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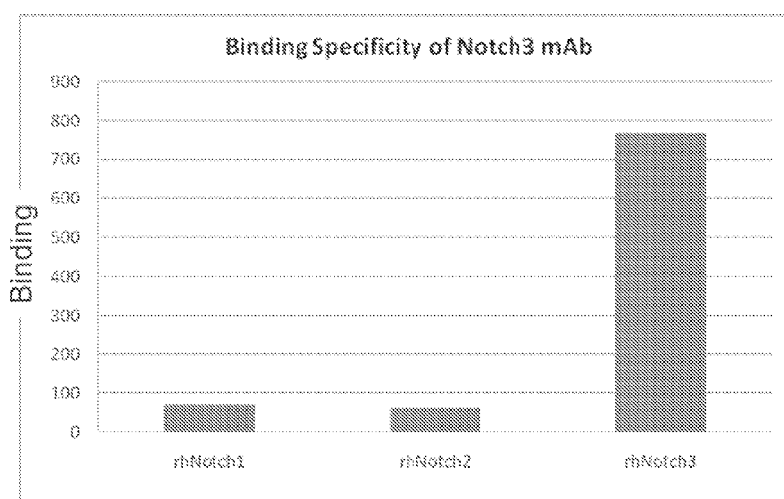
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[Continued on next page]

(54) Title: ANTI-NOTCH3 ANTIBODIES

FIG. 4



(57) Abstract: Monoclonal antibodies that bind and inhibit activation of human Notch3 are disclosed. The antibodies can be used to treat cell proliferative diseases and disorders, including certain forms of cancer, associated with activation of Notch3.

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ANTI-NOTCH3 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 61/866,787, filed August 16, 2013, and U.S. Provisional Patent Application No. 61/739,435, filed December 19, 2012, the entire disclosure of each of which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

5 [0002] The field of the invention is molecular biology, immunology and oncology. More particularly, the field is antibodies that bind human Notch3.

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
10 Notch1-Notch4. All four Notch proteins have a similar domain structure, which includes an extracellular domain, a negative regulatory region (NRR), 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
15 are held together by noncovalent interactions of 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 (*e.g.*, DLL1 and DLL4) and
20 Jagged (also referred to as Jag, *e.g.*, Jag1 and Jag2). When the NICD is released, it travels to the nucleus, where it activates transcription of the Notch-responsive genes, HES1, HES5, NRARP, Deltex1 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.

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[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
5 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-Amézaga *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
10 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
15 (CH₁, CH₂ and CH₃ 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 CDR₁, CDR₂, and CDR₃, contribute to the antibody binding specificity. Naturally occurring antibodies have been used
20 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 Notch3 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 antibodies that specifically bind human
25 Notch3. Antibodies disclosed herein contain Notch3 binding sites based on the CDRs of the anti-Notch3 antibodies described herein. The disclosed antibodies prevent or inhibit activation of human Notch3. They do so by inhibiting Notch3 from binding to Notch ligands, *i.e.*, Jag1, Jag2, DLL1, 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
30 antibodies inhibit or reduce tumor growth in the human patient.

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[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. As used herein, “antibody 4F11” means antibody 4F11, or humanized variants thereof.

DESCRIPTION OF THE DRAWINGS

5 [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.

10 [0012] **FIG. 2** provides the amino acid sequence corresponding to the extracellular domain (ECD; amino acids 40 to 1643) of human Notch3 (SEQ ID NO:95).

[0013] **FIG. 3** provides the amino acid sequence corresponding to EGF-like repeats 1-11 (amino acids 40 to 467 of the extracellular domain shown in **FIG. 2**) of human Notch3 (SEQ ID NO:96).

15 [0014] **FIG. 4** is a bar chart summarizing results of an experiment to determine specificity of antibody 4F11 binding to Fc-bound recombinant human Notch3 (rhNotch3) using Octet. The results demonstrate that the binding specificity of the 4F11 antibody for rhNotch3 is much higher than for rhNotch1 or rhNotch2.

20 [0015] **FIG. 5A** is a bar chart illustrating the ability of antibody 4F11 to neutralize binding of the ligand Jag1 to human Notch3 protein. The chart shows that the antibody 4F11 and a Notch3 Specific Control, but not human immunoglobulin (hIgG), were able to block binding of recombinant human Fc-bound Jag1 (Jag1-Fc) to rhNotch3, as detected by bio-layer interferometry (BLI).

25 [0016] **FIG. 5B** is a bar chart illustrating the ability of antibody 4F11 to neutralize binding of the ligand Jag2 to human Notch3 protein. The chart shows that the antibody 4F11 and a Notch3 Specific Control, but not hIgG, were able to block binding of recombinant human Fc-bound Jag2 (Jag2-Fc) to rhNotch3, as detected by BLI.

[0017] **FIG. 5C** is a bar chart illustrating the ability of antibody 4F11 to neutralize binding of the ligand DLL1 to human Notch3 protein. The chart shows that the antibody 4F11 and a

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Notch3 Specific Control, but not hIgG, block binding of recombinant human Fc-bound DLL1 (DLL1-Fc) to rhNotch3, to a greater extent than IgG, as detected by BLI.

[0018] FIG. 5D is a bar chart illustrating the ability of antibody 4F11 to neutralize binding of the ligand DLL4 to human Notch3 protein. The chart shows that the antibody 4F11 and a Notch3 Specific Control, but not hIgG, were able to block binding of recombinant human Fc-bound DLL4 (DLL4-Fc) to rhNotch3, as detected by BLI.

[0019] FIG. 6A is a dose-response curve summarizing results from a Notch3 reporter assay showing that antibody 4F11 inhibits Notch3-dependent reporter gene expression in the presence of the Fc-bound ligand Jag2 (Jag2-Fc). The graph shows the relationship between inhibition of Jag2-Fc-stimulated reporter activity (% Inhibition) by antibody 4F11 in transduced cells, in relation to the amount of Jag2-Fc ligand. Reporter activity of cells transduced with the RBP-J κ -dependent luciferase reporter, exposed to any activating ligand, and treated with mouse IgG (mIgG) was defined as 100% activity for each ligand. Reporter activity of transduced cells not exposed to ligand but treated with mouse IgG was defined as 0% activity.

[0020] FIG. 6B is a bar chart summarizing results of Notch3 reporter assays showing that antibody 4F11, but not mouse IgG (mIgG), inhibits Notch3-dependent reporter gene expression induced by the ligands hJag1, hJag2, hDLL1 and hDLL4. Reporter activity of cells transduced with the RBP-J κ -dependent luciferase reporter, exposed to any activating ligand, and treated with mouse IgG (mIgG) was defined as 100% activity for each ligand. Reporter activity of transduced cells not exposed to ligand but treated with mouse IgG was defined as 0% activity.

[0021] FIG. 7 is a sequence alignment showing the amino acid sequence of the complete immunoglobulin heavy chain variable region of chimeric 4F11 variable region denoted as Ch4F11 and humanized 4F11 heavy chain variable regions denoted as Sh4F11 Hv3-23, Sh4F11 Hv3-23 A28T S31H T62S, Sh4F11 Hv3-23 S31H T62S, Sh4F11 Hv3-23 A28T S31N T62S, and Sh4F11 Hv3-23 A28T T62S. The amino acid sequences for each heavy chain variable region are aligned against one another, and CDR₁, CDR₂, and CDR₃ sequences (Kabat definition) are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0022] FIG. 8 is a sequence alignment showing the isolated CDR₁, CDR₂, and CDR₃ sequences for each of the immunoglobulin heavy chain variable region sequences in FIG. 7.

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[0023] FIG. 9 is a sequence alignment showing the amino acid sequence of the complete immunoglobulin light chain variable region of chimeric 4F11 denoted as Ch4F11 and humanized 4F11 light chain variable regions denoted as Hu4F11 Kv2D-29, Hu4F11 Kv2D-29 N28H, Hu4F11 Kv2D-29 N28Q, and Hu4F11 Kv2D-29 N28Y. The light chain variable region amino acid sequences are aligned against one another, and CDR₁, CDR₂, and CDR₃ sequences (Kabat definition) are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0024] FIG. 10 is a sequence alignment showing the isolated CDR₁, CDR₂, and CDR₃ sequences for each of the immunoglobulin light chain variable region sequences in FIG. 9.

[0025] FIG. 11 is a graph illustrating the ability of selected humanized 4F11 (*i.e.*, Hu4F11-70, Hu4F11-72, Hu4F11-74, and Hu4F11-78) antibodies to inhibit ligand-induced Notch3 ICD cleavage in MDA-MB-468 cells plated on Jag2mFc. MDA-MB-468 cells exposed to either one of the 4F11 antibodies or hIgG were plated in wells coated with hJag2-mFc, incubated overnight, washed, and their lysates collected for detection of NICD by Western blot. The graph shows intensity of the cleaved NICD band in each sample as detected by Western blot. Blots were also probed with anti- β tubulin as a control, and bands were quantitated using ImageLab software. As a negative control, cells exposed to hIgG were plated in plates coated with mFc (Fc) rather than Jag2mFc.

[0026] FIG. 12A and FIG. 12B are graphs summarizing results of a Notch3 reporter assay showing that the humanized variants of 4F11 inhibit Notch3-dependent reporter gene expression induced by the ligand Jag2. CHO cells transfected with Jag2 cDNA expression vector were incubated for 24 hours with a mixture of HCC1143 reporter cells and 0.1 mg/ml, 1 mg/ml, or 10 mg/ml of 4F11 antibody variants (*i.e.*, Hu4F11-32, Hu4F11-69, Hu4F11-70, Hu4F11-71, Hu4F11-72, Hu4F11-73, Hu4F11-74, Hu4F11-75, Hu4F11-76, Hu4F11-77, Hu4F11-78, Hu4F11-79, or Hu4F11-80), a negative control antibody (Neg Control Ab), or human immunoglobulin G (IgG), or were left untreated. 24 hours after exposing reporter cells to Jag2-expressing CHO cells, cells were processed using the Bright Glo (Promega, Madison, WI) reporter assay protocol and average relative light units (RLU) were detected on a GloMax Luminometer (Promega) (FIG. 12A and FIG. 12B).

[0027] FIG. 13 is a graph illustrating the ability of selected humanized 4F11 antibodies (*i.e.*, Hu4F11-70, Hu4F11-72 and Hu4F11-78) to inhibit activation of Notch3 receptor *in vivo*

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after treatment of mice bearing HCC2429 xenograft tumors. Mice inoculated with matrigel and HCC2429 Notch3-expressing cells were allowed to develop tumors of 300-400mm³, at which point three mice (m1, m2, and m3 in FIG. 13) each were treated with 20mg/kg of hIgG, 4F11, or humanized Notch3 antibody. Tumors were then harvested and lysates collected, and

5 Western blotting was performed using an antibody specific for the Notch3 C-terminus in order to detect the extent of Notch intracellular domain cleavage. Western blot band intensity of the cleaved Notch intracellular domain fragment was calculated, normalizing to β -tubulin loading control.

DETAILED DESCRIPTION

[0028] The antibodies disclosed herein are based on the antigen binding sites of certain
10 monoclonal antibodies that have been selected on the basis of binding and neutralizing the activity of human Notch3. The antibodies contain immunoglobulin variable region CDR sequences that define a binding site for human Notch3.

[0029] 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
15 engineered (*e.g.*, humanized) to minimize or eliminate an immune response when administered as a therapeutic antibody to a human patient. Various features and aspects of the invention are discussed in more detail below.

[0030] 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,
20 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
25 moiety is an example of a chemically conjugated antibody.

I. Antibodies that Bind Human Notch3

[0031] As disclosed herein, the antibodies may comprise: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3} and (b) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3},

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wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human Notch3.

[0032] In some embodiments, the antibody comprises: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-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 Notch3. A CDR_{H1} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60 and SEQ ID NO:61; a CDR_{H2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 and SEQ ID NO:53; and a CDR_{H3} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and SEQ ID NO:16. (The immunoglobulin heavy chain variable regions including the referenced CDR sequences can be found in **Table 10** and **FIGS. 7-8**.)

[0033] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 11, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7.

[0034] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 51 and SEQ ID NO: 54, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7.

[0035] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 51 and SEQ ID NO: 55, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7.

[0036] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the

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group consisting of SEQ ID NO: 52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7.

5 [0037] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7.

10 [0038] Preferably, the CDR_{H1}, CDR_{H2}, and CDR_{H3} sequences are interposed between human or humanized immunoglobulin FR sequences.

15 [0039] 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 Notch3. A CDR_{L1} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:20, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66 and SEQ ID NO:67; a CDR_{L2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:9 or the amino acid sequence of KVS; and a CDR_{L3} comprises an amino acid sequence of SEQ ID NO:10. (The immunoglobulin light chain variable regions including the referenced CDR sequences can be found in **Table 11** and **FIGS. 9-10**.)

[0040] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 8, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10.

25 [0041] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 62, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10.

30 [0042] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 63, a

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CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10.

[0043] In some embodiments, the antibody comprises an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 64, a
5 CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10.

[0044] Preferably, the CDR_{L1}, CDR_{L2}, and CDR_{L3} sequences are interposed between human or humanized immunoglobulin FR sequences.

[0045] In some embodiments, the antibody comprises: (a) an immunoglobulin heavy chain
10 variable region comprising the structure CDR_{H1}-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 Notch3. The CDR_{H1} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:51, SEQ ID
15 NO:52, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60 and SEQ ID NO:61; the CDR_{H2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:15 and SEQ ID NO:53; and the CDR_{H3} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:7 and SEQ ID NO:16. The CDR_{L1} comprises an amino acid
20 sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:20, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66 and SEQ ID NO:67; the CDR_{L2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO:9 or the amino acid sequence of KVS; and the CDR_{L3} comprises an amino acid sequence of SEQ ID NO:10.

25 [0046] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO:11, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:6, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the
30 amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

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[0047] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO:11; a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6; and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 62; a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9; and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10.

[0048] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO:11; a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6; and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 63; a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9; and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10.

[0049] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO:11; a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6; and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 64; a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 9; and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 10.

[0050] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 54, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

[0051] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 54, a CDR_{H2} comprising the amino acid

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sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:62, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

- 5 **[0052]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 54, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:63, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

- 15 **[0053]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 54, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:64, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

- 20 **[0054]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 55, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

- 25 **[0055]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 55, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the
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amino acid sequence of SEQ ID NO:62, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

5 [0056] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 55, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:63, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

10 [0057] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:51 and SEQ ID NO: 55, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:64, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

15 [0058] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

20 [0059] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:62, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

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[0060] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:63, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

[0061] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:64, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

[0062] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

[0063] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:62, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

[0064] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid

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sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:63, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

5 **[0065]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO:7; and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO:64, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO:10.

[0066] The antibodies disclosed herein comprise an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region selected from the group consisting of SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**); SEQ ID NO:34 (**Sh4F11 Hv3-23**); SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**); SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**); SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T S31N T62S**); and SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**); and an immunoglobulin light chain variable region. As a convenience for the reader, certain SEQ ID NOs. are followed by a parenthetical including the antibody designation that was the origin of the sequence. For example, “SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**)” means that SEQ ID NO:2 comes from murine antibody 4F11. SEQ ID NO:2 is also found in the chimeric 4F11 sequence.

[0067] In other embodiments, the antibody comprises an immunoglobulin light chain variable region selected from the group consisting of SEQ ID NO:4 (**4F11, Ch4F11 Chimeric**); SEQ ID NO:44 (**Hu4F11 Kv2D-29**); SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**); SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**); and SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**); and an immunoglobulin heavy chain variable region.

[0068] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region selected from the group consisting of SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**); SEQ ID NO:34 (**Sh4F11 Hv3-23**); SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**); SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**); SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T**

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S31N T62S); and SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain variable region selected from the group consisting of SEQ ID NO:4 (**4F11, Ch4F11 Chimeric**); SEQ ID NO:44 (**Hu4F11 Kv2D-29**); SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**); SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**); and SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**).

5 **[0069]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:4 (**4F11, Ch4F11 Chimeric**).

10 **[0070]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:44 (**Hu4F11 Kv2D-29**).

15 **[0071]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:34 (**Sh4F11 Hv3-23**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:4 (**4F11, Ch4F11 Chimeric**).

20 **[0072]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:34 (**Sh4F11 Hv3-23**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:44 (**Hu4F11 Kv2D-29**).

[0073] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**).

25 **[0074]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**).

30 **[0075]** In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T**

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S31N T62S), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**).

[0076] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**).

[0077] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**).

[0078] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**).

[0079] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**).

[0080] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**).

[0081] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**).

[0082] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**).

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[0083] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**).

- 5 [0084] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**).

- [0085] In certain embodiments, the antibodies disclosed herein comprise an
10 immunoglobulin heavy chain and an immunoglobulin light chain. In some embodiments, the antibody comprises an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO: 27 (**4F11**); SEQ ID NO:74 (**Ch4F11 Chimeric**); SEQ ID NO:76 (**Sh4F11 Hv3-23**); SEQ ID NO:78 (**Sh4F11 Hv3-23 A28T S31H T62S**); SEQ ID NO:80 (**Sh4F11 Hv3-23 S31H T62S**); SEQ ID NO:82 (**Sh4F11 Hv3-23 A28T S31N T62S**); and SEQ ID NO:84 (**Sh4F11 Hv3-23 A28T T62S**); and an immunoglobulin light chain.
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- [0086] In other embodiments, the antibody comprises an immunoglobulin light chain selected from the group consisting of SEQ ID NO:29 (**4F11**); SEQ ID NO:86 (**Ch4F11 Chimeric**); SEQ ID NO:88 (**Hu4F11 Kv2D-29 Kappa**); SEQ ID NO:90 (**Hu4F11 Kv2D-29 N28H Kappa**); SEQ ID NO:92 (**Hu4F11 Kv2D-29 N28Q Kappa**); and SEQ ID NO:94 (**Hu4F11 Kv2D-29 N28Y Kappa**); and an immunoglobulin heavy chain.
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- [0087] In some embodiments, the antibody comprises an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO: 27 (**4F11**); SEQ ID NO:74 (**Ch4F11 Chimeric**); SEQ ID NO:76 (**Sh4F11 Hv3-23**); SEQ ID NO:78 (**Sh4F11 Hv3-23 A28T S31H T62S**); SEQ ID NO:80 (**Sh4F11 Hv3-23 S31H T62S**); SEQ ID NO:82 (**Sh4F11 Hv3-23 A28T S31N T62S**); and SEQ ID NO:84 (**Sh4F11 Hv3-23 A28T T62S**); and an immunoglobulin light chain selected from the group consisting of SEQ ID NO:29 (**4F11**); SEQ ID NO:86 (**Ch4F11 Chimeric**); SEQ ID NO:88 (**Hu4F11 Kv2D-29 Kappa**); SEQ ID NO:90 (**Hu4F11 Kv2D-29 N28H Kappa**); SEQ ID NO:92 (**Hu4F11 Kv2D-29 N28Q Kappa**); and SEQ ID NO:94 (**Hu4F11 Kv2D-29 N28Y Kappa**).
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[0088] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 27 (**4F11**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 29 (**4F11**).

5 [0089] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 74 (**Ch4F11**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 86 (**Ch4F11**).

[0090] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 74 (**Ch4F11**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 88 (**Hu4F11 Kv2D-29**).

10 [0091] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 76 (**Sh4F11 Hv3-23**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 86 (**Ch4F11**).

[0092] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 76 (**Sh4F11 Hv3-23**), and an
15 immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 88 (**Hu4F11 Kv2D-29**).

[0093] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 78 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 90
20 (**Hu4F11 Kv2D-29 N28H Kappa**).

[0094] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 80 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 90 (**Hu4F11 Kv2D-29 N28H Kappa**).

25 [0095] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 82 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 90 (**Hu4F11 Kv2D-29 N28H Kappa**).

[0096] In some embodiments, the antibody comprises an immunoglobulin heavy chain
30 comprising the amino acid sequence of SEQ ID NO: 84 (**Sh4F11 Hv3-23 A28T T62S**), and an

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immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 90 (**Hu4F11 Kv2D-29 N28H Kappa**).

[0097] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 78 (**Sh4F11 Hv3-23 A28T S31H T62S**),
5 and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11 Kv2D-29 N28Q Kappa**).

[0098] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 80 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11**
10 **Kv2D-29 N28Q Kappa**).

[0099] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 82 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11 Kv2D-29 N28Q Kappa**).

15 [00100] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 84 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11 Kv2D-29 N28Q Kappa**).

[00101] In some embodiments, the antibody comprises an immunoglobulin heavy chain
20 comprising the amino acid sequence of SEQ ID NO: 78 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 94 (**Hu4F11 Kv2D-29 N28Y Kappa**).

[00102] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 80 (**Sh4F11 Hv3-23 S31H T62S**), and an
25 immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 94 (**Hu4F11 Kv2D-29 N28Y Kappa**).

[00103] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 82 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 94
30 (**Hu4F11 Kv2D-29 N28Y Kappa**).

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[00104] In some embodiments, the antibody comprises an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 84 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 94 (**Hu4F11 Kv2D-29 N28Y Kappa**).

5 **[00105]** In other embodiments, an isolated antibody that binds human Notch3 comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the FR sequence of SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**); SEQ ID NO:34 (**Sh4F11 Hv3-23**);
 SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**); SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H**
 10 **T62S**); SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T S31N T62S**); or SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**).

[00106] In other embodiments, an isolated antibody that binds human Notch3 comprises an immunoglobulin light chain variable region comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the FR
 15 sequence of SEQ ID NO:4 (**4F11, Ch4F11 Chimeric**); SEQ ID NO:44 (**Hu4F11 Kv2D-29**);
 SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**); SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**); or
 SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**).

[00107] Sequence identity may be determined in various ways that are within the skill of a person skilled in the art, *e.g.*, using publicly available computer software such as BLAST,
 20 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, herein incorporated by reference) are tailored for sequence similarity searching. For a
 25 discussion of basic issues in searching sequence databases see Altschul *et al.*, (1994) NATURE GENETICS 6:119-129, herein 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
 30 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

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BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919, fully incorporated by reference herein). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (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=1; 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.

[00108] 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 Notch3 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.

[00109] In certain embodiments, an isolated antibody binds human Notch3 with a K_D of about 35 nM, 25 nM, 15 nM, 10 nM, 5 nM, 1 nM, 900 pM, 850 pM, 800pM, 750 pM, 700 pM, 650 pM, 600 pM, 500 pM, 400 pM, 300 pM, 250 pM, 200 pM or less. Unless otherwise specified, K_D values are determined by surface plasmon resonance methods under the conditions described in Examples 3 and 9.

[00110] In some embodiments, monoclonal antibodies bind to the same epitope on human Notch3 as antibody 4F11. In some embodiments, monoclonal antibodies compete for binding

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to human Notch3 with antibody 4F11. For example, monoclonal antibodies may compete for binding to the extracellular domain (ECD) of Notch3 with antibody 4F11 (amino acid sequence corresponding to the human Notch3 ECD is shown in **FIG. 2**). In another example, monoclonal antibodies may compete for binding to EGF-like repeats 1-11 of human Notch3 with antibody 4F11 (amino acid sequence corresponding to EGF-like repeats 1-11 of human Notch3 is shown in **FIG. 3**).

[00111] Competition assays for determining whether an antibody binds to the same epitope as, or competes for binding with, antibody 4F11 are known in the art. Exemplary competition assays include immunoassays (*e.g.*, ELISA assays, RIA assays), BIAcore analysis, biolayer interferometry and flow cytometry.

[00112] Typically, a competition assay involves the use of an antigen (*e.g.*, a human Notch3 protein or fragment thereof) bound to a solid surface or expressed on a cell surface, a test anti-Notch3-binding antibody and a reference antibody (*i.e.*, antibody 4F11). The reference antibody is labeled and the test antibody is unlabeled. Competitive inhibition is measured by determining the amount of labeled reference antibody bound to the solid surface or cells in the presence of the test antibody. Usually the test antibody is present in excess (*e.g.*, 1x, 5x, 10x, 20x or 100x). Antibodies identified by competition assay (*i.e.*, competing antibodies) include antibodies binding to the same epitope, or similar (*e.g.*, overlapping) epitopes, as the reference antibody, and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[00113] In an exemplary competition assay, a reference anti-Notch3 antibody (*i.e.*, antibody 4F11) is biotinylated using commercially available reagents. The biotinylated reference antibody is mixed with serial dilutions of the test antibody or unlabeled reference antibody (self-competition control) resulting in a mixture of various molar ratios (*e.g.*, 1x, 5x, 10x, 20x or 100x) of test antibody (or unlabeled reference antibody) to labeled reference antibody. The antibody mixture is added to a human Notch3 (*e.g.*, extracellular domain of human Notch3) polypeptide coated-ELISA plate. The plate is then washed and horseradish peroxidase (HRP)-streptavidin is added to the plate as the detection reagent. The amount of labeled reference antibody bound to the target antigen is detected following addition of a chromogenic substrate (*e.g.*, TMB (3,3',5,5'-tetramethylbenzidine) or ABTS (2,2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate)), which are known in the art. Optical density readings (OD units) are measured

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using a SpectraMax[®] M2 spectrometer (Molecular Devices). OD units corresponding to zero percent inhibition are determined from wells without any competing antibody. OD units corresponding to 100% inhibition, *i.e.*, the assay background are determined from wells without any labeled reference antibody or test antibody. Percent inhibition of labeled reference
5 antibody to Notch3 by the test antibody (or the unlabeled reference antibody) at each concentration is calculated as follows: % inhibition = $(1 - (\text{OD units} - 100\% \text{ inhibition}) / (0\% \text{ inhibition} - 100\% \text{ inhibition})) * 100$. Persons skilled in the art will appreciate that the competition assay can be performed using various detection systems known in the art.

[00114] A competition assay may be conducted in both directions to ensure that the presence
10 of the label does not interfere or otherwise inhibit binding. For example, in the first direction the reference antibody is labeled and the test antibody is unlabeled, and in the second direction, the test antibody is labeled and the reference antibody is unlabeled.

[00115] A test antibody competes with the reference antibody for specific binding to the antigen if an excess of one antibody (*e.g.*, 1x, 5x, 10x, 20x or 100x) inhibits binding of the
15 other antibody, *e.g.*, by at least 50%, 75%, 90%, 95% or 99% as measured in a competitive binding assay.

[00116] Two antibodies may be determined to bind to the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies may be determined to bind to overlapping
20 epitopes if only a subset of the amino acid mutations that reduce or eliminate binding of antibody reduce or eliminate binding of the other.

II. Antibody Production

[00117] 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
25 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
30 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

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sequence information provided herein, or prior art sequence information regarding genes encoding the heavy and light chains of murine antibodies in hybridoma cells.

[00118] 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.

[00119] 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.

[00120] If the engineered gene is to be expressed in eukaryotic host cells, *e.g.*, CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, a poly A sequence, and a stop codon, and, optionally, may contain 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

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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).

[00121] A polypeptide comprising an immunoglobulin heavy chain variable region or light chain variable region can be produced by growing (culturing) 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 or isolated using techniques known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) and histidine tags.

[00122] A monoclonal antibody that binds human Notch3, or an antigen-binding fragment of the antibody, can be produced by growing (culturing) 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 or isolated using techniques 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

[00123] Methods for reducing or eliminating the antigenicity of antibodies and antibody fragments are known in the art. When administered to a human, the disclosed 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.

[00124] 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,

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NATURE 312:604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

[00125] 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-Notch3 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; Verhoeven *et al.* (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS LETT 430: 92-94.

[00126] 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.

[00127] 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).

[00128] 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).

[00129] 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,

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CA). This technology involves the use of a proprietary human “acceptor” library to produce an “epitope focused” library for antibody selection.

[00130] 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
5 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).

[00131] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of an antibody of the invention.

10 [00132] 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

15 [00133] Antibodies disclosed herein may be engineered (*e.g.*, humanized) for administration to humans. 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
20 inhibit cancer cell proliferation by at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99% or 100%.

[00134] In some embodiments, the disclosed antibodies may inhibit or reduce proliferation of a tumor cell by inhibiting binding of human Notch3 to a ligand, *e.g.*, Jag1, Jag2, DLL1, and DLL4. The disclosed antibodies can be used in a method to inhibit tumor growth in a human
25 patient. The method comprises administering to the patient a therapeutically effective amount of the antibody.

[00135] Cancers associated with Notch3 overexpression and/or activation include, but are not limited to, breast cancer, ovarian cancer, prostate cancer, cervical cancer, lung cancer, brain cancer (*e.g.*, glioblastoma, astrocytoma, neuroblastoma), melanomas, gastrointestinal cancers
30 (*e.g.*, colorectal, pancreatic, and gastric), head and neck cancer, sarcomas (*e.g.*

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rhabdomyosarcoma, osteosarcoma), 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)).

[00136] As used herein, “treat”, “treating” and “treatment” mean the treatment of a disease
5 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.

[00137] 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
10 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
15 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, serum half-life of the antibody, 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-
20 based drugs is within ordinary skill in the art. In some embodiments, a monoclonal antibody is lyophilized, and then reconstituted in buffered saline, at the time of administration.

[00138] 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
25 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
30 pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

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[00139] 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 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.

[00140] 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 polyethylene glycol), and suitable mixtures thereof.

[00141] 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

[00142] 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-hNotch3 Monoclonal Antibodies

[00143] Immunizations, fusions, and primary screens were conducted using conventional methods following the Repetitive Immunization Multiple Sites (RIMMS) protocol. Five AJ mice and five Balb/c mice were immunized with a protein containing amino acids 1-428 of human Notch3 fused to the Fc portion of human IgG. In addition, five AJ and five Balb/c mice

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were immunized with a concatemeric protein containing two repeats of the region of Notch3 comprising EGF-like domains 9-12. From each immunization strategy, 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 5,280 supernatants from the cell fusions were screened for binding to human Notch3 on the surface of CHO cells, using a Mesoscale electrochemiluminescence assay (MSD). In total, four hundred and twenty supernatants that bound human Notch3 in this assay were identified from the AJ and Balb/c fusions. These 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. Antibodies were expressed from the hybridoma cell lines and purified by affinity chromatography on Protein G resin under standard conditions.

Example 2: Sequence Analysis of anti-Notch3 Monoclonal Antibodies

[00144] The light chain isotype and heavy chain isotype of the monoclonal antibody, 4F11, in Example 1 was determined using the IsoStrip™ Mouse Monoclonal Antibody Isotyping Kit according to the manufacturer's instructions (Roche Applied Science, Indianapolis, IN). The antibody was determined to be kappa light chain and IgG1 heavy chain.

[00145] The heavy and light chain variable regions of the mouse monoclonal antibody were sequenced using 5' RACE (Rapid Amplification of cDNA Ends). Total RNA was extracted from the 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 the SMARTer™ RACE cDNA Amplification Kit (Clontech, Mountain View, CA) according to the manufacturer's instructions using random primers for 5' RACE.

[00146] The variable regions of the light (kappa) and heavy (IgG1) chains were amplified by PCR, using KOD Hot Start Polymerase (EMD Chemicals, Gibbstown, NJ) according to the manufacturer's instructions. 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: 13) and 5' CTAATACGACTCACTATAGGGC 3' (SEQ ID NO: 17), was used

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as a 5' primer. The heavy chain variable region was amplified using the above 5' primers and a 3' IgG1 constant region specific primer, 5' TATGCAAGGCTTACAACCACA 3' (SEQ ID NO: 18). The kappa chain variable region was amplified with the above 5' primers and a 3' kappa constant region specific primer, 5' CGACTGAGGCACCTCCAGATGTT 3' (SEQ ID NO: 19).

[00147] Individual PCR products were isolated by agarose gel electrophoresis and purified using the Qiaquick Gel Purification kit according to the manufacturer's instructions (Qiagen). The PCR products were subsequently cloned into the pCR[®]4Blunt using the Zero Blunt[®] TOPO[®] PCR Cloning Kit according to the manufacturer's instructions (Invitrogen) and transformed into DH5- α bacteria (Invitrogen) through standard molecular biology techniques. Plasmid DNA isolated from transformed bacterial clones was sequenced using M13 Forward (5' GTAAAACGACGGCCAGT 3') (SEQ ID NO: 21) and M13 Reverse primers (5' CAGGAAACAGCTATGACC 3') (SEQ ID NO: 30) 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 (<http://imgt.cines.fr>) to identify and confirm variable region sequences.

[00148] 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 and are underlined in the amino acid sequences.

[00149] Nucleic Acid Sequence Encoding the Heavy Chain Variable Region of the 4F11 Antibody (SEQ ID NO: 1)

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1  gaagtgcagc  tgggtggagtc  tggggggaggc  ttagtgaagc  ctggagggtc  cctgaaactc
61  tcctgtgcag  cctctggatt  cgctttcagt  agctatgaca  tgtcttgggt  tcgccagact
121  ccggagaaga  ggctggagtg  ggctgcatac  attagtcgtg  gtgggtggtag  cacctactat
181  ccagacactg  tgaagggccg  attcaccatc  tccagagaca  atgccaagaa  caccctgtac
241  ctgcaaata  gcagtctgaa  gtctgaggac  acagccatgt  attactgtgg  aagacatgct
301  actacggcct  actggtactt  cgatgtctgg  ggcgcaggga  ccacggtcac  cgtctcctca

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[00150] Protein Sequence Defining the Heavy Chain Variable Region of the 4F11 Antibody (SEQ ID NO: 2)

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1  evqlvesggg  lvkpqgslkl  scaasgfafs  sydmswvrqt  pekrlewvay  isrgggstyy
61  pdtvkgrfti  srdnakntly  lqmsslksed  tamyycgrha  ttaywyfdvw  gagttvtvss

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[00151] Nucleic Acid Sequence Encoding the Kappa Chain Variable Region of the 4F11Antibody (SEQ ID NO: 3)

5 1 gatgttgtga tgacccaaac tccactctcc ctgcctgtca gtcttgagga tcaagcctcc
 61 atctcttgca gatctagtca gagccttgta cacactaatg gcaacaccta ttacattgg
 121 tacctgcaga agccaggcca gtctccaaaa ctctgatct acaaagtttc caaccgattt
 181 tctgggggtcc cagacagggt cagtggcagt ggatcaggga cagatttcac actcaagatc
 241 agcagagtgg aggctgagga tctgggagtt tatttctgct ctcaaagtac acatgttccg
 301 tggacgttcg gtggaggcac caagctggaa atcaaa

10 **[00152]** Protein Sequence Defining the Kappa Chain Variable Region of the 4F11 Antibody
 (SEQ ID NO: 4)

 1 dvvmtqtpls lpvslgdqas iscrssqslv htngntylhw ylqkpgqspk lliykvsnrff
 61 sgvpdrfsgs gsgtdftlki srveaedlgv yfcsqsthvp wtfgggtkle ik

15 **[00153]** **Table 1** is a concordance chart showing the SEQ ID NO. of each sequence
 discussed in this Example.

Table 1

SEQ. ID NO.	Nucleic Acid or Protein
1	4F11 Heavy Chain Variable Region—nucleic acid
2	4F11 Heavy Chain Variable Region—protein
3	4F11 Light (kappa) Chain Variable Region—nucleic acid
4	4F11 Light (kappa) Chain Variable Region—protein
5	4F11 Heavy Chain CDR ₁
6	4F11 Heavy Chain CDR ₂
7	4F11 Heavy Chain CDR ₃
8	4F11 Light (kappa) Chain CDR ₁
9	4F11 Light (kappa) Chain CDR ₂
10	4F11 Light (kappa) Chain CDR ₃

[00154] Mouse monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and
 20 IMGT definitions) for 4F11 are shown in **Table 2**.

Table 2

	CDR1	CDR2	CDR3
Kabat	SYDMS (SEQ ID NO: 5)	YISRGGGSTYYPDTVKG (SEQ ID NO: 6)	HATTAYWYFDV (SEQ ID NO: 7)
Chothia	GFAFSSY (SEQ ID NO: 11)	SRGGGS (SEQ ID NO: 12)	HATTAYWYFDV (SEQ ID NO: 7)
IMGT	GFAFSSYD (SEQ ID NO: 14)	ISRGGGST (SEQ ID NO: 15)	GRHATTAYWYFDV (SEQ ID NO: 16)

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[00155] Mouse monoclonal antibody kappa light chain CDR sequences (Kabat, Chothia, and IMGT definitions) for 4F11 are shown in **Table 3**.

Table 3

	CDR1	CDR2	CDR3
Kabat/Chothia	RSSQSLVHTNGNTYLH (SEQ ID NO: 8)	KVSNRFS (SEQ ID NO: 9)	SQSTHVPWT (SEQ ID NO: 10)
IMGT	QSLVHTNGNTY (SEQ ID NO: 20)	KVS	SQSTHVPWT (SEQ ID NO: 10)

5

[00156] 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 IgG1 heavy chain constant sequence, and a complete kappa chain comprises a kappa variable sequence followed by the murine kappa light chain constant sequence.

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[00157] Nucleic Acid Sequence Encoding the Murine IgG1 Heavy Chain Constant Region
(SEQ ID NO: 22)

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1  gccaaaacga caccgccatc tgtctatcca ctggcccctg gatctgctgc ccaaactaac
61  tccatggtga cctgggatg cctggtcaag ggctatttcc ctgagccagt gacagtgacc
121 tggaactctg gatccctgtc cagcgggtgtg cacaccttcc cagctgtcct gcagtctgac
181 ctctacactc tgagcagctc agtgactgtc ccctccagca cctggcccag cgagaccgtc
241 acctgcaacg ttgccacccc ggccagcagc accaagggtgg acaagaaaat tgtgcccagg
301 gattgtgggtt gtaagccttg catatgtaca gtcccagaag tatcatctgt cttcatcttc
361 ccccaaagc ccaaggatgt gctcaccatt actctgactc ctaaggtcac gtgtgtgtgtg
421 gtagacatca gcaaggatga tcccagagtc cagttcagct ggtttgtaga tgatgtggag
481 gtgcacacag ctcagacgca accccgggag gagcagttca acagcacttt ccgctcagtc
541 agtgaacttc ccatcatgca ccaggactgg ctcaatggca aggagttcaa atgcagggtc
601 aacagtgcag ctttccctgc ccccatcgag aaaaccatct ccaaaccacaa aggcagaccg
661 aaggctccac aggtgtacac cattccacct cccaaggagc agatggccaa ggataaagtc
721 agtctgacct gcatgataac agacttcttc cctgaagaca ttactgtgga gtggcagtg
781 aatgggcagc cagcggagaa ctacaagaac actcagccca tcatggacac agatggctct
841 tacttctgtc acagcaagct caatgtgcag aagagcaact gggaggcagg aaatactttc
901 acctgctctg ttttacatga gggcctgcac aaccaccata ctgagaagag cctctccac
961 tctcctggtg aa

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[00158] Protein Sequence Defining the Murine IgG1 Heavy Chain Constant Region (SEQ ID NO: 23)

35

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1  akttpsvvyp lapgsaaqtn smvtlglclvk gyfpepvtvt wnsghlssgv htftpavlqsd
61  lytlsssvtv psstwpsetv tcnvahpass tkvdkkivpr dgcgkpcict vpevssvfif
121 pppkpdvlti tltpkvtcvv vdiskddpev qfswfvddve vhtaqtqpre eqfnstfrsv
181 selpimhqdw lngkefkcrv nsaaftpapie ktisktkgrp kapqvtytipp pkeqmakdkv

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241 sltcmtdff peditvewqw ngqpaenykn tqpimtdgs yfvysklvq ksnweagntf
 301 tcsvlheglh nhhtekslsh spgk

[00159] Nucleic Acid Sequence Encoding the Murine Kappa Light Chain Constant Region

5 (SEQ ID NO: 24)

1 cgggctgatg ctgcaccaac tgtatccatc ttcccacccat ccagtgagca gttaacatct
 61 ggaggtgcct cagtcgtgtg cttcttgaac aacttctacc ccaaagacat caatgtcaag
 121 tggaagattg atggcagtga acgacaaaat ggcgtcctga acagttggac tgatcaggac
 181 agcaaagaca gcacctacag catgagcagc accctcacgt tgaccaagga cgagtatgaa
 10 241 cgacataaca gctatacctg tgaggccact cacaagacat caacttcacc cattgtcaag
 301 agcttcaaca ggaatgagtg t

[00160] Protein Sequence Defining the Murine Kappa Light Chain Constant Region (SEQ ID NO: 25)

15 1 radaaptvsi fppsseqqlts ggasvvcfln nfypkdinvk wkidgserqn gvlinswtdqd
 61 skdstysmss tltltkdeye rhnsytceat hktstspivk sfnrnec

[00161] The following sequences represent the actual or contemplated full length heavy and light chain sequences (*i.e.*, containing both the variable and constant regions sequences) for the 4F11 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.

[00162] Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgG1 Constant Region) of 4F11 (SEQ ID NO: 26)

1 gaagtgcagc tgggtggagtc tggggggaggc ttagtgaagc ctggaggggc cctgaaactc
 61 tectgtgcag cctctggatt cgctttcagt agctatgaca tgtcttgggt tcgccagact
 121 ccggagaaga ggctggagtg ggtcgcatac attagtcgtg gtgggtgtag cactactat
 181 ccagacactg tgaaggcccg attcaccatc tccagagaca atgccaagaa caccctgtac
 35 241 ctgcaaatga gcagtctgaa gtctgaggac acagccatgt attactgtgg aagacatgct
 301 actacggcct actggtactt cgatgtcttg ggcgaggga ccacggtcac cgtctcctca
 361 gccaaaacga caccctcacc tgtctatcca ctggcccctg gatctgctgc ccaaactaac
 421 tccatgggtga ccctgggatg cctgggtcaag ggctatttcc ctgagccagt gacagtgacc
 481 tggaactctg gatccctgtc cagcgggtgtg cacaccttcc cagctgtcct gcagtctgac
 40 541 ctctacactc tgagcagctc agtgactgtc ccctccagca cctggcccag cgagaccgtc
 601 acctgcaacg ttgcccaccc ggccagcagc accaaggtgg acaagaaaat tgtgcccagg

- 35 -

5 661 gatttgtggtt gtaagccttg catatgtaca gtcccagaag tatcatctgt cttcatcttc
 721 ccccaaaagc ccaaggatgt gtcaccatt actctgactc ctaaggtcac gtgtgttgtg
 781 gtagacatca gcaaggatga tcccagggtc cagttcagct ggtttgtaga tgatgtggag
 841 gtgcacacag ctcagacgca accccgggag gagcagttca acagcacttt ccgctcagtc
 901 agtgaacttc ccatcatgca ccaggactgg ctcaatggca aggagttcaa atgcagggtc
 961 aacagtgcag ctttcctgc ccccatcgag aaaacatct ccaaaaccaa aggcagaccg
 1021 aaggctccac aggtgtacac cattccacct cccaaggagc agatggccaa ggataaagtc
 1081 agtctgacct gcatgataac agacttcttc cctgaagaca ttactgtgga gtggcagtg
 1141 aatgggcagc cagcggagaa ctacaagaac actcagcca tcatggacac agatggctct
 10 1201 tacttcgtct acagcaagct caatgtgcag aagagcaact gggaggcagg aaatactttc
 1261 acctgctctg tgttacatga gggcctgcac aaccaccata ctgagaagag cctctccac
 1321 tctcctggta aa

[00163] Protein Sequence Defining the Full Length Heavy Chain

15 **Variable Region and IgG1 Constant Region) of 4F11 (SEQ ID NO: 27)**

1 evqlvesggg lvkpqgslkl scaasgfafs sydmswvrt pekrlewvay isrgggstyy
 61 pdtvkgrfti srdaakntly lqmsslkscd tamyycgrha ttaywyfdvw gagttvtvss
 121 akttppsypv lapgsaaqtn smvtlglclvk gyfpepvtvt wnsqslssgv htftpavlqsd
 181 lytlsssvtv psstwpsetv tcnvahpass tkvdskivpr dcgckpcict vpevssvfif
 20 241 ppkpkdvlti ttpkvctcvv vdiskddpev qfswfvddve vhtaqtqpre eqfnstfrsv
 301 selpimhqdw lngkefkcrv nsaaftpaple ktisktkgrp kapqvtytipp pkeqmakdkv
 361 sltcmtdff peditvewqg ngqpaenykn tqpimtdggs yfvysklnvq ksnweagntf
 421 tcsvlheglh nhhtekslsh spgk

25 **[00164] Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa**
Chain Variable Region and Constant Region) of 4F11 (SEQ ID NO: 28)

1 gatgttgtga tgacccaaac tccactctcc ctgcctgtca gtcttgagga tcaagcctcc
 61 atctcttgca gatctagtca gagccttgta cacactaatg gcaacaccta tttacattgg
 121 tacctgcaga agccaggcca gtctccaaaa ctctgatct acaaagtttc caaccgattt
 30 181 tctgggggtcc cagacagggt cagtggcagt ggatcaggga cagatttcac actcaagatc
 241 agcagagtgg aggtcgagga tctgggagtt tatttctgct ctcaaagtac acatgttccg
 301 tggacgttcg gtggaggcac caagctggaa atcaaacggg ctgatgctgc accaactgta
 361 tccatcttcc caccatccag tgagcagtta acatctggag gtgcctcagt cgtgtgcttc
 421 ttgaacaact tctaccccaa agacatcaat gtcaagtggg agattgatgg cagtgaacga
 35 481 caaaatggcg tctgaacag ttgactgat caggacagca aagacagcac ctacagcatg
 541 agcagcacc tcacgttgac caaggacgag tatgaacgac ataacagcta tacctgtgag
 601 gccactcaca agacatcaac ttcaccatt gtcaagagct tcaacaggaa tgagtgt

[00165] Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain

40 **Variable Region and Constant Region) of 4F11 (SEQ ID NO: 29)**

1 dvvmtqtpls lpvslgdqas iscrssqslv htngntylhw ylqkpgqspk lliykvsnrf
 61 sgvpdrfsgs gsgtdftlki srveaedlgv yfcsqsthvp wtfgggkile ikradaaptv
 121 sifppsseql tsggasvvcf lnnfypkdin vkwkidgser qngvlinswd qdskdstysm
 181 sstltltkde yerhnsytce athktstspi vksfnrnec
 45

[00166] Table 4 is a concordance chart showing 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
26	4F11 Heavy Variable + IgG1 Constant—nucleic acid
27	4F11 Heavy Variable + IgG1 Constant—protein
28	4F11 Kappa Variable + Constant—nucleic acid
29	4F11 Kappa Variable + Constant—protein

Example 3: Binding Affinities

[00167] The binding affinity and kinetics of binding of antibody 4F11 to recombinant human Notch3 extracellular domain (containing EGF like domains 1-11) Fc fusion protein (rhNotch3-Fc (R&D Systems, Inc., Minneapolis, MN)) were measured by surface plasmon resonance using a Biacore[®] T100 instrument (GE Healthcare, Piscataway, NJ).

[00168] Rabbit anti-mouse IgGs (GE Healthcare) were immobilized on carboxymethylated dextran CM4 sensor chips (GE Healthcare) by amine coupling, according to a standard protocol. Analyses were performed at 25°C and 37°C using PBS containing 0.05% surfactant P20 as running buffer. The antibody was captured in individual flow cells at a flow rate of 10 µl/minute. Injection time was varied for each antibody to yield an R_{max} between 30 and 60 resonance units (RU). Buffer or rhNotch3-Fc diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 240 seconds at 60 µl/minute. The dissociation phase was monitored for up to 1500 seconds. The surface was then regenerated with two 60-second injections of 10 mM Glycine-HCl, pH 2.25, at a flow rate of 30 µl/minute. The rhNotch3-Fc concentration range tested was 100 nM to 3.125 nM (2 fold dilution).

[00169] Kinetic parameters were determined using the kinetic function of the BIAevaluation software (GE Healthcare) with double reference subtraction. Kinetic parameters for the antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) were determined. Kinetic values of the monoclonal antibody 4F11 on rhNotch3-Fc at 25°C and 37°C are summarized in **Table 5**.

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Table 5
Antibody Binding to rhNotch3-Fc

Temperature	k_a (1/Ms)	k_d (1/s)	K_D (M)	n
25°C	7.0E+04	4.8E-05	8.23E-10	3
37°C	7.5E+04	5.7E-05	7.8E-10	3

[00170] The data in **Table 5** demonstrate that the antibody 4F11 binds rhNotch3-Fc with a K_D of about 1 nM, 900 pM, 850 pM, 800 pM, 750 pM, 700 pM, 650 pM, 600 pM, 500 pM, 400 pM, 300 pM, 250 pM, or 200 pM or less.

[00171] Binding to cell surface human Notch3 by the antibody 4F11 was measured at 4°C, using Fluorescence Activated Cell Sorting (FACS). CHO N3 (Flp-In-CHO cells (Invitrogen) stably transfected with human Notch3), HCC2429 cells, and RL-952 cells expressing human Notch3 were washed once with PBS containing calcium chloride and magnesium chloride (Invitrogen) and harvested using cell dissociation buffer (Invitrogen). Cells were washed a second time with PBS and resuspended in FACS buffer (PBS with 0.5 % BSA (Sigma-Aldrich)) for a final cell concentration of 250,000 cells per well into a 96-well v-bottom plate. Purified antibodies were diluted in FACS buffer over a concentration range of 100 nM to 0.1 nM. Cells were then incubated at 4°C with 100 μ l of antibody for one hour, washed with FACS buffer twice, and resuspended in 100 μ l of goat-anti mouse PE-conjugated antibody (Jackson Immuno Research). Cells were incubated at 4°C for 30 minutes in the dark, washed once with FACS buffer, and then analyzed using a Beckman Coulter Cytomics FC 500 instrument. The geometric mean of the florescent intensity was then calculated for each antibody concentration. These values were then entered into Prism software (GraphPad, La Jolla, Ca) and used to generate a binding curve by plotting geometric mean versus antibody concentration. From the binding curve, the following equation was used to calculate the K_D and K_D range of 4F11 binding to human Notch3 on the cell surface of the three cell lines.

Equation: One site binding (hyperbola)

$$Y = B_{\max} * X / (K_D + X)$$

*describes the binding of a ligand to a receptor that follows the law of mass action. $B_{\max}$ is the maximal binding, and K_D is the concentration of ligand required to reach half-maximal binding.

[00172] Results are summarized in **Table 6**.

Table 6

	K_D (nM)	K_D Range (nM)
CHO N3	0.36	0.21 to 0.51
HCC2429	2.58	1.21 to 3.95
RL-952	0.9633	0.7168 to 1.210

[00173] The results in **Table 6** demonstrate that the antibody 4F11 binds cell surface Notch3 with a K_D of about 1 nM, 750 pM, 650 pM, 600 pM, 500 pM, 400 pM, 300 pM, 250 pM, or
5 200 pM or less.

Example 4: Binding Specificity

[00174] Antibody 4F11 was tested for binding to human Notch1, human Notch2, or human Notch3 proteins. Binding measurements were made by biolayer interferometry (BLI), using a FortéBio Octet® QK instrument (FortéBio, Menlo Park, CA). Anti-human-Fc sensors were
10 soaked in PBS containing 1 mg/ml BSA for five 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 rmNotch3-Fc (R&D Cat. No. 1308-NT-050). Notch protein bound sensors were immersed in antibody
15 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 Notch3 protein, but do not bind to human Notch2 or human Notch1 protein (**FIG. 4**).

[00175] Stable cells lines expressing Notch receptors were produced by transfecting FlpIn™
20 CHO or FlpIn™ 293 cells (Life Technologies, Grand Island, NY) with full length human Notch1, Notch2, Notch3, or Notch4 cDNAs cloned into the pcDNA5FRT vector using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. Twenty-four hours after transfection, CHO cells were split into F12 media containing 10% FBS, 2 mM L-Glutamine and 700 µg/ml hygromycin B (Sigma-Aldrich, St. Louis, MO) to select for
25 transfected cells. 293 cells were split into DMEM media containing 10% FBS, 2mM L-glutamine and 200 µg/ml hygromycin B. Expression of Notch receptors was confirmed by FACS analysis using anti-human Notch1 PE (BioLegend, San Diego, CA), anti-Notch2 PE (eBioscience, San Diego, CA), or anti-human Notch3 PE (BioLegend, San Diego, CA).

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[00176] To determine specificity of binding to cell surface Notch proteins, antibody 4F11 was tested for binding to human Notch1, human Notch2, human Notch3 and human Notch4 expressed on the surface of CHO cells using electrochemiluminescence (Meso Scale Discovery). 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, F12 + 10% FBS). For binding studies, cells were washed in PBS containing calcium and magnesium, and removed from the plate by treatment with Cell Dissociation Buffer (Life Technologies) for ten minutes at 37°C.

[00177] Cells were seeded at a density of 30,000 cells per well, in hybridoma media, in 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 µl hybridoma media, and incubated for one 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 one hour at 4°C. Plates were washed twice with PBS containing 3% BSA, and 150 µl 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 antibody 4F11 binds to human Notch3 displayed on the surfaces of cells, but does not bind to human Notch1, Notch2, or Notch4 displayed on the surfaces of cells. The 4F11 antibody also does not bind CHO-EV (empty vector) cells that express endogenous hamster Notch proteins. These results indicated that antibody 4F11 binds specifically to human Notch3 protein displayed on a cell surface *in vitro*.

Example 5: Inhibition of Notch3-Ligand Binding

[00178] The 4F11 antibody was tested for its ability to inhibit the binding of rhNotch3 binding to human Jag1, Jag2, DLL1 and DLL4. Binding measurements were made by bio-layer interferometry (BLI), using a FortéBio Octet® QK instrument (FortéBio, Menlo Park, CA). The ligands tested were rhJag1-Fc (R&D Cat. No. 1277-JG-050), rhJag2-Fc (R&D Cat. No. 1726-JG-050), rhDLL1-Fc (R&D Cat. No. 5026-DL-050), and His tagged rhDLL4 (R&D Cat. No. 1506-D4-050).

[00179] To determine the degree of inhibition of Notch3-ligand binding by antibody 4F11, the Octet sensors were loaded with recombinant human Notch3, and the antibody was allowed

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to bind, as described in Example 4. In positive control samples, a commercially available Notch3 polyclonal antibody capable of blocking ligand binding to recombinant human Notch3 (Notch3 Specific Control) was used instead of the 4F11 antibody. Then sensors were immersed in 500 µg/ml human IgG, to block non-specific binding. Ligands were prepared at a concentration of 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. The 4F11 antibody blocked binding of all four ligands to rhNotch3-Fc (**FIG. 5A – 5D**).

Example 6: Inhibition of Ligand-Induced Notch3 ICD Cleavage

[00180] Activation of Notch receptors results in cleavage of the Notch intracellular domain (NICD), which can be detected by Western blot. The effect of antibody 4F11 on the activation of Notch3 ICD cleavage was tested.

[00181] To create soluble Notch ligands, PCR was used to amplify sequences corresponding to the extracellular domains of human Jag1 or human Jag2 cDNA and fuse them in-frame to the coding sequence of human IgG Fc. This construct was then subcloned into the pEE14.4 expression vector (Lonza), transfected into CHOK1SV cells, and selected to produce stable cell lines that secrete hJag1-hFc or hJag2-hFc fusion protein. 96-well Immunosorp ELISA plates (Nalgene Nunc, Rochester, NY) were coated with 5 µg/ml anti-human Fc (Jackson ImmunoResearch, West Grove, PA) overnight at 4°C. After washing wells with PBS/0.5% BSA, 5 µg/ml of soluble hJag1-hFc fusion protein was added and allowed to bind at room temperature for two hours. Unbound protein was removed by washing with PBS/0.5% BSA. FlpIn™ 293 cells engineered to express hNotch3 (as described in Example 4) were plated on Jag1-hFc ligand or hFc in the presence of 10 µg/ml 4F11 or mIgG control antibody. Cells were lysed 24 hours later in RIPA buffer (Boston BioProducts, Ashland, MA) containing protease inhibitors. Induction of cleaved NICD was detected by probing the blot with a Notch3 antibody against the C-terminus (Cell Signaling, Danvers, MA) that detects both full length protein and the cleaved ICD. Ligand-induced activation and Notch3 ICD cleavage was inhibited by antibody 4F11.

Example 7: Inhibition of Notch3-Dependent Transcription

[00182] Reporter cell lines dependent upon Notch3 were produced by lentiviral introduction of a RBP-Jκ-dependent luciferase reporter gene (SABiosciences, Frederick, MD) into 293-FlpIn Notch3 cells, RL95-2 endometrial cancer cells, HCC1143 breast cancer cells, and MDA-

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MB-468 breast cancer cells. To activate Notch3-dependent signaling and transcription, cells were plated on ligand-coated wells prepared as described in Example 6. Cells were pre-incubated with a 3-fold dilution series of Notch3 antibody concentrations ranging from 0-300 µg/ml, for one hour at 37°C, before seeding 100 µl 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 twenty-four hours at 37°C, in 5% CO₂. Next, 100 µl 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 µl volume was transferred to white walled plates and read using a luminometer. Polyclonal antibodies against Notch1 (AF1057, R&D Systems), Notch2 (AF1190, 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. As shown in **FIG. 6A**, Notch3 antibody 4F11 inhibited Notch3-dependent transcription stimulated by the ligand Jag2. Activation of Notch3-dependent transcription by each of the ligands Jag1, Jag2, DLL1, and DLL4 was also inhibited by the Notch3 antibody 4F11 (**FIG. 6B** and **Table 7**).

Table 7

Ligand	Jag1	Jag2	DLL1	DLL4
EC₅₀	2.6 nM	0.4 nM	6.0 nM	4.7 nM
Maximum Inhibition	92%	99%	100%	73%

Example 8: Humanization of Anti-Notch3 Antibodies

[00183] This Example describes the humanization and chimerization of the anti-human Notch3 antibody 4F11, and the characterization of the resulting humanized antibodies. The humanized anti-Notch3 antibodies were designed, affinity matured by targeted CDR mutagenesis, and optimized using methods known in the art. The amino acid sequences were converted to codon-optimized DNA sequences and synthesized 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.

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[00184] Chimeric (murine variable region and human constant region) 4F11 heavy (human IgG1) and light (human Kappa) chains were also constructed. To generate chimeric antibodies, the murine variable regions were fused to the human constant region, and codon-optimized DNA sequences were synthesized, 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.

[00185] 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.

[00186] 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 Notch3 was measured as described below. The results are summarized in **Tables 14-16**.

[00187] Exemplary combinations of the chimeric or humanized 4F11 immunoglobulin heavy chain and immunoglobulin light chain variable regions is set forth below in **Table 8**.

Table 8

Antibody Name	Heavy Chain Variable Region	Light Chain Variable Region
Hu4F11-1	Ch4F11 Chimeric Heavy (SEQ ID NO:2)	Ch4F11 Chimeric Kappa (SEQ ID NO:4)
Hu4F11-10	Sh4F11 Hv3-23 Heavy (SEQ ID NO:34)	Ch4F11 Chimeric Kappa (SEQ ID NO:4)
Hu4F11-18	Ch4F11 Chimeric Heavy (SEQ ID NO:2)	Hu4F11 Kv2D-29 Kappa (SEQ ID NO:44)
Hu4F11-32	Sh4F11 Hv3-23 Heavy (SEQ ID NO:34)	Hu4F11 Kv2D-29 Kappa (SEQ ID NO:44)
Hu4F11-69	Sh4F11 Hv3-23 A28T S31N T62S Heavy (SEQ ID NO:40)	Hu4F11 Kv2D-29 N28H Kappa (SEQ ID NO:46)
Hu4F11-70	Sh4F11 Hv3-23 A28T S31H T62S Heavy (SEQ ID NO:36)	Hu4F11 Kv2D-29 N28H Kappa (SEQ ID NO:46)
Hu4F11-71	Sh4F11 Hv3-23 A28T T62S Heavy (SEQ ID NO:42)	Hu4F11 Kv2D-29 N28H Kappa (SEQ ID NO:46)
Hu4F11-72	Sh4F11 Hv3-23 S31H T62S Heavy (SEQ ID NO:38)	Hu4F11 Kv2D-29 N28H Kappa (SEQ ID NO:46)
Hu4F11-73	Sh4F11 Hv3-23 A28T S31N T62S Heavy (SEQ ID NO:40)	Hu4F11 Kv2D-29 N28Y Kappa (SEQ ID NO:50)

Table 8 continued

Antibody Name	Heavy Chain Variable Region	Light Chain Variable Region
Hu4F11-74	Sh4F11 Hv3-23 A28T S31H T62S Heavy (SEQ ID NO:36)	Hu4F11 Kv2D-29 N28Y Kappa (SEQ ID NO:50)
Hu4F11-75	Sh4F11 Hv3-23 A28T T62S Heavy (SEQ ID NO:42)	Hu4F11 Kv2D-29 N28Y Kappa (SEQ ID NO:50)
Hu4F11-76	Sh4F11 Hv3-23 S31H T62S Heavy (SEQ ID NO:38)	Hu4F11 Kv2D-29 N28Y Kappa (SEQ ID NO:50)
Hu4F11-77	Sh4F11 Hv3-23 A28T S31N T62S Heavy (SEQ ID NO:40)	Hu4F11 Kv2D-29 N28Q Kappa (SEQ ID NO:48)
Hu4F11-78	Sh4F11 Hv3-23 A28T S31H T62S Heavy (SEQ ID NO:36)	Hu4F11 Kv2D-29 N28Q Kappa (SEQ ID NO:48)
Hu4F11-79	Sh4F11 Hv3-23 A28T T62S Heavy (SEQ ID NO:42)	Hu4F11 Kv2D-29 N28Q Kappa (SEQ ID NO:48)
Hu4F11-80	Sh4F11 Hv3-23 S31H T62S Heavy (SEQ ID NO:38)	Hu4F11 Kv2D-29 N28Q Kappa (SEQ ID NO:48)

[00188] The nucleic acid sequences and the encoded protein sequences defining variable regions of the chimeric and humanized 4F11 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.

[00189] Nucleic Acid Sequence Encoding the Ch4F11 Chimeric Heavy Chain Variable Region (SEQ ID NO:31)

```

10      1 gaggtacagc ttgtcgagtc gggaggagga ttggtaaaac cgggtgggtc actcaaattg
      61 tcgtgtgctg cgctcgggatt tgcgttttcg tcgtatgata tgcgtgggt ggcgcagacg
      121 ccggaataaac gattggaatg ggtcgcgtat atctcccgag ggggaggttc gacatactat
      181 cccgacacgg tcaaaggcg cttcacgatt tcacgggaca atgcgaaaaa cacgctttat
      241 cttcaaatgt cgtcgttgaa atcggaagat accgcgatgt attactgcg gaggcattgcg
15     301 acgacggcgt attggtatct cgatgtgtgg ggagccggaa cgacggtgac ggtgtcgtcg

```

[00190] Protein Sequence Defining the Ch4F11 Chimeric Heavy Chain Variable Region (SEQ ID NO:2)

```

20      1 evqlvesggg lvkpggslkl scaasgfafs sydmswvrqt pekrlewvay isrgggstyy
      61 pdtvkgrfti srdnakntly lqmsslksed tamyycgrha ttaywyfdw gatttvtvss

```

[00191] Nucleic Acid Sequence Encoding the Sh4F11 Hv3-23 Heavy Chain Variable Region (SEQ ID NO:33)

```

25      1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
      61 tcgtgtgctg cgctcgggatt cgcgttttca tcgtatgaca tgcgtgggt gaggcaggca

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121 ccggggaaaag ggcttgaatg ggtatcgtag atttcgagag ggggaggatc gacgtattac
 181 ccggatacgg tgaaaggaag gtttacgata tcgcgcgaca attcaaagaa tacgctttat
 241 cttcagatga actcgtccg agcggaagat acggcggtat actattgcgg tcgccatgcg
 301 acgacggcgt attggtattt cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg

5

[00192] Protein Sequence Defining the Sh4F11 Hv3-23 Heavy Chain Variable Region (SEQ ID NO:34)

1 evqllesggg lvqpqgslrl scaasgfafs sydmswvrqa pgkglewvsy isrgggstyy
 61 pdtvkgrfti srdnsksntly lqmnsdraed tavyycgrha ttaywyfdvw gqgtmvtvss

10

[00193] Nucleic Acid Sequence Encoding the Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain Variable Region (SEQ ID NO:35)

1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
 61 tcgtgtgagg cgctcgggatt cacccttttca cactatgaca tgctcgtgggt gaggcaggca
 121 ccggggaaaag ggcttgaatg ggtatcgtag atttcgagag ggggaggatc gacgtattac
 181 ccggaattccg tgaaaggaag gtttacgata tcgcgcgaca attcaaagaa tacgctttat
 241 cttcagatga actcgtccg agcggaagat acggcggtat actattgcgg tcgccatgcg
 301 acgacggcgt attggtattt cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg

15

[00194] Protein Sequence Defining the Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain Variable Region (SEQ ID NO:36)

1 evqllesggg lvqpqgslrl scaasgftfs hydmswvrqa pgkglewvsy isrgggstyy
 61 pdsvkgrfti srdnsksntly lqmnsdraed tavyycgrha ttaywyfdvw gqgtmvtvss

[00195] Nucleic Acid Sequence Encoding the Sh4F11 Hv3-23 S31H T62S Heavy Chain Variable Region (SEQ ID NO:37)

1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
 61 tcgtgtgagg cgctcgggatt cgcgttttca cactatgaca tgctcgtgggt gaggcaggca
 121 ccggggaaaag ggcttgaatg ggtatcgtag atttcgagag ggggaggatc gacgtattac
 181 ccggaattccg tgaaaggaag gtttacgata tcgcgcgaca attcaaagaa tacgctttat
 241 cttcagatga actcgtccg agcggaagat acggcggtat actattgcgg tcgccatgcg
 301 acgacggcgt attggtattt cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg

30

[00196] Protein Sequence Defining the Sh4F11 Hv3-23 S31H T62S Heavy Chain Variable Region (SEQ ID NO:38)

1 evqllesggg lvqpqgslrl scaasgfafs hydmswvrqa pgkglewvsy isrgggstyy
 61 pdsvkgrfti srdnsksntly lqmnsdraed tavyycgrha ttaywyfdvw gqgtmvtvss

[00197] Nucleic Acid Sequence Encoding the Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain Variable Region (SEQ ID NO:39)

40

1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
 61 tcgtgtgagg cgctcgggatt cacccttttca aactatgaca tgctcgtgggt gaggcaggca

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5

121 ccggggaaaag ggcttgaatg ggtatcgtac atttcgagag ggggaggatc gacgtattac
 181 ccggattccg tgaaaggaag gtttacgac tcgcgcgaca attcaaagaa tacgctttat
 241 cttcagatga actcgtccg agcggaagat acggcggtat actattgcgg tcgccatgcg
 301 acgacggcgt attggtattt cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg

[00198] Protein Sequence Defining the Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain

Variable Region (SEQ ID NO:40)

10

1 evqllesggg lvqpqgslrl scaasgftfs nydmswvrqa pgkglewvsy isrgggstyy
 61 pdsvkgrfti srdnsksntly lqmnsiraed tavyycgrha ttaywyfdvw gqgtmvtvss

[00199] Nucleic Acid Sequence Encoding the Sh4F11 Hv3-23 A28T T62S Heavy Chain

Variable Region (SEQ ID NO:41)

15

1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
 61 tcgtgtgctg cgctcgggatt cacccttttca tcgtatgaca tgcgtgggt gaggcaggca
 121 ccggggaaaag ggcttgaatg ggtatcgtac atttcgagag ggggaggatc gacgtattac
 181 ccgattccg tgaaaggaag gtttacgac tcgcgcgaca attcaaagaa tacgctttat
 241 cttcagatga actcgtccg agcggaagat acggcggtat actattgcgg tcgccatgcg
 301 acgacggcgt attggtattt cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg

20

[00200] Protein Sequence Defining the Sh4F11 Hv3-23 A28T T62S Heavy Chain Variable Region (SEQ ID NO:42)

1 evqllesggg lvqpqgslrl scaasgftfs sydmswvrqa pgkglewvsy isrgggstyy
 61 pdsvkgrfti srdnsksntly lqmnsiraed tavyycgrha ttaywyfdvw gqgtmvtvss

25

[00201] Nucleic Acid Sequence Encoding the Ch4F11 Chimeric Kappa Chain Variable Region (SEQ ID NO:32)

30

1 gacgtggtaa tgacgcagac gccgttgtcc cttcctgtct cgctcggaga tcaggcgctg
 61 atctcgtgta gaagctcgca gtcactcgtc cataccaacg ggaatacata tcttcaactgg
 121 tattttgcaa agcccggaac gtcaccgaag ctcttgatct acaaagtatc caatcggttt
 181 tcgggggtgc ccgaccgatt ctcgggatcg ggttcgggga cggattttac gttgaagatt
 241 tcgcggttg aagcggagga tctcgggtgc tacttttgtt cgcagtcac gcatgtccc
 301 tggacgttcg gagcgggac aaaacttgag atcaag

[00202] Protein Sequence Defining the Ch4F11 Chimeric Kappa Chain Variable Region

(SEQ ID NO:4)

1 dvvmtqtpls lpvslgdqas iscrssqslv htngntylhw ylqkpgqspk lliykvsnrf
 61 sgvpdrfsgs gsgtdftlki srveadlgy yfcsqsthvp wtfgggkile ik

[00203] Nucleic Acid Sequence Encoding the Hu4F11 Kv2D-29 Kappa Chain Variable

Region (SEQ ID NO:43)

1 gatgtagtca tgacccaaac gccgctttcg ttgtcggatga cggccggaca gcccgcgta
 61 atctcgtgtc ggtcatcgca gtcgttgga cacacaaacg gtaatacgta tctccattgg

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[00204] Protein Sequence Defining the Hu4F11 Kv2D-29 Kappa Chain Variable Region
(SEQ ID NO:44)

10

121 tatctccaga agcccggccca gtcgccgcag ctcttgatct acaaagttag caatcgcttt
181 tcgggggtgc cggatcggtt ctcgggatcg gggtcaggaa cggacttcac gcttaagatt
241 tcgaggggtcg aggcggagga tgtcggagtc tacttttggt cgcagtcac acatgtcccc
301 tggacgtttg ggcaggggac gaaggtggaa atcaag

1 dvvmtqtpls lsvtpgqpas iscrssqslv htngntylhw ylqkpgqspq lliykvsnrnf
61 sgvpdrfsgs gsgtdftlki srveaedvgv yfcsqsthvp wtfgggtkve ik

[00205] Nucleic Acid Sequence Encoding the Hu4F11 Kv2D-29 N28H Kappa Chain
Variable Region (SEQ ID NO:45)

15

1 gatgtagtca tgacccaaac gccgctttcg ttgtcgggtga cggccggaca gcccgcgta
61 atctcgtgtc ggtcatcgca gtcgttggtta cacacacacg gtaatacgta tctccattgg
121 tatctccaga agcccggccca gtcgccgcag ctcttgatct acaaagttag caatcgcttt
181 tcgggggtgc cggatcggtt ctcgggatcg gggtcaggaa cggacttcac gcttaagatt
241 tcgaggggtcg aggcggagga tgtcggagtc tacttttggt cgcagtcac acatgtcccc
301 tggacgtttg ggcaggggac gaaggtggaa atcaag

20 [00206] Protein Sequence Defining the Hu4F11 Kv2D-29 N28H Kappa Chain Variable
Region (SEQ ID NO:46)

1 dvvmtqtpls lsvtpgqpas iscrssqslv htngntylhw ylqkpgqspq lliykvsnrnf
61 sgvpdrfsgs gsgtdftlki srveaedvgv yfcsqsthvp wtfgggtkve ik

25 [00207] Nucleic Acid Sequence Encoding the Hu4F11 Kv2D-29 N28Q Kappa Chain
Variable Region (SEQ ID NO:47)

30

1 gatgtagtca tgacccaaac gccgctttcg ttgtcgggtga cggccggaca gcccgcgta
61 atctcgtgtc ggtcatcgca gtcgttggtta cacacacacg gtaatacgta tctccattgg
121 tatctccaga agcccggccca gtcgccgcag ctcttgatct acaaagttag caatcgcttt
181 tcgggggtgc cggatcggtt ctcgggatcg gggtcaggaa cggacttcac gcttaagatt
241 tcgaggggtcg aggcggagga tgtcggagtc tacttttggt cgcagtcac acatgtcccc
301 tggacgtttg ggcaggggac gaaggtggaa atcaag

35 [00208] Protein Sequence Defining the Hu4F11 Kv2D-29 N28Q Kappa Chain Variable
Region (SEQ ID NO:48)

1 dvvmtqtpls lsvtpgqpas iscrssqslv htngntylhw ylqkpgqspq lliykvsnrnf
61 sgvpdrfsgs gsgtdftlki srveaedvgv yfcsqsthvp wtfgggtkve ik

40 [00209] Nucleic Acid Sequence Encoding the Hu4F11 Kv2D-29 N28Y Kappa Chain
Variable Region (SEQ ID NO:49)

1 gatgtagtca tgacccaaac gccgctttcg ttgtcgggtga cggccggaca gcccgcgta
61 atctcgtgtc ggtcatcgca gtcgttggtta cacacatacg gtaatacgta tctccattgg

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5

[00210] Protein Sequence Defining the Hu4F11 Kv2D-29 N28Y Kappa Chain Variable Region (SEQ ID NO:50)

10

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121 tatctccaga agcccggcca gtcgccgag ctcttgatct acaaagtgag caatcgcttt
181 tcgggggtgc cggatcggtt ctccggatcg gggtcaggaa cggacttcac gcttaagatt
241 tcgaggggtcg aggcggagga tgcggagtc tacttttggt cgcagtccac acatgtcccc
301 tggacgtttg ggcaggggac gaaggtggaa atcaag

```

```

1 dvvmtqtpls lsvtpggpas iscrssqslv htygntylhw ylqkpgqspq lliykvsnrff
61 sgvpdrfsgs gsgtdftlki srveaadv gv yfcsqsthvp wtfgggtkve ik

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[00211] The amino acid sequences defining the immunoglobulin heavy chain variable regions for the antibodies produced in Example 8 are aligned in **FIG. 7**. Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR₁, CDR₂, and CDR₃ (Kabat definition) are identified by boxes. **FIG. 8** show an alignment of the separate CDR₁, CDR₂, and CDR₃ sequences for each of the variable region sequences shown in **FIG. 7**.

[00212] The amino acid sequences defining the immunoglobulin light chain variable regions for the antibodies in Example 8 are aligned in **FIG. 9**. Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR₁, CDR₂ and CDR₃ are identified by boxes. **FIG. 10** shows an alignment of the separate CDR₁, CDR₂, and CDR₃ sequences for each of the variable region sequences shown in **FIG. 9**.

[00213] **Table 9** is a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.

Table 9

SEQ. ID NO.	Nucleic Acid or Protein
31	Ch4F11 Chimeric Heavy Chain Variable Region—nucleic acid
2	Ch4F11 Chimeric Heavy Chain Variable Region—protein
5	Ch4F11 Chimeric Heavy Chain CDR ₁
6	Ch4F11 Chimeric Heavy Chain CDR ₂
7	Ch4F11 Chimeric Heavy Chain CDR ₃
33	Sh4F11 Hv3-23 Heavy Chain Variable Region—nucleic acid
34	Sh4F11 Hv3-23 Heavy Chain Variable Region—protein
5	Sh4F11 Hv3-23 Heavy Chain CDR ₁
6	Sh4F11 Hv3-23 Heavy Chain CDR ₂
7	Sh4F11 Hv3-23 Heavy Chain CDR ₃
35	Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain Variable Region—nucleic acid
36	Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain Variable Region—protein
51	Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain CDR ₁

Table 9 continued

SEQ. ID NO.	Nucleic Acid or Protein
53	Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain CDR ₂
7	Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain CDR ₃
37	Sh4F11 Hv3-23 S31H T62S Heavy Chain Variable Region—nucleic acid
38	Sh4F11 Hv3-23 S31H T62S Heavy Chain Variable Region—protein
51	Sh4F11 Hv3-23 S31H T62S Heavy Chain CDR ₁
53	Sh4F11 Hv3-23 S31H T62S Heavy Chain CDR ₂
7	Sh4F11 Hv3-23 S31H T62S Heavy Chain CDR ₃
39	Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain Variable Region—nucleic acid
40	Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain Variable Region—protein
52	Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain CDR ₁
53	Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain CDR ₂
7	Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain CDR ₃
41	Sh4F11 Hv3-23 A28T T62S Heavy Chain Variable Region—nucleic acid
42	Sh4F11 Hv3-23 A28T T62S Heavy Chain Variable Region—protein
5	Sh4F11 Hv3-23 A28T T62S Heavy Chain CDR ₁
53	Sh4F11 Hv3-23 A28T T62S Heavy Chain CDR ₂
7	Sh4F11 Hv3-23 A28T T62S Heavy Chain CDR ₃
32	Ch4F11 Chimeric Light (kappa) Chain Variable Region—nucleic acid
4	Ch4F11 Chimeric Light (kappa) Chain Variable Region—protein
8	Ch4F11 Chimeric Light (kappa) Chain CDR ₁
9	Ch4F11 Chimeric Light (kappa) Chain CDR ₂
10	Ch4F11 Chimeric Light (kappa) Chain CDR ₃
43	Hu4F11 Kv2D-29 Light (kappa) Chain Variable Region—nucleic acid
44	Hu4F11 Kv2D-29 Light (kappa) Chain Variable Region—protein
8	Hu4F11 Kv2D-29 Light (kappa) Chain CDR ₁
9	Hu4F11 Kv2D-29 Light (kappa) Chain CDR ₂
10	Hu4F11 Kv2D-29 Light (kappa) Chain CDR ₃
45	Hu4F11 Kv2D-29 N28H Light (kappa) Chain Variable Region—nucleic acid
46	Hu4F11 Kv2D-29 N28H Light (kappa) Chain Variable Region—protein
62	Hu4F11 Kv2D-29 N28H Light (kappa) Chain CDR ₁
9	Hu4F11 Kv2D-29 N28H Light (kappa) Chain CDR ₂
10	Hu4F11 Kv2D-29 N28H Light (kappa) Chain CDR ₃
47	Hu4F11 Kv2D-29 N28Q Light (kappa) Chain Variable Region—nucleic acid
48	Hu4F11 Kv2D-29 N28Q Light (kappa) Chain Variable Region—protein
63	Hu4F11 Kv2D-29 N28Q Light (kappa) Chain CDR ₁
9	Hu4F11 Kv2D-29 N28Q Light (kappa) Chain CDR ₂
10	Hu4F11 Kv2D-29 N28Q Light (kappa) Chain CDR ₃
49	Hu4F11 Kv2D-29 N28Y Light (kappa) Chain Variable Region—nucleic acid
50	Hu4F11 Kv2D-29 N28Y Light (kappa) Chain Variable Region—protein
64	Hu4F11 Kv2D-29 N28Y Light (kappa) Chain CDR ₁
9	Hu4F11 Kv2D-29 N28Y Light (kappa) Chain CDR ₂
10	Hu4F11 Kv2D-29 N28Y Light (kappa) Chain CDR ₃

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[00214] Humanized monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in **Table 10**.

Table 10

Kabat				
	CDR1	CDR2	CDR3	Variable Region SEQ ID NO:
Ch4F11 Chimeric	SYDMS (SEQ ID NO: 5)	YISRGGGSTYYPDTVKG (SEQ ID NO: 6)	HATTAYWYFDV (SEQ ID NO: 7)	2
Sh4F11 Hv3-23	SYDMS (SEQ ID NO: 5)	YISRGGGSTYYPDTVKG (SEQ ID NO: 6)	HATTAYWYFDV (SEQ ID NO: 7)	34
Sh4F11 Hv3-23 A28T S31H T62S	HYDMS (SEQ ID NO:51)	YISRGGGSTYYPDSVKG (SEQ ID NO:53)	HATTAYWYFDV (SEQ ID NO: 7)	36
Sh4F11 Hv3-23 S31H T62S	HYDMS (SEQ ID NO:51)	YISRGGGSTYYPDSVKG (SEQ ID NO:53)	HATTAYWYFDV (SEQ ID NO: 7)	38
Sh4F11 Hv3-23 A28T S31N T62S	NYDMS (SEQ ID NO:52)	YISRGGGSTYYPDSVKG (SEQ ID NO:53)	HATTAYWYFDV (SEQ ID NO: 7)	40
Sh4F11 Hv3-23 A28T T62S	SYDMS (SEQ ID NO: 5)	YISRGGGSTYYPDSVKG (SEQ ID NO:53)	HATTAYWYFDV (SEQ ID NO: 7)	42
Chothia				
	CDR1	CDR2	CDR3	Variable Region SEQ ID NO:
Ch4F11 Chimeric	GFAFSSY (SEQ ID NO: 11)	SRGGGS (SEQ ID NO: 12)	HATTAYWYFDV (SEQ ID NO: 7)	2
Sh4F11 Hv3-23	GFAFSSY (SEQ ID NO: 11)	SRGGGS (SEQ ID NO: 12)	HATTAYWYFDV (SEQ ID NO: 7)	34
Sh4F11 Hv3-23 A28T S31H T62S	GFTFSHY (SEQ ID NO:54)	SRGGGS (SEQ ID NO: 12)	HATTAYWYFDV (SEQ ID NO: 7)	36
Sh4F11 Hv3-23 S31H T62S	GFAFSHY (SEQ ID NO:55)	SRGGGS (SEQ ID NO: 12)	HATTAYWYFDV (SEQ ID NO: 7)	38
Sh4F11 Hv3-23 A28T S31N T62S	GFTFSNY (SEQ ID NO:56)	SRGGGS (SEQ ID NO: 12)	HATTAYWYFDV (SEQ ID NO: 7)	40
Sh4F11 Hv3-23 A28T T62S	GFTFSSY (SEQ ID NO:57)	SRGGGS (SEQ ID NO: 12)	HATTAYWYFDV (SEQ ID NO: 7)	42

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Table 10 Continued

IMGT				
	CDR1	CDR2	CDR3	Variable Region SEQ ID NO:
Ch4F11 Chimeric	GFAFSSYD (SEQ ID NO: 14)	ISRGGGST (SEQ ID NO: 15)	GRHATTAYWYFDV (SEQ ID NO: 16)	2
Sh4F11 Hv3-23	GFAFSSYD (SEQ ID NO: 14)	ISRGGGST (SEQ ID NO: 15)	GRHATTAYWYFDV (SEQ ID NO: 16)	34
Sh4F11 Hv3-23 A28T S31H T62S	GFTFSHYD (SEQ ID NO: 58)	ISRGGGST (SEQ ID NO: 15)	GRHATTAYWYFDV (SEQ ID NO: 16)	36
Sh4F11 Hv3-23 S31H T62S	GFAFSHYD (SEQ ID NO: 59)	ISRGGGST (SEQ ID NO: 15)	GRHATTAYWYFDV (SEQ ID NO: 16)	38
Sh4F11 Hv3-23 A28T S31N T62S	GFTFSNYD (SEQ ID NO: 60)	ISRGGGST (SEQ ID NO: 15)	GRHATTAYWYFDV (SEQ ID NO: 16)	40
Sh4F11 Hv3-23 A28T T62S	GFTFSSYD (SEQ ID NO: 61)	ISRGGGST (SEQ ID NO: 15)	GRHATTAYWYFDV (SEQ ID NO: 16)	42

[00215] Humanized monoclonal antibody Kappa light chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in **Table 11**.

5

Table 11

Kabat/Chothia				
	CDR1	CDR2	CDR3	Variable Region SEQ ID NO:
Ch4F11 Chimeric	RSSQSLVHTNGNTYLH (SEQ ID NO: 8)	KVSNRFS (SEQ ID NO: 9)	SQSTHVPWT (SEQ ID NO: 10)	4
Hu4F11 Kv2D-29	RSSQSLVHTNGNTYLH (SEQ ID NO: 8)	KVSNRFS (SEQ ID NO: 9)	SQSTHVPWT (SEQ ID NO: 10)	44
Hu4F11 Kv2D-29 N28H	RSSQSLVHTHGNTYLH (SEQ ID NO: 62)	KVSNRFS (SEQ ID NO: 9)	SQSTHVPWT (SEQ ID NO: 10)	46
Hu4F11 Kv2D-29 N28Q	RSSQSLVHTQGNTYLH (SEQ ID NO: 63)	KVSNRFS (SEQ ID NO: 9)	SQSTHVPWT (SEQ ID NO: 10)	48
Hu4F11 Kv2D-29 N28Y	RSSQSLVHTYGNTYLH (SEQ ID NO: 64)	KVSNRFS (SEQ ID NO: 9)	SQSTHVPWT (SEQ ID NO: 10)	50

Table 11 Continued

IMGT				
	CDR1	CDR2	CDR3	Variable Region SEQ ID NO:
Ch4F11 Chimeric	QSLVHTNGNTY (SEQ ID NO: 20)	KVS	SQSTHVPWT (SEQ ID NO: 10)	4
Hu4F11 Kv2D-29	QSLVHTNGNTY (SEQ ID NO: 20)	KVS	SQSTHVPWT (SEQ ID NO: 10)	44
Hu4F11 Kv2D-29 N28H	QSLVHTHGNTY (SEQ ID NO:65)	KVS	SQSTHVPWT (SEQ ID NO: 10)	46
Hu4F11 Kv2D-29 N28Q	QSLVHTQGNTY (SEQ ID NO:66)	KVS	SQSTHVPWT (SEQ ID NO: 10)	48
Hu4F11 Kv2D-29 N28Y	QSLVHTYGNTY (SEQ ID NO:67)	KVS	SQSTHVPWT (SEQ ID NO: 10)	50

[00216] 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 IgG1 heavy chain constant sequence. A complete kappa chain comprises a kappa variable sequence followed by the human kappa light chain constant sequence.

[00217] Nucleic Acid Sequence Encoding the Human IgG1 Heavy Chain Constant Region
(SEQ ID NO:68)

```

1  gcctcaacaa aaggaccaag tgtgttccca ctgcgcccta gcagcaagag tacatccggg
61  ggcactgcag cactcggtcg cctcgtaag gattattttc cagagccagt aaccgtgagc
121  tggaacagtg gagcactcac ttctgggtgc catacttttc ctgctgtcct gcaaagctct
181  ggccgtgact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
241  tacatctgta atgtaaacca caagcctagc aatactaagg tcgataagcg ggtggaaccc
301  aagagctgcg acaagactca cacttggtccc ccatgccctg cccctgaact tctgggcggt
361  cccagcgtct ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
421  gaggtgacat gtgttggtgt agacgtttcc cacgaggacc cagagggtta gttcaactgg
481  tacgttgatg gagtcgaagt acataatgct aagaccaagc ctagagagga gcagtataat
541  agtacatacc gtgtagtcag tgttctcaca gtgctgcaac aagactggct caacggcaaa
601  gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
661  aaggcaaaag ggcagcctcg tgaaccacag gtgtacactc tgccacccag tagagaggaa
721  atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
781  gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac cccccagtg
841  ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgtctg
901  cagcagggtg acgtcttcag ctgttccgtg atgcacgagg cattgcacaa ccactacacc
961  cagaagtcac tgagcctgag cccagggaag

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[00218] Protein Sequence Defining the Human IgG1 Heavy Chain Constant Region (SEQ ID NO:69)

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1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsгалtsгv htfpavllqss
 61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
 121 psvflfpkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
 181 styrvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
 5 241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
 301 qggnvfscsv mhealhnhyt qkslslspgk

[00219] Nucleic Acid Sequence Encoding the Human Kappa Light Chain Constant Region
(Version 1) (SEQ ID NO:70)

1 cgcacagttg ctgccccag cgtgttcatt ttccaccta gcgatgagca gctgaaaagc
 10 61 ggtactgcct ctgtcgtatg cttgctcaac aacttttacc cacgtgaggc taaggtgcag
 121 tggaaagtgg ataatgcact tcaatctgga aacagtcaag agtccgtgac agaacaggac
 181 agcaaagact caacttattc actctcttcc accctgactc tgtccaaggc agactatgaa
 241 aaacacaagg tatacgcttg cgagggttaca caccagggtt tgtctagtcc tgtcaccaag
 15 301 tccttcaata ggggcgaatg t

[00220] Nucleic Acid Sequence Encoding the Human Kappa Light Chain Constant Region
(Version 2) (SEQ ID NO:71)

1 cgcacagttg cagccccag cgtgttcatt ttccaccta gcgatgagca gctgaaaagc
 20 61 ggtactgcct ctgtcgtatg cttgctcaac aacttttacc cacgtgaggc taaggtgcag
 121 tggaaagtgg ataatgcact tcaatctgga aacagtcaag agtccgtgac agaacaggac
 181 agcaaagact caacttattc actctcttcc accctgactc tgtccaaggc agactatgaa
 241 aaacacaagg tatacgcttg cgagggttaca caccagggtt tgtctagtcc tgtcaccaag
 301 tccttcaata ggggcgaatg t

25 [00221] Protein Sequence Defining the Human Kappa Light Chain Constant Region (nucleic acid versions 1 and 2 encode the same amino acid sequence) (SEQ ID NO:72)

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

30 **[00222]** 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
 35 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 immunoglobulin 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
 40 active full length immunoglobulin heavy and light chains.

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[00223] Nucleic Acid Sequence Encoding the Full Length Ch4F11 Chimeric Heavy Chain
(Mouse Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO:73)

```

1 gaggtacagc ttgtcgagtc gggaggagga ttggtaaaac cgggtgggtc actcaaattg
5 61 tcgtgtgctg cgtcgggatt tgcgttttcg tcgtatgata tgcgtgggt ggcgcagacg
121 ccggaaaaaac gattggaatg ggtcgcgtat atctcccgag ggggaggttc gacatactat
181 ccgcacacgg tcaaagggcg cttcacgatt tcacgggaca atgcgaaaaa cacgctttat
241 cttcaaattgt cgtcgttgaa atcggaagat accgcgatgt attactgcgg gaggcattgcg
301 acgacggcgt attggtatct cgatgtgtgg ggagccggaa cgacgggtgac ggtgtcgtcg
10 361 gcctcaacaa aaggaccaag tgtgttccca ctgcgcccta gcagcaagag tacatccggg
421 ggcaactgcag cactcggtcg cctcgtcaag gattatcttc cagagccagt aaccgtgagc
481 tggaacagtg gagcactcac ttctgggtgc catacttttc ctgctgtcct gcaaagctct
541 ggctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
601 tacatctgta atgtaaacca caagcctagc aatactaagg tcgataagcg ggtggaaccc
661 aagagctgcg acaagactca cacttgtccc ccatgccctg cccctgaact tctgggcggt
15 721 ccagcgtct tttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
781 gaggtgacat gtgtgtgtgt agacgtttcc cacgaggacc cagaggttaa gttcaactgg
841 tacgttgatg gactcgaagt acataatgct aagaccaagc ctagagagga gcagtataat
901 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
961 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
20 1021 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccaccacg tagagaggaa
1081 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
1141 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac cccccagtg
1201 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgtctg
1261 cagcagggtg acgtcttcag ctgttccgtg atgcacgagg cattgcacaa ccactacacc
25 1321 cagaagtcac tgagcctgag cccagggaag

```

[00224] Protein Sequence Defining the Full Length Ch4F11 Chimeric Heavy Chain (Mouse
Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO:74)

```

1 evqlvesggg lvkpggslkl scaasgfafs sydmswvrrt pekrleway isrgggstyy
30 61 pdtvkgrfti srdnakntly lqmsslksed tamyycgrha ttaywyfdvw gagttvtvss
121 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsгалtsgv htfpavlqss
181 glyslssvvt vpssslgtqt yicnvnkps ntkvdkrvep kscdkthtcp pcpapellgg
241 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
301 styrvsvlt vlhqdwlngk eykckvsnka lpapiektis kagqprepq vytlppsree
35 361 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdkstrw
421 qggnvfscsv mhealhnhyt qkslslspgk

```

[00225] Nucleic Acid Sequence Encoding the Full Length Sh4F11 Hv3-23 Heavy Chain
(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID

40 NO:75)

```

1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
61 tcgtgtgctg cgtcgggatt cgcgttttca tcgtatgaca tgcgtgggt gaggcaggca
121 ccggggaaaag ggcttgaatg ggtatcgtag atttcgagag ggggaggatc gacgtattac
181 ccgatacagg tgaaaggaag gtttacgata tcgcgcgaca attcaaagaa tacgctttat
45 241 cttcagatga actcgtccg agcggaagat acggcgggat actattgcgg tcgccatgcg
301 acgacggcgt attggtatct cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg
361 gcctcaacaa aaggaccaag tgtgttccca ctgcgcccta gcagcaagag tacatccggg
421 ggcaactgcag cactcggtcg cctcgtcaag gattatcttc cagagccagt aaccgtgagc
481 tggaacagtg gagcactcac ttctgggtgc catacttttc ctgctgtcct gcaaagctct
50 541 ggctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
601 tacatctgta atgtaaacca caagcctagc aatactaagg tcgataagcg ggtggaaccc

```

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5 661 aagagctgcg acaagactca cacttgctccc ccatgccctg cccctgaact tctgggcggt
 721 cccagcgtct ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
 781 gaggtgacat gtgttggtgt agacgtttcc cacgaggacc cagaggttaa gttcaactgg
 841 tacgttgatg gagtcgaagt acataatgct aagaccaagc ctagagagga gcagtataat
 901 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
 961 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
 1021 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccaccag tagagaggaa
 1081 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
 1141 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac cccccagtg
 10 1201 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgtg
 1261 cagcagggtg acgtcttcag ctgttccgtg atgcacgagg cattgcacaa ccactacacc
 1321 cagaagtcac tgagcctgag cccagggaag

[00226] Protein Sequence Defining the Full Length Sh4F11 Hv3-23 Heavy Chain

15 **(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID**

NO:76)

1 evqllesggg lvqpqgslrl scaasgfafs sydmswvrqa pgkglewvsy isrgggstyy
 61 pdtvmkgrfti srdnskntly lqmnsraed tavyycgrha ttaywyfdvw gqgtmvtvss
 121 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsгалtsgv htfpavlgss
 20 181 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
 241 psvflfpkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
 301 styrvsvlt vlhqdwlngk eykckvsnka lpapiektis kakggprepq vytlppsree
 361 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
 421 qqgnvfscsv mhealhnhyt qkslsispkg

[00227] Nucleic Acid Sequence Encoding the Full Length Sh4F11 Hv3-23 A28T S31H

T62S Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant

Region) (SEQ ID NO:77)

30 1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
 61 tcgtgtgctg cgtcgggatt cactttttca cactatgaca tgcgtgggt gaggcaggca
 121 cgggggaaag ggctgaatg ggtatcgtae atttcgagag ggggaggatc gacgtattac
 181 cgggattccg tgaaaggaag gtttacgata tcgcgcgaca attcaaagaa tacgctttat
 241 cttcagatga actcgtccg agcggaagat acggcgggat actattgcgg tcgccatgctg
 301 acgacggcgt attggtattt cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg
 35 361 gcctcaacaa aaggaccaag tgtgttccca ctgcgcccta gcagcaagag tacatccggg
 421 ggcactgcag cactcggtcg cctcgtcaag gattattttc cagagccagt aaccgtgagc
 481 tggaacagtg gagcactcag ttctggtgct catacttttc ctgctgtcct gcaaagctct
 541 ggcctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
 601 tacatctgta atgtaaacca caagcctagc aataactaagg tcgataagcg ggtggaaccc
 40 661 aagagctgcg acaagactca cacttgctccc ccatgccctg cccctgaact tctgggcggt
 721 cccagcgtct ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
 781 gaggtgacat gtgttggtgt agacgtttcc cacgaggacc cagaggttaa gttcaactgg
 841 tacgttgatg gagtcgaagt acataatgct aagaccaagc ctagagagga gcagtataat
 901 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
 45 961 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
 1021 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccaccag tagagaggaa
 1081 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
 1141 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac cccccagtg
 1201 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgtg
 50 1261 cagcagggtg acgtcttcag ctgttccgtg atgcacgagg cattgcacaa ccactacacc
 1321 cagaagtcac tgagcctgag cccagggaag

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[00228] Protein Sequence Defining the Full Length Sh4F11 Hv3-23 A28T S31H T62S**Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)**

(SEQ ID NO:78)

```

5      1 evqllesggg lvqpqgslrl scaasgftfs hydmswvrqa pgkglewvsy isrgggstyy
      61 pdsvkgrfti srdnskntly lqmnsltraed tavyycgrha ttaywyfdvw gqgtmvtvss
      121 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsгалtsgv htfpavlqss
      181 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
      241 psvflfpkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
      301 styrvsvlt vlhqdwlngk eykckvsnka lpapiektis kakggprepq vytlppsree
10     361 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
      421 qqgnvfscsv mhealhnhyt qkslslspgk

```

[00229] Nucleic Acid Sequence Encoding the Full Length Sh4F11 Hv3-23 S31H T62S**Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)**

15 (SEQ ID NO:79)

```

      1 gaagtacagt tgttgagatc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
      61 tcgtgtgctg cgtcgggatt cgcgttttca cactatgaca tgctcgtggg gaggcaggca
      121 ccggggaaaag ggcttgaatg ggtatcgtac atttcgagag ggggaggatc gacgtattac
      181 ccggattccg tgaaaggaag gtttacgac tcgcgcgaca attcaaagaa tacgctttat
20     241 cttcagatga actcgtcccg agcgggaagat acggcgggat actattgcgg tcgccatgcg
      301 acgacggcgt attggtatatt cgatgtgtgg ggacaaggga cgatggtcac ggtgtcgtcg
      361 gcctcaacaa aaggaccaag tgtgttccca ctgcgcccta gcagcaagag tacatccggg
      421 ggcaactgcag cactcggctg cctcgtcaag gattattttc cagagccagt aaccgtgagc
      481 tggaacagtg gagcactcac ttctggtgtc catacttttc ctgctgtcct gcaaagctct
25     541 ggctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
      601 tacatctgta atgtaaacca caagcctagc aataactaagg tcgataagcg ggtggaaccc
      661 aagagctgcg acaagactca cacttgtccc ccatgccctg cccctgaact tctgggcggt
      721 ccagcgtctt ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
      781 gaggtgacat gtgtgtgtgt agacgtttcc cacgaggacc cagaggttaa gttcaactgg
30     841 tacgttgatg gagtgcgaagt acataatgct aagaccaagc ctagagagga ccagtataat
      901 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
      961 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
      1021 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccaccag tagagaggaa
      1081 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
35     1141 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac cccccagtg
      1201 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgtgtg
      1261 cagcagggtg acgtcttcag ctgttcctg atgcacgagg cattgcacaa ccactacacc
      1321 cagaagtcac tgagcctgag ccagggaag

```

40 [00230] Protein Sequence Defining the Full Length Sh4F11 Hv3-23 S31H T62S Heavy**Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)** (SEQ ID

NO:80)

```

      1 evqllesggg lvqpqgslrl scaasgfafs hydmswvrqa pgkglewvsy isrgggstyy
45     61 pdsvkgrfti srdnskntly lqmnsltraed tavyycgrha ttaywyfdvw gqgtmvtvss
      121 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsгалtsgv htfpavlqss
      181 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
      241 psvflfpkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
      301 styrvsvlt vlhqdwlngk eykckvsnka lpapiektis kakggprepq vytlppsree
      361 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw

```

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421 qqgnvfscsv mhealhnhyt qkslslspgk

[00231] Nucleic Acid Sequence Encoding the Full Length Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO:81)

```

1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
61 tcgtgtgctg cgtcgggatt cacccttttca aactatgaca tgctcgtggg gaggcaggca
121 cgggggaaag ggcttgaatg ggtatcgtac atttcgagag ggggaggatc gacgtattac
181 ccggattccg tgaaaggaag gtttacgac tcgcgcgaca attcaaagaa tacgctttat
10 241 cttcagatga actcgtccg agcggaagat acggcgggat actattgcg tgccatgctg
301 acgacggcgt attggtattt cgtgtgtgtg ggacaaggga cgtatgtcac ggtgtcgtcg
361 gcctcaacaa aaggaccaag tgtgttccca ctgcgcccta gcagcaagag tacatccggg
421 ggcactgcag cactcggctg cctcgtcaag gattattttc cagagccagt aaccgtgagc
481 tggaacagtg gagcactcac ttctgggtgc cactatcttc ctgctgtcct gcaaagctct
15 541 ggctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
601 tacatctgta atgtaaacca caagcctagc aatactaagg tcgataagcg ggtggaaccc
661 aagagctgct acaagactca cacttgtccc ccatgccctg cccctgaact tctgggcggg
721 ccagcgtct ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
781 gaggtgacat gtgtgtgtgt agacgtttcc cagcaggacc cagaggttaa gttcaactgg
20 841 tacgttgatg gagtgcgaag acataatgct aagaccaagc ctagagagga gcagtataat
901 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
961 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
1021 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccaccag tagagaggaa
1081 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
25 1141 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac cccccagtg
1201 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgtctg
1261 cagcagggtg acgtcttcag ctgttcctg atgcacgagg cattgcacaa ccactacacc
1321 cagaagtcac tgagcctgag ccagggaag

```

30 [00232] Protein Sequence Defining the Full Length Sh4F11 A28T S31N T62S Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO:82)

```

1 evqllesggg lvqpqgslrl scaasgftfs nydmswvrqa pgkglewvsy isrgggstyy
61 pdsvkgrfti srdnskntly lqmnslraed tavyycgrha ttaywyfdvw gqgtmvtvss
35 121 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsaltsgv htfpavlqss
181 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
241 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
301 styrvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
361 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
40 421 qqgnvfscsv mhealhnhyt qkslslspgk

```

[00233] Nucleic Acid Sequence Encoding the Full Length Sh4F11 Hv3-23 A28T T62S Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO:83)

```

45 1 gaagtacagt tgttgagtc aggaggaggg ttggtccagc cgggtggatc gttgcggctt
61 tcgtgtgctg cgtcgggatt cacccttttca tcgtatgaca tgctcgtggg gaggcaggca
121 cgggggaaag ggcttgaatg ggtatcgtac atttcgagag ggggaggatc gacgtattac
181 ccggattccg tgaaaggaag gtttacgac tcgcgcgaca attcaaagaa tacgctttat
241 cttcagatga actcgtccg agcggaagat acggcgggat actattgcg tgccatgctg

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301 acgacggcgt attggtattt cgatgtgtgg ggacaaggga cgatgggtcac ggtgtcgtcg
 361 gcctcaacaa aaggaccaag tgtgttccca ctgcgcccta gcagcaagag tacatccggg
 421 ggcactgcag cactcggctg cctcgtcaag gattattttc cagagccagt aaccgtgagc
 481 tggaaacagt gagcactcac ttctggtgtc catacttttc ctgctgtcct gcaaagctct
 5 541 ggctgtact cactcagctc cgctcgtgacc gtgccatctt catctctggg cactcagacc
 601 tacatctgta atgtaaacca caagcctagc aatactaagg tcgataagcg ggtggaaccc
 661 aagagctgcg acaagactca cacttgtccc ccatgccctg cccctgaact tctgggcggg
 721 cccagcgtct ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
 781 gaggtgacat gtgtgttgt agacgtttcc cagcaggacc cagaggtaa gttcaactgg
 10 841 tacgttgatg gagtcgaagt acataatgct aagaccaagc cttagagagga gcagtataat
 901 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
 961 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
 1021 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccaccag tagagaggaa
 1081 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
 15 1141 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac ccccccagtg
 1201 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgtgg
 1261 cagcagggtg acgtcttcag ctgttcctg atgcacgagg cattgcacaa ccactacacc
 1321 cagaagtcac tgagcctgag ccagggaag

20 **[00234] Protein Sequence Defining the Full Length Sh4F11 Hv3-23 A28T T62SHheavy**
Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID
NO:84)

1 evqllesggg lvqpqgslrl scaasgftfs sydmswvrqa pgkglewvsy isrgggstyy
 61 pdsvkgrfti srdnskntly lqmnslraed tavyycgrha ttaywyfdvw gqgtmvtvss
 25 121 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsгалtsqv htfpavlqss
 181 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
 241 psvflfpkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
 301 styrvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
 361 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
 30 421 qqgnvfscsv mhealnhhyt qkslslspgk

[00235] Nucleic Acid Sequence Encoding the Full Length Ch4F11 Chimeric Light Chain
(Mouse Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO:85)

1 gacgtggtaa tgacgcagac gccgttgtec cttcctgtct cgctcggaga tcaggcgctcg
 35 61 atctcgtgta gaagctcgca gtcactcgtc cataccaacg ggaatacata tcttcaactgg
 121 tatttgcaaa agcccggaac gtcaccgaag ctcttgatct acaaagtatc caatcgggtt
 181 tcgggggtgc ccgaccgatt ctcgggatcg ggttcgggga cggattttac gttgaagatt
 241 tcgcggttg aagcggagga tctcgtgtgc tacttttgtt cgcagtcacac gcatgtcccg
 301 tggacgttcg gaggcgggac aaaacttgag atcaagcgca cagttgctgc cccagcgtg
 40 361 ttcatthttcc cacctagcga tgagcagctg aaaagcggta ctgcctctgt cgtatgcttg
 421 ctcaacaact ttaccacacg tgaggctaag gtgcagtgga aagtggataa tgcacttcaa
 481 tctggaaaca gtcaagagtc cgtgacagaa caggacagca aagactcaac ttattcactc
 541 tcttccaccc tgactctgtc caaggcagac tatgaaaaac acaaggtata cgctgcgag
 601 gttacacacc aggtttgtc tagtctgtc accaagtcct tcaatagggg cgaatgt

[00236] Protein Sequence Defining the Full Length Ch4F11 Chimeric Light Chain (Mouse
Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO:86)

1 dvvmtqtpls lpvslgdqas iscrssqslv htngntylhw ylqkpgqspk lliykvsnrff
 61 sgvpdrfsgs gsgtdftlki srveaedlgv yfcsqsthvp wtfgggkile ikrtvaapsv
 50 121 fifppsdeql ksgrtasvvc lnnfybreak vqwkvdnalq sgnsqesvte qdskdstysl
 181 sstltlskad yekhkvyace vthqglsspv tksfnrgec

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[00237] Nucleic Acid Sequence Encoding the Full Length Hu4F11 Kv2D-29 Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region (Version 1))
(SEQ ID NO:87)

```

5      1 gatgtagtca tgacccaaac gccgctttcg ttgtcgggtga cgcccgagaca gcccgcggtca
      61 atctcgtgtc ggtcatcgca gtcggttggtta cacacaaaacg gtaatacgtta tctccattgg
121   121 tatctccaga agcccgagcca gtcgcccagcag ctcttgatct acaaagttag caatcgcttt
181   181 tcggggggtgc cggatcggtt ctccgggatcg gggtcaggaa cggacttcac gcttaagatt
241   241 tcgaggggtcg aggcggagga tgcgggagtc tacttttggt cgcagtcacac acatgtcccc
10    301 tggacgcttg ggcaggggac gaaggtggaa atcaagcgca cagttgctgc cccagcgtg
      361 ttcattttcc cacctagcga tgagcagctg aaaagcggta ctgcctctgt cgtatgcttg
      421 ctcaacaact ttaccacacg tgaggctaag gtgcagtggg aagtggataa tgcacttcaa
      481 tctggaaaca gtcaagagtc cgtgacagaa caggacagca aagactcaac ttattcactc
      541 tcttccaccc tgactctgtc caaggcagac tatgaaaaac acaaggtata cgctgagag
15    601 gttacacacc agggtttgtc tagtctgtc accaagtcct tcaatagggg cgaatgt

```

[00238] Protein Sequence Defining the Full Length Hu4F11 Kv2D-29 Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID
 NO:88)

```

20    1 dvvmtqtpls lsvtpgqpas iscrssqslv htngntylhw ylkpggspq lliykvsnrff
      61 sgvpdrfsgs gsgtdftlki srveaedvgv yfcsqsthvp wtfqggtkve ikrtvaapsv
121   121 fifppsdeql ksgtasvvcl lnnfybreak vqwkvdnalq sgnsqesvte qdskdstysl
181   181 sstltlskad yekhkvyace vthqglsspv tksfnrgec

```

25 [00239] Nucleic Acid Sequence Encoding the Full Length Hu4F11 Kv2D-29 N28H Light
Chain (Humanized Kappa Chain Variable Region and Human Kappa Constant Region (Version
2)) (SEQ ID NO:89)

```

30    1 gatgtagtca tgacccaaac gccgctttcg ttgtcgggtga cgcccgagaca gcccgcggtca
      61 atctcgtgtc ggtcatcgca gtcggttggtta cacacacacg gtaatacgtta tctccattgg
121   121 tatctccaga agcccgagcca gtcgcccagcag ctcttgatct acaaagttag caatcgcttt
181   181 tcggggggtgc cggatcggtt ctccgggatcg gggtcaggaa cggacttcac gcttaagatt
241   241 tcgaggggtcg aggcggagga tgcgggagtc tacttttggt cgcagtcacac acatgtcccc
301   301 tggacgcttg ggcaggggac gaaggtggaa atcaagcgca cagttgcagc cccagcgtg
361   361 ttcattttcc cacctagcga tgagcagctg aaaagcggta ctgcctctgt cgtatgcttg
35    421 ctcaacaact ttaccacacg tgaggctaag gtgcagtggg aagtggataa tgcacttcaa
      481 tctggaaaca gtcaagagtc cgtgacagaa caggacagca aagactcaac ttattcactc
      541 tcttccaccc tgactctgtc caaggcagac tatgaaaaac acaaggtata cgctgagag
601   601 gttacacacc agggtttgtc tagtctgtc accaagtcct tcaatagggg cgaatgt

```

40 [00240] Protein Sequence Defining the Full Length Hu4F11 Kv2D-29 N28H Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID
 NO:90)

```

45    1 dvvmtqtpls lsvtpgqpas iscrssqslv htngntylhw ylkpggspq lliykvsnrff
      61 sgvpdrfsgs gsgtdftlki srveaedvgv yfcsqsthvp wtfqggtkve ikrtvaapsv
121   121 fifppsdeql ksgtasvvcl lnnfybreak vqwkvdnalq sgnsqesvte qdskdstysl
181   181 sstltlskad yekhkvyace vthqglsspv tksfnrgec

```

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[00241] Nucleic Acid Sequence Encoding the Full Length Hu4F11 Kv2D-29 N28Q Light Chain (Humanized Kappa Chain Variable Region and Human Kappa Constant Region (Version 2)) (SEQ ID NO:91)

```

5      1 gatgtagtca tgacccaaac gccgctttcg ttgtcgggtga cgcccggaca gcccgcggtca
      61 atctcgtgtc ggtcatcgca gtcggttggtta cacacacaag gtaatacgta tctccattgg
      121 tatctccaga agcccggcca gtcgccgcag ctcttgatct acaaagttag caatcgcttt
      181 tcggggggtgc cggatcggtt ctccgggatcg gggtcaggaa cggacttcac gcttaagatt
      241 tcgaggggtcg aggcggagga tgcgggagtc tacttttggt cgcagtccac acatgtcccc
10    301 tggacgtttg ggcaggggac gaaggtggaa atcaagcgca cagttgcagc cccagcgtg
      361 ttcattttcc cacctagcga tgagcagctg aaaagcggta ctgcctctgt cgtatgcttg
      421 ctcaacaact ttaccacacg tgaggctaag gtgcagtggg aagtggataa tgcacttcaa
      481 tctggaaaca gtcaagagtc cgtgacagaa caggacagca aagactcaac ttattcactc
      541 tcttccaccc tgactctgtc caaggcagac tatgaaaaac acaaggtata cgctgcgag
15    601 gttacacacc agggtttgtc tagtcctgtc accaagtcct tcaatagggg cgaatgt

```

[00242] Protein Sequence Defining the Full Length Hu4F11 Kv2D-29 N28Q Light Chain (Humanized Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO:92)

```

20    1 dvvmtqtpls lsvtpgqpas iscrssqslv htgntylhw ylqkpgqspq lliykvsnrff
      61 sgvpdrfsgs gsgtdftlki srveaedvgv yfcsqsthvp wtfqggtkve ikrtvaapsv
      121 fifppsdeql ksgtasvvcl lnnfybreak vqwkvdnalq sgnsqesvte qdskdstysl
      181 sstltlskad yekhkvyace vthqglsspv tksfnrgec

```

25 [00243] Nucleic Acid Sequence Encoding the Full Length Hu4F11 Kv2D-29 N28Y Light Chain (Humanized Kappa Chain Variable Region and Human Kappa Constant Region (Version 2)) (SEQ ID NO:93)

```

      1 gatgtagtca tgacccaaac gccgctttcg ttgtcgggtga cgcccggaca gcccgcggtca
      61 atctcgtgtc ggtcatcgca gtcggttggtta cacacatacg gtaatacgta tctccattgg
30    121 tatctccaga agcccggcca gtcgccgcag ctcttgatct acaaagttag caatcgcttt
      181 tcggggggtgc cggatcggtt ctccgggatcg gggtcaggaa cggacttcac gcttaagatt
      241 tcgaggggtcg aggcggagga tgcgggagtc tacttttggt cgcagtccac acatgtcccc
      301 tggacgtttg ggcaggggac gaaggtggaa atcaagcgca cagttgcagc cccagcgtg
      361 ttcattttcc cacctagcga tgagcagctg aaaagcggta ctgcctctgt cgtatgcttg
35    421 ctcaacaact ttaccacacg tgaggctaag gtgcagtggg aagtggataa tgcacttcaa
      481 tctggaaaca gtcaagagtc cgtgacagaa caggacagca aagactcaac ttattcactc
      541 tcttccaccc tgactctgtc caaggcagac tatgaaaaac acaaggtata cgctgcgag
      601 gttacacacc agggtttgtc tagtcctgtc accaagtcct tcaatagggg cgaatgt

```

40 [00244] Protein Sequence Defining the Full Length Hu4F11 Kv2D-29 N28Y Light Chain (Humanized Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO:94)

```

      1 dvvmtqtpls lsvtpgqpas iscrssqslv htygntylhw ylqkpgqspq lliykvsnrff
      61 sgvpdrfsgs gsgtdftlki srveaedvgv yfcsqsthvp wtfqggtkve ikrtvaapsv
45    121 fifppsdeql ksgtasvvcl lnnfybreak vqwkvdnalq sgnsqesvte qdskdstysl
      181 sstltlskad yekhkvyace vthqglsspv tksfnrgec

```

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[00245] Table 12 is a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.

Table 12

SEQ ID NO.	Nucleic Acid or Protein
68	Human IgG1 constant—nucleic acid
69	Human IgG1 constant—protein
70	Human Kappa constant—nucleic acid (version 1)
71	Human Kappa constant—nucleic acid (version 2)
72	Human Kappa constant—protein (nucleic acid versions 1 and 2 encode the same amino acid sequence)
73	Chimeric Ch4F11 Heavy Variable + Human IgG1 constant—nucleic acid
74	Chimeric Ch4F11 Heavy Variable + Human IgG1 constant—protein
75	Humanized Sh4F11 Hv3-23 Heavy Human Variable + Human IgG1 constant—nucleic acid
76	Humanized Sh4F11 Hv3-23 Heavy Human Variable + Human IgG1 constant—protein
77	Humanized Sh4F11 Hv3-23 A28T S31H T62S Heavy Human Variable + Human IgG1 constant—nucleic acid
78	Humanized Sh4F11 Hv3-23 A28T S31H T62S Heavy Human Variable + Human IgG1 constant—protein
79	Humanized Sh4F11 Hv3-23 S31H T62S Heavy Human Variable + Human IgG1 constant—nucleic acid
80	Humanized Sh4F11 Hv3-23 S31H T62S Heavy Human Variable + Human IgG1 constant—protein
81	Humanized Sh4F11 Hv3-23 A28T S31N T62S Heavy Human Variable + Human IgG1 constant—nucleic acid
82	Humanized Sh4F11 Hv3-23 A28T S31N T62S Heavy Human Variable + Human IgG1 constant—protein
83	Humanized Sh4F11 Hv3-23 A28T T62S Heavy Human Variable + Human IgG1 constant—nucleic acid
84	Humanized Sh4F11 Hv3-23 A28T T62S Heavy Human Variable + Human IgG1 constant—protein
85	Chimeric Ch4F11 Kappa Variable + Human Kappa constant—nucleic acid (version 1)
86	Chimeric Ch4F11 Kappa Variable + Human Kappa constant—protein
87	Humanized Hu4F11 Kv2D-29 Kappa Human Variable + Human Kappa constant—nucleic acid (version 1)
88	Humanized Hu4F11 Kv2D-29 Kappa Human Variable + Human Kappa constant—protein
89	Humanized Hu4F11 Kv2D-29 N28H Kappa Human Variable + Human Kappa constant—nucleic acid (version 2)
90	Humanized Hu4F11 Kv2D-29 N28H Kappa Human Variable + Human Kappa constant—protein
91	Humanized Hu4F11 Kv2D-29 N28Q Kappa Human Variable + Human Kappa constant—nucleic acid (version 2)
92	Humanized Hu4F11 Kv2D-29 N28Q Kappa Human Variable + Human Kappa constant—protein

Table 12 continued

SEQ ID NO.	Nucleic Acid or Protein
93	Humanized Hu4F11 Kv2D-29 N28Y Kappa Human Variable + Human Kappa constant—nucleic acid (version 2)
94	Humanized Hu4F11 Kv2D-29 N28Y Kappa Human Variable + Human Kappa constant—protein

[00246] Table 13 below shows antibodies containing chimeric immunoglobulin heavy and light chains and exemplary combinations of the full-length chimeric or humanized

5 immunoglobulin heavy and light chains.

Table 13

Antibody Name	Heavy Chain	Light Chain
Hu4F11-1	Ch4F11 Chimeric Heavy Variable + Human IgG1 constant (SEQ ID NO:74)	Ch4F11 Chimeric Kappa Variable + Human Kappa constant (SEQ ID NO:86)
Hu4F11-10	Humanized Sh4F11 Hv3-23 Heavy Human Variable + Human IgG1 constant (SEQ ID NO:76)	Ch4F11 Chimeric Kappa Variable + Human Kappa constant (SEQ ID NO:86)
Hu4F11-18	Ch4F11 Chimeric Heavy Variable + Human IgG1 constant (SEQ ID NO:74)	Humanized Hu4F11 Kv2D-29 Human Variable + Human Kappa constant (SEQ ID NO:88)
Hu4F11-32	Humanized Sh4F11 Hv3-23 Heavy Human Variable + Human IgG1 constant (SEQ ID NO:76)	Humanized Hu4F11 Kv2D-29 Human Kappa Variable + Human Kappa constant (SEQ ID NO:88)
Hu4F11-69	Humanized Sh4F11 Hv3-23 A28T S31N T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:82)	Humanized Hu4F11 Kv2D-29 N28H Human Kappa Variable + Human Kappa constant (SEQ ID NO:90)
Hu4F11-70	Humanized Sh4F11 Hv3-23 A28T S31H T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:78)	Humanized Hu4F11 Kv2D-29 N28H Human Kappa Variable + Human Kappa constant (SEQ ID NO:90)
Hu4F11-71	Humanized Sh4F11 Hv3-23 A28T T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:84)	Humanized Hu4F11 Kv2D-29 N28H Human Kappa Variable + Human Kappa constant (SEQ ID NO:90)
Hu4F11-72	Humanized Sh4F11 Hv3-23 S31H T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:80)	Humanized Hu4F11 Kv2D-29 N28H Human Kappa Variable + Human Kappa constant (SEQ ID NO:90)
Hu4F11-73	Humanized Sh4F11 Hv3-23 A28T S31N T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:82)	Humanized Hu4F11 Kv2D-29 N28Y Human Kappa Variable + Human Kappa constant (SEQ ID NO:94)

Table 13 continued

Antibody Name	Heavy Chain	Light Chain
Hu4F11-74	Humanized Sh4F11 Hv3-23 A28T S31H T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:78)	Humanized Hu4F11 Kv2D-29 N28Y Human Kappa Variable + Human Kappa constant (SEQ ID NO:94)
Hu4F11-75	Humanized Sh4F11 Hv3-23 A28T T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:84)	Humanized Hu4F11 Kv2D-29 N28Y Human Kappa Variable + Human Kappa constant (SEQ ID NO:94)
Hu4F11-76	Humanized Sh4F11 Hv3-23 S31H T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:80)	Humanized Hu4F11 Kv2D-29 N28Y Human Kappa Variable + Human Kappa constant (SEQ ID NO:94)
Hu4F11-77	Humanized Sh4F11 Hv3-23 A28T S31N T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:82)	Humanized Hu4F11 Kv2D-29 N28Q Human Kappa Variable + Human Kappa constant (SEQ ID NO:92)
Hu4F11-78	Humanized Sh4F11 Hv3-23 A28T S31H T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:78)	Humanized Hu4F11 Kv2D-29 N28Q Human Kappa Variable + Human Kappa constant (SEQ ID NO:92)
Hu4F11-79	Humanized Sh4F11 Hv3-23 A28T T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:84)	Humanized Hu4F11 Kv2D-29 N28Q Human Kappa Variable + Human Kappa constant (SEQ ID NO:92)
Hu4F11-80	Humanized Sh4F11 Hv3-23 S31H T62S Heavy Human Variable + Human IgG1 constant (SEQ ID NO:80)	Humanized Hu4F11 Kv2D-29 N28Q Human Kappa Variable + Human Kappa constant (SEQ ID NO:92)

[00247] The antibody construct containing the full length chimeric heavy and light chains is designated below:

- 5 **Chimeric 4F11 (Hu4F11-1)** = Full Length Ch4F11 Chimeric Heavy Chain (Mouse Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO:74) plus Full Length Ch4F11 Chimeric Light Chain (Mouse Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO:86)

- [00248] Thirteen of the possible antibody constructs containing the full length
10 immunoglobulin heavy and light chains containing humanized variable regions are designated below:

Hu4F11-32 = Full Length Sh4F11 Hv3-23 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO:76) plus Full

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Length Hu4F11 Kv2D-29 Light Chain (Humanized Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO:88)

5 **Hu4F11-69** = Full Length Sh4F11 Hv3-23 A28T S31N T62S_Heavy Chain
(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)
(SEQ ID NO:82) plus Full Length Hu4F11 Kv2D-29 N28H Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region)
(SEQ ID NO:90)

10 **Hu4F11-70** = Full Length Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain
(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)
(SEQ ID NO:78) plus Full Length Hu4F11 Kv2D-29 N28H Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region)
(SEQ ID NO:90)

15 **Hu4F11-71** = Full Length Sh4F11 Hv3-23 A28T T62S Heavy Chain (Humanized
Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID
NO:84) plus Full Length Hu4F11 Kv2D-29 N28H Light Chain (Humanized
Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID
NO:90)

20 **Hu4F11-72** = Full Length Sh4F11 Hv3-23 S31H T62S Heavy Chain (Humanized
Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID
NO:80) plus Full Length Hu4F11 Kv2D-29 N28H Light Chain (Humanized
Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID
NO:90)

25 **Hu4F11-73** = Full Length Sh4F11 Hv3-23 A28T S31N T62S_Heavy Chain
(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)
(SEQ ID NO:82) plus Full Length Hu4F11 Kv2D-29 N28Y Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region)
(SEQ ID NO:94)

30 **Hu4F11-74** = Full Length Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain
(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)
(SEQ ID NO:78) plus Full Length Hu4F11 Kv2D-29 N28Y Light Chain

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(Humanized Kappa Chain Variable Region and Human Kappa Constant Region)
(SEQ ID NO:94)

5 **Hu4F11-75** = Full Length Sh4F11 Hv3-23 A28T T62S Heavy Chain (Humanized
Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID
NO:84) plus Full Length Hu4F11 Kv2D-29 N28Y Light Chain (Humanized
Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID
NO:94)

10 **Hu4F11-76** = Full Length Sh4F11 Hv3-23 S31H T62S Heavy Chain (Humanized
Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID
NO:80) plus Full Length Hu4F11 Kv2D-29 N28Y Light Chain (Humanized
Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID
NO:94)

15 **Hu4F11-77** = Full Length Sh4F11 Hv3-23 A28T S31N T62S Heavy Chain
(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)
(SEQ ID NO:82) plus Full Length Hu4F11 Kv2D-29 N28Q Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region)
(SEQ ID NO:92)

20 **Hu4F11-78** = Full Length Sh4F11 Hv3-23 A28T S31H T62S Heavy Chain
(Humanized Heavy Chain Variable Region and Human IgG1 Constant Region)
(SEQ ID NO:78) plus Full Length Hu4F11 Kv2D-29 N28Q Light Chain
(Humanized Kappa Chain Variable Region and Human Kappa Constant Region)
(SEQ ID NO:92)

25 **Hu4F11-79** = Full Length Sh4F11 Hv3-23 A28T T62S Heavy Chain (Humanized
Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID
NO:84) plus Full Length Hu4F11 Kv2D-29 N28Q Light Chain (Humanized
Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID
NO:92)

30 **Hu4F11-80** = Full Length Sh4F11 Hv3-23 S31H T62S Heavy Chain (Humanized
Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID
NO:80) plus Full Length Hu4F11 Kv2D-29 N28Q Light Chain (Humanized

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Kappa Chain Variable Region and Human Kappa Constant Region) (SEQ ID NO:92)

Example 9: Binding Affinities of Humanized and Chimeric Anti-Notch3 Monoclonal Antibodies

5 [00249] The binding affinities and kinetics of binding of chimeric and humanized antibodies to monomeric recombinant human Notch3 extracellular domain (containing EGF like domains 1-11) fusion protein (monomeric rhNotch3) and monomeric recombinant cynomolgus monkey Notch3 extracellular domain (containing EGF like domains 1-12) fusion protein (monomeric rcNotch3) were measured by surface plasmon resonance, using a BIAcore[®] T100 instrument
10 (GE Healthcare, Piscataway, NJ).

[00250] Goat anti-human IgGs (Fc fragment specific, Jackson ImmunoResearch, West Grove, PA) were immobilized on carboxymethylated dextran CM4 sensor chips by amine coupling, according to a standard protocol. Analyses were performed at 25 and 37°C using PBS containing 0.05% surfactant P20 as running buffer. The antibodies were captured in
15 individual flow cells at a flow rate of 10 µL/minute. Injection time was varied for each antibody to yield an R_{max} between 30 and 60 RU. Buffer or Notch3 protein diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 240 seconds at 60 µL/minute. The dissociation phase was monitored for up to 900 seconds. The surface was then regenerated with two 60-second
20 injections of 10 mM Glycine-HCl, pH 2.25, at a flow rate of 30 µL/minute. The Notch3 concentration range tested was 50 nM to 6.25 nM.

[00251] Kinetic parameters were determined using the kinetic function of the BIAevaluation software (GE Healthcare) with double reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant), and K_D (equilibrium
25 dissociation constant) were determined. Kinetic values of purified monoclonal antibodies on monomeric rcNotch3 at 25°C are summarized in **Table 14**.

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Table 14

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	n
Hu4F11-1	4.8E+05	1.0E-02	2.4E-08	5
Hu4F11-32	7.9E+05	7.5E-03	1.8E-08	8
Hu4F11-69	1.2E+05	9.4E-04	7.7E-09	1
Hu4F11-70	2.0E+05	1.0E-03	5.2E-09	1
Hu4F11-71	1.5E+06	4.0E-03	2.7E-09	1
Hu4F11-72	2.8E+06	4.5E-03	1.6E-09	1
Hu4F11-73	7.6E+05	1.9E-03	2.6E-09	1
Hu4F11-74	9.0E+05	2.5E-03	2.8E-09	1
Hu4F11-75	7.2E+05	2.6E-03	3.7E-09	1
Hu4F11-76	7.8E+05	1.9E-03	2.5E-09	1
Hu4F11-77	9.6E+05	2.5E-03	2.6E-09	1
Hu4F11-78	1.8E+06	3.8E-03	2.2E-09	1
Hu4F11-79	1.3E+06	4.6E-03	3.4E-09	1
Hu4F11-80	8.2E+05	3.2E-03	4.0E-09	1

[00252] Additional kinetic measurements were conducted for each antibody shown in **Table 14**. These measurements confirmed that the antibodies have affinities ranging from about 1nM to about 25 nM for monomeric rcNotch3 at 25°C.

[00253] Kinetic values of purified monoclonal antibodies on monomeric rcNotch3 at 37°C are summarized in **Table 15**.

Table 15

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	n
Hu4F11-32	5.0E+05	1.6E-02	3.2E-08	5
Hu4F11-69	4.0E+05	3.8E-03	1.1E-08	3
Hu4F11-70	3.7E+05	2.9E-03	9.1E-09	3
Hu4F11-71	4.1E+05	5.6E-03	1.4E-08	3
Hu4F11-72	4.4E+05	3.2E-03	9.8E-09	3
Hu4F11-73	5.0E+05	6.3E-03	1.5E-08	3
Hu4F11-74	4.4E+05	4.5E-03	1.2E-08	3
Hu4F11-75	3.8E+05	8.9E-03	2.4E-08	3
Hu4F11-76	5.4E+05	6.2E-03	1.2E-08	3
Hu4F11-77	9.1E+05	1.0E-02	1.2E-08	3
Hu4F11-78	6.6E+05	7.0E-03	1.1E-08	3
Hu4F11-79	9.8E+05	2.1E-02	2.3E-08	3
Hu4F11-80	9.1E+05	1.0E-02	1.2E-08	3

[00254] Additional kinetic measurements were conducted for each antibody shown in **Table 15**. These measurements confirmed that the antibodies have affinities ranging from about 9 nM to about 35 nM for monomeric rcNotch3 at 37°C.

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[00255] Kinetic values of purified monoclonal antibodies on monomeric rhNotch3 at 37°C are summarized in **Table 16**.

Table 16

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	n
Hu4F11-32	1.7E+06	4.6E-03	2.7E-09	1
Hu4F11-69	1.3E+06	3.8E-03	2.9E-09	1
Hu4F11-70	9.4E+05	2.8E-03	3.0E-09	1
Hu4F11-71	1.2E+06	3.7E-03	3.0E-09	1
Hu4F11-72	1.8E+05	9.4E-04	5.2E-09	1
Hu4F11-73	2.1E+05	1.3E-03	5.9E-09	1
Hu4F11-74	5.6E+05	2.2E-03	3.9E-09	1
Hu4F11-75	2.7E+06	9.0E-03	3.4E-09	1
Hu4F11-76	1.7E+06	5.5E-03	3.3E-09	1
Hu4F11-77	2.2E+06	7.3E-03	3.3E-09	1
Hu4F11-78	1.5E+06	5.5E-03	3.8E-09	1
Hu4F11-79	1.8E+06	6.0E-03	3.2E-09	1
Hu4F11-80	1.1E+06	4.1E-03	3.8E-09	1

- 5 [00256] Additional kinetic measurements were conducted for each antibody shown in **Table 16**. The additional measurements were averaged together with those presented in **Table 16**. These combined measurements indicated that the antibodies have affinities ranging from about 7 nM to about 16 nM for monomeric rhNotch3 at 37°C (**Table 17**).

Table 17

Antibody	k_a (1/Ms)	k_d (1/s)	K_D (M)	n
Hu4F11-32	7.4E+06	8.4E-02	7.6E-09	2
Hu4F11-69	7.1E+05	3.7E-03	8.5E-09	3
Hu4F11-70	6.9E+05	3.9E-03	7.5E-09	4
Hu4F11-71	1.1E+06	9.5E-03	9.2E-09	3
Hu4F11-72	7.1E+05	6.9E-03	7.0E-09	4
Hu4F11-73	4.9E+05	4.6E-03	1.1E-08	3
Hu4F11-74	4.9E+05	5.1E-03	1.0E-08	4
Hu4F11-75	1.7E+06	1.3E-02	1.0E-08	3
Hu4F11-76	9.8E+05	6.4E-03	8.2E-09	3
Hu4F11-77	1.2E+06	7.4E-03	8.6E-09	3
Hu4F11-78	8.2E+05	6.0E-03	9.0E-09	4
Hu4F11-79	9.0E+05	9.1E-03	1.6E-08	3
Hu4F11-80	5.9E+05	5.6E-03	1.4E-08	3

10

[00257] The results in **Tables 14-17** demonstrate that the chimeric and each of the humanized antibodies, have fast association rates (k_a), very slow disassociation rates (k_d) and

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very high affinities (K_D). In particular, the antibodies have affinities ranging from about 1 nM to about 35 nM.

Example 10: Binding of Humanized 4F11 Antibodies to Cell Surface-Expressed Notch3

[00258] Binding of humanized 4F11 variants to cell surface Notch3 receptor was confirmed
5 by FACS. FlpIn™ CHO lines stably expressing human Notch3, or FlpIn™ CHO lines
expressing no human Notch receptors, were used for binding experiments. CHO cells were
harvested using Cell Dissociation Buffer (Life Technologies, Grand Island, NY) and
resuspended at 2.5×10^6 cells/ml in PBS/0.5% BSA. 100 μ l of cell suspension per sample was
added to a 96 well v-bottom plate. 4F11 humanized antibodies or human IgG were added at 5
10 μ g/ml to the wells, mixed, and incubated on ice for one hour. After washing with PBS/0.5%
BSA, anti-human PE conjugated secondary antibody was added at a 1:100 dilution in
PBS/0.5% BSA and allowed to incubate on ice in the dark for 30 minutes. Cells were washed
with PBS/0.5% BSA, then resuspended in 300ul PBS/0.5% BSA and FACS analysis was
performed. Expression of human Notch3 receptor was confirmed using anti-human Notch3 PE
15 (BioLegend, San Diego, CA) as a positive control. FACS analysis confirmed that the
humanized 4F11 antibody variants bind to human Notch3 expressed on the cell surface.

Example 11: Inhibition of Ligand-Induced Notch3 ICD Cleavage by Humanized 4F11 Antibodies

[00259] Selected humanized 4F11 variants were tested for their ability to inhibit ligand-
20 induced activation of Notch3, as measured by the presence of cleaved ICD. The MDA-MB-468
breast cancer cell line, which expresses endogenous human Notch3, was plated in 96-well
plates previously coated with hJag2-mFc fusion protein. The wells were prepared by diluting
 α -mouse Fc (Jackson ImmunoResearch, West Grove, PA) to 5 μ g/ml in sterile-filtered
carbonate-bicarbonate coating buffer, pH 9.4 (Thermo Fisher Scientific, Rockland, MD). Then
25 100 μ l 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/0.5% BSA
before adding 100 μ l of soluble Jag2-mFc fusion protein or mouse IgG Fc (Jackson
Immunolabs) diluted to 5 μ g/ml 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/0.5% BSA to
30 remove unbound ligand. MDA-MB-468 cells were counted and resuspended in fresh growth
media at 0.4×10^6 cells/ml. Cells were pre-incubated with 10 μ g/ml of the 4F11 humanized

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antibodies for 30 minutes at 37°C, before seeding 100 µl of the suspension into 96-well plates coated with hJag2-mFc ligand or mFc. Cells were incubated overnight at 37°C. The next day wells were gently washed with ice cold PBS, then cells harvested by adding RIPA buffer containing protease inhibitors directly to the well. Lysates were clarified by centrifugation in a refrigerated microcentrifuge. Supernatants were boiled with 5X SDS sample buffer before loading onto an SDS PAGE gel and Western blotting. Blots were probed with a α -Notch3 antibody to detect the cleaved intracellular domain (Cell Signaling, Danvers, MA). The same blots were also probed with anti- β tubulin (Cell Signaling) for use as a loading control. Bands were quantitated using ImageLab software (BioRad, Hercules, CA) and values adjusted relative to their respective loading control. Each sample was then normalized relative to the value of the Notch3 ICD band from cells plated on wells without ligand in the presence of hIgG control antibody.

[00260] The humanized 4F11 antibody variants significantly inhibit ligand-induced cleavage of the Notch3 ICD as summarized in FIG. 11.

Example 12: Inhibition of Notch3-Dependent Transcription by Humanized 4F11 Antibodies

[00261] Luciferase reporter assays were used to assess the ability of certain humanized 4F11 antibodies to inhibit ligand-dependent Notch3 receptor signaling and transcriptional activity. HCC1143 reporter lines co-cultured with CHO cells expressing Notch ligands were utilized for these assays.

[00262] Stable lines expressing full length Notch ligands at the cell surface were produced by transfecting FlpIn™ CHO cells with Jag1, Jag2, DLL1, or DLL4 cDNAs cloned into the pcDNA5FRT vector using Lipofectamine 2000 (Life Technologies) according to the manufacturer's protocol. Twenty-four hours after transfection, CHO cells were split into F12 media containing 10% FBS, 2 mM L-Glutamine and 700 µg/ml hygromycin B (Sigma-Aldrich, St. Louis, MO) to select for transfected cells. Expression of Notch ligands was confirmed by FACS analysis using antibodies against Jag1 (R&D Systems, Inc., Minneapolis, MN), Jag2 (R&D Systems), DLL1 (R&D Systems), and DLL4 (BioLegend, San Diego, CA).

[00263] FlpIn™ CHO lines stably expressing Notch ligands were trypsinized, counted and seeded in 96-well plates at 60K cells/well in 100 µl of Ham's F12 K media without hygromycin B. The next day, HCC1143 reporter cells were spun down and resuspended in

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RPMI media containing 2% FBS at 0.2×10^6 cells/ml. Antibodies were serially diluted into RPMI without FBS, and 100 μ l of antibody solution was added to an equal volume of reporter cells for 30 minutes at 37°C in a 5% CO₂ incubator. After removing media from the hJag2 CHO cells, 100 μ l of the HCC1143 reporter/antibody mix was added to the ligand-expressing cells and allowed to incubate at 37°C overnight. Twenty-four hours later, 96-well plates placed at room temperature for 20 minutes, then processed with the Bright Glo (Promega, Madison, WI) reporter assay protocol per the manufacturer's instructions. Lysates were transferred to white-walled 96 well plates (Greiner Bio-One, Frickenhausen, Germany) and read on a GloMax Luminometer (Promega) using the Bright Glo program.

- 10 [00264] As shown in **FIG. 12A-B**, the humanized 4F11 antibody variants inhibit activation of ligand-induced transcription in the HCC1143 reporter cell line.

Example 13: Inhibition of Notch3 ICD Cleavage *In Vivo*

- 15 [00265] Selected humanized 4F11 antibodies (*i.e.*, Hu4F11-70, Hu4F11-72, Hu4F11-78) were tested for their ability to inhibit cleavage of Notch3 ICD expressed in HCC2429 tumors *in vivo*.

- 20 [00266] All mice were treated in accordance with the OLAW Public Health Service Policy on Human Care and Use of Laboratory Animals and the ILAR Guide for the Care and Use of Laboratory Animals. All *in vivo* studies were conducted following the protocols approved by the AVEO Institutional Animal Care and Use Committee. HCC2429 is a lung cancer cell line harboring a translocation of chromosome 19 that results in overexpression of the Notch3 receptor. For the Notch3 ICD cleavage experiments, approximately thirteen week old NCR nude mice (Taconic, Germantown, NY) were inoculated subcutaneously into the right flank with 5×10^6 cells in 1:1 DMEM + Matrigel (Invitrogen, Carlsbad, CA)/Matrigel (BD Biosciences, San Jose, CA). Tumor measurements were taken twice weekly, using vernier calipers. Tumor volume was calculated using the formula: $V = 0.5 \times \text{width} \times \text{width} \times \text{length}$. When tumors approached a volume of 300-400 mm³, mice were randomized into groups of three animals each (corresponds to mouse 1 (m1) through mouse 3 (m3), as designated in **FIG. 13**). The next day, mice were treated with 20 mg/kg hIgG (control), or 20 mg/kg of antibodies Hu4F11-70, Hu4F11-72, Hu4F11-78 or murine 4F11 (mu4F11) by intraperitoneal injection.
- 30 Mice were dosed once, and tumors were collected 24 hours later and snap frozen.

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[00267] To assess levels of cleaved Notch3 ICD, tumors were pulverized using a Covaris cryoPREP™ impactor (Covaris, Woburn, MA), resuspended in RIPA buffer (Boston

BioProducts) containing protease inhibitors, and rotated at 4°C for 1 hour. Lysates were

5 clarified by spinning at 14k rpm for 15 minutes in a refrigerated microcentrifuge. Protein

concentration for each sample was measured using the BioRad DC protein assay (BioRad).

Equal concentrations of protein from each sample were loaded onto an SDS PAGE gel and

transferred onto nitrocellulose by Western blotting. Blots were probed with antibody against

10 the C-terminus of Notch3 (Cell Signaling) to detect levels of the cleaved ICD. Blots were also

probed with antibody against β -tubulin (Cell Signaling) for use as a loading control. Bands

were quantified using Image Lab 3.0 software (BioRad), and the intensity of Notch3 ICD bands

were normalized to their respective β -tubulin loading control. All humanized 4F11 antibodies

tested (*i.e.*, Hu4F11-70, Hu4F11-72, Hu4F11-78) significantly inhibit Notch3 activation *in*

vivo, as measured by Notch3 ICD levels present in tumors 24 hours after single dose antibody

treatment (FIG. 13).

INCORPORATION BY REFERENCE

15 [00268] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[00269] 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.

20 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.

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WHAT IS CLAIMED IS:

1 1. An isolated antibody that binds human Notch3 comprising an immunoglobulin heavy chain
2 variable region and an immunoglobulin light chain variable region selected from the group
3 consisting of:

4 (a) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
5 comprising the amino acid sequence of SEQ ID NO:51, a CDR_{H2} comprising the
6 amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino
7 acid sequence of SEQ ID NO:7; and

8 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
9 comprising the amino acid sequence of SEQ ID NO:62, a CDR_{L2} comprising the
10 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
11 sequence of SEQ ID NO:10;

12 (b) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
13 comprising an amino acid sequence selected from the group consisting of SEQ
14 ID NO: 5 and SEQ ID NO:11; a CDR_{H2} comprising the amino acid sequence of
15 SEQ ID NO: 6; and a CDR_{H3} comprising the amino acid sequence of SEQ ID
16 NO: 7; and

17 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
18 comprising the amino acid sequence of SEQ ID NO: 8; a CDR_{L2} comprising the
19 amino acid sequence of SEQ ID NO: 9; and a CDR_{L3} comprising the amino acid
20 sequence of SEQ ID NO: 10;

21 (c) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
22 comprising an amino acid sequence selected from the group consisting of SEQ
23 ID NO: 5 and SEQ ID NO:11; a CDR_{H2} comprising the amino acid sequence of
24 SEQ ID NO: 6; and a CDR_{H3} comprising the amino acid sequence of SEQ ID
25 NO: 7; and

26 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
27 comprising the amino acid sequence of SEQ ID NO: 62; a CDR_{L2} comprising
28 the amino acid sequence of SEQ ID NO: 9; and a CDR_{L3} comprising the amino
29 acid sequence of SEQ ID NO: 10;

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- 30 (d) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
31 comprising an amino acid sequence selected from the group consisting of SEQ
32 ID NO: 5 and SEQ ID NO:11; a CDR_{H2} comprising the amino acid sequence of
33 SEQ ID NO: 6; and a CDR_{H3} comprising the amino acid sequence of SEQ ID
34 NO: 7; and
- 35 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
36 comprising the amino acid sequence of SEQ ID NO: 63; a CDR_{L2} comprising
37 the amino acid sequence of SEQ ID NO: 9; and a CDR_{L3} comprising the amino
38 acid sequence of SEQ ID NO: 10;
- 39 (e) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
40 comprising an amino acid sequence selected from the group consisting of SEQ
41 ID NO: 5 and SEQ ID NO:11; a CDR_{H2} comprising the amino acid sequence of
42 SEQ ID NO: 6; and a CDR_{H3} comprising the amino acid sequence of SEQ ID
43 NO: 7; and
- 44 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
45 comprising the amino acid sequence of SEQ ID NO: 64; a CDR_{L2} comprising
46 the amino acid sequence of SEQ ID NO: 9; and a CDR_{L3} comprising the amino
47 acid sequence of SEQ ID NO: 10;
- 48 (f) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
49 comprising the amino acid sequence of SEQ ID NO:51, a CDR_{H2} comprising the
50 amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino
51 acid sequence of SEQ ID NO:7; and
- 52 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
53 comprising the amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the
54 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
55 sequence of SEQ ID NO:10;
- 56 (g) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
57 comprising the amino acid sequence of SEQ ID NO:51, a CDR_{H2} comprising the
58 amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino
59 acid sequence of SEQ ID NO:7; and
- 60 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
61 comprising the amino acid sequence of SEQ ID NO:63, a CDR_{L2} comprising the

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- 62 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
63 sequence of SEQ ID NO:10;
- 64 (h) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
65 comprising the amino acid sequence of SEQ ID NO:51, a CDR_{H2} comprising the
66 amino acid sequence of SEQ ID NO:53, and a CDR_{H3} comprising the amino
67 acid sequence of SEQ ID NO:7; and
- 68 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
69 comprising the amino acid sequence of SEQ ID NO:64, a CDR_{L2} comprising the
70 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
71 sequence of SEQ ID NO:10;
- 72 (i) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
73 comprising an amino acid sequence selected from the group consisting of SEQ
74 ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of
75 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
76 NO:7; and
- 77 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
78 comprising the amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the
79 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
80 sequence of SEQ ID NO:10;
- 81 (j) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
82 comprising an amino acid sequence selected from the group consisting of SEQ
83 ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of
84 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
85 NO:7; and
- 86 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
87 comprising the amino acid sequence of SEQ ID NO:62, a CDR_{L2} comprising the
88 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
89 sequence of SEQ ID NO:10;
- 90 (k) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
91 comprising an amino acid sequence selected from the group consisting of SEQ
92 ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of

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- 93 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
94 NO:7; and
- 95 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
96 comprising the amino acid sequence of SEQ ID NO:63, a CDR_{L2} comprising the
97 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
98 sequence of SEQ ID NO:10;
- 99 (l) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
100 comprising an amino acid sequence selected from the group consisting of SEQ
101 ID NO:52 and SEQ ID NO: 56, a CDR_{H2} comprising the amino acid sequence of
102 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
103 NO:7; and
- 104 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
105 comprising the amino acid sequence of SEQ ID NO:64, a CDR_{L2} comprising the
106 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
107 sequence of SEQ ID NO:10;
- 108 (m) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
109 comprising an amino acid sequence selected from the group consisting of SEQ
110 ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of
111 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
112 NO:7; and
- 113 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
114 comprising the amino acid sequence of SEQ ID NO:8, a CDR_{L2} comprising the
115 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
116 sequence of SEQ ID NO:10;
- 117 (n) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
118 comprising an amino acid sequence selected from the group consisting of SEQ
119 ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of
120 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
121 NO:7; and
- 122 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
123 comprising the amino acid sequence of SEQ ID NO:62, a CDR_{L2} comprising the

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124 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
 125 sequence of SEQ ID NO:10;

126 (o) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
 127 comprising an amino acid sequence selected from the group consisting of SEQ
 128 ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of
 129 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
 130 NO:7; and

131 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
 132 comprising the amino acid sequence of SEQ ID NO:63, a CDR_{L2} comprising the
 133 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
 134 sequence of SEQ ID NO:10; and

135 (p) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1}
 136 comprising an amino acid sequence selected from the group consisting of SEQ
 137 ID NO:5 and SEQ ID NO: 57, a CDR_{H2} comprising the amino acid sequence of
 138 SEQ ID NO:53, and a CDR_{H3} comprising the amino acid sequence of SEQ ID
 139 NO:7; and

140 (ii) an immunoglobulin light chain variable region comprising a CDR_{L1}
 141 comprising the amino acid sequence of SEQ ID NO:64, a CDR_{L2} comprising the
 142 amino acid sequence of SEQ ID NO:9, and a CDR_{L3} comprising the amino acid
 143 sequence of SEQ ID NO:10.

1 2. The antibody of claim 1, wherein the CDR sequences are interposed between human or
 2 humanized framework sequences.

1 3. An isolated nucleic acid comprising a nucleotide sequence encoding an
 2 immunoglobulin heavy chain variable region of claim 1.

1 4. An isolated nucleic acid comprising a nucleotide sequence encoding an
 2 immunoglobulin light chain variable region of claim 1.

1 5. An expression vector comprising the nucleic acid of claim 3.

1 6. An expression vector comprising the nucleic acid of claim 4.

1 7. The expression vector of claim 6, further comprising the nucleic acid of claim 3.

1 8. A host cell comprising the expression vector of claim 5.

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- 1 9. A host cell comprising the expression vector of claim 6.
- 1 10. A host cell comprising the expression vector of claim 7.
- 1 11. The host cell of claim 9, further comprising the expression vector of claim 5.
- 1 12. A method of producing a polypeptide comprising an immunoglobulin heavy chain
2 variable region or an immunoglobulin light chain variable region, the method comprising:
- 3 (a) growing the host cell of claim 8 or 9 under conditions so that the host cell expresses
4 the polypeptide comprising the immunoglobulin heavy chain variable region or the
5 immunoglobulin light chain variable region; and
- 6 (b) purifying the polypeptide comprising the immunoglobulin heavy chain variable
7 region or the immunoglobulin light chain variable region.
- 1 13. A method of producing an antibody that binds human Notch3 or an antigen binding
2 fragment of the antibody, the method comprising:
- 3 (a) growing the host cell of claim 10 or 11 under conditions so that the host cell
4 expresses a polypeptide or polypeptides comprising the immunoglobulin heavy chain variable
5 region and the immunoglobulin light chain variable region, thereby producing the antibody or
6 the antigen-binding fragment of the antibody; and
- 7 (b) purifying the antibody or the antigen-binding fragment of the antibody.
- 1 14. An isolated antibody that binds human Notch3 comprising an immunoglobulin heavy
2 chain variable region and an immunoglobulin light chain variable region selected from the
3 group consisting of:
- 4 (a) an immunoglobulin heavy chain variable region comprising the amino acid
5 sequence of SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an
6 immunoglobulin light chain variable region comprising the amino acid sequence
7 of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**);
- 8 (b) an immunoglobulin heavy chain variable region comprising the amino acid
9 sequence of SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**), and an immunoglobulin
10 light chain variable region comprising the amino acid sequence of SEQ ID NO:4
11 (**4F11, Ch4F11 Chimeric**);

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- 12 (c) an immunoglobulin heavy chain variable region comprising the amino acid
13 sequence of SEQ ID NO:2 (**4F11, Ch4F11 Chimeric**), and an immunoglobulin
14 light chain variable region comprising the amino acid sequence of SEQ ID NO:44
15 (**Hu4F11 Kv2D-29**);
- 16 (d) an immunoglobulin heavy chain variable region comprising the amino acid
17 sequence of SEQ ID NO:34 (**Sh4F11 Hv3-23**), and an immunoglobulin light
18 chain variable region comprising the amino acid sequence of SEQ ID NO:4
19 (**4F11, Ch4F11 Chimeric**);
- 20 (e) an immunoglobulin heavy chain variable region comprising the amino acid
21 sequence of SEQ ID NO:34 (**Sh4F11 Hv3-23**), and an immunoglobulin light
22 chain variable region comprising the amino acid sequence of SEQ ID NO:44
23 (**Hu4F11 Kv2D-29**);
- 24 (f) an immunoglobulin heavy chain variable region comprising the amino acid
25 sequence of SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**), and an
26 immunoglobulin light chain variable region comprising the amino acid sequence
27 of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**);
- 28 (g) an immunoglobulin heavy chain variable region comprising the amino acid
29 sequence of SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an
30 immunoglobulin light chain variable region comprising the amino acid sequence
31 of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**);
- 32 (h) an immunoglobulin heavy chain variable region comprising the amino acid
33 sequence of SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**), and an
34 immunoglobulin light chain variable region comprising the amino acid sequence
35 of SEQ ID NO:46 (**Hu4F11 Kv2D-29 N28H**);
- 36 (i) an immunoglobulin heavy chain variable region comprising the amino acid
37 sequence of SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an
38 immunoglobulin light chain variable region comprising the amino acid sequence
39 of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**);
- 40 (j) an immunoglobulin heavy chain variable region comprising the amino acid
41 sequence of SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**), and an
42 immunoglobulin light chain variable region comprising the amino acid sequence
43 of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**);

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- 44 (k) an immunoglobulin heavy chain variable region comprising the amino acid
 45 sequence of SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an
 46 immunoglobulin light chain variable region comprising the amino acid sequence
 47 of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**);
- 48 (l) an immunoglobulin heavy chain variable region comprising the amino acid
 49 sequence of SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**), and an
 50 immunoglobulin light chain variable region comprising the amino acid sequence
 51 of SEQ ID NO:48 (**Hu4F11 Kv2D-29 N28Q**);
- 52 (m) an immunoglobulin heavy chain variable region comprising the amino acid
 53 sequence of SEQ ID NO:36 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an
 54 immunoglobulin light chain variable region comprising the amino acid sequence
 55 of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**);
- 56 (n) an immunoglobulin heavy chain variable region comprising the amino acid
 57 sequence of SEQ ID NO:38 (**Sh4F11 Hv3-23 S31H T62S**), and an
 58 immunoglobulin light chain variable region comprising the amino acid sequence
 59 of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**);
- 60 (o) an immunoglobulin heavy chain variable region comprising the amino acid
 61 sequence of SEQ ID NO:40 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an
 62 immunoglobulin light chain variable region comprising the amino acid sequence
 63 of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**); and
- 64 (p) an immunoglobulin heavy chain variable region comprising the amino acid
 65 sequence of SEQ ID NO:42 (**Sh4F11 Hv3-23 A28T T62S**), and an
 66 immunoglobulin light chain variable region comprising the amino acid sequence
 67 of SEQ ID NO:50 (**Hu4F11 Kv2D-29 N28Y**).

1 15. An isolated nucleic acid comprising a nucleotide sequence encoding an
 2 immunoglobulin heavy chain variable region of claim 14.

1 16. An isolated nucleic acid comprising a nucleotide sequence encoding an
 2 immunoglobulin light chain variable region of any one of claims 14.

1 17. An expression vector comprising the nucleic acid of claim 15.

1 18. An expression vector comprising the nucleic acid of claim 16.

1 19. The expression vector of claim 18, further comprising the nucleic acid of claim 15.

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- 1 20. A host cell comprising the expression vector of claim 17.
- 1 21. A host cell comprising the expression vector of claim 18.
- 1 22. A host cell comprising the expression vector of claim 19.
- 1 23. The host cell of claim 21, further comprising the expression vector of claim 17.
- 1 24. A method of producing a polypeptide comprising an immunoglobulin heavy chain
2 variable region or an immunoglobulin light chain variable region, the method comprising:
- 3 (a) growing the host cell of claim 20 or 21 under conditions so that the host cell
4 expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the
5 immunoglobulin light chain variable region; and
- 6 (b) purifying the polypeptide comprising the immunoglobulin heavy chain variable
7 region or the immunoglobulin light chain variable region.
- 1 25. A method of producing an antibody that binds human Notch3 or an antigen binding
2 fragment of the antibody, the method comprising:
- 3 (a) growing the host cell of claim 22 or 23 under conditions so that the host cell
4 expresses a polypeptide or polypeptides comprising the immunoglobulin heavy chain variable
5 region and the immunoglobulin light chain variable region, thereby producing the antibody or
6 the antigen-binding fragment of the antibody; and
- 7 (b) purifying the antibody or the antigen-binding fragment of the antibody.
- 1 26. An isolated antibody that binds human Notch3 comprising an immunoglobulin heavy
2 chain and an immunoglobulin light chain selected from the group consisting of:
- 3 (a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
4 NO: 78 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain
5 comprising the amino acid sequence of SEQ ID NO: 90 (**Hu4F11 Kv2D-29**
6 **N28H Kappa**);
- 7 (b) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
8 NO: 27 (**4F11**), and an immunoglobulin light chain comprising the amino acid
9 sequence of SEQ ID NO: 29 (**4F11**);

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- 10 (c) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
11 NO: 74 (**Ch4F11**), and an immunoglobulin light chain comprising the amino acid
12 sequence of SEQ ID NO: 86 (**Ch4F11**);
- 13 (d) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
14 NO: 74 (**Ch4F11**), and an immunoglobulin light chain comprising the amino acid
15 sequence of SEQ ID NO: 88 (**Hu4F11 Kv2D-29**);
- 16 (e) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
17 NO: 76 (**Sh4F11 Hv3-23**), and an immunoglobulin light chain comprising the
18 amino acid sequence of SEQ ID NO: 86 (**Ch4F11**);
- 19 (f) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
20 NO: 76 (**Sh4F11 Hv3-23**), and an immunoglobulin light chain comprising the
21 amino acid sequence of SEQ ID NO: 88 (**Hu4F11 Kv2D-29**);
- 22 (g) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
23 NO: 80 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain
24 comprising the amino acid sequence of SEQ ID NO: 90 (**Hu4F11 Kv2D-29**
25 **N28H Kappa**);
- 26 (h) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
27 NO: 82 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain
28 comprising the amino acid sequence of SEQ ID NO: 90 (**Hu4F11 Kv2D-29**
29 **N28H Kappa**);
- 30 (i) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
31 NO: 84 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain
32 comprising the amino acid sequence of SEQ ID NO: 90 (**Hu4F11 Kv2D-29**
33 **N28H Kappa**);
- 34 (j) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
35 NO: 78 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain
36 comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11 Kv2D-29**
37 **N28Q Kappa**);
- 38 (k) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
39 NO: 80 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain
40 comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11 Kv2D-29**
41 **N28Q Kappa**);

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- 42 (l) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
 43 NO: 82 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain
 44 comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11 Kv2D-29**
 45 **N28Q Kappa**);
- 46 (m) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
 47 NO: 84 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain
 48 comprising the amino acid sequence of SEQ ID NO: 92 (**Hu4F11 Kv2D-29**
 49 **N28Q Kappa**);
- 50 (n) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
 51 NO: 78 (**Sh4F11 Hv3-23 A28T S31H T62S**), and an immunoglobulin light chain
 52 comprising the amino acid sequence of SEQ ID NO: 94 (**Hu4F11 Kv2D-29 N28Y**
 53 **Kappa**);
- 54 (o) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
 55 NO: 80 (**Sh4F11 Hv3-23 S31H T62S**), and an immunoglobulin light chain
 56 comprising the amino acid sequence of SEQ ID NO: 94 (**Hu4F11 Kv2D-29 N28Y**
 57 **Kappa**);
- 58 (p) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
 59 NO: 82 (**Sh4F11 Hv3-23 A28T S31N T62S**), and an immunoglobulin light chain
 60 comprising the amino acid sequence of SEQ ID NO: 94 (**Hu4F11 Kv2D-29 N28Y**
 61 **Kappa**); and
- 62 (q) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID
 63 NO: 84 (**Sh4F11 Hv3-23 A28T T62S**), and an immunoglobulin light chain
 64 comprising the amino acid sequence of SEQ ID NO: 94 (**Hu4F11 Kv2D-29 N28Y**
 65 **Kappa**).

1 27. An isolated nucleic acid comprising a nucleotide sequence encoding an
 2 immunoglobulin heavy chain of claim 26.

1 28. An isolated nucleic acid comprising a nucleotide sequence encoding an
 2 immunoglobulin light chain of claim 26.

1 29. An expression vector comprising the nucleic acid of claim 27.

1 30. An expression vector comprising the nucleic acid of claim 28.

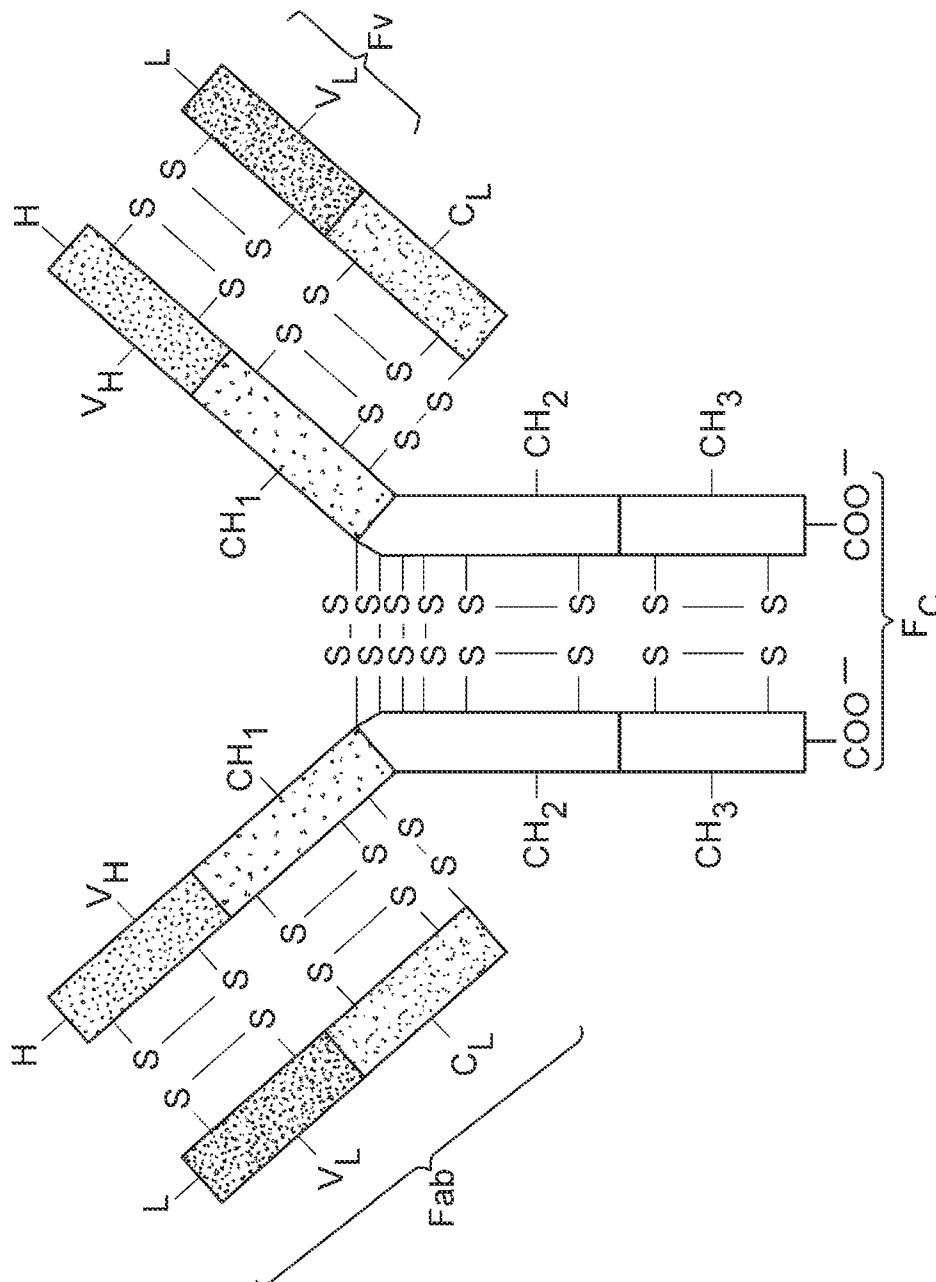
1 31. The expression vector of claim 30, further comprising the nucleic acid of claim 27.

- 83 -

- 1 32. A host cell comprising the expression vector of claim 29.
- 1 33. A host cell comprising the expression vector of claim 30.
- 1 34. A host cell comprising the expression vector of claim 31.
- 1 35. The host cell of claim 33, further comprising the expression vector of claim 29.
- 1 36. A method of producing a polypeptide comprising an immunoglobulin heavy chain
2 variable region or an immunoglobulin light chain variable region, the method comprising:
- 3 (a) growing the host cell of claim 32 or 33 under conditions so that the host cell
4 expresses the polypeptide comprising the immunoglobulin heavy chain variable region or the
5 immunoglobulin light chain variable region; and
- 6 (b) purifying the polypeptide comprising the immunoglobulin heavy chain variable
7 region or the immunoglobulin light chain variable region.
- 1 37. A method of producing an antibody that binds human Notch3 or an antigen binding
2 fragment of the antibody, the method comprising:
- 3 (a) growing the host cell of claim 34 or 35 under conditions so that the host cell
4 expresses a polypeptide or polypeptides comprising the immunoglobulin heavy chain variable
5 region and the immunoglobulin light chain variable region, thereby producing the antibody or
6 the antigen-binding fragment of the antibody; and
- 7 (b) purifying the antibody or the antigen-binding fragment of the antibody.
- 1 38. A method of inhibiting or reducing proliferation of a tumor cell comprising exposing
2 the cell to an effective amount of the antibody of any one of claims 1, 14, or 26 to inhibit or
3 reduce proliferation of the tumor cell.
- 1 39. A method of inhibiting or reducing tumor growth in a mammal, the method comprising
2 exposing the mammal to an effective amount of the antibody of any one of claims 1, 14, or 26
3 to inhibit or reduce proliferation of the tumor.
- 1 40. The method of claim 39, wherein the mammal is a human.
- 1 41. A method of treating cancer in a human subject, the method comprising administering
2 to the subject an effective amount of the antibody of any one of claims 1, 14, or 26 to a human
3 subject in need thereof.

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- 1 42. The method of claim 41, wherein the cancer is selected from the group consisting of
2 breast cancer, ovarian cancer, prostate cancer, cervical cancer, lung cancer, brain cancer, skin
3 cancer, colorectal cancer, pancreatic cancer, gastric cancer, head and neck cancer, and
4 leukemia.
- 1 43. The antibody of any one of claims 1, 14, or 26, wherein the antibody has a K_D of 35 nM
2 or lower as measured by surface plasmon resonance.
- 1 44. The antibody of any one of claims 1, 14, or 26, wherein the antibody has a K_D of 25 nM
2 or lower as measured by surface plasmon resonance.
- 1 45. The antibody of any one of claims 1, 14, or 26, wherein the antibody has a K_D of 15 nM
2 or lower as measured by surface plasmon resonance.
- 1 46. The antibody of any one of claims 1, 14, or 26, wherein the antibody has a K_D of 5 nM
2 or lower as measured by surface plasmon resonance.
- 1 47. The antibody of any one of claims 1, 14, or 26, wherein the antibody has a K_D of 1 nM
2 or lower as measured by surface plasmon resonance.
- 1 48. An isolated antibody that competes with the antibody of claim 1 for binding to the
2 extracellular domain of human Notch3.
- 1 49. An isolated antibody that competes with the antibody of claim 1 for binding to EGF-like
2 repeats 1-11 of human Notch3.
- 1 50. An isolated antibody that binds to the same epitope on human Notch3 as the antibody of
2 claim 1.



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FIG. 2**Human Notch3 ECD (amino acids 40-1643)**

	<u>40</u>	<u>50</u>	<u>60</u>		
	A PPCLDGSPCA NGGRCTQLPS				
<u>70</u>	<u>80</u>	<u>90</u>	<u>100</u>	<u>110</u>	<u>120</u>
REAACLCPPG	WVGERCQLED	PCHSGPCAGR	GVCQSSVVAG	TARFSCRCPR	GFRGPDCLSLP
<u>130</u>	<u>140</u>	<u>150</u>	<u>160</u>	<u>170</u>	<u>180</u>
DPCLSSPCAH	GARCSVGPDG	RFLCSCPPGY	QGRSCRSDVD	ECRVGEPCRH	GGTCLNTPGS
<u>190</u>	<u>200</u>	<u>210</u>	<u>220</u>	<u>230</u>	<u>240</u>
FRCQCPAGYT	GPLCENPAVP	CAPSPCRNGG	TCRQSGDLTY	DCACLPGFEG	QNCEVNVDDC
<u>250</u>	<u>260</u>	<u>270</u>	<u>280</u>	<u>290</u>	<u>300</u>
PGHRCLNGGT	CVDGVNTYNC	QCPPEWTGQF	CTEDVDECQL	QPNACHNGGT	CFNTLGGHSC
<u>310</u>	<u>320</u>	<u>330</u>	<u>340</u>	<u>350</u>	<u>360</u>
VCVNGWTGES	CSQNIDDCAT	AVCFHGATCH	DRVASFYCAC	PMGKTGLLCH	LDDACVSNPC
<u>370</u>	<u>380</u>	<u>390</u>	<u>400</u>	<u>410</u>	<u>420</u>
HEDAICDTNP	VNGRAICTCP	PGFTGGACDQ	DVDECSIGAN	PCEHLGRCVN	TQGSFLCQCG
<u>430</u>	<u>440</u>	<u>450</u>	<u>460</u>	<u>470</u>	<u>480</u>
RGYTGPRCET	DVNECLSGPC	RNQATCLDRI	GQFTCICMAG	FTGTYCEVDI	DECQSSPCVN
<u>490</u>	<u>500</u>	<u>510</u>	<u>520</u>	<u>530</u>	<u>540</u>
GGVCKDRVNG	FSCTCPSGFS	GSTCQLDVDE	CASTPCRNGA	KCVDQPDGYE	CRCAEGFEGT
<u>550</u>	<u>560</u>	<u>570</u>	<u>580</u>	<u>590</u>	<u>600</u>
LCDRNVDDCS	PDPCHHGRCV	DGIASFSCAC	APGYTGTRCE	SQVDECRSQP	CRHGGKCLDL
<u>610</u>	<u>620</u>	<u>630</u>	<u>640</u>	<u>650</u>	<u>660</u>
VDKYLRCRPS	GTTGVNCEVN	IDDCASNPT	FGVCRDGINR	YDCVCQPGFT	GPLCNVEINE
<u>670</u>	<u>680</u>	<u>690</u>	<u>700</u>	<u>710</u>	<u>720</u>
CASSPCGEGG	SCVDGENGFR	CLCPPGSLPP	LCLPPSHPCA	HEPCSHGICY	DAPGGFRCVC
<u>730</u>	<u>740</u>	<u>750</u>	<u>760</u>	<u>770</u>	<u>780</u>
EPGWSGPRCS	QSLARDACES	QPCRAGGTCS	SDGMGFHCTC	PPGVQGRQCE	LLSPCTPNPC

FIG. 2 (cont.)

790	800	810	820	830	840
EHGGRCESAP	GQLPVCSCPQ	GWQGPRCQOD	VDECAGPAPC	GPHGICTNLA	GSFSCTCHGG
850	860	870	880	890	900
YTGPSCDQDI	NDCDPNPCLN	GGSCQDGVGS	FSCSCLPGFA	GPRCARDVDE	CLSNPCGPGT
910	920	930	940	950	960
CTDHVASFTC	TCPPGYGGFH	CEQDLPCDSP	SSCFNGGTCV	DGVNSFSCLC	RPGYTGAHCQ
970	980	990	1000	1010	1020
HEADPCLSRP	CLHGGVCSAA	HPGFRCTCLE	SFTGPQCQTL	VDWCSRQPCQ	NGGRCVQTGA
1030	1040	1050	1060	1070	1080
YCLCPPGWSG	RLCDIRSLPC	REAAAQIGVR	LEQLCQAGGQ	CVDEDSSHYC	VCPEGRTGSH
1090	1100	1110	1120	1130	1140
CEQEVDPCLA	QPCQHGGTCR	GYMGGYMCEC	LPGYNGDNCE	DDVDECASQP	CQHGGSCIDL
1150	1160	1170	1180	1190	1200
VARYLCSCPP	GTLGVLCEIN	EDDCGPGPPL	DSGPRCLHNG	TCVDLVGGFR	CTCPPGYTGL
1210	1220	1230	1240	1250	1260
RCEADINECR	SGACHAAHTR	DCLQDPGGGF	RCLCHAGFSG	PRCQTVLSPC	ESQPCQHGGQ
1270	1280	1290	1300	1310	1320
CRPSPGPGGG	LTFTCHCAQP	FWGPRCERVA	RSCRELQCPV	GVPCQQTTPRG	PRCACPPGLS
1330	1340	1350	1360	1370	1380
GPSCRSFPGS	PPGASNASCA	AAPCLHGGSC	RPAPLAPFFR	CACAQGWGTP	RCEAPAAAPE
1390	1400	1410	1420	1430	1440
VSEEPRCpra	ACQAKRGDQR	CDRECNSPGC	GWDGGDCSLS	VGDPWRQCEA	LQCWRLFNNs
1450	1460	1470	1480	1490	1500
RCDPACSSPA	CLYDNFDCHA	GGRERTCNPV	YEKYCADHFA	DGRCDQGcnt	EECGWDGLDC
1510	1520	1530	1540	1550	1560
ASEVPALLAR	GVLVLTVLLP	PEELLRSSAD	FLQRLSAILR	TSLRFRLDAH	GQAMVFPPYHR

FIG. 2 (cont.)

1570 1580 1590 1600 1610 1620
PSPGSEPRAR RELAPEVIGS VVMLEIDNRL CLQSPENDHC FPDAQSAADY LGALSAVERL
1630 1640
DFPYPLRDVR GEPLPEPEPS VPL

FIG. 3

Human Notch3 EGF-Like Repeats 1-11 (amino acids 40-467)

			<u>40</u>	<u>50</u>	<u>60</u>	
				A	PPCLDGSPCA	NGGRCTQLPS
<u>70</u>	<u>80</u>	<u>90</u>	<u>100</u>	<u>110</u>	<u>120</u>	
REAACLCPPG	WVGERCQLED	PCHSGPCAGR	GVCQSSVVAG	TARFSCRCPR	GFRGPDCLP	
<u>130</u>	<u>140</u>	<u>150</u>	<u>160</u>	<u>170</u>	<u>180</u>	
DPCLSSPCAH	GARCSVGPDG	RFLCSCPPGY	QGRSCRSDVD	ECRVGEPCRH	GGTCLNTPGS	
<u>190</u>	<u>200</u>	<u>210</u>	<u>220</u>	<u>230</u>	<u>240</u>	
FRCQCPAGYT	GPLCENPAVF	CAPSPCRNGG	TCRQSGDLTY	DCACLPGFEG	QNCEVNVDDC	
<u>250</u>	<u>260</u>	<u>270</u>	<u>280</u>	<u>290</u>	<u>300</u>	
PGHRCLNGGT	CVDGVNTYNC	QCPPEWTGQF	CTEDVDECQL	QPNACHNGGT	CFNTLGGHSC	
<u>310</u>	<u>320</u>	<u>330</u>	<u>340</u>	<u>350</u>	<u>360</u>	
VCVNGWTGES	CSQNIDDCAT	AVCFHGATCH	DRVASFYCAC	PMGKTGLLCH	LDDACVSNPC	
<u>370</u>	<u>380</u>	<u>390</u>	<u>400</u>	<u>410</u>	<u>420</u>	
HEDAICDTNP	VNGRAICTCP	PGFTGGACDQ	DVDECSIGAN	PCEHLGRCVN	TQGSFLCQCG	
<u>430</u>	<u>440</u>	<u>450</u>	<u>460</u>			
RGYTGPRCET	DVNECLSGPC	RNQATCLDRI	GQFTCICMAG	FTGTYCE		

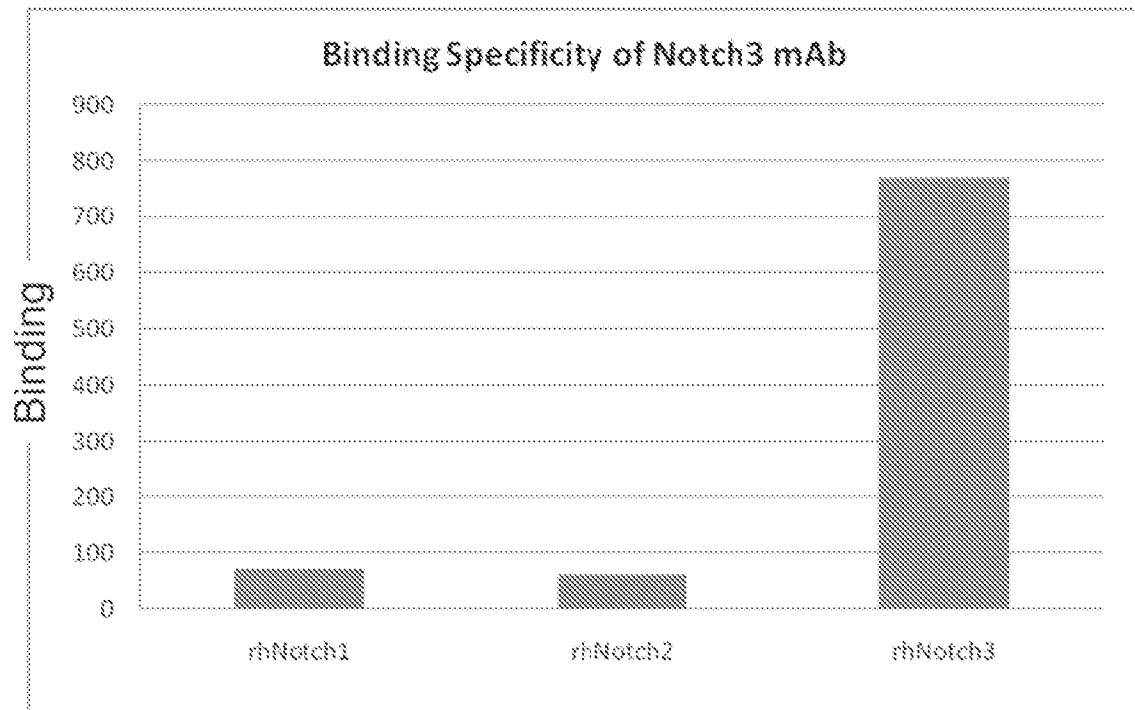
FIG. 4

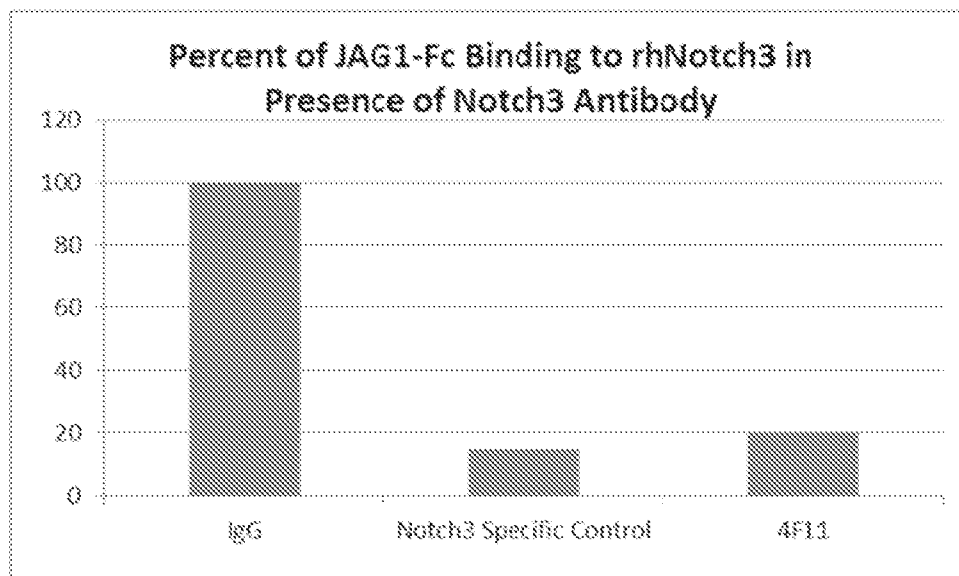
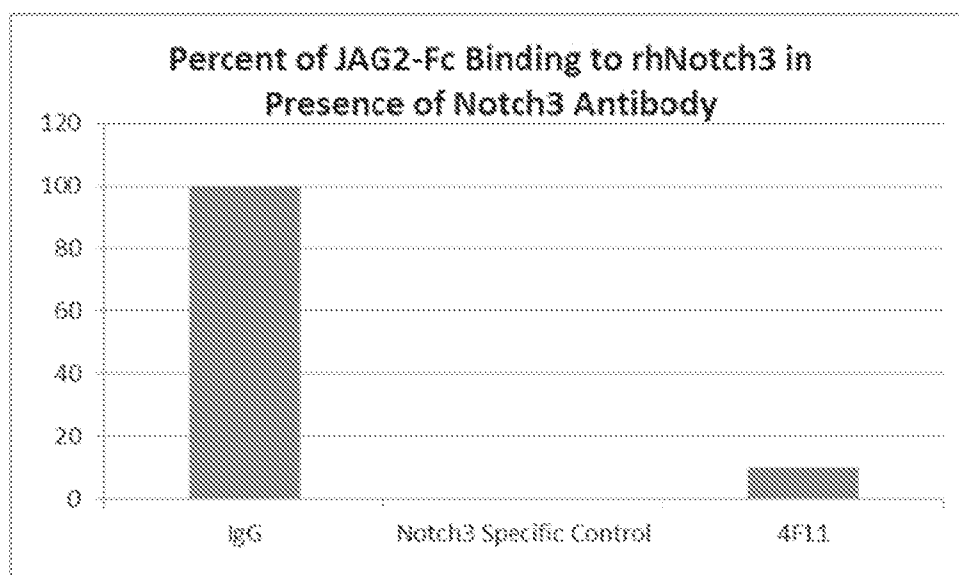
FIG. 5A**FIG. 5B**

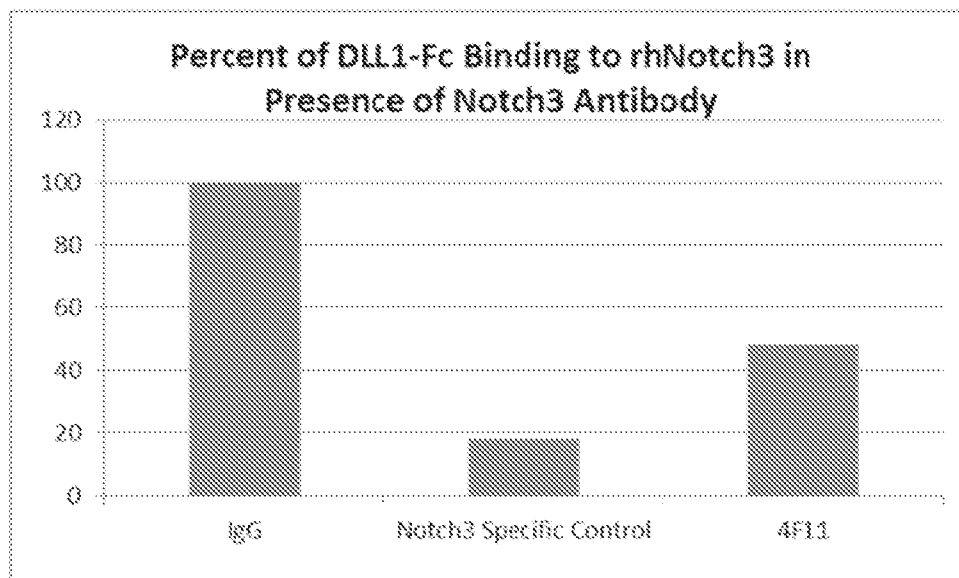
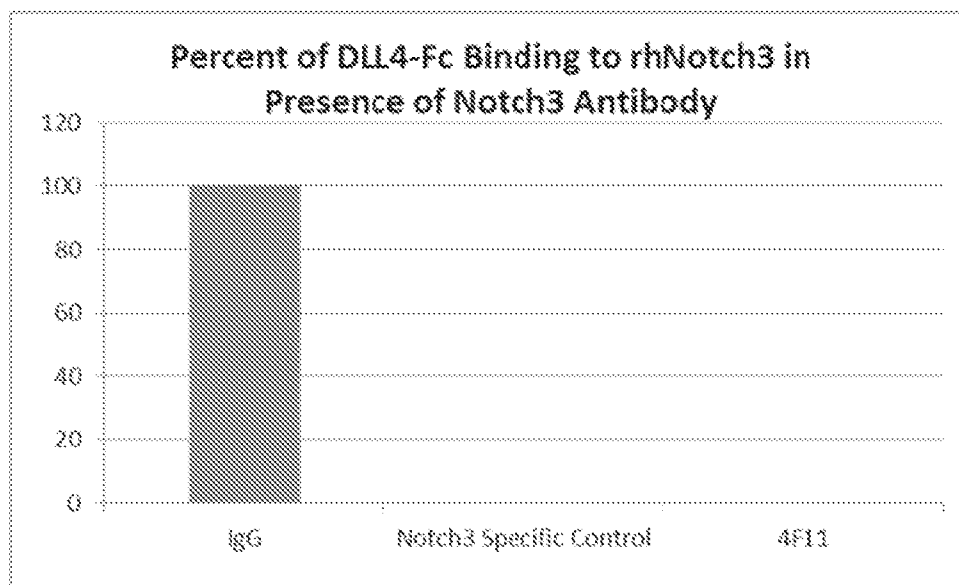
FIG. 5C**FIG. 5D**

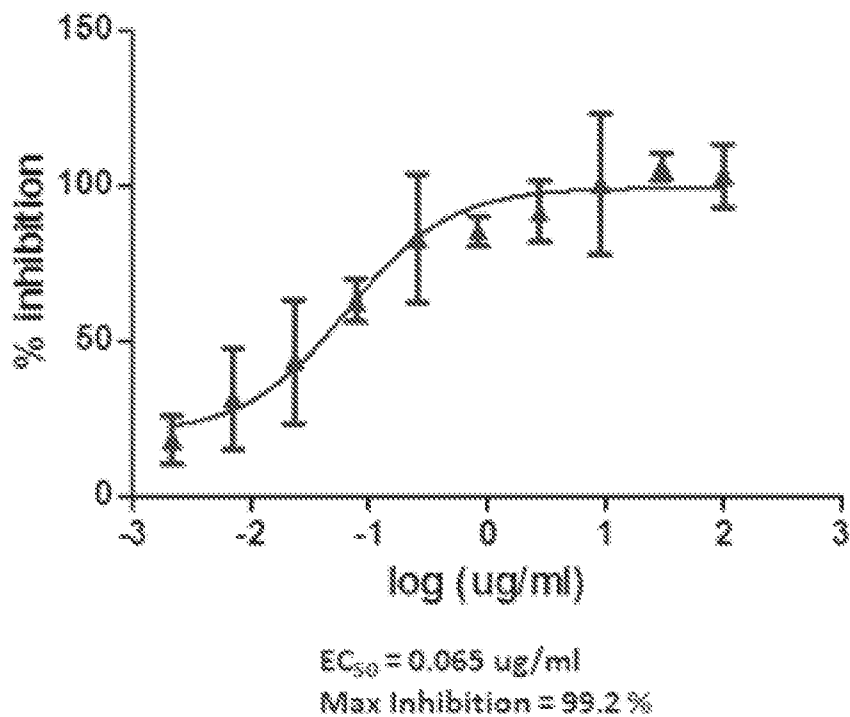
FIG. 6A**Inhibition of JAG2-Fc Stimulated Notch3 Signaling**

FIG. 6B

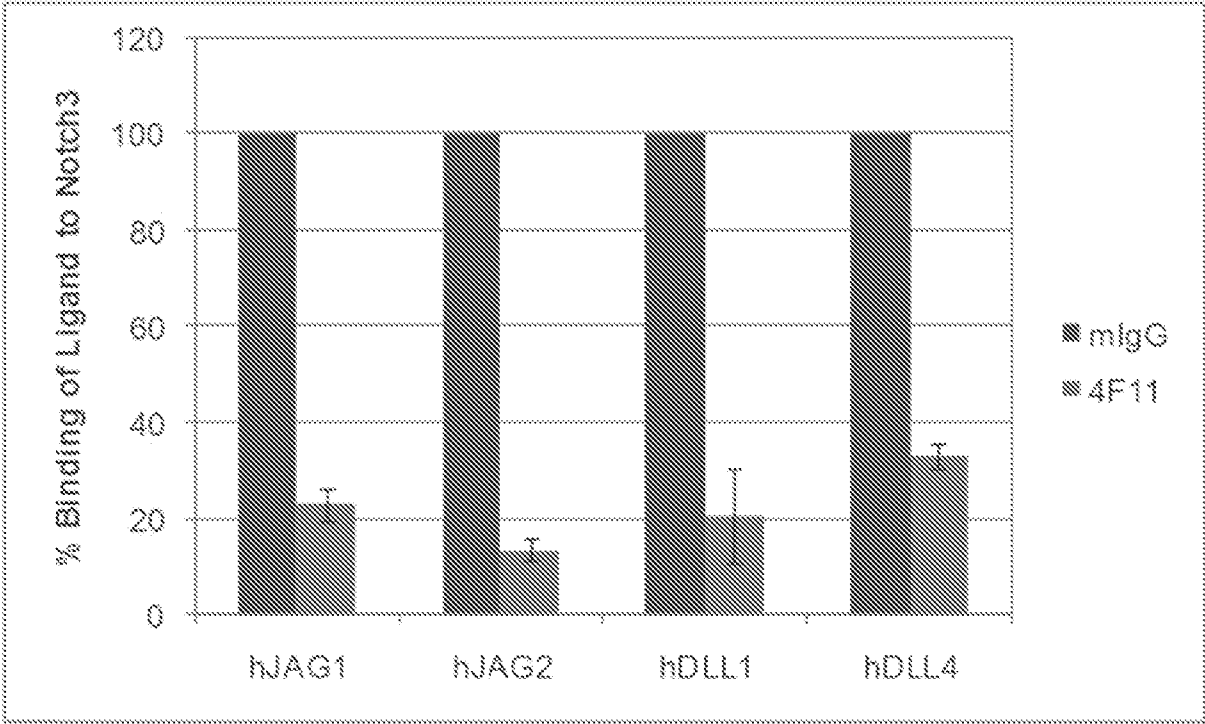


FIG. 7

Complete Heavy Chain Variable Region Amino Acid Alignments

Antibody	CDR1		CDR2	
Ch4F11	EVQLVESGGGLVQPGGSLKLSCAASGFAFS	SYDMS	MVRQTPVKRLEWVSI	SRGGGSTYYPD
Sh4F11 Hv3-23	EVQLVESGGGLVQPGGSLRLS	SYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T	EVQLVESGGGLVQPGGSLRLS	SYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T S31H	EVQLVESGGGLVQPGGSLRLS	HYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T S31H T62S	EVQLVESGGGLVQPGGSLRLS	HYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T S31N	EVQLVESGGGLVQPGGSLRLS	NYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T S31N T62S	EVQLVESGGGLVQPGGSLRLS	NYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T S31N T62S	EVQLVESGGGLVQPGGSLRLS	SYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T S31N T62S	EVQLVESGGGLVQPGGSLRLS	SYDMS	MVRQAPGKLEWVS	SRGGGSTYYPD
Sh4F11 Hv3-23 A28T S31H	SRDNAKNTLYLQMS	HATTAYWYFDV	WGAGT	(SEQ ID NO:2)
Sh4F11 Hv3-23 A28T S31H T62S	SRDNSKNTLYLQMS	HATTAYWYFDV	WGAGT	(SEQ ID NO:34)
Sh4F11 Hv3-23 A28T S31H T62S	SRDNSKNTLYLQMS	HATTAYWYFDV	WGAGT	(SEQ ID NO:36)
Sh4F11 Hv3-23 A28T S31H T62S	SRDNSKNTLYLQMS	HATTAYWYFDV	WGAGT	(SEQ ID NO:38)
Sh4F11 Hv3-23 A28T S31N	SRDNSKNTLYLQMS	HATTAYWYFDV	WGAGT	(SEQ ID NO:40)
Sh4F11 Hv3-23 A28T S31N T62S	SRDNSKNTLYLQMS	HATTAYWYFDV	WGAGT	(SEQ ID NO:42)

FIG. 8

Heavy Chain CDR Amino Acid Alignments

Antibody	CDR1		CDR2	
	(SEQ ID NO: 5)	(SEQ ID NO: 5)	(SEQ ID NO: 6)	(SEQ ID NO: 6)
Ch4F11	SYDMS	YISRGGGSTYYPDTVKG	SYDMS	YISRGGGSTYYPDTVKG
Sh4F11 Hv3-23	SYDMS	YISRGGGSTYYPDTVKG	SYDMS	YISRGGGSTYYPDTVKG
Sh4F11 Hv3-23 A28T S31H T62S	HYDMS	YISRGGGSTYYPDSVKG	HYDMS	YISRGGGSTYYPDSVKG
Sh4F11 Hv3-23 S31H T62S	HYDMS	YISRGGGSTYYPDSVKG	HYDMS	YISRGGGSTYYPDSVKG
Sh4F11 Hv3-23 A28T S31N T62S	NYDMS	YISRGGGSTYYPDSVKG	NYDMS	YISRGGGSTYYPDSVKG
Sh4F11 Hv3-23 A28T T62S	SYDMS	YISRGGGSTYYPDSVKG	SYDMS	YISRGGGSTYYPDSVKG

Antibody	CDR3	
	(SEQ ID NO: 7)	(SEQ ID NO: 7)
Ch4F11	HATTAYWYFDV	HATTAYWYFDV
Sh4F11 Hv3-23	HATTAYWYFDV	HATTAYWYFDV
Sh4F11 Hv3-23 A28T S31H T62S	HATTAYWYFDV	HATTAYWYFDV
Sh4F11 Hv3-23 S31H T62S	HATTAYWYFDV	HATTAYWYFDV
Sh4F11 Hv3-23 A28T S31N T62S	HATTAYWYFDV	HATTAYWYFDV
Sh4F11 Hv3-23 A28T T62S	HATTAYWYFDV	HATTAYWYFDV

FIG. 9
Complete Light (Kappa) Chain Variable Region Amino Acid Alignments

Antibody	CDR1					CDR2				
Ch4F11	DVVM	QTPLSLPVS	LGDAQASIS	CRSSQSLVHTNGNTYLH	WYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGS			
Hu4F11 Kv2D-29	DVVM	QTPLSLSVTPGQ	PASIS	CRSSQSLVHTNGNTYLH	WYLQKPGQSPQLLIY	KVSNRFS	GVPDRFSGS			
Hu4F11 Kv2D-29 N28H	DVVM	QTPLSLSVTPGQ	PASIS	CRSSQSLVHTNGNTYLH	WYLQKPGQSPQLLIY	KVSNRFS	GVPDRFSGS			
Hu4F11 Kv2D-29 N28Q	DVVM	QTPLSLSVTPGQ	PASIS	CRSSQSLVHTNGNTYLH	WYLQKPGQSPQLLIY	KVSNRFS	GVPDRFSGS			
Hu4F11 Kv2D-29 N28Y	DVVM	QTPLSLSVTPGQ	PASIS	CRSSQSLVHTNGNTYLH	WYLQKPGQSPQLLIY	KVSNRFS	GVPDRFSGS			
CDR3										
Ch4F11	GS	GTDF	TLKISR	VEAEDLG	VYFC	SQSTHVPWTF	FGG	GTKLEIK	(SEQ ID NO:4)	
Hu4F11 Kv2D-29	GS	GTDF	TLKISR	VEAEDV	GVYFC	SQSTHVPWTF	FGQ	GTKVEIK	(SEQ ID NO:44)	
Hu4F11 Kv2D-29 N28H	GS	GTDF	TLKISR	VEAEDV	GVYFC	SQSTHVPWTF	FGQ	GTKVEIK	(SEQ ID NO:46)	
Hu4F11 Kv2D-29 N28Q	GS	GTDF	TLKISR	VEAEDV	GVYFC	SQSTHVPWTF	FGQ	GTKVEIK	(SEQ ID NO:48)	
Hu4F11 Kv2D-29 N28Y	GS	GTDF	TLKISR	VEAEDV	GVYFC	SQSTHVPWTF	FGQ	GTKVEIK	(SEQ ID NO:50)	

FIG. 10

Light (Kappa) Chain CDR Amino Acid Alignments

Antibody	CDR1	CDR2
CH4F11	RSSQSLVHTNGNTYLH (SEQ ID NO:8)	KVSNRFS (SEQ ID NO:9)
Hu4F11 Kv2D-29	RSSQSLVHTNGNTYLH (SEQ ID NO:8)	KVSNRFS (SEQ ID NO:9)
Hu4F11 Kv2D-29 N28H	RSSQSLVHTHGNTYLH (SEQ ID NO:62)	KVSNRFS (SEQ ID NO:9)
Hu4F11 Kv2D-29 N28Q	RSSQSLVHTQGNTYLH (SEQ ID NO:63)	KVSNRFS (SEQ ID NO:9)
Hu4F11 Kv2D-29 N28Y	RSSQSLVHTYGNNTYLH (SEQ ID NO:64)	KVSNRFS (SEQ ID NO:9)

Antibody	CDR3
CH4F11	SQSTHVVPWT (SEQ ID NO:10)
Hu4F11 Kv2D-29	SQSTHVVPWT (SEQ ID NO:10)
Hu4F11 Kv2D-29 N28H	SQSTHVVPWT (SEQ ID NO:10)
Hu4F11 Kv2D-29 N28Q	SQSTHVVPWT (SEQ ID NO:10)
Hu4F11 Kv2D-29 N28Y	SQSTHVVPWT (SEQ ID NO:10)

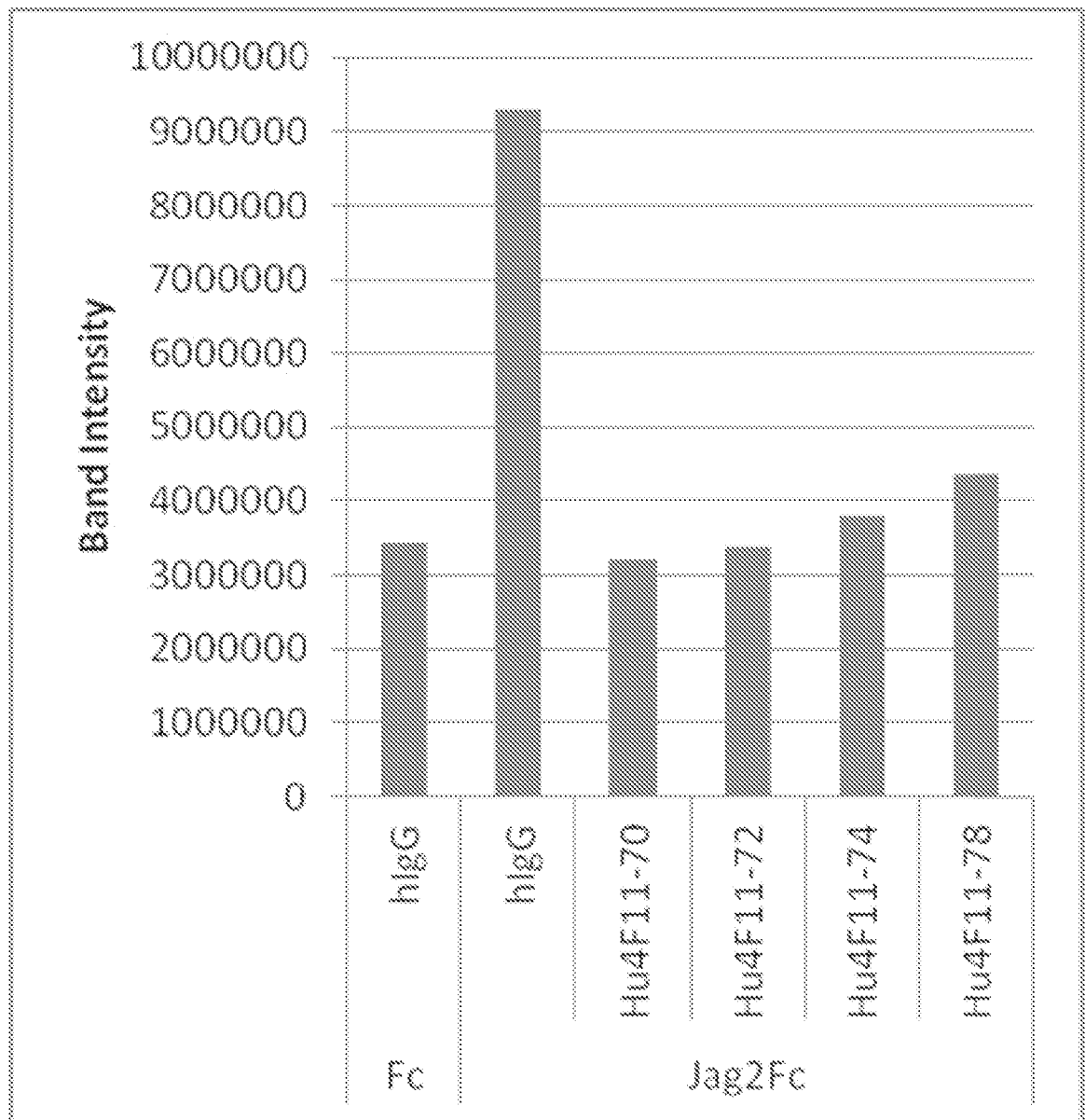
FIG. 11

FIG. 12A

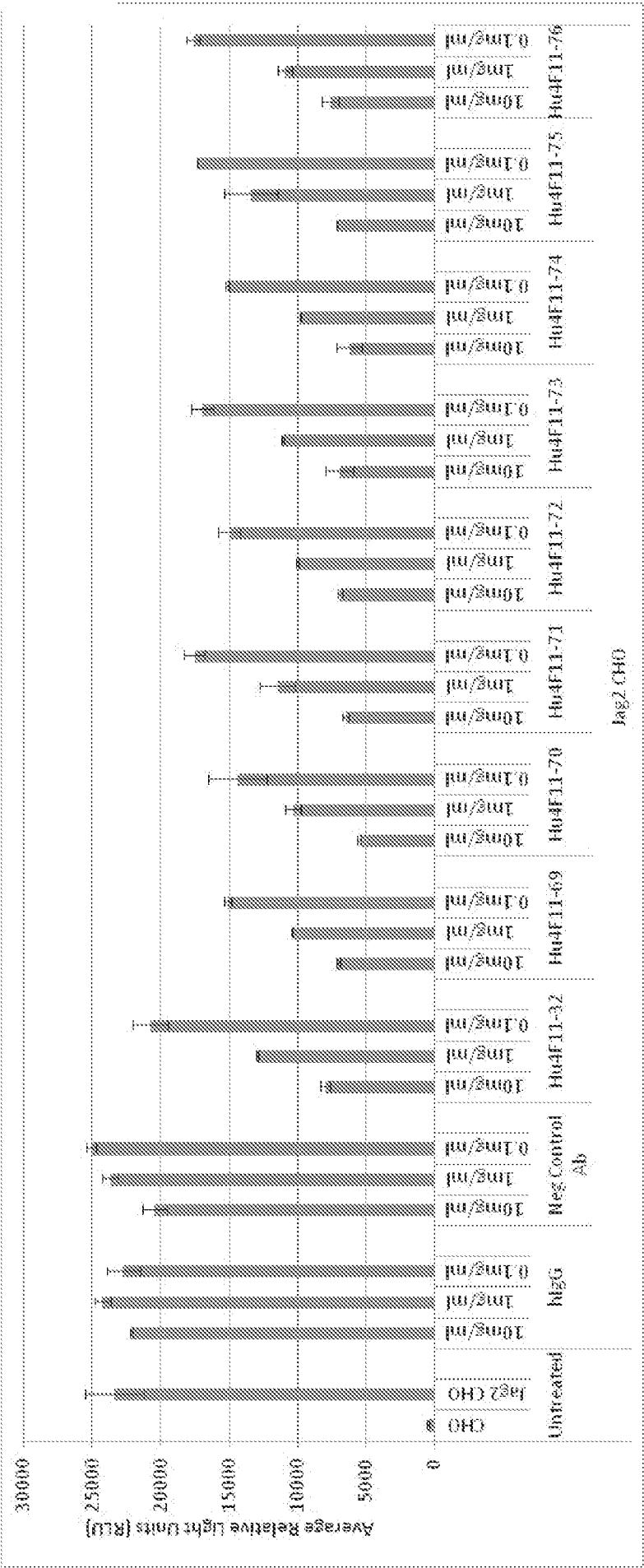


FIG. 12B

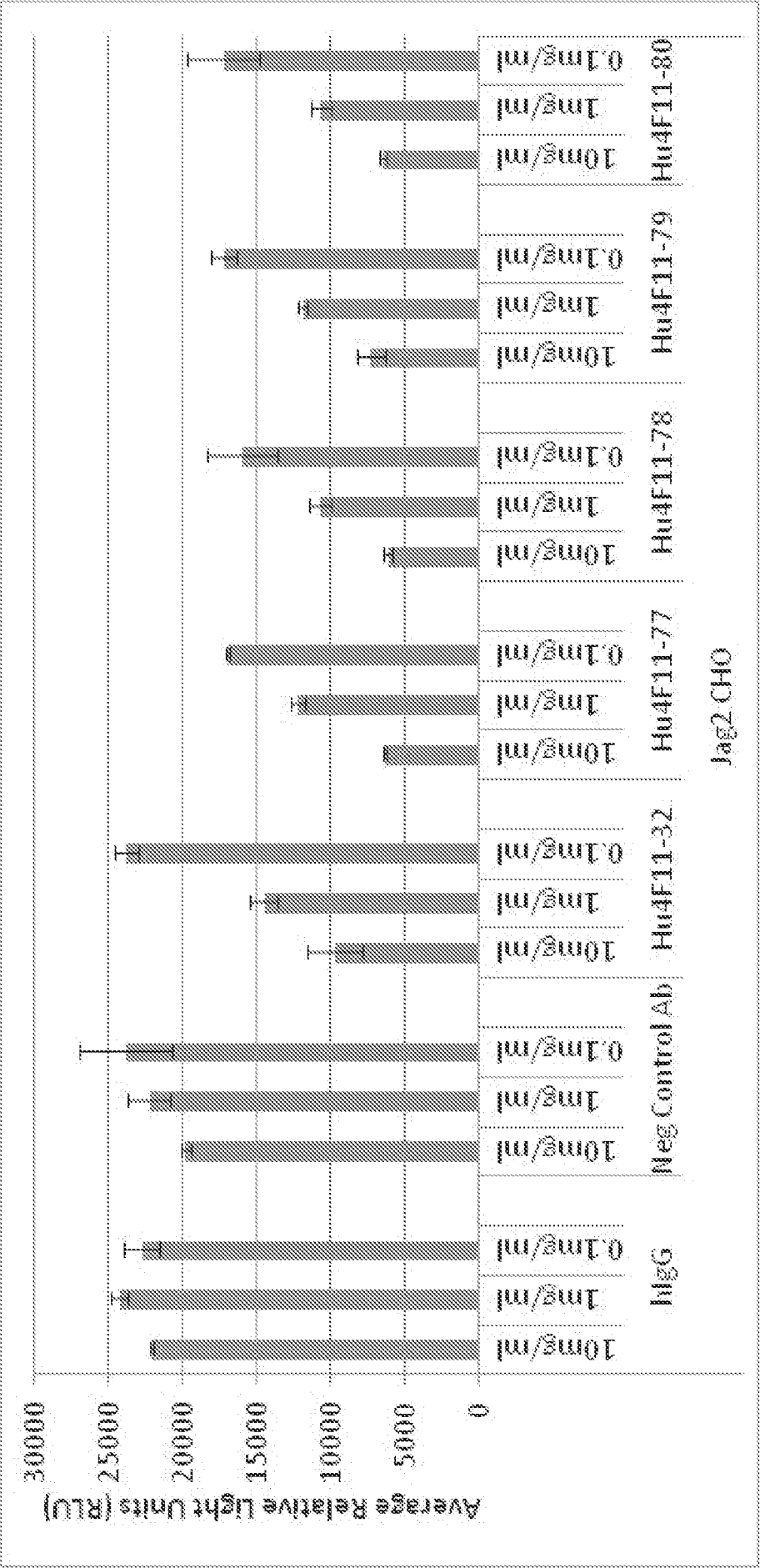
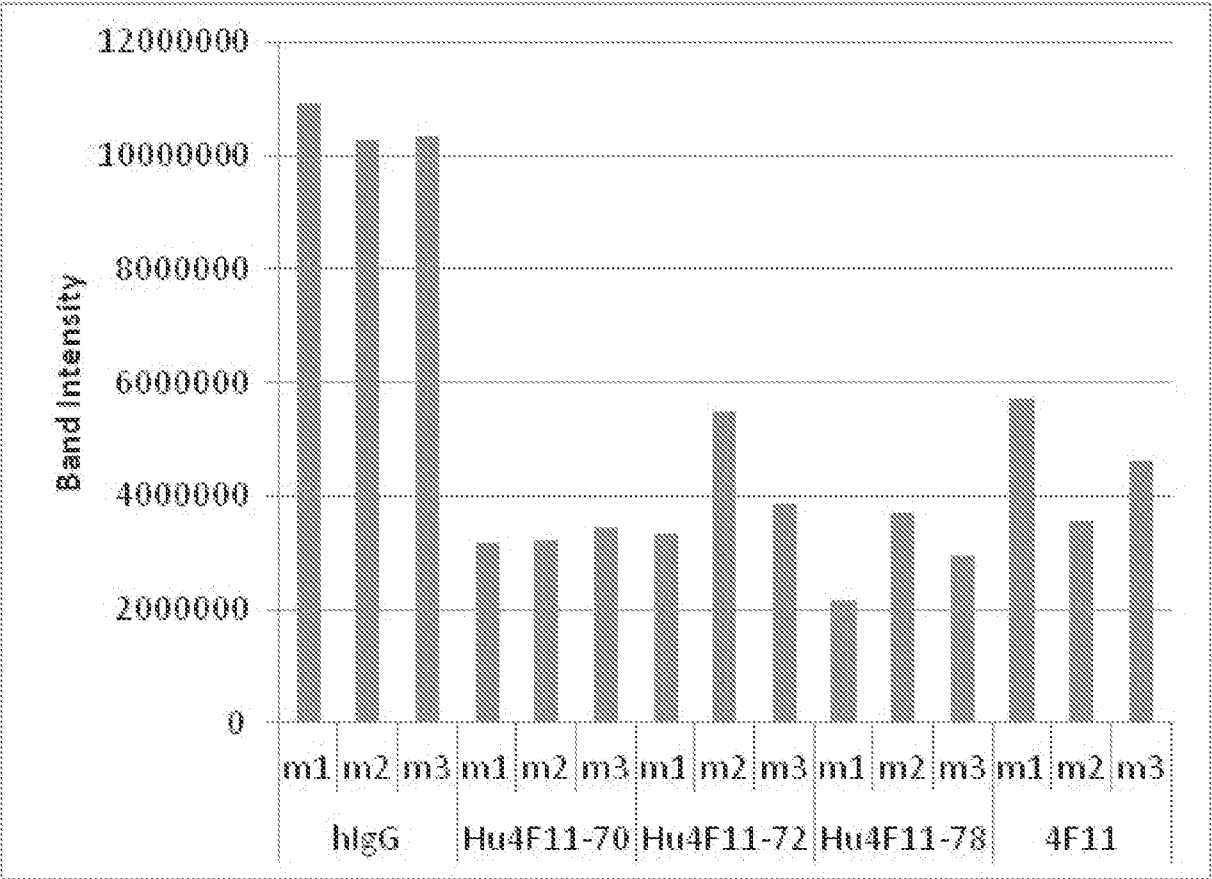


FIG. 13



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/076615

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28
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)
A61K C07K

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 practicable, search terms used)

EP0-Internal, WPI Data, BIOSIS, EMBASE

C. 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 [0018], [0024], [0033], [0165]; claims 23-28, 45; figure 7; examples 2,3,4	1-50
X	WO 2011/041336 A2 (GENENTECH INC [US]; HOFFMANN LA ROCHE [CH]; SIEBEL CHRISTIAN W [US]) 7 April 2011 (2011-04-07) pages 4-6; figure 2 pages 27-31; claims 1-9	1-50
X	WO 2010/005566 A2 (ONCOMED PHARM INC [US]; GURNEY AUSTIN L [US]; HOEY TIMOTHY CHARLES [US]) 14 January 2010 (2010-01-14) paragraphs [0012] - [0014], [0030], [0033]; claims 12, 22; examples 7-8,19	1-50
----- -/--		



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which 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 documents, 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

12 February 2014

Date of mailing of the international search report

08/05/2014

Name and mailing address of the ISA/

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Authorized officer

Aguilera, Miguel

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/076615

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LI KANG ET AL: "Modulation of Notch signaling by antibodies specific for the extracellular negative regulatory region of NOTCH3", JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY FOR BIOCHEMISTRY AND MOLECULAR BIOLOGY, US, vol. 283, no. 12, 21 March 2008 (2008-03-21), pages 8046-8054, XP002481515, ISSN: 0021-9258, DOI: 10.1074/JBC.M800170200 the whole document -----	1-50
A	LIN LUPING ET AL: "Targeting Specific Regions of the Notch3 Ligand-Binding Domain Induces Apoptosis and Inhibits Tumor Growth in Lung Cancer", CANCER RESEARCH, AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 70, no. 2, 15 January 2010 (2010-01-15), pages 632-638, XP009137926, ISSN: 0008-5472, DOI: 10.1158/0008-5472.CAN-09-3293 [retrieved on 2010-01-12] the whole document -----	1-50

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/076615

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-50 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-50(partially)

Isolated anti-Notch3 antibody comprising a VH region comprising CDRs defined by SEQ ID NOs: 51, 53, 7 and a VL region comprising CDRs defined by SEQ ID NOs: 62, 9, 10. Nucleic acids encoding (parts of) said antibodies, expression vectors comprising said nucleic acids, host cells comprising said expression vectors. Methods of production of antibodies using said host cells. Use of said antibodies in the treatment of cancer.

2-12. claims: 1-50(partially)

Isolated anti-Notch3 antibody comprising a VH region comprising CDRs defined by SEQ ID NOs: 5 or 11, 53 and 7 and a VL region comprising CDRs defined by SEQ ID NOs: 8, 9, 10. Nucleic acids encoding (parts of) said antibodies, expression vectors comprising said nucleic acids, host cells comprising said expression vectors. Methods of production of antibodies using said host cells. Use of said antibodies in the treatment of cancer.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/076615

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 2011041336 A2	07-04-2011	AU 2010300747 A1 CA 2775880 A1 CN 102630229 A CO 6531430 A2 CR 20120213 A EP 2483311 A2 JP 2013506675 A KR 20120100952 A MA 33973 B1 PE 09982012 A1 RU 2012117619 A US 2012328608 A1 WO 2011041336 A2	26-04-2012 07-04-2011 08-08-2012 28-09-2012 09-07-2012 08-08-2012 28-02-2013 12-09-2012 01-02-2013 14-08-2012 10-11-2013 27-12-2012 07-04-2011
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/076615

Patent document cited in search report	Publication date	Patent family member(s)	Publication date