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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 November 2008 (06.11.2008)

PCT

(10) International Publication Number
WO 2008/132568 A2

(51) International Patent Classification:
*C07K 16/24* (2006.01) *C12N 15/45* (2006.01)
*C07H 21/04* (2006.01) *C12N 15/62* (2006.01)
*C07K 16/22* (2006.01) *A61K 38/16* (2006.01)
*C07K 19/00* (2006.01) *A61P 35/00* (2006.01)

(21) International Application Number:
PCT/IB2008/000901

(22) International Filing Date:
27 February 2008 (27.02.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/891,769 27 February 2007 (27.02.2007) US

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**
— *without international search report and to be republished upon receipt of that report*
— *with information concerning request for restoration of the right of priority in respect of one or more priority claims*

(54) Title: FUSION PROTEINS BINDING TO GROWTH FACTORS

(57) Abstract: The present application discloses an isolated nucleic acid molecule encoding a polypeptide capable of synchronously binding two different growth factors polypeptide components. Such a polypeptide is capable of synchronously binding VEGF polypeptide and Angiopoietin polypeptide.

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# FUSION PROTEINS BINDING TO GROWTH FACTORS

## BACKGROUND OF THE INVENTION

**[0001]** 1. **Field of the Invention:**

**[0002]** The present invention relates to the field of anti-angiogenesis compositions. The present invention also relates to a composition that binds two different growth factors or cytokines synchronously.

**[0003]** **2. Description of the Background:**

**[0004]** There are three known VEGF receptors, VEGFR1, VEGFR2 and VEGFR3 in humans. Human VEGFR1 consists of 1338 amino acids, separated by three major regions: an extracellular domain consisting of seven immunoglobulin (Ig)-like domains, a transmembrane domain and an intracellular tyrosine kinase domain (UniProtKB/Swiss-Prot entry P17948) (**Figure 1**). VEGFR2 and VEGFR3 are similarly organized and display about 80% identity to VEGFR1 in the tyrosine kinase domain.

**[0005]** Activation of VEGFR1 and VEGFR2 by binding of VEGF-A plays a crucial role for growth, migration and survival of blood endothelial cells, which are essential processes for angiogenesis and vasculogenesis, whereas activation of VEGFR3 by binding of VEGF-C and VEGF-D plays a main role for growth, migration and survival of lymphatic endothelial cells, which are essential processes for lymphangiogenesis (Shibuya M and Claessson-Welsh L, Exp. Cell Research 312:549-560, 2006; Alitalo K, et al., Nature 438:946-953) otherwise specifically indicated.

**[0006]** Affinity of VEGF-A to VEGFR1 is approximately 10 times higher than that to VEGFR2. Among 7 Ig-like domains of VEGR1, the Ig-like domain 2 is essential for VEGF-A binding (**Figure 2**). However, the Ig-like domain 2 of VEGFR1 contains many basic amino acids and its theoretical isoelectric point (pI) is 9.19 (Compute pI/Mw tool for Swiss-Prot/TrEMBL entries, http://kr.expasy.org/tools/pi_tool.html). Therefore, the Ig-like domain 2 of VEGFR1 *per se* cannot be used as therapeutic protein with property of decoy receptor because it has low pharmacokinetic properties.

**[0007]** Tyrosine kinase with immunoglobulin and epidermal growth factor homology domain-2 (Tie2) is a receptor tyrosine kinase (RTK) expressed predominantly on endothelial cells and hematopoietic cells (Dumont DJ, et al., Oncogene 8: 1293-1301, 1993). Tie2 is critical for vasculogenesis, angiogenesis, and hematopoiesis (Yancopoulos GD, et al., *Nature* 407:242-248, 2000). Four Tie2 ligands have been identified: angiopoietin-1 (Ang1),

angiopoietin-2 (Ang2), angiopoietin-3 (Ang3), and angiopoietin-4 (Ang4) (Yancopoulos GD, et al., *Nature* 407:242-248, 2000). Although Angl seems to be an obligate activator of Tie2, Ang2 seems to have context-specific effects, activating this receptor on some cells while blocking Tie2 activation on other cells or under different conditions (Yancopoulos GD, et al., *Nature* 407:242-248, 2000).

**[0008]** Human Tie2 consists of 1124 amino acids, separated by three major regions: an extracellular domain consisting of two Ig-like domain, three EGF-like domain, one Ig-like domain, three fibronectin type-III; a transmembrane domain and an intracellular tyrosine kinase domain (UniProtKB/Swiss-Prot entry Q02763) (**Figure 1**).

**[0009]** Among extracellular subdomains of Tie2, the Ig-like domain 2 is essential for angiopoietin binding, but the Ig-like domain 1 and three EGF-like domain appear to be required for stable binding of angiopoietin **(Figure 2)**. Importantly, these subdomains contain many acidic amino acids and its theoretical isoelectric point (pI) is 6.55 (Compute pI/Mw tool for Swiss-Prot/TrEMBL entries, http://kr.expasy.org/tools/pi_tool.html). Therefore, these domains *per se* could be used as therapeutic protein because it could have a high pharmacokinetic value.

**[0010]** Of VEGF and angiopoietin family proteins, VEGF-A and angiopoietin-2 (Ang2) are critical molecules for tumor angiogenesis (Holash, J. et al., *Science* 1999;284:1994-1998; Holash, J. et al., *Oncogene* 1999;18:5356-5362) and metastasis (Saaristo, A. et al., *Oncogene* 2000;19:6122-6129), age-related macular degeneration (Otani, A. et al., *Invest Ophthalmol. Vis. Sci.*, 1999;40:1912-1920), diabetic retinopathy (Watanabe, D. et al., *Am. J. Ophthalmol.* 2005;139:476-481), rheumatoid arthritis (Fearon, U. et al., *J. Rheumatol.* 2003;30:260-268; Paleolog, E.M. et al., *Arthritis Res.* 2002;4:S81-S90), psoriasis (Kuroda, K. et al., *J. Invest Dermatol.* 2001;116:713-720), acute and chronic inflammation (McDonald, D.M. et al., *Am. J. Respi. Cri. Care Med.* 2001;164:S39-S45; Roviezzo, F. et al., *J. Pharmacol. Exp. Ther.* 2005; 314: 738-744), atherosclerosis (Lim HS, et al., *Atherosclerosis*, 2005;180:113-118) and lymphatic proliferative diseases such as tumor lymphangiogenesis (Scavelli, C. et al., *Leukemia* 2004;18:1054-1058) and lymphatic metastasis (Sfiligoi, C. et al., *Int. J. Cancer* 2003;103:466-474). Therefore, the present invention provides synchronous blockade of VEGF-A and Ang2, preferably with a decoy receptor, intradiabody (double antibody) or RNA interference for treating VEGF-A and/or Ang2-associated diseases.

## SUMMARY OF THE INVENTION

[0011] The present invention also provides for a composition that binds to two different growth factors or cytokines. Preferably, such a composition is a fusion protein capable of binding two proteins simultaneously. In one aspect, such a fusion polypeptide is not an antibody. In a preferred aspect, such a fusion polypeptide includes a full-length form or fragment of the natural binding partners of the growth factors or cytokines. Most preferably, the fusion polypeptide is capable of synchronously binding vascular endothelial growth factor (VEGF) and angiopoietin, namely "double anti-angiogenic proteins (DAAP)". DAAP are disclosed which are therapeutically useful for treating VEGF and angiopoietin-associated conditions and diseases such as cancer, age-related macular degeneration, diabetic retinopathy, rheumatoid arthritis, psoriasis, acute and chronic inflammations, artherosclerosis and lymphatic proliferative diseases.

[0012] In one particular aspect, the present invention is directed to an isolated nucleic acid molecule encoding a polypeptide capable of synchronously binding VEGF polypeptide and angiopoietin polypeptide, which includes a nucleotide sequence encoding a Tie2 component and VEGFR component. The Tie2 and VEGFR components may be operatively linked to a nucleotide sequence encoding a multimerizing component. The VEGFR may be VEGFR1 or VEGFR3, without limitation. And the multimerizing component may be an immunoglobulin domain. In one aspect, the immunoglobulin domain may be the Fc domain of IgG, the heavy chain of IgG, or the light chain of IgG. Further, the Tie2 component may be located upstream or downstream of the VEGFR component.

[0013] In another aspect, in the nucleic acid molecule, the Tie2 component may include a nucleotide sequence encoding the amino acid sequences of Ig-like domain 1, Ig-like domain 2 and three EGF-like domains of the extracellular domain of Tie2. In another aspect, the VEGFR1 component may consist essentially of a nucleotide sequence encoding the amino acid sequences of Ig-like domain 2 of the extracellular domain of VEGFR1; and the VEGFR3 component may consist essentially of the amino acid sequences of Ig-like domain 1, Ig-like domain 2 and Ig-like domain 3 of the extracellular domain of VEGFR3.

[0014] In another aspect, the invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence encoding:

[0015] (a) the nucleotide sequence set forth in Table 1 referred to as DAAP#1;

[0016] (b) the nucleotide sequence set forth in Table 1 referred to as DAAP#2;

[0017] (c) the nucleotide sequence set forth in Table 1 referred to as DAAP#3;

[0018] (d) the nucleotide sequence set forth in Table 1 referred to as DAAP#4;

[0019] (e) the nucleotide sequence set forth in Table 1 referred to as DAAP#11;

[0020] (f) the nucleotide sequence set forth in Table 1 referred to as DAAP#12;

[0021] (g) the nucleotide sequence set forth in Table 1 referred to as DAAP#13;

[0022] (h) the nucleotide sequence set forth in Table 1 referred to as DAAP#14;

[0023] (i) the nucleotide sequence set forth in Table 1 referred to as DAAP#15;

[0024] (j) the nucleotide sequence set forth in Table 1 referred to as DAAP#16;

[0025] (k) the nucleotide sequence set forth in Table 1 referred to as DAAP#17; or

[0026] (l) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucleotide sequence of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), or (k) but which encodes identical amino acid sequence as expressed therefrom.

[0027] The invention is also directed to a vector that includes all of the nucleic acid molecules described above. The vector may be an expression vector.

[0028] The invention is also directed to a host-vector system for the production of a fusion polypeptide which includes the expression vector described above in a suitable host cell. Such a suitable host cell may include a bacterial cell, yeast cell, insect cell, or mammalian cell.

[0029] The invention is also directed to a fusion polypeptide encoded by any of the isolated nucleic acid molecules described above, including, but not limited to the amino acid sequence for DAAP#1-DAAP#4 and DAAP#11-DAAP#17.

[0030] The invention is also directed to a composition capable of synchronously binding VEGF and angiopoietin molecule to form a nonfunctional complex comprising a multimer of the fusion polypeptide described above including, but not limited to, those fusion constructs that use VEGFR1 or VEGFR3 components. The multimer may be a dimer.

[0031] In another aspect, the invention is directed to a method of producing a fusion polypeptide which includes growing cells of the host-vector system described above, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced. Such a fusion polypeptide may be modified by acetylation or pegylation. The acetylation may be accomplished with a molar excess of acetylation reagent ranging from at least about a 10 fold molar excess to about a 100 fold molar excess. The pegylation may be with 10K or 20K PEG.

[0032] In still another aspect, the invention is directed to a method of decreasing or inhibiting plasma leakage in a mammal, which includes administering to a mammal in need thereof an effective amount of the fusion polypeptide described herein. In a preferred embodiment, the leakage may be in the retina.

[0033] In still another aspect, the invention is directed to a method of blocking blood vessel growth in a mammal, which includes administering to a mammal in need thereof an effective amount of the fusion polypeptide described herein. In certain aspects, blood vessel growth blocking activity may be useful for treating cancer, age-related macular degeneration, diabetic retinopathy, rheumatoid arthritis, psoriasis, acute and chronic inflammation, artherosclerosis and lymphatic proliferative diseases, among others.

[0034] In still another aspect, the invention is directed to a method of attenuating or preventing tumor growth in a mammal, which includes administering to a mammal in need thereof an effective amount of the fusion polypeptide described herein.

[0035] In still another aspect, the invention is directed to a method of attenuating or preventing edema in a mammal, which includes administering to a mammal in need thereof an effective amount of the fusion polypeptide described herein. The edema may be retinal edema and brain edema.

[0036] In still another aspect, the invention is directed to a method of attenuating or preventing ascites formation in a mammal, which includes administering to a mammal in need thereof an effective amount of the fusion polypeptide described herein. The ascites may be associated with ovarian cancer. The invention is a also directed to a method of inhibiting VEGF receptor ligand and Tie2 ligand activities in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide described herein.

[0037] In still another aspect, the invention is directed to a method of attenuating or preventing inflammatory angiogenesis in a mammal, which includes administering to a mammal in need thereof an effective amount of the fusion polypeptide described herein. An example of a disorder caused by inflammatory angiogenesis is rheumatoid arthritis. Other examples of disorders caused by inflammatory angiogenesis include without limitation, spondyloarthropathies, psoriasis, diabetic retinopathy, artherosclerosis.

## BRIEF DESCRIPTION OF THE DRAWINGS

[0038] The present invention will become more fully understood from the detailed description given herein below, and the accompanying drawings which are given by way of illustration only, and thus are not limitative of the present invention, and wherein;

[0039] FIGURE 1 shows a schematic diagram of human VEGFR1 separated by three major regions: an extracellular domain consisting of seven immunoglobulin (Ig)-like domains, a transmembrane domain and an intracellular tyrosine kinase domain. FIGURE 1 also shows

a schematic diagram of human Tie2 separated by three major regions: an extracellular domain consisting of two Ig-like domain, three EGF-like domain, one Ig-like domain, three fibronectin type-III; a transmembrane domain and an intracellular tyrosine kinase domain.

**[0040]** FIGURE 2 shows structural interactions for binding between Ang2 and Tie2 and between VEGF and VEGFR1. The Ig-like domain 2 of Tie2 is essential for angiopoietin binding, and the Ig-like domain 1 and three EGF-like domains appear to be required for stable binding of angiopoietin, whereas the Ig-like domain 2 of VEGFR1 is essential for VEGF-A binding.

**[0041]** FIGURES 3A and 3B show schematic diagrams of DAAP (#1, #2, #3 and #4) constructs. FIGURE 3A shows subdomain assemblies from extracellular domains of Tie2 and VEGFR1, and FIGURE 3B shows corresponding amino acids from Tie2 and VEGFR1. EcoR1 and Xho1 are restriction enzyme sites.

**[0042]** FIGURE 4 shows schematic diagrams of gene constructs regarding pCMV-dhfr and pCMV-DAAP-dhfr vectors.

**[0043]** FIGURE 5 shows schematic diagrams of DAAP (#1, #11, #12, #13, #14, #15, #16 and #17) constructs for subdomain assemblies from extracellular domains of Tie2 and VEGFR1, and their corresponding amino acids from Tie2 and VEGFR1.

**[0044]** FIGURE 6 shows schematic diagrams of DAAP (#11, #12, #13, #14, #15, #16 and #17) constructs for subdomain assemblies from extracellular domains of Tie2 and VEGFR1, and their corresponding amino acids from Tie2 and VEGFR1.

**[0045]** FIGURE 7 shows Western blot analysis for DAAP (#1, #2, #3, #4, #11, #12, #13, #14, #15, #16 and #17), VEGF-trap, Tie2-Fc and Fc proteins.

**[0046]** FIGURE 8 shows a schematic diagram of ELISA binding assay for DAAP to VEGF-A.

**[0047]** FIGURE 9 shows a schematic diagram of ELISA binding assay for DAAP to Ang2.

**[0048]** FIGURE 10 shows the results of ELISA binding assay for DAAP to VEGF-A and Ang2.

**[0049]** FIGURES 11A and 11B show binding assay for competitive binding of DAAP to VEGF-A and Ang2.

**[0050]** FIGURES 12A-12D show Western blot analysis after the *in vitro* binding of DAAP to VEGF-A and Ang2.

**[0051]** FIGURE 13 shows a schematic diagram of synchronous binding of VEGF and angiopoietin to DAAP.

**[0052]** FIGURE 14 shows size and dimeric status of IgFc, DAAP#1, VEGF-Trap and Tie2-Fc. The proteins were produced in CHO cells, purified with Protein-A sepharose affinity chromatography, ~2 μg loaded under reducing and unreducing condition into SDS-PAGE, and stained with Coomassie blue.

**[0053]** FIGURES 15A and 15B show ELISA analyses of affinity constants of DAAP#1, VEGF-Trap and Tie2-Fc to (A) VEGF-A and (B) Ang2.

**[0054]** FIGURES 16A and 16B show ELISA analyses of further enhanced binding of DAAP#1 to VEGF-A or Ang2 when DAAP#1 is pre-occupied with (A) Ang2 or (B) VEGF-A.

**[0055]** FIGURE 17 shows *in vitro* pull down assays for detecting physical interactions between DAAP#1, VEGF-Trap or Tie2-Fc and several indicated types of VEGFs and angiopoietins.

**[0056]** FIGURE 18 shows theoretical isoelectric point values of different DAAPs.

**[0057]** FIGURE 19 shows a schematic diagram of extracellular matrix binding assay.

**[0058]** FIGURE 20 shows an ELISA assay for extracellular matrix binding for different DAAPs.

**[0059]** FIGURE 21 shows an ELISA assay for extracellular matrix binding for higher concentrations of DAAP#1, VEGF-Trap and Tie2-Fc.

**[0060]** FIGURE 22 shows a schematic diagram of pharmacokinetic analysis using mouse.

**[0061]** FIGURE 23 shows pharmacokinetic profiles of Fc, DAAP#1, VEGF-Trap and Tie-Fc.

**[0062]** FIGURE 24 shows a schematic diagram of generation of GFP-LLC implantation tumor model and treatment schemes of PBS, Fc, DAAP#1, VEGF-Trap and Tie2-Fc.

**[0063]** FIGURES 25A and 25B show effects of PBS, Fc, DAAP#1, VEGF-Trap and Tie2-Fc on GFP-LLC tumor growth according to FIGURE 24. A. presents photograph of tumor size at 29 day after tumor implantation and at 24 days after the treatments. B. presents changes of tumor volumes (mean± SE) in each group (n=4).

**[0064]** FIGURE 26 shows representative images of blood vessels in the tumors of mice treated with Fc, VEGF-Trap or DAAP#1.

**[0065]** FIGURE 27 shows a schematic diagram of generation of ROP model and treatment schemes of PBS, Fc, DAAP#1, VEGF-Trap and Tie2-Fc.

**[0066]** FIGURES 28A-28F show effects of PBS, Fc, DAAP#1, VEGF-Trap and Tie2-Fc on retinal vasculatures of ROP mouse model. A, B, C and D show retinal vasculatures of the entire retina, optic disc, middle portion and marginal portion. E and F show effects of PBS, Fc, DAAP#1, VEGF-Trap and Tie2-Fc on neovascular tufts area and marginal blood vessel density in the ROP model.

**[0067]** FIGURES 29A-29B show creation of rheumatoid arthritis model animals. A. shows a schematic diagram for generation of experimental mouse model of collagen type-II-induced rheumatoid arthritis (CIA) and time of treatment. B. shows mean clinical arthritis scores in the paws that were determined and quantified based on severity of swelling, erythema, and joint rigidity at indicated times.

**[0068]** FIGURES 30A-30B show treatment of rheumatoid arthritis. A. shows representative radiographic findings of the knee and ankle joints of the RA mice treated with indicated agents. B. shows radiographic scores of bone destruction in the knee, ankle and tarsometatarsal joints of the CIA mice treated with indicated agents. Values are mean ± SD.

**[0069]** FIGURES 31A-31B show treatment of rheumatoid arthritis. A. shows representative histopathologic findings of the knee and ankle joints of the CIA mice treated with indicated agents for 18 days. B. shows histopathologic scores of bone abnormality in the knee and ankle joints of the CIA mice treated with indicated agents for 18 days. Values are mean ± SD.

**[0070]** TABLE 1 shows the nucleic acid and amino acid sequences of DAAP#1, DAAP#2, DAAP#3, DAAP#4, DAAP#11, DAAP#12, DAAP#13, DAAP#14, DAAP#15, DAAP#16, DAAP#17. In particular, SEQ ID NO:1 represents the sense strand of the nucleic acid depicted for DAAP#1. SEQ ID NO:2 represents the amino acid sequence depicted for DAAP#1.

**[0071]** SEQ ID NO:3 represents the sense strand of the nucleic acid depicted for DAAP#2. SEQ ID NO:4 represents the amino acid sequence depicted for DAAP#2.

**[0072]** SEQ ID NO:5 represents the sense strand of the nucleic acid depicted for DAAP#3. SEQ ID NO:6 represents the amino acid sequence depicted for DAAP#3.

**[0073]** SEQ ID NO:7 represents the sense strand of the nucleic acid depicted for DAAP#4. SEQ ID NO:8 represents the amino acid sequence depicted for DAAP#4.

**[0074]** SEQ ID NO:9 represents the sense strand of the nucleic acid depicted for DAAP#11. SEQ ID NO:10 represents the amino acid sequence depicted for DAAP#11.

[0075] SEQ ID NO:11 represents the sense strand of the nucleic acid depicted for DAAP#12. SEQ ID NO:12 represents the amino acid sequence depicted for DAAP#12.

[0076] SEQ ID NO:13 represents the sense strand of the nucleic acid depicted for DAAP#13. SEQ ID NO:14 represents the amino acid sequence depicted for DAAP#13.

[0077] SEQ ID NO:15 represents the sense strand of the nucleic acid depicted for DAAP#14. SEQ ID NO:16 represents the amino acid sequence depicted for DAAP#14.

[0078] SEQ ID NO:17 represents the sense strand of the nucleic acid depicted for DAAP#15. SEQ ID NO:18 represents the amino acid sequence depicted for DAAP#15.

[0079] SEQ ID NO:19 represents the sense strand of the nucleic acid depicted for DAAP#16. SEQ ID NO:20 represents the amino acid sequence depicted for DAAP#16.

[0080] SEQ ID NO:21 represents the sense strand of the nucleic acid depicted for DAAP#17. SEQ ID NO:22 represents the amino acid sequence depicted for DAAP#17.

## DETAILED DESCRIPTION OF THE INVENTION

[0081] In the present application, "a" and "an" are used to refer to both single and a plurality of objects.

[0082] As used herein, "about" or "substantially" generally provides a leeway from being limited to an exact number. For example, as used in the context of the length of a polypeptide sequence, "about" or "substantially" indicates that the polypeptide is not to be limited to the recited number of amino acids. A few amino acids add to or subtracted from the N-terminus or C-terminus may be included so long as the functional activity such as its binding activity is present.

[0083] As used herein, administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[0084] As used herein, "amino acid" and "amino acids" refer to all naturally occurring L-α-amino acids. This definition is meant to include norleucine, ornithine, and homocysteine.

[0085] As used herein, in general, the term "amino acid sequence variant" refers to molecules with some differences in their amino acid sequences as compared to a reference (e.g. native sequence) polypeptide. The amino acid alterations may be substitutions, insertions, deletions or any desired combinations of such changes in a native amino acid sequence.

**[0086]** Substitutional variants are those that have at least one amino acid residue in a native sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

**[0087]** Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, and so on.

**[0088]** Insertional variants are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a native amino acid sequence. Immediately adjacent to an amino acid means connected to either the α-carboxy or α-amino functional group of the amino acid.

**[0089]** Deletional variants are those with one or more amino acids in the native amino acid sequence removed. Ordinarily, deletional variants will have one or two amino acids deleted in a particular region of the molecule.

**[0090]** As used herein, "antagonist" refers to a ligand that tends to nullify the action of another ligand, as a ligand that binds to a cell receptor without eliciting a biological response.

**[0091]** Preferred biological activities of the ligands of the present invention include the ability to inhibit vascular permeability. The ability to inhibit vascular permeability will be useful for treatment of medical conditions and diseases such as diabetic retinopathy, edema, and ascites. Preferred biological activities of the ligands of the present invention include the ability to maintain endothelial cell integrity (including preventing apoptosis). The ability to maintain endothelial cell integrity will be useful for treatment of medical conditions and diseases such as mannitol treatment, irradiation, and sepsis.

[0092] It is also contemplated that DAAP fusion proteins be labeled with a detectable label, such as radioisotope, fluorescent tag, enzymatic tag, or a chemiluminescent tag to determine ligand-receptor binding interaction. As such, assay systems employing the chimeric molecule is also contemplated.

[0093] As used herein, "carriers" include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the pharmaceutically acceptable carrier is an aqueous pH buffered solution. Examples of pharmaceutically acceptable carriers include without limitation buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, polyethylene glycol (PEG), and PLURONICS®.

[0094] As used herein, “consisting essentially of” when used in the context of a nucleic acid sequence or amino acid sequence refers to the sequence that is essential to carry out the intended function of the amino acid encoded by the nucleic acid.

[0095] As used herein, "effective amount" is an amount sufficient to effect beneficial or desired clinical or biochemical results. An effective amount can be administered one or more times. For purposes of this invention, an effective amount of an inhibitor compound is an amount that is sufficient to palliate, ameliorate, stabilize, reverse, slow or delay the progression of the disease state.

[0096] As used herein, “fragments” or “functional derivatives” refers to biologically active amino acid sequence variants and fragments of the native ligands or receptors of the present invention, as well as covalent modifications, including derivatives obtained by reaction with organic derivatizing agents, post-translational modifications, derivatives with nonproteinaceous polymers, and immunoadhesins.

[0097] As used herein, "host cell" includes an individual cell or cell culture which can be or has been a recipient of a vector of this invention. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in total DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation and/or change.

**[0098]** As used herein, "ligand" refers to any molecule or agent, or compound that specifically binds covalently or transiently to a molecule such as a polypeptide. When used in certain context, ligand may include antibody. In other context, "ligand" may refer to a molecule sought to be bound by another molecule with high affinity, such as in a ligand trap.

**[0099]** As used herein, "mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, and so on. Preferably, the mammal is human.

**[00100]** As used herein "pharmaceutically acceptable carrier and/or diluent" includes any and all solvents, dispersion media, coatings antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

**[00101]** 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 material 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 material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired.

**[00102]** The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

**[00103]** As used herein, "sample" or "biological sample" is referred to in its broadest sense, and includes any biological sample obtained from an individual, body fluid, cell line,

tissue culture, or other source which may contain a chimeric Angl binding factor, depending on the type of assay that is to be performed. As indicated, biological samples include body fluids, such as semen, lymph, sera, plasma, urine, synovial fluid, spinal fluid and so on. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art.

**[00104]** As used herein, "subject" is a vertebrate, preferably a mammal, more preferably a human.

**[00105]** As used herein, "synchronous" or "synchronously" binding refers to the binding of the DAAP protein to two or more designated proteins simultaneously if the proteins are available for binding.

**[00106]** As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. "Palliating" a disease means that the extent and/or undesirable clinical manifestations of a disease state are lessened and/or the time course of the progression is slowed or lengthened, as compared to a situation without treatment.

**[00107]** As used herein, "vector", "polynucleotide vector", "construct" and "polynucleotide construct" are used interchangeably herein. A polynucleotide vector of this invention may be in any of several forms, including, but not limited to, RNA, DNA, RNA encapsulated in a retroviral coat, DNA encapsulated in an adenovirus coat, DNA packaged in another viral or viral-like form (such as herpes simplex, and adeno- structures, such as polyamides.

**[00108]** **Inflammatory Angiogenesis.**

**[00109]** Angiogenesis is defined as new vessel formation from pre-existng blood vessels and is an important process in health and disease. The perpetuation of angiogenesis in inflammatory diseases, such as rheumatoid arthritis, spondyloarthropathies, psoriasis, diabetic retinopathy, artherosclerosis, might facilitate the ingress of inflammatory cells to pathological regions. These disorders associated with perpetuated angiogenesis are considered to be "inflammatory angiogenesis" diseases. Several angiogenic mediators, including growth

factors, cytokines, matrix metalloproteinases, matrix macromolecules, cell adhesion receptors, chemokines and chemokine receptors, have been implicated in the inflammatory angiogenesis. There is a regulatory network in inflamed tissues that is involved in the upregulation or downregulation of angiogenesis. (Lainer-Carr and Brahn *Nature Clinical Practice Rheumatology* 3, 434-442, 2007)

**[00110]** The start and propagation of inflammatory angiogenesis may occur according to the following scenario. (1) Different stimuli (autoimmune reaction, acute and chronic inflammation, acute and chronic hypoxia, tumor cell proliferation, necrosis, and apoptosis) cause activation of resident macrophages and other phagocytes causing release of proinflammatory mediators. (2) These phagocyte-derived factors activate the endothelial cells to produce adhesion molecules, chemokines, and cytokines. (3) The adhesion molecules and soluble factors from the activated endothelium recruit and stimulate circulating phagocytes thus increasing the mass of reactive cells. (4)The tissue remodeling–activated phagocytes, in turn, release soluble factors that promote the outgrowth and migration of endothelial cells to form new vessels. The effectiveness of the phagocyte-derived angiogenic stimuli must reach a "critical mass," therefore, step (4) is possible only after additional phagocyte recruitment.

**[00111] Growth Factors and Cytokines**

**[00112]** Any growth factors and cytokines that are desired to be simultaneously bound may be made, including those that have similar effects on a disease progression. Growth factors and cytokines encompassed by the present invention include without limitation, VEGF-A, VEGF-B VEGF-C, VEGF-D, PlGF, PDGF, angiopoietin-1, angiopoietin-2, angiopoietin-3, angiopoietin-4, HGF, EGF, FGF, TNF-α, interleukin-1β, interleukin-6, interleukin-12, TGF-β, RANKL, and so on.

**[00113] Fusion Polypeptide Candidates for Binding to Growth Factors and Cytokines**

**[00114]** In practicing the present invention, one method of making the fusion polypeptides involving the following criteria for selectively making the fusion polypeptide may be used. Binding regions of the different receptors for two or more growth factors with similar effect on disease progression, may be linked to block the actions of the double, triple or multiple growth factors simultaneously. Novel fusion polypeptide candidates can be screened for their synchronous binding affinities and tested for neutralizing activities against growth factors and cytokines for binding of any combination of growth factors and cytokines. For instances, VEGF-A and TNF-α; VEGF-A and interleukin-1β; VEGF-A and VEGF-C; VEGF-C and angiopoietin; VEGF-C and TNF-α; TNF-α and angiopoietin; and VEGF-C and HGF. Any

combination of any growth factors can be created and used in accordance with the present invention. Combinations may be applied to triple or multiple binding to three or more growth factors and not confined to double blockage listed herein.

**[00115] Nucleic Acid Constructs**

**[00116]** Also provided is an expression vector comprising a nucleic acid molecule of the invention as described herein, wherein the nucleic acid molecule is operatively linked to an expression control sequence. Also provided is a host-vector system for the production of a fusion polypeptide which comprises the expression vector of the invention which has been introduced into a host cell suitable for expression of the fusion polypeptide. The suitable host cell may be a bacterial cell such as *E. coli*, a yeast cell, such as *Pichia pastoris*, an insect cell, such as *Spodoptera frugiperda*, or a mammalian cell, such as a COS or CHO cell.

**[00117]** The present invention also provides for methods of producing the fusion polypeptides of the invention by growing cells of the host-vector system described herein, under conditions permitting production of the fusion polypeptide and recovering the fusion polypeptide so produced. The fusion polypeptides useful for practicing the present invention may be prepared by expression in a prokaryotic or eukaryotic expression system.

**[00118]** The recombinant gene may be expressed and the polypeptide purified utilizing any number of methods. The gene may be subcloned into a bacterial expression vector, such as for example, but not by way of limitation, pZErO.

**[00119]** The fusion polypeptides may be purified by any technique which allows for the subsequent formation of a stable, biologically active protein. For example, and not by way of limitation, the factors may be recovered from cells either as soluble proteins or as inclusion bodies, from which they may be extracted quantitatively by 8M guanidinium hydrochloride and dialysis. In order to further purify the factors, any number of purification methods may be used, including but not limited to conventional ion exchange chromatography, affinity chromatography, different sugar chromatography, hydrophobic interaction chromatography, reverse phase chromatography or gel filtration.

**[00120]** When used herein, fusion polypeptide includes functionally equivalent molecules in which amino acid residues are substituted for residues within the sequence resulting in a silent or conservative change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent or conservative alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine,

isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the invention are proteins or fragments or derivatives thereof which exhibit the same or similar biological activity and derivatives which are differentially modified during or after translation, e.g., by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc.

**[00121]** Cells that express the fusion polypeptides of the invention are genetically engineered to produce them by, for example, transfection, transduction, electropration, or microinjection techniques.

**[00122]** In addition, the present invention contemplates use of the fusion polypeptides described herein in tagged form.

**[00123]** Any of the methods known to one skilled in the art for the insertion of DNA fragments into a vector may be used to construct expression vectors encoding the fusion polypeptides of the invention using appropriate transcriptional/translational control signals and protein coding sequences. These methods may include *in vitro* recombinant DNA and synthetic techniques and *in vivo* recombinations (genetic recombination). Expression of nucleic acid sequence encoding the fusion polypeptides of the invention may be regulated by a second nucleic acid sequence so that the fusion polypeptide is expressed in a host transformed with the recombinant DNA molecule. For example, expression of the fusion polypeptides described herein may be controlled by any promoter/enhancer element known in the art. Promoters which may be used to control expression of the fusion polypeptide include, but are not limited to the long terminal repeat as described in Squinto et al., (1991, Cell 65:1-20); the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290:304-310), the CMV promoter, the M-MuLV 5' terminal repeat the promoter contained in the 3'long terminal repeat of Rous sarcoma virus (Yamamoto, et al., 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, Proc. Natl. Acad. Sci. U.S.A. 78:144-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, Nature 296:39-42); prokaryotic expression vectors such as the β-lactamase promoter (Villa-Kamaroff, et al., 1978, Proc. Natl. Acad. Sci. U.S.A. 75:3727-3731), or the tac promoter (DeBoer, et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:21-25), see also "Useful proteins from recombinant bacteria" in Scientific American, 1980, 242:74-94; promoter elements from yeast or other fungi such as the Gal 4 promoter, the ADH (alcohol dehydrogenase) promoter, PGK

(phosphoglycerol kinase) promoter, alkaline phosphatase promoter, and the following animal transcriptional control regions, which exhibit tissue specificity and have been utilized in transgenic animals: elastase I gene control region which is active in pancreatic acinar cells (Swift et al., 1984, Cell 38:639-646; Ornitz et al., 1986, Cold Spring Harbor Symp. Quant. Biol. 50:399-409; MacDonald, 1987, Hepatology 7:425-515); insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, Nature 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl et al., 1984, Cell 38:647-658; Adames et al., 1985, Nature 318:533-538; Alexander et al., 1987, Mol. Cell. Biol. 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder et al., 1986, Cell 45:485-495), albumin gene control region which is active in liver (Pinkert et al., 1987, Genes and Devel. 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf et al., 1985, Mol. Cell. Biol. 5:1639-1648; Hammer et al., 1987, Science 235:53-58); alpha 1-antitrypsin gene control region which is active in the liver (Kelsey et al., 1987, Genes and Devel. 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, Nature 315:338-340; Kollias et al., 1986, Cell 46:89-94); myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, Cell 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Shani, 1985, Nature 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, Science 234:1372-1378).

**[00124]** Thus, according to the invention, expression vectors capable of being replicated in a bacterial or eukaryotic host comprising nucleic acids encoding a fusion polypeptide as described herein, and in particular modified DAAP, are used to transfect the host and thereby direct expression of such nucleic acid to produce fusion polypeptides which may then be recovered in biologically active form. As used herein, a biologically active form includes a form capable of binding to the relevant receptor and causing a differentiated function and/or influencing the phenotype of the cell expressing the receptor. Such biologically active forms would, for example, block phosphorylations of the VEGFR1, VEGFR2, VEGRF3 and Tie2 receptors, or inhibiting of synthesis of cellular DNA.

**[00125]** Expression vectors containing the nucleic acid inserts can be identified by without limitation, at least three general approaches: (a) DNA-DNA hybridization, (b) presence or absence of "marker" gene functions, and (c) expression of inserted sequences. In the first approach, the presence of foreign nucleic acids inserted in an expression vector can be detected by DNA-DNA hybridization using probes comprising sequences that are

homologous to an inserted nucleic acid sequences. In the second approach, the recombinant vector/host system can be identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, transformation phenotype, occlusion body formation in baculovirus, etc.) caused by the insertion of foreign nucleic acid sequences in the vector. For example, if an *efl* nucleic acid sequence is inserted within the marker gene sequence of the vector, recombinants containing the insert can be identified by the absence of the marker gene function. In the third approach, recombinant expression vectors can be identified by assaying the foreign nucleic acid product expressed by the recombinant constructs. Such assays can be based, for example, on the physical or functional properties of the nucleic acid product of interest, for example, by binding of a ligand to a receptor or portion thereof which may be tagged with, for example, a detectable antibody or portion thereof or binding to antibodies produced against the protein of interest or a portion thereof.

**[00126]** The fusion polypeptide, in particular modified DAAP of the present invention, may be expressed in the host cells transiently, constitutively or permanently.

**[00127]** The invention herein further provides for the development of a fusion polypeptide as a therapeutic agent for the treatment of patients suffering from disorders involving cells, tissues or organs which express the VEGFR1, VEGFR2, VEGFR3, or Tie2 receptors. Such molecules may be used in a method of treatment of the human or animal body, or in a method of diagnosis.

**[00128]** Effective doses useful for treating these or other diseases or disorders may be determined using methods known to one skilled in the art (see, for example, Fingl, et al., The Pharmacological Basis of Therapeutics, Goodman and Gilman, eds. Macmillan Publishing Co, New York, pp. 1-46 (1975). Pharmaceutical compositions for use according to the invention include the fusion polypeptides described above in a pharmacologically acceptable liquid, solid or semi-solid carrier, linked to a carrier or targeting molecule (e.g., antibody, hormone, growth factor, etc.) and/or incorporated into liposomes, microcapsules, and controlled release preparation prior to administration *in vivo*. For example, the pharmaceutical composition may comprise a fusion polypeptide in an aqueous solution, such as sterile water, saline, phosphate buffer or dextrose solution. Alternatively, the active agents may be comprised in a solid (e.g. wax) or semi-solid (e.g. gelatinous) formulation that may be implanted into a patient in need of such treatment. The administration route may be any mode of administration known in the art, including but not limited to intravenously,

intrathecally, subcutaneously, intrauterinely, by injection into involved tissue, intraarterially, intranasally, orally, or via an implanted device.

**[00129]** Administration may result in the distribution of the active agent of the invention throughout the body or in a localized area. For example, in some conditions which involve distant regions of the nervous system, intravenous or intrathecal administration of agent may be desirable. In some situations, an implant containing active agent may be placed in or near the lesioned area. Suitable implants include, but are not limited to, gelfoam, wax, spray, or microparticle-based implants.

**[00130]** The present invention also provides for pharmaceutical compositions comprising the fusion polypeptides described herein, in a pharmacologically acceptable vehicle. The compositions may be administered systemically or locally. Any appropriate mode of administration known in the art may be used, incuding, but not limited to, intravenous, intrathecal, intraarterial, intranasal, oral, subcutaneous, intraperitoneal, or by local injection or surgical implant. Sustained release formulations are also provided for.

**[00131]** **Gene Therapy**

**[00132]** In a specific embodiment, nucleic acids comprising sequences encoding the chimeric Angl polypeptide are administered to prevent vascular leakage, and for therapeutic vasculogenesis, 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 protein that mediates a therapeutic effect.

**[00133]** Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

**[00134]** For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical 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); May, TIBTECH 11(5):155-215 (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, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

**[00135]** In a preferred aspect, nucleic acid sequences may encode a chimeric-Angl or Tie2 polypeptide, in which the nucleic acid sequences are part of expression vectors that express the polypeptides in a suitable host. In particular, such nucleic acid sequences have promoters

operably linked to the polypeptide coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the polypeptide coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989).

**[00136]** Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids *in vitro*, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or *ex vivo* gene therapy.

**[00137]** In a specific embodiment, the nucleic acid sequences are directly administered *in vivo*, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors, or by direct injection of naked DNA, or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors) and so on. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor. Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

**[00138]** In a specific embodiment, viral vectors that contain nucleic acid sequences encoding the polypeptide are used. The nucleic acid sequences encoding the polypeptide to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. Retroviral vectors, adenoviral vectors and adeno-associated viruses are examples of viral vectors that may be used. Retroviral vectors contain the components

necessary for the correct packaging of the viral genome and integration into the host cell DNA.

**[00139]** Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia because they naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. In addition, adeno-associated virus (AAV) has also been proposed for use in gene therapy.

**[00140]** Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

**[00141]** In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion and so on. Numerous techniques are known in the art for the introduction of foreign genes into cells and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

**[00142]** Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T-lymphocytes, B-lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, and so on.

**[00143]** In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

**[00144]** In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding the polypeptide are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered *in vivo* for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained *in vitro* can potentially be used in accordance with this embodiment of the present invention.

**[00145]** In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription.

**[00146] Therapeutic Composition**

**[00147]** In one embodiment, the present invention relates to treatment for various diseases that are characterized by vascular leakage or lack of blood vessel formation. In this way, the inventive therapeutic compound may be administered to human patients who are either suffering from, or prone to suffer from the disease by providing compounds that activate Tie2.

**[00148]** The formulation of therapeutic compounds is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, Pa., USA. For example, from about 0.05 μg to about 20 mg per kilogram of body weight per day may be administered. Dosage regime may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The active compound may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intra nasal, intradermal or suppository routes or implanting (eg using slow release molecules by the intraperitoneal route or by using cells e.g. monocytes or dendrite cells sensitised *in vitro* and adoptively transferred to the recipient). Depending on the route of administration, the peptide may be required to be coated in a material to protect it from the action of enzymes, acids and other natural conditions which may inactivate said ingredients.

**[00149]** For example, the low lipophilicity of the peptides will allow them to be destroyed in the gastrointestinal tract by enzymes capable of cleaving peptide bonds and in the stomach by acid hydrolysis. In order to administer peptides by other than parenteral administration, they will be coated by, or administered with, a material to prevent its inactivation. For

example, peptides may be administered in an adjuvant, co-administered with enzyme inhibitors or in liposomes. Adjuvants contemplated herein include resorcinols, non-ionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes.

**[00150]** The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

**[00151]** The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity 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 superfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, chlorobutanol, phenol, sorbic acid, theomersal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the composition of agents delaying absorption, for example, aluminium monostearate and gelatin.

**[00152]** Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterile active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a

powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

**[00153]** When the peptides are suitably protected as described above, the active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

**[00154]** The tablets, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

**[00155] Delivery Systems**

**[00156]** Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis, construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but

are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions 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 addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

**[00157]** In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions 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 during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody or a peptide of the invention, care must be taken to use materials to which the protein does not absorb. In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome. In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, thus requiring only a fraction of the systemic dose.

**[00158]** **Labels**

**[00159]** Suitable enzyme labels include, for example, those from the oxidase group, which catalyze the production of hydrogen peroxide by reacting with substrate. Glucose oxidase is particularly preferred as it has good stability and its substrate (glucose) is readily available. Activity of an oxidase label may be assayed by measuring the concentration of hydrogen peroxide formed by the enzyme-labeled antibody/substrate reaction. Besides enzymes, other suitable labels include radioisotopes, such as iodine ($^{125}I$, $^{121}I$), carbon ($^{14}C$), sulphur ($^{35}S$), tritium ($^{3}H$), indium ($^{112}In$), and technetium ($^{99m}Tc$), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

**[00160]** Further suitable labels for the chimeric-Ang1, Tie2 or chimeric Ang1/Tie2 complex-specific antibodies of the present invention are provided below. Examples of suitable enzyme labels include malate dehydrogenase, δ-5-steroid isomerase, yeast-alcohol dehydrogenase, α-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

**[00161]** Examples of suitable radioisotopic labels include $^{3}H$, $^{111}In$, $^{125}I$, $^{131}I$, $^{32}P$, $^{35}S$, $^{14}C$, $^{51}Cr$, $^{57}To$, $^{58}Co$, $^{59}Fe$, $^{75}Se$, $^{152}Eu$, $^{90}Y$, $^{67}Cu$, $^{217}Ci$, $^{211}At$, $^{212}Pb$, $^{47}Sc$, $^{109}Pd$, etc. $^{111}In$ is preferred isotope where *in vivo* imaging is used since its avoids the problem of dehalogenation of the $^{125}I$ or $^{131}I$-labeled polypeptide by the liver. In addition, this radionucleotide has a more favorable gamma emission energy for imaging. For example, $^{111}In$ coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumors tissues, particularly the liver, and therefore enhances specificity of tumor localization.

**[00162]** Examples of suitable non-radioactive isotopic labels include $^{157}Gd$, $^{55}Mn$, $^{162}Dy$, $52^{Tr}$, and $^{56}Fe$.

**[00163]** Examples of suitable fluorescent labels include an $^{152}Eu$ label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, an o-phthaldehyde label, and a fluorescamine label.

**[00164]** Examples of suitable toxin labels include, Pseudomonas toxin, diphtheria toxin, ricin, and cholera toxin.

**[00165]** Examples of chemiluminescent labels include a luminal label, an isoluminal label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label.

**[00166]** Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron. Deuterium may also be used. Other contrasting agents also exist for EPR, PET or other imaging mechanisms, which are known to persons of skill in the art.

**[00167]** Typical techniques for binding the above-described labels to polypeptides are provided by Kennedy et al. (1976) Clin. Chim. Acta 70:1-31, and Schurs et al. (1977) Clin. Chim. Acta 81:1-40. Coupling techniques include the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

## EXAMPLES

**[00168]** **Example 1** – Experimental Protocol

**[00169]** Gene constructs encoding four different assembled fusion proteins (DAAP#1, DAAP#2, DAAP#3 and DAAP#4) (**Figure 3A** and **3B**) consisting of Ig-like domain 1 of human Tie2, Ig-like domain 2 of human Tie2, three EGF-like domains of human Tie2, Ig-like domain of human VEGFR1 and Fc domain of human IgG were cloned into the pCMV-dhfr vector (Hwang SJ, et al., *Protein Express Purif.* 2005;39:175-183) (**Figure 4**).

**[00170]** Because only DAAP#1 displayed synchronous binding to VEGF-A and Ang2, seven modified forms (DAAP#11, DAAP#12, DAAP#13, DAAP#13, DAAP#14, DAAp#15, DAAP#16 and DAAP#17) of DAAP#1 gene constructs encoding the fusion proteins capable of binding to VEGF-A and Ang2 were further made (**Figure 5** and **Figure 6**).

**[00171]** All DAAP recombinant proteins were obtained by transient expression in HEK293 cells (American Type Culture Collection, Manassas, VA) using Effectene liposomal transfection according to the manufacturer's instructions (Qiagen, Inc., Hilden, Germany). The supernatant was harvested from transfected cells after 48-96 hour. The recombinant proteins were purified using Protein-A sepharose affinity chromatography, with subsequent acid elution and neutralization. After purification, the protein was quantified using the Bradford assay and confirmed with Coomassie blue staining of an SDS-PAGE gel. For the Western blotting analysis, one hundred nanograms of each sample was mixed with sample buffer, heat-denatured for 10 min, run on 10% SDS-PAGE, and electro-blotted onto nitrocellulose membranes. The membrane was blocked with 5% nonfat milk in Tris-buffer solution (50mM Tris, 100 mM NaCl, pH 7.5) containing 0.05% TritonX-100 and Western blotted with horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170) to detect Fc-fused proteins. Signal was visualized by chemiluminescent detection according to the manufacturer's protocol (Amersham Pharmacia Biotech) using chemiluminescence scanner (LAS-1000, Fuji Film, Tokyo). (**Figure 7**).

**[00172]** Binding capability of DAAP recombinant protein to VEGF-A or Ang2 was measured by the enzyme-linked immunosorbent assay (ELISA) (**Figure 8** and **Figure 9**). Two hundred nanograms of VEGF-$A_{165}$ (produced from CHO cell) (hereafter referred to as VEGF-A) or Ang2 (produced from CHO cell) in 100 μl of phosphate buffered saline (PBS) was aliquoted into 96-well plate and incubated at 4°C for overnight. After washing the plate 3 times with each 400 μl of PBS, a blocking was performed with the blocking solution (1%

bovine serum albumin (BSA) in 100 μl PBS) at 37°C for 2 hour. One hundred nanogram of each DAAP protein in 100 μl of blocking solution was added into the plate, and were incubated at 37°C for 2 hour. The same amount of VEGF-trap (Holash, J. et al., *Proc. Natl. Acad. Sci. U. S. A* **99**:11393-11398), Tie2-Fc or Fc recombinant protein was added and incubated as the same manner as a positive and negative control. After washing the plate 3 times with each 400 μl of PBS, 50 μl of horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170) was added into the plate, and were incubated at 37°C for 2 hour. After washing the plate 3 times with each 400 μl of PBS, 50 μl of 3,3',5,5'-tetramethylbenzidine (TMB) solution (Sigma-Aldrich T0440) was added into the plate, and were incubated at room temperature for 10 min. Reaction was stopped by adding 50 μl of 1 M HCl and reactive colors were analyzed at optical density 415 nm by ELISA reader (BioRad M680).

**[00173]** Recombinant Chinese hamster ovary (rCHO) cells expressing VEGF-trap (CHO-VT1) were established following a previously described method (Hwang SJ, et al., *Protein Express Purif.* 2005;39:175-183). Briefly, CHO-VT1 cells were established by transfection of a vector containing the dihydrofolate reductase (*dhfr*) and VEGF-trap (Holash J. et al., PNAS 99:11393-11398, 2002) genes into *dhfr*-deficient CHO cells (CRL-9096, American Type Culture Collection, Manassas, Virginia, USA). This was followed by *dhfr*/methotrexate (MTX)-mediated gene amplification. The three stable rCHO cells secreting VEGF-trap were selected with serial amplified concentrations of MTX (0.02-1.0 μM, Sigma-Aldrich). Among them, one cell line expressing the highest amount of VEGF-trap was chosen and named as "CHO-VT1". CHO-VT1 cells were grown and maintained in Iscove's modified Dulbecco's medium supplemented with 5% dialyzed fetal bovine serum (Invitrogen, Carlsbad, California, USA) and 1 μM MTX (Sigma-Aldrich). For recombinant VEGF-trap protein production, CHO-VT1 cells were inoculated at 2 $x10^5$ cells/mL in 250-ml Erlenmeyer flasks containing 100 ml of medium on an orbital shaker (Vision, Bucheon, Korea) at 110 rpm in a humidified 5% $CO_2$ incubator at 37°C. After indicated days, the culture medium containing VEGF-trap recombinant protein were purified by using Protein-A sepharose affinity chromatography, acid elution and subsequent neutralization. After purification, the protein was quantitated using the Bradford assay and confirmed with Coomassie blue staining of an SDS-PAGE gel. The analysis showed that approximately 10 mg/L of VEGF-trap was harvested.

**[00174]** **Example 2** –

**[00175]** The ELISA analyses indicated that DAAP#1, DAAP#14, DAAP#16 and DAAP#17 were capable of binding VEGF-A and Ang2 (**Figure 10**). DAAP#2, DAAP#3, DAAP#4, DAAP#11 and Fc were incapable of binding to VEGF-A and Ang2 (**Figure 10**). DAAP#12 and VEGF-trap were capable of preferential and selective binding to VEGF-A, whereas DAAP#13 and Tie2-Fc were capable of preferential and selective binding to VEGF-A (**Figure 10**). Because the binding ability of DAAP#1 and DAAP#14 to VEGF-A and/or Ang2 was comparable to that of VEGF-trap or Tie2-Fc, DAAP#1 and DAAP#14 are likely to be the strongest candidates for further development as therapeutic proteins.

**[00176]** To examine whether DAAP recombinant protein are capable of synchronously binding to VEGF and Ang2, 100 ng of each DAAP recombinant protein, (DAAP#1, DAAP#12, DAAP#13, DAAP#14, DAAP#15, DAAP#16 and DAAP#17) was pre-incubated with serial amounts (0 ng, 10 ng, 100 ng, 1,000 ng) of Ang2 or VEGF-A in 100 μl of blocking solution at 37°C for 2 hours before their addition to the plates coated with VEGF-A or Ang2 for the ELISA as described above. The same amount of VEGF-trap, Tie2-Fc or Fc recombinant protein was pre-incubated with the serial amounts of Ang2 or VEGF-A in the same manner as a positive and negative control. The preincubation of Ang2 or VEGF-A occupied the binding site of DAAP proteins to Ang2 or VEGF-A. In the Ang2 preincubation, DAAP#1, DAAP#12, DAAP#14, DAAP#16, DAAP#17 and VEGF-trap were capable of binding VEGF (**Figure 11A**). In comparison, in the VEGF preincubation, DAAP#1, DAAP#13, DAAP#14, DAAP#16, DAAP#17 and Tie2-Fc were capable of binding Ang2 (**Figure 11B**). Therefore, DAAP#1, DAAP#14, DAAP#16 and DAAP#17 are capable of synchronously binding VEGF-A and Ang2 (**Figure 12**).

**[00177]** For further analysis of binding abilities of DAAP#1, DAAP#14, VEGF-trap, and Tie2-Fc to VEGF-A and Ang2, *in vitro* binding assays were performed (**Figure 13**). Five hundred nanograms of each DAAP#1, DAAP#14, VEGF-trap or Tie2-Fc were incubated with 100 ng of FLAG-tagged VEGF-A, FLAG-tagged Ang2, or both VEGF-A and Ang2 in 500 μl Tris-buffer solution (50 mM Tris 100 mM NaCl, pH p.7.5) containing 1.0 % Nonidet P-40 at 4°C for 2 hour. Then, 20 μl of Protein-A agarose beads (Oncogene) were added and incubated at 4°C for another 2 hours. The Protein-A conjugated samples were washed 3 times with 1 ml of Tris-buffer solution containing 1.0% Nonidet P-40. The samples were eluted with sample buffer, and heat-denatured. The samples were separated by 10% SDS-PAGE, and electro-blotted onto nitrocellulose membranes. The membranes were blocked with 5% nonfat milk in Tris-buffer solution (50mM Tris, 100 mM NaCl, pH 7.5) containing 0.05%

TritonX-100 and Western blotted with horseradish-peroxidase (HRP)-conjugated mouse anti-FLAG M2 antibody (1:10,000 dilution; Sigma-Aldrich A8592) to detect the bound FLAG-tagged VEGF-A and FLAG-tagged Ang2. Signal was visualized by chemiluminescent detection according to the manufacturer's protocol (Amersham Pharmacia Biotech) using chemiluminescence scanner (LAS-1000, Fuji Film, Tokyo). The *in vitro* binding indicated that DAAP#1 and DAAP#14 are capable of synchronous binding to VEGF-A and Ang2 (**Figure 12, Figure 13A and 13B**). VEGF-trap was capable of binding to VEGF-A but not to Ang2 (**Figure 13C**) while Tie2-Fc showed binding to Ang2 but not to VEGF-A (**Figure 13D**).

**[00178] Example 3 –**

**[00179]** Based on the above findings, DAAP#1 was further investigated for use as a therapeutic protein. Recombinant Chinese hamster ovary (rCHO) cells expressing DAAP#1 (CHO-DAAP#1) was established following a previously described method (Hwang SJ, et al., *Protein Express Purif.* 2005; 39:175-183). Briefly, CHO-DAAP#1 cells were established by transfection of a vector containing the dihydrofolate reductase (*dhfr*) and DAAP#1 gene construct into *dhfr*-deficient CHO cells (CRL-9096, American Type Culture Collection, Manassas, Virginia, USA). This was followed by *dhfr*/methotrexate (MTX)-mediated gene amplification. The stable rCHO cells secreting DAAP#1 was selected with serial amplified concentrations of MTX (0.02-1.0 μM, Sigma-Aldrich). For recombinant DAAP#1 protein production, CHO-DAAP#1 cells were inoculated at 2 $x10^5$ cells/mL in 250-ml Erlenmeyer flasks containing 100 ml of medium on an orbital shaker (Vision, Bucheon, Korea) at 110 rpm in a humidified 5% $CO_2$ incubator at 37°C. After indicated days, the culture medium containing DAAP#1 recombinant protein were purified by using Protein-A sepharose affinity chromatography, acid elution and subsequent neutralization. After purification, size and dimeric status of DAAP#1 were examined under reducing and non-reducing conditions by SDS-PAGE and Coomassie blue staining (**Figure 14**). The analysis indicates that DAAP#1 is dimeric protein (~150 kDa) (**Figure 14**).

**[00180] Example 4 –**

**[00181]** Binding capabilities of Fc, DAAP#1, VEGF-Trap, and Fc proteins to VEGF-A and Ang2 were measured using the ELISA method. For VEGF-A binding, the 96-well plate was coated with 200 ng of VEGF-A in 100 μl PBS per each well for overnight. After washing the plate 3 times with each 400 μl of PBS, the plate was blocked by 1% bovine serum albumin in 100 μl PBS at 37°C for 2 hrs. After 3 times PBS washing, varying amounts

(0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3 nM) of DAAP#1, Fc, VEGF-Trap or Tie2-Fc protein in blocking solution was incubated at 37°C for 2 hrs. After 3 times of PBS washing, 50 μl of horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170) in blocking solution were incubated at 37°C for 2 hrs. After 3 times of PBS washing, 50 μl of TMB solution (Sigma-Aldrich T0440) was added into each well, and the plate incubated at room temperature for 10 min. Reaction was stopped by adding 50 μl of 1 M HCl and reactive colors were analyzed at optical density 415 nm by ELISA reader (BioRad M680). For Ang2 binding, Ang2 was coated in 96 well plate in the same manner as described above and binding capabilities of Fc, DAAP#1, VEGF-Trap, and Fc proteins to Ang2 were also measured in the same manner as described above.

**[00182]** These assays for binding capabilities revealed that Kd of DAAP#1 to VEGF-A was ~5 pM and Kd of VEGF-Trap to VEGF-A was ~1 pM, whereas Kd of DAAP#1 to Ang2 was ~10 nM and Kd of Tie2-Fc to Ang2 was ~50 nM (**Figure 15**). VEGF-Trap had no binding to Ang2, Tie2-Fc had no binding to VEGF-A (**Figure 15**).

**[00183]** **Example 5 -**

**[00184]** Because DAAP#1 harbors binding sites for both VEGF-A and Ang2, its binding activity to VEGF-A or Ang2 may be affected by its conformational change when one site of DAAP#1 is first occupied or pre-occupied with either Ang2 or VEGF-A.

**[00185]** To test whether pre-occupation of VEGF-A binding site of DAAP#1 with VEGF-A influences Ang2 binding capability, recombinant DAAP#1 protein was pre-incubated with increasing amount (0, 10, 30 100, 300, 1000 ng/μl) of VEGF-A, then the aforementioned Ang2 binding ELISA assay was performed. Conversely, to test whether pre-occupation of Ang2 binding site of DAAP#1 with Ang2 influences VEGF-A binding capability, recombinant DAAP#1 protein was pre-incubated with increasing amount (0, 10, 30 100, 300, 1000 ng/μl) of Ang2, then the aforementioned VEGF binding ELISA assay was performed. As a control, the recombinant VEGF-trap protein was pre-incubated with increasing amount (0, 10, 30 100, 300, 1000 ng/μl) of Ang2, then aforementioned VEGF binding ELISA assay was performed. As a control, the recombinant Tie2-Fc protein was pre-incubated with increasing amount (0, 10, 30 100, 300, 1000 ng/μl) of VEGF-A, then the aforementioned Ang2 binding ELISA assay was performed.

**[00186]** Intriguingly, pre-occupation of Ang2 to DAAP#1 enhanced the binding activity of DAAP#1 to VEGF-A (**Figure 16A**). In addition, pre-occupation of VEGF-A to DAAP#1 enhanced the binding activity of DAAP#1 to Ang2 (**Figure 16B**). In comparison, pre-

occupation of VEGF-A to DAAP#1 inhibited further binding of VEGF-A to DAAP#1, and pre-occupation of Ang2 to DAAP#1 inhibited further binding of Ang2 to DAAP#1 (**Figure 16**). Three repeated experiments showed similar findings. These data indicate that DAAP#1 is capable of simultaneously binding both VEGF-A and Ang2.

**[00187] Example 6 -**

**[00188]** For further analysis of binding ability of DAAP#1, the *in vitro* binding by pull-down assay was performed in the presence of several kinds of VEGF and angiopoietin proteins (n=3) (**Figure 17**). Each 200 ng of FLAG tagged proteins (human VEGF-A165, mouse VEGF-A164, human VEGF-A189, human VEGF-A121, human VEGF-C, VEGF-E, human Ang2, mouse Ang2, human Angl, human Ang4, and mouse Angptl4) was incubated with 500 ng of DAAP#1, VEGF-Trap, and Tie2-Fc in 500 μl of Tris-buffer solution (50 mM Tris, 100 mM NaCl, pH 7.5) containing 1.0% Nonidet P-40 at 4°C for 2 hrs. Then, 20 μl of Protein-A agarose beads (Oncogene) were added and incubated at 4°C for another 2 hrs. The Protein-A conjugated samples were washed 3 times with 1 ml of Tris-buffer solution containing 1.0% Nonidet P-40. The samples were eluted with sample buffer, and heat-denatured. The samples were separated by 10% SDS-PAGE, and electro-blotted onto nitrocellulose membranes. The membranes were blocked with 5% nonfat milk in Tris-buffer solution containing 0.05% Triton X-100 and Western blotted with horseradish-peroxidase (HRP)-conjugated mouse anti-FLAG M2 antibody (1:10,000 dilution; Sigma-Aldrich A8592). Signal was visualized by chemiluminescent detection according to the manufacturer's protocol (Amersham Pharmacia Biotech) using chemiluminescence scanner (LAS-1000, Fuji Film, Tokyo). After stripping, the membrane was reprobed for DAAP, VEGF-Trap, and Tie2-Fc with anti-Fc horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170). DAAP#1 is capable of binding human VEGF-A165, mouse VEGF-A164, human VEGF-A189, human VEGF-A121, human Ang2, mouse Ang2, human Angl and human Ang4, whereas VEGF-Trap is capable of binding human VEGF-A165, mouse VEGF-A164, human VEGF-A189 and human VEGF-A121 and Tie2-Fc is capable of binding human Ang2, mouse Ang2, human Angl and human Ang4 (**Figure 17**).

**[00189]** Theoretical pI values on recombinant DAAP proteins, Ig-like domain 2 of VEGFR1 containing Fc protein [VEGFR1(2)-Fc] , Ig-like domains 2 and 3 of VEGFR1 containing Fc protein [VEGFR1(2-3)-Fc], VEGF-trap and Tie2-Fc are shown in **Figure 18**.

**[00190] Example 7 -**

**[00191]** Generally, higher pI values of proteins correlate with higher extracellular matrix (ECM) binding. The higher ECM binding of proteins also correlates with lower pharmacokinetic properties. ECM coated 96-well plates (Becton Dickinson Cat. No. 354607) were incubated with blocking buffer (1% BSA in PBS) at 37°C for 2 hour, washed 3 times with each 400 µl of PBS. Then varying amounts (0, 0.1, 0.3, 1.0, 3.3, 10, 33, 100 ng) of each DAAP recombinant protein in blocking buffer were added into the plate, and were incubated at 37°C for 2 hour (**Figure 19**). After washing the plate 3 times with each 400 µl of PBS, 50 µl of horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170) was added into the plate, and were incubated at 37°C for 2 hour. After washing the plate 3 times with each 400 µl of PBS, 50 µl of TMB solution (Sigma-Aldrich T0440) was added into the plate, and the plate incubated at room temperature for 10 min. Reaction was stopped by adding 50 µl of 1 M HCl and reactive colors were analyzed at optical density 415 nm by ELISA reader (BioRad M680) (**Figure 19**).

**[00192]** The ECM binding assay revealed that DAAP#1, DAAP#14, DAAP#15 and Tie2-Fc were very low, DAAP#17 and VEGF-trap were relatively low, DAAP#16 was moderate and VEGFR1(2-3)-Fc was very high to ECM binding, respectively (**Figure 20**). Thus, DAAP#1, DAAP#14, DAAP#15 and Tie2-Fc could have relatively high pharmacokinetic properties.

**[00193]** The ECM binding assay was further performed with higher concentrations of DAAP#1, VEGF-Trap and Tie2-Fc proteins. ECM coated 96-well plate (Becton Dickinson Cat. No. 354607) was incubated with 100 µl of blocking buffer (2% BSA in PBS) at 37°C for 2 hr. The plate was washed 3 times with each 400 µl of PBS. Then, varying amounts (0.1, 0.3, 1, 3, 10, 30 nM) of each DAAP#1, VEGF-Trap, Tie2-Fc recombinant proteins in blocking buffer were added into the plate, and were incubated at 37°C for 2 hrs. After 3 times of PBS washing, 50 µl of horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170) was added into the plate, and were incubated at 37°C for 2 hours. After 3 times of PBS washing, 50 µl of horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170) in blocking solution were incubated at 37°C for 2 hrs. After 3 times of PBS washing, 50 µl of TMB solution (Sigma-Aldrich T0440) was added into each well, and the plate incubated at room temperature for 10 min. Reaction was stopped by adding 50 µl of 1 M HCl and reactive colors were analyzed at optical density 415 nm by ELISA reader (BioRad M680). This ECM

binding assay (n=4) revealed that DAAP#1 was low, Tie2-Fc was very low and VEGF-trap relatively high to ECM binding, respectively (**Figure 21**).

**[00194] Example 8 –**

**[00195]** A standard pharmacokinetic analysis was performed (n=3). 100 μg of Fc, DAAP#1, VEGF-Trap or Tie2-Fc recombinant protein was injected subcutaneously into 8-week-old male C57BL/6 mice (~25g of body weight), then blood samples were taken from tail vein at 1, 2, 4, 8, 12, 24, 48, 96, and 144 hours (**Figure 22**). The serum levels of DAAP#1 and VEGF-Trap proteins were measured by the VEGF-A binding ELISA method, and Tie2-Fc was measured by Ang2 binding ELISA method. The serum level of Fc protein was measured by Sandwich ELISA method using mouse monoclonal antibody against Human IgG1 (clone No. 2C11, Abcam AB1927) as capture antibody and horseradish-peroxidase (HRP)-conjugated goat anti-human Fc antibody (1:10,000 dilution; Sigma-Aldrich A0170) as detection antibody. Half-life (T1/2) of Fc, DAAP#1, VEGF-Trap and Tie2-Fc were ~200 hr, ~48 hr, ~24 hr and ~12 hr (**Figure 23**). Thus, disappearance DAAP#1 in the blood was slower than that of VEGF-Trap in the blood after the subcutaneous administration (**Figure 23**).

**[00196] Example 9 -**

**[00197]** To examine the effect of DAAP#1 on tumor growth, 1 x $10^6$ of green fluorescent protein-tagged Lewis lung carcinoma (GFP-LLC) cells were implanted subcutaneously into shaved right flank region of 8-week-old male C57BL/6 mice. Five days after the implantation, mice were divided into 5 groups for treatment: Group 1 (n=4), PBS (100 μl); Group 2 (n=4), Fc (25 mg/kg), Group 3 (n=4), DAAP#1 (25 mg/kg); Group 4 (n=4), VEGF-Trap (25 mg/kg); Group 5 (n=4), Tie2-Fc (25 mg/kg). These agents were given subcutaneous injection in every alternative day (**Figure 24**). Growing tumor size was measured every 6 days by calipers using the formula, width x length x depth x 0.5.

**[00198]** Compared to the control PBS treatment, DAAP#1 and VEGF-Trap produced marked inhibition of LLC tumor growth, while Fc and Tie2-Fc did not produce significant inhibition of LLC tumor growth (**Figure 25**). The tumor growth inhibition by DAAP#1 was more marked than that by VEGF-Trap (**Figure 25**). Thus, potency of tumor growth inhibition is DAAP#1 > VEGF-Trap > Tie2-Fc > Fc > PBS.

**[00199] Example 10 -**

**[00200]** To examine changes of tumor blood vessels, at the indicated days, the mice implanted with GFP-LLC cells were anesthetized by intramuscular injection of a combination

of anesthetics (80 mg/kg ketamine and 12 mg/kg xylazine), and tumors were fixed by systemic vascular perfusion of 1% paraformaldehyde in PBS, removed, embedded with tissue freezing medium (Leica, Nussioch, Germany) and cryo-sectioned at 10 μm thickness. After blocking with 5% donkey serum in PBST (0.3% Triton X-100 in PBS) for 1 hr at room temperature, the sectioned tissues were incubated with anti-mouse PECAM-1 antibody, hamster clone 2H8, 1:1,000 (Chemicon International, Temecula, CA). After several washes in PBST, the samples were incubated for 2 hr at room temperature with Cy3-conjugated anti-hamster IgG antibody, 1:1,000 (Jackson ImmunoResearch). Fluorescent signals were visualized, and digital images were obtained using a Zeiss inverted microscope, a Zeiss ApoTome microscope or a Zeiss LSM 510 confocal microscope equipped with argon and helium-neon lasers (Carl Zeiss). Measurements of morphometry and densities of blood vessels in tumor tissue sections were made with PECAM-1 immunostaining using photographic analysis in ImageJ software (http://rsb.info.nih.gov/ij) or using a Zeiss ApoTome microscope coupled to a monochrome charge-coupled device (CCD) camera and image analysis software (AxioVision, Zeiss).

**[00201]** At 29 days later after the GFP-LLC cells implantation, higher densities and well connected blood vessels were formed in the LLC tumor treated with PBS (**Figure 26**). Pruning of tumor blood vessels was observed by the treatment of VEGF-Trap (**Figure 26**), whereas pruning and narrowing of tumor blood vessels was observed by the treatment of DAAP#1 (**Figure 26**).

**[00202]** **Example 11 –**

**[00203]** Abnormal ocular angiogenesis accompanying vascular leakage and edema in retina is a main cause of diabetic retinopathy and age-related macular degeneration. Mouse model having abnormal ocular angiogenesis can be made by exposure of neonatal mouse to the hyperoxic atmosphere, that is "retina of prematurity (ROP) or oxygen-induced retinopathy" (**Figure 27**). Oxygen-induced retinopathy was introduced in C57/BL6 wild-type mice. Neonatal mice and their nursing mother were exposed to 75% oxygen (PRO-OX 110 chamber oxygen controller used) between postnatal day 7 (P7) and P12 producing vaso-obliteration and cessation of vascular development in the capillary beds of the central retina (**Figure 27**). Return of the animals to normoxia room air condition at P12 renders the ischemic and hypoxic central retina, and results in preretinal neovascularization (**Figure 27**). In each group {PBS control (n=4), Fc (n=4), Tie2-Fc (n=4), VEGF-Trap (n=4), DAAP

(n=4)}, P12 animals received a subcutaneous injection of each protein (25 mg/kg) every other day until P16 and sacrificed on P17 (**Figure 27**).

**[00204]** Then, whole-mounts of retina and immunohistochemical staining for blood vessels were performed as follows. Eyeballs were enucleated from mice immediately and fixed in 4% paraformaldehyde (PFA) at 4 °C for 2 hr. The retinas were isolated in PBS, blocked 1 hr at 25 °C with 0.3% Triton X-100 in TBS (TBS-T) containing 5% donkey serum (Jackson Immuno Research), and stained with PECAM-1 antibody, hamster clone 2H8, 1:1000 dilution (Chemicon International, Temecula, CA) overnight at 4 °C. After six times of washes in TBS-T, samples were incubated with Cy3-conjugated anti-hamster IgG antibody 1:1000 dilution for 4-h at 25 °C. Following another six times of washes in TBS-T, retinas were whole-mounted onto Superfrost/Plus microscope slides (12-550-15, Fisher) with the photoreceptor side down and embedded in VECTASHIELD (Vector) reagent.

**[00205]** P17 retinal vasculature stained with PECAM-1 showed entire blood vessel pattern of retina for each group (**Figure 28A**). In high-magnified optic disc region, increased number of sprouting retinal venules were detected in PBS-, Fc- and Tie2-Fc-treated groups, whereas numbers of sprouting retinal venules were markedly reduced in DAAP#1- and VEGF-Trap-treated groups (**Figure 28B**). Number of vascular micro-tufts in the retinal vessels, a typical feature of retinopathy, were highly increased in PBS-, Fc- and Tie2-Fc-treated groups, whereas number of vascular micro-tufts in the retinal venules were markedly reduced in DAAP#1- and VEGF-Trap-treated groups (**Figure 28C**). Blood vessel density and tortuosity of retinal margin were highly increased in PBS-, Fc- and Tie2-Fc-treated groups, whereas these were markedly reduced in DAAP#1- and VEGF-Trap-treated groups (**Figure 28D**). Quantification analysis revealed that ROP-induced formation of vascular micro-tufts in the retinal vessels and increased marginal retinal vessels density were potently inhibited by the treatments of DAAP#1 and VEGF-trap (**Figure 28E and 28F**). Of these, potency of DAAP#1 was higher than VEGF-Trap. These data indicate that DAAP#1 is more effective than VEGF-Trap for treating patients with diabetic retinopathy and age-related macular degeneration.

**[00206]** Inflammatory angiogenesis is a hallmark and a critical contributing factor in progression of rheumatoid arthritis (Lainer-Carr and Brahn, 2007, Nature Clinical Practice Rhuematology 3:434-442). Therefore, if we inhibit inflammatory angiogenesis, we could reduce progression and joint destruction of RA. For induction of collagen-induced arthritis (CIA) as an experimental mouse model of RA, male DBA/1J mice were immunized intradermally at the base of the tail with bovine type II collagen emulsified in an equal

volume of complete Freund adjuvant (**Figure 29A**). Three weeks later, the mice were boosted in the same manner (**Figure 29A**). The mice with CIA were treated by subcutaneous injections of control buffer (n=5), Fc-protein (25 mg/kg) (n=5), VEGF-Trap (25 mg/kg) (n=6) or DAAP#1 (25 mg/kg) (n=6) twice per week for 18 days (**Figure 29A**). Disease severity was clinically scored for each paw using following system: grade 0, no swelling; grade 1, slight swelling and erythema; 2, pronounced edema; 3, joint rigidity (**Figure 29B**). Radiographic analysis was performed using modified mammographic imaging and the scores were determined in the knee, ankle, and tarsometatarsal joints (**Figure 30**). At 18 days after the start of treatments, histopathological analysis was also performed and the scores were determined (**Figure 31**). Overall, VEGF-Trap and DAAP#1 alleviated clinical scores and radiologic and histological abnormalities, whereas control buffer and Fc-protein did not produce any ameliorative effects in the disease severity and joint destruction of RA. However, notably, effect of DAAP#1 is superior to effect of VEGF-Trap in reducing clinical score and progressions of radiographic and histopathological abnormalities of RA. Thus, DAAP#1 could be potential therapeutic agent to treat the patients of RA.

**[00207]** The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. The following examples are offered by way of illustration of the present invention, and not by way of limitation.

TABLE 1

**DAAP#1**

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           10          20          30          40          50          60
           *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
           *           *           *           *           *           *
GAA GGT GCC ATG GAC TTG ATC TTG ATC AAT TCC CTA CCT CTT GTA TCT GAT GCT GAA ACA
CTT CCA CGG TAC CTG AAC TAG AAC TAG TTA AGG GAT GGA GAA CAT AGA CTA CGA CTT TGT
Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu Pro Leu Val Ser Asp Ala Glu Thr
21__22  23_____25__________hTIE2 IG DOMAIN 1_____35_________________40

           130         140         150         160         170         180
           *           *           *           *           *           *
TCT CTC ACC TGC ATT GCC TCT GGG TGG CGC CCC CAT GAG CCC ATC ACC ATA GGA AGG GAC
AGA GAG TGG ACG TAA CGG AGA CCC ACC GCG GGG GTA CTC GGG TAG TGG TAT CCT TCC CTG
Ser Leu Thr Cys Ile Ala Ser Gly Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp
 41_____________45__________hTIE2 IG DOMAIN 1_____55_________________60

           190         200         210         220         230         240
           *           *           *           *           *           *
TTT GAA GCC TTA ATG AAC CAG CAC CAG GAT CCG CTG GAA GTT ACT CAA GAT GTG ACC AGA
AAA CTT CGG AAT TAC TTG GTC GTG GTC CTA GGC GAC CTT CAA TGA GTT CTA CAC TGG TCT
Phe Glu Ala Leu Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 61_____________65__________hTIE2 IG DOMAIN 1_____75_________________80

           250         260         270         280         290         300
           *           *           *           *           *           *
GAA TGG GCT AAA AAA GTT GTT TGG AAG AGA GAA AAG GCT AGT AAG ATC AAT GGT GCT TAT
CTT ACC CGA TTT TTT CAA CAA ACC TTC TCT CTT TTC CGA TCA TTC TAG TTA CCA CGA ATA
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile Asn Gly Ala Tyr
 81_____________85__________hTIE2 IG DOMAIN 1_____95_________________100

           310         320         330         340         350         360
           *           *           *           *           *           *
TTC TGT GAA GGG CGA GTT CGA GGA GAG GCA ATC AGG ATA CGA ACC ATG AAG ATG CGT CAA
AAG ACA CTT CCC GCT CAA GCT CCT CTC CGT TAG TCC TAT GCT TGG TAC TTC TAC GCA GTT
Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg Ile Arg Thr Met Lys Met Arg Gln
101____________105__________hTIE2 IG DOMAIN 1_____115_________________120

           370         380         390         400         410         420
           *           *           *           *           *           *
CAA GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC
GTT CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG
Gln Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn
121 122________125__________hTIE2 IG DOMAIN 2_____135_________________140

           430         440         450         460         470         480
           *           *           *           *           *           *
ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC
TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG
Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
141____________145__________hTIE2 IG DOMAIN 2_____155_________________160

           490         500         510         520         530         540
           *           *           *           *           *           *
TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT
AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His
161____________165__________hTIE2 IG DOMAIN 2_____175_________________180

           550         560         570         580         590         600
           *           *           *           *           *           *
GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC
CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG
Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr
181____________185__________hTIE2 IG DOMAIN 2_____195_________________200


           610         620         630         640         650         660
           *           *           *           *           *           *
TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA TGC
AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT ACG
Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys
201____________205_____hTIE2 IG DOMAIN 2___213 214_215_________________220
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          670           680           690           700           710           720
           *             *             *             *             *             *
AAC CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA TGC
TTG GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT ACG
Asn His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
221___________225_______hTIE2 EGF LIKE DOMAIN 1_____235_______________240

          730           740           750           760           770           780
           *             *             *             *             *             *
ATT TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG TTT
TAA ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC AAA
Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr Phe
241___________245__hTIE2 EGF LIKE DOMAIN 1___253 254_255_______________260

          790           800           810           820           830           840
           *             *             *             *             *             *
GGC AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC TGT
CCG TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG ACA
Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe Cys
261___________265_______hTIE2 EGF LIKE DOMAIN 2_____275_______________280

          850           860           870           880           890           900
           *             *             *             *             *             *
CTC CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT GAA
GAG GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA CTT
Leu Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu
281___________285_______hTIE2 EGF LIKE DOMAIN 2_____295_______________300

          910           920           930           940           950           960
           *             *             *             *             *             *
GCA TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT GGG
CGT ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA CCC
Ala Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
301___________305_______hTIE2 EGF LIKE DOMAIN 3_____315_______________320

          970           980           990          1000          1010          1020
           *             *             *             *             *             *
GAG ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CCA GGA TGG CAG GGG CTC CAG TGT
CTC TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GGT CCT ACC GTC CCC GAG GTC ACA
Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln Gly Leu Gln Cys
321___________325_______hTIE2 EGF LIKE DOMAIN 3_____335_______________340

          1030          1040          1050          1060          1070          1080
           *             *             *             *             *             *
GAG AGA GAA GGC ATA CCG AGG ATG GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC
CTC TCT CTT CCG TAT GGC TCC TAC CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG
Glu Arg Glu Gly Ile Pro Arg Met Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro
341___________345_________348 349________hVEGFR1 IG DOMAIN 2_____________360

          1090          1100          1110          1120          1130          1140
           *             *             *             *             *             *
GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT
CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA
Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
361___________365__________hVEGFR1 IG DOMAIN 2_______375_______________380

          1150          1160          1170          1180          1190          1200
           *             *             *             *             *             *
AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC
TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg
381___________385__________hVEGFR1 IG DOMAIN 2_______395_______________400


          1210          1220          1230          1240          1250          1260
           *             *             *             *             *             *
ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG
TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC
Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly
401___________405__________hVEGFR1 IG DOMAIN 2_______415_______________420

          1270          1280          1290          1300          1310          1320
           *             *             *             *             *             *
CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT
GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA
Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His
421___________425__________hVEGFR1 IG DOMAIN 2_______435_______________440

          1330          1340          1350          1360          1370          1380
           *             *             *             *             *             *
CGA CAA CTC GAG GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG
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GCT GTT GAG CTC CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC
Arg Gln Leu Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
441_442 443_____445_____________hFC DOMAIN___________455_____________460

          1390          1400          1410          1420          1430          1440
            *             *             *             *             *             *
GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC
CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
461_____________465_____________hFC DOMAIN___________475_____________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC
GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG
Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
481_____________485_____________hFC DOMAIN___________495_____________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC
ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG
Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
501_____________505_____________hFC DOMAIN___________515_____________520

          1570          1580          1590          1600          1610          1620
            *             *             *             *             *             *
AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC
TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
521_____________525_____________hFC DOMAIN___________535_____________540

          1630          1640          1650          1660          1670          1680
            *             *             *             *             *             *
AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC
TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile
541_____________545_____________hFC DOMAIN___________555_____________560

          1690          1700          1710          1720          1730          1740
            *             *             *             *             *             *
TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG
AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTC
Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
561_____________565_____________hFC DOMAIN___________575_____________580

          1750          1760          1770          1780          1790          1800
            *             *             *             *             *             *
GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
581_____________585_____________hFC DOMAIN___________595_____________600


          1810          1820          1830          1840          1850          1860
            *             *             *             *             *             *
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC
TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
601_____________605_____________hFC DOMAIN___________615_____________620

          1870          1880          1890          1900          1910          1920
            *             *             *             *             *             *
GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG
CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg
621_____________625_____________hFC DOMAIN___________635_____________640

          1930          1940          1950          1960          1970          1980
            *             *             *             *             *             *
TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC
ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG
Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
641_____________645_____________hFC DOMAIN___________655_____________660

          1990          2000          2010
            *             *             *
ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA  (SEQ ID NO:1)
TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys ***  (SEQ ID NO:2)
661___________ hFC DOMAIN __________670_____672
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**DAAP#2**

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           10          20          30          40          50          60
           *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
           *           *           *           *           *           *
GAA GGT GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG
CTT CCA CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC
Glu Gly Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val
21__22  23_____25__________hTIE2 IG DOMAIN 2________35_________________40

           130         140         150         160         170         180
           *           *           *           *           *           *
AAC ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT
TTG TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA
Asn Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly
 41_____________45__________hTIE2 IG DOMAIN 2________55_________________60

           190         200         210         220         230         240
           *           *           *           *           *           *
TCC TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT
AGG AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA
Ser Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro
 61_____________65__________hTIE2 IG DOMAIN 2________75_________________80

           250         260         270         280         290         300
           *           *           *           *           *           *
CAT GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC
GTA CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG
His Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe
 81_____________85__________hTIE2 IG DOMAIN 2________95_________________100

           310         320         330         340         350         360
           *           *           *           *           *           *
ACC TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC GGT AGA CCT TTC GTA GAG
TGG AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG CCA TCT GGA AAG CAT CTC
Thr Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gly Arg Pro Phe Val Glu
101____________105________ hTIE2 IG DOMAIN 2_______114 115_________________120

           370         380         390         400         410         420
           *           *           *           *           *           *
ATG TAC AGT GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC
TAC ATG TCA CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG
Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro
121____________125__________hVEGFR1 IG DOMAIN 2_______135_________________140

           430         440         450         460         470         480
           *           *           *           *           *           *
TGC CGG GTT ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG
ACG GCC CAA TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC
Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu
141____________145__________hVEGFR1 IG DOMAIN 2_______155_________________160

           490         500         510         520         530         540
           *           *           *           *           *           *
ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA
TAG GGA CTA CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT
Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala
161____________165__________hVEGFR1 IG DOMAIN 2_______175_________________180

           550         560         570         580         590         600
           *           *           *           *           *           *
ACG TAC AAA GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG
TGC ATG TTT CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC
Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys
181____________185__________hVEGFR1 IG DOMAIN 2_______195_________________200


           610         620         630         640         650         660
           *           *           *           *           *           *
ACA AAC TAT CTC ACA CAT CGA CAA CTC GAG GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA
TGT TTG ATA GAG TGT GTA GCT GTT GAG CTC CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT
Thr Asn Tyr Leu Thr His Arg Gln Leu Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro
201____________205_____208 209_210_____hFC DOMAIN_____________________220

           670         680         690         700         710         720
           *           *           *           *           *           *
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GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC
CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
221 ___________225_____________hFC DOMAIN___________235_____________240

          730           740           750           760           770           780
           *             *             *             *             *             *
CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC
GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
241 ___________245_____________hFC DOMAIN___________255_____________260

          790           800           810           820           830           840
           *             *             *             *             *             *
CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG
GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC
Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
261 ___________265_____________hFC DOMAIN___________275_____________280

          850           860           870           880           890           900
           *             *             *             *             *             *
CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC
GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG
Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
281 ___________285_____________hFC DOMAIN___________295_____________300

          910           920           930           940           950           960
           *             *             *             *             *             *
CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC
GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
301 ___________305_____________hFC DOMAIN___________315_____________320

          970           980           990          1000          1010          1020
           *             *             *             *             *             *
CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC
GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
321 ___________325_____________hFC DOMAIN___________335_____________340

         1030          1040          1050          1060          1070          1080
           *             *             *             *             *             *
CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA
GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
341 ___________345_____________hFC DOMAIN___________355_____________360

         1090          1100          1110          1120          1130          1140
           *             *             *             *             *             *
GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC
CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
361 ___________365_____________hFC DOMAIN___________375_____________380

         1150          1160          1170          1180          1190          1200
           *             *             *             *             *             *
TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC
ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
381 ___________385_____________hFC DOMAIN___________395_____________400


         1210          1220          1230          1240          1250          1260
           *             *             *             *             *             *
ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG
TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
401 ___________405_____________hFC DOMAIN___________415_____________420

         1270          1280          1290          1300          1310
           *             *             *             *             *
GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA (SEQ ID NO:3)
CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys *** (SEQ ID NO:4)
421 ___________425_____________hFC DOMAIN___________435_____438
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**DAAP#3**

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           10          20          30          40          50          60
            *           *           *           *           *           *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu
1_____________5_________hVEGFR1 SIGNAL SEQUENCE________15__________________20

           70          80          90          100         110         120
            *           *           *           *           *           *
ACA GGA TCT AGT TCA GGT GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT
TGT CCT AGA TCA AGT CCA CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG CTT TAA
Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile
21_____________25__26  27_____________hVEGFR1 IG DOMAIN 2_________________40

           130         140         150         160         170         180
            *           *           *           *           *           *
ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC
TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG
Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile
 41_____________45___________hVEGFR1 IG DOMAIN 2________55__________________60

           190         200         210         220         230         240
            *           *           *           *           *           *
ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC
TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG TAT TAG
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
 61_____________65___________hVEGFR1 IG DOMAIN 2________75__________________80

           250         260         270         280         290         300
            *           *           *           *           *           *
TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG
ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu
 81_____________85___________hVEGFR1 IG DOMAIN 2________95__________________100

           310         320         330         340         350         360
            *           *           *           *           *           *
ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA
TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT
Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln
101_____________105___________hVEGFR1 IG DOMAIN 2_______115_________________120

           370         380         390         400         410         420
            *           *           *           *           *           *
GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC ATA
CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG TAT
Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn Ile
121_____________125___________hTIE2 IG DOMAIN 2________135_________________140

           430         440         450         460         470         480
            *           *           *           *           *           *
TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC TTC
AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG AAG
Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser Phe
141_____________145___________hTIE2 IG DOMAIN 2________155_________________160

           490         500         510         520         530         540
            *           *           *           *           *           *
ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT GCT
TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA CGA
Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His Ala
161_____________165___________hTIE2 IG DOMAIN 2________175_________________180

           550         560         570         580         590         600
            *           *           *           *           *           *
CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC TCG
GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG AGC
Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr Ser
181_____________185___________hTIE2 IG DOMAIN 2________195_________________200


           610         620         630         640         650         660
            *           *           *           *           *           *
GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA TGC AAC
CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT ACG TTG
Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys Asn
201_____________205____hTIE2 IG DOMAIN 2____212 213_____215_________________220

           670         680         690         700         710         720
            *           *           *           *           *           *
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CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA TGC ATT
GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT ACG TAA
His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys Ile
221_____225_____hTIE2 EGF LIKE DOMAIN 1_____235_____240

          730       740       750       760       770       780
           *         *         *         *         *         *
TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG TTT GGC
ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC AAA CCG
Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr Phe Gly
241_____hTIE2 EGF LIKE DOMAIN 1_____242 243____245_____260

          790       800       810       820       830       840
           *         *         *         *         *         *
AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC TGT CTC
TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG ACA GAG
Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe Cys Leu
261_____265_____hTIE2 EGF LIKE DOMAIN 2_____275_____280

          850       860       870       880       890       900
           *         *         *         *         *         *
CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT GAA GCA
GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA CTT CGT
Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu Ala
281_____285_____hTIE2 EGF LIKE DOMAIN 2_____295_____299 300

          910       920       930       940       950       960
           *         *         *         *         *         *
TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT GGG GAG
ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA CCC CTC
Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly Glu
301_____305_____hTIE2 EGF LIKE DOMAIN 3_____315_____320

          970       980       990      1000      1010      1020
           *         *         *         *         *         *
ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CTC GAG GAC AAA ACT CAC ACA TGC CCA
TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GAG CTC CTG TTT TGA GTG TGT ACG GGT
Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Leu Glu Asp Lys Thr His Thr Cys Pro
321_____hTIE2 EGF LIKE DOMAIN 3___331 332_____hFC DOMAIN_____340

          1030      1040      1050      1060      1070      1080
           *         *         *         *         *         *
CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC
GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
341_____345_____hFC DOMAIN_____355_____360

          1090      1100      1110      1120      1130      1140
           *         *         *         *         *         *
AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC
TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
361_____365_____hFC DOMAIN_____375_____380

          1150      1160      1170      1180      1190      1200
           *         *         *         *         *         *
CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC
GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
381_____385_____hFC DOMAIN_____385_____400


          1210      1220      1230      1240      1250      1260
           *         *         *         *         *         *
AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC
TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
401_____405_____hFC DOMAIN_____415_____420

          1270      1280      1290      1300      1310      1320
           *         *         *         *         *         *
GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC
CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
421_____425_____hFC DOMAIN_____435_____440

          1330      1340      1350      1360      1370      1380
           *         *         *         *         *         *
CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG
GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
441_____445_____hFC DOMAIN_____455_____460
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          1390          1400          1410          1420          1430          1440
           *             *             *             *             *             *
GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC
CAC ATG TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
461_____________465_____________hFC DOMAIN_____________475_____________480

          1450          1460          1470          1480          1490          1500
           *             *             *             *             *             *
CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG
GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
481_____________485_____________hFC DOMAIN_____________495_____________500

          1510          1520          1530          1540          1550          1560
           *             *             *             *             *             *
GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC
CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
501_____________505_____________hFC DOMAIN_____________515_____________520

          1570          1580          1590          1600          1610          1620
           *             *             *             *             *             *
AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG
TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
521_____________525_____________hFC DOMAIN_____________535_____________540

          1630          1640          1650          1660          1670          1680
           *             *             *             *             *             *
ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA
TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
541_____________545_____________hFC DOMAIN_____________555_____________560

 1683
  *
TGA (SEQ ID NO:5)
ACT
*** (SEQ ID NO:6)
561
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**DAAP#4**

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          10          20          30          40          50          60
           *           *           *           *           *           *
ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG TGC GCG CTG CTC AGC TGT CTG CTT CTC
TAC CAG TCG ATG ACC CTG TGG CCC CAG GAC GAC ACG CGC GAC GAG TCG ACA GAC GAA GAG
Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser Cys Leu Leu Leu
1_____________5_________hVEGFR1 SIGNAL SEQUENCE_______15__________________20

          70          80          90          100         110         120
           *           *           *           *           *           *
ACA GGA TCT AGT TCA GGT GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT
TGT CCT AGA TCA AGT CCA CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG CTT TAA
Thr Gly Ser Ser Ser Gly Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile
21_____________25__26__27_____________hVEGFR1 IG DOMAIN 2_________________40

          130         140         150         160         170         180
           *           *           *           *           *           *
ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC
TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG
Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile
 41_____________45__________hVEGFR1 IG DOMAIN 2________55__________________60

          190         200         210         220         230         240
           *           *           *           *           *           *
ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC
TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG TAT TAG
Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile
 61_____________65__________hVEGFR1 IG DOMAIN 2________75__________________80

          250         260         270         280         290         300
           *           *           *           *           *           *
TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG
ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC
Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu
 81_____________85__________hVEGFR1 IG DOMAIN 2________95__________________100

          310         320         330         340         350         360
           *           *           *           *           *           *
ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA
TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT
Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln
101____________105__________hVEGFR1 IG DOMAIN 2_______115_________________120

          370         380         390         400         410         420
           *           *           *           *           *           *
GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC ATA
CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG TAT
Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn Ile
121____________125__________hTIE2 IG DOMAIN 2________135_________________140

          430         440         450         460         470         480
           *           *           *           *           *           *
TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC TTC
AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG AAG
Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser Phe
141____________145__________hTIE2 IG DOMAIN 2________155_________________160

          490         500         510         520         530         540
           *           *           *           *           *           *
ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT GCT
TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA CGA
Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His Ala
161____________165__________hTIE2 IG DOMAIN 2________175_________________180

          550         560         570         580         590         600
           *           *           *           *           *           *
CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC TCG
GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG AGC
Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr Ser
181____________185__________hTIE2 IG DOMAIN 2________195_________________200


          610         620         630         640         650         660
           *           *           *           *           *           *
GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CTC GAG GAC AAA ACT CAC ACA TGC
CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GAG CTC CTG TTT TGA GTG TGT ACG
Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Leu Glu Asp Lys Thr His Thr Cys
201____________205____hTIE2 IG DOMAIN 2____212 213_______hFC DOMAIN________220

          670         680         690         700         710         720
           *           *           *           *           *           *
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CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA
GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
221_____________225_____________hFC DOMAIN_____________235_____________240

          730           740           750           760           770           780
           *             *             *             *             *             *
CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG
GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
241_____________245_____________hFC DOMAIN_____________255_____________260

          790           800           810           820           830           840
           *             *             *             *             *             *
AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
261_____________265_____________hFC DOMAIN_____________275_____________280

          850           860           870           880           890           900
           *             *             *             *             *             *
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC
CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
281_____________285_____________hFC DOMAIN_____________295_____________300

          910           920           930           940           950           960
           *             *             *             *             *             *
ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
301_____________305_____________hFC DOMAIN_____________315_____________320

          970           980           990          1000          1010          1020
           *             *             *             *             *             *
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA
CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
321_____________325_____________hFC DOMAIN_____________335_____________340

         1030          1040          1050          1060          1070          1080
           *             *             *             *             *             *
CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC
GTC CAC ATG TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
341_____________345_____________hFC DOMAIN_____________355_____________360

         1090          1100          1110          1120          1130          1140
           *             *             *             *             *             *
TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
361_____________365_____________hFC DOMAIN_____________375_____________380

         1150          1160          1170          1180          1190          1200
           *             *             *             *             *             *
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC
GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
381_____________385_____________hFC DOMAIN_____________395_____________400


         1210          1220          1230          1240          1250          1260
           *             *             *             *             *             *
TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC
ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
401_____________405_____________hFC DOMAIN_____________415_____________420

         1270          1280          1290          1300          1310          1320
           *             *             *             *             *             *
GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT
CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
421_____________425_____________hFC DOMAIN_____________435_____________440

    1326
     *
AAA TGA (SEQ ID NO:7)
TTT ACT
Lys *** (SEQ ID NO:8)
441_442
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**DAAP#11**

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           10          20          30          40          50          60
            *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
            *           *           *           *           *           *
GAA GGT GCC ATG GAC TTG ATC TTG ATC AAT TCC CTA CCT CTT GTA TCT GAT GCT GAA ACA
CTT CCA CGG TAC CTG AAC TAG AAC TAG TTA AGG GAT GGA GAA CAT AGA CTA CGA CTT TGT
Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu Pro Leu Val Ser Asp Ala Glu Thr
21__22  23_____25__________hTIE2 IG DOMAIN 1________35_________________40

           130         140         150         160         170         180
            *           *           *           *           *           *
TCT CTC ACC TGC ATT GCC TCT GGG TGG CGC CCC CAT GAG CCC ATC ACC ATA GGA AGG GAC
AGA GAG TGG ACG TAA CGG AGA CCC ACC GCG GGG GTA CTC GGG TAG TGG TAT CCT TCC CTG
Ser Leu Thr Cys Ile Ala Ser Gly Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp
 41_____________45__________hTIE2 IG DOMAIN 1________55_________________60

           190         200         210         220         230         240
            *           *           *           *           *           *
TTT GAA GCC TTA ATG AAC CAG CAC CAG GAT CCG CTG GAA GTT ACT CAA GAT GTG ACC AGA
AAA CTT CGG AAT TAC TTG GTC GTG GTC CTA GGC GAC CTT CAA TGA GTT CTA CAC TGG TCT
Phe Glu Ala Leu Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 61_____________65__________hTIE2 IG DOMAIN 1________75_________________80

           250         260         270         280         290         300
            *           *           *           *           *           *
GAA TGG GCT AAA AAA GTT GTT TGG AAG AGA GAA AAG GCT AGT AAG ATC AAT GGT GCT TAT
CTT ACC CGA TTT TTT CAA CAA ACC TTC TCT CTT TTC CGA TCA TTC TAG TTA CCA CGA ATA
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile Asn Gly Ala Tyr
 81_____________85__________hTIE2 IG DOMAIN 1________95_________________100

           310         320         330         340         350         360
            *           *           *           *           *           *
TTC TGT GAA GGG CGA GTT CGA GGA GAG GCA ATC AGG ATA CGA ACC ATG AAG ATG CGT CAA
AAG ACA CTT CCC GCT CAA GCT CCT CTC CGT TAG TCC TAT GCT TGG TAC TTC TAC GCA GTT
Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg Ile Arg Thr Met Lys Met Arg Gln
101____________105__________hTIE2 IG DOMAIN 1________115________________120

           370         380         390         400         410         420
            *           *           *           *           *           *
CAA GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC
GTT CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG
Gln Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn
121 122________125__________hTIE2 IG DOMAIN 2________135________________140

           430         440         450         460         470         480
            *           *           *           *           *           *
ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC
TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG
Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
141____________145__________hTIE2 IG DOMAIN 2________155________________160

           490         500         510         520         530         540
            *           *           *           *           *           *
TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT
AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His
161____________165__________hTIE2 IG DOMAIN 2________175________________180

           550         560         570         580         590         600
            *           *           *           *           *           *
GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC
CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG
Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr
181____________185__________hTIE2 IG DOMAIN 2________195________________200


           610         620         630         640         650         660
            *           *           *           *           *           *
TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC GGT AGA CCT TTC GTA GAG ATG
AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG CCA TCT GGA AAG CAT CTC TAC
Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gly Arg Pro Phe Val Glu Met
201____________205_____hTIE2 IG DOMAIN 2_______213 214_215________________220

           670         680         690         700         710         720
            *           *           *           *           *           *
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TAC AGT GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC
ATG TCA CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG
Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys
221_____225_____hVEGFR1 IG DOMAIN 2_____215_____240

          730       740       750       760       770       780
           *         *         *         *         *         *
CGG GTT ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC
GCC CAA TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG
Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile
241_____245_____hVEGFR1 IG DOMAIN 2_____255_____260

          790       800       810       820       830       840
           *         *         *         *         *         *
CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG
GGA CTA CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC
Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
261_____265_____hVEGFR1 IG DOMAIN 2_____275_____280

          850       860       870       880       890       900
           *         *         *         *         *         *
TAC AAA GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA
ATG TTT CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr
281_____285_____hVEGFR1 IG DOMAIN 2_____295_____300

          910       920       930       940       950       960
           *         *         *         *         *         *
AAC TAT CTC ACA CAT CGA CAA CTC GAG GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA
TTG ATA GAG TGT GTA GCT GTT GAG CTC CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT
Asn Tyr Leu Thr His Arg Gln Leu Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
301_____305_____307 308_____310_____hFC DOMAIN_____320

          970       980       990      1000      1010      1020
           *         *         *         *         *         *
CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC
GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
321_____325_____hFC DOMAIN_____335_____340

         1030      1040      1050      1060      1070      1080
           *         *         *         *         *         *
ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT
TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
341_____345_____hFC DOMAIN_____355_____360

         1090      1100      1110      1120      1130      1140
           *         *         *         *         *         *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG
CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
361_____365_____hFC DOMAIN_____375_____380

         1150      1160      1170      1180      1190      1200
           *         *         *         *         *         *
CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG
GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
381_____385_____hFC DOMAIN_____395_____400


         1210      1220      1230      1240      1250      1260
           *         *         *         *         *         *
GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC
CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
401_____405_____hFC DOMAIN_____415_____420

         1270      1280      1290      1300      1310      1320
           *         *         *         *         *         *
ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG
TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
421_____425_____hFC DOMAIN_____435_____440

         1330      1340      1350      1360      1370      1380
           *         *         *         *         *         *
CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC
GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
441_____445_____hFC DOMAIN_____455_____460
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          1390          1400          1410          1420          1430          1440
            *             *             *             *             *             *
TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC
AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
461_____________465_____________hFC DOMAIN_____________475_____________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC
TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
481_____________485_____________hFC DOMAIN_____________495_____________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
501_____________505_____________hFC DOMAIN_____________515_____________520

          1570          1580          1590          1600          1610
            *             *             *             *             *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA (SEQ ID NO:9)
GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys *** (SEQ ID NO:10)
521_____________525_____________hFC DOMAIN_____________535_____537
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**DAAP#12**

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           10          20          30          40          50          60
            *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
            *           *           *           *           *           *
GAA GGT GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG
CTT CCA CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC
Glu Gly Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val
21__22  23_____25__________hTIE2 IG DOMAIN 2_________35_________________40

           130         140         150         160         170         180
            *           *           *           *           *           *
AAC ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT
TTG TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA
Asn Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly
 41_____________45__________hTIE2 IG DOMAIN 2_________55_________________60

           190         200         210         220         230         240
            *           *           *           *           *           *
TCC TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT
AGG AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA
Ser Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro
 61_____________65__________hTIE2 IG DOMAIN 2_________75_________________80

           250         260         270         280         290         300
            *           *           *           *           *           *
CAT GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC
GTA CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG
His Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe
 81_____________85__________hTIE2 IG DOMAIN 2_________95_________________100

           310         320         330         340         350         360
            *           *           *           *           *           *
ACC TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA
TGG AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT
Thr Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu
101____________105________hTIE2 IG DOMAIN 2________114 115_________________120

           370         380         390         400         410         420
            *           *           *           *           *           *
TGC AAC CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA
ACG TTG GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT
Cys Asn His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu
121____________125________hTIE2 EGF LIKE DOMAIN 1_____135_________________140

           430         440         450         460         470         480
            *           *           *           *           *           *
TGC ATT TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG
ACG TAA ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC
Cys Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr
141____________145________hTIE2 EGF LIKE DOMAIN 1__154 155_________________160

           490         500         510         520         530         540
            *           *           *           *           *           *
TTT GGC AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC
AAA CCG TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG
Phe Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe
161____________165________hTIE2 EGF LIKE DOMAIN 2_____175_________________180

           550         560         570         580         590         600
            *           *           *           *           *           *
TGT CTC CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT
ACA GAG GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA
Cys Leu Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn
181____________185________hTIE2 EGF LIKE DOMAIN 2________195_________________200

           610         620         630         640         650         660
            *           *           *           *           *           *
GAA GCA TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT
CTT CGT ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA
Glu Ala Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn
201 202________205_______hTIE2 EGF LIKE DOMAIN 3_____215_________________220

           670         680         690         700         710         720
            *           *           *           *           *           *
GGG GAG ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CCA GGA TGG CAG GGG CTC CAG
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CCC CTC TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GGT CCT ACC GTC CCC GAG GTC
Gly Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln Gly Leu Gln
221_____________225_______hTIE2 EGF LIKE DOMAIN 3_______235_________________240

          730           740           750           760           770           780
           *             *             *             *             *             *
TGT GAG AGA GAA GGC ATA CCG AGG ATG GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC
ACA CTC TCT CTT CCG TAT GGC TCC TAC CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG
Cys Glu Arg Glu Gly Ile Pro Arg Met Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile
241_____________245_____________249 250_______hVEGFR1 IG DOMAIN 2___________260

          790           800           810           820           830           840
           *             *             *             *             *             *
CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA
GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT
Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser
261_____________265___________hVEGFR1 IG DOMAIN 2_______275_________________280

          850           860           870           880           890           900
           *             *             *             *             *             *
CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA
GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT
Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys
281_____________285___________hVEGFR1 IG DOMAIN 2_______295_________________300

          910           920           930           940           950           960
           *             *             *             *             *             *
CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA
GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT
Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile
301_____________305___________hVEGFR1 IG DOMAIN 2_______315_________________320

          970           980           990          1000          1010          1020
           *             *             *             *             *             *
GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA
CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT
Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr
321_____________325___________hVEGFR1 IG DOMAIN 2_______335_________________340

         1030          1040          1050          1060          1070          1080
           *             *             *             *             *             *
CAT CGA CAA CTC GAG GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG
GTA GCT GTT GAG CTC CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC
His Arg Gln Leu Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
341_____343 344_345_____________hFC DOMAIN___________355_________________360

         1090          1100          1110          1120          1130          1140
           *             *             *             *             *             *
GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG
CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
361_____________365_____________hFC DOMAIN___________375_________________380

         1150          1160          1170          1180          1190          1200
           *             *             *             *             *             *
ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC
TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
381_____________385_____________hFC DOMAIN___________395_________________400

         1210          1220          1230          1240          1250          1260
           *             *             *             *             *             *
AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG
TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
401_____________405_____________hFC DOMAIN___________415_________________420

         1270          1280          1290          1300          1310          1320
           *             *             *             *             *             *
TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT
ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
421_____________425_____________hFC DOMAIN___________435_________________440

         1330          1340          1350          1360          1370          1380
           *             *             *             *             *             *
GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC
CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
441_____________445_____________hFC DOMAIN___________455_________________460

         1390          1400          1410          1420          1430          1440
           *             *             *             *             *             *
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ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG
TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
461_____________465_____________hFC DOMAIN_____________475_____________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC
CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG
Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
481_____________485_____________hFC DOMAIN_____________495_____________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT
CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
501_____________505_____________hFC DOMAIN_____________515_____________520

          1570          1580          1590          1600          1610          1620
            *             *             *             *             *             *
CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC
GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
521_____________525_____________hFC DOMAIN_____________535_____________540

          1630          1640          1650          1660          1670          1680
            *             *             *             *             *             *
AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC
TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
541_____________545_____________hFC DOMAIN_____________555_____________560

          1690          1700          1710
            *             *             *
TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA (SEQ ID NO:11)
ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys *** (SEQ ID NO:12)
561_____________565___hFC DOMAIN_____________573
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**DAAP#13**

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           10          20          30          40          50          60
           *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
           *           *           *           *           *           *
GAA GGT GCC ATG GAC TTG ATC TTG ATC AAT TCC CTA CCT CTT GTA TCT GAT GCT GAA ACA
CTT CCA CGG TAC CTG AAC TAG AAC TAG TTA AGG GAT GGA GAA CAT AGA CTA CGA CTT TGT
Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu Pro Leu Val Ser Asp Ala Glu Thr
21__22  23_____25__________hTIE2 IG DOMAIN 1________35_________________40

           130         140         150         160         170         180
           *           *           *           *           *           *
TCT CTC ACC TGC ATT GCC TCT GGG TGG CGC CCC CAT GAG CCC ATC ACC ATA GGA AGG GAC
AGA GAG TGG ACG TAA CGG AGA CCC ACC GCG GGG GTA CTC GGG TAG TGG TAT CCT TCC CTG
Ser Leu Thr Cys Ile Ala Ser Gly Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp
 41_____________45__________hTIE2 IG DOMAIN 1________55_________________60

           190         200         210         220         230         240
           *           *           *           *           *           *
TTT GAA GCC TTA ATG AAC CAG CAC CAG GAT CCG CTG GAA GTT ACT CAA GAT GTG ACC AGA
AAA CTT CGG AAT TAC TTG GTC GTG GTC CTA GGC GAC CTT CAA TGA GTT CTA CAC TGG TCT
Phe Glu Ala Leu Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 61_____________65__________hTIE2 IG DOMAIN 1________75_________________80

           250         260         270         280         290         300
           *           *           *           *           *           *
GAA TGG GCT AAA AAA GTT GTT TGG AAG AGA GAA AAG GCT AGT AAG ATC AAT GGT GCT TAT
CTT ACC CGA TTT TTT CAA CAA ACC TTC TCT CTT TTC CGA TCA TTC TAG TTA CCA CGA ATA
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile Asn Gly Ala Tyr
 81_____________85__________hTIE2 IG DOMAIN 1________95_________________100

           310         320         330         340         350         360
           *           *           *           *           *           *
TTC TGT GAA GGG CGA GTT CGA GGA GAG GCA ATC AGG ATA CGA ACC ATG AAG ATG CGT CAA
AAG ACA CTT CCC GCT CAA GCT CCT CTC CGT TAG TCC TAT GCT TGG TAC TTC TAC GCA GTT
Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg Ile Arg Thr Met Lys Met Arg Gln
101____________105__________hTIE2 IG DOMAIN 1________115_________________120

           370         380         390         400         410         420
           *           *           *           *           *           *
CAA GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC
GTT CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG
Gln Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn
121 122________125__________hTIE2 IG DOMAIN 2________135_________________140

           430         440         450         460         470         480
           *           *           *           *           *           *
ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC
TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG
Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
141____________145__________hTIE2 IG DOMAIN 2________155_________________160

           490         500         510         520         530         540
           *           *           *           *           *           *
TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT
AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His
161____________165__________hTIE2 IG DOMAIN 2________175_________________180

           550         560         570         580         590         600
           *           *           *           *           *           *
GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC
CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG
Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr
181____________185__________hTIE2 IG DOMAIN 2________195_________________200


           610         620         630         640         650         660
           *           *           *           *           *           *
TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA TGC
AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT ACG
Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys
201____________205________hTIE2 IG DOMAIN 2____213 214_215_________________220

           670         680         690         700         710         720
           *           *           *           *           *           *
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AAC CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA TGC
TTG GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT ACG
Asn His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
221_____225_____hTIE2 EGF LIKE DOMAIN 1_____235_____240

          730       740       750       760       770       780
           *         *         *         *         *         *
ATT TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG TTT
TAA ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC AAA
Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr Phe
241_____245___hTIE2 EGF LIKE DOMAIN 1___253 254_255_____260

          790       800       810       820       830       840
           *         *         *         *         *         *
GGC AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC TGT
CCG TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG ACA
Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe Cys
261_____265_____hTIE2 EGF LIKE DOMAIN 2_____275_____280

          850       860       870       880       890       900
           *         *         *         *         *         *
CTC CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT GAA
GAG GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA CTT
Leu Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu
281_____285_____hTIE2 EGF LIKE DOMAIN 2_____295_____300

          910       920       930       940       950       960
           *         *         *         *         *         *
GCA TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT GGG
CGT ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA CCC
Ala Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
301_____305_____hTIE2 EGF LIKE DOMAIN 3_____315_____320

          970       980       990      1000      1010      1020
           *         *         *         *         *         *
GAG ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CCA GGA TGG CAG GGG CTC CAG TGT
CTC TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GGT CCT ACC GTC CCC GAG GTC ACA
Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln Gly Leu Gln Cys
321_____325_____hTIE2 EGF LIKE DOMAIN 3_____335_____340

          1030      1040      1050      1060      1070      1080
           *         *         *         *         *         *
GAG AGA GAA GGC ATA CCG AGG ATG TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA GGT AGA
CTC TCT CTT CCG TAT GGC TCC TAC AGA CGT TAG ATA TAT AAA TAA TCA CTA TGT CCA TCT
Glu Arg Glu Gly Ile Pro Arg Met Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg
341_____345_____348 349_____hVEGFR1 IG DOMAIN 2_____360

          1090      1100      1110      1120      1130      1140
           *         *         *         *         *         *
CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG
GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC
Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu
361_____365_____hVEGFR1 IG DOMAIN 2_____375_____380

          1150      1160      1170      1180      1190      1200
           *         *         *         *         *         *
CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA
GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT
Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro
381_____385_____hVEGFR1 IG DOMAIN 2_____395_____400


          1210      1220      1230      1240      1250      1260
           *         *         *         *         *         *
CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC
GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG
Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile
401_____405_____hVEGFR1 IG DOMAIN 2_____415_____420

          1270      1280      1290      1300      1310      1320
           *         *         *         *         *         *
ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG
TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC
Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly
421_____425_____hVEGFR1 IG DOMAIN 2_____435_____440

          1330      1340      1350      1360      1370      1380
           *         *         *         *         *         *
CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA CTC GAG GAC AAA ACT CAC ACA TGC
GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT GAG CTC CTG TTT TGA GTG TGT ACG
His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Leu Glu Asp Lys Thr His Thr Cys
441_____445_____450_____452 453_____hFC DOMAIN_____460
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          1390          1400          1410          1420          1430          1440
            *             *             *             *             *             *
CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA
GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT
Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
461_____________465_____________hFC DOMAIN_____________475_____________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG
GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
481_____________485_____________hFC DOMAIN_____________495_____________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT
TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
501_____________505_____________hFC DOMAIN_____________515_____________520

          1570          1580          1590          1600          1610          1620
            *             *             *             *             *             *
GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC
CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG
Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
521_____________525_____________hFC DOMAIN_____________535_____________540

          1630          1640          1650          1660          1670          1680
            *             *             *             *             *             *
ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA
TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT
Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
541_____________545_____________hFC DOMAIN_____________555_____________560

          1690          1700          1710          1720          1730          1740
            *             *             *             *             *             *
GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA
CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
561_____________565_____________hFC DOMAIN_____________575_____________580

          1750          1760          1770          1780          1790          1800
            *             *             *             *             *             *
CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC
GTC CAC ATG TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG
Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
581_____________585_____________hFC DOMAIN_____________595_____________600


          1810          1820          1830          1840          1850          1860
            *             *             *             *             *             *
TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG
ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
601_____________605_____________hFC DOMAIN_____________615_____________620

          1870          1880          1890          1900          1910          1920
            *             *             *             *             *             *
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC
GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
621_____________625_____________hFC DOMAIN_____________635_____________640

          1930          1940          1950          1960          1970          1980
            *             *             *             *             *             *
TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC
ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
641_____________645_____________hFC DOMAIN_____________655_____________660

          1990          2000          2010          2020          2030          2040
            *             *             *             *             *             *
GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT
CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
661_____________665_____________hFC DOMAIN_____________675_____________680

    2046
      *
AAA TGA (SEQ ID NO:13)
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TTT ACT
Lys *** (SEQ ID NO:14)
681_682

**DAAP#14**

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           10          20          30          40          50          60
           *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE____15_________________20

           70          80          90          100         110         120
           *           *           *           *           *           *
GAA GGT GCC ATG GAC TTG ATC TTG ATC AAT TCC CTA CCT CTT GTA TCT GAT GCT GAA ACA
CTT CCA CGG TAC CTG AAC TAG AAC TAG TTA AGG GAT GGA GAA CAT AGA CTA CGA CTT TGT
Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu Pro Leu Val Ser Asp Ala Glu Thr
21__22  23_____25__________hTIE2 IG DOMAIN 1_____35_________________40

           130         140         150         160         170         180
           *           *           *           *           *           *
TCT CTC ACC TGC ATT GCC TCT GGG TGG CGC CCC CAT GAG CCC ATC ACC ATA GGA AGG GAC
AGA GAG TGG ACG TAA CGG AGA CCC ACC GCG GGG GTA CTC GGG TAG TGG TAT CCT TCC CTG
Ser Leu Thr Cys Ile Ala Ser Gly Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp
 41_____________45__________hTIE2 IG DOMAIN 1_____55_________________60

           190         200         210         220         230         240
           *           *           *           *           *           *
TTT GAA GCC TTA ATG AAC CAG CAC CAG GAT CCG CTG GAA GTT ACT CAA GAT GTG ACC AGA
AAA CTT CGG AAT TAC TTG GTC GTG GTC CTA GGC GAC CTT CAA TGA GTT CTA CAC TGG TCT
Phe Glu Ala Leu Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 61_____________65__________hTIE2 IG DOMAIN 1_____75_________________80

           250         260         270         280         290         300
           *           *           *           *           *           *
GAA TGG GCT AAA AAA GTT GTT TGG AAG AGA GAA AAG GCT AGT AAG ATC AAT GGT GCT TAT
CTT ACC CGA TTT TTT CAA CAA ACC TTC TCT CTT TTC CGA TCA TTC TAG TTA CCA CGA ATA
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile Asn Gly Ala Tyr
 81_____________85__________hTIE2 IG DOMAIN 1_____95_________________100

           310         320         330         340         350         360
           *           *           *           *           *           *
TTC TGT GAA GGG CGA GTT CGA GGA GAG GCA ATC AGG ATA CGA ACC ATG AAG ATG CGT CAA
AAG ACA CTT CCC GCT CAA GCT CCT CTC CGT TAG TCC TAT GCT TGG TAC TTC TAC GCA GTT
Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg Ile Arg Thr Met Lys Met Arg Gln
101____________105__________hTIE2 IG DOMAIN 1_____115_________________120

           370         380         390         400         410         420
           *           *           *           *           *           *
CAA GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC
GTT CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG
Gln Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn
121 122_________125__________hTIE2 IG DOMAIN 2_____135_________________140

           430         440         450         460         470         480
           *           *           *           *           *           *
ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC
TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG
Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
141____________145__________hTIE2 IG DOMAIN 2_____155_________________160

           490         500         510         520         530         540
           *           *           *           *           *           *
TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT
AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His
161____________165__________hTIE2 IG DOMAIN 2_____175_________________180

           550         560         570         580         590         600
           *           *           *           *           *           *
GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC
CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG
Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr
181____________185__________hTIE2 IG DOMAIN 2_____195_________________200


           610         620         630         640         650         660
           *           *           *           *           *           *
TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA TGC
AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT ACG
Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys
201____________205_____hTIE2 IG DOMAIN 2____213 214_215_________________220

           670         680         690         700         710         720
           *           *           *           *           *           *
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AAC CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA TGC
TTG GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT ACG
Asn His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
221_____225_____hTIE2 EGF LIKE DOMAIN 1_____235_____240

          730       740       750       760       770       780
           *         *         *         *         *         *
ATT TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG TTT
TAA ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC AAA
Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr Phe
241_____245___hTIE2 EGF LIKE DOMAIN 1___253 254_255_____260

          790       800       810       820       830       840
           *         *         *         *         *         *
GGC AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC TGT
CCG TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG ACA
Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe Cys
261_____265_____hTIE2 EGF LIKE DOMAIN 2_____275_____280

          850       860       870       880       890       900
           *         *         *         *         *         *
CTC CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT GAA
GAG GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA CTT
Leu Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu
281_____285_____hTIE2 EGF LIKE DOMAIN 2_____295_____300

          910       920       930       940       950       960
           *         *         *         *         *         *
GCA TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT GGG
CGT ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA CCC
Ala Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
301_____305_____hTIE2 EGF LIKE DOMAIN 3_____315_____320

          970       980       990      1000      1010      1020
           *         *         *         *         *         *
GAG ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CCA GGA TGG CAG GGG CTC CAG TGT
CTC TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GGT CCT ACC GTC CCC GAG GTC ACA
Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln Gly Leu Gln Cys
321_____325_____hTIE2 EGF LIKE DOMAIN 3_____335_____340

          1030      1040      1050      1060      1070      1080
           *         *         *         *         *         *
GAG AGA GAA GGC ATA CCG AGG ATG TTT ATT AGT GAT ACA GGT AGA CCT TTC GTA GAG ATG
CTC TCT CTT CCG TAT GGC TCC TAC AAA TAA TCA CTA TGT CCA TCT GGA AAG CAT CTC TAC
Glu Arg Glu Gly Ile Pro Arg Met Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met
341_____345_____348 349_____hVEGFR1 IG DOMAIN 2_____360

          1090      1100      1110      1120      1130      1140
           *         *         *         *         *         *
TAC AGT GAA ATC CCC GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC
ATG TCA CTT TAG GGG CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG
Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys
361_____365_____hVEGFR1 IG DOMAIN 2_____375_____380

          1150      1160      1170      1180      1190      1200
           *         *         *         *         *         *
CGG GTT ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC
GCC CAA TGC AGT GGA TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG
Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile
381_____385_____hVEGFR1 IG DOMAIN 2_____395_____400


          1210      1220      1230      1240      1250      1260
           *         *         *         *         *         *
CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG
GGA CTA CCT TTT GCG TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC
Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr
401_____405_____hVEGFR1 IG DOMAIN 2_____415_____420

          1270      1280      1290      1300      1310      1320
           *         *         *         *         *         *
TAC AAA GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA
ATG TTT CTT TAT CCC GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT
Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr
421_____425_____hVEGFR1 IG DOMAIN 2_____435_____440

          1330      1340      1350      1360      1370      1380
           *         *         *         *         *         *
AAC TAT CTC ACA CAT CGA CAA CTC GAG GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA
TTG ATA GAG TGT GTA GCT GTT GAG CTC CTG TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT
Asn Tyr Leu Thr His Arg Gln Leu Glu Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
441_____445_____447 448_____hFC DOMAIN_____460
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          1390          1400          1410          1420          1430          1440
            *             *             *             *             *             *
CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC
GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
461_____________465_____________hFC DOMAIN_____________475_____________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT
TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
481_____________485_____________hFC DOMAIN_____________495_____________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG
CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
501_____________505_____________hFC DOMAIN_____________515_____________520

          1570          1580          1590          1600          1610          1620
            *             *             *             *             *             *
CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG
GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
521_____________525_____________hFC DOMAIN_____________535_____________540

          1630          1640          1650          1660          1670          1680
            *             *             *             *             *             *
GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC
CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
541_____________545_____________hFC DOMAIN_____________555_____________560

          1690          1700          1710          1720          1730          1740
            *             *             *             *             *             *
ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG
TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
561_____________565_____________hFC DOMAIN_____________575_____________580

          1750          1760          1770          1780          1790          1800
            *             *             *             *             *             *
CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC
GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
581_____________585_____________hFC DOMAIN_____________595_____________600


          1810          1820          1830          1840          1850          1860
            *             *             *             *             *             *
TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC
AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
601_____________605_____________hFC DOMAIN_____________615_____________620

          1870          1880          1890          1900          1910          1920
            *             *             *             *             *             *
AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC
TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr
621_____________625_____________hFC DOMAIN_____________635_____________640

          1930          1940          1950          1960          1970          1980
            *             *             *             *             *             *
GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT
CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
641_____________645_____________hFC DOMAIN_____________655_____________660

          1990          2000          2010          2020          2030
            *             *             *             *             *
CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA (SEQ ID NO:15)
GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys *** (SEQ ID NO:16)
661_____________665_____________hFC DOMAIN_____________675_____677
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**DAAP#15**

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           10          20          30          40          50          60
            *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
            *           *           *           *           *           *
GAA GGT GCC ATG GAC TTG ATC TTG ATC AAT TCC CTA CCT CTT GTA TCT GAT GCT GAA ACA
CTT CCA CGG TAC CTG AAC TAG AAC TAG TTA AGG GAT GGA GAA CAT AGA CTA CGA CTT TGT
Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu Pro Leu Val Ser Asp Ala Glu Thr
21__22  23_____25__________hTIE2 IG DOMAIN 1_____35_________________40

           130         140         150         160         170         180
            *           *           *           *           *           *
TCT CTC ACC TGC ATT GCC TCT GGG TGG CGC CCC CAT GAG CCC ATC ACC ATA GGA AGG GAC
AGA GAG TGG ACG TAA CGG AGA CCC ACC GCG GGG GTA CTC GGG TAG TGG TAT CCT TCC CTG
Ser Leu Thr Cys Ile Ala Ser Gly Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp
 41_____________45__________hTIE2 IG DOMAIN 1_____55_________________60

           190         200         210         220         230         240
            *           *           *           *           *           *
TTT GAA GCC TTA ATG AAC CAG CAC CAG GAT CCG CTG GAA GTT ACT CAA GAT GTG ACC AGA
AAA CTT CGG AAT TAC TTG GTC GTG GTC CTA GGC GAC CTT CAA TGA GTT CTA CAC TGG TCT
Phe Glu Ala Leu Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 61_____________65__________hTIE2 IG DOMAIN 1_____75_________________80

           250         260         270         280         290         300
            *           *           *           *           *           *
GAA TGG GCT AAA AAA GTT GTT TGG AAG AGA GAA AAG GCT AGT AAG ATC AAT GGT GCT TAT
CTT ACC CGA TTT TTT CAA CAA ACC TTC TCT CTT TTC CGA TCA TTC TAG TTA CCA CGA ATA
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile Asn Gly Ala Tyr
 81_____________85__________hTIE2 IG DOMAIN 1_____95_________________100

           310         320         330         340         350         360
            *           *           *           *           *           *
TTC TGT GAA GGG CGA GTT CGA GGA GAG GCA ATC AGG ATA CGA ACC ATG AAG ATG CGT CAA
AAG ACA CTT CCC GCT CAA GCT CCT CTC CGT TAG TCC TAT GCT TGG TAC TTC TAC GCA GTT
Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg Ile Arg Thr Met Lys Met Arg Gln
101_____________105__________hTIE2 IG DOMAIN 1_____115_________________120

           370         380         390         400         410         420
            *           *           *           *           *           *
CAA GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC
GTT CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG
Gln Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn
121 122_________125__________hTIE2 IG DOMAIN 2_____135_________________140

           430         440         450         460         470         480
            *           *           *           *           *           *
ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC
TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG
Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
141_____________145__________hTIE2 IG DOMAIN 2_____155_________________160

           490         500         510         520         530         540
            *           *           *           *           *           *
TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT
AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His
161_____________165__________hTIE2 IG DOMAIN 2_____175_________________180

           550         560         570         580         590         600
            *           *           *           *           *           *
GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC
CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG
Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr
181_____________185__________hTIE2 IG DOMAIN 2_____195_________________200


           610         620         630         640         650         660
            *           *           *           *           *           *
TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA TGC
AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT ACG
Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys
201_____________205________hTIE2 IG DOMAIN 2____213 214_215_________________220

           670         680         690         700         710         720
            *           *           *           *           *           *
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AAC CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA TGC
TTG GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT ACG
Asn His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
221_____225_____hTIE2 EGF LIKE DOMAIN 1_____235_____240

        730       740       750       760       770       780
         *         *         *         *         *         *
ATT TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG TTT
TAA ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC AAA
Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr Phe
241_____245__hTIE2 EGF LIKE DOMAIN 1___253 254_255_____260

          790       800       810       820       830       840
           *         *         *         *         *         *
GGC AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC TGT
CCG TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG ACA
Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe Cys
261_____265_____hTIE2 EGF LIKE DOMAIN 2_____275_____280

          850       860       870       880       890       900
           *         *         *         *         *         *
CTC CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT GAA
GAG GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA CTT
Leu Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu
281_____285_____hTIE2 EGF LIKE DOMAIN 2_____295_____300

          910       920       930       940       950       960
           *         *         *         *         *         *
GCA TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT GGG
CGT ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA CCC
Ala Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
301_____305_____hTIE2 EGF LIKE DOMAIN 3_____315_____320

          970       980       990      1000      1010      1020
           *         *         *         *         *         *
GAG ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CCA GGA TGG CAG GGG CTC CAG TGT
CTC TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GGT CCT ACC GTC CCC GAG GTC ACA
Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln Gly Leu Gln Cys
321_____325_____hTIE2 EGF LIKE DOMAIN 3_____335_____340

         1030      1040      1050      1060      1070      1080
           *         *         *         *         *         *
GAG AGA GAA GGC ATA CCG AGG ATG GAG ATG TAC AGT GAA ATC CCC GAA ATT ATA CAC ATG
CTC TCT CTT CCG TAT GGC TCC TAC CTC TAC ATG TCA CTT TAG GGG CTT TAA TAT GTG TAC
Glu Arg Glu Gly Ile Pro Arg Met Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile His Met
341_____345_____348 349_____hVEGFR1 IG DOMAIN 2_____360

         1090      1100      1110      1120      1130      1140
           *         *         *         *         *         *
ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC ACT GTT ACT
TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA TTG TAG TGA CAA TGA
Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr
361_____365_____hVEGFR1 IG DOMAIN 2_____375_____380

         1150      1160      1170      1180      1190      1200
           *         *         *         *         *         *
TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT
AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG TAT TAG ACC CTG TCA
Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser
381_____385_____hVEGFR1 IG DOMAIN 2_____395_____400


         1210      1220      1230      1240      1250      1260
           *         *         *         *         *         *
AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC TGT GAA
TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC GAA GAC TGG ACA CTT
Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
401_____405_____hVEGFR1 IG DOMAIN 2_____415_____420

         1270      1280      1290      1300      1310      1320
           *         *         *         *         *         *
GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA CTC GAG GAC
CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA GCT GTT GAG CTC CTG
Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg Gln Leu Glu Asp
421_____425_____hVEGFR1 IG DOMAIN 2_____435_____437 438_____440

         1330      1340      1350      1360      1370      1380
           *         *         *         *         *         *
AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC
TTT TGA GTG TGT ACG GGT GGC ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG
Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
441_____445_____hFC DOMAIN_____455_____460
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          1390          1400          1410          1420          1430          1440
            *             *             *             *             *             *
CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC
GAG AAG GGG GGT TTT GGG TTC CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
461_____________465_____________hFC DOMAIN_____________475_____________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
GTG GTG GTG GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC
CAC CAC CAC CTG CAC TCG GTG CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
481_____________485_____________hFC DOMAIN_____________495_____________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT
CAC CTC CAC GTA TTA CGG TTC TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
501_____________505_____________hFC DOMAIN_____________515_____________520

          1570          1580          1590          1600          1610          1620
            *             *             *             *             *             *
GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC
CAC CAG TCG CAG GAG TGG CAG GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG
Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
521_____________525_____________hFC DOMAIN_____________535_____________540

          1630          1640          1650          1660          1670          1680
            *             *             *             *             *             *
AAG GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG
TTC CAG AGG TTG TTT CGG GAG GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC
Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
541_____________545_____________hFC DOMAIN_____________555_____________560

          1690          1700          1710          1720          1730          1740
            *             *             *             *             *             *
CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC
GTC GGG GCT CTT GGT GTC CAC ATG TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
561_____________565_____________hFC DOMAIN_____________575_____________580

          1750          1760          1770          1780          1790          1800
            *             *             *             *             *             *
CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG
GTC CAG TCG GAC TGG ACG GAC CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
581_____________585_____________hFC DOMAIN_____________595_____________600


          1810          1820          1830          1840          1850          1860
            *             *             *             *             *             *
GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC
CTC TCG TTA CCC GTC GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG
Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
601_____________605_____________hFC DOMAIN_____________615_____________620

          1870          1880          1890          1900          1910          1920
            *             *             *             *             *             *
GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC
CCG AGG AAG AAG GAG ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
621_____________625_____________hFC DOMAIN_____________635_____________640

          1930          1940          1950          1960          1970          1980
            *             *             *             *             *             *
GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC
CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
641_____________645_____________hFC DOMAIN_____________655_____________660

          1990          2000
            *             *
TCC CTG TCT CCG GGT AAA TGA (SEQ ID NO:17)
AGG GAC AGA GGC CCA TTT ACT
Ser Leu Ser Pro Gly Lys *** (SEQ ID NO:18)
661_____________665_____667
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**DAAP#16**

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           10          20          30          40          50          60
            *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
            *           *           *           *           *           *
GAA GGT GCC ATG GAC TTG ATC TTG ATC AAT TCC CTA CCT CTT GTA TCT GAT GCT GAA ACA
CTT CCA CGG TAC CTG AAC TAG AAC TAG TTA AGG GAT GGA GAA CAT AGA CTA CGA CTT TGT
Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu Pro Leu Val Ser Asp Ala Glu Thr
21__22 23_____25__________hTIE2 IG DOMAIN 1_____35_________________40

           130         140         150         160         170         180
            *           *           *           *           *           *
TCT CTC ACC TGC ATT GCC TCT GGG TGG CGC CCC CAT GAG CCC ATC ACC ATA GGA AGG GAC
AGA GAG TGG ACG TAA CGG AGA CCC ACC GCG GGG GTA CTC GGG TAG TGG TAT CCT TCC CTG
Ser Leu Thr Cys Ile Ala Ser Gly Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp
 41_____________45__________hTIE2 IG DOMAIN 1_____55_________________60

           190         200         210         220         230         240
            *           *           *           *           *           *
TTT GAA GCC TTA ATG AAC CAG CAC CAG GAT CCG CTG GAA GTT ACT CAA GAT GTG ACC AGA
AAA CTT CGG AAT TAC TTG GTC GTG GTC CTA GGC GAC CTT CAA TGA GTT CTA CAC TGG TCT
Phe Glu Ala Leu Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 61_____________65__________hTIE2 IG DOMAIN 1_____75_________________80

           250         260         270         280         290         300
            *           *           *           *           *           *
GAA TGG GCT AAA AAA GTT GTT TGG AAG AGA GAA AAG GCT AGT AAG ATC AAT GGT GCT TAT
CTT ACC CGA TTT TTT CAA CAA ACC TTC TCT CTT TTC CGA TCA TTC TAG TTA CCA CGA ATA
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile Asn Gly Ala Tyr
 81_____________85__________hTIE2 IG DOMAIN 1_____95_________________100

           310         320         330         340         350         360
            *           *           *           *           *           *
TTC TGT GAA GGG CGA GTT CGA GGA GAG GCA ATC AGG ATA CGA ACC ATG AAG ATG CGT CAA
AAG ACA CTT CCC GCT CAA GCT CCT CTC CGT TAG TCC TAT GCT TGG TAC TTC TAC GCA GTT
Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg Ile Arg Thr Met Lys Met Arg Gln
101_____________105__________hTIE2 IG DOMAIN 1_____115_________________120

           370         380         390         400         410         420
            *           *           *           *           *           *
CAA GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC
GTT CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG
Gln Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn
121 122________125__________hTIE2 IG DOMAIN 2_____135_________________140

           430         440         450         460         470         480
            *           *           *           *           *           *
ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC
TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG
Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
141_____________145__________hTIE2 IG DOMAIN 2_____155_________________160

           490         500         510         520         530         540
            *           *           *           *           *           *
TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT
AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His
161_____________165__________hTIE2 IG DOMAIN 2_____175_________________180

           550         560         570         580         590         600
            *           *           *           *           *           *
GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC
CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG
Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr
181_____________185__________hTIE2 IG DOMAIN 2_____195_________________200


           610         620         630         640         650         660
            *           *           *           *           *           *
TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA TGC
AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT ACG
Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys
201_____________205________hTIE2 IG DOMAIN 2____213 214_215_________________220

           670         680         690         700         710         720
            *           *           *           *           *           *
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AAC CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA TGC
TTG GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT ACG
Asn His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
221_____225_____hTIE2 EGF LIKE DOMAIN 1_____235_____240

          730       740       750       760       770       780
           *         *         *         *         *         *
ATT TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG TTT
TAA ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC AAA
Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr Phe
241_____245__hTIE2 EGF LIKE DOMAIN 1__253 254_255_____260

          790       800       810       820       830       840
           *         *         *         *         *         *
GGC AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC TGT
CCG TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG ACA
Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe Cys
261_____265_____hTIE2 EGF LIKE DOMAIN 2_____275_____280

          850       860       870       880       890       900
           *         *         *         *         *         *
CTC CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT GAA
GAG GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA CTT
Leu Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu
281_____285_____hTIE2 EGF LIKE DOMAIN 2_____295_____300

          910       920       930       940       950       960
           *         *         *         *         *         *
GCA TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT GGG
CGT ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA CCC
Ala Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
301_____305_____hTIE2 EGF LIKE DOMAIN 3_____315_____320

          970       980       990      1000      1010      1020
           *         *         *         *         *         *
GAG ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CCA GGA TGG CAG GGG CTC CAG TGT
CTC TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GGT CCT ACC GTC CCC GAG GTC ACA
Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln Gly Leu Gln Cys
321_____325_____hTIE2 EGF LIKE DOMAIN 3_____335_____340

         1030      1040      1050      1060      1070      1080
           *         *         *         *         *         *
GAG AGA GAA GGC ATA CCG AGG ATG GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC
CTC TCT CTT CCG TAT GGC TCC TAC CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG
Glu Arg Glu Gly Ile Pro Arg Met Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro
341_____345_____348 349_____hVEGFR1 IG DOMAIN 2_____360

         1090      1100      1110      1120      1130      1140
           *         *         *         *         *         *
GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT
CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA
Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
361_____365_____hVEGFR1 IG DOMAIN 2_____375_____380

         1150      1160      1170      1180      1190      1200
           *         *         *         *         *         *
AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC
TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg
381_____385_____hVEGFR1 IG DOMAIN 2_____395_____400


         1210      1220      1230      1240      1250      1260
           *         *         *         *         *         *
ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG
TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC
Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly
401_____405_____hVEGFR1 IG DOMAIN 2_____415_____420

         1270      1280      1290      1300      1310      1320
           *         *         *         *         *         *
CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT
GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA
Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His
421_____425_____hVEGFR1 IG DOMAIN 2_____435_____440

         1330      1340      1350      1360      1370      1380
           *         *         *         *         *         *
CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC AAA TTA CTT
GCT GTT TGG TTA TGT TAG TAT CTA CAG GTT TAT TCG TGT GGT GCG GGT CAG TTT AAT GAA
Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val Lys Leu Leu
441_____445_____448 449_450_____hVEGFR1 IG DOMAIN 3_____460
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          1390          1400          1410          1420          1430          1440
            *             *             *             *             *             *
AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA
TCT CCG GTA TGA GAA CAG GAG TTA ACA TGA CGA TGG TGA GGG AAC TTG TGC TCT CAA GTT
Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln
461_____________465__________hVEGFR1 IG DOMAIN 3_______475_________________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
ATG ACC TGG AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA ATT GAC
TAC TGG ACC TCA ATG GGA CTA CTT TTT TTA TTC TCT CGA AGG CAT TCC GCT GCT TAA CTG
Met Thr Trp Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp
481_____________485__________hVEGFR1 IG DOMAIN 3_______495_________________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT ACT ATT GAC AAA ATG CAG AAC
GTT TCG TTA AGG GTA CGG TTG TAT AAG ATG TCA CAA GAA TGA TAA CTG TTT TAC GTC TTG
Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn
501_____________505__________hVEGFR1 IG DOMAIN 3_______515_________________520

          1570          1580          1590          1600          1610          1620
            *             *             *             *             *             *
AAA GAC AAA GGA CTT TAT ACT TGT CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC
TTT CTG TTT CCT GAA ATA TGA ACA GCA CAT TCC TCA CCT GGT AGT AAG TTT AGA CAA TTG
Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn
521_____________525__________hVEGFR1 IG DOMAIN 3_______535_________________540

          1630          1640          1650          1660          1670          1680
            *             *             *             *             *             *
ACC TCA GTG CAT ATA TAT GAT AAA GCA CTC GAG GAC AAA ACT CAC ACA TGC CCA CCG TGC
TGG AGT CAC GTA TAT ATA CTA TTT CGT GAG CTC CTG TTT TGA GTG TGT ACG GGT GGC ACG
Thr Ser Val His Ile Tyr Asp Lys Ala Leu Glu Asp Lys Thr His Thr Cys Pro Pro Cys
541_____________545_____________549 550___________hFC DOMAIN_____________560

          1690          1700          1710          1720          1730          1740
            *             *             *             *             *             *
CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC
GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC CTG
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
561_____________565_____________hFC DOMAIN_____________575_________________580

          1750          1760          1770          1780          1790          1800
            *             *             *             *             *             *
ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC GAA
TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG CTT
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
581_____________585_____________hFC DOMAIN_____________595_________________600

          1810          1820          1830          1840          1850          1860
            *             *             *             *             *             *
GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG ACA
CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC TGT
Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
601_____________605_____________hFC DOMAIN_____________615_________________620

          1870          1880          1890          1900          1910          1920
            *             *             *             *             *             *
AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG
TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG GAC
Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
621_____________625_____________hFC DOMAIN_____________635_________________640

          1930          1940          1950          1960          1970          1980
            *             *             *             *             *             *
CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC CCA
GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG GGT
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
641_____________645_____________hFC DOMAIN_____________655_________________660

          1990          2000          2010          2020          2030          2040
            *             *             *             *             *             *
GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG TAC
CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC ATG
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
661_____________665_____________hFC DOMAIN_____________675_________________680

          2050          2060          2070          2080          2090          2100
            *             *             *             *             *             *
ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC
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TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG
Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
681_____________685_____________hFC DOMAIN_____________695_____________700

          2110          2120          2130          2140          2150          2160
            *             *             *             *             *             *
AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC
TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC TTG
Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
701_____________705_____________hFC DOMAIN_____________715_____________720

          2170          2180          2190          2200          2210          2220
            *             *             *             *             *             *
AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG
TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG TTC
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
721_____________725_____________hFC DOMAIN_____________735_____________740

          2230          2240          2250          2260          2270          2280
            *             *             *             *             *             *
CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT
GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC GTA
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
741_____________745_____________hFC DOMAIN_____________755_____________760

          2290          2300          2310          2320          2330
            *             *             *             *             *
GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA (SEQ ID NO:19)
CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys *** (SEQ ID NO:20)
761_____________765_____________hFC DOMAIN_____________775_____________779
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**DAAP#17**

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           10          20          30          40          50          60
            *           *           *           *           *           *
ATG GAC TCT TTA GCC AGC TTA GTT CTC TGT GGA GTC AGC TTG CTC CTT TCT GGA ACT GTG
TAC CTG AGA AAT CGG TCG AAT CAA GAG ACA CCT CAG TCG AAC GAG GAA AGA CCT TGA CAC
Met Asp Ser Leu Ala Ser Leu Val Leu Cys Gly Val Ser Leu Leu Leu Ser Gly Thr Val
1_____________5__________hTIE2 SIGNAL SEQUENCE_____15_________________20

           70          80          90          100         110         120
            *           *           *           *           *           *
GAA GGT GCC ATG GAC TTG ATC TTG ATC AAT TCC CTA CCT CTT GTA TCT GAT GCT GAA ACA
CTT CCA CGG TAC CTG AAC TAG AAC TAG TTA AGG GAT GGA GAA CAT AGA CTA CGA CTT TGT
Glu Gly Ala Met Asp Leu Ile Leu Ile Asn Ser Leu Pro Leu Val Ser Asp Ala Glu Thr
21__22  23______25__________hTIE2 IG DOMAIN 1________35_________________40

           130         140         150         160         170         180
            *           *           *           *           *           *
TCT CTC ACC TGC ATT GCC TCT GGG TGG CGC CCC CAT GAG CCC ATC ACC ATA GGA AGG GAC
AGA GAG TGG ACG TAA CGG AGA CCC ACC GCG GGG GTA CTC GGG TAG TGG TAT CCT TCC CTG
Ser Leu Thr Cys Ile Ala Ser Gly Trp Arg Pro His Glu Pro Ile Thr Ile Gly Arg Asp
 41_____________45__________hTIE2 IG DOMAIN 1________55_________________60

           190         200         210         220         230         240
            *           *           *           *           *           *
TTT GAA GCC TTA ATG AAC CAG CAC CAG GAT CCG CTG GAA GTT ACT CAA GAT GTG ACC AGA
AAA CTT CGG AAT TAC TTG GTC GTG GTC CTA GGC GAC CTT CAA TGA GTT CTA CAC TGG TCT
Phe Glu Ala Leu Met Asn Gln His Gln Asp Pro Leu Glu Val Thr Gln Asp Val Thr Arg
 61_____________65__________hTIE2 IG DOMAIN 1________75_________________80

           250         260         270         280         290         300
            *           *           *           *           *           *
GAA TGG GCT AAA AAA GTT GTT TGG AAG AGA GAA AAG GCT AGT AAG ATC AAT GGT GCT TAT
CTT ACC CGA TTT TTT CAA CAA ACC TTC TCT CTT TTC CGA TCA TTC TAG TTA CCA CGA ATA
Glu Trp Ala Lys Lys Val Val Trp Lys Arg Glu Lys Ala Ser Lys Ile Asn Gly Ala Tyr
 81_____________85__________hTIE2 IG DOMAIN 1________95_________________100

           310         320         330         340         350         360
            *           *           *           *           *           *
TTC TGT GAA GGG CGA GTT CGA GGA GAG GCA ATC AGG ATA CGA ACC ATG AAG ATG CGT CAA
AAG ACA CTT CCC GCT CAA GCT CCT CTC CGT TAG TCC TAT GCT TGG TAC TTC TAC GCA GTT
Phe Cys Glu Gly Arg Val Arg Gly Glu Ala Ile Arg Ile Arg Thr Met Lys Met Arg Gln
101____________105__________hTIE2 IG DOMAIN 1________115________________120

           370         380         390         400         410         420
            *           *           *           *           *           *
CAA GCT TCC TTC CTA CCA GCT ACT TTA ACT ATG ACT GTG GAC AAG GGA GAT AAC GTG AAC
GTT CGA AGG AAG GAT GGT CGA TGA AAT TGA TAC TGA CAC CTG TTC CCT CTA TTG CAC TTG
Gln Ala Ser Phe Leu Pro Ala Thr Leu Thr Met Thr Val Asp Lys Gly Asp Asn Val Asn
121 122________125__________hTIE2 IG DOMAIN 2________135________________140

           430         440         450         460         470         480
            *           *           *           *           *           *
ATA TCT TTC AAA AAG GTA TTG ATT AAA GAA GAA GAT GCA GTG ATT TAC AAA AAT GGT TCC
TAT AGA AAG TTT TTC CAT AAC TAA TTT CTT CTT CTA CGT CAC TAA ATG TTT TTA CCA AGG
Ile Ser Phe Lys Lys Val Leu Ile Lys Glu Glu Asp Ala Val Ile Tyr Lys Asn Gly Ser
141____________145__________hTIE2 IG DOMAIN 2________155________________160

           490         500         510         520         530         540
            *           *           *           *           *           *
TTC ATC CAT TCA GTG CCC CGG CAT GAA GTA CCT GAT ATT CTA GAA GTA CAC CTG CCT CAT
AAG TAG GTA AGT CAC GGG GCC GTA CTT CAT GGA CTA TAA GAT CTT CAT GTG GAC GGA GTA
Phe Ile His Ser Val Pro Arg His Glu Val Pro Asp Ile Leu Glu Val His Leu Pro His
161____________165__________hTIE2 IG DOMAIN 2________175________________180

           550         560         570         580         590         600
            *           *           *           *           *           *
GCT CAG CCC CAG GAT GCT GGA GTG TAC TCG GCC AGG TAT ATA GGA GGA AAC CTC TTC ACC
CGA GTC GGG GTC CTA CGA CCT CAC ATG AGC CGG TCC ATA TAT CCT CCT TTG GAG AAG TGG
Ala Gln Pro Gln Asp Ala Gly Val Tyr Ser Ala Arg Tyr Ile Gly Gly Asn Leu Phe Thr
181____________185__________hTIE2 IG DOMAIN 2________195________________200


           610         620         630         640         650         660
            *           *           *           *           *           *
TCG GCC TTC ACC AGG CTG ATA GTC CGG AGA TGT GAA GCC CAG AAG TGG GGA CCT GAA TGC
AGC CGG AAG TGG TCC GAC TAT CAG GCC TCT ACA CTT CGG GTC TTC ACC CCT GGA CTT ACG
Ser Ala Phe Thr Arg Leu Ile Val Arg Arg Cys Glu Ala Gln Lys Trp Gly Pro Glu Cys
201____________205________hTIE2 IG DOMAIN 2____213 214_215________________220

           670         680         690         700         710         720
            *           *           *           *           *           *
```

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AAC CAT CTC TGT ACT GCT TGT ATG AAC AAT GGT GTC TGC CAT GAA GAT ACT GGA GAA TGC
TTG GTA GAG ACA TGA CGA ACA TAC TTG TTA CCA CAG ACG GTA CTT CTA TGA CCT CTT ACG
Asn His Leu Cys Thr Ala Cys Met Asn Asn Gly Val Cys His Glu Asp Thr Gly Glu Cys
221_____225_____hTIE2 EGF LIKE DOMAIN 1_____235_____240

          730       740       750       760       770       780
           *         *         *         *         *         *
ATT TGC CCT CCT GGG TTT ATG GGA AGG ACG TGT GAG AAG GCT TGT GAA CTG CAC ACG TTT
TAA ACG GGA GGA CCC AAA TAC CCT TCC TGC ACA CTC TTC CGA ACA CTT GAC GTG TGC AAA
Ile Cys Pro Pro Gly Phe Met Gly Arg Thr Cys Glu Lys Ala Cys Glu Leu His Thr Phe
241_____245___hTIE2 EGF LIKE DOMAIN 1___253 254_255_____260

          790       800       810       820       830       840
           *         *         *         *         *         *
GGC AGA ACT TGT AAA GAA AGG TGC AGT GGA CAA GAG GGA TGC AAG TCT TAT GTG TTC TGT
CCG TCT TGA ACA TTT CTT TCC ACG TCA CCT GTT CTC CCT ACG TTC AGA ATA CAC AAG ACA
Gly Arg Thr Cys Lys Glu Arg Cys Ser Gly Gln Glu Gly Cys Lys Ser Tyr Val Phe Cys
261_____265_____hTIE2 EGF LIKE DOMAIN 2_____275_____280

          850       860       870       880       890       900
           *         *         *         *         *         *
CTC CCT GAC CCC TAT GGG TGT TCC TGT GCC ACA GGC TGG AAG GGT CTG CAG TGC AAT GAA
GAG GGA CTG GGG ATA CCC ACA AGG ACA CGG TGT CCG ACC TTC CCA GAC GTC ACG TTA CTT
Leu Pro Asp Pro Tyr Gly Cys Ser Cys Ala Thr Gly Trp Lys Gly Leu Gln Cys Asn Glu
281_____285_____hTIE2 EGF LIKE DOMAIN 2_____295_____300

          910       920       930       940       950       960
           *         *         *         *         *         *
GCA TGC CAC CCT GGT TTT TAC GGG CCA GAT TGT AAG CTT AGG TGC AGC TGC AAC AAT GGG
CGT ACG GTG GGA CCA AAA ATG CCC GGT CTA ACA TTC GAA TCC ACG TCG ACG TTG TTA CCC
Ala Cys His Pro Gly Phe Tyr Gly Pro Asp Cys Lys Leu Arg Cys Ser Cys Asn Asn Gly
301_____305_____hTIE2 EGF LIKE DOMAIN 3_____315_____320

          970       980       990      1000      1010      1020
           *         *         *         *         *         *
GAG ATG TGT GAT CGC TTC CAA GGA TGT CTC TGC TCT CCA GGA TGG CAG GGG CTC CAG TGT
CTC TAC ACA CTA GCG AAG GTT CCT ACA GAG ACG AGA GGT CCT ACC GTC CCC GAG GTC ACA
Glu Met Cys Asp Arg Phe Gln Gly Cys Leu Cys Ser Pro Gly Trp Gln Gly Leu Gln Cys
321_____325_____hTIE2 EGF LIKE DOMAIN 3_____335_____340

         1030      1040      1050      1060      1070      1080
           *         *         *         *         *         *
GAG AGA GAA GGC ATA CCG AGG ATG GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC
CTC TCT CTT CCG TAT GGC TCC TAC CCA TCT GGA AAG CAT CTC TAC ATG TCA CTT TAG GGG
Glu Arg Glu Gly Ile Pro Arg Met Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro
341_____345_____348 349_____hVEGFR1 IG DOMAIN 2_____360

         1090      1100      1110      1120      1130      1140
           *         *         *         *         *         *
GAA ATT ATA CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT
CTT TAA TAT GTG TAC TGA CTT CCT TCC CTC GAG CAG TAA GGG ACG GCC CAA TGC AGT GGA
Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr Ser Pro
361_____365_____hVEGFR1 IG DOMAIN 2_____375_____380

         1150      1160      1170      1180      1190      1200
           *         *         *         *         *         *
AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC
TTG TAG TGA CAA TGA AAT TTT TTC AAA GGT GAA CTG TGA AAC TAG GGA CTA CCT TTT GCG
Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg
381_____385_____hVEGFR1 IG DOMAIN 2_____395_____400


         1210      1220      1230      1240      1250      1260
           *         *         *         *         *         *
ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG
TAT TAG ACC CTG TCA TCT TTC CCG AAG TAG TAT AGT TTA CGT TGC ATG TTT CTT TAT CCC
Ile Ile Trp Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly
401_____405_____hVEGFR1 IG DOMAIN 2_____415_____420

         1270      1280      1290      1300      1310      1320
           *         *         *         *         *         *
CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT
GAA GAC TGG ACA CTT CGT TGT CAG TTA CCC GTA AAC ATA TTC TGT TTG ATA GAG TGT GTA
Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His
421_____425_____hVEGFR1 IG DOMAIN 2_____435_____440

         1330      1340      1350      1360      1370      1380
           *         *         *         *         *         *
CGA CAA ACC AAT ACA ATC ATA GAT GTG GTT CTG AGT CCG TCT CAT GGA ATT GAA CTA TCT
GCT GTT TGG TTA TGT TAG TAT CTA CAC CAA GAC TCA GGC AGA GTA CCT TAA CTT GAT AGA
Arg Gln Thr Asn Thr Ile Ile Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser
441_____445_____448 449_450_____hVEGFR2 IG DOMAIN 3_____460
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          1390          1400          1410          1420          1430          1440
            *             *             *             *             *             *
GTT GGA GAA AAG CTT GTC TTA AAT TGT ACA GCA AGA ACT GAA CTA AAT GTG GGG ATT GAC
CAA CCT CTT TTC GAA CAG AAT TTA ACA TGT CGT TCT TGA CTT GAT TTA CAC CCC TAA CTG
Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile Asp
461_____________465__________hVEGFR2 IG DOMAIN 3_______475_________________480

          1450          1460          1470          1480          1490          1500
            *             *             *             *             *             *
TTC AAC TGG GAA TAC CCT TCT TCG AAG CAT CAG CAT AAG AAA CTT GTA AAC CGA GAC CTA
AAG TTG ACC CTT ATG GGA AGA AGC TTC GTA GTC GTA TTC TTT GAA CAT TTG GCT CTG GAT
Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu Val Asn Arg Asp Leu
481_____________485__________hVEGFR2 IG DOMAIN 3_______495_________________500

          1510          1520          1530          1540          1550          1560
            *             *             *             *             *             *
AAA ACC CAG TCT GGG AGT GAG ATG AAG AAA TTT TTG AGC ACC TTA ACT ATA GAT GGT GTA
TTT TGG GTC AGA CCC TCA CTC TAC TTC TTT AAA AAC TCG TGG AAT TGA TAT CTA CCA CAT
Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val
501_____________505__________hVEGFR2 IG DOMAIN 3_______515_________________520

          1570          1580          1590          1600          1610          1620
            *             *             *             *             *             *
ACC CGG AGT GAC CAA GGA TTG TAC ACC TGT GCA GCA TCC AGT GGG CTG ATG ACC AAG AAG
TGG GCC TCA CTG GTT CCT AAC ATG TGG ACA CGT CGT AGG TCA CCC GAC TAC TGG TTC TTC
Thr Arg Ser Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys
521_____________525__________hVEGFR2 IG DOMAIN 3_______535_________________540

          1630          1640          1650          1660          1670          1680
            *             *             *             *             *             *
AAC AGC ACA TTT GTC AGG GTC CAT GAA AAA CTC GAG GAC AAA ACT CAC ACA TGC CCA CCG
TTG TCG TGT AAA CAG TCC CAG GTA CTT TTT GAG CTC CTG TTT TGA GTG TGT ACG GGT GGC
Asn Ser Thr Phe Val Arg Val His Glu Lys Leu Glu Asp Lys Thr His Thr Cys Pro Pro
541____ hVEGFR2 IG DOMAIN 3_________550 551__________hFC DOMAIN_____________560

          1690          1700          1710          1720          1730          1740
            *             *             *             *             *             *
TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG
ACG GGT CGT GGA CTT GAG GAC CCC CCT GGC AGT CAG AAG GAG AAG GGG GGT TTT GGG TTC
Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
561_____________565_____________hFC DOMAIN_____________575_________________580

          1750          1760          1770          1780          1790          1800
            *             *             *             *             *             *
GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC
CTG TGG GAG TAC TAG AGG GCC TGG GGA CTC CAG TGT ACG CAC CAC CAC CTG CAC TCG GTG
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
581_____________585_____________hFC DOMAIN_____________595_________________600

          1810          1820          1830          1840          1850          1860
            *             *             *             *             *             *
GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT GCC AAG
CTT CTG GGA CTC CAG TTC AAG TTG ACC ATG CAC CTG CCG CAC CTC CAC GTA TTA CGG TTC
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
601_____________605_____________hFC DOMAIN_____________615_________________620

          1870          1880          1890          1900          1910          1920
            *             *             *             *             *             *
ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC
TGT TTC GGC GCC CTC CTC GTC ATG TTG TCG TGC ATG GCA CAC CAG TCG CAG GAG TGG CAG
Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
621_____________625_____________hFC DOMAIN_____________635_________________640

          1930          1940          1950          1960          1970          1980
            *             *             *             *             *             *
CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC
GAC GTG GTC CTG ACC GAC TTA CCG TTC CTC ATG TTC ACG TTC CAG AGG TTG TTT CGG GAG
Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
641_____________645_____________hFC DOMAIN_____________655_________________660

          1990          2000          2010          2020          2030          2040
            *             *             *             *             *             *
CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA CCA CAG GTG
GGT CGG GGG TAG CTC TTT TGG TAG AGG TTT CGG TTT CCC GTC GGG GCT CTT GGT GTC CAC
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
661_____________665_____________hFC DOMAIN_____________675_________________680

          2050          2060          2070          2080          2090          2100
            *             *             *             *             *             *
TAC ACC CTG CCC CCA TCC CGG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG
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ATG TGG GAC GGG GGT AGG GCC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
681_____________685_____________hFC DOMAIN_____________695_____________700

          2110          2120          2130          2140          2150          2160
            *             *             *             *             *             *
GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG
CAG TTT CCG AAG ATA GGG TCG CTG TAG CGG CAC CTC ACC CTC TCG TTA CCC GTC GGC CTC
Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
701_____________705_____________hFC DOMAIN_____________715_____________720

          2170          2180          2190          2200          2210          2220
            *             *             *             *             *             *
AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC
TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG AGG AAG AAG GAG ATG TCG
Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
721_____________725_____________hFC DOMAIN_____________735_____________740

          2230          2240          2250          2260          2270          2280
            *             *             *             *             *             *
AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG
TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG AAG AGT ACG AGG CAC TAC
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
741_____________745_____________hFC DOMAIN_____________755_____________760

          2290          2300          2310          2320          2330          2340
            *             *             *             *             *             *
CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG GGT AAA TGA (SEQ ID NO:21)
GTA CTC CGA GAC GTG TTG GTG ATG TGC GTC TTC TCG GAG AGG GAC AGA GGC CCA TTT ACT
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys *** (SEQ ID NO:22)
761_____________765_____________hFC DOMAIN_____________775_____________780
```

**WHAT IS CLAIMED IS:**

1. An isolated nucleic acid molecule encoding a polypeptide having two components, which is capable of synchronously binding two different cytokines.

2. The nucleic acid molecule according to claim 1, encoding a polypeptide capable of synchronously binding VEGF polypeptide and angiopoietin polypeptide.

3. The nucleic acid molecule according to claim 2, comprising wherein the polypeptide is a Tie2 component and VEGFR component.

4. The nucleic acid molecule according to claim 1, wherein the components are operatively linked to a nucleotide sequence encoding a multimerizing component.

5. The nucleic acid molecule according to claim 4, wherein the VEGFR is VEGFR1 or VEGFR3.

6. The nucleic acid molecule according to claim 3, wherein the Tie2 component consists essentially of a nucleotide sequence encoding the amino acid sequences of Ig-like domain 1, Ig-like domain 2 and three EGF-like domains of the extracellular domain of Tie2.

7. The nucleic acid molecule according to claim 5, wherein the VEGFR1 component consists essentially of a nucleotide sequence encoding the amino acid sequences of Ig-like domain 2 of the extracellular domain of VEGFR1; and the VEGFR3 component consists essentially of the amino acid sequences of Ig-like domain 1, Ig-like domain 2 and Ig-like domain 3 of the extracellular domain of VEGFR3.

8. The nucleic acid molecule of claim 3, wherein the nucleotide sequences encoding the Tie2 component are located upstream of the nucleotide sequence encoding the VEGFR component.

9. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequences encoding the Tie2 component are located downstream of the nucleotide sequence encoding the VEGFR component.

10. The nucleic acid molecule of claim 4, wherein the multimerizing component is an immunoglobulin domain.

11. The isolated nucleic acid molecule of claim 10, wherein the immunoglobulin domain is selected from the group consisting of the Fc domain of IgG, the heavy chain of IgG, and the light chain of IgG.

12. An isolated nucleic acid molecule comprising a nucleotide sequence encoding:
(a) the nucleotide sequence set forth in Table 1 referred to as DAAP#1;
(b) the nucleotide sequence set forth in Table 1 referred to as DAAP#2;
(c) the nucleotide sequence set forth in Table 1 referred to as DAAP#3;
(d) the nucleotide sequence set forth in Table 1 referred to as DAAP#4;
(e) the nucleotide sequence set forth in Table 1 referred to as DAAP#11;
(f) the nucleotide sequence set forth in Table 1 referred to as DAAP#12;
(g) the nucleotide sequence set forth in Table 1 referred to as DAAP#13;
(h) the nucleotide sequence set forth in Table 1 referred to as DAAP#14;
(i) the nucleotide sequence set forth in Table 1 referred to as DAAP#15;
(j) the nucleotide sequence set forth in Table 1 referred to as DAAP#16;
(k) the nucleotide sequence set forth in Table 1 referred to as DAAP#17; or
(l) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucleotide sequence of (a), (b), (c), (d), (e), (f), (g), (h), (i), (j), or (k) but which encodes identical amino acid sequence as expressed therefrom.

13. An expression vector comprising a nucleic acid molecule of claim 1, wherein the nucleic acid molecule is operatively linked to an expression control sequence.

14. A host-vector system for the production of a fusion polypeptide which comprises the expression vector of claim 13, in a suitable host cell.

15. The host-vector system of claim 14, wherein the suitable host cell is a bacterial cell, yeast cell, insect cell, or mammalian cell.

16. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 1.

17. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 2.

18. A fusion polypeptide encoded by the isolated nucleic acid molecule of claim 3.

19. A method of producing a fusion polypeptide which comprises growing cells of the host-vector system of claim 14, under conditions permitting production of the fusion polypeptide

and recovering the fusion polypeptide so produced.

20. A fusion polypeptide encoded by the nucleic acid sequence according to claim 1, which has been modified by acetylation or pegylation.

21. A method of decreasing or inhibiting plasma leakage in a mammal comprising administering to a mammal in need thereof an effective amount of the fusion polypeptide of claim 16.

22. The method of claim 21, wherein the mammal is a human.

23. A method of blocking blood vessel growth in a mammal comprising administering to the mammal in need thereof an effective amount of the fusion polypeptide of claim 17.

24. A method of inhibiting VEGF receptor ligand and Tie2 ligand activities in a mammal comprising administering to the mammal an effective amount of the fusion polypeptide of claim 18.

25. A method of attenuating or preventing tumor growth in a mammal, comprising administering to a subject in need thereof a therapeutically effective amount of the fusion polypeptide of claim 16.

26. A method of attenuating or preventing edema in a human comprising administering to a subject in need thereof a therapeutically effective amount of the fusion polypeptide of claim 16.

27. The method of claim 26, wherein the edema is brain edema.

28. A method of attenuating or preventing ascites formation in a human comprising administering to a subject in need thereof a therapeutically effective amount of the fusion polypeptide of claim 16.

29. The method of claim 28, wherein the ascites is associated with ovarian cancer.

30. The nucleic acid according to claim 1, with the proviso that the polypeptide is not an antibody.

31. A method of treating a condition caused by inflammatory angiogenesis in a subject, comprising administering to the subjection the fusion polypeptide according to claim 16.

32. The method according to claim 31, wherein the condition is rheumatoid arthritis.

Figure 1

Schematic diagrams of VEGFR1 and Tie2

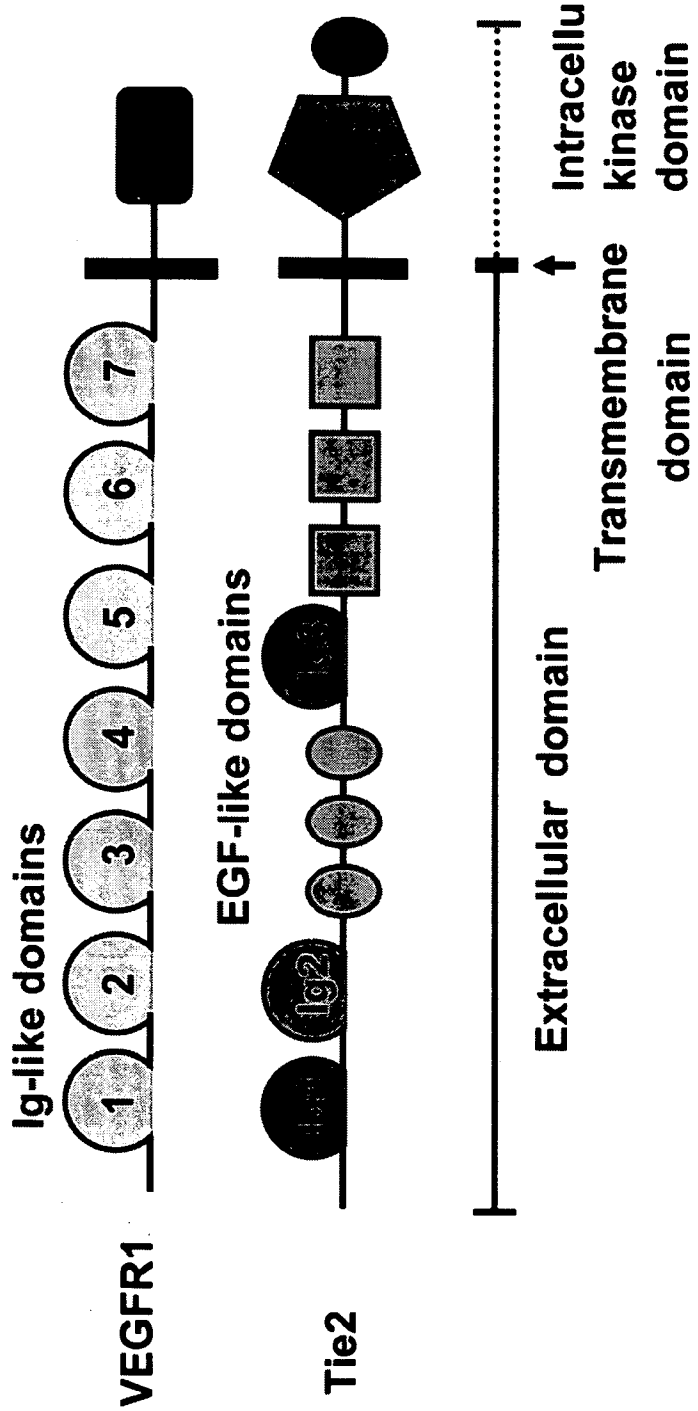


Figure 2

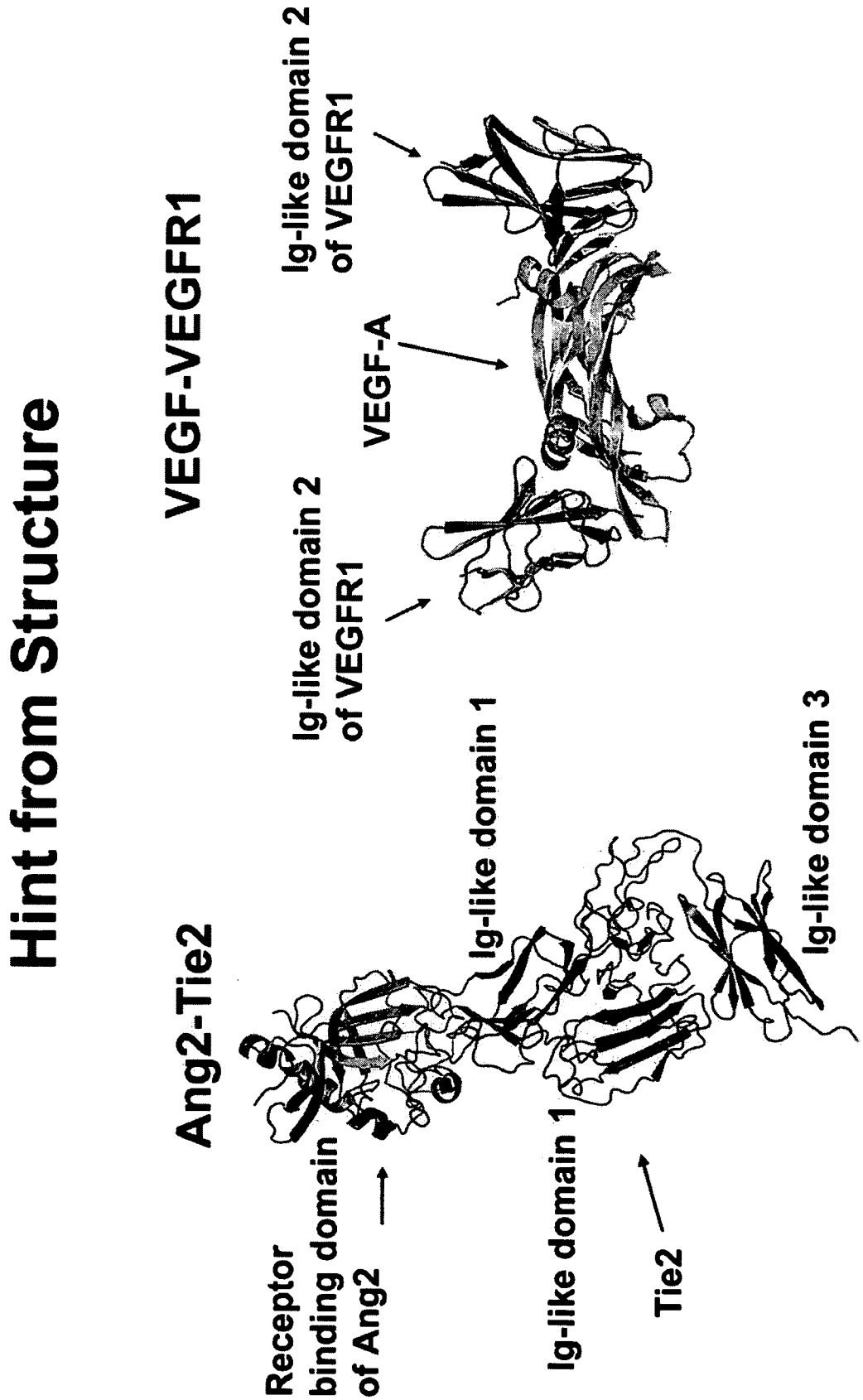

Hint from Structure
VEGF-VEGFR1
Ig-like domain 2 of VEGFR1
VEGF-A
Ig-like domain 2 of VEGFR1
Ang2-Tie2
Ig-like domain 1
Ig-like domain 3
Receptor binding domain of Ang2
Ig-like domain 1
Tie2

Figure 3

Schematic diagrams of DAAP constructs

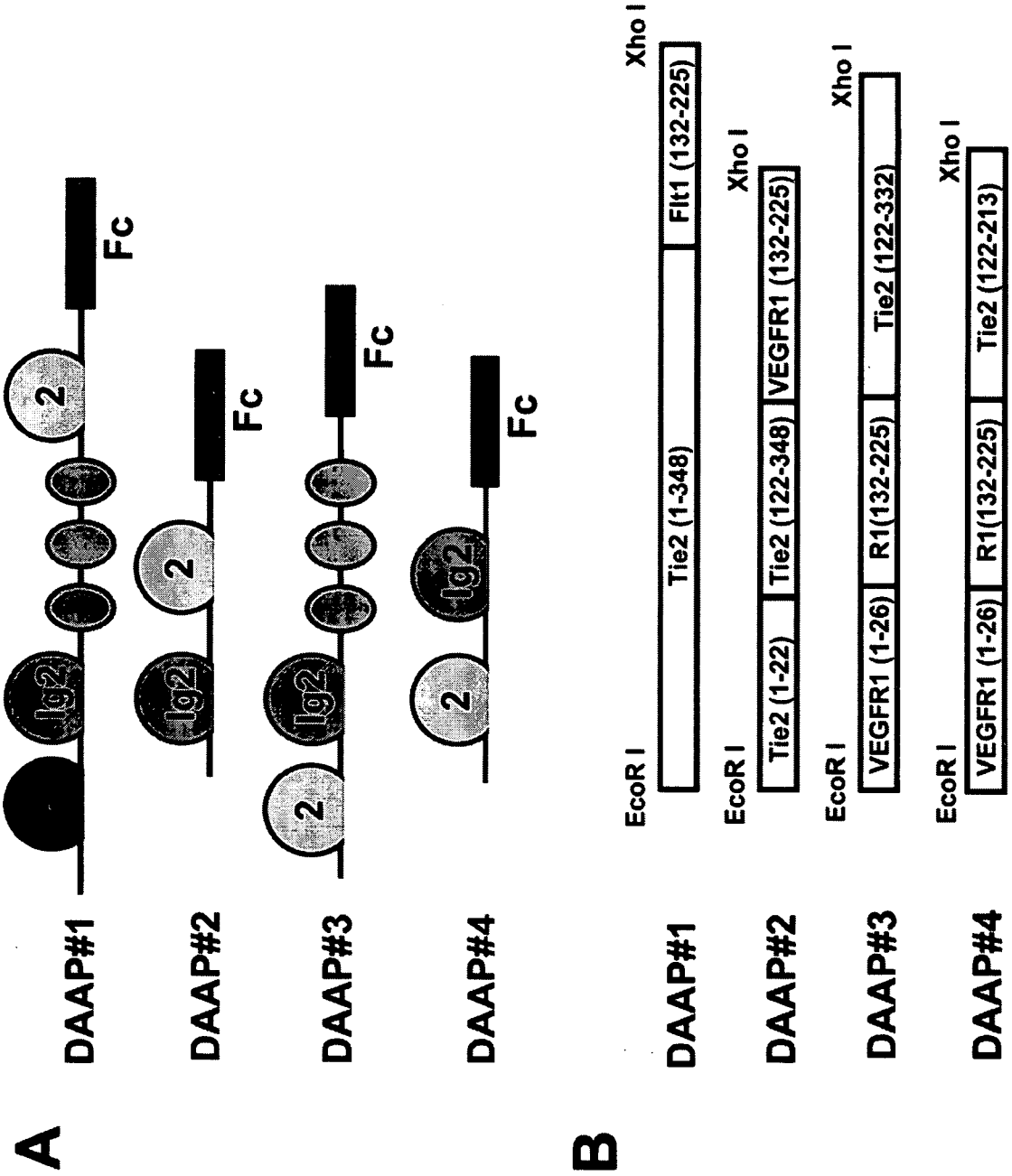


Figure 4

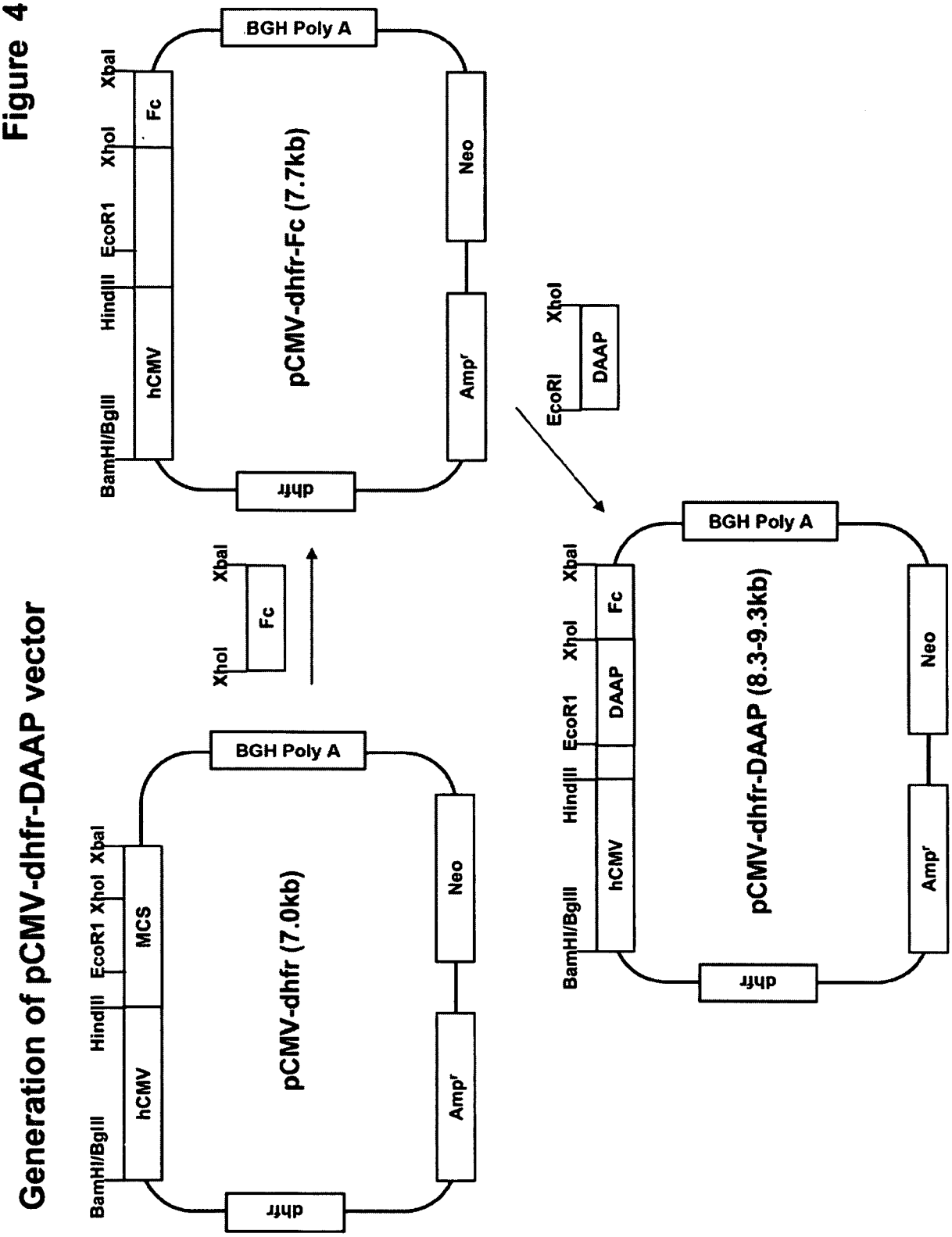

Generation of pCMV-dhfr-DAAP vector
pCMV-dhfr (7.0kb)
BGH Poly A
Neo
Ampr
dhfr
BamHI/BglII
hCMV
HindIII
EcoR1
XhoI
MCS
XbaI
XhoI
Fc
pCMV-dhfr-Fc (7.7kb)
BGH Poly A
Neo
Ampr
dhfr
BamHI/BglII
hCMV
HindIII
EcoR1
XhoI
Fc
XbaI
EcoRI
XhoI
DAAP
pCMV-dhfr-DAAP (8.3-9.3kb)
BGH Poly A
Neo
Ampr
dhfr
BamHI/BglII
hCMV
HindIII
EcoR1
DAAP
XhoI
Fc
XbaI

Figure 5

Schematic diagram of modified DAAP constructs

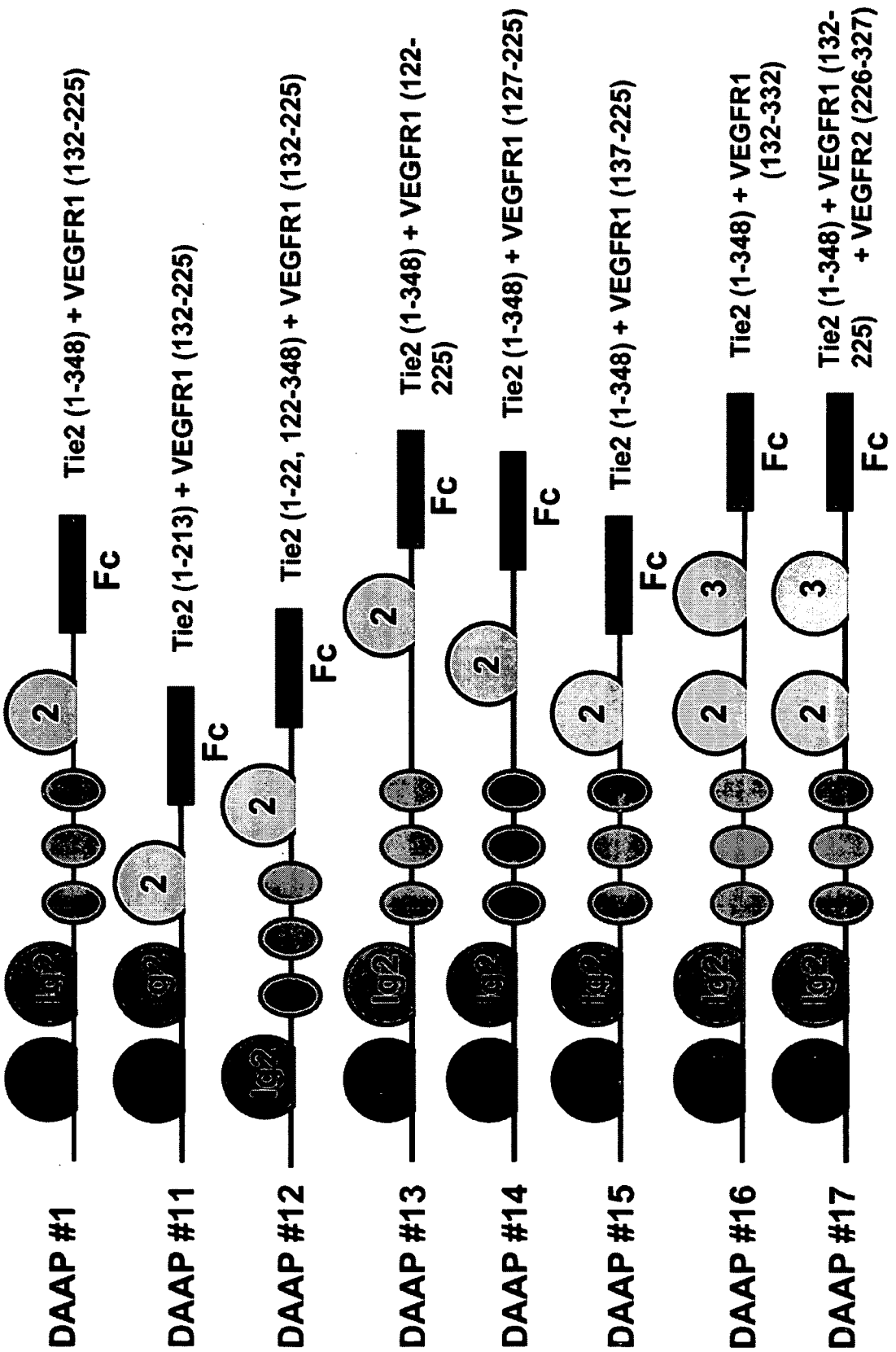


Figure 6

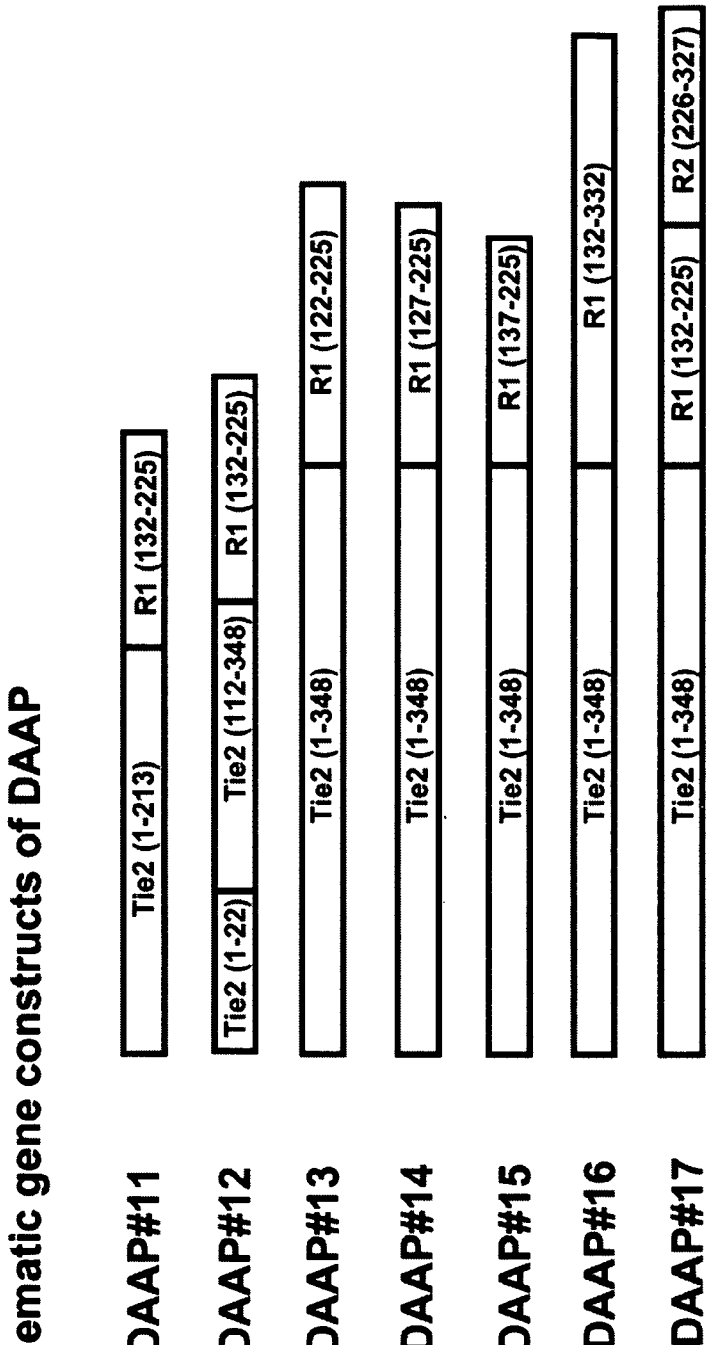

Schematic gene constructs of DAAP
DAAP#11
Tie2 (1-213)
R1 (132-225)
DAAP#12
Tie2 (1-22)
Tie2 (112-348)
R1 (132-225)
DAAP#13
Tie2 (1-348)
R1 (122-225)
DAAP#14
Tie2 (1-348)
R1 (127-225)
DAAP#15
Tie2 (1-348)
R1 (137-225)
DAAP#16
Tie2 (1-348)
R1 (132-332)
DAAP#17
Tie2 (1-348)
R1 (132-225)
R2 (226-327)

Figure 7

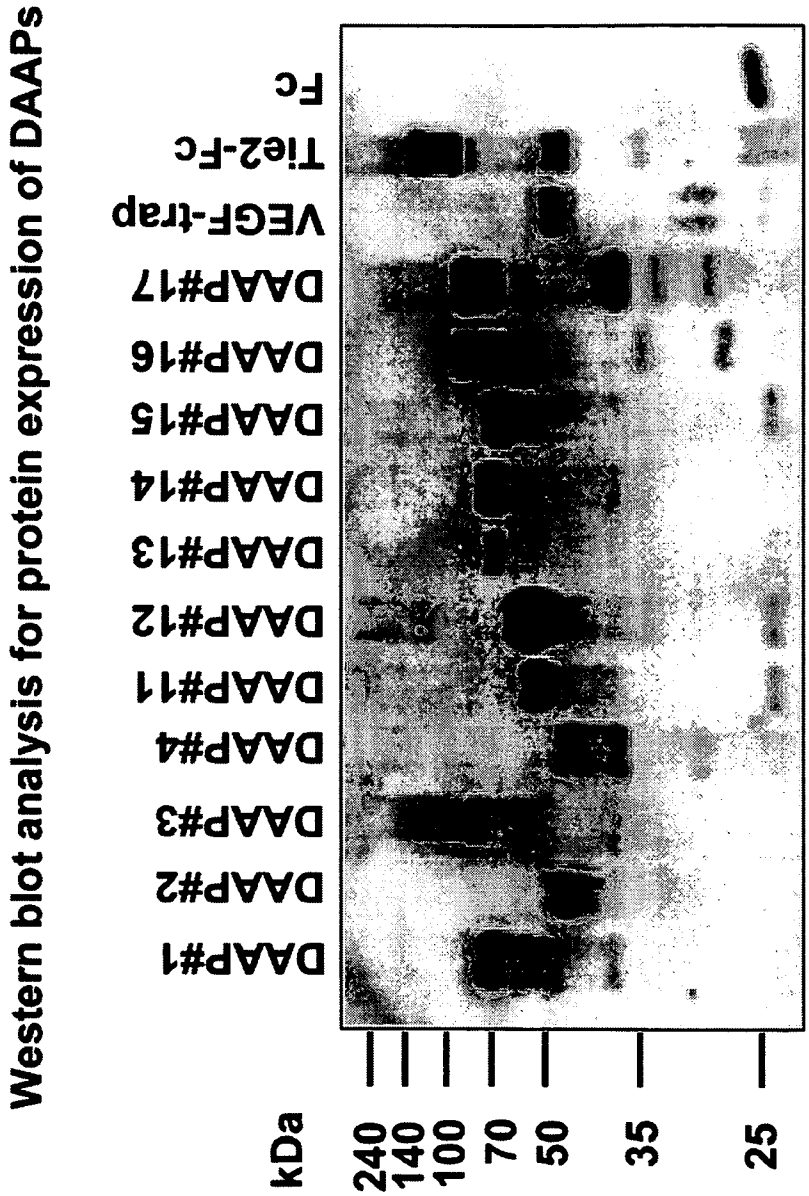

Western blot analysis for protein expression of DAAPs
DAAP#1
DAAP#2
DAAP#3
DAAP#4
DAAP#11
DAAP#12
DAAP#13
DAAP#14
DAAP#15
DAAP#16
DAAP#17
VEGF-trap
Tie2-Fc
Fc
kDa
240
140
100
70
50
35
25

Figure 8

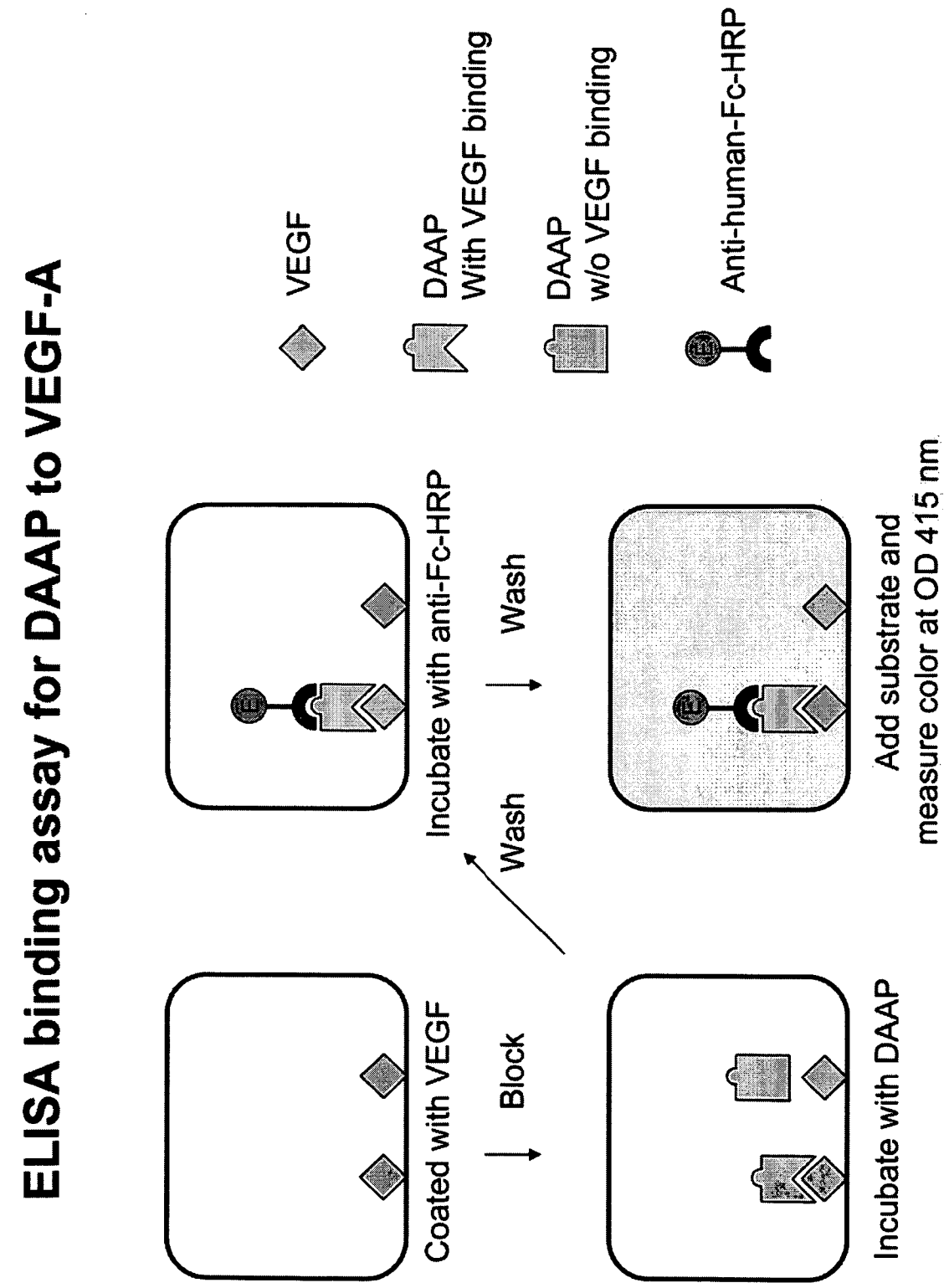

ELISA binding assay for DAAP to VEGF-A
VEGF
DAAP
With VEGF binding
DAAP
w/o VEGF binding
Anti-human-Fc-HRP
Coated with VEGF
Block
Incubate with DAAP
Wash
Incubate with anti-Fc-HRP
Wash
Add substrate and
measure color at OD 415 nm

Figure 9

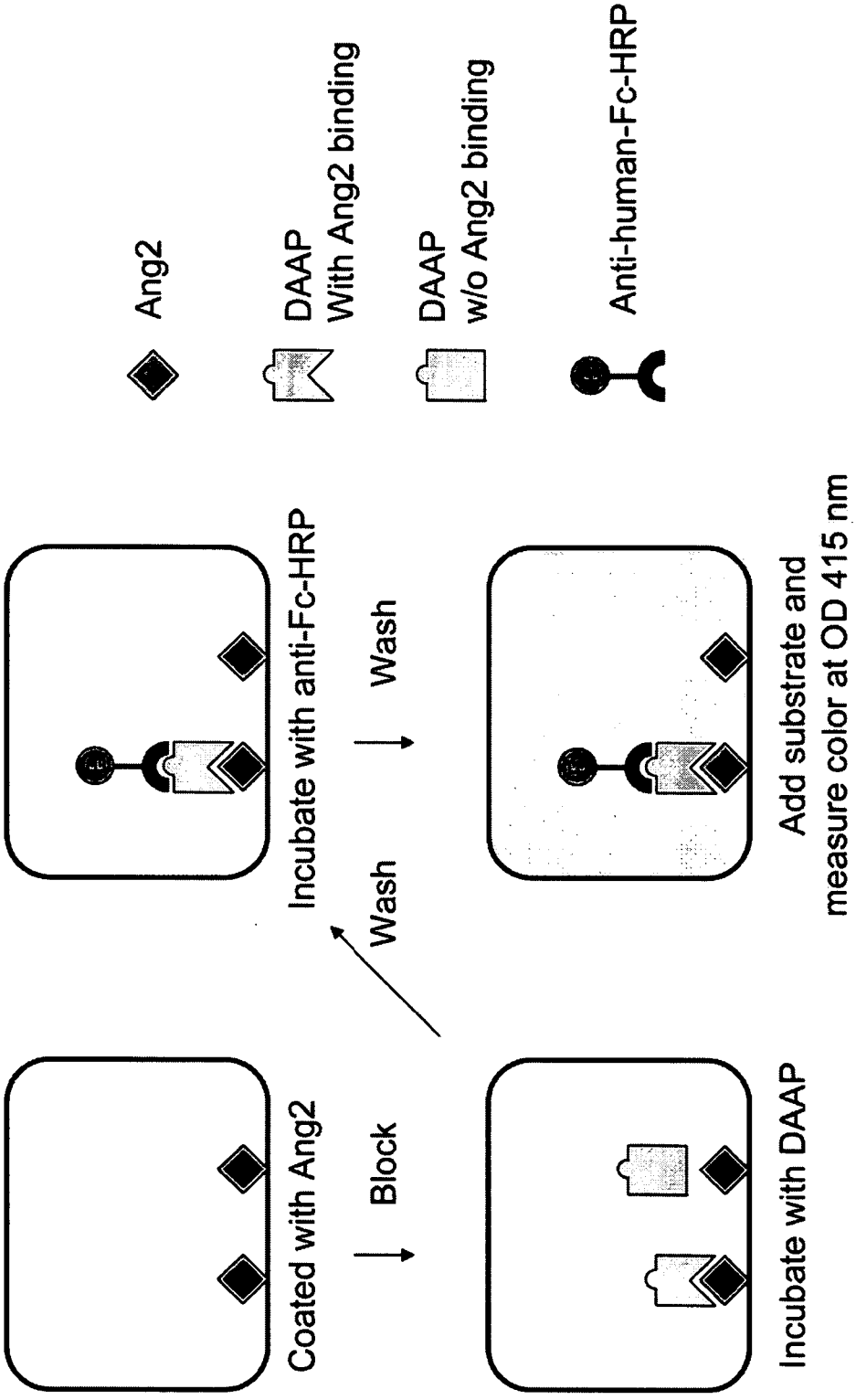

ELISA binding assay for DAAP to Ang2
Ang2
DAAP
With Ang2 binding
DAAP
w/o Ang2 binding
Anti-human-Fc-HRP
Coated with Ang2
Block
Incubate with DAAP
Wash
Incubate with anti-Fc-HRP
Wash
Add substrate and
measure color at OD 415 nm

Figure 10

**DAAP: ELISA binding assay for DAAP to VEGF-A and Ang2**

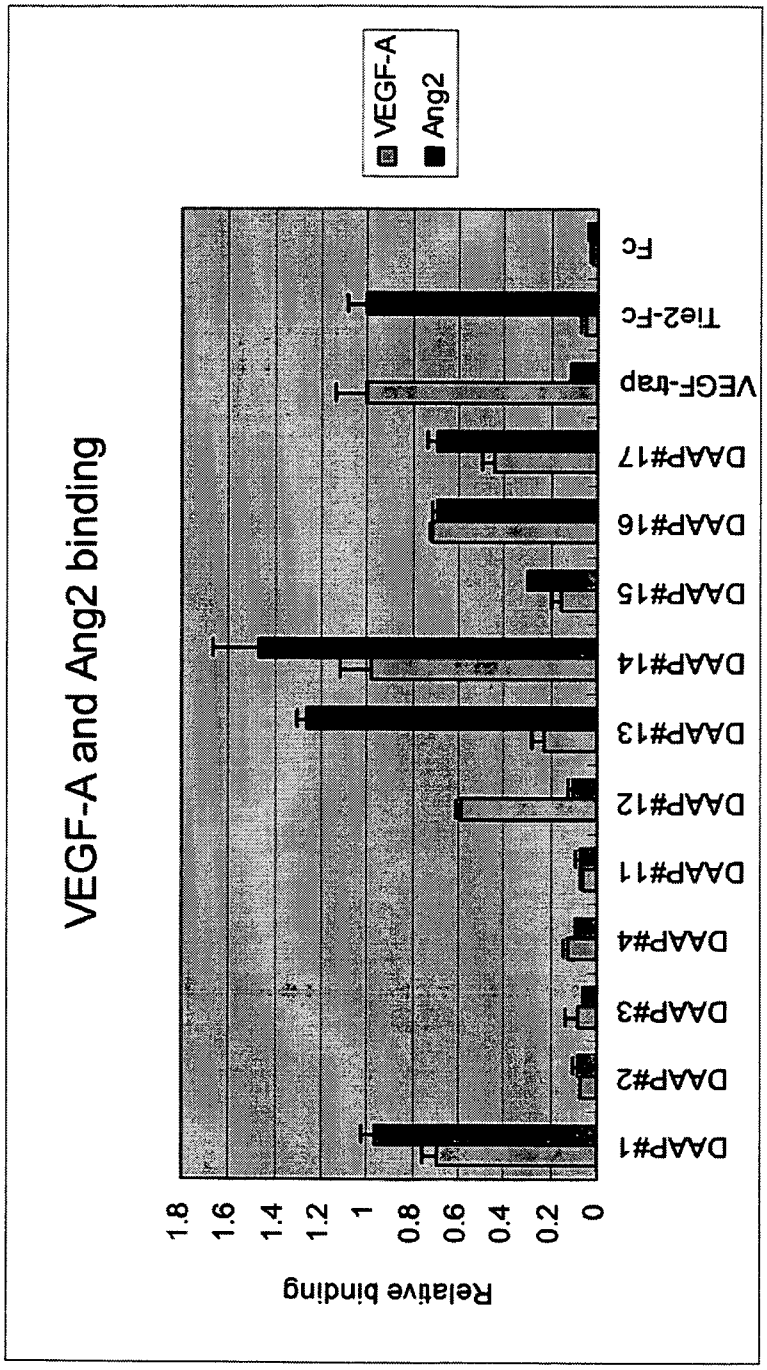


Figure 11

Competitive binding assay of DAAP to VEGF-A and Ang2

ELISA binding assay

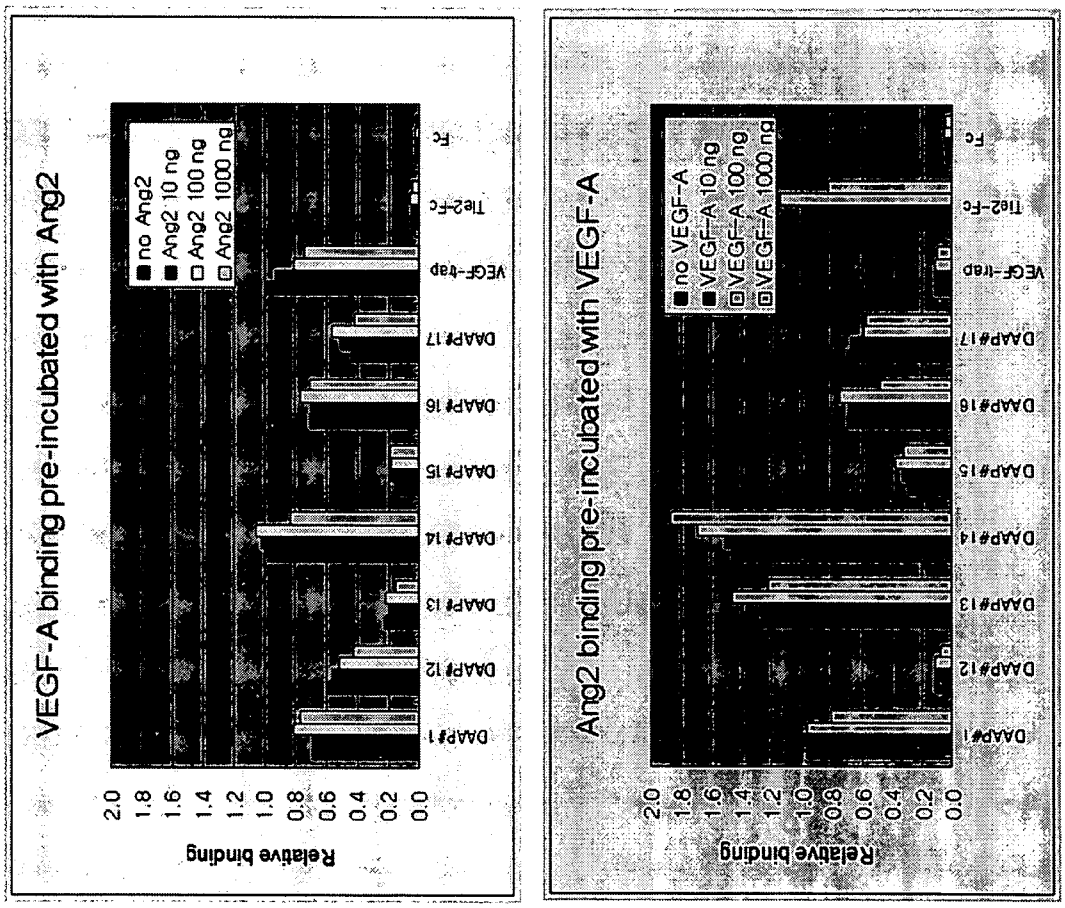


Figure 12

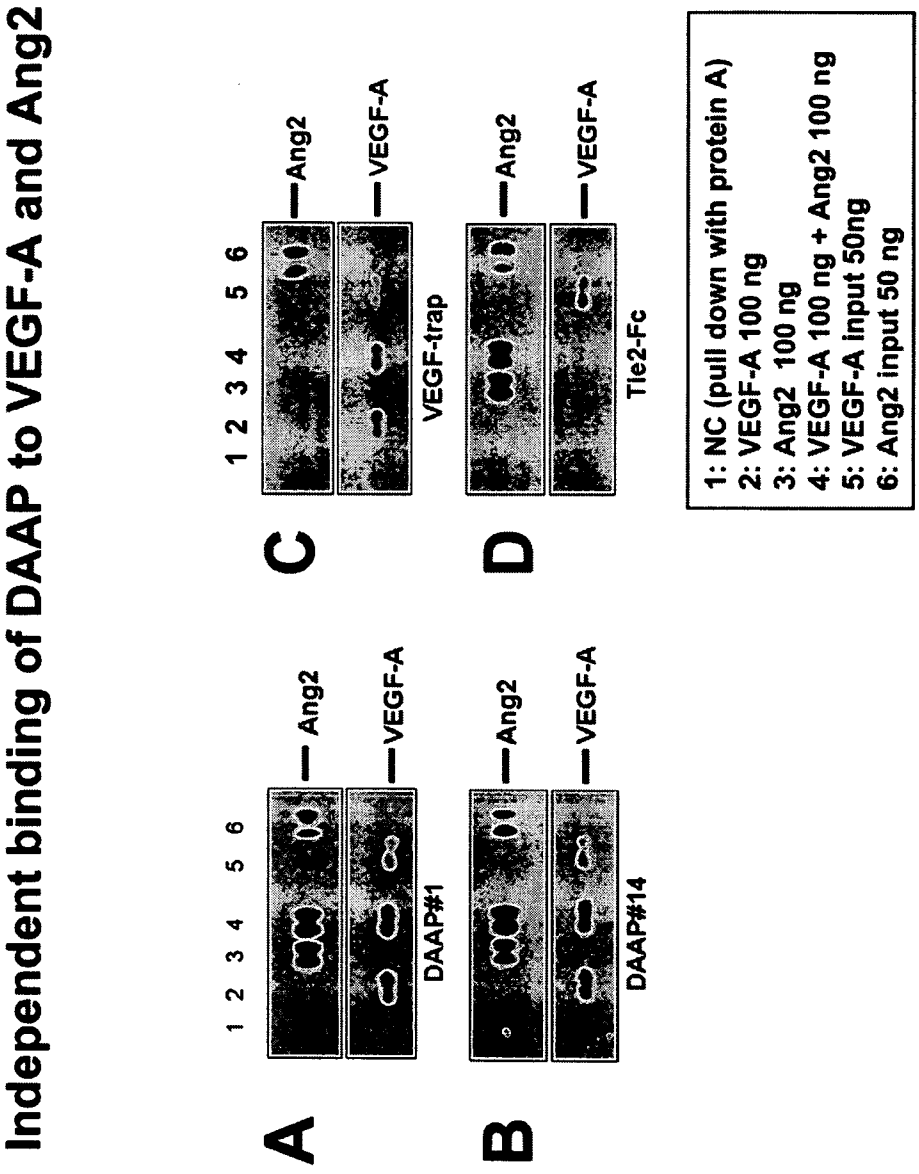

Independent binding of DAAP to VEGF-A and Ang2
A
B
C
D
1 2 3 4 5 6
Ang2
VEGF-A
DAAP#1
DAAP#14
VEGF-trap
Tie2-Fc
1: NC (pull down with protein A)
2: VEGF-A 100 ng
3: Ang2 100 ng
4: VEGF-A 100 ng + Ang2 100 ng
5: VEGF-A input 50ng
6: Ang2 input 50 ng

Figure 13

DAAP capable of synchronous binding of VEGF and angiopoietin

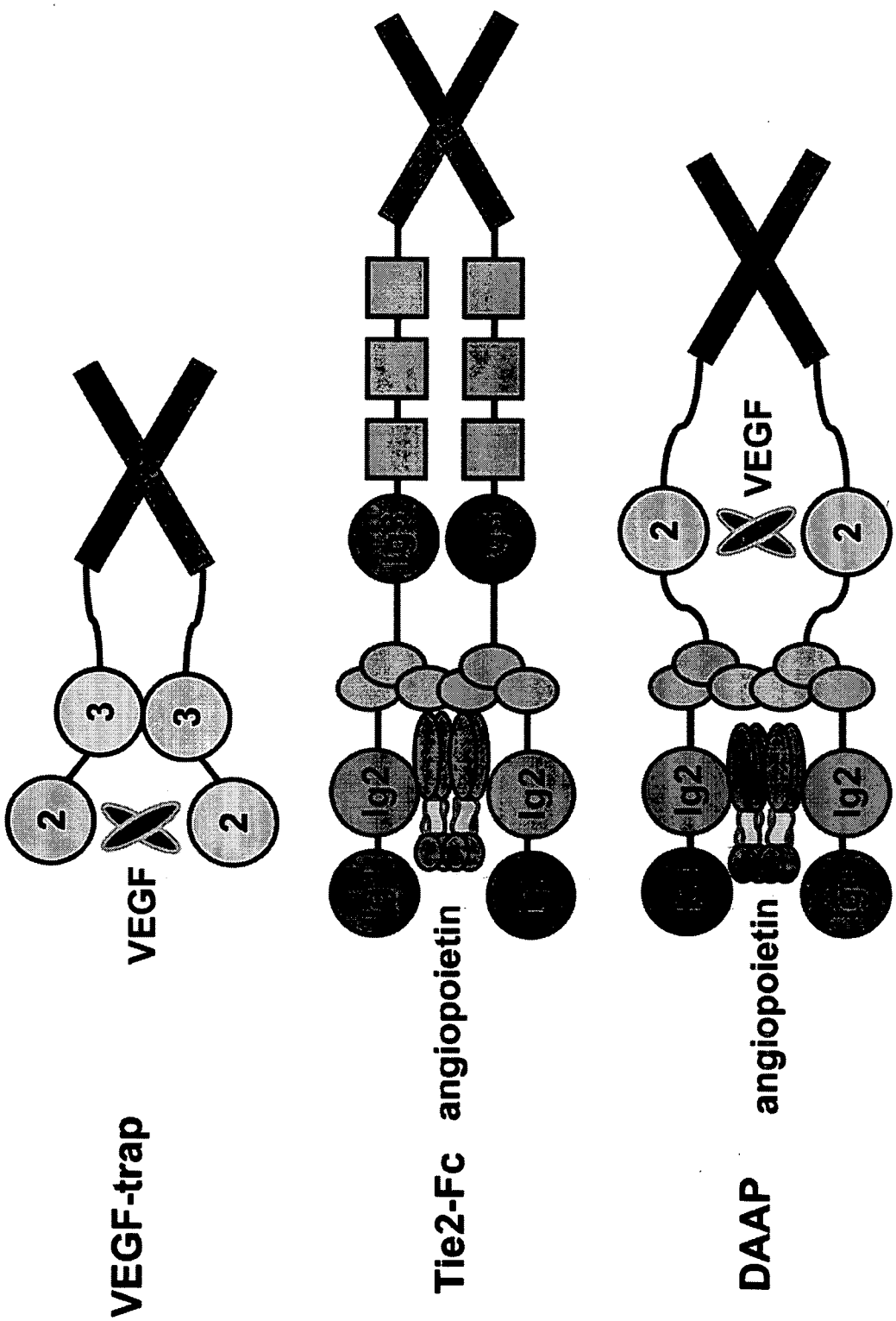


Figure 14

Generation and Purification of DAAP#1

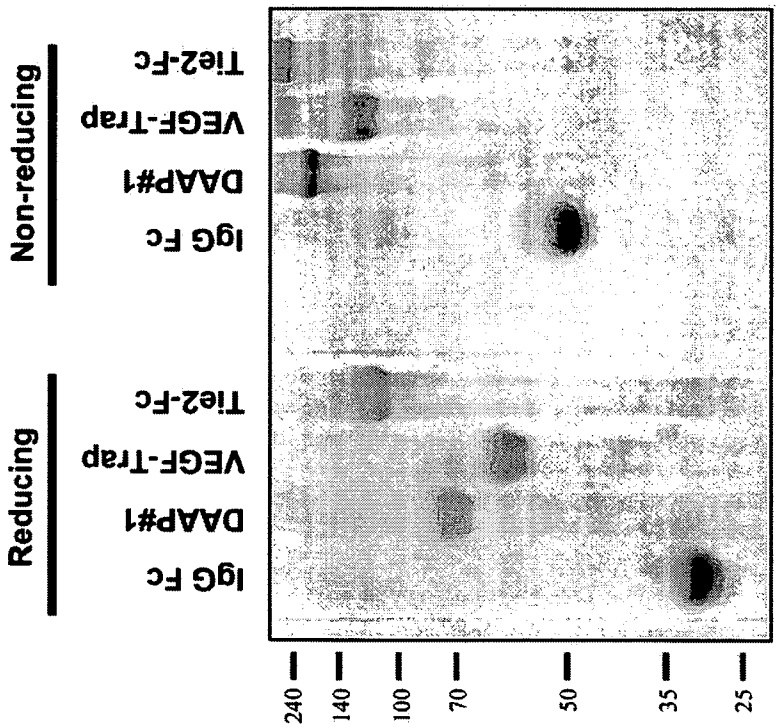


Figure 15

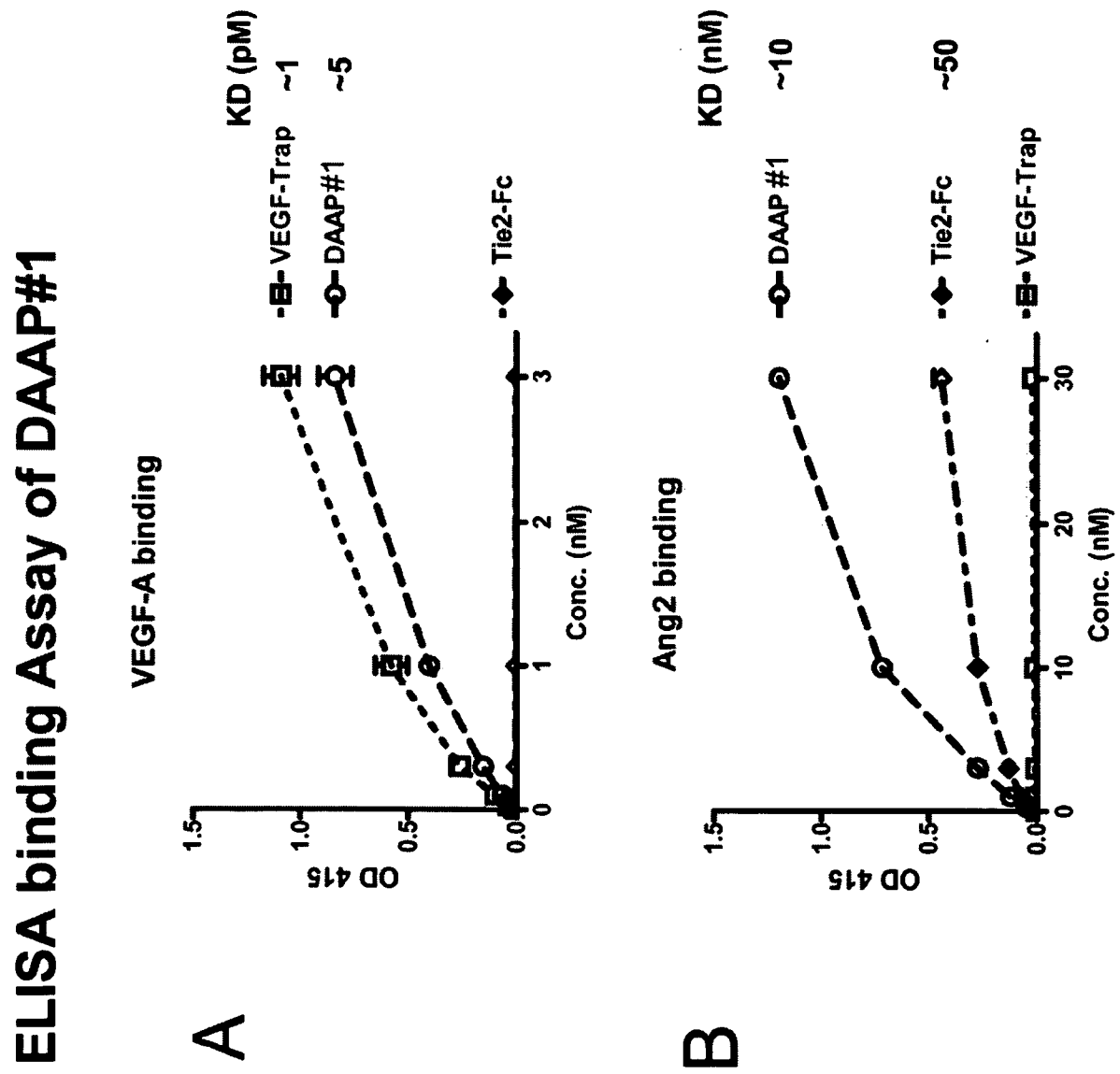

ELISA binding Assay of DAAP#1
A
VEGF-A binding
KD (pM)
VEGF-Trap ~1
DAAP#1 ~5
Tie2-Fc
OD 415
Conc. (nM)
B
Ang2 binding
KD (nM)
DAAP #1 ~10
Tie2-Fc ~50
VEGF-Trap
OD 415
Conc. (nM)

Figure 16

Simultaneous binding of DAAP#1 to VEGF-A and Ang2

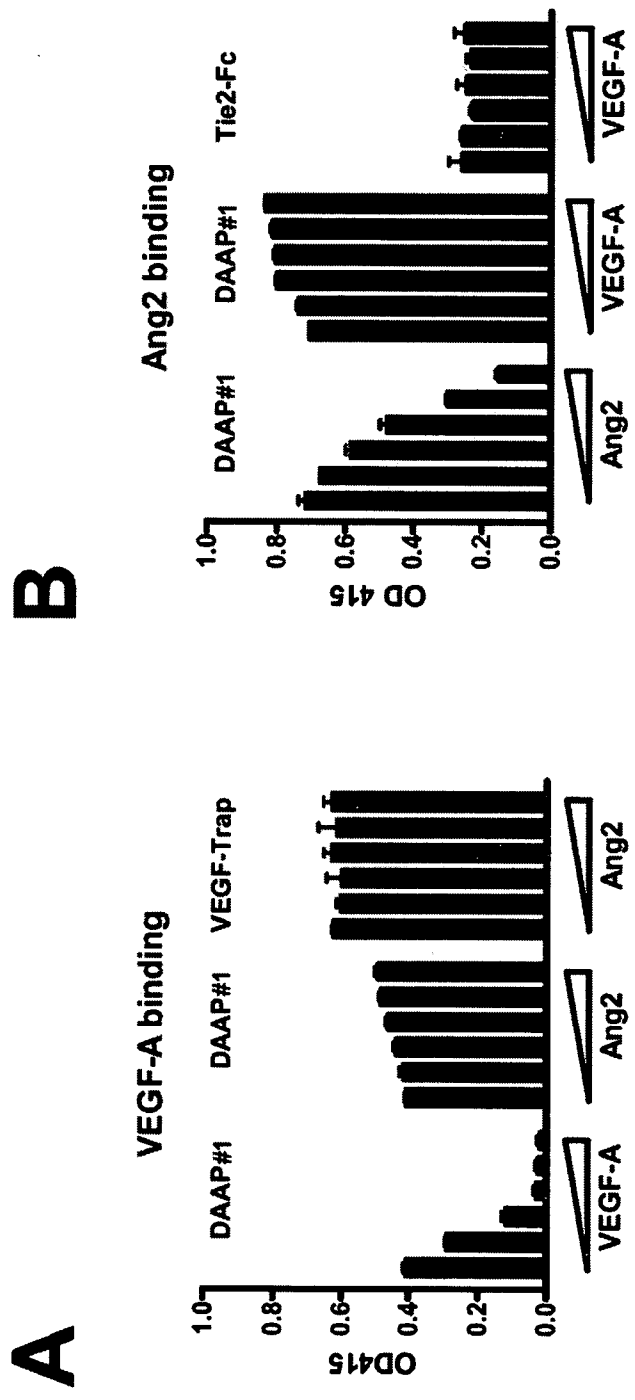


Figure 17

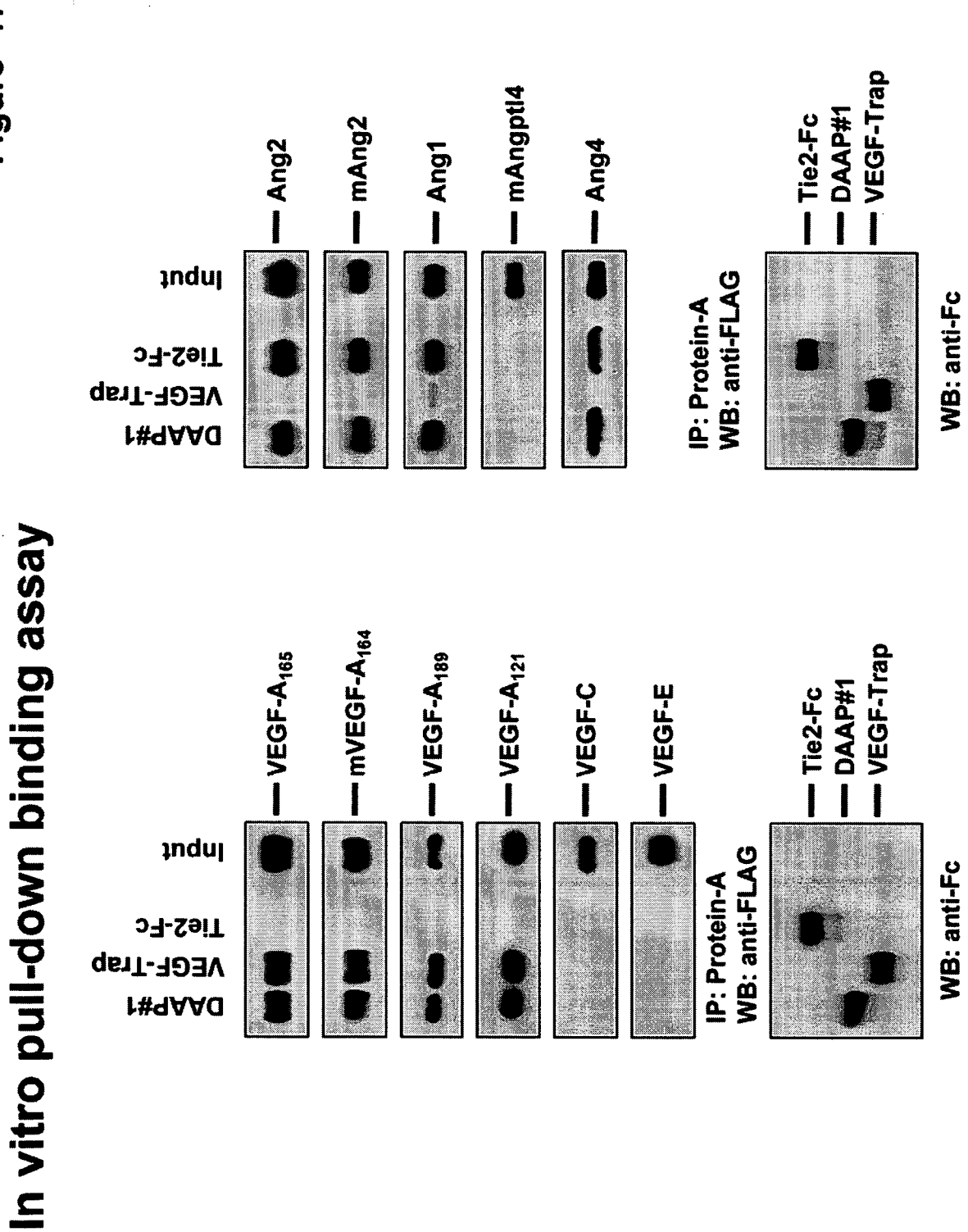

In vitro pull-down binding assay
DAAP#1
VEGF-Trap
Tie2-Fc
Input
VEGF-A165
mVEGF-A164
VEGF-A189
VEGF-A121
VEGF-C
VEGF-E
IP: Protein-A
WB: anti-FLAG
Tie2-Fc
DAAP#1
VEGF-Trap
WB: anti-Fc
DAAP#1
VEGF-Trap
Tie2-Fc
Input
Ang2
mAng2
Ang1
mAngptl4
Ang4
IP: Protein-A
WB: anti-FLAG
Tie2-Fc
DAAP#1
VEGF-Trap
WB: anti-Fc

Figure 18

# Theoretical pI values of DAAP

| | Theoretical pI |
|---|---|
| DAAP#1 | 7.74 |
| DAAP#2 | 8.45 |
| DAAP#3 | 7.17 |
| DAAP#4 | 8.44 |
| DAAP#11 | 8.74 |
| DAAP#12 | 7.18 |
| DAAP#13 | 7.50 |
| DAAP#14 | 7.50 |
| DAAP#15 | 7.50 |
| DAAP#16 | 8.64 |
| DAAP#17 | 8.15 |
| VEGFR1(2) | 9.19 |
| VEGFR1 (2-3) | 9.64 |
| VEGF-trap | 8.64 |
| Tie2-Fc | 6.39 |

Figure 19

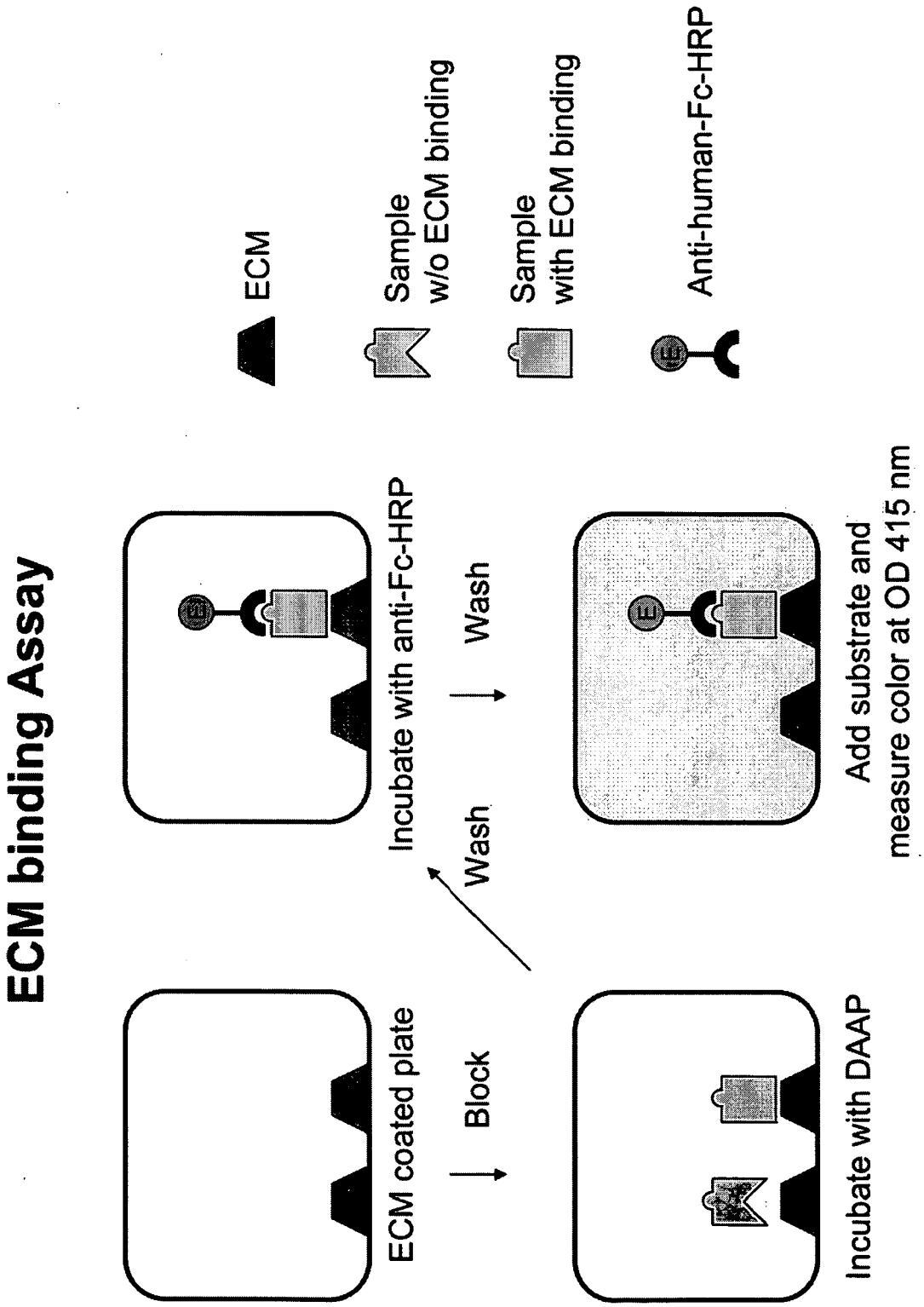

ECM binding Assay
ECM
Sample w/o ECM binding
Sample with ECM binding
Anti-human-Fc-HRP
ECM coated plate
Block
Incubate with DAAP
Incubate with anti-Fc-HRP
Wash
Wash
Add substrate and measure color at OD 415 nm

Figure 20

# ECM binding Assay

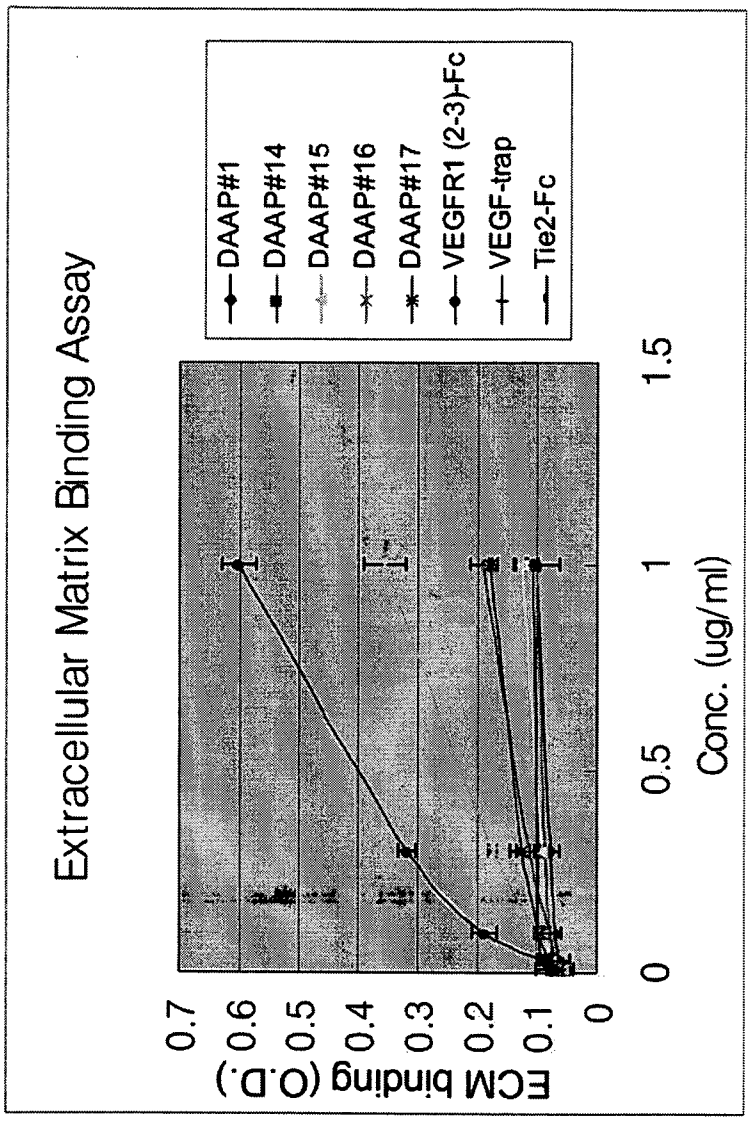


Figure 21

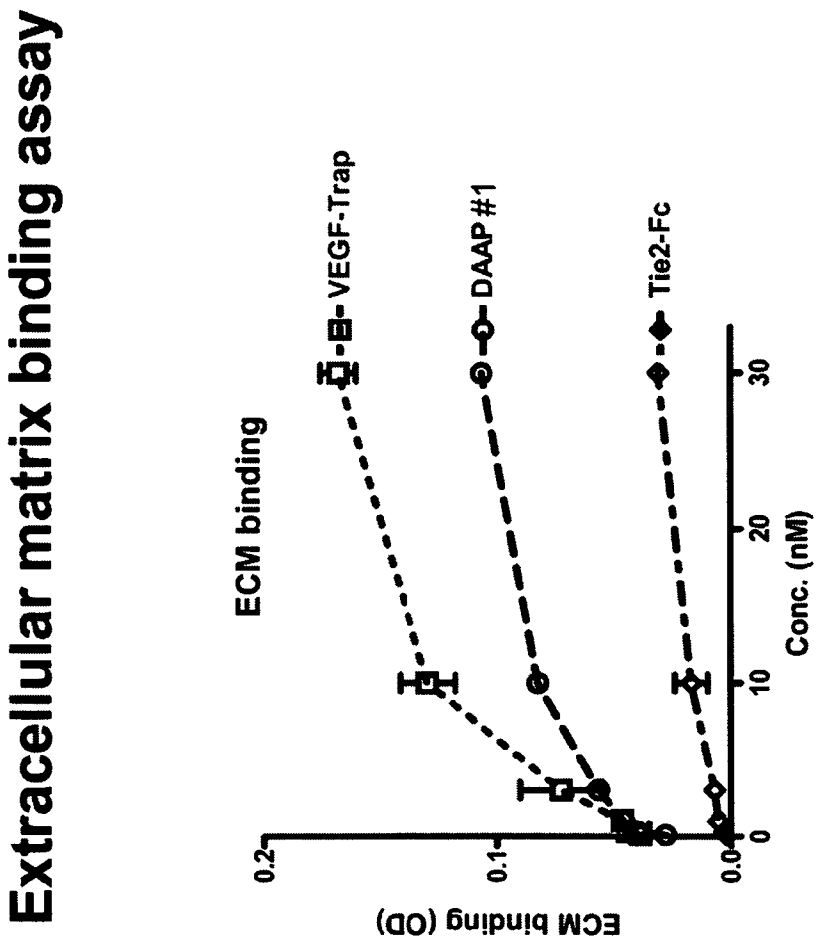

Extracellular matrix binding assay
ECM binding
VEGF-Trap
DAAP #1
Tie2-Fc
ECM binding (OD)
0.2
0.1
0.0
0
10
20
30
Conc. (nM)

Figure 22

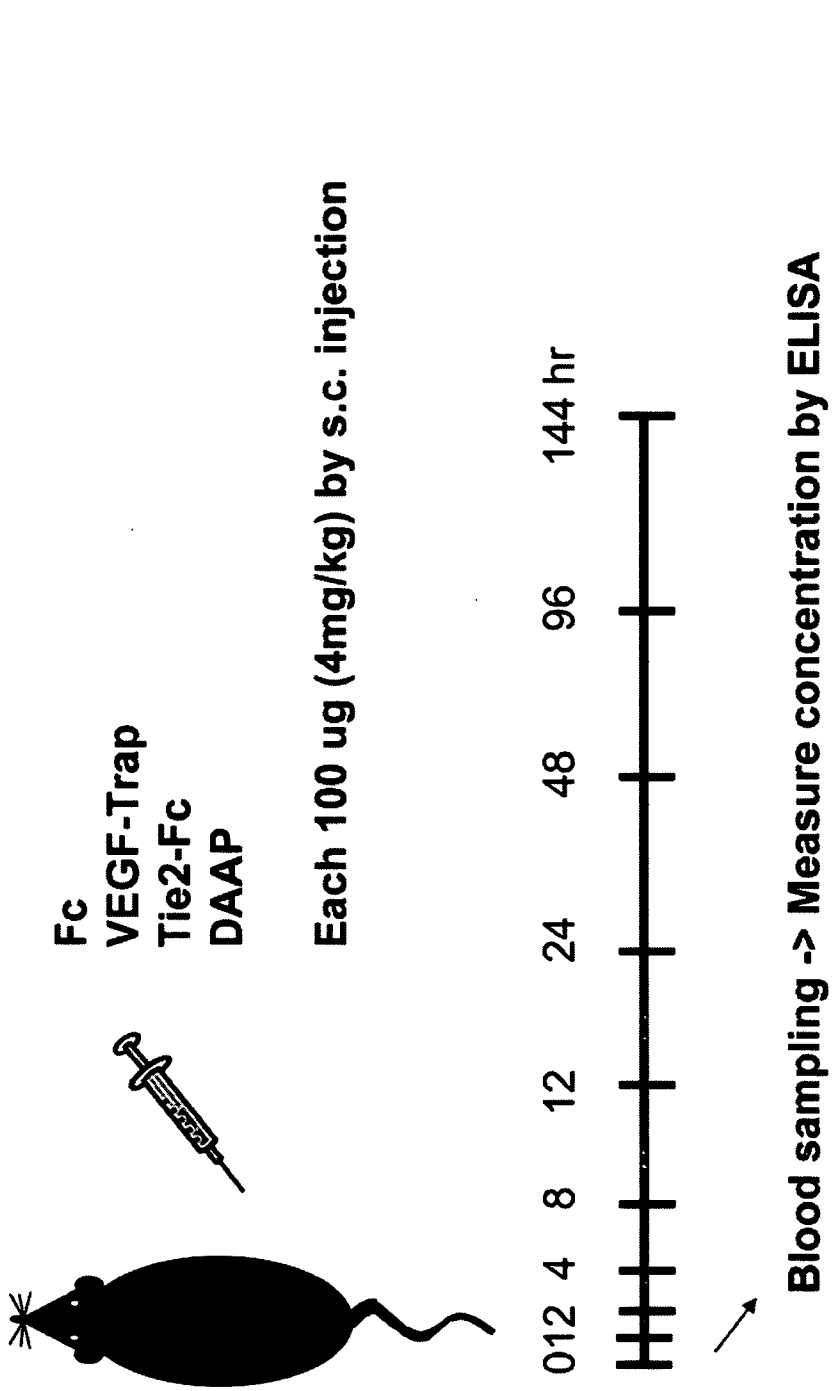

Pharmacokinetic analysis
Fc
VEGF-Trap
Tie2-Fc
DAAP
Each 100 ug (4mg/kg) by s.c. injection
0 1 2 4 8 12 24 48 96 144 hr
Blood sampling -> Measure concentration by ELISA

Figure 23

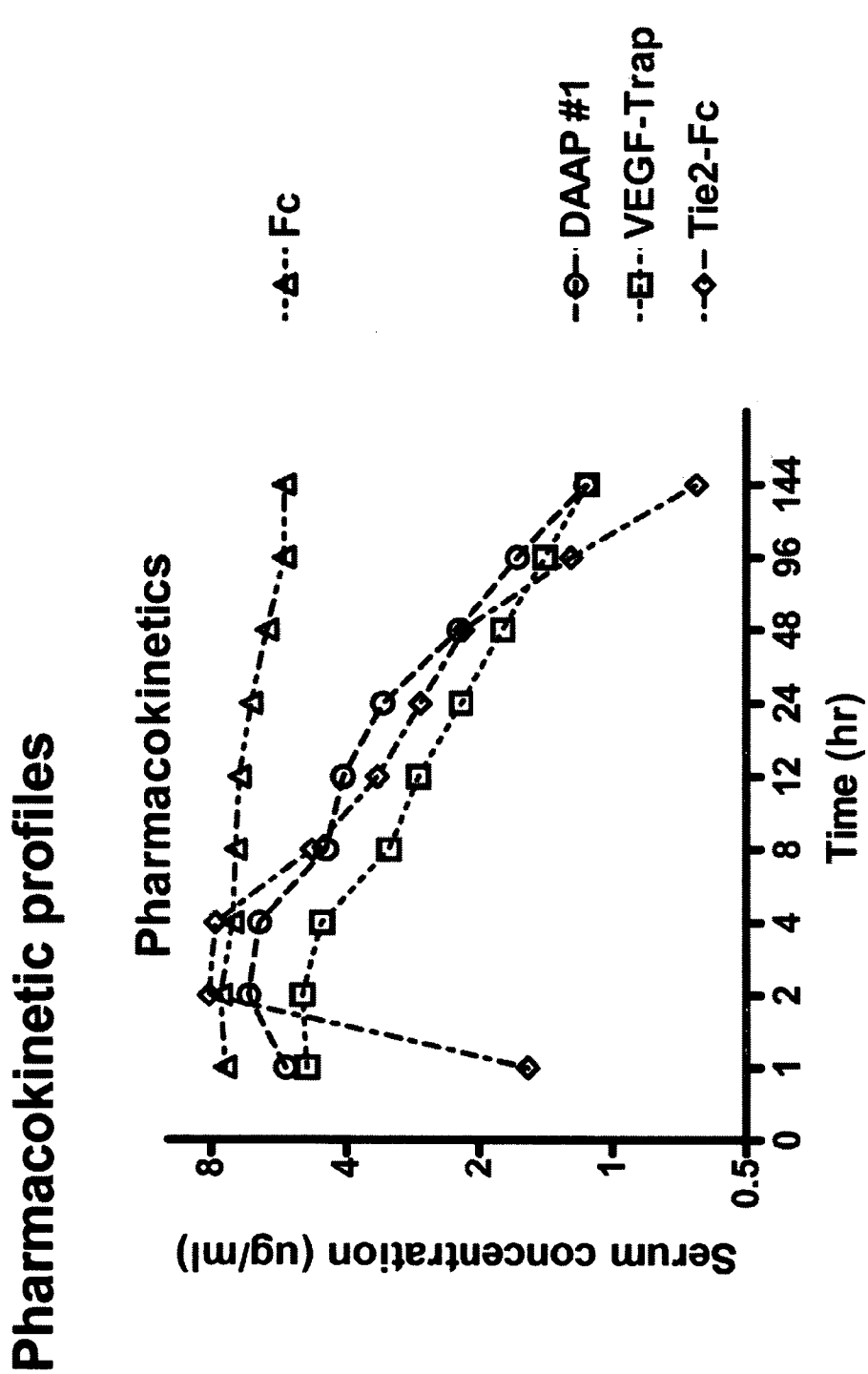

Pharmacokinetic profiles
Pharmacokinetics
Serum concentration (ug/ml)
8
4
2
1
0.5
0
1
2
4
8
12
24
48
96
144
Time (hr)
Fc
DAAP #1
VEGF-Trap
Tie2-Fc

Figure 24

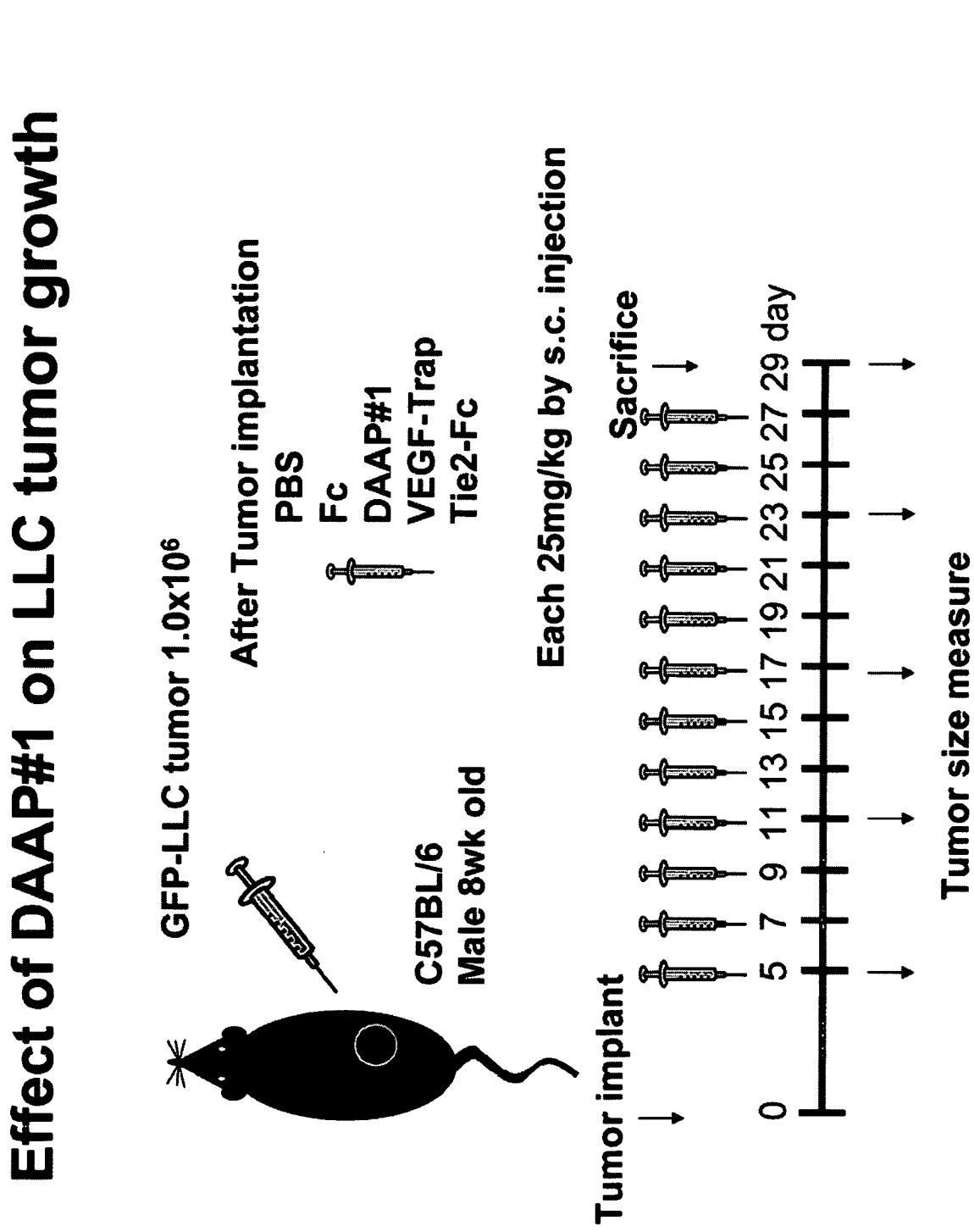

Effect of DAAP#1 on LLC tumor growth
GFP-LLC tumor 1.0x10⁶
After Tumor implantation
PBS
Fc
DAAP#1
VEGF-Trap
Tie2-Fc
C57BL/6
Male 8wk old
Each 25mg/kg by s.c. injection
Tumor implant
Sacrifice
0 5 7 9 11 13 15 17 19 21 23 25 27 29 day
Tumor size measure

Figure 25

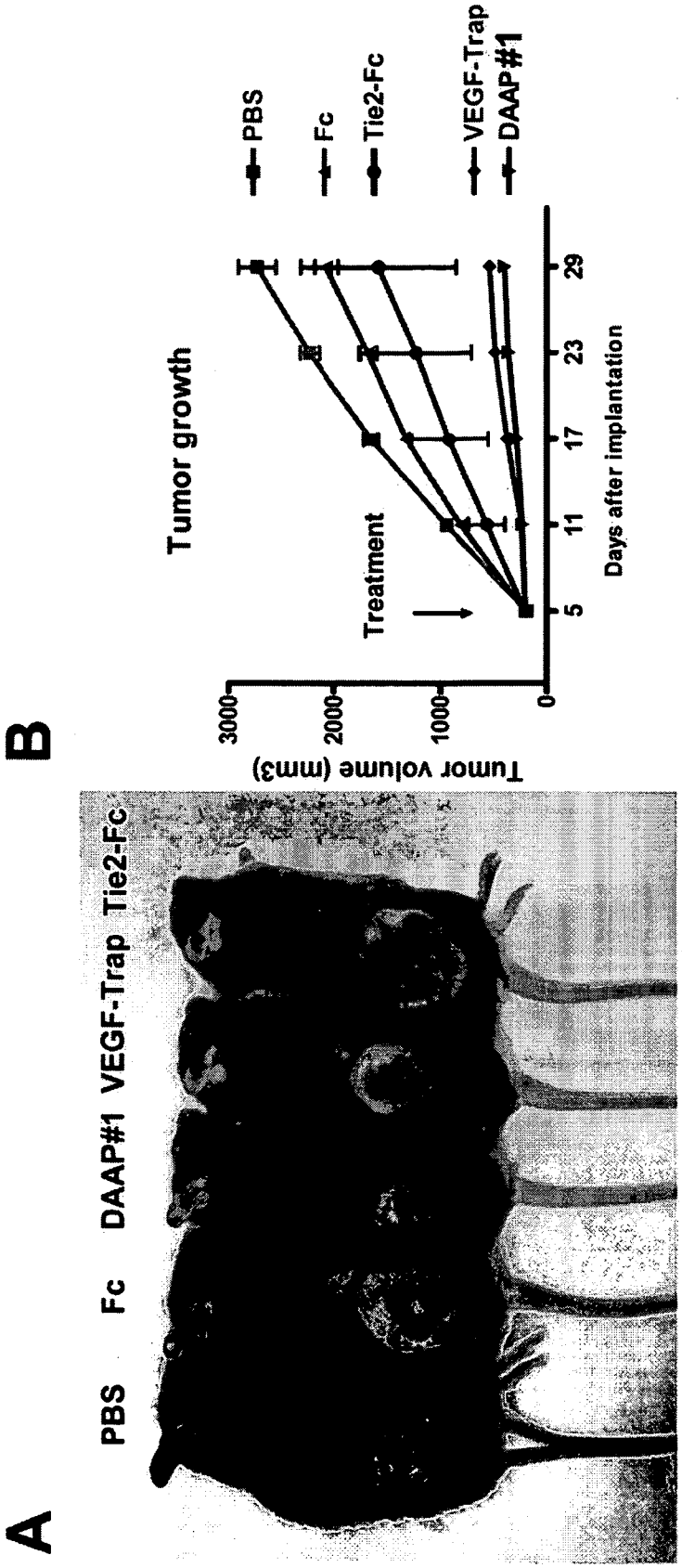

A
PBS
Fc
DAAP#1
VEGF-Trap
Tie2-Fc
B
Tumor growth
Tumor volume (mm3)
3000
2000
1000
0
Treatment
5
11
17
23
29
Days after implantation
PBS
Fc
Tie2-Fc
VEGF-Trap
DAAP#1

Figure 26

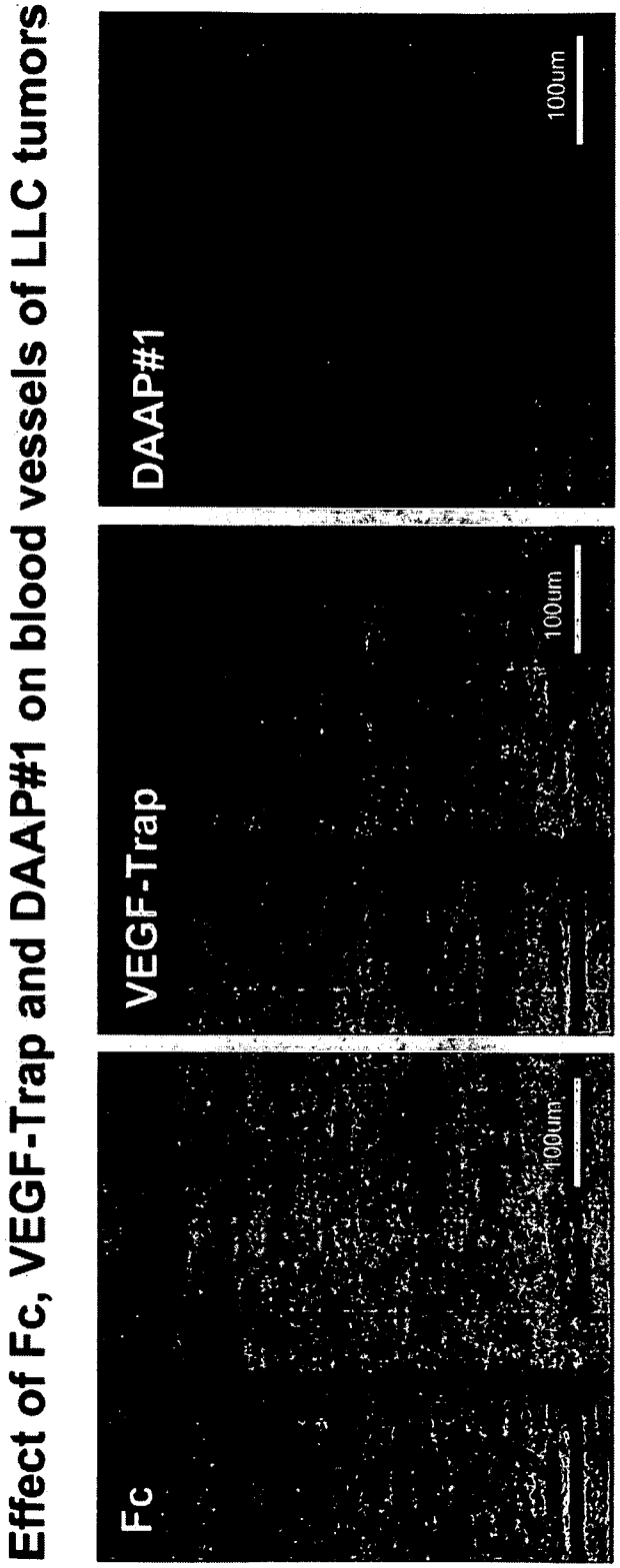

Effect of Fc, VEGF-Trap and DAAP#1 on blood vessels of LLC tumors
Fc
VEGF-Trap
DAAP#1
100um
100um
100um

Figure 27

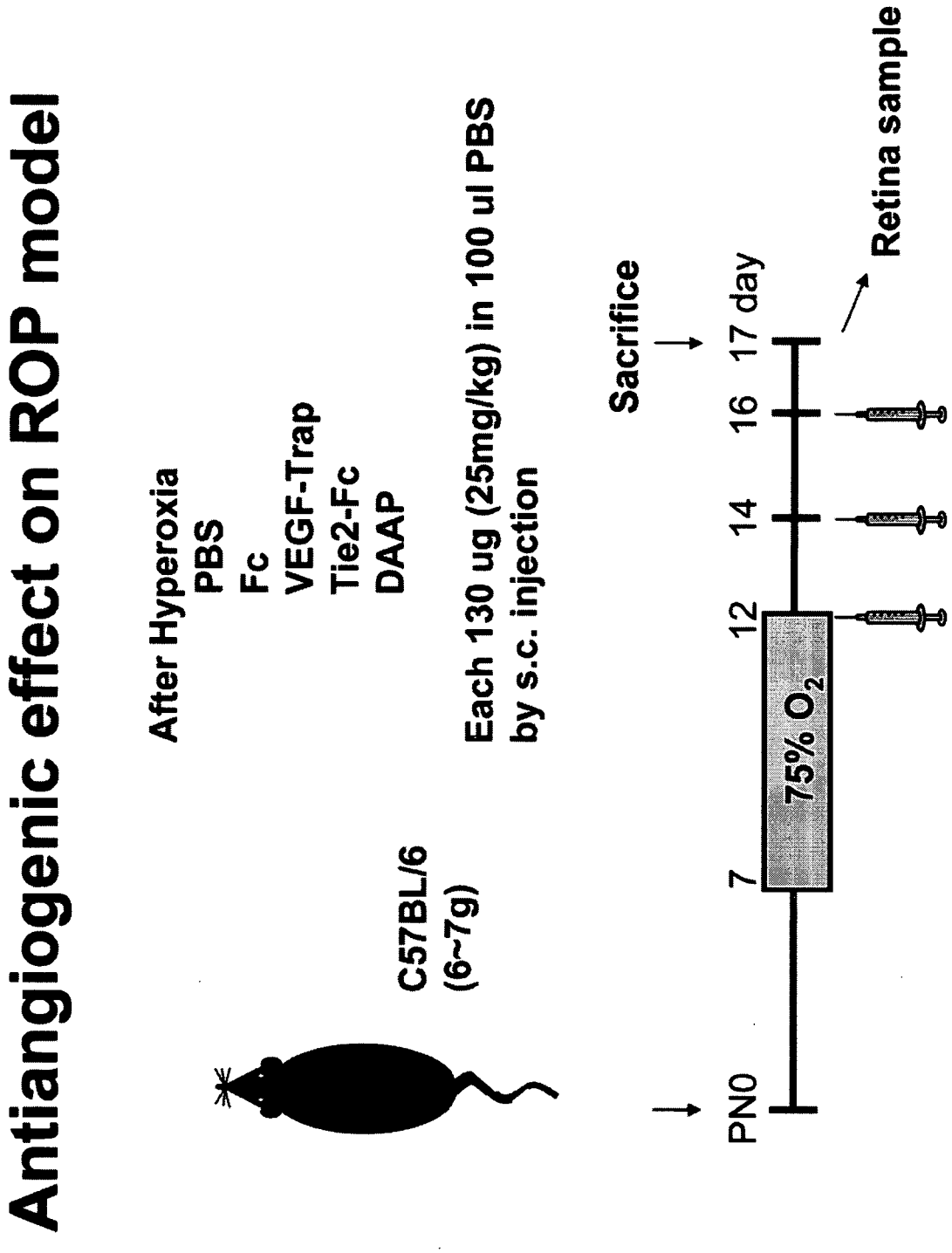

Antiangiogenic effect on ROP model
After Hyperoxia
PBS
Fc
VEGF-Trap
Tie2-Fc
DAAP
Each 130 ug (25mg/kg) in 100 ul PBS
by s.c. injection
C57BL/6
(6~7g)
Sacrifice
Retina sample
PN0
7
12
14
16
17 day
75% O2

Figure 28

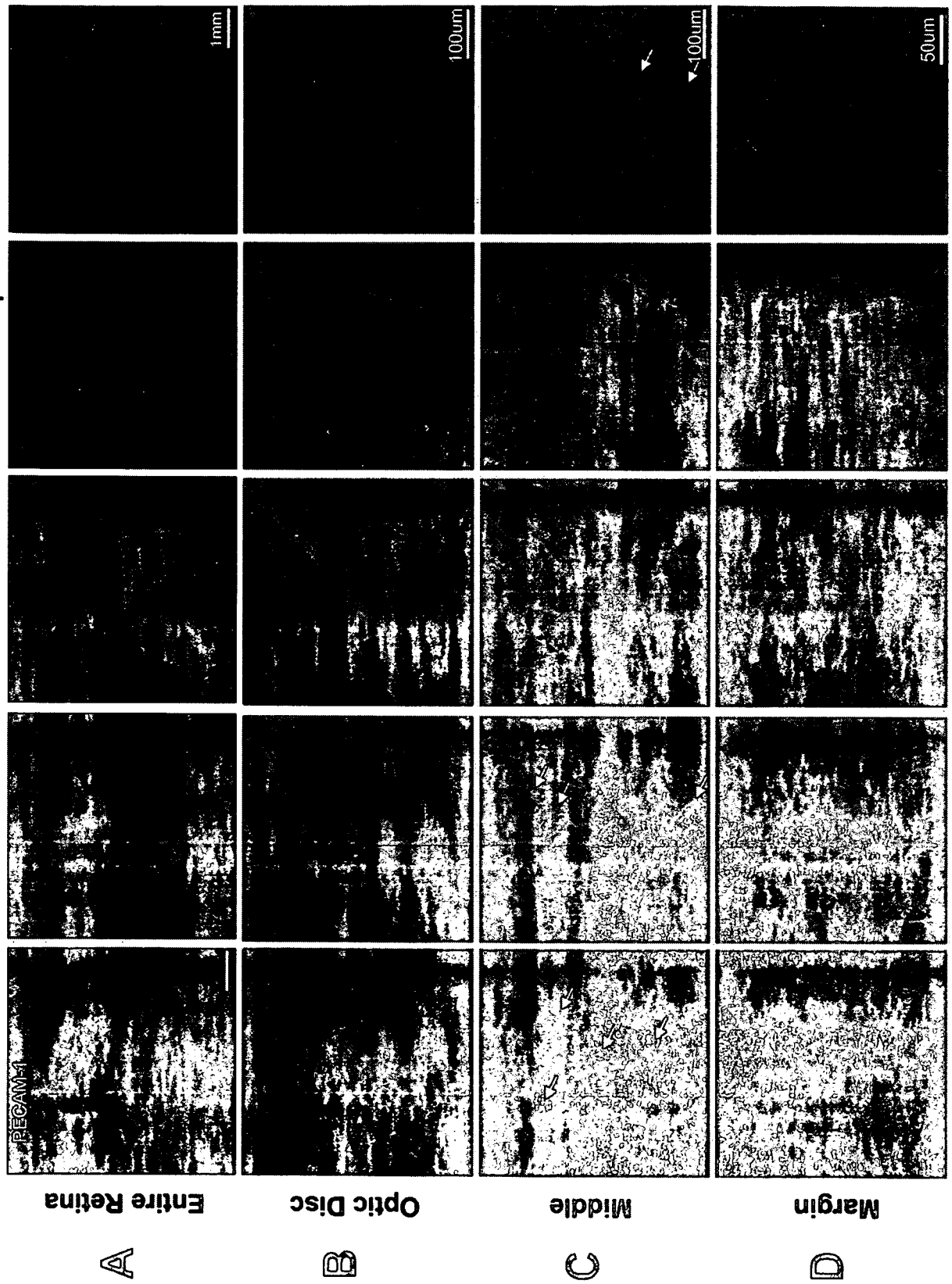
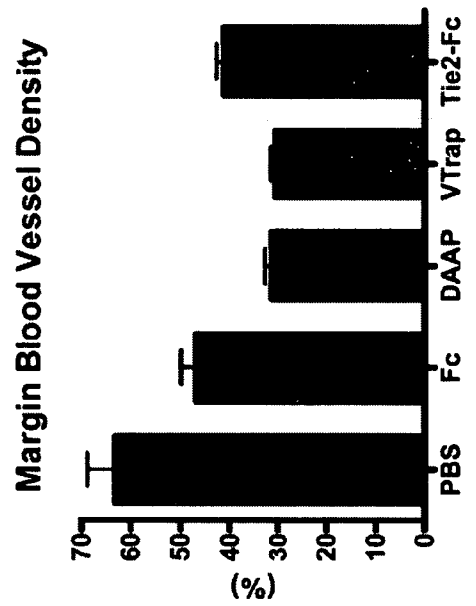

PBS
Fc
DAAP
VEGF-Trap
Tie2-Fc
PECAM-1
1mm
100um
~100um
50um
A
Entire Retina
B
Optic Disc
C
Middle
D
Margin

Figure 28


F
Margin Blood Vessel Density
70
60
50
40
30
20
10
0
(%)
PBS
Fc
DAAP
VTrap
Tie2-Fc

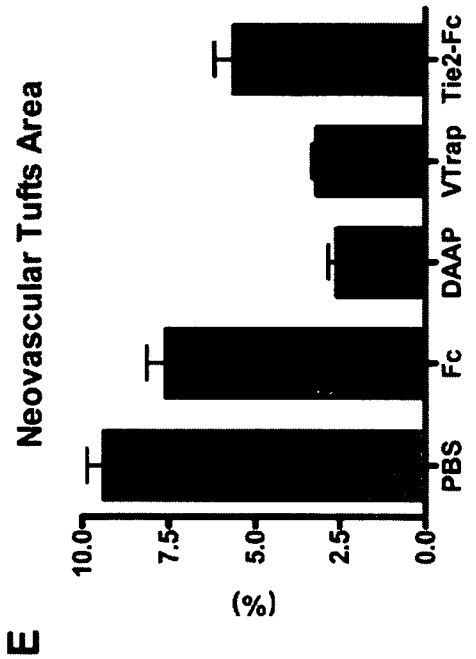

E
Neovascular Tufts Area
10.0
7.5
5.0
2.5
0.0
(%)
PBS
Fc
DAAP
VTrap
Tie2-Fc

Figure 29

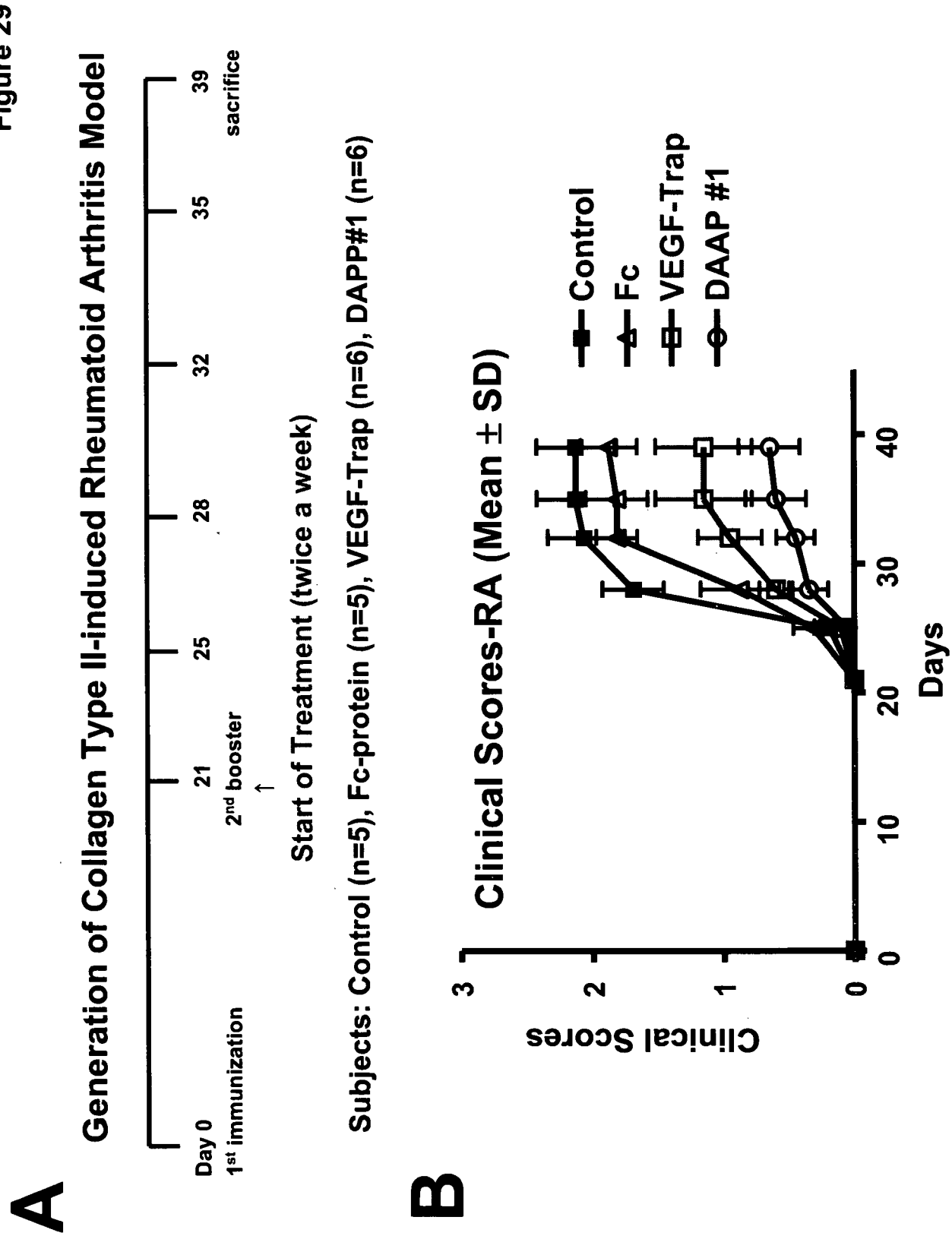

A
Generation of Collagen Type II-induced Rheumatoid Arthritis Model
Day 0
1st immunization
21
2nd booster
25
28
32
35
39
sacrifice
Start of Treatment (twice a week)
Subjects: Control (n=5), Fc-protein (n=5), VEGF-Trap (n=6), DAPP#1 (n=6)
B
Clinical Scores-RA (Mean ± SD)
Control
Fc
VEGF-Trap
DAAP #1
Clinical Scores
Days
0
1
2
3
0
10
20
30
40

Figure 30

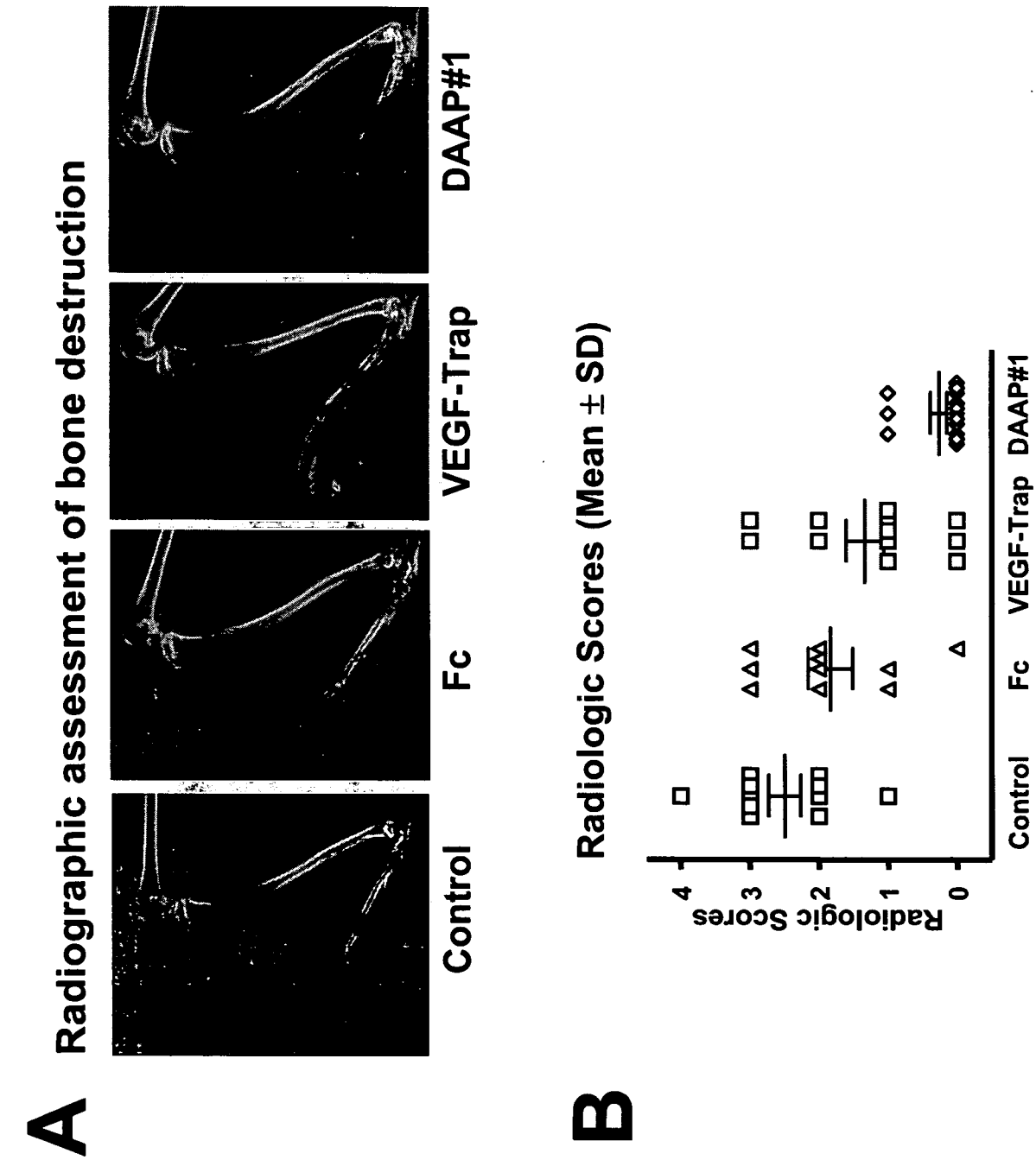
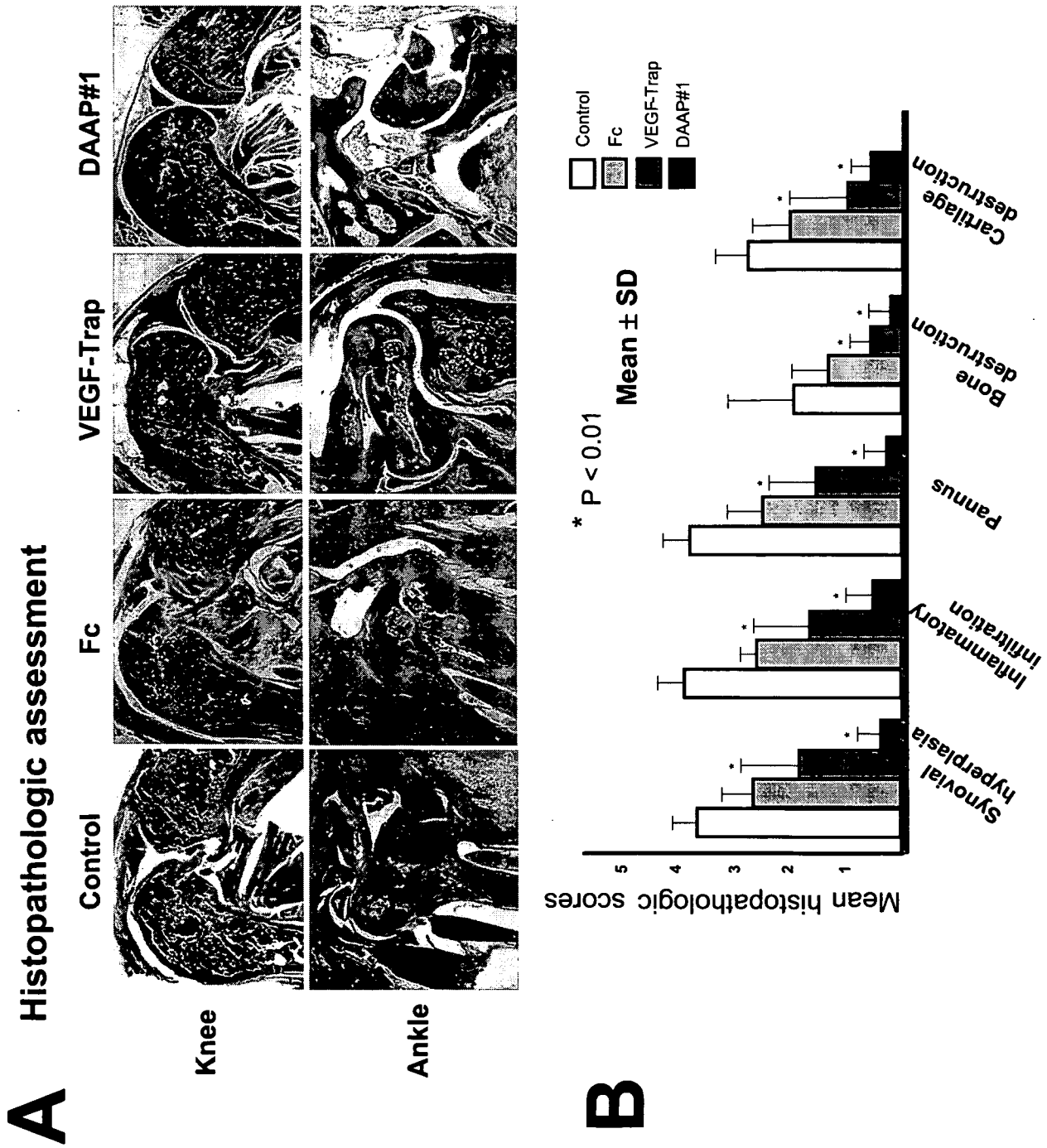

A
Radiographic assessment of bone destruction
Control
Fc
VEGF-Trap
DAAP#1
B
Radiologic Scores (Mean ± SD)
Radiologic Scores
0
1
2
3
4
Control
Fc
VEGF-Trap
DAAP#1

Figure 31


A
Histopathologic assessment
Control
Fc
VEGF-Trap
DAAP#1
Knee
Ankle
B
Control
Fc
VEGF-Trap
DAAP#1
* P < 0.01
Mean ± SD
Mean histopathologic scores
1
2
3
4
5
Synovial hyperplasia
Inflammatory infiltration
Pannus
Bone destruction
Cartilage destruction