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(54) **Title:**
THERAPEUTIC DLL4 BINDING PROTEINS

(57) **Abstract:**

DLL4 binding proteins are described herein, including antibodies, CDR-grafted antibodies, humanized antibodies, and DLL4 binding fragments thereof, proteins that bind DLL4 with high affinity, and DLL4 binding proteins that neutralize DLL4 and/or VEGF activity. The DLL4 binding proteins are useful for treating or preventing cancers and tumors and especially for treating or preventing tumor angiogenesis.

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(54) **Title**: THERAPEUTIC DLL4 BINDING PROTEINS

(57) **Abstract**: DLL4 binding proteins are described herein, including antibodies, CDR-grafted antibodies, humanized antibodies, and DLL4 binding fragments thereof, proteins that bind DLL4 with high affinity, and DLL4 binding proteins that neutralize DLL4 and/or VEGF activity. The DLL4 binding proteins are useful for treating or preventing cancers and tumors and especially for treating or preventing tumor angiogenesis.

THERAPEUTIC DLL4 BINDING PROTEINS

Reference to Related Applications

This application claims priority to US provisional application No. 61/309,494, filed March 2, 2010, the entire contents of which are incorporated herein by reference.

Sequence Listing

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 24, 2011, is named 10920WOO1.txt and is 111,426 bytes in size.

Field of the Invention

The present invention relates to the development and use of DLL4 binding proteins and uses thereof in the inhibition, prevention, and/or treatment of cancers, tumors, other angiogenesis-dependent diseases, angiogenesis-independent diseases, and macular degeneration and age-related macular degeneration diseases characterized by aberrant DLL4 expression or activity.

Background of the Invention

Cell-to-cell communication is required for many biological processes such as differentiation, proliferation, and homeostasis. One system utilized by a wide range of eukaryotes is the Notch-signaling pathway. This pathway, especially the Notch receptor, is also critical for functional tumor angiogenesis. Thus, inhibition of Notch receptor function, blockage of the Notch receptor, and/or blockage of the Notch-signaling pathway are potential strategies for anti-cancer compositions and therapies. Small molecule inhibitors of the Notch receptor have proven to be toxic because they suppress wild type (normal) tissue expression of Notch receptors throughout the body. Thus, different members of the Notch-signaling pathway should be considered as potential targets for therapeutics.

A vasculature ligand for the Notch receptor is Delta 4 or Delta-like 4 (DLL4). Largely expressed in the vasculature, DLL4 is critical for vascular development (Yan et al., *Clin. Cancer Res.*, 13(24): 7243-7246 (2007); Shutter et al., *Genes Dev.*, 14(11): 1313-1318 (2000); Gale et al., *Proc. Natl. Acad. Sci. USA*, 101(45): 15949-15954 (2004); Krebs et al., *Genes Dev.*, 14(11): 1343-1352 (2000)). Mice heterozygous for DLL4 are embryonically lethal due to major defects in vascular development (Gale et al., *Proc. Natl. Acad. Sci. USA*, 101(45): 15949-15954 (2004); Duarte et al., *Genes Dev.*, 18(20): 2474-2478 (2004); Krebs et al., *Genes Dev.*, 18(20): 2469-2473 (2004)). The expression of DLL4 can be induced by VEGF (Liu et al., *Mol. Cell Biol.*, 23(1): 14-25 (2003); Lobov et al., *Proc. Natl. Acad. Sci. USA*, 104(9): 3219-3224 (2007)). In sum, DLL4 can negatively regulate VEGF signaling, in part through repressing VEGFR2 and inducing VEGFR1 (Harrington et al., *Microvasc. Res.*, 75(2): 144-154 (2008); Suchting et al., *Proc. Natl. Acad. Sci. USA*, 104(9): 3225-3230 (2007)). Exquisite coordination between DLL4 and VEGF is essential for functional angiogenesis.

In addition to its physiological role, DLL4 is up-regulated in tumor blood vessels (Gale et al., *Proc. Natl. Acad. Sci. USA*, 101(45): 15949-15954 (2004); Mailhos et al., *Differentiation*, 69(2-3): 135-144 (2001); Patel et al., *Cancer Res.*, 65(19): 8690-8697 (2005); Patel et al., *Clin. Cancer Res.*, 12(16): 4836-4844 (2006); Noguera-Troise et al., *Nature*, 444(7122): 1032-1037 (2006)). Blockade of DLL4 potently inhibited primary tumor growth in multiple models (Noguera-Troise et al., *Nature*, 444(7122): 1032-1037 (2006); Ridgway et al., *Nature*, 444(7122): 1083-1087 (2006); Scehnet et al., *Blood*, 109(11): 4753-4760 (2007)). The inhibition of DLL4 was even effective against tumors that are resistant to anti-VEGF therapy. The combinatorial inhibition of both DLL4 and VEGF provided an enhanced anti-tumor activity. Interestingly, unlike VEGF inhibition that reduces tumor vessel formation, DLL4 blockade leads to an increase in tumor vasculature density wherein the vessels are abnormal, cannot support efficient blood transport, and are effectively nonfunctional. Thus, DLL4 provides a potential target for cancer treatment.

There is a need in the art for therapeutic agents capable of targeting the DLL4-Notch pathway and thereby inhibiting, or even preventing, tumor angiogenesis and growth.

Summary of the Invention

The invention provides proteins that bind human DLL4. DLL4 binding proteins of the invention include, but are not limited to, rat monoclonal antibodies, chimeric antibodies, CDR-grafted antibodies, humanized antibodies, primate-ized antibodies, affinity matured antibodies, and fragments thereof that are capable of binding human DLL4. Preferably, a binding protein described herein binds human DLL4 with high affinity. More preferably, a binding protein according to the invention is capable of neutralizing human DLL4. The invention also provides methods of making and using DLL4 binding proteins.

One aspect of the invention provides a binding protein capable of binding human DLL4, wherein the binding protein comprises at least one amino acid sequence selected from the group of amino acid sequences consisting of SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, and SEQ ID NO:164.

An aspect of this invention pertains to a binding protein comprising an antigen binding domain wherein the binding protein is capable of binding human DLL4, said antigen binding domain comprising at least one or more (i.e., two, three, four, five, or six) CDRs wherein:

CDR-H1 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:151), wherein;

X_1 is N, H, or Y;

X_2 is F;

X_3 is P;

X_4 is M;

X_5 is A or S;

residues 31-35 of SEQ ID NO:157 (CDR-H1 38H12);
residues 31-35 of SEQ ID NO:161 (CDR-H1 37D10);
residues 31-35 of SEQ ID NO:163 (CDR-H1 32C7);
residues 31-35 of SEQ ID NO:165 (CDR-H1 14G1);
residues 31-35 of SEQ ID NO:167 (CDR-H1 14A11);
residues 31-35 of SEQ ID NO:169 (CDR-H1 15D6);
residues 31-35 of SEQ ID NO:171 (CDR-H1 VH.1 1A11);
residues 31-35 of SEQ ID NO:172 (CDR-H1 VH.1a 1A11);
residues 31-35 of SEQ ID NO:173 (CDR-H1 VH.1b 1A11);
residues 31-35 of SEQ ID NO:174 (CDR-H1 VH.2a 1A11);
residues 31-35 of SEQ ID NO:179 (CDR-H1 VH.1 38H12);
residues 31-35 of SEQ ID NO:180 (CDR-H1 VH.1A 38H12);
residues 31-35 of SEQ ID NO:181 (CDR-H1 VH.1b 38H12);
residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);
residues 31-35 of SEQ ID NO:187 (CDR-H1 h1A11VH.1);
residues 31-35 of SEQ ID NO:188 (CDR-H1 h1A11.A6);
residues 31-35 of SEQ ID NO:189 (CDR-H1 h1A11.A8);
residues 31-35 of SEQ ID NO:190 (CDR-H1 h1A11.C6);
residues 31-35 of SEQ ID NO:191 (CDR-H1 h1A11.A11);
residues 31-35 of SEQ ID NO:192 (CDR-H1 h1A11.B5);
residues 31-35 of SEQ ID NO:193 (CDR-H1 h1A11.E12);
residues 31-35 of SEQ ID NO:194 (CDR-H1 h1A11.G3);
residues 31-35 of SEQ ID NO:195 (CDR-H1 h1A11.F5); and
residues 31-35 of SEQ ID NO:196 (CDR-H1 h1A11.H2);

CDR-H2 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17} (SEQ ID NO:152), wherein;

X_1 is T or S;
X_2 is I;
X_3 is S;
X_4 is S or G;
X_5 is S;
X_6 is D;
X_7 is G, A, D, S, or E;
X_8 is T or W;
X_9 is T, P, or A;
X_{10} is Y, S, T, or N;

X_{11} is Y or I;

X_{12} is R or G;

X_{13} is D;

X_{14} is S;

X_{15} is V;

X_{16} is K; and

X_{17} is G;

residues 50-66 of SEQ ID NO:157 (CDR-H2 38H12);

residues 50-68 of SEQ ID NO:161 (CDR-H2 37D10);

residues 50-66 of SEQ ID NO:163 (CDR-H2 32C7);

residues 50-66 of SEQ ID NO:165 (CDR-H2 14G1);

residues 50-66 of SEQ ID NO:167 (CDR-H2 14A11);

residues 50-66 of SEQ ID NO:169 (CDR-H2 15D6);

residues 50-66 of SEQ ID NO:171 (CDR-H2 VH.1 1A11);

residues 50-66 of SEQ ID NO:172 (CDR-H2 VH.1a 1A11);

residues 50-66 of SEQ ID NO:173 (CDR-H2 VH.1b 1A11);

residues 50-66 of SEQ ID NO:174 (CDR-H2 VH.2a 1A11);

residues 50-66 of SEQ ID NO:179 (CDR-H2 VH.1 38H12);

residues 50-66 of SEQ ID NO:180 (CDR-H2 VH.1A 38H12);

residues 50-66 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12);

residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);

residues 50-66 of SEQ ID NO:187 (CDR-H2 h1A11VH.1);

residues 50-66 of SEQ ID NO:188 (CDR-H2 h1A11.A6);

residues 50-66 of SEQ ID NO:189 (CDR-H2 h1A11.A8);

residues 50-66 of SEQ ID NO:190 (CDR-H2 h1A11.C6);

residues 50-66 of SEQ ID NO:191 (CDR-H2 h1A11.A11);

residues 50-66 of SEQ ID NO:192 (CDR-H2 h1A11.B5);

residues 50-66 of SEQ ID NO:193 (CDR-H2 h1A11.E12);

residues 50-66 of SEQ ID NO:194 (CDR-H2 h1A11.G3);

residues 50-66 of SEQ ID NO:195 (CDR-H2 h1A11.F5); and

residues 50-66 of SEQ ID NO:196 (CDR-H2 h1A11.H2);

CDR-H3 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:153), wherein;

X_1 is G;

X_2 is Y;

X_3 is Y;

X_4 is N;

X_5 is S;

X_6 is P;

X_7 is F;

X_8 is A; and

X_9 is Y, F, or S;

residues 99-107 of SEQ ID NO:157 (CDR-H3 38H12);

residues 101-111 of SEQ ID NO:161 (CDR-H3 37D10);

residues 99-105 of SEQ ID NO:163 (CDR-H3 32C7);

residues 99-105 of SEQ ID NO:165 (CDR-H3 14G1);

residues 99-110 of SEQ ID NO:167 (CDR-H3 14A11);

residues 99-110 of SEQ ID NO:169 (CDR-H3 15D6);

residues 99-107 of SEQ ID NO:171 (CDR-H3 VH.1 1A11);

residues 99-107 of SEQ ID NO:172 (CDR-H3 VH.1a 1A11);

residues 99-107 of SEQ ID NO:173 (CDR-H3 VH.1b 1A11);

residues 99-107 of SEQ ID NO:174 (CDR-H3 VH.2a 1A11);

residues 99-107 of SEQ ID NO:179 (CDR-H3 VH.1 38H12);

residues 99-107 of SEQ ID NO:180 (CDR-H3 VH.1A 38H12);

residues 99-107 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12);

residues 99-107 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);

residues 99-107 of SEQ ID NO:187 (CDR-H3 h1A11VH.1);

residues 99-107 of SEQ ID NO:188 (CDR-H3 h1A11.A6);

residues 99-107 of SEQ ID NO:189 (CDR-H3 h1A11.A8);

residues 99-107 of SEQ ID NO:190 (CDR-H3 h1A11.C6);

residues 99-107 of SEQ ID NO:191 (CDR-H3 h1A11.A11);

residues 99-107 of SEQ ID NO:192 (CDR-H3 h1A11.B5);

residues 99-107 of SEQ ID NO:193 (CDR-H3 h1A11.E12);

residues 99-107 of SEQ ID NO:194 (CDR-H3 h1A11.G3);

residues 99-107 of SEQ ID NO:195 (CDR-H3 h1A11.F5);

and

residues 99-107 of SEQ ID NO:196 (CDR-H3 h1A11.H2);

CDR-L1 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11} (SEQ ID NO:154), wherein;

X_1 is R;

X_2 is A;

X_3 is S;

X_4 is E or Q;

X_5 is D or E;
X_6 is I;
X_7 is Y or W;
X_8 is S, I, Y, N, or R;
X_9 is N;
X_{10} is L; and
X_{11} is A;

residues 24-34 of SEQ ID NO:158 (CDR-L1 38H12);
residues 24-34 of SEQ ID NO:162 (CDR-L1 37D10);
residues 24-34 of SEQ ID NO:164 (CDR-L1 32C7);
residues 24-34 of SEQ ID NO:166 (CDR-L1 14G1);
residues 23-37 of SEQ ID NO:168 (CDR-L1 14A11);
residues 23-37 of SEQ ID NO:170 (CDR-L1 15D6);
residues 24-34 of SEQ ID NO:175 (CDR-L1 VL.1 1A11);
residues 24-34 of SEQ ID NO:176 (CDR-L1 VL.1a 1A11);
residues 24-34 of SEQ ID NO:177 (CDR-L1 VL.1b 1A11);
residues 24-34 of SEQ ID NO:178 (CDR-L1 VL.2a 1A11);
residues 24-34 of SEQ ID NO:183 (CDR-L1 VL.1 38H12);
residues 24-34 of SEQ ID NO:184 (CDR-L1 VL.1a 38H12);
residues 24-34 of SEQ ID NO:185 (CDR-L1 VL.1b 38H12);
residues 24-34 of SEQ ID NO:186 (CDR-L1 VL.2a 38H12);
residues 24-34 of SEQ ID NO:197 (CDR-L1 h1A11VL.1);
residues 24-34 of SEQ ID NO:198 (CDR-L1 h1A11.A2);
residues 24-34 of SEQ ID NO:199 (CDR-L1 h1A11.A12);
residues 24-34 of SEQ ID NO:200 (CDR-L1 h1A11.A7);
residues 24-34 of SEQ ID NO:201 (CDR-L1 h1A11.B4);
residues 24-34 of SEQ ID NO:202 (CDR-L1 h1A11.B5); and
residues 24-34 of SEQ ID NO:203 (CDR-L1 h1A11.E12);

CDR-L2 is selected from group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:155), wherein;

X_1 is D;
X_2 is T;
X_3 is N or S;
X_4 is N, D, S, I, Y, or V;
X_5 is L;
X_6 is A; and
X_7 is D;

residues 50-56 of SEQ ID NO:158 (CDR-L2 38H12);
residues 50-56 of SEQ ID NO:162 (CDR-L2 37D10);
residues 50-56 of SEQ ID NO:164 (CDR-L2 32C7);
residues 50-56 of SEQ ID NO:166 (CDR-L2 14G1);
residues 53-59 of SEQ ID NO:168 (CDR-L2 14A11);
residues 53-59 of SEQ ID NO:170 (CDR-L2 15D6);
residues 50-56 of SEQ ID NO:175 (CDR-L2 VL.1 1A11);
residues 50-56 of SEQ ID NO:176 (CDR-L2 VL.1a 1A11);
residues 50-56 of SEQ ID NO:177 (CDR-L2 VL.1b 1A11);
residues 50-56 of SEQ ID NO:178 (CDR-L2 VL.2a 1A11);
residues 50-56 of SEQ ID NO:183 (CDR-L2 VL.1 38H12);
residues 50-56 of SEQ ID NO:184 (CDR-L2 VL.1a 38H12);
residues 50-56 of SEQ ID NO:185 (CDR-L2 VL.1b 38H12);
residues 50-56 of SEQ ID NO:186 (CDR-L2 VL.2a 38H12);
residues 50-56 of SEQ ID NO:197 (CDR-L2 h1A11VL.1);
residues 50-56 of SEQ ID NO:198 (CDR-L2 h1A11.A2);
residues 50-56 of SEQ ID NO:199 (CDR-L2 h1A11.A12);
residues 50-56 of SEQ ID NO:200 (CDR-L2 h1A11.A7);
residues 50-56 of SEQ ID NO:201 (CDR-L2 h1A11.B4);
residues 50-56 of SEQ ID NO:202 (CDR-L2 h1A11.B5); and
residues 50-56 of SEQ ID NO:203 (CDR-L2 h1A11.E12);

and

CDR-L3 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:156), wherein;

X_1 is Q;
X_2 is Q;
X_3 is Y;
X_4 is N, D, or T;
X_5 is N, Y, or W;
X_6 is Y or V;
X_7 is P;
X_8 is P; and
X_9 is T.

residues 89-97 of SEQ ID NO:158 (CDR-L3 38H12);
residues 89-97 of SEQ ID NO:162 (CDR-L3 37D10);
residues 89-97 of SEQ ID NO:164 (CDR-L3 32C7);
residues 89-98 of SEQ ID NO:166 (CDR-L3 14G1);

residues 92-100 of SEQ ID NO:168 (CDR-L3 14A11);
residues 92-100 of SEQ ID NO:170 (CDR-L3 15D6);
residues 89-97 of SEQ ID NO:175 (CDR-L3 VL.1 1A11);
residues 89-97 of SEQ ID NO:176 (CDR-L3 VL.1a 1A11);
residues 89-97 of SEQ ID NO:177 (CDR-L3 VL.1b 1A11);
residues 89-97 of SEQ ID NO:178 (CDR-L3 VL.2a 1A11);
residues 89-97 of SEQ ID NO:183 (CDR-L3 VL.1 38H12);
residues 89-97 of SEQ ID NO:184 (CDR-L3 VL.1a 38H12);
residues 89-97 of SEQ ID NO:185 (CDR-L3 VL.1b 38H12);
residues 89-97 of SEQ ID NO:186 (CDR-L3 VL.2a 38H12);
residues 89-97 of SEQ ID NO:197 (CDR-L3 h1A11VL.1);
residues 89-97 of SEQ ID NO:198 (CDR-L3 h1A11.A2);
residues 89-97 of SEQ ID NO:199 (CDR-L3 h1A11.A12);
residues 89-97 of SEQ ID NO:200 (CDR-L3 h1A11.A7);
residues 89-97 of SEQ ID NO:201 (CDR-L3 h1A11.B4);
residues 89-97 of SEQ ID NO:202 (CDR-L3 h1A11.B5);
residues 89-97 of SEQ ID NO:203 (CDR-L3 h1A11.E12).

Preferably, a DLL4 binding protein of the invention comprises at least one CDR comprising an amino acid sequence selected from the group consisting of:

residues 31-35 of SEQ ID NO:157 (CDR-H1 38H12); residues 50-66 of SEQ ID NO:157 (CDR-H2 38H12); residues 99-107 of SEQ ID NO:157 (CDR-H3 38H12); residues 24-34 of SEQ ID NO:158 (CDR-L1 38H12); residues 50-56 of SEQ ID NO:158 (CDR-L2 38H12); residues 89-97 of SEQ ID NO:158 (CDR-L3 38H12); residues 31-35 of SEQ ID NO:159 (CDR-H1 1A11); residues 50-66 of SEQ ID NO:159 (CDR-H2 1A11); residues 99-107 of SEQ ID NO:159 (CDR-H3 1A11); residues 24-34 of SEQ ID NO:160 (CDR-L1 1A11); residues 50-56 of SEQ ID NO:160 (CDR-L2 1A11); residues 89-97 of SEQ ID NO:160 (CDR-L3 1A11); residues 31-35 of SEQ ID NO:161 (CDR-H1 37D10); residues 50-68 of SEQ ID NO:161 (CDR-H2 37D10); residues 101-111 of SEQ ID NO:161 (CDR-H3 37D10); residues 24-34 of SEQ ID NO:162 (CDR-L1 37D10); residues 50-56 of SEQ ID NO:162 (CDR-L2 37D10); residues 89-97 of SEQ ID NO:162 (CDR-L3 37D10); residues 31-35 of SEQ ID NO:163 (CDR-H1 32C7); residues 50-66 of SEQ ID NO:163 (CDR-H2 32C7); residues 99-105 of SEQ ID NO:163 (CDR-H3 32C7); residues 24-34 of SEQ ID NO:164 (CDR-L1 32C7); residues 50-56 of SEQ ID NO:164 (CDR-L2 32C7); residues 89-98 of SEQ ID NO:164 (CDR-L3 32C7); residues 31-35 of SEQ ID NO:165 (CDR-H1 14G1); residues 50-66 of SEQ ID NO:165 (CDR-H2 14G1); residues 99-105 of SEQ ID NO:165 (CDR-H3 14G1);

residues 24-34 of SEQ ID NO:166 (CDR-L1 14G1); residues 50-56 of SEQ ID NO:166 (CDR-L2 14G1); residues 89-98 of SEQ ID NO:166 (CDR-L3 14G1); residues 31-35 of SEQ ID NO:167 (CDR-H1 14A11); residues 50-66 of SEQ ID NO:167 (CDR-H2 14A11); residues 99-110 of SEQ ID NO:167 (CDR-H3 14A11); residues 23-37 of SEQ ID NO:168 (CDR-L1 14A11); residues 53-59 of SEQ ID NO:168 (CDR-L2 14A11); residues 92-100 of SEQ ID NO:168 (CDR-L3 14A11); residues 31-35 of SEQ ID NO:169 (CDR-H1 15D6); residues 50-66 of SEQ ID NO:169 (CDR-H2 15D6); residues 99-110 of SEQ ID NO:169 (CDR-H3 15D6); residues 23-37 of SEQ ID NO:170 (CDR-L1 15D6); residues 53-59 of SEQ ID NO:170 (CDR-L2 15D6); residues 92-100 of SEQ ID NO:170 (CDR-L3 15D6); residues 31-35 of SEQ ID NO:171 (CDR-H1 VH.1 1A11); residues 50-66 of SEQ ID NO:171 (CDR-H2 VH.1 1A11); residues 99-107 of SEQ ID NO:171 (CDR-H3 VH.1 1A11); residues 31-35 of SEQ ID NO:172 (CDR-H1 VH.1a 1A11); residues 50-66 of SEQ ID NO:172 (CDR-H2 VH.1a 1A11); residues 99-107 of SEQ ID NO:172 (CDR-H3 VH.1a 1A11); residues 31-35 of SEQ ID NO:173 (CDR-H1 VH.1b 1A11); residues 50-66 of SEQ ID NO:173 (CDR-H2 VH.1b 1A11); residues 99-107 of SEQ ID NO:173 (CDR-H3 VH.1b 1A11); residues 31-35 of SEQ ID NO:174 (CDR-H1 VH.2a 1A11); residues 50-66 of SEQ ID NO:174 (CDR-H2 VH.2a 1A11); residues 99-107 of SEQ ID NO:174 (CDR-H3 VH.2a 1A11); residues 24-34 of SEQ ID NO:175 (CDR-L1 VL.1 1A11); residues 50-56 of SEQ ID NO:175 (CDR-L2 VL.1 1A11); residues 89-97 of SEQ ID NO:175 (CDR-L3 VL.1 1A11); residues 24-34 of SEQ ID NO:176 (CDR-L1 VL.1a 1A11); residues 50-56 of SEQ ID NO:176 (CDR-L2 VL.1a 1A11); residues 89-97 of SEQ ID NO:176 (CDR-L3 VL.1a 1A11); residues 24-34 of SEQ ID NO:177 (CDR-L1 VL.1b 1A11); residues 50-56 of SEQ ID NO:177 (CDR-L2 VL.1b 1A11); residues 89-97 of SEQ ID NO:177 (CDR-L3 VL.1b 1A11); residues 24-34 of SEQ ID NO:178 (CDR-L1 VL.2a 1A11); residues 50-56 of SEQ ID NO:178 (CDR-L2 VL.2a 1A11); residues 89-97 of SEQ ID NO:178 (CDR-L3 VL.2a 1A11); residues 31-35 of SEQ ID NO:179 (CDR-H1 VH.1 38H12); residues 50-66 of SEQ ID NO:179 (CDR-H2 VH.1 38H12); residues 99-107 of SEQ ID NO:179 (CDR-H3 VH.1 38H12); residues 31-35 of SEQ ID NO:180 (CDR-H1 VH.1A 38H12); residues 50-66 of SEQ ID NO:180 (CDR-H2 VH.1A 38H12); residues 99-107 of SEQ ID NO:180 (CDR-H3 VH.1A 38H12); residues 31-35 of SEQ ID NO:181 (CDR-H1 VH.1b 38H12); residues 50-66 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12); residues 99-107 of SEQ ID NO:181 (CDR-H3 VH.1b 38H12); residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12); residues 50-66 of SEQ ID NO:182 (CDR-H2 VH.2a 38H12); residues 99-107 of SEQ ID NO:182 (CDR-H3 VH.2a 38H12); residues 24-34 of SEQ ID NO:183 (CDR-L1 VL.1 38H12); residues 50- 56 of SEQ ID

NO:183 (CDR-L2 VL.1 38H12); residues 89-97 of SEQ ID NO:183 (CDR-L3 VL.1 38H12); residues 24-34 of SEQ ID NO:184 (CDR-L1 VL.1a 38H12); residues 50-56 of SEQ ID NO:184 (CDR-L2 VL.1a 38H12); residues 89-97 of SEQ ID NO:184 (CDR-L3 VL.1a 38H12); residues 24-34 of SEQ ID NO:185 (CDR-L1 VL.1b 38H12); residues 50-56 of SEQ ID NO:185 (CDR-L2 VL.1b 38H12); residues 89-97 of SEQ ID NO:185 (CDR-L3 VL.1b 38H12); residues 24-34 of SEQ ID NO:186 (CDR-L1 VL.2a 38H12); residues 50-56 of SEQ ID NO:186 (CDR-L2 VL.2a 38H12); residues 89-97 of SEQ ID NO:186 (CDR-L3 VL.2a 38H12); residues 31-35 of SEQ ID NO:187 (CDR-H1 h1A11VH.1), residues 50-66 of SEQ ID NO:187 (CDR-H2 h1A11VH.1); residues 99-107 of SEQ ID NO:187 (CDR-H3 h1A11VH.1); residues 31-35 of SEQ ID NO:188 (CDR-H1 h1A11.A6), residues 50-66 of SEQ ID NO:188 (CDR-H2 h1A11.A6); residues 99-107 of SEQ ID NO:188 (CDR-H3 h1A11.A6); residues 31-35 of SEQ ID NO:189 (CDR-H1 h1A11.A8), residues 50-66 of SEQ ID NO:189 (CDR-H2 h1A11.A8); residues 99-107 of SEQ ID NO:189 (CDR-H3 h1A11.A8); residues 31-35 of SEQ ID NO:190 (CDR-H1 h1A11.C6), residues 50-66 of SEQ ID NO:190 (CDR-H2 h1A11.C6); residues 99-107 of SEQ ID NO:190 (CDR-H3 h1A11.C6); residues 31-35 of SEQ ID NO:191 (CDR-H1 h1A11.A11), residues 50-66 of SEQ ID NO:191 (CDR-H2 h1A11.A11); residues 99-107 of SEQ ID NO:191 (CDR-H3 h1A11.A11); residues 31-35 of SEQ ID NO:192 (CDR-H1 h1A11.B5), residues 50-66 of SEQ ID NO:192 (CDR-H2 h1A11.B5); residues 99-107 of SEQ ID NO:192 (CDR-H3 h1A11.B5); residues 31-35 of SEQ ID NO:193 (CDR-H1 h1A11.E12), residues 50-66 of SEQ ID NO:193 (CDR-H2 h1A11.E12); residues 99-107 of SEQ ID NO:193 (CDR-H3 h1A11.E12); residues 31-35 of SEQ ID NO:194 (CDR-H1 h1A11.G3), residues 50-66 of SEQ ID NO:194 (CDR-H2 h1A11.G3); residues 99-107 of SEQ ID NO:194 (CDR-H3 h1A11.G3); residues 31-35 of SEQ ID NO:195 (CDR-H1 h1A11.F5), residues 50-66 of SEQ ID NO:195 (CDR-H2 h1A11.F5); residues 99-107 of SEQ ID NO:195 (CDR-H3 h1A11.F5); residues 31-35 of SEQ ID NO:196 (CDR-H1 h1A11.H2), residues 50-66 of SEQ ID NO:196 (CDR-H2 h1A11.H2); residues 99-107 of SEQ ID NO:196 (CDR-H3 h1A11.H2); residues 24-34 of SEQ ID NO:197 (CDR-L1 h1A11VL.1), residues 50-56 of SEQ ID NO:197 (CDR-L2 h1A11VL.1); residues 89-97 of SEQ ID NO:197 (CDR-L3 h1A11VL.1); residues 24-34 of SEQ ID NO:198 (CDR-L1 h1A11.A2), residues 50-56 of SEQ ID NO:198 (CDR-L2 h1A11.A2); residues 89-97 of SEQ ID NO:198 (CDR-L3 h1A11.A2); residues 24-34 of SEQ ID NO:199 (CDR-L1 h1A11.A12), residues 50-56 of SEQ ID NO:199 (CDR-L2 h1A11.A12); residues 89-97 of SEQ ID NO:199 (CDR-L3 h1A11.A12); residues 24-34 of SEQ ID NO:200 (CDR-L1 h1A11.A7), residues 50-56 of SEQ ID NO:200 (CDR-L2 h1A11.A7); residues 89-97 of SEQ ID NO:200 (CDR-L3 h1A11.A7);

residues 24-34 of SEQ ID NO:201 (CDR-L1 h1A11.B4), residues 50-56 of SEQ ID NO:201 (CDR-L2 h1A11.B4); residues 89-97 of SEQ ID NO:201 (CDR-L3 h1A11.B4); residues 24-34 of SEQ ID NO:202 (CDR-L1 h1A11.B5), residues 50-56 of SEQ ID NO:202 (CDR-L2 h1A11.B5); residues 89-97 of SEQ ID NO:202 (CDR-L3 h1A11.B5); residues 24-34 of SEQ ID NO:203 (CDR-L1 h1A11.E12), residues 50-56 of SEQ ID NO:203 (CDR-L2 h1A11.E12); and residues 89-97 of SEQ ID NO:203 (CDR-L3 h1A11.E12).

In an embodiment, a DLL4 binding protein of the invention comprises at least three CDRs described herein (above or below). In a non-limiting example, a DLL4 binding protein of the invention comprises three CDRs described herein, wherein the three CDRs are a CDR-H1, a CDR-H2, and a CDR-H3 as described herein. In another non-limiting example, a DLL4 binding protein of the invention comprising three CDRs described herein, wherein the three CDRs are a CDR-L1, a CDR-L2, and a CDR-L3 as described herein.

In an embodiment, a DLL4 binding protein of the invention comprises one or more CDRs described herein (above or below), such as one, two, three, four, five, or six CDRs described herein. In a preferred embodiment, a DLL4 binding protein according to the invention comprises six CDRs described herein, e.g., a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 as described herein.

In another embodiment, a DLL4 binding protein of the invention comprises three CDRs selected from a set of variable domain CDRs, wherein the set of variable domain CDRs is selected from the group of variable domain CDR sets consisting of:

VH 38H12 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:157
CDR-H2: residues 50-66 of SEQ ID NO:157
CDR-H3 residues 99-107 of SEQ ID NO:157

VL 38H12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:158
CDR-L2: residues 50-56 of SEQ ID NO:158
CDR-L3: residues 89-97 of SEQ ID NO:158

VH 1A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:159
CDR-H2: residues 50-66 of SEQ ID NO:159
CDR-H3: residues 99-107 of SEQ ID NO:159

VL 1A11 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:160
CDR-L2: residues 50-56 of SEQ ID NO:160
CDR-L3: residues 89-97 of SEQ ID NO:160

VH 37D10 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:161
CDR-H2: residues 50-68 of SEQ ID NO:161
CDR-H3: residues 101-111 of SEQ ID NO:161

VL 37D10 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:162
CDR-L2: residues 50-56 of SEQ ID NO:162
CDR-L3: residues 89-97 of SEQ ID NO:162

VH 32C7 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:163
CDR-H2: residues 50-66 of SEQ ID NO:163
CDR-H3: residues 99-105 of SEQ ID NO:163

VL 32C7 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:164
CDR-L2: residues 50-56 of SEQ ID NO:164
CDR-L3: residues 89-98 of SEQ ID NO:164

VH 14G1 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:165
CDR-H2: residues 50-66 of SEQ ID NO:165
CDR-H3: residues 99-105 of SEQ ID NO:165

VL 14G1 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:166
CDR-L2: residues 50-56 of SEQ ID NO:166
CDR-L3: residues 89-97 of SEQ ID NO:166

VH 14A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:167
CDR-H2: residues 50-66 of SEQ ID NO:167
CDR-H3: residues 99-110 of SEQ ID NO:167

VL 14A11 CDR Set

CDR-L1: residues 23-37 of SEQ ID NO:168
CDR-L2: residues 53-59 of SEQ ID NO:168
CDR-L3: residues 92-100 of SEQ ID NO:168

VH 15D6 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:169
CDR-H2: residues 50-66 of SEQ ID NO:169
CDR-H3: residues 99-110 of SEQ ID NO:169

VL 15D6 CDR Set

CDR-L1: residues 23-37 of SEQ ID NO:170
CDR-L2: residues 53-59 of SEQ ID NO:170
CDR-L3: residues 92-100 of SEQ ID NO:170

VH VH.1 1A11 CDR Set
CDR-H1: residues 31-35 o f SEQ ID NO:171
CDR-H2: residues 50-66 of SEQ ID NO:171
CDR-H3: residues 99-107 of SEQ ID NO:171

VH VH.1a 1A11 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:172
CDR-H2: residues 50-66 of SEQ ID NO:172
CDR-H3: residues 99-107 of SEQ ID NO:172

VH VH.1b 1A11 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:173
CDR-H2: residues 50-66 of SEQ ID NO:173
CDR-H3: residues 99-107 of SEQ ID NO:173

VH VH.2a 1A11 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:174
CDR-H2: residues 50-66 of SEQ ID NO:174
CDR-H3: residues 99-107 of SEQ ID NO:174

VL VL.1 1A11 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:175
CDR-L2: residues 50-56 of SEQ ID NO:175
CDR-L3: residues 89-97 of SEQ ID NO:175

VL VL.1a 1A11 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:176
CDR-L2: residues 50-56 of SEQ ID NO:176
CDR-L3: residues 89-97 of SEQ ID NO:176

VL VL.1b 1A11 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:177
CDR-L2: residues 50-56 of SEQ ID NO:177
CDR-L3: residues 89-97 of SEQ ID NO:177

VL VL.2a 1A11 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:178
CDR-L2: residues 50-56 of SEQ ID NO:178
CDR-L3: residues 89-97 of SEQ ID NO:178

VH VH.1 38H12 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:179

CDR-H2: residues 50-66 of SEQ ID NO:179
CDR-H3: residues 99-107 of SEQ ID NO:179

VH VH.1a 38H12 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:180
CDR-H2: residues 50-66 of SEQ ID NO:180
CDR-H3: residues 99-107 of SEQ ID NO:180

VH VH.1b 38H12 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:181
CDR-H2: residues 50-66 of SEQ ID NO:181
CDR-H3: residues 99-107 of SEQ ID NO:181

VH VH.2a 38H12 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:182
CDR-H2: residues 50-66 of SEQ ID NO:182
CDR-H3: residues 99-107 of SEQ ID NO:182

VL VL.1 38H12 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:183
CDR-L2: residues 50-56 of SEQ ID NO:183
CDR-L3: residues 89-97 of SEQ ID NO:183

VL VL.1a 38H12 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:184
CDR-L2: residues 50-56 of SEQ ID NO:184
CDR-L3: residues 89-97 of SEQ ID NO:184

VL VL.1b 38H12 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:185
CDR-L2: residues 50-56 of SEQ ID NO:185
CDR-L3: residues 89-97 of SEQ ID NO:185

VL VL.2a 38H12 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:186
CDR-L2: residues 50-56 of SEQ ID NO:186
CDR-L3: residues 89-97 of SEQ ID NO:186

VH hA11VH.1 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:187
CDR-H2: residues 50-66 of SEQ ID NO:187
CDR-H3: residues 99-107 of SEQ ID NO:187

VH hA11.A6 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:188
CDR-H2: residues 50-66 of SEQ ID NO:188

CDR-H3: residues 99-107 of SEQ ID NO:188

VH hA11.A8 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:189
CDR-H2: residues 50-66 of SEQ ID NO:189
CDR-H3: residues 99-107 of SEQ ID NO:189

VH hA11.C6 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:190
CDR-H2: residues 50-66 of SEQ ID NO:190
CDR-H3: residues 99-107 of SEQ ID NO:190

VH hA11.A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:191
CDR-H2: residues 50-66 of SEQ ID NO:191
CDR-H3: residues 99-107 of SEQ ID NO:191

VH hA11.B5 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:192
CDR-H2: residues 50-66 of SEQ ID NO:192
CDR-H3: residues 99-107 of SEQ ID NO:192

VH hA11.E12 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:193
CDR-H2: residues 50-66 of SEQ ID NO:193
CDR-H3: residues 99-107 of SEQ ID NO:193

VH hA11.G3 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:194
CDR-H2: residues 50-66 of SEQ ID NO:194
CDR-H3: residues 99-107 of SEQ ID NO:194

VH hA11.F5 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:195
CDR-H2: residues 50-66 of SEQ ID NO:195
CDR-H3: residues 99-107 of SEQ ID NO:195

VH hA11.H2 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:196
CDR-H2: residues 50-66 of SEQ ID NO:196
CDR-H3: residues 99-107 of SEQ ID NO:196

VL h1A11VL.1 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:197
CDR-L2: residues 50-56 of SEQ ID NO:197
CDR-L3: residues 89-97 of SEQ ID NO:197

VL h1A11.A2 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:198
CDR-L2: residues 50-56 of SEQ ID NO:198
CDR-L3: residues 89-97 of SEQ ID NO:198

VL h1A11.A12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:199
CDR-L2: residues 50-56 of SEQ ID NO:199
CDR-L3: residues 89-97 of SEQ ID NO:199

VL h1A11.A7 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:200
CDR-L2: residues 50-56 of SEQ ID NO:200
CDR-L3: residues 89-97 of SEQ ID NO:200

VL h1A11.B4 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:201
CDR-L2: residues 50-56 of SEQ ID NO:201
CDR-L3: residues 89-97 of SEQ ID NO:201

VL h1A11.B5 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:202
CDR-L2: residues 50-56 of SEQ ID NO:202
CDR-L3: residues 89-97 of SEQ ID NO:202

and

VL h1A11.E12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:203
CDR-L2: residues 50-56 of SEQ ID NO:203
CDR-L3: residues 89-97 of SEQ ID NO:.203

In an embodiment, a DLL4 binding protein comprises CDRs from at least two sets of variable domain CDRs in the group above.

In another embodiment, a DLL4 binding protein of the invention comprises three CDRs selected from any VH set of three CDRs in the group above and three CDRs selected from any VL set of three CDRs in the group above.

In still another embodiment, a DLL4 binding protein of the invention comprises a VH set of three CDRs as described above and a VL set of three CDRs as described above from a pair of VH and VL sets of CDRs selected from the group consisting of:

VH 38H12 CDR Set and VL 38H12 CDR Set,
VH 1A11 CDR Set and VL 1A11 CDR Set,
VH 37D10 CDR Set and VL 37D10 CDR Set,
VH 32C7 CDR Set and VL 32C7 CDR Set,

VH 14G1 Set and VL 14G1 CDR Set,
VH 14A11 CDR Set and VL 14A11 CDR Set,
VH 15D6 CDR Set and VL 15D6 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.1 38H12 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.1 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH VH.1b 38H12 CDR Set and VL VL.1 38H12 CDR Set,
VH VH.1b 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.1b 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.1b 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.1 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH h1A11.A6 CDR Set and VL h1A11VL.1 CDR Set,
VH h1A11.C6 CDR Set and VL h1A11VL.1 CDR Set,

VH h1A11.A11 CDR Set and VL h1A11VL.1 CDR Set,
VH h1A11.A8 CDR Set and VL h1A11VL.1 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.B4 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.A7 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.A12 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.A2 CDR Set,
VH h1A11.B5 CDR Set and VL h1A11.B5 CDR Set,
VH h1A11.E12 CDR Set and VL h1A11.E12 CDR Set,
VH h1A11.G3 CDR Set and VL h1A11.E12 CDR Set,
VH h1A11.F5 CDR Set and VL h1A11.E12 CDR Set, and
VH h1A11.H2 CDR Set and VL h1A11.E12 CDR Set.

In a preferred embodiment, a DLL4 binding protein possesss a DLL4 antigen binding domain (or binding site) comprising six CDRs, wherein a CDR-H1, CDR-H2, and CDR-H3 are located in a heavy chain variable region (VH) and a CDR-L1, CDR-L2, and CDR-L3 are located in a light chain variable region (VL), and wherein association of the VH and VL regions form a functional DLL4 antigen binding domain of the DLL4 binding protein. In a further non-limiting example of this embodiment, a DLL4 binding protein that possesses two DLL4 antigen binding domains comprises two sets of VH and VL regions and therefore comprises twelve CDRs.

In another embodiment, a DLL4 binding protein possesses a DLL4 antigen binding domain comprising six CDRs, wherein a CDR-H1, CDR-H2, and CDR-H3 are located in a heavy chain variable region (VH) and a CDR-L1, CDR-L2, and CDR-L3 are located in a light chain variable region (VL) and wherein the remaining sequences in each variable region constitute a framework (FR) region such that each CDR is positioned between two FR region sequences for a total of four FR sequences, i.e., FR1, FR2, FR3, and FR4. In this embodiment, the arrangement of FR and CDR sequences in a variable region is FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. In this embodiment, a binding domain formed by association of a VH region and a VL region comprises eight FR sequences and six CDRs.

DLL4 binding proteins of the invention include CDR-grafted antibodies, wherein one or more CDRs of VH and/or VL regions of an antibody of one species (donor species) are grafted into and replace the corresponding CDRs of the VH and/or VL of an antibody of another (acceptor) species using recombinant techniques available in the art. An example of a donor species is a rat anti-human DLL4 monoclonal antibody described herein and an example of an acceptor species is a human immunoglobulin gamma (IgG) molecule, wherein the human FR sequences of the VH and VL regions of the human IgG molecule are human acceptor framework sequences that receive the grafted in CDRs from the donor rat monoclonal antibody. The human acceptor framework sequences of the resulting CDR-grafted antibody may be further mutated to improve one or more properties of the CDR-grafted antibody. By way of non-limiting examples, one or more residues of one or more FR sequences of a CDR-grafted antibody may be mutated to improve DLL4 binding affinity or to lower immunogenicity of the CDR-grafted antibody in a human subject.

In an embodiment of the invention, a DLL4 binding protein comprising one or more CDRs described above further comprises a human acceptor framework sequence. Preferably, a DLL4 binding protein comprises one or more (e.g., one, two, three, four, five, six, seven, or eight) human acceptor framework sequences.

A human acceptor framework sequence present in a DLL4 binding protein of the invention may comprise one or more amino acid residues that have been back mutated to one or more corresponding amino acid residues present in a rat monoclonal antibody that binds DLL4 and/or that have been mutated to one or more amino acid residues that reduce or eliminate a site(s) for an undesirable reaction, for example, to reduce or eliminate a site for undesired glycosylation and/or a site for undesired N-terminal pyroglutamate formation and/or a site for potentially reduced immunogenicity risk.

In an embodiment, a DLL4 binding protein comprising one or more CDRs described above further comprises one or more (e.g., any one, two, three, four, five, six, seven, or eight) of the human acceptor framework sequences selected from the group of human acceptor framework sequences in Tables 3 and 4, below. One or more human acceptor framework sequences from Tables 3 and 4 present in a DLL4 binding protein of the invention may further comprise one or more amino acid residues that have been back mutated to one or more corresponding amino acid residues present in a rat monoclonal antibody that binds DLL4 and/or that have been mutated to one or more amino acids that reduce or eliminate a site(s) for an undesirable reaction, for example, to reduce or eliminate a site for undesired glycosylation and/or a site for undesired N-terminal pyroglutamate formation and/or a site for potentially reduced immunogenicity risk.

In another embodiment, the invention provides a DLL4 binding protein comprising one or more CDRs described above, wherein the binding protein also comprises one or more (e.g., any one, two, three, four, five, six, seven, or eight per binding domain) of the human acceptor framework sequences selected from any framework sequence present in a variable region sequence selected from the group consisting of:

SEQ ID NO:171 VH.1 1A11	SEQ ID NO:180 VH.1a 38H12	SEQ ID NO:188 VH h1A11.A6	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:172 VH.1a 1A11	SEQ ID NO:181 VH.1b 38H12	SEQ ID NO:189 VH h1A11.A8	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:173 VH.1b 1A11	SEQ ID NO:182 VH.2a 38H12	SEQ ID NO:190 VH h1A11.C6	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:174 VH.2a 1A11	SEQ ID NO:183 VL.1 38H12	SEQ ID NO:191 VH h1A11.A11	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:175 VL.1 1A11	SEQ ID NO:184 VL.1a	SEQ ID NO:192 VH h1A11.B5	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:176	SEQ ID NO:185	SEQ ID NO:193	SEQ ID NO:201

VL.1a 1A11	VL.1b	VH h1A11.E12	VL h1A11.B4
SEQ ID NO:177 VL.1b 1A11	SEQ ID NO:186 VL.2a	SEQ ID NO:194 VH h1A11.G3	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:178 VL.2a 1A11	SEQ ID NO:187 VH h1A11VH.1	SEQ ID NO:195 VH h1A11.F5	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:179 VH.1 38H12			

In yet another embodiment of the invention, a DLL4 binding protein further comprises one or more (e.g., any one, two, three, four, five, six, seven, or eight) acceptor framework sequences selected from the group consisting of:

heavy chain framework-1 (H-FR1): E-V-Q-L-V-E-S-G-G-G-L-V-Q-P-G-G-S-L-R-L-S-C-A-A-S-G-F-T-F-X_{30} (SEQ ID NO:143), wherein X_{30} is S, R, or G;

heavy chain framework-2 (H-FR2): W-V-R-Q-A-P-G-K-G-L-E-W-V-A (SEQ ID NO:144);

heavy chain framework-3 (H-FR3): R-F-T-I-S-R-D-N-A-K-X_{11}-S-L-Y-L-Q-M-N-S-L-R-A-E-D-T-A-V-Y-Y-C-X_{31}-R (SEQ ID NO:145), wherein;

X_{11} is N or S; and

X_{31} is A or S;

heavy chain framework-4 (H-FR4): W-G-Q-G-T-L-V-T-V-S-S (SEQ ID NO:146);

light chain framework-1 (L-FR1): D-I-Q-M-T-Q-S-P-S-S-L-S-A-S-V-G-D-R-V-T-I-T-C (SEQ ID NO:147);

light chain framework-2 (L-FR2): W-Y-Q-Q-K-P-G-K-X_9-P-K-L-L-I-X_{15} (SEQ ID NO:148), wherein;

X_9 is A or S; and

X_{15} is F or Y;

light chain framework-3 (L-FR3): G-V-P-S-R-F-S-G-S-G-S-G-T-D-X_{15}-T-L-T-I-S-S-L-Q-P-E-D-F-A-T-Y-Y-C (SEQ ID NO:149), wherein;

X_{15} is F or S; and

light chain framework-4 (L-FR4): F-G-Q-G-T-K-L-E-I-K (SEQ ID NO:150).

In another embodiment, a DLL4 binding protein comprising one or more CDRs described above also comprises a human acceptor framework sequence described above wherein the human acceptor framework sequence comprises at least one framework region amino acid substitution at a key residue, wherein the key residue is selected from the group consisting of a residue adjacent to a CDR, a glycosylation site residue, a rare residue, a residue capable of interacting with human DLL4, a residue capable of interacting with a CDR, a canonical residue, a contact residue between heavy chain variable region and light chain variable region, a residue within a Vernier zone, and a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

In another embodiment, a human acceptor framework sequence of a DLL4 binding protein described herein comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to a sequence of a human germline acceptor framework and comprises at least 70 amino acid residues identical to the human germline acceptor framework. In another embodiment, a DLL4 binding protein of the invention comprises a consensus human variable domain sequence.

In an embodiment, the invention provides a DLL4 binding protein comprises a human acceptor framework sequence, wherein the binding protein comprises at least one variable domain having an amino acid sequence selected from the group consisting of:

SEQ ID NO:171 VH VH.1 1A11	SEQ ID NO:188 VH h1A11.A6
SEQ ID NO:172 VH VH.1a 1A11	SEQ ID NO:189 VH h1A11.A8
SEQ ID NO:173 VH VH.1b 1A11	SEQ ID NO:190 VH h1A11.C6
SEQ ID NO:174 VH VH.2a 1A11	SEQ ID NO:191 VH h1A11.A11
SEQ ID NO:175 VL VL.1 1A11	SEQ ID NO:192 VH h1A11.B5
SEQ ID NO:176 VL VL.1a 1A11	SEQ ID NO:193 VH h1A11.E12
SEQ ID NO:177 VL VL.1b	SEQ ID NO:194 VH h1A11.G3
SEQ ID NO:178 VL VL.2a 1A11	SEQ ID NO:195 VH h1A11.F5
SEQ ID NO:179 VH VH.1 38H12	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:180 VH VH.1a 38H12	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:181 VH VH.1b 38H12	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:182 VH VH.2a 38H12	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:183 VL VL.1 38H12	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:184 VL VL.1a 38H12	SEQ ID NO:201 VL h1A11.B4
SEQ ID NO:185 VL VL.1b 38H12	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:186 VL VL.2a 38H12	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:187 VH h1A11VH.1	

In another embodiment, a DLL4 binding protein of the invention comprises two or more variable domains described above. In a preferred embodiment, a DLL4 binding protein of the invention comprises two variable domains, wherein the two variable domains have amino acid sequences selected from the group consisting of:

SEQ ID NO:171 and SEQ ID NO:175 VH.1 and VL.1 1A11 (Table 12)	SEQ ID NO:180 and SEQ ID NO:186 VH.1a and VL.2a (Table 16)
SEQ ID NO:171 and SEQ ID NO:176 VH.1 and VL.1a 1A11 (Table 12)	SEQ ID NO:181 and SEQ ID NO:183 VH.1b and VL.1 (Table 16)
SEQ ID NO:171 and SEQ ID NO:177 VH.1 and VL.1b 1A11 (Table 12)	SEQ ID NO:181 and SEQ ID NO:184 VH.1b and VL.1a (Table 16)
SEQ ID NO:171 and SEQ ID NO:178 VH.1 and VL.2a 1A11(Table 12)	SEQ ID NO:181 and SEQ ID NO:185 VH.1b and VL.1b (Table 16)
SEQ ID NO:172 and SEQ ID NO:175 VH.1a and VL.1 1A11 (Table 12)	SEQ ID NO:181 and SEQ ID NO:186 VH.1b and VL.2a (Table 16)
SEQ ID NO:172 and SEQ ID NO:176 VH.1a and VL.1a 1A11 (Table 12)	SEQ ID NO:182 and SEQ ID NO:183 VH.2a and VL.1 (Table 16)
SEQ ID NO:172 and SEQ ID NO:177 VH.1a and VL.1b 1A11 (Table 12)	SEQ ID NO:182 and SEQ ID NO:184 VH.2a and VL.1a (Table 16)
SEQ ID NO:172 and SEQ ID NO:178 VH.1a and VL.2a 1A11 (Table 12)	SEQ ID NO:182 and SEQ ID NO:185 VH.2a and VL.1b (Table 16)
SEQ ID NO:173 and SEQ ID NO:175 VH.1b and VL.1 1A11 (Table 12)	SEQ ID NO:182 and SEQ ID NO:186 VH.2a and VL.2a (Table 16)
SEQ ID NO:173 and SEQ ID NO:176 VH.1b and VL.1a 1A11 (Table 12)	SEQ ID NO:188 and SEQ ID NO:197 h1A11.A6 VH and h1A11VL.1 Tables 20/21
SEQ ID NO:173 and SEQ ID NO:177 VH.1b and VL.1b 1A11 (Table 12)	SEQ ID NO:190 and SEQ ID NO:197 h1A11.C6 VH and h1A11VL.1 Tables 20/21
SEQ ID NO:173 and SEQ ID NO:178 VH.1b and VL.2a 1A11 (Table 12)	SEQ ID NO:191 and SEQ ID NO:197 h1A11.All VH and h1A11VL.1 Tables 20/21
SEQ ID NO:174 and SEQ ID NO:175 VH.2a and VL.1 1A11 (Table	SEQ ID NO:189 and SEQ ID NO:197 h1A11.A8 VH and h1A11 VL.1

12)	Tables 20/21
SEQ ID NO:174 and SEQ ID NO:176 VH.2a and VL.1a 1A11 (Table 12)	SEQ ID NO:1878 and SEQ ID NO:201 h1A11VH.1 and h1A11.B4 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:177 VH.2a and VL.1b 1A11 (Table 12)	SEQ ID NO:187 and SEQ ID NO:200 h1A11VH.1 and h1All.A7 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:178 VH.2a and VL.2a 1A11 (Table 12)	SEQ ID NO:187 and SEQ ID NO:199 h1A11VH.1 and h1All.A12 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:183 VH.1 and VL.1 38H12 (Table 16)	SEQ ID NO:187 and SEQ ID NO:198 h1A11VH.1 VH and h1All.A2 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:184 VH.1 and VL.1a 38H12 (Table 16)	SEQ ID NO:192 and SEQ ID NO:202 h1A11.B5 VH and h1All.B5 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:185 VH.1 and VL.1b 38H12 (Table 16)	SEQ ID NO:193 and SEQ ID NO:203 h1A11.E12 VH and h1All.E12 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:186 VH.1 and VL.2a 38H12 (Table 16)	SEQ ID NO:194 and SEQ ID NO:203 h1A11.G3 VH and h1All.E12 VL Tables 20/21
SEQ ID NO:180 and SEQ ID NO:183 VH.1a and VL.1 38H12 (Table 16)	SEQ ID NO:195 and SEQ ID NO:203 h1A11.F5 VH and h1All.E12 VL Tables 20/21
SEQ ID NO:180 and SEQ ID NO:184 VH.1a and VL.1a 38H12 (Table 16)	SEQ ID NO:196 and SEQ ID NO:203 h1A11.H2 VH and h1All.E12 VL Tables 20/21
SEQ ID NO:180 and SEQ ID NO:185 VH.1a and VL.1b 38H12 (Table 16)	

In an embodiment, a DLL4 binding protein of the invention comprises at least one variable domain having an amino acid sequence selected from the group consisting of:

SEQ ID NO:157 VH 38H12	SEQ ID NO:181 VH VH.1b 38H12
SEQ ID NO:158 VL 38H12	SEQ ID NO:182 VH VH.2a 38H12

SEQ ID NO:159 VH 1A11	SEQ ID NO:182 VL VL.1 38H12
SEQ ID NO:160 VL 1A11	SEQ ID NO:184 VL VL.1a 38H12
SEQ ID NO:161 VH 37D10	SEQ ID NO:185 VL VL.1b 38H12
SEQ ID NO:162 VL 37D10	SEQ ID NO:186 VL VL.2a 38H12
SEQ ID NO:163 VH 32C7	SEQ ID NO:187 VH h1A11VH.1
SEQ ID NO:164 VL 32C7	SEQ ID NO:188 VH h1A11.A6
SEQ ID NO:165 VH 14G1	SEQ ID NO:189 VH h1A11.A8
SEQ ID NO:166 VL 14G1	SEQ ID NO:190 VH h1A11.C6
SEQ ID NO:167 VH 14A11	SEQ ID NO:191 VH h1A11.A11
SEQ ID NO:168 VL 14A11	SEQ ID NO:192 VH h1A11.B5
SEQ ID NO:169 VH 15D6	SEQ ID NO:193 VH h1A11.E12
SEQ ID NO:170 VL 15D6	SEQ ID NO:194 VH h1A11.G3
SEQ ID NO:171 VH VH.1 1A11	SEQ ID NO:195 VH h1A11.F5
SEQ ID NO:172 VH VH.1a 1A11	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:173 VH VH.1b 1A11	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:174 VH VH.2a 1A11	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:175 VL VL.1 1A11	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:176 VL VL.1a 1A11	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:177 VL VL.1b	SEQ ID NO:201 VL h1A11.B4
SEQ ID NO:178 VL VL.2a 1A11	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:179 VH VH.1 38H12	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:180 VH VH.1a 38H12	

In another embodiment, a DLL4 binding protein of the invention comprises two variable domains, wherein the two variable domains have the amino acid sequences selected from the group consisting of:

SEQ ID NO:157 and SEQ ID NO:158 38H12	SEQ ID NO:179 and SEQ ID NO:186 VH.1 and VL.2a 38H12 (Table 16)

SEQ ID NO:159 and SEQ ID NO:160 1A11	SEQ ID NO:180 and SEQ ID NO:183 VH.1a and VL.1 38H12 (Table 16)
SEQ ID NO:161 and SEQ ID NO:162 37D10	SEQ ID NO:180 and SEQ ID NO:184 VH.1a and VL.1a 38H12 (Table 16)
SEQ ID NO:163 and SEQ ID NO:164 32C7	SEQ ID NO:180 and SEQ ID NO:185 VH.1a and VL.1b 38H12 (Table 16)
SEQ ID NO:165 and SEQ ID NO:166 14G1	SEQ ID NO:180 and SEQ ID NO:186 VH.1a and VL.2a (Table 16)
SEQ ID NO:167 and SEQ ID NO:168 14A11	SEQ ID NO:181 and SEQ ID NO:183 VH.1b and VL.1 (Table 16)
SEQ ID NO:169 and SEQ ID NO:170 15D6	SEQ ID NO:181 and SEQ ID NO:184 VH.1b and VL.1a (Table 16)
SEQ ID NO:171 and SEQ ID NO:175 VH.1 and VL.1 1A11 (Table 11)	SEQ ID NO:181 and SEQ ID NO:185 VH.1b and VL.1b (Table 16)
SEQ ID NO:171 and SEQ ID NO:176 VH.1 and VL.1a 1A11 (Table 11)	SEQ ID NO:181 and SEQ ID NO:186 VH.1b and VL.2a (Table 16)
SEQ ID NO:171 and SEQ ID NO:177 VH.1 and VL.1b 1A11 (Table 11)	SEQ ID NO:182 and SEQ ID NO:183 VH.2a and VL.1 (Table 16)
SEQ ID NO:171 and SEQ ID NO:178 VH.1 and VL.2a (Table 11)	SEQ ID NO:182 and SEQ ID NO:184 VH.2a and VL.1a (Table 16)
SEQ ID NO:172 and SEQ ID NO:175 VH.1a and VL.1 (Table 11)	SEQ ID NO:182 and SEQ ID NO:185 VH.2a and VL.1b (Table 16)
SEQ ID NO:172 and SEQ ID NO:176 VH.1a and VL.1a (Table 11)	SEQ ID NO:182 and SEQ ID NO:186 VH.2a and VL.2a (Table 16)
SEQ ID NO:172 and SEQ ID NO:177 VH.1a and VL.1b (Table 11)	SEQ ID NO:188 and SEQ ID NO:197 h1A11.A6 VH and h1A11VL.1 Tables 20/21
SEQ ID NO:172 and SEQ ID NO:178 VH.1a and VL.2a (Table 11)	SEQ ID NO:190 and SEQ ID NO:197 h1A11.C6 VH and h1A11VL.1 Tables 20/21
SEQ ID NO:173 and SEQ ID NO:175 VH.1b and VL.1 (Table 11)	SEQ ID NO:191 and SEQ ID NO:197 h1A11.All VH and h1A11VL.1 Tables 20/21
SEQ ID NO:173 and SEQ ID NO:176	SEQ ID NO:189 and SEQ ID NO:197

VH.1b and VL.1a (Table 11)	h1A11.A8 VH and h1A11 VL.1 Tables 20/21
SEQ ID NO:173 and SEQ ID NO:177 VH.1b and VL.1b (Table 11)	SEQ ID NO:187 and SEQ ID NO:201 h1A11VH.1 and h1A11.B4 VL Tables 20/21
SEQ ID NO:173 and SEQ ID NO:178 VH.1b and VL.2a (Table 11)	SEQ ID NO:187 and SEQ ID NO:200 h1A11VH.1 and h1All.A7 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:175 VH.2a and VL.1 (Table 11)	SEQ ID NO:187 and SEQ ID NO:199 h1A11VH.1 and h1All.A12 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:176 VH.2a and VL.1a (Table 11)	SEQ ID NO:187 and SEQ ID NO:198 h1A11VH.1 VH and h1All.A2 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:177 VH.2a and VL.1b (Table 11)	SEQ ID NO:192 and SEQ ID NO:202 h1A11.B5 VH and h1All.B5 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:178 VH.2a and VL.2a (Table 11)	SEQ ID NO:193 and SEQ ID NO:203 h1A11.E12 VH and h1All.E12 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:183 VH.1 and VL.1 **38H12** (Table 16)	SEQ ID NO:194 and SEQ ID NO:203 h1A11.G3 VH and h1All.E12 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:184 VH.1 and VL.1a **38H12** (Table 16)	SEQ ID NO:195 and SEQ ID NO:203 h1A11.F5 VH and h1All.E12 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:185 VH.1 and VL.1b **38H12** (Table 16)	SEQ ID NO:196 and SEQ ID NO:203 h1A11.H2 VH and h1All.E12 VL Tables 20/21

In an embodiment, a DLL4 binding protein of the invention comprises two variable domains having the amino acid sequences selected from the group consisting of:

SEQ ID NO:188 (h1All.A6 VH) and SEQ ID NO:197 (h1A11VL.1),

SEQ ID NO:190 (h1A11.C6 VH) and SEQ ID NO:197 (h1A11VL.1), and

SEQ ID NO:191 (h1A11.A11 VH) and SEQ ID NO:197 (h1A11.VL.1).

In an embodiment, a DLL4 binding protein of the invention comprises two variable domains having the amino acid sequences SEQ ID NO:181 (VH.1b 38H12) and SEQ ID NO:185 (VL.1b 38H12).

According to the invention, variable heavy (VH) domains and variable light (VL) domains of any of the DLL4 binding proteins described herein may also be shuffled using recombinant techniques available in the art to generate and select for additional DLL4 binding proteins that comprise various combinations of VH and VL domains described herein.

In an embodiment, a DLL4 binding protein according to the invention binds human DLL4 (hu DLL4) and at least one other species of DLL4. More preferably, a DLL4 binding protein described herein binds human DLL4 and a DLL4 selected from the group consisting of a cynomolgus monkey DLL4 (cynomolgus DLL4, cyno DLL4), a mouse DLL4 (mu DLL4), a rat DLL4, and combinations thereof.

In another embodiment, a DLL4 binding protein described herein is capable of blocking DLL4 interaction with a Notch protein. Preferably, the Notch protein is selected from the group consisting of Notch-1, Notch-2, Notch-3, Notch-4, and combinations thereof.

In an embodiment, a DLL4 binding protein described herein is capable of modulating, inhibiting, or neutralizing one or more biological functions of human DLL4. More preferably, a DLL4 binding protein of the invention is capable of modulating, inhibiting, or neutralizing an activity of a DLL4 selected from the group consisting of a human DLL4, a cynomolgus DLL4, a monkey DLL4, a rat DLL4, and combinations thereof.

In a further embodiment, a DLL4 binding protein described herein is capable of inhibiting VEGFR2 activity, VEGFR1 activity, or both VEGFR2 and VEGFR1 activities.

In an embodiment, a DLL4 binding protein described herein is capable of inhibiting normal angiogenesis.

In an embodiment, a DLL4 binding protein of the invention has an on rate constant (K_{on}) to DLL4 of at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; or at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance. Preferably, the binding protein of the invention has an on rate constant (K_{on}) to DLL4 between $10^2M^{-1}s^{-1}$ to $10^3M^{-1}s^{-1}$; between $10^3M^{-1}s^{-1}$ to $10^4M^{-1}s^{-1}$; between $10^4M^{-1}s^{-1}$ to $10^5M^{-1}s^{-1}$; or between $10^5M^{-1}s^{-1}$ to $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

In another embodiment, a DLL4 binding protein of the invention has an off rate constant (K_{off}) for DLL4 of at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; or at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance. Preferably, the binding protein of the invention has an off rate constant (K_{off}) to DLL4 of $10^{-3}s^{-1}$ to $10^{-4}s^{-1}$; of $10^{-4}s^{-1}$ to $10^{-5}s^{-1}$; or of $10^{-5}s^{-1}$ to $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

In another embodiment, a DLL4 binding protein of the invention has a dissociation constant (K_D) to DLL4 of at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; or at most 10^{-13} M. Preferably, the binding protein of the invention has a dissociation constant (K_D) to DLL4 of 10^{-7} M to 10^{-8} M; of 10^{-8} M to 10^{-9} M; of 10^{-9} M to 10^{-10} M; of 10^{-10} to 10^{-11} M; of 10^{-11} M to 10^{-12} M; or of 10^{-12} to M 10^{-13} M.

In an embodiment, the invention provides an antibody construct comprising a DLL4 binding protein described above and a linker polypeptide or an immunoglobulin constant domain. In a preferred embodiment, an antibody construct according to the invention is selected from the group consisting of: an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a $F(ab')_2$, an Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, and a bispecific antibody.

In a preferred embodiment, an antibody construct of the invention comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant domain, a human IgG1 constant domain, a human IgG2 constant domain, a human IgG3 constant domain, a human IgG4 constant domain, a human IgE constant domain, and a human IgA constant domain.

In another embodiment, an antibody construct of the invention comprises an immunoglobulin constant region selected from the group consisting of an immunoglobulin gamma-1 (IgG-1) heavy chain constant region (such as SEQ ID NO:3), a mutant IgG-1 heavy chain constant region (such as SEQ ID NO:4), an immunoglobulin kappa light chain constant region (such as SEQ ID NO:5), an immunoglobulin lambda light chain constant region (such as SEQ ID NO:6), and combinations thereof.

In another embodiment, an antibody construct is glycosylated. Preferably, the glycosylation is a human glycosylation pattern.

In an embodiment, the invention provides an antibody conjugate comprising an antibody construct described herein conjugated to an agent. Preferably, the agent is selected from the group consisting of: an imaging agent, a therapeutic agent, a cytotoxic agent, and an immunoadhesion molecule. In a preferred embodiment, an imaging agents is selected from the group consisting of: a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. More preferably the imaging agent is a radiolabel selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm. In a preferred embodiment, the therapeutic or cytotoxic agent is selected from the group consisting of: an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

In another embodiment, a DLL4 binding protein, an antibody construct, or antibody conjugate described above exists as a crystal. Preferably, the crystal is a carrier-free pharmaceutical controlled release crystal. In another embodiment, such a crystallized binding protein, crystallized antibody construct, or crystallized antibody conjugate has a greater half life *in vivo* than its soluble counterpart. In a preferred embodiment, a crystallized binding protein, crystallized antibody construct, or crystallized antibody conjugate retains the biological activity of the soluble or non-crystal form of the binding protein, antibody construct, or antibody conjugate after crystallization.

In an embodiment, the invention provides an isolated nucleic acid encoding one or more amino acid sequences of a DLL4 binding protein (including any antibody construct or antibody conjugate) described herein

In a preferred embodiment, the invention provides an isolated nucleic acid encoding a polypeptide selected from the group consisting of: a polypeptide comprising a heavy chain variable domain, wherein the heavy chain variable domain comprises one or more of a CDR-H1, a CDR-H2, and a CDR-H3 as described above; a polypeptide comprising a light chain variable domain, wherein the light chain variable domain comprises one or more of a CDR-L1, a CDR-L2, and a CDR-L3 as described above; and a combination of both polypeptides.

One aspect of the invention pertains to an isolated nucleic acid encoding a DLL4 binding protein, an antibody construct, a DLL4 binding antibody conjugate, or DLL4 binding portion thereof. Particularly preferred is an isolated nucleic acid that encodes a polypeptide selected from the group consisting of: a polypeptide comprising a heavy chain variable domain, wherein the heavy chain variable domain comprises a CDR-H1, a CDR-H2, or a CDR-H3 described above; a polypeptide comprising a light chain variable domain, wherein the light chain variable domain comprises a CDR-L1, a CDR-L2, or a CDR-L3 as described above; or a combination of both polypeptides.

A further embodiment provides a vector comprising an isolated nucleic acid described herein. In a preferred embodiment, the vector is selected from the group consisting of: pcDNA, pTT (Durocher et al., *Nucl. Acids Res.*, 30(2e9): 1-9 (2002)), pTT3 (pTT with additional multiple cloning sites), pEFBOS (Mizushima et al., *Nucl. Acids. Res.*, 18 (17): 5322 (1990)), pBV, pJV, and pBJ.

In another aspect of the invention there is provided a host cell transformed with the vector described above. The host cell can be a prokaryotic or eukaryotic cell. A preferred prokaryotic host cell is *Escherichia coli*. Preferably, the eukaryotic cell is selected from the group consisting of: a protist cell, an animal cell, a plant cell, and a fungal cell. More preferably, the host cell is a mammalian cell including, but not limited to, CHO and COS cells. A preferred fungal cell is *Saccharomyces cerevisiae*. A preferred insect cell is an Sf9 cell.

In another aspect of the invention there is provided a method of producing a binding protein that binds human DLL4 comprising the step of culturing any one of the host cells described above in a culture medium under conditions sufficient to produce a binding protein that binds human DLL4.

One embodiment provides a composition for the release of a DLL4 binding protein according to the invention wherein the composition comprises a formulation that comprises a crystallized DLL4 binding protein, a crystallized antibody construct, or a crystallized antibody conjugate as described above and an ingredient, and further at least one polymeric carrier. Preferably, the polymeric carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride- alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polysaccharides, blends and copolymers thereof. Preferably, the ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-β-cyclodextrin, methoxypolyethylene glycol and polyethylene glycol.

Another embodiment provides a method for treating a mammal comprising the step of administering to the mammal an effective amount of a composition comprising a crystallized DLL4 binding protein, a crystallized antibody construct, or a crystallized antibody conjugates described above.

The invention also provides a pharmaceutical composition comprising a DLL4 binding protein as described above (including an antibody construct or an antibody conjugate as described above) and a pharmaceutically acceptable carrier. In a further embodiment, the pharmaceutical composition comprises at least one additional agent. The additional agent may be a therapeutic agent for treating a disorder in which DLL4 is detrimental. Preferably, a pharmaceutical composition of the invention comprises an additional agent selected from the group consisting of: a therapeutic agent; an imaging agent; an antineoplastic agent; a chemotherapeutic agent; an angiogenesis inhibitor; an anti-VEGF antibody; an anti-EGFR antibody; an anti-cMet antibody; an anti-ErbB3 antibody; an anti-HER2 antibody; an anti-CD20 antibody; a VEGF-trap molecule; a kinase inhibitor; a co-stimulation molecule blocker; an anti-B7.2 antibody; a CTLA4-Ig; an adhesion molecule blocker; an anti-E selectin antibody; an anti-L selectin antibody; an anti-cytokine antibody or functional fragment thereof; an anti-IL-18 antibody; an anti-TNF antibody; anti-IL-6 antibody; methotrexate; a corticosteroid; a cyclosporin; a rapamycin; FK506; a DNA alkylating agent; cisplatin; carboplatin; an anti-tubulin agent; paclitaxel; docetaxel; doxorubicin; gemcitabine; gemzar; an anthracycline; adriamycin; a topoisiomersase I inhibitor; a topoisomerase II inhibitor; 5-fluorouracil (5-FU); leucovorin; irinotecan; a receptor tyrosine kinase inhibitor; an apoptosis inhibitor; a Bcl2/Bclx inhibitor; erlotinib; gefitinib; a COX-2 inhibitor; celecoxib; cyclosporin; rapamycin; a detectable label or reporter molecule; a TNF antagonist; an antirheumatic; a muscle relaxant; a narcotic; an analgesic; an anesthetic; a sedative; a local anesthetic; a neuromuscular blocker; an antimicrobial agent; an antipsoriatic agent; a corticosteroid; an anabolic steroid; an erythropoietin; an immunization; an immunoglobulin; an immunosuppressive agent; a growth hormone; a hormone replacement drug; a radiopharmaceutical drug; an antidepressant; an antipsychotic drug; a stimulant; an asthma medication; a beta agonist; an inhaled steroid; an epinephrine; an epinephrine analog thereof; a cytokine; and a cytokine antagonist.

In another aspect, the invention provides a method for inhibiting human DLL4 activity comprising contacting human DLL4 with a binding protein disclosed above such that human DLL4 is inhibited or neutralized. In a related aspect, the invention provides a method for inhibiting DLL4 activity in a human subject suffering from a disorder in which DLL4 is detrimental, comprising administering to the human subject a binding protein disclosed above such that human DLL4 in the human subject is inhibited and treatment is achieved. Preferably, the disorder is selected from the group comprising primary and metastatic cancers, including carcinomas of breast, colon, rectum, lung, oropharynx, hypopharynx, esophagus, stomach, pancreas, liver, gallbladder and bile ducts, small intestine, urinary tract (including kidney, bladder, and urothelium), female genital tract (including cervix, uterus, and ovaries as well as choriocarcinoma and gestational trophoblastic disease), male genital tract (including prostate, seminal vesicles, testes, and germ cell tumors), endocrine glands (including the thyroid, adrenal, and pituitary glands), and skin, as well as hemangiomas, melanomas, sarcomas (including those arising from bone and soft tissues as well as Kaposi's sarcoma), tumors of the brain, nerves, eyes, and meninges (including astrocytomas, gliomas, glioblastomas, retinoblastomas, neuromas, neuroblastomas, Schwannomas, and meningiomas), solid tumors arising from hematopoietic malignancies such as leukemias and lymphomas (both Hodgkin's and non-Hodgkin's lymphomas), tumor metastases, ocular neovascularization (including diabetic blindness, retinopathies, age-induced macular degeneration and rubeosis), edema, rheumatoid arthritis, atherosclerotic plaques, refractory ascites, psoriasis, pancreatitis, polycystic ovarian disease (POD), endometriosis, uterine fibroids, benign prostate hypertrophy, T-cell acute lymphoblastic leukemia (T-ALL), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), multiple sclerosis (MS), tetralogy of Fallot (TOF), Alagille syndrome (AS), macular degeneration and age-related macular degeneration diseases, and other angiogenesis independent and dependent diseases characterized by aberrant DLL4 expression or activity.

In another aspect, the invention provides a method of treating a patient suffering from a disorder in which human DLL4 is detrimental comprising the step of administering any one of the binding proteins disclosed above before, concurrent, or after the administration of a therapeutically effective amount of a second agent. In a preferred embodiment, the second agent is selected from the group consisting of: a radiotherapeutic agent; an antineoplastic agent; a chemotherapeutic agent; a DNA alkylating agent; cisplatin; carboplatin; an anti-tubulin agent; paclitaxel; docetaxel; taxol; doxorubicin; gemcitabine; gemzar; an anthracycline; adriamycin; a topoisomerase I inhibitor; a topoisomerase II inhibitor; 5-fluorouracil (5-FU); leucovorin; irinotecan; a receptor tyrosine kinase inhibitor; an apoptosis inhibitor; a Bcl2/Bclx inhibitor; erlotinib; gefitinib; a COX-2 inhibitor; celecoxib; a kinase inhibitor; an angiogenesis inhibitor; an anti-VEGF antibody; anti-EGFR antibody; an anti-cMet antibody; an anti-ErbB3 antibody; an anti-HER2 antibody; an anti-CD20 antibody; VEGF-Trap (aflibercept); a co-stimulation molecule blocker; an anti-B7.1 antibody; an anti-B7.2 antibody; CTLA4-Ig; an adhesion molecule blocker; an anti-LFA-1 antibody; an anti-E selectin antibody; an anti-L selectin antibody; a small molecule inhibitor; an anti-cytokine antibody or functional fragment thereof; an anti-IL-18 antibody; an anti-TNF antibody; an anti-IL-6 antibody; an anti-cytokine receptor antibody; methotrexate; cyclosporin; rapamycin; FK506; a detectable label or reporter; a TNF antagonist; an antirheumatic; a muscle relaxant; a narcotic; a non-steroid anti-inflammatory drug (NSAID); an analgesic; an anesthetic; a sedative; a local anesthetic; a neuromuscular blocker; an antimicrobial agent; an antipsoriatic drug; a corticosteroid; an anabolic steroid; an erythropoietin; an immunization; an immunoglobulin; an immunosuppressive agent; a growth hormone; a hormone replacement drug; a radiopharmaceutical drug; an antidepressant; an antipsychotic drug; a stimulant; an asthma medication; a beta agonist; an inhaled steroid; an epinephrine; an epinephrine analog; a cytokine; and a cytokine antagonist.

In a preferred embodiment, the pharmaceutical compositions disclosed above are administered to the subject by at least one mode selected from the group consisting of: parenteral, subcutaneous, intramuscular, intravenous, intraarterial, intraarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

Another aspect of the invention provides at least one DLL4 anti-idiotype antibody to at least one DLL4 binding protein of the present invention. The anti-idiotype antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, and any portion thereof, that can be incorporated into a binding protein of the present invention.

Any of a variety of immunodetection assay formats may be adapted to employ a DLL4 binding protein of the invention to detect or measure DLL4 in a mixture, solution, or biological sample. Such immunodetection assay formats include but are not limited to radioimmunoassay (RIA), immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), immunoblot (e.g., Western), immunostrips (e.g., immunodipsticks) comprising a DLL4 binding protein of the invention adsorbed or immobilized to substrate, FACS, and the like. Detection of DLL4 using a DLL4 binding protein of the invention may be conducted *in vitro* on a mixture, solution, or in biological sample. A biological sample that may be contacted with a binding protein of the invention to detect or measure DLL4 in the sample includes, but is not limited to, urine, saliva, oral swab (buccal, lingual, or throat swab), dermal swab, dermal scrape, rectal swab, vaginal swab, whole blood sample, plasma sample, serum sample, tissue biopsy, and any other sample obtained from an individual by a procedure known in the art. In another embodiment, a DLL4 binding protein may be employed to detect DLL4 *in vivo* such as various tomography and scanning methods, including but not limited to X-ray computer assisted tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET).

Detailed Description of the Invention

This invention pertains to DLL4 binding proteins, particularly anti-DLL4 antibodies, or antigen-binding portions thereof that bind DLL4. An amino acid sequence (SEQ ID NO:1) for human DLL4 is shown in Table 1 along with a corresponding DLL4 nucleotide coding sequence (SEQ ID NO:2). Various aspects of the invention relate to antibodies and antibody fragments, and pharmaceutical compositions thereof, as well as nucleic acids, recombinant expression vectors, and host cells for making such antibodies and fragments. Methods of using the antibodies of the invention to detect human DLL4 or murine DLL4, methods to inhibit human or mouse DLL4 and/or human or mouse VEGFR2 or VEGFR1 activity, either *in vitro* or *in vivo*, and methods to regulate gene expression are also encompassed by the invention.

Table 1. Amino acid and nucleotide coding sequences for human DLL4.

Kind of Sequence	Sequence Identifier	Sequence
		123456789012345678901234567890
Human DLL4 Amino Acid Sequence	SEQ ID NO:1	MAAASRSASGWALLLLVALWQQRAAGSGVF QLQLQEFINERGVLASGRPCEPGCRTFFRV CLKHFQAVVSPGPCTFGTVSTPVLGTNSFA VRDDSSGGGRNPLQLPFNFTWPGTFSLIIE AWHAPGDDLRPEALPPDALISKIAIQGSLA VGQNWLLDEQTSTLTRLRYSYRVICSDNYY GDNCSRLCKKRNDHFGHYVCQPDGNLSCLP GWTGEYCQQPICLSGCHEQNGYCSKPAECL CRPGWQGRLCNECIPHNGCRHGTCSTPWQC TCDEGWGGLFCDQDLNYCTHHSPCKNGATC SNSGQRSYTCTCRPGYTGVDCELELSECDS NPCRNGGSCKDQEDGYHCLCPPGYYGLHCE

Kind of Sequence	Sequence Identifier	Sequence
		123456789012345678901234567890
		HSTLSCADSPCFNGGSCRERNQGANYACEC PPNFTGSNCEKKVDRCTSNPCANGGQCLNR GPSRMCRCRPGFTGTYCELHVSDCARNPCA HGGTCHDLENGLMCTCPAGFSGRRCEVRTS IDACASSPCFNRATCYTDLSTDTFVCNCPY GFVGSRCEFPVGLPPSFPWVAVSLGVGLAV LLVLLGMVAVAVRQLRLRRPDDGSREAMNN LSDFQKDNLIPAAQLKNTNQKKELEVDCGL DKSNCGKQQNHTLDYNLAPGPLGRGTMPGK FPHSDKSLGEKAPLRLHSEKPECRISAICS PRDSMYQSVCLISEERNECVIATEV
Human DLL4 Nucleotide Coding Sequence	SEQ ID NO:2	atggcggcagcgtcccggagcgcctctggc tgggcgctactgctgctggtggcactttgg cagcagcgcgcggccggctccggcgtcttc cagctgcagctgcaggagttcatcaacgag cgcggcgtactggccagtgggcggccttgc gagcccggctgccggactttcttccgcgtc tgccttaagcacttccaggcggtcgtctcg cccggaccctgcaccttcgggaccgtctcc acgccggtattgggcaccaactccttcgct gtccgggacgacagtagcggcgggggcgc aaccctctccaactgcccttcaatttcacc tggccgggtaccttctcgctcatcatcgaa gcttggcacgcgccaggagacgacctgcgg ccagaggccttgccaccagatgcactcatc agcaagatcgccatccagggctccctagct gtgggtcagaactggttattggatgagcaa accagcaccctcacaaggctgcgctactct taccgggtcatctgcagtgacaactactat ggagacaactgctcccgcctgtgcaagaag cgcaatgaccacttcggccactatgtgtgc cagccagatggcaacttgtcctgcctgccc ggttggactggggaatattgccaacagcct atctgtctttcgggctgtcatgaacagaat ggctactgcagcaagccagcagagtgcctc tgccgcccaggctggcagggccggctgtgt aacgaatgcatcccccacaatggctgtcgc cacggcacctgcagcactccctggcaatgt acttgtgatgagggctggggaggcctgttt tgtgaccaagatctcaactactgcacccac cactccccatgcaagaatggggcaacgtgc tccaacagtgggcagcgaagctacacctgc acctgtcgcccaggctacactggtgtggac tgtgagctggagctcagcgagtgtgacagc aacccctgtcgcaatggaggcagctgtaag gaccaggaggatggctaccactgcctgtgt cctccgggctactatggcctgcattgtgaa cacagcaccttgagctgcgccgactccccc tgcttcaatgggggctcctgccgggagcgc aaccagggggccaactatgcttgtgaatgt ccccccaacttcaccggctccaactgcgag

Kind of Sequence	Sequence Identifier	Sequence
		123456789012345678901234567890
		aagaaagtggacaggtgcaccagcaacccc tgtgccaacgggggacagtgcctgaaccga ggtccaagccgcatgtgccgctgccgtcct ggattcacgggcacctactgtgaactccac gtcagcgactgtgcccgtaacccttgcgcc cacggtggcacttgccatgacctggagaat gggctcatgtgcacctgccctgccggcttc tctggccgacgctgtgaggtgcggacatcc atcgatgcctgtgcctcgagtccctgcttc aacagggccacctgctacaccgacctctcc acagacacctttgtgtgcaactgcccttat ggctttgtgggcagccgctgcgagttcccc gtgggcttgccgcccagcttcccctgggtg gccgtctcgctgggtgtggggctggcagtg ctgctggtactgctgggcatggtggcagtg gctgtgcggcagctgcggcttcgacggccg gacgacggcagcagggaagccatgaacaac ttgtcggacttccagaaggacaacctgatt cctgccgcccagcttaaaaacacaaaccag aagaaggagctggaagtggactgtggcctg gacaagtccaactgtggcaaacagcaaaac cacacattggactataatctggccccaggg cccctggggcggggaccatgccaggaaag tttccccacagtgacaagagcttaggagag aaggcgccactgcggttacacagtgaaaag ccagagtgtcggatatcagcgatatgctcc cccagggactccatgtaccagtctgtgtgt ttgatatcagaggagaggaatgaatgtgtc attgccacggaggtataa

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear, however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including," as well as other forms, such as "includes" and "included," is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

That the present invention may be more readily understood, select terms are defined below.

The term "polypeptide" as used herein, refers to any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric. Use of "polypeptide" herein is intended to encompass polypeptide and fragments and variants (including fragments of variants) thereof, unless otherwise stated. For an antigenic polypeptide, a fragment of polypeptide optionally contains at least one contiguous or nonlinear epitope of polypeptide. The precise boundaries of the at least one epitope fragment can be confirmed using ordinary skill in the art. The fragment comprises at least about 5 contiguous amino acids, such as at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, or at least about 20 contiguous amino acids. A variant of polypeptide is as described herein.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

The term "recovering" as used herein, refers to the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

The term "human DLL4" (abbreviated herein as "hDLL4" or "huDLL4"), as used herein, includes several EGF-like domains and a DSL domain that is required for receptor binding. The term includes a protein comprising about 74-75 kDa. The structure and deduced DNA and protein sequences of human DLL4 is described further in, for example, Shutter et al., *Genes & Dev.*, 4: 1313-1318 (2000). The term "human DLL4" is intended to include recombinant human DLL4 (rh DLL4), which can be prepared by standard recombinant expression methods.

"Biological activity", as used herein with respect to DLL4, refers to all inherent biological properties of DLL4. Biological properties of DLL4 include, but are not limited to, binding a Notch receptor, activating a Notch receptor, negatively regulating VEGF signaling, repressing VEGFR2, and inducing VEGR1.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, means that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

A "binding protein" is a monomeric or multimeric protein that binds to and forms a complex with a binding partner, which may be a polypeptide, an antigen, a chemical compound or other molecule, or a substrate of any kind. A binding protein specifically binds a binding partner. Binding proteins include antibodies and other molecules comprising one or more antigen-binding domains that bind to an antigen molecule or a particular site (epitope) on the antigen molecule. A binding protein includes an antibody or any of its antigen-binding fragments, and various forms and derivatives of antibodies known in the art and described below. Accordingly, a binding protein includes, but is not limited to, an antibody, a tetrameric immunoglobulin, an IgG molecule, an IgG_1 molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, an affinity matured antibody, and fragments of any such antibodies that retain the ability to bind to an antigen.

The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. Nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain, which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain. Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (US Patent Nos. 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions, e.g., cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC), and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for a therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment, at least one amino acid residue is replaced in the constant region of the antibody, for example, the Fc region of the antibody, such that effector functions of the antibody are altered. The dimerization of two identical heavy chains of an immunoglobulin is mediated by the dimerization of CH3 domains and is stabilized by the disulfide bonds within the hinge region (Huber et al., *Nature*, 264: 415-420 (1976); Thies et al., *J. Mol. Biol.*, 293: 67-79 (1999)). Mutation of cysteine residues within the hinge regions to prevent heavy chain-heavy chain disulfide bonds will destabilize dimerization of CH3 domains. Residues responsible for CH3 dimerization have been identified (Dall'Acqua, *Biochem.*, 37: 9266-9273 (1998)). Therefore, it is possible to generate a monovalent half-Ig. Interestingly, these monovalent half Ig molecules have been found in nature for both IgG and IgA subclasses (Seligman, *Ann. Immunol.*, 129: 855-70 (1978); Biewenga et al., *Clin. Exp. Immunol.*, 51: 395-400 (1983)). The stoichiometry of FcRn: Ig Fc region has been determined to be 2:1 (West et al., *Biochem.*, 39: 9698-9708 (2000)), and half Fc is sufficient for mediating FcRn binding (Kim et al., *Eur. J. Immunol.*, 24: 542-548 (1994)). Mutations to disrupt the dimerization of CH3 domain may not have greater adverse effect on its FcRn binding as the residues important for CH3 dimerization are located on the inner interface of CH3 b sheet structure, whereas the region responsible for FcRn binding is located on the outside interface of CH2-CH3 domains. However, the half Ig molecule may have certain advantages in tissue penetration due to its smaller size in comparison to that of a regular antibody. In one embodiment, at least one amino acid residue is replaced in the constant region of a binding protein of the invention, for example the Fc region, such that the dimerization of the heavy chains is disrupted, resulting in half Ig molecules. The anti-inflammatory activity of IgG is completely dependent on sialylation of the N-linked glycan of the IgG Fc fragment. The precise glycan requirements for anti-inflammatory activity has been determined, such that an appropriate IgG1 Fc fragment can be created, thereby generating a fully recombinant, sialylated IgG1 Fc with greatly enhanced potency (Anthony et al., *Science*, 320: 373-376 (2008)).

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retains the ability to bind specifically to an antigen (i.e., to a particular epitope of an antigen, such as an epitope of DLL4). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens (or two or more different epitopes of the same antigen). Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL, and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment consisting of the VH and CH1 domains; (iv) an Fv fragment consisting of the VL and VH domains of a single arm of an antibody; (v) a dAb fragment (Ward et al., *Nature*, 341:544-546 (1989); PCT Publication No. WO 90/05144 A1), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see, e.g., Bird et al., *Science*, 242: 423-426 (1988); Huston et al., *Proc. Natl. Acad. Sci. USA*, 85: 5879-5883 (1988)). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies, are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see, e.g., Holliger et al., *Proc. Natl. Acad. Sci. USA*, 90: 6444-6448 (1993); Poljak, R.J., *Structure*, 2: 1121-1123 (1994)). Such antibody binding portions are known in the art (see, Kontermann and Dubel eds., Antibody Engineering (Springer-Verlag. New York, 2001), p. 790 (ISBN 3-540-41354-5)). In addition, single chain antibodies also include "linear antibodies" comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata et al. *Protein Eng.*, 8(10): 1057-1062 (1995); and US Patent No. 5,641,870).

The term "antibody construct" (or "DLL4 antibody construct") as used herein refers to a polypeptide comprising one or more the antigen binding portions of the invention linked to a linker polypeptide or an immunoglobulin constant domain. Linker polypeptides comprise two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see e.g., Holliger et al., *Proc. Natl. Acad. Sci. USA*, 90: 6444-6448 (1993); Poljak, R.J., *Structure*, 2: 1121-1123 (1994)). An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art and represented in Table 2.

Table 2. Sequence of human IgG heavy chain constant domain and light chain constant domain

Protein	Sequence Identifier	Sequence
		12345678901234567890123456789012
Ig gamma-1 constant region	SEQ ID NO:3	ASTKGPSVFFLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
Ig gamma-1 constant region mutant	SEQ ID NO:4	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
Ig Kappa constant region	SEQ ID NO:5	TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDST YSLSSTLTLSKADYEKHKVYACEVTHQGLSSP VTKSFNRGEC
Ig Lambda constant region	SEQ ID NO:6	QPKAAPSVTLFPPSSEELQANKATLVCLISDF YPGAVTVAWKADSSPVKAGVETTTPSKQSNNK YAASSYLSLTPEQWKSHRSYSCQVTHEGSTVE KTVAPTECS

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecule, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov et al., *Human Antibodies and Hybridomas*, 6: 93-101 (1995)) and use of a cysteine residue, a marker peptide, and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov et al., *Mol. Immunol.*, 31: 1047-1058 (1994)). Antibody portions, such as Fab and F(ab')2 fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions, and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein and known in the art.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds hDLL4 is substantially free of antibodies that specifically bind antigens other than hDLL4). An isolated antibody that specifically binds hDLL4 may, however, have cross-reactivity to other antigens, such as DLL4 molecules from other species (e.g., muDLL4). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "monoclonal antibody" and abbreviations "MAb" and "mAb", as used herein, refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created, or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell, antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom, *Trends Biotechnol.*, 15:62-70 (1997); Azzazy and Highsmith, *Clin. Biochem.*, 35: 425-445 (2002);

Gavilondo and Larrick, *BioTechniques*, 29: 128-145 (2000); Hoogenboom and Chames, *Immunol. Today*, 21: 371-378 (2000)), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see, Taylor et al., *Nucl. Acids Res.*, 20: 6287-6295 (1992); Kellermann and Green, *Curr. Opin. Biotechnol.*, 13: 593-597 (2002); Little et al., *Immunol. Today*, 21: 364-370 (2000)) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, in vivo somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

As used herein, the term "CDR" refers to a complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated "CDR1", "CDR2", and "CDR3", for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single variable region that binds the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as "Kabat CDRs". Chothia and coworkers (Chothia and Lesk, *J. Mol. Biol.*, 196: 901-917 (1987); Chothia et al., *Nature*, 342: 877-883 (1989)) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as "L1", "L2", and "L3", or "H1", "H2", and "H3", where the "L" and the "H" designate the light chain and the heavy chain regions, respectively. These regions may be referred to as "Chothia CDRs", which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan, *FASEB J.*, 9: 133-139 (1995) and MacCallum, *J. Mol. Biol.*, 262(5): 732-745 (1996). Still other CDR boundary definitions may not strictly follow one of the herein systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although certain embodiments use Kabat or Chothia defined CDRs.

The terms "Kabat numbering," "Kabat definitions", and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat et al., *Ann. NY Acad. Sci.*, 190: 382-391 (1971) and Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)). For the heavy chain variable region (VH), the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region (VL), the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The growth and analysis of extensive public databases of amino acid sequences of variable heavy and light regions over the past twenty years have led to the understanding of the typical boundaries between framework regions (FR) and CDR sequences within variable region sequences and enabled persons skilled in this art to accurately determine the CDRs according to Kabat numbering, Chothia numbering, or other systems. See, e.g., Martin, "Protein Sequence and Structure Analysis of Antibody Variable Domains," *In* Kontermann and Dübel, eds., Antibody Engineering (Springer-Verlag, Berlin, 2001), chapter 31, pages 432-433. A useful method of determining the amino acid sequences of Kabat CDRs, and thereby sequences of Kabat FRs as well, within the amino acid sequences of variable heavy (VH) and variable light (VL) regions is provided below:

To identify a CDR-L1 amino acid sequence:

Starts approximately 24 amino acid residues from the amino terminus of the VL region;

Residue before the CDR-L1 sequence is always cysteine (C);

Residue after the CDR-L1 sequence is always tryptophan (W), typically Trp-Tyr-Gln (W-Y-Q), but also Trp-Leu-Gln (W-L-Q), Trp-Phe-Gln (W-F-Q), and Trp-Tyr-Leu (W-Y-L);

Length is typically 10 to 17 amino acid residues.

To identify a CDR-L2 amino acid sequence:

Starts always 16 residues after the end of CDR-L1;

Residues before the CDR-L2 sequence are generally Ile-Tyr (I-Y), but also Val-Tyr (V-Y), Ile-Lys (I-K), and Ile-Phe (I-F);

Length is always 7 amino acid residues.

To identify a CDR-L3 amino acid sequence:

Starts always 33 amino acids after the end of CDR-L2;

Residue before the CDR-L3 amino acid sequence is always a cysteine (C);

Residues after are always Phe-Gly-X-Gly (F-G-X-G) (SEQ ID NO:7), where X is any amino acid;

Length is typically 7 to 11 amino acid residues.

To identify a CDR-H1 amino acid sequence:

Starts approximately 31 amino acid residues from amino terminus of VH region and always 9 residues after a cysteine (C);

Residues before are always Cys-X-X-X-X-X-X-X-X (SEQ ID NO:8), where X is any amino acid;

Residue after is always a Trp (W), typically Trp-Val (W-V), but also Trp-Ile (W-I), and Trp-Ala (W-A);

Length is typically 5 to 7 amino acid residues.

To identify a CDR-H2 amino acid sequence:

Starts always 15 amino acid residues after the end of CDR-H1;

Residues before are typically Leu-Glu-Trp-Ile-Gly (L-E-W-I-G) (SEQ ID NO:9), but other variations also;

Residues after are Lys/Arg-Leu/Ile/Val/Phe/Thr/Ala-Thr/Ser/Ile/Ala (K/R-L/I/V/F/T/A-T/S/I/A);

Length is typically 16 to 19 amino acid residues.

To identify a CDR-H3 amino acid sequence:

Starts always 33 amino acid residues after the end of CDR-H2 and always 3 after a cysteine (C)

Residues before are always Cys-X-X (C-X-X), where X is any amino acid, typically Cys-Ala-Arg (C-A-R);

Residues after are always Trp-Gly-X-Gly (W-G-X-G) (SEQ ID NO:10), where X is any amino acid;

Length is typically 3 to 25 amino acid residues.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (e.g., CDR3) has been replaced with human CDR sequences.

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like," i.e., more similar to human germline variable sequences. A "humanized antibody" is an antibody or a variant, derivative, analog, or fragment thereof, which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. In an embodiment, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

A humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA, and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3, and IgG4. A humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well known in the art.

The framework regions and CDRs of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion, and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See, e.g., Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, 1987)). A "consensus immunoglobulin sequence" may thus comprise a "consensus framework region(s)" and/or a "consensus CDR(s)". In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

An "affinity matured" antibody is an antibody with one or more alterations in one or more CDRs thereof which result in an improvement in the affinity of the antibody for a target antigen, compared to a parent antibody which does not possess the alteration(s). Exemplary affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. A variety of procedures for producing affinity matured antibodies are known in the art. For example, Marks et al., *BioTechnology*, 10: 779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by Barbas et al., *Proc. Nat. Acad. Sci. USA*, 91: 3809-3813 (1994); Schier et al., *Gene*, 169: 147-155 (1995); Yelton et al., *J. Immunol.*, 155: 1994-2004 (1995); Jackson et al., *J. Immunol.*, 154(7): 3310-3319 (1995); Hawkins et al, *J. Mol. Biol.*, 226: 889-896 (1992). Selective mutation at selective mutagenesis positions and at contact or hypermutation positions with an activity enhancing amino acid residue is described in US Patent No. 6,914,128 B1.

The term "multivalent binding protein" denotes a binding protein comprising two or more antigen binding sites (also referred to herein as "antigen binding domains"). A multivalent binding protein is preferably engineered to have three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein capable of binding two or more related or unrelated targets, including a binding protein capable of binding two or more different epitopes of the same target molecule.

The term "bispecific antibody", as used herein, refers to full-length antibodies that are generated by quadroma technology (see Milstein et al., *Nature*, 305(5934): 537-540 (1983)), by chemical conjugation of two different monoclonal antibodies (see, Staerz et al., *Nature*, 314(6012): 628-631 (1985)), or by knob-into-hole or similar approaches which introduces mutations in the Fc region (see Holliger et al., *Proc. Natl. Acad. Sci. USA*, 90(14): 6444-6448 (1993)), resulting in multiple different immunoglobulin species of which only one is the functional bispecific antibody. By molecular function, a bispecific antibody binds one antigen (or epitope) on one of its two binding arms (one pair of HC/LC), and binds a different antigen (or epitope) on its second arm (a different pair of HC/LC). By this definition, a bispecific antibody has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen it binds to.

The term "dual-specific antibody", as used herein, refers to full-length antibodies that can bind two different antigens (or epitopes) in each of its two binding arms (a pair of HC/LC) (see PCT publication WO 02/02773). Accordingly a dual-specific binding protein has two identical antigen binding arms, with identical specificity and identical CDR sequences, and is bivalent for each antigen to which it binds.

"Dual variable domain" ("DVD") binding proteins of the invention comprise two or more antigen binding sites and may be divalent (two antigen binding sites), tetravalent (four antigen binding sites), or multivalent binding proteins. DVDs may be monospecific, i.e., capable of binding one antigen (or one specific epitope), or multispecific, i.e., capable of binding two or more antigens (i.e., two or more epitopes of the same target antigen molecule or two or more epitopes of different target antigens). A preferred DVD binding protein comprises two heavy chain DVD polypeptides and two light chain DVD polypeptides is referred to as a "DVD immunoglobulin" or "DVD-Ig". Such a DVD-Ig binding protein is thus tetrameric and reminiscent of an IgG molecule, but provides more antigen binding site than an IgG molecule. Thus, each half of a tetrameric DVD-Ig molecule is reminiscent of one half of an IgG molecule and comprises a heavy chain DVD polypeptide and a light chain DVD polypeptide, but unlike a pair of heavy and light chains of an IgG molecule that provide a single antigen bindind domain, a pair of heavy and light chains of a DVD-Ig provide two or more antigen binding sites.

Each antigen binding site of a DVD-Ig binding protein is derived from a donor ("parental") monoclonal antibody and thus comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) with a total of six CDRs involved in antigen binding per antigen binding site. Accordingly, a DVD-Ig binding protein that binds two different epitopes (i.e., two different epitopes of two different antigen molecules or two different epitopes of the same antigen molecule) comprises an antigen binding site derived from a first parental monoclonal antibody and an antigen binding site of a second parental monoclonal antibody.

A description of the design, expression, and characterization of DVD-Ig binding molecules is provided in PCT Publication No. WO 2007/024715, US Patent No. 7,612,181, and Wu et al., *Nature Biotech.*, 25: 1290-1297 (2007). A preferred example of such DVD-Ig molecules comprises a heavy chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, X2 is an Fc region, and n is 0 or 1, but preferably 1; and a light chain that comprises the structural formula VD1-(X1)n-VD2-C-(X2)n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region; and n is 0 or 1, but preferably 1. Such a DVD-Ig may comprise two such heavy chains and two such light chains, wherein each chain comprises variable domains linked in tandem without an intervening constant region between variable regions, wherein a heavy chain and a light chain associate to form tandem functional antigen binding sites, and a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with four functional antigen binding sites. In another example, a DVD-Ig molecule may comprise heavy and light chains that each comprise three variable domains (VD1, VD2, VD3) linked in tandem without an intervening constant region between variable domains, wherein a pair of heavy and light chains may associate to form three antigen binding sites, and wherein a pair of heavy and light chains may associate with another pair of heavy and light chains to form a tetrameric binding protein with six antigen binding sites.

In a preferred embodiment, a DVD-Ig binding protein according to the invention not only binds the same target molecules bound by its parental monoclonal antibodies, but also possesses one or more desirable properties of one or more of its parental monoclonal antibodies. Preferably, such an additional property is an antibody parameter of one or more of the parental monoclonal antibodies. Antibody parameters that may be contributed to a DVD-Ig binding protein from one or more of its parental monoclonal antibodies include, but are not limited to, antigen specificity, antigen affinity, potency, biological function, epitope recognition, protein stability, protein solubility, production efficiency, immunogenicity, pharmacokinetics, bioavailability, tissue cross reactivity, and orthologous antigen binding.

A DVD-Ig binding protein according to the invention binds at least one epitope of a human DLL4 protein. Non-limiting examples of a DVD-Ig binding protein according to the invention include a DVD-Ig binding protein that binds one or more epitopes of human DLL4, a DVD-Ig binding protein that binds an epitope of a human DLL4 and an epitope of a DLL4 of another species (for example, mouse), and a DVD-Ig binding protein that binds an epitope of a human DLL4 and an epitope of another target molecule (for example, VEGFR2 or VEGFR1).

A "functional antigen binding site" of a binding protein is one that is capable of binding a target antigen. The antigen binding affinity of the antigen binding site is not necessarily as strong as the parent antibody from which the antigen binding site is derived, but the ability to bind antigen must be measurable using any one of a variety of methods known for evaluating antibody binding to an antigen. Moreover, the antigen binding affinity of each of the antigen binding sites of a multivalent antibody herein need not be quantitatively the same.

As used herein, the terms "acceptor" and "acceptor antibody" refer to an antibody or nucleic acid sequence providing or encoding at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions (FRs). In some embodiments, the term "acceptor" refers to the antibody amino acid or nucleic acid sequence providing or encoding the constant region(s). In yet another embodiment, the term "acceptor" refers to the antibody amino acid or nucleic acid sequence providing or encoding one or more of the framework regions and the constant region(s). In a specific embodiment, the term "acceptor" refers to a human antibody amino acid or nucleic acid sequence that provides or encodes at least 80%, preferably, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions. In accordance with this embodiment, an acceptor may contain at least 1, at least 2, at least 3, least 4, at least 5, or at least 10 amino acid residues that does (do) not occur at one or more specific positions of a human antibody. An acceptor framework region and/or acceptor constant region(s) may be, e.g., derived or obtained from a germline antibody gene, a mature antibody gene, a functional antibody (e.g., antibodies well-known in the art, antibodies in development, or antibodies commercially available).

As used herein, the term "canonical" residue refers to a residue in a CDR or framework that defines a particular canonical CDR structure as defined by Chothia et al. (*J. Mol. Biol.*, 196: 901-917 (1987); Chothia et al., *J. Mol. Biol.*, 227: 799-817 (1992), both are incorporated herein by reference). According to Chothia et al., critical portions of the CDRs of many antibodies have nearly identical peptide backbone confirmations despite great diversity at the level of amino acid sequence. Each canonical structure specifies primarily a set of peptide backbone torsion angles for a contiguous segment of amino acid residues forming a loop.

As used herein, the terms "donor" and "donor antibody" refer to an antibody providing one or more CDRs. In a preferred embodiment, the donor antibody is an antibody from a species different from the antibody from which the framework regions are obtained or derived. In the context of a humanized antibody, the term "donor antibody" refers to a non-human antibody providing one or more CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems (for example, see above), the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3, and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3, or FR4, a framework region, as referred by others, represents the combined FRs within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub- regions constituting a framework region.

Human heavy chain and light chain framework (FR) sequences are known in the art that can be used as heavy chain and light chain "acceptor" framework sequences (or simply, "acceptor" sequences) to humanize a non-human antibody using techniques known in the art. In an embodiment of the invention, human heavy chain and light chain acceptor sequences are selected from the framework sequences listed in publicly available databases such as V-base (hypertext transfer protocol://vbase.mrc-cpe.cam.ac.uk/) or in the international ImMunoGeneTics® (IMGT®) information system (hypertext transfer protocol://imgt.cines.fr/texts/IMGTrepertoire/LocusGenes/). Table 3, below, provides a non-limiting list of examples of human heavy chain acceptor sequences known in the art. Table 4, below, provides a non-limiting list of examples of human light chain acceptor sequences known in the art. In an embodiment of the invention, human heavy chain and light chain acceptor sequences are selected from the amino acid sequences described in Table 3 and Table 4, below, however, other human heavy chain and light acceptors sequences not listed in Tables 3 and 4 may also be used to humanize an antibody according to the invention.

Table 3. Heavy Chain Acceptor Sequences.

SEQ ID NO:	Protein region/ Closest Germline Family	Amino Acid Sequence
		12345678901234567890123456789012
11	VH3-7 FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
12	VH3-7 FR2	WVRQAPGKGLEWVA
13	VH3-7 FR3	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
14	JH4 FR4	WGQGTLVTVSS
15	VH3 CONSENUSUS FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
16	VH3 CONSENUSUS FR2	WVRQAPGKGLEWVS
17	VH3 CONSENUSUS FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
18	JH4 FR4	WGQGTLVTVSS
19	VH1-46 FR1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT
20	VH1-46 FR2	WVRQAPGQGLEWMG
21	VH1-46 FR3	RVTMTRDTSTSTVYMELSSLRSEDTAVYYCAR
22	JH4 FR4	WGQGTLVTVSS
23	VH3-30 FR1	QVQLVESGGGVVQPGRSLRLSCAASGFTFS
24	VH3-30 FR2	WVRQAPGKGLEWVA
25	VH3-30 FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
26	JH3 FR4	WGQGTMVTVSS
27	VH3 CONSENUSUS FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
28	VH3 CONSENUSUS FR2	WVRQAPGKGLEWVS
29	VH3 CONSENUSUS FR3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR
30	JH3 FR4	WGQGTMVTVSS
31	VH2-70/JH6 FR1	EVTLRESGPALVKPTQTLTLTCTFSGFSLS
32	VH2-70/JH6 FR2	WIRQPPGKALEWLA
33	VH2-70/JH6 FR3	RLTISKDTSKNQVVLTMTNMDPVDTATYYCAR
34	VH2-70/JH6 FR4	WGQGTTVTVSS
35	VH2-26/JH6 FR1	EVTLKESGPVLVKPTETLTLTCTVSGFSLS
36	VH2-26/JH6 FR2	WIRQPPGKALEWLA
37	VH2-26/JH6 FR3	RLTISKDTSKSQVVLTMTNMDPVDTATYYCAR
38	VH2-26/JH6 FR4	WGQGTTVTVSS
39	VH3-72/JH6 FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
40	VH3-72/JH6 FR2	WVRQAPGKGLEWVG
41	VH3-72/JH6 FR3	RFTISRDDSKNSLYLQMNSLKTEDTAVYYCAR
42	VH3-72/JH6 FR4	WGQGTTVTVSS
43	VH3-21/JH6 FR1	EVQLVESGGGLVKPGGSLRLSCAASGFTFS
44	VH3-21/JH6 FR2	WVRQAPGKGLEWVS
45	VH3-21/JH6 FR3	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
46	VH3-21/JH6 FR4	WGQGTTVTVSS
47	VH1-69/JH6 FR1	EVQLVQSGAEVKKPGSSVKVSCKASGGTFS

SEQ ID NO:	Protein region/ Closest Germline Family	Amino Acid Sequence
48	VH1-69/JH6 FR2	WVRQAPGQGLEWMG
49	VH1-69/JH6 FR3	RVTITADKSTSTAYMELSSLRSEDTAVYYCAR
50	VH1-69/JH6 FR4	WGQGTTVTVSS
51	VH1-18/JH6 FR1	EVQLVQSGAEVKKPGASVKVSCKASGYTFT
52	VH1-18/JH6 FR2	WVRQAPGQGLEWMG
53	VH1-18/JH6 FR3	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR
54	VH1-18/JH6 FR4	WGQGTTVTVSS
55	IGHV4-59 FR1	EVQLQESGPGLVKPSETLSLTCTVSGGSIS
56	IGHV4-59 FR2	WIRQPPGKGLEWIG
57	IGHV4-59 FR3	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
58	IGHV4-59/JH FR4	WGQGTLVTVSS
59	IGHV3-66 FR1	EVQLVESGGGLVQPGGSLRLSCAVSGGSIS
60	IGHV3-66 FR2	WIRQAPGKGLEWIG
61	IGHV3-66 FR3	RVTISVDTSKNSFYLQMNSLRAEDTAVYYCAR
62	IGHV3-66/JH FR4	WGQGTLVTVSS
63	IGHV4-59 FR1	EVQLQESGPGLVKPGETLSLTCTVSGGSIS
64	IGHV4-59 FR2	WIRQAPGKGLEWIG
65	IGHV4-59 FR3	RVTISVDTSKNQFYLKLSSVRAEDTAVYYCAR
66	IGHV4-59/JH FR4	WGQGTLVTVSS
67	IGHV5-51 FR1	EVQLVQSGTEVKKPGESLKISCKVSGGSIS
68	IGHV5-51 FR2	WIRQMPGKGLEWIG
69	IGHV5-51 FR3	QVTISVDTSFNTFFLQWSSLKASDTAMYYCAR
70	IGHV5-51/JH FR4	WGQGTMVTVSS
71	IGHV2-70 FR1	EVTLRESGPALVKPTQTLTLTCTVSGGSIS
72	IGHV2-70 FR2	WIRQPPGKGLEWIG
73	IGHV2-70 FR3	RVTISVDTSKNQFVLTMTNMDPVDTATYYCAR
74	IGHV2-70/JH FR4	WGQGTTVTVSS
75	IGHV3-15 FR1	EVQLLESGGGLVKSGGSLRLSCAASGFTFR
76	IGHV3-15 FR2	WVRQAPGKGLEWVA
77	IGHV3-15 FR3	RFTISRDNSKNTLYLQLNSLRAEDTAVYYCAK
78	IGHV3-15/JH FR4	WGQGTMVTVSS
79	IGHV3-43 FR1	EVQLVESGGGVVQPGGSLRLSCAASGFTFG
80	IGHV3-43 FR2	WVRQAPGKGLEWVA
81	IGHV3-43 FR3	RFTISRDNSKNTLYLQLNSLRAEDTAVYYCAK
82	IGHV3-43/JH FR4	WGQGTMVTVSS

Table 4. Light Chain Acceptor Sequences

SEQ ID NO.:	Protein region/ Closest Germline Family	Sequence
		12345678901234567890123456789012
83	O2 FR1	DIQMTQSPSSLSASVGDRVTITC
84	O2 FR2	WYQQKPGKAPKLLIY
85	O2 FR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
86	JK2 FR4	FGQGTKLEIK
87	L2 FR1	EIVMTQSPATLSVSPGERATLSC
88	L2 FR2	WYQQKPGQAPRLLIY
89	L2 FR3	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC
90	JK2 FR4	FGQGTKLEIK
91	B3/JK4 FR1	DIVMTQSPDSLAVSLGERATINC

SEQ ID NO.:	Protein region/ Closest Germline Family	Sequence
92	B3/JK4 FR2	WYQQKPGQPPKLLIY
93	B3/JK4 FR3	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC
94	B3/JK4 FR4	FGGGTKVEIKR
95	L2/JK4 FR1	EIVMTQSPATLSVSPGERATLSC
96	L2/JK4 FR2	WYQQKPGQAPRLLIY
97	L2/JK4 FR3	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC
98	L2/JK4 FR4	FGGGTKVEIKR
99	L15/JK4 FR1	DIQMTQSPSSLSASVGDRVTITC
100	L15/JK4 FR2	WYQQKPEKAPKSLIY
101	L15/JK4 FR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
102	L15/JK4 FR4	FGGGTKVEIKR
103	L5/JK4 FR1	DIQMTQSPSSVSASVGDRVTITC
104	L5/JK4 FR2	WYQQKPGKAPKLLIY
105	L5/JK4 FR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
106	L5/JK4 FR4	FGGGTKVEIKR
107	IGLV3-1 FR1	SYELTQPPSVSVSPGQTASITC
108	IGLV3-1 FR2	WYQQKPGQSPVLVIY
109	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQPMDEADYYC
110	IGLV3-1/JL FR4	FGYGTKVTVL
111	IGLV3-1 FR1	SYELTQPPSVSVSPGQTASITC
112	IGLV3-1 FR2	WYQQKPGQSPVLVIY
113	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQPMDEADYYC
114	IGLV3-1/JL FR4	GGGTKLTVLG
115	IGLV3-1 FR1	YELTQPPSVSVSPGQTASITC
116	IGLV3-1 FR2	WYQQKPGQSPVLVIY
117	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQPMDEADYYC
118	IGLV3-1/JL FR4	GGGTKLTVLG
119	IGLV3-1 FR1	LYVLTQPPSVSVSPGQTASITC
120	IGLV3-1 FR2	WYQQKPGQSPVLVIY
121	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQTMDEADYLC
122	IGLV3-1/JL FR4	FGGGTKVTVLG
123	IGKV6D-21 FR1	EYVLTQSPDFQSVTPKEKVTITC
124	IGKV6D-21 FR2	WYQQKPDQSPKLVIY
125	IGKV6D-21 FR3	GVPSRFSGSNSGDDATLTINSLEAEDAATYYC
126	IGKV6D-21/JK FR4	FGQGTKVEIKR
127	IGKV3D-15 FR1	EYVLTQSPATLSVSPGERATLSC
128	IGKV3D-15 FR2	WYQQKPGQSPRLVIY
129	IGKV3D-15 FR3	DIPARFSGSNSGDEATLTISSLQSEDFAVYYC
130	IGKV3D-15/JK FR4	FGQGTRLEIKR
131	IGKV4-1 FR1	DYVLTQSPDSLAVSLGERATINC
132	IGKV4-1 FR2	WYQQKPGQSPKLVIY
133	IGKV4-1 FR3	GIPDRFSGSNSGDDATLTISSLQAEDVAVYYC
134	IGKV4-1/JK FR4	FGGGTKVEIKR
135	IGLV3-1 FR1	LPVLTQPPSVSVSPGQTASITC
136	IGLV3-1 FR2	WYQQKPGQSPVLVIY
137	IGLV3-1 FR3	GIPERFSGSNSGNTATLTISGTQTMDEADYLC
138	IGLV3-1/JL FR4	FGGGTKVTVL
139	IGLV3-1 FR1	SYELTQPPSVSVSPGQTASITC

SEQ ID NO.:	Protein region/ Closest Germline Family	Sequence
140	IGLV3-1 FR2	WYQQKPGQSPVLVIY
141	IGLV3-1 FR3	GIPERFSGSNSGNTATLTISGTQTMDEADYLC
142	IGLV3-1/JL FR4	FGGGTKLTVL

In an embodiment, heavy chain human acceptor framework sequences from Table 3 for use in generating humanized antibodies that bind DLL4 according to the invention include a set consisting of the VH3-7 FR1, the VH3-7 FR2, the VH3-7 FR3, and the JH4 FR4 acceptor sequences; a set consisting of the VH3 consensus FR1, the VH3 consensus FR2, the VH3 consensus FR3, and the JH4 FR4 acceptor sequences; a set consisting of the VH1-46 FR1, the VH1-46 FR2, the VH1-46 FR3, and the JH4 FR4 acceptor sequences; a set consisting of the VH3-30 FR1, the VH3-30 FR2, the VH3-30 FR3, and the JH3 FR4 acceptor sequences; and a set consisting of the VH3 consensus FR1, the VH3 consensus FR2, the VH3 consensus FR3, and the JH3 FR4 acceptor sequences.

In an embodiment, light chain human acceptor framework sequences from Table 4 for use in generating humanized antibodies that bind DLL4 according to the invention include a set consisting of the O2 FR1, O2 FR2, O2 FR3, and JK2 FR4 acceptors sequences and a set a consisting of the L2 FR1, L2 FR2, L2 FR3, and JK2 FR4 acceptor sequences.

In an embodiment, a set of human acceptor framework sequences for use in generating a humanized antibody that binds DLL4 according to the invention comprises one or more (e.g., any one, two, three, four, five, six, seven, or eight per binding domain) of the acceptor framework sequences selected from the group consisting of:

heavy chain framework-1 (H-FR1):
E-V-Q-L-V-E-S-G-G-G-L-V-Q-P-G-G-S-L-R-L-S-C-A-A-S-G-F-T-F-X_{30} (SEQ ID NO:143), wherein X_{30} is S, R, or G;

heavy chain framework-2 (H-FR2): W-V-R-Q-A-P-G-K-G-L-E-W-V-A (SEQ ID NO:144);

heavy chain framework-3 (H-FR3):
R-F-T-I-S-R-D-N-A-K-X_{11}-S-L-Y-L-Q-M-N-S-L-R-A-E-D-T-A-V-Y-Y-C-X_{31}-R (SEQ ID NO:145), wherein;

X_{11} is N or S; and

X_{31} is A or S;

heavy chain framework-4 (H-FR4): W-G-Q-G-T-L-V-T-V-S-S (SEQ ID NO:146);

light chain framework-1 (L-FR1):
D-I-Q-M-T-Q-S-P-S-S-L-S-A-S-V-G-D-R-V-T-I-T-C (SEQ ID NO:147);

light chain framework-2 (L-FR2): W-Y-Q-Q-K-P-G-K-X_9-P-K-L-L-I-X_{15} (SEQ ID NO:148), wherein;

X_9 is A or S; and

X_{15} is F or Y;

light chain framework-3 (L-FR3): G-V-P-S-R-F-S-G-S-G-S-G-T-D-X_{15}-T-L-T-I-S-S-L-Q-P-E-D-F-A-T-Y-Y-C (SEQ ID NO:149), wherein;

X_{15} is F or S; and

light chain framework-4 (L-FR4): F-G-Q-G-T-K-L-E-I-K (SEQ ID NO:150).

In a preferred embodiment, an antibody that binds DLL4 according to the invention is humanized using a set of human acceptor sequences consisting of an H-FR1, H-FR2, H-FR3, H-FR-4, L-FR1, L-FR2, L-FR3, and L-FR4 acceptor sequence described above.

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non-lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al., *Crit. Rev. Immunol.*, 22(3): 183-200 (2002); Marchalonis et al., *Adv. Exp. Med. Biol.*, 484:13-30 (2001)). One of the advantages provided by various embodiments of the present invention stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "key residue" refers to certain residues within the variable region that have more impact on the binding specificity and/or affinity of an antibody, in particular a humanized antibody. A key residue includes, but is not limited to, one or more of the following: a residue that is adjacent to a CDR, a potential glycosylation site (can be either N- or O-glycosylation site), a rare residue, a residue capable of interacting with the antigen, a residue capable of interacting with a CDR, a canonical residue, a contact residue between heavy chain variable region and light chain variable region, a residue within the Vernier zone, and a residue in the region that overlaps between the Chothia definition of a variable heavy chain CDR1 and the Kabat definition of the first heavy chain framework.

As used herein, "Vernier" zone refers to a subset of framework residues that may adjust CDR structure and fine-tune the fit to antigen as described by Foote and Winter (*J. Mol. Biol.*, 224: 487-499 (1992)). Vernier zone residues form a layer underlying the CDRs and may impact on the structure of CDRs and the affinity of the antibody.

As used herein, the term "neutralizing" refers to counteracting the biological activity of an antigen when a binding protein specifically binds the antigen. In an embodiment, the neutralizing binding protein binds an antigen and reduces its biological activity by at least about 20%, 40%, 60%, 80%, 85%, 90%, 95%, or more.

The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen, for example, an anti-hDLL4 antibody that binds to an DLL4 antigen and/or the neutralizing potency of an antibody, or an anti-hDLL4 antibody whose binding to hDLL4 inhibits the biological activity of hDLL4, e.g. inhibition of PHA blast proliferation or inhibition of receptor binding in a human Notch receptor binding assay, or PHA blast interferon-gamma induction assay.

The term "epitope" includes any polypeptide determinant that specifically binds to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. An epitope thus consists of the amino acid residues of a region of an antigen (or fragment thereof) known to bind to the complementary site on the specific binding partner. An antigen or antigenic fragment can contain more than one epitope. Thus, it is understood by persons skilled in this art that every "antigen binding site" of an antibody molecule binds an epitope of an antigen molecule and every antigen molecule may have one, two, several, or many epitopes. Moreover, it is understood by persons skilled in this art that two independently isolated antibodies to an antigen molecule may bind at the same epitope or at two different epitopes on the antigen molecule.

In certain embodiments, an antibody is said to specifically bind an antigen when it recognizes its target antigen in a complex mixture of proteins and/or macromolecules. Antibodies are said to "bind to the same epitope" if the antibodies cross-compete (one prevents the binding or modulating effect of the other). In addition, structural definitions of epitopes (overlapping, similar, identical) are informative, but functional definitions are often more relevant as they encompass structural (binding) and functional (modulation, competition) parameters.

The term "surface plasmon resonance," as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore® system (BIAcore International AB, a GE Healthcare company, Uppsala, Sweden and Piscataway, New Jersey, US). For further descriptions, see Jönsson et al., *Ann. Biol. Clin.*, 51: 19-26 (1993); Jönsson et al. *BioTechniques*, 11: 620-627 (1991); Johnsson et al., *J. Mol. Recognit.*, 8: 125-131 (1995); and Johnsson et al., *Anal. Biochem.*, 198: 268-277 (1991).

The term "K_{on}", as used herein, is intended to refer to the on rate constant for association of a binding protein (e.g., an antibody) to a cognate partner (e.g., an antigen) to form a binding partner/cognate partner (e.g., antibody/antigen) complex as is known in the art. The "K_{on}" also is known by the terms "association rate constant," or "k_a," as used interchangeably herein. This value indicating the binding rate of an antibody to its target antigen or the rate of complex formation between an antibody and antigen also is shown by the equation:

$$\text{Antibody ("Ab")} + \text{Antigen ("Ag")} \rightarrow \text{Ab-Ag}.$$

The term "K_{off}," as used herein, is intended to refer to the off rate constant for dissociation of a binding protein (e.g., an antibody) from the, e.g., antibody/antigen complex as is known in the art. The "K_{off}" also is known by the terms "dissociation rate constant" or “k_d" as used interchangeably herein. This value indicates the dissociation rate of an antibody from its target antigen or separation of Ab-Ag complex over time into free antibody and antigen as shown by the equation below:

$$\text{Ab} + \text{Ag} \leftarrow \text{Ab-Ag}.$$

The terms "equilibrium dissociation constant" or "K_D", as used interchangeably herein, refer to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant (K_{off}) by the association rate constant (K_{on}). The association rate constant, the dissociation rate constant, and the equilibrium dissociation constant are used to represent the binding affinity of an antibody to an antigen. Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence-based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIAcore® surface plasmon resonance (biomolecular interaction analysis) assay can be used (e.g., instrument available from BIAcore International AB, a GE Healthcare company, Uppsala, Sweden). Additionally, a KinExA® (Kinetic Exclusion Assay) assay, available from Sapidyne Instruments (Boise, Idaho) can also be used.

"Label" and "detectable label" mean a moiety attached to a specific binding partner, such as an antibody or an analyte bound by the antibody, e.g., to render the reaction between members of a specific binding pair, such as an antibody and an analyte, detectable. The specific binding partner, e.g., antibody or analyte, so labeled is referred to as "detectably labeled". Thus, the term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. In an embodiment, the label is a detectable marker that can produce a signal that is detectable by visual or instrumental means, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by a marked avidin (e.g., an avidin or a streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H,

^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm); chromogens, fluorescent labels (e.g., FITC, rhodamine, and lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, and epitope tags); and magnetic agents, such as gadolinium chelates. Representative examples of labels commonly employed for immunoassays include moieties that produce light, e.g., acridinium compounds, and moieties that produce fluorescence, e.g., fluorescein. Other labels are known in the art or described herein. In this regard, the moiety itself may not be detectably labeled but may become detectable upon reaction with yet another moiety. Use of "detectably labeled" is intended to encompass the latter type of detectable labeling.

The term "antibody conjugate" refers to a binding protein, such as an antibody, chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. Preferably, the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

The terms "crystal" and "crystallized" as used herein, refer to a binding protein (e.g., an antibody), or antigen binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes, including Fab/antigen complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. See, Giegé et al., *In* Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ed., (Ducruix and Giegé, eds.) (Oxford University Press, New York, 1999), chapter 1, pages 1-16.

The term "polynucleotide" means a polymeric form of two or more nucleotides, either ribonucleotides or deoxyribonucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

The term "isolated polynucleotide" shall mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, by virtue of its origin, the "isolated polynucleotide" is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector," is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions. RNA versions of vectors (including RNA viral vectors) may also find use in the invention.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked" sequences include expression control sequences that are contiguous with a gene of interest, expression control sequences that act *in trans*, i.e., located on a different nucleic acid molecule than a gene of interest, as well as expression control sequences that are located on the same nucleic acid molecule as, but at a distance from, a gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include a promoter, a ribosomal binding site, and a transcription termination sequence; in eukaryotes, generally, such control sequences include a promoter and a transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Transformation," refers to any process by which exogenous nucleic acid (e.g., a DNA molecule) enters a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, plasmid uptake across a cellular membrane, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. They also include cells which transiently express the inserted DNA or RNA for limited periods of time.

The term "recombinant host cell" (or simply "host cell"), is intended to refer to a cell into which exogenous DNA has been introduced. In an embodiment, the host cell comprises two or more (e.g., multiple) nucleic acids encoding antibodies, such as, by way of non-limiting example, the host cells described in US Patent No. 7,262,028. Such terms are intended to refer not only to the particular subject cell, but, also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. In an embodiment, host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. In another embodiment, eukaryotic cells include protist, fungal, plant and animal cells. In another embodiment, host cells include but are not limited to prokaryotic species, such *Escherichia coli*; mammalian cell lines, such as CHO, HEK 293, COS, NS0, SP2, and PER.C6; the insect cell line Sf9; and fungal cell species, such as *Saccharomyces cerevisiae*.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. See, e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, second ed. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1989).

"Transgenic organism," as known in the art, refers to an organism having cells that contain a transgene, wherein the transgene introduced into the organism (or an ancestor of the organism) expresses a polypeptide not naturally expressed in the organism. A "transgene" is a DNA construct, which is stably and operably integrated into the genome of a cell from which a transgenic organism develops, directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic organism.

The term "regulate" and “modulate” are used interchangeably, and, as used herein, refers to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of hDLL4). Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction.

Correspondingly, the term "modulator," as used herein, is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of hDLL4). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies have been described. See, e.g., PCT Publication No. WO01/83525.

The term "agonist", as used herein, refers to a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the agonist. Particular agonists of interest may include, but are not limited to, members of the Notch-signaling pathway, DLL4 polypeptides and nucleic acids, carbohydrates, or any other molecules that bind to DLL4.

The term "antagonist" or "inhibitor", as used herein, refers to a modulator that, when contacted with a molecule of interest causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Particular antagonists of interest include those that block or modulate the biological or immunological activity of DLL4, especially human DLL4 (hDLL4). Antagonists and inhibitors of hDLL4 may include, but are not limited to, proteins, nucleic acids, carbohydrates, or any other molecule, which binds to hDLL4 and/or rodent DLL4.

As used herein, the term "effective amount" refers to the amount of a therapy that is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof; inhibit or prevent the advancement of a disorder; cause regression of a disorder; inhibit or prevent the recurrence, development, onset, or progression of one or more symptoms associated with a disorder; detect a disorder; or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent).

"Patient" and "subject" may be used interchangeably herein to refer to an animal, such as a mammal, including a primate (for example, a human, a monkey, and a chimpanzee), a non-primate (for example, a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, and a whale), a bird (e.g., a duck or a goose), and a shark. Preferably, a patient or subject is a human, such as a human being treated or assessed for a disease, disorder, or condition; a human at risk for a disease, disorder, or condition; a human having a disease, disorder, or condition; and/or human being treated for a disease, disorder, or condition. More preferably, a patient or subject is being treated or assessed for cancer or other disease in which the existing aberrant DLL4 expression supports the cancer or other disease and inhibition or disruption of DLL4 activity is desirable to treat the cancer or other disease.

The term "sample," as used herein, is used in its broadest sense. A "biological sample," as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, rats, monkeys, dogs, rabbits and other animals. Such substances include, but are not limited to, blood, (e.g., whole blood), plasma, serum, urine, amniotic fluid, synovial fluid, endothelial cells, leukocytes, monocytes, other cells, organs, tissues, bone marrow, lymph nodes and spleen.

"Component," "components," and "at least one component," refer generally to a capture antibody, a detection or conjugate antibody, a control, a calibrator, a series of calibrators, a sensitivity panel, a container, a buffer, a diluent, a salt, an enzyme, a co-factor for an enzyme, a detection reagent, a pretreatment reagent/solution, a substrate (e.g., as a solution), a stop solution, and the like that can be included in a kit for assay of a test sample, such as a patient urine, serum or plasma sample, in accordance with the methods described herein and other methods known in the art. Thus, in the context of the present disclosure, "at least one component," "component," and "components" can include a polypeptide or other analyte as above, such as a composition comprising an analyte such as a polypeptide, which is optionally immobilized on a solid support, such as by binding to an anti-analyte (e.g., anti-polypeptide) antibody. Some components can be in solution or lyophilized for reconstitution for use in an assay.

"Risk" refers to the possibility or probability of a particular event occurring either presently or at some point in the future. "Risk stratification" refers to an array of known clinical risk factors that allows physicians to classify patients into a low, moderate, high or highest risk of developing a particular disease, disorder or condition.

“Specific" and "specificity" in the context of an interaction between members of a specific binding pair (e.g., an antigen or fragment thereof and an antibody or antigen binding fragment thereof) refer to the selective reactivity of the interaction. The phrase "specifically binds to" and analogous phrases refer to the ability of binding proteins, such as antibodies (or antigen binding fragments thereof), to bind specifically to a molecule of interest (or a fragment thereof) and not bind specifically to other entities.

"Specific binding partner" is a member of a specific binding pair. A specific binding pair comprises two different molecules, which specifically bind to each other through chemical or physical means. Therefore, in addition to antigen and antibody specific binding pairs, other specific binding pairs can include biotin and avidin (or streptavidin), carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, and antibodies, including monoclonal and polyclonal antibodies as well as complexes, fragments, and variants (including fragments of variants) thereof, whether isolated or recombinantly produced.

"Variant" as used herein means a polypeptide that differs from a given polypeptide (e.g., DLL4 polypeptide or anti-DLL4 antibody) in amino acid sequence by the addition (e.g., insertion), deletion, or conservative substitution of amino acids, but that retains the biological activity of the given polypeptide (e.g., a variant DLL4 may compete with a wildtype DLL4 for binding with an anti-DLL4 antibody if the variant DLL4 retains the original antibody binding site (epitope) of the wildtype DLL4). A conservative substitution of an amino acid, i.e., replacing an amino acid with a different amino acid of similar properties (e.g., hydrophilicity and degree and distribution of charged regions) is recognized in the art as typically involving a minor change. These minor changes can be identified, in part, by considering the hydropathic index of amino acids, as understood in the art (see, e.g., Kyte et al., *J. Mol. Biol.*, 157: 105-132 (1982)). The hydropathic index of an amino acid is based on a consideration of its hydrophobicity and charge. It is known in the art that amino acids of similar hydropathic indexes can be substituted and still retain protein function. In one aspect, amino acids having hydropathic indexes of ± 2 are substituted. The hydrophilicity of amino acids also can be used to reveal substitutions that would result in proteins retaining biological function. A consideration of the hydrophilicity of amino acids in the context of a peptide permits calculation of the greatest local average hydrophilicity of that peptide, a useful measure that has been reported to correlate well with antigenicity and immunogenicity (see, e.g., US Patent No. 4,554,101). Substitution of amino acids having similar hydrophilicity values can result in peptides retaining biological activity, for example immunogenicity, as is understood in the art. In one aspect, substitutions are performed with amino acids having hydrophilicity values within ± 2 of each other. Both the hydrophobicity index and the hydrophilicity value of amino acids are influenced by the particular side chain of that amino acid. Consistent with that observation, amino acid substitutions that are compatible with biological function are understood to depend on the relative similarity of the amino acids, and particularly the side chains of those amino acids, as revealed by the hydrophobicity, hydrophilicity, charge, size, and other properties. "Variant" also can be used to describe a polypeptide or fragment thereof that has been differentially processed, such as by proteolysis, phosphorylation, or other post-translational modification, yet retains its biological activity or antigen reactivity, e.g., the ability to bind to DLL4. Use of "variant" herein is intended to encompass fragments of a variant unless otherwise contradicted by context.

The term "sample", as used herein, is used in its broadest sense. A “biological sample”, as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, rats, monkeys, dogs, rabbits and other animals. Such substances include, but are not limited to, blood, serum, urine, synovial fluid, cells, organs, tissues, bone marrow, lymph nodes, and spleen.

I. Antibodies That Bind Human DLL4.

One aspect of the present invention provides isolated rat monoclonal antibodies, or antigen-binding portions thereof, that bind to DLL4 with high affinity, a slow off rate, and/or high neutralizing capacity. Another aspect of the invention provides chimeric antibodies that bind DLL4. In another aspect, the invention provides CDR grafted antibodies, or antigen-binding portions thereof, that DLL4. Another aspect of the invention provides humanized antibodies, or antigen-binding portions thereof, that bind DLL4. In an embodiment, the antibodies, or portions thereof, are isolated antibodies or isolated portions thereof. In another embodiment, the antibodies, or antigen-binding portions thereof, of the invention are neutralizing anti-DLL antibodies. Advantageously, such antibodies or antigen-binding portions thereof that bind DLL4 find use as therapeutic agents that can be administered to an individual (human or other mammal). Preferably, the antibodies or antigen-binding portions thereof of the invention are neutralizing anti-DLL4 and/or anti-VEGFR2 antibodies.

A. Method of making anti-DLL4 antibodies.

Antibodies of the present invention may be made by any of a number of techniques known in the art. Aspects of various techniques that may be employed to obtain DLL4 monoclonal antibodies according to the invention are described below.

1. Anti-DLL4 monoclonal antibodies using hybridoma technology.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, second edition, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1988); Hammerling, et al., In Monoclonal Antibodies and T-Cell Hybridomas, (Elsevier, New York, 1981). It is also noted that the term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

In an embodiment, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from an animal, e.g., a rat or a mouse, immunized with DLL4 with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention. Briefly, rats can be immunized with a DLL4 antigen (see, Examples, below). In a preferred embodiment, the DLL4 antigen is administered with an adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks; however, a single administration of the polypeptide may also be used.

After immunization of an animal with a DLL4 antigen, antibodies and/or antibody-producing cells may be obtained from the animal. An anti-DLL4 antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-DLL4 antibodies may be purified from the serum. Serum or immunoglobulins obtained in this manner are polyclonal, thus having a heterogeneous array of properties.

Once an immune response is detected, e.g., antibodies specific for the antigen DLL4 are detected in the rat serum, the rat spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection (ATCC, Manassas, Virginia, US). Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding DLL4. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing rats with positive hybridoma clones.

In another embodiment, antibody-producing immortalized hybridomas may be prepared from the immunized animal. After immunization, the animal is sacrificed and the splenic B cells are fused to immortalized myeloma cells as is well known in the art. See, e.g., Harlow and Lane, *supra*. In a preferred embodiment, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After fusion and antibiotic selection, the hybridomas are screened using DLL4, or a portion thereof, or a cell expressing DLL4. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunosorbent assay (ELISA) or a radioimmunoassay (RIA), preferably an ELISA. An example of ELISA screening is provided in PCT Publication No. WO 00/37504.

Anti-DLL4 antibody-producing hybridomas are selected, cloned, and further screened for desirable characteristics, including robust hybridoma growth, high antibody production, and desirable antibody characteristics, as discussed further below. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

In a preferred embodiment, hybridomas are rat hybridomas, as described herein. In another embodiment, hybridomas are produced in a non-human, non-rat species such as mice, sheep, pigs, goats, cattle, or horses. In yet another preferred embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an anti-DLL4 antibody.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce two identical Fab fragments) or pepsin (to produce a $F(ab')_2$ fragment). A $F(ab')_2$ fragment of an IgG molecule retains the two antigen-binding sites of the larger ("parent") IgG molecule, including both light chains (containing the variable light chain and constant light chain regions), the CH1 domains of the heavy chains, and a disulfide-forming hinge region of the parent IgG molecule. Accordingly, a $F(ab')_2$ fragment is still capable of crosslinking antigen molecules like the parent IgG molecule.

2. Anti-DLL4 monoclonal antibodies using SLAM.

In another aspect of the invention, recombinant antibodies are generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in US Patent No. 5,627,052; PCT Publication No. WO 92/02551; and Babcook et al., *Proc. Natl. Acad. Sci. USA*, 93: 7843-7848 (1996). In this method, single cells secreting antibodies of interest, e.g., lymphocytes derived from any one of the immunized animals described in Section I.A.1 (above), are screened using an antigen-specific hemolytic plaque assay, wherein the antigen DLL4, a subunit of DLL4, or a fragment thereof, is coupled to sheep red blood cells using a linker, such as biotin, and used to identify single cells that secrete antibodies with specificity for DLL4. Following identification of antibody-secreting cells of interest, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR (RT-PCR) and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (e.g., human constant regions), in mammalian host cells, such as COS or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from in vivo selected lymphocytes, can then undergo further analysis and selection *in vitro*, for example, by panning the transfected cells to isolate cells expressing antibodies to DLL4. The amplified immunoglobulin sequences further can be manipulated *in vitro*, such as by *in vitro* affinity maturation method. See, for example, PCT Publication No. WO 97/29131 and PCT Publication No. WO 00/56772.

3. Anti-DLL4 monoclonal antibodies using transgenic animals.

In another embodiment of the instant invention, antibodies are produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with a DLL4 antigen. In an embodiment, the non-human animal is a XENOMOUSE® transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. See, e.g., Green et al., *Nature Genetics*, 7: 13-21 (1994) and US Patent Nos. 5,916,771; 5,939,598; 5,985,615; 5,998,209; 6,075,181; 6,091,001; 6,114,598; and 6,130,364. See also PCT Publication Nos. WO 91/10741; WO 94/02602; WO 96/34096; WO 96/33735; WO 98/16654; WO 98/24893; WO 98/50433; WO 99/45031; WO 99/53049; WO 00/09560; and WO 00/37504. The XENOMOUSE® transgenic mouse produces an adult-like human repertoire of fully human antibodies, and generates antigen-specific human monoclonal antibodies. The XENOMOUSE® transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. See Mendez et al., *Nature Genetics*, 15: 146-156 (1997), Green and Jakobovits, *J. Exp. Med.*, 188: 483-495 (1998), the disclosures of which are hereby incorporated by reference.

4. Anti-DLL4 monoclonal antibodies using recombinant antibody libraries.

In vitro methods also can be used to make the antibodies of the invention, wherein an antibody library is screened to identify an antibody having the desired DLL4-binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, US Patent No. 5,223,409 (Ladner et al.); PCT Publication No. WO 92/18619 (Kang et al.); PCT Publication No. WO 91/17271 (Dower et al.); PCT Publication No. WO 92/20791 (Winter et al.); PCT Publication No. WO 92/15679 (Markland et al.); PCT Publication No. WO 93/01288 (Breitling et al.); PCT Publication No. WO 92/01047 (McCafferty et al.); PCT Publication No. WO 92/09690 (Garrard et al.); Fuchs et al., *Bio/Technology*, 9: 1369-1372 (1991); Hay et al., *Hum. Antibod. Hybridomas*, 3: 81-85 (1992); Huse et al., *Science*, 246: 1275-1281 (1989); McCafferty et al., *Nature*, 348: 552-554 (1990); Griffiths et al., *EMBO J.*, 12: 725-734 (1993); Hawkins et al., *J. Mol. Biol.*, 226: 889-896 (1992); Clackson et al., *Nature*, 352: 624-628 (1991); Gram et al., *Proc. Natl. Acad. Sci. USA*, 89: 3576-3580 (1992); Garrard et al., *Bio/Technology*, 9: 1373-1377 (1991); Hoogenboom et al., *Nucl. Acids Res.*, 19: 4133-4137 (1991); Barbas et al., *Proc. Natl. Acad. Sci. USA*, 88: 7978-7982

(1991); US Patent Application Publication No. 2003/0186374; and PCT Publication No. WO 97/29131, the contents of each of which are incorporated herein by reference.

The recombinant antibody library may be from a subject immunized with DLL4, or a portion of DLL4. Alternatively, the recombinant antibody library may be from a naïve subject, i.e., one who has not been immunized with DLL4, such as a human antibody library from a human subject who has not been immunized with human DLL4. Antibodies of the invention are selected by screening the recombinant antibody library with the peptide comprising human DLL4 to thereby select those antibodies that recognize DLL4. Methods for conducting such screening and selection are well known in the art, such as described in the references in the preceding paragraph. To select antibodies of the invention having particular binding affinities for DLL4, such as those that dissociate from human DLL4 with a particular K_{off} rate constant, the art-known method of surface plasmon resonance can be used to select antibodies having the desired K_{off} rate constant. To select antibodies of the invention having a particular neutralizing activity for hDLL4, such as those with a particular IC_{50}, standard methods known in the art for assessing the inhibition of DLL4 activity may be used.

In one aspect, the invention pertains to an isolated antibody, or an antigen-binding portion thereof, that binds human DLL4. Preferably, the antibody is a neutralizing antibody. In various embodiments, the antibody is a recombinant antibody or a monoclonal antibody.

For example, antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. Such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv, or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkmann et al., *J. Immunol. Methods*, 182: 41-50 (1995); Ames et al., *J. Immunol. Methods*, 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.*, 24: 952-958 (1994); Persic et al., *Gene*, 187: 9-18 (1997); Burton et al., *Advances in Immunology*, 57: 191-280 (1994); PCT Publication No. WO 92/01047; PCT Publication Nos. WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and US Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743; and 5,969,108.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab', and F(ab')$_2$ fragments can also be employed using methods known in the art such as those disclosed in PCT publication No. WO 92/22324; Mullinax et al., *BioTechniques*, 12(6): 864-869 (1992); Sawai et al., *Am. J. Reprod. Immunol.*, 34: 26-34 (1995); and Better et al., *Science*, 240: 1041-1043 (1988). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in US Patent No. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology*, 203: 46-88 (1991); Shu et al., *Proc. Natl. Acad. Sci. USA*, 90: 7995-7999 (1993); and Skerra et al., *Science*, 240: 1038-1041 (1988).

Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of antibodies of the invention. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in PCT Publication No. WO 98/31700 (Szostak and Roberts), and in Roberts and Szostak, *Proc. Natl. Acad. Sci. USA*, 94: 12297-12302 (1997). In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by *in vitro* translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (e.g., a combinatorial library) based on the properties of the encoded peptide or protein, e.g., antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described above (e.g., in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected sequence(s), or by other methods for affinity maturation in vitro of recombinant antibodies, as described above. A preferred example of this methodology, is the PROfusion display technology employed in the Examples (*infra*).

In another approach the antibodies of the present invention can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Examples of yeast display methods that can be used to make the antibodies of the present invention include those disclosed in US Patent No. 6,699,658 (Wittrup et al.) incorporated herein by reference.

B. Production of recombinant DLL4 antibodies

Antibodies of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

Exemplary mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77: 4216-4220 (1980), used with a DHFR selectable marker, e.g., as described in Kaufman and Sharp, *J. Mol. Biol.*, 159: 601-621 (1982), NS0 myeloma cells, COS cells, and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention (i.e., binds human DLL4) and the other heavy and light chain are specific for an antigen other than human DLL4 by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant antibody of the invention is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

1. Anti-DLL4 antibodies.

Amino acid sequences of VH and VL regions of isolated rat monoclonal antibodies that bind human DLL4 are shown for clones 38H12, 1A11, 37D10, 32C7, 14G1, 14A11, and 15D6 in Table 9 (See, Example 4, below). The isolated anti-DLL4 antibody CDR sequences described herein establish a family of DLL4 binding proteins, isolated in accordance with this invention, and comprising polypeptides that include the CDR sequences derived therefrom and affinity matured clones thereof. Sequences of variable regions and CDRs of the monoclonal antibodies and affinity matured derivatives thereof are listed in Tables 9, 11, 16, 20, and 21. To generate and to select CDRs for binding proteins according to the invention having preferred DLL4 binding and/or neutralizing activity with respect to human DLL4, standard methods known in the art for generating binding proteins of the present invention and assessing the DLL4 binding and/or neutralizing characteristics of those binding protein may be used, including but not limited to those specifically described herein.

Based on an alignment of the amino acid sequences of the CDRs of the heavy chain variable regions (VH) and the light chain variable regions (VL) of the anti-DLL4 antibody clones described herein, the invention provides a DLL4 binding protein comprising an antigen binding domain capable of binding human DLL4, said antigen binding domain comprising at least one or more of the six CDRs, i.e., CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDRL-3, defined below:

CDR-H1 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:151), wherein;

X_1 is N, H, or Y;

X_2 is F;

X_3 is P;

X_4 is M; and

X_5 is A or S;

residues 31-35 of SEQ ID NO:157 (CDR-H1 38H12);

residues 31-35 of SEQ ID NO:161 (CDR-H1 37D10);

residues 31-35 of SEQ ID NO:163 (CDR-H1 32C7);

residues 31-35 of SEQ ID NO:165 (CDR-H1 14G1);

residues 31-35 of SEQ ID NO:167 (CDR-H1 14A11);

residues 31-35 of SEQ ID NO:169 (CDR-H1 15D6);

residues 31-35 of SEQ ID NO:171 (CDR-H1 VH.1 1A11);

residues 31-35 of SEQ ID NO:172 (CDR-H1 VH.1a 1A11);

residues 31-35 of SEQ ID NO:173 (CDR-H1 VH.1b 1A11);

residues 31-35 of SEQ ID NO:174 (CDR-H1 VH.2a 1A11);

residues 31-35 of SEQ ID NO:179 (CDR-H1 VH.1 38H12);

residues 31-35 of SEQ ID NO:180 (CDR-H1 VH.1A 38H12);

residues 31-35 of SEQ ID NO:181 (CDR-H1 VH.1b 38H12);

residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);

residues 31-35 of SEQ ID NO:187 (CDR-H1 h1A11VH.1);

residues 31-35 of SEQ ID NO:188 (CDR-H1 h1A11.A6);

residues 31-35 of SEQ ID NO:189 (CDR-H1 h1A11.A8);

residues 31-35 of SEQ ID NO:190 (CDR-H1 h1A11.C6);

residues 31-35 of SEQ ID NO:191 (CDR-H1 h1A11.A11);

residues 31-35 of SEQ ID NO:192 (CDR-H1 h1A11.B5);

residues 31-35 of SEQ ID NO:193 (CDR-H1 h1A11.E12);

residues 31-35 of SEQ ID NO:194 (CDR-H1 h1A11.G3);

residues 31-35 of SEQ ID NO:195 (CDR-H1 h1A11.F5); and

residues 31-35 of SEQ ID NO:196 (CDR-H1 h1A11.H2);

CDR-H2 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17} (SEQ ID NO:152), wherein;

X_1 is T or S;

X_2 is I;

X_3 is S;

X_4 is S or G;

X_5 is S;

X_6 is D;

X_7 is G, A, D, S, or E;

X_8 is T or W;

X_9 is T, P, or A;

X_{10} is Y, S, T, or N;

X_{11} is Y or I;

X_{12} is R or G;

X_{13} is D;

X_{14} is S;

X_{15} is V;

X_{16} is K; and

X_{17} is G;

residues 50-66 of SEQ ID NO:157 (CDR-H2 38H12);

residues 50-68 of SEQ ID NO:161 (CDR-H2 37D10);

residues 50-66 of SEQ ID NO:163 (CDR-H2 32C7);

residues 50-66 of SEQ ID NO:165 (CDR-H2 14G1);

residues 50-66 of SEQ ID NO:167 (CDR-H2 14A11);

residues 50-66 of SEQ ID NO:169 (CDR-H2 15D6);

residues 50-66 of SEQ ID NO:171 (CDR-H2 VH.1 1A11);

residues 50-66 of SEQ ID NO:172 (CDR-H2 VH.1a 1A11);

residues 50-66 of SEQ ID NO:173 (CDR-H2 VH.1b 1A11);

residues 50-66 of SEQ ID NO:174 (CDR-H2 VH.2a 1A11);

residues 50-66 of SEQ ID NO:179 (CDR-H2 VH.1 38H12);

residues 50-66 of SEQ ID NO:180 (CDR-H2 VH.1A 38H12);

residues 50-66 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12);

residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);

residues 50-66 of SEQ ID NO:187 (CDR-H2 h1A11VH.1);

residues 50-66 of SEQ ID NO:188 (CDR-H2 h1A11.A6);

residues 50-66 of SEQ ID NO:189 (CDR-H2 h1A11.A8);

residues 50-66 of SEQ ID NO:190 (CDR-H2 h1A11.C6);

residues 50-66 of SEQ ID NO:191 (CDR-H2 h1A11.A11);

residues 50-66 of SEQ ID NO:192 (CDR-H2 h1A11.B5);

residues 50-66 of SEQ ID NO:193 (CDR-H2 h1A11.E12);

residues 50-66 of SEQ ID NO:194 (CDR-H2 h1A11.G3);

residues 50-66 of SEQ ID NO:195 (CDR-H2 h1A11.F5); and

residues 50-66 of SEQ ID NO:196 (CDR-H2 h1A11.H2);

CDR-H3 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:153), wherein;

X_1 is G;

X_2 is Y;

X_3 is Y;

X_4 is N;

X_5 is S;

X_6 is P;

X_7 is F;

X_8 is A; and

X_9 is Y, F, or S;

residues 99-107 of SEQ ID NO:157 (CDR-H3 38H12);

residues 101-111 of SEQ ID NO:161 (CDR-H3 37D10);

residues 99-105 of SEQ ID NO:163 (CDR-H3 32C7);

residues 99-105 of SEQ ID NO:165 (CDR-H3 14G1);

residues 99-110 of SEQ ID NO:167 (CDR-H3 14A11);

residues 99-110 of SEQ ID NO:169 (CDR-H3 15D6);

residues 99-107 of SEQ ID NO:171 (CDR-H3 VH.1 1A11);

residues 99-107 of SEQ ID NO:172 (CDR-H3 VH.1a 1A11);

residues 99-107 of SEQ ID NO:173 (CDR-H3 VH.1b 1A11);

residues 99-107 of SEQ ID NO:174 (CDR-H3 VH.2a 1A11);

residues 99-107 of SEQ ID NO:179 (CDR-H3 VH.1 38H12);

residues 99-107 of SEQ ID NO:180 (CDR-H3 VH.1A 38H12);

residues 99-107 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12);

residues 99-107 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);

residues 99-107 of SEQ ID NO:187 (CDR-H3 h1A11VH.1);

residues 99-107 of SEQ ID NO:188 (CDR-H3 h1A11.A6);

residues 99-107 of SEQ ID NO:189 (CDR-H3 h1A11.A8);

residues 99-107 of SEQ ID NO:190 (CDR-H3 h1A11.C6);

residues 99-107 of SEQ ID NO:191 (CDR-H3 h1A11.A11);

residues 99-107 of SEQ ID NO:192 (CDR-H3 h1A11.B5);

residues 99-107 of SEQ ID NO:193 (CDR-H3 h1A11.E12);

residues 99-107 of SEQ ID NO:194 (CDR-H3 h1A11.G3);

residues 99-107 of SEQ ID NO:195 (CDR-H3 h1A11.F5); and

residues 99-107 of SEQ ID NO:196 (CDR-H3 h1A11.H2);

CDR-L1 is selected from the group consisting of:

$X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}$ (SEQ ID NO:154), wherein;

X_1 is R;

X_2 is A;

X_3 is S;

X_4 is E or Q;

X_5 is D or E;

X_6 is I;

X_7 is Y or W;

X_8 is S, I, Y, N, or R;

X_9 is N;

X_{10} is L; and

X_{11} is A;

residues 24-34 of SEQ ID NO:158 (CDR-L1 38H12);

residues 24-34 of SEQ ID NO:162 (CDR-L1 37D10);

residues 24-34 of SEQ ID NO:164 (CDR-L1 32C7);

residues 24-34 of SEQ ID NO:166 (CDR-L1 14G1);

residues 23-37 of SEQ ID NO:168 (CDR-L1 14A11);

residues 23-37 of SEQ ID NO:170 (CDR-L1 15D6);

residues 24-34 of SEQ ID NO:175 (CDR-L1 VL.1 1A11);

residues 24-34 of SEQ ID NO:176 (CDR-L1 VL.1a 1A11);

residues 24-34 of SEQ ID NO:177 (CDR-L1 VL.1b 1A11);

residues 24-34 of SEQ ID NO:178 (CDR-L1 VL.2a 1A11);

residues 24-34 of SEQ ID NO:183 (CDR-L1 VL.1 38H12);

residues 24-34 of SEQ ID NO:184 (CDR-L1 VL.1a 38H12);

residues 24-34 of SEQ ID NO:185 (CDR-L1 VL.1b 38H12);

residues 24-34 of SEQ ID NO:186 (CDR-L1 VL.2a 38H12);

residues 24-34 of SEQ ID NO:197 (CDR-L1 h1A11VL.1);

residues 24-34 of SEQ ID NO:198 (CDR-L1 h1A11.A2);

residues 24-34 of SEQ ID NO:199 (CDR-L1 h1A11.A12);

residues 24-34 of SEQ ID NO:200 (CDR-L1 h1A11.A7);

residues 24-34 of SEQ ID NO:201 (CDR-L1 h1A11.B4);

residues 24-34 of SEQ ID NO:202 (CDR-L1 h1A11.B5); and

residues 24-34 of SEQ ID NO:203 (CDR-L1 h1A11.E12);

CDR-L2 is selected from group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:155), wherein;

X_1 is D;

X_2 is T;

X_3 is N or S;

X_4 is N, D, S, I, Y, or V;

X_5 is L;

X_6 is A; and

X_7 is D;

residues 50-56 of SEQ ID NO:158 (CDR-L2 38H12);

residues 50-56 of SEQ ID NO:162 (CDR-L2 37D10);

residues 50-56 of SEQ ID NO:164 (CDR-L2 32C7);

residues 50-56 of SEQ ID NO:166 (CDR-L2 14G1);

residues 53-59 of SEQ ID NO:168 (CDR-L2 14A11);

residues 53-59 of SEQ ID NO:170 (CDR-L2 15D6);

residues 50-56 of SEQ ID NO:175 (CDR-L2 VL.1 1A11);

residues 50-56 of SEQ ID NO:176 (CDR-L2 VL.1a 1A11);

residues 50-56 of SEQ ID NO:177 (CDR-L2 VL.1b 1A11);

residues 50-56 of SEQ ID NO:178 (CDR-L2 VL.2a 1A11);

residues 50-56 of SEQ ID NO:183 (CDR-L2 VL.1 38H12);

residues 50-56 of SEQ ID NO:184 (CDR-L2 VL.1a 38H12);

residues 50-56 of SEQ ID NO:185 (CDR-L2 VL.1b 38H12);

residues 50-56 of SEQ ID NO:186 (CDR-L2 VL.2a 38H12);

residues 50-56 of SEQ ID NO:197 (CDR-L2 h1A11VL.1);

residues 50-56 of SEQ ID NO:198 (CDR-L2 h1A11.A2);

residues 50-56 of SEQ ID NO:199 (CDR-L2 h1A11.A12);

residues 50-56 of SEQ ID NO:200 (CDR-L2 h1A11.A7);

residues 50-56 of SEQ ID NO:201 (CDR-L2 h1A11.B4);

residues 50-56 of SEQ ID NO:202 (CDR-L2 h1A11.B5); and

residues 50-56 of SEQ ID NO:203 (CDR-L2 h1A11.E12);

and

CDR-L3 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:156), wherein;

X_1 is Q;

X_2 is Q;

X_3 is Y;

X_4 is N, D, or T;

X_5 is N, Y, or W;

X_6 is Y or V;

X_7 is P;

X_8 is P; and

X_9 is T;

residues 89-97 of SEQ ID NO:158 (CDR-L3 38H12);

residues 89-97 of SEQ ID NO:162 (CDR-L3 37D10);

residues 89-97 of SEQ ID NO:164 (CDR-L3 32C7);
residues 89-98 of SEQ ID NO:166 (CDR-L3 14G1);
residues 92-100 of SEQ ID NO:168 (CDR-L3 14A11);
residues 92-100 of SEQ ID NO:170 (CDR-L3 15D6);
residues 89-97 of SEQ ID NO:175 (CDR-L3 VL.1 1A11);
residues 89-97 of SEQ ID NO:176 (CDR-L3 VL.1a 1A11);
residues 89-97 of SEQ ID NO:177 (CDR-L3 VL.1b 1A11);
residues 89-97 of SEQ ID NO:178 (CDR-L3 VL.2a 1A11);
residues 89-97 of SEQ ID NO:183 (CDR-L3 VL.1 38H12);
residues 89-97 of SEQ ID NO:184 (CDR-L3 VL.1a 38H12);
residues 89-97 of SEQ ID NO:185 (CDR-L3 VL.1b 38H12);
residues 89-97 of SEQ ID NO:186 (CDR-L3 VL.2a 38H12);
residues 89-97 of SEQ ID NO:197 (CDR-L3 h1A11VL.1);
residues 89-97 of SEQ ID NO:198 (CDR-L3 h1A11.A2);
residues 89-97 of SEQ ID NO:199 (CDR-L3 h1A11.A12);
residues 89-97 of SEQ ID NO:200 (CDR-L3 h1A11.A7);
residues 89-97 of SEQ ID NO:201 (CDR-L3 h1A11.B4);
residues 89-97 of SEQ ID NO:202 (CDR-L3 h1A11.B5); and
residues 89-97 of SEQ ID NO:203 (CDR-L3 h1A11.E12).

Preferably, a DLL4 binding protein comprises at least one CDR described above, more preferably any two CDRs described above, more preferably any three CDRs described above, even more preferably any four CDRs described above, still more preferably any five CDRs described above, and most preferably any six CDRs described above (i.e., CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 as described above). A particularly preferred DLL4 binding protein comprising three CDRs comprises CDR-H1, CDR-H2, and CDR-H3 as described above.

Preferably, a DLL4 binding protein comprising one or more CDRs described above binds human ("hu", "h") DLL4 and also one or more DLL4 proteins selected from the group consisting of: mouse ("murine", "mu") DLL4, cynomolgus monkey ("cynomolgus", "cyno") DLL4, and rat DLL4.

Preferably, a DLL4 binding protein comprising one or more CDRs described above binds human ("hu") DLL4 and also cynomolgus monkey ("cynomolgus", "cyno") DLL4.

2. Anti-DLL4 chimeric antibodies.

A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. See e.g., Morrison, *Science*, 229: 1202-1207 (1985); Oi et al., *BioTechniques*, 4: 214 (1986); Gillies et al., *J. Immunol.*

Methods, 125: 191-202 (1989); US Patent Nos. 5,807,715; 4,816,567; and 4,816,397. In addition, techniques developed for the production of "chimeric antibodies" by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. See, for example, Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81: 6851-6855 (1984); Neuberger et al., *Nature*, 312: 604-608 (1984); Takeda et al., *Nature*, 314: 452-454 (1985), which are incorporated herein by reference in their entireties.

3. Anti-DLL4 CDR grafted antibodies.

The isolated anti-DLL4 antibody CDR sequences of the invention may be used to make CDR-grafted antibodies to modulate the properties of the original antibody. Such properties include but are not limited to binding kinetics, affinity, biological activities, species cross-reactivity, molecule cross-reactivity, epitope, physicochemical properties, pharmacokinetic properties, pharmacodynamic properties, or pharmacological properties. CDR-grafted antibodies comprise heavy and light chain variable region sequences from a human antibody or a non-human primate antibody wherein one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of the original anti-DLL4 antibody. A framework sequence from any human or non-human primate antibody may serve as the template for CDR grafting. However, straight chain replacement onto such a framework often leads to some loss of binding affinity to the antigen. The more homologous a human, or other species, antibody is to the original human antibody, the less likely the possibility that combining the CDRs with the new human framework or non-human primate framework will introduce distortions in the CDRs that could reduce affinity or other properties. Therefore, it is preferable that the variable framework that is chosen to replace the human variable region framework apart from the CDRs has at least a 30% sequence identity with the human antibody variable region framework. It is more preferable that the variable region framework that is chosen to replace the human variable region framework apart from the CDRs has at least a 40% sequence identity with the human antibody variable region framework. It is more preferable that the variable region framework that is chosen to replace the human variable framework apart from the CDRs has at least a 50% sequence identity with the human antibody variable region framework. It is more preferable that the variable region framework that is chosen to replace the human variable framework apart from the CDRs has at least a 60% sequence identity with the human antibody variable region framework. It is more preferable that the new human or non-human primate and the original human variable region framework apart from the CDRs has at least 70% sequence identity. It is even more preferable that the new human or non-human primate and the original human variable region framework apart from the CDRs has at least 75% sequence identity. It is most preferable that the new human or non-human primate and the original human variable region framework apart from the CDRs has at least 80% sequence identity. Even using a highly homologous human or non-human primate framework to graft CDRs of the original human anti-DLL4 antibody, the resulting grafted antibody may still lose binding affinity to antigen to some degree. In this case, to regain the affinity it is necessary to include at least one or more key framework residue(s) substitution of the original antibody to the corresponding position of the newly grafted antibody. Such a key residue may be selected from the group consisting of:

a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with human DLL4
a canonical residue;
a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone; and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

4. Anti-DLL4 humanized antibodies.

While the compositions of the present invention eliminate the requirement to make humanized antibodies, humanized DLL4 antibodies may be prepared using compositions of the invention. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Known human Ig sequences are disclosed at web sites available via the world wide web (www.), e.g., ncbi.nlm.nih.gov/entrez/query.fcgi; atcc.org/phage/hdb.html; sciquest.com/; abcam.com/; antibodyresource.com/onlinecomp.html; public.iastate.edu/.about.pedro-/research_tools.html; mgen.uniheidelberg.de/SD/IT/IT.html; whfreeman.com/immunology-/CH05/kuby05.htm; library.thinkquest.org/12429/Immune/Antibody.html; hhmi.org/grants/lectures/1996/vlab/; path.-cam.ac.uk/.about.mrc7/mikeimages.html; antibodyresource.com/; mcb.harvard.edu/BioLinks-/Immunology.html; immunologylink.com/; pathbox.wustl.edu/.about.hcenter/index.html; bio-tech.ufl.edu/.about.hcl/; pebio.com/pa/340913-/340913.html; nal.usda.gov/awic/pubs/antibody/; m.ehimeu.acjp/.about.yasuhito-/Elisa.html; biodesign.com/table.asp; icnet.uk/axp/facs/davies/lin-ks.html; biotech.ufl.edu-/.about.fccl/protocol.html; isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/.about.rek/AEP-Start.html; baserv.uci.kun.nl/.about.jraats/linksl.html; recab.uni-hd.de/immuno.bme.nwu.edu/; mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; ibt.unam.mx/-vir/V_mice.html; imgt.cnusc.fr:8104/; biochem.ucl.ac.uk/.about.martin/abs/index.html; anti-body.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; unizh.ch/.about.honegger/AHO-seminar/Slide01.html; cryst.bbk.ac.uk/.about.ubcg07s/; nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; path.cam.ac.uk/.about.mrc7/humanisation/TAHHP.html; ibt.unam.mx/vir/structure/stat_aim.-

html; biosci.missouri.edu/smithgp/index.html; cryst.bioc.cam.ac.uk/.about.fmolina/Webpages-/Pept/spottech.html; jerini.de/frroducts.htm; patents.ibm.com/ibm.html. Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference. Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art.

Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., US Patent No. 5,585,089 (Queen et al.); Riechmann et al., *Nature*, 332: 323-327 (1988), which are incorporated herein by reference in their entireties.) Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, such as but not limited to those described in Jones et al., *Nature*, 321: 522-525 (1986); Verhoeyen et al., *Science*, 239: 1534-1536 (1988), Sims et al., *J. Immunol.*, 151: 2296-2308 (1993); Chothia and Lesk, *J. Mol. Biol.*, 196: 901-917 (1987), Carter et al., *Proc. Natl. Acad. Sci. USA*, 89: 4285-4289 (1992); Presta et al., *J. Immunol.*, 151: 2623-2632 (1993), Padlan, E.A., *Molecular Immunology*, 28(4/5): 489-498 (1991); Studnicka et al., *Protein Engineering*, 7(6): 805-814 (1994); Roguska. et al., *Proc. Natl. Acad. Sci. USA*, 91:969-973 (1994); PCT Publication Nos. WO 91/09967, WO 99/06834 (PCT/US98/16280), WO 97/20032 (PCT/US96/18978), WO 92/11272 (PCT/US91/09630), WO 92/03461 (PCT/US91/05939), WO 94/18219 (PCT/US94/01234), WO 92/01047 (PCT/GB91/01134), WO 93/06213 (PCT/GB92/01755), WO90/14443, WO90/14424, and WO90/14430; European Publication Nos. EP 0 592 106, EP 0 519 596, and EP 0 239 400; US Patent Nos. 5,565,332; 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567, each entirely incorporated herein by reference, included references cited therein.

C. Production of antibodies and antibody-producing cell lines.

Preferably, anti-DLL4 antibodies of the present invention exhibit a high capacity to reduce or to neutralize tumor angiogenesis activity, e.g., as assessed by any one of several *in vitro* and *in vivo* assays known in the art. For example, these antibodies neutralize DLL4 interaction in the Notch-signaling pathway with IC_{50} values in DLL4 in the range of at least about 10^{-7} M, or about 10^{-8} M. Preferably, anti-DLL4 antibodies of the present invention also exhibit a high capacity to reduce or to neutralize DLL4 activity.

In preferred embodiments, an isolated antibody, or antigen-binding portion thereof, binds human DLL4, wherein the antibody, or antigen-binding portion thereof, dissociates from human DLL4 with a K_{off} rate constant of about 0.1 s^{-1} or less, as determined by surface plasmon resonance, or which inhibits DLL4 and/or human DLL4 activity with an IC_{50} of about 1×10^{-6} M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human DLL4 with a K_{off} rate constant of about 1×10^{-2} s^{-1} or less, as determined by surface plasmon resonance, or may inhibit human DLL4 and/or human DLL4 activity with an IC_{50} of about 1×10^{-7} M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human DLL4 with a K_{off} rate constant of about 1×10^{-3} s^{-1} or less, as determined by surface plasmon resonance, or may inhibit human DLL4 with an IC_{50} of about 1×10^{-8} M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human DLL4 with a K_{off} rate constant of about 1×10^{-4} s^{-1} or less, as determined by surface plasmon resonance, or may inhibit DLL4 activity with an IC_{50} of about 1×10^{-9} M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human DLL4 with a K_{off} rate constant of about 1×10^{-5} s^{-1} or less, as determined by surface plasmon resonance, or may inhibit DLL4 and/or human DLL4 activity with an IC_{50} of about 1×10^{-10} M or less. Alternatively, the antibody, or an antigen-binding portion thereof, may dissociate from human DLL4 with a K_{off} rate constant of about 1×10^{-5} s^{-1}or less, as determined by surface plasmon resonance, or may inhibit DLL4 and/or human DLL4 activity with an IC_{50} of about 1×10^{-11} M or less.

In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, or IgD constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. Preferably, the antibody comprises a kappa light chain constant region. Alternatively, the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (see, US Patent Nos. 5,648,260 and 5,624,821 (Winter et al.)). The Fc portion of an antibody mediates several important effector functions e.g. cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC), and half-life/clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered.

One embodiment provides a labeled binding protein wherein an antibody or antibody portion of the invention is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally linking an antibody or antibody portion of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

Another embodiment of the invention provides a crystallized DLL4 binding protein. Preferably, the invention relates to crystals of DLL4 binding proteins described herein, including whole anti-DLL4 antibodies, fragments thereof, as well as antibody constructs and binding protein conjugates (including antibody conjugates) as disclosed herein, and formulations and compositions comprising such crystals. In one embodiment, the crystallized binding protein has a greater half-life *in vivo* than the soluble counterpart of the binding protein. In another embodiment the binding protein retains biological activity after crystallization. Crystallized binding proteins of the invention may be produced according methods known in the art and as disclosed in PCT Publication No. WO 02/72636, incorporated herein by reference.

Another embodiment of the invention provides a glycosylated binding protein wherein the antibody or antigen-binding portion thereof comprises one or more carbohydrate residues. Nascent in vivo protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (e.g., glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. Preferably the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

It is known to those skilled in the art that differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life in vivo after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using techniques known in the art a practitioner may generate antibodies or antigen-binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (US Patent Application Publication Nos. 2004/0018590 and 2002/0137134).

Further, it will be appreciated by one skilled in the art that a protein of interest may be expressed using a library of host cells genetically engineered to express various glycosylation enzymes, such that member host cells of the library produce the protein of interest with variant glycosylation patterns. A practitioner may then select and isolate the protein of interest with particular novel glycosylation patterns. Preferably, the protein having a particularly selected novel glycosylation pattern exhibits improved or altered biological properties.

D. Uses of DLL4 binding proteins.

Given their ability to bind to human DLL4 and murine DLL4, the DLL4 binding proteins described herein, including antibodies and portions thereof, can be used to detect or measure DLL4 in a sample (e.g., in a mixture, solution, or biological sample, such as blood, serum, or plasma), using any of the conventional immunoassays known in the art, such as an enzyme linked immunosorbent assays (ELISA), a radioimmunoassay (RIA), or a tissue immunohistochemistry. The invention provides a method for detecting human DLL4 and/or murine DLL4 in a sample comprising contacting a sample with a DLL4 binding protein and detecting either the DLL4 binding protein bound to human DLL4 and/or murine DLL4 or the unbound binding protein to thereby detect human DLL4 and/or murine DLL4 in the sample. A DLL4 binding protein described herein can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound DLL4 binding protein. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

Biological samples that can be assayed for DLL4 include urine, feces, blood, serum, plasma, perspiration, saliva, oral swab (cheek, tongue, throat), vaginal swab, rectal swab, dermal swab, dermal scrape, tissue biopsy, as well as any other tissue sample that can be obtained by methods available in the art.

Alternative to labeling the binding protein, human DLL4 can be assayed in biological fluids by a competition immunoassay utilizing recombinant human (rh) DLL4 standards labeled with a detectable substance and an unlabeled DLL4 binding protein described herein. In this assay, the biological sample, the labeled rhDLL4 standards, and the DLL4 binding protein are combined and the amount of labeled rhDLL4 standard bound to the unlabeled binding protein is determined. The amount of human DLL4 in the biological sample is inversely proportional to the amount of labeled rhDLL4 standard bound to the DLL4 binding protein. Similarly, human DLL4 can also be assayed in biological fluids by a competition immunoassay utilizing rhDLL4 standards labeled with a detectable substance and an unlabeled DLL4 binding protein described herein.

The DLL4 binding proteins of the invention preferably are capable of neutralizing DLL4 activity, in particular hDLL4 activity, both *in vitro* and *in vivo*. Accordingly, such binding proteins of the invention can be used to inhibit DLL4 activity, e.g., in a cell culture containing DLL4, in human subjects, or in other mammalian subjects expressing a DLL4 with which a binding protein of the invention cross-reacts. In one embodiment, the invention provides a method for inhibiting DLL4 activity comprising contacting a DLL4 with a DLL4 antibody or antibody portion of the invention such that DLL4 activity is inhibited. For example, in a cell culture containing or suspected of containing DLL4, an antibody or antibody portion of the invention can be added to the culture medium to inhibit DLL4 activity in the culture.

In another embodiment, the invention provides a method for reducing DLL4 activity in a subject, advantageously from a subject suffering from a disease or disorder in which DLL4 or DLL4 activity is detrimental. The invention provides methods for reducing DLL4 or DLL4 activity in a subject suffering from such a disease or disorder, which method comprises administering to the subject a DLL4 binding protein of the invention such that DLL4 or DLL4 activity in the subject is reduced. Preferably, the DLL4 is human DLL4, and the subject is a human subject. Alternatively, the subject can be a mammal expressing a DLL4 to which a DLL4 binding protein of the invention is capable of binding. Still further, the subject can be a mammal into which DLL4 has been introduced (e.g., by administration of DLL4 or by expression of a DLL4 transgene). An antibody or other DLL4 binding protein of the invention can be administered to a human subject for therapeutic purposes. Moreover, a DLL4 binding protein of the invention can be administered to a non-human mammal expressing a DLL4 with which the binding protein is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies and other DLL4 binding proteins of the invention (e.g., testing of dosages and time courses of administration).

As used herein, the term "a disorder in which DLL4 and/or Notch signaling activity is detrimental" is intended to include diseases, such as cancer, and other disorders in which the presence of DLL4 and/or Notch signaling activity in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which DLL4 and/or Notch signaling activity is detrimental is a disorder in which reduction of DLL4 and/or Notch signaling activity is expected to alleviate the symptoms and/or progression of the disorder (e.g., tumor growth). Such disorders may be evidenced, for example, by an increase in angiogenesis in a subject suffering from the disorder (e.g., an increase in the concentration of various proteins known in the art to increase in serum, plasma, synovial fluid, etc., of the subject during tumor growth and formation), which can be detected, for example, using an anti-DLL4 antibody as described above. Non-limiting examples of disorders that can be treated with the antibodies of the invention include those disorders discussed in the section below pertaining to pharmaceutical compositions of the antibodies of the invention.

II. Pharmaceutical Compositions and Therapeutic Uses.

The invention also provides pharmaceutical compositions comprising a DLL4 binding protein of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising DLL4 binding proteins of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder; in preventing, treating, managing, or ameliorating a disorder or one or more symptoms thereof; and/or in research. In a specific embodiment, a composition comprises one or more DLL4 binding proteins of the invention. In another embodiment, the pharmaceutical composition comprises one or more binding proteins of the invention and one or more prophylactic or therapeutic agents other than binding proteins of the invention for treating a disorder in which DLL4 and/or DLL4 activity is detrimental. Preferably, the prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder, such as cancer or a tumor, or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent, or excipient.

The binding proteins of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises a DLL4 binding protein (or DLL4 binding portion thereof) of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

Various delivery systems are known and can be used to administer one or more DLL4 binding proteins of the invention or the combination of one or more binding proteins of the invention and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., reducing tumor angiogenesis, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the DLL4 binding protein, receptor- mediated endocytosis (see, e.g., Wu and Wu, *J.*

Biol. Chem., 262: 4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, and mucosal administration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., US Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934, 272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In one embodiment, a DLL4 binding protein of the invention, combination therapy, or a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Massachusetts, US). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more DLL4 binding proteins of the invention antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more DLL4 binding proteins of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than a binding protein of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

In another embodiment, the prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see, Langer (*Science*, 249: 1527-1533 (1990)); Sefton, *CRC Crit. Ref. Biomed. Eng.*, 14: 201-240 (1987); Buchwald et al., *Surgery*, 88: 507-516 (1980); Saudek et al., *N. Engl. J. Med.*, 321: 574-579 (1989)). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention. See, e.g., Goodson, J.M, *In* Medical Applications of Controlled Release, Vol. II, Applications and

Evaluations, (Langer and Wise, eds.), (CRC Press Inc., Boca Raton, 1984), chapter 6, pages 115-138; Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.) (Wiley, New York, 1984); Langer and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem. Phys.*, C23: 61-126 (1983); see also, Levy et al., *Science*, 228: 190-192 (1985); During et al., *Ann. Neurol.*, 25: 351-356 (1989); Howard et al., *J. Neurosurg.*, 71: 105-112 (1989); US Patent Nos. 5,679,377; 5,916,597; 5,912,015; 5,989,463; and 5,128,326; and PCT Publication Nos. WO 99/15154 and WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N- vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, *In* Medical Applications of Controlled Release, (1984), pages 115-138).

Controlled release systems are discussed in the review by Langer (*Science*, 249: 1527-1533 (1990)). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. See, e.g., U. S. Patent No. 4,526,938; PCT Publication Nos. WO 91/05548 and WO 96/20698; Ning et al., "Intratumoral Radioimmunotherapy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiother. Oncol.,* 39: 179-189 (1996); Song et al., "Antibody Mediated Lung Targeting of Long- Circulating Emulsions," *PDA J. Pharm. Sci.Tech.*, 50: 372-377 (1996); Cleek et al., "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," *Proceed. Intl. Symp. Control. Rel. Bioact. Mater.*, 24: 853-854 (1997), and Lam et al., "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," *Proceed. Intl. Symp. Control Rel. Bioact. Mater.*: 24: 759-760 (1997), each of which is incorporated herein by reference in their entireties.

In a specific embodiment, where the composition of the invention is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered *in vivo* to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see US Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, DuPont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA*, 88:

1864-1868 (1991)). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral (e.g., intravenous), intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocamne to ease pain at the site of the injection.

If compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., (Mack Publishing Co., Easton, Pennsylvania, 1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as FREON®) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art.

If a method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

If a method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g., pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

A method of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. See, e.g., US Patent Nos. 6,019,968; 5,985,320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In a specific embodiment, an antibody of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Massachusetts, US).

A method of the invention may comprise administration of a composition formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use.

A method of the invention may additionally comprise administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

Methods of the invention encompass administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, a composition can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

In particular, the invention also provides that one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between 2°C and 8°C in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, preferably within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, more preferably at least 0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml, or at least 100 mg/ml. The liquid form should be stored at between 2°C and 8°C in its original container.

The binding proteins of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the binding protein will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampoule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10 mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, a DLL4 binding protein described herein is administered by intravenous infusion or injection. In another preferred embodiment, a DLL4 binding protein is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The DLL4 binding proteins of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection, or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J.R. Robinson, ed., (Marcel Dekker, Inc., New York, 1978).

In certain embodiments, a binding protein of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, a binding protein of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which DLL4 activity is detrimental. For example, an anti-huDLL4 antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface molecules). Furthermore, one or more binding proteins of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, a DLL4 binding protein of the invention is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in US Patent No. 6,660,843 B1 and published PCT Publication No. WO 99/25044, which are hereby incorporated by reference.

In a specific embodiment, nucleic acid sequences comprising nucleotide sequences encoding a binding protein of the invention or another prophylactic or therapeutic agent of the invention are administered to treat, prevent, manage, or ameliorate a disorder or one or more symptoms thereof by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded binding protein or prophylactic or therapeutic agent of the invention that mediates a prophylactic or therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. For general reviews of the methods of gene therapy, see Goldspiel et al., *Clin. Pharmacy*, 12: 488-505 (1993); Wu and Wu, *Biotherapy*, 3: 87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.*, 32: 573-596 (1993); Mulligan, *Science*, 260: 926- 932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.*, 62: 191-217 (1993); Robinson, C., *Trends Biotechnol.*, 11(5):155 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology (John Wiley & Sons, New York, 1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, (Stockton Press, New York, 1990). Detailed descriptions of various methods of gene therapy are disclosed in US Patent Application Publication No. 20050042664 A1, which is incorporated herein by reference.

In another aspect, this invention provides a method of treating (e.g. curing, suppressing, ameliorating, delaying, or preventing the onset of, or preventing recurrence or relapse of) or preventing a DLL4-associated tumor in a subject. The method includes administering to a subject a DLL4 binding protein, e.g., an anti-DLL4 antibody or fragment thereof as described herein, in an amount sufficient to treat or prevent the DLL-associated tumor or cancer. The DLL4 antagonist, i.e., the anti-DLL4 antibody or fragment thereof, may be administered to a subject alone or in combination with other therapeutic modalities as described herein.

DLL4 plays a critical role in the pathology associated with a variety of diseases involving immune and inflammatory elements, in particular cancer and tumor angiogenesis. Examples of DLL4-associated disorders include, but are not limited to, those disorders that adversely affect the following biological processes: neuronal function and development; stabilization of arterial endothelial fate and angiogenesis; regulation of crucial cell communication events between endocardium and myocardium during both the formation of the valve primordial and ventricular development and differentiation; cardiac valve homeostasis, as well as implications in other human disorders involving the cardiovascular system; timely cell lineage specification of both endocrine and exocrine pancreas; influencing of binary fate decisions of cells that must choose between the secretory and absorptive lineages in the gut; expansion of the hematopoietic stem cell compartment during bone development and participation in commitment to the osteoblastic lineage such as osteoporosis; regulation of cell-fate decision in mammary glands at several distinct development stages; and certain non-nuclear mechanisms, such as control of the actin cytoskeleton through the tyrosine kinase Abl. More specifically, DLL4-associated disorders include, but are not limited to, cancers, T-ALL (T-cell acute lymphoblastic leukemia), CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), MS (multiple sclerosis), tetralogy of Fallot (TOF), and Alagille syndrome (AS), macular degeneration and age-related macular degeneration diseases, and other angiogenesis independent and dependent diseases characterized by aberrant DLL4 expression or activity.

Preferably, DLL4 binding proteins, such as antibodies and antigen-binding portions thereof as described herein, are used to treat cancers and tumors.

Binding proteins according to the invention can be used alone or in combination, i.e., more than one DLL4-binding protein described herein, to treat a cancer, a tumor, or other disorder in which binding to, inhibition of, and/or neutralization of DLL4 is considered desirable or otherwise beneficial to the health of an individual.

It should be understood that DLL4 binding proteins of the invention can also be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled practitioner for its intended purpose. For example, the additional agent can be a therapeutic agent that is recognized in the art as being useful to treat a cancer, tumor, or other disease or condition in which binding to or inhibition of DLL4 is considered to be desirable or advantageous for treating the cancer, tumor, or other disease or condition. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition, e.g., an agent which affects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents, if the combination is such that the formed composition can perform its intended function.

Preferred combinations of therapeutic agents may interfere at different points in the pro-tumorigenic or pro-angiogenic signaling pathways. Preferred examples of therapeutic agents useful in the methods and compositions of the invention include antineoplastic agents, radiotherapy, and chemotherapy such as DNA alkylating agents, cisplatin, carboplatin, anti-tubulin agents, paclitaxel, docetaxel, taxol, doxorubicin, gemcitabine, gemzar, anthracyclines, adriamycin, topoisomerase I inhibitors, topoisomerase II inhibitors, 5-fluorouracil (5-FU), leucovorin, irinotecan, receptor tyrosine kinase inhibitors (e.g., erlotinib, gefitinib), COX-2 inhibitors (e.g., celecoxib), and kinase inhibitors.

The DLL4 binding proteins of the invention may also be administered in combination with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquinine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, colchicine, corticosteroids (oral, inhaled and local injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adensosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signaling by proinflammatory cytokines such as TNFα or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFα converting enzyme (TACE) inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g., soluble p55 or p75 TNF receptors and the derivatives p75TNFRIgG (Enbrel™) and p55TNFRIgG (Lenercept), sIL-1RI, sIL-1RII, sIL-6R), antiinflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-13 and TGFβ), celecoxib, folic acid, hydroxychloroquine sulfate, rofecoxib, etanercept, infliximab, naproxen, valdecoxib, sulfasalazine, methylprednisolone, meloxicam, methylprednisolone acetate, gold sodium thiomalate, aspirin, triamcinolone acetonide, propoxyphene napsylate/apap, folate, nabumetone, diclofenac, piroxicam, etodolac, diclofenac sodium, oxaprozin, oxycodone hcl, hydrocodone bitartrate/apap, diclofenac sodium/misoprostol, fentanyl, anakinra, human recombinant, tramadol hcl, salsalate, sulindac, cyanocobalamin/fa/pyridoxine, acetaminophen, alendronate sodium, prednisolone, morphine sulfate, lidocaine hydrochloride, indomethacin, glucosamine sulf/chondroitin, amitriptyline HCl, sulfadiazine, oxycodone HCl/acetaminophen, olopatadine hcl, misoprostol, naproxen sodium, omeprazole, cyclophosphamide, rituximab, IL-1 TRAP, MRA, CTLA4-IG, IL-18 BP, anti-IL-18, anti-IL15, BIRB-796, SCIO-469, VX-702, AMG-548, VX-740, Roflumilast, IC-485, CDC-801, and Mesopram.

Non-limiting examples of therapeutic agents for cancers with which a DLL4 binding protein of the invention can be co-administered or used in combination include the following: budenoside; epidermal growth factor; sulfasalazine; aminosalicylates; 6-mercaptopurine;

azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1β monoclonal antibodies; anti-IL-6 monoclonal antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; and antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-17, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90, or their ligands.

Other examples of therapeutic agents with which a DLL4 binding protein of the invention can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, D2E7 (PCT Publication No. WO 97/29131; HUMIRA®), CA2 (REMICADE®), CDP 571, TNFR-Ig constructs (p75TNFRIgG (ENBREL®) and p55TNFRIgG (LENERCEPT)), and PDE4 inhibitors. Binding proteins of the invention can be combined with mesalamine, prednisone, azathioprine, mercaptopurine, infliximab, methylprednisolone sodium succinate, diphenoxylate/atrop sulfate, loperamide hydrochloride, methotrexate, omeprazole, folate, ciprofloxacin/dextrose-water, hydrocodone bitartrate/apap, tetracycline hydrochloride, fluocinonide, metronidazole, thimerosal/boric acid, cholestyramine/sucrose, ciprofloxacin hydrochloride, hyoscyamine sulfate, meperidine hydrochloride, midazolam hydrochloride, oxycodone hcl/acetaminophen, promethazine hydrochloride, sodium phosphate, sulfamethoxazole/trimethoprim, celecoxib, polycarbophil, propoxyphene napsylate, hydrocortisone, multivitamins, balsalazide disodium, codeine phosphate/apap, colesevelam hcl, cyanocobalamin, folic acid, levofloxacin, methylprednisolone, natalizumab, and interferon-gamma.

Non-limiting examples of therapeutic agents with which a binding protein of the invention can be combined include the following: aspirin, nitroglycerin, isosorbide mononitrate, metoprolol succinate, atenolol, metoprolol tartrate, amlodipine besylate, diltiazem hydrochloride, isosorbide dinitrate, clopidogrel bisulfate, nifedipine, atorvastatin calcium, potassium chloride, furosemide, simvastatin, verapamil hcl, digoxin, propranolol hydrochloride, carvedilol, lisinopril, spironolactone, hydrochlorothiazide, enalapril maleate, nadolol, ramipril, enoxaparin sodium, heparin sodium, valsartan, sotalol hydrochloride, fenofibrate, ezetimibe, bumetanide, losartan potassium, lisinopril/hydrochlorothiazide, felodipine, captopril, and bisoprolol fumarate.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of a binding protein of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the binding protein may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the binding protein to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the binding protein are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of a DLL4 binding protein of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

III. Use in Immunotechniques.

Any of a variety of immunodetection assay formats may be adapted to employ a DLL4 binding protein of the invention for use in detecting DLL4 present in a mixture, solution, or biological sample. Such immunodetection assay formats include but are not limited to radioimmunoassay (RIA), immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), immunoblot (e.g., Western blot), immunostrips (e.g., immunodipsticks) that comprise a DLL4 binding protein of the invention adsorbed or immobilized to substrate, fluorescence activated cell sorting (FACS), and the like. A DLL4 binding protein described herein can be adsorbed or immobilized to a substrate, e.g., a resin particle or other material, for use in an affinity column or any other affinity format available in the art to purify DLL4 from a sample. Detection of DLL4 using a DLL4 binding protein of the invention can be conducted *in vitro* on a mixture, solution, or in biological sample. A biological sample that can be contacted with a DLL4 binding protein of the invention to detect or measure DLL4 in the sample includes, but is not limited to, urine, saliva, oral swab (buccal, lingual, or throat swab), dermal swab, dermal scrape, rectal swab, vaginal swab, whole blood sample, plasma sample, serum sample, tissue biopsy, and any other sample obtained from an individual by a procedure known in the art. In another embodiment, a DLL4 binding protein may be employed to detect DLL4 *in vivo* such as various tomography and scanning methods, including but not limited to X-ray computer assisted tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET).

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

Examples

Example 1: *In vitro* Assays Used to Determine the Functional Activity of DLL4 Antibodies.

Example 1.1: Affinity Determination Using BIACORE® Surface Plasmon Resonance Technology.

The BIACORE® surface plasmon resonance assay (Biacore, Inc., Piscataway, New Jersey, US) determines the affinity of antibodies with kinetic measurements of on-rate and off-rate constants. Binding of DLL4 antibodies to a purified recombinant DLL4 extracellular domain (ECD) was determined by surface plasmon resonance-based measurements with a Biacore® instrument (either a Biacore 2000, Biacore 3000, or Biacore T100; GE Healthcare, Piscataway, New Jersey, US) using running buffer HBS-EPB (10 mM HEPES [pH 7.4], 150 mM NaCl, 3 mM EDTA, 0.1 mg/ml BSA and 0.005% surfactant P20) at 25°C. For example, approximately 9000 RU of goat anti-human Fc specific polyclonal antibody (Thermo Fisher Scientific Inc., Rockford, Illinois, US) diluted in 10 mM sodium acetate (pH 4.5) is directly immobilized across a CM5 research grade biosensor chip using a standard amine coupling kit according to manufacturer's instructions and procedures at 25 μg/ml. Unreacted moieties on the biosensor surface were blocked with ethanolamine. For kinetic analysis, rate equations derived from the 1:1 Langmuir binding model were fitted simultaneously to multiple antigen injections (using global fit analysis) with the use of Scrubber 2 (BioLogic Software), Biacore Biaevaluation 4.0.1 software or Biacore T100 Evaluation software. Purified antibodies were diluted in running buffer for capture across goat anti-human Fc reaction surfaces. Antibodies to be captured as a ligand (1 μg/ml) were injected over reaction matrices at a flow rate of 10 µl/min. During the assay, all measurements were referenced against the capture surface alone (i.e., with no captured anti-DLL4 antibody). The association and dissociation rate constants, K_{on} ($M^{-1}s^{-1}$) and K_{off} (s^{-1}) were determined under a continuous flow rate of 80 µl/min. Rate constants were derived by making kinetic binding measurements at different antigen concentrations ranging from 1.23 – 900 nM, as a 3-fold dilution series, and included buffer-only injections (to be used for double referencing). The equilibrium dissociation constant K_D (M) of the reaction between antibodies and the target antigen was then calculated from the kinetic rate constants by the following formula: $K_D = K_{off}/K_{on}$. Binding was recorded as a function of time and kinetic rate constants were calculated. In this assay, on-rates as fast as 10^6 $M^{-1}s^{-1}$ and off-rates as slow as 10^{-6} s^{-1} could be measured.

Example 1.2: Binding of DLL4 Antibodies to Soluble DLL4 Extracellular Domain as Determined by ELISA.

Method 1 (Capture ELISA).

96-well Nunc-Immuno plates (#439454) were coated with 5 µg/ml antibody against human IgG (Fcg fragment specific, Jackson ImmunoResearch, #109-005-098, 100 µl/well) in D-PBS (Gibco #14190) and incubated overnight at 4°C. ELISA plates were washed 3 times with wash buffer (PBS, 0.05% Tween-20) and then blocked with 200 ml/well blocking buffer (D-PBS, 1% BSA, 1 mM $CaCl_2$, 0.05% Tween-20) for 1 hour at 25°C. Plates were washed 3 times and incubated with 100 µl/well DLL4 antibodies (0.0001-100 nM, 10-fold serial dilution in blocking buffer) for 1 hour at 25°C, and then washed again 3 times. Plates containing captured DLL4 antibody were incubated with biotin-labeled human DLL4 extracellular domain (10 nM in blocking buffer, 100 µl/well) for 1 hour at 25°C, washed 3 times, and incubated with streptavidin conjugated with HRP (KPL #474-3000, 1:10,000 dilution in blocking buffer, 100 µl/well) for 1 hour at 25°C. After the final wash, plates were incubated with 100 µl/well ELISA substrate (1-Step Ultra TMB-ELISA, Pierce #340280). The reaction was stopped after 2 minutes at 25°C with 100 µl/well 2 N H_2SO_4 and the absorbance was read at 450 nm. Data were analyzed using Graphpad Prism software and EC_{50} values were reported.

Method 2 (Copper Coated Plate).

96-well copper-coated plates (Thermo Scientific #15143) were washed 3 times with wash buffer (PBS, 0.05% Tween-20) before use and then incubated with 100 µl/well of 6xHis-tagged recombinant DLL4 extracellular domain (ECD) ("6xHis" disclosed as SEQ ID NO: 206) at 1 µg/ml in PBS, 1 hour at 25°C with shaking. Plates were then washed 3 times. 100 µl/well of recombinant rat/human chimeric or recombinant human anti-DLL4 antibodies were then added to the plate (0.00164-27 nM, 4-fold serial dilution in ELISA buffer = PBST, 10% Superblock (Pierce #37515)) for 1 hour at 25°C with shaking and then washed again 3 times. Plates were incubated with goat anti-human HRP (Pierce #31412) (1:40,000 dilution in ELISA buffer, 100 μl/well) for 1 hour at 25°C with shaking, then washed 3 times. After the final wash, plates were incubated with 100 μl/well ELISA substrate (Sigma #T8665). The reaction was stopped after 8 minutes at 25°C with 100 μl/well 1N HCl and the absorbance was read at 450 nm. Data were analyzed using Graphpad Prism software, and EC50 values were reported.

Example 1.3: Binding of DLL4 Monoclonal Antibodies to the Surface of Human Tumor Cell Lines as Assessed by Flow Cytometry (FACS).

Stable cell lines overexpressing cell-surface DLL4 were harvested from tissue culture flasks, washed four times and resuspended in phosphate buffered saline (PBS) containing 1% bovine serum albumin and 1 mM $CaCl_2$ (FACS buffer). 1.5 $x10^5$ cells were incubated with antibodies at various concentrations in FACS buffer for 60 minutes on ice. Cells were washed twice and 50 μL of R-phycoerythrin-conjugated anti-rat IgG, $F(ab')_2$ fragment (1:200 dilution in FACS buffer) (Jackson ImmunoResearch, West Grove, PA, Cat.#112-116-072) were added. Following an incubation on ice (4°C, 60 minutes), cells were washed three times and resuspended in FACS buffer. Fluorescence was measured using a Becton Dickinson FACSCalibur-HTS (Becton Dickinson, San Jose, California, US). Data were analyzed using Graphpad Prism software and EC_{50} values were reported as the concentration of antibody to achieve 50% of maximal DLL4 antibodies binding to DLL4 expressing cells.

Example 1.4: Inhibition of Notch-1 Interaction with Soluble DLL4 Extracellular Domain by DLL4 Antibodies (competition ELISA).

96-well Nunc-Immuno plates (#439454 for huDLL4 ELISA) and 96-well Costar plates (#9018 for muDLL4 ELISA) were coated with 16 nM human Notch-1 (R&D Systems #3647-TK, 100 μl/well in D-PBS) and incubated overnight at 4°C. Plates were then washed 3 times with wash buffer (PBS, 0.05% Tween-20) and blocked with 200 μl/well blocking buffer (D-PBS, 1% BSA, 1 mM $CaCl_2$, 0.05% Tween-20) for 1 hour at 25°C. While blocking, biotin labeled DLL4 extracellular domain (14 nM) was mixed with antibody (30 pM-66 nM, 3-fold serial dilution in blocking buffer) for 1 hour at 25°C with shaking. Assay plates were washed after blocking, and incubated with DLL4/antibody mixtures (100 μl/well, 1 hour at 25°C with shaking). Plates were washed again and 100 μl/well streptavidin conjugated with HRP (Fitzgerald #65R-S104PHRPx, diluted 1:5,000 in blocking buffer) was added for 1 hour at 25°C with shaking. After a final wash, plates were developed using 100 μl/well substrate (TMB Sigma #T8665), and the reaction was stopped using 100 μl/well 1N HCl, and the absorbance was read at 450 nm. Data were analyzed using Graphpad Prism software and IC_{50} values were reported as the concentration of antibody to achieve 50% reduction of DLL4 bound to Notch1.

Example 1.5: Blocking of Soluble Notch Binding to DLL4-overexpressing 293G Cells by Anti-DLL4 Monoclonal Antibodies as Assessed by Flow Cytometry (competition FACS).

Notch blocking assay: Briefly, stable cell lines overexpressing cell-surface DLL4 were harvested from tissue culture flasks and re-suspended in phosphate buffered saline (PBS) containing 1% bovine serum albumin and 1 mM $CaCl_2$ (FACS buffer). HEK293G-DLL4 cells were dispensed into 96-well plate (v-bottom) at $1.5x10^5$ cells/well in FACS buffer. After spinning down cells and discarding the supernatant, 50 µL of purified IgG with appropriate dilution were added to each well, and incubated on ice at 4°C for 60 minutes, followed by addition of 50 µL/well of Notch1-biotin at 0.2 µg/mL for human DLL4-293G or 2.0 µg/mL for mouse DLL4-293G (1.0 or 0.1 µg/mL final) for an additional 1 hour incubation ice at 4°C. After washing the cells two times with FACS buffer, 50 µL of R-phycoerythrin-conjugated streptavidin (1:150 dilution in FACS buffer) (Jackson ImmunoResearch, West Grove, PA, Cat.#016-110-084) were added. Following an incubation on ice (4°C, 60 minutes), cells were washed three times and resuspended in FACS buffer. Fluorescence was measured using a Becton Dickinson FACSCalibur-HTS (Becton Dickinson, San Jose, CA). Data were analyzed using Graphpad Prism software, and IC_{50} values were reported as the concentration of antibody to achieve 50% reduction of Notch1 bound to DLL4 expressing cells.

Example 1.6: Inhibition of DLL4-Dependent Notch Activation in EA.hy926 Cells by DLL4 Antibodies using Notch Reporter Assay.

96-well black clear-bottom tissue culture plates were seeded overnight with 7,000 cells/well engineered EA.hy926 cells expressing luciferase driven by a Notch-responsive promoter. Antibodies serially diluted from 200 nM were mixed for 15 minutes with equal volumn of 5,000 HEK293G cells/well expressing full-length DLL4. The 293G/DLL4 cells were co-cultured with EA.hy926 Notch reporter cells for 24 hours in the presence of testing antibodies. Luciferase activity was analyzed by Promega's substrate (Promega # E2940). Data was analyzed using Graphpad Prism software, and IC_{50} values were reported as the concentration of antibody to achieve 50% reduction of DLL4-induced Notch activation.

Example 1.7: Analytical Methods and Techniques for Physicochemical property characterizations.

PEG Precipitation Method.

The use of PEG for inducing phase separation of a solid protein according to principles of volume exclusion represents a feasible approach to assess the solubility of a protein. PEG has several advantages over other precipitants, including minimal denaturation of proteins at ambient temperatures (does not affect tertiary structure of proteins) and within the range of 4°C to 30°C temperature control is not required, i.e., precipitation studies can be performed at ambient temperature at the laboratory bench.

Generally, the precipitation of proteins by PEGs is explained on the basis of volume exclusion effects. According to this theory, proteins are sterically excluded from the regions of solvent that are occupied by PEG linear chains. As a result, proteins are concentrated and eventually precipitated when their solubility is exceeded. In thermodynamic terms, the steric exclusion leads to an increase in the chemical potential of the protein until it exceeds that of the pure solid state, resulting in protein precipitation. This happens mainly because of a large unfavorable free energy of interaction between PEG and proteins, reducing the preferential hydration of protein due to steric exclusion effects. In aqueous solutions, preferential hydration helps to maintain the native structure of proteins. Generally, volume exclusion has been shown to become more effective with increasing molecular weight of the PEG, i.e., less PEG is needed to precipitate proteins with increasing PEG molecular weight.

A PEG molecular weight of 3000 was chosen for estimating the solubility of the antibodies covered by this patent. A 50% PEG solution was made by dissolving PEG in deionized water in the ratio of one gram of PEG to 1 mL of water. The PEG solution was then added to a solution of antibody which was initially at a concentration of less than or equal to 0.5 mg/ml and a volume of 0.5 mL. The PEG solution was continually added and mixed until the first instance of cloudiness persists. The percentage of PEG 3000 needed to cause this precipitation is calculated as (50) x (volume of PEG 3000 solution added)/(initial volume of antibody solution before PEG addition).

The percentage of PEG 3000 needed for precipitation was compared to the percentage needed for precipitation of protein with known water solubility. For example, the water solubility of adalimumab exceeds 200 mg/mL. Consequently, if the percentage of PEG 3000 required to precipitate a protein of interest is similar to the percentage needed to precipitate adalimumab, then the predicted solubility of that protein will be similar to the solubility adalimumab.

Real Solubility Method.

Real solubility is determined by using Amicon centrifugal filters to concentrate a protein in solution until the protein is observed to precipitate out of solution or until the minimum volume to which the protein can be concentrated within the filter unit is reached. For the latter, 15 mL Amicon centrifugal filters have a minimum volume of ~50 µl while 4 mL Amicon centrifugal filters have a minimum volume of ~15 µl.

First, a protein was dialyzed into a specific formulation(s). For these studies, the antibody amount was 10 mg or much less. Then the protein solution was inserted into the Amicon centrifugal filter retentate chamber. The chamber was lined with a nitrocellulose membrane with pores that permitted molecules of less than 10 to 30 kilodaltons to pass when subjected to centrifugal force. Antibodies, which were typically above 140 kilodaltons, were retained while water, buffer molecules, small excipients, and salts passed through. The centrifugal filter was then centrifuged according to manufacturer specifications until the protein was observed to precipitate out of solution or until the minimum volume to which the protein can be concentrated within the filter unit was reached.

After centrifugation, the protein solution was removed from the retentate chamber, and the concentration was measured by ultraviolet absorbance. The solution was then kept at 25°C and 5°C for 1 to 2 days and was monitored for signs of precipitation.

Near UV-CD Technique.

Near UV-CD spectroscopy provides important information about the tertiary structure of proteins and was one of the most used techniques in this regards. CD refers to the differential absorption of the left and right circularly polarized components of plane polarized radiation. For proteins, the chromophores in the near UVCD region (250 – 320 nm) are the aromatic amino acids (i.e., tryptophan, tyrosine, and phenylalanine) and the disulfide bonds, and the CD effect occurs when the chromophores are present in an asymmetric (buried) environment. Signals in the region from 250-270 nm are attributable to phenylalanine residues, signals from 270-290 nm are attributable to tyrosine, and those from 280-300 nm are attributable to tryptophan. Disulfide bonds give rise to broad weak signals throughout the near-UV spectrum. The near-UV CD spectrum can be sensitive to small changes in tertiary structure such as those due to protein-protein interactions and/or changes in formulation conditions.

There are a number of other factors that can influence the CD spectra of aromatic amino acids. Among these are (1) the rigidity of the protein, (2) the nature of hydrogen bonding, and (3) interactions between various aromatic amino acids. Additionally, proteins with large number of such amino acids can have smaller CD bands due to the cancellation of the positive and negative bands.

Briefly, a protein dialyzed into the desired formulation(s) at 1 mg/ml and was scanned from 250-320 nm or 240-320 nm with a Jasco 800 CD spectrometer. The corresponding formulation without protein was also scanned, and the readings subtracted from that of the scan of the protein solution. A near UV-CD spectra was a plot of molar ellipticities versus wavelength from 250 or 240 to 320 nm.

For antibodies in general, a near UV-CD spectrum with a semi-sigmoidal profile indicates good tertiary structure folding while a flatter and less featured profile indicates a greater tendency to unfold. Compact folding is associated with good stability while poor folding exposes the hydrophobic interior which may lead to hydrophobic interactions among protein molecules resulting in the formation of undesired aggregates.

DSC Technique.

The thermal stability of the antibodies was assessed using a differential scanning calorimetry (DSC) instrument. The DSC instrument used was an automated VP-DSC equipment with Capillary Cell (Microcal, GE Healthcare Ltd./Microcal, Buckinghamshire, UK). Unfolding of molecules was studied applying a 1°C/minute scan rate over a 25°C - 95°C temperature range for samples at 1 mg/mL. Additional measurement parameters applied were a fitting period of 16 seconds, a pre-scan wait time of 10 minutes, and measurements were performed in nonefeedback mode. Per individual measurement, 420 μL of sample/blank were filled into the DSC measurement sample holder, with a plate fill scheme as provided below. The thermograms obtained were fitted to a non two state model to obtain the midpoint temperatures and enthalpies of the different transitions.

An additional requirement for successful biologics development candidate is that the protein remains in its native state and conformation. A protein in aqueous solution is in equilibrium between the native (folded) conformation and its denatured (unfolded) conformation. The stability of the native state is based on the magnitude of the Gibbs free energy (DG) of the system and the thermodynamic relationship between enthalpy (DH) and entropy (DS) changes. A positive DG indicates the native state is more stable than the denatured state – the more positive the DG, the greater the stability. For a protein to unfold, stabilizing forces need to be broken. Conformational entropy overcomes stabilizing forces allowing the protein to unfold at temperatures where entropy becomes dominant. DSC measures DH of protein unfolding due to heat denaturation. As a general rule, it can be stated that the higher the transition midpoint (the Tm), the more stable the protein at lower temperatures. During the same experiment, DSC also measures the change in heat capacity (DCp) for protein denaturation. Heat capacity changes associated with protein unfolding are primarily due to changes in hydration of side chains that were buries in the native state, but become solvent exposed in the denatured state. DSC has been shown to be a valuable predictor of liquid formulation stability for proteins and other biological macromolecules (Remmele, R.L. Jr., Gombotz, W.R., BioPharm 13, 36-46, 2000, and; Remmele, R.L. Jr., Nightlinger, N.S., Srinivasen, S., Gombotz, W.R., Pharm. Res. 15, 200-208, 1998).

SEC Technique.

Size exclusion chromatography (SEC) was used to separate proteins based on size. Proteins are carried in an aqueous mobile phase and through a porous stationary phase resin packed in a column. The retention time in the column is a function of the hydrodynamic size of the protein and the size of the pores in the packed resin bed. Smaller molecules can penetrate into smaller pores in the resin and are retained longer than larger molecules. Upon elution from the column, the proteins are detected by UV absorbance. The SEC method used a TSK gel guard (TOSOH Biosciences, Montgomeryville, PA, cat. no. 08543) and a TSK gel G3000SWxL (TOSOH Biosciences, Montgomeryville, PA, cat. no. 08541). The mobile phase was 100 mM Na_2HPO_4, 200 mM Na_2SO_4, pH 6.8. The flow rate was 0.25 mL/minute. Injection volume was 20 μL of 1 mg/mL sample. The column temperature was room temperature. The autosampler temperature was 2-8°C. The total run time was 55 minutes. The detection was based on UV absorbance at 214 nm wavelength, with band width set at 8 nm, using reference wavelength at 360 nm with band width 100 nm.

Freeze-Thaw Method.

Antibody solutions at 1 mg/ml in the desired formulation(s) were frozen at -80°C for at least 4 hours and then thawed at 30°C in a water bath. The solutions were then refrozen at -80°C. This was repeated for 5 cycles. After certain freeze-thaw cycles, e.g., second and fourth, a portion of the solution was withdrawn for analysis by SEC before refreezing. Freeze-thaw stability testing was done at low protein concentration in order obtain a "worse-case scenario" due to greater exposure of protein molecules to the denaturing ice-water interfaces. At higher concentrations, proportionally less protein encounters the ice-water interface, instead interacting with other protein molecules.

Accelerated Stability Method.

Antibody solutions at 1 mg/ml in the desired formulation(s) were passed through 0.22 μm PVDF filters under sterile conditions and incubated at 40°C and/or 50°C for at least 21 days. At 7 days and 21 days, aliquots were withdrawn under sterile conditions and subjected to analysis by SEC. Solutions were then returned to incubation.

Example 2: Generation of Rat Anti-DLL4 Monoclonal Antibodies by Rat Hybridoma Technology.

Rats were immunized according to the methods known in the art (for example, E Harlow, D. Lane, Antibody: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998). A human cell line expressing full-length human DLL4 as well as recombinant mouse DLL4-ECD (DLL4 extracellular domain) proteins was used as immunogen. Mouse cell lines expressing either human DLL4 or mouse DLL4 were used for determining anti-sera titer and for screening hybridomas secreting antigen-specific antibodies. Immunizing dosages contained 1 x 10^6 cells/rat/injection for both primary and boost immunizations. To increase immune response to mouse DLL4, the rats were further boosted with recombinant mouse DLL4-ECD in emulsion form with an incomplete Freud's adjuvant (Sigma, St. Louis, MO, US). Briefly, adjuvant-antigen mixture was prepared by first gently mixing the adjuvant in a vial using a vortex. The desired amount of adjuvant was removed from the vial and put into an autoclaved 1.5 mL microcentrifuge tube. The antigen was prepared in PBS or saline with concentration ranging from 0.5-1.0 mg/ml. The calculated amount of antigen was then added to the microcentrifuge tube with the adjuvant, and the solution was mixed by gently vortexing for 2 minutes to generate water-in-oil emulsion. The adjuvant-antigen solution was then drawn into the proper syringe for animal injection. A total of 50 μg of antigen was injected in a volume of 50-100 μl. Each animal was immunized, and then boosted for 2 to 3 times depending on the titer. Animals with good titers were given a final subcutaneous boost with cell line expressing human DLL4 before fusion.

Hybridoma fusion and screening.

Cells of murine myeloma cell line (SP2/0-Ag14, ATCC CRL-1581) were cultured to reach the log phase stage right before fusion. Immunized rat spleen cells were prepared sterilely and fused with myeloma cells according to the methods known in the art (for example, E Harlow, D. Lane, Antibody: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1998); Kohler G, and Milstein C., "Continuous cultures of fused cell secreting antibody of predefined specificity," *Nature*, 256: 495-497 (1975)). Fused "hybrid cells" were subsequently dispensed into 96-well plates in DMEM/20%FCS/HAT media. Surviving hybridoma colonies were observed under the microscope seven to ten days post-fusion. After two weeks, the supernatant from each well was subjected to cell-based binding screening using mouse cell lines expressing either recombinant human DLL4 or mouse DLL4. Briefly, the mixture (1:1 ratio) of mouse cell lines expressing either human or mouse DLL4 was dispensed into a 96-well (round bottom) plate at 1 x 10^6 cells/well and incubated with hybridoma supernatant (50 μl) at 4°C for 1 hour. Cells were then washed 3 times with FACS buffer (PBS+2% BSA), HRP-goat anti-rat Ig-PE (phycoerythrin) was used for detection in FACS machine. Hybridomas were screened using ELISA format according the following procedure. ELISA plates were coated with 50 μl of either human DLL4 or mouse DLL4 (2.0 μg/ml in PBS) overnight at 4°C. Plates were washed 3 times with 250 μl PBS/0.5%Tween$_{20}$ and blocked with 200 μl blocking buffer (2% BSA in PBS with 0.5%Tween$_{20}$). Diluted sera or hybridoma supernatant (100 μl) was added to each well, and incubated at room temperature for 1 hour. Plates were then washed 3 times with PBS/0.5%Tween$_{20}$, HRP-goat anti-rat-IgG was used for detection, and binding ODs were observed at 450 nm. Positive hybridoma secreting antibody that binds to either human DLL4 or mouse DLL4 or both were then selected and transferred to 24-well plates and subcloned by limiting dilution to ensure the clonality of the cell line. The isotype of each monoclonal antibody was determined using the Zymed's Mouse MonoAb-ID Kit. Hybridoma clones producing antibodies that showed high specific binding activity were subcloned and purified (Table 5), and affinity (Biacore) and potency (Notch blocking FACS and reporter assay) of the antibodies were characterized as follows.

Table 5. A List of Anti-DLL4 Antibodies Generated Using Rat Hybridoma Technology

Hybridoma	**Isotype**	**FACS binding**	
		Human DLL4	**Mouse DLL4**
MC10-37D10.1C2	IgG2a/k	+	+
MC10-40B10.3C3	IgG2a/k	+	+
MC10-32C7.5A4	IgG2a/k	+	+
MC10-38H12.2G8	IgG2a/k	+	−

MC13-14A11.3A4	IgG2a/K	+	+
MC13-14G1.1B4	IgG2a/K	+	–
MC13-1A11.2E1	IgG1/K	+	+
MC13-13E4.4A3	IgG1/K	+	+
MC13-15D6.1G7	IgG2a/K	+	+

"+" indicates antibody bound to cells; "–" indicates antibody did not bind to cells

Example 3. *In Vitro* Characterization of Anti-DLL4 Rat Monoclonal Antibodies.

The antigen binding affinities of these rat monoclonal antibodies (mAbs) were determined by the BIACORE technology as described in Example 1.1, and are shown in Tables 6 and 7, below. Their *in vitro* activities were further examined using other methods described in Example 1, and are summarized in Table 8. Further characterization determined 37D10 and 40B10 were identical rat mAbs.

Table 6. Biacore Kinetics of Anti-DLL4 Rat Hybridoma Antibodies Binding to Human and Cynomolgus Monkey DLL4.

Clone	**Kinetics on Biacore**					
	huDLL4 ECD			**cynoDLL4 ECD**		
	ka ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)	*ka* ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)
38H12	$4.0 \times 10^{+5}$	1.1×10^{-4}	0.3	$2.6 \times 10^{+5}$	9.8×10^{-5}	0.4
1A11	$1.9 \times 10^{+5}$	1.1×10^{-3}	5.8	$1.1 \times 10^{+5}$	9.3×10^{-4}	8.1
37D10 = 40B10	$1.4 \times 10^{+5}$	7.0×10^{-2}	484	$4.0 \times 10^{+4}$	Too fast*	<617
32C7	$2.8 \times 10^{+5}$	7.0×10^{-6}	0.03	$2.2 \times 10^{+5}$	6.3×10^{-6}	0.03
15D6	$1.1 \times 10^{+5}$	1.0×10^{-3}	9.2	$5.6 \times 10^{+4}$	8.3×10^{-4}	14.9
14A11	$1.2 \times 10^{+5}$	1.2×10^{-3}	10.3	$1.1 \times 10^{+5}$	1.2×10^{-3}	11.6
14G1	$5.4 \times 10^{+4}$	3.9×10^{-4}	7.2	$3.9 \times 10^{+4}$	4.1×10^{-4}	10.5
13E4	$2.3 \times 10^{+5}$	3.9×10^{-4}	1.7	$1.5 \times 10^{+5}$	4.2×10^{-4}	2.8

hu = human; cyno = cynomolgus monkey; * = falls out of measurement range

Table 7. Biacore Kinetics of Anti-DLL4 Rat Hybridoma Antibodies Binding Murine and Rat DLL4.

	Kinetics on Biacore

Clone	**muDLL4 ECD**			**ratDLL4 ECD**		
	ka ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)	*ka* ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)
38H12	N/B	N/B	N/B	N/B	N/B	N/B
1A11	$1.3 \times 10^{+5}$	2.9×10^{-3}	23	N/B	N/B	N/B
37D10 = 40B10	$1.0 \times 10^{+5}$	1.35×10^{-2}	135	N/D	N/D	N/D
32C7	$4.8 \times 10^{+4}$	4.1×10^{-3}	88	N/D	N/D	N/D
15D6	$7.4 \times 10^{+4}$	5.1×10^{-5}	0.7	N/B	N/B	N/B
14A11	7.0×10^{-4}	9.0×10^{-5}	1.3	N/B	N/B	N/B
14G1	N/B	N/B	N/B	N/B	N/B	N/B
13E4	$1.8 \times 10^{+5}$	1.9×10^{-4}	1.1	N/B	N/B	N/B

N/B = no significant binding; N/D = not determined, mu = mouse

Table 8. ***In Vitro*** **Characterization of Rat Hybridoma Derived Anti-DLL4 Antibodies.**

	Direct Binding Assays				**Functional Blockade Assays**				
Rat mAb	Binding ELISA (EC_{50}, nM)		FACS (EC_{50}, nM)		Competition ELISA (IC_{50}, nM)		Competition FACS (IC_{50}, nM)		Notch activation Inhibition (IC_{50}, nM)
	DLL4 ECD		DLL4 Cells		DLL4 ECD/ huNotch-1		huNotch-1/ DLL4 cells		huDLL4 cell/ Notch reporter cells
	hu	mu	hu	mu	hu	mu	hu	mu	
1A11	0.06	0.11	34.8	0.8	0.78	0.79	8.4	0.8	2.9
38H12	0.06	–	1.2	–	3.63	–	1.7	–	0.5
37D10 = 40B10	0.12	0.14	2.3	0.7	5.39	3.36	5.5	3.8	–
32C7	0.08	0.15	3.1	0.8	–	–	–	–	Agonist
14G1	N/D	N/D	8.6	>50	N/D	N/D	4.2	–	6.1
14A11	N/D	N/D	>50	2.1	–	19.8	205	1.6	2.7
15D6	N/D	N/D	15	1.6	–	–	25	1.5	3.2
13E4	N/D	N/D	26	1.3	–	–	5.2	> 200	–

"–" = no activity or under detection limit of the assays used; "N/D" = not determined;

hu = human; cyno = cynomolgus monkey; mu = mouse.

Example 4. Deduction of Variable Region Protein Sequences of Anti-DLL4 Rat Monoclonal Antibodies by DNA Cloning and Sequencing.

Total RNA was extracted from hybridoma cell pellets using RNeasy mini kit (Qiagen, catalog # 74104) using the following protocol. 600 µl of buffer RLT were added to disrupt cells by pipetting up and down several times. The cell lysate was homogenized by passing it 10 times through a 20-gauge needle fitted to an RNase-free syringe. One volume of 70% ethanol was added to the homogenized lysate and mixed well by pipetting. Up to 700 µl at a time of the sample were added to an RNeasy spin column and spun for 15 seconds at 10,000 rpm, discarding flow through. 700 µl of buffer RW1 were added to the column and spun for 15 seconds at 10,000 rpm, discarding flow through. 500 µl of buffer RPE were added to wash the column membrane and spun for 15 seconds at 10,000 rpm, discarding flow through. The same step was repeated one more time, but the column was centrifuged for 2 minutes. Sample was then centrifuged for 1 minute at 10,000 rpm to eliminate any carryover of buffer RPE. RNA was eluted with 30 µl of RNase-free water by centrifuging for 1 minute at 10,000 rpm. Subsequently, 2 µg of total RNA were used to synthesize first-strand cDNA using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen, catalog # 11904-018) according to following protocol: 2 µg of RNA + 2 µl dNTP + 2 µl Oligo (dT) + DEPC-H_2O (to 20 µl) were incubated at 65°C for 5 minutes, then transferred to ice for at least 1 minute. The sample was then added to the following mixture: 4 µl of 10X RT buffer + 8 µl 25 mM $MgCl_2$ + 4 µl 0.1 M DTT + 2 µl RNase OUT and incubated at 42°C for 2 minutes. Then, 2 µl of SuperScript II RT were added to the sample and incubated at 42°C for 50 minutes. Sample was then incubated at 70°C for 15 minutes and chilled on ice. 2 µl of RNase H were then added and the sample was incubated at 37°C for 20 minutes. cDNA was then used as template for PCR amplification of variable regions of antibodies. PCR was performed using first-strand cDNA, primers from Mouse Ig-Primer Set (Novagen, catalog # 69831-3) and Platinum Super Mix High Fidelity (Invitrogen, catalog # 12532-016). To amplify heavy chain variable regions, PCR samples were assembled as follows: 22.5 µl PCR Super Mix + 0.25 µl reverse primer MuIgG V_H3'-2 + 1 µl cDNA + 1.25 µl of one the forward primers (VH-A, VH-B) or 0.5 µl of one of the forward primers (VH-C, VH-D, VH-E, VH-F). To amplify light chain variable regions PCR samples were assembled as follows: 22.5 µl PCR Super Mix + 0.25 µl reverse primer MuIgKV$_L$-3'-1 + 1 µl cDNA + 1.25 µl of one the forward primers (VL-A, VL-B) or 0.5 µl of one of the forward primers (VL-C, VL-D, VL-E, VL-F, VL-G).

For samples with primers VH-A, VH-B, VL-A and VL-B, the following PCR cycles were used (40-45 cycles, steps 2 through 4):

1-Denature 94°C 2 min.

2-Denature 94°C 30 sec.

3-Anneal 50°C 30 sec.

4-Extend 68°C 1 min.

5-Final extension 68°C 5 min.

6-Cool 4°C forever

For samples with primers VH-C through VH-F, and VL-C through VL-G, the following PCR cycles were used (40-45 cycles, steps 2 through 4):

1-Denature 94°C 2 min.

2-Denature 94°^{0}C 30 sec.

3-Anneal 60°C 30 sec.

4-Extend 68°C 1 min.

5-Final extension 68°C 5 min.

6-Cool 4°C forever

PCR products were run on 1.2% agarose gel, and bands migrating at the expected size (400-500 bp) were excised for DNA extraction. DNA was purified using QIAquick Gel Extraction Kit (Qiagen, catalog # 28704) according to the following protocol: gel slices were weighed. 3 volumes of buffer QG to 1 volume of gel were added to each gel slice. Samples were incubated at 50°C for 10 minutes until gel slices were completely dissolved, mixing every 2-3 minutes. One gel volume of isopropanol was then added to each sample and mixed. Samples were then applied to QIAquick column and centrifuged for 1 minute at 13000 rpm. To wash, 750 µl of buffer PE were added to samples and spun for 1 minute at 13000 rpm. Columns were then centrifuged for an additional minute at 13,000 rpm to completely remove residual ethanol. DNA was eluted by adding 30 µl of H_2O to each column and by spinning 1 minute at 13,000 rpm. Purified PCR products were then sequenced to identify variable region sequences (Table 9, below).

Table 9. VH and VL Amino Acid Sequences of Rat Anti-DLL4 Monoclonal Antibodies.

SEQ ID NO:	**Protein region**		**Sequence**
			123456789**0**123456789**0**123456789**0**
157	**VH 38H12**		EVQLVESGGGLVQPGRSLKLSCAASGFTFS **NYGMY**WIRQAPTKGLQWVA**FISHGGGITYY** **RDSVKG**RFTISRDNAKSTLYLQMDSLRSED TATYHCAA**LNWELGIDY**WGQGVMVTVSS
	VH 38H12 CDR-H1	Residues 31-35 of SEQ ID NO:157	**NYGMY**
	VH 38H12 CDR-H2	Residues 50-66 of SEQ ID NO:**157**	**FISHGGGITYYRDSVKG**

SEQ ID NO:	**Protein region**		**Sequence**
			123456789**0**123456789**0**123456789**0**
	VH 38H12 CDR-H3	Residues 99-107 of SEQ ID NO:**157**	**LNWELGIDY**
			123456789**0**123456789**0**123456789**0**
158	**VL 38H12**		DIQMTQSPASLSASLGETISIEC**RASEDIYSNLA**WYQKKSGKSPQLLIY**AANRLQD**GVPSRFSGSGSGTQYSLKISGMQPEDEGDYFC**LQGSKFPLT**FGSGTKLEIKR
	VL 38H12 CDR-L1	Residues 24-34 of SEQ ID NO:**158**	**RASEDIYSNLA**
	VL 38H12 CDR-L2	Residues 50-56 of SEQ ID NO:**158**	**AANRLQD**
	VL 38H12 CDR-L3	Residues 89-97 of SEQ ID NO:**158**	**LQGSKFPLT**
			123456789**0**123456789**0**123456789**0**
159	**VH 1A11**		EVQLVESGGGLVQPGRSMKLSCAASGFTFR**NFPMA**WVRQAPTRGLEWVA**TISSSDGTTYYRDSVKG**RFTISRDNAKSTLYLQVNSLRSEDTATYYCSR**GYYNSPFAY**WGQGTLVTVSS
	VH 1A11 CDR-H1	Residues 31-35 of SEQ ID NO:**159**	**NFPMA**
	VH 1A11 CDR-H2	Residues 50-66 of SEQ ID NO:**159**	**TISSSDGTTYYRDSVKG**
	VH 1A11 CDR-H3	Residues 99-107 of SEQ ID NO:**159**	**GYYNSPFAY**
			123456789**0**123456789**0**123456789**0**
160	**VL 1A11**		DIQMTQSPASLSASLGETVTIEC**RASEDIYSNLA**WYQQKPGNSPQLLIF**DTNNLAD**GVPSRFSGSGSGTQSSLKINSLQSEDVASYFC**QQYNNYPPT**FGGGTKLELKR
	VL 1A11 CDR-L1	Residues 24-34 of SEQ ID NO:**160**	**RASEDIYSNLA**
	VL 1A11 CDR-L2	Residues 50-56 of SEQ ID NO:**160**	**DTNNLAD**
	VL 1A11 CDR-L3	Residues 89-97 of SEQ ID NO:**160**	**QQYNNYPPT**
			123456789**0**123456789**0**123456789**0**
161	**VH 37D10**		AVQLVESGGGLVQPKESLKISCAASGFTFS**NAAMY**WVRLAPGKGLEWVA**RIRTKPNNYATYYAESVKG**RFTISRDDSKSMVYVQMDNLKTEDTAMYYCTA**APWRDSYAHVY**WGQGVMVTVSS
	VH 37D10 CDR-H1	Residues 31-35 of SEQ ID NO:**161**	**NAAMY**

SEQ ID NO:	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
	VH 37D10 CDR-H2	Residues 50-68 of SEQ ID NO:**161**	**RIRTKPNNYATYYAESVKG**
	VH 37D10 CDR-H3	Residues 101-111 of SEQ ID NO:**161**	**APWRDSYAHVY**
			123456789**0**123456789**0**123456789**0**
162	**VL 37D10**		DIQMTQSPPVLSASVGDRVTLSC**KASQNIHKNLD**WYQQKHGDAPKLLIY**YTDHLQT**GVPSRFSGSGSATDYTLTISSLQPEDVATYYC**YQYNGGPFT**FGSGTKLEIKR
	VL 37D10 CDR-L1	Residues 24-34 of SEQ ID NO:**162**	**KASQNIHKNLD**
	VL 37D10 CDR-L2	Residues 50-56 of SEQ ID NO:**162**	**YTDHLQT**
	VL 37D10 CDR-L3	Residues 89-97 of SEQ ID NO:**162**	**YQYNGGPFT**
			123456789**0**123456789**0**123456789**0**
163	**VH 32C7**		EVQLVESGGGLVQPGRSLKLSCLASGFPFS**SVWMT**WIRQAPGKGLEWIA**TITNSGASTYYSASVKG**RFTISRDNVKSTLYLQMTSLGSEDTATYYCTR**VGTSFDY**WGQGVMVTVSS
	VH 32C7 CDR-H1	Residues 31-35 of SEQ ID NO:163	**SVWMT**
	VH 32C7 CDR-H2	Residues 50-66 of SEQ ID NO:**163**	**TITNSGASTYYSASVKG**
	VH 32C7 CDR-H3	Residues 99-105 of SEQ ID NO:**163**	**VGTSFDY**
			123456789**0**123456789**0**123456789**0**
164	**VL 32C7**		DIQMTQSPASLSASLGETVTIEC**RASDDIYNGLA**WFQQKPGKSPQLLIY**DANTLHT**GVPSRFSGSGSGTQFSLKINSLQSEDVASYFC**QQFYDYPPYT**FGAGTKLELKR
	VL 32C7 CDR-L1	Residues 24-34 of SEQ ID NO:**164**	**RASDDIYNGLA**
	VL 32C7 CDR-L2	Residues 50-56 of SEQ ID NO:**164**	**DANTLHT**
	VL 32C7 CDR-L3	Residues 89-98 of SEQ ID NO:**164**	**QQFYDYPPYT**
			123456789**0**123456789**0**123456789**0**

SEQ ID NO:	**Protein region**		**Sequence**
			123456789**0**123456789**0**123456789**0**
165	**VH 14G1**		EVQLQQSGAELAKPGSSVKISCKASGYTFT**NYDIS**WIKQTNGQGLEYLG**YINTGSGGIYSNEKFKG**KATLTVDKSSNTAFMQLSSLTPEDTAVYYCVR**EGNNFDH**WGQGVKVTVSS
	VH 14G1 CDR-H1	Residues 31-35 of SEQ ID NO:**165**	**NYDIS**
	VH 14G1 CDR-H2	Residues 50-66 of SEQ ID NO:**165**	**YINTGSGGIYSNEKFKG**
	VH 14G1 CDR-H3	Residues 99-105 of SEQ ID NO:**165**	**EGNNFDH**
			123456789**0**123456789**0**123456789**0**
166	**VL 14G1**		DTVMTQSPASMSTSVGERVTVNC**KASQSVGTIVA**WFQQKPGQSPKRLIY**LATYRHT**GVPDRFIGSGFGRDFTLTISNVEAEDLAVYYC**LQYGSRPFT**FGAGTKLEIKR
	VL 14G1 CDR-L1	Residues 24-34 of SEQ ID NO:**166**	**KASQSVGTIVA**
	VL 14G1 CDR-L2	Residues 50-56 of SEQ ID NO:**166**	**LATYRHT**
	VL 14G1 CDR-L3	Residues 89-97 of SEQ ID NO:**166**	**LQYGSRPFT**
			123456789**0**123456789**0**123456789**0**
167	**VH 14A11**		EVQLQQSGPELAKPGSSVKISCKASGYTFT**NSYIS**WIKQTTGQGLEYVG**YINTGSGGADYNEKFKG**KATLTVDKSSRTAFMQLSSLTPGDSAVYYCAK**SILLGSTCYFDY**WGQGVLVTVSS
	VH 14A11 CDR-H1	Residues 31-35 of SEQ ID NO:**167**	**NSYIS**
	VH 14A11 CDR-H2	Residues 50-66 of SEQ ID NO:**167**	**YINTGSGGADYNEKFKG**
	VH 14A11 CDR-H3	Residues 99-110 of SEQ ID NO:**167**	**SILLGSTCYFDY**
			123456789**0**123456789**0**123456789**0**
168	**VL 14A11**		NTVLTQSPALAVSLGQRVTISC**KASRSVSSPMYSYIY**WYQQKPGQQPKLLIY**RASTLAS**GVPARFSGSGSGTDFTLNIDPVEADDIATYFC**QQSWSDPFT**FGSGTKLEIKR
	VL 14A11 CDR-L1	Residues 23-37 of SEQ ID NO:168	**KASRSVSSPMYSYIY**

SEQ ID NO:	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
	VL 14A11 CDR-L2	Residues 53-59 of SEQ ID NO:**168**	**RASTLAS**
	VL 14A11 CDR-L3	Residues 92-100 of SEQ ID NO:**168**	**QQSWSDPFT**
			123456789**0**123456789**0**123456789**0**
169	**VH 15D6**		EVQLQQSGPELAKPGSSVKISCKASGYTFT **SSYIS**WIKQTTGQGLEYIG**YINTGSGGTDY** **NEKFKD**KATLTVDKSSRTVFMQLSSLTPGD SAVYYCAK**SILLGSTYYLDY**WGQGVMVTVS S
	VH 15D6 CDR-H1	Residues 31-35 of SEQ ID NO:169	**SSYIS**
	VH 15D6 CDR-H2	Residues 50-66 of SEQ ID NO:**169**	**YINTGSGGTDYNEKFKD**
	VH 15D6 CDR-H3	Residues 99-110 of SEQ ID NO:**169**	**SILLGSTYYLDY**
			123456789**0**123456789**0**123456789**0**
170	**VL 15D6**		DTVLTQSPALAVSLGQRVTISC**KASRSLSS** **PMYSYIY**WYQQKLGQQPRLLIY**RASTLAS**G VPARFSGSGSGTDFTLNIDPVEADDIATYF C**QQSWSDPFT**FGSGTKLEIKR
	VL 15D6 CDR-L1	Residues 23-37 of SEQ ID NO:**170**	**KASRSLSSPMYSYIY**
	VL 15D6 CDR-L2	Residues 53-59 of SEQ ID NO:**170**	**RASTLAS**
	VL 15D6 CDR-L3	Residues 92-100 of SEQ ID NO:**170**	**QQSWSDPFT**

Example 5. Generation of Chimeric Antibodies.

The variable domains of the heavy and light chain of the anti-DLL4 rat mAbs (Table 9, above) were cloned in-frame to mutant human IgG1 (L234, 235A) heavy-chain and kappa light-chain constant regions, respectively. The activities of the resulting chimeric antibodies were confirmed in FACS-based binding and competition assays (Table 10, below), and were comparable to their parental rat mAbs.

Table 10. FACS-Based Binding and Neutralizing Activity of Recombinant Chimeric Antibodies Containing the Variable Domains of the Anti-DLL4 Rat mAbs.

	FACS binding (EC_{50} nM)	Competition FACS (IC_{50} nM)
	DLL4 cells	huNotch-1/DLL4 cells

Chimera of	Human DLL4	Mouse DLL4	Human DLL4	Mouse DLL4
1A11	19.59	0.74	2.338	0.682
38H12	1.468	N/D	1.443	N/D
32C7	3.706	4.114	ND	ND
37D10	2.32	0.99	5.951	4.395
14G1	0.994	N/D	4.11	N/D
14A11	1.613	2.139	4.025	1.391
15D6	1.715	1.817	10.48	1.49

N/D = not determined.

Example 6. Humanization of Anti-DLL4 Rat Monoclonal Antibody 1A11.

1A11 rat anti-DLL4 antibody (Table 9, above) was humanized. Humanized variant amino acid sequences VH.1, VH.1a, VH.1b, VH.2a, VL.1, VL.1a, VL.1b, and VL.2a (Table 11, below) were converted to DNA sequence based on the most homologous human germlines and synthesized. For the heavy chain variants, human germline heavy chain acceptor sequences VH3-7 FR1, VH3-7 FR2, VH3-7 FR 3, and JH4 FR4 were used (see, Table 3, above). For light chain variants VL.1, VL.1a, and VL.1b, human germline light chain acceptor sequences O2 FR1, O2 FR2, O2 FR3, and JK2 FR4 were used (see, Table 3, above). For light chain variant VL.2a, human germline light chain acceptor sequences L2 FR1, L2 FR2, L2 FR3, and JK2 FR4 were used (see, Table 4, above). Individual constructs were sequence verified to check for accuracy. Positive variants were then inoculated into 250 mls Luria broth plus ampicillin and cultured overnight at 37°C. DNA was extracted from variant cultures using the Qiagen Hi speed maxi prep kit (12662).

Table 11. VH and VL Amino Acid Sequences of Humanized Versions of Rat Anti-DLL4 Monoclonal Antibody 1A11.

SEQ ID NO:	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
171	**VH.1 1A11**		EVQLVESGGGLVQPGGSLRLSCAASGFTFS **NFPMA**WVRQAPGKGLEWVA**TISSSDGTTYY** **RDSVKG**RFTISRDNAKNSLYLQMNSLRAED TAVYYCAR**GYYNSPFAY**WGQGTLVTVSS
	VH.1 1A11 CDR-H1	Residues 31-35 of SEQ ID NO:171	**NFPMA**
	VH.1 1A11 CDR-H2	Residues 50-66 of SEQ ID NO:171	**TISSSDGTTYYRDSVKG**
	VH.1 1A11 CDR-H3	Residues 99-107 of SEQ ID NO:171	**GYYNSPFAY**
			123456789**0**123456789**0**123456789**0**

SEQ ID NO:	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
172	**VH.1a 1A11**		EVQLVESGGGLVQPGGSLRLSCAASGFTFS **NFPMA**WVRQAPGKGLEWVA**TISSSDGTTYY** **RDSVKG**RFTISRDNAKSSLYLQMNSLRAED TAVYYCSR**GYYNSPFAY**WGQGTLVTVSS
	VH.1a 1A11 CDR-H1	Residues 31-35 of SEQ ID NO:172	**NFPMA**
	VH.1a 1A11 CDR-H2	Residues 50-66 of SEQ ID NO:172	**TISSSDGTTYYRDSVKG**
	VH.1a 1A11 CDR-H3	Residues 99-107 of SEQ ID NO:172	**GYYNSPFAY**
173	**VH.1b 1A11**		EVQLVESGGGLVQPGGSLRLSCAASGFTFS **NFPMA**WVRQAPGKGLEWVA**TISSSDGTTYY** **RDSVKG**RFTISRDNAKNSLYLQMNSLRAED TAVYYCSR**GYYNSPFAY**WGQGTLVTVSS
	VH.1b 1A11 CDR-H1	Residues 31-35 of SEQ ID NO:173	**NFPMA**
	VH.1b 1A11 CDR-H2	Residues 50-66 of SEQ ID NO:173	**TISSSDGTTYYRDSVKG**
	VH.1b 1A11 CDR-H3	Residues 99-107 of SEQ ID NO:173	**GYYNSPFAY**
			123456789**0**123456789**0**123456789**0**
174	**VH.2a 1A11**		EVQLVESGGGLVQPGGSLRLSCAASGFTFS **NFPMA**WVRQAPGKGLEWVA**TISSSDGTTYY** **RDSVKG**RFTISRDNSKSTLYLQMNSLRAED TAVYYCSR**GYYNSPFAY**WGQGTLVTVSS
	VH.2a 1A11 CDR-H1	Residues 31-35 of SEQ ID NO:174	**NFPMA**
	VH.2a 1A11 CDR-H2	Residues 50-66 of SEQ ID NO:174	**TISSSDGTTYYRDSVKG**
	VH.2a 1A11 CDR-H3	Residues 99-107 of SEQ ID NO:174	**GYYNSPFAY**
			123456789**0**123456789**0**123456789**0**

SEQ ID NO:	**Protein region**		**Sequence**
			123456789**0**123456789**0**123456789**0**
175	**VL.1 1A11**		DIQMTQSPSSLSASVGDRVTITC**RASEDIYSNLA**WYQQKPGKAPKLLIY**DTNNLAD**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQYNNYPPT**FGQGTKLEIKR
	VL.1 1A11 CDR-L1	Residues 24-34 of SEQ ID NO:175	**RASEDIYSNLA**
	VL.1 1A11 CDR-L2	Residues 50-56 of SEQ ID NO:175	**DTNNLAD**
	VL.1 1A11 CDR-L3	Residues 89-97 of SEQ ID NO:175	**QQYNNYPPT**
			123456789**0**123456789**0**123456789**0**
176	**VL.1a 1A11**		DIQMTQSPSSLSASVGDRVTITC**RASEDIYSNLA**WYQQKPGKSPKLLIF**DTNNLAD**GVPSRFSGSGSGTDSTLTISSLQPEDFATYFC**QQYNNYPPT**FGQGTKLEIKR
	VL.1a 1A11 CDR-L1	Residues 24-34 of SEQ ID NO:176	**RASEDIYSNLA**
	VL.1a 1A11 CDR-L2	Residues 50-56 of SEQ ID NO:176	**DTNNLAD**
	VL.1a 1A11 CDR-L3	Residues 89-97 of SEQ ID NO:176	**QQYNNYPPT**
			123456789**0**123456789**0**123456789**0**
177	**VL.1b 1A11**		DIQMTQSPSSLSASVGDRVTITC**RASEDIYSNLA**WYQQKPGKAPKLLIF**DTNNLAD**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQYNNYPPT**FGQGTKLEIKR
	VL.1b 1A11 CDR-L1	Residues 24-34 of SEQ ID NO:177	**RASEDIYSNLA**
	VL.1b 1A11 CDR-L2	Residues 50-56 of SEQ ID NO:177	**DTNNLAD**
	VL.1b 1A11 CDR-L3	Residues 89-97 of SEQ ID NO:177	**QQYNNYPPT**
			123456789**0**123456789**0**123456789**0**
178	**VL.2A 1A11**		EIVMTQSPATLSVSPGERATLSC**RASEDIYSNLA**WYQQKPGQSPRLLIF**DTNNLAD**GVPARFSGSGSGTESTLTISSLQSEDFAVYFC**QQYNNYPPT**FGQGTKLEIKR

SEQ ID NO:	Protein region		Sequence
			123456789012345678901234567890
	VL.2a 1A11 CDR-L1	Residues 24-34 of SEQ ID NO:178	**RASEDIYSNLA**
	VL.2a 1A11 CDR-L2	Residues 50-56 of SEQ ID NO:178	**DTNNLAD**
	VL.2a 1A11 CDR-L3	Residues 89-97 of SEQ ID NO:178	**QQYNNYPPT**

Humanized antibodies were generated by combining each heavy chain variant with each light chain variant for a total of 16 variants (Table 12, below). Variants 1-4 each contained VH.1 paired with each VL variant: VL.1: 0 back mutations, VL.1a: 4 back mutations, VL.1b: 1 back mutation, and VL.2a: 5 back mutations. Variants 5-8 each contained VH.1a paired with each VL variant: VL.1: 2 back mutations, VL.1a: 6 back mutations, VL.1b:3 back mutations, and VL.2a: 7 back mutations. Variants 9-12 each contained VH.1b paired with each VL variant: VL.1: 1 back mutation, VL.1a: 5 back mutations, VL.1b: 2 back mutations, and VL.2a: 6 back mutations. Variants 13-16 each contained VH.2a paired with each VL variant: VL.1: 3 back mutations, VL.1a: 7 back mutations, VL.1b: 4 back mutations, VL.2a: 8 back mutations.

Table 12. Summary of Humanized 1A11 Antibodies Generated and Back Mutations.

Name	VH/VL Combination	Back Mutations in Variable Regions† (VH/VL)
h1A11.1	VH.1/VL.1	0/0
h1A11.2	VH.1/VL.1a	0/A43S, Y49F, F71S, Y87F
h1A11.3	VH.1/VL.1b	0/Y49F
h1A11.4	VH.1/VL.2a	0/A43S, Y49F, I58V, F71S, Y87F
h1A11.5	VH.1a/VL.1	N76S, A93S/0
h1A11.6	VH.1a/VL.1a	N76S, A93S/A43S, Y49F, F71S, Y87F
h1A11.7	VH.1a/VL.1b	N76S, A93S/Y49F
h1A11.8	VH.1a/VL.2a	N76S, A93S/A43S, Y49F, I58V, F71S, Y87F
h1A11.9	VH.1b/VL.1	A93S/0

h1A11.10	VH.1b/VL.1a	A93S/A43S, Y49F, F71S, Y87F
h1A11.11	VH.1b/VL.1b	A93S/Y49F
h1A11.12	VH.1b/VL.2a	A93S/A43S, Y49F, I58V, F71S, Y87F
h1A11.13	VH.2a/VL.1	S49A, N76S, A93S/0
h1A11.14	VH.2a/VL.1a	S49A, N76S, A93S,/A43S, Y49F, F71S, Y87F
h1A11.15	VH.2a/VL.1b	S49A, N76S, A93S/ Y49F
h1A11.16	VH.2a/VL.2a	S49A, N76S, A93S/A43S, Y49F, I58V, F71S, Y87F

† Kabat numbering used.

All 16 variants were transiently transfected into 50 mls of HEK 293 6e suspension cell cultures in a ratio of 60% to 40% light to heavy chain construct. 1 mg/ml PEI was used to transfect the cells. Cell supernatants were harvested after six days in shaking flasks, spun down to pellet cells, and filtered through 0.22 μm filters to separate IgG from culture contaminates. Variant binding to human DLL4 was initially assessed through a capture binding ELISA (Example 1.2), which utilized a goat anti human-Fc capture antibody (Jackson ImmunoResearch, 109-005-008) to capture IgG within filtered 293 6e cell supernatants (Table 13, below). All 16 variants had very comparable affinities and were purified for further characterization.

All 16 variants (h1A11.1-h1A11.16) were batch purified by adding 1 supernatant volume of protein A IgG binding buffer (Thermo Scientific 21001) and 1 ml of rProteinA sepharose fast flow beads (GE Healthcare, 17-1279-04). Supernatants, with beads and buffer added, were rocked overnight at 4°C, and the day after beads were collected by gravity over poly prep chromatography columns (Bio Rad, 731-1550). Once supernatants had passed through the columns the beads were washed with 10 column volumes of binding buffer, and IgG was eluted with Immunopure IgG elution buffer (Pierce, 185 1520) and collected in 1 ml aliquots. Fractions containing IgG were pooled and dialyzed in PBS overnight at 4°C.

Purified variants were further characterized for their affinities for human, murine and cynomolgus DLL4 by binding ELISA (Example 1.2, Method 2), by Biacore (Example 1.1), and by Flow Cytometry (FACS). All 16 variants showed comparable affinities to the parent recombinant antibody 1A11 in all the three assays (Table 13). The humanized variants were then tested for their functionality with human and murine DLL4 by competition ELISA (Example 1.3) and by Notch reporter assay (Example 1.6). All 16 variants showed comparable potencies to the parent recombinant antibody 1A11 in both assays (Table 14, below).

Table 13. Summary of Binding Activities of Humanized 1A11 mAbs.

Binding Data							
	Human DLL4			**Mouse DLL4**			**Cyno DLL4**
mAb	Binding ELISA (EC50, nM)	Biacore (KD, M)	Binding FACS (EC50, nM)	Binding ELISA (EC50, nM)	Biacore (KD, M)	Binding FACS (EC50, nM)	Binding ELISA (EC50, nM)
h1A11-1	0.08	1.5×10^{-8}	3.13	0.09	3.8×10^{-8}	0.21	0.13
h1A11-2	0.08	1.0×10^{-8}	2.85	0.09	$1.9 \times 10^{-8*}$	0.21	0.13
h1A11-3	0.07	1.5×10^{-8}	3.51	0.08	3.7×10^{-8}	0.24	0.16
h1A11-4	0.08	1.6×10^{-8}	3.61	0.09	3.9×10^{-8}	0.22	0.17
h1A11-5	0.07	0.96×10^{-8}	3.61	0.08	2.2×10^{-8}	0.21	0.16
h1A11-6	0.08	1.13×10^{-8}	3.74	0.10	3.1×10^{-8}	0.15	0.13
h1A11-7	0.06	1.3×10^{-8}	3.71	0.09	3.5×10^{-8}	0.18	0.14
h1A11-8	0.06	1.1×10^{-8}	3.34	0.09	2.4×10^{-8}	0.18	0.17
h1A11-9	0.07	1.3×10^{-8}	3.28	0.09	3.4×10^{-8}	0.18	0.15
h1A11-10	0.06	1.3×10^{-8}	nt	0.09	3.5×10^{-8}	nt	0.15
h1A11-11	0.07	1.2×10^{-8}	nt	0.09	2.7×10^{-8}	nt	0.15
h1A11-12	0.06	1.4×10^{-8}	nt	0.08	3.5×10^{-8}	nt	0.16
h1A11-13	0.06	1.4×10^{-8}	nt	0.09	3.7×10^{-8}	nt	0.16
h1A11-14	0.055	1.3×10^{-8}	nt	0.07	2.8×10^{-8}	nt	0.14
h1A11-15	0.07	1.4×10^{-8}	nt	0.09	3.7×10^{-8}	nt	0.13
h1A11-16	0.06	1.5×10^{-8}	nt	0.09	3.9×10^{-8}	nt	0.12
1A11 (chimeric)	0.2	0.9×10^{-8}	6.29	0.3	2.4×10^{-8}	0.53	0.33
1A11 (rat mAb)		0.6×10^{-8}					

nt = not tested

Table 14. Summary of *In Vitro* Functional Potency of Humanized 1A11 mAbs.

Functional Data			
	Human DLL4		Mouse DLL4
mAb	Competition ELISA (IC50, nM)	Reporter Assay (EC50, nM)	Competition ELISA (IC50, nM)
h1A11-1	0.4	2.53	1.3
h1A11-2	0.3	3.92	0.9
h1A11-3	0.2	2.53	0.9
h1A11-4	0.3	3.28	0.9
h1A11-5	0.3	3.8	0.8
h1A11-6	0.4	1.45	0.8
h1A11-7	0.5	4.84	0.9
h1A11-8	0.35	4.24	0.9
h1A11-9	0.3	3.18	0.9
h1A11-10	0.35	5.88	0.9
h1A11-11	0.4	3.73	0.8
h1A11-12	0.4	2.89	0.9
h1A11-13	0.3	10.42	1
h1A11-14	0.25	4.1	0.7
h1A11-15	0.2	5.4	0.7
h1A11-16	0.3	2.61	0.7
1A11 (chimera)	1.8	5.98	3.5
1A11 (rat hybridoma mAb)	0.5		1.1

Additional Designs for Humanizing Anti-DLL4 1A11 Antibodies.

Additional VH and VL designs for humanizing anti-DLL4 rat monoclonal antibody 1A11 are shown in the table below.

Table 15. Additional VH and VL Designs for Humanizing 1A11 Antibodies.

VH or VL Design	Acceptor Framework Sequence	Back Mutations†
h1A11VH.2	VH3 CONSENSUS + JH4	0

h1A11VH.2b	VH3 CONSENSUS + JH4	S49A, A93S
h1A11VH.3	VH1-46 + JH4 (with Q1E to prevent N-terminal pyroglutamate formation)	0
h1A11VH.3b	VH1-46 + JH4 (Q1E)	Y27F, M48V, G49A, A93S
h1A11VH.3c	VH1-46 + JH4 (Q1E)	Y27F, M48V, G49A, V67F, M69I, T73N, V78L, A93S, and T75I (to avoid undesirable N-glycosylation signal)
h1A11VH.3d	VH1-46 + JH4 (Q1E)	Y27F, M48V, G49A, V67F, M69I, V78L, A93S (T73N omitted to avoid undesirable N-glycosylation signal)
h1A11VL.1c	O2 + JK2	Y49F, F71S
h1A11VL.2	3-15/L2 + JK2	0
h1A11VL.2b	3-15/L2 + JK2	Y49F
h1A11VL.2c	3-15/L2 + JK2	Y49F, F71S

† Kabat numbering used.

Example 7. Humanization of Anti-DLL4 Rat mAb 38H12.

The 38H12 rat anti-DLL4 antibody (Table 9, above) was humanized. Humanized variant amino acid sequences VH.1, VH.1a, VH.1b, VH.2a, VL.1, VL.1a, VL.1b, and VL.2a (Table 16, below) were converted to DNA sequence, based on the most homologous human germlines, and synthesized. Human germline heavy chain acceptor sequences VH3-30 FR1, VH3-30 FR2, VH3-30 FR3, and JH3 FR4 (see, Table 3) were used for generating the humanized heavy chain variants shown in Table 16. Human germline light chain acceptor sequences O2 FR1, O2 FR2, O2 FR3, and JK2 FR4 (see, Table 4) were used for generating the humanized light chain variants shown in

Table 16. Individual constructs were sequence verified to check for accuracy. Positive variants were then inoculated into 150 mls Luria broth plus ampicillin and cultured overnight at 37°C. DNA was extracted from variant cultures using the Qiagen Hi speed maxi prep kit (12662).

Table 16. VH and VL Amino Acid Sequences of Humanized Rat Anti-DLL4 Monoclonal Antibody 38H12.

SEQ ID NO:	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
179	**VH.1 38H12**		EVQLVESGGGVVQPGRSLRLSCAASGFTFS **NYGMY**WVRQAPGKGLEWVA**FISHGGGITYY** **RDSVKG**RFTISRDNSKNTLYLQMNSLRAED TAVYYCAR**LNWELGIDY**WGQGTMVTVSS
	VH.1 38H12 CDR-H1	Residues 31-35 of SEQ ID NO:179	**NYGMY**
	VH.1 38H12 CDR-H2	Residues 50-66 of SEQ ID NO:179	**FISHGGGITYYRDSVKG**
	VH.1 38H12 CDR-H3	Residues 99-107 of SEQ ID NO:179	**LNWELGIDY**
			123456789**0**123456789**0**123456789**0**
180	**VH.1a 38H12**		EVQLVESGGGVVQPGRSLRLSCAASGFTFS **NYGMY**WIRQAPGKGLEWVA**FISHGGGITYY** **RDSVKG**RFTISRDNSKSTLYLQMNSLRAED TAVYHCAA**LNWELGIDY**WGQGTMVTVSS
	VH.1a 38H12 CDR-H1	Residues 31-35 of SEQ ID NO:180	**NYGMY**
	VH.1a 38H12 CDR-H2	Residues 50-66 of SEQ ID NO:180	**FISHGGGITYYRDSVKG**
	VH.1a 38H12 CDR-H3	Residues 99-107 of SEQ ID NO:180	**LNWELGIDY**
			123456789**0**123456789**0**123456789**0**
181	**VH.1b 38H12**		EVQLVESGGGVVQPGRSLRLSCAASGFTFS **NYGMY**WVRQAPGKGLEWVA**FISHGGGITYY** **RDSVKG**RFTISRDNSKNTLYLQMNSLRAED TAVYYCAA**LNWELGIDY**WGQGTMVTVSS
	VH.1b 38H12 CDR-H1	Residues 31-35 of SEQ ID NO:181	**NYGMY**
	VH.1b 38H12 CDR-H2	Residues 50-66 of SEQ ID NO:181	**FISHGGGITYYRDSVKG**
	VH.1b 38H12 CDR-H3	Residues 99-107 of SEQ ID NO:181	**LNWELGIDY**
			123456789**0**123456789**0**123456789**0**

SEQ ID NO:	**Protein region**		**Sequence**
			123456789**0**123456789**0**123456789**0**
182	**VH.2a 38H12**		EVQLVESGGGLVQPGGSLRLSCAASGFTFS **NYGMY**WIRQAPGKGLEWVA**FISHGGGITYY** **RDSVKG**RFTISRDNSKSTLYLQMNSLRAED TAVYHCAA**LNWELGIDY**WGQGTMVTVSS
	VH.2a 38H12 CDR-H1	Residues 31-35 of SEQ ID NO:182	**NYGMY**
	VH.2a 38H12 CDR-H2	Residues 50-66 of SEQ ID NO:182	**FISHGGGITYYRDSVKG**
	VH.2a 38H12 CDR-H3	Residues 99-107 of SEQ ID NO:182	**LNWELGIDY**
			123456789**0**123456789**0**123456789**0**
183	**VL.1 38H12**		DIQMTQSPSSLSASVGDRVTITC**RASEDIY** **SNLA**WYQQKPGKAPKLLIY**AANRLQD**GVPS RFSGSGSGTDFTLTISSLQPEDFATYYC**LQ** **GSKFPLT**FGQGTKLEIKR
	VL.1 38H12 CDR-L1	Residues 24-34 of SEQ ID NO:183	**RASEDIYSNLA**
	VL.1 38H12 CDR-L2	Residues 50-56 of SEQ ID NO:183	**AANRLQD**
	VL.1 38H12 CDR-L3	Residues 89-97 of SEQ ID NO:183	**LQGSKFPLT**
			123456789**0**123456789**0**123456789**0**
184	**VL.1a 38H12**		DIQMTQSPSSLSASVGDRVTITC**RASEDIY** **SNLA**WYQKKPGKSPKLLIY**AANRLQD**GVPS RFSGSGSGTDYTLTISSLQPEDFATYFC**LQ** **GSKFPLT**FGQGTKLEIKR
	VL.1a 38H12 CDR-L1	Residues 24-34 of SEQ ID NO:184	**RASEDIYSNLA**
	VL.1a 38H12 CDR-L2	Residues 50-56 of SEQ ID NO:184	**AANRLQD**
	VL.1a 38H12 CDR-L3	Residues 89-97 of SEQ ID NO:184	**LQGSKFPLT**
			123456789**0**123456789**0**123456789**0**
185	**VL.1b 38H12**		DIQMTQSPSSLSASVGDRVTITC**RASEDIY** **SNLA**WYQQKPGKAPKLLIY**AANRLQD**GVPS RFSGSGSGTDYTLTISSLQPEDFATYYC**LQ** **GSKFPLT**FGQGTKLEIKR
	VL.1b 38h12 CDR-L1	Residues 24-34 of SEQ ID NO:185	**RASEDIYSNLA**
	VL.1b 38H12 CDR-L2	Residues 50-56 of SEQ ID NO:185	**AANRLQD**
	VL.1b 38H12 CDR-L3	Residues 89-97 of SEQ ID NO:185	**LQGSKFPLT**
			123456789**0**123456789**0**123456789**0**
186	**VL.2a 38H12**		EIVMTQSPATLSVSPGERATLSC**RASEDIY** **SNLA**WYQKKPGQSPRLLIY**AANRLQD**GVPA RFSGSGSGTEYTLTISSLQSEDFAVYFC**LQ** **GSKFPLT**FGQGTKLEIKR
	VL.2a 38H12 CDR-L1	Residues 24-34 of SEQ ID NO:186	**RASEDIYSNLA**
	VL.2a 38H12 CDR-L2	Residues 50-56 of SEQ ID NO:186	**AANRLQD**
	VL.2a 38H12 CDR-L3	Residues 89-97 of SEQ ID NO:186	**LQGSKFPLT**

Humanized antibodies were generated by combining each heavy chain variant with each light chain variant for a total of 16 variants (Table 17, below). Variants 1-4 each contained VH.1 paired with each VL variant: VL.1: 0 back mutations, VL.1a: 4 back mutations, VL.1b: 1 back mutation, and VL.2a: 5 back mutations. Variants 5-8 each contained VH.1a paired with each VL variant: VL.1: 4 back mutations, VL.1a: 8 back mutations, VL.1b: 5 back mutations, and VL.2a: 9 back mutations. Variants 9-12 each contained VH.1b paired with each VL variant: VL.1: 1 back mutation, VL.1a: 5 back mutations, VL.1b: 2 back mutations, and VL.2a: 5 back mutations. Variants 13-16 each contained VH.2a paired with each VL variant: VL.1: 5 back mutations, VL.1a: 9 back mutations, VL.1b: 6 back mutations, VL.2a:10 back mutations.

Table 17. Summary of Humanized 38H12 Antibodies Generated and Back Mutations.

Name	VH/VL Combination	Back Mutations in Variable Regions† (VH/VL)
h38H12.1	VH.1/VL.1	0/0 (Q1E in VH to prevent N-terminal pyroglutamate formation)
h38H12.2	VH.1/VL.1a	0/Q38K, A43S, F71Y, Y87F (Q1E in VH as noted above)
h38H12.3	VH.1/VL.1b	0/F71Y (Q1E in VH as noted above)
h38H12.4	VH.1/VL.2a	0/Q38K, A43S, I58V, F71Y, Y87F (Q1E in VH as noted above)
h38H12.5	VH.1a/VL.1	V37I, N76S, Y91H, R94A/0
h38H12.6	VH.1a/VL.1a	V37I, N76S, Y91H, R94A/Q38K, A43S, F71Y, Y87F
h38H12.7	VH.1a/VL.1b	V37I, N76S, Y91H, R94A/F71Y

h38H12.8	VH.1a/VL.2a	V37I, N76S, Y91H, R94A/Q38K, A43S, I58V, F71Y, Y87F
h38H12.9	VH.1b/VL.1	R94A/0
h38H12.10	VH.1b/VL.1a	R94A/Q38K, A43S, F71Y, Y87F
h38H12.11	VH.1b/VL.1b	R94A/F71Y
h38H12.12	VH.1b/VL.2a	R94A/Q38K, A43S, I58V, F71Y, Y87F
h38H12.13	VH.2a/VL.1	V37I, S49A, N76S, Y91H, R94A/0
h38H12.14	VH.2a/VL.1a	V37I, S49A, N76S, Y91H, R94A/Q38K, A43S, F71Y, Y87F
h38H12.15	VH.2a/VL.1b	V37I, S49A, N76S, Y91H, R94A/F71Y
h38H12.16	VH.2a/VL.2a	V37I, S49A, N76S, Y91H, R94A/ Q38K, A43S, I58V, F71Y, Y87F

† Kabat numbering used.

All 16 variants were transiently transfected into 50 mls of HEK 293 6e suspension cell cultures in a ratio of 60% to 40% light to heavy chain construct. 1 mg/ml PEI was used to transfect the cells. Cell supernatants were harvested after seven days in shaking flasks, spun down to pellet cells, and filtered through 0.22 μm filters to separate IgG from culture contaminates. Variant binding to human DLL4 was initially assessed through a capture binding ELISA (Example 1.2), which utilized a goat anti human-Fc capture antibody (Jackson immuno research, 109-005-008) to capture IgG within filtered 293 6e cell supernatants (see, ELISA binding EC_{50}, Table 18, below). Variants containing VH.1 exhibited the lowest binding affinities as compared to the other variants and were considered out of the screening. VH.1 is CDR-grafted with no framework back mutations.

Good binders (h38H12.5-h38H12.16) were then batch purified by adding 1 supernatant volume of protein A IgG binding buffer (Thermo Scientific 21001) and 800 µl of rProteinA sepharose fast flow beads (GE Healthcare, 17-1279-04). Supernatants, with beads and buffer added, were stirred at room temperature for 4 hours, and beads were collected by gravity over poly prep chromatography columns (Bio Rad, 731-1550). Once supernatants had passed through the columns the beads were washed with 10 mls binding buffer and IgG was eluted with Immunopure IgG elution buffer (Pierce, 185 1520) and collected in 1 ml aliquots neutralized with 100 µl 1M Tris, pH 8.

Purified variants were further characterized in human Notch-1 competition ELISAs (Example 1.4), which used a format of plating Notch-1 Fc onto ELISA plates and pre-incubating biotinylated human DLL4 plus titrated antibody. Signal was assessed by free biotinylated DLL4 binding to Notch-1 Fc. Strong binders inhibited signal at low antibody concentration. h38H12.5 through h38H12.7 exhibited lower competition potencies as compared to the other variants (see, Notch Competition ELISA EC_{50}, Table 18, below).

The good binder variants, as determined by binding ELISA, were assessed by Biacore (Example 1.1) concurrently with cell based assay screens. The K_D for human DLL4 was similar for all variants (see, Biacore, K_D in Table 18, below). Variants were screened in cell-based assays that examined direct binding to human DLL4 (Example 1.3; FACS binding EC_{50} in Table 18, below) and inhibition of Notch-1 signaling (Example 1.6; Notch Reporter Assay EC_{50} in Table 18, below).

Table 18. Summary of *In Vitro* Activities Against Human DLL4 of Humanized 38H12 mAbs.

Name	ELISA binding EC_{50} (nM)	Notch Competition ELISA EC_{50} (nM)	FACS binding EC_{50} (nM)	Notch Reporter Assay EC_{50} (nM)	Biacore, K_D (nM)
h38H12.1	21.5		17.84		
h38H12.2	26.88				
h38H12.3	5.57				
h38H12.4	20.65				
h38H12.5	0.2015	14.81	77.15	7.307	0.401
h38H12.6	0.1584	26.96	12.29	6.317	0.434
h38H12.7	0.1798	12.49	19.06	2.598	0.34
h38H12.8	0.1972	8.315	20.02	5.557	0.397

h38H12.9	0.1155	4.158	3.71	1.436	0.986
h38H12.10	0.1226	3.902	2.489	0.7861	0.578
h38H12.11	0.1264	3.8	2.477	0.6572	0.554
h38H12.12	0.1651	3.228	1.478	1.062	0.819
h38H12.13	0.1534	5.287	2.556	0.7943	0.507
h38H12.14	0.146	5.839	1.04	1.014	0.303
h38H12.15	0.0904	5.714	2.369	0.837	0.355
h38H12.16	0.1696	3.766	2.914	1.185	0.392

Additional Designs for Humanizing Anti-DLL4 38H12 Antibodies.

Additional VH and VL designs for humanizing anti-DLL4 rat monoclonal antibody 38H12 are shown in the table below.

Table 19. Additional VH and VL Designs for Humanizing 38H12 Antibodies.

VH or VL Design	**Germline Acceptor Framework Sequences**	**Back Mutations†**
h38H12VH.2	VH3 CONSENSUS + JH3	0
h38H12VH.2b	VH3 CONSENSUS + JH3	S49A, R94A
h38H12VH.3	VH1-46 + JH3	0 (Q1E in VH to prevent N-terminal pyroglutamate formation)
h38H12VH.3b	VH1-46 + JH3	Y27F, M48V, G49A, R94A (Q1E in VH as noted above)
h38H12VH.3c	VH1-46 + JH3	Y27F, Y37I, M48V, G49A, V67F, M69I, T73N, V78L, Y91H, R94A (T75I to eliminate undesirable N-glycosylation signal, Q1E in VH as noted above)

h38H12VH.3d	VH1-46 + JH3	Y27F, Y37I, M48V, G49A, V67F, M69I, V78L, Y91H, R94A (Q1E in VH as noted above)
h38H12VL.2	3-15/L2 + JK2	0
h38H12VL.2b	3-15/L2 + JK2	F71Y

† Kabat numbering used.

Example 8. Affinity Maturation of h1A11.1.

Humanized antibody h1A11.1 was used as a template for affinity maturation. A description of the design of the library is provided below. Numbering of variable region sequences of monoclonal antibodies was annotated with Kabat numbering (as described above; or see worldwide website bioinf.org.uk/abs/#kabatnum) and was used in generating the libraries described below.

Three libraries were made as described below.

H1+H2 library (doping: 76080808):

Doped 11 residues at 30, 31, 32, 35, 50, 52, 52a, 55, 56, 57, and 58.

Toggle between germline and h1A11 sequence at position 76(V/I).

A 10^9 library sampled mutants with 4 or fewer mutated residues at least 3.7 times.

The majority of the library with mutants carrying 4 to 6 residues was mutated by doping.

H3 library (doping: 70101010)

Doped 8 residues @ 95, 96, 97, 98, 99, 100, 100a, and 102.

Toggle between germline and h1A11 sequence at position 93(A/S) and 101 (D/A).

A 10^9 library sampled mutants with 4 or fewer mutated residues at least 4.7 times.

The majority of the library with mutants carrying 4 to 5 residues was mutated by doping.

LC library (doping: 70101010):

Doped 9 residues at 28, 30, 31, 50, 53, 92, 93, 94, and 96.

Toggle between germline and h1A11 sequence at 7 positions 27(Q/E), 43(A/S), 49(Y/F), 52(S/N), 71(F/S), 87(Y/F), and 91(S/Y).

A 10^9 library sampled mutants with 4 or fewer mutated residues at least 1 time.

The majority of the library with mutants carrying 4 to 6 residues was mutated by doping.

rHC library: recombine outputs of H1+H2 and H3 libraries.

rHCLC library: recombine outputs of H1+H2, H3, and LC libraries.

Both VH and VL framework germlining reduced predicted immunogenicity. The most desirable germlining mutation in the h1A11VL.1a was the S43A and S71F

Used codons specified here for residues that were doped:

If a proline is to be doped, the doping oligo will have $C_{(5-85-5-5)}C_{(5-85-5-5)}S$ codon regardless of the original codon in the antibody sequence. These codons were selected based on the following criteria:

1. increase non-synonymous mutation
2. increase coverage of more amino acids when mutated
3. uses high frequency codons
4. avoid SSS and WWW codons.

Doping order was A-C-G-T

$A_{(85-5-5-5)}$, $A_{(70-10-10-10)}$
$C_{(5-85-5-5)}$, $C_{(10-70-10-10)}$
$G_{(5-5-85-5)}$, $G_{(10-10-70-10)}$
$T_{(5-5-5-85)}$, $T_{(10-10-10-70)}$

Alanine (A):		
GCN	$G_{(10-10-70-10)}C_{(10-70-10-10)}S$	$G_{(5-5-85-5)}C_{(5-85-5-5)}S$
Threonine (T):		
ACN	$A_{(70-10-10-10)}C_{(10-70-10-10)}S$	$A_{(85-5-5-5)}C_{(5-85-5-5)}S$
Proline (P):		
CCN	$C_{(10-70-10-10)}C_{(10-70-10-10)}S$	$C_{(5-85-5-5)}C_{(5-85-5-5)}S$
Serine (S):		
If TCN	$T_{(10-10-10-70)}C_{(10-70-10-10)}S$	$T_{(5-5-5-85)}C_{(5-85-5-5)}S$
If AGY	$A_{(70-10-10-10)}G_{(10-10-70-10)}C_{(10-70-10-10)}$	$A_{(85-5-5-5)}G_{(5-5-85-5)}C_{(5-85-5-5)}$
Valine (V):		
GTN	$G_{(10-10-70-10)}T_{(10-10-10-70)}S$	$G_{(5-5-85-5)}T_{(5-5-5-85)}S$
Glycine (G):		
GGN	$G_{(10-10-70-10)}G_{(10-10-70-10)}S$	$G_{(5-5-85-5)}G_{(5-5-85-5)}S$
Leucine (L):		
If CTN	$C_{(10-70-10-10)}T_{(10-10-10-70)}S$	$C_{(5-85-5-5)}T_{(5-5-5-85)}S$
If TTR	$T_{(10-10-10-70)}T_{(10-10-10-70)}G_{(10-10-70-10)}$	$T_{(5-5-5-85)}T_{(5-5-5-85)}G_{(5-5-85-5)}$
Arginine (R):		
If CGN	$C_{(10-70-10-10)}G_{(10-10-70-10)}S$	$C_{(5-85-5-5)}G_{(5-5-85-5)}S$
If AGR	$A_{(70-10-10-10)}G_{(10-10-70-10)}G_{(10-10-70-10)}$	$A_{(85-5-5-5)}G_{(5-5-85-5)}G_{(5-5-85-5)}$
Methionine (M):		
ATG	$A_{(70-10-10-10)}T_{(10-10-10-70)}G_{(10-10-70-10)}$	$A_{(85-5-5-5)}T_{(5-5-5-85)}G_{(5-5-85-5)}$
Tryptophan (W):		

TGG	$T_{(10-10-10-70)}G_{(10-10-70-10)}G_{(10-10-70-10)}$	$T_{(5-5-5-85)}G_{(5-5-85-5)}G_{(5-5-85-5)}$
Pheylalanine (F):		
TTY	$T_{(10-10-10-70)}T_{(10-10-10-70)}C_{(10-70-10-10)}$	$T_{(5-5-5-85)}T_{(5-5-5-85)}C_{(5-85-5-5)}$
Isoleucine (I): need two oligos		
50% ATY	$A_{(70-10-10-10)}T_{(10-10-10-70)}C_{(10-70-10-10)}$	$A_{(85-5-5-5)}T_{(5-5-5-85)}C_{(5-85-5-5)}$
50% ATA	$A_{(70-10-10-10)}T_{(10-10-10-70)}A_{(70-10-10-10)}$	$A_{(85-5-5-5)}T_{(5-5-5-85)}A_{(85-5-5-5)}$
Tyrosine (Y):		
TAY	$T_{(10-10-10-70)}A_{(70-10-10-10)}C_{(10-70-10-10)}$	$T_{(5-5-5-85)}A_{(85-5-5-5)}C_{(5-85-5-5)}$
Histidine (H):		
CAY	$C_{(10-70-10-10)}A_{(70-10-10-10)}C_{(10-70-10-10)}$	$C_{(5-85-5-5)}A_{(85-5-5-5)}C_{(5-85-5-5)}$
Glutamine (Q):		
CAR	$C_{(10-70-10-10)}A_{(70-10-10-10)}G_{(10-10-70-10)}$	$C_{(5-85-5-5)}A_{(85-5-5-5)}G_{(5-5-85-5)}$
Asparagines (N):		
AAY	$A_{(70-10-10-10)}A_{(70-10-10-10)}C_{(10-70-10-10)}$	$A_{(85-5-5-5)}A_{(85-5-5-5)}C_{(5-85-5-5)}$
Lysine (K):		
AAR	$A_{(70-10-10-10)}A_{(70-10-10-10)}G_{(10-10-70-10)}$	$A_{(85-5-5-5)}A_{(85-5-5-5)}G_{(5-5-85-5)}$
Aspartic Acid (D):		
GAY	$G_{(10-10-70-10)}A_{(70-10-10-10)}C_{(10-70-10-10)}$	$G_{(5-5-85-5)}A_{(85-5-5-5)}C_{(5-85-5-5)}$
Glutamic acid (E):		
GAR	$G_{(10-10-70-10)}A_{(70-10-10-10)}G_{(10-10-70-10)}$	$G_{(5-5-85-5)}A_{(85-5-5-5)}G_{(5-5-85-5)}$
Cysteine (C):		
TGY	always NNS	

The h1A11.1 libraries were transformed into yeast cells and displayed on the cell surface to be selected against low concentration of biotinylated DLL4 extracellular domain by magnetic then fluorescence activated cell sorting. Selection for improved on-rate or off-rate or both were carried out, and antibody protein sequences of affinity-modulated hu1A11 clones (Tables 20 and 21, below) were recovered from yeast cells for converting back to IgG format for further characterization (see, summary of clones in Table 22). Table 23 lists the amino acids observed during the affinity maturation selection in both framework regions (FR) and CDRs.

Table 20. VH sequences of Affinity Matured Humanized 1A11.1 Clones

SEQ ID NO:	Protein region			Sequence
				123456789012345678901234567890
187	**h1A11VH.1 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFS**NFPMA**WVRQAPGKGLEWVA**TISSSDGTTYYRDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR**GYYNSPFAY**WGQGTLVTVSS
	h1A11VH.1 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:187	**NFPMA**
	h1A11VH.1 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:187	**TISSSDGTTYYRDSVKG**
	h1A11VH.1 VH	**CDR-H3**	Residues 99-107 of SEQ ID NO:187	**GYYNSPFAY**
188	**h1A11.A6 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFR**HFPMA**WVRQAPGKGLEWVA**TISSSDAWPSYRDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYYCSR**GYYNSPFAY**WGQGTLVTVSS
	h1A11.A6 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:188	**HFPMA**
	h1A11.A6 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:188	**TISSSDAWPSYRDSVKG**
	h1A11.A6 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:188	**GYYNSPFAY**
189	**h1A11.A8 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFG**NFPMS**WVRQAPGKGLEWVA**SISSSDSWATIGDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYYCSR**GYYNSPFAY**WGQGTLVTVSS
	h1A11.A8 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:189	**NFPMS**
	h1A11.A8 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:189	**SISSSDSWATIGDSVKG**
	h1A11.A8 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:189	**GYYNSPFAY**

SEQ ID NO:	Protein region			Sequence
				1234567890**123456789**0**123456789**0
190	**h1A11.C6 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFR**NFPMA**WVRQAPGKGLEWVA**TISSSDGWPTYRDSVKG**RFTISRDNAKSSLYLQMNSLRAEDTAVYYCSR**GYYNSPFAY**WGQGTLVTVSS
	h1A11.C6 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:190	**NFPMA**
	h1A11.C6 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:190	**TISSSDGWPTYRDSVKG**
	h1A11.C6 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:190	**GYYNSPFAY**
191	**h1A11.A11 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFR**HFPMA**WVRQAPGKGLEWVA**TISSSDDWPNYRDSVKG**RFTISRDNAKSSLYLQMNSLRAEDTAVYYCSR**GYYNSPFAY**WGQGTLVTVSS
	h1A11.A11 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:191	**HFPMA**
	h1A11.A11 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:191	**TISSSDDWPNYRDSVKG**
	h1A11.A11 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:191	**GYYNSPFAY**
192	**h1A11.B5 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFR**YFPMS**WVRQAPGKGLEWVA**SISGSDGWASYRDSVKG**RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR**GYYNSPFAS**WGQGTLVTVSS
	h1A11.B5 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:192	**YFPMS**
	h1A11.B5 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:192	**SISGSDGWASYRDSVKG**
	h1A11.B5 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:192	**GYYNSPFAS**

SEQ ID NO:	Protein region			Sequence
				123456789012345678901234567890
193	**h1A11.E12 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFR **YFPMA**WVRQAPGKGLEWVA**TISGSDEWPNY** **RDSVKG**RFTISRDNAKNSLYLQMNSLRAED TAVYYCAR**GYYNSPFAF**WGQGTLVTVSS
	H1A11.E12 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:193	**YFPMA**
	h1A11.E12 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:193	**TISGSDEWPNYRDSVKG**
	h1A11.E12 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:193	**GYYNSPFAF**
194	**h1A11.G3 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFR **YFPMS**WVRQAPGKGLEWVA**SISGSDGWASY** **RDSVKG**RFTISRDNAKNSLYLQMNSLRAED TAVYYCAR**GYYNSPFAY**WGQGTLVTVSS
	h1A11.G3 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:194	**YFPMS**
	h1A11.G3 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:194	**SISGSDGWASYRDSVKG**
	h1A11.G3 VH	**CDR-H3**	Residues 99-107 of SEQ ID NO:194	**GYYNSPFAY**
195	**h1A11.F5 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFR **HFPMA**WVRQAPGKGLEWVA**TISSSDAWPSY** **RDSVKG**RFTISRDNAKNSLYLQMNSLRAED TAVYYCAR**GYYNSPFAY**WGQGTLVTVSS
	h1A11.F5 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:195	**HFPMA**
	h1A11.F5 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:195	**TISSSDAWPSYRDSVKG**
	h1A11.F5 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:195	**GYYNSPFAY**

SEQ ID NO:	Protein region			Sequence
				1234567890**123456789**0**123456789**0
196	**h1A11.H2 VH**			EVQLVESGGGLVQPGGSLRLSCAASGFTFG **NFPMS**WVRQAPGKGLEWVA**SISSSDSWATI** **GDSVKG**RFTISRDNAKNSLYLQMNSLRAED TAVYYCAR**GYYNSPFAF**WGQGTLVTVSS
	h1A11.H2 VH	**CDR-H1**	Residues 31-35 of SEQ ID NO:196	**NFPMS**
	h1A11.H2 VH	**CDR-H2**	Residues 50-66 of SEQ ID NO:196	**SISSSDSWATIGDSVKG**
	h1A11.H2 VH	**CDR-H3**	Residues 99 -107 of SEQ ID NO:196	**GYYNSPFAF**

Table 21. VL Sequences of Affinity Matured Humanized 1A11.1 Clones.

SEQ ID NO:	Protein region			Sequence
				1234567890**123456789**0**123456789**0
197	**h1A11VL.1 VL**			DIQMTQSPSSLSASVGDRVTITC**RASEDIY** **SNLA**WYQQKPGKAPKLLIY**DTNNLAD**GVPS RFSGSGSGTDFTLTISSLQPEDFATYYC**QQ** **YNNYPPT**FGQGTKLEIK
	h1A11VL.1 VL	**CDR-L1**	Residues 24-34 of SEQ ID NO:197	**RASEDIYSNLA**
	h1A11VL.1 VL	**CDR-L2**	Residues 50-56 of SEQ ID NO:197	**DTNNLAD**
	h1A11VL.1 VL	**CDR-L3**	Residues 89 -97 of SEQ ID NO:197	**QQYNNYPPT**
198	**h1A11.A2 VL**			DIQMTQSPSSLSASVGDRVTITC**RASQDIY** **INLA**WYQQKPGKSPKLLIF**DTNDLAD**GVPS RFSGSGSGTDFTLTISSLQPEDFATYYC**QQ** **YDYVPPT**FGQGTKLEIK
	h1A11.A2 VL	**CDR-L1**	Residues 24-34 of SEQ ID NO:198	**RASQDIYINLA**

SEQ ID NO:	Protein region			Sequence
				123456789012345678901234567890
	h1A11.A2 VL	**CDR-L2**	Residues 50-56 of SEQ ID NO:198	**DTNDLAD**
	h1A11.A2 VL	**CDR-L3**	Residues 89 -97 of SEQ ID NO:198	**QQYDYVPPT**
199	**h1A11.A12 VL**			DIQMTQSPSSLSASVGDRVTITC**RASQDIYYNLA**WYQQKPGKSPKLLIF**DTSSLAD**GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC**QQYDWYPPT**FGQGTKLEIK
	h1A11.A12 VL	**CDR-L1**	Residues 24-34 of SEQ ID NO:199	**RASQDIYYNLA**
	h1A11.A12 VL	**CDR-L2**	Residues 50-56 of SEQ ID NO:199	**DTSSLAD**
	h1A11.A12 VL	**CDR-L3**	Residues 89 -97 of SEQ ID NO:199	**QQYDWYPPT**
200	**h1A11.A7 VL**			DIQMTQSPSSLSASVGDRVTITC**RASQDIYINLA**WYQQKPGKAPKLLIF**DTSDLAD**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQYDYYPPT**FGQGTKLEIK
	h1A11.A7 VL	**CDR-L1**	Residues 24-34 of SEQ ID NO:200	**RASQDIYINLA**
	h1A11.A7 VL	**CDR-L2**	Residues 50-56 of SEQ ID NO:200	**DTSDLAD**
	h1A11.A7 VL	**CDR-L3**	Residues 89 -97 of SEQ ID NO:200	**QQYDYYPPT**
201	**h1A11.B4 VL**			DIQMTQSPSSLSASVGDRVTITC**RASQDIYYNLA**WYQQKPGKAPKLLIF**DTNILAD**GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC**QQYDYVPPT**FGQGTKLEIK
	h1A11.B4 VL	**CDR-L1**	Residues 24-34 of SEQ ID NO:201	**RASQDIYYNLA**

SEQ ID NO:	Protein region			Sequence
				1234567890**123456789**0**123456789**0
	h1A11.B4 VL	**CDR-L2**	Residues 50-56 of SEQ ID NO:201	**DTNILAD**
	h1A11.B4 VL	**CDR-L3**	Residues 89 -97 of SEQ ID NO:201	**QQYDYVPPT**
202	**h1A11.B5 VL**			DIQMTQSPSSLSASVGDRVTITC**RASQDIWNNLA**WYQQKPGKSPKLLIF**DTSYLAD**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQYDWYPPT**FGQGTKLEIK
	h1A11.B5 VL	**CDR-L1**	Residues 24-34 of SEQ ID NO:202	**RASQDIWNNLA**
	h1A11.B5 VL	**CDR-L2**	Residues 50-56 of SEQ ID NO:202	**DTSYLAD**
	h1A11.B5 VL	**CDR-L3**	Residues 89-97 of SEQ ID NO:202	**QQYDWYPPT**
203	**h1A11.E12 VL**			DIQMTQSPSSLSASVGDRVTITC**RASQEIYRNLA**WYQQKPGKSPKLLIF**DTSVLAD**GVPSRFSGSGSGTDSTLTISSLQPEDFATYYC**QQYTYYPPT**FGQGTKLEIK
	h1A11.E12 VL	**CDR-L1**	Residues 24-34 of SEQ ID NO:203	**RASQEIYRNLA**
	h1A11.E12 VL	**CDR-L2**	Residues 50-56 of SEQ ID NO:203	**DTSVLAD**
	h1A11.E12 VL	**CDR-L3**	Residues 89 -97 of SEQ ID NO:203	**QQYTYYPPT**

Table 22. Summary of Affinity Matured h1A11.1 Converted Clones.

Clone name	VH	VL
h1A11.A6	h1A11.A6 VH	h1A11VL.1
h1A11.C6	h1A11.C6 VH	h1A11VL.1

h1A11.A11	h1A11.A11 VH	h1A11VL.1
h1A11.A8	h1A11.A8 VH	h1A11VL.1
h1A11.B4	h1A11VH.1	h1A11.B4 VL
h1A11.A7	h1A11VH.1	h1A11.A7 VL
h1A11.A12	h1A11VH.1	h1A11.A12 VL
h1A11.A2	h1A11VH.1	h1A11.A2 VL
h1A11.B5	h1A11.B5 VH	h1A11.B5 VL
h1A11.E12	h1A11.E12 VH	h1A11.E12 VL
h1A11.G3	h1A11.G3 VH	h1A11.E12 VL
h1A11.F5	h1A11.F5 VH	h1A11.E12 VL
h1A11.H2	h1A11.H2 VH	h1A11.E12 VL

Table 23. Amino acids observed during affinity maturation selections of h1A11.1 in framework regions (FRs) and each of the CDRs for VH (SEQ ID NO:204) and VL (SEQ ID NO:205) regions.

SEQ ID NO:204

VH

```
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNFPMAWVRQAPGKGLEWVATISSSDGTTYYRDSVKG
                             RYY  S              S  G  SSASIG
                             NH   T              A     AFDN
                             GS                        EWST
                             KA                        D PA
                             T                         F  D
                             L                         Q
                                                       C

RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGYYNSPFAYWGQGTLVTVSS
          SL          D                DF
                                        S
```

SEQ ID NO:205

```
VL

DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKAPKLLIYDTNNLAD
                         TQE WN           S     F  NS
                           T EI                     D
                             DT                     Q
                             NR                     T
                              M                     V
                              G                     E

GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNNYPPTFGQGTKLEIK
              S               F   SDWV  P
                                   TY
                                   YI
                                    F
                                    P
```

The hu1A11.1 affinity matured clones (Table 22) were expressed, purified, and further characterized *in vitro*. Their antigen binding affinities were determined by Biacore technology as described in Example 1.1, and are shown in Tables 24 and 25 (below). Their activities of binding to cell-bound DLL4 and inhibiting cell-bound DLL4-induced Notch activation were further examined using methods described in Examples 1.3 and 1.6, and are summarized in Table 26 (below).

Table 24. Biacore Kinetics of Affinity-Matured Humanized 1A11.1 Antibodies Binding to Human and Cynomolgus Monkey DLL4.

Clone	Kinetics on Biacore					
	Human DLL4			Cyno DLL4		
	ka ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)	*ka* ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)
h1A11.A6	$1.82 \times 10^{+5}$	6.62×10^{-6}	3.6×10^{-11}	$1.71 \times 10^{+5}$	2.10×10^{-5}	1.2×10^{-10}
h1A11.C6	$1.78 \times 10^{+5}$	7.18×10^{-6}	4.0×10^{-11}	$1.71 \times 10^{+5}$	3.12×10^{-5}	1.8×10^{-10}
h1A11-G3	$1.09 \times 10^{+5}$	9.39×10^{-6}	8.7×10^{-11}	$9.90 \times 10^{+4}$	1.73×10^{-5}	1.7×10^{-10}
h1A11-F5	$1.31 \times 10^{+5}$	9.82×10^{-6}	7.5×10^{-11}	$1.18 \times 10^{+5}$	2.00×10^{-5}	1.7×10^{-10}
h1A11.A8	$1.74 \times 10^{+5}$	1.08×10^{-5}	6.2×10^{-11}	$1.60 \times 10^{+5}$	2.40×10^{-5}	1.5×10^{-10}
h1A11.A11	$1.83 \times 10^{+5}$	2.66×10^{-5}	1.5×10^{-10}	$1.70 \times 10^{+5}$	3.35×10^{-5}	2.0×10^{-10}
h1A11-E12	$1.49 \times 10^{+5}$	3.26×10^{-5}	2.2×10^{-10}	$1.37 \times 10^{+5}$	3.84×10^{-5}	2.8×10^{-10}
h1A11-H2	$1.43 \times 10^{+5}$	3.85×10^{-5}	2.7×10^{-10}	$1.31 \times 10^{+5}$	4.60×10^{-5}	3.5×10^{-10}

h1A11-B5	$1.30 \times 10^{+5}$	1.34×10^{-4}	1.0×10^{-9}	$1.17 \times 10^{+5}$	1.78×10^{-4}	1.5×10^{-9}
h1A11.A2	$1.42 \times 10^{+5}$	4.21×10^{-4}	3.0×10^{-9}	$1.34 \times 10^{+5}$	5.27×10^{-4}	3.9×10^{-9}
h1A11-B4	$1.57 \times 10^{+5}$	8.23×10^{-4}	5.2×10^{-9}	$1.43 \times 10^{+5}$	9.90×10^{-4}	6.9×10^{-9}
h1A11.A7	$1.70 \times 10^{+5}$	9.73×10^{-4}	5.7×10^{-9}	$1.58 \times 10^{+5}$	1.24×10^{-3}	7.8×10^{-9}
h1A11.A12	$1.73 \times 10^{+5}$	1.32×10^{-3}	7.6×10^{-9}	$1.58 \times 10^{+5}$	1.62×10^{-3}	1.0×10^{-8}
h1A11.1	$1.58 \times 10^{+5}$	2.12×10^{-3}	1.3×10^{-8}	$1.44 \times 10^{+5}$	2.55×10^{-3}	1.8×10^{-8}

Table 25. Biacore Kinetics of Affinity-Matured Humanized 1A11.1 Antibodies Binding to Mouse and Rat DLL4.

Clone	Kinetics on Biacore					
	Mouse DLL4			Rat DLL4		
	ka ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)	*ka* ($M^{-1}s^{-1}$)	*kd* (s^{-1})	K_D (nM)
h1A11.A6	$1.98 \times 10^{+5}$	3.12×10^{-5}	1.6×10^{-10}	$1.29 \times 10^{+5}$	7.72×10^{-4}	6.0×10^{-9}
h1A11.C6	$2.03 \times 10^{+5}$	2.34×10^{-5}	1.2×10^{-10}	$1.69 \times 10^{+5}$	3.05×10^{-3}	1.8×10^{-8}
h1A11-G3	$1.17 \times 10^{+5}$	4.04×10^{-5}	3.5×10^{-10}	$1.18 \times 10^{+5}$	1.01×10^{-3}	8.6×10^{-9}
h1A11-F5	$1.43 \times 10^{+5}$	3.97×10^{-5}	2.8×10^{-10}	$1.16 \times 10^{+5}$	6.79×10^{-4}	5.8×10^{-9}
h1A11.A8	$1.87 \times 10^{+5}$	3.27×10^{-5}	1.8×10^{-10}	$1.39 \times 10^{+5}$	6.50×10^{-3}	4.7×10^{-8}
h1A11.A11	$1.98 \times 10^{+5}$	3.54×10^{-5}	1.8×10^{-10}	$1.16 \times 10^{+5}$	1.02×10^{-3}	8.8×10^{-9}
h1A11-E12	$1.56 \times 10^{+5}$	5.44×10^{-5}	3.5×10^{-10}	$1.08 \times 10^{+5}$	1.75×10^{-4}	1.6×10^{-9}
h1A11-H2	$1.54 \times 10^{+5}$	5.07×10^{-5}	3.3×10^{-10}	$1.78 \times 10^{+5}$	2.83×10^{-3}	1.6×10^{-8}
h1A11-B5	$1.45 \times 10^{+5}$	1.66×10^{-4}	1.2×10^{-9}	$9.82 \times 10^{+4}$	3.97×10^{-2}	4.1×10^{-7}
h1A11.A2	$1.81 \times 10^{+5}$	9.04×10^{-4}	5.0×10^{-9}	NB	NB	NB
h1A11-B4	$4.79 \times 10^{+5}$	2.51×10^{-3}	5.2×10^{-9}	NB	NB	NB
h1A11.A7	poor binding		1.3×10^{-8}	NB	NB	NB
h1A11.A12	poor binding		1.6×10^{-8}	NB	NB	NB

h1A11.1	1.56 x 10^{+5}	4.98 x 10^{-3}	3.2 x 10^{-8}	NB	NB	NB

NB = no observable binding

Table 26. *In Vitro* Activities Against Cell-Bound DLL4 of Affinity-Matured Humanized 1A11.1 Antibodies.

	Direct Binding to DLL4 cells, FACS (nM)		**Inhibition of Notch Activation via DLL4 cells, Notch reporter assay (nM)**	
	Human DLL4	Mouse DLL4	Human DLL4	Mouse DLL4
h1A11.A6	2.227	0.636	0.746	1.168
h1A11.C6	2.452	0.517	0.894	1.188
h1A11-G3	3.592	1.397	1.845	2.353
h1A11-F5	1.171	0.460	0.484	0.649
h1A11.A8	3.160	0.744	1.331	1.247
h1A11.A11	2.480	0.500	0.904	1.175
h1A11-E12	0.996	1.615	0.208	0.266
h1A11-H2	1.977	0.420	0.856	0.586
h1A11.A2	2.375	0.634	3.681	0.854
h1A11-B4	2.145	0.665	3.280	1.079
h1A11.A7	2.174	0.625	2.920	1.788
h1A11.A12	1.768	0.568	1.662	0.832

Example 9. Molecular Identity and Physicochemical Properties of Rat Hybridoma Antibodies.

The identity of monoclonal antibodies specific to DLL4 was determined by mass spectrometry as described below.

Mass Spectrometry Analysis of h1A11.1

The light chain molecular weight of 23,501 Daltons matched well with the theoretical value. The heavy chain molecular weights matched well with the theoretical values. The observed molecular weights were 50,190 Daltons; 50,352 Daltons; and 50,514 Daltons, with the difference corresponding to 162 Daltons as the result of different glycosylation.

Mass Spectrometry Analysis of h38H12.11

The light chain molecular weight of 23,408 Daltons matched well with the theoretical value. The heavy chain molecular weights matched well with the theoretical values. The observed molecular weights were 50,368 Dalton; 50,530 Daltons; and 50,692 Daltons; with the difference corresponding to 162 Daltons as the result of different glycosylation.

The solubilities of the antibodies were estimated by polyethylene glycol (PEG) 3000 precipitation. They were also directly determined, i.e., real solubility, by concentrating the antibodies in a specific solution and/or buffer with Amicon centrifugal filters and then observed for any precipitation at 25°C and 5°C. Stability was inferred by near ultra-violet circular (UV-CD) and differential scanning calorimetry (DSC). Stability to freezing and thawing and at elevated temperatures (accelerated stability) was assessed by size exclusion chromatography (SEC). The details of the techniques were described in Example 1.7, and the results are described below.

<u>Real Solubility Screening Results for 1A11</u>

For a series of 1A11 clones, including hu1A11.1, hu1A11.3, hu1A11.9, hu1A11.11, and 1A11 recombinant, 2 mg of each were concentrated with Amicon centrifugal filters to above 60 mg/ml. No precipitation or cloudiness was observed at 25°C or after storage for 1 day at 5°C. The concentrations of each were 63 mg/ml for 1A11.1, 76 mg/ml for 1A11.3, 63 mg/ml for 1A11.9, 69 mg/ml for 1A11.11, and 76 mg/ml for chimeric 1A11.

Example 10. Anti-DLL4 Antibody Epitope Grouping by Biacore Technology.

Epitope grouping was performed with the use of Biacore 2000, 3000, and T100 instruments. Antibodies of interest were directly immobilized on the CM5 chip surface via amino coupling. Flow cell one with similarly immobilized irrelevant IgG served as a reference surface. First, immobilized monoclonal antibodies (mAbs) were allowed to bind recombinant antigen (at concentrations of at least 200 nM) for 120 seconds at 50 μl/min. Then, another antibody was injected at 50 μl/ml for 120-240 seconds to monitor its ability to bind to the antigen that is already bound to the immobilized mAbs. The absence of additional binding response on the sensogram constituted overlap in the epitopes of the two mAbs (the one immobilized on the chip and the one introduced in liquid phase). Orientation of the assay was then switched in such a way that the antibody that was in a liquid phase was immobilized and *vice versa*. The pairs of mAbs that did not allow for additional antibody binding in both orientations of the assay were grouped as truly overlapping in these experiments. The resulting grouping of mAbs with overlapping epitopes is shown in Table 27, below.

Table 27. Anti-DLL4 Antibody Epitope Grouping By BIAcore Technology.

Immobilized antibody	**1st Injection**	**2nd Injection**						
	huDLL4	**38H12**	**15D6**	**13E4**	**1A11**	**14G1**	**14A11**	**37D10**
38H12	+	–	+	+	+	+	+	+
15D6	+	+	–	–	–	–	–	
13E4	+	+	–	–	–	+	–	
1A11	+	+	–	–	–	–	–	
14G1	+	+	+	+	+	–	–	–

14A11	+	+	–	–	–	–	–	

"+" indicates binding; "-" indicates no binding

Example 11. Activities of DLL4 Antibodies in Endothelial Cell Sprouting Assay *In Vitro*.

Fibrin gel beads sprouting assay was carried out to examine the *in vitro* angiogenesis activity of HUVEC (passage 2-3, Lonza) as described (Nakatsu, M. N. et al. 2003 Microvasc. Res. 66, 102–112). Briefly, fibrinogen solution was reconstituted with aprotinin (4 units/ml) and thrombin (50 units/ml). Cytodex 3 beads (Amersham Pharmacia Biotech) were coated with 350–400 HUVECs per bead for over night. About 20 HUVEC-coated beads were imbedded in the fibrin clot per well of a 96-well tissue culture plate. Conditioned medium derived from normal human fibroblasts (NHLF, Lonza) at 80% confluence was plated on top of the gel. DLL4 antibody and control antibody KLH at 15 μg/ml were added onto the well. At day 10 and 12, images were taken with inverted microscope and Nikon CCD camera. Table 28 summarizes the activities of some DLL4 antibodies to enhance endothelial cell sprouting *in vitro*. (Nakatsu et al., "Angiogenic sprouting and capillary lumen formation modeled by human umbilical vein endothelial cells (HUVEC) in fibrin gels: the role of fibroblasts and Angiopoietin-1," *Microvasc. Res.* 66, 102–112 (2003)).

Table 28. Activities of DLL4 antibodies to stimulate endothelial cell sprouting.

Tested antibody	Stimulate HUVEC sprouting
38H12 rat mAb	Yes
1A11 rat mAb	Yes
h1A11.1	Yes
40B10 rat mAb	Not observed
32C7 rat mAb	Not observed

Example 12. Rodent PK Assessment of Hybridoma-Derived Antibodies.

To assess pharmacokinetics properties of anti-DLL4 antibodies, SCID-Beige mice (n=3 per antibody) were administered a single intraperitoneal (IP) dose of antibody at either 1, 5, 10, or 30 mg/kg concentration, depending on cross-reactivity of antibody to murine DLL4. Longitudinal serum samples (5 μl of whole blood diluted 1:50 in HBS-EP+ buffer per time point) were collected from each animal over 21 days. Serum concentrations were determined using a DLL4-specific Biacore platform. Briefly, human DLL4 was immobilized to a sensorchip and samples were injected over the flowcell at 5 μl per minute for 5 minutes with the resulting binding levels measured and compared to standards. Serum concentration time profiles were used to estimate the pharmacokinetic parameters of C_{max} (peak serum concentration), CL (clearance), and $t_{1/2}$ (antibody half-life), summarized in Table 29, below.

Table 29. Pharmacokinetic Parameters of Anti-DLL4 Antibodies in SCID-Beige Mice Following a Single IP Dose.

Antibody	Dose (mg/kg)	Cmax (μg/mL)	CL (mL/hr/kg)	$t_{1/2}$ (d)
38H12 rat mAb	5	30.2	0.3	20-29
h1A11.1	30	163	0.44	11.3
h1A11.1	10	49.9	0.50	9.9
h1A11.1	5	11.0	1.78	6.3
h1A11.1	1	3.1	2.16	4.4

Example 13. DLL4 Antibody Treatment Inhibited Tumor Growth *In Vivo*.

The effect of anti-DLL4 antibodies on tumor growth was evaluated on subcutaneous Calu-6 xenograft tumors implanted in SCID-Beige mice. Briefly, 2 x 10^6 cells were inoculated subcutaneously into the right hind flank of female SCID-Beige mice. Tumors were allowed to establish for 14-18 days, at which point tumor volume was determined using electronic caliper measurements. Tumor size was calculated using the formula: L x $W^2/2$. Mice were allocated into treatment groups (n = 10 per group) so that each cohort of animals had equivalent mean tumor volume prior to initiation of therapy (typically between 180 and 250 mm^3). Animals were then dosed intraperitoneally either twice a week for two weeks (total of 4 doses) or weekly for four weeks (total of 4 doses) with anti-DLL4 antibodies. Tumor volume was measured on average twice a week for the duration of the experiment until the mean tumor volume in each group reached an endpoint of ≥ 2,000 mm^3. Results are shown in Table 30, below.

Table 30. Efficacy of Anti-DLL4 Antibodies in the Calu-6 Human Non-Small Cell Lung Cancer Subcutaneous Xenograft Model.

Treatment	Dose, Route, Regimen	%T/C[a]	%ILS[b]
1A11 rat mAb	30 mg/kg, IP, 2X/weekX2	37**	89**
1A11 rat mAb	10 mg/kg, IP, 2X/weekX2	47**	39**
1A11 rat mAb	5 mg/kg, IP, 2X/weekX2	43**	57**
14A11 rat mAb	10 mg/kg, IP, 2X/weekX2	37**	57**
40B10 rat mAb	30 mg/kg, IP, 2X/weekX2	29**	89**
32C7 rat mAb	30 mg/kg, IP,	65*	28*

	2X/weekX2		
14A11 chimera	10 mg/kg, IP, q7dX4	32**	114**
15D6 chimera	10 mg/kg, IP, q7dX4	47**	57**
40B10 chimera	10 mg/kg, IP, q7dX4	43**	73**
32C7 chimera	10 mg/kg, IP, q7dX4	71*	18*
h1A11.1	10 mg/kg, IP, q7dX4	34**	75**
h1A11.1	5 mg/kg, IP, q7dX4	31**	80**
h1A11.1	1 mg/kg, IP, q7dX4	43**	36**
h1A11.1	0.5 mg/kg, IP, q7dX4	62**	25*

[a] %T/C = mean tumor volume of treatment group/tumor volume of treatment control group x 100. P values (as indicated by asterisks) were derived from Student's T test comparison of treatment group versus treatment control group. Based on day 25/26/27 measurements.

[b] %ILS = (T - C)/C x 100, where T = median time to endpoint of treatment group and C = median time to endpoint of treatment control group. P values (as indicated by asterisks) were derived from Kaplan Meier log-rank comparison of treatment group versus treatment control group. Based on an endpoint of 2000 mm^3.

*p < 0.05; **p < 0.001

Incorporation by Reference

The contents of all cited references (including literature references, patents, patent applications, and websites) that maybe cited throughout this application are hereby expressly incorporated by reference in their entirety for any purpose, as are the references cited therein.

Equivalents

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 of the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are therefore intended to be embraced herein.

What is claimed is:

1. A binding protein capable of binding DLL4, comprising at least one amino acid sequence selected from the group of amino acid sequences consisting of SEQ ID NO:157, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:160, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:163, and SEQ ID NO:164.

2. A binding protein comprising an antigen binding domain wherein the binding protein is capable of binding human DLL4, said antigen binding domain comprising at least one CDR, wherein:

CDR-H1 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:151), wherein;

X_1 is N, H, or Y;

X_2 is F;

X_3 is P;

X_4 is M; and

X_5 is A or S;

residues 31-35 of SEQ ID NO:157 (CDR-H1 38H12);

residues 31-35 of SEQ ID NO:161 (CDR-H1 37D10);

residues 31-35 of SEQ ID NO:163 (CDR-H1 32C7);

residues 31-35 of SEQ ID NO:165 (CDR-H1 14G1);

residues 31-35 of SEQ ID NO:167 (CDR-H1 14A11);

residues 31-35 of SEQ ID NO:169 (CDR-H1 15D6);

residues 31-35 of SEQ ID NO:171 (CDR-H1 VH.1 1A11);

residues 31-35 of SEQ ID NO:172 (CDR-H1 VH.1a 1A11);

residues 31-35 of SEQ ID NO:173 (CDR-H1 VH.1b 1A11);

residues 31-35 of SEQ ID NO:174 (CDR-H1 VH.2a 1A11);

residues 31-35 of SEQ ID NO:179 (CDR-H1 VH.1 38H12);

residues 31-35 of SEQ ID NO:180 (CDR-H1 VH.1A 38H12);

residues 31-35 of SEQ ID NO:181 (CDR-H1 VH.1b 38H12);

residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);

residues 31-35 of SEQ ID NO:187 (CDR-H1 h1A11VH.1);

residues 31-35 of SEQ ID NO:188 (CDR-H1 h1A11.A6);

residues 31-35 of SEQ ID NO:189 (CDR-H1 h1A11.A8);

residues 31-35 of SEQ ID NO:190 (CDR-H1 h1A11.C6);

residues 31-35 of SEQ ID NO:191 (CDR-H1 h1A11.A11);

residues 31-35 of SEQ ID NO:192 (CDR-H1 h1A11.B5);

residues 31-35 of SEQ ID NO:193 (CDR-H1 h1A11.E12);

residues 31-35 of SEQ ID NO:194 (CDR-H1 h1A11.G3);

residues 31-35 of SEQ ID NO:195 (CDR-H1 h1A11.F5); and

residues 31-35 of SEQ ID NO:196 (CDR-H1 h1A11.H2);

CDR-H2 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17} (SEQ ID NO:152), wherein;

X_1 is T or S;

X_2 is I;

X_3 is S;

X_4 is S or G;

X_5 is S;

X_6 is D;

X_7 is G, A, D, S, or E;

X_8 is T or W;

X_9 is T, P, or A;

X_{10} is Y, S, T, or N;

X_{11} is Y or I;

X_{12} is R or G;

X_{13} is D;

X_{14} is S;

X_{15} is V;

X_{16} is K; and

X_{17} is G;

residues 50-66 of SEQ ID NO:157 (CDR-H2 38H12);

residues 50-68 of SEQ ID NO:161 (CDR-H2 37D10);

residues 50-66 of SEQ ID NO:163 (CDR-H2 32C7);

residues 50-66 of SEQ ID NO:165 (CDR-H2 14G1);

residues 50-66 of SEQ ID NO:167 (CDR-H2 14A11);

residues 50-66 of SEQ ID NO:169 (CDR-H2 15D6);

residues 50-66 of SEQ ID NO:171 (CDR-H2 VH.1 1A11);

residues 50-66 of SEQ ID NO:172 (CDR-H2 VH.1a 1A11);

residues 50-66 of SEQ ID NO:173 (CDR-H2 VH.1b 1A11);

residues 50-66 of SEQ ID NO:174 (CDR-H2 VH.2a 1A11);

residues 50-66 of SEQ ID NO:179 (CDR-H2 VH.1 38H12);

residues 50-66 of SEQ ID NO:180 (CDR-H2 VH.1A 38H12);

residues 50-66 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12);

residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);

residues 50-66 of SEQ ID NO:187 (CDR-H2 h1A11VH.1);
residues 50-66 of SEQ ID NO:188 (CDR-H2 h1A11.A6);
residues 50-66 of SEQ ID NO:189 (CDR-H2 h1A11.A8);
residues 50-66 of SEQ ID NO:190 (CDR-H2 h1A11.C6);
residues 50-66 of SEQ ID NO:191 (CDR-H2 h1A11.A11);
residues 50-66 of SEQ ID NO:192 (CDR-H2 h1A11.B5);
residues 50-66 of SEQ ID NO:193 (CDR-H2 h1A11.E12);
residues 50-66 of SEQ ID NO:194 (CDR-H2 h1A11.G3);
residues 50-66 of SEQ ID NO:195 (CDR-H2 h1A11.F5); and
residues 50-66 of SEQ ID NO:196 (CDR-H2 h1A11.H2);

CDR-H3 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:153), wherein;

X_1 is G;
X_2 is Y;
X_3 is Y;
X_4 is N;
X_5 is S;
X_6 is P;
X_7 is F;
X_8 is A; and
X_9 is Y, F, or S;

residues 99-107 of SEQ ID NO:157 (CDR-H3 38H12);
residues 101-111 of SEQ ID NO:161 (CDR-H3 37D10);
residues 99-105 of SEQ ID NO:163 (CDR-H3 32C7);
residues 99-105 of SEQ ID NO:165 (CDR-H3 14G1);
residues 99-110 of SEQ ID NO:167 (CDR-H3 14A11);
residues 99-110 of SEQ ID NO:169 (CDR-H3 15D6);
residues 99-107 of SEQ ID NO:171 (CDR-H3 VH.1 1A11);
residues 99-107 of SEQ ID NO:172 (CDR-H3 VH.1a 1A11);
residues 99-107 of SEQ ID NO:173 (CDR-H3 VH.1b 1A11);
residues 99-107 of SEQ ID NO:174 (CDR-H3 VH.2a 1A11);
residues 99-107 of SEQ ID NO:179 (CDR-H3 VH.1 38H12);
residues 99-107 of SEQ ID NO:180 (CDR-H3 VH.1A 38H12);
residues 99-107 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12);
residues 99-107 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12);
residues 99-107 of SEQ ID NO:187 (CDR-H3 h1A11VH.1);
residues 99-107 of SEQ ID NO:188 (CDR-H3 h1A11.A6);

residues 99-107 of SEQ ID NO:189 (CDR-H3 h1A11.A8);
residues 99-107 of SEQ ID NO:190 (CDR-H3 h1A11.C6);
residues 99-107 of SEQ ID NO:191 (CDR-H3 h1A11.A11);
residues 99-107 of SEQ ID NO:192 (CDR-H3 h1A11.B5);
residues 99-107 of SEQ ID NO:193 (CDR-H3 h1A11.E12);
residues 99-107 of SEQ ID NO:194 (CDR-H3 h1A11.G3);
residues 99-107 of SEQ ID NO:195 (CDR-H3 h1A11.F5);
and
residues 99-107 of SEQ ID NO:196 (CDR-H3 h1A11.H2);

CDR-L1 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11} (SEQ ID NO:154), wherein;

X_1 is R;
X_2 is A;
X_3 is S;
X_4 is E or Q;
X_5 is D or E;
X_6 is I;
X_7 is Y or W;
X_8 is S, I, Y, N, or R;
X_9 is N;
X_{10} is L; and
X_{11} is A;

residues 24-34 of SEQ ID NO:158 (CDR-L1 38H12);
residues 24-34 of SEQ ID NO:162 (CDR-L1 37D10);
residues 24-34 of SEQ ID NO:164 (CDR-L1 32C7);
residues 24-34 of SEQ ID NO:166 (CDR-L1 14G1);
residues 23-37 of SEQ ID NO:168 (CDR-L1 14A11);
residues 23-37 of SEQ ID NO:170 (CDR-L1 15D6);
residues 24-34 of SEQ ID NO:175 (CDR-L1 VL.1 1A11);
residues 24-34 of SEQ ID NO:176 (CDR-L1 VL.1a 1A11);
residues 24-34 of SEQ ID NO:177 (CDR-L1 VL.1b 1A11);
residues 24-34 of SEQ ID NO:178 (CDR-L1 VL.2a 1A11);
residues 24-34 of SEQ ID NO:183 (CDR-L1 VL.1 38H12);
residues 24-34 of SEQ ID NO:184 (CDR-L1 VL.1a 38H12);
residues 24-34 of SEQ ID NO:185 (CDR-L1 VL.1b 38H12);
residues 24-34 of SEQ ID NO:186 (CDR-L1 VL.2a 38H12);

residues 24-34 of SEQ ID NO:197 (CDR-L1 h1A11VL.1);

residues 24-34 of SEQ ID NO:198 (CDR-L1 h1A11.A2);

residues 24-34 of SEQ ID NO:199 (CDR-L1 h1A11.A12);

residues 24-34 of SEQ ID NO:200 (CDR-L1 h1A11.A7);

residues 24-34 of SEQ ID NO:201 (CDR-L1 h1A11.B4);

residues 24-34 of SEQ ID NO:202 (CDR-L1 h1A11.B5); and

residues 24-34 of SEQ ID NO:203 (CDR-L1 h1A11.E12);

CDR-L2 is selected from group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:155), wherein;

X_1 is D;

X_2 is T;

X_3 is N or S;

X_4 is N, D, S, I, Y, or V;

X_5 is L;

X_6 is A; and

X_7 is D;

residues 50-56 of SEQ ID NO:158 (CDR-L2 38H12);

residues 50-56 of SEQ ID NO:162 (CDR-L2 37D10);

residues 50-56 of SEQ ID NO:164 (CDR-L2 32C7);

residues 50-56 of SEQ ID NO:166 (CDR-L2 14G1);

residues 53-59 of SEQ ID NO:168 (CDR-L2 14A11);

residues 53-59 of SEQ ID NO:170 (CDR-L2 15D6);

residues 50-56 of SEQ ID NO:175 (CDR-L2 VL.1 1A11);

residues 50-56 of SEQ ID NO:176 (CDR-L2 VL.1a 1A11);

residues 50-56 of SEQ ID NO:177 (CDR-L2 VL.1b 1A11);

residues 50-56 of SEQ ID NO:178 (CDR-L2 VL.2a 1A11);

residues 50-56 of SEQ ID NO:183 (CDR-L2 VL.1 38H12);

residues 50-56 of SEQ ID NO:184 (CDR-L2 VL.1a 38H12);

residues 50-56 of SEQ ID NO:185 (CDR-L2 VL.1b 38H12);

residues 50-56 of SEQ ID NO:186 (CDR-L2 VL.2a 38H12);

residues 50-56 of SEQ ID NO:197 (CDR-L2 h1A11VL.1);

residues 50-56 of SEQ ID NO:198 (CDR-L2 h1A11.A2);

residues 50-56 of SEQ ID NO:199 (CDR-L2 h1A11.A12);

residues 50-56 of SEQ ID NO:200 (CDR-L2 h1A11.A7);

residues 50-56 of SEQ ID NO:201 (CDR-L2 h1A11.B4);

residues 50-56 of SEQ ID NO:202 (CDR-L2 h1A11.B5); and

residues 50-56 of SEQ ID NO:203 (CDR-L2 h1A11.E12);

and

CDR-L3 is selected from the group consisting of:

X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:156), wherein;

X_1 is Q;
X_2 is Q;
X_3 is Y;
X_4 is N, D, or T;
X_5 is N, Y, or W;
X_6 is Y or V;
X_7 is P;
X_8 is P; and
X_9 is T.

residues 89-97 of SEQ ID NO:158 (CDR-L3 38H12);
residues 89-97 of SEQ ID NO:162 (CDR-L3 37D10);
residues 89-97 of SEQ ID NO:164 (CDR-L3 32C7);
residues 89-98 of SEQ ID NO:166 (CDR-L3 14G1);
residues 92-100 of SEQ ID NO:168 (CDR-L3 14A11);
residues 92-100 of SEQ ID NO:170 (CDR-L3 15D6);
residues 89-97 of SEQ ID NO:175 (CDR-L3 VL.1 1A11);
residues 89-97 of SEQ ID NO:176 (CDR-L3 VL.1a 1A11);
residues 89-97 of SEQ ID NO:177 (CDR-L3 VL.1b 1A11);
residues 89-97 of SEQ ID NO:178 (CDR-L3 VL.2a 1A11);
residues 89-97 of SEQ ID NO:183 (CDR-L3 VL.1 38H12);
residues 89-97 of SEQ ID NO:184 (CDR-L3 VL.1a 38H12);
residues 89-97 of SEQ ID NO:185 (CDR-L3 VL.1b 38H12);
residues 89-97 of SEQ ID NO:186 (CDR-L3 VL.2a 38H12);
residues 89-97 of SEQ ID NO:197 (CDR-L3 h1A11VL.1);
residues 89-97 of SEQ ID NO:198 (CDR-L3 h1A11.A2);
residues 89-97 of SEQ ID NO:199 (CDR-L3 h1A11.A12);
residues 89-97 of SEQ ID NO:200 (CDR-L3 h1A11.A7);
residues 89-97 of SEQ ID NO:201 (CDR-L3 h1A11.B4);
residues 89-97 of SEQ ID NO:202 (CDR-L3 h1A11.B5); and
residues 89-97 of SEQ ID NO:203 (CDR-L3 h1A11.E12).

3. The binding protein according to claim 2, wherein said at least one CDR comprises an amino acid sequence selected from the group consisting of:

residues 31-35 of SEQ ID NO:157 (CDR-H1 38H12); residues 50-66 of SEQ ID NO:157 (CDR-H2 38H12); residues 99-107 of SEQ ID NO:157 (CDR-H3 38H12); residues 24-34 of SEQ ID NO:158 (CDR-L1 38H12); residues 50-56 of SEQ ID NO:158 (CDR-L2 38H12); residues 89-97 of SEQ ID NO:158 (CDR-L3 38H12); residues 31-35 of SEQ ID NO:159 (CDR-H1 1A11); residues 50-66 of SEQ ID NO:159 (CDR-H2 1A11); residues 99-107 of SEQ ID NO:159 (CDR-H3 1A11); residues 24-34 of SEQ ID NO:160 (CDR-L1 1A11); residues 50-56 of SEQ ID NO:160 (CDR-L2 1A11); residues 89-97 of SEQ ID NO:160 (CDR-L3 1A11); residues 31-35 of SEQ ID NO:161 (CDR-H1 37D10); residues 50-68 of SEQ ID NO:161 (CDR-H2 37D10); residues 101-111 of SEQ ID NO:161 (CDR-H3 37D10); residues 24-34 of SEQ ID NO:162 (CDR-L1 37D10); residues 50-56 of SEQ ID NO:162 (CDR-L2 37D10); residues 89-97 of SEQ ID NO:162 (CDR-L3 37D10); residues 31-35 of SEQ ID NO:163 (CDR-H1 32C7); residues 50-66 of SEQ ID NO:163 (CDR-H2 32C7); residues 99-105 of SEQ ID NO:163 (CDR-H3 32C7); residues 24-34 of SEQ ID NO:164 (CDR-L1 32C7); residues 50-56 of SEQ ID NO:164 (CDR-L2 32C7); residues 89-98 of SEQ ID NO:164 (CDR-L3 32C7); residues 31-35 of SEQ ID NO:165 (CDR-H1 14G1); residues 50-66 of SEQ ID NO:165 (CDR-H2 14G1); residues 99-105 of SEQ ID NO:165 (CDR-H3 14G1); residues 24-34 of SEQ ID NO:166 (CDR-L1 14G1); residues 50-56 of SEQ ID NO:166 (CDR-L2 14G1); residues 89-98 of SEQ ID NO:166 (CDR-L3 14G1); residues 31-35 of SEQ ID NO:167 (CDR-H1 14A11); residues 50-66 of SEQ ID NO:167 (CDR-H2 14A11); residues 99-110 of SEQ ID NO:167 (CDR-H3 14A11); residues 23-37 of SEQ ID NO:168 (CDR-L1 14A11); residues 53-59 of SEQ ID NO:168 (CDR-L2 14A11); residues 92-100 of SEQ ID NO:168 (CDR-L3 14A11); residues 31-35 of SEQ ID NO:169 (CDR-H1 15D6); residues 50-66 of SEQ ID NO:169 (CDR-H2 15D6); residues 99-110 of SEQ ID NO:169 (CDR-H3 15D6); residues 23-37 of SEQ ID NO:170 (CDR-L1 15D6); residues 53-59 of SEQ ID NO:170 (CDR-L2 15D6); residues 92-100 of SEQ ID NO:170 (CDR-L3 15D6); residues 31-35 of SEQ ID NO:171 (CDR-H1 VH.1 1A11); residues 50-66 of SEQ ID NO:171 (CDR-H2 VH.1 1A11); residues 99-107 of SEQ ID NO:171 (CDR-H3 VH.1 1A11); residues 31-35 of SEQ ID NO:172 (CDR-H1 VH.1a 1A11); residues 50-66 of SEQ ID NO:172 (CDR-H2 VH.1a 1A11); residues 99-107 of SEQ ID NO:172 (CDR-H3 VH.1a 1A11); residues 31-35 of SEQ ID NO:173 (CDR-H1 VH.1b 1A11); residues 50-66 of SEQ ID NO:173 (CDR-H2 VH.1b 1A11); residues 99-107 of SEQ ID NO:173 (CDR-H3 VH.1b 1A11); residues 31-35 of SEQ ID NO:174 (CDR-H1 VH.2a 1A11); residues 50-66 of SEQ ID NO:174 (CDR-H2 VH.2a 1A11); residues 99-107 of SEQ ID NO:174 (CDR-H3 VH.2a 1A11); residues 24-34 of SEQ ID

NO:175 (CDR-L1 VL.1 1A11); residues 50-56 of SEQ ID NO:175 (CDR-L2 VL.1 1A11); residues 89-97 of SEQ ID NO:175 (CDR-L3 VL.1 1A11); residues 24-34 of SEQ ID NO:176 (CDR-L1 VL.1a 1A11); residues 50-56 of SEQ ID NO:176 (CDR-L2 VL.1a 1A11); residues 89-97 of SEQ ID NO:176 (CDR-L3 VL.1a 1A11); residues 24-34 of SEQ ID NO:177 (CDR-L1 VL.1b 1A11); residues 50-56 of SEQ ID NO:177 (CDR-L2 VL.1b 1A11); residues 89-97 of SEQ ID NO:177 (CDR-L3 VL.1b 1A11); residues 24-34 of SEQ ID NO:178 (CDR-L1 VL.2a 1A11); residues 50-56 of SEQ ID NO:178 (CDR-L2 VL.2a 1A11); residues 89-97 of SEQ ID NO:178 (CDR-L3 VL.2a 1A11); residues 31-35 of SEQ ID NO:179 (CDR-H1 VH.1 38H12); residues 50-66 of SEQ ID NO:179 (CDR-H2 VH.1 38H12); residues 99-107 of SEQ ID NO:179 (CDR-H3 VH.1 38H12); residues 31-35 of SEQ ID NO:180 (CDR-H1 VH.1A 38H12); residues 50-66 of SEQ ID NO:180 (CDR-H2 VH.1A 38H12); residues 99-107 of SEQ ID NO:180 (CDR-H3 VH.1A 38H12); residues 31-35 of SEQ ID NO:181 (CDR-H1 VH.1b 38H12); residues 50-66 of SEQ ID NO:181 (CDR-H2 VH.1b 38H12); residues 99-107 of SEQ ID NO:181 (CDR-H3 VH.1b 38H12); residues 31-35 of SEQ ID NO:182 (CDR-H1 VH.2a 38H12); residues 50-66 of SEQ ID NO:182 (CDR-H2 VH.2a 38H12); residues 99-107 of SEQ ID NO:182 (CDR-H3 VH.2a 38H12); residues 24-34 of SEQ ID NO:183 (CDR-L1 VL.1 38H12); residues 50-56 of SEQ ID NO:183 (CDR-L2 VL.1 38H12); residues 89-97 of SEQ ID NO:183 (CDR-L3 VL.1 38H12); residues 24-34 of SEQ ID NO:184 (CDR-L1 VL.1a 38H12); residues 50-56 of SEQ ID NO:184 (CDR-L2 VL.1a 38H12); residues 89-97 of SEQ ID NO:184 (CDR-L3 VL.1a 38H12); residues 24-34 of SEQ ID NO:185 (CDR-L1 VL.1b 38H12); residues 50-56 of SEQ ID NO:185 (CDR-L2 VL.1b 38H12); residues 89-97 of SEQ ID NO:185 (CDR-L3 VL.1b 38H12); residues 24-34 of SEQ ID NO:186 (CDR-L1 VL.2a 38H12); residues 50-56 of SEQ ID NO:186 (CDR-L2 VL.2a 38H12); residues 89-97 of SEQ ID NO:186 (CDR-L3 VL.2a 38H12); residues 31-35 of SEQ ID NO:187 (CDR-H1 h1A11VH.1), residues 50-66 of SEQ ID NO:187 (CDR-H2 h1A11VH.1); residues 99-107 of SEQ ID NO:187 (CDR-H3 h1A11VH.1); residues 31-35 of SEQ ID NO:188 (CDR-H1 h1A11.A6), residues 50-66 of SEQ ID NO:188 (CDR-H2 h1A11.A6); residues 99-107 of SEQ ID NO:188 (CDR-H3 h1A11.A6); residues 31-35 of SEQ ID NO:189 (CDR-H1 h1A11.A8), residues 50-66 of SEQ ID NO:189 (CDR-H2 h1A11.A8); residues 99-107 of SEQ ID NO:189 (CDR-H3 h1A11.A8); residues 31-35 of SEQ ID NO:190 (CDR-H1 h1A11.C6), residues 50-66 of SEQ ID NO:190 (CDR-H2 h1A11.C6); residues 99-107 of SEQ ID NO:190 (CDR-H3 h1A11.C6); residues 31-35 of SEQ ID NO:191 (CDR-H1 h1A11.A11), residues 50-66 of SEQ ID NO:191 (CDR- H2 h1A11.A11); residues 99-107 of SEQ ID NO:191 (CDR-H3 h1A11.A11); residues 31-35 of SEQ ID NO:192 (CDR-H1 h1A11.B5), residues 50-66 of SEQ ID NO:192 (CDR-H2 h1A11.B5); residues 99-107 of SEQ ID NO:192 (CDR-H3 h1A11.B5); residues 31-35 of SEQ ID NO:193 (CDR-H1 h1A11.E12), residues 50-66 of SEQ ID NO:193 (CDR-H2 h1A11.E12); residues 99-107 of SEQ ID NO:193 (CDR-H3 h1A11.E12); residues 31-35 of SEQ ID NO:194 (CDR-H1 h1A11.G3), residues 50-66 of SEQ ID NO:194 (CDR-H2 h1A11.G3); residues 99-107 of SEQ ID NO:194 (CDR-H3 h1A11.G3); residues 31-35 of SEQ ID NO:195 (CDR-H1 h1A11.F5), residues 50-66 of SEQ ID NO:195 (CDR-H2 h1A11.F5); residues 99-107 of SEQ ID NO:195 (CDR-H3 h1A11.F5); residues 31-35 of SEQ ID NO:196 (CDR-H1 h1A11.H2), residues 50-66 of SEQ ID NO:196 (CDR-H2 h1A11.H2); residues 99-107 of SEQ ID NO:196 (CDR-H3 h1A11.H2); residues 24-34 of SEQ ID NO:197 (CDR-L1 h1A11VL.1), residues 50-56 of SEQ ID NO:197 (CDR-L2 h1A11VL.1); residues 89-97 of SEQ ID NO:197 (CDR-L3 h1A11VL.1); residues 24-34 of SEQ ID NO:198 (CDR-L1 h1A11.A2), residues 50-56 of SEQ ID NO:198 (CDR-L2 h1A11.A2); residues 89-97 of SEQ ID NO:198 (CDR-L3 h1A11.A2); residues 24-34 of SEQ ID NO:199 (CDR-L1 h1A11.A12), residues 50-56 of SEQ ID NO:199 (CDR-L2 h1A11.A12); residues 89-97 of SEQ ID NO:199 (CDR-L3 h1A11.A12); residues 24-34 of SEQ ID NO:200 (CDR-L1 h1A11.A7), residues 50-56 of SEQ ID NO:200 (CDR-L2 h1A11.A7); residues 89-97 of SEQ ID NO:200 (CDR-L3 h1A11.A7); residues 24-34 of SEQ ID NO:201 (CDR-L1 h1A11.B4), residues 50-56 of SEQ ID NO:201 (CDR-L2 h1A11.B4); residues 89-97 of SEQ ID NO:201 (CDR-L3 h1A11.B4); residues 24-34 of SEQ ID NO:202 (CDR-L1 h1A11.B5), residues 50-56 of SEQ ID NO:202 (CDR-L2 h1A11.B5); residues 89-97 of SEQ ID NO:202 (CDR-L3 h1A11.B5); residues 24-34 of SEQ ID NO:203 (CDR-L1 h1A11.E12), residues 50-56 of SEQ ID NO:203 (CDR-L2 h1A11.E12); and residues 89-97 of SEQ ID NO:203 (CDR-L3 h1A11.E12).

4. The binding protein according to claim 2, wherein said binding protein comprises at least three CDRs.

5. The binding protein according to claim 4, wherein said at least three CDRs are selected from a variable domain CDR set selected from the group consisting of:

VH 38H12 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:157
CDR-H2: residues 50-66 of SEQ ID NO:157
CDR-H3 residues 99-107 of SEQ ID NO:157
VL 38H12 CDR Set
CDR-L1: residues 24-34 of SEQ ID NO:158

CDR-L2: residues 50-56 of SEQ ID NO:158

CDR-L3: residues 89-97 of SEQ ID NO:158

VH 1A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:159

CDR-H2: residues 50-66 of SEQ ID NO:159

CDR-H3: residues 99-107 of SEQ ID NO:159

VL 1A11 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:160

CDR-L2: residues 50-56 of SEQ ID NO:160

CDR-L3: residues 89-97 of SEQ ID NO:160

VH 37D10 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:161

CDR-H2: residues 50-68 of SEQ ID NO:161

CDR-H3: residues 101-111 of SEQ ID NO:161

VL 37D10 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:162

CDR-L2: residues 50-56 of SEQ ID NO:162

CDR-L3: residues 89-97 of SEQ ID NO:162

VH 32C7 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:163

CDR-H2: residues 50-66 of SEQ ID NO:163

CDR-H3: residues 99-105 of SEQ ID NO:163

VL 32C7 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:164

CDR-L2: residues 50-56 of SEQ ID NO:164

CDR-L3: residues 89-98 of SEQ ID NO:164

VH 14G1 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:165

CDR-H2: residues 50-66 of SEQ ID NO:165

CDR-H3: residues 99-105 of SEQ ID NO:165

VL 14G1 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:166

CDR-L2: residues 50-56 of SEQ ID NO:166

CDR-L3: residues 89-97 of SEQ ID NO:166

VH 14A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:167

CDR-H2: residues 50-66 of SEQ ID NO:167

CDR-H3: residues 99-110 of SEQ ID NO:167

VL 14A11 CDR Set

CDR-L1: residues 23-37 of SEQ ID NO:168

CDR-L2: residues 53-59 of SEQ ID NO:168

CDR-L3: residues 92-100 of SEQ ID NO:168

VH 15D6 CDR Set

CDR-H1: residues 31-35 o f SEQ ID NO:169

CDR-H2: residues 50-66 of SEQ ID NO:169

CDR-H3: residues 99-110 of SEQ ID NO:169

VL 15D6 CDR Set

CDR-L1: residues 23-37 of SEQ ID NO:170

CDR-L2: residues 53-59 of SEQ ID NO:170

CDR-L3: residues 92-100 of SEQ ID NO:170

VH VH.1 1A11 CDR Set

CDR-H1: residues 31-35 o f SEQ ID NO:171

CDR-H2: residues 50-66 of SEQ ID NO:171

CDR-H3: residues 99-107 of SEQ ID NO:171

VH VH.1a 1A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:172

CDR-H2: residues 50-66 of SEQ ID NO:172

CDR-H3: residues 99-107 of SEQ ID NO:172

VH VH.1b 1A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:173

CDR-H2: residues 50-66 of SEQ ID NO:173

CDR-H3: residues 99-107 of SEQ ID NO:173

VH VH.2a 1A11 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:174

CDR-H2: residues 50-66 of SEQ ID NO:174

CDR-H3: residues 99-107 of SEQ ID NO:174

VL VL.1 1A11 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:175

CDR-L2: residues 50-56 of SEQ ID NO:175

CDR-L3: residues 89-97 of SEQ ID NO:175

VL VL.1a 1A11 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:176

CDR-L2: residues 50-56 of SEQ ID NO:176

CDR-L3: residues 89-97 of SEQ ID NO:176

VL VL.1b 1A11 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:177

CDR-L2: residues 50-56 of SEQ ID NO:177

CDR-L3: residues 89-97 of SEQ ID NO:177

VL VL.2a 1A11 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:178

CDR-L2: residues 50-56 of SEQ ID NO:178

CDR-L3: residues 89-97 of SEQ ID NO:178

VH VH.1 38H12 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:179

CDR-H2: residues 50-66 of SEQ ID NO:179

CDR-H3: residues 99-107 of SEQ ID NO:179

VH VH.1a 38H12 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:180

CDR-H2: residues 50-66 of SEQ ID NO:180

CDR-H3: residues 99-107 of SEQ ID NO:180

VH VH.1b 38H12 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:181

CDR-H2: residues 50-66 of SEQ ID NO:181

CDR-H3: residues 99-107 of SEQ ID NO:181

VH VH.2a 38H12 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:182

CDR-H2: residues 50-66 of SEQ ID NO:182

CDR-H3: residues 99-107 of SEQ ID NO:182

VL VL.1 38H12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:183

CDR-L2: residues 50-56 of SEQ ID NO:183

CDR-L3: residues 89-97 of SEQ ID NO:183

VL VL.1a 38H12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:184

CDR-L2: residues 50-56 of SEQ ID NO:184

CDR-L3: residues 89-97 of SEQ ID NO:184

VL VL.1b 38H12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:185

CDR-L2: residues 50-56 of SEQ ID NO:185

CDR-L3: residues 89-97 of SEQ ID NO:185

VL VL.2a 38H12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:186
CDR-L2: residues 50-56 of SEQ ID NO:186
CDR-L3: residues 89-97 of SEQ ID NO:186

VH hA11VH.1 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:187
CDR-H2: residues 50-66 of SEQ ID NO:187
CDR-H3: residues 99-107 of SEQ ID NO:187

VH hA11.A6 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:188
CDR-H2: residues 50-66 of SEQ ID NO:188
CDR-H3: residues 99-107 of SEQ ID NO:188

VH hA11.A8 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:189
CDR-H2: residues 50-66 of SEQ ID NO:189
CDR-H3: residues 99-107 of SEQ ID NO:189

VH hA11.C6 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:190
CDR-H2: residues 50-66 of SEQ ID NO:190
CDR-H3: residues 99-107 of SEQ ID NO:190

VH hA11.A11 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:191
CDR-H2: residues 50-66 of SEQ ID NO:191
CDR-H3: residues 99-107 of SEQ ID NO:191

VH hA11.B5 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:192
CDR-H2: residues 50-66 of SEQ ID NO:192
CDR-H3: residues 99-107 of SEQ ID NO:192

VH hA11.E12 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:193
CDR-H2: residues 50-66 of SEQ ID NO:193
CDR-H3: residues 99-107 of SEQ ID NO:193

VH hA11.G3 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:194
CDR-H2: residues 50-66 of SEQ ID NO:194
CDR-H3: residues 99-107 of SEQ ID NO:194

VH hA11.F5 CDR Set
CDR-H1: residues 31-35 of SEQ ID NO:195

CDR-H2: residues 50-66 of SEQ ID NO:195

CDR-H3: residues 99-107 of SEQ ID NO:195

VH hA11.H2 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:196

CDR-H2: residues 50-66 of SEQ ID NO:196

CDR-H3: residues 99-107 of SEQ ID NO:196

VL h1A11VL.1 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:197

CDR-L2: residues 50-56 of SEQ ID NO:197

CDR-L3: residues 89-97 of SEQ ID NO:197

VL h1A11.A2 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:198

CDR-L2: residues 50-56 of SEQ ID NO:198

CDR-L3: residues 89-97 of SEQ ID NO:198

VL h1A11.A12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:199

CDR-L2: residues 50-56 of SEQ ID NO:199

CDR-L3: residues 89-97 of SEQ ID NO:199

VL h1A11.A7 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:200

CDR-L2: residues 50-56 of SEQ ID NO:200

CDR-L3: residues 89-97 of SEQ ID NO:200

VL h1A11.B4 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:201

CDR-L2: residues 50-56 of SEQ ID NO:201

CDR-L3: residues 89-97 of SEQ ID NO:201

VL h1A11.B5 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:202

CDR-L2: residues 50-56 of SEQ ID NO:202

CDR-L3: residues 89-97 of SEQ ID NO:202

and

VL h1A11.E12 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:203

CDR-L2: residues 50-56 of SEQ ID NO:203

CDR-L3: residues 89-97 of SEQ ID NO:.203

6. The binding protein according to claim 5, comprising CDRs from at least two variable domain CDR sets.

7. The binding protein according to claim 6, wherein said at least two variable domain CDR sets are a pair of CDR sets selected from the group consisting of:
VH 38H12 CDR Set and VL 38H12 CDR Set,
VH 1A11 CDR Set and VL 1A11 CDR Set,
VH 37D10 CDR Set and VL 37D10 CDR Set,
VH 32C7 CDR Set and VL 32C7 CDR Set,
VH 14G1 Set and VL 14G1 CDR Set,
VH 14A11 CDR Set and VL 14A11 CDR Set,
VH 15D6 CDR Set and VL 15D6 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.1 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.1a 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.1b 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.1 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.1a 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.1b 1A11 CDR Set,
VH VH.2a 1A11 CDR Set and VL VL.2a 1A11 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.1 38H12 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.1 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.1 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.1a 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH VH.1b 38H12 CDR Set and VL VL.1 38H12 CDR Set,

VH VH.1b 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.1b 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.1b 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.1 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.1a 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.1b 38H12 CDR Set,
VH VH.2a 38H12 CDR Set and VL VL.2a 38H12 CDR Set,
VH h1A11.A6 CDR Set and VL h1A11VL.1 CDR Set,
VH h1A11.C6 CDR Set and VL h1A11VL.1 CDR Set,
VH h1A11.A11 CDR Set and VL h1A11VL.1 CDR Set,
VH h1A11.A8 CDR Set and VL h1A11VL.1 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.B4 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.A7 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.A12 CDR Set,
VH h1A11VH.1 CDR Set and VL h1A11.A2 CDR Set,
VH h1A11.B5 CDR Set and VL h1A11.B5 CDR Set,
VH h1A11.E12 CDR Set and VL h1A11.E12 CDR Set,
VH h1A11.G3 CDR Set and VL h1A11.E12 CDR Set,
VH h1A11.F5 CDR Set and VL h1A11.E12 CDR Set, and
VH h1A11.H2 CDR Set and VL h1A11.E12 CDR Set.

8. The binding protein according to claim 4, further comprising a human acceptor framework sequence.

9. The binding protein according to claim 5, further comprising a human acceptor framework sequence.

10. The binding protein according to claim 6, further comprising a human acceptor framework sequence.

11. The binding protein according to claim 7, further comprising a human acceptor framework sequence.

12. The binding protein according to claim 8, wherein said human acceptor framework sequence is selected from the group of acceptor sequences listed in Tables 3 and 4.

13. The binding protein according to claim 9, wherein said human acceptor framework sequence is selected from the group of acceptor sequences listed in Tables 3 and 4.

14. The binding protein according to claim 10, wherein said human acceptor framework sequence is selected from the group of acceptor sequences listed in Tables 3 and 4

15. The binding protein according to claim 11, wherein said human acceptor framework sequence is selected from the group of acceptor sequences listed in Tables 3 and 4.

16. The binding protein according to claim 8, wherein said human acceptor framework sequence is selected from any framework sequence in a variable region sequence selected from the group consisting of:

SEQ ID NO:171 VH.1 1A11	SEQ ID NO:180 VH.1a 38H12	SEQ ID NO:188 VH h1A11.A6	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:172 VH.1a 1A11	SEQ ID NO:181 VH.1b 38H12	SEQ ID NO:189 VH h1A11.A8	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:173 VH.1b 1A11	SEQ ID NO:182 VH.2a 38H12	SEQ ID NO:190 VH h1A11.C6	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:174 VH.2a 1A11	SEQ ID NO:183 VL.1 38H12	SEQ ID NO:191 VH h1A11.A11	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:175 VL.1 1A11	SEQ ID NO:184 VL.1a	SEQ ID NO:192 VH h1A11.B5	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:176 VL.1a 1A11	SEQ ID NO:185 VL.1b	SEQ ID NO:193 VH h1A11.E12	SEQ ID NO:201 VL h1A11.B4
SEQ ID NO:177 VL.1b 1A11	SEQ ID NO:186 VL.2a	SEQ ID NO:194 VH h1A11.G3	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:178 VL.2a 1A11	SEQ ID NO:187 VH h1A11VH.1	SEQ ID NO:195 VH h1A11.F5	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:179 VH.1 38H12			

17. The binding protein according to claim 9, wherein said human acceptor framework sequence is selected from any framework sequence in a variable region sequence selected from the group consisting of:

SEQ ID NO:171 VH.1 1A11	SEQ ID NO:180 VH.1a 38H12	SEQ ID NO:188 VH h1A11.A6	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:172 VH.1a 1A11	SEQ ID NO:181 VH.1b 38H12	SEQ ID NO:189 VH h1A11.A8	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:173 VH.1b 1A11	SEQ ID NO:182 VH.2a 38H12	SEQ ID NO:190 VH h1A11.C6	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:174 VH.2a 1A11	SEQ ID NO:183 VL.1 38H12	SEQ ID NO:191 VH h1A11.A11	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:175 VL.1 1A11	SEQ ID NO:184 VL.1a	SEQ ID NO:192 VH h1A11.B5	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:176 VL.1a 1A11	SEQ ID NO:185 VL.1b	SEQ ID NO:193 VH h1A11.E12	SEQ ID NO:201 VL h1A11.B4

SEQ ID NO:177 VL.1b 1A11	SEQ ID NO:186 VL.2a	SEQ ID NO:194 VH h1A11.G3	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:178 VL.2a 1A11	SEQ ID NO:187 VH h1A11VH.1	SEQ ID NO:195 VH h1A11.F5	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:179 VH.1 38H12			

18. The binding protein according to claim 10, wherein said human acceptor framework sequence is selected from any framework sequence in a variable region sequence selected from the group consisting of:

SEQ ID NO:171 VH.1 1A11	SEQ ID NO:180 VH.1a 38H12	SEQ ID NO:188 VH h1A11.A6	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:172 VH.1a 1A11	SEQ ID NO:181 VH.1b 38H12	SEQ ID NO:189 VH h1A11.A8	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:173 VH.1b 1A11	SEQ ID NO:182 VH.2a 38H12	SEQ ID NO:190 VH h1A11.C6	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:174 VH.2a 1A11	SEQ ID NO:183 VL.1 38H12	SEQ ID NO:191 VH h1A11.A11	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:175 VL.1 1A11	SEQ ID NO:184 VL.1a	SEQ ID NO:192 VH h1A11.B5	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:176 VL.1a 1A11	SEQ ID NO:185 VL.1b	SEQ ID NO:193 VH h1A11.E12	SEQ ID NO:201 VL h1A11.B4
SEQ ID NO:177 VL.1b 1A11	SEQ ID NO:186 VL.2a	SEQ ID NO:194 VH h1A11.G3	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:178 VL.2a 1A11	SEQ ID NO:187 VH h1A11VH.1	SEQ ID NO:195 VH h1A11.F5	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:179 VH.1 38H12			

19. The binding protein according to claim 11, wherein said human acceptor framework sequence is selected from any framework sequence in a variable region sequence selected from the group consisting of:

SEQ ID NO:171 VH.1 1A11	SEQ ID NO:180 VH.1a 38H12	SEQ ID NO:188 VH h1A11.A6	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:172 VH.1a 1A11	SEQ ID NO:181 VH.1b 38H12	SEQ ID NO:189 VH h1A11.A8	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:173 VH.1b 1A11	SEQ ID NO:182 VH.2a 38H12	SEQ ID NO:190 VH h1A11.C6	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:174 VH.2a 1A11	SEQ ID NO:183 VL.1 38H12	SEQ ID NO:191 VH h1A11.A11	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:175 VL.1 1A11	SEQ ID NO:184 VL.1a	SEQ ID NO:192 VH h1A11.B5	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:176 VL.1a 1A11	SEQ ID NO:185 VL.1b	SEQ ID NO:193 VH h1A11.E12	SEQ ID NO:201 VL h1A11.B4
SEQ ID NO:177 VL.1b 1A11	SEQ ID NO:186 VL.2a	SEQ ID NO:194 VH h1A11.G3	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:178 VL.2a 1A11	SEQ ID NO:187 VH h1A11VH.1	SEQ ID NO:195 VH h1A11.F5	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:179 VH.1 38H12			

20. The binding protein according to claim 8, wherein said binding protein comprises at least one acceptor framework sequence selected from the group consisting of:
heavy chain framework-1 (H-FR1):
E-V-Q-L-V-E-S-G-G-G-L-V-Q-P-G-G-S-L-R-L-S-C-A-A-S-G-F-T-F-X_{30} (SEQ ID NO:143), wherein X_{30} is S, R, or G;
heavy chain framework-2 (H-FR2): W-V-R-Q-A-P-G-K-G-L-E-W-V-A (SEQ ID NO:144);
heavy chain framework-3 (H-FR3):
R-F-T-I-S-R-D-N-A-K-X_{11}-S-L-Y-L-Q-M-N-S-L-R-A-E-D-T-A-V-Y-Y-C-X_{31}-R (SEQ ID NO:145), wherein;
X_{11} is N or S; and
X_{31} is A or S;
heavy chain framework-4 (H-FR4): W-G-Q-G-T-L-V-T-V-S-S (SEQ ID NO:146);
light chain framework-1 (L-FR1):
D-I-Q-M-T-Q-S-P-S-S-L-S-A-S-V-G-D-R-V-T-I-T-C (SEQ ID NO:147);
light chain framework-2 (L-FR2): W-Y-Q-Q-K-P-G-K-X_9-P-K-L-L-I-X_{15} (SEQ ID NO:148), wherein;
X_9 is A or S; and
X_{15} is F or Y;
light chain framework-3 (L-FR3):
G-V-P-S-R-F-S-G-S-G-S-G-T-D-X_{15}-T-L-T-I-S-S-L-Q-P-E-D-F-A-T-Y-Y-C (SEQ ID NO:149), wherein;
X_{15} is F or S; and
light chain framework-4 (L-FR4): F-G-Q-G-T-K-L-E-I-K (SEQ ID NO:150).

21. The binding protein according to claim 9, wherein said binding protein comprises at least one acceptor framework sequence selected from the group consisting of:
heavy chain framework-1 (H-FR1):
E-V-Q-L-V-E-S-G-G-G-L-V-Q-P-G-G-S-L-R-L-S-C-A-A-S-G-F-T-F-X_{30} (SEQ ID NO:143), wherein X_{30} is S, R, or G;
heavy chain framework-2 (H-FR2): W-V-R-Q-A-P-G-K-G-L-E-W-V-A (SEQ ID NO:144);
heavy chain framework-3 (H-FR3):
R-F-T-I-S-R-D-N-A-K-X_{11}-S-L-Y-L-Q-M-N-S-L-R-A-E-D-T-A-V-Y-Y-C-X_{31}-R (SEQ ID NO:145), wherein;
X_{11} is N or S; and

X_{31} is A or S;
heavy chain framework-4 (H-FR4): W-G-Q-G-T-L-V-T-V-S-S (SEQ ID NO:146);
light chain framework-1 (L-FR1):
D-I-Q-M-T-Q-S-P-S-S-L-S-A-S-V-G-D-R-V-T-I-T-C (SEQ ID NO:147);
light chain framework-2 (L-FR2): W-Y-Q-Q-K-P-G-K-X_9-P-K-L-L-I-X_{15} (SEQ ID NO:148), wherein;
X_9 is A or S; and
X_{15} is F or Y;
light chain framework-3 (L-FR3):
G-V-P-S-R-F-S-G-S-G-S-G-T-D-X_{15}-T-L-T-I-S-S-L-Q-P-E-D-F-A-T-Y-Y-C (SEQ ID NO:149), wherein;
X_{15} is F or S; and
light chain framework-4 (L-FR4): F-G-Q-G-T-K-L-E-I-K (SEQ ID NO:150).

22. The binding protein according to claim 10, wherein said binding protein comprises at least one acceptor framework sequence selected from the group consisting of:
heavy chain framework-1 (H-FR1):
E-V-Q-L-V-E-S-G-G-G-L-V-Q-P-G-G-S-L-R-L-S-C-A-A-S-G-F-T-F-X_{30} (SEQ ID NO:143), wherein X_{30} is S, R, or G;
heavy chain framework-2 (H-FR2): W-V-R-Q-A-P-G-K-G-L-E-W-V-A (SEQ ID NO:144);
heavy chain framework-3 (H-FR3):
R-F-T-I-S-R-D-N-A-K-X_{11}-S-L-Y-L-Q-M-N-S-L-R-A-E-D-T-A-V-Y-Y-C-X_{31}-R (SEQ ID NO:145), wherein;
X_{11} is N or S; and
X_{31} is A or S;
heavy chain framework-4 (H-FR4): W-G-Q-G-T-L-V-T-V-S-S (SEQ ID NO:146);
light chain framework-1 (L-FR1):
D-I-Q-M-T-Q-S-P-S-S-L-S-A-S-V-G-D-R-V-T-I-T-C (SEQ ID NO:147);
light chain framework-2 (L-FR2): W-Y-Q-Q-K-P-G-K-X_9-P-K-L-L-I-X_{15} (SEQ ID NO:148), wherein;
X_9 is A or S; and
X_{15} is F or Y;
light chain framework-3 (L-FR3):
G-V-P-S-R-F-S-G-S-G-S-G-T-D-X_{15}-T-L-T-I-S-S-L-Q-P-E-D-F-A-T-Y-Y-C (SEQ ID NO:149), wherein;
X_{15} is F or S; and

light chain framework-4 (L-FR4): F-G-Q-G-T-K-L-E-I-K (SEQ ID NO:150).

23. The binding protein according to claim 11, wherein said binding protein comprises at least one acceptor framework sequence selected from the group consisting of:
heavy chain framework-1 (H-FR1):
E-V-Q-L-V-E-S-G-G-G-L-V-Q-P-G-G-S-L-R-L-S-C-A-A-S-G-F-T-F-X_{30} (SEQ ID NO:143), wherein X_{30} is S, R, or G;
heavy chain framework-2 (H-FR2): W-V-R-Q-A-P-G-K-G-L-E-W-V-A (SEQ ID NO:144);
heavy chain framework-3 (H-FR3):
R-F-T-I-S-R-D-N-A-K-X_{11}-S-L-Y-L-Q-M-N-S-L-R-A-E-D-T-A-V-Y-Y-C-X_{31}-R (SEQ ID NO:145), wherein;
X_{11} is N or S; and
X_{31} is A or S;
heavy chain framework-4 (H-FR4): W-G-Q-G-T-L-V-T-V-S-S (SEQ ID NO:146);
light chain framework-1 (L-FR1):
D-I-Q-M-T-Q-S-P-S-S-L-S-A-S-V-G-D-R-V-T-I-T-C (SEQ ID NO:147);
light chain framework-2 (L-FR2): W-Y-Q-Q-K-P-G-K-X_9-P-K-L-L-I-X_{15} (SEQ ID NO:148), wherein;
X_9 is A or S; and
X_{15} is F or Y;
light chain framework-3 (L-FR3):
G-V-P-S-R-F-S-G-S-G-S-G-T-D-X_{15}-T-L-T-I-S-S-L-Q-P-E-D-F-A-T-Y-Y-C (SEQ ID NO:149), wherein;
X_{15} is F or S; and
light chain framework-4 (L-FR4): F-G-Q-G-T-K-L-E-I-K (SEQ ID NO:150).

24. The binding protein according to claim 2, wherein said binding protein comprises a human acceptor framework sequence and at least one variable domain having an amino acid sequence selected from the group consisting of:

SEQ ID NO:171 VH VH.1 1A11	SEQ ID NO:188 VH h1A11.A6
SEQ ID NO:172 VH VH.1a 1A11	SEQ ID NO:189 VH h1A11.A8
SEQ ID NO:173 VH VH.1b 1A11	SEQ ID NO:190 VH h1A11.C6
SEQ ID NO:174 VH VH.2a 1A11	SEQ ID NO:191 VH h1A11.A11

SEQ ID NO:175 VL VL.1 1A11	SEQ ID NO:192 VH h1A11.B5
SEQ ID NO:176 VL VL.1a 1A11	SEQ ID NO:193 VH h1A11.E12
SEQ ID NO:177 VL VL.1b	SEQ ID NO:194 VH h1A11.G3
SEQ ID NO:178 VL VL.2a 1A11	SEQ ID NO:195 VH h1A11.F5
SEQ ID NO:179 VH VH.1 38H12	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:180 VH VH.1a 38H12	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:181 VH VH.1b 38H12	SEQ ID NO:198 VL h1A11.A2
SEQ ID NO:182 VH VH.2a 38H12	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:183 VL VL.1 38H12	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:184 VL VL.1a 38H12	SEQ ID NO:201 VL h1A11.B4
SEQ ID NO:185 VL VL.1b 38H12	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:186 VL VL.2a 38H12	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:187 VH h1A11VH.1	

25. The binding protein according to claim 24, wherein said binding protein comprises two variable domains, wherein said two variable domains have amino acid sequences selected from the group consisting of:

SEQ ID NO:171 and SEQ ID NO:175 VH.1 and VL.1 1A11 (Table 12)	SEQ ID NO:180 and SEQ ID NO:186 VH.1a and VL.2a (Table 16)
SEQ ID NO:171 and SEQ ID NO:176 VH.1 and VL.1a 1A11 (Table 12)	SEQ ID NO:181 and SEQ ID NO:183 VH.1b and VL.1 (Table 16)
SEQ ID NO:171 and SEQ ID NO:177 VH.1 and VL.1b 1A11 (Table 12)	SEQ ID NO:181 and SEQ ID NO:184 VH.1b and VL.1a (Table 16)
SEQ ID NO:171 and SEQ ID NO:178 VH.1 and VL.2a 1A11(Table 12)	SEQ ID NO:181 and SEQ ID NO:185 VH.1b and VL.1b (Table 16)
SEQ ID NO:172 and SEQ ID NO:175 VH.1a and VL.1 1A11 (Table 12)	SEQ ID NO:181 and SEQ ID NO:186 VH.1b and VL.2a (Table 16)
SEQ ID NO:172 and SEQ ID NO:176 VH.1a and VL.1a 1A11 (Table	SEQ ID NO:182 and SEQ ID NO:183 VH.2a and VL.1 (Table 16)

12)	
SEQ ID NO:172 and SEQ ID NO:177 VH.1a and VL.1b 1A11 (Table 12)	SEQ ID NO:182 and SEQ ID NO:184 VH.2a and VL.1a (Table 16)
SEQ ID NO:172 and SEQ ID NO:178 VH.1a and VL.2a 1A11 (Table 12)	SEQ ID NO:182 and SEQ ID NO:185 VH.2a and VL.1b (Table 16)
SEQ ID NO:173 and SEQ ID NO:175 VH.1b and VL.1 1A11 (Table 12)	SEQ ID NO:182 and SEQ ID NO:186 VH.2a and VL.2a (Table 16)
SEQ ID NO:173 and SEQ ID NO:176 VH.1b and VL.1a 1A11 (Table 12)	SEQ ID NO:188 and SEQ ID NO:197 h1A11.A6 VH and h1A11VL.1 Tables 20/21
SEQ ID NO:173 and SEQ ID NO:177 VH.1b and VL.1b 1A11 (Table 12)	SEQ ID NO:190 and SEQ ID NO:197 h1A11.C6 VH and h1A11VL.1 Tables 20/21
SEQ ID NO:173 and SEQ ID NO:178 VH.1b and VL.2a 1A11 (Table 12)	SEQ ID NO:191 and SEQ ID NO:197 h1A11.All VH and h1A11VL.1 Tables 20/21
SEQ ID NO:174 and SEQ ID NO:175 VH.2a and VL.1 1A11 (Table 12)	SEQ ID NO:189 and SEQ ID NO:197 h1A11.A8 VH and h1A11 VL.1 Tables 20/21
SEQ ID NO:174 and SEQ ID NO:176 VH.2a and VL.1a 1A11 (Table 12)	SEQ ID NO:187 and SEQ ID NO:201 h1A11VH.1 and h1A11.B4 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:177 VH.2a and VL.1b 1A11 (Table 12)	SEQ ID NO:187 and SEQ ID NO:200 h1A11VH.1 and h1All.A7 VL Tables 20/21
SEQ ID NO:174 and SEQ ID NO:178 VH.2a and VL.2a 1A11 (Table 12)	SEQ ID NO:187 and SEQ ID NO:199 h1A11VH.1 and h1All.A12 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:183 VH.1 and VL.1 38H12 (Table 16)	SEQ ID NO:187 and SEQ ID NO:198 h1A11VH.1 VH and h1All.A2 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:184 VH.1 and VL.1a 38H12 (Table 16)	SEQ ID NO:192 and SEQ ID NO:202 h1A11.B5 VH and h1All.B5 VL Tables 20/21
SEQ ID NO:179 and SEQ ID NO:185 VH.1 and VL.1b 38H12 (Table 16)	SEQ ID NO:193 and SEQ ID NO:203 h1A11.E12 VH and h1All.E12 VL Tables 20/21

SEQ ID NO:179 and SEQ ID NO:186 VH.1 and VL.2a 38H12 (Table 16)	SEQ ID NO:194 and SEQ ID NO:203 h1A11.G3 VH and h1A11.E12 VL Tables 20/21
SEQ ID NO:180 and SEQ ID NO:183 VH.1a and VL.1 38H12 (Table 16)	SEQ ID NO:195 and SEQ ID NO:203 h1A11.F5 VH and h1A11.E12 VL Tables 20/21
SEQ ID NO:180 and SEQ ID NO:184 VH.1a and VL.1a 38H12 (Table 16)	SEQ ID NO:196 and SEQ ID NO:203 h1A11.H2 VH and h1A11.E12 VL Tables 20/21
SEQ ID NO:180 and SEQ ID NO:185 VH.1a and VL.1b 38H12 (Table 16)	

26. The binding protein according to claim 8, wherein said human acceptor framework sequence comprises at least one Framework Region amino acid substitution at a key residue, said key residue selected from the group consisting of:
a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with human DLL4;
a residue capable of interacting with a CDR;
a canonical residue;
a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone; and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

27. The binding protein according to claim 11, wherein said human acceptor framework sequence comprises at least one Framework Region amino acid substitution at a key residue, said key residue selected from the group consisting of:
a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with human DLL4;
a residue capable of interacting with a CDR;
a canonical residue;

a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone; and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

28. The binding protein according to claim 25, wherein said human acceptor framework sequence comprises at least one Framework Region amino acid substitution at a key residue, said key residue selected from the group consisting of:
a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with human DLL4;
a residue capable of interacting with a CDR;
a canonical residue;
a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone; and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

29. The binding protein according to claim 26, wherein the binding protein comprises a consensus human variable domain sequence.

30. The binding protein according to claim 27, wherein the binding protein comprises a consensus human variable domain sequence.

31. The binding protein according to claim 28, wherein the binding protein comprises a consensus human variable domain sequence.

32. The binding protein according to claim 8, wherein said human acceptor framework comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to a sequence of a human germline acceptor framework and comprises at least 70 amino acid residues identical to the human germline acceptor framework.

33. The binding protein according to claim 11, wherein said human acceptor framework comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to a sequence of a human germline

acceptor framework and comprises at least 70 amino acid residues identical to the human germline acceptor framework.

34. The binding protein according to claim 25, wherein said human acceptor framework comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to a sequence of a human germline acceptor framework and comprises at least 70 amino acid residues identical to the human germline acceptor framework.

35. The binding protein according to claim 2, wherein said binding protein comprises at least one variable domain having an amino acid sequence selected from the group consisting of:

SEQ ID NO:157 VH 38H12	SEQ ID NO:181 VH VH.1b 38H12
SEQ ID NO:158 VL 38H12	SEQ ID NO:182 VH VH.2a 38H12
SEQ ID NO:159 VH 1A11	SEQ ID NO:182 VL VL.1 38H12
SEQ ID NO:160 VL 1A11	SEQ ID NO:184 VL VL.1a 38H12
SEQ ID NO:161 VH 37D10	SEQ ID NO:185 VL VL.1b 38H12
SEQ ID NO:162 VL 37D10	SEQ ID NO:186 VL VL.2a 38H12
SEQ ID NO:163 VH 32C7	SEQ ID NO:187 VH h1A11VH.1
SEQ ID NO:164 VL 32C7	SEQ ID NO:188 VH h1A11.A6
SEQ ID NO:165 VH 14G1	SEQ ID NO:189 VH h1A11.A8
SEQ ID NO:166 VL 14G1	SEQ ID NO:190 VH h1A11.C6
SEQ ID NO:167 VH 14A11	SEQ ID NO:191 VH h1A11.A11
SEQ ID NO:168 VL 14A11	SEQ ID NO:192 VH h1A11.B5
SEQ ID NO:169 VH 15D6	SEQ ID NO:193 VH h1A11.E12
SEQ ID NO:170 VL 15D6	SEQ ID NO:194 VH h1A11.G3
SEQ ID NO:171 VH VH.1 1A11	SEQ ID NO:195 VH h1A11.F5
SEQ ID NO:172 VH VH.1a 1A11	SEQ ID NO:196 VH h1A11.H2
SEQ ID NO:173 VH VH.1b 1A11	SEQ ID NO:197 VL h1A11VL.1
SEQ ID NO:174	SEQ ID NO:198

VH VH.2a 1A11	VL h1A11.A2
SEQ ID NO:175 VL VL.1 1A11	SEQ ID NO:199 VL h1A11.A12
SEQ ID NO:176 VL VL.1a 1A11	SEQ ID NO:200 VL h1A11.A7
SEQ ID NO:177 VL VL.1b	SEQ ID NO:201 VL h1A11.B4
SEQ ID NO:178 VL VL.2a 1A11	SEQ ID NO:202 VL h1A11.B5
SEQ ID NO:179 VH VH.1 38H12	SEQ ID NO:203 VL h1A11.E12
SEQ ID NO:180 VH VH.1a 38H12	

36. The binding protein according to claim 35, wherein said binding protein comprises two variable domains, wherein said two variable domains have amino acid sequences selected from the group consisting of:

SEQ ID NO:157 and SEQ ID NO:158 38H12	SEQ ID NO:179 and SEQ ID NO:186 VH.1 and VL.2a 38H12
SEQ ID NO:159 and SEQ ID NO:160 1A11	SEQ ID NO:180 and SEQ ID NO:183 VH.1a and VL.1 38H12
SEQ ID NO:161 and SEQ ID NO:162 37D10	SEQ ID NO:180 and SEQ ID NO:184 VH.1a and VL.1a 38H12
SEQ ID NO:163 and SEQ ID NO:164 32C7	SEQ ID NO:180 and SEQ ID NO:185 VH.1a and VL.1b 38H12
SEQ ID NO:165 and SEQ ID NO:166 14G1	SEQ ID NO:180 and SEQ ID NO:186 VH.1a and VL.2a 38H12
SEQ ID NO:167 and SEQ ID NO:168 14A11	SEQ ID NO:181 and SEQ ID NO:183 VH.1b and VL.1 38H12
SEQ ID NO:169 and SEQ ID NO:170 15D6	SEQ ID NO:181 and SEQ ID NO:184 VH.1b and VL.1a 38H12
SEQ ID NO:171 and SEQ ID NO:175 VH.1 and VL.1 1A11 (Table 11)	SEQ ID NO:181 and SEQ ID NO:185 VH.1b and VL.1b 38H12
SEQ ID NO:171 and SEQ ID NO:176 VH.1 and VL.1a 1A11	SEQ ID NO:181 and SEQ ID NO:186 VH.1b and VL.2a 38H12
SEQ ID NO:171 and SEQ ID NO:177 VH.1 and VL.1b 1A11	SEQ ID NO:182 and SEQ ID NO:183 VH.2a and VL.1 38H12
SEQ ID NO:171 and SEQ ID NO:178 VH.1 and VL.2a	SEQ ID NO:182 and SEQ ID NO:184 VH.2a and VL.1a 38H12
SEQ ID NO:172 and SEQ ID	SEQ ID NO:182 and SEQ ID

NO:175 VH.1a and VL.1	NO:185 VH.2a and VL.1b 38H12
SEQ ID NO:172 and SEQ ID NO:176 VH.1a and VL.1a	SEQ ID NO:182 and SEQ ID NO:186 VH.2a and VL.2a 38H12
SEQ ID NO:172 and SEQ ID NO:177 VH.1a and VL.1b	SEQ ID NO:188 and SEQ ID NO:197 h1A11.A6 VH and h1A11VL.1
SEQ ID NO:172 and SEQ ID NO:178 VH.1a and VL.2a	SEQ ID NO:190 and SEQ ID NO:197 h1A11.C6 VH and h1A11VL.1
SEQ ID NO:173 and SEQ ID NO:175 VH.1b and VL.1	SEQ ID NO:191 and SEQ ID NO:197 h1A11.All VH and h1A11VL.1
SEQ ID NO:173 and SEQ ID NO:176 VH.1b and VL.1a	SEQ ID NO:189 and SEQ ID NO:197 h1A11.A8 VH and h1A11 VL.1
SEQ ID NO:173 and SEQ ID NO:177 VH.1b and VL.1b	SEQ ID NO:187 and SEQ ID NO:201 h1A11VH.1 and h1A11.B4 VL
SEQ ID NO:173 and SEQ ID NO:178 VH.1b and VL.2a	SEQ ID NO:187 and SEQ ID NO:200 h1A11VH.1 and h1All.A7 VL
SEQ ID NO:174 and SEQ ID NO:175 VH.2a and VL.1	SEQ ID NO:187 and SEQ ID NO:199 h1A11VH.1 and h1All.A12 VL
SEQ ID NO:174 and SEQ ID NO:176 VH.2a and VL.1a	SEQ ID NO:187 and SEQ ID NO:198 h1A11VH.1 VH and h1All.A2 VL
SEQ ID NO:174 and SEQ ID NO:177 VH.2a and VL.1b	SEQ ID NO:192 and SEQ ID NO:202 h1A11.B5 VH and h1All.B5 VL
SEQ ID NO:174 and SEQ ID NO:178 VH.2a and VL.2a	SEQ ID NO:193 and SEQ ID NO:203 h1A11.E12 VH and h1All.E12 VL
SEQ ID NO:179 and SEQ ID NO:183 VH.1 and VL.1 38H12	SEQ ID NO:194 and SEQ ID NO:203 h1A11.G3 VH and h1All.E12 VL
SEQ ID NO:179 and SEQ ID NO:184 VH.1 and VL.1a 38H12	SEQ ID NO:195 and SEQ ID NO:203 h1A11.F5 VH and h1All.E12 VL
SEQ ID NO:179 and SEQ ID NO:185 VH.1 and VL.1b 38H12	SEQ ID NO:196 and SEQ ID NO:203 h1A11.H2 VH and h1All.E12 VL

37. The DLL4 binding protein according to claim 2, wherein said binding protein is capable of blocking DLL4 interaction with a Notch protein.

38. The DLL4 binding protein according to claim 36, wherein the binding protein is capable of blocking DLL4 interaction with a Notch protein selected from the group consisting of Notch-1, Notch-2, Notch-3, Notch-4, and combinations thereof.

39. The DLL4 binding protein according to claim 2, wherein said binding protein is capable of modulating a biological function of DLL4.

40. The DLL4 binding protein according to claim 2, wherein said binding protein is capable of neutralizing a biological function of DLL4.

41. The DLL4 binding protein according to claim 2, wherein said binding protein is capable of inhibiting VEGFR2 activity, VEGFR1 activity, or both.

42. The DLL4 binding protein according to claim 2, wherein said binding protein is capable of diminishing the ability of DLL4 to bind to its receptor.

43. The DLL4 binding protein according to claim 2, wherein said binding protein is capable of inhibiting normal angiogenesis.

44. The DLL4 binding protein according to claim 2, wherein said binding protein has an on rate constant (Kon) to DLL4 selected from the group consisting of: at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; and at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

45. The DLL4 binding protein according to claim 8, wherein said binding protein has an on rate constant (Kon) to DLL4 selected from the group consisting of: at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; and at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

46. The DLL4 binding protein according to claim 24, wherein said binding protein has an on rate constant (Kon) to DLL4 selected from the group consisting of: at least about $10^2M^{-1}s^{-1}$; at least about $10^3M^{-1}s^{-1}$; at least about $10^4M^{-1}s^{-1}$; at least about $10^5M^{-1}s^{-1}$; and at least about $10^6M^{-1}s^{-1}$, as measured by surface plasmon resonance.

47. The DLL4 binding protein according to claim 2, wherein said binding protein has an off rate constant (Koff) to DLL4 selected from the group consisting of: at most about $10^{-3}s^{-1}$;

at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

48. The DLL4 binding protein according to claim 8, wherein said binding protein has an off rate constant (Koff) to DLL4 selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

49. The DLL4 binding protein according to claim 24, wherein said binding protein has an off rate constant (Koff) to DLL4 selected from the group consisting of: at most about $10^{-3}s^{-1}$; at most about $10^{-4}s^{-1}$; at most about $10^{-5}s^{-1}$; and at most about $10^{-6}s^{-1}$, as measured by surface plasmon resonance.

50. The DLL4 binding protein according to claim 2, wherein said binding protein has a dissociation constant (K_D) to DLL4 selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M.

51. The DLL4 binding protein according to claim 8, wherein said binding protein has a dissociation constant (K_D) to DLL4 selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M.

52. The DLL4 binding protein according to claim 24, wherein said binding protein has a dissociation constant (K_D) to DLL4 selected from the group consisting of: at most about 10^{-7} M; at most about 10^{-8} M; at most about 10^{-9} M; at most about 10^{-10} M; at most about 10^{-11} M; at most about 10^{-12} M; and at most 10^{-13} M.

53. An antibody construct comprising a binding protein described in claim 2, said antibody construct further comprising a linker polypeptide or an immunoglobulin constant domain.

54. The antibody construct according to claim 53, selected from the group consisting of:
    an immunoglobulin molecule,
    a monoclonal antibody,
    a chimeric antibody,
    a CDR-grafted antibody,
    a Fab,

a Fab',
a $F(ab')_2$,
an Fv,
a disulfide linked Fv,
an scFv,
a single domain antibody,
a diabody,
a multispecific antibody,
a dual specific antibody, and
a bispecific antibody.

55. The antibody construct according to claim 53, wherein said antibody construct comprises a heavy chain immunoglobulin constant domain selected from the group consisting of:
a human IgM constant domain,
a human IgG1 constant domain,
a human IgG2 constant domain,
a human IgG3 constant domain,
a human IgG4 constant domain,
a human IgE constant domain,
and
a human IgA constant domain.

56. The antibody construct according to claim 53, comprising an immunoglobulin constant domain having an amino acid sequence selected from the group consisting of:
SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and combinations thereof.

57. An antibody conjugate comprising an antibody construct as described in any one of claims 53-56, said antibody conjugate further comprising an agent selected from the group consisting of: an imaging agent, a therapeutic agent, a cytotoxic agent, and an immunoadhesion molecule.

58. The antibody conjugate according to claim 57, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

59. The antibody conjugate according to claim 57, wherein said imaging agent is a radiolabel selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm.

60. The antibody conjugate according to claim 57, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of: an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, a toxin, and an apoptotic agent.

61. The antibody construct according to claim 53, wherein said binding protein possesses a human glycosylation pattern.

62. The antibody conjugate according to claim 57, wherein said binding protein possesses a human glycosylation pattern.

63. The binding protein according to claim 4, wherein said binding protein exists as a crystal.

64. The antibody construct according to claim 53, wherein said antibody construct exists as a crystal.

65. The antibody conjugate according to claim 57, wherein said antibody construct exists as a crystal.

66. The binding protein according to claim 63, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

67. The antibody construct according to claim 64, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

68. The antibody conjugate according to claim 65, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

69. The binding protein according to claim 63, wherein said binding protein crystal has a greater half life *in vivo* than the soluble counterpart of the binding protein.

70. The antibody construct according to claim 64, wherein said antibody construct crystal has a greater half life *in vivo* than the soluble counterpart of the antibody construct.

71. The antibody conjugate according to claim 65, wherein said antibody conjugate crystal has a greater half life *in vivo* than the soluble counterpart of the antibody conjugate.

72. The binding protein according to claim 63, wherein said binding protein crystal retains biological activity of the non-crystal form of the binding protein.

73. The antibody construct according to claim 64, wherein said antibody construct crystal retains biological activity of the non-crystal form of the antibody construct.

74. The antibody conjugate according to claim 65, wherein said antibody conjugate crystal retains biological activity of the non-crystal form of the antibody conjugate.

75. An isolated nucleic acid encoding a binding protein amino acid sequence described in claim 2.

76. An isolated nucleic acid encoding a polypeptide selected from the group consisting of: a polypeptide comprising a heavy chain variable domain, wherein the heavy chain variable domain comprises one or more of a CDR-H1, a CDR-H2, and a CDR-H3 as described in claim 2; a polypeptide comprising a light chain variable domain, wherein the light chain variable domain comprises one or more of a CDR-L1, a CDR-L2, and a CDR-L3 as described in claim 2; and a combination of both polypeptides.

77. A vector comprising the isolated nucleic acid according to claim 76.

78. The vector according to claim 77, wherein the vector is selected from the group consisting of: pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, and pBJ.

79. A host cell comprising the vector described in claim 77.

80. The host cell according to claim 79, wherein said host cell is a prokaryotic cell.

81. The host cell according to claim 80, wherein said host cell is *Escherichia coli*.

82. The host cell according to claim 79, wherein said host cell is a eukaryotic cell.

83. The host cell according to claim 82, wherein said eukaryotic cell is selected from the group consisting of: a protist cell, an animal cell, a plant cell, and a fungal cell.

84. The host cell according to claim 83, wherein said eukaryotic cell is an animal cell selected from the group consisting of: a mammalian cell, an avian cell, and an insect cell.

85. The host cell according to claim 84, wherein said mammalian cell is a CHO cell.

86. The host cell according to claim 84, wherein said mammalian cell is a COS cell.

87. The host cell according to claim 83, wherein said fungal cell is *Saccharomyces cerevisiae*.

88. The host cell according to claim 84, wherein said insect cell is an Sf9 cell.

89. A method of producing a binding protein that binds human DLL4, comprising culturing the host cell as described in claim 79 in a culture medium under conditions sufficient to produce a binding protein that binds human DLL4.

90. A DLL4 binding protein produced according to the method of claim 89.

91. A composition for the release of a binding protein said composition comprising:
   (a) a formulation, wherein said formulation comprises a crystallized binding protein according as described in any one of claims 63-65, and an ingredient; and
   (b) at least one polymeric carrier.

92. The composition according to claim 93, wherein said polymeric carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone), poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride- alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polysaccharides, blends and copolymers thereof.

93. The composition according to claim 91, wherein said ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-β-cyclodextrin, methoxypolyethylene glycol and polyethylene glycol.

94. A method for treating a mammal comprising the step of administering to the mammal an effective amount of the composition as described in claim 91.

95. A pharmaceutical composition comprising a DLL4 binding protein as described in claim 2, and a pharmaceutically acceptable carrier.

96. The pharmaceutical composition of claim 95, further comprising at least one additional agent for treating a disorder in which DLL4 activity is detrimental.

97. The pharmaceutical composition of claim 95, wherein said additional agent is selected from the group consisting of: a therapeutic agent; an imaging agent; an antineoplastic agent; a chemotherapeutic agent; an angiogenesis inhibitor; an anti-VEGF antibody; an anti-EGFR antibody; an anti-cMet antibody; an anti-ErbB3 antibody; an anti-HER2 antibody; an anti-CD20 antibody; aflibercept; a kinase inhibitor; a co-stimulation molecule blocker; an anti-B7.2 antibody; a CTLA4-Ig; an adhesion molecule blocker; an anti-E selectin antibody; an anti-L selectin antibody; an anti-cytokine antibody or functional fragment thereof; an anti-IL-18 antibody; an anti-TNF antibody; anti-IL-6 antibody; methotrexate; a corticosteroid; a cyclosporin; a rapamycin; FK506; a DNA alkylating agent; cisplatin; carboplatin; an anti-tubulin agent; paclitaxel; docetaxel; doxorubicin; gemcitabine; gemzar; an anthracycline; adriamycin; a topoisiomersase I inhibitor; a topoisomerase II inhibitor; 5-fluorouracil (5-FU); leucovorin; irinotecan; a receptor tyrosine kinase inhibitor, an apoptosis inhibitor; a Bcl2/Bclx inhibitor; erlotinib, gefitinib, a COX-2 inhibitor, celecoxib, cyclosporin; rapamycin; a detectable label or reporter molecule; a TNF antagonist; an antirheumatic; a muscle relaxant; a narcotic; an analgesic; an anesthetic; a sedative; a local anesthetic; a neuromuscular blocker; an antimicrobial agent; an antipsoriatic agent; a corticosteroid; an anabolic steroid; an erythropoietin; an immunization; an immunoglobulin; an immunosuppressive agent; a growth hormone; a hormone replacement drug; a radiopharmaceutical drug; an antidepressant; an antipsychotic drug; a stimulant; an asthma medication; a beta agonist; an inhaled steroid; an epinephrine; an epinephrine analog thereof; a cytokine; and a cytokine antagonist.

98. A method for reducing human DLL4 activity comprising contacting human DLL4 with the binding protein as described in claim 2 such that human DLL4 activity is reduced.

99. A method for reducing human DLL4 activity in a human subject suffering from a disorder in which DLL4 activity is detrimental, comprising administering to the human subject a binding protein as described in claim 2 such that human DLL4 activity in the human subject is reduced.

100. A method for treating a subject for a disease or a disorder in which DLL4 activity is detrimental by administering to the subject a DLL4 binding protein as described in claim 2 such that treatment is achieved.

101. The method of claim 100, wherein said disorder is selected from the group consisting of: breast cancer, colon cancer, rectal cancer, lung cancer, oropharynx cancer, hypopharynx cancer, esophageal cancer, stomach cancer, pancreas cancer, liver cancer, gallbladder cancer, bile duct cancer, small intestine cancer, urinary tract cancer, female genital tract cancer, male genital tract cancer, endocrine gland cancer, skin cancer, hemangioma, melanoma, sarcoma, brain tumor, nerve cancer, eye tumor, meninges cancer, solid tumors from hematopoietic malignancy, tumor metastases, ocular neovascularization, edema, rheumatoid arthritis, atherosclerotic plaques, Crohn's disease, inflammatory bowel disease, refractory ascites, psoriasis, sarcoidosis, arterial arteriosclerosis, sepsis, peptic ulcers, burns, pancreatitis, polycystic ovarian disease (POD), endometriosis, uterine fibroid, benign prostate hypertrophy, T-cell acute lymphoblastic leukemia (T-ALL), cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), multiple sclerosis (MS), tetralogy of Fallot (TOF), Alagille syndrome (AS), macular degeneration and age-related macular degeneration diseases, and other angiogenesis independent and dependent diseases characterized by aberrant DLL4 activity.

102. The method according to Claim 101, wherein the disorder is a primary and metastatic cancer.

103. The method according to Claim 101, wherein the urinary tract cancer is selected from the group consisting of renal cancer, bladder cancer, and urothelium cancer.

104. The method according to Claim 101, wherein the female genital tract cancer is selected from the group consisting of cervical cancer, uterine cancer, ovarian cancer, choriocarcinoma, and gestational trophoblastic disease.

105. The method according to Claim 101, wherein the male genital tract cancer is selected from the group consisting of prostate cancer, seminal vesicles cancer, testicular cancer, and germ cell tumor.

106. The method according to Claim 101, wherein the endocrine gland cancer is selected from the group consisting of thyroid cancer, adrenal cancer, and pituitary gland cancer.

107. The method according to Claim 101, wherein the sarcoma is selected from the group consisting of a bone sarcoma, a soft tissue sarcoma, and Kaposi's sarcoma.

108. The method according to Claim 101, wherein the meninges cancer is selected from the group consisting of an astrocytoma, a glioma, a glioblastoma, a retinoblastoma, a neuroma, a neuroblastoma, a Schwannoma, and a meningioma.

109. The method according to Claim 101, wherein the solid tumor from a hematopoietic malignancy is a leukemia, a Hodgkin's leukemia, a non-Hodgkin's leukemia, a lymphoma, a Hodgkin's lymphoma, and a non-Hodgkin's lymphomas.

110. The method according to Claim 101, wherein the ocular neovascularization is selected from the group consisting of diabetic blindness, a retinopathy, an age-related macular degeneration, and a rubeosis.

111. The method according to Claim 101, wherein said administering to the subject is by at least one mode selected from the group consisting of: parenteral, subcutaneous, intramuscular, intravenous, intraarterial, intraarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

112. A method of treating a patient suffering from a disorder in which DLL4 is detrimental comprising the step of administering a DLL4 binding protein as described in claim 2 before, concurrent with, or after the administration of a therapeutically effective amount of a second agent, wherein the second agent is selected from the group consisting of an antibody or fragment thereof capable of binding human VEGFR2; methotrexate; an antibody or fragment thereof capable of binding human TNF; a corticosteroid; a cyclosporine; a rapamycin; FK506; a non-steroidal anti-inflammatory agent (NSAID); a radiotherapeutic agent; an antineoplastic agent; a chemotherapeutic agent; a DNA alkylating agent; cisplatin; carboplatin; an anti-tubulin agent; paclitaxel; docetaxel; taxol; doxorubicin; gemcitabine; gemzar; an anthracycline; adriamycin; a topoisomerase I inhibitor; a topoisomerase II inhibitor; 5-fluorouracil (5-FU); leucovorin; irinotecan; a receptor tyrosine kinase inhibitor; erlotinib; gefitinib; a COX-2 inhibitor; celecoxib; a kinase inhibitor; an angiogenesis inhibitor; an anti-VEGF antibody; aflibercept; a co-stimulation molecule blocker; an anti-B7.1 antibody; an anti-B7.2 antibody; a CTLA4-Ig; an anti-CD20 antibody; an adhesion molecule blocker; an anti-LFA-1 antibody; an anti-E selectin antibody; and anti-L selectin antibody; a small molecule inhibitor; an anti-cytokine antibody or functional fragment thereof; an anti-IL-18 antibody; anti-TNF antibody; an anti-IL-6 antibody; an anti-cytokine receptor antibody; a detectable label or reporter; a TNF antagonist; an antirheumatic; a muscle relaxant; a narcotic; an analgesic; an anesthetic; a sedative; a local anesthetic; a neuromuscular blocker; an antimicrobial agent; an antipsoriatic drug; a corticosteroid; an anabolic steroid; an erythropoietin; an immunization; an immunoglobulin; an immunosuppressive agent; a growth hormone; a hormone replacement drug; a radiopharmaceutical drug; an antidepressant; an antipsychotic drug; a stimulant; an asthma medication; a beta agonist; an inhaled steroid; an epinephrine; an epinephrine analog; a cytokine; and a cytokine antagonist.

113. A binding protein according to claim 2, wherein said binding protein is an antibody.

114. The antibody according to claim 113, wherein the antibody is selected from the group consisting of a monoclonal antibody, a full-length tetrameric immunoglobulin, an IgG molecule, an IgG_1 molecule, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, and an affinity matured antibody.

115. The antibody according to claim 114, wherein the antibody is a monoclonal antibody.

116. The binding protein according to claim 36, wherein said two variable domains have the amino acid sequences selected from the group consisting of:

SEQ ID NO:188 and SEQ ID NO:197,
SEQ ID NO:190 and SEQ ID NO:197, and
SEQ ID NO:191 and SEQ ID NO:197.

117. The binding protein according to claim 36, wherein said two variable domains have the amino acid sequences of SEQ ID NO:181 and SEQ ID NO:185.