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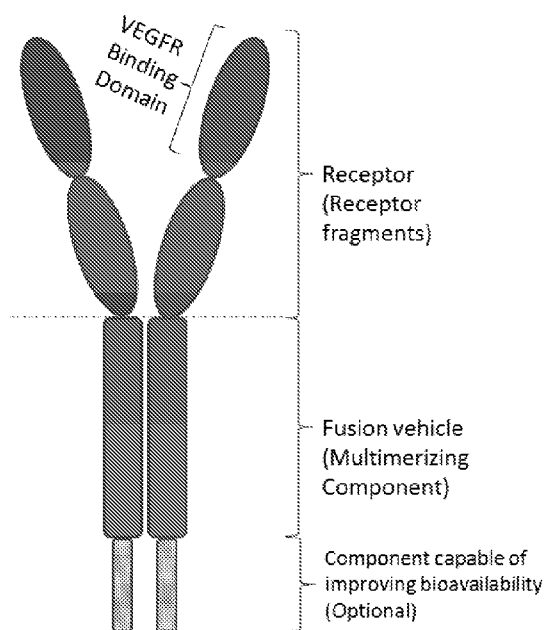


Figure 2B

(57) Abstract: Disclosed is an isolated polypeptide comprising $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$, wherein A comprises an immunoglobulin-like domain of VEGFR1 (Vascular Endothelial Growth Factor Receptor 1), B comprises an immunoglobulin-like domain of VEGFR2 (Vascular Endothelial Growth Factor Receptor 2), C comprises an immunoglobulin-like domain of VEGFR3 (Vascular Endothelial Growth Factor Receptor 3), D comprises a multimerizing component, x is 1 or 2, y is 1 or 0, z is 2 or 3, w is 0 or 1, and n is 2, or 3, or 4, or 5. Also disclosed are nucleic acids encoding the polypeptides, expression vectors, host cells, pharmaceutical compositions, kits, and methods of treating angiogenesis-related disorder and/or vascularization-related disorder. Also disclosed is the use of the isolated polypeptides.

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW,
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PEPTIDE MULTI-TRAP

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of Singapore patent application No. 10201610530P, filed 15 December 2016, the contents of it being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention generally relates to polypeptide. In particular, the present invention relates to fusion protein for use in therapy.

BACKGROUND OF THE INVENTION

[0003] Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates the formation of blood vessels. The VEGF family comprises five members namely, VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF (Platelet Induced Growth Factor), which are important signaling proteins involved in both vasculogenesis (the *de novo* formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature). VEGF's is important in the creation of new blood vessels during embryonic development, creation of new blood vessel after injury, creation of muscles following exercise, or creation of collateral circulation (which are new vessels to bypass blocked vessels). However, overexpression of VEGF can contribute to development of various diseases such as angiogenesis-related disorder, vascularization-related disorder, proliferative disorder, and eye disorder. Therefore, in order to control the overexpression of VEGF proteins, it is useful to design an agent that can bind the overexpressed VEGF protein and that can bind all members of the VEGF family.

[0004] Currently, there are several inhibitors known in the art that can bind some members of the VEGF protein. Examples of those inhibitors are aflibercept (also known as Eylea® or Zaltrap®), conbercept, and OPT-302. Aflibercept and conbercept are fusion proteins each comprising the binding domains of VEGFR1 and VEGFR2 and the constant fragment of immunoglobulin G 1 (i.e. Fc fragment of IgG1). Aflibercept and conbercept target VEGF-A, VEGF-B, and PIGF but it does not target VEGF-C and VEGF-D. Another type of VEGF inhibitor is OPT-302, which is a soluble form of VEGFR3. It targets VEGF-C

and VEGF-D but it does not target VEGF-A, VEGF-B, and PlGF. Because inhibitors that are currently available do not target all members of the VEGF family that are implicated in various angiogenesis-related disorders and vascularization-related disorders, the clinical response of current VEGF inhibitor for cancer therapy may be limited.

- 5 [0005] In view of the above, there is a need to provide an alternative polypeptide that can bind to all members of the VEGF family.

SUMMARY OF THE INVENTION

- [0006] In one aspect, there is provided an isolated polypeptide comprising: $(C_{(w)} - A_{(x)} -$
 10 $B_{(y)} - C_{(z)} - D)_n$, wherein A comprises an immunoglobulin-like domain of VEGFR1 (Vascular Endothelial Growth Factor Receptor 1), B comprises an immunoglobulin-like domain of VEGFR2 (Vascular Endothelial Growth Factor Receptor 2), C comprises an immunoglobulin-like domain of VEGFR3 (Vascular Endothelial Growth Factor Receptor 3), D comprises a multimerizing component, x is 1 or 2, y is 1 or 0, z is 2 or 3, w is 0 or 1, and n
 15 is 2, or 3, or 4, or 5. In one embodiment, A, B, and C are independently ligand binding domain of the respective VEGFR. In another embodiment, (i) each A is independently selected from the group consisting of Domain 1 (D1) of VEGFR1 and Domain 2 (D2) of VEGFR1, and/or (ii) B is Domain 3 (D3) of VEGFR2, and/or (iii) each C is independently selected from the group consisting of Domain 1 (D1) of VEGFR3, Domain 2 (D2) of
 20 VEGFR3, and Domain 3 (D3) of VEGFR3.

[0007] In yet another embodiment, the polypeptide is selected from the group consisting of:

- (i) $(A_{(1)} - B_{(1)} - C_{(2)} - D)_n$,
 (ii) $(A_{(1)} - C_{(2)} - D)_n$,
 25 (iii) $(A_{(2)} - B_{(1)} - C_{(2)} - D)_n$,
 (iv) $(A_{(2)} - C_{(2)} - D)_n$,
 (v) $(C_{(1)} - A_{(1)} - B_{(1)} - C_{(2)} - D)_n$, and
 (vi) $(C_{(1)} - A_{(1)} - C_{(2)} - D)_n$.

- [0008] In yet another embodiment, the polypeptide is selected from the group consisting
 30 of:

- (i) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,

- (ii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,
- (iii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,
- 5 (iv) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,
- (v) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n, and
- (vi) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n.

[0009] In yet another embodiment, (i) the amino acid of the Domain 1 (D1) of VEGFR1 has at least 90% identity to a sequence of SEQ ID NO: 1, and/or (ii) the amino acid of the Domain 2 (D2) of VEGFR1 has at least 90% identity to a sequence of SEQ ID NO: 2, and/or (iii) the amino acid of the Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 has at least 90% identity to a sequence of SEQ ID NO: 12, and/or (iv) the amino acid of the Domain 3 (D3) of VEGFR2 has at least 90% identity to a sequence of SEQ ID NO: 3, and/or (v) the amino acid of the Domain 1 (D1) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 6, and/or (vi) the amino acid of the Domain 2 (D2) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 4, and/or (vii) the amino acid of the Domain 3 (D3) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 5, and/or (viii) the amino acid of the Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 13.

[0010] In yet another embodiment, (i) the amino acid of the Domain 1 (D1) of VEGFR1 has a sequence of SEQ ID NO: 1, and/or (ii) the amino acid of the Domain 2 (D2) of VEGFR1 has a sequence of SEQ ID NO: 2, and/or (iii) the amino acid of the Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 has a sequence of SEQ ID NO: 12, and/or (iv) the amino acid of the Domain 3 (D3) of VEGFR2 has a sequence of SEQ ID NO: 3, and/or (v) the amino acid of the Domain 1 (D1) of VEGFR3 has a sequence of SEQ ID NO: 6, and/or (vi) the amino acid of the Domain 2 (D2) of VEGFR3 has a sequence of SEQ ID NO: 4, and/or (vii) the amino acid of the Domain 3 (D3) of VEGFR3 has a sequence of SEQ ID NO: 5, and/or (viii) the amino acid of the Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 has a sequence of SEQ ID NO: 13.

[0011] In yet another embodiment, the multimerizing component comprises at least one selected from the group consisting of: Fc portion of Human Immunoglobulin constant Fragment, Mannose-binding protein C (Uniprot: P11226), Human Ficolin2 protein, and Cartilage oligomeric matrix protein (Uniprot: P49747). In yet another embodiment, the multimerizing component is Fc portion of Human Immunoglobulin constant Fragment or wherein the amino acid of the Fc portion of Human Immunoglobulin constant Fragment has at least 90% identity to a sequence of SEQ ID NO: 7 or wherein the amino acid of the Fc portion of Human Immunoglobulin constant Fragment has a sequence of SEQ ID NO: 7. In yet another embodiment, the multimerizing component is Human Ficolin2 protein or wherein the amino acid of the Human Ficolin2 protein has at least 90% identity to a sequence of SEQ ID NO: 8 or wherein the amino acid of the Human Ficolin2 protein has a sequence of SEQ ID NO: 8.

[0012] In yet another embodiment, the polypeptide is selected from the group consisting of

(i) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(ii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(iii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;

(iv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;

(v) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(vi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(vii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;

- (viii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
- (ix) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (x) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- (xi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (xiii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- (xiv) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xv) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;
- (xvi) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
- (xvii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (xviii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;

- (xix) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xx) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (xxi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- (xxii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xxiii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (xxiv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- (xxv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xxvi) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;
- (xxvii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
- (xxviii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (xxix) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3; and

(xxx) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5.

[0013] In yet another embodiment, the polypeptide is selected from the group consisting of

(i) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(ii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(iii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;

(iv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;

(v) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(vi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(vii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3; and

(viii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3.

[0014] In yet another embodiment, (i) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 16, or (ii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 20, or (iii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 17, or (iv) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 21, or (v) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 14, or (vi) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 18, or (vii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 15, or (viii) the

polypeptide has at least 90% identity to a sequence of SEQ ID NO: 19. In yet another embodiment, (i) the polypeptide has a sequence of SEQ ID NO: 16, or (ii) the polypeptide has a sequence of SEQ ID NO: 20, or (iii) the polypeptide has a sequence of SEQ ID NO: 17, or (iv) the polypeptide has a sequence of SEQ ID NO: 21, or (v) the polypeptide has a sequence of SEQ ID NO: 14, or (vi) the polypeptide has a sequence of SEQ ID NO: 18, or (vii) the polypeptide has a sequence of SEQ ID NO: 15, or (viii) the polypeptide has a sequence of SEQ ID NO: 19.

[0015] In yet another embodiment, the polypeptide is conjugated to Human Serum Albumin or wherein the amino acid of the Human Serum Albumin has at least 90% identity to a sequence of SEQ ID NO: 23. In yet another embodiment, (i) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 26, or (ii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 30, or (iii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 27, or (iv) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 31, or (v) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 24, or (vi) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 28, or (vii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 25, or (viii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 29. In yet another embodiment, (i) the polypeptide has a sequence of SEQ ID NO: 26, or (ii) the polypeptide has a sequence of SEQ ID NO: 30, or (iii) the polypeptide has a sequence of SEQ ID NO: 27, or (iv) the polypeptide has a sequence of SEQ ID NO: 31, or (v) the polypeptide has a sequence of SEQ ID NO: 24, or (vi) the polypeptide has a sequence of SEQ ID NO: 28, or (vii) the polypeptide has a sequence of SEQ ID NO: 25, or (viii) the polypeptide has a sequence of SEQ ID NO: 29.

[0016] In another aspect, there is provided a nucleic acid encoding one or more polypeptides as described herein.

[0017] In yet another aspect, there is provided an expression vector comprising the nucleic acid as described herein.

[0018] In yet another aspect, there is provided a host cell comprising the nucleic acid as described herein or the expression vector as described herein.

[0019] In yet another aspect, there is provided a polypeptide as described herein for use in therapy or medicine.

[0020] In yet another aspect, there is provided a pharmaceutical composition comprising one or more polypeptide(s) as described herein.

[0021] In yet another aspect, there is provided a method of treating angiogenesis-related disorder and/or vascularization-related disorder in a subject in need thereof, comprising
5 administering a therapeutically effective amount of the polypeptide as described herein or a therapeutically effective amount of the composition as described herein into the subject.

[0022] In yet another aspect, there is provided use of the polypeptide as described herein or the composition as described herein in the manufacture of a medicament for treating angiogenesis-related disorder and/or vascularization-related disorder. In yet another
10 embodiment, the angiogenesis-related disorder and/or vascularization-related disorder is cancer or wherein the cancer is selected from the group consisting of non-small cell lung cancer, gastric cancer, gastric tumors, and breast cancer. In yet another embodiment, the angiogenesis-related disorder and/or vascularization-related disorder is eye disorder or wherein the eye disorder is a retinal angiogenic disease or wherein the retinal angiogenic
15 disease is selected from the group consisting of age-related macular degeneration, macular edema, diabetic retinopathy, and diabetic macular edema.

[0023] In yet another aspect, there is provided a kit comprising one or more polypeptide(s) as described herein.

20 BRIEF DESCRIPTION OF THE DRAWINGS

[0024] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0025] **Figure 1A** shows a graphical illustration depicting typical members of Vascular
25 Endothelial Growth Factor Receptors (VEGFR) on cell membrane, which are VEGFR1, VEGFR2, and VEGFR3. **Figure 1A** also depicts the types of Vascular Endothelial Growth Factor (VEGF), which are VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E, that binds to each VEGFR. **Figure 1B** shows a graphical illustration showing that each member of the VEGFR family comprises seven extracellular domains (denoted as D1, D2, D3, D4, D5, D6,
30 and D7). The two circles that connect the pair of D2 represent VEGF molecules (the binding target of VEGFR) that bind to D2 of the VEGFR.

[0026] **Figure 1B** also shows that in addition to binding VEGFs, VEGFR1 also binds Platelet Induced Growth Factor (PIGF). Thus, **Figure 1** illustrates that all three VEGFRs bind specific types of VEGFs and PIGF.

[0027] **Figure 2** shows a set of graphical illustrations depicting the strategy used in preparing the polypeptides of the present disclosure and the typical structure of the polypeptides of the present disclosure. **Figure 2A** shows a graphical illustration depicting four different sets of receptors fragments (denoted as Gryff, Slyth, Huffl, and Ravcl) that are combined with either one of the five different types of multimerizing components (denoted as Fragments M1, M2, M3, M4, and M5) thereby obtaining 20 different possibilities of polypeptides. Within the receptor fragments, the box labeled “1A” denotes Domain 1 (D1) of VEGFR1, “1B” denotes Domain 2 (D2) of VEGFR1, “2A” denotes Domain 3 (D3) of VEGFR2, “3A” denotes Domain 2 (D2) of VEGFR3, “3B” denotes Domain 3 (D3) of VEGFR3, and “3C” denotes Domain 1 (D1) of VEGFR3. **Figure 2B** shows a graphical illustration depicting exemplary structure of the polypeptides of the present disclosure. Even though the illustration depicts a dimer, the peptide of the present disclosure can also form other multimers. Thus, **Figure 2** shows that at least 20 possible polypeptides or 20 possible combinations of the receptors fragments and multimerizing components have been prepared.

[0028] **Figure 3A** shows a set of graphical illustration depicting combinations of four different receptors fragments (denoted as Gryff, Slyth, Huffl, and Ravcl) with a multimerizing component (such as Fc portion of Human Immunoglobulin constant Fragment) that allows for the dimerization of the polypeptides. **Figure 3B** shows a set of graphical illustration depicting combinations of four different receptors fragments (denoted as Gryff, Slyth, Huffl, and Ravcl) with a multimerizing component (such as Mannose-binding protein C (Uniprot: P11226) or Human Ficolin2 protein) that allows for the trimerization of the polypeptides. Thus, **Figure 3** illustrates that different types of multimerizing components allow for formation of different multimers of the polypeptide described herein.

[0029] **Figure 4** shows a set of data comparing the expression level of four different polypeptides that are combinations of four different receptors fragments (denoted as Gryff, Slyth, Huffl, and Ravcl) with multimerizing component M1 (i.e. Fc portion of Human Immunoglobulin constant Fragment). **Figure 4A** is a graphical illustration showing the four different polypeptides tested and their expected molecular weight in kDa. **Figure 4B** is a photograph of a protein gel depicting samples obtained on day 2 post transfection. The gel

comprises four different polypeptide tested (lanes 1 to 4). The molecular weight marker used (lane not shown) is Bio-Rad Dual or Kaleidoscope and the approximate molecular weight of the marker is indicated on the left. Relatively larger and more intense bands were observed on lane 1 (labelled “Gryff-IgG”) at approximately 86kDa and on lane 3 (labelled “Huffl-IgG”) at approximately 74kDa. **Figure 4C** is a photograph of a protein gel depicting samples obtained on day 6 post transfection. The gel comprises molecular ladder Bio-Rad Dual or Kaleidoscope (lanes 5 and 6; molecular weights from top to bottom are 250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa), the cell lysates from the cells expressing the four different polypeptides tested (lanes 1 to 4), and the supernatant of the four different polypeptide tested (lanes 7 to 10). Relatively larger and more intense bands were observed on lane 7 (labelled “Supernatant; Gryff-IgG”) at approximately 86kDa and on lane 9 (labelled “Supernatant; Huffl-IgG”) at approximately 74kDa. Thus, **Figure 4** illustrates that polypeptides Gryff-IgG (MW: 86kDa) and Huffl-IgG (MW: 74kDa) are expressed at higher amount compared to the other polypeptides and that the cells secreted the expressed polypeptides into the supernatant.

[0030] **Figure 5** shows a set of data comparing the expression level of five different polypeptides that are combinations of a type of receptors fragments (denoted as “Gryff” and comprised Domain 1 (D1) of VEGFR1, Domain 2 (D2) of VEGFR1, Domain 3 (D3) of VEGFR2, Domain 2 (D2) of VEGFR3 and Domain 3 (D3) of VEGFR3) with five different types of multimerizing components (denoted as Fragments M1, M2, M3, M4, and M5).

Figure 5A is a graphical illustration showing the five different polypeptides tested and their expected molecular weight in kDa. **Figures 5B and 5D** are photographs of protein gels depicting samples (supernatants) obtained on day 2 post transfection (**Figure 5B**) and on day 7 post transfection (**Figure 5D**). The gel comprises molecular ladder Bio-Rad Dual or Kaleidoscope (lane 1; molecular weights from top to bottom are 250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa) and the five different polypeptides tested (lanes 2 to 6). Relatively larger and more intense bands were observed on lane 2 (labelled “Gryff-IgG”) at approximately 86kDa and on lane 5 (labelled “Gryff-FCN2”) at approximately 85kDa. **Figures 5C and 5E** are pairs of blots depicting result of western blot experiment using samples obtained on day 2 post transfection (**Figure 5C**) and on day 7 post transfection (**Figure 5E**). The blots were probed using anti-6His-HRP antibody that recognizes the histidine tag on the polypeptides of interest. Chemiluminescent detection was performed using Luminata Forte (Millipore) or SuperSignal West Pico. The blot on the left shows

normal exposure, the blot on the right shows higher exposure. Each blot comprises molecular ladder Bio-Rad Dual or Kaleidoscope (lane 1; molecular weights are indicated with plus (+) and minus (-) signs; molecular weights from top to bottom are 250, 150, 100, 75 (marked with +), 50, 37, 25 (marked with +), 20, 15, and 10 kDa) and the five different polypeptides tested (lanes 2 to 6). Relatively larger and more intense bands were observed on lane 2 (labelled “Gryff-IgG”) and on lane 5 (labelled “Gryff-FCN2”). Thus, **Figure 5** illustrates that polypeptides Gryff-IgG (MW: 86kDa) and Gryff-FCN2 (MW: 85kDa) are expressed at higher amount compared to the other polypeptides and that the cells secreted the expressed polypeptides into the supernatant.

Figure 6 shows a set of data comparing the expression level of five different polypeptides that are combinations of a type of receptors fragments (denoted as “Huffl” and comprised Domain 1 (D1) of VEGFR1, Domain 2 (D2) of VEGFR1, Domain 2 (D2) of VEGFR3 and Domain 3 (D3) of VEGFR3) with five different types of multimerizing components (denoted as Fragments M1, M2, M3, M4, and M5). **Figure 6A** is a graphical illustration showing the five different polypeptides tested and their expected molecular weight in kDa. **Figures 6B and 6D** are photographs of protein gels depicting samples (supernatants) obtained on day 2 post transfection (**Figure 6B**) and on day 5 post transfection (**Figure 6D**). The gel comprises molecular ladder Bio-Rad Dual or Kaleidoscope (lanes 1, 7, and 8; molecular weights from top to bottom are 250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa) and the five different polypeptides tested (lanes 2 to 6). The largest and most intense band was observed on lane 2 (labelled “Huffl-IgG”) at approximately 74kDa. Another relatively larger and more intense band was observed on lane 5 (labelled “Huffl-FCN2”) at approximately 73kDa. **Figure 6C** is a blot depicting result of western blot experiment using samples (supernatants) obtained on day 2 post transfection. The blot was probed using anti-6His-HRP antibody that recognizes the histidine tag on the polypeptides of interest. Chemiluminescent detection was performed using Luminata Forte (Millipore) or SuperSignal West Pico. The blot comprises molecular ladder Bio-Rad Dual or Kaleidoscope (lane 1; molecular weights are indicated with plus (+) and minus (-) signs; molecular weights from top to bottom are 250, 150, 100, 75 (marked with +), 50, 37, 25 (marked with +), 20, 15, and 10 kDa) and the five different polypeptides tested (lanes 2 to 6). The largest and most intense band was observed on lane 2 (labelled “Huffl-IgG”). Another relatively larger and more intense band was observed on lane 5 (labelled “Huffl-FCN2”). **Figure 6E** are a pair of blots

depicting result of western blot experiment using samples (left blot: supernatants; right blot: cell lysates) obtained on day 5 post transfection. The blots were probed using anti-6His-HRP antibody that recognizes the histidine tag on the polypeptides of interest. Chemiluminescent detection was performed using Luminata Forte (Millipore) or SuperSignal West Pico. The blot comprises molecular ladder Bio-Rad Dual or Kaleidoscope (lane 1 on left blot and lane 6 on right blot; molecular weights are indicated with plus (+) and minus (-) signs; molecular weights from top to bottom are 250, 150, 100, 75 (marked with +), 50, 37, 25 (marked with +), 20, 15, and 10 kDa) and the five different polypeptides tested (lanes 2 to 6 on left blot and lanes 1 to 5 on right blot). The largest and most intense band was observed on lane 2 (labelled “Supernatant; Huffl-IgG”). Another relatively larger and more intense band was observed on lane 5 (labelled “Supernatant; Huffl-FCN2”). Thus, **Figure 6** illustrates that polypeptides Huffl-IgG (MW: 74kDa) and Huffl-FCN2 (MW: 73kDa) are expressed at higher amount compared to the other polypeptides and that the cells secreted the expressed polypeptides into the supernatant.

[0032] **Figure 7** shows a set of data corresponding to the various samples obtained from the purification of polypeptide Huffl-IgG (i.e. a polypeptide comprising Domain 1 (D1) of VEGFR1, Domain 2 (D2) of VEGFR1, Domain 2 (D2) of VEGFR3, Domain 3 (D3) of VEGFR3, and Fc portion of Human Immunoglobulin constant Fragment) using Ni-NTA purification system. **Figure 7A** is a photograph of a protein gel whereas **Figure 7B** is a blot depicting result of western blot experiment. The gel and the blot each comprises molecular ladder Bio-Rad Dual or Kaleidoscope (lanes 1 and 6; molecular weights from top to bottom are 250, 150, 100, 75, 50, 37, 25, 20, 15, and 10 kDa), supernatant input (lane 2), supernatant post binding to column or supernatant output (lane 3), beads after elution of polypeptide of interest (lane 4), and eluate (lane 5). Thus, **Figure 7** shows that the polypeptide of interest (such as Huffl-IgG) can be purified using standard purification system such as Ni-NTA system.

[0033] **Figure 8** shows a set of data depicting the final purification result of four different polypeptides. **Figure 8A** is a graphical illustration depicting polypeptides that are highly expressed such as Gryff-IgG dimer, Huffl-IgG dimer, Gryff-FCN2 trimer, and Huffl-FCN2 trimer. **Figure 8B** is a blot depicting result of western blot experiment using eluate of polypeptides of interest. Even though the volume of polypeptides added to each lane is the same, the μ g amount of the polypeptides added to each lane is not identical. That is because

the lanes represent typical yield of various polypeptides obtained from similar volume of cultures. The blot was probed using anti-6His-HRP antibody that recognizes the histidine tag on the polypeptides of interest. Chemiluminescent detection was performed using Luminata Forte (Millipore) or SuperSignal West Pico. Thus, **Figure 8** shows that in addition to Huffl-IgG, other polypeptide of interest (such as Gryff-IgG, Gryff-FCN2, and Huffl-FCN2) can also be purified using standard purification system.

[0034] **Figure 9** shows a set of bar graphs depicting the result of bioavailability experiments. **Figure 9A** shows a bar graph depicting the result of bioavailability experiment of Gryff-FCN2 (or GF2), Gryff-IgG (or GI), Huffl-FCN2 (or HF2), and Huffl-IgG (or HI).

Figure 9B shows a bar graph depicting the result of bioavailability experiment of shorter forms of Gryff-IgG (or sGI), Huffl-IgG (or sHI), Gryff-FCN2 (or sGF2), and Huffl-FCN2 (or sHF2) in comparison to the bioavailability of aflibercept (or AFLI). Thus, **Figure 9** shows that Huffl-IgG (or HI) have better bioavailability among the longer polypeptides and that shorter form of Huffl-IgG (or sHI) has better bioavailability among the shorter polypeptides.

[0035] **Figure 10** shows a set of graphical illustrations depicting the difference between the longer (the full length) and the shorter version of Gryff-IgG, Huffl-IgG, Gryff-FCN2, and Huffl-FCN2. The shorter version of the polypeptides generally has better bioavailability than the longer version because the shorter version of the polypeptides do not comprise domain 1 of VEGFR1 (denoted as 1A). Domain 1 of VEGFR1 comprises many residues (such as arginine, lysine, histidine, aspartic acid and glutamic acid) that may cause the polypeptide to be bind to extracellular matrix (ECM) instead of to the target (e.g. VEGFs and PIGF). When the polypeptide is bound to the ECM, the polypeptide becomes unavailable in general blood circulation and thus it becomes less bioavailable.

[0036] **Figure 11** shows a set of data depicting the result of tube formation assay or the inhibition of the tube formation of Human Umbilical Vein Endothelial Cells (HUVEC) using Gryff-IgG, Huffl-IgG, Gryff-FCN2, and Huffl-FCN2. **Figure 11A** shows a set of light microscopy photographs depicting negative control, cells treated with 1 μ g/well of Gryff-IgG, cells treated with 1 μ g/well of Huffl-IgG, cells treated with 1 μ g/well of Gryff-FCN2, and cells treated with 1 μ g/well of Huffl-FCN2. **Figure 11B** shows a bar graph depicting the number of master segments formed upon treatment of various concentrations of Gryff-IgG, Huffl-IgG, Gryff-FCN2, and Huffl-FCN2. **Figure 11C** shows a bar graph depicting the total length of master segments formed upon treatment of various concentrations of Gryff-IgG,

Huffl-IgG, Gryff-FCN2, and Huffl-FCN2. Thus, **Figure 11** illustrates higher concentration of Gryff-IgG, Huffl-IgG, Gryff-FCN2, and Huffl-FCN2 is more effective in inhibiting angiogenesis.

[0037] **Figure 12** shows a pair of line graphs depicting the amount of HUVEC cells growth after treatments using various concentrations of aflibercept (denoted as “AFLI”), Gryff-IgG (also denoted as “GI”), Huffl-IgG (also denoted as “HI”), Gryff-FCN2 (also denoted as “GF2”), Huffl-FCN2 (also denoted as “HF2”), Gryff-IgG-HSA (also denoted as “GIH”), and Huffl-IgG-HSA (also denoted as “HIH”). Gryff-IgG-HSA and Huffl-IgG-HSA are Gryff-IgG and Huffl-IgG polypeptides that are conjugated with Human Serum Albumin (HSA). The treatments in **Figures 12A** and **12B** are performed for 18 hours. The concentration on **Figure 12A** is displayed in amount of polypeptide added (in μg) per 100 μL whereas the concentration on **Figure 12B** is displayed in μM of molecules. The treatment in **Figure 12C** is performed for 24 hours and the concentration is displayed in nM of molecules. Thus, **Figure 12** shows that the polypeptides of the present disclosure are more effective in inhibiting the growth of HUVEC cells when compared to the drug known in the art (i.e. aflibercept).

[0038] **Figure 13** shows a graphical illustration depicting the combinations of receptor fragments and the multimerizing component of the peptides of the present disclosure that are considered to be lead candidates. Within the receptor fragments, the box labeled “1A” denotes Domain 1 (D1) of VEGFR1, “1B” denotes Domain 2 (D2) of VEGFR1, “2A” denotes Domain 3 (D3) of VEGFR2, “3A” denotes Domain 2 (D2) of VEGFR3, “3B” denotes Domain 3 (D3) of VEGFR3, and “3C” denotes Domain 1 (D1) of VEGFR3. For the multimerizing component, the box labeled “IgG” denotes Fc portion of Human Immunoglobulin constant Fragment or Fragment M1 and “FCN2” denotes Human Ficolin2 protein or Fragment M2. The box labeled “HSA” denotes Human Serum Albumin.

[0039] **Figure 14** shows a line graph showing the binding affinity of aflibercept (or AFLI), short Gryff-IgG (or sGI), short Huffl-IgG (or sHI), short Gryff-FCN2 (or sGF2), short Huffl-FCN2 (or sHF2), and negative control (Bovine Serum Albumin or BSA) to VEGF-A. The Dissociation Constants (or K_d) that are listed on the table below the graph is determined using Graphpad Prism (fitted non-linear regression analysis, Binding saturation, One-site). Thus, **Figure 14** illustrates that the polypeptide of the present disclosures (i.e. sGI, sHI,

sGF2, and sHF2) has higher binding affinity to VEGF-A when compared to the drug known in the art (i.e. aflibercept).

[0040] **Figure 15** shows a line graph depicting the amount of HUVEC cells growth after 24 hours of treatment using various concentrations of aflibercept (or AFLI), short Gryff-IgG (or sGI), short Huffl-IgG (or sHI), short Gryff-FCN2 (or sGF2), or short Huffl-FCN2 (or sHF2). Thus, **Figure 15** shows that the short version of the polypeptides of the present disclosure is as effective in inhibiting the growth of HUVEC cells when compared to the drug known in the art (i.e. aflibercept).

[0041] **Figure 16** shows a set of data depicting the result of tube formation assay or the inhibition of the tube formation of Human Umbilical Vein Endothelial Cells (HUVEC) using short Gryff-IgG, short Huffl-IgG, short Gryff-FCN2, and short Huffl-FCN2. **Figure 16A** shows a set of light microscopy photographs depicting negative control (PBS), cells treated with aflibercept (denoted as “AFLI”), short Gryff-IgG (denoted as “PP1”), short Huffl-IgG (denoted as “PP2”), short Gryff-FCN2 (denoted as “PP3”), and short Huffl-FCN2 (denoted as “PP4”) at various concentration. **Figure 16B** shows a graphical illustration depicting the set-up of tube formation assay. Briefly, HUVEC cells are seeded homogenously on gel matrix and were incubated for 6 hours. After 6 hours, the cells were observed using light microscope and the total segment lengths of the tubes formed are calculated. **Figure 19C** shows a bar graph depicting the total length of segments formed upon treatment of various concentrations of aflibercept, short Gryff-IgG, short Huffl-IgG, short Gryff-FCN2, and short Huffl-FCN2. Thus, **Figure 16** illustrates that short Gryff-IgG, short Huffl-IgG, short Gryff-FCN2, and short Huffl-FCN2 are better in terms capability to inhibit tube formation in HUVECs than Aflibercept..

[0042] **Figure 17** shows a set of data depicting the inhibition of growth of subcutaneous tumor in mice. **Figure 17A** is a graphical illustration depicting the location of the subcutaneous injection of melanoma cells (5×10^5 of B16-F10 melanoma cells) to the mice. **Figure 17B** is a set of photographs depicting the subcutaneous tumors that are excised from the mice. Mice on first row are negative control (injected with PBS). Mice on second to fourth rows are injected with 500 μ g of aflibercept (denoted as “AFLI”), short-Gryff-IgG (denoted as “sGI”), or short-Huffl-IgG (denoted as “sHI”). Injections were performed twice a week for two weeks. **Figure 17C** is a bar graph depicting average weight of tumors that were excised from the mice on Day 15. **Figure 17D** is a set of microscopy photographs depicting a

cross-section of tumors that were excised from the mice on Day 15. The block arrows indicate the location of tumor vascularization and the asterisk indicates the location of necrotic tumor tissue. Thus, **Figure 17** illustrates that the polypeptides of the present disclosure (such as short-Gryff-IgG and short-Huffl-IgG) are equally effective in reducing the size of the subcutaneous tumors when compared to the drug known in the art (i.e. aflibercept).

[0043] **Figure 18** shows a set of data depicting the inhibition of metastasis of intravenously injected tumor cells in mice. **Figure 18A** is a graphical illustration depicting the intravenous injection of melanoma cells into the tail vein of mice. **Figure 18B** is a set of photographs depicting the lungs that are dissected from the mice. Lungs on the first row are negative control (i.e. from mice injected with PBS). Lungs on second to fourth rows are from injected with 500µg of aflibercept (denoted as “AFLI”), short-Gryff-IgG (denoted as “sGI”), or short-Huffl-IgG (denoted as “sHI”). Injections were performed on Day 4, Day 8, Day 11, and Day 15. The line on the bottom right corner indicates a scale of 1cm. **Figure 18C** is a bar graph depicting average number of nodules on the surface of the lungs. Counting was performed via visual observation of the lungs. Thus, **Figure 18** illustrates that the effectiveness of short-Huffl-IgG (sHI) and short-Gryff-IgG (sGI) in inhibiting metastasis on lungs is comparable to the drug known in the art (i.e. aflibercept).

[0044] **Figure 19** shows a flowchart depicting the process for preparing and systematically screening polypeptides that can be used to target and to bind to VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF.

[0045] **Figure 20** shows a line graph showing the binding affinity of aflibercept (or AFLI), short Gryff-IgG (or sGI), short Huffl-IgG (or sHI), short Gryff-FCN2 (or sGF2), short Huffl-FCN2 (or sHF2), and negative control (Bovine Serum Albumin or BSA) to VEGF-C and VEGF-D. Thus, **Figure 20** illustrates that some of the polypeptide of the present disclosures (e.g. sGI and sHI), besides binding to VEGF-A, also bind to both VEGF-C and VEGF-D. On the other hand, the drug known in the art (i.e. aflibercept) is not able to bind to VEGF-C and VEGF-D.

[0046] **Figure 21** shows a set of data depicting the improvement of expression level of short-Gryff-FCN2 (sGF2). **Figure 21A** is a graphical illustration depicting different combinations of signal peptide, polypeptide sequence between domains 1B and 2A (i.e. Domain 2 (D2) of VEGFR1 and Domain 3 (D3) of VEGFR2), and expression vector tested.

Combination labeled “SEGF-pci” is sGF2 polypeptide that has IL2 signal peptide, has NTII sequence between domains 1B and 2A, and is expressed using pCI-puro expression vector (i.e. modified vector pCI-neo (Promega) with the neo resistant gene cassette replaced with puro resistance gene cassette). Combination labeled “SEGF-pCD” is sGF2 polypeptide that
5 has IL2 signal peptide, has NTII sequence between domains 1B and 2A, and is expressed using pCDNA3.4 expression vector (Thermo). Combination labeled “ISP-sGF2” is sGF2 polypeptide that has VEGFR1 signal peptide (i.e. innate signal peptide), has YRIY sequence between domains 1B and 2A, and is expressed using pCDNA3.4 expression vector (Thermo). Combination labeled “ISP-sGF2 AFmod” is sGF2 polypeptide that has VEGFR1 signal
10 peptide (i.e. innate signal peptide), has NTII sequence between domains 1B and 2A, and is expressed using pCDNA3.4 expression vector (Thermo). **Figure 21B** is a blot depicting result of western blot experiment comparing the expression level of various combinations described in **Figure 21A**. The blot was probed using anti-6His-HRP antibody that recognizes the histidine tag on the polypeptides of interest. Chemiluminescent detection was performed
15 using Luminata Forte (Millipore) or SuperSignal West Pico. Thus, **Figure 21** illustrates that selection of the signal peptide, polypeptide sequence between domains 1B and 2A (i.e. Domain 2 (D2) of VEGFR1 and Domain 3 (D3) of VEGFR2), and expression vector can influence the expression level of sGF2.

[0047] **Figure 22** is a graphical illustration depicting the protein purification process for
20 preparing very pure polypeptides for ocular or intra-ocular injections.

BRIEF DESCRIPTION OF THE TABLES

[0048] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying tables,
25 in which:

[0049] **Table 1** shows a summary of the level of expressions of the polypeptides described herein.

[0050] **Table 2** shows the yield of some of the polypeptides described herein.

[0051] **Table 3** shows the yield of and the approximate cost for producing some of the
30 polypeptides described herein.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

[0052] Overexpression of members of the VEGF family is known to contribute to development of various diseases such as angiogenesis-related disorder, vascularization-related disorder, proliferative disorder, and eye disorder. The VEGF family comprises five members namely, VEGF-A, VEGF-B, VEGF-C, VEGF-D and PIGF (Platelet Induced Growth Factor). VEGF family is typically processed by three different VEGF receptors (VEGFR), namely VEGFR1, VEGFR2, and VEGFR3. VEGFR1 (Flt-1) binds to VEGF-A, VEGF-B, and PIGF. VEGFR2 (KDR/Flk-1) appears to mediate almost all of the known cellular responses to VEGF and it binds to VEGF-A, VEGF-C, VEGF-D, and VEGF-E. VEGFR3 (Flt4), which mediates lymphangiogenesis, binds to VEGF-C and VEGF-D, but not VEGF-A. As illustrated in **Figure 1**, the relationship between various VEGFRs and VEGFs is not simple. Accordingly, to provide an agent that can inhibit VEGF, one must be aware of the fact that (1) there are multiple VEGF receptors and (2) each member of the VEGF family binds to one or more VEGF receptors.

[0053] Therefore, there is a need to provide an agent that can target various different members of the VEGF family. Currently, there are several inhibitors in the market that can bind some members of the VEGF protein (e.g. aflibercept and conbercept that binds VEGF-A, VEGF-B, and PIGF and OPT-302 that binds VEGF-C and VEGF-D). However, none of those inhibitors can bind to all five members of the VEGF family.

[0054] Without wishing to be bound by theory, the inventors of the present invention believes that having an agent comprising binding domains of all three VEGF receptors may be useful in targeting all of the members of VEGFs family. By doing so, such agent may be useful in ameliorating diseases caused by overexpression of VEGFs. Therefore, the inventors of the present invention has provided an alternative fusion protein or polypeptide that comprises binding domains or the immunoglobulin-like domains of VEGF receptors (i.e. VEGFR1, VEGFR2, and VEGFR3) that can target VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF.

[0055] Thus, in one aspect, the present disclosure provides an isolated polypeptide comprising or consisting of $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$, wherein A comprises an immunoglobulin-like domain of VEGFR1 (Vascular Endothelial Growth Factor Receptor 1), B comprises an immunoglobulin-like domain of VEGFR2 (Vascular Endothelial Growth Factor Receptor 2), C comprises an immunoglobulin-like domain of VEGFR3 (Vascular Endothelial Growth Factor Receptor 3), D comprises a multimerizing component, x is 1 or 2,

y is 0 or 1, z is 2 or 3, w is 0 or 1, and n is 2, or 3, or 4, or 5. As used herein, the term “isolated” refers to a material that encompasses both purified and recombinant polypeptides. In other examples, the term “isolated or recombinant” means the material is separated from constituents, cellular and otherwise, in which the cell, tissue, polynucleotide, peptide, polypeptide, protein, antibody or fragment(s) thereof, which are normally associated in nature and/or the material has been altered by a person from its natural state. Thus, as used herein, the term “isolated polypeptide” refers to a polypeptide that does not naturally occur and that has to be expressed or synthesized using any method known in the art. In one example, A, B, and C of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ are independently extracellular domain of the respective VEGFR. As used herein, the term “extracellular domain” refers to portions of VEGFR that are located outside of a cell and that comprise seven immunoglobulin-like domains. The term “extracellular domain” does not refer to portions of VEGFR that are located within a cell membrane (such as a single transmembrane spanning region) or inside of a cell (such as an intracellular portion containing a split tyrosine-kinase domain).

[0056] Further to the above and as shown for example in **Figure 1B**, a VEGFR typically comprises an extracellular domain that is divided into seven immunoglobulin-like domains. These immunoglobulin-like domains are also known as domains 1 to 7 (i.e. D1 to D7). As used herein, the term and “immunoglobulin-like domains” refer to a type of protein domain that consists of a 2-layer sandwich of 7-9 antiparallel β -strands arranged in two β -sheets with a Greek key topology and typically comprises of about 125 amino acids. Immunoglobulin-like domains may or may not be involved in protein-protein interaction or binding of other proteins. Immunoglobulin-like domains that are involved in protein-protein interaction or binding of other proteins (e.g. binding of any members of the VEGF family and/or PIGF) is also referred as “binding domains”. Thus, in one example, A, B, and C of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ are independently ligand binding domain of the respective VEGFR such as VEGFR1, VEGFR2, and VEGFR3.

[0057] In one example, each A of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is independently selected from the group that include, but is not limited to, Domain 1 (D1) of VEGFR1 (for example as shown on SEQ ID NO: 35), Domain 2 (D2) of VEGFR1 (for example as shown on SEQ ID NO: 36), Domain 3 (D3) of VEGFR1 (for example as shown on SEQ ID NO: 37), Domain 4 (D4) of VEGFR1 (for example as shown on SEQ ID NO: 38), Domain 5 (D5) of VEGFR1 (for example as shown on SEQ ID NO: 39), Domain 6 (D6) of

VEGFR1 (for example as shown on SEQ ID NO: 40), Domain 7 (D7) of VEGFR1 (for example as shown on SEQ ID NO: 41), and the like. In one example, each A of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is independently selected from the group that include, but is not limited to, Domain 1 (D1) of VEGFR1 (for example as shown on SEQ ID NO: 35), Domain 2 (D2) of VEGFR1 (for example as shown on SEQ ID NO: 36), and the like.

[0058] In one example, each B of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is independently selected from the group that include, but is not limited to, Domain 1 (D1) of VEGFR2 (for example as shown on SEQ ID NO: 42), Domain 2 (D2) of VEGFR2 (for example as shown on SEQ ID NO: 43), Domain 3 (D3) of VEGFR2 (for example as shown on SEQ ID NO: 44), Domain 4 (D4) of VEGFR2 (for example as shown on SEQ ID NO: 45), Domain 5 (D5) of VEGFR2 (for example as shown on SEQ ID NO: 46), Domain 6 (D6) of VEGFR2 (for example as shown on SEQ ID NO: 47), and Domain 7 (D7) of VEGFR2 (for example as shown on SEQ ID NO: 48), and the like. In one example, each B of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is independently selected from the group that include, but is not limited to, Domain 3 (D3) of VEGFR2 (for example as shown on SEQ ID NO: 44) and the like.

[0059] In one example, each C of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is independently selected from the group that include, but is not limited to, Domain 1 (D1) of VEGFR3 (for example as shown on SEQ ID NO: 49), Domain 2 (D2) of VEGFR3 (for example as shown on SEQ ID NO: 50), Domain 3 (D3) of VEGFR3 (for example as shown on SEQ ID NO: 51), Domain 4 (D4) of VEGFR3 (for example as shown on SEQ ID NO: 52), Domain 5 (D5) of VEGFR3 (for example as shown on SEQ ID NO: 53), Domain 6 (D6) of VEGFR3 (for example as shown on SEQ ID NO: 54), and Domain 7 (D7) of VEGFR3 (for example as shown on SEQ ID NO: 55) and the like. In one example, each C of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is independently selected from the group that include, but is not limited to, Domain 1 (D1) of VEGFR3 (for example as shown on SEQ ID NO: 49), Domain 2 (D2) of VEGFR3 (for example as shown on SEQ ID NO: 50), Domain 3 (D3) of VEGFR3 (for example as shown on SEQ ID NO: 51), and the like.

[0060] The inventors have also surprisingly found that the specific combination and the specific order of the binding domains within the isolated polypeptide, especially the location of the binding domains from VEGFR3, are important. That is because the systematic screen (the process of which is depicted in **Figure 19**) shows that incorporation of the

immunoglobulin-like domains in other order than that described (i.e. the order of $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$) would result in either a non-secreted fusion protein or unstable fusion protein prone to degradation. Some other fusion proteins (i.e. fusion protein not having the above combination or order) also fail to express in mammalian cell culture and or have relatively low bioavailability *in vivo*.

[0061] In addition to having specific combination and order, the polypeptide described herein comprises of certain numbers of the binding domains or immunoglobulin-like domains of VEGFR1, VEGFR2, and VEGFR3. Thus, in one example, the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is selected from the group that includes, but is not limited to, (i) $(A_{(2)} - B_{(1)} - C_{(2)} - D)_n$, (ii) $(A_{(1)} - B_{(1)} - C_{(2)} - D)_n$, (iii) $(C_{(1)} - A_{(1)} - B_{(1)} - C_{(2)} - D)_n$, (iv) $(A_{(2)} - C_{(2)} - D)_n$, (v) $(A_{(1)} - C_{(2)} - D)_n$, (vi) $(C_{(1)} - A_{(1)} - C_{(2)} - D)_n$, and the like. Additionally and as shown for example in **Figure 2**, each A, B, and C of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ corresponds to specific binding domains or immunoglobulin-like domains of VEGFR1, VEGFR2, and VEGFR3. Thus, in one example, the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is selected from the group that includes but are not limited to (i) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n, (ii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n, (iii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n, (iv) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n, (v) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n, (vi) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n.

[0062] Each of the binding domains or immunoglobulin-like domains of VEGFR1, VEGFR2, and VEGFR3 that forms the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ may comprise specific amino acid sequence. Thus, in one example, the amino acid of the Domain 1 (D1) of VEGFR1 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 1. In one example, the amino acid of

the Domain 1 (D1) of VEGFR1 of the polypeptide described herein has a sequence of SEQ ID NO: 1.

[0063] In one example, the amino acid of the Domain 2 (D2) of VEGFR1 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 2. In one example, the amino acid of the Domain 2 (D2) of VEGFR1 of the polypeptide described herein has a sequence of SEQ ID NO: 2.

[0064] In one example, the amino acid of the Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 12. In one example, the amino acid of the Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 of the polypeptide described herein has a sequence of SEQ ID NO: 12.

[0065] In one example, the amino acid of the Domain 3 (D3) of VEGFR2 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 3. In one example, the amino acid of the Domain 3 (D3) of VEGFR2 of the polypeptide described herein has a sequence of SEQ ID NO: 3.

[0066] In one example, the amino acid of the Domain 1 (D1) of VEGFR3 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 6. In one example, the amino acid of the Domain 1 (D1) of VEGFR3 of the polypeptide described herein has a sequence of SEQ ID NO: 6.

[0067] In one example, the amino acid of the Domain 2 (D2) of VEGFR3 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 4. In one example, the amino acid of the Domain 2 (D2) of VEGFR3 of the polypeptide described herein has a sequence of SEQ ID NO: 4.

[0068] In one example, the amino acid of the Domain 3 (D3) of VEGFR3 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 5. In one example, the amino acid of the Domain 3 (D3) of VEGFR3 of the polypeptide described herein has a sequence of SEQ ID NO: 5.

[0069] In one example, the amino acid of the Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 13. In one example, the amino acid of the Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 of the polypeptide described herein has a sequence of SEQ ID NO: 13.

[0070] In addition to providing polypeptides having specific orders and combinations of various binding domains or immunoglobulin-like domains of VEGFR1, VEGFR2, and VEGFR3, the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein also comprises at least one multimerizing component (which is denoted by D in the formula). As used herein, the term “multimerizing component” refers to any natural or synthetic sequence capable of interacting with another multimerizing component to form a higher order structure such as a dimer, a trimer, a tetramer, a pentamer, a hexamer, a heptamer, an octamer, a nonamer, a decamer, and the like. Thus, in one example, the multimerizing component of the polypeptide as described herein comprises one, or at least one, or at least two, or at least three, or at least four, or more selected from the group that

includes, but is not limited to, Fc portion of Human Immunoglobulin constant Fragment (i.e. IgG, Ig, Fc), Mannose-binding protein C (Uniprot: P11226) (such as MBL2(101-138) or MBL2S and MBL2(101-248) or MBL2L), Human Ficolin2 protein (i.e. FCN2, FCN, F2), Cartilage oligomeric matrix protein (Uniprot: P49747) (i.e. COMP), and the like. Having said
5 the above, the inventors of the present disclosure have surprisingly found that not all known multimerizing components are suitable for the preparation of the polypeptide of the present disclosure. In one example, the use of C-reactive protein (Uniprot: P02741) (i.e. CRP), Human Ficolin1 protein, or Human Ficolin3 protein as multimerizing components could result in non-expression or non-secretion of the polypeptide of the present disclosure.
10 Therefore, in one example, the multimerizing component of the polypeptide as described herein does not comprise C-reactive protein (Uniprot: P02741) (i.e. CRP).

[0071] Each of the multimerizing component that forms the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ may comprise specific amino acid sequence. Thus, in one example, the multimerizing component of the polypeptide described herein is Fc portion of
15 Human Immunoglobulin constant Fragment (i.e. IgG, Ig, Fc). In one example, the amino acid of the Fc portion of Human Immunoglobulin constant Fragment of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5%
20 identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 7. In one example, the amino acid of the Fc portion of Human Immunoglobulin constant Fragment of the polypeptide described herein has a sequence of SEQ ID NO: 7.

[0072] In one example, the multimerizing component of the polypeptide described herein is Human Ficolin2 protein (i.e. FCN2, FCN, F2). In one example, the amino acid of the
25 Human Ficolin2 protein of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 8. In one example, the amino acid of the
30 Human Ficolin2 protein of the polypeptide described herein has a sequence of SEQ ID NO: 8.

[0073] In one example, the multimerizing component of the polypeptide described herein is Mannose-binding protein C (Uniprot: P11226). In one example, the multimerizing component of the polypeptide described herein is Mannose-binding protein C (Uniprot: P11226) comprising an amino acid sequence of position 101 – 138 (i.e. MBL2(101-138),
5 MBL2S). In one example, the amino acid of the Mannose-binding protein C (Uniprot: P11226), position 101 – 138 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or
10 at least 99.5% identity, to a sequence of SEQ ID NO: 9. In one example, the amino acid of the Mannose-binding protein C (Uniprot: P11226), position 101 – 138 of the polypeptide described herein has a sequence of SEQ ID NO: 9. In one example, the multimerizing component of the polypeptide described herein is Mannose-binding protein C (Uniprot: P11226) comprising an amino acid sequence of position 101 – 248 (i.e. MBL2(101-248),
15 MBL2L). In one example, the amino acid of the Mannose-binding protein C (Uniprot: P11226), position 101 – 248 of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or
20 at least 99.5% identity, to a sequence of SEQ ID NO: 10. In one example, the amino acid of the Mannose-binding protein C (Uniprot: P11226), position 101 – 248 of the polypeptide described herein has a sequence of SEQ ID NO: 10.

[0074] In one example, the multimerizing component of the polypeptide described herein is Cartilage oligomeric matrix protein (Uniprot: P49747) (i.e. COMP). In one example, the
25 amino acid of the Cartilage oligomeric matrix protein (Uniprot: P49747) of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID
30 NO: 11. In one example, the amino acid of Cartilage oligomeric matrix protein (Uniprot: P49747) of the polypeptide described herein has a sequence of SEQ ID NO: 11.

[0075] The polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein is a fusion protein or polypeptide comprising various binding domains or immunoglobulin-like domains of VEGFR1, VEGFR2, and VEGFR3 and at least one multimerizing component. The terms “fusion protein” and “fusion polypeptide” are used interchangeably throughout the specification. As used herein, the terms “fusion protein” and “fusion polypeptide” refer to a protein or polypeptide which comprises amino acid sequences from at least two different proteins. In order to ensure that the amino acid sequences from different proteins are expressed and are functional, they have to be operably linked. As used herein, the term “operably linked” is intended to mean that the amino acid sequences from at least two different proteins (such as the various binding domains or immunoglobulin-like domains of VEGFR1, VEGFR2, and VEGFR3 and the multimerizing component) are connected in a manner such that each amino acid sequences can serve its intended function. The connector between the amino acid sequences from different proteins is termed as a linker. Thus, in one example, one, or at least one, or at least two, or at least three, or all of A, B, C, or D of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein further comprises a linker. In one example of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein, C is operably linked to A by a linker; and/or A is operably linked to A by a linker; and/or A is operably linked to B by a linker; and/or B is operably linked to C by a linker; and/or C is operably linked to C by a linker. The linker that operably links C to A, and/or A to A, and/or A to B, and/or B to C, and/or C to C of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein can be any linker that is commonly used in the art. In one example, the linker that operably links C to A, and/or A to A, and/or A to B, and/or B to C, and/or C to C of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein is a native IgG-like domain linker. In one example of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein, D is operably linked to C by a linker. The linker that operably links D to C of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein can be any linker that is commonly used in the art. In one example, the linker that operably links D to C of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein is a polypeptide linker. Without wishing to be bound by theory, the polypeptide linker that operably links D to C of the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein is capable of improving or improves the conformational flexibility

between C and D. As used herein, the term “conformational flexibility” refers to the ability of a macromolecule (such as protein or polypeptide) to change its shape in response to various environmental factors (such as changes in temperature, pH, and the like) or upon binding to a ligand. In one example, the amino acid of the polypeptide linker that operably links D to C in the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein comprises a sequence of SEQ ID NO: 22 (GGGS). In one example, the amino acid of the polypeptide linker that operably links D to C in the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein has a sequence of SEQ ID NO: 22 (GGGS). The inventors have surprisingly found that even though the polypeptide described herein can still be expressed and secreted from the host cells, removal of linker having a sequence of SEQ ID NO: 22 (GGGS) from the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein may confer different structural changes to the polypeptide and hence affect its functionality.

[0076] As described above, the multimerizing component comprised within the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein allows the polypeptide to form a higher order structure. As illustrated on **Figure 3A**, in one example, wherein when n of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is 2, the polypeptide is a dimer. The multimerizing component that allows for the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ to form dimer can be any suitable multimerizing component that is commonly used in the art. In one example, when n of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is 2, the multimerizing component is Fc portion of Human Immunoglobulin constant Fragment (i.e. IgG, Ig, Fc). As illustrated on **Figure 3B**, in one example, when n of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is 3, the polypeptide is a trimer. The multimerizing component that allows for the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ to form trimer can be any suitable multimerizing component that is commonly used in the art. In one example, when n of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is 3, the multimerizing component is any one selected from the group that includes, but is not limited to, Human Ficolin2 protein (i.e. FCN2, FCN, F2) and Mannose-binding protein C (Uniprot: P11226) (i.e. MBL2(101-138), MBL2S and MBL2(101-248), MBL2L). In one example, when n of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is 5, the polypeptide is a pentamer. The multimerizing component that allows for the polypeptide having the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ to form pentamer can be any suitable multimerizing component that is commonly used in the

art. In one example, when n of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ is 5, the multimerizing component is Cartilage oligomeric matrix protein (Uniprot: P49747) (i.e. COMP).

[0077] Further to the above and as shown for example in **Figures 2 and 10**, the inventors of the present disclosure have prepared polypeptides from six different sets of receptor fragments (i.e. four full length receptor fragments and two shortened receptor fragments) that are combined with either one of the five different types of multimerizing components. Thus, in one example, the polypeptide having formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein is selected from the group that includes, but is not limited to,

- 10 (1a) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT123-Fc; Gryff-Fc; Gryff-IgG; GI), wherein $n=2$;
- (1b) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT123-F2; Gryff-F2; Gryff-FCN2; GF2), wherein $n=3$;
- 15 (1c) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n (i.e. VMT123-MBL2S; Gryff-MBL2S), wherein $n=3$;
- 20 (1d) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n (i.e. VMT123-MBL2L; Gryff-MBL2L), wherein $n=3$;
- 25 (1e) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n (i.e. VMT123-COMP; Gryff-COMP), wherein $n=5$;
- (2a) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT123-Fc-S; Gryff-Fc-S, short/only one domain of VEGFR1; Short-Gryff-IgG; sGI), wherein $n=2$;
- 30

(2b) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT123-F2-S; Gryff-F2-S, short/only one domain of VEGFR1; Short-Gryff-FCN2; sGF2), wherein n=3;

(2c) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n (i.e. VMT123-MBL2S-S; Gryff-MBL2S-S, short/only one domain of VEGFR1), wherein n=3;

(2d) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n (i.e. VMT123-MBL2L-S; Gryff-MBL2L-S, short/only one domain of VEGFR1), wherein n=3;

(2e) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n (i.e. VMT123-COMP-S; Gryff-COMP-S, short/only one domain of VEGFR1), wherein n=5;

(3a) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT3123-Fc; Slyth-Fc), wherein n=2;

(3b) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT3123-F2; Slyth-F2), wherein n=3;

(3c) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n (i.e. VMT3123-MBL2S; Slyth-MBL2S), wherein n=3;

(3d) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n (i.e. VMT3123-MBL2L; Slyth-MBL2L), wherein n=3;

(3e) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n (i.e. VMT3123-COMP; Slyth-COMP), wherein n=5;

- (4a) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT13-Fc; Huffl-Fc; Huffl-IgG; HI), wherein n=2;
- 5 (4b) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT13-F2; Huffl-F2; Huffl-FCN2; HF2), wherein n=3;
- (4c) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n (i.e. VMT13-MBL2S; Huffl-MBL2S), wherein n=3;
- 10 (4d) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n (i.e. VMT13-MBL2L; Huffl-MBL2L), wherein n=3;
- (4e) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n (i.e. VMT13-COMP; Huffl-COMP), wherein n=5;
- 15 (5a) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT13-Fc-S; Huffl-Fc-S, short/only one domain of VEGFR1; Short-Huffl-IgG; sHI), wherein n=2;
- (5b) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT13-F2-S; Huffl-F2-S, short/only one domain of VEGFR1; Short-Huffl-FCN2; sHF2), wherein n=3;
- 20 (5c) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n (i.e. VMT13-MBL2S-S; Huffl-MBL2S-S, short/only one domain of VEGFR1), wherein n=3;
- 25 (5d) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n (i.e. VMT13-MBL2L-S; Huffl-MBL2L-S, short/only one domain of VEGFR1), wherein n=3;
- (5e) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n (i.e. VMT13-COMP-S; Huffl-COMP-S, short/only one domain of VEGFR1), wherein n=5;
- 30

(6a) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT313-Fc; Ravcl-Fc), wherein n=2;

(6b) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT313-F2; Ravcl-F2), wherein n=3;

(6c) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n (i.e. VMT313-MBL2S; Ravcl-MBL2S), wherein n=3;

(6d) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n (i.e. VMT313-MBL2L; Ravcl-MBL2L), wherein n=3;

(6e) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n (i.e. VMT313-COMP; Ravcl-COMP), wherein n=5; and the like.

[0078] As shown for example in **Figures 4 to 6**, the inventors have found that polypeptides Gryff-IgG (or GI), Huffl-IgG (or HI), Gryff-FCN2 (or GF2), and Huffl-FCN2 (of HF2) are expressed at higher amount when compared to the other polypeptides. Additionally, as shown for example in **Figure 9**, the inventors have surprisingly found that the shorter forms of the polypeptides, which are Short-Gryff-IgG (or sGI), Short-Huffl-IgG (or sHI), Short-Gryff-FCN2 (or sGF2), and Short-Huffl-FCN2 (of sHF2), have even higher bioavailability when compared to the full length polypeptides (i.e. GI, HI, GF2, and HF2). Thus, in one example, the polypeptide having formula (C_(w) – A_(x) – B_(y) – C_(z) – D)_n as described herein is selected from the group that includes, but is not limited to,

(1a) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT123-Fc; Gryff-Fc; Gryff-IgG; GI), wherein n=2;

(1b) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT123-F2; Gryff-F2; Gryff-FCN2; GF2), wherein n=3;

- (2a) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT123-Fc-S; Gryff-Fc-S, short/only one domain of VEGFR1; Short-Gryff-IgG; sGI), wherein n=2;
- 5 (2b) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT123-F2-S; Gryff-F2-S, short/only one domain of VEGFR1; Short-Gryff-FCN2; sGF2), wherein n=3;
- (3a) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant
10 Fragment)_n (i.e. VMT13-Fc; Huffl-Fc; Huffl-IgG; HI), wherein n=2;
- (3b) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT13-F2; Huffl-F2; Huffl-FCN2; HF2), wherein n=3;
- (4a) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of
15 VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n (i.e. VMT13-Fc-S; Huffl-Fc-S, short/only one domain of VEGFR1; Short-Huffl-IgG; sHI), wherein n=2;
- (4b) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n (i.e. VMT13-F2-S; Huffl-F2-S, short/only one domain of VEGFR1; Short-Huffl-FCN2; sHF2), wherein n=3; and the like.
- 20 [0079] Each of the polypeptide of the formula (C_(w) – A_(x) – B_(y) – C_(z) – D)_n as described herein may comprise specific amino acid sequence. Thus, in one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity,
25 or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 14. In one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at
30 least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 15. In one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity,

or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 16. In one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 17. In one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 18. In one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 19. In one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 20. In one example, the amino acid of the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 21. In one example, the amino acid of the polypeptide described herein has a sequence selected from the group that include, but is not limited to, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and the like.

[0080] In order to further improve the bioavailability of the polypeptides, in addition to preparing shorter forms of several polypeptides (as shown for example in **Figure 10**), the

inventors have also conjugated or fused the polypeptides with a component that is able to improve bioavailability. Thus, in one example, the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein is conjugated to a component capable of improving or which improves bioavailability. As used herein, the term “bioavailability” refers to the concentration of polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein in the serum of a subject after administration of said polypeptide into the subject. The component capable of improving bioavailability that is conjugated to the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein can be any component that is commonly used in the art. In one example, the component capable of improving bioavailability that is conjugated to the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein is Human Serum Albumin (i.e. HSA). In one example, the amino acid of the Human Serum Albumin that is conjugated to the polypeptide described herein has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 23. In one example, the amino acid of the Human Serum Albumin that is conjugated to the polypeptide described herein has a sequence of SEQ ID NO: 23. As shown in **Figure 13**, in one example, the Human Serum Albumin is conjugated to the multimerizing domains of the peptide.

[0081] Each of the polypeptide of the formula $(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n$ as described herein and that is conjugated to Human Serum Albumin may comprise specific amino acid sequence. Thus, in one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 24. In one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 25. In one example, the amino acid of the polypeptide

described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 26. In one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 27. In one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 28. In one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 29. In one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 30. In one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has at least 90% identity, or at least 91% identity, or at least 92% identity, or at least 93% identity, or at least 94% identity, or at least 95% identity, or at least 96% identity, or at least 97% identity, or at least 97.5% identity, or at least 98% identity, or at least 98.5% identity, or at least 99% identity, or at least 99.5% identity, to a sequence of SEQ ID NO: 31. In one example, the amino acid of the polypeptide described herein and that is conjugated to Human Serum Albumin has a sequence selected

from the group that include, but is not limited to, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and the like.

[0082] As explained above, the polypeptides of the present disclosure is surprisingly
5 capable of targeting VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF. Thus, in one example, the polypeptides of the present disclosure are capable of binding at least one, or at least two, or at least three, or at least four, or all of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF (Platelet Induced Growth Factor). In one example, the polypeptides of the present disclosure are capable of binding all of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PIGF
10 (Platelet Induced Growth Factor). The binding affinity of the peptides of the present disclosure to one of the member of VEGF family (i.e. VEGF-A or VEGF-165) is shown for example on **Figure 14**. The binding affinity of the peptides of the present disclosure to VEGF-C and VEGF-D is shown for example on **Figure 20**.

[0083] The expression of the polypeptides of the present disclosure can be performed
15 using any method known in the art. In one example, the production or the expression of the polypeptides is performed using cell-based system. In cell-based system, one or more expression vectors comprising one or more nucleic acids that encode for the polypeptide is inserted into a host cell. The host cell comprising the expression vector is then allowed to express or to overexpress the polypeptide of the present disclosure in a media that is suitable
20 for the expression or the overexpression of the polypeptides as described herein. The polypeptides that have been expressed may be secreted out into the media. Thus, in another aspect, the present disclosure provides one or more nucleic acid(s) encoding the polypeptide as described herein. In yet another aspect, the present disclosure provides one or more expression vector(s) comprising the nucleic acid as described herein. In yet another aspect,
25 the present disclosure provides a host cell comprising the nucleic acid as described herein and/or the expression vector as described herein. Any type of host cells that is suitable for the production or expression or overexpression of the polypeptides described herein can be used. The host cell include, but are not limited to, mammalian cells, bacterial cells, yeast cells, insect cells, and the like. In one example, the host cell used for the production or expression
30 or overexpression of the polypeptides described herein is mammalian cells. The mammalian cells include, but are not limited to, Expi293F (HEK 293 cells), ExpiCHO (CHO cells), and the like.

[0084] As explained above, the polypeptides of the present disclosure can be used to bind or to target VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF. Overexpression of members of VEGF family is often implicated in the development of various disorders. Therefore, by binding and targeting members of the VEGF family and PlGF, the polypeptides of the present disclosure may be used as an alternative therapy or medicine for disorders that are related to overexpression of VEGF-A, VEGF-B, VEGF-C, VEGF-D, and PlGF. Thus, in yet another aspect, the present disclosure provides a polypeptide as described herein for use in therapy or medicine. In yet another aspect, the present disclosure provides a pharmaceutical composition comprising one or more polypeptide(s) as described herein.

[0085] VEGF signaling is involved in vasculogenesis and angiogenesis. As used herein, the term “vasculogenesis” or “vascularization” refers to the differentiation of precursor cells (angioblasts) into endothelial cells and the *de novo* formation of a primitive vascular network). As used herein, the term “angiogenesis” refers to the growth of new capillaries from pre-existing blood vessels. Overexpression of VEGF has been found to cause various angiogenesis-related and vascularization-related disorders such as cancer, retinal angiogenic diseases, tumor metastasis, and the like because it allows for the formation of new blood vessels that can supply nutrient into the tumor. Without wishing to be bound by theory, treatment of tumor using anti-angiogenesis agent (such as the polypeptides of the present disclosure) can prevent the overexpressed VEGF to bind to VEGF receptors and thereby causing the blood vessels in the tumor to regress. Regression of blood vessel in a tumor can cause the tumor to shrink because it is no longer supplied with nutrient required to support its growth. Therefore, inhibition of overexpressed VEGF using the polypeptide of the present disclosure may lead to reduction in tumor growth. Thus, in yet another aspect, the present disclosure provides a method of treating angiogenesis-related disorder and/or vascularization-related disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the polypeptide as described herein or a therapeutically effective amount of the composition as described herein into the subject. As shown for example in **Figure 16**, the polypeptide of the present disclosure is able to inhibit angiogenesis because it is able to prevent HUVEC cells from forming of capillary-like structures (or tubes) that represent blood vessels. The lengths of the capillary-like structure is shorter at higher concentration of polypeptides of the present disclosure signifying that higher concentration of the polypeptides is more effective in inhibiting angiogenesis.

[0086] In one example, the angiogenesis-related disorder and/or vascularization-related disorder that can be treated using the polypeptides as described herein is selected from the group that include, but are not limited to, proliferative disorder, eye disorder, and the like. In one example, the proliferative disorder that can be treated using the polypeptides as described

5 herein is selected from the group that includes, but is not limited to, cancer, tumor, and the like. As shown for example on **Figure 17**, the weight of the tumor is reduced after treatment using the polypeptides of the present disclosure. In one example, the cancer or tumor that can be treated using the polypeptides as described herein is selected from the group that includes, but is not limited to, actinic keratosis, AIDS-Related Kaposi's Sarcoma, basal cell carcinoma,

10 breast cancer, cervical cancer, colorectal cancer, differentiated thyroid carcinoma, Follicular Lymphoma, gastric cancer, gastric tumors, gastric or gastroesophageal junction adenocarcinoma, gastrointestinal stromal tumor, glioblastoma, Hairy Cell Leukemia, hepatocellular carcinoma, mantle cell lymphoma, melanoma, Myelodysplastic Syndrome, Multiple myeloma, neoplasm, neuroendocrine tumors, Non-Hodgkins Lymphoma, ovarian

15 cancer, pancreatic neuroendocrine tumors, renal cell carcinoma, skin cancer, subependymal giant cell astrocytoma, medullary thyroid cancer, thyroid cancer, non-small cell lung cancer, and the like. In one example, the cancer that can be treated using the polypeptides as described herein is selected from the group that includes, but is not limited to, non-small cell lung cancer, gastric cancer, gastric tumors, breast cancer, and the like. In one example, the

20 cancer that can be treated using the polypeptides as described herein is lung cancer. As shown for example on **Figure 18**, the number of nodules on the lung of a mice treated with the polypeptide of the present disclosure (such as short-Gryff-IgG or sGI) is lower than on the control thereby indicating that the polypeptide of the present disclosure can prevent metastasis. In one example, the eye disorder that can be treated using the polypeptides as

25 described herein is retinal angiogenic disease. In one example, the eye disorder that can be treated using the polypeptides as described herein is selected from the group that includes, but is not limited to, age-related macular degeneration (such as wet or dry age-related macular degeneration), branch retinal vein occlusion, central retinal vein occlusion, corneal neovascularization (trachoma), diabetic retinopathy, diabetic macular edema, macular edema,

30 Myopic Choroidal Neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, Pterygium, Retinal Vein Occlusion, Uveitis, and the like. In one example, the eye disorder that can be treated using the polypeptides as described herein is selected from

the group that includes, but is not limited to, age-related macular degeneration (such as wet or dry age-related macular degeneration), macular edema, diabetic retinopathy, diabetic macular edema, and the like.

[0087] When used in therapy or medicine or when used in treating angiogenesis-related disorder and/or vascularization-related disorder, the polypeptides as described herein or the pharmaceutical composition comprising the polypeptides as described herein can be administered using any administration route known in the art. In one example, the polypeptides as described herein or the pharmaceutical composition can be injected directly into the cancer or tumor. In another example and as shown for example on **Figure 18**, the polypeptides as described herein or the pharmaceutical composition can be injected directly into the vein of the subject (or injected intravenously). In one example, the administration of the polypeptides as described herein or the pharmaceutical composition comprising the polypeptides as described herein is via intra-vital injection. The administration of the polypeptides as described herein or the pharmaceutical composition comprising the polypeptides as described herein is repeated according to the individual requirements of the subject being treated. In one example, the administration of the polypeptides as described herein or the pharmaceutical composition comprising the polypeptides as described herein is repeated every month (or monthly), or every two months (or bi-monthly), or every six months, or every year (or yearly), or every two years, and the like.

[0088] In yet another aspect, the present invention provides a kit comprising one or more polypeptide(s) as described herein. In one example, the kit comprising one or more polypeptide(s) as described herein is an enzyme-linked immunosorbent assay (ELISA) kit.

[0089] As used in this application, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a polypeptide” includes a plurality of polypeptides, including mixtures and combinations thereof.

[0090] As used herein, the terms “increase” and “decrease” refer to the relative alteration of a chosen trait or characteristic in a subset of a population in comparison to the same trait or characteristic as present in the whole population. An increase thus indicates a change on a positive scale, whereas a decrease indicates a change on a negative scale. The term “change”, as used herein, also refers to the difference between a chosen trait or characteristic of an

isolated population subset in comparison to the same trait or characteristic in the population as a whole. However, this term is without valuation of the difference seen.

[0091] As used herein, the term "about" in the context of concentration of a substance, size of a substance, length of time, or other stated values means +/- 5% of the stated value, or +/- 4% of the stated value, or +/- 3% of the stated value, or +/- 2% of the stated value, or +/- 1% of the stated value, or +/- 0.5% of the stated value.

[0092] Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0093] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0094] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0095] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

5

EXPERIMENTAL SECTION

[0096] **Example 1: Gel Electrophoresis and Western Blot**

[0097] Gel electrophoresis and western blot experiment were performed in order to determine the expression level of various polypeptides that were tested. Expi293F cells
10 (Thermo Scientific) was transfected with plasmid DNA carrying the transgene (that can express polypeptides of interest) using Expifectamine Kit (Thermo Scientific) according to the manufacturer's protocol. Transfected cells were centrifuged at 4000rpm for 30 mins. The cells in the pellet was then washed once with PBS and then resuspended in 1x Protein Loading Dye containing SDS and boiled for 5 to 10 mins before loading onto SDS-PAGE gel
15 for analysis. As the proteins produced by the transfected cells are secreted, they are found in the cell media or supernatant. The supernatant was prepared from cell suspension that was obtained at various days post-transfection (as indicated in the figures). To obtain the supernatant, the cell suspension was centrifuged at 3000g for 30 minutes, followed by passing through a 0.45um PVDF filter. Centrifugation and filtration was performed to remove
20 the cells and cellular debris from the supernatant. Protein loading dye containing 5% beta-mercaptoethanol was added to 10µl of the supernatant and boiled for 5 mins before separation on SDS-PAGE gel. The molecular weight marker used was Bio-Rad Dual or Kaleidoscope. The gel was stained with Instant-Blue (Expedeon) or transferred onto a PVDF membrane and blotted with anti-6His-HRP (Roche) (1:500). Chemiluminescent detection of the polypeptides
25 of interest was performed using Luminata Forte (Millipore) that was diluted with water at 1:10 or using SuperSignal West Pico that was diluted with water at 1:5.

[0098] The results of the gel electrophoresis and western blot experiments were shown on **Figures 4 to 6**. As shown on **Figure 4**, four different combinations of VEGFR domains were tested. Two polypeptides (denoted as "Gryff-IgG" and "Huffl-IgG") were found to be
30 expressed at higher concentration than the other polypeptides. After the two combinations of VEGFR domains were selected, various multimerizing components were also tested. The various multimerizing components were fused with each one of the two combinations of

VEGFR domains. The expression level of polypeptides having the various multimerizing components were shown on **Figures 5** and **6**. As shown on **Figures 5** and **6**, the polypeptides that were expressed at higher amount compared to other polypeptides are polypeptides having Fc portion of Human Immunoglobulin constant fragment (i.e. denoted as “Gryff-IgG” and “Huffl-IgG” on the figures) and polypeptides having Human Ficolin2 protein (i.e. denoted as “Gryff-FCN2” and “Huffl-FCN2”). The expression levels of all of the polypeptides tested were also summarized on **Table 1** below.

[0099] **Table 1**

	VMT123 (Gryff)	VMT3123 (Slyth)	VMT13 (Huffl)	VMT313 (Rafcl)
IgG	High Expression Completed 30ml	Low Expression	High Expression Completed 30ml	Low Expression
FCN2	High Expression Completed 30ml	Low/No Expression	Moderate Expression	Low/No Expression
MBL2S	Moderate Expression Completed 30ml	Low/No Expression	Low Expression	Low/No Expression
MBL2L	Moderate Expression Completed 30ml	Low/No Expression	Low Expression	Low/No Expression
COMP	Low Expression	Low/No Expression	Low Expression	Low/No Expression
CRP	No Expression	No Expression	No Expression	No Expression

Note:

- “No Expression” refers to the observation that no band is detected in whole supernatant on Coomassie-blue stained SDS-PAGE gel, no band is detected in whole supernatant analyzed with Western Blot, and no appreciable amount of protein is purified from supernatant.
- “Low Expression” refers to the observation that very faint band is detected in whole supernatant on Coomassie-blue stained SDS-PAGE gel, very faint band is detected in whole supernatant analyzed with Western Blot, and the amount protein yield is less than 0.5mg/30mL (i.e. 16.7mg/L).

[00100] In addition for determining the expression level of all of the polypeptides tested, western blot were also used for optimization of expression of sGF2 (also denoted as “short-Gryff-FCN2”). As shown on **Figure 10**, sGF2 is a version of GF2 (also denoted as “Gryff-FCN2”) that does not comprise Domain 1 (D1) of VEGFR1. As indicated in **Figure 21**, sGF2 was initially expressed using pci-puro expression vector (modified pCI-neo expression vector

(Promega) with the neomycin resistant gene cassette replaced with puromycin resistant gene cassette). Because expression level was low, the pci-puro expression vector was replaced with pCDNA3.4 expression vector. Additionally, different signaling peptide and different polypeptide sequence between domains 1B and 2A (i.e. Domain 2 (D2) of VEGFR1 and Domain 3 (D3) of VEGFR2) was also tested. **Figure 21** also showed that changes in expression vector, signaling peptide, and polypeptide sequence used can improve the expression level of sGF2.

[00101] **Example 2: Polypeptides Expression and Purification**

[00102] Expi293F cells (Thermo Scientific) was transfected with plasmid DNA carrying the transgene using Expifectamine Kit (Thermo Scientific) according to the manufacturer's protocol. Supernatant was obtained by centrifuging the cell suspension obtained 7 days post transfection at 3000g for 30 minutes, followed by passing through a 0.45µm PVDF filter. The supernatant was then allowed to bind to Ni-NTA beads (Qiagen) at 4°C overnight. Polypeptides bound on the beads were then washed with Wash Buffer (1x PBS, 200mM Sodium Chloride, 20mM Imidazole, 0.05% Tween-20) before eluting with Elution Buffer (1x PBS, 200mM Sodium Chloride, 500mM Imidazole, 0.05% Tween-20). Analysis of the polypeptides and other proteins contained in the supernatant before and after purification using Ni-NTA beads was shown on **Figure 7**. Eluted polypeptides were then dialyzed against 1xPBS before concentrating to 1µg/µl. The concentrated polypeptides were used for further experiments or were stored at -80°C. If polypeptides of higher purity was required (e.g. for intra-ocular injection), the concentrated polypeptides was further purified using Fast Protein Liquid Chromatography (FPLC) as outlined on **Figure 22**.

[00103] As listed on **Tables 2** and **3** below, amounts of polypeptides obtained after the expression and purification (using Ni-NTA beads) varies. The differences in the expression levels of polypeptides listed on **Table 2** was further illustrated in **Figure 8**. In this figure, similar volumes of cultures were added to each lane of the gel. However, because the expression level of the polypeptides and the polypeptides concentration in each sample was different, the size and the thickness of the bands corresponding to the polypeptides of interest also vary.

[00104] **Table 2**

Protein Name	Identity	Yield per 30ml (mg)	Yield per liter (mg/L)
Gryff.IgG	VMT123-IgG	3.5	116.67

Gryff.F2	VMT123-FCN2	2.0	66.67
Huffl.IgG	VMT13-IgG	6.3	210.00
Huffl.F2	VMT13-FCN2	2.1	70.00

[00105] **Table 3**

Protein Name	Yield per liter (mg/L)	Effective production cost/mg*
short.Gryff.IgG (sGI)	120 mg/L	\$13/mg
short.Huffl.IgG (sHI)	120 mg/L	\$13/mg
short.Gryff.F2 (sGF2)	50 mg/L	\$32/mg
short.Huffl.F2 (sGH2)	50 mg/L	\$32/mg

*Approximate cost for producing 1mg of protein in the system

5 [00106] **Example 3: Bioavailability**

[00107] The purpose of the bioavailability experiment is to measure the amount of polypeptides in mice serum after subcutaneous administration of the polypeptides. Briefly, Balb/C mice were subcutaneously injected with the polypeptide at a dose of 4mg/kg (4mg of polypeptide per kg of mice). The mice were bled at 24 hours post injection. Serum was then
 10 obtained from the blood obtained and was analyzed for polypeptide concentrations using Simple Step VEGFR1 ELISA kit (Abcam) according to manufacturer's instructions. The result of the bioavailability experiment is shown on **Figure 9**. Huffl-IgG (or HI) was found to have higher bioavailability among all of the longer version of polypeptides tested. Short-Huffl-IgG (of sHI) was found to have higher bioavailability among all of the shorter version
 15 of polypeptides tested.

[00108] **Example 4: Tube Formation Assay**

[00109] The purpose of performing tube formation assay was to determine whether the polypeptides described herein are able to inhibit formation of blood vessels. Briefly, the wells of 96 well plates were coated with 40μl of Matrigel without Growth Factors (Corning).
 20 Human Umbilical Vein Endothelial Cells (HUVEC) cells were then plated at 7000 cells per well in complete Endothelial Cell Growth Medium or EGM-2 (Lonza) with either PBS (control) or various concentrations of polypeptides in PBS. The cells are then allowed for form tubes for 6 hours, fixed, and visualized either by phase microscopy or stained briefly with 1% Crystal Violet and visualized by light microscopy.

[00110] Analysis of the image obtained (in order to determine the number of master segments and the length of master segments) was performed using Angiogenesis analyzer for Image J (<http://image.bio.methods.free.fr/ImageJ/?-Home->). The image analyzer detected pixels that have at least three neighbors on the image and defined them as nodes. A junction was considered a node or fused nodes. The segments corresponded to elements that were limited by two junctions/nodes while the branches were elements delimited by a junction and one extremity. The master segments were considered pieces of three, delimited by two junctions, but not exclusively implicated with one branch (master junctions). The master junctions linked at least 3 master segments. Fewer and shorter master segments indicate better inhibition of tube formation that translates to better inhibition of angiogenesis. As shown on **Figures 11** and **16**, higher concentrations of the polypeptides tested are effective in reducing length of segments and/or master segments formed by the HUVEC cells. Therefore, the polypeptides tested are effective in inhibiting angiogenesis.

[00111] **Example 5: Cell Viability**

[00112] The HUVEC cells (10,000 cells) were plated in EGM-2 (Lonza) with either PBS (control) or various concentrations of polypeptides in PBS. The cells were then incubated at 37°C, 5% CO₂ for 24 hours. Cell viability is determined using CellTiter 96[®] AQueous One Solution Cell Proliferation Assay (MTS) (Promega) according to manufacturer's protocol. The results of the cell viability experiment were shown on **Figures 12** and **15**. These figures shows that the polypeptides tested are as effective as or more effective than aflibercept in inhibiting the growth of HUVEC cells.

[00113] **Example 6: Binding Affinity to VEGF**

[00114] The purpose of the binding affinity assays was to determine (1) whether the polypeptides bind to VEGF, (2) whether the polypeptides have a comparable affinity to commercially available Aflibercept (AF) and (3) whether the polypeptides that form trimers (i.e. polypeptides having Human Ficolin2 protein as multimerizing component) have a higher binding capacity. To perform the binding affinity assays, ELISA plates were first coated with 20µg/ml of VEGF-A (VEGF-165), VEGF-C, or VEGF-D in coating buffer (100mM Sodium Carbonate/Sodium Bicarbonate) overnight. After coating, the plates were washed with 1x PBS several times and blocked with 5% BSA/PBS for 1 hour at room temperature (RT). Serial dilutions of polypeptides or BSA (negative control) were made in 5%BSA/PBS and allowed to bind onto plates for 2 hours at RT. The plates were then washed and incubated

with anti-6His-HRP (Roche) at 1:1000 for 1 hour at RT. The plates were then washed before colorimetric detection using 3, 3',5, 5'-Tetramethylbenzidine Liquid Substrate (Sigma). The reaction was quenched with 0.1M Sulfuric acid and endpoint absorbance at 490nm was measured using Biotek Synergy HT plate reader.

- 5 [00115] The polypeptides binding affinity to VEGF-A was shown on **Figure 14**. All of the polypeptides tested were able to bind to VEGF-A. As predicted, the polypeptides that form trimers (such as sGF2 and sHF2) had higher binding affinity to VEGF-A when compared to aflibercept. Besides binding to VEGF-A, the polypeptides tested also bind to VEGF-C and VEGF-D. On the other hand, aflibercept is not able to bind to VEGF-C and VEGF-D. The
- 10 polypeptides binding affinity to VEGF-C and VEGF-D was shown on **Figure 20**.

[00116] **Example 7: Effect of polypeptide in subcutaneous tumor in mice**

- [00117] Inhibition of angiogenesis (as shown on Example 4 above) may lead to reduction of tumor size. In order to study the reduction in tumor size, a model wherein BL mice were subcutaneously injected with tumor cells was used. Tumor in BL6 mice (9 to 11 weeks old;
- 15 obtained from In Vivos) was induced by subcutaneously injecting the mice with B16F10 cells (50,000 cells) in 50% Matrigel (Corning)/PBS at the left flank. 500µg of polypeptide (per mice per injection) was then subcutaneously injected to the right flank of the mice on Day 1, 4, 7, and 10 post-injection. The injection of the polypeptides on the opposite flank will eliminate the possibility that physical injection actually disrupts the tumor. Furthermore, the
- 20 effects observed on the tumor after injection of the polypeptide serve as an indirect evidence of bioavailability of the peptide. The mice were then sacrificed on Day 15 post injection and the tumors were weighed. Tumors excised were then fixed with Neutral-Buffered Formalin (Sigma) overnight, followed by embedding in paraffin and H&E (Haematoxylin and Eosin) staining. As the tumors were not perfused prior to fixing, the blood/vasculature that was intra-
- 25 tumor can be visualized by the red blood cells (RBCs). The result of this experiment was shown on **Figure 17**. Because the average weights of tumor on Day 15 for the mice injected with aflibercept were about the same as those injected with the polypeptides tested, the polypeptides tested were equally effective in reducing the size of the subcutaneous tumors when compared to aflibercept.

30 [00118] **Example 8: Polypeptides for Metastasis Inhibition in mice**

[00119] Besides reducing size of tumor, inhibition of angiogenesis (as shown on Example 4 above) may also lead to inhibition of metastasis. In order to study the inhibition of tumor

metastasis, a model wherein BL6 mice were intravenously injected with tumor cells was used. Lung metastasis was induced by intravenously injecting B16F10 cells (500,000 cells; resuspended in 100µl of PBS) into 9 to 11 weeks old BL6 mice from In Vivos. The mice were then treated either with PBS (control) or 500µg of polypeptide (per mice per injection) subcutaneously on Day 4, 8, 11, and 15 post-injection. Mice were then sacrificed on Day 18 and the lungs were dissected and fixed in Fekete's solution (55% Ethanol, 3% Formaldehyde, 4% glacial acetic acid) overnight and the nodules were counted. The result of this experiment was shown on **Figure 18**. Because the average number of nodules for the mice injected with aflibercept were about the same as those injected with the polypeptides tested, the polypeptides tested were equally effective in inhibiting metastasis on lungs when compared to aflibercept.

Claims

1. An isolated polypeptide comprising:

$$(C_{(w)} - A_{(x)} - B_{(y)} - C_{(z)} - D)_n,$$

5 wherein

A comprises an immunoglobulin-like domain of VEGFR1 (Vascular Endothelial Growth Factor Receptor 1),

B comprises an immunoglobulin-like domain of VEGFR2 (Vascular Endothelial Growth Factor Receptor 2),

10 C comprises an immunoglobulin-like domain of VEGFR3 (Vascular Endothelial Growth Factor Receptor 3),

D comprises a multimerizing component,

x is 1 or 2,

y is 1 or 0,

15 z is 2 or 3,

w is 0 or 1, and

n is 2, or 3, or 4, or 5.

- 20 2. The polypeptide of claim 1, wherein A, B, and C are independently ligand binding domain of the respective VEGFR.

3. The polypeptide of claim 1 or 2, wherein

(i) each A is independently selected from the group consisting of Domain 1 (D1) of VEGFR1 and Domain 2 (D2) of VEGFR1, and/or

25 (ii) B is Domain 3 (D3) of VEGFR2, and/or

(iii) each C is independently selected from the group consisting of Domain 1 (D1) of VEGFR3, Domain 2 (D2) of VEGFR3, and Domain 3 (D3) of VEGFR3.

- 30 4. The polypeptide of any one of the preceding claims, wherein the polypeptide is selected from the group consisting of

(i) $(A_{(1)} - B_{(1)} - C_{(2)} - D)_n,$

(ii) $(A_{(1)} - C_{(2)} - D)_n,$

- (iii) $(A_{(2)} - B_{(1)} - C_{(2)} - D)_n$,
- (iv) $(A_{(2)} - C_{(2)} - D)_n$,
- (v) $(C_{(1)} - A_{(1)} - B_{(1)} - C_{(2)} - D)_n$, and
- (vi) $(C_{(1)} - A_{(1)} - C_{(2)} - D)_n$.

5

5. The polypeptide of any one of the preceding claims, wherein the polypeptide is selected from the group consisting of

- (i) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,
- 10 (ii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,
- (iii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,
- (iv) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n,
- 15 (v) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n, and
- (vi) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – D)_n.

20

6. The polypeptide of any one of claims 3 to 5, wherein

- (i) the amino acid of the Domain 1 (D1) of VEGFR1 has at least 90% identity to a sequence of SEQ ID NO: 1, and/or
- (ii) the amino acid of the Domain 2 (D2) of VEGFR1 has at least 90% identity to a sequence of SEQ ID NO: 2, and/or
- 25 (iii) the amino acid of the Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 has at least 90% identity to a sequence of SEQ ID NO: 12, and/or
- (iv) the amino acid of the Domain 3 (D3) of VEGFR2 has at least 90% identity to a sequence of SEQ ID NO: 3, and/or
- 30 (v) the amino acid of the Domain 1 (D1) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 6, and/or

(vi) the amino acid of the Domain 2 (D2) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 4, and/or

(vii) the amino acid of the Domain 3 (D3) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 5, and/or

5 (viii) the amino acid of the Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 has at least 90% identity to a sequence of SEQ ID NO: 13.

7. The polypeptide of any one of claims 3 to 6, wherein

10 (i) the amino acid of the Domain 1 (D1) of VEGFR1 has a sequence of SEQ ID NO: 1, and/or

(ii) the amino acid of the Domain 2 (D2) of VEGFR1 has a sequence of SEQ ID NO: 2, and/or

(iii) the amino acid of the Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 has a sequence of SEQ ID NO: 12, and/or

15 (iv) the amino acid of the Domain 3 (D3) of VEGFR2 has a sequence of SEQ ID NO: 3, and/or

(v) the amino acid of the Domain 1 (D1) of VEGFR3 has a sequence of SEQ ID NO: 6, and/or

20 (vi) the amino acid of the Domain 2 (D2) of VEGFR3 has a sequence of SEQ ID NO: 4, and/or

(vii) the amino acid of the Domain 3 (D3) of VEGFR3 has a sequence of SEQ ID NO: 5, and/or

(viii) the amino acid of the Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 has a sequence of SEQ ID NO: 13.

25

8. The polypeptide of any one of the preceding claims, wherein the multimerizing component comprises at least one selected from the group consisting of:

Fc portion of Human Immunoglobulin constant Fragment,

Mannose-binding protein C (Uniprot: P11226),

30 Human Ficolin2 protein, and

Cartilage oligomeric matrix protein (Uniprot: P49747).

9. The polypeptide of any one of the preceding claims, wherein the multimerizing component is Fc portion of Human Immunoglobulin constant Fragment or wherein the amino acid of the Fc portion of Human Immunoglobulin constant Fragment has at least 90% identity to a sequence of SEQ ID NO: 7 or wherein the amino acid of the Fc portion of Human Immunoglobulin constant Fragment has a sequence of SEQ ID NO: 7.
10. The polypeptide of any one of claims 1 to 8, wherein the multimerizing component is Human Ficolin2 protein or wherein the amino acid of the Human Ficolin2 protein has at least 90% identity to a sequence of SEQ ID NO: 8 or wherein the amino acid of the Human Ficolin2 protein has a sequence of SEQ ID NO: 8.
11. The polypeptide of any one of the preceding claims, wherein the polypeptide is selected from the group consisting of
- (i) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;
 - (ii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;
 - (iii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
 - (iv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
 - (v) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;
 - (vi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

- (vii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
- 5 (viii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
- (ix) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- 10 (x) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- (xi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- 15 (xii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (xiii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- 20 (xiv) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xv) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;
- 25 (xvi) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
- 30 (xvii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;

- (xviii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- 5 (xix) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xx) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- 10 (xxi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- (xxii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- 15 (xxiii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- (xxiv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3;
- 20 (xxv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5;
- (xxvi) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;
- 25 (xxvii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;
- (xxviii) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 138)_n, wherein n=3;
- 30

(xxix) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Mannose-binding protein C (Uniprot: P11226), position 101 – 248)_n, wherein n=3; and

(xxx) (Domain 1 (D1) of VEGFR3 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Cartilage oligomeric matrix protein (Uniprot: P49747))_n, wherein n=5.

12. The polypeptide of any one of the preceding claims, wherein the polypeptide is selected from the group consisting of

(i) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(ii) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(iii) (Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;

(iv) (Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3;

(v) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(vi) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Fc portion of Human Immunoglobulin constant Fragment)_n, wherein n=2;

(vii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 3 (D3) of VEGFR2 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3; and

(viii) (Domain 1 (D1) of VEGFR1 – Domain 2 (D2) of VEGFR1 – Domain 2 (D2) of VEGFR3 – Domain 3 (D3) of VEGFR3 – Human Ficolin2 protein)_n, wherein n=3.

13. The polypeptide of any one of the preceding claims, wherein

- 5 (i) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 16, or
(ii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 20, or
(iii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 17, or
(iv) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 21, or
(v) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 14, or
(vi) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 18, or
(vii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 15, or
(viii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 19.
- 10 14. The polypeptide of any one of the preceding claims, wherein
(i) the polypeptide has a sequence of SEQ ID NO: 16, or
(ii) the polypeptide has a sequence of SEQ ID NO: 20, or
(iii) the polypeptide has a sequence of SEQ ID NO: 17, or
(iv) the polypeptide has a sequence of SEQ ID NO: 21, or
15 (v) the polypeptide has a sequence of SEQ ID NO: 14, or
(vi) the polypeptide has a sequence of SEQ ID NO: 18, or
(vii) the polypeptide has a sequence of SEQ ID NO: 15, or
(viii) the polypeptide has a sequence of SEQ ID NO: 19.
- 20 15. The polypeptide of any one of the preceding claims, wherein the polypeptide is
conjugated to Human Serum Albumin or wherein the amino acid of the Human Serum
Albumin has at least 90% identity to a sequence of SEQ ID NO: 23.
- 25 16. The polypeptide of any one of the preceding claims, wherein
(i) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 26, or
(ii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 30, or
(iii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 27, or
(iv) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 31, or
(v) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 24, or
30 (vi) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 28, or
(vii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 25, or
(viii) the polypeptide has at least 90% identity to a sequence of SEQ ID NO: 29.

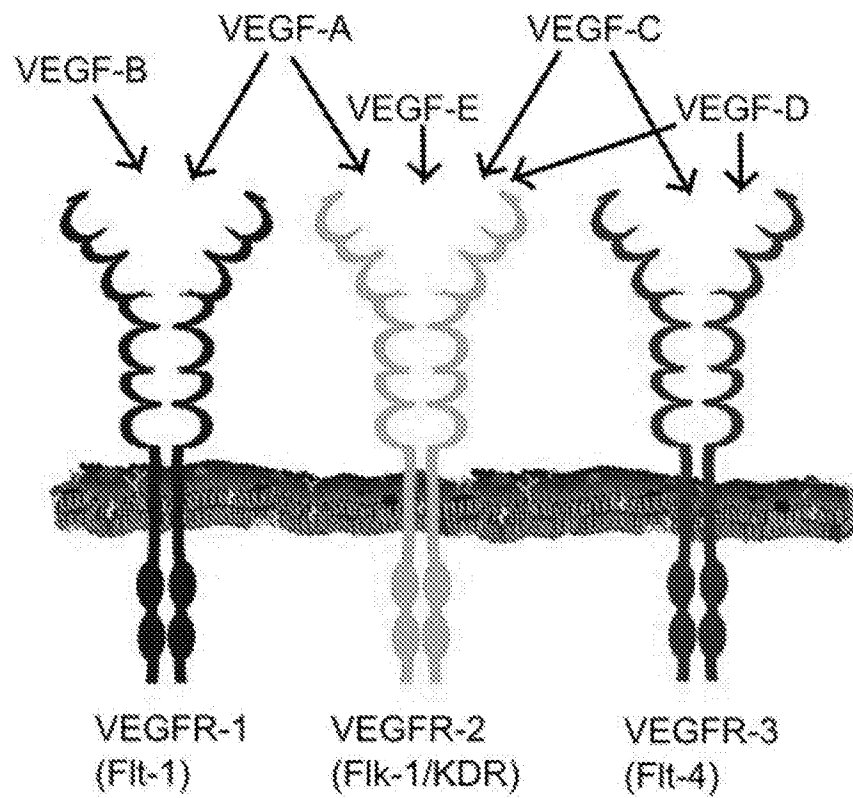
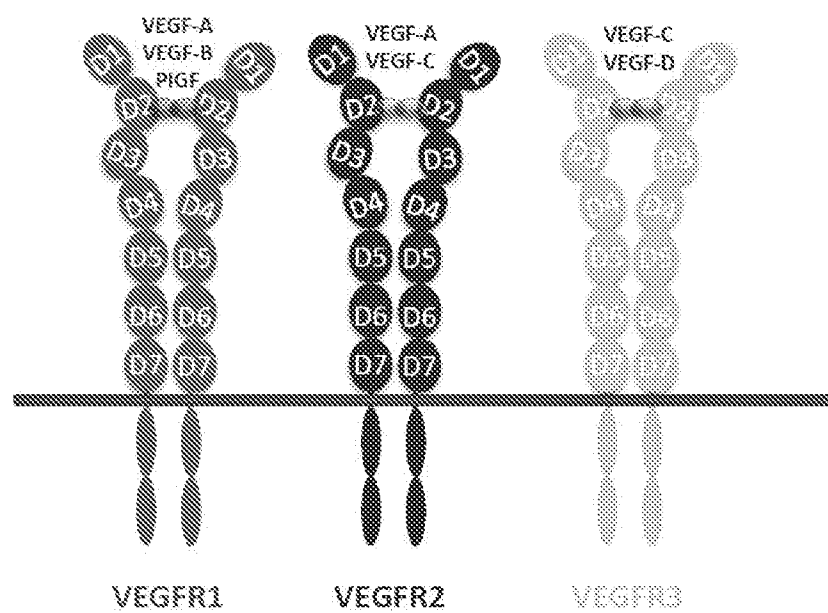
17. The polypeptide of any one of the preceding claims, wherein
- (i) the polypeptide has a sequence of SEQ ID NO: 26, or
 - (ii) the polypeptide has a sequence of SEQ ID NO: 30, or
 - 5 (iii) the polypeptide has a sequence of SEQ ID NO: 27, or
 - (iv) the polypeptide has a sequence of SEQ ID NO: 31, or
 - (v) the polypeptide has a sequence of SEQ ID NO: 24, or
 - (vi) the polypeptide has a sequence of SEQ ID NO: 28, or
 - (vii) the polypeptide has a sequence of SEQ ID NO: 25, or
 - 10 (viii) the polypeptide has a sequence of SEQ ID NO: 29.
18. A nucleic acid encoding one or more polypeptides as claimed in any of the preceding claims.
- 15 19. An expression vector comprising the nucleic acid of claim 18.
20. A host cell comprising the nucleic acid as claimed in claim 18 or the expression vector of claim 19.
- 20 21. A polypeptide as claimed in claims 1 to 17 for use in therapy or medicine.
22. A pharmaceutical composition comprising one or more polypeptide(s) as claimed in claims 1 to 17.
- 25 23. A method of treating angiogenesis-related disorder and/or vascularization-related disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the polypeptide as claimed in claims 1 to 17 or a therapeutically effective amount of the composition as claimed in claim 22 into the subject.
- 30 24. Use of the polypeptide as claimed in claims 1 to 17 or the composition as claimed in claim 22 in the manufacture of a medicament for treating angiogenesis-related disorder and/or vascularization-related disorder.

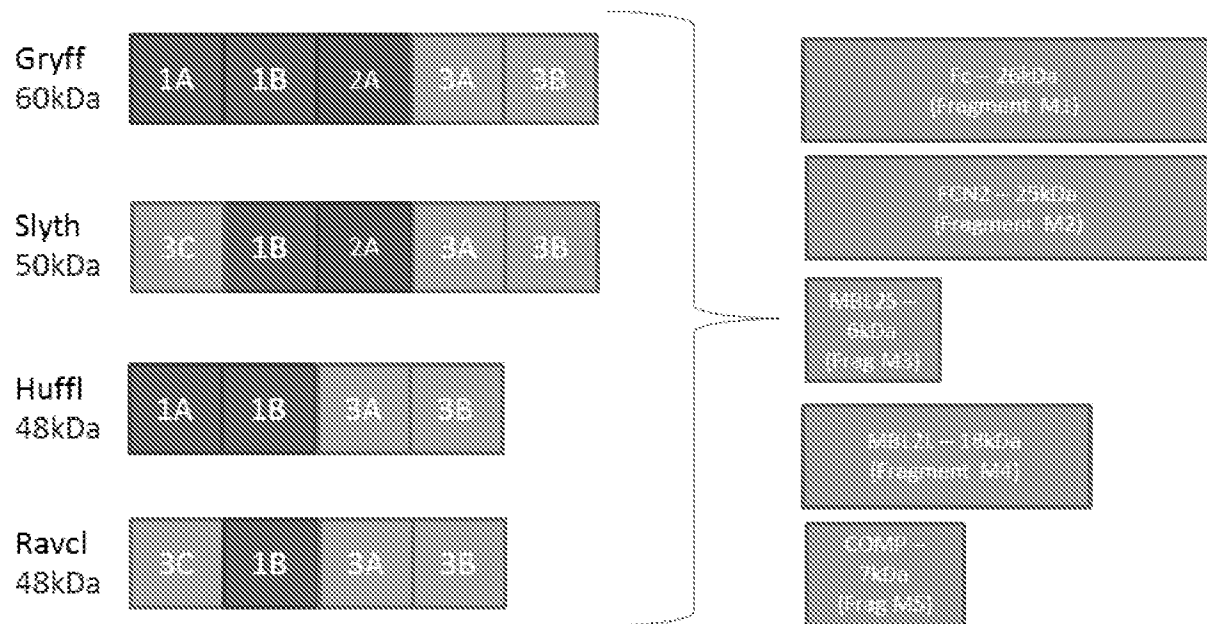
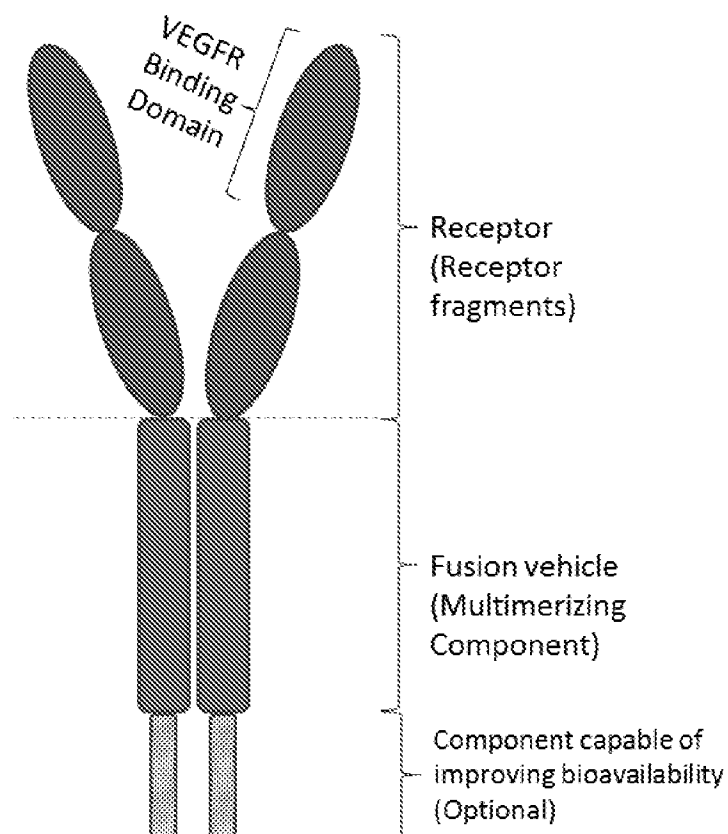
25. The method of claim 23 of the use of claim 24, wherein the angiogenesis-related disorder and/or vascularization-related disorder is cancer or wherein the cancer is selected from the group consisting of non-small cell lung cancer, gastric cancer,
5 gastric tumors, and breast cancer.

26. The method of claim 23 of the use of claim 24, wherein the angiogenesis-related disorder and/or vascularization-related disorder is eye disorder or wherein the eye disorder is a retinal angiogenic disease or wherein the retinal angiogenic disease is
10 selected from the group consisting of age-related macular degeneration, macular edema, diabetic retinopathy, and diabetic macular edema.

27. A kit comprising one or more polypeptide(s) of any one of claims 1 to 17.

15

**Figure 1A****Figure 1B**

**Figure 2A****Figure 2B**

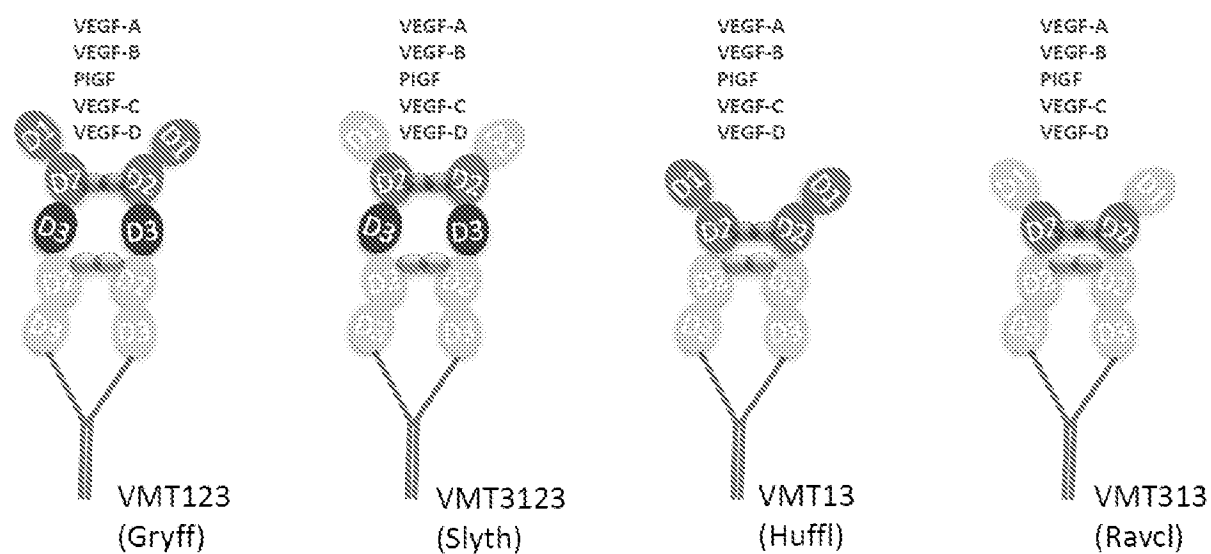


Figure 3A

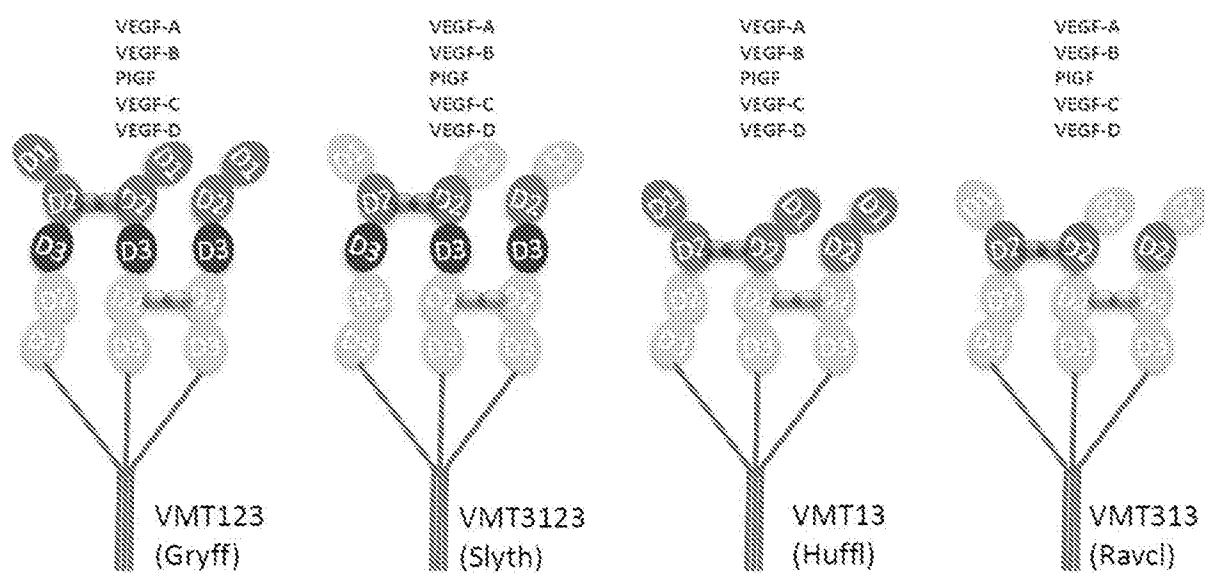


Figure 3B



Figure 4A

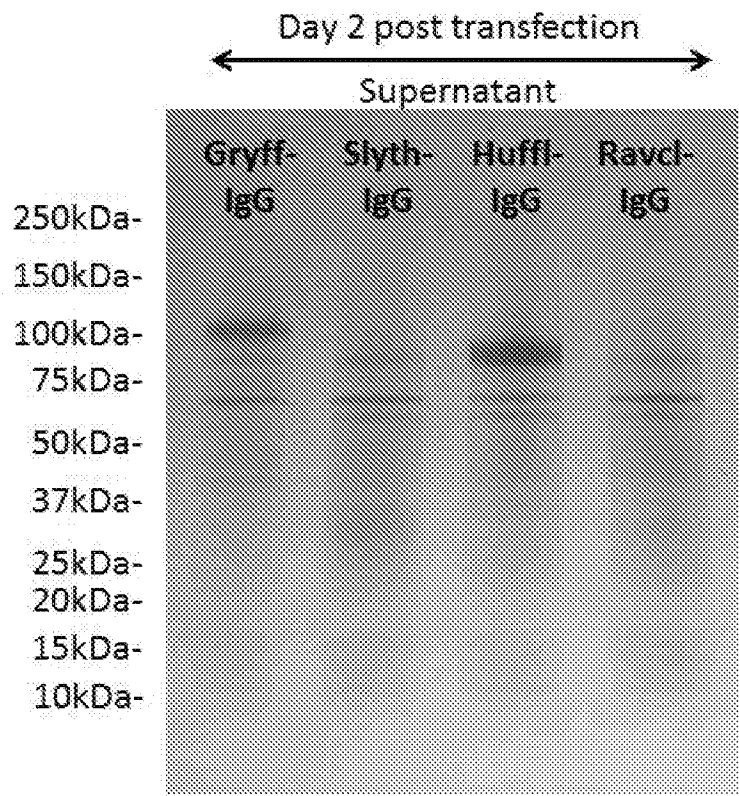


Figure 4B

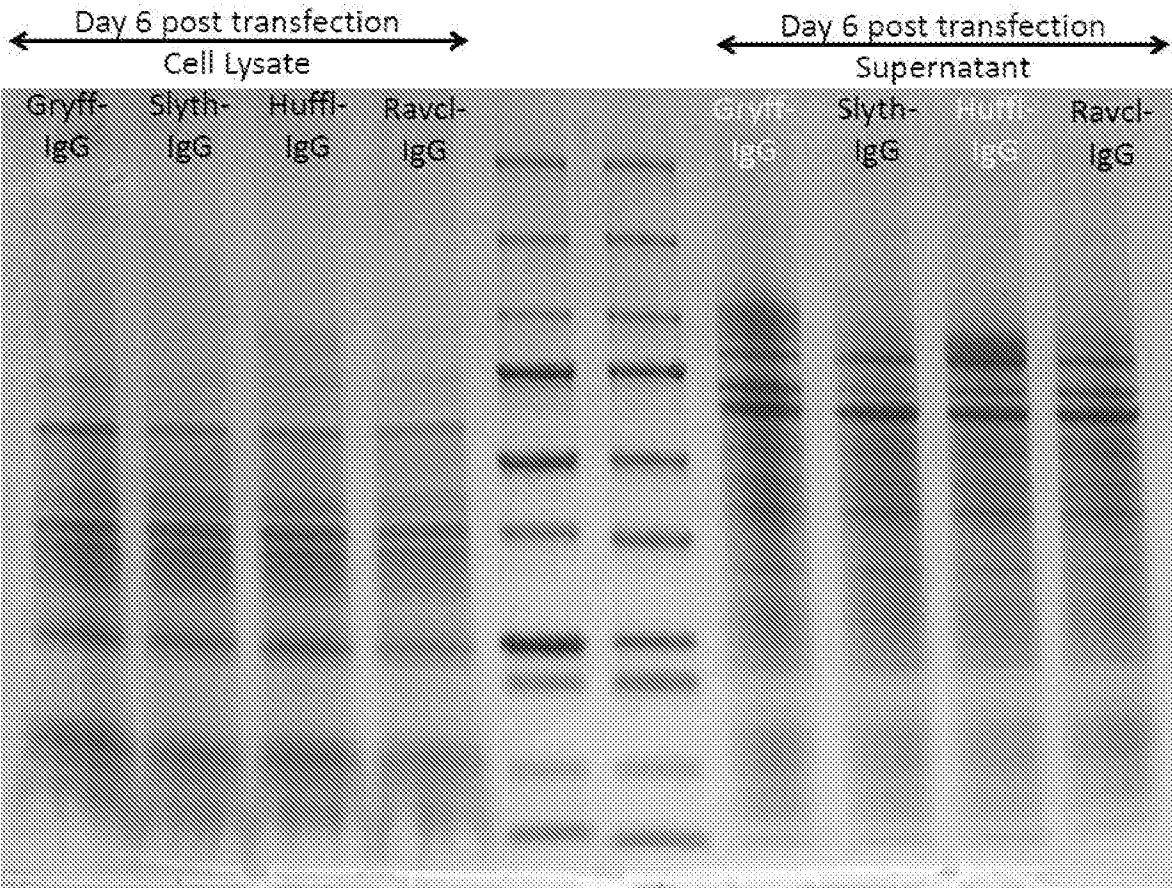


Figure 4C

Gryff-IgG	1A	1B	2A	3A	3B	Fc – 26kDa (Fragment M1)	86kDa
Gryff-FCN2	1A	1B	2A	3A	3B	FCN2 – 25kDa (Fragment M2)	85kDa
Gryff-MBL2S	1A	1B	2A	3A	3B	MBL2S – 6kDa (Frag M3)	66kDa
Gryff-MBL2L	1A	1B	2A	3A	3B	MBL2L – 18kDa (Fragment M4)	78kDa
Gryff-COMP	1A	1B	2A	3A	3B	COMP – 7kDa (Frag M5)	67kDa

Figure 5A

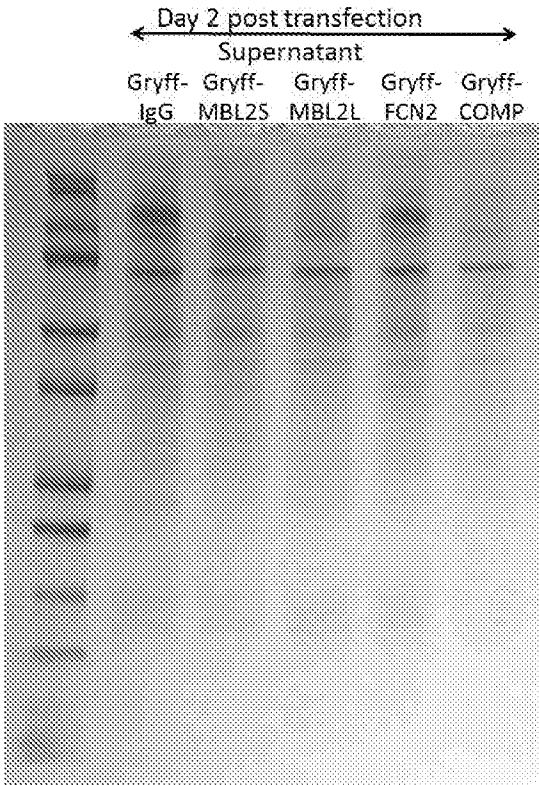


Figure 5B

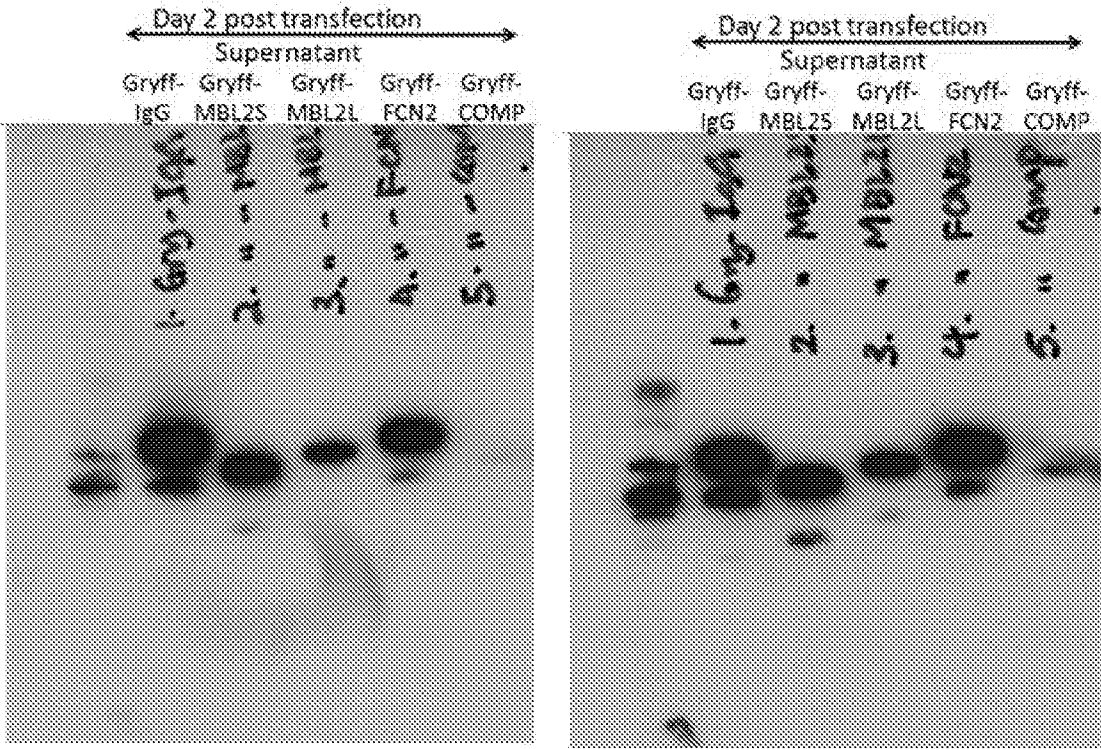


Figure 5C

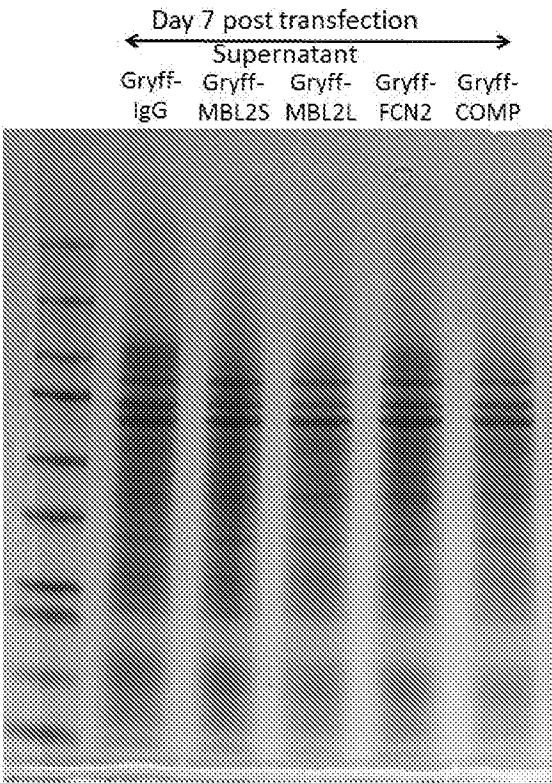


Figure 5D

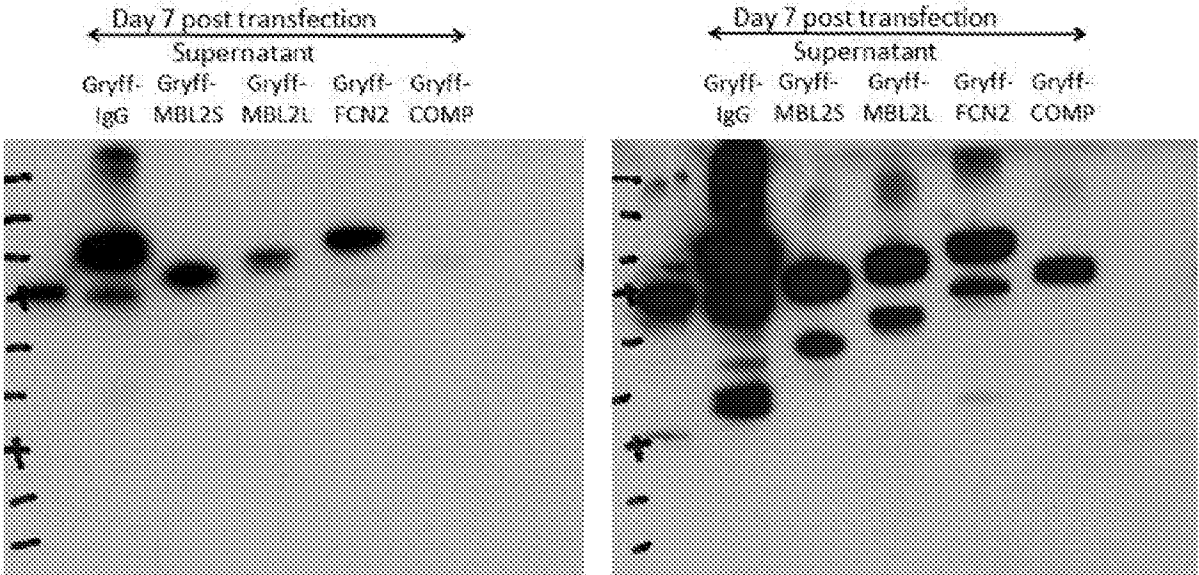


Figure 5E

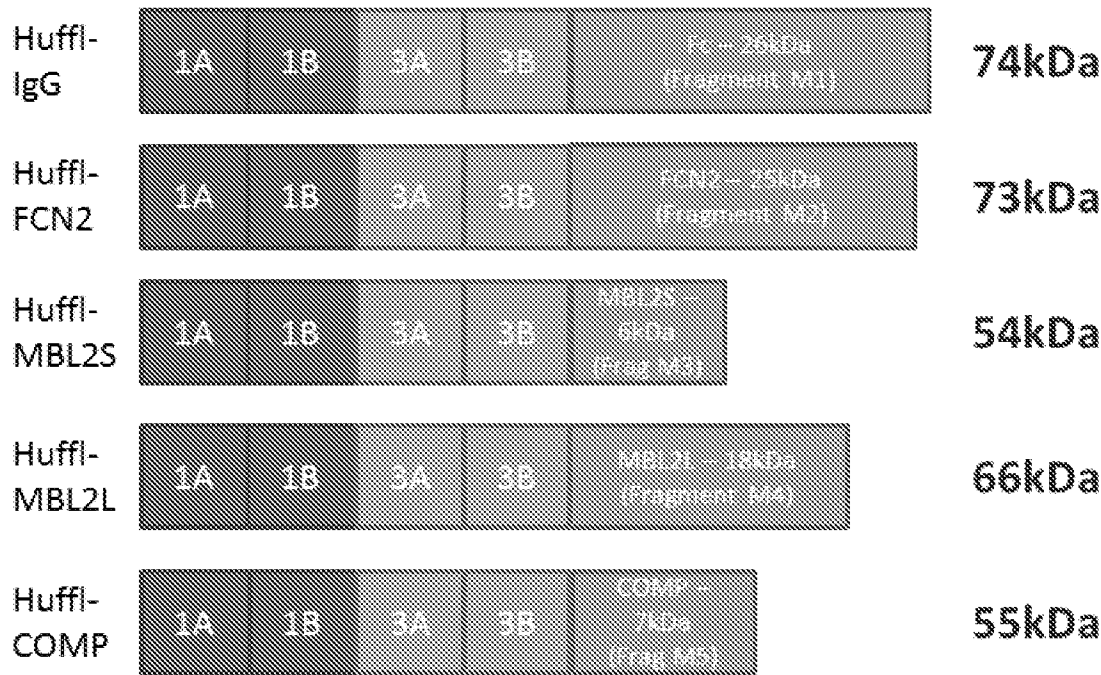


Figure 6A

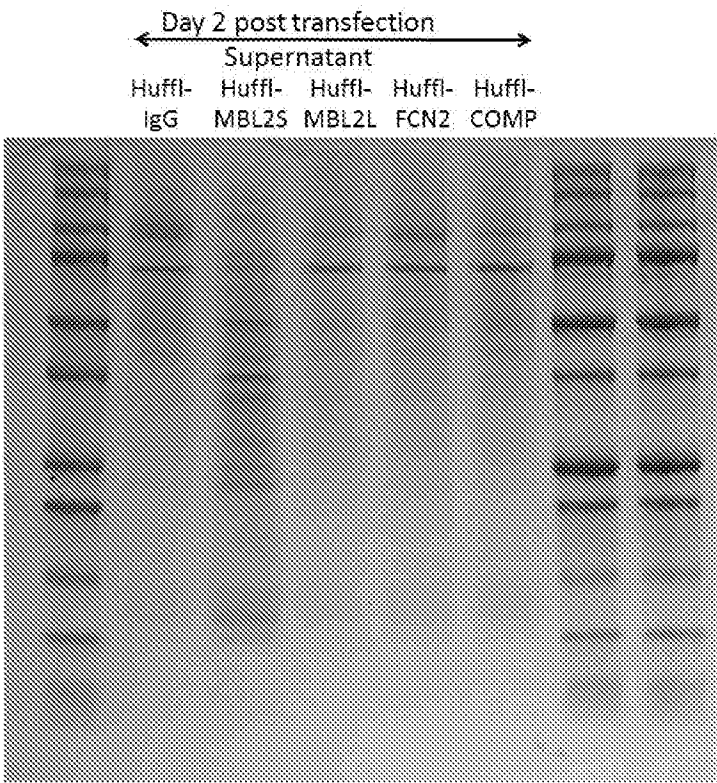


Figure 6B

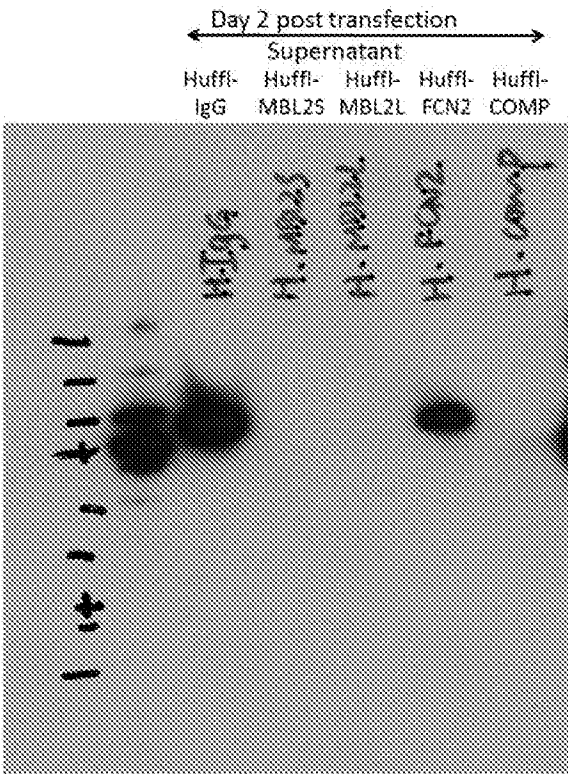


Figure 6C

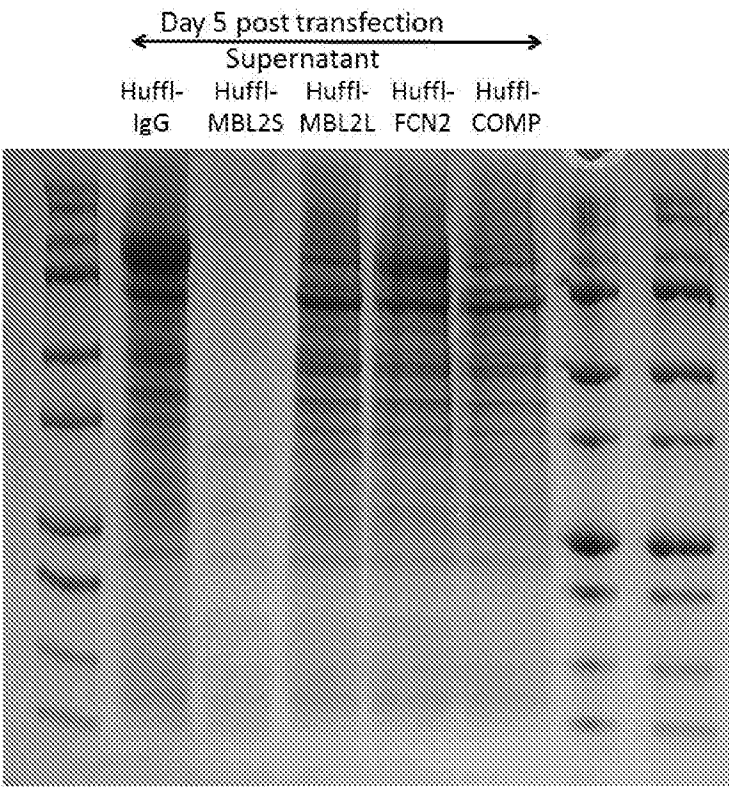


Figure 6D

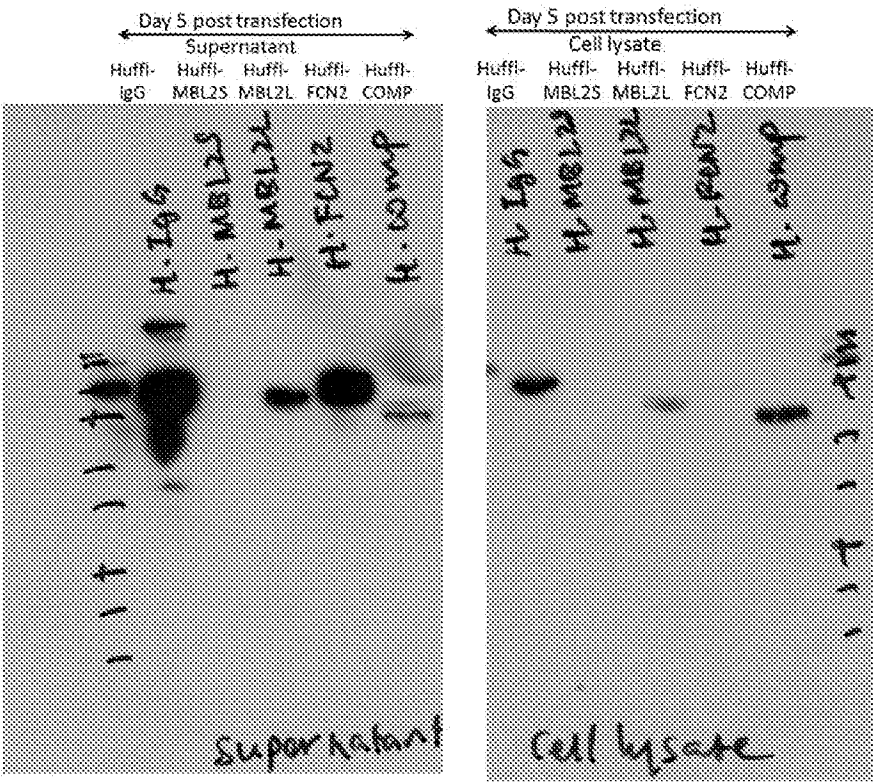


Figure 6E

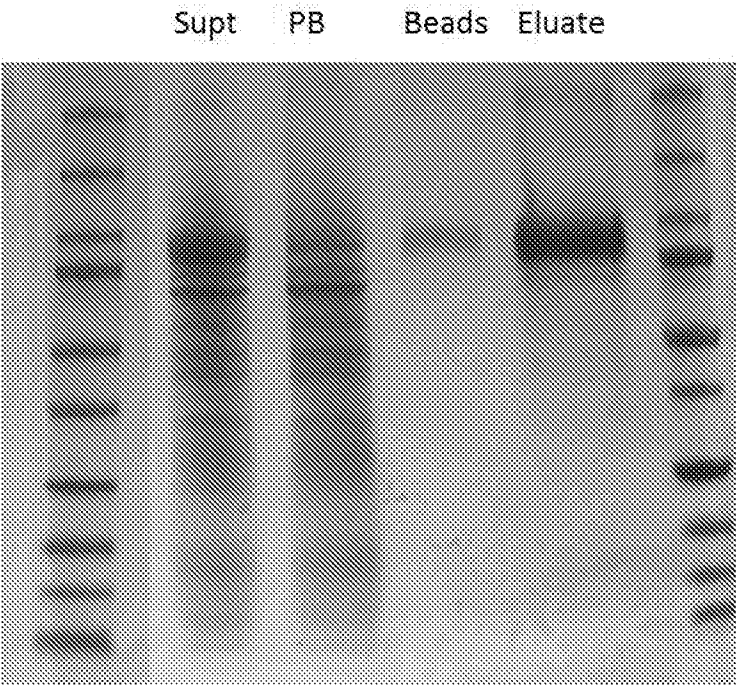


Figure 7A

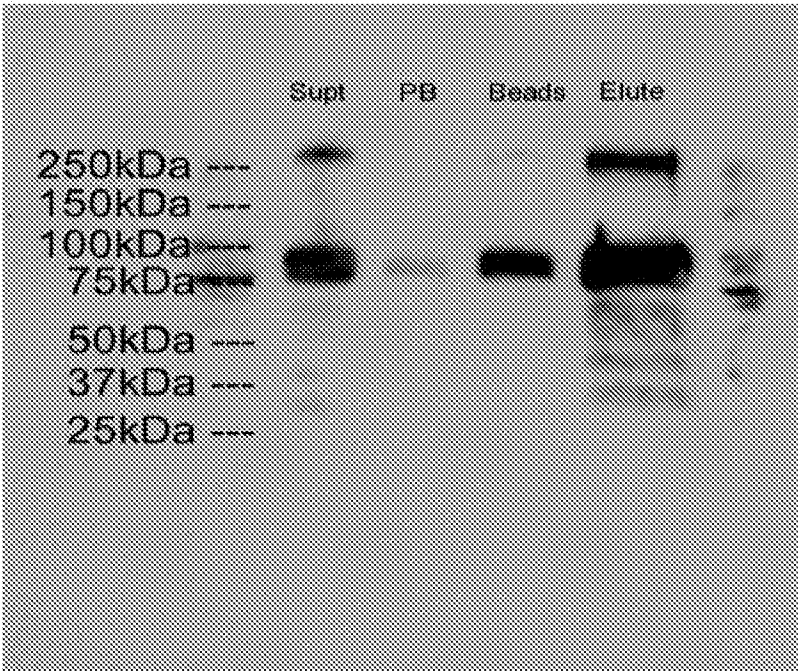


Figure 7B

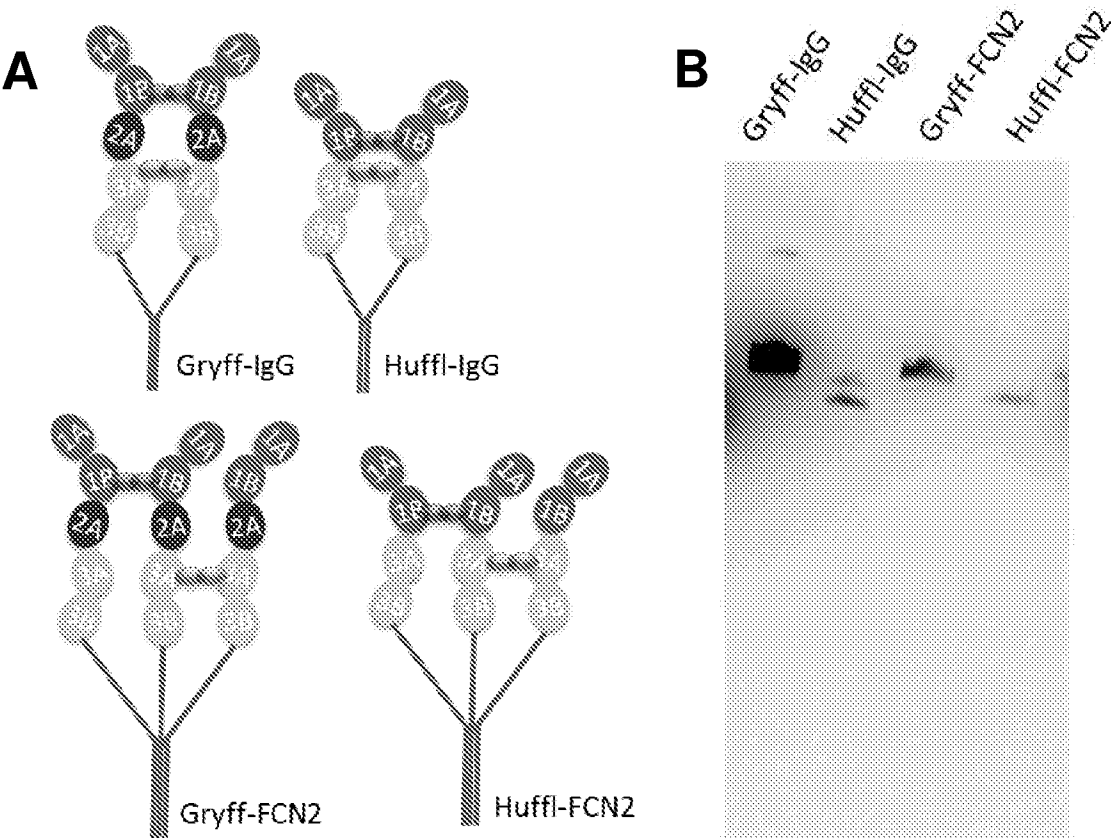


Figure 8

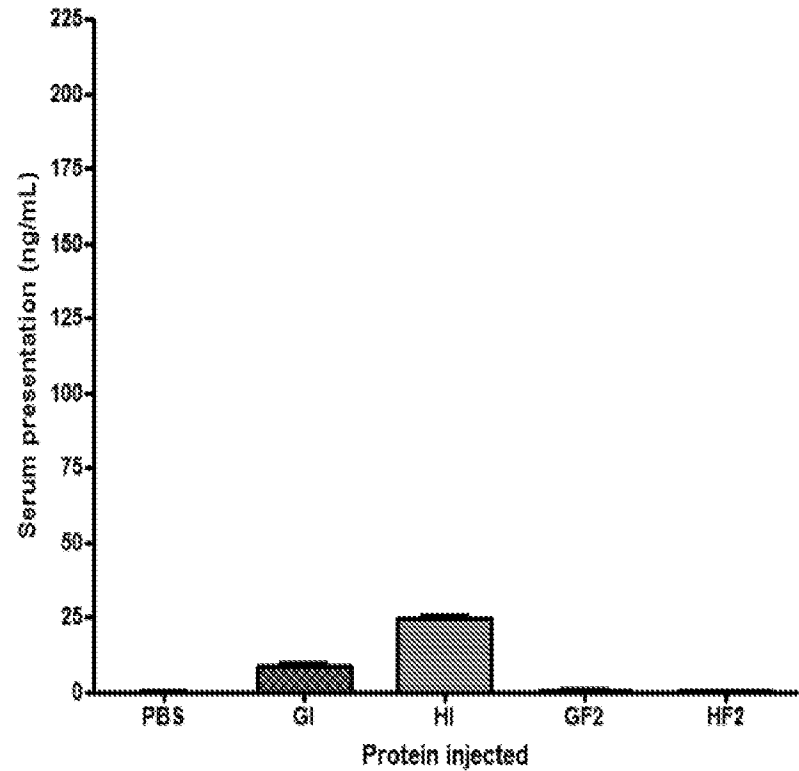


Figure 9A

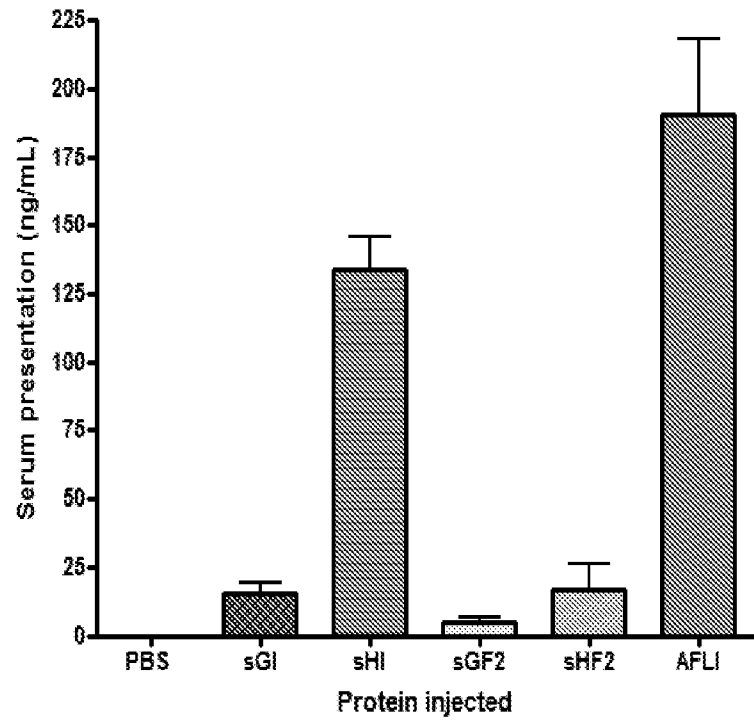
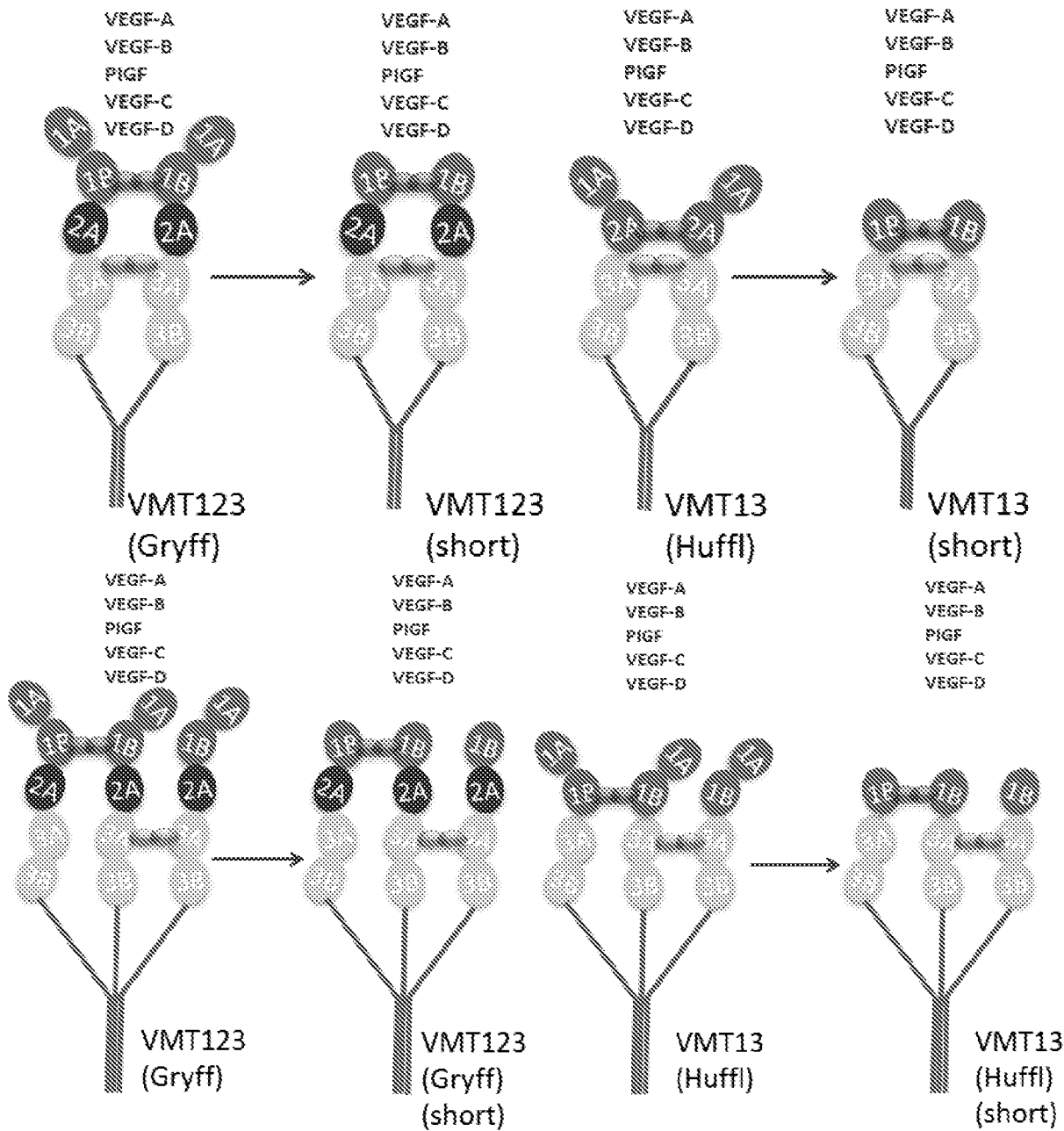
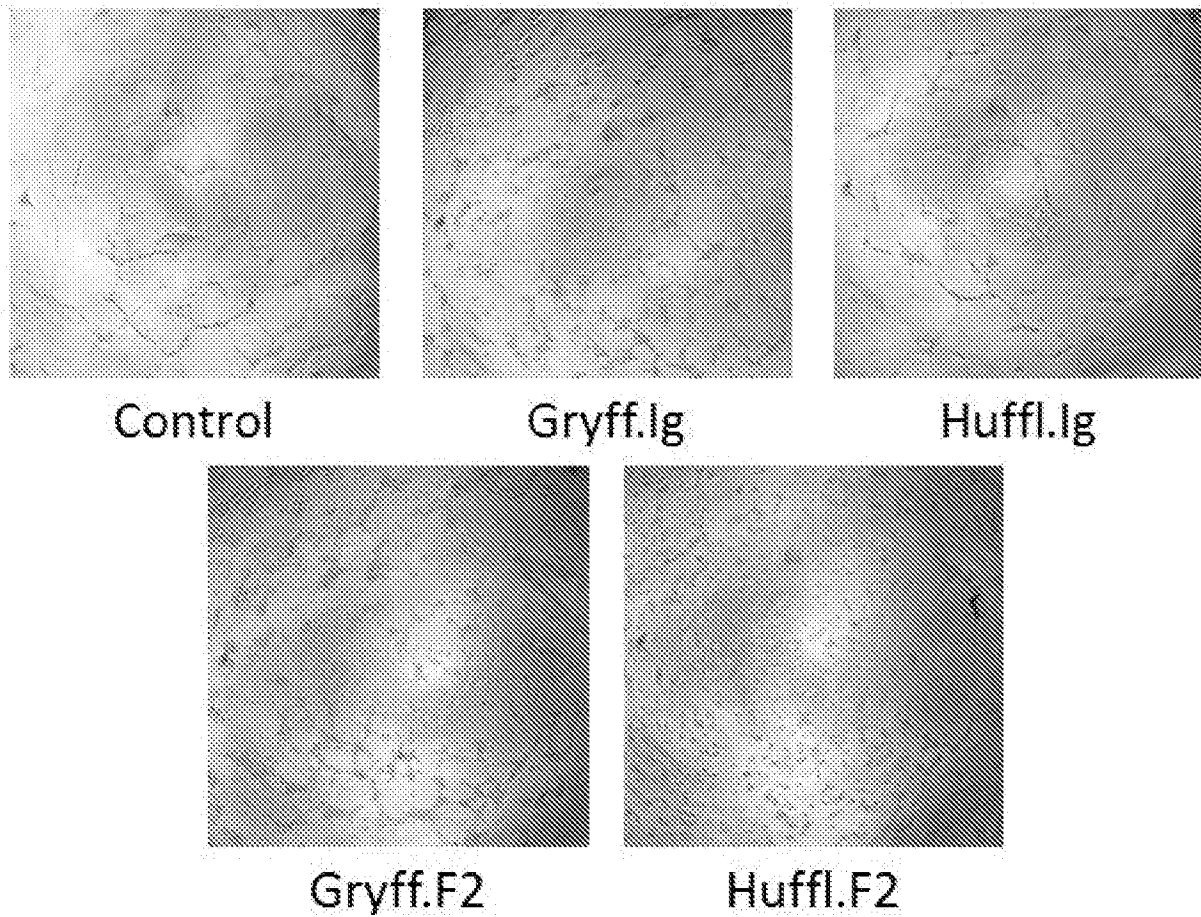
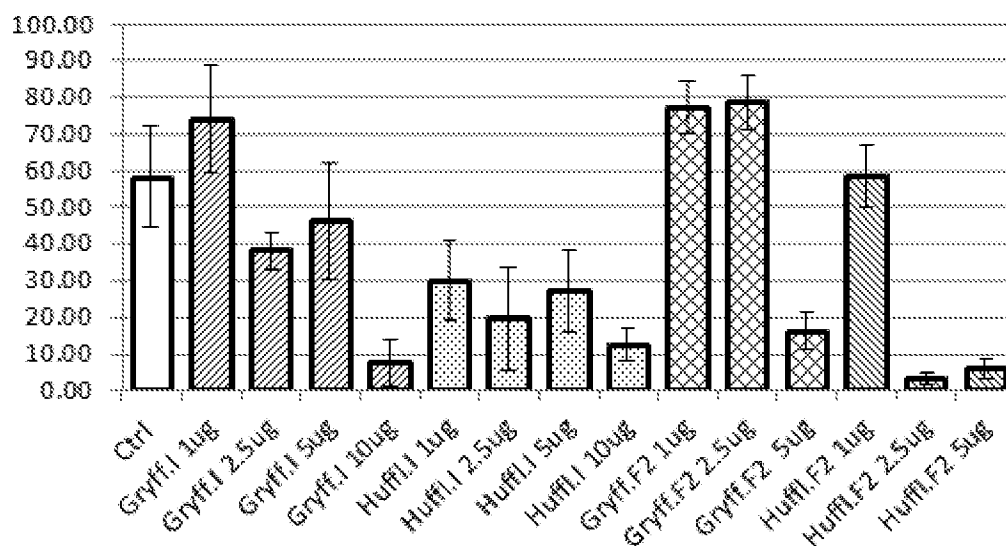
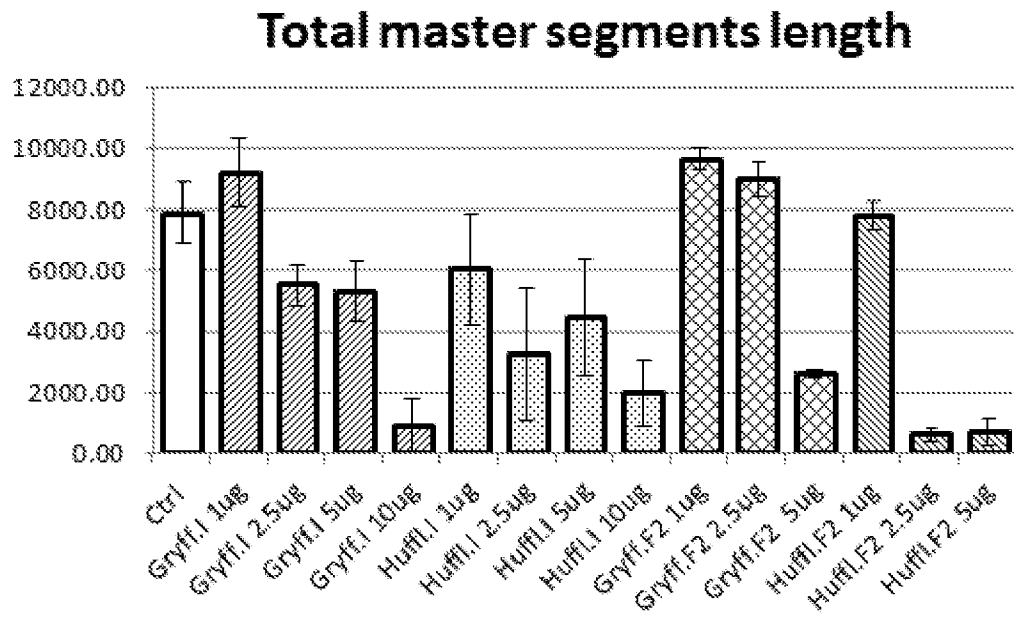


Figure 9B



**Figure 11A****No. of master segments****Figure 11B**

**Figure 11C**

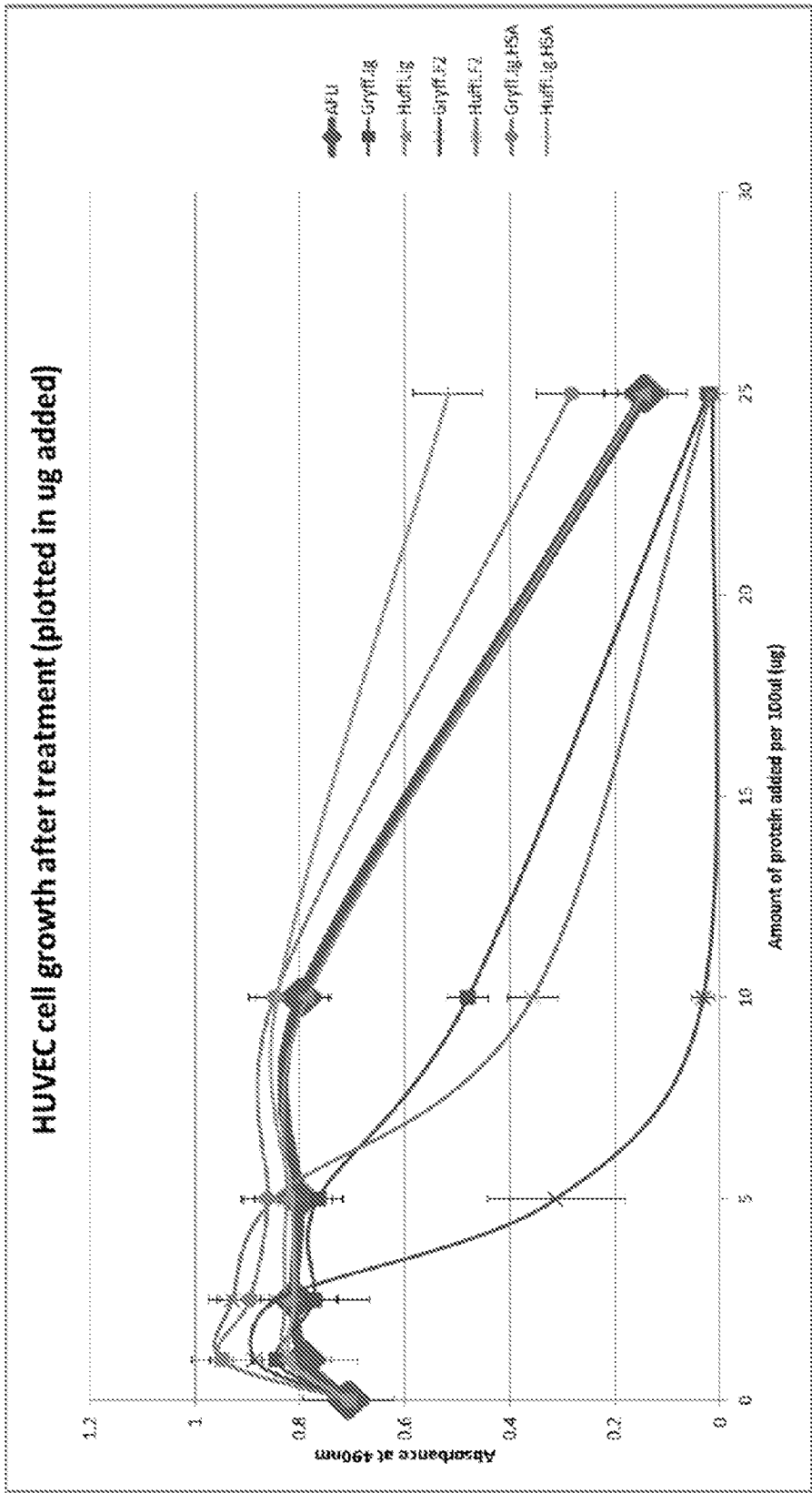


Figure 12A

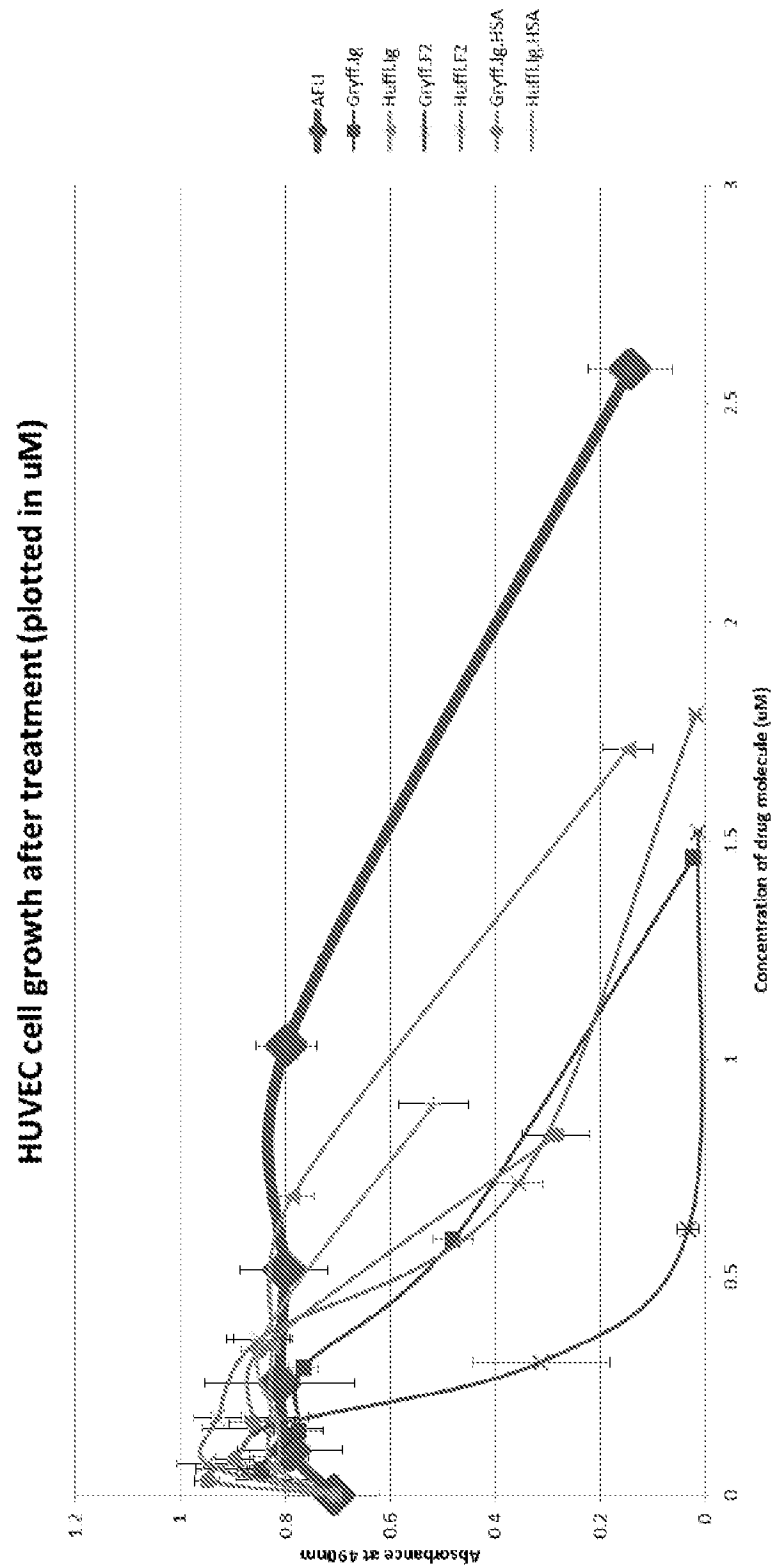


Figure 12B

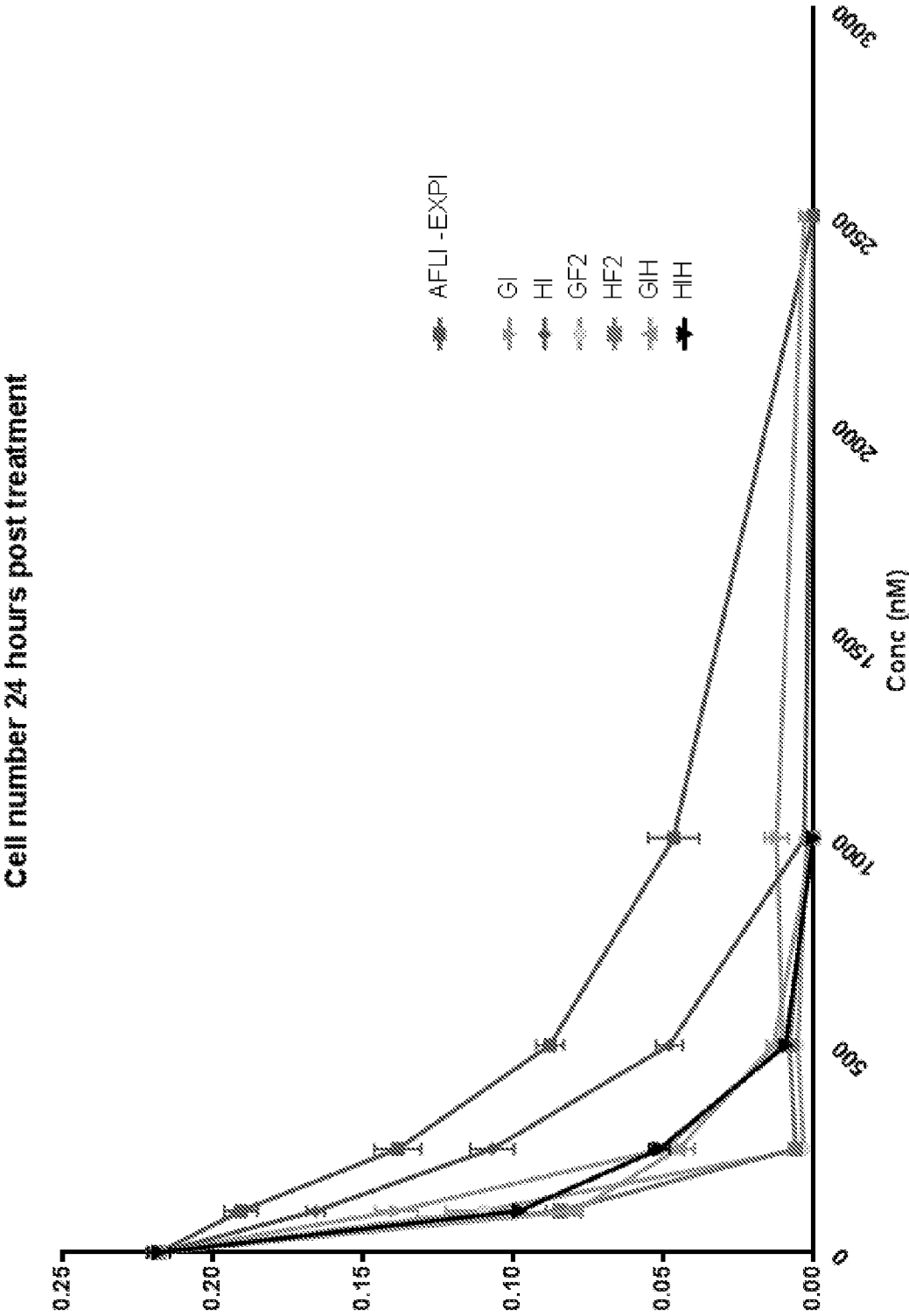
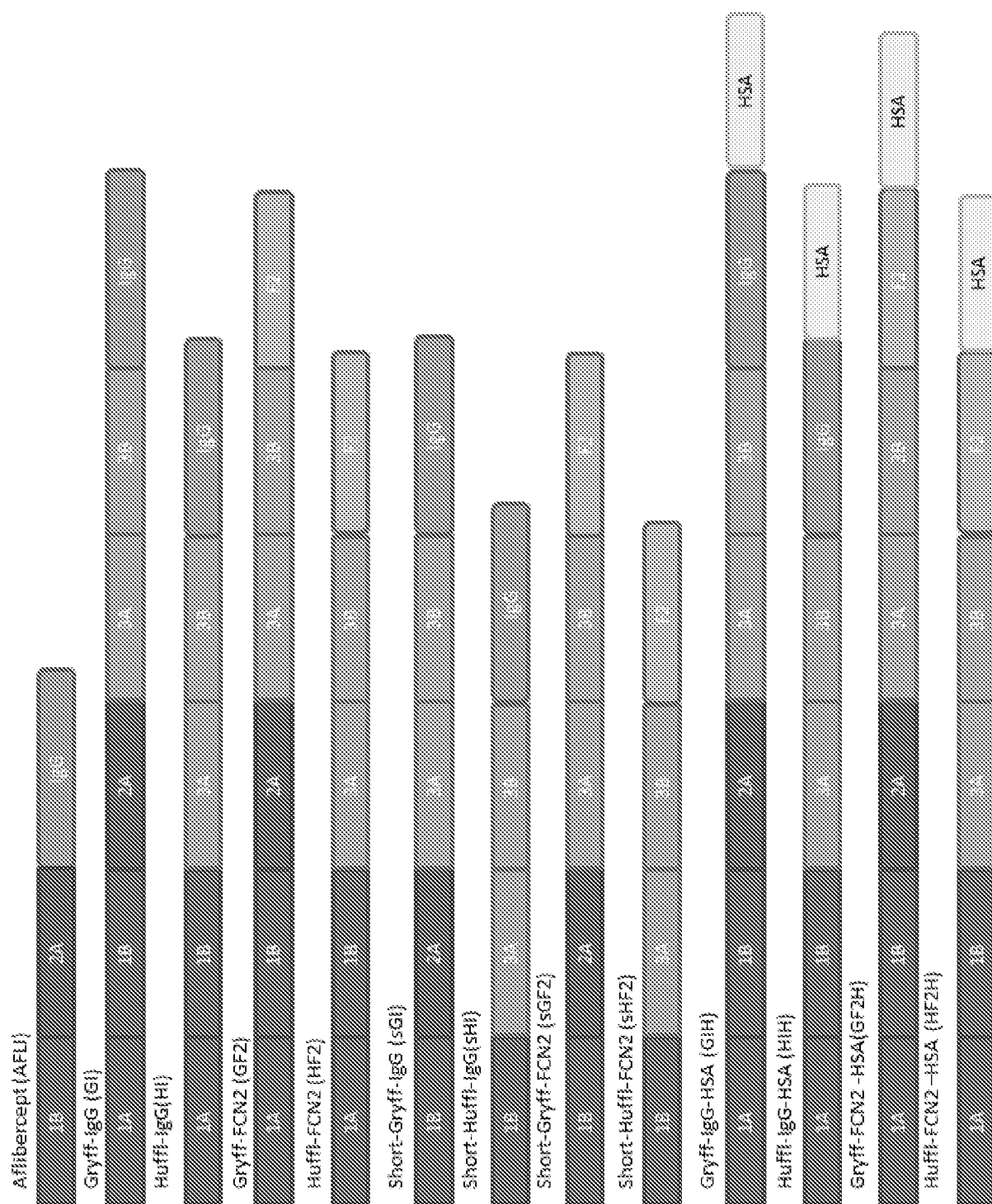


Figure 12C



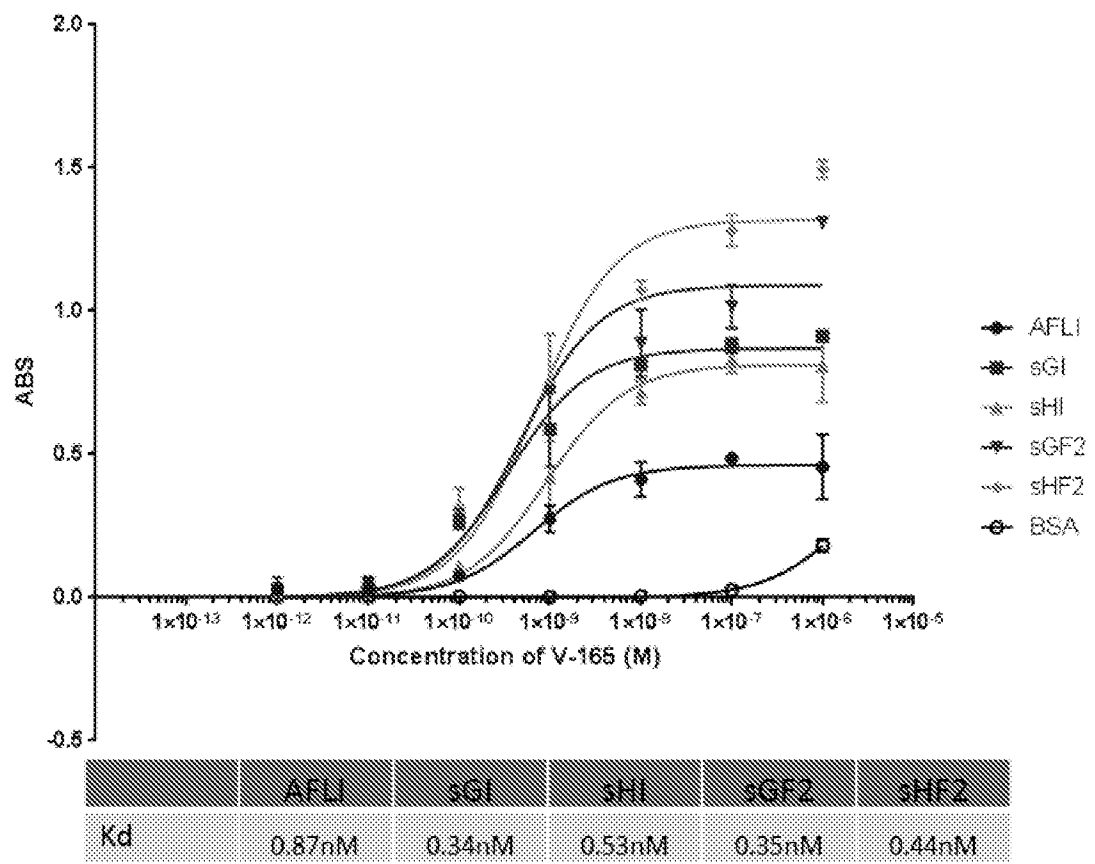


Figure 14

HUVEC (PS) cell growth 24 hours post treatment

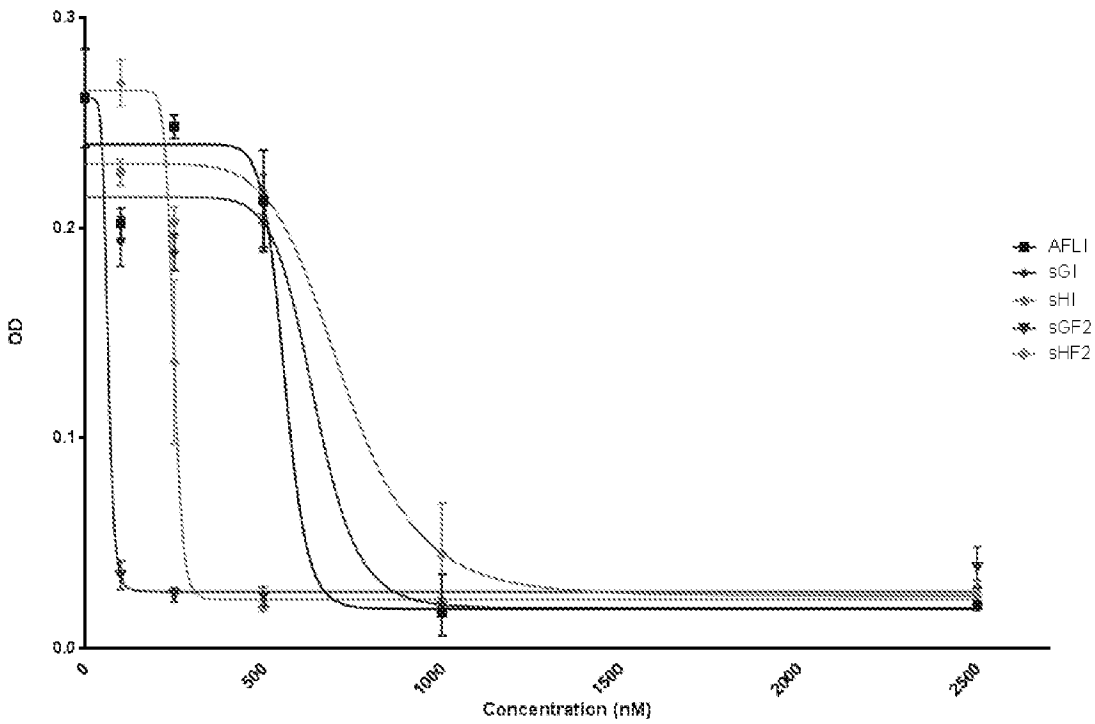


Figure 15

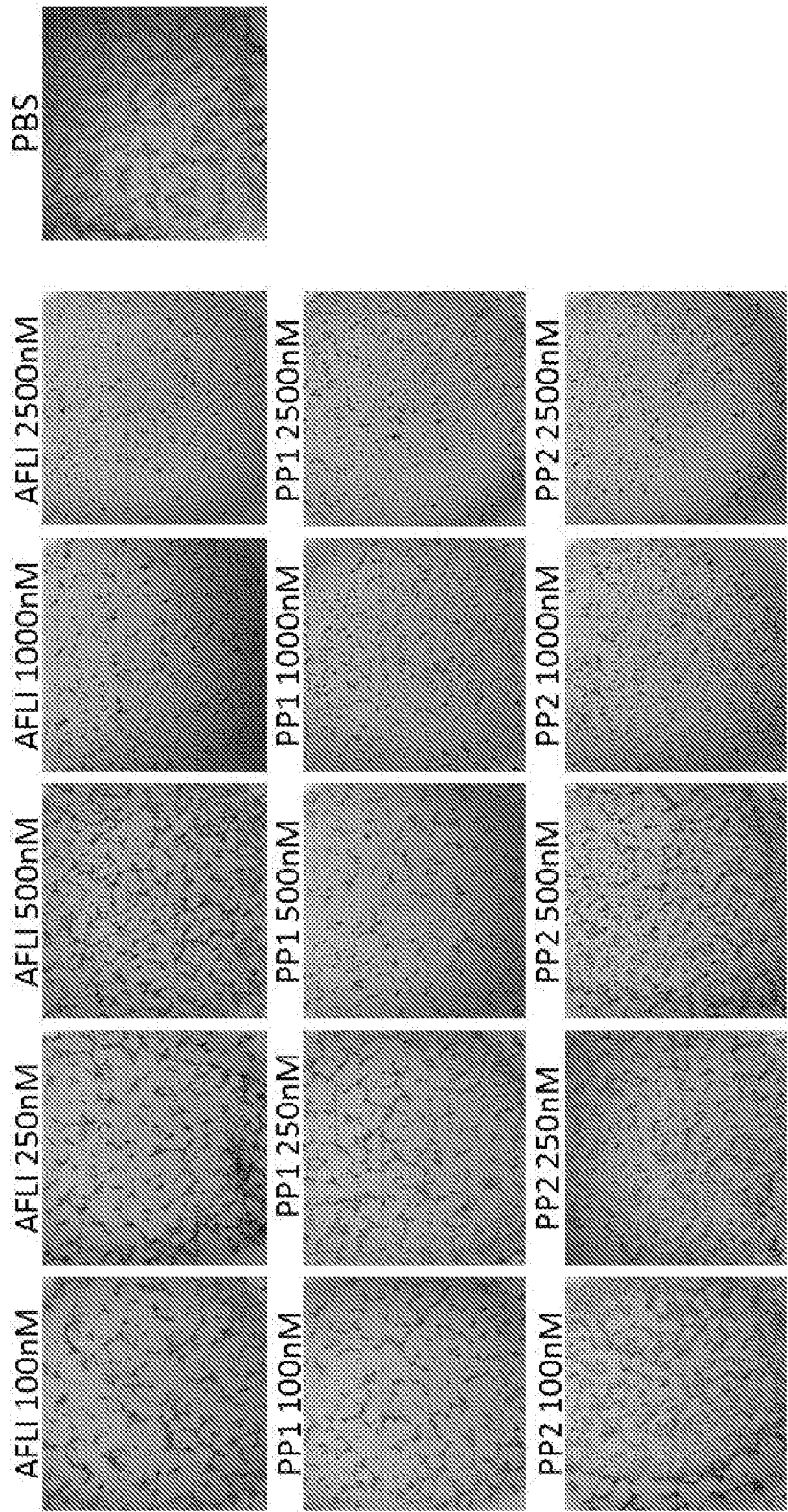


Figure 16A (cont.)

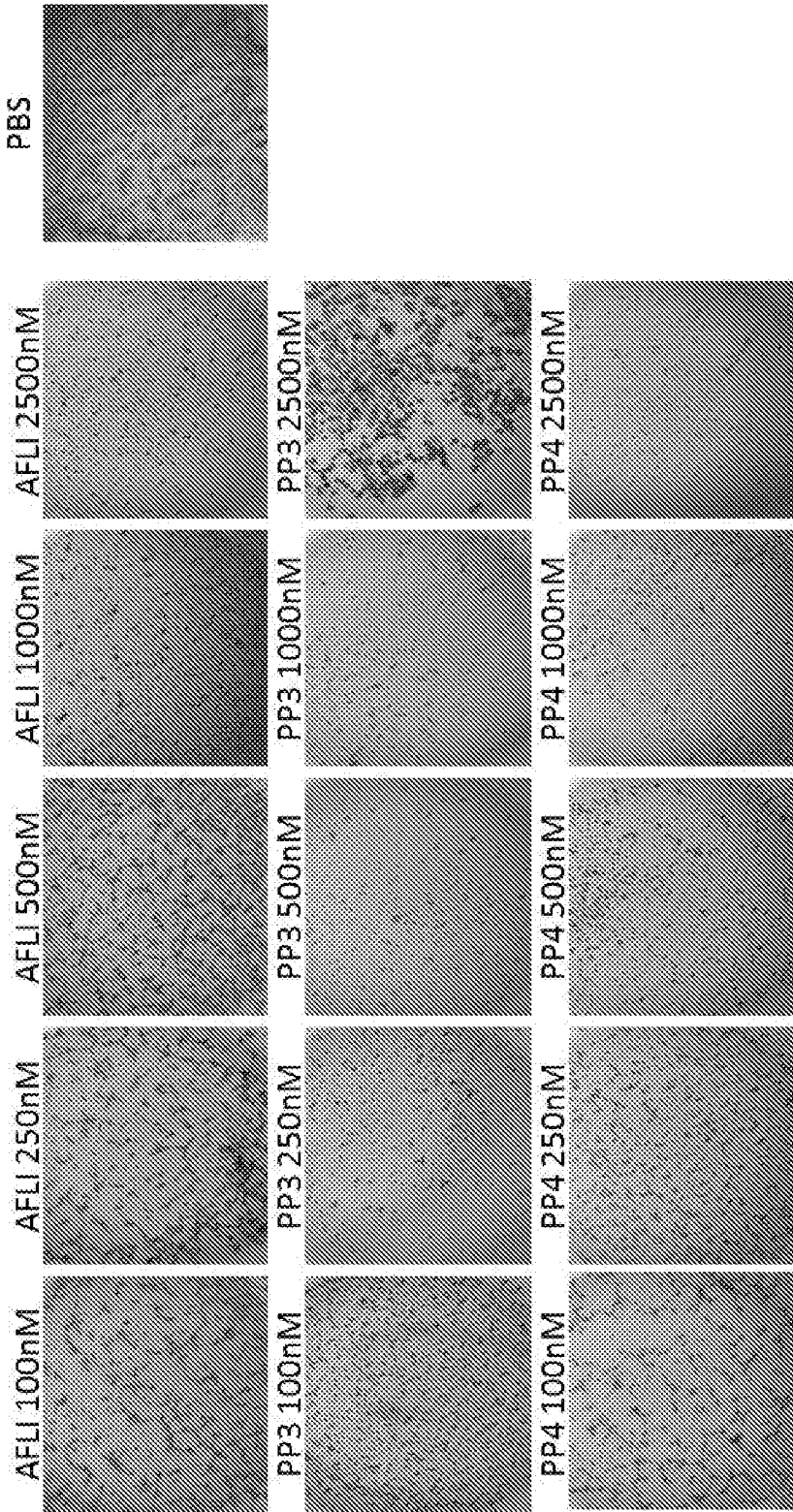


Figure 16A

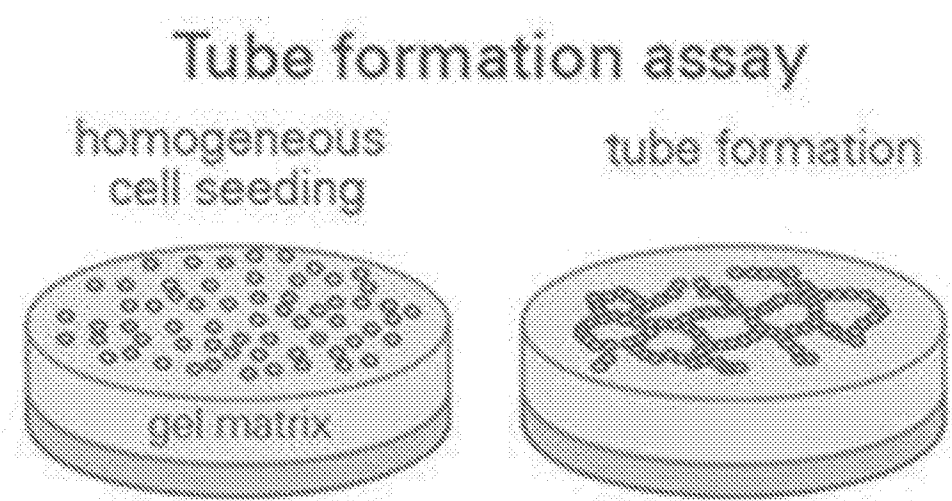


Figure 16B

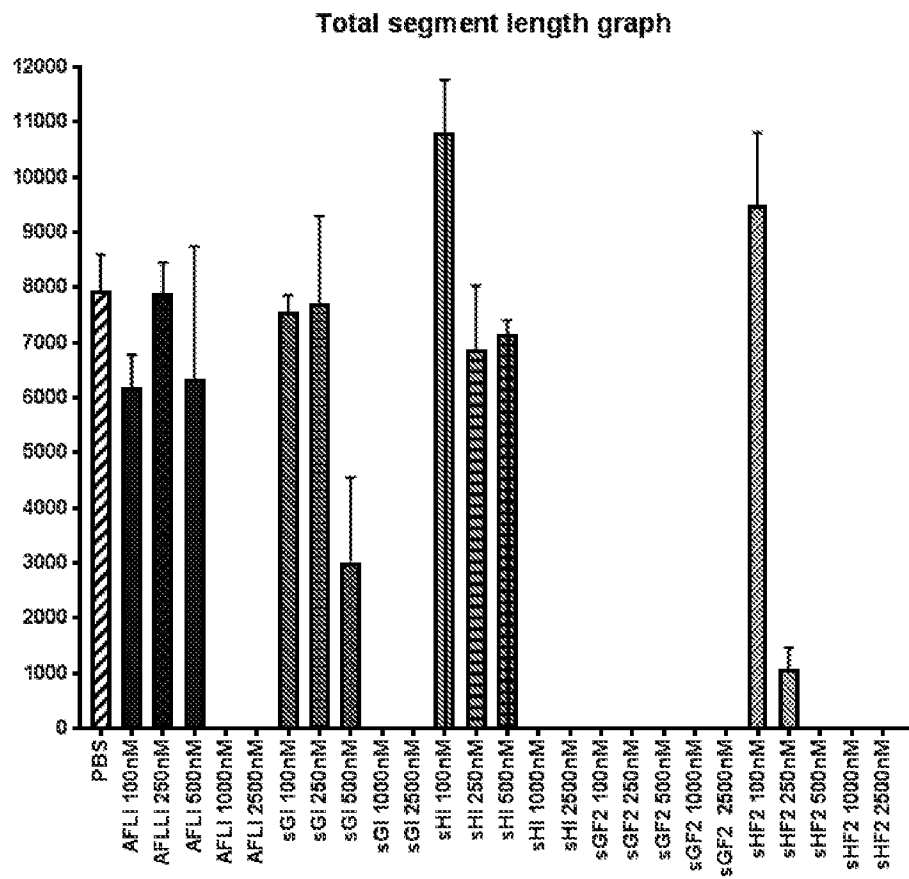
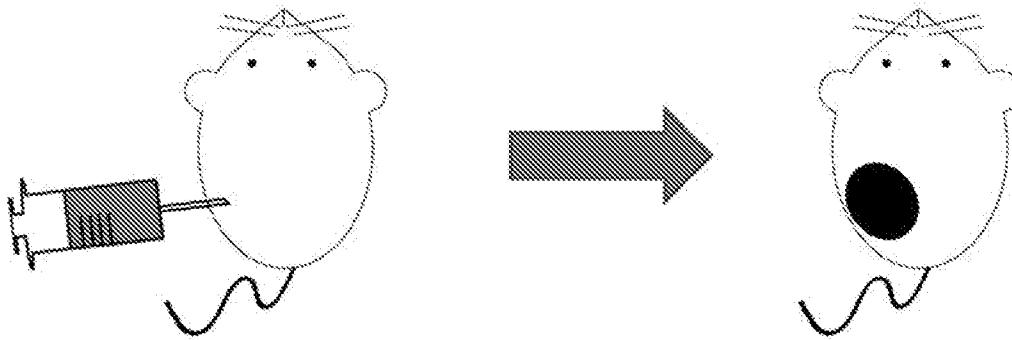
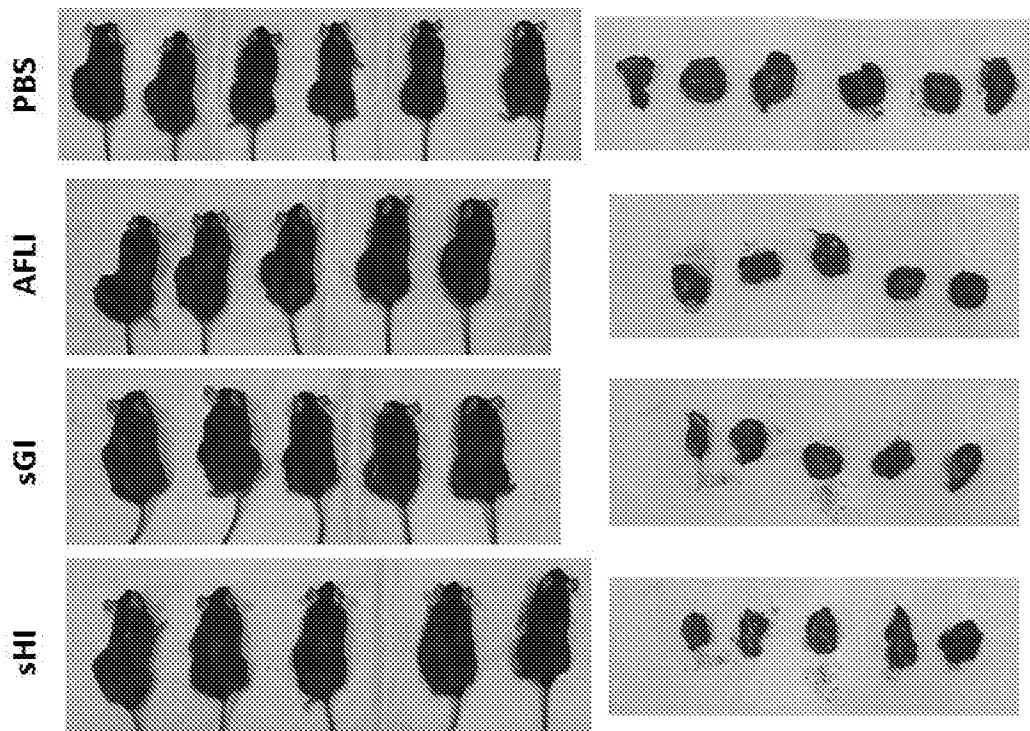


Figure 16C

**Figure 17A****Figure 17B**

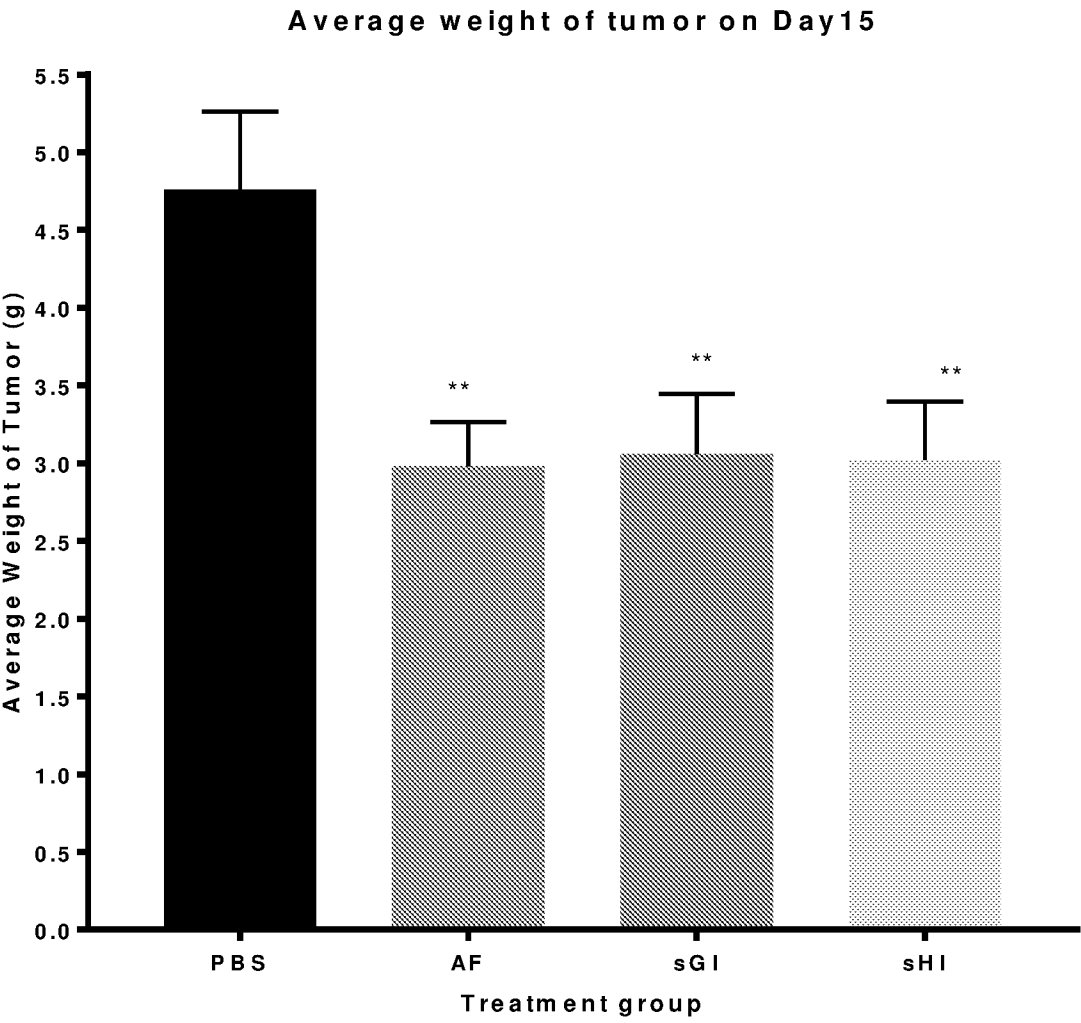


Figure 17C

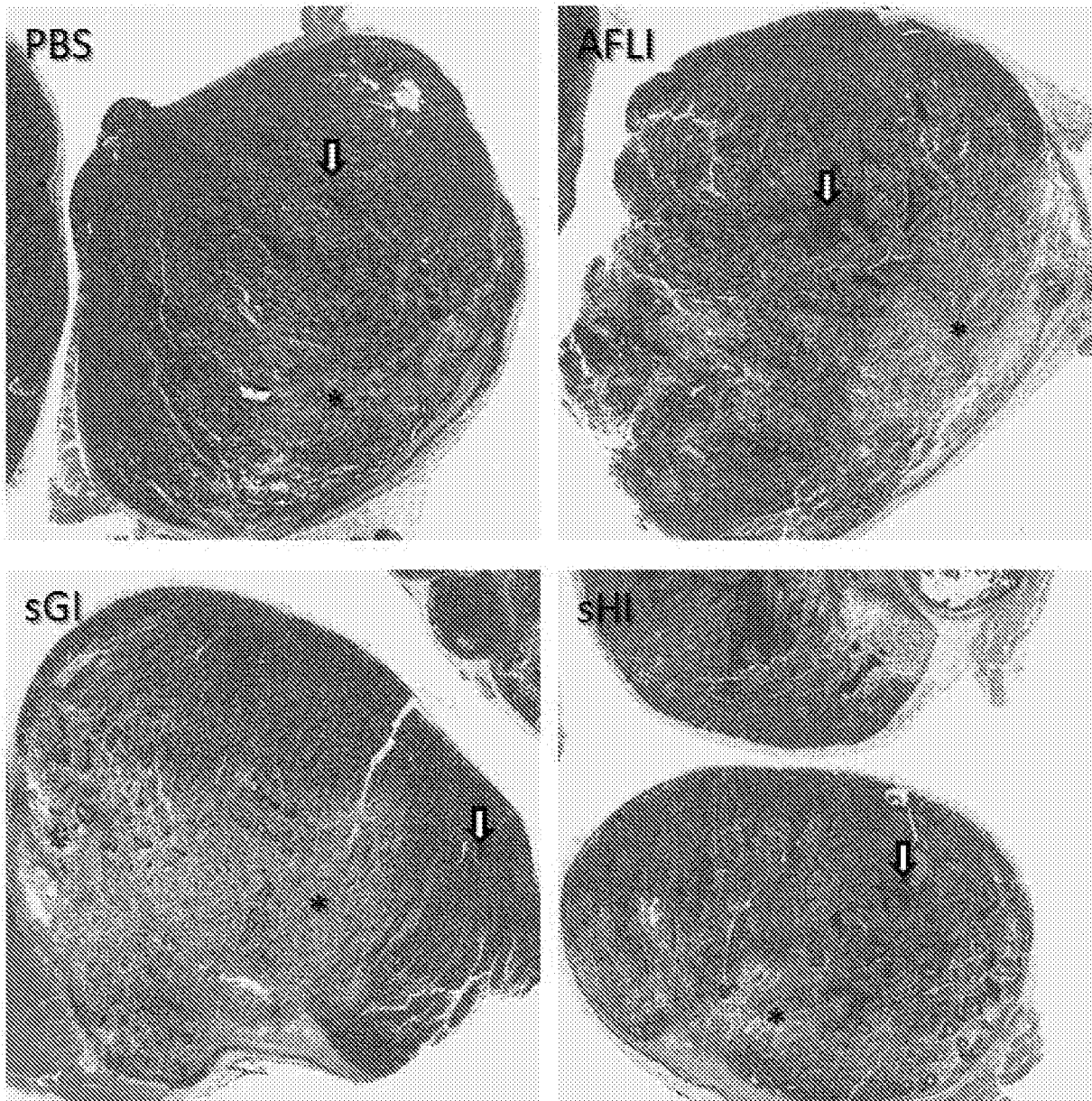


Figure 17D

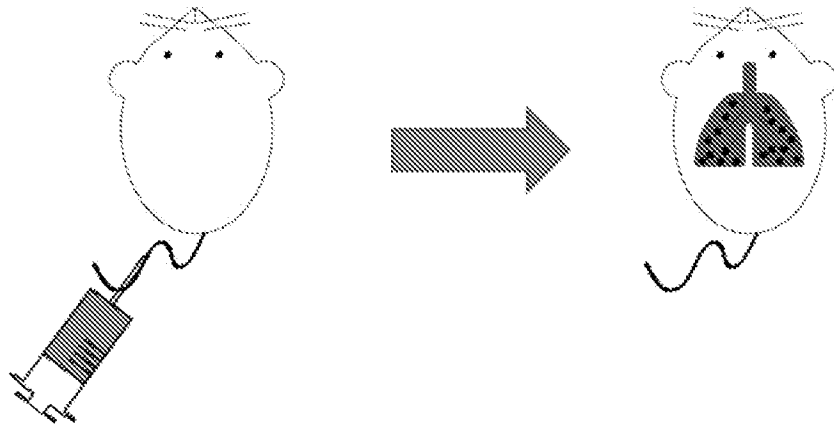


Figure 18A

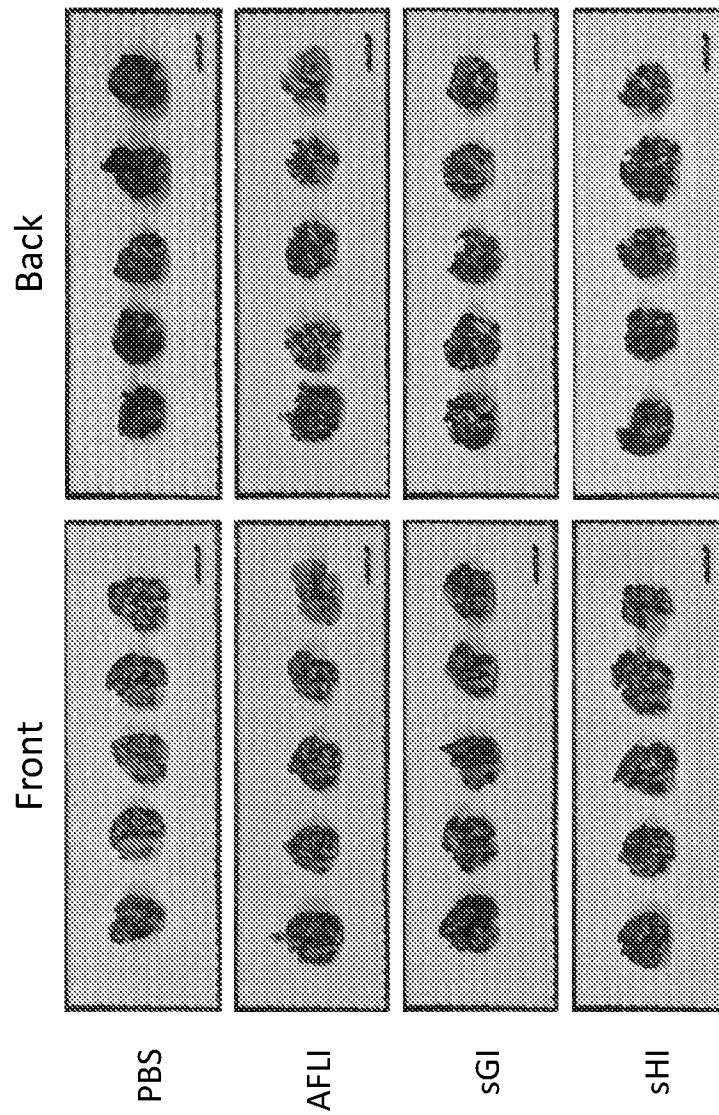


Figure 18B

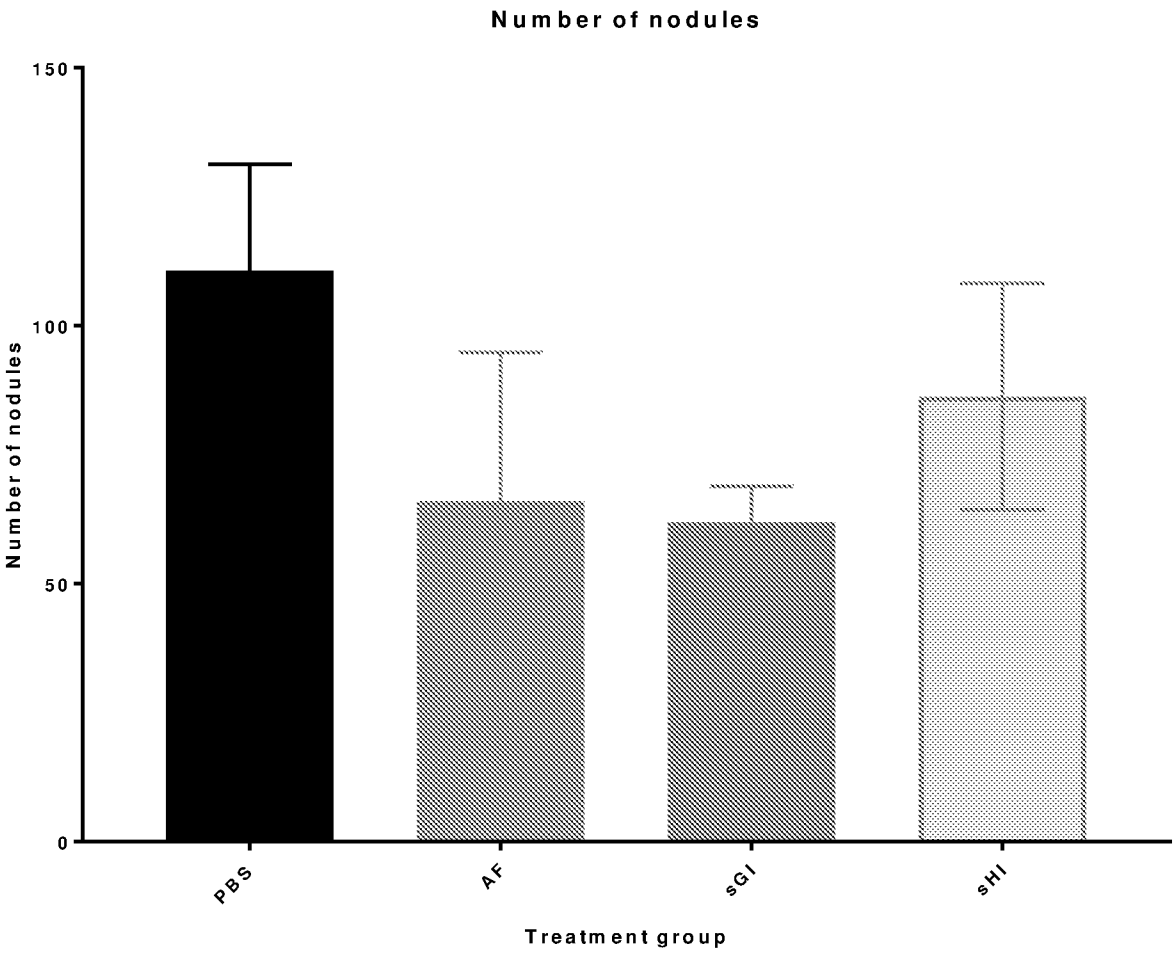
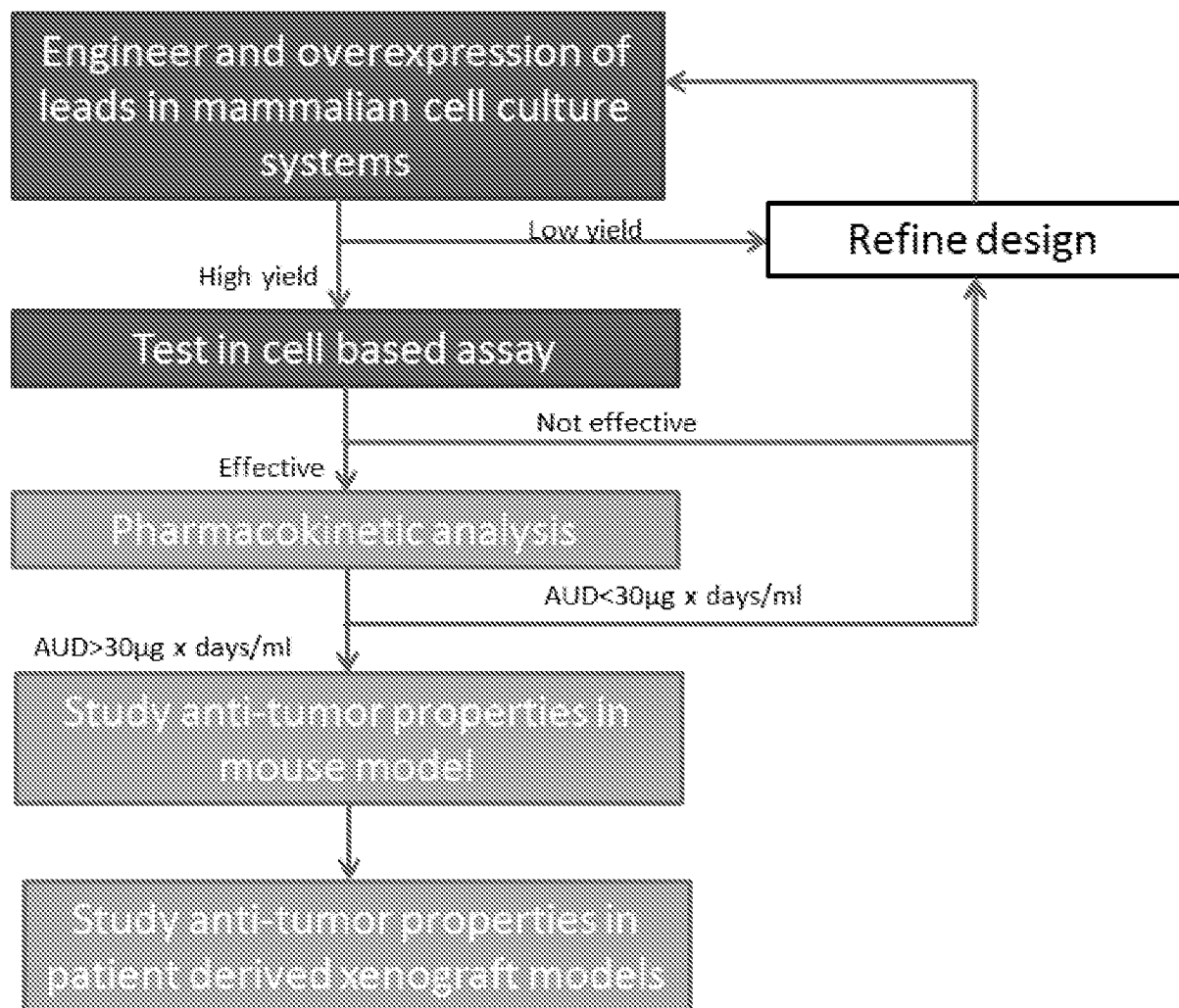


Figure 18C

**Figure 19**

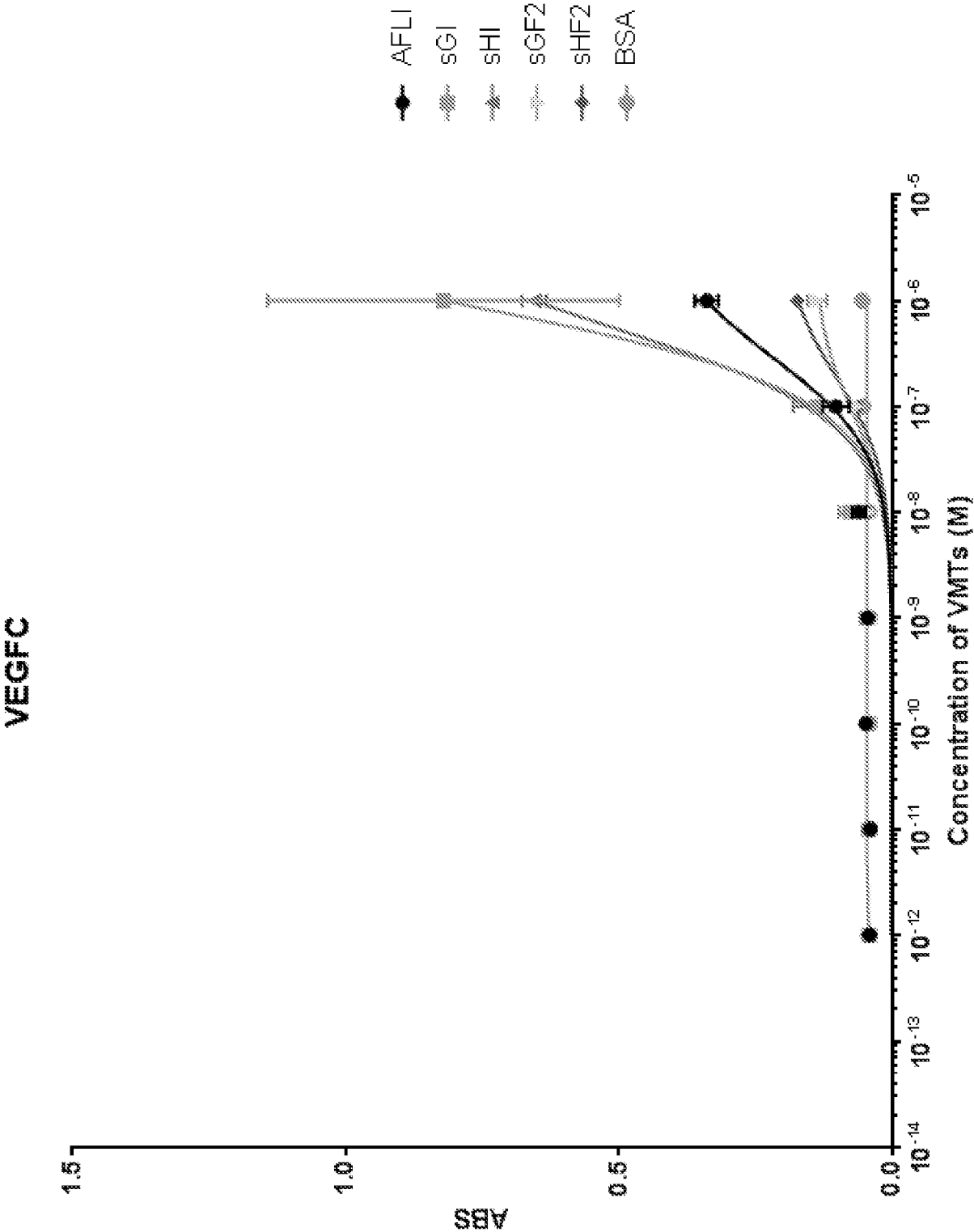


Figure 20 (cont.)

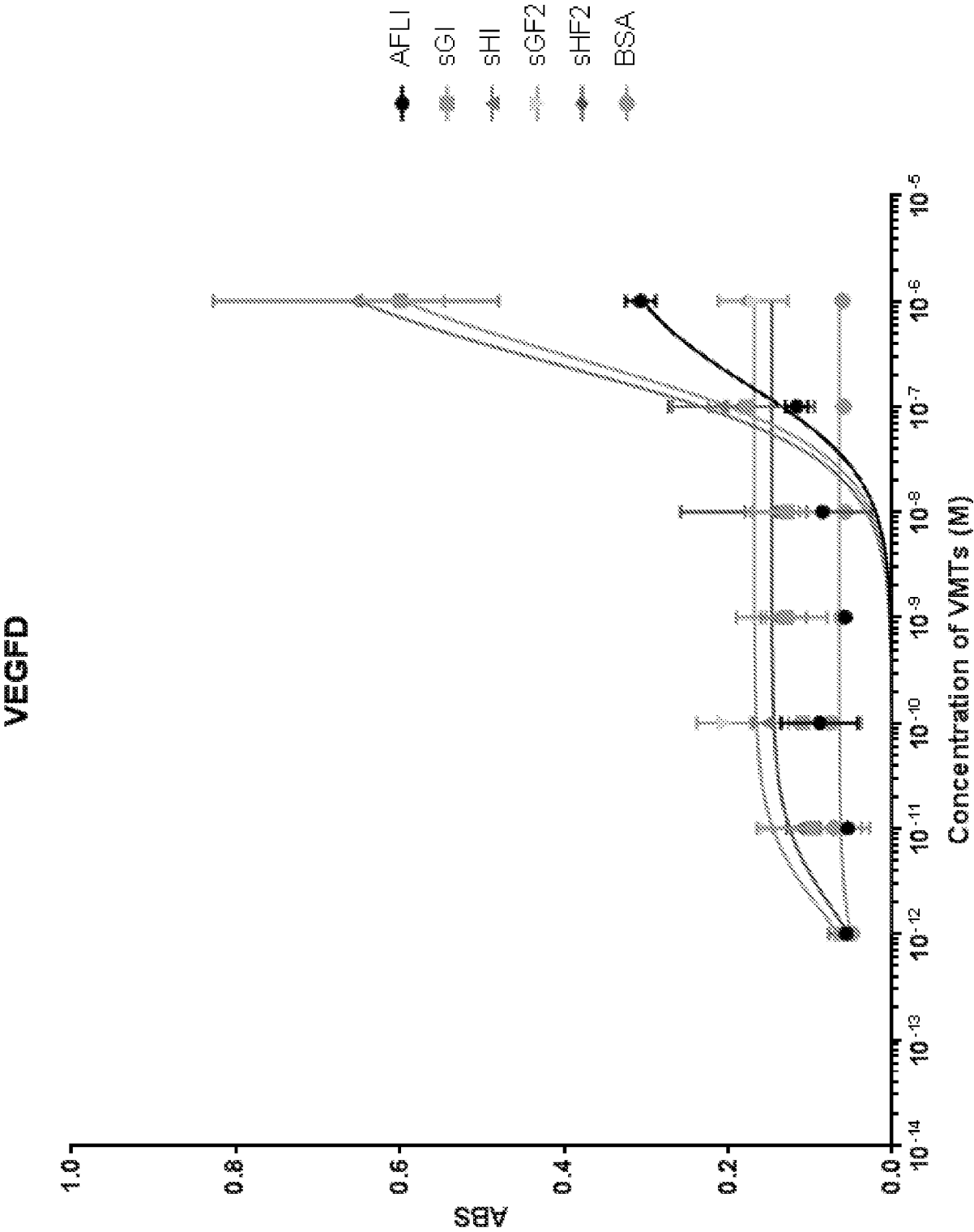
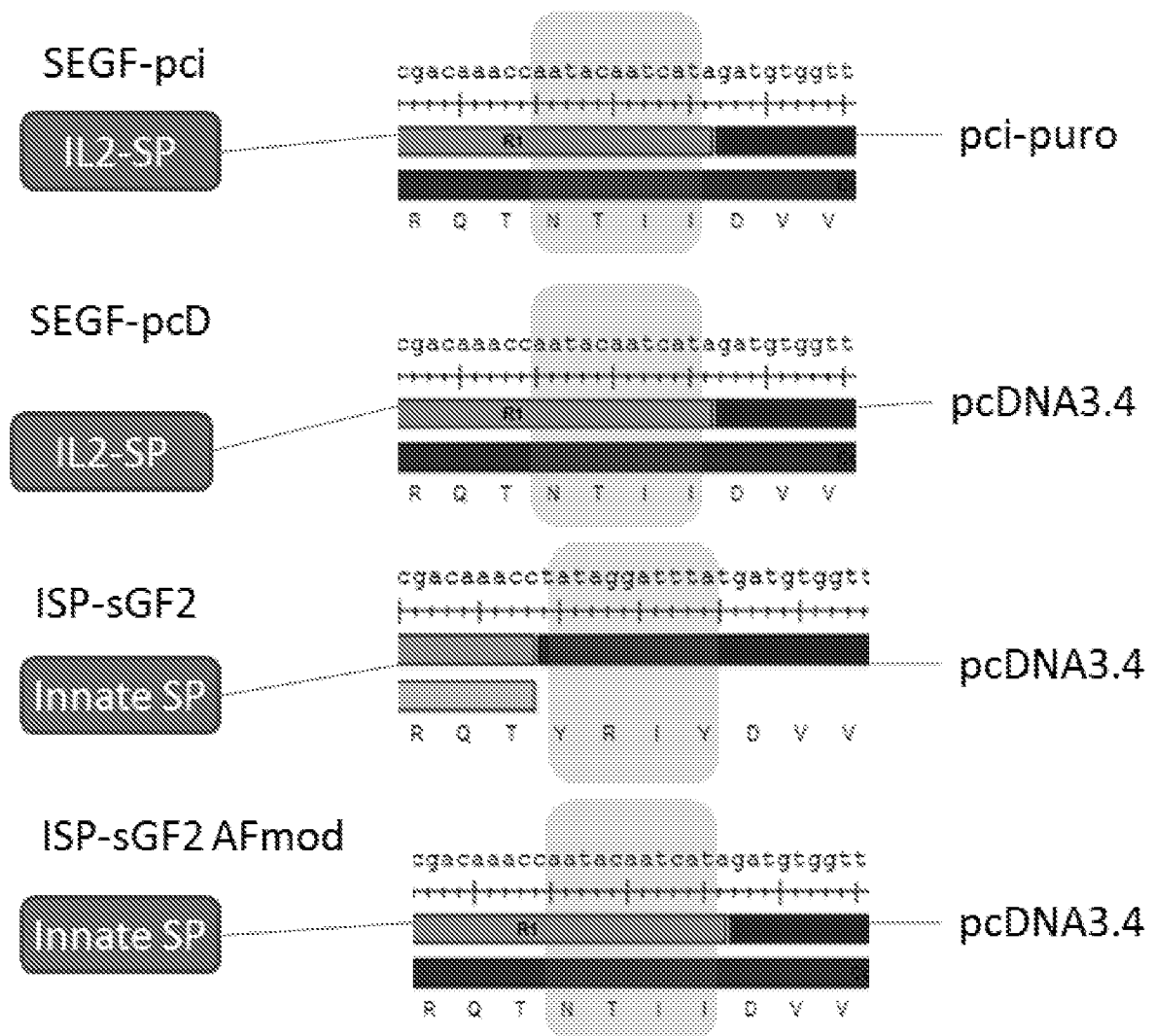
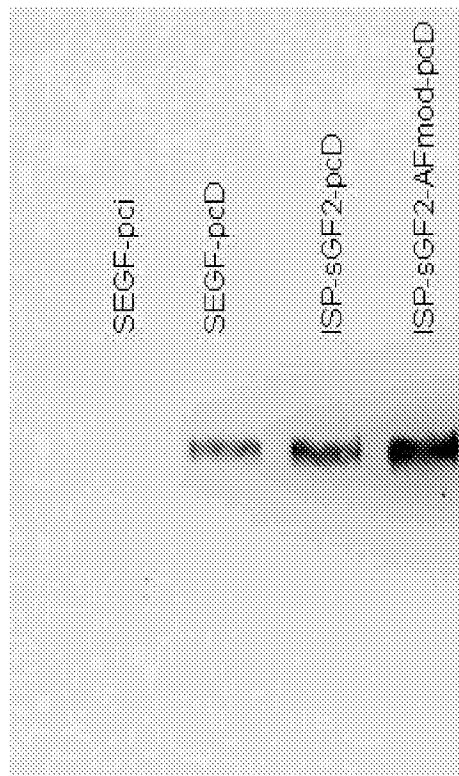
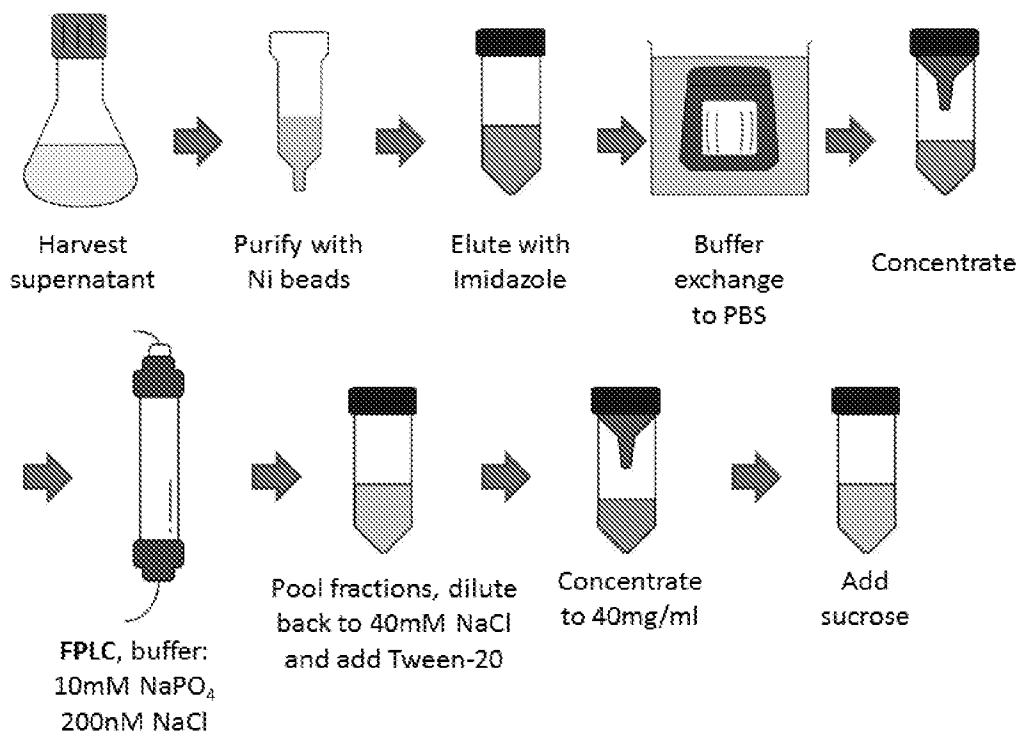


Figure 20

**Figure 21A**

**Figure 21B****Figure 22**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2017/050623

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/17 (2006.01) C07K 14/71 (2006.01) C07K 19/00 (2006.01) A61P 35/00 (2006.01)

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, FAMPAT, MEDLINE, EMBASE, BIOSIS

VEGF receptor, VEGFR1, VEGFR2, VEGFR3, Flk-1, KDR, VEGF Trap, VEGF decoy, FLT-4, FLT-1, immunoglobulin-like, ectodomain, extracellular domain, chimeric, fusion, recombinant and related terms

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 102134277 A (SHANGHAI NATIONAL ENGINEERING RESEARCH CENTER OF ANTIBODY MEDICINE CO., LTD) 27 July 2011 (Paragraphs [0006], [0009]-[0010], [0013], [0018], [0029]-[0030], [0034]; Experimental Examples 1-6; Figure 6) of the machine translation	1-27
A	CN 103304668 B (JIANGSU JIANDE BIOLOGICAL PHARMACEUTICAL CO., LTD) 28 October 2015 (Paragraphs [0017], [0037]; Examples 1-3; Figures 1, 6) of the machine translation	1-27
A	US 2005/0260203 A1 (WIEGAND S.J. ET AL.) 24 November 2005 (Paragraph [0044]; Example 1)	1-27

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

*Special categories of cited documents:

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

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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

15/02/2018

(day/month/year)

Date of mailing of the international search report

06/03/2018

(day/month/year)

Name and mailing address of the ISA/SG



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Singapore 189554

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Authorized officer

Terence Tan (Dr)

IPOS Customer Service Tel. No.: (+65) 6339 8616

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2017/050623

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOLASH J. ET AL., VEGF-Trap: a VEGF blocker with potent antitumor effects. <i>Proc Natl Acad Sci USA</i> , 12 August 2002, Vol. 99, No. 17, pages 11393-11398 [Retrieved on 2018-02-15] <DOI: 10.1073/PNAS.172398299> (Figure 1A)	1-27
A	WANG Q. ET AL., Novel VEGF decoy receptor fusion protein conbercept targeting multiple VEGF isoforms provide remarkable anti-angiogenesis effect in vivo. 12 August 2013, Vol. 8, No. 8, pages e70544:1-7 [Retrieved on 2018-02-15] <DOI: 10.1371/JOURNAL.PONE.0070544> (Figure 1)	1-27

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/SG2017/050623

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 102134277 A	27/07/2011	NONE	
CN 103304668 B	28/10/2015	CN 103304668 A	18/09/2013
US 2005/0260203 A1	24/11/2005	NONE	