THERAPEUTIC DLL4 BINDING PROTEINS

Reference to Related Applications

This application claims priority to US provisional application No. 61/238,152, filed August 29, 2009, and to US provisional application No. 61/261,728, filed November 16, 2009.

Field of the Invention

The present invention relates to the development and use of improved DLL4 binding proteins and uses thereof in the inhibition, prevention, and/or treatment of cancers, tumors, and/or other angiogenesis-dependent diseases such as ocular neovascularization, or angiogenesis-independent diseases characterized by aberrant DLL4 expression or activity such as autoimmune disorders.

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 turn, DLL4 can negatively regulate VEGF signaling, in part through repressing VEGFR2 and inducing VEGR1 (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 Disclosure

The disclosure provides proteins that bind DLL4, including antibodies, CDR-grafted antibodies, and binding fragments thereof, that are capable of binding DLL4. Preferably, a binding protein described herein binds DLL4 with high affinity. More preferably, a binding protein according to the disclosure is capable of neutralizing DLL4. The disclosure also provides methods of making and using DLL4 binding proteins, including human DLL4 binding proteins. Advantageously, the disclosure eliminates the need to prepare humanized DLL4 binding proteins; thereby, eliminating the complications associated with humanized DLL4 binding proteins.

One aspect of this disclosure pertains to a binding protein comprising an antigen binding domain capable of binding human DLL4, said antigen binding domain comprising at least one or more CDRs selected from the group consisting of:

CDR-H1: X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:99), wherein;

X_1 is S or N;

X_2 is S, G, or N;

X_3 is S, N, T, G, or R;

X_4 is Y;

X_5 is Y or H;

X_6 is W; and

X_7 is G;

CDR-H2: 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} (SEQ ID NO:100), wherein;

X_1 is D;

X_2 is I;

X_3 is Y, N, or S;

X_4 is Y;

X_5 is T, N, A, I, S, or R;

X_6 is G;

X_7 is S, N, T, or G;

X_8 is T;

X_9 is Y;

X_{10} is Y;

X_{11} is N;

X_{12} is P;

X_{13} is S;

X_{14} is L;

X_{15} is K; and

X_{16} is S, N, D, or G;

CDR-H3: 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:101), wherein;

X_1 is E, Y, F, Q, W, L, or A;

X_2 is D, A, S, G, V, E, or N;

X_3 is V, M, L, P, or A;

X_4 is I, A, P, R, S, K, Q, V, G, M, or E;

X_5 is L, Y, F, or M;

X_6 is R, G, S, Q, or A;

X_7 is G;

X_8 is G, A, or S;

X_9 is S, A, L, V, R, or G;

X_{10} is D; and

X_{11} is Y, D, S, N, H, E, R, L, P, C, I, M, T, Q, or K;

CDR-L1: 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:102), wherein;

X_1 is S;

X_2 is G;

X_3 is Q, E, or D;

X_4 is R, S, G, M, K, L, or T;

X_5 is L;

X_6 is G;

X_7 is D or E;

X_8 is K;

X_9 is Y;

X_{10} is A or V; and

X_{11} is S;

CDR-L2: X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:103), wherein;

X_1 is E or Q;

X_2 is D;

X_3 is S, L, T, A, E, or F;

X_4 is K, T, E, N, Q, S, or M;

X_5 is R;

X_6 is P; and

X_7 is S;

and

CDR-L3: X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:104), wherein;

X_1 is Q;

X_2 is A;

X_3 is W;

X_4 is D;

X_5 is R, S, M, E, N, G, or K;

X_6 is D or E;

X_7 is T, V, A, S, or M;

X_8 is G, A, or C; and

X_9 is V.

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

residues 31-37 of SEQ ID NO:1 (CDR-H1); residues 52-67 of SEQ ID NO:1 (CDR-H2); residues 100-110 of SEQ ID NO:1 (CDR-H3);

residues 23-33 of SEQ ID NO:111 (CDR-L1); residues 49-55 of SEQ ID NO:111 (CDR-L2); residues 88-96 of SEQ ID NO:111 (CDR-L3);

SEQ ID NO:117 (CDR-H1); SEQ ID NO:118 (CDR-H2); SEQ ID NO:119 (CDR-H3); SEQ ID NO:121 (CDR-H1); SEQ ID NO:122 (CDR-H2); SEQ ID NO:123 (CDR-H3); SEQ ID NO:125 (CDR-H1); SEQ ID NO:126 (CDR-H2); SEQ ID NO:127 (CDR-H3); SEQ ID NO:129 (CDR-H1); SEQ ID NO:130 (CDR-H2); SEQ ID NO:131 (CDR-H3); SEQ ID NO:133 (CDR-H1); SEQ ID NO:134 (CDR-H2); SEQ ID NO:135 (CDR-H3); SEQ ID NO:137 (CDR-H1); SEQ ID NO:138 (CDR-H2); SEQ ID NO:139 (CDR-H3); SEQ ID NO:141 (CDR-H1); SEQ ID NO:142 (CDR-H2); SEQ ID NO:143 (CDR-H3); SEQ ID NO:145 (CDR-H1); SEQ ID NO:146 (CDR-H2); SEQ ID NO:147 (CDR-H3); SEQ ID NO:149 (CDR-H1); SEQ ID NO:150 (CDR-H2); SEQ ID NO:151 (CDR-H3); SEQ ID NO:153 (CDR-H1); SEQ ID NO:154 (CDR-H2); SEQ ID NO:155 (CDR-H3);

SEQ ID NO:157 (CDR-H1); SEQ ID NO:158 (CDR-H2); SEQ ID NO:159 (CDR-H3);
SEQ ID NO:161 (CDR-H1); SEQ ID NO:162 (CDR-H2); SEQ ID NO:163 (CDR-H3);
SEQ ID NO:165 (CDR-H1); SEQ ID NO:166 (CDR-H2); SEQ ID NO:167 (CDR-H3);
SEQ ID NO:169 (CDR-H1); SEQ ID NO:170 (CDR-H2); SEQ ID NO:171 (CDR-H3);
SEQ ID NO:173 (CDR-H1); SEQ ID NO:174 (CDR-H2); SEQ ID NO:175 (CDR-H3);
SEQ ID NO:177 (CDR-H1); SEQ ID NO:178 (CDR-H2); SEQ ID NO:179 (CDR-H3);
SEQ ID NO:181 (CDR-H1); SEQ ID NO:182 (CDR-H2); SEQ ID NO:183 (CDR-H3);
SEQ ID NO:185 (CDR-H1); SEQ ID NO:186 (CDR-H2); SEQ ID NO:187 (CDR-H3);
SEQ ID NO:189 (CDR-H1); SEQ ID NO:190 (CDR-H2); SEQ ID NO:191 (CDR-H3);
SEQ ID NO:193 (CDR-H1); SEQ ID NO:194 (CDR-H2); SEQ ID NO:195 (CDR-H3);
SEQ ID NO:197 (CDR-H1); SEQ ID NO:198 (CDR-H2); SEQ ID NO:199 (CDR-H3);
SEQ ID NO:201 (CDR-H1); SEQ ID NO:202 (CDR-H2); SEQ ID NO:203 (CDR-H3);
SEQ ID NO:205 (CDR-H1); SEQ ID NO:206 (CDR-H2); SEQ ID NO:207 (CDR-H3);
SEQ ID NO:209 (CDR-H1); SEQ ID NO:210 (CDR-H2); SEQ ID NO:211 (CDR-H3);
SEQ ID NO:213 (CDR-H1); SEQ ID NO:214 (CDR-H2); SEQ ID NO:215 (CDR-H3);
SEQ ID NO:217 (CDR-L1); SEQ ID NO:218 (CDR-L2); SEQ ID NO:219 (CDR-L3);
SEQ ID NO:221 (CDR-L1); SEQ ID NO:222 (CDR-L2); SEQ ID NO:223 (CDR-L3);
SEQ ID NO:225 (CDR-L1); SEQ ID NO:226 (CDR-L2); SEQ ID NO:227 (CDR-L3);
SEQ ID NO:229 (CDR-L1); SEQ ID NO:230 (CDR-L2); SEQ ID NO:231 (CDR-L3);
SEQ ID NO:233 (CDR-L1); SEQ ID NO:234 (CDR-L2); SEQ ID NO:235 (CDR-L3);
SEQ ID NO:237 (CDR-L1); SEQ ID NO:238 (CDR-L2); SEQ ID NO:239 (CDR-L3);
SEQ ID NO:241 (CDR-L1); SEQ ID NO:242 (CDR-L2); SEQ ID NO:243 (CDR-L3);
SEQ ID NO:245 (CDR-L1); SEQ ID NO:246 (CDR-L2); SEQ ID NO:247 (CDR-L3);
SEQ ID NO:249 (CDR-L1); SEQ ID NO:250 (CDR-L2); SEQ ID NO:251 (CDR-L3);
SEQ ID NO:253 (CDR-L1); SEQ ID NO:254 (CDR-L2); SEQ ID NO:255 (CDR-L3);
SEQ ID NO:257 (CDR-L1); SEQ ID NO:258 (CDR-L2); SEQ ID NO:259 (CDR-L3);
SEQ ID NO:261 (CDR-L1); SEQ ID NO:262 (CDR-L2); SEQ ID NO:263 (CDR-L3);
SEQ ID NO:265 (CDR-L1); SEQ ID NO:266 (CDR-L2); SEQ ID NO:267 (CDR-L3);
SEQ ID NO:269 (CDR-L1); SEQ ID NO:270 (CDR-L2); SEQ ID NO:271 (CDR-L3);
SEQ ID NO:273 (CDR-L1); SEQ ID NO:274 (CDR-L2); SEQ ID NO:275 (CDR-L3);
SEQ ID NO:277 (CDR-L1); SEQ ID NO:278 (CDR-L2); SEQ ID NO:279 (CDR-L3);
SEQ ID NO:281 (CDR-L1); SEQ ID NO:282 (CDR-L2); SEQ ID NO:283 (CDR-L3);
SEQ ID NO:285 (CDR-L1); SEQ ID NO:286 (CDR-L2); SEQ ID NO:287 (CDR-L3);
SEQ ID NO:289 (CDR-L1); SEQ ID NO:290 (CDR-L2); SEQ ID NO:291 (CDR-L3);
SEQ ID NO:293 (CDR-L1); SEQ ID NO:294 (CDR-L2); SEQ ID NO:295 (CDR-L3);
SEQ ID NO:297 (CDR-L1); SEQ ID NO:298 (CDR-L2); SEQ ID NO:299 (CDR-L3);
SEQ ID NO:301 (CDR-L1); SEQ ID NO:302 (CDR-L2); SEQ ID NO:303 (CDR-L3);

SEQ ID NO:305 (CDR-L1); SEQ ID NO:306 (CDR-L2); SEQ ID NO:307 (CDR-L3); SEQ ID NO:309 (CDR-L1); SEQ ID NO:310 (CDR-L2); SEQ ID NO:311 (CDR-L3); SEQ ID NO:313 (CDR-L1); SEQ ID NO:314 (CDR-L2); SEQ ID NO:315 (CDR-L3); residues 31-37 of SEQ ID NO:334 (CDR-H1); residues 52-67 of SEQ ID NO:334 (CDR-H2); residues 100-110 of SEQ ID NO:334 (CDR-H3);

residues 23-33 of SEQ ID NO:335 (CDR-L1); residues 49-55 of SEQ ID NO:335 (CDR-L2); residues 88-96 of SEQ ID NO:335 (CDR-L3);

residues 31-37 of SEQ ID NO:336 (CDR-H1); residues 52-67 of SEQ ID NO:336 (CDR-H2); residues 100-110 of SEQ ID NO:336 (CDR-H3);

residues 23-33 of SEQ ID NO:337 (CDR-L1); residues 49-55 of SEQ ID NO:337 (CDR-L2); residues 88-96 of SEQ ID NO:337 (CDR-L3);

residues 31-37 of SEQ ID NO:338 (CDR-H1); residues 52-67 of SEQ ID NO:338 (CDR-H2); residues 100-110 of SEQ ID NO:338 (CDR-H3);

residues 23-33 of SEQ ID NO:339 (CDR-L1); residues 49-55 of SEQ ID NO:339 (CDR-L2); residues 88-96 of SEQ ID NO:339 (CDR-L3);

residues 31-37 of SEQ ID NO:340 (CDR-H1); residues 52-67 of SEQ ID NO:340 (CDR-H2); residues 100-110 of SEQ ID NO:340 (CDR-H3);

residues 23-33 of SEQ ID NO:341 (CDR-L1); residues 49-55 of SEQ ID NO:341 (CDR-L2); residues 88-96 of SEQ ID NO:341 (CDR-L3);

residues 31-37 of SEQ ID NO:342 (CDR-H1); residues 52-67 of SEQ ID NO:342 (CDR-H2); residues 100-110 of SEQ ID NO:342 (CDR-H3);

residues 23-33 of SEQ ID NO:343 (CDR-L1); residues 49-55 of SEQ ID NO:343 (CDR-L2); residues 88-96 of SEQ ID NO:343 (CDR-L3);

residues 31-37 of SEQ ID NO:344 (CDR-H1); residues 52-67 of SEQ ID NO:344 (CDR-H2); residues 100-110 of SEQ ID NO:344 (CDR-H3);

residues 24-34 of SEQ ID NO:345 (CDR-L1); residues 50-56 of SEQ ID NO:345 (CDR-L2); residues 89-97 of SEQ ID NO:345 (CDR-L3);

residues 31-37 of SEQ ID NO:346 (CDR-H1); residues 52-67 of SEQ ID NO:346 (CDR-H2); residues 100-110 of SEQ ID NO:346 (CDR-H3);

residues 23-33 of SEQ ID NO:347 (CDR-L1); residues 49-55 of SEQ ID NO:347 (CDR-L2); residues 88-96 of SEQ ID NO:347 (CDR-L3);

residues 31-37 of SEQ ID NO:348 (CDR-H1); residues 52-67 of SEQ ID NO:348 (CDR-H2); residues 100-110 of SEQ ID NO:348 (CDR-H3);

residues 24-34 of SEQ ID NO:349 (CDR-L1); residues 50-56 of SEQ ID NO:349 (CDR-L2); residues 89-97 of SEQ ID NO:349 (CDR-L3);

residues 31-37 of SEQ ID NO:350 (CDR-H1); residues 52-67 of SEQ ID NO:350 (CDR-H2); residues 100-110 of SEQ ID NO:350 (CDR-H3);

residues 24-34 of SEQ ID NO:351 (CDR-L1); residues 50-56 of SEQ ID NO:351 (CDR-L2); residues 89-97 of SEQ ID NO:351 (CDR-L3);
residues 31-37 of SEQ ID NO:352 (CDR-H1); residues 52-67 of SEQ ID NO:352 (CDR-H2); residues 100-110 of SEQ ID NO:352 (CDR-H3);
residues 24-34 of SEQ ID NO:353 (CDR-L1); residues 50-56 of SEQ ID NO:353 (CDR-L2); residues 89-97 of SEQ ID NO:353 (CDR-L3);
residues 31-37 of SEQ ID NO:354 (CDR-H1); residues 52-67 of SEQ ID NO:354 (CDR-H2); residues 100-110 of SEQ ID NO:354 (CDR-H3);
residues 24-34 of SEQ ID NO:355 (CDR-L1); residues 50-56 of SEQ ID NO:355 (CDR-L2); residues 89-97 of SEQ ID NO:355 (CDR-L3);
residues 31-37 of SEQ ID NO:356 (CDR-H1); residues 52-67 of SEQ ID NO:356 (CDR-H2); residues 100-110 of SEQ ID NO:356 (CDR-H3);
residues 23-33 of SEQ ID NO:357 (CDR-L1); residues 49-55 of SEQ ID NO:357 (CDR-L2); residues 88-96 of SEQ ID NO:357 (CDR-L3);
residues 31-37 of SEQ ID NO:358 (CDR-H1); residues 52-67 of SEQ ID NO:358 (CDR-H2); residues 100-110 of SEQ ID NO:358 (CDR-H3);
residues 23-33 of SEQ ID NO:359 (CDR-L1); residues 49-55 of SEQ ID NO:359 (CDR-L2); residues 88-96 of SEQ ID NO:359 (CDR-L3);
residues 31-37 of SEQ ID NO:360 (CDR-H1); residues 52-67 of SEQ ID NO:360 (CDR-H2); residues 100-110 of SEQ ID NO:360 (CDR-H3);
residues 23-33 of SEQ ID NO:361 (CDR-L1); residues 49-55 of SEQ ID NO:361 (CDR-L2); residues 88-96 of SEQ ID NO:361 (CDR-L3).

In one embodiment, there is provided a binding protein comprising an antigen binding domain, said binding protein being

capable of binding human DLL4, said antigen binding domain comprising six CDRs: CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, wherein:

CDR-H1 is selected from the group consisting of:

residues 31-37 of SEQ ID NO:1;
SEQ ID NO:117;
SEQ ID NO:121;
SEQ ID NO:125;
SEQ ID NO:129;
SEQ ID NO:133;
SEQ ID NO:137;
SEQ ID NO:141;
SEQ ID NO:145;
SEQ ID NO:149;
SEQ ID NO:153;
SEQ ID NO:157;
SEQ ID NO:161;
SEQ ID NO:165;
SEQ ID NO:169;
SEQ ID NO:173;
SEQ ID NO:177;
SEQ ID NO:181;
SEQ ID NO:185;
SEQ ID NO:189;
SEQ ID NO:193;
SEQ ID NO:197;
SEQ ID NO:201;
SEQ ID NO:205;
SEQ ID NO:209;
SEQ ID NO:213;
residues 31-37 of SEQ ID NO:334;
residues 31-37 of SEQ ID NO:336;
residues 31-37 of SEQ ID NO:338;
residues 31-37 of SEQ ID NO:340;
residues 31-37 of SEQ ID NO:342;

residues 31-37 of SEQ ID NO:344;
residues 31-37 of SEQ ID NO:346;
residues 31-37 of SEQ ID NO:348;
residues 31-37 of SEQ ID NO:350;
residues 31-37 of SEQ ID NO:352;
residues 31-37 of SEQ ID NO:354;
residues 31-37 of SEQ ID NO:356;
residues 31-37 of SEQ ID NO:358; and
residues 31-37 of SEQ ID NO:360;

CDR-H2, selected from the group consisting of:

residues 52-67 of SEQ ID NO:1;
SEQ ID NO:118;
SEQ ID NO:122;
SEQ ID NO:126;
SEQ ID NO:130;
SEQ ID NO:134;
SEQ ID NO:138;
SEQ ID NO:142;
SEQ ID NO:146;
SEQ ID NO:150;
SEQ ID NO:154;
SEQ ID NO:158;
SEQ ID NO:162;
SEQ ID NO:166;
SEQ ID NO:170;
SEQ ID NO:174;
SEQ ID NO:178;
SEQ ID NO:182;
SEQ ID NO:186;
SEQ ID NO:190;
SEQ ID NO:194;
SEQ ID NO:198;
SEQ ID NO:202;
SEQ ID NO:206;
SEQ ID NO:210;
SEQ ID NO:214;
residues 52-67 of SEQ ID NO:334;

residues 52-67 of SEQ ID NO:336;
residues 52-67 of SEQ ID NO:338;
residues 52-67 of SEQ ID NO:340;
residues 52-67 of SEQ ID NO:342;
residues 52-67 of SEQ ID NO:344;
residues 52-67 of SEQ ID NO:346;
residues 52-67 of SEQ ID NO:348;
residues 52-67 of SEQ ID NO:350;
residues 52-67 of SEQ ID NO:352;
residues 52-67 of SEQ ID NO:354;
residues 52-67 of SEQ ID NO:356;
residues 52-67 of SEQ ID NO:358; and
residues 52-67 of SEQ ID NO:360;

CDR-H3 is selected from the group consisting of:

residues 100-110 of SEQ ID NO:1;
SEQ ID NO:119;
SEQ ID NO:123;
SEQ ID NO:127;
SEQ ID NO:131;
SEQ ID NO:135;
SEQ ID NO:139;
SEQ ID NO:143;
SEQ ID NO:147;
SEQ ID NO:151;
SEQ ID NO:155;
SEQ ID NO:159;
SEQ ID NO:163;
SEQ ID NO:167;
SEQ ID NO:171;
SEQ ID NO:175;
SEQ ID NO:179;
SEQ ID NO:183;
SEQ ID NO:187;
SEQ ID NO:191;
SEQ ID NO:195;
SEQ ID NO:199;
SEQ ID NO:203;

SEQ ID NO:207;

SEQ ID NO:211;

SEQ ID NO:215;

residues 100-110 of SEQ ID NO:334;

residues 100-110 of SEQ ID NO:336;

residues 100-110 of SEQ ID NO:338;

residues 100-110 of SEQ ID NO:340;

residues 100-110 of SEQ ID NO:342;

residues 100-110 of SEQ ID NO:344;

residues 100-110 of SEQ ID NO:346;

residues 100-110 of SEQ ID NO:348;

residues 100-110 of SEQ ID NO:350;

residues 100-110 of SEQ ID NO:352;

residues 100-110 of SEQ ID NO:354;

residues 100-110 of SEQ ID NO:356;

residues 100-110 of SEQ ID NO:358; and

residues 100-110 of SEQ ID NO:360;

CDR-L1 is selected from the group consisting of:

residues 23-33 of SEQ ID NO:111;

SEQ ID NO:217;

SEQ ID NO:221;

SEQ ID NO:225;

SEQ ID NO:229;

SEQ ID NO:233;

SEQ ID NO:237;

SEQ ID NO:241;

SEQ ID NO:245;

SEQ ID NO:249;

SEQ ID NO:253;

SEQ ID NO:257;

SEQ ID NO:261;

SEQ ID NO:265;

SEQ ID NO:269;

SEQ ID NO:273;

SEQ ID NO:277;

SEQ ID NO:281;

SEQ ID NO:285;

SEQ ID NO:289;

SEQ ID NO:293;

SEQ ID NO:297;

SEQ ID NO:301;

SEQ ID NO:305;

SEQ ID NO:309;

SEQ ID NO:313;

residues 23-33 of SEQ ID NO:335;

residues 23-33 of SEQ ID NO:337;

residues 23-33 of SEQ ID NO:339;

residues 23-33 of SEQ ID NO:341;

residues 23-33 of SEQ ID NO:343;

residues 24-34 of SEQ ID NO:345;

residues 23-33 of SEQ ID NO:347;

residues 24-34 of SEQ ID NO:349;

residues 24-34 of SEQ ID NO:351;

residues 24-34 of SEQ ID NO:353;

residues 24-34 of SEQ ID NO:355;

residues 23-33 of SEQ ID NO:357;

residues 23-33 of SEQ ID NO:359; and

residues 23-33 of SEQ ID NO:361;

CDR-L2 is selected from the group consisting of:

residues 49-55 of SEQ ID NO:111;

SEQ ID NO:230;

SEQ ID NO:234;

SEQ ID NO:238;

SEQ ID NO:242;

SEQ ID NO:246;

SEQ ID NO:250;

SEQ ID NO:254;

SEQ ID NO:258;

SEQ ID NO:262;

SEQ ID NO:266;

SEQ ID NO:270;

SEQ ID NO:218;

SEQ ID NO:222;

SEQ ID NO:226;

SEQ ID NO:274;

SEQ ID NO:278;

SEQ ID NO:302;

SEQ ID NO:294;

SEQ ID NO:282;

SEQ ID NO:290;

SEQ ID NO:298;

SEQ ID NO:286;

SEQ ID NO:306;

SEQ ID NO:310;

SEQ ID NO:314;

residues 49-55 of SEQ ID NO:335;

residues 49-55 of SEQ ID NO:337;

residues 49-55 of SEQ ID NO:339;

residues 49-55 of SEQ ID NO:341;

residues 49-55 of SEQ ID NO:343;

residues 50-56 of SEQ ID NO:345;

residues 49-55 of SEQ ID NO:347;

residues 50-56 of SEQ ID NO:349;

residues 50-56 of SEQ ID NO:351;

residues 50-56 of SEQ ID NO:353;

residues 50-56 of SEQ ID NO:355;

residues 49-55 of SEQ ID NO:357;

residues 49-55 of SEQ ID NO:359; and

residues 49-55 of SEQ ID NO:361;

and

CDR-L3 is selected from the group consisting of:

residues 88-96 of SEQ ID NO:111;

SEQ ID NO:231;

SEQ ID NO:235;

SEQ ID NO:239;

SEQ ID NO:243;

SEQ ID NO:247;

SEQ ID NO:251;

SEQ ID NO:255;

SEQ ID NO:259;

SEQ ID NO:263;

SEQ ID NO:267;
SEQ ID NO:271;
SEQ ID NO:219;
SEQ ID NO:223;
SEQ ID NO:227;
SEQ ID NO:275;
SEQ ID NO:279;
SEQ ID NO:303;
SEQ ID NO:295;
SEQ ID NO:283;
SEQ ID NO:291;
SEQ ID NO:299;
SEQ ID NO:287;
SEQ ID NO:307;
SEQ ID NO:311;
SEQ ID NO:315;
residues 88-96 of SEQ ID NO:335;
residues 88-96 of SEQ ID NO:337;
residues 88-96 of SEQ ID NO:339;
residues 88-96 of SEQ ID NO:341;
residues 88-96 of SEQ ID NO:343;
residues 89-97 of SEQ ID NO:345;
residues 88-96 of SEQ ID NO:347;
residues 89-97 of SEQ ID NO:349;
residues 89-97 of SEQ ID NO:351;
residues 89-97 of SEQ ID NO:353;
residues 89-97 of SEQ ID NO:355;
residues 88-96 of SEQ ID NO:357;
residues 88-96 of SEQ ID NO:359; and
residues 88-96 of SEQ ID NO:361.

In another embodiment, the binding protein comprises at least 3 CDRs disclosed above.

Preferably, a DLL4 binding protein according to the disclosure comprises one or more CDRs disclosed above. More preferably, the binding protein comprises three or more CDRs disclosed above. Most preferably, a DLL4 binding protein according to the disclosure comprises six CDRs described above, i.e., a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 described above.

In a preferred embodiment, the binding protein comprises at least 3 CDRs selected from the group consisting of the sequences disclosed above.

More preferably, the binding protein comprises 3 CDRs selected from a set of variable domain CDRs selected from the group below.

VH E9 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:1

CDR-H2: residues 52-67 of SEQ ID NO:1

CDR-H3 residues 100-110 of SEQ ID NO:1

VL E9 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:111

CDR-L2: residues 49-55 of SEQ ID NO:111

CDR-L3: residues 88-96 of SEQ ID NO:111

VH E9.4 CDR Set

CDR-H1: SEQ ID NO:117

CDR-H2: SEQ ID NO:118

CDR-H3: SEQ ID NO:119

VL E9.4 CDR Set

CDR-L1: SEQ ID NO:229

CDR-L2: SEQ ID NO:230

CDR-L3: SEQ ID NO:231

VH E9.11 CDR Set

CDR-H1: SEQ ID NO:121

CDR-H2: SEQ ID NO:122

CDR-H3: SEQ ID NO:123

VL E9.11 CDR Set

CDR-L1: SEQ ID NO:233

CDR-L2: SEQ ID NO:234

CDR-L3: SEQ ID NO:235

VH E9.14 CDR Set

CDR-H1: SEQ ID NO:125

CDR-H2: SEQ ID NO:126

CDR-H3: SEQ ID NO:127

VL E9.14 CDR Set

CDR-L1: SEQ ID NO:237

CDR-L2: SEQ ID NO:238

CDR-L3: SEQ ID NO:239

VH E9.17 CDR Set

CDR-H1: SEQ ID NO:129

CDR-H2: SEQ ID NO:130

CDR-H3: SEQ ID NO:131

VL E9.17 CDR Set

CDR-L1: SEQ ID NO:241

CDR-L2: SEQ ID NO:242

CDR-L3: SEQ ID NO:243

VH E9.18 CDR Set

CDR-H1: SEQ ID NO:133

CDR-H2: SEQ ID NO:134

CDR-H3: SEQ ID NO:135

VL E9.18 CDR Set

CDR-L1: SEQ ID NO:245

CDR-L2: SEQ ID NO:246

CDR-L3: SEQ ID NO:247

VH E9.19 CDR Set

CDR-H1: SEQ ID NO:137

CDR-H2: SEQ ID NO:138

CDR-H3: SEQ ID NO:139

VL E9.19 CDR Set

CDR-L1: SEQ ID NO:249

CDR-L2: SEQ ID NO:250

CDR-L3: SEQ ID NO:251

VH E9.22 CDR Set

CDR-H1: SEQ ID NO:141

CDR-H2: SEQ ID NO:142

CDR-H3: SEQ ID NO:143

VL E9.22 CDR Set

CDR-L1: SEQ ID NO:253

CDR-L2: SEQ ID NO:254

CDR-L3: SEQ ID NO:255

VH E9.48 CDR Set

CDR-H1: SEQ ID NO:145

CDR-H2: SEQ ID NO:146

CDR-H3: SEQ ID NO:147

VL E9.48 CDR Set

CDR-L1: SEQ ID NO:257

CDR-L2: SEQ ID NO:258

CDR-L3: SEQ ID NO:259

VH E9.65 CDR Set

CDR-H1: SEQ ID NO:149

CDR-H2: SEQ ID NO:150

CDR-H3: SEQ ID NO:151

VL E9.65 CDR Set

CDR-L1: SEQ ID NO:261

CDR-L2: SEQ ID NO:262

CDR-L3: SEQ ID NO:263

VH E9.66 CDR Set

CDR-H1: SEQ ID NO:153

CDR-H2: SEQ ID NO:154

CDR-H3: SEQ ID NO:155

VL E9.66 CDR Set

CDR-L1: SEQ ID NO:265

CDR-L2: SEQ ID NO:266

CDR-L3: SEQ ID NO:267

VH E9.71 CDR Set

CDR-H1: SEQ ID NO:157

CDR-H2: SEQ ID NO:158

CDR-H3: SEQ ID NO:159

VL E9.71 CDR Set

CDR-L1: SEQ ID NO:269

CDR-L2: SEQ ID NO:270

CDR-L3: SEQ ID NO:271

VH E9.13 CDR Set

CDR-H1: SEQ ID NO:161

CDR-H2: SEQ ID NO:162

CDR-H3: SEQ ID NO:163

VL E9.13 CDR Set

CDR-L1: SEQ ID NO:217

CDR-L2: SEQ ID NO:218

CDR-L3: SEQ ID NO:219

VH E9.16 CDR Set

CDR-H1: SEQ ID NO:165

CDR-H2: SEQ ID NO:166

CDR-H3: SEQ ID NO:167

VL E9.16 CDR Set

CDR-L1: SEQ ID NO:221

CDR-L2: SEQ ID NO:222

CDR-L3: SEQ ID NO:223

VH E9.38 CDR Set

CDR-H1: SEQ ID NO:169

CDR-H2: SEQ ID NO:170

CDR-H3: SEQ ID NO:171

VL E9.38 CDR Set

CDR-L1: SEQ ID NO:225

CDR-L2: SEQ ID NO:226

CDR-L3: SEQ ID NO:227

VH E9.2B CDR Set

CDR-H1: SEQ ID NO:173

CDR-H2: SEQ ID NO:174

CDR-H3: SEQ ID NO:175

VL E9.2B CDR Set

CDR-L1: SEQ ID NO:273

CDR-L2: SEQ ID NO:274

CDR-L3: SEQ ID NO:275

VH E9.1F CDR Set

CDR-H1: SEQ ID NO:177

CDR-H2: SEQ ID NO:178

CDR-H3: SEQ ID NO:179

VL E9.1F CDR Set

CDR-L1: SEQ ID NO:277

CDR-L2: SEQ ID NO:278

CDR-L3: SEQ ID NO:279

VH E9.10H CDR Set

CDR-H1: SEQ ID NO:181

CDR-H2: SEQ ID NO:182

CDR-H3: SEQ ID NO:183

VL E9.10H CDR Set

CDR-L1: SEQ ID NO:301

CDR-L2: SEQ ID NO:302

CDR-L3: SEQ ID NO:303

VH E9.5E CDR Set

CDR-H1: SEQ ID NO:185

CDR-H2: SEQ ID NO:186

CDR-H3: SEQ ID NO:187

VL E9.5E CDR Set

CDR-L1: SEQ ID NO:293

CDR-L2: SEQ ID NO:294

CDR-L3: SEQ ID NO:295

VH E9.10C CDR Set

CDR-H1: SEQ ID NO:189

CDR-H2: SEQ ID NO:190

CDR-H3: SEQ ID NO:191

VL E9.10C CDR Set

CDR-L1: SEQ ID NO:281

CDR-L2: SEQ ID NO:282

CDR-L3: SEQ ID NO:283

VH E9.7E CDR Set

CDR-H1: SEQ ID NO:193

CDR-H2: SEQ ID NO:194

CDR-H3: SEQ ID NO:195

VL E9.7E CDR Set

CDR-L1: SEQ ID NO:289

CDR-L2: SEQ ID NO:290

CDR-L3: SEQ ID NO:291

VH E9.12B CDR Set

CDR-H1: SEQ ID NO:197

CDR-H2: SEQ ID NO:198

CDR-H3: SEQ ID NO:199

VL E9.12B CDR Set

CDR-L1: SEQ ID NO:297

CDR-L2: SEQ ID NO:298

CDR-L3: SEQ ID NO:299

VH E9.10E CDR Set

CDR-H1: SEQ ID NO:201

CDR-H2: SEQ ID NO:202

CDR-H3: SEQ ID NO:203

VL E9.10E CDR Set

CDR-L1: SEQ ID NO:285

CDR-L2: SEQ ID NO:286

CDR-L3: SEQ ID NO:287

VH E9.6A CDR Set

CDR-H1: SEQ ID NO:205

CDR-H2: SEQ ID NO:206

CDR-H3: SEQ ID NO:207

VL E9.6A CDR Set

CDR-L1: SEQ ID NO:305

CDR-L2: SEQ ID NO:306

CDR-L3: SEQ ID NO:307

VH E9.7A CDR Set

CDR-H1: SEQ ID NO:209

CDR-H2: SEQ ID NO:210

CDR-H3: SEQ ID NO:211

VL E9.7A CDR Set

CDR-L1: SEQ ID NO:309

CDR-L2: SEQ ID NO:310

CDR-L3: SEQ ID NO:311

VH E9.8H CDR Set

CDR-H1: SEQ ID NO:213

CDR-H2: SEQ ID NO:214

CDR-H3: SEQ ID NO:215

VL E9.8H CDR Set

CDR-L1: SEQ ID NO:313

CDR-L2: SEQ ID NO:314

CDR-L3: SEQ ID NO:315

VH E9.1 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:334

CDR-H2: residues 52-67 of SEQ ID NO:334

CDR-H3: residues 100-110 of SEQ ID NO:334

VL E9.1 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:335

CDR-L2: residues 49-55 of SEQ ID NO:335

CDR-L3: residues 88-96 of SEQ ID NO:335

VH E9-SE1 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:336

CDR-H2: residues 52-67 of SEQ ID NO:336

CDR-H3: residues 100-110 of SEQ ID NO:336

VL E9-SE1 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:337

CDR-L2: residues 49-55 of SEQ ID NO:337

CDR-L3: residues 88-96 of SEQ ID NO:337

VH E9-SE2 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:338

CDR-H2: residues 52-67 of SEQ ID NO:338

CDR-H3: residues 100-110 of SEQ ID NO:338

VL E9-SE2 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:339

CDR-L2: residues 49-55 of SEQ ID NO:339

CDR-L3: residues 88-96 of SEQ ID NO:339

VH E9-SE3 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:340

CDR-H2: residues 52-67 of SEQ ID NO:340

CDR-H3: residues 100-110 of SEQ ID NO:340

VL E9-SE3 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:341

CDR-L2: residues 49-55 of SEQ ID NO:341

CDR-L3: residues 88-96 of SEQ ID NO:341

VH E9-SE4 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:342

CDR-H2: residues 52-67 of SEQ ID NO:342

CDR-H3: residues 100-110 of SEQ ID NO:342

VL E9-SE4 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:343

CDR-L2: residues 49-55 of SEQ ID NO:343

CDR-L3: residues 88-96 of SEQ ID NO:343

VH E9-SE5 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:344

CDR-H2: residues 52-67 of SEQ ID NO:344

CDR-H3: residues 100-110 of SEQ ID NO:344

VL E9-SE5 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:345

CDR-L2: residues 50-56 of SEQ ID NO:345

CDR-L3: residues 89-97 of SEQ ID NO:345

VH E9-SE6 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:346

CDR-H2: residues 52-67 of SEQ ID NO:346

CDR-H3: residues 100-110 of SEQ ID NO:346

VL E9-SE6 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:347

CDR-L2: residues 49-55 of SEQ ID NO:347

CDR-L3: residues 88-96 of SEQ ID NO:347

VH E9-SE7 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:348

CDR-H2: residues 52-67 of SEQ ID NO:348

CDR-H3: residues 100-110 of SEQ ID NO:348

VL E9-SE7 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:349

CDR-L2: residues 50-56 of SEQ ID NO:349

CDR-L3: residues 89-97 of SEQ ID NO:349

VH E9-SE8 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:350

CDR-H2: residues 52-67 of SEQ ID NO:350

CDR-H3: residues 100-110 of SEQ ID NO:350

VL E9-SE8 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:351

CDR-L2: residues 50-56 of SEQ ID NO:351

CDR-L3: residues 89-97 of SEQ ID NO:351

VH E9-FR1 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:352

CDR-H2: residues 52-67 of SEQ ID NO:352

CDR-H3: residues 100-110 of SEQ ID NO:352

VL E9-FR1 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:353

CDR-L2: residues 50-56 of SEQ ID NO:353

CDR-L3: residues 89-97 of SEQ ID NO:353

VH E9-FR2 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:354

CDR-H2: residues 52-67 of SEQ ID NO:354

CDR-H3: residues 100-110 of SEQ ID NO:354

VL E9-FR2 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:355

CDR-L2: residues 50-56 of SEQ ID NO:355

CDR-L3: residues 89-97 of SEQ ID NO:355

VH E9.71 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:356

CDR-H2: residues 52-67 of SEQ ID NO:356

CDR-H3: residues 100-110 of SEQ ID NO:356

VL E9.71 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:357

CDR-L2: residues 49-55 of SEQ ID NO:357

CDR-L3: residues 88-96 of SEQ ID NO:357

VH E9.71(M) CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:358

CDR-H2: residues 52-67 of SEQ ID NO:358

CDR-H3: residues 100-110 of SEQ ID NO:358

VL E9.71(M) CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:359

CDR-L2: residues 49-55 of SEQ ID NO:359

CDR-L3: residues 88-96 of SEQ ID NO:359

VH E9.71(L) CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:360

CDR-H2: residues 52-67 of SEQ ID NO:360

CDR-H3: residues 100-110 of SEQ ID NO:360

VL E9.71(L) CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:361

CDR-L2: residues 49-55 of SEQ ID NO:361

CDR-L3: residues 88-96 of SEQ ID NO:361

In another embodiment, a binding protein comprises a variable heavy chain (VH) set of 3 CDRs selected from any VH set of 3 CDRs in the group above and a variable light chain (VL) set of 3 CDRS selected from any VL set of 3 CDRs in the group above.

In still another embodiment a binding protein comprises a named VH set of 3 CDRs and a correspondingly named VL set of 3 CDRs from the group below. Preferably, a binding protein according to the disclosure comprises at least two variable domain CDR sets selected from the group of variable domain CDR sets consisting of:

VH E9 CDR Set and VL E9 CDR Set,

VH E9.4 CDR Set and VL E9.4 CDR Set,

VH E9.11 CDR Set and VL E9.11 CDR Set,
VH E9.14 CDR Set and VL E9.14 CDR Set,
VH E9.17 CDR Set and VL E9.17 CDR Set,
VH E9.18 CDR Set and VL E9.18 CDR Set,
VH E9.19 CDR Set and VL E9.19 CDR Set,
VH E9.22 CDR Set and VL E9.22 CDR Set,
VH E9.48 CDR Set and VL E9.48 CDR Set,
VH E9.65 CDR Set and VL E9.65 CDR Set,
VH E9.66 CDR Set and VL E9.66 CDR Set,
VH E9.71 CDR Set and VL E9.71 CDR Set,
VH E9.13 CDR Set and VL E9.13 CDR Set,
VH E9.16 CDR Set and VL E9.16 CDR Set,
VH E9.38 CDR Set and VL E9.38 CDR Set,
VH E9.2B CDR Set and VL E9.2B CDR Set,
VH E9.1F CDR Set and VL E9.1F CDR Set,
VH E9.10H CDR Set and VL E9.10H CDR Set,
VH E9.5E CDR Set and VL E9.5E CDR Set,
VH E9.10C CDR Set and VL E9.10C CDR Set,
VH E9.7E CDR Set and VL E9.7E CDR Set,
VH E9.12B CDR Set and VL E9.12B CDR Set,
VH E9.10E CDR Set and VL E9.10E CDR Set,
VH E9.6A CDR Set and VL E9.6A CDR Set,
VH E9.7A CDR Set and VL E9.7A CDR Set,
VH E9.8H CDR Set and VL E9.8H CDR Set,
VH E9-SE1 CDR Set and VL E9-SE1 CDR Set,
VH E9-SE2 CDR Set and VL E9-SE2 CDR Set,
VH E9-SE3 CDR Set and VL E9-SE3 CDR Set,
VH E9-SE4 CDR Set and VL E9-SE4 CDR Set,
VH E9-SE5 CDR Set and VL E9-SE5 CDR Set,
VH E9-SE6 CDR Set and VL E9-SE6 CDR Set,
VH E9-SE7 CDR Set and VL E9-SE7 CDR Set,
VH E9-SE8 CDR Set and VL E9-SE8 CDR Set,
VH E9-FR1 CDR Set and VL E9-FR1 CDR Set,
VH E9-FR2 CDR Set and VL E9-FR2 CDR Set,
VH E9.71 CDR Set and VL E9.71 CDR Set,
VH E9.71(M) CDR Set and VL E9.71(M) CDR Set, and
VH E9.71(L) CDR Set and VL E9.71(L) CDR Set.

In yet another embodiment, a binding protein described above further comprises a human acceptor framework. Preferably, the human acceptor framework comprises an amino acid sequence selected from the group consisting of:

heavy chain acceptor framework sequences SEQ ID NOS:6-22,
heavy chain acceptor sequences SEQ ID NOS:35-62,
light chain acceptor sequences SEQ ID NOS:23-34, and
light chain acceptor sequences SEQ ID NOS:63-98.

In another embodiment a binding protein described above comprises a human acceptor framework, which comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of said human acceptor framework and comprising at least 70 amino acid residues identical to said human acceptor framework.

In another embodiment a binding protein described herein comprises a human acceptor framework that 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 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.

Preferably, the key residue is selected from the group consisting of: 2H, 4H, 24H, 26H, 27H, 29H, 34H, 35H, 37H, 39H, 44H, 45H, 47H, 48H, 49H, 50H, 51H, 58H, 59H, 60H, 63H, 67H, 69H, 71H, 73H, 76H, 78H, 91H, 93H, 94H, 2L, 4L, 25L, 29L, 27bL, 33L, 34L, 36L, 38L, 43L, 44L, 46L, 47L, 48L, 49L, 55L, 58L, 62L, 64L, 71L, 87L, 89L, 90L, 91L, 94L, 95L.

In another embodiment, a binding protein described herein comprises a consensus human variable domain.

In a preferred embodiment, a binding protein described above comprises at least one variable domain having amino acid sequence selected from the group consisting of:
SEQ ID NOS:1, 111, 116, 228, 120, 232, 124, 236, 128, 240, 132, 244, 136, 248, 140, 252, 144, 256, 148, 260, 152, 264, 156, 268, 160, 216, 164, 220, 168, 224, 172, 272, 176, 276, 180, 300, 184, 292, 188, 280, 192, 288, 196, 296, 200, 284, 204, 304, 208, 308, 212, 312, 334, 335, 336,

337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, and 361.

In another embodiment, a binding protein according to the disclosure comprises two variable domains, wherein said two variable domains have amino acid sequences selected from the group consisting of: SEQ ID NOS:1 and 111, SEQ ID NOS:116 and 228, SEQ ID NOS:120 and 232, SEQ ID NOS:124 and 236, SEQ ID NOS:128 and 240, SEQ ID NOS:132 and 244, SEQ ID NOS:136 and 248, SEQ ID NOS:140 and 252, SEQ ID NOS:144 and 256, SEQ ID NOS:148 and 260, SEQ ID NOS:152 and 264, SEQ ID NOS:156 and 268, SEQ ID NOS: 160 and 216, SEQ ID NOS: 164 and 220, SEQ ID NOS:168 and 224, SEQ ID NOS:172 and 272, SEQ ID NOS:176 and 276, SEQ ID NOS:180 and 300, SEQ ID NOS:184 and 292, SEQ ID NOS:188 and 280, SEQ ID NOS:192 and 288, SEQ ID NOS:196 and 296, SEQ ID NOS:200 and 284, SEQ ID NOS:204 and 304, SEQ ID NOS:208 and 308, SEQ ID NOS:212 and 312, SEQ ID NOS:334 and 335, SEQ ID NOS:336 and 337, SEQ ID NOS:338 and 339, SEQ ID NOS:340 and 341, SEQ ID NOS:342 and 343, SEQ ID NOS:344 and 345, SEQ ID NOS:346 and 347, SEQ ID NOS:348 and 349, SEQ ID NOS:350 and 351, SEQ ID NOS:352 and 353, SEQ ID NOS:354 and 355, SEQ ID NOS:356 and 357, SEQ ID NOS:358 and 359, SEQ ID NOS:360 and 361.

In an embodiment, a binding protein according to the disclosure comprises heavy chain variable domain (V_H), preferably wherein the V_H comprises an amino acid sequence selected from the group consisting of:

SEQ ID NOS:1, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, and 360.

In yet another embodiment, a binding protein according to the disclosure comprises a light chain variable domain (a V_L), preferably wherein the V_L comprises an amino acid sequence selected from the group consisting of:

SEQ ID NOS:111, 228, 232, 236, 240, 244, 248, 252, 256, 260, 264, 268, 216, 220, 224, 272, 276, 300, 292, 280, 288, 296, 284, 304, 308, 312, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, and 361.

In a preferred embodiment, a binding protein according to the disclosure comprises a V_H and a V_L, preferably wherein V_H and a V_L are any of those sequences disclosed above.

In another embodiment, the disclosure provides a binding protein capable of binding human DLL-4, said binding protein comprising:

an Ig constant heavy region having an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3;

an Ig constant light region having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5;

an Ig variable heavy region having an amino acid sequence selected from the group consisting:

SEQ ID NOS:1, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, and 360; and

an Ig variable light region having an amino acid sequence selected from the group consisting:

SEQ ID NOS:111, 228, 232, 236, 240, 244, 248, 252, 256, 260, 264, 268, 216, 220, 224, 272, 276, 300, 292, 280, 288, 296, 284, 304, 308, 312, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, and 361.

Another aspect of the disclosure pertains to a binding protein comprising an antigen binding domain capable of binding human DLL4, said antigen binding domain comprising at least one or more CDRs selected from the group consisting of:

CDR-H1: X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:105), wherein;

X_1 is S, N, or D;

X_2 is H or Y;

X_3 is W;

X_4 is M;

X_5 is S or H;

CDR-H2: 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:106), wherein;

X_1 is I, D, M, or T;

X_2 is I;

X_3 is S;

X_4 is Y, N, S, Q, V, T, H, or D;

X_5 is D;

X_6 is G;

X_7 is S, R, I, T, G, K, H, or N;

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

X_9 is K, M, N, Q, E, T, R, S, A, or L;

X_{10} is Y, D, or E;

X_{11} is S or Y;

X_{12} is A;

X_{13} is D;

X_{14} is S;

X_{15} is V;

X_{16} is K; and

X_{17} is G;

CDR-H3: X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10} (SEQ ID NO:107), wherein;

X_1 is A;

X_2 is G, A, or R;

X_3 is G;

X_4 is G, S, or A;

X_5 is N;

X_6 is V or M;

X_7 is G;

X_8 is F, L, Y, or M;

X_9 is D; and

X_{10} is I, S, or L;

CDR-L1: 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:108), wherein;

X_1 is S;

X_2 is A or G;

X_3 is D;

X_4 is K, N, L, Q, M, E, S, T, G, or D;

X_5 is L;

X_6 is G;

X_7 is T, S, N, A, G, or E;

X_8 is K, Q, N, or R;

X_9 is Y;

X_{10} is V or I; and

X_{11} is S;

CDR-L2: X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:109), wherein;

X_1 is Q;

X_2 is D;

X_3 is A, G, W, S, or D;

X_4 is K, M, Q, N, L, T, I, or E;

X_5 is R;

X_6 is P; and

X_7 is S;

and

CDR-L3: X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:110), wherein;

X_1 is Q;

X_2 is S or A;
X_3 is W;
X_4 is D;
X_5 is R, S, Q, P, A, V, W, or M;
X_6 is S, G, I, N, R, or T;
X_7 is D or G;
X_8 is V, A, P, or E; and
X_9 is V.

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

residues 31-35 of SEQ ID NO:112 (CDR-H1); residues 50-66 of SEQ ID NO:112 (CDR-H2); residues 99-108 of SEQ ID NO:112 (CDR-H3);
residues 23-33 of SEQ ID NO:113 (CDR-L1); residues 49-55 of SEQ ID NO:113 (CDR-L2); residues 88-96 of SEQ ID NO:113 (CDR-L3);
residues 31-35 of SEQ ID NO:316 (CDR-H1); residues 50-66 of SEQ ID NO:316 (CDR-H2); residues 99-108 of SEQ ID NO:316 (CDR-H3);
residues 31-35 of SEQ ID NO:317 (CDR-H1); residues 50-66 of SEQ ID NO:317 (CDR-H2); residues 99-108 of SEQ ID NO:317 (CDR-H3);
residues 31-35 of SEQ ID NO:318 (CDR-H1); residues 50-66 of SEQ ID NO:318 (CDR-H2); residues 99-108 of SEQ ID NO:318 (CDR-H3);
residues 31-35 of SEQ ID NO:319 (CDR-H1); residues 50-66 of SEQ ID NO:319 (CDR-H2); residues 99-108 of SEQ ID NO:319 (CDR-H3);
residues 31-35 of SEQ ID NO:320 (CDR-H1); residues 50-66 of SEQ ID NO:320 (CDR-H2); residues 99-108 of SEQ ID NO:320 (CDR-H3);
residues 31-35 of SEQ ID NO:321 (CDR-H1); residues 50-66 of SEQ ID NO:321 (CDR-H2); residues 99-108 of SEQ ID NO:321 (CDR-H3);
residues 31-35 of SEQ ID NO:322 (CDR-H1); residues 50-66 of SEQ ID NO:322 (CDR-H2); residues 99-108 of SEQ ID NO:322 (CDR-H3);
residues 31-35 of SEQ ID NO:323 (CDR-H1); residues 50-66 of SEQ ID NO:323 (CDR-H2); residues 99-108 of SEQ ID NO:323 (CDR-H3);
residues 31-35 of SEQ ID NO:324 (CDR-H1); residues 50-66 of SEQ ID NO:324 (CDR-H2); residues 99-108 of SEQ ID NO:324 (CDR-H3);
residues 31-35 of SEQ ID NO:325 (CDR-H1); residues 50-66 of SEQ ID NO:325 (CDR-H2); residues 99-108 of SEQ ID NO:325 (CDR-H3);
residues 31-35 of SEQ ID NO:326 (CDR-H1); residues 50-66 of SEQ ID NO:326 (CDR-H2); residues 99-108 of SEQ ID NO:326 (CDR-H3);

residues 23-33 of SEQ ID NO:327 (CDR-L1); residues 49-55 of SEQ ID NO:327 (CDR-L2); residues 88-96 of SEQ ID NO:327 (CDR-L3);
residues 23-33 of SEQ ID NO:328 (CDR-L1); residues 49-55 of SEQ ID NO:328 (CDR-L2); residues 88-96 of SEQ ID NO:328 (CDR-L3);
residues 23-33 of SEQ ID NO:329 (CDR-L1); residues 49-55 of SEQ ID NO:329 (CDR-L2); residues 88-96 of SEQ ID NO:329 (CDR-L3);
residues 23-33 of SEQ ID NO:330 (CDR-L1); residues 49-55 of SEQ ID NO:330 (CDR-L2); residues 88-96 of SEQ ID NO:330 (CDR-L3);
residues 23-33 of SEQ ID NO:331 (CDR-L1); residues 49-55 of SEQ ID NO:331 (CDR-L2); residues 88-96 of SEQ ID NO:331 (CDR-L3);
residues 23-33 of SEQ ID NO:332 (CDR-L1); residues 49-55 of SEQ ID NO:332 (CDR-L2); residues 88-96 of SEQ ID NO:332 (CDR-L3);
residues 23-33 of SEQ ID NO:333 (CDR-L1); residues 49-55 of SEQ ID NO:333 (CDR-L2); residues 88-96 of SEQ ID NO:333 (CDR-L3).

In another embodiment, the binding protein comprises at least 3 CDRs disclosed above.

Preferably, a DLL4 binding protein according to the disclosure comprises one or more CDRs disclosed above. More preferably, the binding protein comprises three or more CDRs disclosed above. Most preferably, a DLL4 binding protein according to the disclosure comprises six CDRs described above, i.e., a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 described above.

In a preferred embodiment, the binding protein comprises at least 3 CDRs selected from the group consisting of the sequences disclosed above.

In another preferred embodiment, a binding protein comprises 3 CDRs selected from a set of variable domain CDRs selected from the group below.

VH A10 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:112
CDR-H2: residues 50-66 of SEQ ID NO:112
CDR-H3: residues 99-108 of SEQ ID NO:112

VL A10 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:113
CDR-L2: residues 49-55 of SEQ ID NO:113
CDR-L3: residues 88-96 of SEQ ID NO:113

VH A10.3 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:316
CDR-H2: residues 50-66 of SEQ ID NO:316
CDR-H3: residues 99-108 of SEQ ID NO:316

VL A10.3 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:327

CDR-L2: residues 49-55 of SEQ ID NO:327

CDR-L3: residues 88-96 of SEQ ID NO:327

VH A10.K30 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:317

CDR-H2: residues 50-66 of SEQ ID NO:317

CDR-H3: residues 99-108 of SEQ ID NO:317

VH A10.K42 CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:318

CDR-H2: residues 50-66 of SEQ ID NO:318

CDR-H3: residues 99-108 of SEQ ID NO:318

VH A10.9A CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:319

CDR-H2: residues 50-66 of SEQ ID NO:319

CDR-H3: residues 99-108 of SEQ ID NO:319

VH A10.8A CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:320

CDR-H2: residues 50-66 of SEQ ID NO:320

CDR-H3: residues 99-108 of SEQ ID NO:320

VH A10.1A CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:321

CDR-H2: residues 50-66 of SEQ ID NO:321

CDR-H3: residues 99-108 of SEQ ID NO:321

VH A10.5D CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:322

CDR-H2: residues 50-66 of SEQ ID NO:322

CDR-H3: residues 99-108 of SEQ ID NO:322

VH A10.3A CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:323

CDR-H2: residues 50-66 of SEQ ID NO:323

CDR-H3: residues 99-108 of SEQ ID NO:323

VL A10.3A CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:330

CDR-L2: residues 49-55 of SEQ ID NO:330

CDR-L3: residues 88-96 of SEQ ID NO:330

VH A10.6B CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:324

CDR-H2: residues 50-66 of SEQ ID NO:324

CDR-H3: residues 99-108 of SEQ ID NO:324

VL A10.6B CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:331

CDR-L2: residues 49-55 of SEQ ID NO:331

CDR-L3: residues 88-96 of SEQ ID NO:331

VH A10.3D CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:325

CDR-H2: residues 50-66 of SEQ ID NO:325

CDR-H3: residues 99-108 of SEQ ID NO:325

VL A10.3D CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:332

CDR-L2: residues 49-55 of SEQ ID NO:332

CDR-L3: residues 88-96 of SEQ ID NO:332

VH A10.4C CDR Set

CDR-H1: residues 31-35 of SEQ ID NO:326

CDR-H2: residues 50-66 of SEQ ID NO:326

CDR-H3: residues 99-108 of SEQ ID NO:326

VL A10.4C CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:333

CDR-L2: residues 49-55 of SEQ ID NO:333

CDR-L3: residues 88-96 of SEQ ID NO:333

VL A10.L45 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:328

CDR-L2: residues 49-55 of SEQ ID NO:328

CDR-L3: residues 88-96 of SEQ ID NO:328

VL A10.L73 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:329

CDR-L2: residues 49-55 of SEQ ID NO:329

CDR-L3: residues 88-96 of SEQ ID NO:329

In another embodiment, a binding protein comprises a variable heavy chain (VH) set of 3 CDRs selected from any VH set of 3 CDRs in the group above and a variable light chain (VL) set of 3 CDRS selected from any VL set of 3 CDRs in the group above.

In still another embodiment a binding protein comprises a named VH set of 3 CDRs and a correspondingly named VL set of 3 CDRs from the group below. Preferably, a binding protein according to the disclosure comprises at least two variable domain CDR sets selected from the group of variable domain CDR sets consisting of:

VH A10 CDR Set and VL A10 CDR Set;
VH A10.3 CDR Set and VL A10.3 CDR Set:
VH A10.3A CDR Set and VL A10.3A Set;
VH A10.6B CDR Set and VL A10.6B Set;
VH A10.3D CDR Set and VL A10.3D CDR Set;
VH A10.4C CDR Set and VL A10.4C CDR Set;
VH A10.K30 CDR Set and VL A10.3 CDR Set;
VH A10.K42 CDR Set and VL A10.3 CDR Set;
VH A10.3 CDR Set and VL A10.L45 CDR Set;
VH A10.3 CDR Set and VL A10.L73 CDR Set;
VH A10.9A CDR Set and VL A10.3 CDR Set;
VH A10.8A CDR Set and VL A10.3 CDR Set;
VH A10.1A CDR Set and VL A10.3 CDR Set; and
VH A10.5D CDR Set and VL A10.3 CDR Set.

In yet another embodiment, a binding protein described above further comprises a human acceptor framework. Preferably, the human acceptor framework comprises an amino acid sequence selected from the group consisting of:

heavy chain acceptor framework sequences SEQ ID NOS:6-22,
heavy chain acceptor sequences SEQ ID NOS:35-62,
light chain acceptor sequences SEQ ID NOS:23-34, and
light chain acceptor sequences SEQ ID NOS:63-98.

In another embodiment a binding protein described above comprises a human acceptor framework, which comprises at least one framework region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of said human acceptor framework and comprising at least 70 amino acid residues identical to said human acceptor framework.

In another embodiment a binding protein described herein comprises a human acceptor framework that 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 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.

Preferably, the key residue is selected from the group consisting of: 2H, 4H, 24H, 26H, 27H, 29H, 34H, 35H, 37H, 39H, 44H, 45H, 47H, 48H, 49H, 50H, 51H, 58H, 59H, 60H, 63H, 67H, 69H, 71H, 73H, 76H, 78H, 91H, 93H, 94H, 2L, 4L, 25L, 29L, 27bL, 33L, 34L, 36L, 38L, 43L, 44L, 46L, 47L, 48L, 49L, 55L, 58L, 62L, 64L, 71L, 87L, 89L, 90L, 91L, 94L, 95L.

In another embodiment, a binding protein described herein comprises a consensus human variable domain.

In a preferred embodiment, a binding protein described above comprises at least one variable domain having amino acid sequence selected from the group consisting of: SEQ ID NOS:112, 113, 316, 327, 317, 318, 319, 320, 321, 322, 323, 330, 324, 331, 325, 332, 326, 333, 328, and 329.

In another embodiment, a binding protein described above comprises two variable domains, wherein said two variable domains have amino acid sequences selected from the group consisting of: SEQ ID NOS:112 and 113, SEQ ID NOS:316 and 327, SEQ ID NOS:323 and 330, SEQ ID NOS:324 and 331, SEQ ID NOS:325 and 332, and SEQ ID NOS:326 and 333.

In an embodiment, a binding protein according to the disclosure comprises heavy chain variable domain (V_H), preferably wherein the V_H comprises an amino acid sequence selected from the group consisting of:

SEQ ID NOS:112, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, and 326.

In yet another embodiment, a binding protein according to the disclosure comprises a light chain variable domain (a V_L), preferably wherein the V_L comprises an amino acid sequence selected from the group consisting of:

SEQ ID NOS:113, 327, 328, 329, 330, 331, 332, and 333.

In another embodiment, the disclosure provides a binding protein capable of binding human DLL-4, said binding protein comprising:

an Ig constant heavy region having an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3;

an Ig constant light region having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5;

an Ig variable heavy region having an amino acid sequence selected from the group consisting:

SEQ ID NOS: SEQ ID NOS:112, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, and 326; and

an Ig variable light region having an amino acid sequence selected from the group consisting:

SEQ ID NOS: 113, 327, 328, 329, 330, 331, 332, and 333.

In one embodiment, there is provided a binding protein capable of binding human DLL-4, said binding protein comprising:

an Ig constant heavy region having an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3;

an Ig constant light region having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5;

an Ig variable heavy region having an amino acid sequence selected from the group consisting:

SEQ ID NOS:1, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, and 360; and

an Ig variable light region having an amino acid sequence selected from the group consisting:

SEQ ID NOS:111, 228, 232, 236, 240, 244, 248, 252, 256, 260, 264, 268, 216, 220, 224, 272, 276, 300, 292, 280, 288, 296, 284, 304, 308, 312, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, and 361.

According to the disclosure, variable heavy (VH) domains and variable light (VL) domains of 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 a preferred embodiment, a DLL4 binding protein according to the disclosure 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 mouse DLL4 (mu DLL4), a cynomolgus monkey DLL4 (cynomolgus DLL4, cyno DLL4), a rat DLL4, and combinations thereof.

In another embodiment, a DLL4 binding protein is a fully human antibody or antigen binding portion thereof.

In another embodiment, a DLL4 binding protein is a CDR grafted antibody. More preferably, a DLL4 binding protein is a CDR-grafted antibody or antigen binding portion thereof comprising one or more CDRs described above.

Still more preferably, the CDR grafted antibody or antigen binding portion thereof comprise a variable domain described above. More preferably, a CDR grafted antibody or antigen binding portion thereof comprises two variable domains described above. Preferably, the CDR grafted antibody or antigen binding portion thereof comprises a human acceptor framework. More preferably, the human acceptor framework is any one of the human acceptor frameworks described above.

More preferably, a binding protein is capable of neutralizing an activity of a DLL4 selected from the group consisting of human DLL4, mouse DLL4, cynomolgus monkey DLL4, rat DLL4, and combinations thereof. Evaluating the neutralization of activity of DLL4 can be assessed via several *in vitro* and *in vivo* assays know in the art. Exemplary parameters for assessing neutralization of DLL4 activity include, but are not limited to, antibodies that inhibit DLL4 interaction with the Notch receptor, and/or Notch-signaling pathway with an IC_{50} values of about at least 10^{-6} M; at least 10^{-7} M, or at least 10^{-8} M.

In one embodiment, the binding protein of the disclosure 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 disclosure 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, the binding protein of the disclosure 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, the binding protein of the disclosure 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 disclosure 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 one embodiment, there is provided a labeled binding protein comprising a binding protein as defined herein, wherein said binding protein is conjugated to a detectable label.

One embodiment of the disclosure provides an antibody construct comprising any one of the DLL4 binding proteins disclosed above and a linker polypeptide or an immunoglobulin constant domain.

In one embodiment, there is provided an antibody construct comprising a binding protein as defined herein and further comprising a linker polypeptide or an immunoglobulin constant domain.

In a preferred embodiment, an antibody construct according to the disclosure 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 $F(ab')_2$, a 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 disclosure 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, and mutants of the above Ig isotypes which may alter Fc gamma receptor binding, FcRn binding, C1q binding and may alter pharmacokinetic properties and/or Fc effector functions.

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

In another embodiment, a DLL4 binding protein described herein is conjugated to an agent. Binding protein conjugates of the disclosure include antibody conjugates in which an antibody construct described herein is conjugated to an agent. Preferably, the agent is selected from the group consisting of: an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.

In one embodiment, there is provided an antibody conjugate comprising an antibody construct as defined herein, wherein said antibody construct is conjugated to a therapeutic or cytotoxic agent.

In a preferred embodiment, the imaging agent 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 a binding protein conjugate (including antibody conjugates) disclosed above exists as a crystal. Preferably, the crystal is a carrier-free pharmaceutical controlled release crystal. In a preferred 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 another preferred embodiment, the crystallized binding protein, crystallized antibody construct, or crystallized binding protein conjugate (including antibody conjugate) retains biological activity after crystallization.

One aspect of the disclosure 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.

In one embodiment there is provided 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 CDR-H1, a CDR-H2, or a CDR-H3 as defined herein; a polypeptide comprising a light chain variable domain, wherein the light chain variable domain comprises one or more of CDR-L1, a CDR-L2, or a CDR-L3 as defined herein; or a combination of both polypeptides.

A further embodiment provides a vector comprising an isolated nucleic acid disclosed above. 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)), pHybE, pBV, pJV, and pBJ, and any other expression vectors suitable for prokaryotic or eukaryotic cells.

In another aspect of the disclosure there is provided a host cell is transformed with the vector disclosed above. The host cell may be 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, but not limited to, *Saccharomyces cerevisiae*. A preferred insect cell is an Sf9 cell.

Another aspect of the disclosure provides a method of producing a binding protein that binds human DLL4 comprising the step of culturing any one of the host cells disclosed above in a culture medium under conditions sufficient to produce a binding protein that binds human DLL4. Another embodiment provides a binding protein produced according to the method disclosed above.

One embodiment provides a composition for the release of a DLL4 binding protein according to the disclosure wherein the composition comprises a formulation which in turn

comprises a crystallized DLL4 binding protein, crystallized antibody construct, or crystallized binding protein conjugate (including antibody conjugates) as disclosed 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 polyeaccharides, 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 protein conjugate (including antibody conjugates) disclosed above.

The disclosure also provides a pharmaceutical composition comprising a DLL4 binding protein, an antibody construct, or a binding protein conjugate (including antibody conjugates) as disclosed 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 comprises an additional agent selected from the group consisting of: a therapeutic agent; an imaging agent; an antineoplastic agent; a chemtherapeutic agent (such as 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), and a receptor tyrosine kinase inhibitors (e.g., erlotinib, gefitinib), a COX-2 inhibitor (e.g., celecoxib), a kinase inhibitor, and an angiogenesis inhibitor (including but not limited to an anti-VEGF antibody or VEGF-trap); a co-stimulation molecule blocker (including but not limited to anti-B7.1 antibody, anti-B7.2 antibody, CTLA4-Ig, anti-CD20 antibody); an adhesion molecule blocker (including but not limited to an anti-LFA-1 antibody, an anti-E/L selectin antibody, and a small molecule inhibitor); anti-cytokine antibody or functional fragment thereof (including but not limited to an anti-IL-18, an anti-TNF, and an anti-IL-6/cytokine receptor antibody); methotrexate; cyclosporine; rapamycin; FK506; a detectable label or reporter molecule; 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 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 or analog thereof; a cytokine; and a cytokine antagonist.

In another aspect, the disclosure 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, multiple sclerosis, atherosclerotic plaques, Crohn's disease, inflammatory bowel disease, refractory ascites, psoriasis, sarcoidosis, arterial arteriosclerosis, sepsis, peptic ulcers, burns, and pancreatitis, polycystic ovarian disease (POD), endometriosis, uterine fibroids, benign prostate hypertrophy, and other angiogenesis independent and dependent diseases characterized by abberant DLL4 activity.

In another aspect the disclosure 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 second agent, as discussed above. In a preferred embodiment, the second agent is selected from the group consisting of: radiotherapeutic agent; an antineoplastic agent; a chemotherapeutic agent (such as 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 (e.g., erlotinib, gefitinib), a COX-2 inhibitor (e.g., celecoxib), a kinase inhibitor, and an angiogenesis inhibitor (including but not limited to an anti-VEGF antibody or VEGF-trap); a co-stimulation molecule blocker (including but not limited to anti-B7.1, anti-B7.2, CTLA4-Ig, anti-CD20); an adhesion molecule blocker (including but not limited to an anti-LFA-1 antibody, an anti-E/L selectin antibody, a small molecule inhibitor); anti-cytokine antibody or functional fragment thereof (including but not limited to anti-IL-18, anti-TNF, anti-IL-6/cytokine receptor antibodies); methotrexate; cyclosporine; rapamycin; FK506; 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 or analog thereof; a cytokine; and a cytokine antagonist.

In one embodiment, there is provided the use of the binding protein as defined herein, in the manufacture of a pharmaceutical composition for reducing human DLL4 activity.

In one embodiment, there is provided the use of the binding protein as defined herein, in the manufacture of a pharmaceutical composition for reducing human DLL4 activity in a human subject suffering from a disorder in which DLL4 activity is detrimental.

In one embodiment, there is provided the use of the binding protein as defined herein, in the manufacture of a pharmaceutical composition for treating a patient suffering from a disorder in which DLL4 is detrimental, wherein said composition is for administration before, concurrent with, or after the administration 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; corticosteroids, cyclosporine, rapamycin, FK506, and non-steroidal anti-inflammatory agents.

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, intrarticular, 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 disclosure provides at least one DLL4 anti-idiotype antibody to at least one DLL4 binding protein of the present disclosure. 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 disclosure.

Any of a variety of immunodetection assay formats may be adapted to employ a DLL4 binding protein of the disclosure to detect 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 disclosure adsorbed or immobilized to substrate, FACS, and the like. Detection of DLL4 using a DLL4 binding protein of the disclosure may be conducted *in vitro* on a mixture, solution, or in biological sample. A biological sample that may be contacted with 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. 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 VEGR1 activity, either *in vitro* or *in vivo*, and methods to regulate gene expression are also encompassed by the invention.

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.

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 complementary 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 complementary 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 1.

Table 1: **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:2	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKG

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

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, 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 complementary 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 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) residue, 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:374), 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:375), 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:376), 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:377), 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 FR's 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 acceptor sequences are known in the art. In one embodiment of the invention the human heavy chain and light chain acceptor sequences are selected from the sequences described in Table 2 and Table 3.

Table 2. Heavy Chain Acceptor Sequences

SEQ ID NO.:	Protein region/ Closest Germline Family	Sequence
		123456789012345678901234567890 12
6	VH2-70/JH6 FR1	EVTLRESGPALVKPTQTLTLTCTFSGFSLS
7	VH2-70/JH6 FR2	WIRQPPGKALEWLA
8	VH2-70/JH6 FR3	RLTISKDTSKNQVVLTMTNMDPVDTATYYCAR
9	VH2-70/JH6 FR4	WGQGTTVTVSS
10	VH2-26/JH6 FR1	EVTLKESGPVLVKPTETLTLTCTVSGFSLS
7	VH2-26/JH6 FR2	WIRQPPGKALEWLA
11	VH2-26/JH6 FR3	RLTISKDTSKSQVVLTMTNMDPVDTATYYCAR
9	VH2-26/JH6 FR4	WGQGTTVTVSS
12	VH3-72/JH6 FR1	EVQLVESGGGLVQPGGSLRLSCAASGFTFS
13	VH3-72/JH6 FR2	WVRQAPGKGLEWVG
14	VH3-72/JH6 FR3	RFTISRDDSKNSLYLQMNSLKTEDTAVYYCAR
9	VH3-72/JH6 FR4	WGQGTTVTVSS
15	VH3-21/JH6 FR1	EVQLVESGGGLVKPGGSLRLSCAASGFTFS
16	VH3-21/JH6 FR2	WVRQAPGKGLEWVS
17	VH3-21/JH6 FR3	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR
9	VH3-21/JH6 FR4	WGQGTTVTVSS
18	VH1-69/JH6 FR1	EVQLVQSGAEVKKPGSSVKVSCKASGGTFS
19	VH1-69/JH6 FR2	WVRQAPGQGLEWMG
20	VH1-69/JH6 FR3	RVTITADKSTSTAYMELSSLRSEDTAVYYCAR
9	VH1-69/JH6 FR4	WGQGTTVTVSS

SEQ ID NO.:	Protein region/ Closest Germline Family	Sequence
21	VH1-18/JH6 FR1	EVQLVQSGAEVKKPGASVKVSCKASGYTFT
19	VH1-18/JH6 FR2	WVRQAPGQGLEWMG
22	VH1-18/JH6 FR3	RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR
9	VH1-18/JH6 FR4	WGQGTTVTVSS
35	IGHV4-59 FR1	EVQLQESGPGLVKPSETLSLTCTVSGGSIS
36	IGHV4-59 FR2	WIRQPPGKGLEWIG
37	IGHV4-59 FR3	RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR
38	IGHV4-59/JH FR4	WGQGTLVTVSS
39	IGHV3-66 FW1	EVQLVESGGGLVQPGGSLRLSCAVSGGSIS
40	IGHV3-66 FW2	WIRQAPGKGLEWIG
41	IGHV3-66 FW3	RVTISVDTSKNSFYLQMNSLRAEDTAVYYCAR
42	IGHV3-66/JH FW4	WGQGTLVTVSS
43	IGHV4-59 FR1	EVQLQESGPGLVKPGETLSLTCTVSGGSIS
44	IGHV4-59 FR2	WIRQAPGKGLEWIG
45	IGHV4-59 FR3	RVTISVDTSKNQFYLKLSSVRAEDTAVYYCAR
46	IGHV4-59/JH FR4	WGQGTLVTVSS
47	IGHV5-51 FR1	EVQLVQSGTEVKKPGESLKISCKVSGGSIS
48	IGHV5-51 FR2	WIRQMPGKGLEWIG
49	IGHV5-51 FR3	QVTISVDTSFNTFFLQWSSLKASDTAMYYCAR
50	IGHV5-51/JH FR4	WGQGTMVTVSS
51	IGHV2-70 FR1	EVTLRESGPALVKPTQTLTLTCTVSGGSIS
52	IGHV2-70 FR2	WIRQPPGKGLEWIG
53	IGHV2-70 FR3	RVTISVDTSKNQFVLTMTNMDPVDTATYYCAR
54	IGHV2-70/JH FR4	WGQGTTVTVSS
55	IGHV3-15 FR1	EVQLLESGGGLVKSGGSLRLSCAASGFTFR
56	IGHV3-15 FR2	WVRQAPGKGLEWVA
57	IGHV3-15 FR3	RFTISRDNSKNTLYLQLNSLRAEDTAVYYCAK
58	IGHV3-15/JH FR4	WGQGTMVTVSS
59	IGHV3-43 FR1	EVQLVESGGGVVQPGGSLRLSCAASGFTFG
60	IGHV3-43 FR2	WVRQAPGKGLEWVA
61	IGHV3-43 FR3	RFTISRDNSKNTLYLQLNSLRAEDTAVYYCAK
62	IGHV3-43/JH FR4	WGQGTMVTVSS

Table 3. Light Chain Acceptor Sequences

SEQ ID NO.:	Protein region/ Closest Germline Family	Sequence
		123456789012345678901234567890 12
23	B3/JK4 FR1	DIVMTQSPDSLAVSLGERATINC
24	B3/JK4 FR2	WYQQKPGQPPKLLIY
25	B3/JK4 FR3	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC
26	B3/JK4 FR4	FGGGTKVEIKR
27	L2/JK4 FR1	EIVMTQSPATLSVSPGERATLSC
28	L2/JK4 FR2	WYQQKPGQAPRLLIY
29	L2/JK4 FR3	GIPARFSGSGSGTEFTLTISSLQSEDFAVYYC
26	L2/JK4 FR4	FGGGTKVEIKR
30	L15/JK4 FR1	DIQMTQSPSSLSASVGDRVTITC

SEQ ID NO.:	Protein region/ Closest Germline Family	Sequence
31	L15/JK4 FR2	WYQQKPEKAPKSLIY
32	L15/JK4 FR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
26	L15/JK4 FR4	FGGGTKVEIKR
33	L5/JK4 FR1	DIQMTQSPSSVSASVGDRVTITC
34	L5/JK4 FR2	WYQQKPGKAPKLLIY
32	L5/JK4 FR3	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC
26	L5/JK4 FR4	FGGGTKVEIKR
63	IGLV3-1 FR1	SYELTQPPSVSVSPGQTASITC
64	IGLV3-1 FR2	WYQQKPGQSPVLVIY
65	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQPMDEADYYC
66	IGLV3-1/JL FR4	FGYGTKVTVL
67	IGLV3-1 FR1	SYELTQPPSVSVSPGQTASITC
68	IGLV3-1 FR2	WYQQKPGQSPVLVIY
69	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQPMDEADYYC
70	IGLV3-1/JL FR4	GGGTKLTVLG
71	IGLV3-1 FR1	YELTQPPSVSVSPGQTASITC
72	IGLV3-1 FR2	WYQQKPGQSPVLVIY
73	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQPMDEADYYC
74	IGLV3-1/JL FR4	GGGTKLTVLG
75	IGLV3-1 FR1	LYVLTQPPSVSVSPGQTASITC
76	IGLV3-1 FR2	WYQQKPGQSPVLVIY
77	IGLV3-1 FR3	GIPERFSGSNSGDTATLTISGTQTMDEADYLC
78	IGLV3-1/JL FR4	FGGGTKVTVLG
79	IGKV6D-21 FR1	EYVLTQSPDFQSVTPKEKVTITC
80	IGKV6D-21 FR2	WYQQKPDQSPKLVIY
81	IGKV6D-21 FR3	GVPSRFSGSNSGDDATLTINSLEAEDAATYYC
82	IGKV6D-21/JK FR4	FGQGTKVEIKR
83	IGKV3D-15 FR1	EYVLTQSPATLSVSPGERATLSC
84	IGKV3D-15 FR2	WYQQKPGQSPRLVIY
85	IGKV3D-15 FR3	DIPARFSGSNSGDEATLTISSLQSEDFAVYYC
86	IGKV3D-15/JK FR4	FGQGTRLEIKR
87	IGKV4-1 FR1	DYVLTQSPDSLAVSLGERATINC
88	IGKV4-1 FR2	WYQQKPGQSPKLVIY
89	IGKV4-1 FR3	GIPDRFSGSNSGDDATLTISSLQAEDVAVYYC
90	IGKV4-1/JK FR4	FGGGTKVEIKR
91	IGLV3-1 FR1	LPVLTQPPSVSVSPGQTASITC
92	IGLV3-1 FR2	WYQQKPGQSPVLVIY
93	IGLV3-1 FR3	GIPERFSGSNSGNTATLTISGTQTMDEADYLC
94	IGLV3-1/JL FR4	FGGGTKVTVL
95	IGLV3-1 FR1	SYELTQPPSVSVSPGQTASITC
96	IGLV3-1 FR2	WYQQKPGQSPVLVIY
97	IGLV3-1 FR3	GIPERFSGSNSGNTATLTISGTQTMDEADYLC
98	IGLV3-1/JL FR4	FGGGTKLTVL

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 reduces its biologically activity by at least about 20%, 40%, 60%, 80%, 85%, 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 receptor binding in a ligand-receptor binding assay or inhibition of receptor activation in a human Notch reporter assay, or stimulation of endothelial cell proliferation in a endothelial cell sprouting 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 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 human monoclonal antibodies, or antigen-binding portions thereof, that bind to DLL4 with high affinity, a slow off rate, and/or high neutralizing capacity. Advantageously, such human antibodies or antigen-binding portions thereof that bind DLL4 find use as human therapeutic agents that can be administered to a human

patient with minimal or no response by the patient's immune system toward the administered therapeutic DLL4 binding protein. Accordingly, a patient may obtain the benefit of such fully human DLL4 binding proteins over the course of repeated administrations. Other aspects of the invention provide chimeric antibodies that bind DLL4 and CDR grafted antibodies, or antigen-binding portions thereof, that bind DLL4. Preferably, the antibodies, or portions thereof, are isolated antibodies. Preferably, the antibodies of the invention are neutralizing human anti-DLL4 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. A preferred method is PROfusion mRNA display technology as exemplified in Example 2 herein. Another method is to immunize a transgenic rodent (e.g., a transgenic mouse) that carries a functional complement of human immunoglobulin genes with human DLL4 or antigenic portion thereof followed by standard hybridoma technology to generate hybridomas that express fully human monoclonal antibodies that bind human DLL4. Recombinant human antibodies obtain by such a method have variable and constant regions derived from human germline immunoglobulin sequences. Such methods provide fully human DLL4 binding proteins and eliminate the need to otherwise carry out one or more rounds of humanization to reduce the sources of non-human antigenicity of the monoclonal DLL4 antibody molecules. Accordingly, techniques utilizing material from multiple species are less preferred but may be used.

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.

Additional aspects of various techniques that may be employed to obtain DLL4 monoclonal antibody molecules 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, pages 563-681 (Elsevier, New York, 1981) (said references incorporated by reference in their entireties). Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In one 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 a mouse immunized with an antigen of the invention 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, mice can be immunized with an DLL4 antigen. In a preferred embodiment, the DLL4 antigen is administered with a 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 mouse serum, the mouse 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 mice 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, incorporated herein by reference.

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 mouse hybridomas, as described above. In another embodiment, hybridomas are produced in a non-human, non-mouse species such as rats, 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 Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region, and the CHI domain of the heavy chain.

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 an DLL4 antigen. In a preferred 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, the 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; each of which is incorporated herein by reference in its entirety.

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) (said references incorporated by reference in their entireties). 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.

Preferred 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 and the other heavy and light chain are specific for an antigen other than the antigens of interest 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 fully human antibodies that bind human DLL4 are shown for clones E9 and A10 in Table 4 (See, Examples, below). The isolated anti-DLL4 antibody CDR sequences of the E9 and A10 antibodies establish two novel families of DLL4 binding proteins, isolated in accordance with this invention, and comprising polypeptides that include the CDR sequences derived from E9 and affinity matured clones thereof or derived from A10 and affinity matured clones thereof. The variable regions and CDRs of the E9 monoclonal antibody and affinity matured derivatives thereof are listed in Tables 4, 8, 14, 18, and 19. The variable regions and CDRs of the A10 monoclonal antibody and affinity matured derivatives thereof are listed in Table 4, 9, and 10. 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 E9 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 CDRs selected from the group consisting of:

CDR-H1: X_1- X_2 - X_3 - X_4 - X_5 - X_6 - X_7 (SEQ ID NO:99), wherein;

X_1 is S or N;

X_2 is S, G or N;

X_3 is S, N, T, G or R;

X_4 is Y;

X_5 is Y or H;

X_6 is W; and

X_7 is G;

CDR-H2: 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} (SEQ ID NO:100), wherein;

X_1 is D;

X_2 is I;

X_3 is Y, N, or S;

X_4 is Y;

X_5 is T, N, A, I, S or R;

X_6 is G;

X_7 is S, N, T or G;

X_8 is T;

X_9 is Y;

X_{10} is Y;

X_{11} is N;

X_{12} is P;

X_{13} is S;

X_{14} is L;

X_{15} is K; and

X_{16} is S, N, D or G;

CDR-H 3: 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:101), wherein;

X_1 is E, Y, F, Q, W, L, or A;

X_2 is D, A, S, G, V, E or N;

X_3 is V, M, L, P, or A;

X_4 is I, A, P, R, S, K, Q, V, G, M or E;

X_5 is L, Y, F or M;

X_6 is R, G, S, Q or A

X_7 is G;

X_8 is G, A or S;

X_9 is S, A, L, V, R or G;

X_{10} is D; and

X_{11} is Y, D, S, N, H, E, R, L, P, C, I M, T, Q, or K;

CDR-L1: 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:102), wherein;

X_1 is S;

X_2 is G;

X_3 is Q, E or D;

X_4 is R, S, G, M, K, L or T;

X_5 is L;

X_6 is G;

X_7 is D or E;

X_8 is K;

X_9 is Y;

X_{10} is A or V; and

X_{11} is S;

CDR-L2: X_1- X_2 - X_3 - X_4 - X_5 - X_6 - X_7 (SEQ ID NO:103), wherein;

X_1 is E or Q;

X_2 is D;

X_3 is S, L, T, A, E or F;

X_4 is K, T, E, N, Q, S, or M;

X_5 is R;

X_6 is P; and

X_7 is S;

and

CDR-L3: X_1- X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 – X_9 (SEQ ID NO:104), wherein;

X_1 is Q;

X_2 is A;

X_3 is W;

X_4 is D;

X_5 is R, S, M, E, N, G, or K;

X_6 is D or E

X_7 is T, V, A, S or M;

X_8 is G, A or C; and

X_9 is V.

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.

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 A10 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 CDRs selected from the group consisting of:

CDR-H1: X_1- X_2 - X_3 - X_4 - X_5 (SEQ ID NO: 105), wherein;

X_1 is S, N, or D;

X_2 is H or Y;

X_3 is W;

X_4 is M; and

X_5 is S or H;

CDR-H2: 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:106), wherein;

X_1 is I, D, M, or T;

X_2 is I;

X_3 is S;

X_4 is Y, N, S, Q, V, T, H, or D;

X_5 is D;

X_6 is G;

X_7 is S, R, I, T, G, K, H, or N;

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

X_9 is K, M, N, Q, E, T, R, S, A, or L;

X_{10} is Y, D, or E;

X_{11} is S or Y;

X_{12} is A;

X_{13} is D;

X_{14} is S;

X_{15} is V;

X_{16} is K; and

X_{17} is G;

CDR-H3: X_1- X_2 - X_3 - X_4 - X_5 - X_6 - X_7 – X_8 – X_9- X_{10} (SEQ ID NO:107), wherein;

X_1 is A;

X_2 is G, A, or R;

X_3 is G;

X_4 is G, S, or A;

X_5 is N;

X_6 is V or M;

X_7 is G;

X_8 is F, L, Y, or M;

X_9 is D; and

X_{10} is I, S, or L;

CDR-L1: 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:108), wherein;

X_1 is S;

X_2 is A or G;

X_3 is D;

X_4 is K, N, L, Q, M, E, S, T, G, or D;

X_5 is L;

X_6 is G;

X_7 is T, S, N, A, G, or E;

X_8 is K, Q, N, or R;

X_9 is Y;

X_{10} is V or I; and

X_{11} is S;

CDR-L2: X_1- X_2 - X_3 - X_4 - X_5 - X_6 - X_7 (SEQ ID NO:109), wherein;

X_1 is Q;

X_2 is D;

X_3 is A, G, W, S, or D;

X_4 is K, M, Q, N, L, T, I, or E;

X_5 is R;

X_6 is P; and

X_7 is S;

and

CDR-L3: X_1- X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 – X_9 (SEQ ID NO:110), wherein;

X_1 is Q;

X_2 is S or A;

X_3 is W;

X_4 is D;

X_5 is R, S, Q, P, A, V, W, or M;

X_6 is S, G, I, N, R, or T

X_7 is D or G;

X_8 is V, A, P, or E; and

X_9 is V.

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, which are incorporated herein by reference in their entireties. 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 complementary 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. Evaluating the neutralization of activity of DLL4 can be assessed via several *in vitro* and *in vivo* assays know in the art. Exemplary parameters for

assessing neutralization of DLL4 activity include, but are not limited to, antibodies that inhibit DLL4 interaction with the Notch receptor, and/or Notch-signaling pathway with an IC_{50} values of about at least 10^{-6} M; at least 10^{-7} M, or at least 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, the 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 $1 \times 10^{-2} s^{-1}$ 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 $1 \times 10^{-3} s^{-1}$ 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 $1 \times 10^{-4} s^{-1}$ 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 $1 \times 10^{-5} s^{-1}$ 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 $1 \times 10^{-5} s^{-1}$ 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 alteration 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.

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 U. S. 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, Pennsylavania, 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 effect 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 Sub-cortical Infarcts and Leukoencephalopathy), MS (Multiple Sclerosis), Tetralogy of Fallot, and Alagille syndrome. Preferably, 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 are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS, which include drugs like ibuprofen. Other preferred combinations are corticosteroids including prednisolone; the well known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the

anti-DLL4 antibodies of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, interferons, 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, CD80 (B7.1), CD86 (B7.2), CD90, CTLA or their ligands including CD154 (gp39 or CD40L).

Preferred combinations of therapeutic agents may interfere at different points in the pro-tumorigenic or pro-angiogenic signaling pathways. Preferred examples 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 combined 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 or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1bTNFα converting enzyme (TACE) inhibitors, T-cellconverting enzyme inhibitors, TNFα 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. Preferred combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Non-limiting examples of therapeutic agents for cancers with which a DLL4 binding protein of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporin; 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. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with or IL-1 (e.g., IRAK, NIK,signaling by proinflammatory cytokines such as TNFα IKK, p38 or MAP kinase inhibitors), IL-1β converting enzyme inhibitors, TNFα converting enzyme inhibitors, T-cell signalling 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, sIL-1RI, sIL-1RII, sIL-6R), and anti-inflammatory cytokines (e.g., IL-4, IL-10, IL-11, IL-13, and TGFβ).

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 corticosteroids, for example, budenoside and dexamethasone. Binding proteins of the invention may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid, and olsalazine, and agents which interfere

with synthesis or action of proinflammatory cytokines such as IL-1, for example, IL-1β converting enzyme inhibitors and IL-1ra. DLL4 binding proteins of the invention may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-mercaptopurines. DLL4 binding proteins of the invention can be combined with IL-11. 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.

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 is 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 are blocked with ethanolamine. For kinetic analysis, rate equations derived from the 1:1 Langmuir binding model are 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 are diluted in running buffer for capture across goat anti-human Fc reaction surfaces. Antibodies to be captured as a ligand (1 μg/ml) are 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}) are determined under a continuous flow rate of 80 μl/min. Rate constants are 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 is then calculated from the kinetic rate constants by the following formula: $K_D = K_{off}/K_{on}$. Binding is recorded as a function of time and kinetic rate constants are 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} can 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 human DLL4-his or mouse DLL4-his or cyno DLL4-his 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, Pennsylvania, US, Cat.#112-116-072) was 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 human 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 after an 8-minute incubation at 25°C (for muDLL4 ELISA) and after 20 minute incubation at 25°C (for huDLL4 ELISA) 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). HEK293-hDLL4 or HEK293-mDLL4 cells were dispensed into 96-well plate (v-bottom) at 1.5 x 10^5 cells/well in FACS buffer. After spinning down cells and discarding the supernatant, 50 µL of purified IgG with appropriate dilution was 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 hDLL4-293G or 2.0 µg/mL for mDLL4-293G (1.0 or 0.1 µg/mL final) for 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, Pennsylvania, US, catalog no. 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 Increase of sVEGFR1 (sFLT1) in EA.hy926 Cells by DLL4 Antibodies.

Tissue culture plates, 96-well, were coated with 100 µl/well human DLL4 extracellular domain at 1.67 mg/ml in D-PBS (Gibco #14190) and incubated overnight at 4°C. Plates were washed once with D-PBS and 4000 EA.hy926 cells/well were seeded in the absence or presence of antibodies. Cell proliferation was measured four days later using the CyQUANT Cell Proliferation Assay Kit (Invitrogen, #C35007). sVEGFR1 expression in the conditioned media was detected by an ELISA kit per the manufacturer's recommendations (R&D Systems #DVR100B). Levels of sVEGFR1 were normalized to the RFU determined by CyQUANT assay to account for differences in cell proliferation.

Example 1.7: 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 7000 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 volume of 5000 HEK293G cells/well expressing full-length DLL4. The 293G/DLL4 cells were co-cultured with EA.hy926 Notch reporter cells for 24 hrs in the presence of testing antibodies. Luciferase activity was analyzed by Promega's substrate (Promega # E2940).

Example 1.8: Analytical methods and techniques for molecule identity and 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 is then added to a solution of antibody which is initially at a concentration of less than or equal to 0.5 mg/ml and a volume of 0.5 mL. The PEG solution is 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 is 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 approximately 50 µl while 4 mL Amicon centrifugal filters have a minimum volume of approximately 15 µl.

First a protein is dialyzed into a specific formulation(s). For these studies, the antibody amount was 10 mg or much less. Then the protein solution is inserted into the Amicon centrifugal filter retentate chamber. The chamber is lined with a nitrocellulose membrane with pores that permit molecules of less than 10 to 30 kilodaltons to pass when subjected to centrifugal force. Antibodies which are typically above 140 kilodaltons will be retained while water, buffer molecules, small excipients, and salts will pass through. The centrifugal filter is then centrifuged according to manufacturer specifications 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.

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

Near UV-CD Technique.

Near UV-CD spectroscopy provides important information about the tertiary structure of proteins and is one of the most used techniques in this regard. 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 phenyl-alanine, 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 is scanned from 250-320 nm or 240-320 nm with a Jasco 800 CD spectrometer. The corresponding formulation without protein is also scanned and the readings subtracted from that of the scan of the protein solution. A near UV-CD spectra is 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 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 none- feedback 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 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 and Gombotz, *BioPharm.*, 13: 36-46 (2000), and Remmele et al., *Pharm. Res.*, 15: 200-208 (1998)).

SEC Technique.

Size exclusion chromatography 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, Pennsylvania, US, cat. no. 08543) and a TSK gel G3000SWxL (TOSOH Biosciences, Montgomeryville, Pennsylvania, US, 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) are frozen at -80°C for at least 4 hours and are then thawed at 30°C in a water bath. The solution is then refrozen at -80°C. This is repeated for 5 cycles. After certain freeze-thaw cycles, e.g., second and fourth, a portion of the solution may be withdrawn for analysis by SEC before refreezing. Freeze-thaw stability testing is 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) are 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 are withdrawn under sterile conditions and subjected to analysis by SEC. Solutions are then returned to incubation.

Example 2: Generation and Isolation of Anti-DLL4 Human Monoclonal Antibodies E9 and A10 by PROfusion mRNA Display Technology.

Using PROfusion mRNA display technology (see, Chung-Ming Hsieh et al., US Patent Application Publication No. 2010/0099103), pooled human spleen and lymph node antibody libraries were selected seven rounds against DLL4 antigens: 100 nM biotin-labeled human DLL4 extracellular domain (round 1 and 2), a mixture of 50 nM biotin-labeled human DLL4 extracellular domain, and 50 nM biotin-labeled mouse DLL4 extracellular domain (round 3), 100 nM biotin-labeled human DLL4 extracellular domain (round 4 and 5), 293G cells stably

expressing human DLL4 and 100 nM biotin-labeled human DLL4 extracellular domain (round 6), BAF3 cells stably expressing human and mouse DLL4 (round 7). Both A10 and E9 were identified from the round 7 selection of the antibody libraries (Table 4). When constructed in wild type human IgG1, they are renamed A10.1 and E9.1, respectively.

Table 4. Anti-DLL4 PROfusion Fully Human Antibody Clones E9 and A10 Sequence Information (Kabat numbered CDRs are indicated by underlining)

Clone	Source	Isotype	V Region	Germline	Sequence
					12345678901234567890
E9	Human Lymph Node	huIgG1: VL(λ), VH(L234, 235A)	VH	VH4-39	EVQLQESGPGLVKPSETLSL TCTVSGGSISSSSYYWGWIR QPPGKGLEWIGDIYYTGSTY YNPSLKSRVTISVDTSKNQF SLKLSSVTAADTAVYYCARE DVILRGGSDYWGQGTLVTVS S (SEQ ID NO:1)
			VL	V2-1	SYELTQPPSVSVSPGQTASI TCSGQRLGDKYASWYQQKPG QSPVLVIYEDSKRPSGIPER FSGSNSGDTATLTISGTQPM DEADYYCQAWDRDTGVFGYG TRVTVL (SEQ ID NO:111)
A10	Human Spleen	huIgG1: VL(λ), VH(L234, 235A)	VH	VH3-30	EVQLLESGGGLVKSGGSLRL SCAASGFTFRSHWMSWVRQA PGKGLEWVAIISYDGSNKYS ADSVKGRFTISRDNSKNTLY LQLNSLRAEDTAVYYCAKAG GGNVGFDIWGQGTMVTVSS (SEQ ID NO:112)
			VL	V2-1	LPVLTQPPSVSVSPGQTASI TCSADKLGTKYVSWYQQKPG QSPVLVIYQDAKRPSGIPER FSGSNSGNTATLTISGTQTM DEADYLCQSWDRSDVVFGGG TKVTVL (SEQ ID NO:113)

Example 3: *In Vitro* Characterization of PROfusion Antibodies E9 and A10.

The DLL4 antigen binding affinities for E9 and A10 were determined by the BIACORE technology as described in Example 1.1. As shown in Table 5, below, E9 and A10 have similar equilibrium dissociation constant values against human DLL4 (K_D of 3.36 and 6.68 nM, respectively) and cynomolgus monkey DLL4 (K_D of 4.2 and 7.8 nM, respectively). E9 also cross-reacts with mouse and rat DLL4 (K_D of 16 and 15 nM, respectively).

Table 5. Biacore Kinetics on Anti-DLL4 PROfusion Antibodies.

	Kinetics on Biacore											
MAb	huDLL4 ECD			cynoDLL4 ECD			muDLL4 ECD			ratDLL4 ECD		
	Ka	Kd	K_D	Ka	Kd	K_D	Ka	Kd	K_D	Ka	Kd	K_D
E9	2.0 E+04	6.6 E-05	3.36	1.8 E+04	7.4 E-05	4.2	1.8 E+04	2.6 E-04	16	1.9 E+04	2.9 E-04	15
A10	2.2 E+04	1.5 E-04	6.68	1.9 E+04	1.5 E-04	7.8	--	--	NB	--	--	NB

MAb = monoclonal antibody; E = multiply by 10 to indicated exponent; Ka ($M^{-1}s^{-1}$); Kd (s^{-1}); K_D (nM); NB = no binding (900 nM DLL4)

Antibody-antigen binding activity was also evaluated using ELISA and FACS based assays (described in Example 1.2, 1.3 and EC_{50} values reported in Table 3). In addition to binding to recombinant DLL4 extracellular domain (ECD), E9 and A10 can both bind DLL4 expressed at cell surface (Table 6).

The ability of the antibodies to block DLL4 interaction with its receptor Notch1 was assessed with ELISA and FACS based competition assays as described in Example 1.4 and 1.5. As shown in Table 6, E9 and A10 efficiently blocked the interaction of Notch1 with DLL4 (ECD and cell-bound form). In addition, cell-based functional assays were developed to further determine the ability of the antibodies to neutralize DLL4-mediated cellular activity *in vitro* (as described in Examples 1.6 and 1.7). Both E9 and A10 inhibited DLL4-induced Notch activation and sVEGFR1 expression in EA.hy926 cells (Table 6).

Table 6. PROfusion DLL4 antibody *in vitro* potency.

	Direct Binding Assays					Functional Blockade Assays							
MAb	Capture ELISA (EC_{50}, nM)			FACS (EC_{50}, nM)		Competition ELISA (IC_{50}, nM)			Competition FACS (IC_{50}, nM)		sVEGFR1 Inhibition (IC_{50}, nM)		Inhibition of Notch activation via huDLL4 cells, coculture (IC_{50}, nM)
	DLL4 ECD			DLL4 Cells		DLL4 ECD/ huNotch-1			huNotch-1/ DLL4 cells		DLL4 ECD/ EaHy cells		
	hu	mu	cyno	hu	mu	hu	mu	cyno	hu	mu	hu	mu	
E9	0.17	0.18	0.40	7.23	0.32	1.7	2.1	2.1	23.4	3.2	1.0	0.4	6.2
A10	0.17	-	0.44	0.93	-	2.6	-	5.1	4.4	-	1.5	-	7.3

MAb = monoclonal antibody; hu = human; mu = murine; cyno = cynomolgus monkey

Example 4: Affinity Maturation of PROfusion Antibodies E9 and A10.

Anti-DLL4 E9 Affinity Maturation.

Sequence alignment showed that the DLL4 antibody E9 shares the highest identity to human germlines VH4-39/JH4 and V2-1/JL6. To improve the affinity of E9 to DLL4, hypermutated CDR residues were identified from other human antibody sequences in the

IgBLAST database that also shared high identity to germlines VH4-39 and V2-1. The corresponding E9 CDR residues were then subjected to limited mutagenesis by PCR with primers having low degeneracy at these positions to create three antibody libraries in the scFv format suitable for use an affinity maturation procedure. The first library contained mutations at residues 30, 31, 32, 33, 50, 54, 56, and 65 in the VH CDR1 and 2 (Kabat numbering); the second library at residues 95 to 100, 100a, 100b, 100c, and 102 in VH CDR3; and the third library at residues 27, 30, 31, 33, 52, 53, 93 to 96 in the three VL CDRs. To further increase the identity of E9 to the human germline framework sequences, an Arg at VL position 103 was mutated to a Lys and a binary degeneracy at VL positions 80 (A/P) and 100 (S/Y) was also introduced into the third library (Table 7).

Table 7. Mutations in E9 VH and VL Amino Acid Sequences for Affinity Maturation.

Mutated E9 VH Sequence (SEQ ID NO:114):

```
EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYYTGSTYY
                               NNGN        S           N N N
                               G NT        L             A T
                               R  G                      I G
                                  R                      S
                                                         R


NPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDYWGQGTLVTVSS
     N                         E          YAMAYGGAA D
     D                                    FSLPFS SL S
     G                                    QGPRMQ  V N
                                          WV S A  R H
                                          LE K      E
                                          AN Q      R
                                             V      L
                                             G      P
                                             M      C
                                             E      I
                                                    M
```

Mutated E9 VL Sequence (SEQ ID NO:115):

```
SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPSGIPER
                         ES  E  V                Q LT
                         DG                        TE
                          M                        AN
                          K                        EQ
                          L                        FS
                          T                         M

FSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTRVTVL
                    A           SEVA   S  K
                                M AC
                                E S
                                N M
                                K
```

These E9 libraries were transformed into cells and displayed on cell surfaces to be selected against a low concentration of biotinylated DLL4 extracellular domain by magnetic then fluorescence activated cell sorting (FACS). Selection for improved on-rate, off-rate, or both were carried out and antibody protein sequences of affinity-modulated E9 clones (Table 8) were recovered for converting back to IgG format for further characterization.

Table 8. Protein sequences of antibody clones identified from affinity maturation libraries for anti-DLL4 antibody E9.

Affinity Matured Clones: Heavy Chain (VH) Regions			
E9.4	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARYDVSLGGSSDH WGQGTLVTVSS (SEQ ID NO:116)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:117)	DIYYTGSTYYNPSLKS (SEQ ID NO:118)	YDVSLGGSSDH (SEQ ID NO:119)
E9.11	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREAVPLGGGSDY WGQGTLVTVSS (SEQ ID NO:120)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:121)	DIYYTGSTYYNPSLKS (SEQ ID NO:122)	EAVPLGGGSDY (SEQ ID NO:123)
E9.14	EVQLQESGPGLVKPSETLSLTCTVSGGSISNSRYHWGWIRQSPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAEDTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:124)		
	CDR1	CDR2	CDR3
	NSRYHWG (SEQ ID NO:125)	DIYYTGSTYYNPSLKS (SEQ ID NO:126)	EDVILRGGSDY (SEQ ID NO:127)
E9.17	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREEAILGGGSDY WGQGTLVTVSS (SEQ ID NO:128)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:129)	DIYYTGSTYYNPSLKS (SEQ ID NO:130)	EEAILGGGSDY (SEQ ID NO:131)
E9.18	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSGYYWGWIRQPPGKGLEWIGDINY AGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:132)		
	CDR1	CDR2	CDR3
	SSGYYWG (SEQ ID NO:133)	DINYAGSTYYNPSLKS (SEQ ID NO:134)	EDVILRGGSDY (SEQ ID NO:135)
E9.19	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARFDVSLGGGSDT WGQGTLVTVSS (SEQ ID NO:136)		

	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:137)	DIYYTGSTYYNPSLKS (SEQ ID NO:138)	FDVSLGGGSDT (SEQ ID NO:139)
E9.22	EVQLQESGPGLVKPSETLSLTCTVSGGSISNSRYHWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKGRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:140)		
	CDR1	CDR2	CDR3
	NSRYHWG (SEQ ID NO:141)	DIYYTGSTYYNPSLKG (SEQ ID NO:142)	EDVILRGGSDY (SEQ ID NO:143)
E9.48	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSGYYWGWIRQPPGKGLEWIGDINY RGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:144)		
	CDR1	CDR2	CDR3
	SSGYYWG (SEQ ID NO:145)	DINYRGSTYYNPSLKS (SEQ ID NO:146)	EDVILRGGSDY (SEQ ID NO:147)
E9.65	EVQLQESGPGLVKPSETLSLTCTVSGGSIRNSRYHWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKGRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:148)		
	CDR1	CDR2	CDR3
	NSRYHWG (SEQ ID NO:149)	DIYYTGSTYYNPSLKG (SEQ ID NO:150)	EDVILRGGSDY (SEQ ID NO:151)
E9.66	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREGVPLGGGADK WGQGTLVTVSS (SEQ ID NO:152)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:153)	DIYYTGSTYYNPSLKS (SEQ ID NO:154)	EGVPLGGGADK (SEQ ID NO:155)
E9.71	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARQALAMGGGSDK WGQGTLVTVSS (SEQ ID NO:156)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:157)	DIYYTGSTYYNPSLKS (SEQ ID NO:158)	QALAMGGGSDK (SEQ ID NO:159)
E9.13	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:160)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:161)	DIYYTGSTYYNPSLKS (SEQ ID NO:162)	EDVILRGGSDY (SEQ ID NO:163)
E9.16	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:164)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:165)	DIYYTGSTYYNPSLKS (SEQ ID NO:166)	EDVILRGGSDY (SEQ ID NO:167)
E9.38	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSSYYWGWIRQPPGKGLEWIGDIYY		

	TGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREDVILRGGSDY WGQGTLVTVSS (SEQ ID NO:168)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:169)	DIYYTGSTYYNPSLKS (SEQ ID NO:170)	EDVILRGGSDY (SEQ ID NO:171)
E9.2B	EVQLQESGPGLVKPSETLSLTCTVSGGSISSSNYYWGWIRQPPGKGLEWIGDINY NGNTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREAVALGGGADD WGQGTLVTVSS (SEQ ID NO:172)		
	CDR1	CDR2	CDR3
	SSNYYWG (SEQ ID NO:173)	DINYNGNTYYNPSLKS (SEQ ID NO:174)	EAVALGGGADD (SEQ ID NO:175)
E9.1F	EVQLQESGPGLVKPSETLSLTCTVSGGSISSGSYYWGWIRQPPGKGLEWIGDINY IGSTYYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCAREAVSFGGGADS WGQGTLVTVSS (SEQ ID NO:176)		
	CDR1	CDR2	CDR3
	SGSYYWG (SEQ ID NO:177)	DINYIGSTYYNPSLKS (SEQ ID NO:178)	EAVSFGGGADS (SEQ ID NO:179)
E9.10H	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SSGYYWG**WIRQPPGKGLEWIG**DIYY TGNTYYNPSLKN**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**EEVILGGGADQ** WGQGTLVTVSS (SEQ ID NO:180		
	CDR1	CDR2	CDR3
	SSGYYWG (SEQ ID NO:181)	**DIYYTGNTYYNPSLKN** (SEQ ID NO:182)	**EEVILGGGADQ** (SEQ ID NO:183)
E9.5E	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SSSYYWG**WIRQPPGKGLEWIG**DINY IGSTYYNPSLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**ESVPLGGGADE** WGQGTLVTVSS (SEQ ID NO:184)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:185)	**DINYIGSTYYNPSLKS** (SEQ ID NO:186)	**ESVPLGGGADE** (SEQ ID NO:187)
E9.10C	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SGSYYWG**WIRQPPGKGLEWIG**DIYY TGSTYYNPSLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**QAVMYGGGSDN** WGQGTLVTVSS (SEQ ID NO:188)		
	CDR1	CDR2	CDR3
	SGSYYWG (SEQ ID NO:189)	**DIYYTGSTYYNPSLKS** (SEQ ID NO:190)	**QAVMYGGGSDN** (SEQ ID NO:191)
E9.7E	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SSSYYWG**WIRQPPGKGLEWIG**DIYY AGSTYYNPSLKD**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**EDMILGGGADN** WGQGTLVTVSS (SEQ ID NO:192)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:193)	**DIYYAGSTYYNPSLKD** (SEQ ID NO:194)	**EDMILGGGADN** (SEQ ID NO:195)
E9.12B	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SSNYYWG**WIRQPPGKGLEWIG**DIYY TGSTYYNPSLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**EAVSFGGGADS** WGQGTLVTVSS (SEQ ID NO:196)		
	CDR1	CDR2	CDR3
	SSNYYWG (SEQ ID NO:197)	**DIYYTGSTYYNPSLKS** (SEQ ID NO:198)	**EAVSFGGGADS** (SEQ ID NO:199)

E9.10E	EVQLQESGPGLVKPSETLSLTCTVSGGSIN**SGNYYWG**WIRQPPGKGLEWIG**DISY** **TGSTYYNPSLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**EDVMYGGGGDS** WGQGTLVTVSS (SEQ ID NO:200)		
	CDR1	CDR2	CDR3
	SGNYYWG (SEQ ID NO:201)	**DISYTGSTYYNPSLKS** (SEQ ID NO:202)	**EDVMYGGGGDS** (SEQ ID NO:203)
E9.6A	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SSSYYWG**WIRQPPGKGLEWIG**DINY** **AGSTYYNPSLKN**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**EAVALGGGADS** WGQGTLVTVSS (SEQ ID NO:204)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:205)	**DINYAGSTYYNPSLKN** (SEQ ID NO:206)	**EAVALGGGADS** (SEQ ID NO:207)
E9.7A	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SSSYYWG**WIRQPPGKGLEWIG**DINY** **AGSTYYNPSLKS**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**EDVKFGGGADL** WGQGTLVTVSS (SEQ ID NO:208)		
	CDR1	CDR2	CDR3
	SSSYYWG (SEQ ID NO:209)	**DINYAGSTYYNPSLKS** (SEQ ID NO:210)	**EDVKFGGGADL** (SEQ ID NO:211)
E9.8H	EVQLQESGPGLVKPSETLSLTCTVSGGSIS**SGSYYWG**WIRQPPGKGLEWIG**DIYY** **TGSTYYNPSLKN**RVTISVDTSKNQFSLKLSSVTAADTAVYYCAR**ESVPLGGGADN** WGQGTLVTVSS (SEQ ID NO:212)		
	CDR1	CDR2	CDR3
	SGSYYWG (SEQ ID NO:213)	**DIYYTGSTYYNPSLKN** (SEQ ID NO:214)	**ESVPLGGGADN** (SEQ ID NO:215)
Affinity Matured Clones: Light Chain (VL) Regions			
E9.13	SYELTQPPSVSVSPGQTASITCSGDTLGDKYVSWYQQKPGQSPVLVIYEDSERPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDSETGVFGSGTKVTVL (SEQ ID NO:216)		
	CDR1	CDR2	CDR3
	SGDTLGDKYVS (SEQ ID NO:217)	EDSERPS (SEQ ID NO:218)	QAWDSETGV (SEQ ID NO:219)
E9.16	SYELTQPPSVSVSPGQTASITCSGERLGDKYVSWYQQKPGQSPVLVIYEDFKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:220)		
	CDR1	CDR2	CDR3
	SGERLGDKYVS (SEQ ID NO:221)	EDFKRPS (SEQ ID NO:222)	QAWDRDTGV (SEQ ID NO:223)
E9.38	SYELTQPPSVSVSPGQTASITCSGQRLGDKYVSWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDVGVFGSGTKVTVL (SEQ ID NO:224)		
	CDR1	CDR2	CDR3
	SGQRLGDKYVS (SEQ ID NO:225)	EDSKRPS (SEQ ID NO:226)	QAWDRDVGV (SEQ ID NO:227)
E9.4	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS		

	GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:228)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:229)	EDSKRPS (SEQ ID NO:230)	QAWDRDTGV (SEQ ID NO:231)
E9.11	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:232)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:233)	EDSKRPS (SEQ ID NO:234)	QAWDRDTGV (SEQ ID NO:235)
E9.14	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:236)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:237)	EDSKRPS (SEQ ID NO:238)	QAWDRDTGV (SEQ ID NO:239)
E9.17	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:240)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:241)	EDSKRPS (SEQ ID NO:242)	QAWDRDTGV (SEQ ID NO:243)
E9.18	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:244)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:245)	EDSKRPS (SEQ ID NO:246)	QAWDRDTGV (SEQ ID NO:247)
E9.19	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:248)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:249)	EDSKRPS (SEQ ID NO:250)	QAWDRDTGV (SEQ ID NO:251)
E9.22	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:252)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:253)	EDSKRPS (SEQ ID NO:254)	QAWDRDTGV (SEQ ID NO:255)
E9.48	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:256)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:257)	EDSKRPS (SEQ ID NO:258)	QAWDRDTGV (SEQ ID NO:259)

E9.65	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDS KRPSGIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGT KVTVL (SEQ ID NO: 260)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO: 261)	EDSKRPS (SEQ ID NO: 262)	QAWDRDTGV (SEQ ID NO: 263)
E9.66	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:264)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:265)	EDSKRPS (SEQ ID NO:266)	QAWDRDTGV (SEQ ID NO:267)
E9.71	SYELTQPPSVSVSPGQTASITCSGQRLGDKYASWYQQKPGQSPVLVIYEDSKRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDRDTGVFGYGTKVTVL (SEQ ID NO:268)		
	CDR1	CDR2	CDR3
	SGQRLGDKYAS (SEQ ID NO:269)	EDSKRPS (SEQ ID NO:270)	QAWDRDTGV (SEQ ID NO:271)
E9.2B	SYELTQPPSVSVSPGQTASITCSGEGLGDKYVSWYQQKPGQSPVLVIYEDSTRPS GIPERFSGSNSGDTATLTISGTQPMDEADYYCQAWDSETGVFGSGTKVTVL (SEQ ID NO:272)		
	CDR1	CDR2	CDR3
	SGEGLGDKYVS (SEQ ID NO:273)	EDSTRPS (SEQ ID NO:274)	QAWDSETGV (SEQ ID NO:275)
E9.1F	SYELTQPPSVSVSPGQTASITCSGDRLGDKYVSWYQQKPGQSPVLVIYEDSQRPS GIPERFSGSNSGDTATLTISGTQAMDEADYYCQAWDMEAGVFGSGTKVTVL (SEQ ID NO:276)		
	CDR1	CDR2	CDR3
	SGDRLGDKYVS (SEQ ID NO:277)	EDSQRPS (SEQ ID NO:278)	QAWDMEAGV (SEQ ID NO:279)
E9.10 C	SYELTQPPSVSVSPGQTASITC**SGDSLGDKYVS**WYQQKPGQSPVLVIY**EDSERPS** GIPERFSGSNSGDTATLTISGTQAMDEADYYC**QAWDSETGV**FGSGTKVT (SEQ ID NO:280)		
	CDR1	CDR2	CDR3
	SGDSLGDKYVS (SEQ ID NO:281)	**EDSERPS** (SEQ ID NO:282)	**QAWDSETGV** (SEQ ID NO:283)
E9.10 E	SYELTQPPSVSVSPGQTASITC**SGEGLGDKYVS**WYQQKPGQSPVLVIY**EDSERPS** GIPERFSGSNSGDTATLTISGTQAMDEADYYC**QAWDSEAGV**FGSGTKVT (SEQ ID NO:284)		
	CDR1	CDR2	CDR3
	SGEGLGDKYVS (SEQ ID NO:285)	**EDSERPS** (SEQ ID NO:286)	**QAWDSEAGV** (SEQ ID NO:287)
E9.7E	SYELTQPPSVSVSPGQTASITC**SGDRLGDKYVS**WYQQKPGQSPVLVIY**ED SERPS**GIPERFSGSNSGDTATLTISGTQAMDEADYYC**QAWDSEAGV**FGSG TKVT (SEQ ID NO:288)		

	CDR1	CDR2	CDR3
	SGDRLGDKYVS (SEQ ID NO:289)	**EDSERPS** (SEQ ID NO:290)	**QAWDSEAGV** (SEQ ID NO:291)
E9.5E	SYELTQPPSVSVSPGQTASITC**SGDMLGDKYVS**WYQQKPGQSPVLVIY**EDSQRPS** GIPERFSGSNSGDTATLTISGTQPMDEADYYC**QAWDSETGV**FGSGTKVT (SEQ ID NO:292)		
	CDR1	CDR2	CDR3
	SGDMLGDKYVS (SEQ ID NO:293)	**EDSQRPS** (SEQ ID NO:294)	**QAWDSETGV** (SEQ ID NO:295)
E9.12 B	SYELTQPPSVSVSPGQTASITC**SGDGLGDKYVS**WYQQKPGQSPVLVIY**EDSTRPS** GIPERFSGSNSGDTATLTISGTQPMDEADYYC**QAWDSESGV**FGSGTKVT (SEQ ID NO:296)		
	CDR1	CDR2	CDR3
	SGDGLGDKYVS (SEQ ID NO:297)	**EDSTRPS** (SEQ ID NO:298)	**QAWDSESGV** (SEQ ID NO:299)
E9.10 H	SYELTQPPSVSVSPGQTASITC**SGESLGDKYVS**WYQQKPGQSPVLVIY**EDSKRPS** GIPERFSGSNSGDTATLTISGTQAMDEADYYC**QAWDGETGV**FGSGTKVT (SEQ ID NO:300)		
	CDR1	CDR2	CDR3
	SGESLGDKYVS (SEQ ID NO:301)	**EDSKRPS** (SEQ ID NO:302)	**QAWDGETGV** (SEQ ID NO:303)
E9.6A	SYELTQPPSVSVSPGQTASITC**SGDMLGDKYVS**WYQQKPGQSPVLVIY**EDTNRPS** GIPERFSGSNSGDTATLTISGTQAMDEADYYC**QAWDSETGV**FGSGTKVT (SEQ ID NO:304)		
	CDR1	CDR2	CDR3
	SGDMLGDKYVS (SEQ ID NO:305)	**EDTNRPS** (SEQ ID NO:306)	**QAWDSETGV** (SEQ ID NO:307)
E9.7A	SYELTQPPSVSVSPGQTASITC**SGESLGDKYVS**WYQQKPGQSPVLVIY**QDAMRPS** GIPERFSGSNSGDTATLTISGTQAMDEADYYC**QAWDMETGV**FGSGTKVT (SEQ ID NO:308)		
	CDR1	CDR2	CDR3
	SGESLGDKYVS (SEQ ID NO:309)	**QDAMRPS** (SEQ ID NO:310)	**QAWDMETGV** (SEQ ID NO:311)
E9.8H	SYELTQPPSVSVSPGQTASITC**SGESLGDKYVS**WYQQKPGQSPVLVIY**EDSMRPS** GIPERFSGSNSGDTATLTISGTQPMDEADYYC**QAWDSEVGV**FGSGTKVT (SEQ ID NO:312)		
	CDR1	CDR2	CDR3
	SGESLGDKYVS (SEQ ID NO:313)	**EDSMRPS** (SEQ ID NO:314)	**QAWDSEVGV** (SEQ ID NO:315)

Anti-DLL4 A10 affinity maturation.

In a manner similar to E9 affinity maturation described above, sequence alignment showed that the DLL4 antibody A10 shared the highest identity to human germlines VH3-30 and V2-1. Human VH and Vλ sequences derived from VH3-30 and V2-1, respectively, were

downloaded from NCBI IgBlast database to generate sequence logos. These sequence logos were used to decide which positions would be doped to generate the affinity matured libraries.

The A10 libraries were transformed into 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 A10 clones were recovered for converting back to IgG format for further characterization. The heavy chain (VH) regions of the affinity matured clones are shown below in Table 9 and the light chain (VL) regions of the affinity matured clones are shown below in Table 10.

Table 9. Variable Heavy Chain Regions (VH) of Affinity Matured A10 Clones.

Protein Region			Sequence
			12345678901234567890123456789O
A10.3 VH			EVQLVESGGGLVKSGGSLRLSCAASGFTFR **SHWMS**WVRQAPGKGLEWVA**IISYDGSNKYS ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:316)
A10.3 VH	CDR-H1	Residues 31-35 of SEQ ID NO.:316	**SHWMS**
A10.3 VH	CDR-H2	Residues 50-66 of SEQ ID NO.:316	**IISYDGSNKYSADSVKG**
A10.3 VH	CDR-H3	Residues 99 - 108 of SEQ ID NO.:316	**AGGGNVGFDI**
A10.K30 VH			EVQLVESGGGVVQPGGSLRLSCAASGFTFG **NHWMS**WVRQAPGKGLEWVA**DISSDGRYKYY ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:317)
A10.K30 VH	CDR-H1	Residues 31-35 of SEQ ID NO.:317	**NHWMS**
A10.K30 VH	CDR-H2	Residues 50-66 of SEQ ID NO.:317	**DISSDGRYKYYADSVKG**
A10.K30 VH	CDR-H3	Residues 99 - 108 of SEQ ID NO.:317	**AGGGNVGFDI**
A10.K42 VH			EVQLVESGGGLVQPGGSLRLSCAASGFTFQ **SHWMS**WVRQAPGKGLEWVA**MISYDGTIKYY ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:318)
A10.K42 VH	CDR-H1	Residues 31-35 of SEQ ID NO.:318	**SHWMS**
A10.K42 VH	CDR-H2	Residues 50-66 of SEQ ID NO.:318	**MISYDGTIKYYADSVKG**

Protein Region			Sequence
			12345678901234567890123456789 0
A10.K42 VH	CDR-H3	Residues 99 - 108 of SEQ ID NO.:318	**AGGGNVGFDI**
A10.9A VH			EVQLVESGGGVVQPGRSLRLSCAASGFTFR **SYWMS**WVRQAPGKGLEWVA**TISYDGRNKDY ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:319)
A10.9A VH		Residues 31-35 of SEQ ID NO.: 319	**SYWMS**
A10.9A VH		Residues 50-66 of SEQ ID NO.:319	**TISYDGRNKDYADSVKG**
A10.9A VH		Residues 99 - 108 of SEQ ID NO.: 319	**AGGGNVGFDI**
A10.8A VH			EVQLVESGGGVVQPGGSLRLSCAASGFTFG **NHWMS**WVRQAPGKGLEWVA**DISSDGRYKYY ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:320)
A10.8A VH		Residues 31-35 of SEQ ID NO.: 320	**NHWMS**
A10.8A VH		Residues 50-66 of SEQ ID NO.:320	**DISSDGRYKYYADSVKG**
A10.8A VH		Residues 99 - 108 of SEQ ID NO.: 320	**AGGGNVGFDI**
A10.1A VH			EVQLVESGGGVVQPGGSLRLSCAASGFTFH **SHWMS**WVRQAPGKGLEWVA**MISDDGRNKDY ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAR**AAGGNVGLDI**WGQGTMVTVSS (SEQ ID NO:321)
A10.1A VH		Residues 31-35 of SEQ ID NO.: 321	**SHWMS**
A10.1A VH		Residues 50-66 of SEQ ID NO.: 321	**MISDDGRNKDYADSVKG**
A10.1A VH		Residues 99 - 108 of SEQ ID NO.: 321	**AAGGNVGLDI**
A10.5D VH			EVQLVESGGGVVQSGGSLRLSCAASGFTFG **SHWMS**WVRQAPGKGLEWVA**DISVDGSNKYS ADSVKG**RFTISRDNSKNTLYLQMNSLRAED TAVYYCAR**AAGGNVGLDS**WGQGTMVTVSS (SEQ ID NO:322)
A10.5D VH		Residues 31-35 of SEQ ID NO.: 322	**SHWMS**
A10.5D VH		Residues 50-66 of SEQ ID NO.: 322	**DISVDGSNKYSADSVKG**

Protein Region			Sequence
			123456789012345678901234567890
A10.5D VH		Residues 99 - 108 of SEQ ID NO.: 322	**AAGGNVGLDS**
A10.3A VH			EVQLVESGGGVVQPGGSLRLSCAASGFTFG **NHWMS**WVRQAPGKGLEWVA**DISSDGRYKYY ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:323)
A10.3A VH		Residues 31-35 of SEQ ID NO.: 323	**NHWMS**
A10.3A VH		Residues 50-66 of SEQ ID NO.: 323	**DISSDGRYKYYADSVKG**
A10.3A VH		Residues 99 - 108 of SEQ ID NO.: 323	**AGGGNVGFDI**
A10.6B VH			EVQLVESGGGVVQPGGSLRLSCAASGFTFG **NHWMS**WVRQAPGKGLEWVA**DISSDGRYKYY ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:324)
A10.6B VH		Residues 31-35 of SEQ ID NO.: 324	**NHWMS**
A10.6B VH		Residues 50-66 of SEQ ID NO.: 324	**DISSDGRYKYYADSVKG**
A10.6B VH		Residues 99 - 108 of SEQ ID NO.: 324	**AGGGNVGFDI**
A10.3D VH			EVQLVESGGGVVQPGRSLRLSCAASGFTFR **SHWMS**WVRQAPGKGLEWVA**DISQDGSYKYY ADSVKG**RFTISRDNSKNTLYLQMNSLRAED TAVYYCAR**AAGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:325)
A10.3D VH		Residues 31-35 of SEQ ID NO.: 325	**SHWMS**
A10.3D VH		Residues 50-66 of SEQ ID NO.: 325	**DISQDGSYKYYADSVKG**
A10.3D VH		Residues 99 - 108 of SEQ ID NO.: 325	**AAGGNVGFDI**
A10.4C VH			EVQLVESGGGVVQPGGSLRLSCAASGFTFG **SHWMS**WVRQAPGKGLEWVA**DISNDGRYAYS ADSVKG**RFTISRDNSKNTLYLQLNSLRAED TAVYYCAK**AGGGNVGFDI**WGQGTMVTVSS (SEQ ID NO:326)
A10.4C VH		Residues 31-35 of SEQ ID NO.: 326	**SHWMS**
A10.4C VH		Residues 50-66 of SEQ ID NO.: 326	**DISNDGRYAYSADSVKG**

Protein Region			Sequence
			123456789012345678901234567890
A10.4C VH		Residues 99 - 108 of SEQ ID NO.: 326	**AGGGNVGFDI**

Table 10. Variable Light Chain Regions (VL) of Affinity Matured A10 Clones.

Protein region			Sequence
			123456789012345678901234567890
A10.3 VL			SYELTQPPSVSVSPGQTASITC**SADKLGTK YVS**WYQQKPGQSPVLVIY**QDAKRPS**GIPER FSGSNSGNTATLTISGTQTMDEADYLC**QSW DRSDVV**FGGGTKLTVL (SEQ ID NO:327)
A10.3 VL	CDR-L1	Residues 23-33 of SEQ ID NO.: 327	**SADKLGTKYVS**
A10.3 VL	CDR-L2	Residues 49-55 of SEQ ID NO.: 327	**QDAKRPS**
A10.3 VL	CDR-L3	Residues 88 - 96 of SEQ ID NO.: 327	**QSWDRSDVV**
A10.L45 VL			SYELTQPPSVSVSPGQTASITC**SADELGTQ YVS**WYQQKPGQSPVLVIY**QDATRPS**GIPER FSGSNSGNTATLTISGTQAMDEADYYC**QAW DRSGVV**FGGGTKLTVL (SEQ ID NO:328)
A10.L45 VL	CDR-L1	Residues 23-33 of SEQ ID NO.: 328	**SADELGTQYVS**
A10.L45 VL	CDR-L2	Residues 49-55 of SEQ ID NO.: 328	**QDATRPS**
A10.L45 VL	CDR-L3	Residues 88 - 96 of SEQ ID NO.: 328	**QAWDRSGVV**
A10.L73 VL			SYELTQPPSVSVSPGQTASITC**SGDNLGSQ YVS**WYQQKPGQSPVLVIY**QDAQRPS**GIPER FSGSNSGNTATLTISGTQAMDEADYYC**QAW DRSGVV**FGGGTKLTVL (SEQ ID NO:329)
A10.L73 VL	CDR-L1	Residues 23-33 of SEQ ID NO.: 329	**SGDNLGSQYVS**
A10.L73 VL	CDR-L2	Residues 49-55 of SEQ ID NO.: 329	**QDAQRPS**
A10.L73 VL	CDR-L3	Residues 88 - 96 of SEQ ID NO.: 329	**QAWDRSGVV**
A10.3A VL			SYELTQPPSVSVSPGQTASITC**SADNLGEK YVS**WYQQKPGQSPVLVIY**QDATRPS**GIPER FSGSNSGNTATLTISGTQAMDEADYYC**QSW DSSGAV**FGGGTKLTVL (SEQ ID NO:330)
A10.3A VL	CDR-L1	Residues 23-33 of SEQ ID NO.: 330	**SADNLGEKYVS**

Protein region			Sequence
			12345678901234567890123456789 0
A10.3A VL	CDR-L2	Residues 49-55 of SEQ ID NO.: 330	**QDATRPS**
A10.3A VL	CDR-L3	Residues 88 - 96 of SEQ ID NO.: 330	**QSWDSSGAV**
A10.6B VL			SYELTQPPSVSVSPGQTASITC**SADNLGNQ YVS**WYQQKPGQSPVLVIY**QDGMRPS**GIPER FSGSNSGNTATLTISGTQAMDEADYYC**QAW DWSGEV**FGGGTKLTVL (SEQ ID NO:331)
A10.6B VL	CDR-L1	Residues 23-33 of SEQ ID NO.: 331	**SADNLGNQYVS**
A10.6B VL	CDR-L2	Residues 49-55 of SEQ ID NO.: 331	**QDGMRPS**
A10.6B VL	CDR-L3	Residues 88 - 96 of SEQ ID NO.: 331	**QAWDWSGEV**
A10.3D VL			SYELTQPPSVSVSPGQTASITC**SADKLGTK YVS**WYQQKPGQSPVLVIY**QDGNRPS**GIPER FSGSNSGNTATLTISGTQAMDEADYYC**QSW DQSGAV**FGGGTKLTVL (SEQ ID NO:332)
A10.3D VL	CDR-L1	Residues 23-33 of SEQ ID NO.: 332	**SADKLGTKYVS**
A10.3D VL	CDR-L2	Residues 49-55 of SEQ ID NO.: 332	**QDGNRPS**
A10.3D VL	CDR-L3	Residues 88 - 96 of SEQ ID NO.: 332	**QSWDQSGAV**
A10.4C VL			SYELTQPPSVSVSPGQTASITC**SADNLGNQ YVS**WYQQKPGQSPVLVIY**QDGMRPS**GIPER FSGSNSGNTATLTISGTQAMDEADYYC**QAW DSSGAV**FGGGTKLTVL (SEQ ID NO:333)
A10.4C VL	CDR-L1	Residues 23-33 of SEQ ID NO.: 333	**SADNLGNQYVS**
A10.4C VL	CDR-L2	Residues 49-55 of SEQ ID NO.: 333	**QDGMRPS**
A10.4C VL	CDR-L3	Residues 88 - 96 of SEQ ID NO.: 333	**QAWDSSGAV**

Example 5: Construction of CDR-Grafted E9 Antibodies.

CDRs of E9 VH were grafted onto VH3 consensus framework (grafted VH = E9vh3g2) and CDRs of E9 VL were grafted onto VL2-1 framework of anti-DLL4 A10 antibody (grafted VL = E9a10vlg2) and onto VK framework of A10 germline (the closest germline in homology to the E9 VL) (grafted VL= E9AVK). Alternatively framework (FW) repairing was also performed

with the E9 VH; its FW was maintained but amino acids that may cause antibody instability were replaced, (repaired VH = E9VH4r2). Framework back-mutations were incorporated in both CDR-grafting and FW-repairing to maintain antibody structure and functionality (Table 11 and Table 14).

CDRs of E9 were also grafted on the frameworks of anti-IL-18 and anti-IL-12 antibodies. Specifically CDRs of E9 VH were grafted onto VH5-51 framework of anti-IL-18 (grafted VH = E9VH325) and onto VH2-70 framework of anti-IL-12 (grafted VH = E9VH1D4.1). CDRs of E9 VL were grafted onto VK L2/L16 framework of anti-IL-12 (grafted VL = E9VL325) and onto VK B3 framework of anti-IL-12 (grafted VL = E9VL1D4.1). Framework back-mutations were incorporated in the CDR-grafting to maintain antibody structure and functionality (Table 15 and Table 18).

In silico constructed CDR grafted antibodies described above were synthesized directly in the plasmid of choice by Blue Heron Biotechnology. The variable heavy region was inserted in-frame onto a cDNA fragment encoding the wild type human IgG1 constant region and onto the human IgG1 constant region containing two hinge-region amino acid mutations. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al., *J. Immunol.*, 147: 2657 (1991)). The variable light chain region was inserted in-frame with the human lambda constant region and with the human kappa constant region. Upon receipt of synthesized constructs from Blue Heron, DNA was scaled up and sequence confirmed. Correct CDR-grafted heavy and light chains corresponding to each antibody (Tables 11 and 14) (Tables 15 and 18) were co-transfected into HEK-293-6E cells to transiently produce full-length CDR-grafted anti-human DLL4 antibodies. Table 11 summarizes all the E9 antibody variants generated and HEK-293-6e expression data. Cell supernatants containing recombinant human antibody were purified by Protein A Sepharose chromatography and bound antibody eluted by addition of acid buffer. Antibodies were dialyzed into PBS.

The ability of purified CDR grafted antibodies to bind to DLL4 or to inhibit DLL4 activity was determined using different types of assays like ELISA (Example 1.2, Method 2), Biacore (Example 1.2) and Flow Cytometry (FACS) (Example 1.3). Table 12 and Table 16 show EC50 values from the ELISA assays and the FACS assays and the affinity determined by Biacore of the CDR grafted antibodies described respectively in Table 11 and Table 15 for human DLL4, murine DLL4 and cynomolgus DLL4. Table 13 and Table 17 show IC50 values from the blocking ELISA (Example 1.4) and blocking FACS (Example 1.5) with human DLL4 and murine DLL4 for the CDR-grafted antibodies described respectively in Table 11 and Table 15.

Table 11. Summary of the E9 antibody variants generated and expression data

Antibody	VH name	VL name	HEK-293-6e titer (mg/L)
E9-SE1	E9vh3g2	E9a10vlg2	104
E9-SE2	E9vh3g2	E9.1	87

E9-SE3	E9vh4r2	E9.1	89
E9-SE4	E9.1	E9a10vlg2	66
E9-SE5	E9.1	E9AVK	79
E9-SE6	E9vh4r2	E9a10vlg2	125
E9-SE7	E9vh3g2	E9AVK	56
E9-SE8	E9vh4r2	E9AVK	85

Table 12. Binding affinities of the E9 antibody variants for human, mouse, and cynomolgus DLL4 as determined by ELISA, Biacore, and FACS.

	Binding Data							
	Human DLL4			Mouse DLL4			Cyno DLL4	
Antibody	Binding ELISA (EC50, nM)	Biacore (Kd, nM)	Binding FACS (Kd, nM)	Binding ELISA (EC50, nM)	Biacore (Kd, nM)	Binding FACS (Kd, nM)	Binding ELISA (EC50, nM)	Biacore (Kd, nM)
E9-SE1	0.16	15.2*	3.45	0.19	12.83	1.34	0.14	5.42
E9-SE2	0.18	2.43	5.2	0.20	15.09	0.87	0.14	3.68
E9-SE3	0.18	2.26	2.29	0.20	12.1	0.74	0.14	3.42
E9-SE4	0.17	2.38	nc	0.20	10.52	1.15	0.14	3.92
E9-SE5	0.16	1.57	7.81	0.19	12.77	2.9	0.13	6.7
E9-SE6	0.17	0.64	4.25	0.19	10.04	1.18	0.13	6.55
E9-SE7	0.17	1.69	2.28	0.17	16.22	2.24	0.12	7.53
E9-SE8	0.18	2.00	2.67	0.19	13.03	2.32	0.14	3.41
E9.1	0.29	2.38	0.35	0.34	12.77	1.6*	0.24	4.08

*historic data; nc = not calculated

Table 13. Neutralizing activities of the E9 antibody variants for human and mouse DLL4 as determined by ELISA and FACS.

	Functional Data			
	Human DLL4		Mouse DLL4	
Antibody	Blocking ELISA (IC50, nM)	Blocking FACS (IC50, nM)	Blocking ELISA (IC50, nM)	Blocking FACS (IC50, nM)
E9-SE1	2.7	8.51	2.9	1.71
E9-SE2	2.6	5.61	2.2	1.23
E9-SE3	2.5	6.75	2.1	1.25
E9-SE4	2.7	5.82	2.9	1.71
E9-SE5	2.6	6.38	2.4	1.76
E9-SE6	2.5	8.16	2.9	1.53
E9-SE7	2.4	7.81	2.4	1.94
E9-SE8	2.5	9.25	2.6	2.3
E9.1	2.5	4.55	2.5	1.02

Table 14. VH and VL amino acid sequences of human CDR-grafted E9 antibodies.

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
334	**VH E9.1**		EVQLQESGPGLVKPSETLSLTCTVSGGSIS **SSSYYWG**WIRQPPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFSLKLSSVTAA DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9.1 CDR-H1	Residues 31-37 of SEQ ID NO.:334	**SSSYYWG**
	VH E9.1 CDR-H2	Residues 52-67 of SEQ ID NO.:334	**DIYYTGSTYYNPSLKS**
	VH E9.1 CDR-H3	Residues 100-110 of SEQ ID NO.:334	**EDVILRGGSDY**
			123456789012345678901234567890
335	**VL E9.1**		SYELTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQPMDEADYYC**QAW DRDTGV**FGYGTRVTVLG
	VL E9.1 CDR-L1	Residues 23-33 of SEQ ID NO.:335	**SGQRLGDKYAS**
	VL E9.1 CDR-L2	Residues 49-55 of SEQ ID NO.:335	**EDSKRPS**
	VL E9.1 CDR-L3	Residues 88-96 of SEQ ID NO.:335	**QAWDRDTGV**
			123456789012345678901234567890
336	**VH E9-SE1**		EVQLVESGGGLVQPGGSLRLSCAVSGGSIS **SSSYYWG**WIRQAPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNSFYLQMNSLRAE DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE1 CDR-H1	Residues 31-37 of SEQ ID NO.:336	**SSSYYWG**
	VH E9-SE1 CDR-H2	Residues 52-67 of SEQ ID NO.:336	**DIYYTGSTYYNPSLKS**
	VH E9-SE1 CDR-H3	Residues 100-110 of SEQ ID NO.:336	**EDVILRGGSDY**
			123456789012345678901234567890
337	**VL E9-SE1**		LYVLTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQTMDEADYLC**QAW DRDTGV**FGGGTKVTVLG

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
	VL E9-SE1 CDR-L1	Residues 23-33 of SEQ ID NO.:337	**SGQRLGDKYAS**
	VL E9-SE1 CDR-L2	Residues 49-55 of SEQ ID NO.:337	**EDSKRPS**
	VL E9-SE1 CDR-L3	Residues 88-96 of SEQ ID NO.:337	**QAWDRDTGV**
			123456789012345678901234567890
338	**VH E9-SE2**		EVQLVESGGGLVQPGGSLRLSCAVSGGSIS **SSSYYWG**WIRQAPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNSFYLQMNSLRAE DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE2 CDR-H1	Residues 31-37 of SEQ ID NO.:338	**SSSYYWG**
	VH E9-SE2 CDR-H2	Residues 52-67 of SEQ ID NO.:338	**DIYYTGSTYYNPSLKS**
	VH E9-SE2 CDR-H3	Residues 100-110 of SEQ ID NO.:338	**EDVILRGGSDY**
			123456789012345678901234567890
339	**VL E9-SE2**		SYELTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQPMDEADYYC**QAW DRDTGV**FGYGTRVTVLG
	VL E9-SE2 CDR-L1	Residues 23-33 of SEQ ID NO.:339	**SGQRLGDKYAS**
	VL E9-SE2 CDR-L2	Residues 49-55 of SEQ ID NO.:339	**EDSKRPS**
	VL E9-SE2 CDR-L3	Residues 88-96 of SEQ ID NO.:339	**QAWDRDTGV**
			123456789012345678901234567890
340	**VH E9-SE3**		EVQLQESGPGLVKPGETLSLTCTVSGGSIS **SSSYYWG**WIRQAPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFYLKLSSVRAE DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE3 CDR-H1	Residues 31-37 of SEQ ID NO.:340	**SSSYYWG**
	VH E9-SE3 CDR-H2	Residues 52-67 of SEQ ID NO.:340	**DIYYTGSTYYNPSLKS**

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
	VH E9-SE3 CDR-H3	Residues 100-110 of SEQ ID NO.:340	**EDVILRGGSDY**
			123456789012345678901234567890
341	**VL E9-SE3**		SYELTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQPMDEADYYC**QAW DRDTGV**FGYGTRVTVLG
	VL E9-SE3 CDR-L1	Residues 23-33 of SEQ ID NO.:341	**SGQRLGDKYAS**
	VL E9-SE3 CDR-L2	Residues 49-55 of SEQ ID NO.:341	**EDSKRPS**
	VL E9-SE3 CDR-L3	Residues 88-96 of SEQ ID NO.:341	**QAWDRDTGV**
			123456789012345678901234567890
342	**VH E9-SE4**		EVQLQESGPGLVKPSETLSLTCTVSGGSIS **SSSYYWG**WIRQPPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFSLKLSSVTAA DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE4 CDR-H1	Residues 31-37 of SEQ ID NO.:342	**SSSYYWG**
	VH E9-SE4 CDR-H2	Residues 52-67 of SEQ ID NO.:342	**DIYYTGSTYYNPSLKS**
	VH E9-SE4 CDR-H3	Residues 100-110 of SEQ ID NO.:342	**EDVILRGGSDY**
			123456789012345678901234567890
343	**VL E9-SE4**		LYVLTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQTMDEADYLC**QAW DRDTGV**FGGGTKVTVLG
	VL E9-SE4 CDR-L1	Residues 23-33 of SEQ ID NO.:343	**SGQRLGDKYAS**
	VL E9-SE4 CDR-L2	Residues 49-55 of SEQ ID NO.:343	**EDSKRPS**
	VL E9-SE4 CDR-L3	Residues 88-96 of SEQ ID NO.:343	**QAWDRDTGV**
			123456789012345678901234567890

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
344	**VH E9-SE5**		EVQLQESGPGLVKPSETLSLTCTVSGGSIS **SSSYYWG**WIRQPPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFSLKLSSVTAA DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE5 CDR-H1	Residues 31-37 of SEQ ID NO.:344	**SSSYYWG**
	VH E9-SE5 CDR-H2	Residues 52-67 of SEQ ID NO.:344	**DIYYTGSTYYNPSLKS**
	VH E9-SE5 CDR-H3	Residues 100-110 of SEQ ID NO.:344	**EDVILRGGSDY**
345			123456789012345678901234567890
	VL E9-SE5		EYVLTQSPDFQSVTPKEKVTITC**SGQRLGD KYAS**WYQQKPDQSPKLVIY**EDSKRPS**GVPS RFSGSNSGDDATLTINSLEAEDAATYYC**QA WDRDTGV**FGQGTKVEIKR
	VL E9-SE5 CDR-L1	Residues 24-34 of SEQ ID NO.:345	**SGQRLGDKYAS**
	VL E9-SE5 CDR-L2	Residues 50-56 of SEQ ID NO.:345	**EDSKRPS**
	VL E9-SE5 CDR-L3	Residues 89-97 of SEQ ID NO.:345	**QAWDRDTGV**
346			123456789012345678901234567890
	VH E9-SE6		EVQLQESGPGLVKPGETLSLTCTVSGGSIS **SSSYYWG**WIRQAPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFYLKLSSVRAE DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE6 CDR-H1	Residues 31-37 of SEQ ID NO.:346	**SSSYYWG**
	VH E9-SE6 CDR-H2	Residues 52-67 of SEQ ID NO.346	**DIYYTGSTYYNPSLKS**
	VH E9-SE6 CDR-H3	Residues 100-110 of SEQ ID NO.:346	**EDVILRGGSDY**
347			123456789012345678901234567890
	VL E9-SE6		LYVLTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQTMDEADYLC**QAW DRDTGV**FGGGTKVTVLG

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
	VL E9-SE6 CDR-L1	Residues 23-33 of SEQ ID NO.:347	**SGQRLGDKYAS**
	VL E9-SE6 CDR-L2	Residues 49-55 of SEQ ID NO.:347	**EDSKRPS**
	VL E9-SE6 CDR-L3	Residues 88-96 of SEQ ID NO.:347	**QAWDRDTGV**
348			
	VH E9-SE7		EVQLVESGGGLVQPGGSLRLSCAVSGGSIS **SSSYYWG**WIRQAPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNSFYLQMNSLRAE DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE7 CDR-H1	Residues 31-37 of SEQ ID NO.:348	**SSSYYWG**
	VH E9-SE7 CDR-H2	Residues 52-67 of SEQ ID NO.:348	**DIYYTGSTYYNPSLKS**
	VH E9-SE7 CDR-H3	Residues 100-110 of SEQ ID NO.:348	**EDVILRGGSDY**
349			123456789012345678901234567890
	VL E9-SE7		EYVLTQSPDFQSVTPKEKVTITC**SGQRLGD KYAS**WYQQKPDQSPKLVIY**EDSKRPS**GVPS RFSGSNSGDDATLTINSLEAEDAATYYC**QA WDRDTGV**FGQGTKVEIKR
	VL E9-SE7 CDR-L1	Residues 24-34 of SEQ ID NO.:349	**SGQRLGDKYAS**
	VL E9-SE7 CDR-L2	Residues 50-56 of SEQ ID NO.:349	**EDSKRPS**
	VL E9-SE7 CDR-L3	Residues 89-97 of SEQ ID NO.:349	**QAWDRDTGV**
350			123456789012345678901234567890
	VH E9-SE8		EVQLQESGPGLVKPGETLSLTCTVSGGSIS **SSSYYWG**WIRQAPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFYLKLSSVRAE DTAVYYCAR**EDVILRGGSDY**WGQGTLVTVS S
	VH E9-SE8 CDR-H1	Residues 31-37 of SEQ ID NO.:350	**SSSYYWG**
	VH E9-SE8 CDR-H2	Residues 52-67 of SEQ ID NO.:350	**DIYYTGSTYYNPSLKS**

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
	VH E9-SE8 CDR-H3	Residues 100-110 of SEQ ID NO.:350	**EDVILRGGSDY**
			123456789012345678901234567890
351	**VL E9-SE8**		EYVLTQSPDFQSVTPKEKVTITC**SGQRLGD KYAS**WYQQKPDQSPKLVIY**EDSKRPS**GVPS RFSGSNSGDDATLTINSLEAEDAATYYC**QA WDRDTGV**FGQGTKVEIKR
	VL E9-SE8 CDR-L1	Residues 24-34 of SEQ ID NO.:351	**SGQRLGDKYAS**
	VL E9-SE8 CDR-L2	Residues 50-56 of SEQ ID NO.:351	**EDSKRPS**
	VL E9-SE8 CDR-L3	Residues 89-97 of SEQ ID NO.:351	**QAWDRDTGV**

Table 15. Summary of the E9 antibody variants generated and expression data.

Antibody	VH name	VL name	HEK-293-6e titer (mg/L)
E9-FR1	E9VH325	E9VL325	29
E9-FR2	E9VH1D4.1	E9VL1D4.1	44

Table 16. Binding affinities of the E9 CDR-grafted antibodies for human, mouse and cynomolgus DLL4 as determined by ELISA, Biacore and FACS.

	Binding Data							
	Human DLL4			Mouse DLL4			Cyno DLL4	
MAb	Binding ELISA (EC50, nM)	Biacore (Kd, nM)	Binding FACS (Kd, nM)	Binding ELISA (EC50, nM)	Biacore (Kd, nM)	Binding FACS (Kd, nM)	Binding ELISA (EC50, nM)	Biacore (Kd, nM)
E9-FR1	0.11	0.72	4	0.13	1.6	2.51	0.12	0.74
E9-FR2	0.105	0.31	6.2	0.125	0.69	3.57	0.12	0.3
E9.1	0.03	0.52	3.62	0.04	1.1	1.66	0.035	0.47

Table 17. Neutralizing activities of the E9 CDR-grafted antibodies for human and mouse DLL4 as determined by ELISA and FACS.

	Functional Data			
	Human DLL4		Mouse DLL4	
MAb	Blocking ELISA (IC50, nM)	Blocking FACS (IC50, nM)	Blocking ELISA (IC50, nM)	Blocking FACS (IC50, nM)
E9-FR1	1.54	1.57	3.35	0.98
E9-FR2	1.52	3.03	4.35	1.07
E9.1	2.2	1.62	5.85	1.06

Table 18. VH and VL amino acid sequences of human CDR-grafted E9 antibodies.

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
352	**VH E9-FR1**		EVQLVQSGTEVKKPGESLKISCKVSGGSIS **SSSYYWG**WIRQMPGKGLEWIG**DIYYTGSTY YNPSLKS**QVTISVDTSFNTFFLQWSSLKAS DTAMYYCAR**EDVILRGGSDY**WGQGTMVTVS S
	VH E9-FR1 CDR-H1	Residues 31-37 of SEQ ID NO.:352	**SSSYYWG**
	VH E9-FR1 CDR-H2	Residues 52-67 of SEQ ID NO.:352	**DIYYTGSTYYNPSLKS**
	VH E9-FR1 CDR-H3	Residues 100-110 of SEQ ID NO.:352	**EDVILRGGSDY**
			123456789012345678901234567890
353	**VL E9-FR1**		EYVLTQSPATLSVSPGERATLSC**SGQRLGD KYAS**WYQQKPGQSPRLVIY**EDSKRPS**DIPA RFSGSNSGDEATLTISSLQSEDFAVYYC**QA WDRDTGV**FGQGTRLEIKR
	VL E9-FR1 CDR-L1	Residues 24-34 of SEQ ID NO.:353	**SGQRLGDKYAS**
	VL E9-FR1 CDR-L2	Residues 50-56 of SEQ ID NO.:353	**EDSKRPS**
	VL E9-FR1 CDR-L3	Residues 89-97 of SEQ ID NO.:353	**QAWDRDTGV**
			123456789012345678901234567890
354	**VH E9-FR2**		EVTLRESGPALVKPTQTLTLTCTVSGGSIS **SSSYYWG**WIRQPPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFVLTMTNMDPV DTATYYCAR**EDVILRGGSDY**WGQGTTVTVS S
	VH E9-FR2 CDR-H1	Residues 31-37 of SEQ ID NO.:354	**SSSYYWG**
	VH E9-FR2 CDR-H2	Residues 52-67 of SEQ ID NO.:354	**DIYYTGSTYYNPSLKS**
	VH E9-FR2 CDR-H3	Residues 100-110 of SEQ ID NO.:354	**EDVILRGGSDY**
			123456789012345678901234567890
355	**VL E9-FR2**		DYVLTQSPDSLAVSLGERATINC**SGQRLGD KYAS**WYQQKPGQSPKLVIY**EDSKRPS**GIPD RFSGSNSGDDATLTISSLQAEDVAVYYC**QA WDRDTGV**FGGGTKVEIKR

SEQ ID No.	Protein region		Sequence
			12345678901234567890123456789O
	VL E9-FR2 CDR-L1	Residues 24-34 of SEQ ID NO.:355	**SGQRLGDKYAS**
	VL E9-FR2 CDR-L2	Residues 50-56 of SEQ ID NO.:355	**EDSKRPS**
	VL E9-FR2 CDR-L3	Residues 89-97 of SEQ ID NO.:355	**QAWDRDTGV**

Example 6: Further Engineering of Affinity-Matured Antibody E9-71.

E9-71(M) and E9-71(L).

Both heavy chain and light chain of affinity-matured anti-DLL4 antibody E9-71 were further engineered. The methionine (M) in CDR-H3 of E9-71 heavy chain was mutated to leucine (L) by designing forward and reverse overlapping primers containing the mutated nucleotides. Polymerase chain reaction (PCR) was performed in two sequential steps to amplify the entire variable region gene using the two primers carrying the mutated nucleotides and two outermost primers containing overhanging sequences complementary to the receiving vector.

The signal peptide used for the light chain of E9-71(M) and E9-71(L) is called lambda 1a signal peptide. The framework 4 (FW4) region hJL-1 of E9-71 light chain was changed to hJL2 to be more compatible with the huCL2 constant region of the antibody. Forward and reverse primers were designed containing the mutated nucleotides. Polymerase chain reaction (PCR) was performed in two sequential steps to amplify the entire variable region gene using the two primers carrying the mutated nucleotides and two outermost primers containing overhanging sequences complementary to the receiving vector.

The PCR products derived from each cDNA assembly was separated on an agarose gel and the band corresponding to the predicted variable region cDNA size excised and purified. The variable heavy region were inserted in-frame onto a cDNA fragment encoding the human IgG1 constant region containing two hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al., *J. Immunol.*, 147: 2657 (1991)). The variable light chain region were inserted in-frame between the human lambda 1a signal peptide and the human lambda constant region by homologous recombination. Bacterial colonies were isolated and plasmid DNA extracted; cDNA inserts were sequenced in their entirety. Correct heavy and light chains corresponding to each antibody (Table 19) were co-transfected into HEK-293-6E cells to transiently produce full-length E9-71(M) or E9-71(L) anti-human DLL4 antibodies. Both E9.71(M) and E9.71(L) share the same light chain. Cell supernatants containing

recombinant human antibody may be purified by Protein A Sepharose chromatography and bound antibody eluted by addition of acid buffer. Antibodies were neutralized and dialyzed into PBS.

Table 19. VH and VL Amino Acid Sequences of Human E9.71 engineered antibodies

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
356	**VH E9.71**		EVQLQESGPGLVKPSETLSLTCTVSGGSIS **SSSYYWG**WIRQPPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFSLKLSSVTAA DTAVYYCAR**QALAMGGGSDK**WGQGTLVTVS S
	VH E9.71 CDR-H1	Residues 31-37 of SEQ ID NO.:356	**SSSYYWG**
	VH E9.71 CDR-H2	Residues 52-67 of SEQ ID NO.:356	**DIYYTGSTYYNPSLKS**
	VH E9.71 CDR-H3	Residues 100-110 of SEQ ID NO.:356	**QALAMGGGSDK**
			123456789012345678901234567890
357	**VL E9.71**		SYELTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQPMDEADYYC**QAW DRDTGV**FGYGTKVTVLG
	VL E9.71 CDR-L1	Residues 23-33 of SEQ ID NO.:357	**SGQRLGDKYAS**
	VL E9.71 CDR-L2	Residues 49-55 of SEQ ID NO.:357	**EDSKRPS**
	VL E9.71 CDR-L3	Residues 88-96 of SEQ ID NO.:357	**QAWDRDTGV**
			123456789012345678901234567890
358	**VH E9.71(M)**		EVQLQESGPGLVKPSETLSLTCTVSGGSIS **SSSYYWG**WIRQPPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFSLKLSSVTAA DTAVYYCAR**QALAMGGGSDK**WGQGTLVTVS S
	VH E9.71(M) CDR-H1	Residues 31-37 of SEQ ID NO.:358	**SSSYYWG**
	VH E9.71(M) CDR-H2	Residues 52-67 of SEQ ID NO.:358	**DIYYTGSTYYNPSLKS**
	VH E9.71(M) CDR-H3	Residues 100-110 of SEQ ID NO.:358	**QALAMGGGSDK**
			123456789012345678901234567890

SEQ ID No.	Protein region		Sequence
			123456789012345678901234567890
359	**VL E9.71(M)**		SYELTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQPMDEADYYC**QAW DRDTGV**FGGGTKLTVLG
	VL E9.71(M) CDR-L1	Residues 23-33 of SEQ ID NO.:359	**SGQRLGDKYAS**
	VL E9.71(M) CDR-L2	Residues 49-55 of SEQ ID NO.:359	**EDSKRPS**
	VL E9.71(M) CDR-L3	Residues 88-96 of SEQ ID NO.:359	**QAWDRDTGV**
360			123456789012345678901234567890
	VH E9.71(L)		EVQLQESGPGLVKPSETLSLTCTVSGGSIS **SSSYYWG**WIRQPPGKGLEWIG**DIYYTGSTY YNPSLKS**RVTISVDTSKNQFSLKLSSVTAA DTAVYYCAR**QALALGGGSDK**WGQGTLVTVS S
	VH E9.71(L) CDR-H1	Residues 31-37 of SEQ ID NO.:360	**SSSYYWG**
	VH E9.71(L) CDR-H2	Residues 52-67 of SEQ ID NO.:360	**DIYYTGSTYYNPSLKS**
	VH E9.71(L) CDR-H3	Residues 100-110 of SEQ ID NO.:360	**QALALGGGSDK**
361			123456789012345678901234567890
	VL E9.71(L)		SYELTQPPSVSVSPGQTASITC**SGQRLGDK YAS**WYQQKPGQSPVLVIY**EDSKRPS**GIPER FSGSNSGDTATLTISGTQPMDEADYYC**QAW DRDTGV**FGGGTKLTVLG
	VL E9.71(L) CDR-L1	Residues 23-33 of SEQ ID NO.:361	**SGQRLGDKYAS**
	VL E9.71(L) CDR-L2	Residues 49-55 of SEQ ID NO.:361	**EDSKRPS**
	VL E9.71(L) CDR-L3	Residues 88-96 of SEQ ID NO.:361	**QAWDRDTGV**

Example 7: E9-71(M) Signal Peptide Engineering.

Signal peptide lambda 1a was used for the generation of anti-DLL4 antibody E9-71(M). Alternative signal peptides were also investigated. The prediction of the percentage of the correct antibody cleavage during mammalian expression of *in silico* constructed amino acid sequences of E9-71(M) N-terminal variable region with different signal peptides from the lambda and kappa

signal peptide families is known in the art using the Signal IP 3.0 Server available on the Internet (e.g., worldwide website cbs.dtu.dk/services/SignalP/) or numerous other equivalent software. The signal peptides with the highest predicted percentages of correct cleavage, one from each family were chosen: the lambda 3p from the lambda family and the L23 from the kappa family. A mutated version of the original lambda 1a signal peptide was also selected, with two amino acid changes (glycine to arginine and serine to valine). For the construction of the light chain containing the lambda 1a signal peptide polymerase chain reaction (PCR) was performed in one step to amplify the entire variable region gene using the two outermost primers containing overhanging sequences complementary to the receiving vector with one of them containing the mutated nucleotide sequence. For the construction of the light chain containing the lambda 3p signal peptide and the kappa L23 signal peptide two overlapping primers were designed to construct the signal peptide region then polymerase chain reaction (PCR) was performed to amplify the entire variable region gene using the two outermost primers containing overhanging sequences complementary to the receiving vector. Two versions of the E9-71 variable regions were generated using the lambda 3p and the kappa L23 signal peptides: one with the full length variable region and another one with the first serine (S) at the variable region N-terminus missing. Only the full length variable region was generated with the lambda 1a signal peptide. The PCR products derived from each cDNA assembly were separated on an agarose gel and the band corresponding to the predicted variable region cDNA size excised and purified. The variable heavy region was inserted in-frame onto a cDNA fragment encoding the human IgG1 constant region containing two hinge-region amino acid mutations by homologous recombination in bacteria. These mutations are a leucine to alanine change at position 234 (EU numbering) and a leucine to alanine change at position 235 (Lund et al., *J. Immunol.*, 147: 2657 (1991)). The variable light chain region was inserted in-frame with the human lambda constant region by homologous recombination. Bacterial colonies were isolated, plasmid DNA extracted, and cDNA inserts were sequenced in their entirety. Correct heavy and light chains corresponding to each antibody were co-transfected into HEK-293-6E cells to transiently produce full-length E9-71(M) anti-human DLL4 antibodies. Cell supernatants containing recombinant human antibody were purified by Protein A Sepharose chromatography and bound antibody eluted by addition of acid buffer. Antibodies were dialyzed into PBS. Purified E9.71(M) antibodies were analyzed by Mass Spectrometry (MS) for confirmation of intact antibody sequence. Table 20, below, shows the amino acid sequence of the different signal peptides used for the generation of E9.71(M). Table 21, below, shows the E9.71(M) cleavage sites analyzed by Mass Spectrometry.

Table 20. Amino acid sequence of the signal peptides used for the generation of E9.71(M)

Antibody	Signal Peptide	Signal Peptide Sequence	VL Region
E9.71(M)	λ 1a	MAWSPLFLTLITHCAGSWA (SEQ ID NO:362)	Full Length
E9.71(M)-1	λ 1a (GS to RV)	MAWSPLFLTLITHCARVWA	Full Length

		(SEQ ID NO:363)	
E9.71(M)-2	λ 3p	MAWTPLLLPLLTFCTVSEA (SEQ ID NO:364)	Full Length
E9.71(M)-3	λ 3p	MAWTPLLLPLLTFCTVSEA (SEQ ID NO:365)	N-terminus S missing
E9.71(M)-4	Kappa L23	MDMRVPAQRLGLLLLWFPGARC (SEQ ID NO:366)	Full Length
E9.71(M)-5	Kappa L23	MDMRVPAQRLGLLLLWFPGARC (SEQ ID NO:367)	N-terminus S missing

Table 21. E9.71(M) antibody cleavage sites analyzed by Mass Spectrometry (MS)

Antibody	Antibody Major Cleavage Site	% Major Peak (↓)
E9.71(M)	MAWSPLFLTLITHCAG↓SWA↓SYELTQPPSVS (SEQ ID NO:368)	95
E9.71(M)-1	MAWSPLFLTLITHCARVWA↓SYELTQPPSVS (SEQ ID NO:369)	96
E9.71(M)-2	MAWTPLLLPLLTFCTVSEA↓SYELTQPPSVS (SEQ ID NO:370)	96
E9.71(M)-3	MAWTPLLLPLLTFCTVSEA↓YELTQPPSVS (SEQ ID NO:371)	97
E9.71(M)-4	MDMRVPAQRLGLLLLWFPGARC↓SYELTQPPSVS (SEQ ID NO:372)	Not determined
E9.71(M)-5	MDMRVPAQRLGLLLLWFPGARC↓YELTQPPSVS (SEQ ID NO:373)	Not determined

Example 8. *In Vitro* Characterization of Engineered PROfusion Antibodies.

The antigen binding affinities of these engineered PROfusion antibodies were determined by the BIACORE technology as described in Example 1.1, and are shown in Table 22. The *in vitro* activities of the representative ones were further evaluated using other methods described in Example 1, with results shown in Table 23.

Table 22. Biacore kinetics of engineered anti-DLL4 PROfusion antibodies.

	Binding kinetics								
	Human DLL4 ECD			Mouse DLL4 ECD			Cyno DLL4 ECD		
Antibody	K_a ($M^{-1}S^{-1}$) E+04	K_d (S^{-1}) E-05	K_D (nM)	K_a ($M^{-1}S^{-1}$) E+04	K_d (S^{-1}) E-05	K_D (nM)	K_a ($M^{-1}S^{-1}$) E+04	K_d (S^{-1}) E-05	K_D (nM)
E9.4	5.67	3.31	0.58	5.38	6.01	1.12	1.37	3.44	0.25
E9.11	3.63	1.74	0.48	2.77	1.39	0.5	5.31	0.89	0.17
E9.14	3.99	36.5	9.14	3.89	13.6	3.49	5.72	38.6	6.75
E9.17	4.39	0.44	0.1	3.7	1.41	0.38	7.51	1.23	0.16
E9.18	1.57	1.84	1.18	2.32	1.68	0.73	2.63	2.63	1

E9.19	7.49	5.17	0.69	6.7	20.6	3.07	1.92	4.86	0.25
E9.22	3.91	33.1	8.46	3.78	13.3	3.5	5.62	35.2	6.26
E9.48	1.1	7.59	6.92	1.65	4.17	2.53	1.68	7.91	4.71
E9.65	2.59	36.3	14	2.57	13.6	5.27	3.21	41.4	12.9
E9.66	3.26	0.25	0.078	2.89	1.44	0.50	6.55	0.91	0.14
E9.71	3.88	3.51	0.91	3.55	21.6	6.08	7.75	3.79	0.49
E9.13	3.74	4.97	1.33	4.34	21.2	4.89	7.92	4.95	0.63
E9.16	1.29	3.15	2.45	1.83	14.8	8.1	2.49	3.79	1.52
E9-38	1.27	3.48	2.75	1.84	17.1	9.27	2.42	3.47	1.44
E9.2B	35.6	0.79	0.02	N/D	N/D	N/D	N/D	N/D	N/D
E9.1F	21.8	1.16	0.05	N/D	N/D	N/D	N/D	N/D	N/D
E9-12B	15.1	0.31	0.02	8.35	1.47	0.18	N/D	N/D	N/D
E9-10H	10.4	5.9	0.57	6.53	2.23	0.34	N/D	N/D	N/D
E9-5E	15	0.6	0.04	8.9	0.2	0.02	N/D	N/D	N/D
E9-10C	12	4.59	0.38	7.37	12.1	1.6	N/D	N/D	N/D
E9-10E	21.8	4.62	0.21	15.1	9.41	0.62	N/D	N/D	N/D
E9-7E	15.2	4.12	0.27	9.53	22.1	2.3	N/D	N/D	N/D
A10.K30	3.57	27.4	7.69	NB	NB	NB	N/D	N/D	N/D
A10.K42	10	40.6	4.06	NB	NB	NB	N/D	N/D	N/D
A10.L45	4.4	3.16	0.72	NB	NB	NB	N/D	N/D	N/D
A10.L73	5.07	0.95	0.19	NB	NB	NB	N/D	N/D	N/D

MAb = monoclonal antibody; N/D = not determined, NB = no binding

Table 23. Characterization of selected engineered PROfusion DLL4 antibody.

	Direct Binding Assays					Functional Blockade Assays					
Antibody	Capture ELISA (EC_{50}, nM)			FACS (EC_{50}, nM)		Competition ELISA (IC_{50}, nM)			Competition FACS (IC_{50} nM)		Inhibition of Notch activation via huDLL4 cells, coculture (IC_{50} nM)
	DLL4 ECD			DLL4 Cells		DLL4 ECD/ huNotch-1			huNotch-1/ DLL4 cells		
	hu	mu	cyno	hu	mu	hu	mu	cyno	hu	mu	
E9-2B	0.16	0.16	0.45	1.78	0.35	2.3	2.8	2.2	2.63	0.34	2.1
E9-71	0.16	0.17	0.44	1.82	0.32	2.0	1.3	2.0	3.22	0.24	2.1

E9-19	0.17	0.17	0.46	2.79	0.54	1.8	1.3	1.5	4.90	0.44	4.4
E9-4	N/D	N/D	N/D	1.63	0.63	N/D	N/D	N/D	4.3	0.3	N/D
E9-11	N/D	N/D	N/D	4.46	1.45	N/D	N/D	N/D	14.75	2.06	N/D
E9-16	N/D	N/D	N/D	1.7	>50	N/D	N/D	N/D	12.6	1.1	N/D
E9-17	N/D	N/D	N/D	8	2.57	N/D	N/D	N/D	19.79	N/D	1
E9-22	N/D	N/D	N/D	34.34	37.35	N/D	N/D	N/D	21.05	2.63	N/D
E9-38	N/D	N/D	N/D	1.66	0.13	N/D	N/D	N/D	7.76	1.2	N/D
E9-48	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	9.12	1.3	N/D
E9-66	N/D	N/D	N/D	2.02	0.7	N/D	N/D	N/D	5.03	0.99	N/D
E9-1F	N/D	N/D	N/D	2.65	0.37	N/D	N/D	N/D	6.55	0.65	N/D
E9-5E	N/D	N/D	N/D	2.7	0.89	N/D	N/D	N/D	3.76	0.34	N/D
E9-7E	N/D	N/D	N/D	4.46	1.94	N/D	N/D	N/D	4	0.33	0.41
E9-10C	N/D	N/D	N/D	3.15	0.88	N/D	N/D	N/D	3.03	0.26	0.85
E9-10E	N/D	N/D	N/D	5.1	1.19	N/D	N/D	N/D	3.83	0.36	N/D
E9-10H	N/D	N/D	N/D	3.52	0.82	N/D	N/D	N/D	5.65	0.6	N/D
E9-12B	N/D	N/D	N/D	3.34	0.71	N/D	N/D	N/D	4.44	0.37	N/D
E9-71(M)	N/D	N/D	N/D	0.27	0.29	N/D	N/D	N/D	2.12	0.18	N/D
E9-71(L)	N/D	N/D	N/D	0.22	0.47	N/D	N/D	N/D	1.82	0.21	2.4
E9-71(M)-3	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	N/D	0.65

hu = human; mu = murine; cyno = cynomolgus monkey; N/D = not determined

Example 8: Physicochemical Properties of Selected PROfusion Antibodies.

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

Mass Spectrometry Analysis of E9-71.

Light chain and heavy chain molecular weight analysis: E9-71 sample was diluted to 1 mg/mL with Milli-Q water. 1 µL of 1 M DTT was added to 20 µL of diluted sample. The sample was incubated at 37°C for 30 minutes. 1 µL of the reduced samples was injected onto the Agilent 6510 Q-TOF LC/MS system with a Varian Diphenyl column. Buffer A was 0.02% trifluoroacetic acid (TFA), 0.08% formic acid (FA) in water. Buffer B was 0.02% TFA, 0.08% FA in acetonitrile. The gradient started at 5%B, increased to 35%B in 5 minutes, and increased to 38%B in 15 minutes. The gradient then increased to 95% B in 1 minute and stayed at 95%B for 4 minutes, and decreased to 5%B in 1 minute. The flow rate was 50 µL/min. The mass spectrometer was operated at 5 kvolts spray voltage and scan range was from 600 to 3200 mass to

charge ratio. The light chain molecular weight of 22649 Dalton matched well with the theoretical value with the amino acid Y being the N-terminus. Three minor peaks were observed at molecular weight 22014 Dalton, 22737 Dalton, and 22937 Dalton. The peak at 22014 Dalton was consistent with N-terminal -6 amino acid fragment. This peak was most probably caused by the in-source fragmentation from the full length light chain, as lowering the mass spectrometer "fragmentor value" can lead to the disappearance of this peak. The 22737 Dalton was consistent with the theoretical value with the amino acid S being the N-terminus. The 22937 Dalton was consistent with the light chain with signal peptide extension of amino acids "LS" on the N-terminal. The heavy chain molecular weights matched well with the theoretical values. The observed molecular weights were 50263 Dalton, 50426 Dalton, and 50588 Dalton, with the difference corresponding to 162 Dalton as result of different glycosylation.

Mass Spectrometry Analysis of E9-71(M).

The same method described in "Mass spectrometry analysis of E9-71" was used to analyze the E9-71(M) sample. The light chain molecular weight of 22645 Dalton matched well with the theoretical value with the amino acid S being the N-terminus. A small peak with molecular weight 22989 Dalton was observed, corresponding to the light chain with signal peptide extension of amino acids "SWA" on the N-terminal. A very small peak with molecular weight 21923 Dalton was also observed, although it was highly likely caused by in-source fragmentation as lowering the mass spectrometer "fragmentor value" led to disappearance of this peak. The heavy chain molecular weights matched well with the theoretical values. The observed molecular weights were 50263 Dalton, 50426 Dalton, and 50588 Dalton, with the difference corresponding to 162 Dalton as result of different glycosylation.

Mass Spectrometry Analysis of E9-71(L).

The same method described in "Mass spectrometry analysis of E9-71" was used to analyze the E9-71(L) sample. The light chain molecular weight of 22645 Dalton matched well with the theoretical value with the amino acid S being the N-terminus. A small peak with molecular weight 22989 Dalton was observed, corresponding to the light chain with signal peptide extension of amino acids "SWA" on the N-terminal. A very small peak with molecular weight 21923 Dalton was also observed, although it was highly likely caused by in-source fragmentation as lowering the mass spectrometer "fragmentor value" led to disappearance of this peak. The heavy chain molecular weights matched well with the theoretical values. The observed molecular weights were 50245 Dalton, 50407 Dalton, and 50569 Dalton, with the difference corresponding to 162 Dalton as result of different glycosylation.

Mass Spectrometry Analysis of E9-71(M)-3.

The same method described in "Mass spectrometry analysis of E9-71" was used to analyze the E9-71(M)-3 sample. The light chain molecular weight of 22558 Dalton matched well with the theoretical value. A very small peak with molecular weight 21923 Dalton was also

observed, although it was highly likely caused by in-source fragmentation as lowering the mass spectrometer "fragmentor value" led to disappearance of this peak. The heavy chain molecular weights matched well with the theoretical values. The observed molecular weights were 50263 Dalton, 50426 Dalton, and 50588 Dalton, with the difference corresponding to 162 Dalton as 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 detail techniques were described in Example 1.8 and the results are described below: Solubility Estimation by PEG Precipitation Results.

Tables 24 and 26 shows the percentage of PEG 3000 needed to induce precipitation for a series of E9 clones. The clones and the Adalimumab reference were formulated at 0.2 mg/ml. According to the results, clones such as E9-4, E9-14, E9-22, and E9-19 are estimated to have solubilities similar to that of adalimumab (approximately 200 mg/ml) while clones such as E9-11 and E9-17 are estimated to have much lower solubilities.

Table 25 shows the percentage of PEG 3000 needed to induce precipitation for a series of stability engineered E9 clones. The clones and the adalimumab reference were formulated at 0.2 mg/ml. According to the results, clones such as E9-SE1 have the highest solubilities in the series but are not expected to have solubilities similar to that of adalimumab (approximately 200 mg/ml) while clones such as E9-SE5 are estimated to have much lower solubilities.

Table 24. Percentage of PEG 3000 needed to induce precipitation for a series of E9 antibodies. (The antibodies were formulated at 0.2 mg/ml.)

A-Number	Lot #	Antibody	% PEG 3000
A-1242367.0	1718299	DLL4-E9-11 hIgG1/L	3.00
A-1242368.0	1718300	DLL4-E9-17 hIgG1/L	3.00
		E9.1 IgG2	3.00
A-1242369.0	1718301	DLL4-E9-18 hIgG1/L	4.00
A-1242370.0	1718302	DLL4-E9-48 hIgG1/L	4.00
		E9.1 IgG4	5.00
		DLL4-E9-3 hIgG/L	6.00
A-1242371.0	1718303	DLL4-E9-66 hIgG1/L	8.00
A-1241120.0	1716682	DLL4-E9-16 hIgG1/L	10.00
A-1242795.0	1718785	DLL4-E9-13 hIgG1/L	10.00
A-1241121.0	1716683	DLL4-E9-38 hIgG1/L	11.00
A-1242800.0	1718790	DLL4-E9-71 hIgG1/L	11.00
A-1242794.0	1718784	DLL4-E9-4 hIgG1/L	12.00
A-1242796.0	1718786	DLL4-E9-14 hIgG1/L	12.00
A-1242798.0	1718788	DLL4-E9-22 hIgG1/L	12.00

A-1242797.0	1718787	DLL4-E9-19 hIgG1/L	14.00
		adalimumab	14.00

Table 25. Percentage of PEG 3000 needed to induce precipitation for a series of E9 stability engineered antibodies. (The antibodies were formulated at 0.2 mg/ml.)

Antibody	% PEG 3000
E9-SE5	6.00
E9-SE7	7.00
E9-SE4	7.50
E9-SE8	7.50
E9-SE2	9.00
E9-SE3	9.00
E9-SE6	9.50
E9-SE1	10.00
(+) E9.1	9.00
(+) E9	10.50
adalimumab	13.00

Table 26. Percentage of PEG 3000 needed to induce precipitation for a series of E9 antibodies. (The antibodies were formulated at 0.2 mg/ml.)

Antibody	% PEG 3000
E9-2B	8.00
E9-1F	8.00
E9	10
adalimumab	14

Real Solubility Screening Results: E9, E9-19, E9-71, E9-2B, E9-1F.

Solutions containing 12 mg of E9, E9-71, E9-1F, and E9-19 and 5.5 mg of E9-2B were obtained. E9 is the IgG1 mutant isotype. The volumes of all solutions were reduced below 1 ml by ultra-centrifugation with Amicon 30K 15 ml tubes.

After this step, the following was observed: E9-2B and E9-19 were clear. E9, E9-71, and E9-1F were slightly cloudy.

10 ml of 15 mM histidine buffer at pH 5.03 was added to each tube and the solutions re-concentrated to 1 ml. The solutions were then transferred to Amicon 30K 4 ml tubes and concentrated to as low a volume as possible.

After this step, the following was observed at room temperature:

E9: 163 mg/ml; vol=0.05 ml; pH = 5.12

E9-19: 132 mg/ml; vol=0.05 ml; pH = 5.06

E9-71: 193 mg/ml; vol=0.05 ml; pH = 5.32

E9-2B: 64 mg/ml; vol=0.1 ml; pH=5.29

E9-1F: 100 mg/ml; vol=0.1 ml; pH=5.31

Both E9-2B and E9-1F required much longer to concentrate than the other three. This may suggest that their viscosity is high under the formulation conditions.

The solutions were then placed at 5°C for two days to assess solubility at this temperature. The following was observed:

E9: remained clear at 5°C and when brought back to room temperature

E9-71: remained clear at 5°C and when brought back to room temperature

E9-1F: showed an extremely slight amount of cloudiness at 5°C that cleared when brought to room temperature after 20 minutes

E9-2B: showed a slight amount of cloudiness at 5°C that cleared when brought to room temperature after 20 minutes

E9-19: showed apparent cloudiness at 5°C that cleared when brought to room temperature after 20 minutes.

Real Solubility Screening Results for E9-71.

For E9-71, 4 mg in solution was concentrated with Amicon centrifugal filters to 60 mg/ml. No precipitation or cloudiness was observed at 25°C nor after storage for 1 day at 5°C.

Tertiary Structure Characterization by Near UV-CD Results.

Near UV-CD was performed on E9, E9-19, E9-71, E9-2B, and E9-1F samples at 1 mg/ml. The profiles of the spectra show a sigmoidal pattern typically observed for properly folded antibodies. From this technique, no indication of misfolding is observed for the antibodies.

Intrinsic Stability Characterization by Differential Scanning Calorimetry (DSC).

DSC was performed on E9, E9-19, E9-71, E9-2B, and E9-1F samples at 1 mg/ml. The results are given in Table 27. The onset is the temperature at which unfolding initiates. Also, an IgG antibody typically shows three unfolding transitions (Tm): unfolding of the intact antibody is associated with the melting of the CH2 domain in the Fc fragment, melting of the CH3 domain in the Fc fragment, and melting of the Fab fragment. Onset values suggest E9, E9-19, and E9-71 are most stable. Typically, clones with higher Tm values are preferred over those with lower values.

Table 27. Intrinsic stability of anti-DLL4 E9 clones via DSC at 1 mg/ml.

Antibody	Tm1 (°C)	Tm2 (°C)	Tm3 (°C)	Onset (°C)
E9	64.45	72.12	80.04	54
E9-19	65.66	77	---	56
E9-71	65.65	75.36	81.24	54
E9-2B	64.74	80.58	83.27	52.5
E9-1F	63.84	80.51	83.26	51.3

Evaluation of Stability to Freeze-Thaw Stress

Stability to freeze-thaw stress was evaluated for E9, E9-19, E9-71, E9-2B, and E9-1F samples at 1 mg/ml. Table 28 shows the results of SEC analysis of samples after five freeze-thaw

cycles. All the antibodies tested were stable to freeze-thaw stress. No apparent loss of monomer was observed even after 5 freeze-thaw cycles.

Table 28. Percentage of monomer species quantitated by SEC before freezing at -80°C and after five cycles of freezing at -80°C and thawing at 30°C in water bath for anti-DLL4 clones.

Antibody	% monomer before freezing	% monomer after 5th freeze-thaw cycle
E9	99.2	99.3
E9-19	99.3	99.1
E9-71	96.9	96.8
E9-2B	99.0	98.9
E9-1F	98.6	98.5

Evaluation of Stability at Elevated Temperatures (Accelerated Stability).

Stability at elevated temperatures was evaluated for E9, E9-19, E9-71, E9-2B, and E9-1F samples at 1 mg/ml. Table 29 shows the results of SEC analysis of samples at time zero and after 7 and 21 days at 40°C and 50°C. The degradation kinetics for all of the antibodies revealed an approximately 8% reduction in monomer percentage after 21 days 50°C.

Table 29. Percentage of different species quantitated by SEC for anti-DLL4 clones at time zero and after incubation for 7 and 21 days at 40°C and 50°C. (Samples were formulated at 1 mg/ml.)

Antibody	% monomer at time zero	% monomer at 7 days at 40°C	% monomer at 7 days at 50°C	% monomer at 21 days at 40°C	% monomer at 21 days at 50°C
E9	99.2	98.6	86.8	90.9	88.1
E9-19	99.3	98.7	96.8	97.4	91.9
E9-71	96.9	96.4	95.4	95.0	91.0
E9-2B	99.0	98.6	97.0	97.1	92.4
E9-1F	98.6	98.2	96.5	96.8	91.9
Antibody	% aggregate at time zero	% aggregate at 7 days at 40°C	% aggregate at 7 days at 50°C	% aggregate at 21 days at 40°C	% aggregate at 21 days at 50°C
E9	0.3	0.3	0.3	0.5	0.6
E9-19	0.3	0.3	0.4	0.7	1.4
E9-71	2.4	2.3	1.8	2.9	2.8
E9-2B	0.5	0.4	0.5	0.9	1.7
E9-1F	0.3	0.3	0.3	0.6	1.6
Antibody	% fragment at time zero	% fragment at 7 days at 40°C	% fragment at 7 days at 50°C	% fragment at 21 days at 40°C	% fragment at 21 days at 50°C
E9	0.5	1.2	12.9	8.7	11.3
E9-19	0.4	1.0	2.8	1.9	6.7
E9-71	0.7	1.3	2.8	2.1	6.2
E9-2B	0.6	1.0	2.5	2.0	5.9
E9-1F	1.1	1.5	3.2	2.5	6.5

Example 9: Rodent PK Assessment of Anti-DLL4 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 5 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 30. For both E9 and A10 PROfusion antibodies, their pharmacokinetics properties were improved through CDR-engineering during the process of affinity maturation.

Table 30. Pharmacokinetic parameters of anti-DLL4 antibodies in SCID-beige mice.

Antibody	Dose (mg/kg)	Cmax (µg/mL)	CL (mL/hr/kg)	$t_{1/2}$ (d)
E9	30	201	3.10	1.3
E9-10C	30	165	0.82	5.2
E9-10E	30	263	0.97	3.8
E9-10H	30	235	1.49	3.1
E9-12B	30	146	1.32	3.8
E9-19	30	179	1.08	4.4
E9-1F	30	269	0.89	4.2
E9-2B	30	165	0.65	5.5
E9-5E	30	234	0.95	3.0
E9-66	30	114	2.90	1.9
E9-71	30	102	1.10	4.2
E9-71(L)	30	145	0.83	4.1
E9-71(M)	30	113	1.07	4.3
E9-7E	30	130	1.23	5.8
A10	5	10.5	5.80	3.1
A10.K30	5	15.6	0.69	17.7
A10.K42	5	12.5	0.93	13.8
A10.L45	5	18.6	0.65	13.4

Example 10: Anti-DLL4 Antibody Treatment Increased Endothelial Cell Sprouting *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 et al., *Microvasc. Res.*, 66: 102–112 (2003)). Briefly, fibrinogen solution was reconstituted with aprotinin (4 U/ml) and thrombin (50 U/ml). Cytodex 3 beads (Amersham Pharmacia Biotech) were coated with 350 to 400 HUVECs per bead for overnight. 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. DLL4 inhibition with E9 and A10 antibodys results in enhancement of endothelial cell sprouting *in vitro* (data not shown).

Example 11: 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, $2x10^6$ 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: $L \times W^2/2$. 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 twice a week for two 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 $\geq 2,000$ mm^3. Results are shown in Table 31. For E9 series of PROfusion antibodies, those with improved pharmacokinetics (as shown in Example 9) tend to have stronger anti-tumor activity *in vivo*.

Table 31. Efficacy of anti-DLL4 antibodies in the Calu-6 human non-small cell lung cancer xenograft model.

Treatment	Dose Route, Regimen	%T/C a	%ILSb
E9	10 mg/kg IP, 2X/week X2	43**	52**
E9-10C	10 mg/kg IP, 2X/week X2	26**	81**
E9-2B	10 mg/kg IP, 2X/week X2	28**	76**
E9-10E	10 mg/kg IP, 2X/week X2	30**	70**
E9-19	10 mg/kg IP, 2X/week X2	31**	64**
E9-5E	10 mg/kg IP, 2X/week X2	30**	57**
E9-71	10 mg/kg IP, 2X/week X2	34**	63**
E9-1F	10 mg/kg IP, 2X/week X2	34**	57**
E9-12B	10 mg/kg IP, 2X/week X2	38**	52**
E9-7E	10 mg/kg IP, 2X/week X2	38**	44**
E9-10H	10 mg/kg IP, 2X/week X2	41**	52**
E9-66	10 mg/kg IP, 2X/week X2	43**	32*

a. %T/C = mean tumor volume of treatment group/ tumor volume of treatment control group x 100. P values (as indicated by asterisks) are derived from Student's T test comparison of treatment group vs. 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) derived from Kaplan Meier log-rank comparison of treatment group vs. treatment control group. Based on an endpoint of 2000 mm^3.
* $p < 0.05$; ** $p < 0.01$

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.

WE CLAIM:

1. A binding protein comprising an antigen binding domain, said binding protein being capable of binding human DLL4, said antigen binding domain comprising six CDRs: CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3, wherein:
CDR-H1 is selected from the group consisting of:
residues 31-37 of SEQ ID NO:1;
SEQ ID NO:117;
SEQ ID NO:121;
SEQ ID NO:125;
SEQ ID NO:129;
SEQ ID NO:133;
SEQ ID NO:137;
SEQ ID NO:141;
SEQ ID NO:145;
SEQ ID NO:149;
SEQ ID NO:153;
SEQ ID NO:157;
SEQ ID NO:161;
SEQ ID NO:165;
SEQ ID NO:169;
SEQ ID NO:173;
SEQ ID NO:177;
SEQ ID NO:181;
SEQ ID NO:185;
SEQ ID NO:189;
SEQ ID NO:193;
SEQ ID NO:197;
SEQ ID NO:201;
SEQ ID NO:205;
SEQ ID NO:209;
SEQ ID NO:213;
residues 31-37 of SEQ ID NO:334;
residues 31-37 of SEQ ID NO:336;
residues 31-37 of SEQ ID NO:338;
residues 31-37 of SEQ ID NO:340;
residues 31-37 of SEQ ID NO:342;

residues 31-37 of SEQ ID NO:344;
residues 31-37 of SEQ ID NO:346;
residues 31-37 of SEQ ID NO:348;
residues 31-37 of SEQ ID NO:350;
residues 31-37 of SEQ ID NO:352;
residues 31-37 of SEQ ID NO:354;
residues 31-37 of SEQ ID NO:356;
residues 31-37 of SEQ ID NO:358; and
residues 31-37 of SEQ ID NO:360;

CDR-H2, selected from the group consisting of:

residues 52-67 of SEQ ID NO:1;
SEQ ID NO:118;
SEQ ID NO:122;
SEQ ID NO:126;
SEQ ID NO:130;
SEQ ID NO:134;
SEQ ID NO:138;
SEQ ID NO:142;
SEQ ID NO:146;
SEQ ID NO:150;
SEQ ID NO:154;
SEQ ID NO:158;
SEQ ID NO:162;
SEQ ID NO:166;
SEQ ID NO:170;
SEQ ID NO:174;
SEQ ID NO:178;
SEQ ID NO:182;
SEQ ID NO:186;
SEQ ID NO:190;
SEQ ID NO:194;
SEQ ID NO:198;
SEQ ID NO:202;
SEQ ID NO:206;
SEQ ID NO:210;
SEQ ID NO:214;
residues 52-67 of SEQ ID NO:334;

residues 52-67 of SEQ ID NO:336;
residues 52-67 of SEQ ID NO:338;
residues 52-67 of SEQ ID NO:340;
residues 52-67 of SEQ ID NO:342;
residues 52-67 of SEQ ID NO:344;
residues 52-67 of SEQ ID NO:346;
residues 52-67 of SEQ ID NO:348;
residues 52-67 of SEQ ID NO:350;
residues 52-67 of SEQ ID NO:352;
residues 52-67 of SEQ ID NO:354;
residues 52-67 of SEQ ID NO:356;
residues 52-67 of SEQ ID NO:358; and
residues 52-67 of SEQ ID NO:360;

CDR-H3 is selected from the group consisting of:

residues 100-110 of SEQ ID NO:1;
SEQ ID NO:119;
SEQ ID NO:123;
SEQ ID NO:127;
SEQ ID NO:131;
SEQ ID NO:135;
SEQ ID NO:139;
SEQ ID NO:143;
SEQ ID NO:147;
SEQ ID NO:151;
SEQ ID NO:155;
SEQ ID NO:159;
SEQ ID NO:163;
SEQ ID NO:167;
SEQ ID NO:171;
SEQ ID NO:175;
SEQ ID NO:179;
SEQ ID NO:183;
SEQ ID NO:187;
SEQ ID NO:191;
SEQ ID NO:195;
SEQ ID NO:199;
SEQ ID NO:203;

SEQ ID NO:207;

SEQ ID NO:211;

SEQ ID NO:215;

residues 100-110 of SEQ ID NO:334;

residues 100-110 of SEQ ID NO:336;

residues 100-110 of SEQ ID NO:338;

residues 100-110 of SEQ ID NO:340;

residues 100-110 of SEQ ID NO:342;

residues 100-110 of SEQ ID NO:344;

residues 100-110 of SEQ ID NO:346;

residues 100-110 of SEQ ID NO:348;

residues 100-110 of SEQ ID NO:350;

residues 100-110 of SEQ ID NO:352;

residues 100-110 of SEQ ID NO:354;

residues 100-110 of SEQ ID NO:356;

residues 100-110 of SEQ ID NO:358; and

residues 100-110 of SEQ ID NO:360;

CDR-L1 is selected from the group consisting of:

residues 23-33 of SEQ ID NO:111;

SEQ ID NO:217;

SEQ ID NO:221;

SEQ ID NO:225;

SEQ ID NO:229;

SEQ ID NO:233;

SEQ ID NO:237;

SEQ ID NO:241;

SEQ ID NO:245;

SEQ ID NO:249;

SEQ ID NO:253;

SEQ ID NO:257;

SEQ ID NO:261;

SEQ ID NO:265;

SEQ ID NO:269;

SEQ ID NO:273;

SEQ ID NO:277;

SEQ ID NO:281;

SEQ ID NO:285;

SEQ ID NO:289;
SEQ ID NO:293;
SEQ ID NO:297;
SEQ ID NO:301;
SEQ ID NO:305;
SEQ ID NO:309;
SEQ ID NO:313;
residues 23-33 of SEQ ID NO:335;
residues 23-33 of SEQ ID NO:337;
residues 23-33 of SEQ ID NO:339;
residues 23-33 of SEQ ID NO:341;
residues 23-33 of SEQ ID NO:343;
residues 24-34 of SEQ ID NO:345;
residues 23-33 of SEQ ID NO:347;
residues 24-34 of SEQ ID NO:349;
residues 24-34 of SEQ ID NO:351;
residues 24-34 of SEQ ID NO:353;
residues 24-34 of SEQ ID NO:355;
residues 23-33 of SEQ ID NO:357;
residues 23-33 of SEQ ID NO:359; and
residues 23-33 of SEQ ID NO:361;

CDR-L2 is selected from the group consisting of:
residues 49-55 of SEQ ID NO:111;
SEQ ID NO:230;
SEQ ID NO:234;
SEQ ID NO:238;
SEQ ID NO:242;
SEQ ID NO:246;
SEQ ID NO:250;
SEQ ID NO:254;
SEQ ID NO:258;
SEQ ID NO:262;
SEQ ID NO:266;
SEQ ID NO:270;
SEQ ID NO:218;
SEQ ID NO:222;
SEQ ID NO:226;

SEQ ID NO:274;
SEQ ID NO:278;
SEQ ID NO:302;
SEQ ID NO:294;
SEQ ID NO:282;
SEQ ID NO:290;
SEQ ID NO:298;
SEQ ID NO:286;
SEQ ID NO:306;
SEQ ID NO:310;
SEQ ID NO:314;
residues 49-55 of SEQ ID NO:335;
residues 49-55 of SEQ ID NO:337;
residues 49-55 of SEQ ID NO:339;
residues 49-55 of SEQ ID NO:341;
residues 49-55 of SEQ ID NO:343;
residues 50-56 of SEQ ID NO:345;
residues 49-55 of SEQ ID NO:347;
residues 50-56 of SEQ ID NO:349;
residues 50-56 of SEQ ID NO:351;
residues 50-56 of SEQ ID NO:353;
residues 50-56 of SEQ ID NO:355;
residues 49-55 of SEQ ID NO:357;
residues 49-55 of SEQ ID NO:359; and
residues 49-55 of SEQ ID NO:361;

and

CDR-L3 is selected from the group consisting of:

residues 88-96 of SEQ ID NO:111;
SEQ ID NO:231;
SEQ ID NO:235;
SEQ ID NO:239;
SEQ ID NO:243;
SEQ ID NO:247;
SEQ ID NO:251;
SEQ ID NO:255;
SEQ ID NO:259;
SEQ ID NO:263;

SEQ ID NO:267;
SEQ ID NO:271;
SEQ ID NO:219;
SEQ ID NO:223;
SEQ ID NO:227;
SEQ ID NO:275;
SEQ ID NO:279;
SEQ ID NO:303;
SEQ ID NO:295;
SEQ ID NO:283;
SEQ ID NO:291;
SEQ ID NO:299;
SEQ ID NO:287;
SEQ ID NO:307;
SEQ ID NO:311;
SEQ ID NO:315;
residues 88-96 of SEQ ID NO:335;
residues 88-96 of SEQ ID NO:337;
residues 88-96 of SEQ ID NO:339;
residues 88-96 of SEQ ID NO:341;
residues 88-96 of SEQ ID NO:343;
residues 89-97 of SEQ ID NO:345;
residues 88-96 of SEQ ID NO:347;
residues 89-97 of SEQ ID NO:349;
residues 89-97 of SEQ ID NO:351;
residues 89-97 of SEQ ID NO:353;
residues 89-97 of SEQ ID NO:355;
residues 88-96 of SEQ ID NO:357;
residues 88-96 of SEQ ID NO:359; and
residues 88-96 of SEQ ID NO:361.

2. *The binding protein according to claim 1, wherein said binding protein comprises* at least 3 CDRs selected from the group of variable domain CDR sets consisting of:

VH E9 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:1

CDR-H2: residues 52-67 of SEQ ID NO:1

CDR-H3 residues 100-110 of SEQ ID NO:1

VL E9 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:111

CDR-L2: residues 49-55 of SEQ ID NO:111

CDR-L3: residues 88-96 of SEQ ID NO:111

VH E9.4 CDR Set

CDR-H1: SEQ ID NO:117

CDR-H2: SEQ ID NO:118

CDR-H3: SEQ ID NO:119

VL E9.4 CDR Set

CDR-L1: SEQ ID NO:229

CDR-L2: SEQ ID NO:230

CDR-L3: SEQ ID NO:231

VH E9.11 CDR Set

CDR-H1: SEQ ID NO:121

CDR-H2: SEQ ID NO:122

CDR-H3: SEQ ID NO:123

VL E9.11 CDR Set

CDR-L1: SEQ ID NO:233

CDR-L2: SEQ ID NO:234

CDR-L3: SEQ ID NO:235

VH E9.14 CDR Set

CDR-H1: SEQ ID NO:125

CDR-H2: SEQ ID NO:126

CDR-H3: SEQ ID NO:127

VL E9.14 CDR Set

CDR-L1: SEQ ID NO:237

CDR-L2: SEQ ID NO:238

CDR-L3: SEQ ID NO:239

VH E9.17 CDR Set

CDR-H1: SEQ ID NO:129

CDR-H2: SEQ ID NO:130

CDR-H3: SEQ ID NO:131

VL E9.17 CDR Set

CDR-L1: SEQ ID NO:241

CDR-L2: SEQ ID NO:242

CDR-L3: SEQ ID NO:243

VH E9.18 CDR Set

CDR-H1: SEQ ID NO:133

CDR-H2: SEQ ID NO:134

CDR-H3: SEQ ID NO:135

VL E9.18 CDR Set

CDR-L1: SEQ ID NO:245

CDR-L2: SEQ ID NO:246

CDR-L3: SEQ ID NO:247

VH E9.19 CDR Set

CDR-H1: SEQ ID NO:137

CDR-H2: SEQ ID NO:138

CDR-H3: SEQ ID NO:139

VL E9.19 CDR Set

CDR-L1: SEQ ID NO:249

CDR-L2: SEQ ID NO:250

CDR-L3: SEQ ID NO:251

VH E9.22 CDR Set

CDR-H1: SEQ ID NO:141

CDR-H2: SEQ ID NO:142

CDR-H3: SEQ ID NO:143

VL E9.22 CDR Set

CDR-L1: SEQ ID NO:253

CDR-L2: SEQ ID NO:254

CDR-L3: SEQ ID NO:255

VH E9.48 CDR Set

CDR-H1: SEQ ID NO:145

CDR-H2: SEQ ID NO:146

CDR-H3: SEQ ID NO:147

VL E9.48 CDR Set

CDR-L1: SEQ ID NO:257

CDR-L2: SEQ ID NO:258

CDR-L3: SEQ ID NO:259

VH E9.65 CDR Set

CDR-H1: SEQ ID NO:149

CDR-H2: SEQ ID NO:150

CDR-H3: SEQ ID NO:151

VL E9.65 CDR Set

CDR-L1: SEQ ID NO:261

CDR-L2: SEQ ID NO:262

CDR-L3: SEQ ID NO:263

VH E9.66 CDR Set

CDR-H1: SEQ ID NO:153

CDR-H2: SEQ ID NO:154

CDR-H3: SEQ ID NO:155

VL E9.66 CDR Set

CDR-L1: SEQ ID NO:265

CDR-L2: SEQ ID NO:266

CDR-L3: SEQ ID NO:267

VH E9.71 CDR Set

CDR-H1: SEQ ID NO:157

CDR-H2: SEQ ID NO:158

CDR-H3: SEQ ID NO:159

VL E9.71 CDR Set

CDR-L1: SEQ ID NO:269

CDR-L2: SEQ ID NO:270

CDR-L3: SEQ ID NO:271

VH E9.13 CDR Set

CDR-H1: SEQ ID NO:161

CDR-H2: SEQ ID NO:162

CDR-H3: SEQ ID NO:163

VL E9.13 CDR Set

CDR-L1: SEQ ID NO:217

CDR-L2: SEQ ID NO:218

CDR-L3: SEQ ID NO:219

VH E9.16 CDR Set

CDR-H1: SEQ ID NO:165

CDR-H2: SEQ ID NO:166

CDR-H3: SEQ ID NO:167

VL E9.16 CDR Set

CDR-L1: SEQ ID NO:221

CDR-L2: SEQ ID NO:222

CDR-L3: SEQ ID NO:223

VH E9.38 CDR Set

CDR-H1: SEQ ID NO:169

CDR-H2: SEQ ID NO:170

CDR-H3: SEQ ID NO:171

VL E9.38 CDR Set

CDR-L1: SEQ ID NO:225

CDR-L2: SEQ ID NO:226

CDR-L3: SEQ ID NO:227

VH E9.2B CDR Set

CDR-H1: SEQ ID NO:173

CDR-H2: SEQ ID NO:174

CDR-H3: SEQ ID NO:175

VL E9.2B CDR Set

CDR-L1: SEQ ID NO:273

CDR-L2: SEQ ID NO:274

CDR-L3: SEQ ID NO:275

VH E9.1F CDR Set

CDR-H1: SEQ ID NO:177

CDR-H2: SEQ ID NO:178

CDR-H3: SEQ ID NO:179

VL E9.1F CDR Set

CDR-L1: SEQ ID NO:277

CDR-L2: SEQ ID NO:278

CDR-L3: SEQ ID NO:279

VH E9.10H CDR Set

CDR-H1: SEQ ID NO:181

CDR-H2: SEQ ID NO:182

CDR-H3: SEQ ID NO:183

VL E9.10H CDR Set

CDR-L1: SEQ ID NO:301

CDR-L2: SEQ ID NO:302

CDR-L3: SEQ ID NO:303

VH E9.5E CDR Set

CDR-H1: SEQ ID NO:185

CDR-H2: SEQ ID NO:186

CDR-H3: SEQ ID NO:187

VL E9.5E CDR Set

CDR-L1: SEQ ID NO:293

CDR-L2: SEQ ID NO:294

CDR-L3: SEQ ID NO:295

VH E9.10C CDR Set

CDR-H1: SEQ ID NO:189

CDR-H2: SEQ ID NO:190

CDR-H3: SEQ ID NO:191

VL E9.10C CDR Set

CDR-L1: SEQ ID NO:281

CDR-L2: SEQ ID NO:282

CDR-L3: SEQ ID NO:283

VH E9.7E CDR Set

CDR-H1: SEQ ID NO:193

CDR-H2: SEQ ID NO:194

CDR-H3: SEQ ID NO:195

VL E9.7E CDR Set

CDR-L1: SEQ ID NO:289

CDR-L2: SEQ ID NO:290

CDR-L3: SEQ ID NO:291

VH E9.12B CDR Set

CDR-H1: SEQ ID NO:197

CDR-H2: SEQ ID NO:198

CDR-H3: SEQ ID NO:199

VL E9.12B CDR Set

CDR-L1: SEQ ID NO:297

CDR-L2: SEQ ID NO:298

CDR-L3: SEQ ID NO:299

VH E9.10E CDR Set

CDR-H1: SEQ ID NO:201

CDR-H2: SEQ ID NO:202

CDR-H3: SEQ ID NO:203

VL E9.10E CDR Set

CDR-L1: SEQ ID NO:285

CDR-L2: SEQ ID NO:286

CDR-L3: SEQ ID NO:287

VH E9.6A CDR Set

CDR-H1: SEQ ID NO:205

CDR-H2: SEQ ID NO:206

CDR-H3: SEQ ID NO:207

VL E9.6A CDR Set

CDR-L1: SEQ ID NO:305

CDR-L2: SEQ ID NO:306

CDR-L3: SEQ ID NO:307

VH E9.7A CDR Set

CDR-H1: SEQ ID NO:209

CDR-H2: SEQ ID NO:210

CDR-H3: SEQ ID NO:211

VL E9.7A CDR Set

CDR-L1: SEQ ID NO:309

CDR-L2: SEQ ID NO:310

CDR-L3: SEQ ID NO:311

VH E9.8H CDR Set

CDR-H1: SEQ ID NO:213

CDR-H2: SEQ ID NO:214

CDR-H3: SEQ ID NO:215

VL E9.8H CDR Set

CDR-L1: SEQ ID NO:313

CDR-L2: SEQ ID NO:314

CDR-L3: SEQ ID NO:315

VH E9.1 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:334

CDR-H2: residues 52-67 of SEQ ID NO:334

CDR-H3: residues 100-110 of SEQ ID NO:334

VL E9.1 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:335

CDR-L2: residues 49-55 of SEQ ID NO:335

CDR-L3: residues 88-96 of SEQ ID NO:335

VH E9-SE1 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:336

CDR-H2: residues 52-67 of SEQ ID NO:336

CDR-H3: residues 100-110 of SEQ ID NO:336

VL E9-SE1 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:337

CDR-L2: residues 49-55 of SEQ ID NO:337

CDR-L3: residues 88-96 of SEQ ID NO:337

VH E9-SE2 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:338

CDR-H2: residues 52-67 of SEQ ID NO:338

CDR-H3: residues 100-110 of SEQ ID NO:338

VL E9-SE2 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:339

CDR-L2: residues 49-55 of SEQ ID NO:339

CDR-L3: residues 88-96 of SEQ ID NO:339

VH E9-SE3 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:340

CDR-H2: residues 52-67 of SEQ ID NO:340

CDR-H3: residues 100-110 of SEQ ID NO:340

VL E9-SE3 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:341

CDR-L2: residues 49-55 of SEQ ID NO:341

CDR-L3: residues 88-96 of SEQ ID NO:341

VH E9-SE4 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:342

CDR-H2: residues 52-67 of SEQ ID NO:342

CDR-H3: residues 100-110 of SEQ ID NO:342

VL E9-SE4 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:343

CDR-L2: residues 49-55 of SEQ ID NO:343

CDR-L3: residues 88-96 of SEQ ID NO:343

VH E9-SE5 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:344

CDR-H2: residues 52-67 of SEQ ID NO:344

CDR-H3: residues 100-110 of SEQ ID NO:344

VL E9-SE5 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:345

CDR-L2: residues 50-56 of SEQ ID NO:345

CDR-L3: residues 89-97 of SEQ ID NO:345

VH E9-SE6 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:346

CDR-H2: residues 52-67 of SEQ ID NO:346

CDR-H3: residues 100-110 of SEQ ID NO:346

VL E9-SE6 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:347

CDR-L2: residues 49-55 of SEQ ID NO:347

CDR-L3: residues 88-96 of SEQ ID NO:347

VH E9-SE7 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:348

CDR-H2: residues 52-67 of SEQ ID NO:348

CDR-H3: residues 100-110 of SEQ ID NO:348

VL E9-SE7 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:349

CDR-L2: residues 50-56 of SEQ ID NO:349

CDR-L3: residues 89-97 of SEQ ID NO:349

VH E9-SE8 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:350

CDR-H2: residues 52-67 of SEQ ID NO:350

CDR-H3: residues 100-110 of SEQ ID NO:350

VL E9-SE8 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:351

CDR-L2: residues 50-56 of SEQ ID NO:351

CDR-L3: residues 89-97 of SEQ ID NO:351

VH E9-FR1 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:352

CDR-H2: residues 52-67 of SEQ ID NO:352

CDR-H3: residues 100-110 of SEQ ID NO:352

VL E9-FR1 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:353

CDR-L2: residues 50-56 of SEQ ID NO:353

CDR-L3: residues 89-97 of SEQ ID NO:353

VH E9-FR2 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:354

CDR-H2: residues 52-67 of SEQ ID NO:354

CDR-H3: residues 100-110 of SEQ ID NO:354

VL E9-FR2 CDR Set

CDR-L1: residues 24-34 of SEQ ID NO:355

CDR-L2: residues 50-56 of SEQ ID NO:355

CDR-L3: residues 89-97 of SEQ ID NO:355

VH E9.71 CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:356

CDR-H2: residues 52-67 of SEQ ID NO:356

CDR-H3: residues 100-110 of SEQ ID NO:356

VL E9.71 CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:357

CDR-L2: residues 49-55 of SEQ ID NO:357

CDR-L3: residues 88-96 of SEQ ID NO:357

VH E9.71 (M) CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:358

CDR-H2: residues 52-67 of SEQ ID NO:358

CDR-H3: residues 100-110 of SEQ ID NO:358

VL E9.71 (M) CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:359

CDR-L2: residues 49-55 of SEQ ID NO:359

CDR-L3: residues 88-96 of SEQ ID NO:359

VH E9.71 (L) CDR Set

CDR-H1: residues 31-37 of SEQ ID NO:360

CDR-H2: residues 52-67 of SEQ ID NO:360

CDR-H3: residues 100-110 of SEQ ID NO:360

VL E9.71 (L) CDR Set

CDR-L1: residues 23-33 of SEQ ID NO:361

CDR-L2: residues 49-55 of SEQ ID NO:361

CDR-L3: residues 88-96 of SEQ ID NO:361

3. The binding protein according to claim 2, comprising at least two variable domain CDR sets.

4. The binding protein according to claim 3, wherein said at least two variable domain CDR sets are selected from a group consisting of:

VH E9 CDR Set and VL E9 CDR Set,

VH E9.4 CDR Set and VL E9.4 CDR Set,

VH E9.11 CDR Set and VL E9.11 CDR Set,

VH E9.14 CDR Set and VL E9.14 CDR Set,

VH E9.17 CDR Set and VL E9.17 CDR Set,

VH E9.18 CDR Set and VL E9.18 CDR Set,

VH E9.19 CDR Set and VL E9.19 CDR Set,

VH E9.22 CDR Set and VL E9.22 CDR Set,

VH E9.48 CDR Set and VL E9.48 CDR Set,

VH E9.65 CDR Set and VL E9.65 CDR Set,
VH E9.66 CDR Set and VL E9.66 CDR Set,
VH E9.71 CDR Set and VL E9.71 CDR Set,
VH E9.13 CDR Set and VL E9.13 CDR Set,
VH E9.16 CDR Set and VL E9.16 CDR Set,
VH E9.38 CDR Set and VL E9.38 CDR Set,
VH E9.2B CDR Set and VL E9.2B CDR Set,
VH E9.1F CDR Set and VL E9.1F CDR Set,
VH E9.10H CDR Set and VL E9.10H CDR Set,
VH E9.5E CDR Set and VL E9.5E CDR Set,
VH E9.10C CDR Set and VL E9.10C CDR Set,
VH E9.7E CDR Set and VL E9.7E CDR Set,
VH E9.12B CDR Set and VL E9.12B CDR Set,
VH E9.10E CDR Set and VL E9.10E CDR Set,
VH E9.6A CDR Set and VL E9.6A CDR Set,
VH E9.7A CDR Set and VL E9.7A CDR Set,
VH E9.8H CDR Set and VL E9.8H CDR Set,
VH E9-SE1 CDR Set and VL E9-SE1 CDR Set,
VH E9-SE2 CDR Set and VL E9-SE2 CDR Set,
VH E9-SE3 CDR Set and VL E9-SE3 CDR Set,
VH E9-SE4 CDR Set and VL E9-SE4 CDR Set,
VH E9-SE5 CDR Set and VL E9-SE5 CDR Set,
VH E9-SE6 CDR Set and VL E9-SE6 CDR Set,
VH E9-SE7 CDR Set and VL E9-SE7 CDR Set,
VH E9-SE8 CDR Set and VL E9-SE8 CDR Set,
VH E9-FR1 CDR Set and VL E9-FR1 CDR Set,
VH E9-FR2 CDR Set and VL E9-FR2 CDR Set,
VH E9.71 CDR Set and VL E9.71 CDR Set,
VH E9.71(M) CDR Set and VL E9.71(M) CDR Set, and
VH E9.71(L) CDR Set and VL E9.71(L) CDR Set.

5. The binding protein according to any one of Claims 1-4, further comprising a human acceptor framework.

6. The binding protein according to claim 4, wherein said human acceptor framework comprises an amino acid sequence selected from the group consisting of:

SEQ ID NO.: 6, SEQ ID NO.: 7, SEQ ID NO.: 8, SEQ ID NO.: 9,

SEQ ID NO.: 10, SEQ ID NO.: 11, SEQ ID NO.: 12, SEQ ID NO.: 13,

SEQ ID NO.: 14, SEQ ID NO.: 15, SEQ ID NO.: 16, SEQ ID NO.: 17,

SEQ ID NO.: 18, SEQ ID NO.: 19 SEQ ID NO.: 20, SEQ ID NO.: 21,

SEQ ID NO.: 22, SEQ ID NO.: 35, SEQ ID NO.: 36, SEQ ID NO.: 37,

SEQ ID NO.: 38, SEQ ID NO.: 39, SEQ ID NO.: 40, SEQ ID NO.: 41,

SEQ ID NO.: 42, SEQ ID NO.: 43, SEQ ID NO.: 44, SEQ ID NO.: 45,

SEQ ID NO.: 46, SEQ ID NO.: 47, SEQ ID NO.: 48, SEQ ID NO.: 49,

SEQ ID NO.: 50, SEQ ID NO.: 51, SEQ ID NO.: 52, SEQ ID NO.: 53,

SEQ ID NO.: 54, SEQ ID NO.: 55, SEQ ID NO.: 56, SEQ ID NO.: 57,

SEQ ID NO.: 58, SEQ ID NO.: 59, SEQ ID NO.: 60, SEQ ID NO.: 61,

SEQ ID NO.: 62,
SEQ ID NO.: 23, SEQ ID NO.: 24, SEQ ID NO.: 25, SEQ ID NO.: 26,

SEQ ID NO.: 27, SEQ ID NO.: 28, SEQ ID NO.: 29, SEQ ID NO.: 30,

SEQ ID NO.: 31, SEQ ID NO.: 32, SEQ ID NO.: 33, SEQ ID NO.: 34,

SEQ ID NO.: 63, SEQ ID NO.: 64, SEQ ID NO.: 65, SEQ ID NO.: 66,

SEQ ID NO.: 67, SEQ ID NO.: 68, SEQ ID NO.: 69, SEQ ID NO.: 70,

SEQ ID NO.: 71, SEQ ID NO.: 72, SEQ ID NO.: 73, SEQ ID NO.: 74,

SEQ ID NO.: 75, SEQ ID NO.: 76, SEQ ID NO.: 77, SEQ ID NO.: 78,

SEQ ID NO.: 79, SEQ ID NO.: 80, SEQ ID NO.: 81, SEQ ID NO.: 82,

SEQ ID NO.: 83, SEQ ID NO.: 84, SEQ ID NO.: 85, SEQ ID NO.: 86,

SEQ ID NO.: 87, SEQ ID NO.: 88, SEQ ID NO.: 89, SEQ ID NO.: 90,

SEQ ID NO.: 91, SEQ ID NO.: 92, SEQ ID NO.: 93, SEQ ID NO.: 94,

SEQ ID NO.: 95, SEQ ID NO.: 96, SEQ ID NO.: 97, and SEQ ID NO.: 98.

7. The binding protein according to claim 6, wherein said human acceptor framework 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 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.

8. The binding protein according to claim 7, wherein said key residue is selected from the group consisting of: 2H, 4H, 24H, 26H, 27H, 29H, 34H, 35H, 37H, 39H, 44H, 45H, 47H, 48H, 49H, 50H, 51H, 58H, 59H, 60H, 63H, 67H, 69H, 71H, 73H, 76H, 78H, 91H, 93H, 94H, 2L, 4L, 25L, 29L, 27bL, 33L, 34L, 36L, 38L, 43L, 44L, 46L, 47L, 48L, 49L, 55L, 58L, 62L, 64L, 71L, 87L, 89L, 90L, 91L, 94L, and 95L.

9. The binding protein according to claim 1, wherein said binding protein comprises at least one variable domain having amino acid sequence selected from the group consisting of:

SEQ ID NOS:1, 111, 116, 228, 120, 232, 124, 236, 128, 240, 132, 244, 136, 248, 140, 252, 144, 256, 148, 260, 152, 264, 156, 268, 160, 216, 164, 220, 168, 224, 172, 272, 176, 276, 180, 300, 184, 292, 188, 280, 192, 288, 196, 296, 200, 284, 204, 304,

208, 308, 212, 312, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, and 361.

10. The binding protein according to claim 9, 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 NOS:1 and 111, SEQ ID NOS:116 and 228, SEQ ID NOS:120 and 232, SEQ ID NOS:124 and 236, SEQ ID NOS:128 and 240, SEQ ID NOS:132 and 244, SEQ ID NOS:136 and 248, SEQ ID NOS:140 and 252, SEQ ID NOS:144 and 256, SEQ ID NOS:148 and 260, SEQ ID NOS:152 and 264, SEQ ID NOS:156 and 268, SEQ ID NOS: 160 and 216, SEQ ID NOS: 164 and 220, SEQ ID NOS:168 and 224, SEQ ID NOS:172 and 272, SEQ ID NOS:176 and 276, SEQ ID NOS:180 and 300, SEQ ID NOS:184 and 292, SEQ ID NOS:188 and 280, SEQ ID NOS:192 and 288, SEQ ID NOS:196 and 296, SEQ ID NOS:200 and 284, SEQ ID NOS:204 and 304, SEQ ID NOS:208 and 308, SEQ ID NOS:212 and 312, SEQ ID NOS:334 and 335, SEQ ID NOS:336 and 337, SEQ ID NOS:338 and 339, SEQ ID NOS:340 and 341, SEQ ID NOS:342 and 343, SEQ ID NOS:344 and 345, SEQ ID NOS:346 and 347, SEQ ID NOS:348 and 349, SEQ ID NOS:350 and 351, SEQ ID NOS:352 and 353, SEQ ID NOS:354 and 355, SEQ ID NOS:356 and 357, SEQ ID NOS:358 and 359, SEQ ID NOS:360 and 361.

11. The binding protein according to claim 8, wherein said binding protein comprises at least one variable domain having an amino acid sequence selected from the group consisting of: SEQ ID NOS:1, 111, 116, 228, 120, 232, 124, 236, 128, 240, 132, 244, 136, 248, 140, 252, 144, 256, 148, 260, 152, 264, 156, 268, 160, 216, 164, 220, 168, 224, 172, 272, 176, 276, 180, 300, 184, 292, 188, 280, 192, 288, 196, 296, 200, 284, 204, 304, 208, 308, 212, 312, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, and 361.

12. The binding protein according to claim 1, further comprising 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.

13. The binding protein according to claim 9, wherein said heavy chain immunoglobulin constant domain is a human IgG1 constant domain, said human IgG1 constant

domain comprising amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3.

14. The binding protein according to claim 1, further comprising a light chain immunoglobulin constant domain, wherein said light chain immunoglobulin constant domain is a human Ig kappa constant domain comprising amino acid sequence SEQ ID NO:4.

15. The binding protein according to claim 1, further comprising a light chain immunoglobulin constant domain, wherein said light chain immunoglobulin constant domain is a human Ig lambda constant domain comprising amino acid sequence SEQ ID NO:5.

16. The binding protein according to Claim 1 wherein said binding protein is selected from the group consisting of: an immunoglobulin molecule, an scFv, a monoclonal antibody, a human antibody, a chimeric antibody, a humanized antibody, a single domain antibody, a Fab fragment, a Fab' fragment, an $F(ab')_2$, an Fv, a disulfide linked Fv, a single domain antibody, a diabody, a multispecific antibody, a bispecific antibody, and a dual specific antibody.

17. The binding protein according to claim 16, wherein said binding protein is a human antibody.

18. A binding protein capable of binding human DLL-4, said binding protein comprising:
an Ig constant heavy region having an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:3;
an Ig constant light region having an amino acid sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:5;
an Ig variable heavy region having an amino acid sequence selected from the group consisting:
SEQ ID NOS:1, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176, 180, 184, 188, 192, 196, 200, 204, 208, 212, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, and 360; and
an Ig variable light region having an amino acid sequence selected from the group consisting:

SEQ ID NOS:111, 228, 232, 236, 240, 244, 248, 252, 256, 260, 264, 268, 216, 220, 224, 272, 276, 300, 292, 280, 288, 296, 284, 304, 308, 312, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, and 361.

19. The binding protein according to Claim 18, wherein the Ig constant light region is SEQ ID NO:5.

20. The binding protein according to any one of Claims 1 to 19, 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.

21. The binding protein according to Claim 20, wherein the binding protein is capable of blocking DLL4 interaction with Notch-1 and Notch-4.

22. The binding protein according to any one of Claims 1 to 21, wherein the binding protein is capable of modulating a biological function of DLL4.

23. The binding protein according to any one of Claims 1 to 21, wherein said binding protein is capable of neutralizing a DLL4.

24. The binding protein according to claim 23, wherein said DLL4 is selected from the group consisting of: human DLL4, mouse DLL4, cynomolgus DLL4, and rat DLL4.

25. The binding protein according to claim 23, wherein said binding protein is capable of reducing normal angiogenesis.

26. The binding protein according to claim 23, wherein said binding protein has a dissociation constant (K_D) 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.

27. The binding protein according to claim 23, wherein said binding protein has an on rate 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 $10^6M^{-1}s^{-1}$.

28. The binding protein according to claim 23, wherein said binding protein has an off rate 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 $10^{-6}s^{-1}$.

29. A labeled binding protein comprising a binding protein of Claim 16, wherein said binding protein is conjugated to a detectable label.

30. An antibody construct comprising a binding protein described in Claim 1 and further comprising a linker polypeptide or an immunoglobulin constant domain.

31. The antibody construct according to claim 30, 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 $F(ab')_2$,
a Fv,
a disulfide linked Fv,
a scFv,
a single domain antibody,
a diabody,
a multispecific antibody,
a dual specific antibody, and
a bispecific antibody.

32. The antibody construct according to claim 30, 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,

a human IgA constant domain,
and an IgG constant domain variant with one or more mutations altering binding strength to Fc neonatal receptor, Fc gamma receptors, or C1q.

33. An antibody conjugate comprising an antibody construct as described in Claim 30, wherein said antibody construct is conjugated to a therapeutic or cytotoxic agent.

34. The antibody conjugate of Claim 33, wherein said 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.

35. 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 CDR-H1, a CDR-H2, or a CDR-H3 as described in Claim 1; a polypeptide comprising a light chain variable domain, wherein the light chain variable domain comprises one or more of CDR-L1, a CDR-L2, or a CDR-L3 as described in Claim 1; or a combination of both polypeptides.

36. A vector comprising an isolated nucleic acid according to Claim 35.

37. A host cell comprising a vector according to Claim 36, wherein said cell is not a transformed host cell within a human.

38. The host cell according to claim 37, wherein said host cell is a eukaryotic cell selected from the group consisting of: a protist cell, an animal cell, a plant cell, and a fungal cell.

39. The host cell according to claim 38, wherein said eukaryotic cell is an animal cell selected from the group consisting of: a mammalian cell, an avian cell, and an insect cell.

40. The host cell according to claim 39, wherein said host cell is a CHO cell, a COS, a Sf9 cell, or a yeast cell.

41. A method of producing a binding protein that binds human DLL4, comprising culturing the host cell of Claim 37 in a culture medium under conditions sufficient to produce a binding protein that binds human DLL4.

42. A binding protein produced according to the method of claim 41.

43. A crystallized binding protein comprising a binding protein according to Claim 1, wherein said binding protein exists as a crystal.

44. A pharmaceutical composition comprising the binding protein of any one of Claims 1 to 19 or 42, and a pharmaceutically acceptable carrier, and optionally comprising an additional therapeutic agent.

45. The pharmaceutical composition of claim 44, wherein said additional agent is selected from the group consisting of: angiogenesis inhibitors; kinase inhibitors; co-stimulation molecule blockers; adhesion molecule blockers; anti-cytokine antibody or functional fragment thereof; methotrexate; corticosteroids; cyclosporine; rapamycin; FK506; and non-steroidal anti-inflammatory agents.

46. Use of the binding protein of any one of Claims 1 to 19, or 42, in the manufacture of a pharmaceutical composition for reducing human DLL4 activity.

47. Use of the binding protein of any one of Claims 1 to 19, or 42, in the manufacture of a pharmaceutical composition for reducing human DLL4 activity in a human subject suffering from a disorder in which DLL4 activity is detrimental.

48. The use of Claim 47, 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, hemangiomas, melanomas, sarcomas, brain tumor, nerve cancer, eye tumor, meninges cancer, solid tumors from hematopoietic malignancies, tumor metastases, ocular neovascularization, edema, rheumatoid arthritis, multiple sclerosis, atherosclerotic plaques, Crohn's disease, inflammatory bowel disease, refractory ascites, psoriasis, sarcoidosis, arterial arteriosclerosis, sepsis, peptic ulcers, burns, and pancreatitis, polycystic ovarian disease (POD), endometriosis,

uterine fibroids, benign prostate hypertrophy, and other angiogenesis independent and dependent diseases characterized by abberant DLL4 activity.

49. Use of the binding protein of any one of Claims 1 to 19, or 42, in the manufacture of a pharmaceutical composition for treating a patient suffering from a disorder in which DLL4 is detrimental, wherein said composition is for administration before, concurrent with, or after the administration 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; corticosteroids, cyclosporine, rapamycin, FK506, and non-steroidal anti-inflammatory agents.