_1
101	Hu2E6_Kv2 Light (kappa) Chain CDR_2
20	Hu2E6_Kv2 Light (kappa) Chain CDR_3

[0202] Humanized monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 9.

Table 9

	Kabat		
	CDR1	CDR2	CDR3
2E6	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFKG (SEQ ID NO: 16)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv1	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFKG (SEQ ID NO: 16)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv1 T57A	SYWMH (SEQ ID NO: 15)	AVYPRNNDATYNQKFKG (SEQ ID NO: 94)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv2	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFQG (SEQ ID NO: 95)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv2 T57A	SYWMH (SEQ ID NO: 15)	AVYPRNNDATYNQKFQG (SEQ ID NO: 96)	FNYNFDY (SEQ ID NO: 17)
	Chothia		
	CDR1	CDR2	CDR3
2E6	GYTFTSY (SEQ ID NO: 40)	YPRNNDT (SEQ ID NO: 41)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv1	GYTFTSY (SEQ ID NO: 40)	YPRNNDT (SEQ ID NO: 41)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv1 T57A	GYTFTSY (SEQ ID NO: 40)	YPRNNDA (SEQ ID NO: 97)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv2	GYTFTSY (SEQ ID NO: 40)	YPRNNDT (SEQ ID NO: 41)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv2 T57A	GYTFTSY (SEQ ID NO: 40)	YPRNNDA (SEQ ID NO: 97)	FNYNFDY (SEQ ID NO: 17)
	IMGT		
	CDR1	CDR2	CDR3
2E6	GYTFTSYW (SEQ ID NO: 49)	VYPRNNDT (SEQ ID NO: 50)	LYFNYNFDY (SEQ ID NO: 51)
Hu2E6_Hv1	GYTFTSYW (SEQ ID NO: 49)	VYPRNNDT (SEQ ID NO: 50)	LYFNYNFDY (SEQ ID NO: 51)
Hu2E6_Hv1 T57A	GYTFTSYW (SEQ ID NO: 49)	VYPRNNDA (SEQ ID NO: 98)	LYFNYNFDY (SEQ ID NO: 51)
Hu2E6_Hv2	GYTFTSYW (SEQ ID NO: 49)	VYPRNNDT (SEQ ID NO: 50)	LYFNYNFDY (SEQ ID NO: 51)
Hu2E6_Hv2 T57A	GYTFTSYW (SEQ ID NO: 49)	VYPRNNDA (SEQ ID NO: 98)	LYFNYNFDY (SEQ ID NO: 51)

[0203] Humanized monoclonal antibody Kappa light chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 10.

Table 10

	Kabat/Chothia		
	CDR1	CDR2	CDR3
2E6	SASSSVSYMH (SEQ ID NO: 18)	DTSNLAS (SEQ ID NO: 19)	QQWSSYPYT (SEQ ID NO: 20)
Hu2E6_Kv1	RASSSVSYMH (SEQ ID NO: 99)	DTSNRAS (SEQ ID NO: 100)	QQWSSYPYT (SEQ ID NO: 20)
Hu2E6_Kv2	RASSSVSYMH (SEQ ID NO: 99)	DTSNRAT (SEQ ID NO: 101)	QQWSSYPYT (SEQ ID NO: 20)
	IMGT		
	CDR1	CDR2	CDR3
2E6	SSVSY (SEQ ID NO: 59)	DTS	QQWSSYPYT (SEQ ID NO: 20)
Hu2E6_Kv1	SSVSY (SEQ ID NO: 59)	DTS	QQWSSYPYT (SEQ ID NO: 20)
Hu2E6_Kv2	SSVSY (SEQ ID NO: 59)	DTS	QQWSSYPYT (SEQ ID NO: 20)

[0204] To create the complete chimeric and humanized heavy or kappa chain antibody sequences, each variable sequence above is combined with its respective human constant region. For example, a complete heavy chain comprises a heavy variable sequence followed by a human IgGl heavy chain constant sequence. A complete kappa chain comprises a kappa variable sequence followed by the human kappa light chain constant sequence.

[0205] Nucleic Acid Sequence Encoding the Human IgGl Heavy Chain Constant Region (SEQ ID NO: 114)

```
   1 gcctcaacaa aaggaccaag tgtgttccca ctcgccccta gcagcaagag tacatccggg
  61 ggcactgcag cactcggctg cctcgtcaag gattattttc cagagccagt aaccgtgagc
 121 tggaacagtg gagcactcac ttctggtgtc catacttttc ctgctgtcct gcaaagctct
 181 ggcctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
 241 tacatctgta atgtaaacca caagcctagc aatactaagg tcgataagcg ggtggaaccc
 301 aagagctgcg acaagactca cacttgtccc ccatgccctg cccctgaact tctgggcggt
 361 cccagcgtct ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
 421 gaggtgacat gtgttgttgt agacgtttcc cacgaggacc cagaggttaa gttcaactgg
 481 tacgttgatg gagtcgaagt acataatgct aagaccaagc ctagagagga gcagtataat
 541 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
 601 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
 661 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccacccag tagagaggaa
 721 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
 781 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac ccccccagtg
 841 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgctgg
 901 cagcagggta acgtcttcag ctgttccgtg atgcacgagg cattgcacaa ccactacacc
 961 cagaagtcac tgagcctgag cccagggaag
```

[0206] Protein Sequence Defining the Human IgGl Heavy Chain Constant Region (SEQ ID NO: 115)

```
  1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
 61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
301 qqgnvfscsv mhealhnhyt qkslslspgk
```

[0207] Nucleic Acid Sequence Encoding the Human Kappa Light Chain Constant Region (used for chimeric antibodies) (SEQ ID NO: 116)

```
  1 cgcacagtcg ccgctccctc cgtgttcatc tttccaccaa gtgatgagca actgaagtct
 61 ggtactgctt cagtcgtgtg tctgctgaac aatttctacc ctcgagaagc caaagtccaa
121 tggaaggtag acaacgcact gcagtccggc aatagccaag aatcagttac cgaacaggat
181 tcaaaggaca gtacatattc cctgagcagc actctgaccc tgtcaaaggc cgattacgag
241 aaacacaagg tctatgcttg cgaagtgaca catcagggac tgtccagccc agtgacaaaa
301 tcttttaacc gtggggagtg t
```

[0208] Nucleic Acid Sequence Encoding the Human Kappa Light Chain Constant Region (used for humanized antibodies) (SEQ ID NO: 117)

```
  1 cgcacagttg ctgcccccag cgtgttcatt ttcccaccta gcgatgagca gctgaaaagc
 61 ggtactgcct ctgtcgtatg cttgctcaac aacttttacc cacgtgaggc taaggtgcag
121 tggaaagtgg ataatgcact tcaatctgga aacagtcaag agtccgtgac agaacaggac
181 agcaaagact caacttattc actctcttcc accctgactc tgtccaaggc agactatgaa
241 aaacacaagg tatacgcctg cgaggttaca caccagggtt tgtctagtcc tgtcaccaag
301 tccttcaata ggggcgaatg t
```

[0209] Protein Sequence Defining the Human Kappa Light Chain Constant Region (used for chimeric and humanized antibodies) (SEQ ID NO: 118)

```
  1 rtvaapsvfi fppsdeqlks gtasvvclln nfypreakvq wkvdnalqsg nsqesvteqd
 61 skdstyslss tltlskadye khkvyacevt hqglsspvtk sfnrgec
```

[0210] The following sequences represent the actual or contemplated full length heavy and light chain sequence *(i.e.,* containing both the variable and constant regions sequences) for each antibody described in this Example. Signal sequences for proper secretion of the antibodies *(e.g.,* signal sequences at the 5' end of the DNA sequences or the amino terminal end of the protein sequences) are not shown in the full length heavy and light chain sequences disclosed herein and are not included in the final secreted protein. Also not shown are stop codons for termination of translation required at the 3' end of the DNA sequences. It is within ordinary skill in the art to select a signal sequence and/or a stop codon for expression of the disclosed full length IgG heavy chain and light chain sequences. It is also contemplated that the variable

region sequences can be ligated to other constant region sequences to produce active full length IgG heavy and light chains.

[0211] Nucleic Acid Sequence Encoding the Full Length Chimeric 2E6 Heavy Chain (Mouse Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 119)

```
   1 gaggttcagc tccagcagtc tggggctgag ctggcaagac ctggggcttc agtgaagatg
  61 tcctgcaagg cttctggcta cacctttacc agctactgga tgcactgggt aaaacagagg
 121 cctggacagg gtctggaatg gattggcgct gtttatccta gaaacaatga tactacttac
 181 aatcagaagt tcaagggcaa ggccaagctg actgctgtca catccgccag cactgcctac
 241 atggcactca gcagcctaac aaatgaggac tctgcggtct attactgtct ttattttaac
 301 tacaactttg actactgggg ccaaggcacc actctcacag tctcctcagc ctcaacaaaa
 361 ggaccaagtg tgttcccact cgcccctagc agcaagagta catccggggg cactgcagca
 421 ctcggctgcc tcgtcaagga ttattttcca gagccagtaa ccgtgagctg gaacagtgga
 481 gcactcactt ctggtgtcca tacttttcct gctgtcctgc aaagctctgg cctgtactca
 541 ctcagctccg tcgtgaccgt gccatcttca tctctgggca ctcagaccta catctgtaat
 601 gtaaaccaca agcctagcaa tactaaggtc gataagcggg tggaacccaa gagctgcgac
 661 aagactcaca cttgtccccc atgccctgcc cctgaacttc tgggcggtcc cagcgtcttt
 721 ttgttcccac caaagcctaa agatactctg atgataagta gaacacccga ggtgacatgt
 781 gttgttgtag acgtttccca cgaggaccca gaggttaagt tcaactggta cgttgatgga
 841 gtcgaagtac ataatgctaa gaccaagcct agagaggagc agtataatag tacataccgt
 901 gtagtcagtg ttctcacagt gctgcaccaa gactggctca acggcaaaga atacaaatgc
 961 aaagtgtcca acaaagcact cccagcccct atcgagaaga ctattagtaa ggcaaagggg
1021 cagcctcgtg aaccacaggt gtacactctg ccacccagta gagaggaaat gacaaagaac
1081 caagtctcat tgacctgcct ggtgaaaggc ttctacccca gcgacatcgc cgttgagtgg
1141 gagagtaacg gtcagcctga gaacaattac aagacaaccc ccccagtgct ggatagtgac
1201 gggtctttct ttctgtacag taagctgact gtggacaagt cccgctggca gcagggtaac
1261 gtcttcagct gttccgtgat gcacgaggca ttgcacaacc actacaccca gaagtcactg
1321 agcctgagcc cagggaag
```

[0212] Protein Sequence Defining the Full Length Chimeric 2E6 Heavy Chain (Mouse Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 120)

```
   1 evqlqqsgae larpgasvkm sckasgytft sywmhwvkqr pgqglewiga vyprnndtty
  61 nqkfkgkakl tavtsastay malssltned savyyclyfn ynfdywgqgt tltvssastk
 121 gpsvfplaps skstsggtaa lgclvkdyfp epvtvswnsg altsgvhtfp avlqssglys
 181 lssvvtvpss slgtqtyicn vnhkpsntkv dkrvepkscd kthtcppcpa pellggpsvf
 241 lfppkpkdtl misrtpevtc vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr
 301 vvsvltvlhq dwlngkeykc kvsnkalpap iektiskakg qprepqvytl ppsreemtkn
 361 qvsltclvkg fypsdiavew esngqpenny kttppvldsd gsfflysklt vdksrwqqgn
 421 vfscsvmhea lhnhytqksl slspgk
```

[0213] Nucleic Acid Sequence Encoding the Full Length Chimeric 2E6 Light Chain (Mouse Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO: 121)

```
   1 caaattgttc tcacccagtc tccagcaatc atgtctgctt ctccagggga gaaggtcacc
  61 atgacctgca gtgccagctc aagtgtaagt tacatgcact ggtaccagca gaagccagga
 121 tcctccccca gactcctgat ttatgacaca tccaacctgg cttctggagt ccctgtgcac
 181 ttcagtggca gtgggtctgg gacctcttac tctctcacaa tcatccgaat ggaggctgaa
 241 gatgctgcca cttattactg ccagcagtgg agtagttacc cgtacacgtt cggaggggg
 301 accaagctgg aaataaaacg cacagtcgcc gctccctccg tgttcatctt tccaccaagt
 361 gatgagcaac tgaagtctgg tactgcttca gtcgtgtgtc tgctgaacaa tttctaccct
 421 cgagaagcca aagtccaatg gaaggtagac aacgcactgc agtccggcaa tagccaagaa
```

```
481 tcagttaccg aacaggattc aaaggacagt acatattccc tgagcagcac tctgaccctg
541 tcaaaggccg attacgagaa acacaaggtc tatgcttgcg aagtgacaca tcagggactg
601 tccagcccag tgacaaaatc ttttaaccgt ggggagtgt
```

[0214] Protein Sequence Defining the Full Length Chimeric 2E6 Light Chain (Mouse Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO: 122)

```
  1 qivltqspai msaspgekvt mtcsasssvs ymhwyqqkpg ssprlliydt snlasgvpvh
 61 fsgsgsgtsy sltiirmeae daatyycqqw ssypytfggg tkleikrtva apsvfifpps
121 deqlksgtas vvcllnnfyp reakvqwkvd nalqsgnsqe svteqdskds tyslsstltl
181 skadyekhkv yacevthqgl sspvtksfnr gee
```

[0215] Nucleic Acid Sequence Encoding the Full Length Humanized Hu2E6_Hyl_Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl_Constant Region) (SEQ ID NO: 123)

```
   1 gaagtgcagt tggtacaaag tggggccgaa gttgeaaage caggggcctc agtgaagatg
  61 tettgeaagg ettceggata cacattcact tcatattgga tgcactgggt gaagcaagct
 121 cccggccagg gtctggagtg gateggegea gtctacccta gaaacaacga taccacctat
 181 aaccagaaat tcaagggcaa ggccaccctc accgctgaca ctagcacatc cacagcatac
 241 atggagctgc gctctcttcg gagegacgat acagccgtct attactgtct gtatttcaat
 301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttccgc ctcaacaaaa
 361 ggaccaagtg tgttcccact cgcccctagc agcaagagta catcegggggg cactgcagca
 421 ctcggctgcc tegtcaagga ttattttcca gagecagtaa ccgtgagctg gaacagtgga
 481 gcactcactt ctggtgtcca tacttttcct gctgtcctgc aaagctctgg cctgtactca
 541 ctcagctccg tcgtgaccgt gccatcttca tctctgggca ctcagaccta catctgtaat
 601 gtaaaccaca agectagcaa tactaaggtc gataageggg tggaacccaa gagctgegae
 661 aagactcaca cttgtccccc atgccctgcc cctgaacttc tgggeggtec cagegtcttt
 721 ttgttcccac caaagectaa agatactctg atgataagta gaacacccga ggtgacatgt
 781 gttgttgtag acgtttccca cgaggaccca gaggttaagt tcaactggta cgttgatgga
 841 gtcgaagtac ataatgetaa gaccaagcct agagaggagc agtataatag tacataccgt
 901 gtagtcagtg ttctcacagt gctgcaccaa gactggctca aeggcaaaga atacaaatgc
 961 aaagtgtcca acaaagcact cccagcccct atcgagaaga ctattagtaa ggcaaagggg
1021 cagcctcgtg aaccacaggt gtacactctg ccacccagta gagaggaaat gacaaagaac
1081 caagtctcat tgacctgcct ggtgaaaggc ttctacccca gcgacatcgc cgttgagtgg
1141 gagagtaacg gtcagcctga gaacaattac aagacaaccc ccccagtgct ggatagtgac
1201 gggtctttct ttctgtacag taagctgact gtggacaagt cccgctggca gcagggtaac
1261 gtcttcagct gttccgtgat gcacgaggca ttgcacaacc actacaccca gaagtcactg
1321 agcctgagcc cagggaag
```

[0216] Protein Sequence Defining the Full Length Humanized Hu2E6_Hyl_Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl_Constant Region) (SEQ ID NO: 124)

```
  1 evqlvqsgae vakpgasvkm sckasgytft sywmhwvkqa pgqglewiga vyprnndtty
 61 nqkfkgkatl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvssastk
121 gpsvfplaps skstsggtaa lgclvkdyfp epvtvswnsg altsgvhtfp avlqssglys
181 lssvvtvpss slgtqtyicn vnhkpsntkv dkrvepkscd kthtcppcpa pellggpsvf
241 lfppkpkdtl misrtpevtc vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr
301 vvsvltvlhq dwlngkeykc kvsnkalpap iektiskakg qprepqvytl ppsreemtkn
361 qvsltclvkg fypsdiavew esngqpenny kttppvldsd gsfflysklt vdksrwqqgn
421 vfscsvmhea lhnhytqksl slspgk
```

[0217] Nucleic Acid Sequence Encoding the Full Length Humanized Hu2E6 Hyl T57A Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 125)

```
   1 gaagtgcagt tggtacaaag tggggccgaa gttgcaaagc caggggcctc agtgaagatg
  61 tcttgcaagg cttccggata cacattcact tcatattgga tgcactgggt gaagcaagct
 121 cccggccagg gtctggagtg gatcggcgca gtctacccta gaaacaacga tgccacctat
 181 aaccagaaat tcaagggcaa ggccaccctc accgctgaca ctagcacatc cacagcatac
 241 atggagctgc gctctcttcg gagcgacgat acagccgtct attactgtct gtatttcaat
 301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttccgc ctcaacaaaa
 361 ggaccaagtg tgttcccact cgcccctagc agcaagagta catccggggg cactgcagca
 421 ctcggctgcc tcgtcaagga ttattttcca gagccagtaa ccgtgagctg gaacagtgga
 481 gcactcactt ctggtgtcca tacttttcct gctgtcctgc aaagctctgg cctgtactca
 541 ctcagctccg tcgtgaccgt gccatcttca tctctgggca ctcagaccta catctgtaat
 601 gtaaaccaca agcctagcaa tactaaggtc gataagcggg tggaacccaa gagctgcgac
 661 aagactcaca cttgtccccc atgccctgcc cctgaacttc tgggcggtcc cagcgtcttt
 721 ttgttcccac caaagcctaa agatactctg atgataagta gaacacccga ggtgacatgt
 781 gttgttgtag acgtttccca cgaggaccca gaggttaagt tcaactggta cgttgatgga
 841 gtcgaagtac ataatgctaa gaccaagcct agagaggagc agtataatag tacataccgt
 901 gtagtcagtg ttctcacagt gctgcaccaa gactggctca acggcaaaga atacaaatgc
 961 aaagtgtcca acaaagcact cccagcccct atcgagaaga ctattagtaa ggcaaagggg
1021 cagcctcgtg aaccacaggt gtacactctg ccacccagta gagaggaaat gacaaagaac
1081 caagtctcat tgacctgcct ggtgaaaggc ttctacccca gcgacatcgc cgttgagtgg
1141 gagagtaacg gtcagcctga gaacaattac aagacaaccc ccccagtgct ggatagtgac
1201 gggtctttct ttctgtacag taagctgact gtggacaagt cccgctggca gcagggtaac
1261 gtcttcagct gttccgtgat gcacgaggca ttgcacaacc actacaccca gaagtcactg
1321 agcctgagcc cagggaag
```

[0218] Protein Sequence Defining the Full Length Humanized Hu2E6 Hyl T57A Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 126)

```
   1 evqlvqsgae vakpgasvkm sckasgytft sywmhwvkqa pgqglewiga vyprnndaty
  61 nqkfkgkatl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvssastk
 121 gpsvfplaps skstsggtaa lgclvkdyfp epvtvswnsg altsgvhtfp avlqssglys
 181 lssvvtvpss slgtqtyicn vnhkpsntkv dkrvepkscd kthtcppcpa pellggpsvf
 241 lfppkpkdtl misrtpevtc vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr
 301 vvsvltvlhq dwlngkeykc kvsnkalpap iektiskakg qprepqvytl ppsreemtkn
 361 qvsltclvkg fypsdiavew esngqpenny kttppvldsd gsfflysklt vdksrwqqgn
 421 vfscsvmhea lhnhytqksl slspgk
```

[0219] Nucleic Acid Sequence Encoding the Full Length Humanized Hu2E6 Hv2 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 127)

```
   1 caggtgcagt tggtacaaag tggggccgaa gttaagaagc caggggcctc agtgaagatg
  61 tcttgcaagg cttccggata cacattcact tcatattgga tgcactgggt gaggcaagct
 121 cccggccagg gtctggagtg gatcggcgca gtctacccta gaaacaacga taccacctat
 181 aaccagaaat tccagggcag ggccaccctc accgctgaca ctagcacatc cacagcatac
 241 atggagctgc gctctcttcg gagcgacgat acagccgtct attactgtct gtatttcaat
```

```
 301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttccgc ctcaacaaaa
 361 ggaccaagtg tgttcccact cgcccctagc agcaagagta catccggggg cactgcagca
 421 ctcggctgcc tcgtcaagga ttattttcca gagccagtaa ccgtgagctg gaacagtgga
 481 gcactcactt ctggtgtcca tacttttcct gctgtcctgc aaagctctgg cctgtactca
 541 ctcagctccg tcgtgaccgt gccatcttca tctctgggca ctcagaccta catctgtaat
 601 gtaaaccaca agcctagcaa tactaaggtc gataagcggg tggaacccaa gagctgcgac
 661 aagactcaca cttgtccccc atgccctgcc cctgaacttc tgggcggtcc cagcgtcttt
 721 ttgttcccac caaagcctaa agatactctg atgataagta gaacacccga ggtgacatgt
 781 gttgttgtag acgtttccca cgaggaccca gaggttaagt tcaactggta cgttgatgga
 841 gtcgaagtac ataatgctaa gaccaagcct agagaggagc agtataatag tacataccgt
 901 gtagtcagtg ttctcacagt gctgcaccaa gactggctca acggcaaaga atacaaatgc
 961 aaagtgtcca acaaagcact cccagcccct atcgagaaga ctattagtaa ggcaaagggg
1021 cagcctcgtg aaccacaggt gtacactctg ccacccagta gagaggaaat gacaaagaac
1081 caagtctcat tgacctgcct ggtgaaaggc ttctacccca gcgacatcgc cgttgagtgg
1141 gagagtaacg gtcagcctga gaacaattac aagacaaccc ccccagtgct ggatagtgac
1201 gggtctttct ttctgtacag taagctgact gtggacaagt cccgctggca gcagggtaac
1261 gtcttcagct gttccgtgat gcacgaggca ttgcacaacc actacaccca gaagtcactg
1321 agcctgagcc cagggaag
```

[0220] Protein Sequence Defining the Full Length Humanized Hu2E6_Hv2 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1_Constant Region) (SEQ ID NO: 128)

```
   1 qvqlvqsgae vkkpgasvkm sckasgytft sywmhwvrqa pgqglewiga vyprnndtty
  61 nqkfqgratl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvssastk
 121 gpsvfplaps skstsggtaa lgclvkdyfp epvtvswnsg altsgvhtfp avlqssglys
 181 lssvvtvpss slgtqtyicn vnhkpsntkv dkrvepkscd kthtcppcpa pellggpsvf
 241 lfppkpkdtl misrtpevtc vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr
 301 vvsvltvlhq dwlngkeykc kvsnkalpap iektiskakg qprepqvytl ppsreemtkn
 361 qvsltclvkg fypsdiavew esngqpenny kttppvldsd gsfflysklt vdksrwqqgn
 421 vfscsvmhea lhnhytqksl slspgk
```

[0221] Nucleic Acid Sequence Encoding the Full Length Humanized Hu2E6_Hv2 T57A Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1_Constant Region) (SEQ ID NO: 129)

```
   1 caggtgcagt tggtacaaag tggggccgaa gttaagaagc caggggcctc agtgaagatg
  61 tcttgcaagg cttccggata cacattcact tcatattgga tgcactgggt gaggcaagct
 121 cccggccagg gtctggagtg gatcggcgca gtctacccta gaaacaacga tgccacctat
 181 aaccagaaat tccagggcag ggccaccctc accgctgaca ctagcacatc cacagcatac
 241 atggagctgc gctctcttcg gagcgacgat acagccgtct attactgtct gtatttcaat
 301 tacaatttcg actactgggg acagggtact ctcctgaccg ttagttccgc ctcaacaaaa
 361 ggaccaagtg tgttcccact cgcccctagc agcaagagta catccggggg cactgcagca
 421 ctcggctgcc tcgtcaagga ttattttcca gagccagtaa ccgtgagctg gaacagtgga
 481 gcactcactt ctggtgtcca tacttttcct gctgtcctgc aaagctctgg cctgtactca
 541 ctcagctccg tcgtgaccgt gccatcttca tctctgggca ctcagaccta catctgtaat
 601 gtaaaccaca agcctagcaa tactaaggtc gataagcggg tggaacccaa gagctgcgac
 661 aagactcaca cttgtccccc atgccctgcc cctgaacttc tgggcggtcc cagcgtcttt
 721 ttgttcccac caaagcctaa agatactctg atgataagta gaacacccga ggtgacatgt
 781 gttgttgtag acgtttccca cgaggaccca gaggttaagt tcaactggta cgttgatgga
 841 gtcgaagtac ataatgctaa gaccaagcct agagaggagc agtataatag tacataccgt
 901 gtagtcagtg ttctcacagt gctgcaccaa gactggctca acggcaaaga atacaaatgc
 961 aaagtgtcca acaaagcact cccagcccct atcgagaaga ctattagtaa ggcaaagggg
```

```
1021 cagcctcgtg aaccacaggt gtacactctg ccacccagta gagaggaaat gacaaagaac
1081 caagtctcat tgacctgcct ggtgaaaggc ttctacccca gcgacatcgc cgttgagtgg
1141 gagagtaacg gtcagcctga gaacaattac aagacaaccc ccccagtgct ggatagtgac
1201 gggtctttct ttctgtacag taagctgact gtggacaagt cccgctggca gcagggtaac
1261 gtcttcagct gttccgtgat gcacgaggca ttgcacaacc actacaccca gaagtcactg
1321 agcctgagcc cagggaag
```

[0222] Protein Sequence Defining the Full Length Humanized Hu2E6 Hv2 T57A Heavy Chain (Humanized Heavy Chain Variable Region and Human IgGl Constant Region) (SEQ ID NO: 130)

```
   1 qvqlvqsgae vkkpgasvkm sckasgytft sywmhwvrqa pgqglewiga vyprnndaty
  61 nqkfqgratl tadtststay melrslrsdd tavyyclyfn ynfdywgqgt lltvssastk
 121 gpsvfplaps skstsggtaa lgclvkdyfp epvtvswnsg altsgvhtfp avlqssglys
 181 lssvvtvpss slgtqtyicn vnhkpsntkv dkrvepkscd kthtcppcpa pellggpsvf
 241 lfppkpkdtl misrtpevtc vvvdvshedp evkfnwyvdg vevhnaktkp reeqynstyr
 301 vvsvltvlhq dwlngkeykc kvsnkalpap iektiskakg qprepqvytl ppsreemtkn
 361 qvsltclvkg fypsdiavew esngqpenny kttppvldsd gsfflysklt vdksrwqqgn
 421 vfscsvmhea lhnhytqksl slspgk
```

[0223] Nucleic Acid Sequence Encoding the Full Length Humanized Hu2E6 Kyl Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 131)

```
   1 gaaattgtcc tgacacagtc acccgcaaca atgtctgcct ctccaggcga gagagtcacc
  61 atgtcttgca gggcttcctc ctctgtgagc tacatgcatt ggtaccagca aaagccaggt
 121 cagtcccctc ggctgcttat ctatgacacc tccaaccgag cctctggagt tcccgcccac
 181 ttcagcggca gcgggagtgg gacagattac actctgacca taagttcaat ggagcctgag
 241 gactttgcaa cctattactg ccagcaatgg agcagttatc cctatacttt cggccaggga
 301 accaaactcg aaatcaagcg cacagttgct gcccccagcg tgttcatttt cccacctagc
 361 gatgagcagc tgaaaagcgg tactgcctct gtcgtatgct tgctcaacaa cttttaccca
 421 cgtgaggcta aggtgcagtg gaaagtggat aatgcacttc aatctggaaa cagtcaagag
 481 tccgtgacag aacaggacag caaagactca acttattcac tctcttccac cctgactctg
 541 tccaaggcag actatgaaaa acacaaggta tacgcctgcg aggttacaca ccagggtttg
 601 tctagtcctg tcaccaagtc cttcaatagg ggcgaatgt
```

[0224] Protein Sequence Defining the Full Length Humanized Hu2E6 Kyl Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 132)

```
   1 eivltqspat msaspgervt mscrasssvs ymhwyqqkpg qsprlliydt snrasgvpah
  61 fsgsgsgtdy tltissmepe dfatyycqqw ssypytfgqg tkleikrtva apsvfifpps
 121 deqlksgtas vvcllnnfyp reakvqwkvd nalqsgnsqe svteqdskds tyslsstltl
 181 skadyekhkv yacevthqgl sspvtksfnr gee
```

[0225] Nucleic Acid Sequence Encoding the Full Length Humanized Hu2E6 Kv2 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 133)

```
   1 gaaattgtcc tgacacagtc acccgcaaca ttgtctgcct ctccaggcga gagagtcacc
```

```
  61 atgtcttgca gggcttcctc ctctgtgagc tacatgcatt ggtaccagca aaagccaggt
 121 caggctcctc ggctgcttat ctatgacacc tccaaccgag ccactggagt tcccgccagg
 181 ttcagcggca gcgggagtgg gacagattac actctgacca taagttcaat ggagcctgag
 241 gactttgcaa cctattactg ccagcaatgg agcagttatc cctatacttt cggccaggga
 301 accaaactcg aaatcaagcg cacagttgct gcccccagcg tgttcatttt cccacctagc
 361 gatgagcagc tgaaaagcgg tactgcctct gtcgtatgct tgctcaacaa cttttaccca
 421 cgtgaggcta aggtgcagtg gaaagtggat aatgcacttc aatctggaaa cagtcaagag
 481 tccgtgacag aacaggacag caaagactca acttattcac tctcttccac cctgactctg
 541 tccaaggcag actatgaaaa acacaaggta tacgcctgcg aggttacaca ccagggtttg
 601 tctagtcctg tcaccaagtc cttcaatagg ggcgaatgt
```

[0226] Protein Sequence Defining the Full Length Humanized Hu2E6 Kv2 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 134)

```
   1 eivltqspat lsaspgervt mscrasssvs ymhwyqqkpg qaprlliydt snratgvpar
  61 fsgsgsgtdy tltissmepe dfatyycqqw ssypytfgqg tkleikrtva apsvfifpps
 121 deqlksgtas vvcllnnfyp reakvqwkvd nalqsgnsqe svteqdskds tyslsstltl
 181 skadyekhkv yacevthqgl sspvtksfnr gee
```

[0227] For convenience, Table 11 provides a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.

Table 11

SEQ ID NO.	Nucleic Acid or Protein
114	Human IgG1 constant—nucleic acid
115	Human IgG1 constant—protein
116	Human Kappa constant (used for chimeric antibodies)—nucleic acid
117	Human Kappa constant (used for humanized antibodies)—nucleic acid
118	Human Kappa constant (used for chimeric and humanized antibodies)——protein
119	Chimeric 2E6 Mouse Heavy Chain Variable + Human IgG1 constant—nucleic acid
120	Chimeric 2E6 Mouse Heavy Chain Variable + Human IgG1 constant—protein
121	Chimeric 2E6 Mouse Light Chain Variable + Human Kappa constant—nucleic acid
122	Chimeric 2E6 Mouse Light Chain Variable + Human Kappa constant—protein
123	Humanized Hu2E6_Hv1 Heavy Human Variable + Human IgG1 constant—nucleic acid
124	Humanized Hu2E6_Hv1 Heavy Human Variable + Human IgG1 constant—protein
125	Humanized Hu2E6_Hv1 T57A Heavy Human Variable + Human IgG1 constant—nucleic acid
126	Humanized Hu2E6_Hv1 T57A Heavy Human Variable + Human IgG1 constant—protein
127	Humanized Hu2E6_Hv2 Heavy Human Variable + Human IgG1 constant—nucleic acid
128	Humanized Hu2E6_Hv2 Heavy Human Variable + Human IgG1 constant—protein
129	Humanized Hu2E6_Hv2 T57A Heavy Human Variable + Human IgG1 constant—nucleic acid
130	Humanized Hu2E6_Hv2 T57A Heavy Human Variable + Human IgG1 constant—

SEQ ID NO.	Nucleic Acid or Protein
	protein
131	Humanized Hu2E6_Kv1 Human Variable + Human Kappa constant—nucleic acid
132	Humanized Hu2E6_Kv1 Human Variable + Human Kappa constant—protein
133	Humanized Hu2E6_Kv2 Human Variable + Human Kappa constant—nucleic acid
134	Humanized Hu2E6_Kv2 Human Variable + Human Kappa constant- - protein

[0228] Table 12 below shows antibodies containing chimeric immunoglobulin heavy and light chains and each of the possible combinations of the full-length humanized immunoglobulin heavy and light chains.

Table 12

Antibody Name	Light Chain	Heavy Chain
Hu2E6-1	2E6 Chimeric Kappa (SEQ ID NO: 122)	2E6 Chimeric Heavy IgG1 (SEQ ID NO: 120)
Hu2E6-56	Hu2E6_Kv2 Kappa (SEQ ID NO: 134)	Hu2E6_Hv2 IgG1 (SEQ ID NO: 128)
Hu2E6-57	Hu2E6_Kv1 Kappa (SEQ ID NO: 132)	Hu2E6_Hv1 IgG1 (SEQ ID NO: 124)
Hu2E6-58	Hu2E6_Kv2 Kappa (SEQ ID NO: 134)	Hu2E6_Hv1 IgG1 (SEQ ID NO: 124)
Hu2E6-76	Hu2E6_Kv1 Kappa (SEQ ID NO: 132)	Hu2E6_Hv2 IgG1 (SEQ ID NO: 128)
Hu2E6-62	Hu2E6_Kv1 Kappa (SEQ ID NO: 132)	Hu2E6_Hv1 T57A IgG1 (SEQ ID NO: 126)
Hu2E6-74	Hu2E6_Kv2 Kappa (SEQ ID NO: 134)	Hu2E6_Hv1 T57A IgG1 (SEQ ID NO: 126)
Hu2E6-75	Hu2E6_Kv2 Kappa (SEQ ID NO: 134)	Hu2E6_Hv2 T57A IgG1 (SEQ ID NO: 130)
Hu2E6-77	Hu2E6_Kv1 Kappa (SEQ ID NO: 132)	Hu2E6_Hv2 T57A IgG1 (SEQ ID NO: 130)

[0229] The antibody construct containing the full length chimeric heavy and light chains is designated below:

Chimeric 2E6 (also referred to as Hu2E6-l) = Full Length Chimeric 2E6 Heavy Chain (Mouse Variable Region and Human IgGl Constant Region) (SEQ ID NO: 120) plus Full Length Chimeric 2E6 Light Chain (Mouse Variable Region and Human Kappa Constant Region) (SEQ ID NO: 122)

[0230] Two of the possible antibody constructs containing the full length immunoglobulin heavy and light chains containing humanized variable regions are designated below:

Hu2E6-62 = Humanized Hu2E6_Hvl T57A Heavy Chain Variable Region and Human IgGl Constant Region (SEQ ID NO: 126) plus Hu2E6_Kvl Light Chain Variable Region and Human Kappa Constant Region (SEQ ID NO: 132)

Hu2E6-74 = Humanized Hu2E6_Hvl T57A Heavy Chain Variable Region and Human IgGl Constant Region (SEQ ID NO: 126) plus Hu2E6_Kv2 Light Chain Variable Region and Human Kappa Constant Region (SEQ ID NO: 134)

B. Binding Affinities of Humanized and Chimeric Anti-NOTCHl Monoclonal Antibodies

[0231] The binding affinities and kinetics of interaction of monoclonal antibodies produced in Example 14 against recombinant human Notch 1 (monomeric protein containing EGF-Like repeats 1-13) were measured by surface plasmon resonance using a Biacore T100 (Biacore (GE Healthcare), Piscataway, NJ) instrument.

[0232] Goat anti-human IgG Fc (Jackson ImmunoResearch, Catalog No. 109-005-098) was immobilized on carboxymethylated dextran CM4 sensor chips (Biacore, Catalog No. BR-1005-34) by amine coupling (Biacore, Catalog No. BR-1000-50) using a standard coupling protocol according to the vendor's instructions. The analyses were performed at 37°C using PBS (Invitrogen, Catalog No. 14040-133) containing 0.05% surfactant P20 (Biacore, Catalog No. BR-1000-54) as running buffer.

[0233] The antibodies were captured in individual flow cells at a flow rate of 10 µï/minute. Injection time was varied for each antibody to yield an R_{max} between 30 and 60 RU. Buffer or recombinant human Notch 1 monomer diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 240 sec at 60 µï/minute. The dissociation phase was monitored for up to 1200 sec. The surface was then regenerated with two 60 second injections of Glycine pH 2.25 (made from Glycine pH 2.0 (Biacore, Catalog No. BR-1003-55) and pH 2.5 (Biacore, Catalog No. BR-1003-56) at 30 µï/minute. Concentrations of recombinant human Notchl tested were between 30 nM and 3.75 nM (a 2-fold serial dilution) (results are summarized in Table 13).

[0234] Kinetic parameters were determined using the kinetic function of the BIAevaluation software (Biacore) with double reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) were determined. The kinetic values of the monoclonal antibodies on recombinant human Notch 1 at 25℃ are summarized in Tables 13.

Table 13

Antibody	ka (1/Ms)	kd (1/s)	KD (M)	n
Hu2E6-1	2.2E+05	1.3E-03	6.8E-09	3
Hu2E6-56	1.0E+05	4.6E-04	5.8E-09	3
Hu2E6-57	1.5E+05	3.6E-04	3.4E-09	3
Hu2E6-58	1.5E+05	4.1E-04	3.2E-09	3
Hu2E6-62	1.8E+05	4.3E-04	2.7E-09	4
Hu2E6-74	1.8E+05	4.3E-04	2.8E-09	3
Hu2E6-75	5.6E+04	3.4E-04	8.0E-09	3

[0235] The results in Table 13 demonstrate that the purified antibodies have affinities ranging from about 2.7 nM to about 8.0 nM when test at 25°C.

[0236] The kinetic values of the monoclonal antibodies on recombinant human Notch 1 at 37°C are summarized in Table 14.

Table 14

Antibody	ka (1/Ms)	kd (1/s)	KD (M)	n
Hu2E6-1	2.1E+06	2.9E-02	1.2E-08	3
Hu2E6-56	1.3E+06	1.0E-02	7.4E-09	2
Hu2E6-57	3.6E+05	2.3E-03	6.7E-09	3
Hu2E6-58	3.3E+05	2.1E-03	6.9E-09	2
Hu2E6-62	3.1E+05	2.2E-03	8.0E-09	3
Hu2E6-74	4.1E+05	2.8E-03	7.6E-09	3
Hu2E6-75	6.8E+05	6.3E-03	9.8E-09	2

[0237] The results in Table 14 demonstrate that the purified antibodies have affinities ranging from about 6.7 nM to about 12 nM when test at 37°C.

[0238] The results in Table 13 and 14 demonstrate that the chimeric and each of the humanized 2E6 antibodies have fast association rates (k_a), very slow disassociation rates (k_d)

and very high affinities (K_D). The affinity of humanized variants *(e.g.,* Hu2E6-56, Hu2E6-57, Hu2E6-58, Hu2E6-62, and Hu2E6-74) for monomeric Notch 1 is consistently better than the affinity of chimeric 2E6 (Hu2E6-l). Overall, these results show that the K_D of the humanized antibodies *(e.g.,* Hu2E6-56, Hu2E6-57, Hu2E6-58, Hu2E6-62, and Hu2E6-74) was smaller *(i.e.,* higher affinity) than the K_D for Hu2E6-l (chimeric 2E6).

C. Comparison with Another Notch 1 Antibody

[0239] A2-NRR1, as disclosed in Wu *et al,* (2010) NATURE 464: 1052-57, is an antibody known to inhibit the function of human Notch 1. The binding specificities of antibodies mu2E6, Hu2E6-62, and A2-NRR1 against human Notch 1 protein expressed on the surface of the T-ALL cell line Karpas45s were measured as described above *(See* Example 4). Results are summarized in Table 15.

Table 15

		mu2E6	Hu2E6-62	A2-NRR1
K_D	Cell Surface	1.0 nM	0.11 nM	0.12 nM

[0240] The results in Table 15 demonstrate that Hu2E6-62 and A2-NRR1 have similar binding specificities for human Notch 1 protein. Further, both antibodies exhibited higher specificities than mu2E6.

Example 15: Inhibition of Notchl-Ligand Binding

[0241] Antibodies mu2E6 and Hu2E6-62 were tested for their ability to inhibit the binding of rhNotchl-Fc to human Jagl, Jag2, DLLl and DLL4. Binding measurements were made by bio-layer interferometry (BLI), using a ForteBio Octet® QK instrument as described in Example 5. The ligands tested were rhJagl-Fc (R&D Cat. No. 1277-JG-050), rhJag2-Fc (R&D Cat. No. 1726-JG-050), rhDLLl-Fc (R&D Cat. No. 5026-DL-050), and His tagged rhDLL4 (R&D Cat. No. 1506-D4-050). The inhibitory activities of the antibodies on Notch 1-ligand binding are summarized in Table 16.

Table 16

	mu2E6	Hu2E6-62
Jag1	95%	98%
Jag2	ND	95%
DLL1	95%	82%
DLL4	96%	89%

[0242] As shown in Table 16, Hu2E6-62 blocked binding of all four ligands to rhNotch1-Fc.

Example 16: Inhibition of Notch1-Dependent Signaling and Transcription

[0243] Antibodies mu2E6, Hu2E6-62, and A2-NRR1 were tested for their ability to inhibit Notch 1-dependent signaling and transcription in the presence of DLL4 as described in Example 7. Results are shown in **Fig. 16** and demonstrate that Hu2E6-62 is approximately three times more potent than mu2E6 in inhibiting Notch 1-dependent transcription. Further, antibodies Hu2E6-62 and A2-NRR1 are equally effective in their inhibitory activities.

[0244] Notch 1 antibodies mu2E6, Hu2E6-62, and A2-NRR1 were tested for their ability to inhibit Notch 1-dependent transcription by each of the ligands Jag1, Jag2, DLL1 and DLL4 as described in Example 7. The inhibitory activities of antibodies mu2E6, Hu2E6-62, and A2-NRR1 on Notch 1-dependent transcription are summarized in Table 17.

Table 17

		mu2E6		Hu21E6-61		A2-NKKI	
Inhibition of Notch1 Signaling		**EC50**	**Max Inhibition**	EC50	**Max Inhibition**	EC50	**Max Inhibition**
	Jag1	0.4 **nM**	100%	0.9 **nM**	95%	0.1 **nM**	92%
	Jag2	0.1 **nM**	85%	0.5 **nM**	97%	**NA**	**NA**
	DLI4	0.2 **nM**	90%	0.1 **nM**	96%	**NA**	**NA**
	DLL4	0.1 **nM**	93%	0.02 **nM**	100%	0.06 **nM**	100%

[0245] The data in Table 17 shows that Hu2E6-62 inhibits activation of transcription of Notch 1-dependent reporter gene by Jag1, Jag2, DLL1 or DLL4. The mu2E6 and Hu2E6-62 antibodies appeared to show equivalent inhibition of Jag1-dependent Notch 1 signaling.

Further, the mu2E6 and Hu2E6-62 antibodies showed equivalent inhibition of Jagl or DLL4-dependent Notch 1 signaling when compared to the A2-NRR1 antibody.

[0246] A reporter cell line dependent upon Notch 1 was produced by lentiviral introduction of a RBP-**jK** -dependent luciferase reporter gene (SABiosciences, Frederick, MD) into DU4475 cells. To activate Notch 1-dependent signaling and transcription, cells were plated on ligand-coated wells prepared, as described in Example 6 (above). Cells were pre-incubated with a 3-fold dilution series of antibody Hu2E6-62 concentrations ranging from 0-300 μg/ml, for one hour at 37°C, before seeding 100 μï of the suspension into 96-well plates coated with ligand or hFc. Cells were incubated in ligand-coated or human-Fc-coated wells for four or 24 hours at 37°C, in 5% $C0_2$. Next, 100 μï of Promega Bright Glo™ (Promega, Madison, WI) was added to each well. The reaction was allowed to proceed for five minutes in the dark, and then the entire 200 μï volume was transferred to white walled plates and read using a luminometer. Polyclonal antibody against Notch 1 (AF1057, R&D Systems) was used as controls to confirm that ligand-stimulated reporter activity in each cell line was specifically dependent upon the introduced Notch receptor. Results demonstrate that antibody Hu2E6-62 specifically inhibited Notch 1-dependent transcription **(FIG. 17A).**

[0247] To determine the effect of antibody Hu2E6-62 on transcription of endogenous Notch 1 target genes, Notch 1 signaling was activated by Jagl in DU4475 cells, as described above *(See* Example 7). The effect on expression of endogenous Notchl targets, as a result of treatment with IgG control or antibody Hu2E6-62 was assessed by quantitative RT-PCR. DU4475 cells were seeded into 6-well plates, in 2 ml of media. Replicate wells of cells were treated with antibody Hu2E6-62, IgG control, or vehicle control (DMSO), immediately after seeding. Cells were incubated at 37°C, 5% $C0_2$ for 20 hours after treatment, collected, and rinsed with PBS. Cell pellets were frozen on dry ice and stored at -80°C. RNA was prepared using Qiagen RNeasy™ miniprep columns (Qiagen GR8RNA). Quantitative RT-PCR was performed to analyze Notch target gene expression, using a commercial kit according to the kit vendor's instructions (Quantitect SYBR GREEN RT-PCR Kit; Qiagen). Results were analyzed using the comparative Ct method. Beta actin was used as an internal standard, and Stratagene Universal Human Reference RNA (Stratagene 740000) was used as an external standard for measurement of expression levels of the genes investigated. Results as shown in **FIG. 17B**

showed that antibody Hu2E6-62 inhibited transcription of endogenous Notch target genes, including Heyl, Hey 2, HeyL, and Hes5.

Example 17: Inhibition of T-cell Fate Specification *In Vivo*

[0248] The antibodies Hu2E6-62 and Nrrl were tested for inhibition of thymocyte development and T-cell fate specification in humanized Notch 1 mice as described above *(See* Example 10).

[0249] As shown in **Fig. 18,** antibody Hu2E6-62 reduced the total number of thymocytes by greater than 95%. Similar levels of thymocyte depletion were observed with A2-NRR1. The results indicate that antibodies Hu2E6-62 and A2-NRR1 inhibited the *in vivo* function of Notch 1 in thymocyte development to equivalent extents.

Example 18: Lack of Toxicity

[0250] To determine if the antibodies were associated with toxicity, mice were treated with 20 mg/kg of antibodies Hu2E6-62, or a IgG control three times per week, or 5 mg/kg of A2-NRR1 twice per week, as described in Example 10. As shown in **Fig. 19A,** the Hu2E6-62 treated animals exhibited normal weight gain indicating a lack of toxicity of these antibodies. However, the A2-NRR1 treated mice exhibited significant weight loss over the period of treatment, accompanied by diarrhea.

[0251] After 18 days, animals were sacrificed, small intestines were collected, fixed and embedded in paraffin. To observe goblet cells in the small intestine, sections of small intestine from antibody-treated and IgG-treated animals were stained with Alcian Blue (Diagnostic Biosystems, Cat. No. KT 003). As shown in **FIG. 19B,** mice treated with antibody Hu2E6-62 showed no increase in goblet cell numbers compared to control animals treated with IgG. By contrast, small intestines from animals treated with A2-NRR1 showed extensive Alcian Blue staining. These results indicate that antibody Hu2E6-62 did not lead to goblet cell metaplasia, and had little or no intestinal toxicity in treated mice. By contrast, A2-NRR1 treatment led to dramatic goblet cell hyperplasia indicative of severe intestinal toxicity. A2-NRR1 treated animals also exhibited diarrhea, significant weight loss (FIG. 19A), and approximately 30% of the animals died within 18 days of treatment. Upon necropsy the animals that died during treatment with A2-NRRlwere found to have bloated intestines, consistent with goblet cell

hyperplasia, and similar to the gross morphological phenotype observed in intestines of mice treated with gamma-secretase inhibitors (Example 12 and Fig 9B).

Example 19: Inhibition of Angiogenesis *in vivo*

[0252] The effect of antibody Hu2E6-62 on functional angiogenesis induced by bFGF was determined using an *in vivo* matrigel plug assay as described in Example 13. Briefly, mice were treated intraperitoneally with 20 mg/kg of antibody Hu2E6-62 or a IgG control on day 0, and every 3 days thereafter. No significant loss of body weight was observed during these experiments. Animals were sacrificed after 7 days. Plugs were removed and processed (as described above in Example 13), to determine hemoglobin concentration. The results as shown in **FIG. 20** indicated that Hu2E6-62 inhibited bFGF-induced angiogenesis in humanized ($Notch1^{hll2/hl\ 12}$ knock-in) mice.

[0253] The effect of antibody Hu2E6-62 on functional angiogenesis induced by human lung cancer cells (Calu-6) was determined, using an *in vivo* matrigel plug assay as described in Example 13. Briefly, 129Sv/Ev or immunocompromised SCID mice were treated intraperitoneally with 20 mg/kg of antibody Hu2E6-62, or a IgG control on day 0, and every 3 days thereafter. No significant loss of body weight was observed during these experiments. Animals were sacrificed after 7 days. Plugs were removed and processed (as described above in Example 13), to determine hemoglobin concentration. The results of this experiment indicated that antibody Hu2E6-62 inhibited angiogenesis induced by the human lung cancer Calu-6 cells **(FIG. 21).**

[0254] A second matrigel plug was obtained from each bFGF treated mouse and processed for histologic analysis, in parallel with the hemoglobin measurements. Plugs were removed, fixed over night in 10% buffered formalin at room temperature, embedded in paraffin, and 10-20 um sections were prepared for immunohistochemistry. To detect blood vessels present in the matrigel plug, thin sections were stained for CD31, using an anti-mouse-CD3 1 antibody (Biocare Medical, Cat. Nos. CM303 and RT517SK) according to the vendor's instructions. CD31 staining of the matrigel plugs demonstrated increased vessel branching and smaller vessels after treatment with antibody Hu2E6-62, when compared to mice treated with IgG control. (Data not shown). These data indicated that antibody Hu2E6-62 promoted vascular branching in the treated mice. However, despite the increase in vascular branching, the decrease in blood content (as measured by hemoglobin content) suggested that antibody

Hu2E6-62 decreased the function of the vessels that were present, and thus inhibited functional angiogenesis. This apparent decrease in functional angiogenesis caused by antibody Hu2E6-62 is consistent with the increased branching and decreased vascular function associated with a genetic loss of function of the Notch pathway in endothelial tissue.

INCORPORATION BY REFERENCE

[0255] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0256] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and the range of equivalency of the claims are intended to be embraced therein.

[0257] **WHAT IS CLAIMED IS :**

1. An isolated antibody that binds human Notch 1 comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region selected from the group consisting of:

(a) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H} I comprising the amino acid sequence of SEQ ID NO: 15 **(2E6),** a CDR_{H2} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 94 **(Hu2E6_Hvl T57A),** SEQ ID NO: 95 **(Hu2E6_Hv2),** SEQ ID NO: 96 **(Hu2E6_Hv2 T57A)** and SEQ ID NO: 97 **(Hu2E6_Hvl T57A, Hu2E6_Hv2 T57A),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 17 **(2E6);** and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2),** a CDR_{L2} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 100 **(Hu2E6_Kvl)** and SEQ ID NO: 101 **(Hu2E6_Kv2),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6);**

(b) (i) an immunoglobulin heavy chain variable region comprising a **CDR_{H}i** comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15 **(2E6)** and SEQ ID NO: 40 **(2E6),** a CDR_{H2} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16 **(2E6)** and SEQ ID NO: 41 **(2E6),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 17 **(2E6);** and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L} I comprising the amino acid sequence of SEQ ID NO: 18 **(2E6),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 19 **(2E6),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6);**

(c) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H} I comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 **(2G10)** and SEQ ID NO: 38 **(2G10),** a CDR_{H2} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 6 **(2G10)** and SEQ ID NO: 39 **(2G10),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7 **(2G10);** and

(ii) an immunoglobulin light chain variable region comprising a $CDR_{L}I$ comprising the amino acid sequence of SEQ ID NO: 8 **(2G10),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9 **(2G10),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10 **(2G10);**

(d) (i) an immunoglobulin heavy chain variable region comprising a $CDR_{H}I$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 25 **(2A11)** and SEQ ID NO: 42 **(2A11),** a CDR_{H2} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 26 **(2A11)** and SEQ ID NO: 43 **(2A11),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 27 **(2A11);** and

(ii) an immunoglobulin light chain variable region comprising a $CDR_{L}I$ comprising the amino acid sequence of SEQ ID NO: 18 **(2A11),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 19 **(2A11),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2A11);** and

(e) (i) an immunoglobulin heavy chain variable region comprising a $CDR_{H}I$ comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 32 **(2D11)** and SEQ ID NO: 44 **(2D11),** a CDR_{H2} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 33 **(2D11)** and SEQ ID NO: 45 **(2D11),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 34 **(2D11);** and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 35 **(2D11),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 36 **(2D11),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 37 **(2D11).**

2. The antibody of claim 1, wherein the immunoglobulin heavy chain variable region comprises a **CDR_{H}i** comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 15 **(2E6)** and SEQ ID NO: 40 **(2E6),** a CDR_{H2} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16 **(2E6)** and SEQ ID NO: 41 **(2E6),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 17 **(2E6);** and

the immunoglobulin light chain variable region comprises a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 18 **(2E6),** a CDR_{L2} comprising the amino acid sequence

of SEQ ID NO: 19 **(2E6),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6).**

3. The antibody of claim 1, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 15 **(2E6),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 94 **(Hu2E6_Hvl T57A),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 17 **(2E6);** and

the immunoglobulin light chain variable region comprises a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 100 **(Hu2E6_Kvl),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6).**

4. The antibody of claim 1, wherein the immunoglobulin heavy chain variable region comprises a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 15 **(2E6),** a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 94 **(Hu2E6_Hvl T57A),** and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 17 **(2E6);** and

the immunoglobulin light chain variable region comprises a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 99 **(Hu2E6_Kvl, Hu2E6_Kv2),** a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 101 **(Hu2E6_Kv2),** and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 20 **(2E6).**

5. The antibody of any one of claims 1-4, wherein the CDR sequences are interposed between human and humanized framework sequences.

6. The antibody of any one of claims 1-4, wherein the antibody is an antigen-binding fragment.

7. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain variable region of any one of claims 1-4.

8. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain variable region of any one of claims 1-4.

9. An expression vector comprising the nucleic acid of claim 7.

10. An expression vector comprising the nucleic acid of claim 8.

11. The expression vector of claim 10, further comprising the nucleic acid of claim 7.

12. A host cell comprising the expression vector of claim 9.

13. A host cell comprising the expression vector of claim 10.

14. A host cell comprising the expression vector of claim 11.

15. The host cell of claim 13, further comprising the expression vector of claim 9.

16. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:

(a) growing the host cell of claim 12 or 13 under conditions so that the host cell expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and

(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

17. A method of producing an antibody that binds human Notch1 or an antigen binding fragment of the antibody, the method comprising:

(a) growing the host cell of claim 14 or 15 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and

(b) purifying the antibody or the antigen-binding fragment of the antibody.

18. An isolated antibody that binds human Notch1, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region selected from the group consisting of:

(a) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ **ID** NO: 12 **(2E6),** and

an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ **ID** NO: 14 **(2E6);**

(b) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ **ID** NO: 2 **(2G10),** and

an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 **(2G10);**

(c) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 22 **(2A11),** and

an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 24 **(2A11);**

(d) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29 **(2D11),** and

an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 31 **(2D11);**

(e) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** and

an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 111 **(Hu2E6_Kvl);** and

(f) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** and

an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 113 **(Hu2E6_Kv2).**

19. The antibody of claim 18, wherein the immunoglobulin heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 12 **(2E6),** and the immunoglobulin light chain variable region comprises the amino acid sequence of SEQ ID NO: 14 **(2E6).**

20. The antibody of claim 18, wherein the immunoglobulin heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** and the immunoglobulin light chain variable region comprises the amino acid sequence of SEQ ID NO: 111 **(Hu2E6_Kvl).**

21. The antibody of claim 18, wherein the immunoglobulin heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 105 **(Hu2E6_Hvl T57A),** and the immunoglobulin light chain variable region comprises the amino acid sequence of SEQ ID NO: 113 **(Hu2E6_Kv2).**

22. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain variable region of any one of claims 18-21.

23. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain variable region of any one of claims 18-21.

24. An expression vector comprising the nucleic acid of claim 22.

25. An expression vector comprising the nucleic acid of claim 23.

26. The expression vector of claim 25, further comprising the nucleic acid of claim 22.

27. A host cell comprising the expression vector of claim 24.

28. A host cell comprising the expression vector of claim 25.

29. A host cell comprising the expression vector of claim 26.

30. The host cell of claim 28, further comprising the expression vector of claim 24.

31. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:

(a) growing the host cell of claim 27 or 28 under conditions so that the host cell expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and

(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

32. A method of producing an antibody that binds human Notch 1 or an antigen binding fragment of the antibody, the method comprising:

(a) growing the host cell of claim 29 or 30 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and

(b) purifying the antibody or the antigen-binding fragment of the antibody.

33. An isolated antibody that binds human Notch 1 comprising an immunoglobulin heavy chain and an immunoglobulin light chain selected from the group consisting of:

(a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 73 **(2E6),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 75 **(2E6);**

(b) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 69 **(2G10),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 71 **(2G10);**

(c) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 77 **(2A11),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 79 **(2A11);**

(d) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 81 **(2D11),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 83 **(2D11);**

(e) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 126 **(Hu2E6_Hvl T57A IgGl),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 132 **(Hu2E6_Kvl Kappa);** and

(f) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 126 **(Hu2E6_Hvl T57A IgGl),** and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 134 **(Hu2E6_Kv2 Kappa).**

34. The antibody of claim 33, wherein the immunoglobulin heavy chain comprises the amino acid sequence of SEQ ID NO: 73 **(2E6),** and the immunoglobulin light chain comprises the amino acid sequence of SEQ ID NO: 75 **(2E6).**

35. The antibody of claim 33, wherein the immunoglobulin heavy chain comprises the amino acid sequence of SEQ ID NO: 126 **(Hu2E6_Hvl T57A IgGl),** and the immunoglobulin light chain comprises the amino acid sequence of SEQ ID NO: 132 **(Hu2E6_Kvl Kappa).**

36. The antibody of claim 33, wherein the immunoglobulin heavy chain comprises the amino acid sequence of SEQ ID NO: 126 **(Hu2E6_Hvl T57A IgGl),** and the immunoglobulin light chain comprises the amino acid sequence of SEQ ID NO: 134 **(Hu2E6_Kv2 Kappa).**

37. The antibody of claim 18 or 33, wherein the antibody is an antigen-binding fragment.

38. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain of claim 33.

39. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain of claim 33.

40. An expression vector comprising the nucleic acid of claim 38.

41. An expression vector comprising the nucleic acid of claim 39.

42. The expression vector of claim 41, further comprising the nucleic acid of claim 38.

43. A host cell comprising the expression vector of claim 40.

44. A host cell comprising the expression vector of claim 41.

45. A host cell comprising the expression vector of claim 42.

46. The host cell of claim 44, further comprising the expression vector of claim 40.

47. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:

(a) growing the host cell of claim 43 or 44 under conditions so that the host cell expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and

(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

48. A method of producing an antibody that binds human Notch 1 or an antigen binding fragment of the antibody, the method comprising:

(a) growing the host cell of claim 45 or 46 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and the immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and

(b) purifying the antibody or the antigen-binding fragment of the antibody.

49. The antibody of any one of claims 1-6, 18-21, or 33-37, wherein the antibody binds human Notch1 with a K_D of 12 nM or lower as measured by surface plasmon resonance.

50. A method of inhibiting or reducing proliferation of a tumor cell comprising exposing the cell to an effective amount of the antibody of any one of claims 1-6, 18-21, 33-37 or 49 to inhibit or reduce proliferation of the tumor cell.

51. A method of inhibiting or reducing tumor growth in a mammal, the method comprising exposing the mammal to an effective amount of the antibody of any one of claims 1-6, 18-21, 33-37 or 49 to inhibit or reduce proliferation of the tumor.

52. A method of treating cancer in a human patient, the method comprising administering an effective amount of the antibody of any one of claims 1-6, 18-21, 33-37 or 49 to a mammal in need thereof.

53. The method of claim 52, wherein the cancer is selected from the group consisting of breast cancer, ovarian cancer, prostate cancer, cervical cancer, lung cancer, brain cancers, melanomas, gastrointestinal cancers, head and neck cancer, and hematopoietic cell cancers.

54. The antibody of any one of claims 1-6, 18-21, 33-37 or 49 for use in therapy.

55. The antibody of any one of claims 1-6, 18-21, 33-37 or 49 for use in inhibiting or reducing proliferation of a tumor cell.

56. The antibody of any one of claims 1-6, 18-21, 33-37 or 49 for use in inhibiting or reducing tumor growth in a mammal.

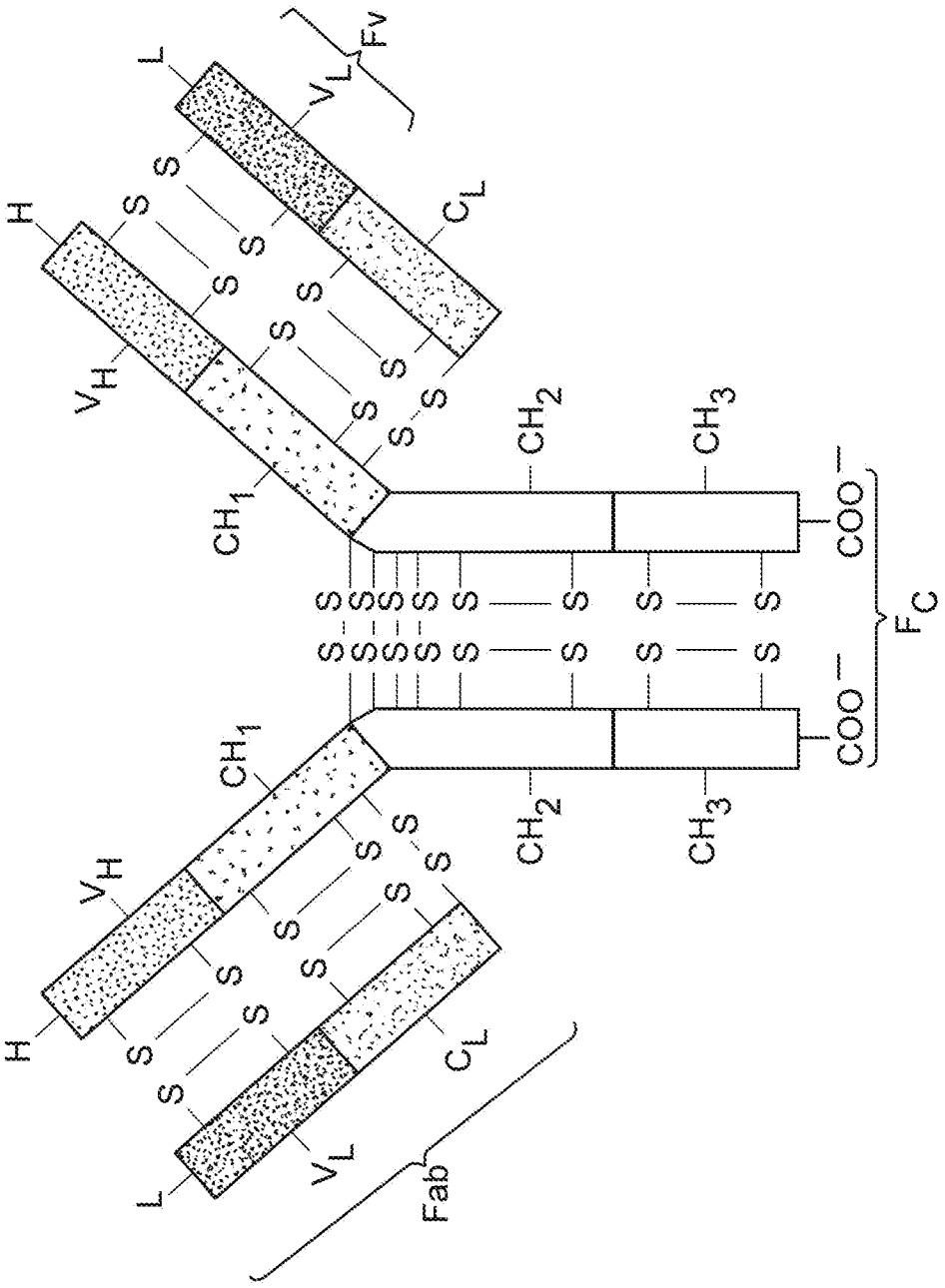


FIG. 1

Complete Heavy Chain Variable Region Amino Acid Alignments

```
Antibody                                          CDR1                    CDR2
2G10     (1) DVQLVESGGVLVQPGGSRKLSCTASGFTFSSFGMHWVRQAPEKGLEWVAYISSGSKTIYYADTMKGRFTI
2E6      (1) EVQLQQSGAELARPGASVKMSCKASGYTFTSYWMHWVKQRPGQGLEWIGAVYPRNNDTTYNQKFKGKAKL
2A11     (1) QVQLQQSGPELVKPGASVKISCKASGYAFSSSWMNWVKQRPGKGLEWIGRIYPGDGDTNYNGKFKGKATL
2D11     (1) EVQLQQSGAELARPGASVKMSCKASGYTFTRYWMHWVKQRPGQGLEWIGAIYPGNSDTTYNQKFKGKAKL

                                                  CDR3
2G10    (71) SRDNPKNTLFLQMTSLRSEDTAIYYCARSYG--------YFDVWGAGTTVTVSS  (SEQ ID NO: 2)
2E6     (71) TAVTSASTAYMALSSLTNEDSAVYYCLYFN--------YNFDYWGQGTTLTVSS  (SEQ ID NO: 12)
2A11    (71) TADKSSSTAYMQLSSLTSEDSAVYFCARSGSIYYGNHGDYFDYWGQGTTLTVSS  (SEQ ID NO: 22)
2D11    (71) TAVTSASTAYMELSSLTNEDSAVYYCIYPY--------DYLDYWGQGTTLTVSS  (SEQ ID NO: 29)
```

Fig.2

Heavy Chain CDR Amino Acid Alignments

Antibody	CDR1	CDR2	CDR3
2G10	SFGMH (SEQ ID NO: 5)	YISSGSKTIYYADTMKG (SEQ ID NO: 6)	SYG--------YFDV (SEQ ID NO: 7)
2E6	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFKG (SEQ ID NO: 16)	FN--------YNFDY (SEQ ID NO: 17)
2A11	SSWMN (SEQ ID NO: 25)	RIYPGDGDTNYNGKFKG (SEQ ID NO: 26)	SGSIYYGNHGDYFDY (SEQ ID NO: 27)
2D11	RYWMH (SEQ ID NO: 32)	AIYPGNSDTTYNQKFKG (SEQ ID NO: 33)	PY--------DYLDY (SEQ ID NO: 34)

Fig.3

Complete Light (Kappa) Chain Variable Region Amino Acid Alignments

Antibody		CDR1	CDR2
2G10	(1)	DIVMTQSQKFMSTSVGDRVSVTC**KASQNVGTNVA**WYQQKPGQSPKVLIY**SASYRYS**GVPDRFTGSGSGTD	
2E6	(1)	QIVLTQSPAIMSASPGEKVTMTC**SASS-SVSYMH**WYQQKPGSSPRLLIY**DTSNLAS**GVPVHFSGSGSGTS	
2A11	(1)	QIVLTQSPAIMSASPGEKVTMTC**SASS-SVSYMH**WYQQKPGSSPRLLIY**DTSNLAS**GVPVHFSGSGSGTS	
2D11	(1)	QIVLTQSPAIMSASPGEKVTMTC**SASS-SLSYMH**WYQQKPGTSPKRWVY**DTSKLAS**GVPARFSGSGSGTS	

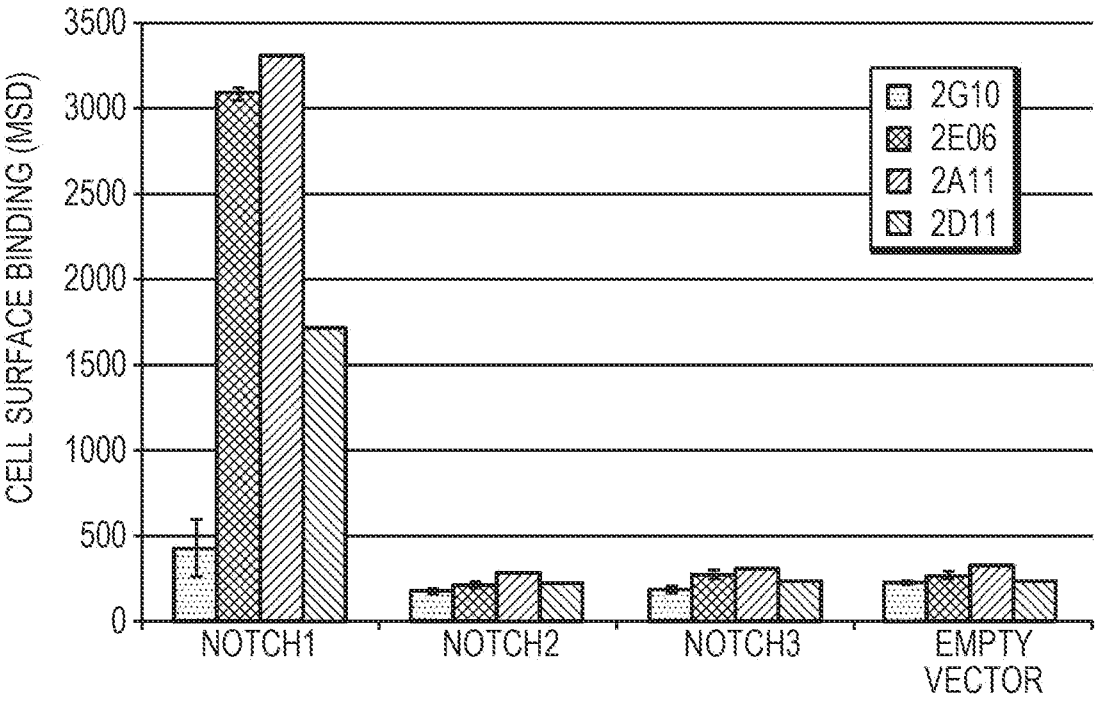
		CDR3	
2G10	(71)	FTLTIANVQSEDLAEYFC**QQYDSYPRT**FGGVTKLEIK	(SEQ ID NO: 4)
2E6	(70)	YSLTIIRMEAEDAATYYC**QQWSSYPYT**FGGGTKLEIK	(SEQ ID NO: 14)
2A11	(70)	YSLTIIRMEAEDAATYYC**QQWSSYPYT**FGGGTKLEIK	(SEQ ID NO: 24)
2D11	(70)	YSLTISSMEAEDAATYYC**HQRSSYPYT**FGGGTKLEIK	(SEQ ID NO: 31)

Fig.4

Light (Kappa) Chain CDR Amino Acid Alignments

Antibody	CDR1	CDR2	CDR3
2G10	KASQNVGTNVA (SEQ ID NO: 8)	SASYRYS (SEQ ID NO: 9)	QQYDSYPRT (SEQ ID NO: 10)
2E6	SASS-SVSYMH (SEQ ID NO: 18)	DTSNLAS (SEQ ID NO: 19)	QQWSSYPYT (SEQ ID NO: 20)
2A11	SASS-SVSYMH (SEQ ID NO: 18)	DTSNLAS (SEQ ID NO: 19)	QQWSSYPYT (SEQ ID NO: 20)
2D11	SASS-SLSYMH (SEQ ID NO: 35)	DTSKLAS (SEQ ID NO: 36)	HQRSSYPYT (SEQ ID NO: 37)

Fig.5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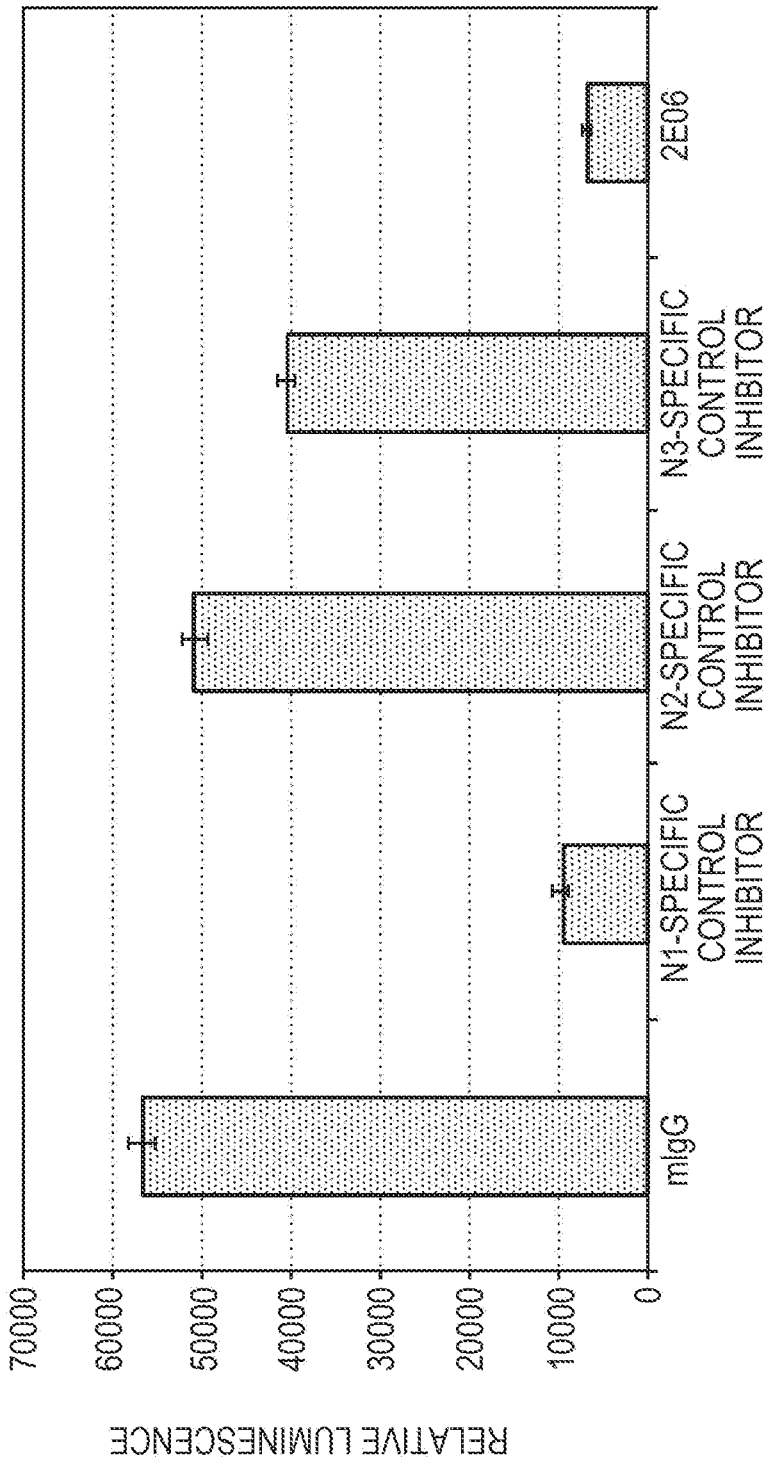
3500
3000
2500
2000
1500
1000
500
0
2G10
2E06
2A11
2D11
NOTCH1
NOTCH2
NOTCH3
EMPTY VECTOR

FIG. 6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10000
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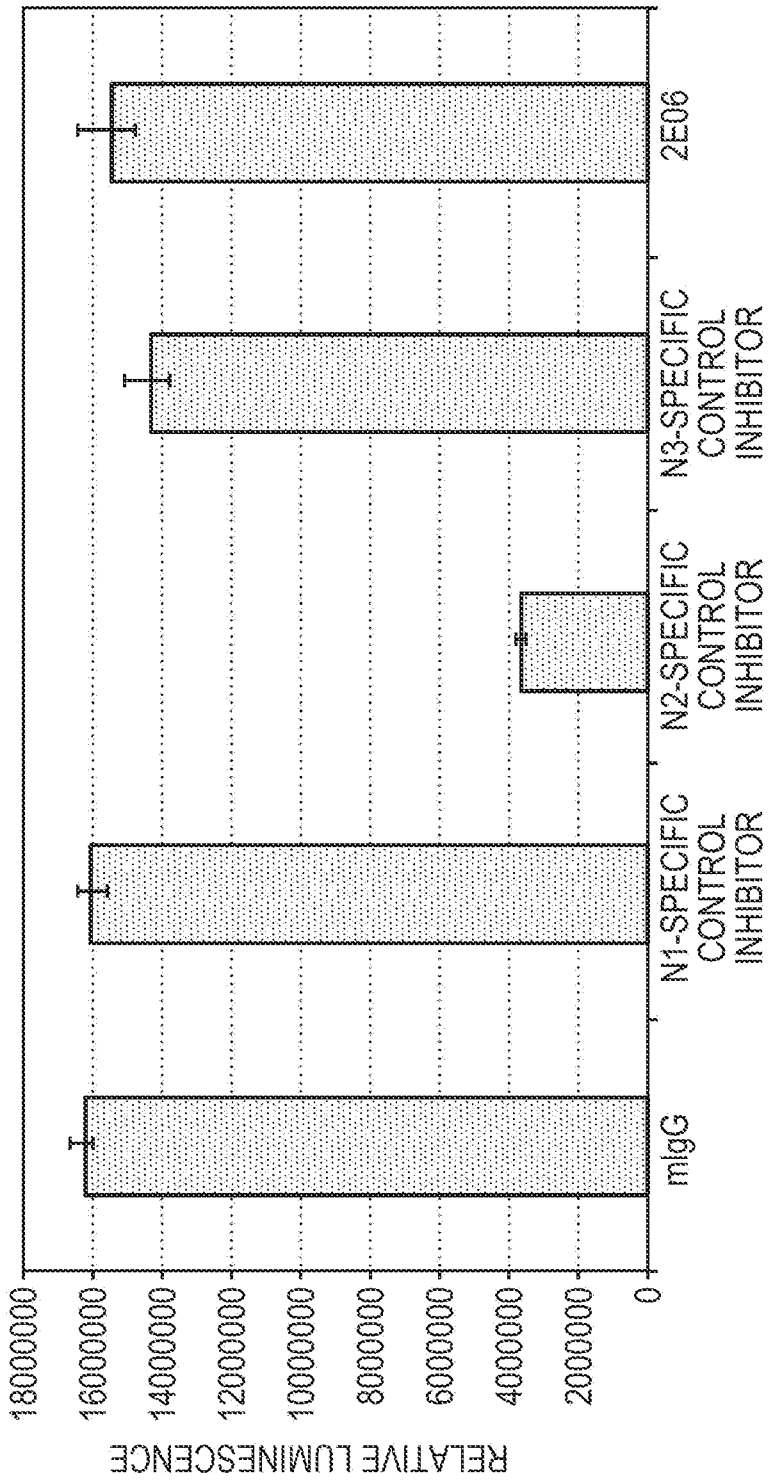
N1-SPECIFIC CONTROL INHIBITOR
N2-SPECIFIC CONTROL INHIBITOR
N3-SPECIFIC CONTROL INHIBITOR
2E06

FIG. 7A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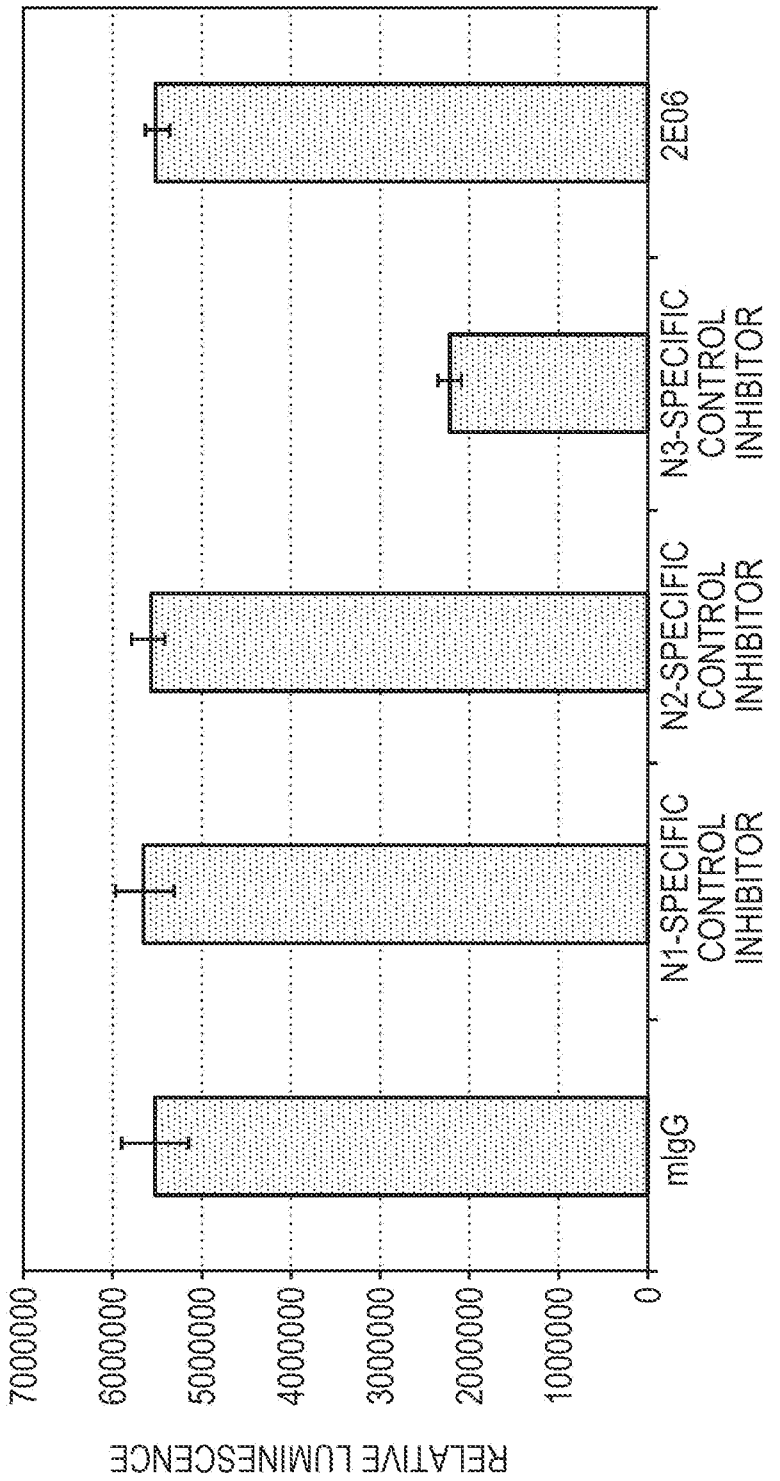
N1-SPECIFIC CONTROL INHIBITOR
N2-SPECIFIC CONTROL INHIBITOR
N3-SPECIFIC CONTROL INHIBITOR
2E06

FIG. 7B


RELATIVE LUMINESCENCE
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0
mIgG
N1-SPECIFIC CONTROL INHIBITOR
N2-SPECIFIC CONTROL INHIBITOR
N3-SPECIFIC CONTROL INHIBITOR
2E06

FIG. 7C

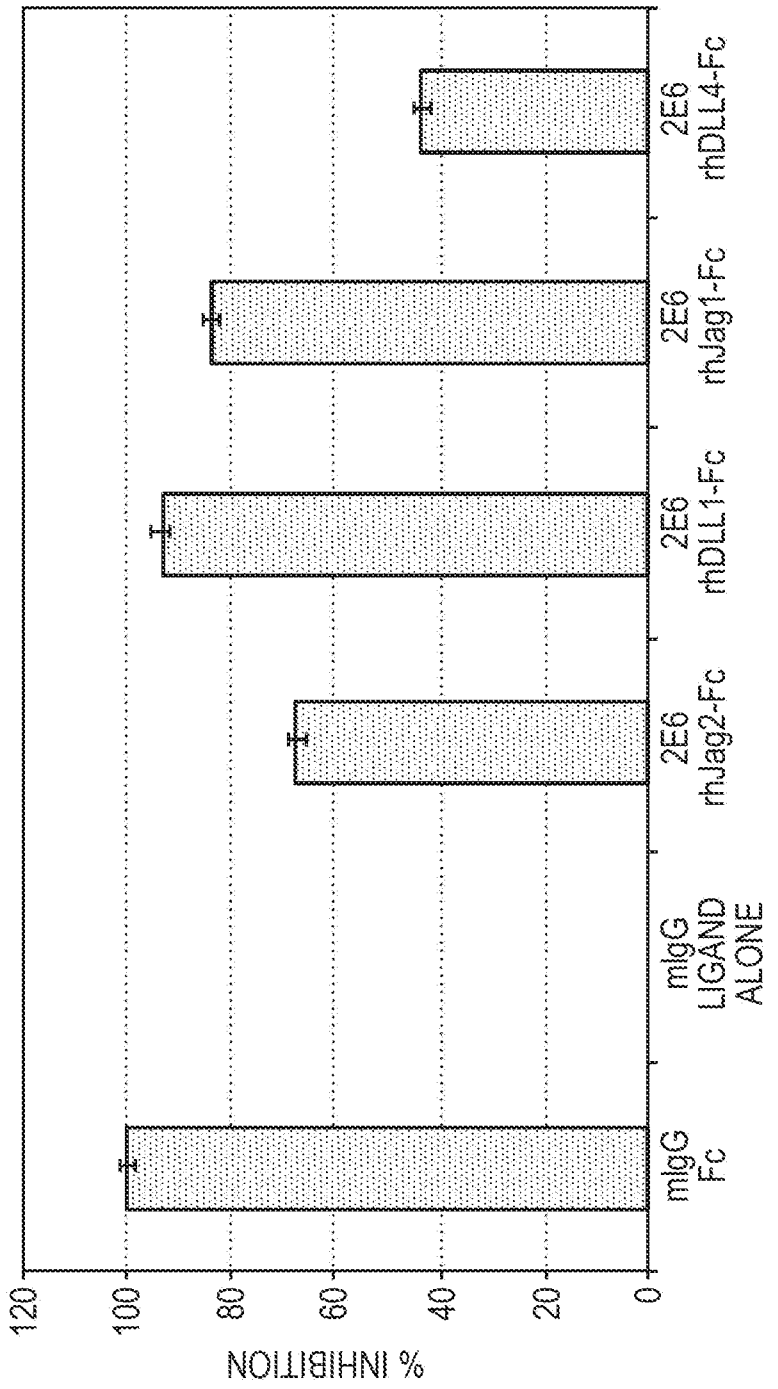

% INHIBITION
120
100
80
60
40
20
0
mIgG Fc
mIgG LIGAND ALONE
2E6 rhJag2-Fc
2E6 rhDLL1-Fc
2E6 rhJag1-Fc
2E6 rhDLL4-Fc

FIG. 8A

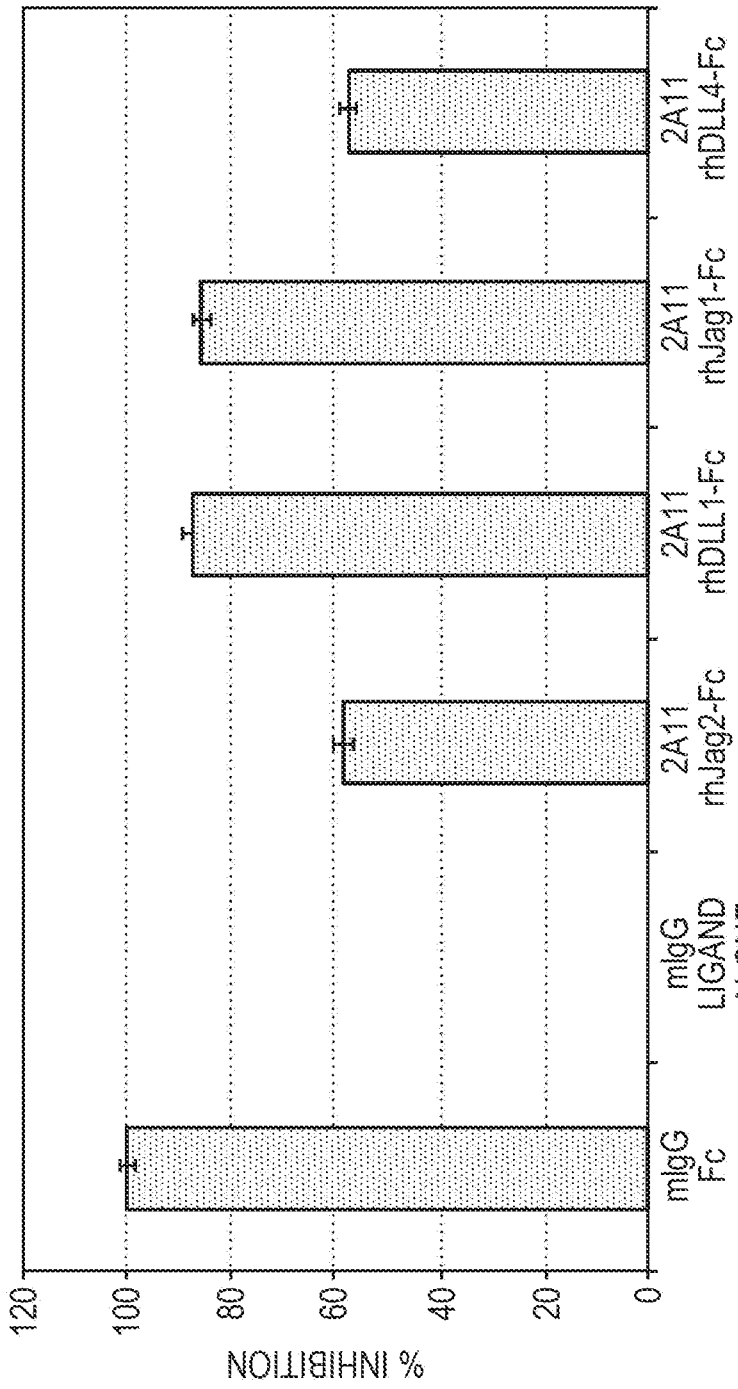

% INHIBITION
120
100
80
60
40
20
0
mIgG Fc
mIgG LIGAND ALONE
2A11 rhJag2-Fc
2A11 rhDLL1-Fc
2A11 rhJag1-Fc
2A11 rhDLL4-Fc

FIG. 8B

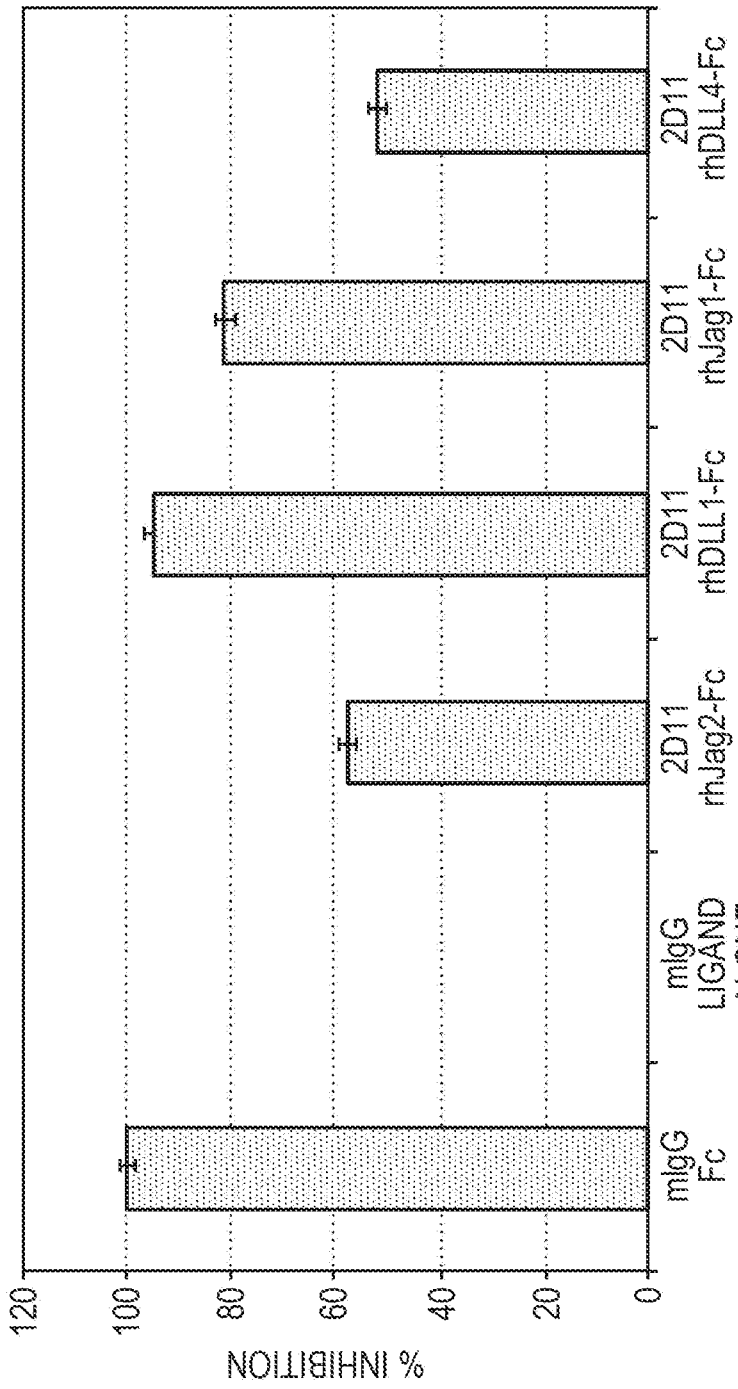

% INHIBITION
120
100
80
60
40
20
0
mIgG Fc
mIgG LIGAND ALONE
2D11 rhJag2-Fc
2D11 rhDLL1-Fc
2D11 rhJag1-Fc
2D11 rhDLL4-Fc

FIG. 8C

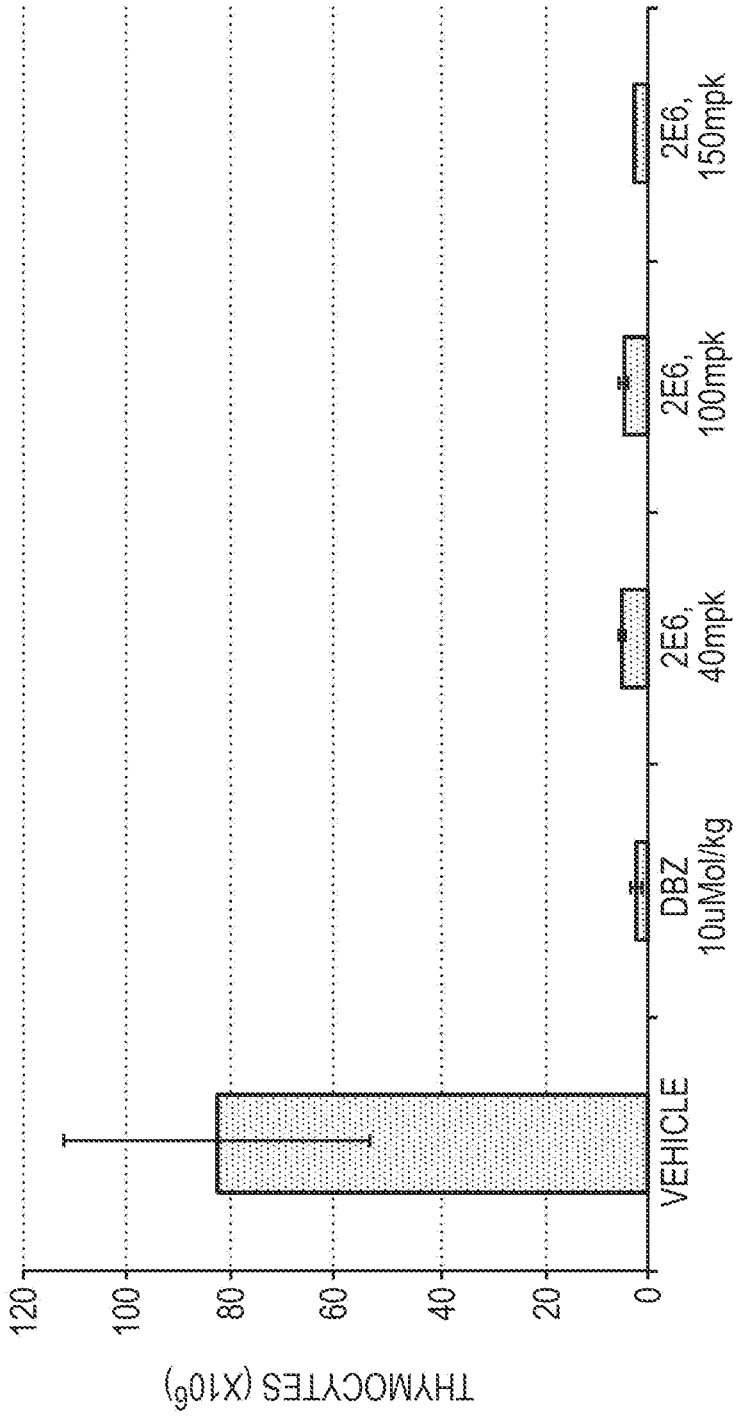

THYMOCYTES (X10⁶)
120
100
80
60
40
20
0
VEHICLE
DBZ 10uMol/kg
2E6, 40mpk
2E6, 100mpk
2E6, 150mpk

FIG. 9A

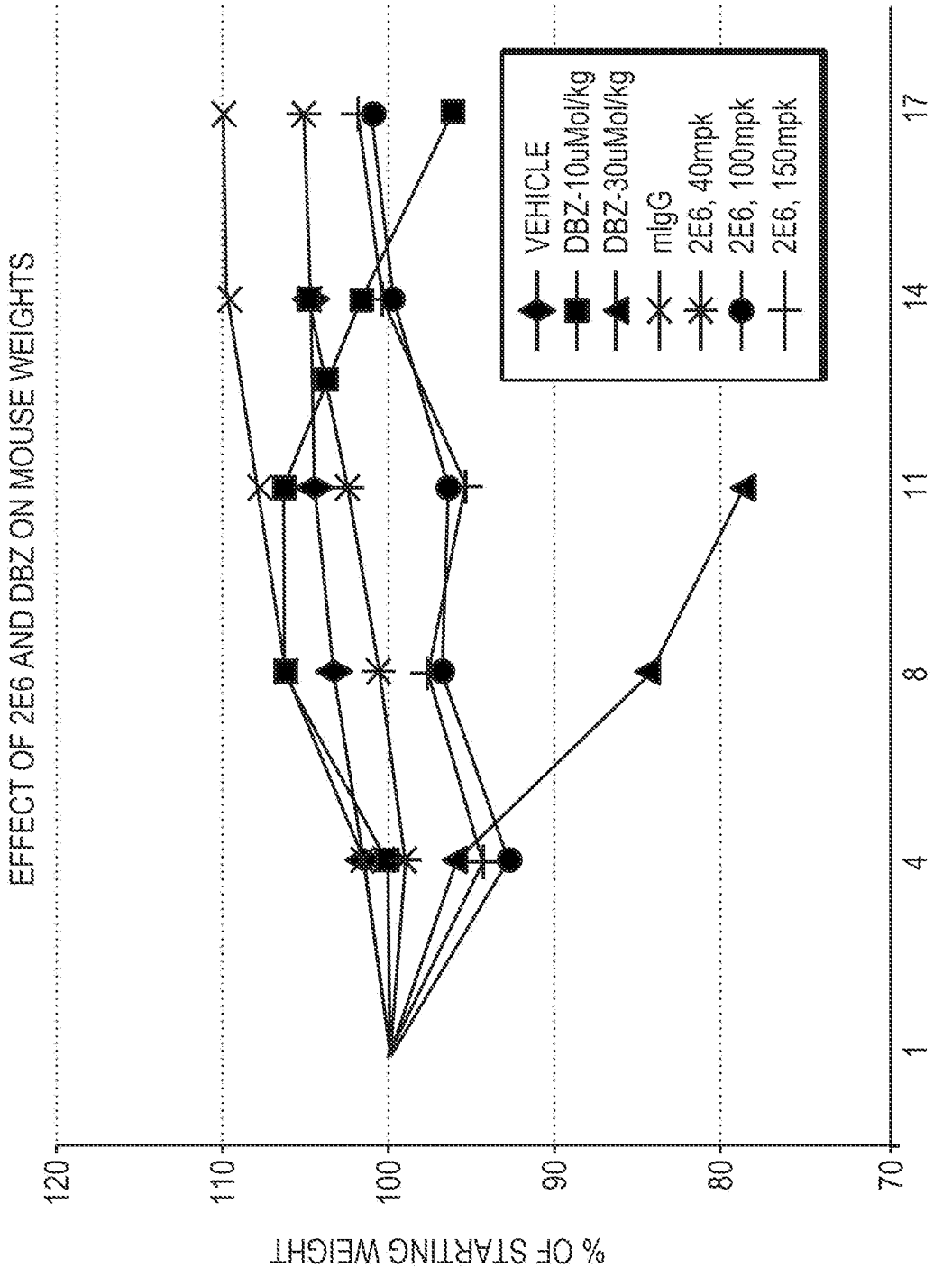

EFFECT OF 2E6 AND DBZ ON MOUSE WEIGHTS
% OF STARTING WEIGHT
120
110
100
90
80
70
1
4
8
11
14
17
DAYS OF TREATMENT
VEHICLE
DBZ-10uMol/kg
DBZ-30uMol/kg
mIgG
2E6, 40mpk
2E6, 100mpk
2E6, 150mpk

FIG. 9B

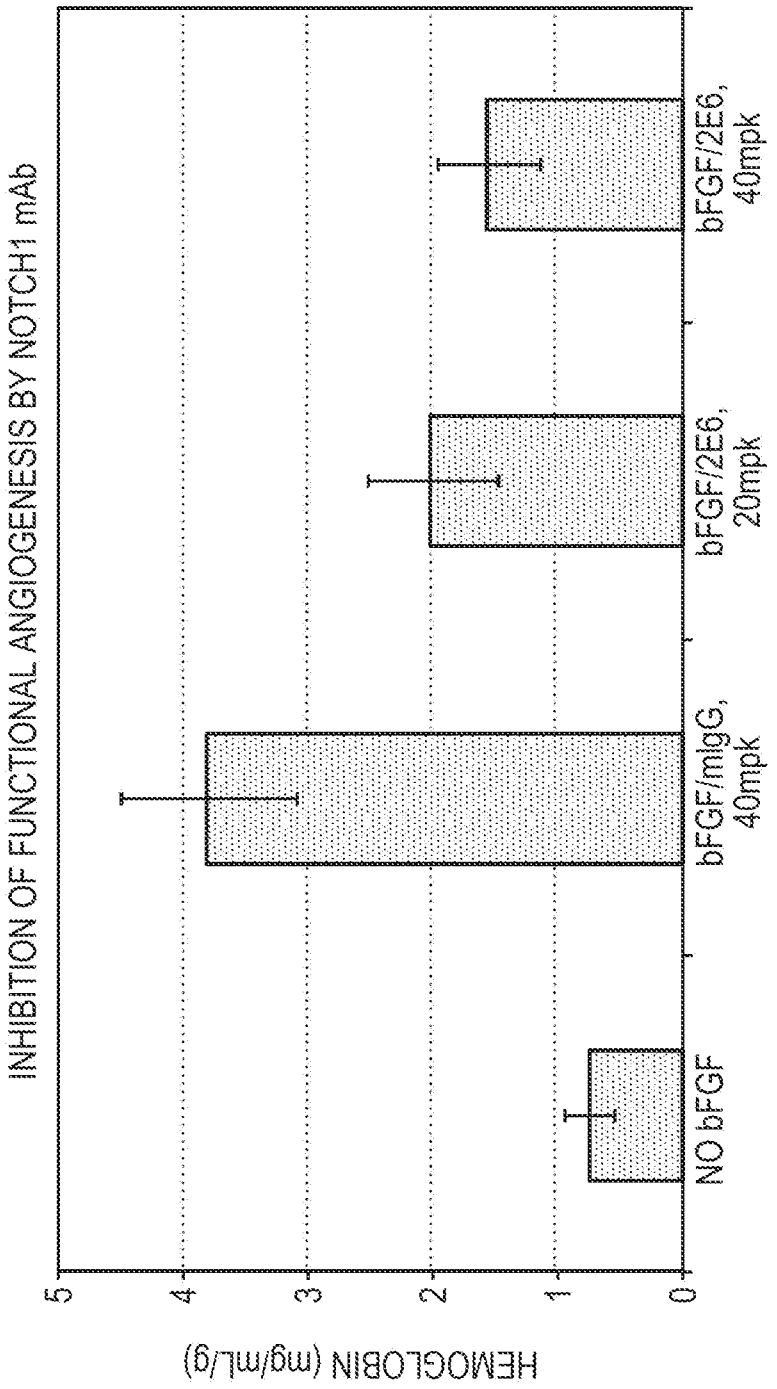

INHIBITION OF FUNCTIONAL ANGIOGENESIS BY NOTCH1 mAb
HEMOGLOBIN (mg/mL/g)
0
1
2
3
4
5
NO bFGF
bFGF/mIgG, 40mpk
bFGF/2E6, 20mpk
bFGF/2E6, 40mpk
N1h112/h112

FIG. 10

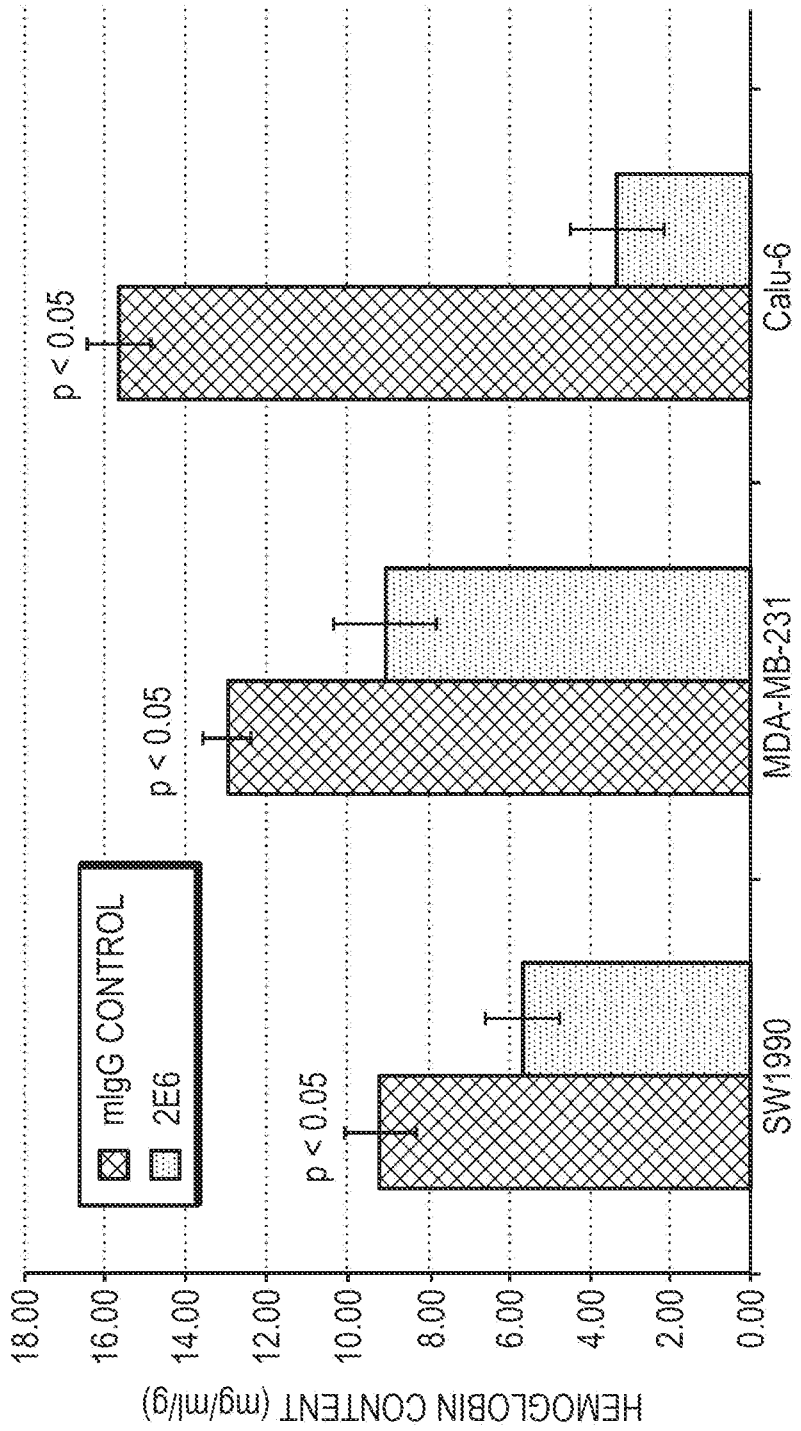

HEMOGLOBIN CONTENT (mg/ml/g)
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
mIgG CONTROL
2E6
p < 0.05
p < 0.05
p < 0.05
SW1990
MDA-MB-231
Calu-6

FIG. 11

Complete Heavy Chain Variable Region Amino Acid Alignments

Antibody		CDR1	CDR2	
2E6	EVQLQQSGAELARPGASVKMSCKASGYTFT	SYWMH	WVKQRPGQGLEWIG	AVYPRNNDTTYNQKFKG KAKL
Hu2E6_Hv1	EVQLVQSGAEVAKPGASVKMSCKASGYTFT	SYWMH	WVKQAPGQGLEWIG	AVYPRNNDTTYNQKFKG KATL
Hu2E6_Hv1 T57A	EVQLVQSGAEVAKPGASVKMSCKASGYTFT	SYWMH	WVKQAPGQGLEWIG	AVYPRNNDATYNQKFKG KATL
Hu2E6_Hv2	QVQLVQSGAEVKKPGASVKMSCKASGYTFT	SYWMH	WVRQAPGQGLEWIG	AVYPRNNDTTYNQKFQG RATL
Hu2E6_Hv2 T57A	QVQLVQSGAEVKKPGASVKMSCKASGYTFT	SYWMH	WVRQAPGQGLEWIG	AVYPRNNDATYNQKFQG RATL

	CDR3		
2E6	TAVTSASTAYMALSSLTNEDSAVYYCLY	FNYNFDY	WGQGTTLTVSS (SEQ ID NO: 12)
Hu2E6_Hv1	TADTSTSTAYMELRSLRSDDTAVYYCLY	FNYNFDY	WGQGTLLTVSS (SEQ ID NO: 103)
Hu2E6_Hv1 T57A	TADTSTSTAYMELRSLRSDDTAVYYCLY	FNYNFDY	WGQGTLLTVSS (SEQ ID NO: 105)
Hu2E6_Hv2	TADTSTSTAYMELRSLRSDDTAVYYCLY	FNYNFDY	WGQGTLLTVSS (SEQ ID NO: 107)
Hu2E6_Hv2 T57A	TADTSTSTAYMELRSLRSDDTAVYYCLY	FNYNFDY	WGQGTLLTVSS (SEQ ID NO: 109)

Fig. 12

Heavy Chain CDR Amino Acid Alignments

Antibody	CDR1	CDR2	CDR3
2E6	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFKG (SEQ ID NO: 16)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv1	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFKG (SEQ ID NO: 16)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv1 T57A	SYWMH (SEQ ID NO: 15)	AVYPRNNDATYNQKFKG (SEQ ID NO: 94)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv2	SYWMH (SEQ ID NO: 15)	AVYPRNNDTTYNQKFQG (SEQ ID NO: 95)	FNYNFDY (SEQ ID NO: 17)
Hu2E6_Hv2 T57A	SYWMH (SEQ ID NO: 15)	AVYPRNNDATYNQKFQG (SEQ ID NO: 96)	FNYNFDY (SEQ ID NO: 17)

Fig. 13

Complete Light (Kappa) Chain Variable Region Amino Acid Alignments

Antibody	CDR1	CDR2
2E6	QIVLTQSPAIMSASPGEKVTMTC**SASSSVSYMH**WYQQKPGSSPRLLIY**DTSNLAS**GVPVHFSGSGSGTSY	
Hu2E6_Kv1	EIVLTQSPATMSASPGERVTMSC**RASSSVSYMH**WYQQKPGQSPRLLIY**DTSNRAS**GVPAHFSGSGSGTDY	
Hu2E6_Kv2	EIVLTQSPATLSASPGERVTMSC**RASSSVSYMH**WYQQKPGQAPRLLIY**DTSNRAT**GVPARFSGSGSGTDY	

	CDR3	
2E6	SLTIIRMEAEDAATYYC**QQWSSYPYT**FGGGTKLEIK	(SEQ ID NO: 14)
Hu2E6_Kv1	TLTISSMEPEDFATYYC**QQWSSYPYT**FGQGTKLEIK	(SEQ ID NO: 111)
Hu2E6_Kv2	TLTISSMEPEDFATYYC**QQWSSYPYT**FGQGTKLEIK	(SEQ ID NO: 113)

Fig. 14

Light (Kappa) Chain CDR Amino Acid Alignments

Antibody	CDR1	CDR2	CDR3
2E6	SASSSVSYMH (SEQ ID NO: 18)	DTSNLAS (SEQ ID NO: 19)	QQWSSYPYT (SEQ ID NO: 20)
Hu2E6_Kv1	RASSSVSYMH (SEQ ID NO: 99)	DTSNRAS (SEQ ID NO: 100)	QQWSSYPYT (SEQ ID NO: 20)
Hu2E6_Kv2	RASSSVSYMH (SEQ ID NO: 99)	DTSNRAT (SEQ ID NO: 101)	QQWSSYPYT (SEQ ID NO: 20)

Fig. 15

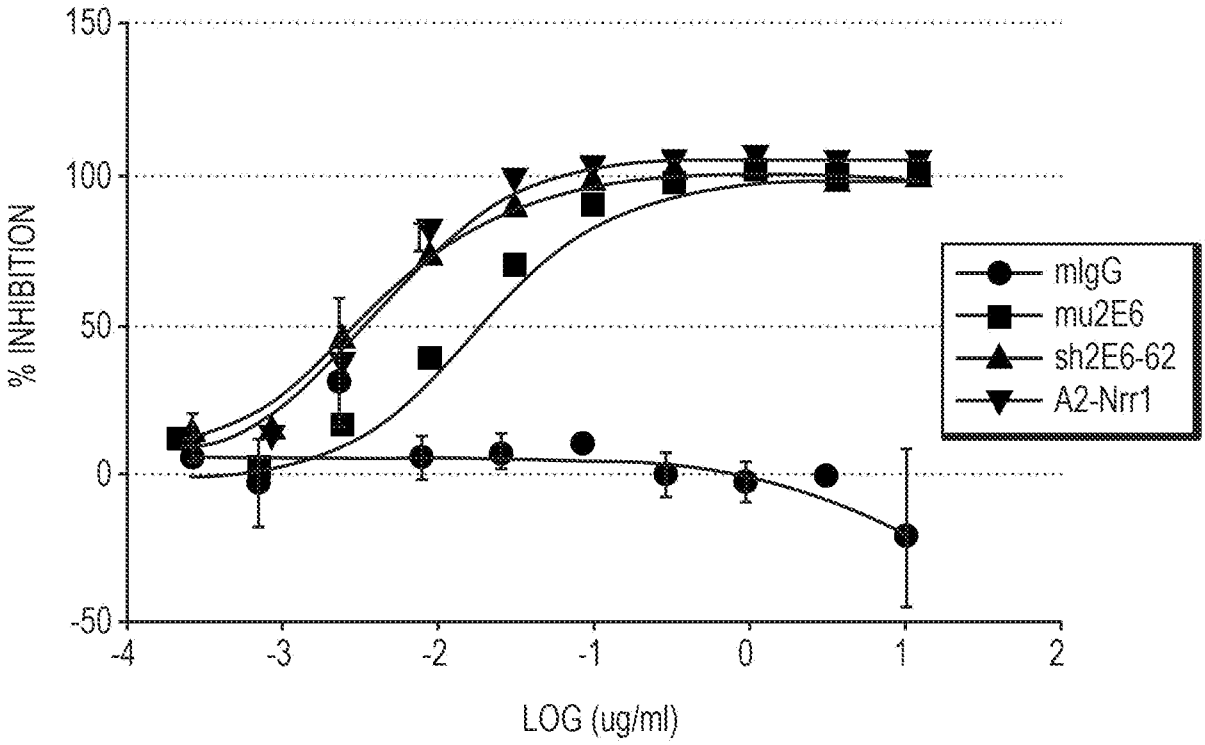

150
100
50
0
-50
% INHIBITION
-4
-3
-2
-1
0
1
2
LOG (ug/ml)
mIgG
mu2E6
sh2E6-62
A2-Nrr1

FIG. 16

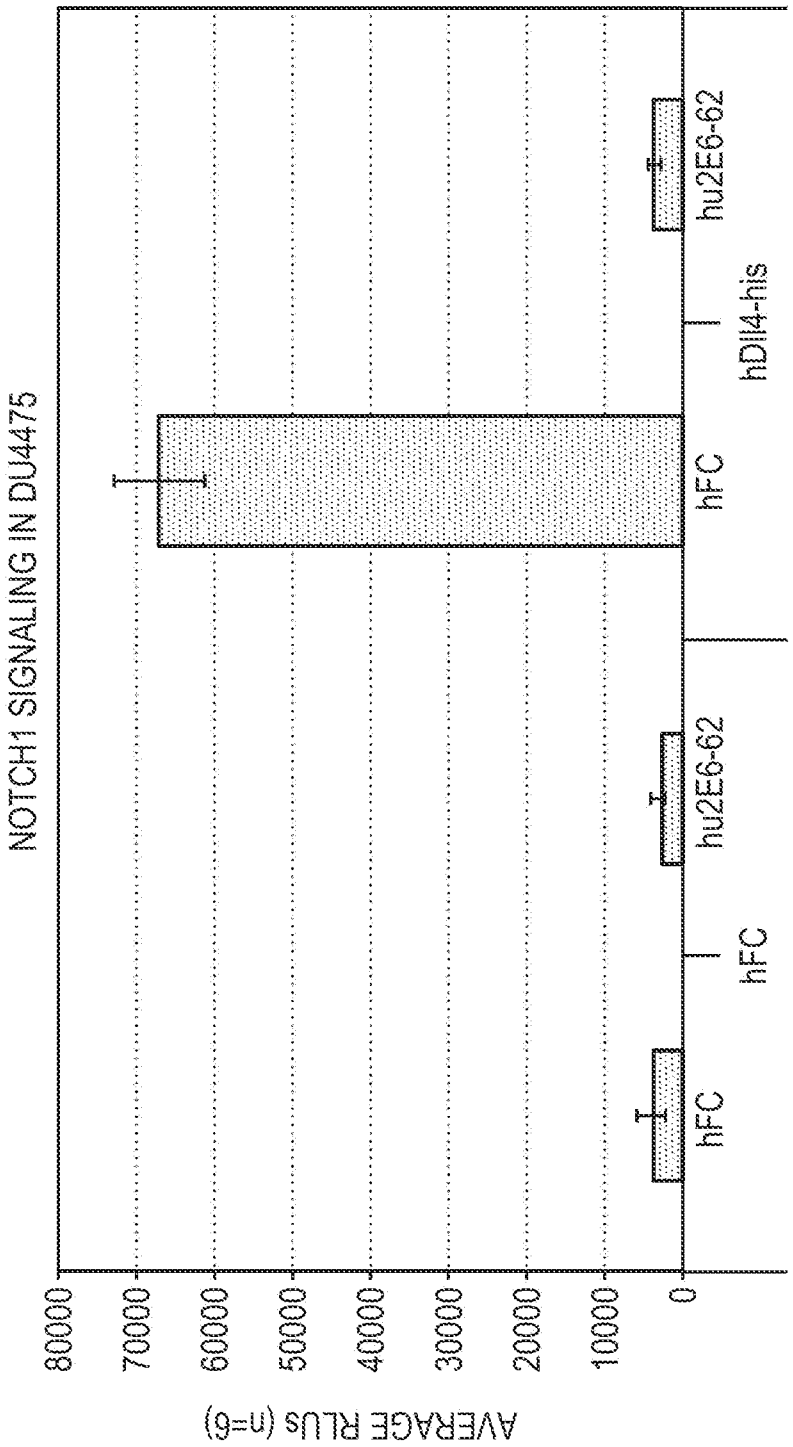

NOTCH1 SIGNALING IN DU4475
AVERAGE RLUs (n=6)
80000
70000
60000
50000
40000
30000
20000
10000
0
hFC
hFC
hu2E6-62
hFC
hDll4-his
hu2E6-62

FIG. 17A

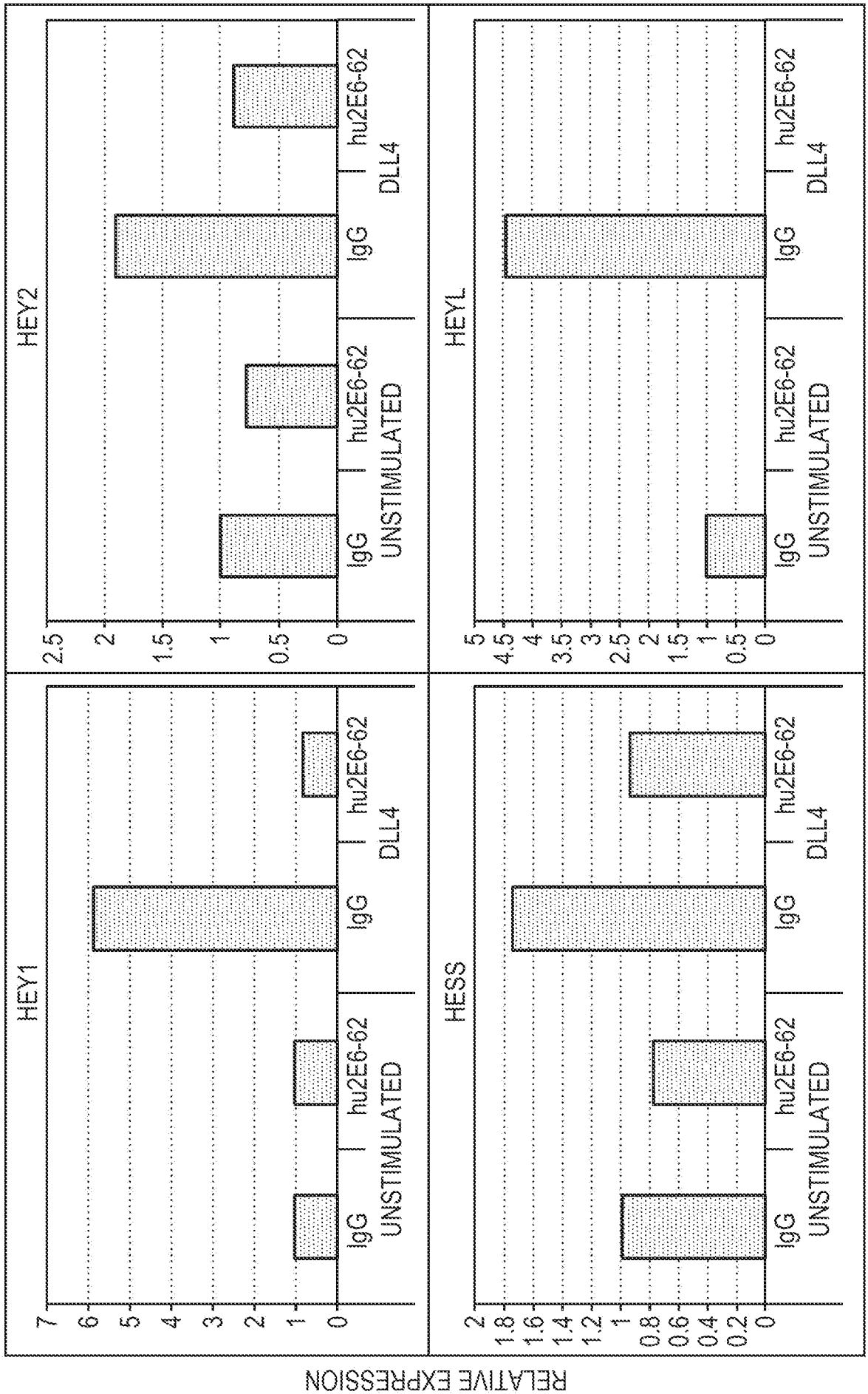

HEY2
HEY1L
HEY1
HESS
RELATIVE EXPRESSION
IgG
hu2E6-62
UNSTIMULATED
DLL4

FIG. 17B

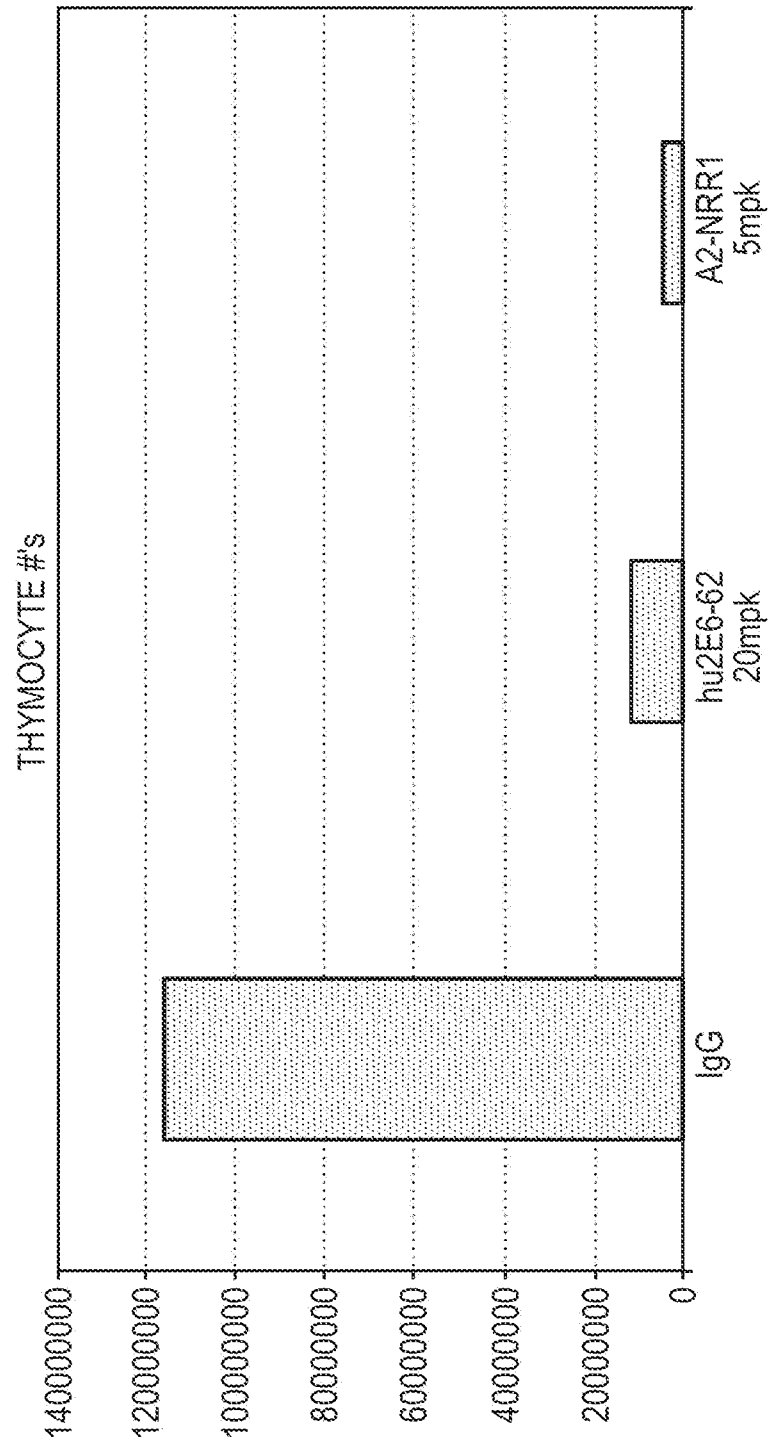

THYMOCYTE #'s
140000000
120000000
100000000
80000000
60000000
40000000
20000000
0
IgG
hu2E6-62
20mpk
A2-NRR1
5mpk

FIG. 18

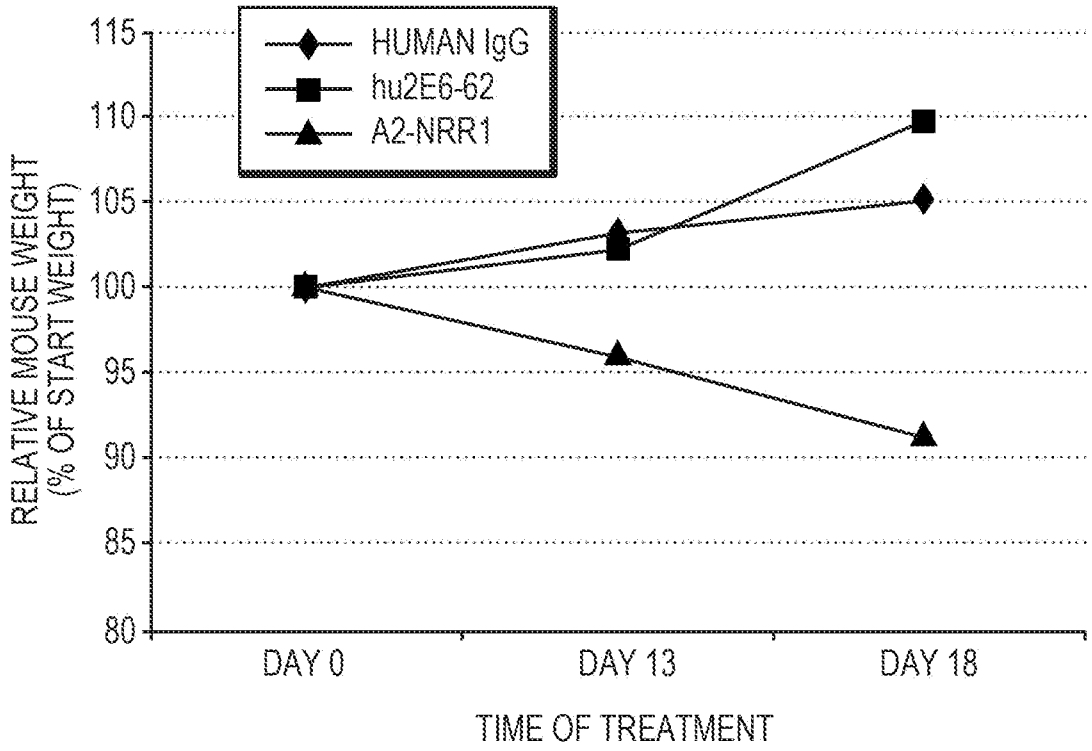
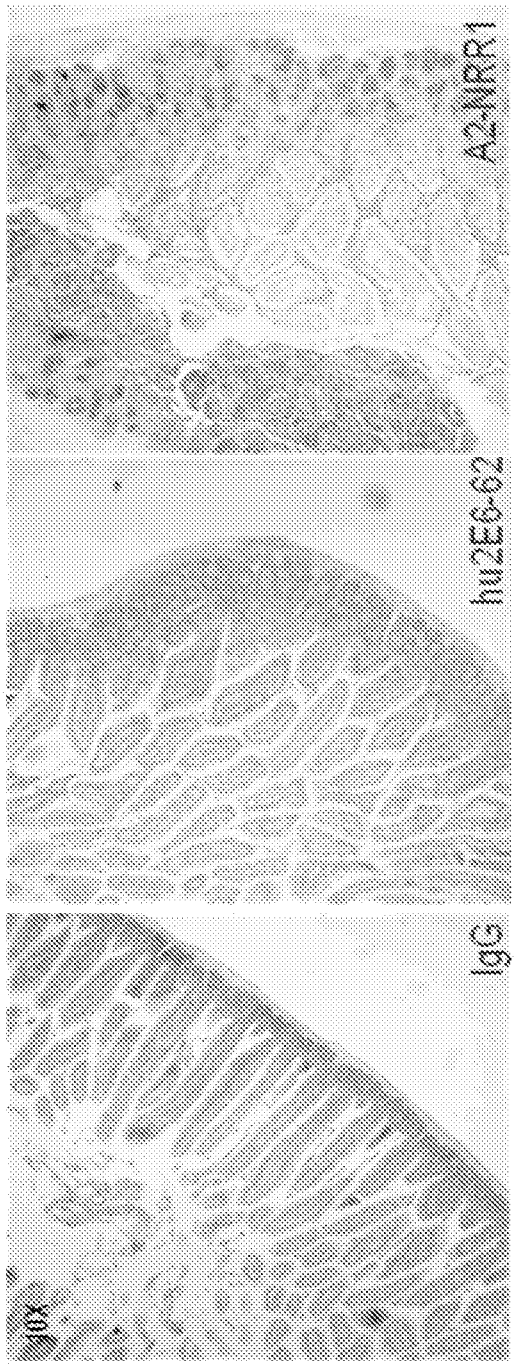

HUMAN IgG
hu2E6-62
A2-NRR1
RELATIVE MOUSE WEIGHT
(% OF START WEIGHT)
115
110
105
100
95
90
85
80
DAY 0
DAY 13
DAY 18
TIME OF TREATMENT

FIG. 19A

FIG. 19B



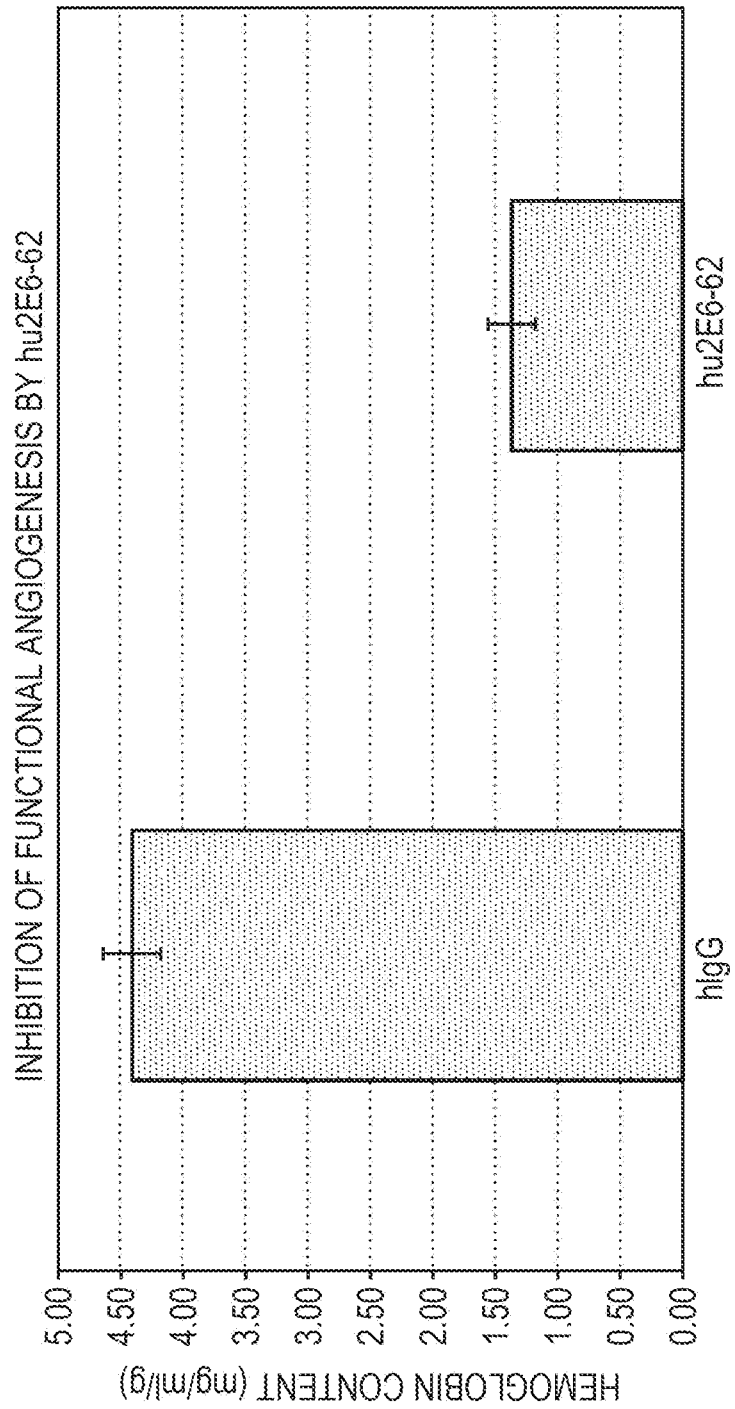

INHIBITION OF FUNCTIONAL ANGIOGENESIS BY hu2E6-62
HEMOGLOBIN CONTENT (mg/ml/g)
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
hIgG
hu2E6-62

FIG. 20

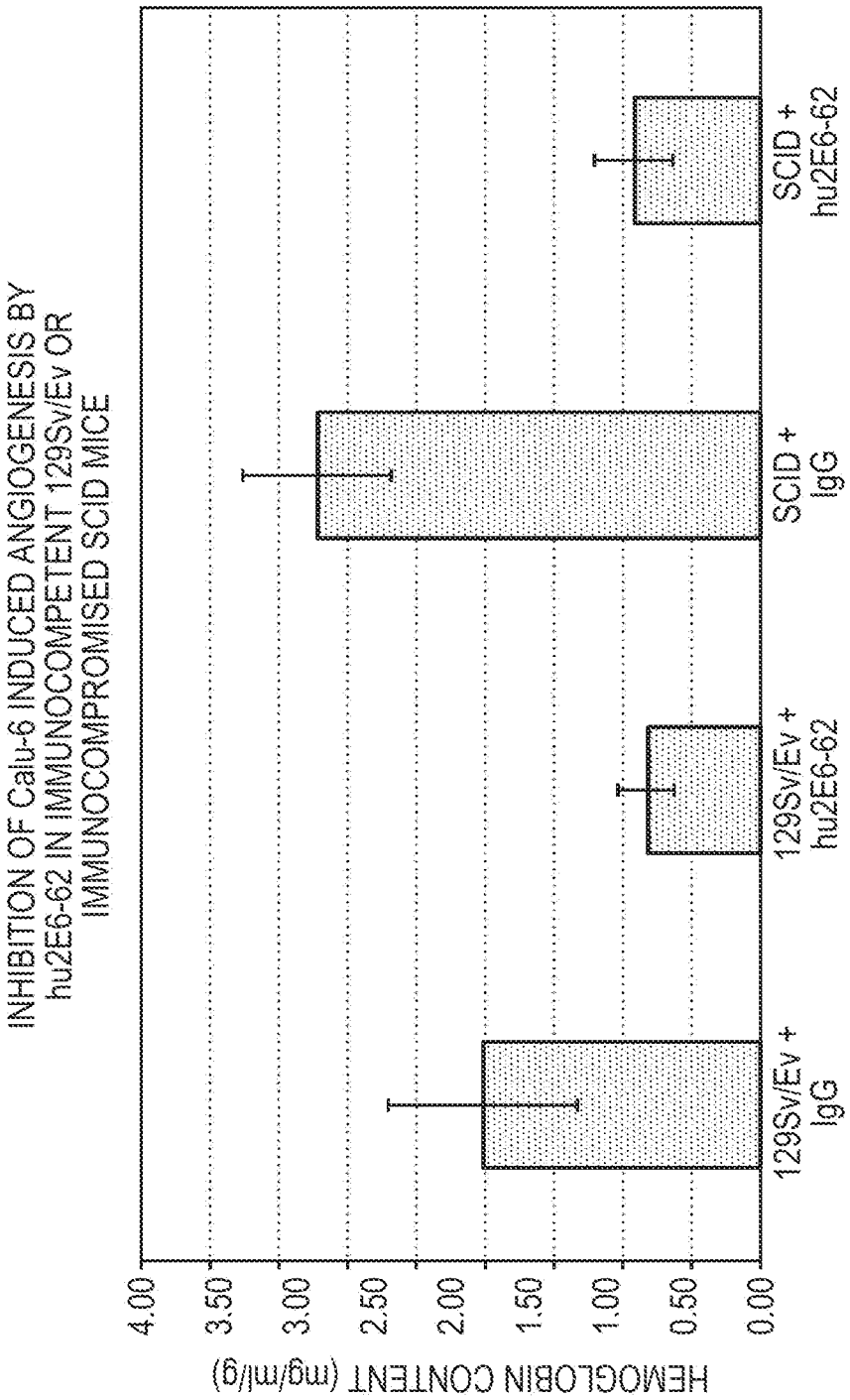

INHIBITION OF Calu-6 INDUCED ANGIOGENESIS BY
hu2E6-62 IN IMMUNOCOMPETENT 129Sv/Ev OR
IMMUNOCOMPROMISED SCID MICE
HEMOGLOBIN CONTENT (mg/ml/g)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
129Sv/Ev + IgG
129Sv/Ev + hu2E6-62
SCID + IgG
SCID + hu2E6-62

FIG. 21

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/042843

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K39/395 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal , BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 00/20576 A2 (US HEALTH [US] ; MI ELE LUCIO [US] ; SHI ELDS LESLI E S [US] ; FUCHS CHANA [) 13 Apri l 2000 (2000-04-13) page 4, l ine 31 - page 5, l ine 6; cl aims 19-21 , 25, 32-34	1-56

X	W0 2007/145840 A2 (0NC0MED PHARM INC [US] ; LEWICKI JOHN [US] ; GURNEY AUSTIN [US] ; H0EY TI) 21 December 2007 (2007-12-21) paragraph [0231] - paragraph [0232] ; cl aims 15-1723 ,36-38; f i gure 6b paragraph [0242] paragraph [0250]	1-56

	-/- ·	

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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"L" documentwhich may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu¬ments, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2011	21/10/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Si aterl i , Mari a

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/042843

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/091641 A2 (ONCOMED PHARM INC [US] ; GURNEY AUSTIN [US] ; SATO AARON [US] ; FITCH-BRU) 31 July 2008 (2008-07-31) paragraphs [0173] , [0181] , [0183] , [0183] - paragraph [0190] ; claims 1-17 paragraph [0196]	1-56
X	0KAMURA HEIDI ET AL: "Monoclonal antibodies to Notch receptors inhibit tumor maintenance" , PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol . 51, April 2010 (2010-04) , page 1254, XP002661144, & 101ST ANNUAL MEETING OF THE AMERICAN-ASSOCIATION-F0R-CANCER- RESEARCH ; WASHINGTON , DC, USA; APRIL 17 -21 , 2010 ISSN : 0197-016X the whole document	1-56

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/042843

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 6289499 A	26-04-2000
		CA 2343963 Al	13-04-2000
		EP 1117778 A2	25-07-2001
		JP 2002526109 A	20-08-2002
Wo 2007145840 A2	21-12-2007	CA 2655362 Al	21-12-2007
		EP 2032166 A2	11-03-2009
		JP 2009539403 A	19-11-2009
		US 2008131434 Al	05-06-2008
		US 2011195065 Al	11-08-2011
WO 2008091641 A2	31-07-2008	AU 2008209482 Al	31-07-2008
		CA 2676008 Al	31-07-2008
		EP 2125887 A2	02-12-2009
		JP 2010517513 A	27-05-2010
		US 2009047285 Al	19-02-2009