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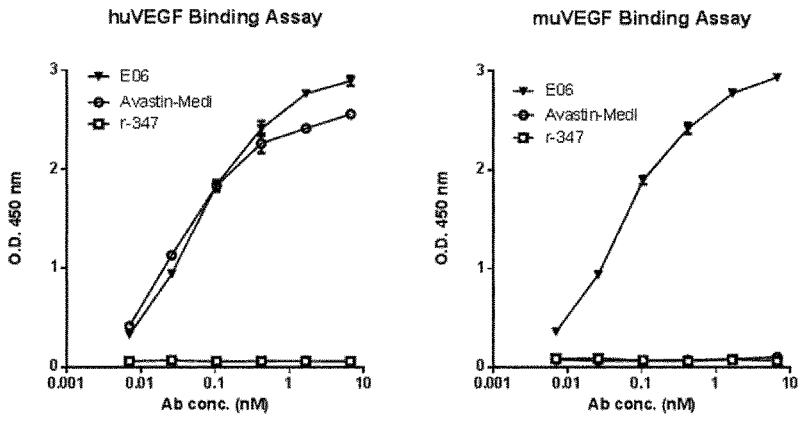
(54) Title: ANTI-VEGF-A ANTIBODIES AND USES THEREOF

Figure 1



(57) Abstract: The present invention relates to antibodies having activity against a vascular endothelial growth factor (VEGF), and methods of making and using such antibodies.

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ANTI-VEGF-A ANTIBODIES AND USES THEREOF

Field of the Invention

The invention relates to antibodies having activity against vascular endothelial growth factor (VEGF) and uses of such antibodies.

Background to the Invention

Angiogenesis, the formation of new blood vessels from existing vasculature, is a complex biological process required for the formation and physiological functions of virtually all the organs. It is an essential element of embryogenesis, normal physiological growth, repair and pathological processes such as tumour expansion. Normally, angiogenesis is tightly regulated by the local balance of angiogenic and angiostatic factors in a multi-step process involving vessel sprouting, branching and tubule formation by endothelial cells (involving processes such as activation of endothelial cells (ECs), vessel destabilisation, synthesis and release of degradative enzymes, EC migration, EC proliferation, EC organisation and differentiation and vessel maturation).

In the adult, physiological angiogenesis is largely confined to wound healing and several components of female reproductive function and embryonic development. In disease-related angiogenesis which includes any abnormal, undesirable or pathological angiogenesis, the local balance between angiogenic and angiostatic factors is dysregulated leading to inappropriate and/or structurally abnormal blood vessel formation. Pathological angiogenesis has been associated with disease states including diabetic retinopathy, psoriasis, cancer, rheumatoid arthritis, atheroma, Kaposi's sarcoma and haemangioma (Fan et al, 1995, Trends Pharmacology. Science. 16: 57-66; Folkman, 1995, Nature Medicine 1: 27-31). In cancer, growth of primary and secondary tumours beyond 1-2 mm3 requires angiogenesis (Folkman, J. New England Journal of Medicine 1995; 33, 1757-1763).

VEGF is a potent and ubiquitous vascular growth factor. Prior to identification of the role of VEGF as a secreted mitogen for endothelial cells, it was identified as a vascular permeability factor, highlighting VEGF's ability to control many distinct aspects of endothelial cell behaviour, including proliferation, migration, specialization and survival (Ruhrberg, 2003 BioEssays 25:1052-1060). VEGF-A was the first member of the VEGF family of structurally related dimeric

glycoproteins belonging to the platelet-derived growth factor superfamily to be identified. Beside the founding member, VEGF-A, the VEGF family includes VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor (PIGF) and endocrine gland-derived VEGF (EG-VEGF). Active forms of VEGF are synthesised either as homodimers or heterodimers with other VEGF family members. Human VEGF-A exists in six isoforms generated by alternative splicing: VEGF121, VEGF145, VEGF165, VEGF183, VEGF189 and VEGF206. These isoforms differ primarily in their bioavailability, with VEGF165 being the predominant isoform (Podar, et al. 2005 Blood 105(4):1383-1395) but with the others also having biological activity. The regulation of splicing during embryogenesis to produce stage- and tissue-specific ratios of the various isoforms creates rich potential for distinct and context dependent behavior of endothelial cells in response to VEGF.

VEGF is believed to be an important stimulator of both normal and disease-related angiogenesis (Jakeman, et al. 1993 Endocrinology: 133,848-859; Kolch, et al. 1995 Breast Cancer Research and Treatment: 36,139-155) and vascular permeability (Connolly, et al. 1989 J. Biol. Chem: 264,20017-20024). Antagonism of VEGF action by sequestration of VEGF with antibodies can result in reduction of tumor growth (Kim, et al. 1993 Nature: 362,841-844). Heterozygous disruption of the VEGF gene resulted in fatal deficiencies in vascularisation (Carmeliet, et al. 1996 Nature 380:435-439; Ferrara, et al. 1996 Nature 380:439-442).

There is at least one commercially marketed anti-VEGF-A antibody, which is Avastin®. However, there are serious, sometimes fatal, toxicities associated with its use, including non-gastrointestinal fistulas, thromboembolic events, hypertension, reversible posterior leukoencephalopathy syndrome, etc. As such, there is an unmet need at least as it relates to improving the safety associated with targeting VEGF-A. To this end, the antibodies of the invention have binding characteristics that support such an improvement over the art, including the ability of these antibodies to differentially bind VEGF-A isoforms.

Summary of the Invention

The invention relates to binding molecules, including antibodies, that bind to VEGF-A. The invention further relates to binding molecules, including antibodies, that bind to one or more VEGF-A isoforms with greater affinity when compared to one or more other VEGF-A isoforms. The invention also relates to binding molecules, including antibodies, that bind to VEGF-A and reduce the activity of at least one biological activity of VEGF-A.

In one aspect, the invention provides an antibody comprising heavy chain complementarity determining regions 1 – 3 (HCDR1, HCDR2, and HCDR3) and light chain complementarity determining regions 1 – 3 (LCDR1, LCDR2, and LCDR3), wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively, wherein the antibody comprises a heavy chain and a light chain comprising SEQ ID NOs: 73 and 77, respectively.

Brief Description of the Drawings

Figure 1. Representative data demonstrating binding to human VEGF165 and mouse VEGF164.

Figure 2. Representative data demonstrating lack of binding to VEGF121.

Figure 3. Sequence alignment of clone E06 and the most sequence homologous germline genes.

Figure 4. Representative data demonstrating improved binding of affinity optimized variants.

Figure 5. Representative data demonstrating improved binding of affinity optimized variants as Fabs and IgGs. Fabs are shown in the top two graphs. IgGs are shown in the bottom two graphs.

Figure 6. Representative data demonstrating binding of affinity optimized variants to murine VEGF164.

Figure 7. Representative data demonstrating lack of binding of affinity optimized variants to VEGF121.

Figure 8 A – B. Representative data demonstrating activity of affinity optimized variants in functional cell-based assays.

Figure 9. Representative data demonstrating activity of an affinity optimized variant in a retinal vasculogenesis model.

Detailed Description

Definitions

Before describing the present invention in detail, it is to be understood that this invention is not limited to specific compositions or process steps, as such can vary. As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein. Further it is understood that

wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

As used herein, the term "binding molecule" refers to a molecule that is capable of binding to a target molecule or antigen in a manner similar to that of an antibody binding to an antigen. Examples of binding molecules include full-length antibodies and antigen-binding fragments. Examples of "antigen-binding fragments" of an antibody include (i) a Fab fragment, a monovalent fragment that includes a VL, VH, CL and CH1 domain of an antibody; (ii) a F(ab')2 fragment, a bivalent fragment that includes two Fab fragments linked by a disulfide bridge at a hinge region; (iii) a Fd fragment that includes the VH and CH1 domains; (iv) a Fv fragment that includes VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which includes a VH domain; and (vi) an isolated complementarity determining region (CDR). Antigen-binding fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins. In one embodiment, the antigen-binding fragment includes a single chain antibody, including, for example, a "single-chain variable fragment" or "scFv." scFv refers to a fusion protein that includes at least one variable region of a heavy chain (VH) and at least one variable region of a light chain (VL) of an immunoglobulin. These single chain antibody fragments can be obtained using conventional techniques known to those with skill in the art. For example, the VH and VL domains of a Fv fragment, which are encoded by separate genes, can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single polypeptide chain in which the VH and VL regions pair to form a monovalent molecule (See, Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883).

Complementarity determining regions (CDRs) are responsible for antibody binding to its antigen. CDRs are determined by a number of methods in the art (including Kabat (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)); Chothia (Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)); IMGT (ImMunoGeneTics) (Lefranc, M.P. et al., Dev. Comp. Immunol. 27: 55-77 (2003)); and other methods). Although specific CDR sequences are mentioned and claimed herein, the invention also encompasses CDR sequences defined by any method known in the art.

As use herein, the term "subject" refers to any member of the subphylum cordata, including, without limitation, humans and other primates, including non-human primates such as

chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like are also non-limiting examples.

Binding Molecules

A binding molecule can include a full length or intact antibody, an antibody fragment, including an antigen binding fragment, a human, humanized, post-translationally modified, chimeric or fusion antibody, immunoconjugate, or a functional fragment thereof.

Suitable immunoglobulin molecules or portions thereof of the invention (i.e., binding molecules) can be or are derived from any isotype (e.g., IgG, IgE, IgM, IgD, IgA and IgY), sub-isotype (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or allotype (e.g., Gm, e.g., G1m(f, z, a or x), G2m(n), G3m(g, b, or c), Am, Em, and Km(1, 2 or 3)). Immunoglobulin molecules can include light chains classified as either lambda chains or kappa chains based on the amino acid sequence of the light chain constant region.

Production of Binding Molecules

Recombinant DNA methods for producing and screening for polypeptides, such as the binding molecules described herein, are known in the art (e.g. U.S. Patent No. 4,816,567). DNA encoding the binding molecules or fragments thereof, for example, DNA encoding a VH domain, a VL domain, an scFv, or combinations thereof can be inserted into a suitable expression vector, which can then be transfected into a suitable host cell, such as E. coli cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce an antibody protein, to obtain the binding molecule.

Suitable expression vectors are known in the art. An expression vector can contain a polynucleotide that encodes an antibody linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., U.S. Patent Nos. 5,981,216; 5,591,639; 5,658,759 and 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains. The expression vector can be transferred to a host cell by

conventional techniques and the transfected cells can be cultured by conventional techniques to produce the binding molecule.

Mammalian cell lines suitable as hosts for expression of recombinant antibodies are known in the art and include many immortalized cell lines available from the American Type Culture Collection, including but not limit to CHO cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), human epithelial kidney 293 cells, and a number of other cell lines. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the binding molecule. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT2O and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any functional immunoglobulin chains), SP20, CRL7O3O and HsS78Bst cells. Human cell lines developed by immortalizing human lymphocytes can be used to recombinantly produce monoclonal antibodies. The human cell line PER.C6®. (Crucell, Netherlands) can be used to recombinantly produce monoclonal antibodies. Additional cell lines which may be used as hosts for expression of recombinant antibodies include insect cells (e.g. Sf21/Sf9, Trichoplusia ni Bti-Tn5b1-4) or yeast cells (e.g. S. cerevisiae, Pichia, US7326681; etc.), plants cells (US20080066200); and chicken cells (WO2008142124).

Antibodies can be stably expressed in a cell line using methods known in the art. Stable expression can be used for long-term, high-yield production of recombinant proteins. For stable expression, host cells can be transformed with an appropriately engineered vector that includes expression control elements (e.g., promoter, enhancer, transcription terminators, polyadenylation sites, etc.), and a selectable marker gene. Following the introduction of the foreign DNA, cells are allowed to grow for 1-2 days in an enriched media, and are then switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells that have stably integrated the plasmid into their chromosomes to grow and form foci which in turn can be cloned and expanded into cell lines. Methods for producing stable cell lines with a high yield are known in the art and reagents are generally available commercially. Transient

expression can also be carried out by using methods known in the art. Transient transfection is a process in which the nucleic acid introduced into a cell does not integrate into the genome or chromosomal DNA of that cell and is maintained as an extra-chromosomal element in the cell (e.g., as an episome).

A cell line, either stable or transiently transfected, is maintained in cell culture medium and conditions known in the art resulting in the expression and production of the binding molecule. Cell culture media can be based on commercially available media formulations, including, for example, DMEM or Ham's F12. In addition, the cell culture media can be modified to support increases in both cell growth and biologic protein expression. As used herein, the terms "cell culture medium," "culture medium," and "medium formulation" refer to a nutritive solution for the maintenance, growth, propagation, or expansion of cells in an artificial in vitro environment outside of a multicellular organism or tissue. Cell culture medium may be optimized for a specific cell culture use, including cell culture growth medium which is formulated to promote cellular growth or cell culture production medium which is formulated to promote recombinant protein production. The terms nutrient, ingredient, and component are used interchangeably herein to refer to the constituents that make up a cell culture medium. Cell lines can be maintained using a fed batch method. As used herein, "fed batch method," refers to a method by which a cell culture is supplied with additional nutrients after first being incubated with a basal medium. For example, a fed batch method may include adding supplemental media according to a determined feeding schedule within a given time period. Thus, a "fed batch cell culture" refers to a cell culture wherein the cells, typically mammalian, and culture medium are supplied to the culturing vessel initially and additional culture nutrients are fed, continuously or in discrete increments, to the culture during culturing, with or without periodic cell and/or product harvest before termination of culture.

Cell culture media and the nutrients contained therein are known to one of skilled in the art. The cell culture medium may include a basal medium and at least one hydrolysate, e.g., soy-based hydrolysate, a yeast-based hydrolysate, or a combination of the two types of hydrolysates resulting in a modified basal medium. The additional nutrients may include only a basal medium, such as a concentrated basal medium, or may include only hydrolysates, or concentrated hydrolysates. Suitable basal media include, but are not limited to Dulbecco's Modified Eagle's Medium (DMEM), DME/F12, Minimal Essential Medium (MEM), Basal Medium Eagle (BME), RPMI 1640, F-10, F-12, α-Minimal Essential Medium (α-MEM), Glasgow's Minimal Essential

Medium (G-MEM), PF CHO (see, e.g., CHO protein free medium (Sigma) or EX-CELL™ 325 PF CHO Serum-Free Medium for CHO Cells Protein-Free (SAFC Bioscience), and Iscove's Modified Dulbecco's Medium. Other examples of basal media which may be used in the invention include BME Basal Medium (Gibco-Invitrogen; see also Eagle, H (1965) Proc. Soc. Exp. Biol. Med. 89, 36); Dulbecco's Modified Eagle Medium (DMEM, powder) (Gibco-Invitrogen (# 31600); see also Dulbecco and Freeman (1959) Virology. 8:396; Smith et al. (1960) Virology. 12:185. Tissue Culture Standards Committee, In Vitro 6:2, 93); CMRL 1066 Medium (Gibco-Invitrogen (#11530); see also Parker et al. (1957) Special Publications, N.Y. Academy of Sciences, 5:303).

The basal medium may be serum-free, meaning that the medium contains no serum (e.g., fetal bovine serum (FBS), horse serum, goat serum, or any other animal-derived serum known to one skilled in the art) or animal protein free media or chemically defined media.

The basal medium may be modified in order to remove certain non-nutritional components found in standard basal medium, such as various inorganic and organic buffers, surfactant(s), and sodium chloride. Removing such components from basal cell medium allows an increased concentration of the remaining nutritional components, and may improve overall cell growth and protein expression. In addition, omitted components may be added back into the cell culture medium containing the modified basal cell medium according to the requirements of the cell culture conditions. The cell culture medium may contain a modified basal cell medium, and at least one of the following nutrients, an iron source, a recombinant growth factor; a buffer; a surfactant; an osmolarity regulator; an energy source; and non-animal hydrolysates. In addition, the modified basal cell medium may optionally contain amino acids, vitamins, or a combination of both amino acids and vitamins. A modified basal medium may further contain glutamine, e.g, L-glutamine, and/or methotrexate.

Purification and Isolation

Once a binding molecule has been produced, it may be purified by methods known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigens Protein A or Protein G, and sizing column chromatography), centrifugation, differential solubility, or by any other standard

technique for the purification of proteins. Further, the binding molecules of the invention may be fused to heterologous polypeptide sequences (referred to herein as "tags") to facilitate purification.

Uses

Binding molecules of the invention can be used in a number of ways. For example, antibodies of the invention can be used to bind to VEGF-A and thereby reduce at least one biological activity of VEGF-A. More particularly, the antibodies of the invention can be used to bind to VEGF-165 and thereby reduce at least one biological activity of VEGF-165, which may include a reduction in activation or phosphorylation of its receptor, a reduction in angiogenesis in connection with cellular dysregulation, a reduction in tumor growth, a reduction in tumor volume, and/or reduction in tumor growth and tumor volume.

Exemplary Embodiments

An embodiment of the invention relates to a binding molecule comprising heavy chain complementarity determining regions 1 – 3 (i.e., HCDR1, HCDR2, and HCDR3) and light chain complementarity determining regions 1 – 3 (i.e., LCDR1, LCDR2, and LCDR3) of an antibody described herein.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF189.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121 and VEGF189.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding

molecule reduces human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces angiogenesis.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces tumor growth, reduces tumor volume, or reduces tumor growth and tumor volume as a result of being provided to a subject having a tumor.

Another embodiment relates to a binding molecule comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule has one or more or any combination of the characteristics described herein, including binding VEGF165, binding VEGF165 with greater affinity compared to VEGF121, binding VEGF165 with greater affinity compared to VEGF189, binding VEGF165 with greater affinity compared to VEGF121 and VEGF189, reducing human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation, reducing angiogenesis, or reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume as a result of being provided to a subject having a tumor.

An embodiment of the invention relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and a light chain variable domain comprising

LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF189.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121 and VEGF189.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and comprising a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces angiogenesis.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and comprising a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces tumor growth, reduces tumor volume, or reduces tumor growth and tumor volume as a result of being provided to a subject having a tumor.

Another embodiment relates to a binding molecule comprising a heavy chain variable domain comprising HCDR1, HCDR2, and HCDR3 and comprising a light chain variable domain comprising LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule has one or more or any combination of the characteristics described herein, including binding VEGF165, binding VEGF165 with greater affinity compared to VEGF121, binding VEGF165 with greater affinity compared to VEGF189, binding VEGF165 with greater affinity compared to VEGF121 and VEGF189, reducing human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation, reducing angiogenesis, or reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume as a result of being provided to a subject having a tumor.

An embodiment of the invention relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF189.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121 and VEGF189.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces angiogenesis.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces tumor growth, reduces tumor volume, or reduces tumor growth and tumor volume as a result of being provided to a subject having a tumor.

Another embodiment relates to a binding molecule comprising a full-length antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody

described herein, wherein the binding molecule has one or more or any combination of the characteristics described herein, including binding VEGF165, binding VEGF165 with greater affinity compared to VEGF121, binding VEGF165 with greater affinity compared to VEGF189, binding VEGF165 with greater affinity compared to VEGF121 and VEGF189, reducing human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation, reducing angiogenesis, or reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume as a result of being provided to a subject having a tumor.

An embodiment of the invention relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF189.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121 and VEGF189.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces angiogenesis.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces tumor growth, reduces tumor volume, or reduces tumor growth and tumor volume as a result of being provided to a subject having a tumor.

Another embodiment relates to a binding molecule comprising a full-length IgG1 antibody comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule has one or more or any combination of the characteristics described herein, including binding VEGF165, binding VEGF165 with greater affinity compared to VEGF121, binding VEGF165 with greater affinity compared to VEGF189, binding VEGF165 with greater affinity compared to VEGF121 and VEGF189, reducing human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation, reducing angiogenesis, or reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume as a result of being provided to a subject having a tumor.

An embodiment of the invention relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1,

LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF189.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule binds VEGF165 with greater affinity compared to VEGF121 and VEGF189.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces angiogenesis.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule reduces tumor growth, reduces tumor volume, or reduces tumor growth and tumor volume as a result of being provided to a subject having a tumor.

Another embodiment relates to a binding molecule which is a full-length antibody, including a full-length IgG1 antibody, comprising HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 of an antibody described herein, wherein the binding molecule has one or more or any combination of the characteristics described herein, including binding VEGF165, binding VEGF165 with greater affinity compared to VEGF121, binding VEGF165 with greater affinity compared to VEGF189, binding VEGF165 with greater affinity compared to VEGF121 and VEGF189, reducing human VEGFR2 phosphorylation, murine VEGFR2 phosphorylation, or both human and murine VEGFR2 phosphorylation, reducing angiogenesis, or reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume as a result of being provided to a subject having a tumor.

In a specific embodiment, there is an antibody comprising an HCDR1, HCDR2, and HCDR3 and an LCDR1, LCDR2, and LCDR3, wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively.

In another specific embodiment, there is an antibody comprising a heavy chain and a light chain comprising SEQ ID NOs: 73 and 77, respectively.

In another specific embodiment, there is an antibody comprising a heavy chain amino acid sequence comprising SEQ ID NO: 71 and a light chain amino acid sequence comprising SEQ ID NO: 75.

In another specific embodiment, there is an antibody comprising an HCDR1, HCDR2, and HCDR3 and an LCDR1, LCDR2, and LCDR3, wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively, and wherein the antibody is a monoclonal antibody.

In another specific embodiment, there is a nucleic acid sequence comprising polynucleotides encoding an antibody comprising an HCDR1, HCDR2, and HCDR3 and an LCDR1, LCDR2, and LCDR3, wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively.

In another specific embodiment, there is a vector comprising polynucleotides encoding an antibody comprising an HCDR1, HCDR2, and HCDR3 and an LCDR1, LCDR2, and LCDR3, wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively.

In another specific embodiment, there is a cell comprising a vector comprising polynucleotides encoding an antibody comprising an HCDR1, HCDR2, and HCDR3 and an LCDR1, LCDR2, and LCDR3, wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively.

In another specific embodiment, there is a method of making an antibody comprising culturing a cell comprising a vector comprising polynucleotides encoding an antibody comprising an HCDR1, HCDR2, and HCDR3 and an LCDR1, LCDR2, and LCDR3, wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively.

In another specific embodiment, there is a method of reducing angiogenesis comprising providing an antibody to a subject wherein the antibody comprises an HCDR1, HCDR2, and

HCDR3 and an LCDR1, LCDR2, and LCDR3, wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively.

Sequences

SEQ ID NO	SEQUENCE	DESCRIPTION
1	EVQLLESGGGLVQPGGSLRLSCAASGFTFSWYEMYWVRQA PGKGLEWVSSISPSGGWTMYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCATPLYSSDGLSAGDIWGQGTMVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK	Amino acid sequence of the heavy chain of E06
2	GAGGTGCAGCTGCTGGAGAGCGGCGGCGGCCTGG TGCAGCCCGGCGGCAGCCTGAGGCTGAGCTGCGCCGCCA GCGGCTTCACCTTCAGCTGGTACGAGATGTACTGGGTGA GGCAGGCCCCCGGCAAGGGCCTGGAGTGGGTGAGCAGCA TCAGCCCCAGCGGCGGCTGGACCATGTACGCCGACAGCG TGAAGGGCAGGTTCACCATCAGCAGGGACAACAGCAAGA ACACCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAGG ACACCGCCGTGTACTACTGCGCCACCCCCCTGTACAGCAG CGACGGCCTGAGCGCCGGCGACATCTGGGGCCAGGGCAC CATGGTGACCGTGAGCAGCGCCAGCACCAAGGGCCCCAG CGTGTTCCCCCTGGCCCCCAGCAGCAAGAGCACCAGCGG CGGCACCGCCGCCCTGGGCTGCCTGGTGAAGGACTACTT CCCCGAGCCCGTGACCGTGAGCTGGAACAGCGGCGCCCT GACCAGCGGCGTGCACACCTTCCCCGCCGTGCTGCAGAG CAGCGGCCTGTACAGCCTGAGCAGCGTGGTGACCGTGCC CAGCAGCAGCCTGGGCACCCAGACCTACATCTGCAACGT GAACCACAAGCCCAGCAACACCAAGGTGGACAAGAGGG TGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCCC CCTGCCCCGCCCCCGAGCTGCTGGGCGGCCCCAGCGTGTT CCTGTTCCCCCCCAAGCCCAAGGACACCCTGATGATCAGC AGGACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGAGC CACGAGGACCCCGAGGTGAAGTTCAACTGGTACGTGGAC GGCGTGGAGGTGCACAACGCCAAGACCAAGCCCAGGGA GGAGCAGTACAACAGCACCTACAGGGTGGTGAGCGTGCT GACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTA CAAGTGCAAGGTGAGCAACAAGGCCCTGCCCGCCCCCAT CGAGAAGACCATCAGCAAGGCCAAGGGCCAGCCCAGGG AGCCCCAGGTGTACACCCTGCCCCCCAGCAGGGAGGAGA TGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTGAAGG GCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCA ACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCCG TGCTGGACAGCGACGGCAGCTTCTTCCTGTACAGCAAGCT GACCGTGGACAAGAGCAGGTGGCAGCAGGGCAACGTGTT	Nucleotide sequence of the heavy chain of E06

SEQ ID NO	SEQUENCE	DESCRIPTION
	CAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTA CACCCAGAAGAGCCTGAGCCTGAGCCCCGGCAAG	
3	EVQLLESGGGLVQPGGSLRLSCAASGFTFSWYEMYWVRQA PGKGLEWVSSISPSGGWTMYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCATPLYSSDGLSAGDIWGQGTMVTVS S	Amino acid sequence of the heavy chain variable domain of E06
4	GAGGTGCAGCTGCTGGAGAGCGGCGGCGGCCTGGTGCAG CCCGGCGGCAGCCTGAGGCTGAGCTGCGCCGCCAGCGGC TTCACCTTCAGCTGGTACGAGATGTACTGGGTGAGGCAG GCCCCCGGCAAGGGCCTGGAGTGGGTGAGCAGCATCAGC CCCAGCGGCGGCTGGACCATGTACGCCGACAGCGTGAAG GGCAGGTTCACCATCAGCAGGGACAACAGCAAGAACACC CTGTACCTGCAGATGAACAGCCTGAGGGCCGAGGACACC GCCGTGTACTACTGCGCCACCCCCCTGTACAGCAGCGAC GGCCTGAGCGCCGGCGACATCTGGGGCCAGGGCACCATG GTGACCGTGAGCAGC	Nucleotide sequence of the heavy chain variable domain of E06
5	DIQMTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKP GQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF ATYYCQQSYSTPSFGQGTRLEITRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of E06
6	GACATCCAGATGACCCAGAGCCCCGCCACCCTGA GCCTGAGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGG CCAGCCAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACC AGCAGAAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACG GCGCCAGCAGCAGGGCCACCGGCATCCCCGACAGGTTCA GCGGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCA GCAGGCTGGAGCCCGAGGACTTCGCCACCTACTACTGCC AGCAGAGCTACAGCACCCCCAGCTTCGGCCAGGGCACCA GGCTGGAGATCACCAGGACCGTGGCCGCCCCCAGCGTGT TCATCTTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCA CCGCCAGCGTGGTGT GCCTGCTGAACAACTTCTACCCCAGGGAGGCCAAGGTGC AGTGGAAGGTGGACAACGCCCTGCAGAGCGGCAACAGCC AGGAGAGCGTGACCGAGCAGGACAGCAAGGACAGCACC TACAGCCTGAGCAGCACCCTGACCCTGAGCAAGGCCGAC TACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCAC CAGGGCCTGAGCAGCCCCGTGACCAAGAGCTTCAACAGG GGCGAGTGC	Nucleotide sequence of the light chain of E06
7	DIQMTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKP GQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDF ATYYCQQSYSTPSFGQGTRLEIT	Amino acid sequence of the light chain variable domain of E06
8	GACATCCAGATGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAG AGCTACAGCACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACC	Nucleotide sequence of the light chain variable domain of E06

SEQ ID NO	SEQUENCE	DESCRIPTION
9	WYEMY	Amino acid sequence of HCDR1 of E06
10	SISPSGGWTMYADSVKG	Amino acid sequence of HCDR2 of E06
11	PLYSSDGLSAGDI	Amino acid sequence of HCDR3 of E06
12	RASQSVSSSYLA	Amino acid sequence of LCDR1 of E06
13	GASSRAT	Amino acid sequence of LCDR2 of E06
14	QQSYSTPS	Amino acid sequence of LCDR3 of E06
15	SAME AS E06	Amino acid sequence of the heavy chain of E06 germline M4
16	SAME AS E06	Nucleotide sequence of the heavy chain of E06 germline M4
17	SAME AS E06	Amino acid sequence of the heavy chain variable domain of E06 germline M4
18	SAME AS E06	Nucleotide sequence of the heavy chain variable domain of E06 germline M4
19	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYSTPSFGQGTRLEITRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of E06 germline M4
20	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGCACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACCAGGACCGTGGCCGCCCCCAGCGTGTTCATC TTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCACCGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCAGGGAG GCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGC GGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAA GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAG CAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA GGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAG CTTCAACAGGGGCGAGTGC	Nucleotide sequence of the light chain of E06 germline M4
21	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYSTPSFGQGTRLEIT	Amino acid sequence of the light chain variable domain of E06 germline M4
22	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC	Nucleotide sequence of the light chain variable domain of E06 germline M4

SEQ ID NO	SEQUENCE	DESCRIPTION
	AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGCACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACC	
23	SAME AS E06 HCDR1	Amino acid sequence of HCDR1 of E06 germline M4
24	SAME AS E06 HCDR2	Amino acid sequence of HCDR2 of E06 germline M4
25	SAME AS E06 HCDR3	Amino acid sequence of HCDR3 of E06 germline M4
26	SAME AS E06 LCDR1	Amino acid sequence of LCDR1 of E06 germline M4
27	SAME AS E06 LCDR2	Amino acid sequence of LCDR2 of E06 germline M4
28	SAME AS E06 LCDR3	Amino acid sequence of LCDR3 or E06 germline M4
29	SAME AS E06	Amino acid sequence of the heavy chain of D04
30	SAME AS E06	Nucleotide sequence of the heavy chain of D04
31	SAME AS E06	Amino acid sequence of the heavy chain variable domain of D04
32	SAME AS E06	Nucleotide sequence of the heavy chain variable domain of D04
33	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYSTPSFGQGTRLEITRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of D04
34	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGCACAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGCACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACCAGGACCGTGGCCGCCCCCAGCGTGTTCATC TTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCACCGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCAGGGAG GCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGC GGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAA GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAG CAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA GGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAG CTTCAACAGGGGCGAGTGC	Nucleotide sequence of the light chain of D04
35	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYSTPSFGQGTRLEIT	Amino acid sequence of the light chain variable domain of D04

SEQ ID NO	SEQUENCE	DESCRIPTION
36	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGCACAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGCACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACC	Nucleotide sequence of the light chain variable domain of D04
37	SAME AS E06 HCDR1	Amino acid sequence of HCDR1 of D04
38	SAME AS E06 HCDR2	Amino acid sequence of HCDR2 of D04
39	SAME AS E06 HCDR3	Amino acid sequence of HCDR3 of D04
40	RASQSVHSSYLA	Amino acid sequence of LCDR1 of D04
41	SAME AS E06 LCDR2	Amino acid sequence of LCDR2 of D04
42	SAME AS E06 LCDR3	Amino acid sequence of LCDR3 of D04
43	SAME AS E06	Amino acid sequence of the heavy chain of J05
44	SAME AS E06	Nucleotide sequence of the heavy chain of J05
45	SAME AS E06	Amino acid sequence of the heavy chain variable domain of J05
46	SAME AS E06	Nucleotide sequence of the heavy chain variable domain of J05
47	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYRTPSFGQGTRLEITRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of J05
48	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGGACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACCAGGACCGTGGCCGCCCCCAGCGTGTTCATC TTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCACCGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCAGGGAG GCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGC GGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAA GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAG CAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA GGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAG CTTCAACAGGGGCGAGTGC	Nucleotide sequence of the light chain of J05

SEQ ID NO	SEQUENCE	DESCRIPTION
49	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYRTPSFGQGTRLEIT	Amino acid sequence of the light chain variable domain of J05
50	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGGACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACC	Nucleotide sequence of the light chain variable domain of J05
51	SAME AS E06 HCDR1	Amino acid sequence of HCDR1 of J05
52	SAME AS E06 HCDR2	Amino acid sequence of HCDR2 of J05
53	SAME AS E06 HCDR3	Amino acid sequence of HCDR3 of J05
54	SAME AS E06 LCDR1	Amino acid sequence of LCDR1 of J05
55	SAME AS E06 LCDR2	Amino acid sequence of LCDR2 of J05
56	QQSYRTPS	Amino acid sequence of LCDR3 of J05
57	SAME AS E06	Amino acid sequence of the heavy chain of I20
58	SAME AS E06	Nucleotide sequence of the heavy chain of I20
59	SAME AS E06	Amino acid sequence of the heavy chain variable domain of I20
60	SAME AS E06	Nucleotide sequence of the heavy chain variable domain of I20
61	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQDYSTPSFGQGTRLEITRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of I20
62	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG GACTACAGCACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACCAGGACCGTGGCCGCCCCCAGCGTGTTCATC TTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCACCGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCAGGGAG GCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGC GGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAA GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAG	Nucleotide sequence of the light chain of I20

SEQ ID NO	SEQUENCE	DESCRIPTION
	CAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA GGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAG CTTCAACAGGGGCGAGTGC	
63	EIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQDYSTPSFGQGTRLEIT	Amino acid sequence of the light chain variable domain of I20
64	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGAGCAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG GACTACAGCACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACC	Nucleotide sequence of the light chain variable domain of I20
65	SAME AS E06 HCDR1	Amino acid sequence of HCDR1 of I20
66	SAME AS E06 HCDR2	Amino acid sequence of HCDR2 of I20
67	SAME AS E06 HCDR3	Amino acid sequence of HCDR3 of I20
68	SAME AS E06 LCDR1	Amino acid sequence of LCDR1 of I20
69	SAME AS E06 LCDR2	Amino acid sequence of LCDR2 of I20
70	QQDYSTPS	Amino acid sequence of LCDR3 of I20
71	SAME AS E06	Amino acid sequence of the heavy chain of H1R
72	SAME AS E06	Nucleotide sequence of the heavy chain of H1R
73	SAME AS E06	Amino acid sequence of the heavy chain variable domain of H1R
74	SAME AS E06	Nucleotide sequence of the heavy chain variable domain of H1R
75	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYRTPSFGQGTRLEITRTVAAPSVFIFPPSDEQLKSG TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of H1R
76	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGCACAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGGACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACCAGGACCGTGGCCGCCCCCAGCGTGTTCATC TTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCACCGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCAGGGAG	Nucleotide sequence of the light chain of H1R

SEQ ID NO	SEQUENCE	DESCRIPTION
	GCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGC GGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAA GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAG CAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA GGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAG CTTCAACAGGGGCGAGTGC	
77	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYRTPSFGQGTRLEIT	Amino acid sequence of the light chain variable domain of H1R
78	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGCACAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG AGCTACAGGACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACC	Nucleotide sequence of the light chain variable domain of H1R
79	SAME AS E06 HCDR1	Amino acid sequence of HCDR1 of H1R
80	SAME AS E06 HCDR2	Amino acid sequence of HCDR2 of H1R
81	SAME AS E06 HCDR3	Amino acid sequence of HCDR3 of H1R
82	RASQSVHSSYLA	Amino acid sequence of LCDR1 of H1R
83	SAME AS E06 LCDR2	Amino acid sequence of LCDR2 of H1R
84	QQSYRTPS	Amino acid sequence of LCDR3 of H1R
85	EVQLLESGGGLVQPGGSLRLSCAASGFTFSWYEMYWVRQA PGKGLEWVSSISPSGGWTMYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCATPLYSSDGLSAGDIWGQGTMVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC NVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GK	Amino acid sequence of the heavy chain of H1RK
86	GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGC CTGGTGGTTCTTTACGTCTTTCTTGCGCTGCTTCCGGATTC ACTTTCTCTTGGTACGAGATGTATTGGGTTCGCCAAGCTC CTGGTAAAGGTTTGGAGTGGGTTTCTTCTATCTCTCCTTCT GGTGGCTGGACTATGTATGCTGACTCCGTTAAAGGTCGCT TCACTATCTCTAGAGACAACTCTAAGAATACTCTCTACTT GCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTA TTACTGTGCGACCCCCTTGTATAGCAGTGACGGGCTTTCG GCGGGGGATATCTGGGGCCAAGGGACAATGGTCACCGTC TCAAGCGCGTCGACCAAGGGCCCATCCGTCTTCCCCCTGG	Nucleotide sequence of the heavy chain of H1RK

SEQ ID NO	SEQUENCE	DESCRIPTION
	CACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCC TGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGA CGGTGTCCTGGAACTCAGGCGCTCTGACCAGCGGCGTGC ACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTC CCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGG CACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAG CAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTG TGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGA ACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAA CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAG CACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCC AACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCACAGGTCTACACC CTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACA TCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGC TCCTTCTTCCTCTATAGCAAGCTCACCGTGGACAAGAGCA GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCT CCCTGTCTCCGGGTAAA	
87	EVQLLESGGGLVQPGGSLRLSCAASGFTFSWYEMYWVRQA PGKGLEWVSSISPSGGWTMYADSVKGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCATPLYSSDGLSAGDIWGQGTMVTVS S	Amino acid sequence of the heavy chain variable domain of H1RK
88	GAAGTTCAATTGTTAGAGTCTGGTGGCGGTCTTGTTCAGC CTGGTGGTTCTTTACGTCTTTCTTGCGCTGCTTCCGGATTC ACTTTCTCTTGGTACGAGATGTATTGGGTTCGCCAAGCTC CTGGTAAAGGTTTGGAGTGGGTTTCTTCTATCTCTCCTTCT GGTGGCTGGACTATGTATGCTGACTCCGTTAAAGGTCGCT TCACTATCTCTAGAGACAACTCTAAGAATACTCTCTACTT GCAGATGAACAGCTTAAGGGCTGAGGACACGGCCGTGTA TTACTGTGCGACCCCCTTGTATAGCAGTGACGGGCTTTCG GCGGGGGATATCTGGGGCCAAGGGACAATGGTCACCGTC TCAAGC	Nucleotide sequence of the heavy chain variable domain of H1RK
89	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYRTPSFGQGTRLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of H1RK
90	GAGATCGTGCTGACCCAGTCTCCAGCCACCCTCTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTC AGAGTGTTCACAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTTTACTACTGTCAACAGAGTT ACCGCACCCCTTCCTTCGGCCAAGGGACACGACTGGAGA	Nucleotide sequence of the light chain of H1RK

SEQ ID NO	SEQUENCE	DESCRIPTION
	TTAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCC GCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGTT GTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAA GTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAAC TCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAG CACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGC AGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCAC CCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAA CAGGGGAGAGTGT	
91	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQSYRTPSFGQGTRLEIK	Amino acid sequence of the light chain variable domain of H1RK
92	GAGATCGTGCTGACCCAGTCTCCAGCCACCCTCTCTTTGT CTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTC AGAGTGTTCACAGCAGCTACTTAGCCTGGTACCAGCAGA AACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATC CAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAG TGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTG GAGCCTGAAGATTTTGCAGTTTACTACTGTCAACAGAGTT ACCGCACCCCTTCCTTCGGCCAAGGGACACGACTGGAGA TTAAA	Nucleotide sequence of the light chain variable domain of H1RK
93	WYEMY	Amino acid sequence of HCDR1 of H1RK
94	SISPSGGWTMYADSVKG	Amino acid sequence of HCDR2 of H1RK
95	PLYSSDGLSAGDI	Amino acid sequence of HCDR3 of H1RK
96	RASQSVHSSYLA	Amino acid sequence of LCDR1 of H1RK
97	GASSRAT	Amino acid sequence of LCDR2 of H1RK
98	QQSYRTPS	Amino acid sequence of LCDR3 of H1RK
99	SAME AS E06	Amino acid sequence of the heavy chain of H1DR
100	SAME AS E06	Nucleotide sequence of the heavy chain of H1DR
101	SAME AS E06	Amino acid sequence of the heavy chain variable domain of H1DR
102	SAME AS E06	Nucleotide sequence of the heavy chain variable domain of H1DR
103	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQDYRTPSFGQGTRLEITRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC	Amino acid sequence of the light chain of H1DR
104	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGCACAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC	Nucleotide sequence of the light chain of H1DR

SEQ ID NO	SEQUENCE	DESCRIPTION
	AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG GACTACAGGACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACCAGGACCGTGGCCGCCCCCAGCGTGTTCATC TTCCCCCCCAGCGACGAGCAGCTGAAGAGCGGCACCGCC AGCGTGGTGTGCCTGCTGAACAACTTCTACCCCAGGGAG GCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGAGC GGCAACAGCCAGGAGAGCGTGACCGAGCAGGACAGCAA GGACAGCACCTACAGCCTGAGCAGCACCCTGACCCTGAG CAAGGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGA GGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGAG CTTCAACAGGGGCGAGTGC	
105	EIVLTQSPATLSLSPGERATLSCRASQSVHSSYLAWYQQKPG QAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFA VYYCQQDYRTPSFGQGTRLEIT	Amino acid sequence of the light chain variable domain of H1DR
106	GAGATCGTGCTGACCCAGAGCCCCGCCACCCTGAGCCTG AGCCCCGGCGAGAGGGCCACCCTGAGCTGCAGGGCCAGC CAGAGCGTGCACAGCAGCTACCTGGCCTGGTACCAGCAG AAGCCCGGCCAGGCCCCCAGGCTGCTGATCTACGGCGCC AGCAGCAGGGCCACCGGCATCCCCGACAGGTTCAGCGGC AGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGG CTGGAGCCCGAGGACTTCGCCGTGTACTACTGCCAGCAG GACTACAGGACCCCCAGCTTCGGCCAGGGCACCAGGCTG GAGATCACC	Nucleotide sequence of the light chain variable domain of H1DR
107	SAME AS E06 HCDR1	Amino acid sequence of HCDR1 of H1DR
108	SAME AS E06 HCDR2	Amino acid sequence of HCDR2 of H1DR
109	SAME AS E06 HCDR3	Amino acid sequence of HCDR3 of H1DR
110	RASQSVHSSYLA	Amino acid sequence of LCDR1 of H1DR
111	SAME AS E06 LCDR2	Amino acid sequence of LCDR2 of H1DR
112	QQDYRTPS	Amino acid sequence of LCDR3 of H1DR

Examples

For the experiments described herein various antibodies were used including, Avastin® (Ferrara, N et al. Biochem Biophys Res Comm, 333:328-335, 2005), G6-31 (Liang, WC et al. J Biol Chem, 281: 951-961, 2006), B20-4.1 (Liang, WC et al. J Biol Chem, 281: 951-961, 2006), and an isotype control, designated R347, as a monospecific or a bispecific antibody as needed. An anti-VEGF IgG1 antibody capable of binding all VEGF isoforms that is not cross-reactive with mouse can used as a positive control for some binding and functional studies. Where cross

reactivity to mouse VEGF is needed the antibodies G6-31 and B20-4.1 can be used as a positive control.

EXAMPLE 1: IDENTIFICATION OF ANTI-VEGF ANTIBODIES FROM PHAGE ANTIBODY DISPLAY LIBRARIES

Solution phage panning was applied to isolate anti-VEGF antibodies from antibody phage libraries. The phage libraries were incubated with biotinylated VEGF165 (PeproTech, NJ) at the final concentration of 4 µg/ml (human VEGF165 for first and third round and mVEGF164 for the second and fourth round panning). After incubation for 30 minutes, the VEGF bound phages were captured from the solution by adding straptavidin beads (Invitrogen), which were prewashed with phosphate buffer saline (PBS) and blocked with 3% bovine serum albumin (BSA) in PBS. The beads captured phages were eluted with 100 mM TEA buffer, neutralized with 1 M Tris-HCI (pH8.0), and then 1 ml of eluted phage were used to infect 5 ml of log phase TG1 and 4 ml of 2YT medium (Teknova) for phage amplification. After incubation at 37° C for 30 minutes in water bath, the cells were spun down at 4000g, resuspended in 2YT, spread on 2YT agar plates (Teknova) with 100 µg/ml of carbenicillin and 2% glucose, and incubated at 30° C overnight. On the second day, the colonies were collected from the agar plates, inoculated into 2YT medium at the final density of OD600 = 0.1, grown at 37° C until log phase, infected with helper phage (Invitrogen) and then let it grow overnight in 2YT with carbenicillin (Invitrogen) and kanamycin (Sigma) at 30° C to generate high titer phages. The amplified phages were precipitated with PEG8000, resuspended in PBS and used for the next round of panning using the standard procedure. A total of four rounds of panning were applied to isolate VEGF specific antibodies. A significant enrichment of output phage numbers was observed from 2 x 10^5 pfu in the first round to 4 x 10^7 pfu in the fourth round of panning.

EXAMPLE 2: SCREENING OF VEGF SPECIFIC ANTIBODIES

Specificity of individual phage Fab from the fourth round of panning was assessed by phage ELISA. The 96-well microtiter plates were coated with human VEGF165 at a concentration of 5 µg/ml in PBS at 40° C overnight. After being washed three times with PBS, the wells were blocked with the blocking buffer (4% skimmed milk in PBS) for 1 h at 37° C. Then, 50 µl/well phage was added and incubated for 1 h at 37° C. After washing, 50 µl of horseradish peroxidase

(HRP)-conjugated mouse anti-M13 (Amersham Pharmacia) in blocking buffer with 1:1,000 dilution was added for 30 minutes at 37° C. For detection, 50 µl/well of SureBlue Reserve TMB substrate (KPL) for 5 minutes at room temperature and the reaction was stopped by adding 50 µL of TMB Stop Solution (KPL). The absorbance was read at 450 nm. After four rounds of panning, more than 50% of clones are VEGF specific with absorbance higher than 1, which is 20-fold higher than the nonspecific background reading approximately 0.05. Representative data is show in Table 1.

Table 1

1	2	3	4	5	6	7	8	9	10	11	12
0.091	2.276	2.406	0.111	0.116	0.116	0.087	2.534	0.059	2.478	0.098	0.193
0.091	2.755	3.161	1.508	0.054	3.166	0.062	0.066	0.061	3.2	2.354	1.971
2.328	0.078	2.697	2.919	3.216	3.258	3.133	2.948	2.859	2.6	1.314	2.595
2.209	3.056	0.084	3.139	3.181	0.055	3.336	2.472	3.074	0.062	2.741	0.073
0.05	0.052	0.053	3.02	3.57	0.049	0.047	2.862	2.761	2.609	0.051	0.05
0.05	0.05	0.053	2.861	3.469	3.155	0.057	0.387	1.325	0.052	3.067	2.446
1.963	2.641	3.27	3.349	3.246	0.047	3.509	2.727	0.046	2.67	2.679	0.053
0.044	0.05	3.105	0.053	3.215	2.483	0.059	3.004	2.294	2.994	3.091	1.336

EXAMPLE 3 - BINDING OF CLONE E06 TO HUMAN AND MOUSE VEGF

After initial phage screening, one clone (E06) with cross-reactivity to mouse VEGF164 was further converted and expressed as the full length IgG using standard molecular biology materials and methods. The 96-well microtiter plates (Corning) were coated with 50 µl /well of human and mouse VEGF165 or VEGF121 at a concentration of 5 µg/ml in PBS at 4° C overnight. After being washed three times with PBS containing 0.1% Tween-20 (PBST), the wells were blocked with blocking buffer (4% skimmed milk in PBS). After 1 h incubation at room temperature, the plates were washed with PBST and a 2-fold serial dilution of the antibodies (starting from 8 nM) in blocking buffer were added and incubated for 1 hour at room temperature. The antibody solution was removed by washing with PBST followed by 1 hour incubation at room temperature with a 1:3000 dilution of an anti-human-Fc-HRP antibody (Thermo Scientific) prepared in PBST. Binding was visualized with the addition of 50 µL of SureBlue Reserve TMB substrate (KPL) for 5 minutes at room temperature and the reaction was stopped by adding 50 µL of TMB Stop Solution (KPL). The absorbance at 450 nm was measured using a microtiter plate reader. The data were analyzed using Prism 5 software (GraphPad).

Similar binding activity of antibody E06 to human VEGF165 and mouse VEGF164 was observed with an EC50 of 0.048 nM and 0.049 nM, respectively. As expected, Avastin® (an anti-VEGF antibody) showed strong binding to human VEGF165 but no detectable binding to mouse VEGF164. The isotype control antibody R347 did not bind either human or mouse forms of VEGF. Representative data are shown in Figure 1.

To evaluate whether antibody E06 binds to a different epitope than Avastin® (an anti-VEGF antibody), an ELISA assay was conducted using human VEGF121. Avastin® (an anti-VEGF antibody) showed very strong binding to human VEGF121. However, no binding was deteceted for E06, indicating antibody E06 binds to a different epitope than Avastin® (an anti-VEGF antibody). The isotype control antibody R347 also did not demonstrate any binding to human VEGF121. Representative data are shown in Figure 2.

EXAMPLE 4 – GERMLINE OF CLONE E06

Framework sequences of clone E06 were engineered to match to its closest germline sequences. A sequence analysis against the IgG germline gene database showed that the E06 VL sequence best matches to the germline gene VK3-NL5*01 with 4 amino acid differences at positions 1, 3, 4 and 86 of the VL. Although the VH sequence is identical to the germline gene VH3-23*2, the T98 differs from most conserved germline amino acid of R or K at this position. To germline E06, four variants were designed and expressed. These variants substitute partially or totally with the germline gene encoded amino acids at the positions that E06 sequence differs from the best matched germline genes. For instance, M1 contains D1E/Q3V/M4L substitutions in the VL and T98R substitution in the VH; M4 contains D1E/Q3V/M4L/T86V substitutions in the VL; M7 contains D1E/Q3V/M4L/T86V substitutions in the VL and T98R substitution in the VH; and F1 contains the D1E/Q3V/M4L substitutions in the VL, where the first letter represents the one letter amino acid code of the original and the second letter represents the one letter amino acid code of the germline sequence. Figure 3 shows the sequence alignment of the parental E06 and the most homologous germline genes.

The germline variants were expressed and purified as Fabs and their binding to the recombinant VEGF165 was determined by ELISA. The binding results showed that the germline variants M4 and F1, which contain the full or partial VL germline amino acid substitutions retained E06 WT Fab binding activity. In addition, M4 showed similar activity compared to the WT E06

Fab in pVEGFR2 assay using HUVECs. On the other hand, germline variants M1 and M7 containing the VH germline amino acid substitution at position T98 demonstrated drastically reduced binding and pVEGFR2 phosphorylation, indicating that T98 participated in binding and activity and must be retained. The germline clone, M4, was used as the template for further affinity optimization.

EXAMPLE 5 – AFFINITY OPTIMIZATION

Each amino acid of all 6 CDRs of germline clone M4 was individually mutated to the other 20 amino acids using a hybridization mutagenesis method (Kunkel, Proc. Natl. Acad. Sci. USA Vol. 82, pp. 488-492, 1985). Two sets of DNA primers, one containing a NSS codon encoding 8 amino acids and the other containing a NWS codon encoding 12 different amino acids, were used to introduce mutations to each targeted CDR position. The individual degenerate primers were used in hybridization mutagenesis reactions. Briefly, each degenerate primer was phosphorylated and used in a 10:1 ratio with the uridinylated M4 Fab ssDNA. The mixture was heated to 95° C then cooled down to 55° C over 1 hour. Thereafter, T4 ligase and T7 DNA polymerase were added and the mix was incubated for 1.5 hours at 37° C. Synthesis products for VH and VL CDRs were pooled respectively; however, NSS and NWS libraries were kept separate and screened independently. Typically, 1 μL of the pooled library DNA was electroporated into XL1-Blue for plaque formation on XL1-Blue bacterial lawn or for production of Fab fragments (Wu H, An LL. Tailoring kinetics of antibodies using focused combinatorial libraries. Methods Mol Biol 2003; 207:213-33). These mutants were then screened for activity.

The primary screen consisted of a single point ELISA (SPE) assay which was carried out using culture supernatant of bacteria grown in 96-well plates (deep well) and infected with individual recombinant M13 clones as described elsewhere (Wu H, An LL. Tailoring kinetics of antibodies using focused combinatorial libraries. Methods Mol Biol 2003; 207:213-33). Briefly, this capture ELISA involved coating individual wells of a 96-well Maxisorp Immunoplate with approximately 50 ng of a sheep anti-human Fd antibody (Biodesign International, ME) in a carbonate buffer at pH 8.5 overnight at 4° C. The next day, the plate was blocked with 3% BSA in PBS buffer for 1 hour at room temperature. Fab supernatant was then added to the plate and incubated at room temperature for 1 hour. After washing, the biotinylated VEGF165 protein was added to the well and the mixture was incubated for 1.5 hours at room temperature. This was

followed by incubation with neutravidin-horseradish peroxydase (HRP) conjugate (Pierce, IL) for approximately 40 minutes at room temperature. HRP activity was detected with tetra-methyl-benzidine (TMB) substrate and the reaction quenched with 0.2 M H2SO4. Plates were read at 450 nm.

Clones exhibiting an optical density (OD) signal at 450 nm greater than the parental clone M4 Fab were picked and regrown (15 mL) (Wu H, An LL. Tailoring kinetics of antibodies using focused combinatorial libraries. Methods Mol Biol 2003; 207:213-33) and re-assayed by ELISA (as described above) in duplicate to confirm positive results. Clones that repeatedly exhibited a signal greater than that of the M4 Fab were sequenced. The Fab protein concentration of each clone that had a CDR change was then determined by a quantitative Fab ELISA, where a Fab with known concentration was used as a reference. The Fab concentration was determined by comparing the ELISA signals with the signals generated by the reference Fab. The binding assay was repeated once more for all positive variants under normalized Fab concentrations in order to determine the relative binding affinity of the mutant Fabs and the parental M4 Fab.

Many point mutations showed binding improvements over M4 to VEGF165. Among those mutants, D04, J05, and I20 showed in excess of 10-fold improvement in EC50 compared to germline E06 as Fabs. Representative data are shown in Figure 4. Sequence analysis revealed that amino acid substitutions that benefit to the VEGF165 binding are found in the VL, especially in the VL-CDR3. For example, a point mutation S94R in mutant J05 improved binding approximately 25-fold over E06, indicating key contributions of this amino acid in binding.

The point mutants demonstrating improved binding were then combined using site-directed mutagenesis methods. The combination mutants were expressed as Fabs and IgGs and tested in a VEGF165 ELISA. Representative data are shown in Figure 5.

Combination mutants showed significantly higher binding than a point mutant, J05, in an ELISA assay. The apparent binding affinity of the combination mutants was improved approximately 3- to 10-fold over J05 as Fabs. Similarly, all combination mutants tested as IgGs showed significant binding improvement compared to the parental clone E06. The equilibrium binding constants (KD) of the affinity optimized clones were measurements using KinExA. Representative data are shown in Table 2. Sequences of point mutants as well as combination mutants are shown in Table 3.

Table 2

IgG	K_D, pM (95% CI) (Std. Aff. model - ref [VEGF165])	FoldΔ K_D vs. EO6-wt
H1R	23.2 (10.8-40.9)	109
J05	220.3 (179.1-268.4)	11
Wt (E06)	2520 (1800-4260)	-
Avastin®	99.9 (71.1-137.0)	25

Table 3

VL	CDR 1												CDR 3							
E06	R	A	S	Q	S	V	S	S	S	Y	L	A	Q	Q	S	Y	S	T	P	S
D04	R	A	S	Q	S	V	*H*	S	S	Y	L	A	Q	Q	S	Y	S	T	P	S
J05	R	A	S	Q	S	V	S	S	S	Y	L	A	Q	Q	S	Y	*R*	T	P	S
I20	R	A	S	Q	S	V	S	S	S	Y	L	A	Q	Q	*D*	Y	S	T	P	S
H1R	R	A	S	Q	S	V	*H*	S	S	Y	L	A	Q	Q	S	Y	*R*	T	P	S
H1DR	R	A	S	Q	S	V	*H*	S	S	Y	L	A	Q	Q	*D*	Y	*R*	T	P	S

EXAMPLE 6 - MEASUREMENT OF KD FOR THE BINDING OF AVASTIN, E06 AND AFFINITY OPTIMIZED E06 VARIANTS TO HUMAN VEGF165.

Equilibrium binding constant (KD) measurements were performed on KinExA 3000 and 3200 instruments (Sapidyne Instruments, Boise, ID). Human VEGF 165 (huVEGF) protein was coated onto UltraLink® Biosupport beads (PIERCE, Rockford, IL) at concentrations of 2 ug/mL, 3 ug/mL, and 30 ug/mL in coating buffer (50 mM sodium carbonate buffer, pH 9). Coated beads were then separated (spin) from unreacted huVEGF protein solution, and blocked with 1M Tris, pH 8, containing BSA at 10 mg/mL), for approximately 15 minutes at room temperature. After

this, the bead slurry was spun to remove the blocking solution, and then the block step was repeated for approximately 2 hours using fresh block buffer, and stored at 4° C until used. Prior to use, the huVEGF-coated beads were transferred to a bead vial, resuspended in approximately 27 mLs of instrument buffer (10mM HEPES+300mM NaCl+5mM $CaCl_2$+0.05% P20+0.02% NaN_3, pH8), and affixed to the KinExA instrument. For the KD measurements, separate solutions of antibodies were prepared at 100 pM and 2.5 nM concentrations in instrument buffer containing BSA (mg/mL), then dispensed into two separate series of 13 tubes. These concentrations of mAbs were chosen to allow each KD measurement to be made under both receptor- and KD -controlled conditions, leading to more rigorous estimations of reagent activity and affinity, respectively. Owing to its relatively weak KD, a 3rd concentration series (50 nM) for mAb EO6-wt was also prepared to satisfy the requirement for a fully receptor-controlled measurement. Two-fold serial dilutions of huVEGF protein were then titrated across 9 of the tubes in each mAb series, followed by two additional 10-fold-dilutions, leaving one tube as the mAb-only, "zero" control. In so doing, this yielded concentration series' of huVEGF protein that ranged from 488 fM – 25 nM (100 pM mAb experiments), 3.91 pM – 200 nM (2.5 nM mAb experiments), and 9.77 pM – 500 nM (50 nM mAb experiment). Based on theory curve simulations available through the vendor software (Sapidyne Instruments, Boise, Idaho), the mixtures were incubated 1 – 4 days at room temperature to allow binding to reach equilibrium. At the end of this time, signal-testing experiments were conducted to determine the appropriate run conditions for each set of measurements. Detection of free antibody was made possible using a species-specific, secondary antibody reagent (Goat Anti-Human IgG (H+L)-DyLight649, Part #109-495-088, Jackson ImmunoResearch Laboratories), employed at 0.75 ug/mL, 1.0 ug/mL or 2 ug/mL in instrument buffer containing BSA at 1 mg/mL. Data obtained for each mAb/huVEGF interaction was then simultaneously fit to a one-site binding model using the vendor's software to obtain the equilibrium KDs. The KD of the affinity optimized clones were measured using KinExA and with results summarized in Table 2.

EXAMPLE 7 - ASSAYING AFFINITY OPTIMIZED VARIANTS FOR BINDING TO MURINE VEGF164.

Affinity optimized variants were screened to confirm binding to murine VEGF164, similarly as previously described for VEGF165 ELISA except with murine VEGF164. EC50 values were determined using non-linear regression analysis (log dose response, 4-parameter fit

curves) in GraphPad Prism, version 5.01 (San Diego, CA). Representative data are shown in Figure 6. All affinity optimized variants exhibited strong binding to murine VEGF164, similar to the parental E06 antibody.

EXAMPLE 8 - ASSAYING AFFINITY OPTIMIZED VARIANTS FOR REDUCED BINDING TO VEGF121.

Affinity optimized variants were screened to confirm reduced binding to human VEGF121 in an ELISA, similarly as previously described for VEGF165 ELISA. EC50 values were determined using non-linear regression analysis (log dose response, 4-parameter fit curves) in GraphPad Prism, version 5.01 (San Diego, CA). Representative data are shown in Figure 7. VEGF121 binding was reduced for J05, H1R, and H1DR in contrast to the VEGF positive control antibody with strong binding to VEGF121 (EC50 of 0.0063 nM).

EXAMPLE 9 - ASSAYING AFFINITY OPTIMIZED VARIANTS FOR BINDING TO VEGF189.

Affinity optimized variant screening for binding to VEGF189 is in an ELISA format. 96-well half well maxisorp plates will be coated with 25 µl of 2 µg/mL human VEGF189 (R&D Systems), diluted in PBS without Ca++ or Mg++, and refrigerated overnight. Plates will be decanted, then blocked for 1.5 hours at 37° C with 180 µl of Blocking Buffer containing 3% BSA (Sigma, Cat #A-3059) and 0.1% Tween-20 in 1X PBS. Plates will be washed 3 times with 1 X PBS containing 0.1% Tween-20. 50 µl of 6.7 nM and serial dilutions of affinity optimized variants, a positive control, and a negative control in blocking buffer is added in duplicate and incubated at 37° C for 1 hour. Plates will be washed 3 times with wash buffer, then 50 µl of 1:5000 goat anti-human HRP IgG H+L (Jackson Immunoresearch) is added to each well and incubated at room temperature for 1 hour. Plates will developed by adding 50 µl of TMB solution (KPL) to each well, followed by stopping the reaction with 50 µl of 1M phosphoric acid. Plates will be read at 450nm using a microplate reader. Affinity optimized variants show a decrease in binding to VEGF189.

EXAMPLE 10 - FUNCTIONAL CELL-BASED ASSAYS TO COMPARE POTENCY OF AFFINITY OPTIMIZED VARIANTS.

Human and murine pVEGFR2 cell based assays were performed as previously described to confirm potency of affinity optimized variants. EC50 values were determined using non-linear regression analysis (log dose response, 4-parameter fit curves) in GraphPad Prism, version 5.01 (San Diego, CA). Representative data are shown in Figure 8A and 8B. Clones J05, H1R, and H1DR exhibited up to 20-fold improvement (EC50 range 2.6-10.15 nM) vs. the E06 parental control (EC50= 52.25 nM) in the human pVEGFR2 assay (Figure 8A), while improvement in the mouse pVEGFR2 assay was up to 2-fold compared to the parental antibody (EC50 range 2.38-3.57 nM vs 5.34 nM (Figure 8B)).

EXAMPLE 11 – IN VIVO ACTIVITY OF AFFINITY OPTIMIZED E06 VARIANTS.

Affinity optimized variant testing for in vivo activity will be carried out in a retinal vasculogenesis model, and a 786-0 renal cell carcinoma and BxPC3 pancreatic carcinoma model. In addition to these models, evaluation in a thrombocytopenia model will be carried out.

For the retinal vasculogenesis model, CD1 mice were intraperitoneally dosed at birth, days 1, 3, and 5. At day 8 the mice were anesthetized and were infused with fluorescein-labeled dextran. Eyes were removed and fixed with 10% formalin before preparation of flat mounts. Flat mounts were examined by fluorescence microscopy. Neonatal retinal angiogenesis is comprised of two processes, namely, vessel migration from the optic nerve to the edge of the retina and branching. There is a decrease in branching in the presence of H1RK compared to the untreated group. Representative data are shown in Figure 9. H1RK differs from H1R in that the light chain at position 107 (germline corrected position at 107) contains a threonine instead of a lysine.

For the 786-0 renal cell carcinoma model, 786-0 fragments will be implanted subcutaneously into the right flank. After tumor volume reaches approximately 200 mm^3, mice will be put on treatment. Mice will be treated 2X per week for a total of 6 doses. The affinity optimized variants demonstrate effectiveness at reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume. In addition to reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume, the affinity optimized variants also reduce tumor vasculature. Briefly, mice will be pretreated with heparin to prevent blood clotting 15 minutes prior to euthanasia. A solution of 0.1mM sodium nitroprusside will be perfused at a rate of approximately 6 mL/min. Microfil MV-122 will be prepared by mixing 8 mL of lates, 10 mL of diluent and 900 uL of cure. After the mixture settles (1 minute) it will be perfused at a rate

of approximately 2mL/min until a total volume of 17 mL is administered. After 60-90 minutes the tumor will be dissected and immersed in 10% NBF for 24 hours. The sample will then be transferred to 25% ETOH/PBS, 50% ETOH/PBS, 75% ETOH/PBS, 95% ETOH, and then 100 % ETOH for 24 hours each. After the final incubation the sample will be immersed in methyl salicylate to clear the dehydrated tumor sample before imaging by light microscopy.

For the BxPC3 pancreatic carcinoma model, female SCID mice will be implanted subcutaneously into the right flank. After tumor volume reaches approximately 200 mm^3, mice will be put on treatment. Mice will be treated 2X per week for a total of 6 doses. The affinity optimized variants demonstrate effectiveness at reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume. In addition to reducing tumor growth, reducing tumor volume, or reducing tumor growth and tumor volume, the affinity optimized variants also reduce tumor vasculature.

For the thrombocytopenia model, a method will be used that is adopted from Meyer et al, 2009 (J Thromb Haemost 7:171-81, 2009). Briefly FC gamma receptor 2A transgenic mice, 8-16 weeks old will be injected with premixed $VEGF_{165,}$ 0.6 units heparin, and an affinity optimized variant into the lateral tail vein. Mice will then be observed for behavioral signs of distress and scored as: (-) stopped and moved constantly from corner to corner, breathing normal, (+) signs of lethargy, stopped and moved in longer duration, breathing shallow, (++) very lethargic, stopped moving, staying in mostly one side of the box, breathing deeply, (+++) sever thrombotic event-twitching and twirling, (++++) death. The affinity optimized variants demonstrate a reduction in thrombocytopenia as compared to the anti-VEGF control.

Incorporation by Reference

All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entireties for all purposes.

Equivalents

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the embodiments. It will be appreciated, however, that no matter how detailed

the foregoing may appear in text, the embodiments may be practiced in many ways and the claims include any equivalents thereof.

Throughout the description and claims of the specification, the word "comprise" and variations of the word, such as "comprising" and "comprises", is not intended to exclude other additives, components, integers or steps.

A reference herein to a patent document or other matter which is given as prior art is not to be taken as admission that the document or matter was known or that the information it contains was part of the common general knowledge as at the priority date of any of the claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A antibody comprising heavy chain complementarity determining regions 1 – 3 (HCDR1, HCDR2, and HCDR3) and light chain complementarity determining regions 1 – 3 (LCDR1, LCDR2, and LCDR3), wherein HCDR1, HCDR2, and HCDR3 and LCDR1, LCDR2, and LCDR3 comprise SEQ ID NOs: 79 – 84, respectively, wherein the antibody comprises a heavy chain and a light chain comprising SEQ ID NOs: 73 and 77, respectively.

2. The antibody of claim 1, wherein the heavy chain amino acid sequence comprises SEQ ID NO: 71 and the light chain amino acid sequence comprises SEQ ID NO: 75.

3. The antibody of claim 1, wherein the antibody is a monoclonal antibody.

4. A nucleic acid sequence comprising polynucleotides encoding the antibody of claim 1.

5. A vector comprising the nucleotide sequence of claim 4.

6. A cell comprising the vector of claim 5.

7. A method of making an antibody of claim 1 comprising culturing a cell comprising a vector of claim 5.

8. A method of reducing angiogenesis in a subject in need thereof comprising providing an antibody of claim 1 to the subject, wherein the subject exhibits aberrant angiogenesis.

Figure 1

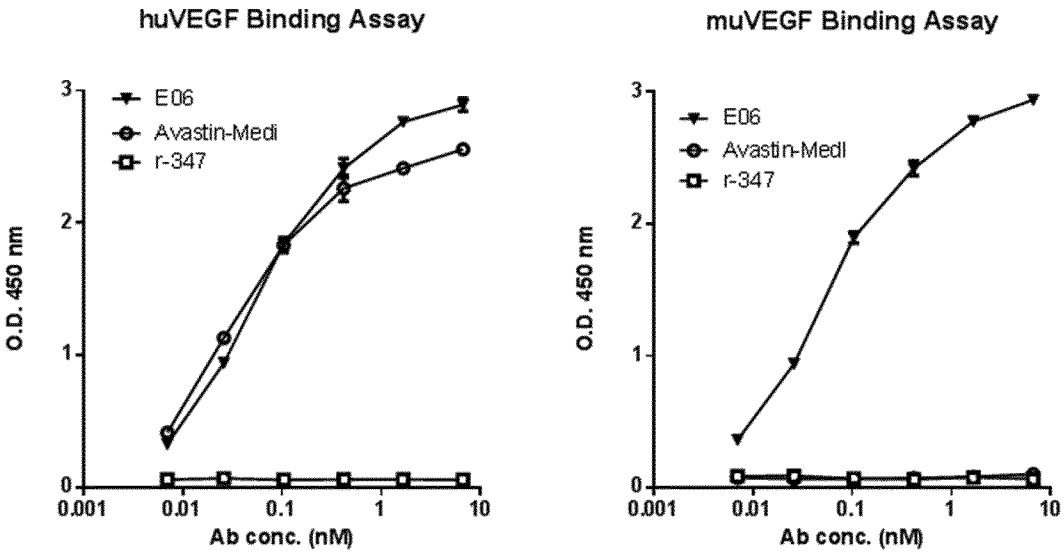

huVEGF Binding Assay
E06
Avastin-Medi
r-347
O.D. 450 nm
Ab conc. (nM)
muVEGF Binding Assay
E06
Avastin-Medi
r-347
O.D. 450 nm
Ab conc. (nM)

Figure 2

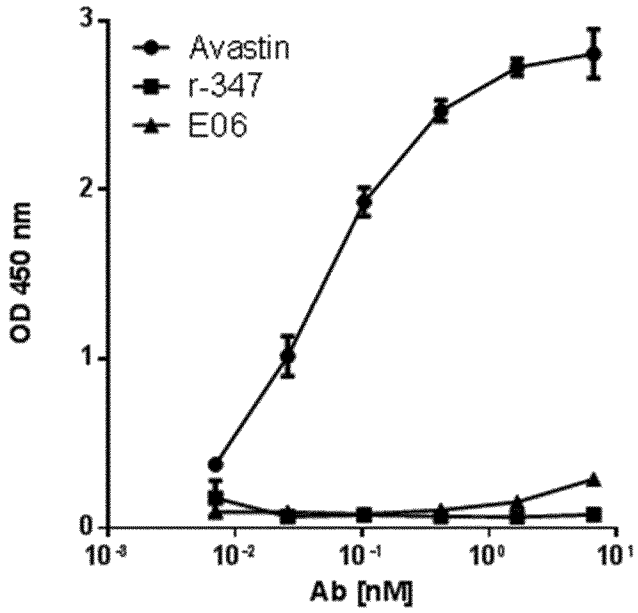
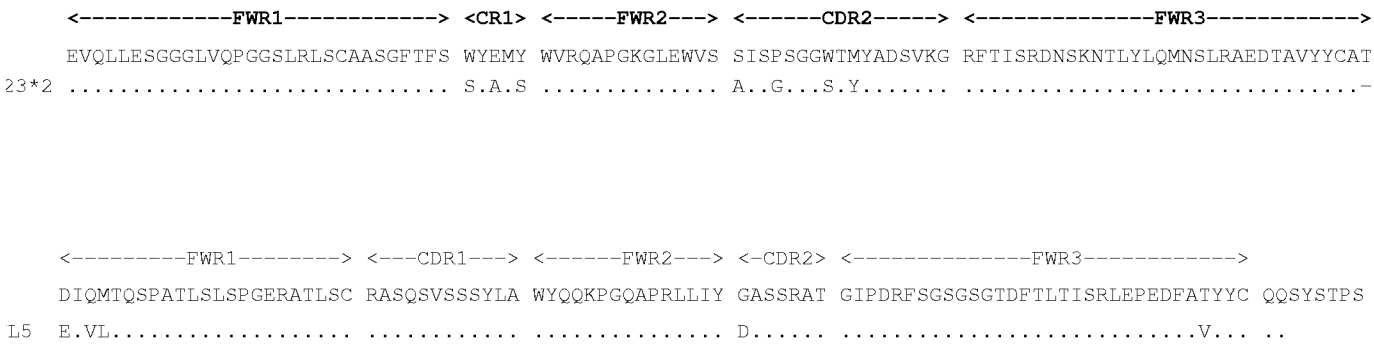


Figure 3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EVQLLESGGGLVQPGGSLRLSCAASGFTFS WYEMY WVRQAPGKGLEWVS SISPSGGWTMYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAT 98
23*2 ..... S.A.S ..... A..G...S.Y..... .....-
<---------FWR1--------> <---CDR1---> <------FWR2---> <-CDR2> <--------------FWR3----------->
DIQMTQSPATLSLSPGERATLSC RASQSVSSSYLA WYQQKPGQAPRLLIY GASSRAT GIPDRFSGSGSGTDFTLTISRLEPEDFATYYC QQSYSTPS 97
L5 E.VL..... .... D..... .....V... ..

Figure 4

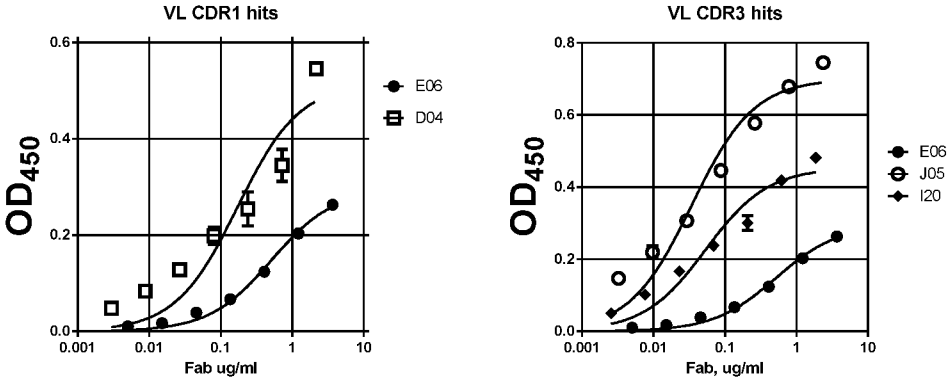

VL CDR1 hits
OD450
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
Fab ug/ml
E06
D04
VL CDR3 hits
OD450
0.8
0.6
0.4
0.2
0.0
0.001
0.01
0.1
1
10
Fab, ug/ml
E06
J05
I20

Figure 5

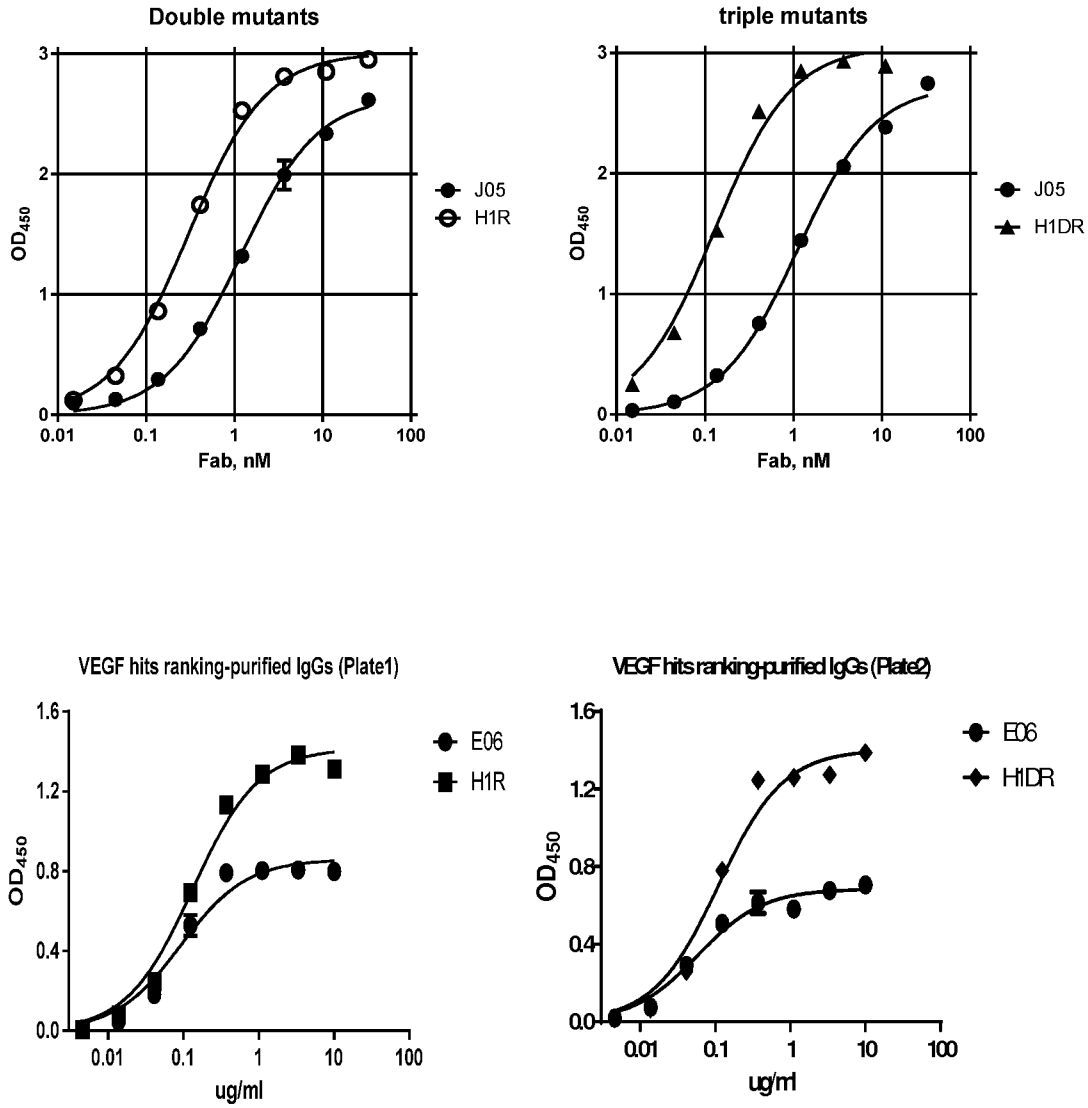

Double mutants
triple mutants
OD450
Fab, nM
J05
H1R
H1DR
VEGF hits ranking-purified IgGs (Plate1)
VEGF hits ranking-purified IgGs (Plate2)
E06
ug/ml

Figure 6

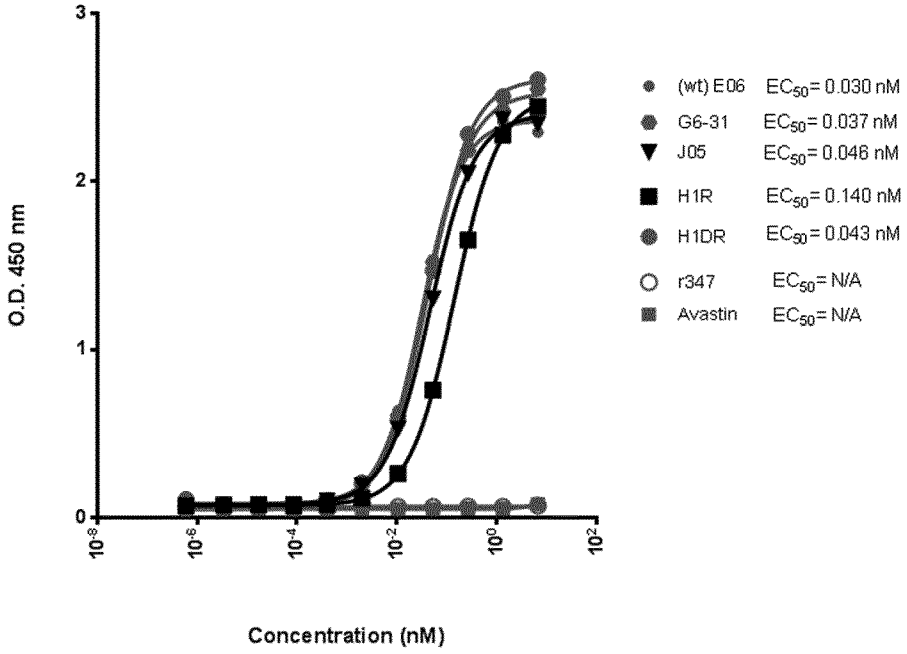

O.D. 450 nm
3
2
1
0
10^{-8}
10^{-6}
10^{-4}
10^{-2}
10^{0}
10^{2}
Concentration (nM)
(wt) E06 EC_{50}= 0.030 nM
G6-31 EC_{50}= 0.037 nM
J05 EC_{50}= 0.046 nM
H1R EC_{50}= 0.140 nM
H1DR EC_{50}= 0.043 nM
r347 EC_{50}= N/A
Avastin EC_{50}= N/A

Figure 7

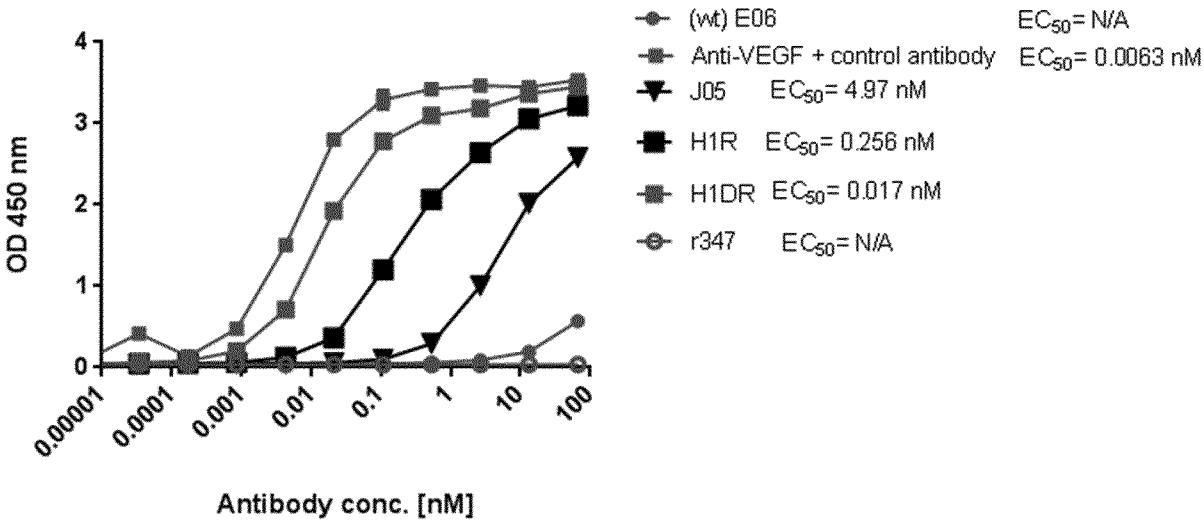
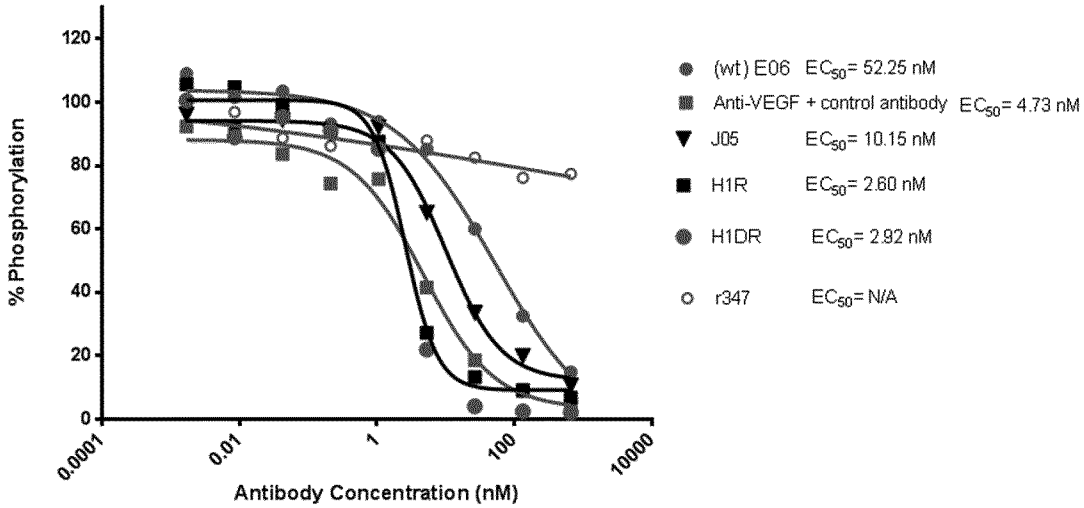

OD 450 nm
4
3
2
1
0
0.00001
0.0001
0.001
0.01
0.1
1
10
100
Antibody conc. [nM]
(wt) E06 EC50= N/A
Anti-VEGF + control antibody EC50= 0.0063 nM
J05 EC50= 4.97 nM
H1R EC50= 0.256 nM
H1DR EC50= 0.017 nM
r347 EC50= N/A

Figure 8

A)


% Phosphorylation
120
100
80
60
40
20
0
0.0001
0.01
1
100
10000
Antibody Concentration (nM)
(wt) E06 EC50= 52.25 nM
Anti-VEGF + control antibody EC50= 4.73 nM
J05 EC50= 10.15 nM
H1R EC50= 2.60 nM
H1DR EC50= 2.92 nM
r347 EC50= N/A

B)

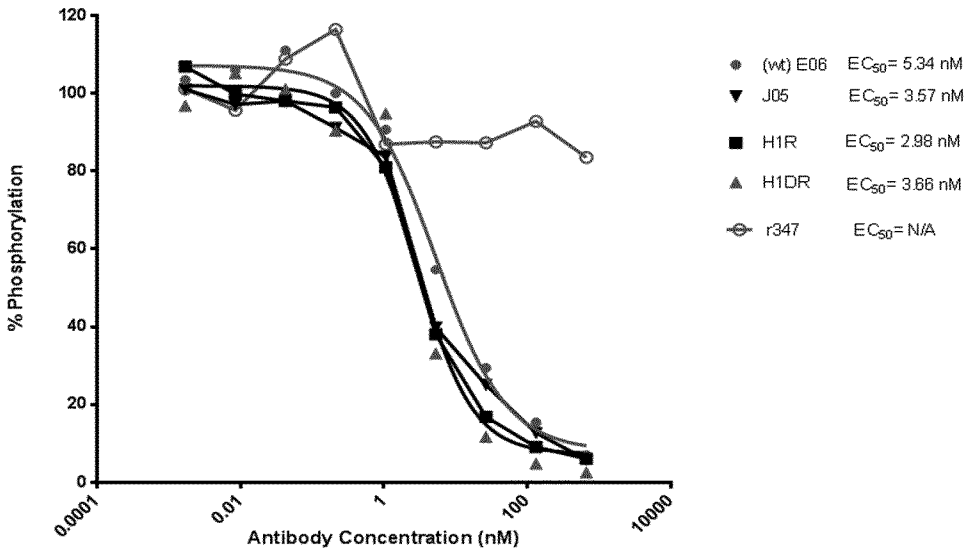
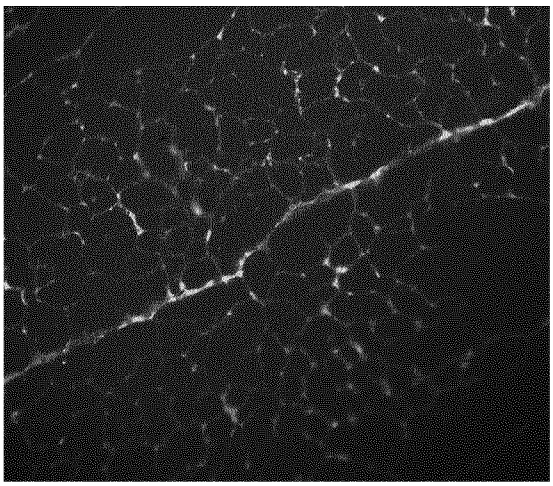

% Phosphorylation
120
100
80
60
40
20
0
0.0001
0.01
1
100
10000
Antibody Concentration (nM)
(wt) E06 EC50= 5.34 nM
J05 EC50= 3.57 nM
H1R EC50= 2.98 nM
H1DR EC50= 3.66 nM
r347 EC50= N/A

Figure 9

(-) H1RK



(+) H1RK