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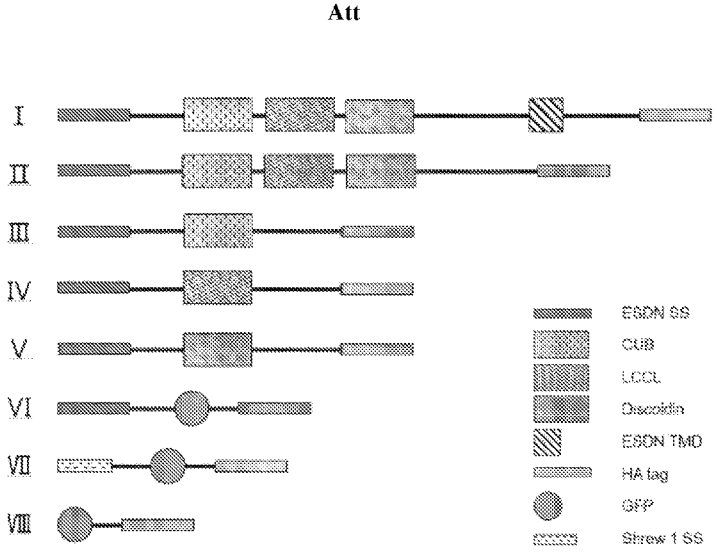

Fig. 1A

(57) Abstract: Described herein is a method of promoting neovascularization in a subject and/or a method of promoting wound healing in a subject. Both methods include administering to the subject a compound including a peptide, which includes a poly-leucine section having a length ranging from about 6 amino acid residues to about 14 amino acid residues, and a leucine content of 80% or higher.

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PEPTIDES FOR PROMOTING NEOVASCULARIZATION AND WOUND HEALING AND METHODS USING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Patent Application No. 63/599,066, filed November 15, 2023, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[002] This invention was made with government support under HL114703, HL112992, and HL162792 awarded by National Institutes of Health. This invention was made with government support under I0-BX001750 awarded by Department of Veterans Affairs. The government has certain rights in the invention.

SEQUENCE LISTING

[003] The XML file named "047162-7411WO1(02467)_Seq Listing.xml" created on November 8, 2024, comprising 19,408 bytes, is hereby incorporated by reference in its entirety.

BACKGROUND

[004] Lack of blood supply is implicated in many types of diseases and disorders, such as certain cardiovascular conditions (e.g., peripheral arterial disease and coronary ischemia) and wound healing disorders (e.g., diabetic wounds).

[005] Neovascularization is the process of forming new blood vessels. Neovascularization includes: vasculogenesis, the *de novo* formation of blood vessels; angiogenesis, the formation of new vessels from pre-existing vessels; and arteriogenesis, the remodeling of existing vasculature to create collateral arteries. Methods of promoting neovascularization in areas lacking blood supply are expected to be useful for treating both cardiovascular condition and wound healing disorders.

[006] Currently, methods for promoting neovascularization or chronic wound healing are lacking, and methods of improving blood supply are mostly limited to surgical means such as vascular bypass and angioplasty. As such, there is a need for novel methods of promoting

neovascularization and/or methods for promoting wound healing. The present invention addresses this need.

SUMMARY

[007] In some aspects, the present invention is directed to the following non-limiting embodiments:

Method of promoting neovascularization and/or hair growth

[008] In some aspects, the present invention is directed to a method of promoting neovascularization and/or hair growth in a subject in need thereof.

[009] In some embodiments, the method comprises: administering to the subject a compound comprising a polypeptide that promotes neovascularization and/or hair growth.

[0010] In some embodiments, the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues.

[0011] In some embodiments, the polypeptide comprises the amino acid set forth in SEQ ID NO:5, 6, 8, or 9.

[0012] In some embodiments, the compound is soluble in water or insoluble in water.

[0013] In some embodiments, the compound is soluble in water, and the compound further comprises a hydrophilic motif.

[0014] In some embodiments, the hydrophilic motif is at least one selected from the group consisting of: a second peptide which is hydrophilic; and one or more polyethylene glycol (PEG) groups.

[0015] In some embodiments, the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

[0016] In some embodiments, the method is a method of promoting neovascularization, and wherein the method promotes in the subject at least one selected from the group consisting of angiogenesis and arteriogenesis.

[0017] In some embodiments, the subject suffers from a chronic wound healing disorder, and wherein the method promotes neovascularization in or near at least one chronic wound of the subject.

[0018] In some embodiments, the subject suffers from an acute wound, and wherein the method promotes neovascularization which speeds up the healing of the acute wound.

[0019] In some embodiments, the chronic wound comprises at least one of the following:

(a) a nonhealing wound;

(b) an infected wound, optionally an infected surgical wound or an infected traumatic wound;

(c) an ulcer, optionally a diabetic ulcer such as a diabetic foot ulcer, an arterial ulcer, a venous ulcer, a pressure ulcer, or an ischemic ulcer; or

(d) a wound associated with immunosuppression or radiation.

[0020] In some embodiments, the compound reduces phosphatase activity exerted by protein-tyrosine phosphatase 1B (PTP1B) and/or T-cell protein tyrosine phosphatase (TC-PTP) on vascular endothelial growth factor receptor 2 (VEGFR2).

[0021] In some embodiments, the method further comprises administering to the subject an effective amount of vascular endothelial growth factor (VEGF).

Method of promoting wound healing

[0022] In some aspects, the present invention is directed to a method of promoting wound healing in a subject in need thereof.

[0023] In some embodiments, the method comprises administering to the subject an effective amount of a compound comprises a polypeptide.

[0024] In some embodiments, the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues.

[0025] In some embodiments, the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

[0026] In some embodiments, the compound is soluble in water or insoluble in water.

[0027] In some embodiments, the compound is soluble in water, and the compound further comprises a hydrophilic motif.

[0028] In some embodiments, the hydrophilic motif is at least one selected from the group consisting of: (a) a second peptide which is hydrophilic; and (b) one or more polyethylene glycol (PEG) groups.

[0029] In some embodiments, the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

[0030] In some embodiments, the method promotes wound healing by promoting in the subject at least one selected from the group consisting of angiogenesis and arteriogenesis.

[0031] In some embodiments, the wound is a chronic wound or an acute wound.

[0032] In some embodiments, the chronic wound comprises at least one of the following:

(a) a nonhealing wound;

(b) an infected wound, optionally an infected surgical wound or an infected traumatic wound;

(c) an ulcer, optionally a diabetic ulcer such as a diabetic foot ulcer, an arterial ulcer, a venous ulcer, a pressure ulcer, or an ischemic ulcer; or

(d) a wound associated with immunosuppression or radiation.

[0033] In some embodiments, the compound reduces phosphatase activity exerted by protein-tyrosine phosphatase 1B (PTP1B) and/or T-cell protein tyrosine phosphatase (TC-PTP) on vascular endothelial growth factor receptor 2 (VEGFR2).

[0034] In some embodiments, the method further comprises administering to the subject an effective amount of vascular endothelial growth factor (VEGF).

Compound

[0035] In some aspects, the present invention is directed to a compound comprising a polypeptide and a hydrophilic motif, or a salt or solvate thereof.

[0036] In some embodiments, the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues.

[0037] In some embodiments, the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

[0038] In some embodiments, the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

Wound dressing

[0039] In some aspects, the present invention is directed to a wound dressing.

[0040] In some embodiments, the wound dressing comprises a base dressing material, and a compound comprising a polypeptide.

[0041] In some embodiments, the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues.

[0042] In some embodiments, the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

[0043] In some embodiments, the compound is soluble in water or insoluble in water.

[0044] In some embodiments, the compound is soluble in water, and wherein the compound further comprises a hydrophilic motif.

[0045] In some embodiments, the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

[0046] In some embodiments, the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

[0047] In some embodiments, the base dressing material comprises at least one selected from the group consisting of an alginate dressing material, a biosynthetic dressing material, a collagen dressing material, a composite dressing material, a contact layer dressing material, a gauze, a hydrocolloid dressing material, a hydrofiber dressing material, a hydrogel, a hydropolymer dressing material, a polyurethane foam dressing material, a skin substitute dressing material, a superabsorbent dressing material, a transparent film dressing material, a wound filler, a wound pouch.

[0048] In some embodiments, the compound comprising the polypeptide is on a surface of or impregnated into the base dressing material.

[0049] In some embodiments, the wound dressing further comprises an adhesive substrate.

BRIEF DESCRIPTION OF THE DRAWINGS

[0050] The following detailed description of exemplary embodiments will be better understood when read in conjunction with the appended drawings. For the purpose of illustrating, non-limiting embodiments are shown in the drawings. It should be understood,

however, that the present specification is not limited to the precise arrangements and instrumentalities of the embodiments shown in the drawings.

[0051] Figs. 1A-1E demonstrates that the endothelial and smooth muscle cell-derived neuropilin-like molecule (ESDN, also called DCBLD2) signal sequence is the key part for its interaction with vascular endothelial growth factor receptor 2 (VEGFR2), in accordance with some embodiments. Fig. 1A: Schematic representation of the constructs named with Roman numerals I to VIII. Construct I represents the full-length ESDN fused with an heme agglutinin (HA) tag. Construct II represents the extracellular part of ESDN fused with an HA tag. Construct III, IV, and V represent different ESDN key domains (CUB, LCCL, Discoidin) connected to ESDN signal sequence and fused with an HA tag, respectively. Construct VI and VII represent the signal sequence of ESDN (VI)/ Shrew 1 (VII) fused with green fluorescent protein (GFP) and HA tag, and construct VIII represents GFP-HA alone as a negative control. Fig. 1B: Interaction of key ESDN domains with VEGFR2. Construct I- V co-transfected with VEGFR2 to HEK 293T cells, respectively. Western blot analysis of VEGFR2 confirmed their successful expression (upper panel). VEGFR2 immunoprecipitation was followed by Western blot detection of HA tag in the product protein of each construct (lower panel). Fig. 1C: Effect of key ESDN domains on VEGF-mediated ERK phosphorylation. Western blot example of phosphorylated ERK / total ERK in wild type (WT_ and *Esdn*$^{-/-}$ (*Dcbld2*$^{-/-}$) murine lung endothelial cells (MLECs_ transfected with constructs I-V (upper panel). Western blot analysis of HA tag to confirm protein expression of each construct (lower panel). Fig. 1D: Interaction of ESDN signal sequence with VEGFR2. Construct VI, VII, and VIII products co-immunoprecipitation with VEGFR2 in HEK 293T cells. Fig. 1E: Effect of ESDN signal sequence on VEGF-mediated ERK phosphorylation. Western blot of phosphorylated ERK / total ERK in human umbilical vein endothelial cells (HUVEs) transfected with siRNAs siESDN / siCon and construct VI / pcDNA vector, followed by 10 ng/ml VEGF treatment for five minutes or not.

[0052] Figs. 2A-2E demonstrate that the traC subdomain is the key part of ESDN SS that interacts with VEGFR2, in accordance with some embodiments. Fig. 2A: The predicted 'NtraC' subdomain combination of ESDN signal sequence (residues 1-66 of SEQ ID NO:1). Figs. 2B-2C: Significant differences between ESDN signal sequence subdomains. Hydrophobicity analysis performed by ProtScale (Fig. 2B) and transmembrane helices prediction performed by DeepTMHMM (Fig. 2C). Fig. 2D: Schematic diagram of constructs named with roman numerals I to IV. Construct I- III represent full length ESDN signal sequence / the N subdomain / the traC subdomain fused with GFP and HA tags, respectively.

Construct IV represents GFP-HA alone as a negative control. Fig. 2E: Interaction of key ESDN signal sequence subdomains with VEGFR2. Construct I to IV products co-immunoprecipitation with VEGFR2 in HEK 293T cells.

[0053] Figs. 3A-3F demonstrate that traC increases VEGF/VEGFR2 signaling in endothelial cells, in accordance with some embodiments. Figs. 3A-3B: Immunofluorescence staining of traC treated HUVECs. FITC-traC is in green. Nuclei was stained with DAPI in blue. VEGFR2 was stained in red. Arrowheads indicate traC co-localized with VEGFR2 on cell membrane. Scale bar: 20μm. Fig. 3C: Western blot example and quantification of the effect of traC on VEGF/VEGFR2 signaling. Serum starved HUVECs were treated with 1μM traC / 1% DMSO for 15min, followed by 10ng/ml VEGF treatment for 5min or not. The levels of VEGFR2 Tyr1175 phosphorylation, ERK Thr202/Tyr204 phosphorylation, and Akt Ser473 phosphorylation, and their corresponding total proteins were detected. $^{**}p < 0.01$, $^{***}p < 0.001$. Fig. 3D: Western blot example and quantification of the effect of different concentrations of traC on VEGF/VEGFR2 signaling. Serum starved HUVECs were treated with 0/0.1/1μM traC for 15min, followed by 10ng/ml VEGF stimulation for 5min or not. $^{*}p < 0.1$, $^{**}p < 0.01$. Fig. 3E: Western blot example and quantification of VEGF/VEGFR2 signaling in WT / ESDN deficient HUVECs following treatment with / without traC / VEGF. $^{*}p < 0.1$. Fig. 3F: traC's effect on the interaction between VEGFR2 and phosphatases in HUVECs. VEGFR2 immunoprecipitation was followed by Western blot detection of PTP1B and TCPTP.

[0054] Fig. 4: Specificity of traC-VEGFR2 interaction, in accordance with some embodiments. Following overexpression of different tyrosine kinase receptors in HEK 293 cells, their expression was confirmed by Western blotting (Fig. 4, right panel). After adding FITC-traC for 15 minutes to the cultures, VEGFR2, but not VEGFR1 or PDGFRβ, could be co- immunoprecipitated with FITC-traC (Fig. 4, left panel),

[0055] Figs. 5A-5B demonstrate that traC promotes *in vivo* matrigel angiogenesis, in accordance with some embodiments. Fig. 5A: Examples of matrigel plugs harvested 5 days after 1 μM traC / 1% DMSO in 300μL growth factor reduced matrigel with or without 2 ug/ml VEGF-A were subcutaneously injected into the opposite iliac regions of C57BL/6J mice. Fig. 5B: Examples and quantification of CD31 immunofluorescence staining (in red) of plug sections. Scale bar: 200μm. $^{**}p < 0.01$.

[0056] Figs. 6A-6B demonstrate that traC promotes *in vivo* cornea angiogenesis, in accordance with some embodiments. Fig. 6A: Examples of cornea pictures taken before tissue collection on the 13th day. Small pellets containing traC / DMSO with or without

VEGF were implanted into a 1 mm pocket incision in the cornea about 1-1.3 mm from the limbus. Fig. 6B: Examples and quantification of CD31 whole mount immunostaining (in red) of cornea tissue. The white dashed circles represent the location where the pellets were implanted. Scale bar: 500 μm. ****$p < 0.0001$.

[0057] Figs. 7A-7D demonstrate that traC facilitates ear wound healing, in accordance with some embodiments. Fig. 7A: Schematic diagram of the ear wound healing model. A 2mm diameter circular wound was punched in the center of both ears. FITC-traC 10 μg / ear or 1% DMSO were injected around the ear wounds of each mouse every other day for 20 days. Fig. 7B: Typical pictures of ear wounds of albino mice on the 1st and 20th days. Fig. 7C: Quantification of wound area (n=9). Fig. 7D: Examples and quantification of CD31 immunostaining (in red) of ear tissues collected on day 8 and day 20. FITC-traC is seen in green. Nuclei were stained with DAPI in blue. Scale bar: 300 μm. *$p < 0.1$.

[0058] Figs. 8A-8G: Poly-leucine interact with VEGFR2, in accordance with some embodiments. Fig. 8A: Co-Immunoprecipitation of traC / traC part 1(CSNSSSFSMPLF, residues 39-50 of the sequence set forth in SEQ ID NO:1) / traC part 2 (LLLLLVLLLLLEDAGA, residues 51-66 of the sequence set forth in SEQ ID NO:1) with VEGFR2. Fig. 8B: Co-Immunoprecipitation of traC part 2 (LLLLLVLLLLLEDAGA, residues 51-66 of the sequence set forth in SEQ ID NO:1) / LLLLLVLLLL (residues 51-61 of the sequence set forth in SEQ ID NO:1) / EDAGA (residues 62-66 of the sequence set forth in SEQ ID NO:1) / GFP (negative control) with VEGFR2. Fig. 8C: Summary of the interaction between traC segments and VEGFR2. Fig. 8D: Co-Immunoprecipitation of LLLLLVLLLL (residues 51-61 of the sequence set forth in SEQ ID NO:1) /LLLLLLLLL (9L) / LLLLLLLLLLLLLLL (16L) with VEGFR2. Fig. 8E: Summary of the interaction between traC part 2 segments and VEGFR2. Fig. 8F: Co-Immunoprecipitation of 7L / 8L / 9L / 10L / 11L / 12L peptides with VEGFR2. Fig. 8G: Western blot of the effect of a 10L synthetic peptide (FITC-L10 connected to EDAGA to increase water solubility) at 1 μM and 10 μM on VEGF signaling in endothelial cells.

[0059] The peptides listed in Fig. 8E are also listed in Table 1:

Table 1

LLLLLVLLLLLEDAGA (SEQ ID NO:1)
LLLLLVLLLLLLLLL (SEQ ID NO:2)
EDAGA (SEQ ID NO:3)
DAG (SEQ ID NO:4)

LLLLLVLLLLL (SEQ ID NO:5)
LLLLLLLLL (SEQ ID NO:6)
LLLLLLLLLLLLLLL (SEQ ID NO:7)

[0060] Fig. 9 recites of the sequences of the TracC, scrambled (Scr, SEQ ID NO:7), and hydrophobic control (SEQ ID NO:6) peptides, in accordance with some embodiments. The sequences shown in Fig. 9 is also listed below:

CSNSSSFSMPLFLLLLLVLLLLLEDAGA (SEQ ID NO:8)
CSNSSSFSMPIFIIIIIVIIIIIEDAGA (SEQ ID NO:9)
LSLESALMLFLCLDLSGVFLSLPLSNL (SEQ ID NO:10)

[0061] Figs. 10A-10C illustrate certain aspects of the effect of Scrambled (Scr) peptide and traC on VEGF induced VEGFR2 signaling in HUVEC, in accordance with some embodiments. Specifically, HUVECs were treated with DMSO, Scr peptide, or traC peptide for 15 min followed by 5 mins of VEGF(10 ng/ml) treatment. traC peptide enhances VEGF-induced VEGFR2 and ERK phosphorylation compared to DMSO. Scr peptide increases VEGF-induced VEGFR2 phosphorylation (albeit less than traC) compared to DMSO. Fig. 10A: western blot panels. Fig. 10B: quantification of p-VEGFR2/Total VEGFR2. Fig. 10C: quantification of p-EER/Total ERK. N=3.

[0062] Figs. 11A-11B illustrate certain aspects of the effect of traC in comparison with DMSO on VEGF induced VEGFR2 signaling in HUVEC, in accordance with some embodiments. Specifically, HUVECs were treated with DMSO, or traC peptide for 15 min followed by VEGF(10 ng/ml). traC peptide enhances VEGF-induced VEGFR2, AKT and ERK phosphorylation compared to DMSO. No difference in the time course of VEGF-induced VEGF signaling between DMSO and traC treated samples. N=3. Fig. 11A: Western blot panels. Fig. 11B: quantification bar charts of pVEGFR2, pERK and pAKT.

[0063] Fig. 12 demonstrates the different kinetics of VEGF-induced VEGFR2 signaling in HUVEC in the presence of scrambled (Scr) peptide or traC, in accordance with some embodiments. Specifically, HUVECs were treated with scr peptide or traC peptide for 15 min followed by 0-30mins of VEGF(10ng/ml). traC and Scr peptides increase VEGFR2 phosphorylation with different time courses.

[0064] Figs. 13A-13E demonstrate the different kinetics of VEGF-induced VEGFR2 signaling in HUVEC in the presence of control peptide (CP) or traC, in accordance with some embodiments. Specifically, HUVECs were treated with CP or traC for 15 min followed by 0-

30mins of VEGF(10ng/ml) treatment. traC and CP increase VEGFR2 phosphorylation with different time courses.

[0065] Figs. 14A-14B demonstrate that FITC-traC, but not FITC-CP or FITC-Scr peptides, are internalized in HUVEC, in accordance with some embodiments. Specifically, HUVECs were treated with Scr, CP or traC for 15 min followed by 0-30mins of VEGF(10ng/ml) treatment. After washing the cells, the FITC signal was only detectable in FITC-traC treated cells. A separate gel with the three peptides showed similar protein and fluorescence intensities on the gel and after transfer to a membrane.

[0066] Figs. 15A-15G demonstrate that the DCBLD2 SS traC subdomain is involved in DCBLD2 interaction with VEGFR2, in accordance with some embodiments. Fig. 15A: Schematic representation of constructs I-VIII, each with various combinations of DCBLD2 domains tagged with HA (I-V), DCBLD2, or Shrew-1 SS fused with GFP and HA tags (VI, VII) or GFP-HA alone (VIII). Fig. 15B: Co-immunoprecipitation of the products of constructs I-V with VEGFR2 following transfection of each construct with VEGFR2 (or transfection of construct I alone without VEGFR2) in HEK 293T cells. VEGFR2 immunoprecipitation is followed by Western blotting for HA and VEGFR2. Fig. 15C: Western blot analysis of the effects of constructs I-V on VEGF-induced ERK phosphorylation following their transfection in *Dcbld2*$^{-/-}$ murine lung endothelial cells transfected. Fig. 15D: Co-immunoprecipitation of the products of constructs VI-VIII with VEGFR2 following their transfection in HEK 293T cells. HA immunoprecipitation is followed by Western blotting for HA and VEGFR2. Fig. 15E: NtraC model of DCBLD2 SS. Fig. 15F: Schematic representation of constructs IX-XI encompassing different DCBLD2 SS subdomains fused with GFP and HA tags (IX-XI) or GFP-HA alone (VIII). Fig. 15G: Co-immunoprecipitation of the products of constructs IX-XI with VEGFR2 following their transfection in HEK 293T cells. HA immunoprecipitation is followed by Western blotting for HA and VEGFR2. SS: signal sequence; TMD: transmembrane domain. HA: Hemagglutinin; GFP: green fluorescent protein; IP: immunoprecipitation; WB: western blot; WT: wild type. The sequence shown in Fig. 15E is:

DCBLD2 SS subdomains
MASRAVVRARRCPQCPQVRAAAAAPAWAALPLSRSLPPCSNSSSFSMPLFLLLLL VLLLLLEDAGA (SQ ID NO:X)

[0067] Figs. 16A-16F demonstrate that FITC-traC enhances VEGF/VEGFR2 signaling in endothelial cells, in accordance with some embodiments. Fig. 16A: Illustrative

immunofluorescence images showing FITC-traC (green) peptide penetration into the cells over time. Nuclei are stained blue with DAPI. Bar = 20μm. Fig. 16B: Illustrative immunofluorescent images showing co-localization of FITC-traC (green) with VEGFR2 (red) on the cell membrane (arrowheads) 15 minutes after adding FITC-traC to endothelial cells. Nuclei are stained blue with DAPI. Bar = 20μm. Fig. 16C: Western blot analysis of VEGF induced VEGFR2, ERK, and Akt phosphorylation with and without FITC-traC (n=7). Fig. 16D: Western blot analysis of the effect of FITC-traC on VEGF induced VEGFR2, ERK, and Akt phosphorylation in endothelial cells following siRNA-mediated DCBLD2 downregulation (n=5). Fig. 16E: Co-immunoprecipitation of FITC-traC with VEGFR2 but not PDGFRβ following the addition of FITC-traC to endothelial cell cultures. FITC immunoprecipitation is followed by Western blotting for VEGFR2, PDGFRβ, or FITC. Fig. 16F: Effect of FITC-traC on protein tyrosine phosphatases PTP1B and TCPTP interaction with VEGFR2 in endothelial cells. VEGFR2 immunoprecipitation is followed by Western blotting for VEGFR2, PTP1B or TCPTP. PTP1B: Protein tyrosine phosphatase 1B; TCPTP: T cell protein tyrosine phosphatase. ns: not significant; *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0001$. Statistical significance was determined by two-way ANOVA and Tukey's multiple comparison post hoc test (Fig. 16C) or by repeated measures one-way ANOVA and Holm-Šídák's multiple comparisons post hoc test (Fig. 16D).

[0068] Fig. 17A-17G demonstrate that the DCBLD2 SS FITC-traC peptide promotes VEGF and ischemia-induced angiogenesis *in vivo*, in accordance with some embodiments. Figs. 17A-17B: Illustrative photographs (Fig. 17A) and CD31 immunofluorescent staining (red, n=5, Fig. 17B) of matrigel plugs with or without VEGF and FITC-traC, collected after 5 days post-implantation. Nuclei are stained blue with DAPI. Bar = 200μm. Figs. 17C-17D: Illustrative slit-lamp photographs (Fig. 17C) and CD31 immunofluorescent staining (red, n=10, Fig. 17D) of corneas implanted with sustained- release pellets containing either FITC-traC or DMSO and with or without VEGF. Bar= 1mm. Figs. 17E-17G: Illustrative laser Doppler images and quantification of hindlimb blood flow recovery after femoral artery ligation in mice treated with FITC-traC compared to control animals. L: ligated, NL: non-ligated, T: tail. (n=5, Fig. 17E). Illustrative examples of the thigh (Fig. 17F) and calf (Fig. 17G) CD31 immunofluorescent staining (red) and its quantification, as well as Gapdh-normalized Cdh5 expression by real time PCR at day 7 post femoral artery ligation in mice treated with FITC-traC compared to control animals (n=5). Nuclei are stained blue with DAPI. Bar=200μm. *: $P<0.05$, **: $P<0.01$. Statistical significance was assessed by two-way

ANOVA and Tukey's multiple comparison test (Figs. 17B and 17D) or Student t-test (Figs. 17E, 17F and 17G).

[0069] Figs. 18A-18E demonstrate that a non-limiting poly leucine decapeptide mimics the effect of DCBLD2 SS traC on VEGF signaling, in accordance with some embodiments. Fig. 18A: Co-immunoprecipitation of the products of HA-tagged traC Part 1 or Part 2 constructs with VEGFR2 following their co-transfection in HEK 293T cells. HA immunoprecipitation is followed by western blotting for HA and VEGFR2. Fig. 18B: Co-immunoprecipitation of the products of HA-tagged traC Part 2 or its derivative constructs or a control HA-tagged GFP with VEGFR2 following their co-transfection in HEK 293T cells. HA immunoprecipitation is followed by western blotting for HA and VEGFR2. Fig. 18C: Co-immunoprecipitation of the products of HA- tagged traC Part 2 L5VL5 sequence, or 9 or 16 poly-L-leucine constructs with VEGFR2 following their co-transfection in HEK 293T cells. HA immunoprecipitation is followed by western blotting for HA and VEGFR2. Fig. 18D: Co-immunoprecipitation of the products of HA- tagged polyleucine constructs with VEGFR2 following their co-transfection in HEK 293T cells. HA immunoprecipitation is followed by western blotting for HA and VEGFR2. Fig. 18E: Western blot analysis of VEGF induced VEGFR2 and ERK phosphorylation with and without FITC-L10 peptide (n=5). GAPDH was used as a loading control, with separate blots for total proteins (tp) and phosphorylated proteins (pp). *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0001$. Statistical significance was determined by two-way ANOVA and Tukey's multiple comparison post-hoc test. The sequences shown in Figs. 18A-18B are also listed below:

tra-C peptide CSNSSSFSMPLFLLLLLVLLLLLEDAGA (SEQ ID NO:8)
Part 2 of tra-C peptide LLLLLVLLLLLEDAGA (SEQ ID NO:1)

[0070] Fig.19 illustrates certain aspects of the expression of DCBLD2 constructs in HEK 293T cells, in accordance with some embodiments. Western blot of protein extracts from HEK 293T cells transfected with DCBLD2 constructs I-V, showing expression of appropriate size products.

[0071] Figs. 20A-20B illustrate certain aspects of the signal peptide cleavage prediction, in accordance with some embodiments. SignalP 6.0 prediction of signal peptide cleavage sites

of DCBLD2 (Fig. 20A) and SHREW1 (Fig. 20B), showing the low probability of DCBLD2 SS cleavage. The sequences shown in Figs. 20A-20B are also listed below:

Human DCBLD2, N-terminal portion MASRAVVRARRCPQCPQVRAAAAAPAWAALPLSRSLPPCSNSSSFSMPLFLLLLL VLLLLLEDAGAQQGD (SEQ ID NO:11)
Human SHREW1, N-terminal portion MWIQQLLGLSSMSIRWPGRPLGSHAWILIAMFQLAVDLPACEALGPGPEFWLLPRS PPRPPRLWSFRSGQ (SEQ ID NO:12)

[0072] Fig. 21 illustrates certain aspects of the DCBLD2 SS cleavage determination, in accordance with some embodiments. HA immunoprecipitation of dually (N-terminal FLAG and C-terminal HA) tagged DCBLD2 and its mutant variants, where the one or both alanine residues in the putative A-X-A signal peptidase cleavage site of DCBLD2 is replaced with glycine, transfected in HEK293T cells followed by FLAG, HA and DCBLD2 immunoblotting.

[0073] Fig. 22 demonstrates that VEGF-induced ERK phosphorylation in Dcbld2-/- MLEC transfected with DCBLD2-SS-GFP-HA or GFP-HA constructs, in accordance with some embodiments.

[0074] Fig. 23 illustrates certain aspects of the hydrophobicity analysis of DCBLD2 SS subdomains by ProtScale, in accordance with some embodiments. The sequence shown in Fig. 23 is also produced below:

DCBLD2 SS subdomains MASRAVVRARRCPQCPQVRAAAAAPAWAALPLSRSLPPCSNSSSFSMPLFLLLLL VLLLLLEDAGA (SQ ID NO:11)

[0075] Fig. 24 illustrates certain aspects of the DeepTMHMM prediction of transmembrane regions within DCBLD2 SS subdomains, in accordance with some embodiments.

[0076] Fig. 25 illustrates certain aspects of the VEGF signaling in endothelial cells pretreated with FITC-traC or a scrambled homologue (n=3), in accordance with some embodiments. ns: not significant, **: $P<0.01$, ****: $P<0.0001$. Statistical significance was determined by two-way ANOVA and Tukey's multiple comparison post hoc test.

[0077] Fig. 26 illustrates certain aspects of the FITC-traC dose-response on VEGF signaling in endothelial cells, in accordance with some embodiments. Illustrative Western

blot and quantitative analysis of VEGF-induced VEGFR2, AKT, and ERK phosphorylation (n=4). *: P<0.05, **: P<0.01. Statistical significance was determined by two-way ANOVA and Tukey's multiple comparison post hoc test.

[0078] Fig. 27 provides the Western blots showing the effect of pre-heating (120 °C, 50 minutes) on FITC-traC potentiation of VEGF signaling in endothelial cells, in accordance with some embodiments.

[0079] Fig. 28 provides the Western blots showing the effect of FITC-antennapedia and FITC-traC on VEGF signaling in endothelial cells, in accordance with some embodiments.

[0080] Fig. 29 illustrates certain aspects of the specificity of FITC-traC interaction with VEGFR2, in accordance with some embodiments. Co-immunoprecipitation of FITC-traC with VEGFR2 but not VEGFR1 or PDGFRβ following the addition of FITC-traC to HEK293T cells cotransfected with VEGFR1, VEGFR2, or PDGFRβ. FITC immunoprecipitation is followed by Western blotting for VEGFR2, VEGFR1, PDGFRβ, or FITC.

[0081] Figs. 30A-30B illustrate certain aspects of the quantitative reverse transcription PCR analysis of Gapdh-normalized Cdh5 expression in the contralateral non-ligated thighs (Fig. 30A) and calves (Fig. 30B) at day 7 post femoral artery ligation in mice treated with FITC-traC compared to control animals (n=5). Statistical significance was evaluated using the Student t-test.

[0082] Figs. 31A-31B show an alignment of rat, mouse and human DCBLD2 protein sequences, in accordance with some embodiments. The mouse and rat sequences contain a L9 sequence instead of the L5VL5 sequence in human DCBLD2. The sequences shown in Figs. 31A-31B are also listed below:

Rat DCBLD2 protein:
MASRAPLRAARSPQDPGGRAAPAATGRAPLPSAGWCPLPPGRNSSSRPRLLLLLLL
LLPDAGAQKGDGCGHTVLGPESGTLTSINYPHTYPNSTVCKWEIRVKTGERIRIKF
GDFDIEDSDYCHLNYLKIFNGIGVSRTEIGKYCGLGLQMNQSIESKGSEITVLFMSGI
HASGRGFLASYSVIDKQDLITCLDTVSNFLEPEFSKYCPAGCLLPFAEISGTIPHGYR
DSSPLCMAGIHAGVVSDVLGGQISVVISKGTPYYESSLANNVTSMVGYLSTSLFTF
KTSGCYGTLGMESGVIADPQITASSVLEWTDHMGQENSWKPEKARLRKPGPPWA
AFATDEHQWLQIDLNKEKKITGIVTTGSTLIEHNYYVSAYRVLYSDDGQKWTVYR
EPGAAQDKIFQGNKDYHKDVRNNFLPPIIARFIRVNPVQWQQKIAMKVELLGCQF
TLKGRLPKLTQPPPPRNSNNLKNTTVHPKLGRAPKFTQALQPRSRNDLPLLPAQTT

ATPDVKNTTVTPSVTKDVALAAVLVPVLVMALTTLILILVCAWHWRNRKKKAEG TYDLPHWDRAGWWKGVKQLLPAKSVEHEETPVRYSNSEVSHLSPREVTTVLQAD SAEYAQPLVGGIVGTLHQRSTFKPEEGKEASYADLDPYNAPVQEVYHAYAEPLPV TGPEYATPIVMDMSGHSTASVGLPSTSTFRTAGNQPPALVGTYNTLLSRTDSCSSG QAQYDTPKGGKPAAAPEELVYQVPQSTQEASGAGRDEKFDAFKETL (SEQ ID NO:13)
Mouse DCBLD2 protein: MASRAPLRAARSPQGPGGPAAPAATGRAALPSAGCCPLPPGRNSSSRPRLLLLLLL LLQDAGGQQGDGCGHTVLGPESGTLTSINYPHTYPNSTVCEWEIRVRTGERIRIKF GDFDIEDSDYCHLNYLKIFNGIGVSRTEIGKYCGLGLQMNQSIESKGSEVTVLFMS GTHAAGRGFLASYSVIDKEDLITCLDTVSNFLEPEFSKYCPAGCLLPFAEISGTIPHG YRDSSPLCMAGIHAGVVSNVLGGQISIVISKGTPYYESSLANNVTSTVGYLSASLFT FKTSGCYGTLGMESGVIADPQITASSALEWTDHMGQENSWTAEKARLRKPGPPW AAFATDEHQWLQIDLNKEKKITGIVTTGSTMIEHSYYVSAYRVLYSDDGQRWTVY REPGVDQDKIFQGNKDYHKDVRNNFLPPIIARFIRVNPVQWQQKIAMKVELLGCQ FTLKGRLPKLTPPPRNGNNLRNTTARPKLGKGRAPKFTQVLQPRSRNELPVQPAET TTTPDIKNTTVTPSVTKDVALAAVLVPVLVMALTTLILILVCAWHWRNRKKKTEG AYDLPHWDRAGWWKGMKQLLPAKSVDHEETPVRYSTSEVSHLSAREVTTVLQA DSAEYAQPLVGGIVGTLHQRSTFKPEEGKEAGYADLDPYNSPMQEVYHAYAEPLP VTGPEYATPIVMDMSGHPTASVGLPSTSTFKTAGTQPHALVGTYNTLLSRTDSCSS GQAQYDTPKGGKSAATPEELVYQVPQSTQELSGAGRDEKFDAFKEIL (SEQ ID NO:14)
Human DCBLD2 protein: MASRAVVRARRCPQCPQVRAAAAAPAWAALPLSRSLPPCSNSSSFSMPLFLLLLL VLLLLLEDAGAQQGDGCGHTVLGPESGTLTSINYPQTYPNSTVCEWEIRVKMGER VRIKFGDFDIEDSDSCHFNYLRIYNGIGVSRTEIGKYCGLGLQMNHSIESKGNEITLL FMSGIHVSGRGFLASYSVIDKQDLITCLDTASNFLEPEFSKYCPAGCLLPFAEISGTIP HGYRDSSPLCMAGVHAGVVSNTLGGQISVVISKGIPYYESSLANNVTSVVGHLSTS LFTFKTSGCYGTLGMESGVIADPQITASSVLEWTDHTGQENSWKPKKARLKKPGP PWAAFATDEYQWLQIDLNKEKKITGIITTGSTMVEHNYYVSAYRILYSDDGQKWT VYREPGVEQDKIFQGNKDYHQDVRNNFLPPIIARFIRVNPTQWQQKIAMKMELLG CQFIPKGRPPKLTQPPPPRNSNDLKNTTAPPKIAKGRAPKFTQPLQPRSSNEFPAQTE QTTASPDIRNTTVTPNVTKDVALAAVLVPVLVMVLTTLILILVCAWHWRNRKKKT

EGTYDLPYWDRAGWWKGMKQFLPAKAVDHEETPVRYSSSEVNHLSPREVTTVL QADSAEYAQPLVGGIVGTLHQRSTFKPEEGKEAGYADLDPYNSPGQEVYHAYAEP LPITGPEYATPIIMDMSGHPTTSVGQPSTSTFKATGNQPPPLVGTYNTLLSRTDSCSS AQAQYDTPKAGKPGLPAPDELVYQVPQSTQEVSGAGRDGECDVFKEIL (SEQ ID NO:15)

DETAILED DESCRIPTION

[0083] The following disclosure provides many different embodiments, or examples, for implementing different features of the provided subject matter. Specific examples of components and arrangements are described below to simplify the present disclosure. These are, of course, merely examples and are not intended to be limiting. For example, the formation of a first feature over or on a second feature in the description that follows may include embodiments in which the first and second features are formed in direct contact, and may also include embodiments in which additional features may be formed between the first and second features, such that the first and second features may not be in direct contact. In addition, the present disclosure may repeat reference numerals and/or letters in the various examples. This repetition is for the purpose of simplicity and clarity and does not in itself dictate a relationship between the various embodiments and/or configurations discussed.

Definitions

[0084] As used herein, each of the following terms has the meaning associated with it in this section. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Generally, the nomenclature used herein and the laboratory procedures in animal pharmacology, pharmaceutical science, peptide chemistry, and organic chemistry are those well-known and commonly employed in the art. It should be understood that the order of steps or order for performing certain actions is immaterial, so long as the present teachings remain operable. Any use of section headings is intended to aid reading of the document and is not to be interpreted as limiting; information that is relevant to a section heading may occur within or outside of that particular section. All publications, patents, and patent documents referred to in this document are incorporated by reference herein in their entirety, as though individually incorporated by reference.

[0085] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the

element or component can be any one of the recited elements or components and can be selected from a group consisting of two or more of the recited elements or components.

[0086] In the methods described herein, the acts can be carried out in any order, except when a temporal or operational sequence is explicitly recited. Furthermore, specified acts can be carried out concurrently unless explicit claim language recites that they be carried out separately. For example, a claimed act of doing X and a claimed act of doing Y can be conducted simultaneously within a single operation, and the resulting process will fall within the literal scope of the claimed process.

[0087] In this document, the terms "a," "an," or "the" are used to include one or more than one unless the context clearly dictates otherwise. The term "or" is used to refer to a nonexclusive "or" unless otherwise indicated. The statement "at least one of A and B" or "at least one of A or B" has the same meaning as "A, B, or A and B."

[0088] "About" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±20% or ±10%, in certain embodiments ±5%, in certain embodiments ±1%, in certain embodiments ±0.1% from the specified value, as such variations are appropriate to perform the disclosed methods.

[0089] A "disease" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

[0090] A "disorder" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

[0091] A disease or disorder is "alleviated" if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

[0092] In one aspect, the terms "co-administered" and "co-administration" as relating to a subject refer to administering to the subject a compound and/or composition of the disclosure along with a compound and/or composition that may also treat or prevent a disease or disorder contemplated herein. In certain embodiments, the co-administered compounds and/or compositions are administered separately, or in any kind of combination as part of a single therapeutic approach. The co-administered compound and/or composition may be formulated in any kind of combinations as mixtures of solids and liquids under a variety of solid, gel, and liquid formulations, and as a solution.

[0093] As used herein, the term "pharmaceutical composition" or "composition" refers to a mixture of at least one compound useful within the disclosure with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient. Multiple techniques of administering a compound exist in the art including, but not limited to, subcutaneous, intravenous, oral, aerosol, inhalational, rectal, vaginal, transdermal, intranasal, buccal, sublingual, parenteral, intrathecal, intragastrical, ophthalmic, pulmonary, and topical administration.

[0094] As used herein, the term "pharmaceutically acceptable" refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

[0095] As used herein, the term "pharmaceutically acceptable carrier" means a pharmaceutically acceptable material, composition or carrier, such as a liquid or solid filler, stabilizer, dispersing agent, suspending agent, diluent, excipient, thickening agent, solvent or encapsulating material, involved in carrying or transporting a compound useful within the disclosure within or to the patient such that it may perform its intended function. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation, including the compound useful within the disclosure, and not injurious to the patient. Some examples of materials that may serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives. As used herein, "pharmaceutically acceptable carrier" also includes any and all coatings, antibacterial and antifungal agents, and absorption delaying agents, and the like that are compatible with the activity of the compound useful within the disclosure, and are physiologically acceptable to the patient. The "pharmaceutically acceptable carrier" may further include a pharmaceutically acceptable salt of the compound useful within the disclosure. Other additional ingredients that may be included in the pharmaceutical compositions used in the practice of the disclosure are known in the art and described, for example in Remington's Pharmaceutical Sciences (Genaro, Ed., Mack Publishing Co., 1985, Easton, PA), which is incorporated herein by reference.

[0096] As used herein, the language "pharmaceutically acceptable salt" refers to a salt of the administered compound prepared from pharmaceutically acceptable non-toxic acids and bases, including inorganic acids, inorganic bases, organic acids, inorganic bases, solvates, hydrates, and clathrates thereof.

[0097] As used herein, a "pharmaceutically effective amount," "therapeutically effective amount," or "effective amount" of a compound is that amount of compound that is sufficient to provide a beneficial effect to the subject to which the compound is administered.

[0098] As used herein, the term "prevent" or "prevention" means no disorder or disease development if none had occurred, or no further disorder or disease development if there had already been development of the disorder or disease. Also considered is the ability of one to prevent some or all of the symptoms associated with the disorder or disease.

[0099] As used herein, the terms "subject" and "individual" and "patient" can be used interchangeably and may refer to a human or non-human mammal or a bird. Non-human mammals include, for example, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. In certain embodiments, the subject is human.

[00100] As used herein, the term "treatment" or "treating" is defined as the application or administration of a therapeutic agent, *i.e.*, a compound useful within the disclosure (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (*e.g.*, for diagnosis or *ex vivo* applications), who has a disease or disorder and/or a symptom of a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder and/or the symptoms of the disease or disorder. Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

Method of Promoting Neovascularization and/or Hair Growth

[00101] In the present study, the signal sequence of endothelial and smooth muscle cell-derived neuropilin-like molecule (ESDN) were found to be able to interact with vascular endothelial growth factor receptor 2 (VEGFR2), activating VEGFR2 signaling, and promote neovascularization. Mapping studies of the signal sequence led to the discovery that the minimum essential sequence in the signal sequence is an eleven-residue long section including ten leucine residues and one valine residues (see e.g., Figs. 8D-8E). Further studies demonstrated that any peptides that include a section of certain length range, and has a sufficiently high leucine content, are able to interact with VEGFR2 and activate VEGFR2 signaling (see e.g., Figs. 8D-8G).

[00102] Furthermore, when performing experiments of the present study, it was observed that mice that were administered some of the peptides herein topically grew hair faster . It has

been reported that VEGF pathway and VEGF-mediated angiogenesis promote hair growth, as well. (Yano *et al.*, *J Clin Invest.* 2001 Feb;107(4):409-17).

[00103] Accordingly, in some aspect, the present invention is directed to a method of promoting neovascularization in a subject in need thereof. In some aspects, the present invention is directed to a method of promoting hair growth in a subject in need thereof.

[00104] In some embodiments, the method includes: administering to the subject a compound including a polypeptide. In some embodiments, the compound and/or the polypeptide is/are capable of activating VEGFR2 signaling. In some embodiments, the polypeptide includes a first peptide capable for activating VEGFR2 signaling. In some embodiments, the first peptide contain a poly-leucine. It is worth noting that nucleic acids encoding the polypeptide herein are considered within the scope of the present invention. In some embodiments, the compound is formulated as a composition, such as a pharmaceutical composition with one or more pharmaceutically acceptable carriers.

[00105] The present study discovered that poly-leucine sections of certain lengths are able to interact with VEGFR2, activate VEGFR2 signaling, and promote neovascularization.

[00106] Accordingly, in some embodiments, the first peptide ranges in length from about 6 amino acid residues to about 14 amino acid residues, such as about 7 amino acid residues to about 12 amino acid residues, or about 8 amino acid residues to about 11 amino acid residues. In some embodiments, the peptide has a length of about 6 amino acid residues, about 7 amino acid residues, about 8 amino acid residues, about 9 amino acid residues, about 10 amino acid residues, about 11 amino acid residues, about 12 amino acid residues, about 13 amino acid residues, about 14 amino acid residues, or any ranges therebetween.

[00107] In some embodiments, the percentage of the amino acid residues in the first peptide that are leucine residues is 80% or higher, such as 85% or higher, 90% or higher, 95% or higher, or 100%. In some embodiments, the first peptide comprises no non-leucine amino acid residue (i.e., all residues of the first peptide are leucine. In some embodiments, the first peptide comprises one non-leucine amino acid residue. In some embodiments, the first peptide comprises two independently selected non-leucine amino acid residue. In some embodiments, the first peptide comprises three independently selected non-leucine amino acid residue. In some embodiments, the first peptide comprises four independently selected non-leucine amino acid residue. In some embodiments, the first peptide comprises five independently selected non-leucine amino acid residue.

[00108] The present study discovered that, when the poly-leucine section in the peptide is too long, such as longer than 14 amino acid residues, the abilities of the poly-leucine section

to interact with VEGFR2 and activate VEGFR2 signaling become diminished. Accordingly, in some embodiments, the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than 80% or higher (such as 85% or higher, 90% or higher, 95% or higher, or 100%) of the amino acid residues as leucine residues. Alternatively, in some embodiments, the compound does not comprise a peptide sequence that includes more than 14 continuous leucine residues.

[00109] It should be noted that a polypeptide can include one, two, or more of the poly-leucine sections, as long as the poly-leucine sections are not close enough to each other to create an uninterrupted poly-leucine section of 14 amino acids or more. Accordingly, in some embodiments, the polypeptide herein comprises a plurality of the first peptides as contemplated herein.

[00110] One of ordinary skill in the art would understand that the local environments in the body of the subject where neovascularization is needed are sometimes aqueous in nature (i.e., where the predominant solvent is water). Accordingly, in some embodiments, the compound is soluble in water.

[00111] As used herein, the term "soluble in water" means having a solubility of 1 mg/ml or larger, such as 2 mg/ml or larger, 5 mg/ml or larger, 10 mg/ml or larger, 20 mg/ml or larger, 50 mg/ml or larger, 100 mg/ml or larger, 200 mg/ml or larger, or 500 mg/ml or larger.

[00112] One of ordinary skill in the art would understand that leucine is hydrophobic, and a peptide in which 80% or more of the amino acid residues are leucine is unlikely to be soluble in water. Accordingly, in some embodiments, the compound further includes a hydrophilic motif. In certain embodiments, the hydrophilic motif increases solubility of the compound in water.

[00113] In some embodiments, the hydrophilic motif is a second peptide which is hydrophilic. In some embodiments, the hydrophilic motif includes one or more peptides, for example, attached to either or both of the N-terminal and the C-terminal of the first peptide. In some embodiments, the hydrophilic motif and the first peptide are of the same peptide chain in which all of the amino acid residues are linked via peptide bonds. In some embodiments, the hydrophilic motif and the first peptide are linked via chemical bonds other than peptide bonds.

[00114] The present study has discovered that the "traC" peptide, which includes only 17 residues apart from the poly-leucine section is able to interact and activate VEGFR2 (see e.g., Fig. 3C-E). The present study has further discovered that even a protein as large as the green fluorescence protein (GFP), which composed of 238 amino acids and has a molecular weight

of 27 kDa (i.e., significantly larger than the poly-leucine section), can be attached to the poly-leucine section without affecting the interaction between the poly-leucine section and VEGFR2 (see e.g., Figs. 2D-2E). As such, the hydrophilic motif is not particularly limited. Among the 20 amino acids, arginine (R), asparagine (N), aspartic acid (D), glutamine (Q), glutamic acid (E), lysine (K), serine (S), and threonine (T) are considered hydrophilic. In some embodiments, the presence of the hydrophilic motif brings a percentage of hydrophilic residues in the polypeptide of the compound to about 20% or higher, such as about 30% or higher, about 40% or higher, about 50% or higher, or about 60% or higher. In some embodiments, the hydrophilic motif has a tertiary structure such that some of the hydrophobic amino acid residues are buried inside the tertiary structure. According to these embodiments, the percentage of hydrophilic residues in the polypeptide of the compound may be lower than 20% as the percentage of the residues exposed on the surface of the tertiary structure, and therefore the overall hydrophilicity, are higher than the raw number indicates.

[00115] In some embodiments, the hydrophilic motif includes one or more polyethylene glycol (PEG) groups, one or more saccharide groups such as chitosan groups, one of more bis-ethylene glycol groups, tri-ethylene glycol group, sulfonation (such as on the tyrosine residues). Other non-limiting hydrophilic motifs that can be attached to increase the hydrophilicity include those described in US 2004/0147728 A1, the entirety of the reference is hereby incorporated herein by reference. In some embodiments, the hydrophilic motifs are attached to the peptide via a covalent bond.

[00116] In some embodiments, the compound is not soluble or has low solubility in water. Since transdermal or transcutaneous applications would benefit from hydrophobicity which is an inherent characteristic of peptides rich in leucine residues, in some embodiments, the peptide is not attached to a hydrophilic motif, or is attached to a hydrophobic motif.

[00117] Regardless of the solubility, the compound can be formulated with a delivery vehicle. In some embodiments, the delivery vehicle is a particle having a hydrophilic outer surface, and a hydrophobic inner side for containing hydrophobic compounds. None limiting examples of vehicles for delivering compounds, such as hydrophobic compounds with low solubility in water includes pluronic gel, methylcellulose, hydroxyethylcellulose, various scaffolds, Matrigel, or nanoparticles such as liposomes and micelles.

[00118] In some embodiments, the polypeptide and/or first peptide of the compound includes the complete or part of the amino acid sequence of endothelial and smooth muscle cell-derived neuropilin-like molecule (ESDN) protein. In some embodiments, ESDN is the

protein having the sequence set forth in SEQ ID NO:15, isoforms thereof or orthologs in non-human animals.

Human ESDN protein (NP_563615.3)
MASRAVVRARRCPQCPQVRAAAAAPAWAALPLSRSLPPCSNSSSFSMPLFLLLLL VLLLLLEDAGAQQGDGCGHTVLGPESGTLTSINYPQTYPNSTVCEWEIRVKMGER VRIKFGDFDIEDSDSCHFNYLRIYNGIGVSRTEIGKYCGLGLQMNHSIESKGNEITLL FMSGIHVSGRGFLASYSVIDKQDLITCLDTASNFLEPEFSKYCPAGCLLPFAEISGTIP HGYRDSSPLCMAGVHAGVVSNTLGGQISVVISKGIPYYESSLANNVTSVVGHLSTS LFTFKTSGCYGTLGMESGVIADPQITASSVLEWTDHTGQENSWKPKKARLKKPGP PWAAFATDEYQWLQIDLNKEKKITGIITTGSTMVEHNYYVSAYRILYSDDGQKWT VYREPGVEQDKIFQGNKDYHQDVRNNFLPPIIARFIRVNPTQWQQKIAMKMELLG CQFIPKGRPPKLTQPPPPRNSNDLKNTTAPPKIAKGRAPKFTQPLQPRSSNEFPAQTE QTTASPDIRNTTVTPNVTKDVALAAVLVPVLVMVLTTLILILVCAWHWRNRKKKT EGTYDLPYWDRAGWWKGMKQFLPAKAVDHEETPVRYSSSEVNHLSPREVTTVL QADSAEYAQPLVGGIVGTLHQRSTFKPEEGKEAGYADLDPYNSPGQEVYHAYAEP LPITGPEYATPIIMDMSGHPTTSVGQPSTSTFKATGNQPPPLVGTYNTLLSRTDSCSS AQAQYDTPKAGKPGLPAPDELVYQVPQSTQEVSGAGRDGECDVFKEIL (SEQ ID NO:15)

[00119] In some embodiments, the polypeptide and/or first peptide includes residues 1-66 of the sequence set forth in SEQ ID NO:15. In some embodiments, the polypeptide and/or first peptide includes residues 39-66 of the sequence set forth in SEQ ID NO:15. In some embodiments, the polypeptide and/or first peptide includes residues 51-66 of the sequence set forth in SEQ ID NO:15. In some embodiments, the polypeptide and/or first peptide includes residues 51-61 of the sequence set forth in SEQ ID NO:15.

[00120] In some embodiments, the method promotes at least one selected from the group consisting of angiogenesis and arteriogenesis in the subject.

[00121] Impaired wound revascularization is a hallmark of chronic wounds. Accordingly, in some embodiments, the subject suffers from a chronic wound. In some embodiments, the method promotes neovascularization in or near the chronic wound. As used herein, the term "chronic wound" refers to a wound that has shown no significant progress toward healing (such as failed to achieve sufficient healing) in a prolonged time period, such as about 7 days

or longer, about 10 days or longer, about 2 weeks or longer, about 15 days or longer, about 20 days or longer, about 3 weeks or longer, about 4 weeks or longer, or about 30 days or longer.

[00122] Non-limiting examples of chronic wounds includes nonhealing wounds; infected wounds, such as infected surgical wounds or infected traumatic wounds; or ulcers, such as diabetic ulcer (e.g., diabetic foot ulcer), arterial ulcers, venous ulcers, pressure ulcers, ischemic ulcers, wounds associated with immunosuppression or radiation, and the like.

[00123] In some embodiments, the subject is suffering from an acute wound, which would benefit from faster healings, as well.

[00124] Without wishing to be bound by theory, it is hypothesized that peptides including poly-leucine are able to compete with protein-tyrosine phosphatase 1B (PTP1B) or T-cell protein tyrosine phosphatase (TCPTP) for interaction with vascular endothelial growth factor receptor 2 (VEGFR2), thereby reducing the phosphate activity exert on VEGFR2 by PTP1B or TCPTP. Since PTP1B and TCPTP counter the tyrosine phosphorylation of VEGFR2 required for kinase activation, peptides including poly-leucine are able to activate VEGFR2. Accordingly, in some embodiments, compound reduces phosphatase activity exerted on VEGFR2 by PTP1B or TCPTP.

[00125] The present study discovered that, when traC peptide (a non-limiting example of the compound that includes a poly-leucine section including peptide) is co-administered with vascular endothelial growth factor (VEGF), the vascularization effects were significantly stronger than VEGF along or traC alone (see e.g., Figs. 5A-5B, 6A-6B). Accordingly, in some embodiments, the method further includes administering to the subject an effective amount of vascular endothelial growth factor (VEGF).

[00126] In some embodiments, the subject is a mammal, such as a human.

Method of Promoting Wound Healing

[00127] In the present study, the signal sequence of endothelial and smooth muscle cell-derived neuropilin-like molecule (ESDN) were found to be able to interact with vascular endothelial growth factor receptor 2 (VEGFR2), activating VEGFR2 signaling, and promote wound healing (see e.g, Figs. 7A-7D). Mapping studies of the signal sequence led to the discovery that the minimum essential sequence in the signal sequence is an eleven-residue long section including ten leucine residues and one valine residues (see e.g., Figs. 8C-8E). Further studies demonstrated that any peptides that include a section of certain length range, and has a sufficiently high leucine content are able to interact with VEGFR2 and activate VEGFR2 signaling (see e.g., Figs. 8D-8G).

[00128] Accordingly, in some embodiments, the present specification is directed to a method of promoting wound healing in a subject in need thereof.

[00129] In some embodiments, the method includes administering to the subject an effective amount of a compound and/or composition that promotes wound healing. In some embodiments, the compound, the composition, as well as the formulations thereof, is the same as or similar to those detailed elsewhere herein, such as in the "Method of Promoting Neovascularization" section.

[00130] In some embodiments, the type of the wound and/or the nature of the subject are the same as or similar to those detailed elsewhere herein, such as in the "Method of Promoting Neovascularization and/or Hair Growth" section.

[00131] In some embodiments, the method further includes administering to the subject an effective amount of vascular endothelial growth factor (VEGF).

Wound Dressing

[00132] In the present study, it was discovered that compounds (such as polypeptides) including leucine rich peptides of certain length ranges herein are able to promote neovascularization and wound healing. The compounds herein can be administered topically, and are therefore suitable for being included in wound dressings that are placed directly against the wound to speed up wound healing.

[00133] Accordingly, in some aspects, the present invention is directed to a wound dressing.

[00134] In some embodiments, the wound dressing includes a base dressing material and a compound for promoting neovascularization and/or wound healing.

[00135] In some embodiments, the compound for promoting neovascularization and/or wound healing is the same as or similar to those described in the "Method of Promoting Neovascularization and/or Hair Growth" section.

[00136] The base dressing material suitable for the wound dressing is not limited, as long as the base dressing material allows the compound attached thereto to be in contact with the wound when the wound dressing is applied. None limiting examples of base dressing material includes alginate dressing materials, biosynthetic dressing materials, collagen dressing materials, composite dressing materials, contact layer dressing materials, gauzes, hydrocolloid dressing materials, hydrofiber dressing materials, hydrogels, hydropolymer dressing materials, polyurethane foam dressing materials, skin substitute dressing materials, superabsorbent dressing materials, transparent film dressing materials, wound filles, wound pouches, or the like.

[00137] In some embodiments, the compound including the polypeptide herein is on a surface of or impregnated into the base dressing material.

[00138] In some embodiments, the wound dressing further includes an adhesive substrate, such that the wound dressing can be used as adhesive bandage, such as a Band-Aid.

Combination Therapies

[00139] In some embodiments, the method of treating, ameliorating, and/or preventing the disease/disorder includes administering to the subject the effective amount of at least one compound and/or composition contemplated within the disclosure.

[00140] In some embodiments, the composition for treating the disease/disorder herein includes at least one compound and/or composition contemplated within the disclosure.

[00141] In some embodiments, the subject is further administered at least one additional agent that treats, ameliorates, and/or prevents a disease and/or disorder contemplated herein. In other embodiments, the compound and the at least one additional agent are co-administered to the subject. In yet other embodiments, the compound and the at least one additional agent are co-formulated.

[00142] The compounds contemplated within the disclosure are intended to be useful in combination with one or more additional compounds. These additional compounds may comprise compounds of the present disclosure and/or at least one additional agent for treating the disease/disorder, and/or at least one additional agent that treats one or more diseases or disorders contemplated herein.

[00143] A synergistic effect may be calculated, for example, using suitable methods such as, for example, the Sigmoid-E_{max} equation (Holford & Scheiner, 1981, Clin. Pharmacokinet. 6:429-453), the equation of Loewe additivity (Loewe & Muischnek, 1926, Arch. Exp. Pathol Pharmacol. 114:313-326) and the median-effect equation (Chou & Talalay, 1984, Adv. Enzyme Regul. 22:27-55). Each equation referred to above may be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

Administration/Dosage/Formulations

[00144] The regimen of administration may affect what constitutes an effective amount. The therapeutic formulations contemplated within the disclosure may be administered to the subject either prior to or after the onset of a disease and/or disorder contemplated herein.

Further, several divided dosages, as well as staggered dosages may be administered daily or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, the dosages of the therapeutic formulations contemplated within the disclosure may be proportionally increased or decreased as indicated by the exigencies of the therapeutic or prophylactic situation.

[00145] Administration of the compositions contemplated within the disclosure to a patient, preferably a mammal, more preferably a human, may be carried out using known procedures, at dosages and for periods of time effective to treat a disease and/or disorder contemplated herein in the patient. An effective amount of the therapeutic compound necessary to achieve a therapeutic effect may vary according to factors such as the state of the disease or disorder in the patient; the age, sex, and weight of the patient; and the ability of the therapeutic compound contemplated within the disclosure to treat a disease and/or disorder contemplated herein in the patient. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A non-limiting example of an effective dose range for a therapeutic compound contemplated within the disclosure is from about 0.001 and 5,000 mg/kg of body weight/per day. One of ordinary skill in the art would be able to study the relevant factors and make the determination regarding the effective amount of the therapeutic compound without undue experimentation.

[00146] Actual dosage levels of the active ingredients in the pharmaceutical compositions contemplated within the disclosure may be varied so as to obtain an amount of the active ingredient that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[00147] In particular, the selected dosage level depends upon a variety of factors including the activity of the particular compound employed, the time of administration, the rate of excretion of the compound, the duration of the treatment, other drugs, compounds or materials used in combination with the compound, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well, known in the medical arts.

[00148] A medical doctor, *e.g.*, physician or veterinarian, having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds contemplated within the disclosure employed in the pharmaceutical composition at levels

lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[00149] In particular embodiments, it is especially advantageous to formulate the compound in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the patients to be treated; each unit containing a predetermined quantity of therapeutic compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical vehicle. The dosage unit forms contemplated within the disclosure are dictated by and directly dependent on (a) the unique characteristics of the therapeutic compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding/formulating such a therapeutic compound for the treatment of a disease and/or disorder contemplated herein.

[00150] In certain embodiments, the compounds of the disclosure are formulated as a composition with one or more pharmaceutically acceptable excipients or carriers. In certain embodiments, the pharmaceutical compositions of the disclosure comprise a therapeutically effective amount of a compound of the disclosure and a pharmaceutically acceptable carrier.

[00151] The carrier may be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it is preferable to include isotonic agents, for example, sugars, sodium chloride, or polyalcohols such as mannitol and sorbitol, in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate or gelatin.

[00152] In certain embodiments, the compositions of the disclosure are administered to the patient in dosages that range from one to five times per day or more. In another embodiment, the compositions of the disclosure are administered to the patient in range of dosages that include, but are not limited to, once every day, every two, days, every three days to once a week, and once every two weeks. It is readily apparent to one skilled in the art that the frequency of administration of the various combination compositions of the disclosure varies from individual to individual depending on many factors including, but not limited to, age,

disease or disorder to be treated, gender, overall health, and other factors. Thus, the disclosure should not be construed to be limited to any particular dosage regime and the precise dosage and composition to be administered to any patient is determined by the attending physical taking all other factors about the patient into account.

[00153] Compounds of the disclosure for administration may be in the range of from about 1 μg to about 10,000 mg, about 20 μg to about 9,500 mg, about 40 μg to about 9,000 mg, about 75 μg to about 8,500 mg, about 150 μg to about 7,500 mg, about 200 μg to about 7,000 mg, about 3050 μg to about 6,000 mg, about 500 μg to about 5,000 mg, about 750 μg to about 4,000 mg, about 1 mg to about 3,000 mg, about 10 mg to about 2,500 mg, about 20 mg to about 2,000 mg, about 25 mg to about 1,500 mg, about 30 mg to about 1,000 mg, about 40 mg to about 900 mg, about 50 mg to about 800 mg, about 60 mg to about 750 mg, about 70 mg to about 600 mg, about 80 mg to about 500 mg, and any and all whole or partial increments therebetween.

[00154] In some embodiments, the dose of a compound of the disclosure is from about 1 mg and about 2,500 mg. In some embodiments, a dose of a compound of the disclosure used in compositions described herein is less than about 10,000 mg, or less than about 8,000 mg, or less than about 6,000 mg, or less than about 5,000 mg, or less than about 3,000 mg, or less than about 2,000 mg, or less than about 1,000 mg, or less than about 500 mg, or less than about 200 mg, or less than about 50 mg. Similarly, in some embodiments, a dose of a second compound as described herein is less than about 1,000 mg, or less than about 800 mg, or less than about 600 mg, or less than about 500 mg, or less than about 400 mg, or less than about 300 mg, or less than about 200 mg, or less than about 100 mg, or less than about 50 mg, or less than about 40 mg, or less than about 30 mg, or less than about 25 mg, or less than about 20 mg, or less than about 15 mg, or less than about 10 mg, or less than about 5 mg, or less than about 2 mg, or less than about 1 mg, or less than about 0.5 mg, and any and all whole or partial increments thereof.

[00155] In certain embodiments, the present disclosure is directed to a packaged pharmaceutical composition comprising a container holding a therapeutically effective amount of a compound of the disclosure, alone or in combination with a second pharmaceutical agent; and instructions for using the compound to treat, prevent, or reduce one or more symptoms of the disease/disorder herein in a patient.

[00156] Formulations may be employed in admixtures with conventional excipients, *i.e.*, pharmaceutically acceptable organic or inorganic carrier substances suitable for intracranially, oral, parenteral, nasal, intravenous, subcutaneous, enteral, or any other suitable

mode of administration, known to the art. The pharmaceutical preparations may be sterilized and if desired mixed with auxiliary agents, *e.g.*, lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure buffers, coloring, flavoring and/or aromatic substances and the like. They may also be combined where desired with other active agents, *e.g.*, other analgesic agents.

[00157] Routes of administration of any of the compositions of the disclosure include oral, nasal, rectal, intravaginal, parenteral, buccal, sublingual or topical. The compounds for use in the disclosure may be formulated for administration by any suitable route, such as for oral or parenteral, for example, transdermal, transmucosal (*e.g.*, sublingual, lingual, (trans)buccal, (trans)urethral, vaginal (*e.g.*, trans- and perivaginally), (intra)nasal and (trans)rectal), intravesical, intrapulmonary, intraduodenal, intragastrical, intrathecal, subcutaneous, intramuscular, intradermal, intra-arterial, intravenous, intrabronchial, inhalation, and topical administration.

[00158] Suitable compositions and dosage forms include, for example, tablets, capsules, caplets, pills, gel caps, troches, dispersions, suspensions, solutions, syrups, granules, beads, transdermal patches, gels, powders, pellets, magmas, lozenges, creams, pastes, plasters, lotions, discs, suppositories, liquid sprays for nasal or oral administration, dry powder or aerosolized formulations for inhalation, compositions and formulations for intravesical administration and the like. It should be understood that the formulations and compositions that would be useful in the present disclosure are not limited to the particular formulations and compositions that are described herein.

Oral Administration

[00159] For oral application, particularly suitable are tablets, dragees, liquids, drops, suppositories, or capsules, caplets and gelcaps. The compositions intended for oral use may be prepared according to any method known in the art and such compositions may contain one or more agents selected from the group consisting of inert, non-toxic pharmaceutically excipients that are suitable for the manufacture of tablets. Such excipients include, for example an inert diluent such as lactose; granulating and disintegrating agents such as cornstarch; binding agents such as starch; and lubricating agents such as magnesium stearate. The tablets may be uncoated or they may be coated by known techniques for elegance or to delay the release of the active ingredients. Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert diluent.

[00160] For oral administration, the compounds of the disclosure may be in the form of tablets or capsules prepared by conventional means with pharmaceutically acceptable

excipients such as binding agents (*e.g.*, polyvinylpyrrolidone, hydroxypropylcellulose or hydroxypropylmethylcellulose); fillers (*e.g.*, cornstarch, lactose, microcrystalline cellulose or calcium phosphate); lubricants (*e.g.*, magnesium stearate, talc, or silica); disintegrates (*e.g.*, sodium starch glycollate); or wetting agents (*e.g.*, sodium lauryl sulphate). If desired, the tablets may be coated using suitable methods and coating materials such as OPADRY™ film coating systems available from Colorcon, West Point, Pa. (*e.g.*, OPADRY™ OY Type, OYC Type, Organic Enteric OY-P Type, Aqueous Enteric OY-A Type, OY-PM Type and OPADRY™ White, 32K18400). Liquid preparation for oral administration may be in the form of solutions, syrups or suspensions. The liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, methyl cellulose or hydrogenated edible fats); emulsifying agent (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters or ethyl alcohol); and preservatives (*e.g.*, methyl or propyl p-hydroxy benzoates or sorbic acid).

[00161] The present disclosure also includes a multi-layer tablet comprising a layer providing for the delayed release of one or more compounds of the disclosure, and a further layer providing for the immediate release of another medication. Using a wax/pH-sensitive polymer mix, a gastric insoluble composition may be obtained in which the active ingredient is entrapped, ensuring its delayed release.

Parenteral Administration

[00162] For parenteral administration, the compounds of the disclosure may be formulated for injection or infusion, for example, intravenous, intramuscular or subcutaneous injection or infusion, or for administration in a bolus dose and/or continuous infusion. Suspensions, solutions or emulsions in an oily or aqueous vehicle, optionally containing other formulatory agents such as suspending, stabilizing and/or dispersing agents may be used.

Additional Administration Forms

[00163] Additional dosage forms of this disclosure include dosage forms as described in U.S. Patents Nos. 6,340,475; 6,488,962; 6,451,808; 5,972,389; 5,582,837; and 5,007,790. Additional dosage forms of this disclosure also include dosage forms as described in U.S. Patent Applications Nos. 20030147952; 20030104062; 20030104053; 20030044466; 20030039688; and 20020051820. Additional dosage forms of this disclosure also include dosage forms as described in PCT Applications Nos. WO 03/35041; WO 03/35040; WO 03/35029; WO 03/35177; WO 03/35039; WO 02/96404; WO 02/32416; WO 01/97783; WO 01/56544; WO 01/32217; WO 98/55107; WO 98/11879; WO 97/47285; WO 93/18755; and WO 90/11757.

Controlled Release Formulations and Drug Delivery Systems

[00164] In certain embodiments, the formulations of the present disclosure may be, but are not limited to, short-term, rapid-offset, as well as controlled, for example, sustained release, delayed release and pulsatile release formulations.

[00165] The term sustained release is used in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that may, although not necessarily, result in substantially constant blood levels of a drug over an extended time period. The period of time may be as long as a month or more and should be a release which is longer that the same amount of agent administered in bolus form.

[00166] For sustained release, the compounds may be formulated with a suitable polymer or hydrophobic material which provides sustained release properties to the compounds. As such, the compounds for use the method of the disclosure may be administered in the form of microparticles, for example, by injection or in the form of wafers or discs by implantation.

[00167] In certain embodiments of the disclosure, the compounds of the disclosure are administered to a patient, alone or in combination with another pharmaceutical agent, using a sustained release formulation.

[00168] The term delayed release is used herein in its conventional sense to refer to a drug formulation that provides for an initial release of the drug after some delay following drug administration and that mat, although not necessarily, includes a delay of from about 10 minutes up to about 12 hours.

[00169] The term pulsatile release is used herein in its conventional sense to refer to a drug formulation that provides release of the drug in such a way as to produce pulsed plasma profiles of the drug after drug administration.

[00170] The term immediate release is used in its conventional sense to refer to a drug formulation that provides for release of the drug immediately after drug administration.

[00171] As used herein, short-term refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes and any or all whole or partial increments thereof after drug administration after drug administration.

[00172] As used herein, rapid-offset refers to any period of time up to and including about 8 hours, about 7 hours, about 6 hours, about 5 hours, about 4 hours, about 3 hours, about 2 hours, about 1 hour, about 40 minutes, about 20 minutes, or about 10 minutes, and any and all whole or partial increments thereof after drug administration.

Dosing

[00173] The therapeutically effective amount or dose of a compound of the present disclosure depends on the age, sex and weight of the patient, the current medical condition of the patient and the progression of disease/disorder in the patient being treated. The skilled artisan is able to determine appropriate dosages depending on these and other factors.

[00174] A suitable dose of a compound of the present disclosure may be in the range of from about 0.01 mg to about 5,000 mg per day, such as from about 0.1 mg to about 1,000 mg, for example, from about 1 mg to about 500 mg, such as about 5 mg to about 250 mg per day. The dose may be administered in a single dosage or in multiple dosages, for example from 1 to 4 or more times per day. When multiple dosages are used, the amount of each dosage may be the same or different. For example, a dose of 1 mg per day may be administered as two 0.5 mg doses, with about a 12-hour interval between doses.

[00175] It is understood that the amount of compound dosed per day may be administered, in non-limiting examples, every day, every other day, every 2 days, every 3 days, every 4 days, or every 5 days. For example, with every other day administration, a 5 mg per day dose may be initiated on Monday with a first subsequent 5 mg per day dose administered on Wednesday, a second subsequent 5 mg per day dose administered on Friday, and so on.

[00176] In the case wherein the patient's status does improve, upon the doctor's discretion the administration of the modulator of the disclosure is optionally given continuously; alternatively, the dose of drug being administered is temporarily reduced or temporarily suspended for a certain length of time (*i.e.*, a "drug holiday"). The length of the drug holiday optionally varies between 2 days and 1 year, including by way of example only, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 12 days, 15 days, 20 days, 28 days, 35 days, 50 days, 70 days, 100 days, 120 days, 150 days, 180 days, 200 days, 250 days, 280 days, 300 days, 320 days, 350 days, or 365 days. The dose reduction during a drug holiday includes from 10%-100%, including, by way of example only, 10%, 15%,20%,25%,30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 100%.

[00177] Once improvement of the patient's conditions has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, is reduced, as a function of the patient's condition, to a level at which the improved disease is retained. In certain embodiments, patients require intermittent treatment on a long-term basis upon any recurrence of symptoms and/or infection.

[00178] The compounds for use in the method of the disclosure may be formulated in unit dosage form. The term "unit dosage form" refers to physically discrete units suitable as

unitary dosage for patients undergoing treatment, with each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, optionally in association with a suitable pharmaceutical carrier. The unit dosage form may be for a single daily dose or one of multiple daily doses (*e.g.*, about 1 to 4 or more times per day). When multiple daily doses are used, the unit dosage form may be the same or different for each dose.

[00179] Toxicity and therapeutic efficacy of such therapeutic regimens are optionally determined in cell cultures or experimental animals, including, but not limited to, the determination of the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between the toxic and therapeutic effects is the therapeutic index, which is expressed as the ratio between LD_{50} and ED_{50}. Capsid assembly modulators exhibiting high therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are optionally used in formulating a range of dosage for use in human. The dosage of such capsid assembly modulators lies preferably within a range of circulating concentrations that include the ED_{50} with minimal toxicity. The dosage optionally varies within this range depending upon the dosage form employed and the route of administration utilized.

[00180] Those skilled in the art recognizes, or is able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures, embodiments, claims, and examples described herein. Such equivalents were considered to be within the scope of this disclosure and covered by the claims appended hereto. For example, it should be understood, that modifications in assay and/or reaction conditions, with art-recognized alternatives and using no more than routine experimentation, are within the scope of the present application.

[00181] It is to be understood that wherever values and ranges are provided herein, all values and ranges encompassed by these values and ranges, are meant to be encompassed within the scope of the present disclosure. Moreover, all values that fall within these ranges, as well as the upper or lower limits of a range of values, are also contemplated by the present application.

Examples

[00182] The present specification further describes in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless so specified. Thus, the present specification should in no way be construed as being limited to the following examples, but rather, should be

construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1-1: Post-targeting functions of a long signal sequence in angiogenesis

[00183] Angiogenesis, the process of new blood vessel formation from existing vessels, plays a key role in a number of physiological processes, including development and growth, adult organ regeneration, and wound healing . Angiogenesis is tightly controlled through numerous stimulators and inhibitors and its dysregulation, whether insufficient or excessive, contributes to a wide variety of diseases, ranging from malignancy, ischemic heart and peripheral artery disease, and neurodegeneration to proliferative retinopathy. Accordingly, modulation of this process provides several therapeutic opportunities.

[00184] Discoidin, CUB and LCCL domain containing 2 (DCBLD2), also known as endothelial and smooth muscle cell–derived neuropilin-like protein (ESDN) or CLCP1, was initially cloned from human coronary artery and highly metastatic lung cancer cells as a protein with an exceptionally long secretory signal sequence among eukaryotes. DCBLD2 is highly conserved among vertebrates and closely resembles the structure of neuropilins, major co-receptors for vascular endothelial cell growth factor (VEGF) receptors (VEGFRs), which play a critical role in angiogenesis. DCBLD2 is upregulated in human, rat, and mouse remodeling arteries, and regulates VEGF-induced endothelial cell (EC) proliferation, migration, and signal transduction, as well as developmental and adult angiogenesis.

[00185] Targeting of proteins to the endoplasmic reticulum (ER) and eventually cell membrane is mediated by signal sequences (SS). For type I transmembrane and secretory proteins, SS are typically 16 to 30 amino acid N-terminal extensions of the nascent proteins (After targeting the proteins, many SS are cleaved off by signal peptidase within the endoplasmic reticulum as a signal peptide (SP), while others remain an integral part of the mature protein, as signal anchor sequences. Generally, cleaved SP are rapidly degraded. However, some SP and their fragments are retained and have specific, post-targeting functions on their own. To date, however, most known examples of such SS post-targeting functions are of viral origin.

[00186] A typical SP has a tripartite structure with a hydrophobic, α-helical core (*h* region) surrounded by an N-terminal polar *n* region, and a C-terminal *c* region that contains the signal peptidase cleavage site. Based on proteome analysis by machine-learning systems, it is suggested that exceptionally long SS may have a bipartite 'NtraC' domain structure with potentially functional N-terminal (N-domain, 'N') and C-terminal (C-domain, 'C') domains

connected by a turn-rich linker area (transition area, 'tra'). The 66 amino acid SS of DCBLD2 is predicted to fit the 'NtraC' model.

[00187] The present study identified the DCBLD2 SS, and more specifically its 'traC' segment, as a key domain of DCBLD2 that interacts with VEGFR2 and promotes VEGF/VEGFR2 signaling in EC. Furthermore, an exogenous synthetic traC peptide promoted angiogenesis in vivo. The smallest unit in DCBLD2 SS that interacted with VEGFR2 was the 5LV5L sequence. As such, in addition to establishing a novel post-targeting function of signal sequences in regulating growth factor signaling, these findings introduce the DCBLD2 traC and its derivatives as promising candidates for therapeutic angiogenesis.

Example 1-2: The ESDN (DCBLD2) signal sequence is involved in interaction with VEGFR2

[00188] Distinct functional and structural unites of DCBLD2 include an unusually long SS, which in tandem with CUB, LCCL, and Discoidin domains form the extracellular segment. The transmembrane and intracellular domains comprise the rest of the molecule. To identify the key domain(s) of human DCBLD2 that interact with VEGFR2, the present study generated multiple plasmids containing various combinations of DCBLD2 domains linked to a C-terminal hemagglutinin (HA) tag (Fig. 1A I-V).

[00189] Upon co-transfection with human VEGFR2, each of these constructs expressed the appropriate-size protein in HEK 293T cells. Surprisingly, co-immunoprecipitation with an anti-VEGFR2 antibody showed that all of these products complex with VEGFR2 (Fig. 1B). Furthermore, while as reported previously, VEGF signaling was diminished in *Dcbld2*-deficient murine lung endothelial cells (MLEC), all these DCBLD2 constructs enhanced VEGF-induced ERK phosphorylation upon transfection in *Dcbld2*$^{-/-}$ MLEC (Fig. 1C). This led us to speculate that a common component of the constructs, i.e., the SS, might be involved in its interaction with VEGFR2 and enhancing VEGFR2 signaling.

[00190] To test this possibility, and to rule out HA as the VEGFR2 interacting component, the present study constructed additional plasmids composed of DCBLD2 SS, Shrew1 SS (another unusually long SS with a similar 'NtraC' domain structure (11)), or nothing in tandem with C-terminal green fluorescent protein (GFP)-HA tags (Fig. 1A VI - VIII). Upon co-transfection of these constructs with VEGFR2 in HEK 293T cells and HA immunoprecipitation, VEGFR2 could be pulled down by GFP-HA-tagged DCBLD2 SS, but not Shrew 1 SS or GFP-HA without any SS (Fig. 1D).

[00191] Next, the effect of DCBLD2 (ESDN) SS on VEGF/VEGFR2 signaling was evaluated. As expected, in HUVEC transfected with control siRNA (siCon) and pcDNA, VEGF stimulation increased ERK phosphorylation. In HUVEC transfected with ESDN siRNA (siESDN) and pcDNA, ERK phosphorylation in response to exogenous VEGF was attenuated. However, co-transfection with ESDN SS instead of pcDNA, reversed the impaired VEGF-induced ERK phosphorylation in ESDN deficient HUVECs. (Fig. 1E). Taken together, those data suggest the DCBLD2 SS is a key domain in its interaction with VEGFR2 and affects downstream VEGF signaling.

Example 1-3: The traC subdomain of ESDN SS interacts with VEGFR2

[00192] Based on the 'NtraC' model, the DCBLD2 SS 'N' subdomain contains 38 amino acid residues, and the 24 amino acid C-terminal residues constitute the 'C' subdomain, with those two separated by a short 'tra' subdomain (Fig. 2A). Hydrophobicity analysis by ProtScale (Fig. 2B) and the transmembrane helix prediction by DeepTMHMM (Fig. 2C) indicated significant differences between different DCBLD2 SS subdomains, with the 'C' subdomain flanked by 'tra' predicted to be hydrophobic and transmembrane.

[00193] To identify the subdomain of DCBLD2 SS which interacts with VEGFR2, the present study synthesized additional constructs encompassing different DCBLD2 SS subdomains (Fig. 2D), which were co-transfected with VEGFR2 into HEK 293T cells. Co-immunoprecipitation studies indicated that the 'traC' subdomain, but not the 'N' subdomain, is involved in the DCBLD2-VEGFR2 interaction (Fig. 2E).

Example 1-4: FITC-TraC enhances VEGF/VEGFR2 signaling in endothelial cells

[00194] Given the interaction between the DCBLD2 SS traC fragment and VEGFR2, the present study sought to investigate whether a synthetic traC peptide, labeled with FITC for tracking purposes, can modulate VEGF signaling in EC. As a first step in evaluating the effects of FITC-traC in EC, the present study followed by fluorescence imaging the fate of the labeled peptide added to EC culture media. Interestingly, FITC-traC was rapidly taken up by HUVEC with the peptide detected in the cytoplasm as early as 1 minute after its addition to the medium. The cytoplasmic accumulation of the peptide increased over time and by 15 minutes it was also detectable in the cell membrane (Fig. 3A), where it co-localized with VEFR2 (Fig. 3B). Accordingly, the 15-minute time point was selected for the next set of studies.

[00195] Next, the present study evaluated the effect of synthetic DCBLD2 traC on VEGF signaling. Pretreatment of HUVEC with FITC-traC peptide (1μM in 1% DMSO, 15 minutes) significantly increased VEGF-induced VEGFR2, ERK and AKT phosphorylation compared to HUVEC pre-treated with vehicle control (Fig. 3C). A dose-response study showed that the effect of FITC-traC peptide on VEGF signaling is concentration-dependent (Fig.3D). Of note, higher concentrations of FITC-traC induced AKT and ERK phosphorylation independent of VEGF. Pre-heating of FITC-traC peptide abrogated its effect on VEGF signaling. To explore the molecular mechanisms of this observation, the present study first determined whether the effect of FITC-traC is simply related to its cell penetrating property. A similar set of experiments showed that unlike FITC-traC, pre-treatment of HUVEC with a classical cell penetrating peptide, antennapedia, does not affect downstream VEGF signaling. Next, the present study sought to determine whether FITC-traC peptide can reverse the inhibitory effect of DCBLD2 downregulation on VEGF signaling in EC. As expected, DCBLD2 siRNA significantly reduced VEGF-induced VEGFR2, ERK and AKT phosphorylation in HUVEC (Fig. 3E). This impaired VEGF/VEGFR2 signaling was fully restored upon pre-treatment of cells with FITC-traC peptide (Fig. 3E).

[00196] As the effect of DCBLD2 in regulating VEGF signaling has been linked to regulation of VEGFR2-protein tyrosine phosphatase interaction, the present study evaluated the effect of synthetic FITC-traC peptide on VEGFR2 interactions with protein tyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TCPTP). Addition of FITC-traC peptide to HUVEC markedly reduced PTP1B and TCPTP co-precipitation with VEGFR2 (Fig. 3F), suggesting that the effect of DCBLD2 traC on VEGF signaling parallels, at least in part, the effect of endogenous DCBLD2 on VEGF signaling.

[00197] The specificity of VEGFR2 interaction with FITC-traC was confirmed following overexpression of different tyrosine kinase receptors in HEK 293 cells. Importantly, VEGFR2, but not PDGFRβ, could be immunoprecipitated from FITC-traC, when the cells were pretreated with the peptide for 15 minutes (Fig. 4), indicating that like endogenous DCBLD2, the exogenous peptide interacts with VEGFR2.

Example 1-5: DCBLD2 SS traC peptide promotes VEGF-induced angiogenesis in vivo

[00198] The effect of DCBLD2 SS traC peptide on in vivo response to exogenous VEGF was evaluated in matrigel plug and cornea micropocket angiogenesis assays. Visual inspection of matrigel plugs showed to contain more neovascularization in the presence of FITC-traC and VEGF, compared to VEGF alone (Fig. 5A). This was confirmed by

immunostaining which showed significantly more CD31 staining in matrigel plugs containing FITC-traC and VEGF, compared to VEGF alone, while in the absence in VEGF, there was no difference between FITC-traC and control matrigel plugs (Fig. 5B).

[00199] Similarly, in the cornea micropocket assay, FITC-traC enhanced angiogenesis in the presence of VEGF. This difference was readily detectable on visual inspection of corneas and was confirmed by CD31 immunostaining (Fig. 6A-B).

[00200] To investigate the effect of FITC-traC peptide on ear wound healing, ears of albino mice (16 weeks old, n=9 in each group) to induce 2mm diameter circular wounds. FITCtraC 10 ug/ear or 1% DMSO were injected around the wound edges of opposite ears in each mouse every other day for 20 days (Fig. 7A). Follow up measurement of the wound area showed that the wound area of ears injected with traC was significantly reduced compared with that of the control ears injected with DMSO (Fig. 7B-C), indicating that traC can significantly promote wound healing in mouse ears. Immunofluorescence staining of the ear tissue collected on day 8 and day showed that the ears injected with traC had significantly more CD31 signal, indicative of neovascularization, on both timepoints (Fig. 7D).

Example 1-6: A poly leucine decapeptide mimics the effect of DCBLD2 SS traC on VEGF signaling

[00201] As a prelude for further evaluation of DCBLD2 SS traC peptide in therapeutic applications, the present study sought to determine its minimal sequence that interacts with VEGFR2. The DCBLD2 SS traC was split into two parts with the C-terminal part 2 (LLLLLVLLLLLEDAGA) encompassing the poly-leucine sequence. Following co-transfection of VEGFR2 with GFP-HA-tagged Part 1 (CSNSSSFSMPLF) or Part 2 into HEK 293 cells, Part 2, but not Part 1 could be co-immunoprecipitated with VEGFR2 (Fig. 8A). Part 2 was then split into two sub-parts: LLLLLVLLLLL (5LV5L) and EDAGA, each connected to a C-terminal GFP-HA tag. Following co-transfection with VEGFR2 in HEK 293 cells, only the sequence 5LV5L, and not EDAGA, could co-precipitate with VEGFR2, indicating that this segment of the DCBLD2 signal sequence is involved in its macromolecular interactions with VEGFR2 (Fig. 8B).

[00202] A comparison of the rat, mouse and human DCBLD2 amino acid sequences showed that while the three proteins share a poly-L segment in their N-terminal, the 5LV5L is replaced by 9L in murine and rat proteins. The present study therefore designed poly-leucine sequences with 9 or 16 amino acids, connected to C-terminal GFP-HA tags (Fig. 8C). Following co-transfection with VEGFR2 in HEK 293 cells, co-immunoprecipitation studies

that removal of the valine in the middle of the '5LV5L' sequence did not affect its interaction with VEGFR2. (Fig. 8D-E). Interestingly however, the 16L sequence did not co-immunoprecipitate with VEGFR2 (Fig. 8D-E). Evaluation of additional constructs encompassing 7 to 12 leucine residues showed that the 9 to 11-leucine sequences appear to have the strongest interaction with VEGFR2, and this interaction diminishes as the number of leucine residues increases or decreases (Fig. 8F).

[00203] Finally, to evaluate the functional significance of this interaction, the effect of a 10L synthetic peptide (FITC-L10 connected to EDAGA to increase water solubility) on VEGF signaling was evaluated. Similar to FITC-traC, the FITC-10L peptide (1 μM, 15 minutes) enhanced VEG-induced VEGFR2, ERK and AKT phosphorylation in HUVEC (Fig. 8G).

[00204] In summary, the exceptionally long signal sequence of DCBLD2/ESDN contains a hydrophobic and transmembrane subdomain, 'traC', which specifically interacts with VEGFR2. 'traC' promotes VEGF/VEGFR2 signaling in EC and enhances *in vivo* angiogenesis and promotes ear wound healing. The minimum essential sequence of traC for interacting with VEGFR2 is the polyleucine segment.

Example 1-7: traC peptide effect on VEGFR2 signaling in comparison with control peptide and scrambled peptide in endothelial cells

[00205] Referring to Figs. 10A-13E, treating human umbilical vein endothelial cells (HUVEC) with traC peptide resulted in the activation of VEGFR2 signaling (upregulation of p-VEGFR2, p-ERK and p-AKT). The scrambled peptide and control peptide also increased VEGF signaling in endothelial cells, albeit to a smaller intensity and different kinetics of activation, than the traC peptide.

Example 1-8: traC but not control peptide and scrambled peptide, is internalized by endothelial cells

[00206] Referring to Figs. 14A-14B, upon contact with HUVEC, traC peptide is internalized. In contrast, a control peptide having similar sequence to traC (CSNSSSFSMPIFIIIIIVIIIIIEDAGA, SEQ ID NO:9, leucine residues of traC are replaced with isoleucine residues) and a scramble were not.

Example 1-9: Materials and methods

Reagents

[00207] Recombinant Human VEGF 165 was obtained from R&D System (Catalog No. 293-VE). The antibodies for phospho–VEGFR2 (Tyr1175, 19A10, Catalog No. 2478S), total VEGFR2 (55B11, Catalog No. 2479S), phospho-ERK 1/2 (Thr202/Tyr204, Catalog No. 9101S), total ERK 1/2 (Catalog No. 9102S), phospho-Akt (Ser473, D9E, Catalog No. 4060S), total Akt (Catalog No. 9272S), HA Tag (C29F4, Catalog No. 3724S), FLAG Tag (Catalog No. 2368S), GAPDH (14C10, Catalog No. 2118L) were purchased from Cell Signaling Technology. Anti-FITC antibody (Catalog No. PA1-26793) was obtained from Invitrogen Technology. Anti-PDGFRβ (Catalog No. sc-432), and anti-TCPTP (S-17, Catalog No. sc-102129) antibodies were obtained from Santa Cruz Biotechnology. The anti-PTP1B antibody (EP1837Y, Catalog No. ab52650) was obtained from Abcam. Rat anti–mouse CD31 antibody (Catalog No. ab553369) and anti-CD102 antibody (Catalog No. ab553325) were obtained from BD Biosciences.

Cell culture

[00208] HEK 293T cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Catalog No. 11965-092) with 10% heat-inactivated fetal Bovine serum (FBS, Sigma-Aldrich, Catalog No. 12306C) and 1% penicillin-streptomycin (Gibco, Catalog No. 15140-122).

[00209] Endothelial cells were cultured on 0.5% gelatin (Alfa Aesar, Catalog No. J62699) coated plates in Medium 199 (Gibco, Catalog No. 11150-059) supplemented with 20% FBS, 1% endothelial cell growth supplement (ECGS), and 1% penicillin-streptomycin. Subcultures were obtained by trypsinization (0.25% Trypsin-EDTA, Gibco, Catalog No. 25200-056) and used at up to passage five. HUVECs were provided by the tissue culture core laboratory of Yale Vascular Biology and Therapeutics Program. Mouse lung endothelial cells (MLECs) were isolated from 4-week-old WT and *Dcbld2*$^{-/-}$ mice by two rounds of immunoselection with anti-CD31 and anti-CD102 conjugated magnetic beads according established procedures. The purity of MLEC isolations, as assessed by flow cytometry using CD31, was consistently >95%. Furthermore, there was no difference in cell purity between knockout and WT cells.

[00210] Transfection of the cells with Lipofectamine® RNAiMAX Reagent (Thermo Fisher Scientific, Catalog No. 13778-150) was carried out according to the manufacturer's recommendations. For VEGF/VEGFR2 signaling assays, endothelial cells were transfected with either ESDN siRNA (Horizon, Catalog No. L-016714-00-0005) or a non-targeting control siRNA (Horizon, Catalog No. D-001810-01-05) for forty-eight hours. The cells were

then starved in Medium 199 with 1% FBS for 2 hours and treated with / without traC (1uM for 15min, unless stated otherwise) or VEGF (10 ng/ml for 10min, unless stated otherwise) as indicated.

Plasmid construction

[00211] The genes encoding different protein fragments tagged with a GFP / HA / His were cloned into a pCDNA3.0 plasmid using HindIII and KpnI restriction enzymes. All constructs were confirmed by PCR or DNA sequencing.

Peptide synthesis

[00212]

The traC peptide was synthesized by Thermo Fisher Scientific following a sequence of FITC-Acp-CSNSSSFSMPLFLLLLLVLLLLLEDAG (SEQ ID NO:19), with a purity of over 90%. The formula of this peptide is $C_{141}H_{231}N_{29}O_{39}S_2$, with a molecular weight of 3410.09. The traC peptide is very hydrophobic, so the peptide was initially dissolved in 100% DMSO.

Western blotting

[00213] Cells were washed with ice-cold PBS twice and lysed in RIPA buffer (Thermoscientific, Catalog No. 89901) supplemented with complete proteinase inhibitor (Roche Applied Sciences, Catalog No. 11873580001) and phosphatase inhibitor cocktails (Roche Applied Sciences, Catalog No. 04906837001) for five minutes. The cell homogenates were centrifuged (12,000 rpm, 15 minutes, 4°C), the supernatants were collected, and protein concentrations were determined (Protein Assay Dye Reagent Concentrate, Bio-Rad, Catalog No.5000006). Equal amounts of protein were resolved by SDS-PAGE and transferred onto an Immun-Blot PVDF membrane (Bio-Rad, Catalog No. 1620174). The membranes were probed with primary antibodies, followed by horseradish peroxidase–conjugated secondary antibodies. The membranes probed with phosphorylated protein antibodies were stripped using restore plus Western blot stripping buffer (Thermo Fisher Scientific, Catalog No. 46430) and re-probed with total protein antibodies. The blots were imaged using Bio-Rad ChemiDoc MP Imaging System and quantified using Image J software.

Immunoprecipitation

[00214] Cell lysates (containing 500µg protein) were incubated with 50ul (1.5mg) Dynabeads protein A (Invitrogen, Catalog No. 10006D) for one hour at 4°C, after which the

beads were removed by magnet to clear the lysate. The precleared lysates were then incubated with respective Dynabeads-antibody complex at 4°C overnight and washed gently with washing buffer three times. After eluting the target antigen from the immunocomplexes, the beads were removed with a magnet, and the supernatant was stored for further analysis.

Immunofluorescence staining

[00215] Cells cultured on coverslips or five μm thick cryostat sections were fixed with 4% paraformaldehyde in PBS for 20 min at room temperature, followed by blocking with 10% normal goat serum in PBS for one hour. These were next incubated with primary antibodies overnight at 4°C, followed by fluorescent secondary antibodies (Thermo Fisher Scientific) for one hour, and DAPI staining (Thermo Fisher Scientific, Catalog No. 62248) for five minutes. After washing, the slides were mounted, and fluorescence images were acquired using a Nikon Spinning disk confocal microscope. The positive staining area was quantified using ImageJ software.

Cornea pocket assay

[00216] Cornea pocket assay was performed as described in Tang et al. (*J Vis Exp.* 2011(54).) with minor modifications. Using a micro-knife (Ambler Surgical, Catalog No. 3401E) a 1 mm incision was made in the cornea about 1-1.3 mm from the limbus. The small pellets freshly prepared by mixing poly-HEMA (Sigma, Catalog No. P3932), sucralfate (Cayman Chemical, Catalog No. 27882), with combinations of recombinant human VEGF 165 and traC /DMSO (1%) were applied to the pockets, which were allowed to be closed over the pellets. Thirteen days after the pellets were implanted, corneas were collected and cut into four equal sectors, followed by whole mount immunostaining using antibodies against CD31 (R&D Systems, Catalog No. AF3628). Corneas were then flat mounted using ProLong Glass Antifade Mountant (Thermo Fisher Scientific, Catalog No. P36980) on a glass slide (epithelium up) and sealed under a #1.5 thick coverslip for imaging. Quantification of angiogenesis was performed by defining the fraction of CD31 positive area in each sector.

Matrigel angiogenesis assay

[00217] 1 μM FITC-traC/DMSO with or without 2 μg/ml VEGF were mixed into 300μl growth factor reduced matrigel (Corning, Catalog No. 354230) at 4°C, and then subcutaneously injected into the opposite iliac regions of C57BL/6 mice, where it rapidly solidified. The animals were euthanized after five days, and the plugs were excised for

immunostaining. When imaging, the present study selected the area that contains the most CD31 positive signals at the edge of each section, and quantified angiogenesis by defining the percentage of the CD31 positive area.

Ear wound healing model

[00218] A finger loop ear punch was used to perforate 2mm diameter circular wounds in the center of both ears of albino mice. FITC- traC (10 µg) dissolved in 10µl PBS was injected into the left ear wound edge every other day, and 1% DMSO in 10µl PBS was injected into the right ear as control. The two ears of each mouse were photographed daily to track their healing. After 20 days, the animals were euthanized, and ear tissues were harvested. For each ear, the wound sections with the largest diameters were imaged and used for measuring the size of the remaining hole.

Statistics

[00219] All values are expressed as mean ± SEM. The significance of the results was assessed by 2-tailed ratio t test or 1-way / 2-way ANOVA with Bonferroni post hoc test (for >2 groups). A P value less than 0.05 was considered significant.

Example 2: Regulation of angiogenesis by signal sequence-derived peptides

[00220] The neuropilin-like, Discoidin, CUB and LCCL domain containing 2 (DCBLD2) is a transmembrane protein with an unusually long signal sequence (SS) composed of N-terminal (N) and C-terminal (C) subdomains, separated by a transition (tra) subdomain. DCBLD2 interacts with VEGFR-2 and regulates VEGF-induced endothelial cell signaling, proliferation and migration, as well as angiogenesis. The exact mechanisms by which DCBLD2 interacts with VEGFR2 to modulate VEGF signaling remain unclear.

[00221] Searching for VEGFR2 interacting DCBLD2 domains, the present study generated various constructs containing different DCBLD2 domain combinations and conducted co-immunoprecipitation and signaling studies in HEK 293T and endothelial cells. Several peptides were synthesized based on the identified domain, and their effect on VEGF signaling was assessed in vitro in cell culture and in vivo using matrigel plug and corneal micropocket assays. The effect of the lead peptide was further evaluated using a murine hindlimb ischemia model.

[00222] DCBLD2 SS interacted with VEGFR2 and promoted VEGF signaling. SS was not cleaved in the mature DCBLD2 and its hydrophobic transmembrane 'traC' segment, but not

the 'N' subdomain, was involved in DCBLD2-VEGFR2 interaction. The smallest unit in DCBLD2 SS that interacts with VEGFR2 was the L5VL5 sequence. Even after the central valine was removed, the L10 sequence mimicked the DCBLD2 SS traC's effect on VEGF-signaling, while shorter or longer poly-leucine sequences were less effective. Finally, a synthetic traC peptide enhanced VEGF signaling in vitro, promoted VEGF-induced angiogenesis in vivo, and improved blood flow recovery following hindlimb ischemia.

[00223] DCBLD2 SS along with its derivative peptides can promote VEGFR2 signaling and angiogenesis. Synthetic peptides based on DCBLD2 SS hold promise as therapeutic agents for regulating angiogenesis. Importantly these findings refine the traditional view of signal sequences as mere targeting elements, revealing a role in cellular signaling. This opens new avenues for research and therapeutic strategies.

Example 2-1:

[00224] Targeting of newly synthesized proteins to the endoplasmic reticulum (ER) and eventually the plasma membrane of eucaryotic cells is mediated by signal sequences (SS). For type I transmembrane and secretory proteins, SS are typically 15 to 40 amino acid N-terminal extensions of the nascent proteins. During or after completion of ER translocation, many SS are cleaved by signal peptidase to generate a signal peptide (SP), while others remain an integral part of the mature protein, as signal anchor sequences. Generally, cleaved SPs are rapidly degraded. However, some SPs and their fragments are retained and have specific, post-targeting functions on their own. To date, however, most known examples of such SS post-targeting functions are of viral origin.

[00225] A typical SP has a tripartite structure with a hydrophobic, α-helical core (*h* region) surrounded by an N-terminal polar *n* region, and a C-terminal *c* region that contains the signal peptidase cleavage site. Based on proteome analysis by machine-learning systems, it is suggested that exceptionally long SS may have a bipartite 'NtraC' domain structure with potentially functional N-terminal (N-domain, 'N') and C-terminal (C-domain, 'C') domains connected by a turn-rich linker area (transition area, 'tra').

[00226] One such protein featuring an exceptionally long SS is Discoidin, CUB and LCCL domain containing 2 (DCBLD2), whose 66 amino acid SS is predicted to fit the 'NtraC' model. The gene encoding DCBLD2, also known as endothelial and smooth muscle cell–derived neuropilin-like protein (ESDN) or CLCP1, was initially cloned from human coronary artery and highly metastatic lung cancer cells as a protein with the longest known secretory signal sequence among eukaryotes. DCBLD2 is highly conserved among vertebrates and

closely resembles the structure of neuropilins, major co-receptors for vascular endothelial cell growth factor (VEGF) receptors (VEGFRs), which play a critical role in angiogenesis. DCBLD2 is upregulated in human, rat, and mouse remodeling arteries, and regulates VEGF-induced endothelial cell (EC) proliferation, migration, and signal transduction, as well as developmental and adult angiogenesis.

[00227] Angiogenesis, the process of new blood vessel formation from existing vessels, plays a key role in a number of physiological processes, including development and growth, adult organ regeneration, and wound healing. Angiogenesis is tightly controlled through numerous stimulators and inhibitors and its dysregulation, whether insufficient or excessive, contributes to a wide variety of diseases, including malignancy, ischemic heart and peripheral artery disease, neurodegeneration, and proliferative retinopathy. Accordingly, modulation of this process provides several therapeutic opportunities.

[00228] The present study identified the DCBLD2 SS, and more specifically its 'traC' segment, as a key domain of DCBLD2 that interacts with VEGFR2 and promotes VEGF/VEGFR2 signaling in EC. Furthermore, an exogenous synthetic FITC-traC peptide promoted angiogenesis in vivo. The smallest unit in DCBLD2 SS that interacted with VEGFR2 was the L5VL5 sequence. Even after the middle valine was removed, the L10 sequence could still mimic the effect of DCBLD2 SS traC on VEGF signaling. As such, in addition to establishing a novel post-targeting function of SS in regulating growth factor signaling, these findings introduce the DCBLD2 traC and its derivatives as promising candidates for therapeutic angiogenesis.

Example 2-2: Materials and Methods

Sex as a biological variable

[00229] *In vivo* studies exclusively used male mice to reduce variability. In vitro studies, performed with mixed populations of cells, suggest that the findings are sex independent.

Cell culture

[00230] HEK 293T cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% heat inactivated fetal bovine serum (FBS, Sigma Aldrich) and 1% penicillin/streptomycin (Gibco). For endothelial cell culture, primary human umbilical vein endothelial cells (HUVECs) were obtained from Yale Vascular Biology & Therapeutics Program Tissue Culture program and cultured on gelatin-coated cultureware with Medium 199 (Gibco) supplemented with 20% FBS, 1% endothelial cell growth

supplement (ECGS), 2 mM L- glutamine, and 1% penicillin/streptomycin. MLEC were isolated from 4-week-old wild-type and *Dcbld2*$^{-/-}$ mice. Mice were anesthetized and lungs harvested, rinsed in Hank's Balanced Salt Solution (HBSS, Sigma Aldrich), cut into small pieces, and subjected to enzymatic digestion with a 1mg/ml collagenase solution for 45 minutes. The tissue solution was then mechanically disrupted, passed through a 70 µm strainer, and cells were collected by centrifugation at 350g for 5 minutes and resuspended in complete Medium 199. CD31-positive cells were separated using immunomagnetic separation with CD31-conjugated beads, followed by detachment of the cells from the beads and cultured until 70-80% confluent. Finally, cells were subjected to another round of immunoselection with CD102-conjugated beads following the same procedure and detachments. The purity of isolated MLECs was verified by assessing the expression of CD31 with flow cytometry. Preparation of antibody conjugated magnetic beads was carried out according to manufacturer's (Thermo Fisher) instructions. A 0.25% trypsin-EDTA (Gibco) solution was used together with standard lab protocols for passing adherent cells.

Plasmid construction and transfection

[00231] The constructs used in this study are schematically shown in Fig. 15A and 15F. The genes encoding different protein fragments were cloned into a pCDNA3.0 plasmid between HindIII and KpnI restriction sites. All constructs were confirmed by PCR or DNA sequencing. Transfection of cells with plasmid DNA was carried out using Lipofectamine 3000 (Thermo Fisher) according to the manufacturer's instructions. 24 hours after transfection, cells were washed and cultured in DMEM supplemented with 10% FBS for another 24 hours before use.

Immunoblotting and immunoprecipitation

[00232] Cells were lysed on ice using ice-cold RIPA buffer (Thermo Fisher) supplemented with protease and phosphatase inhibitor cocktails (Roche). The lysates were centrifuged at 14,000g for 15 minutes at 4°C, and supernatant was collected. Protein concentrations were determined using BCA assays (Thermo Fisher). Equal amounts of protein were subjected to SDS-PAGE and transferred onto 0.2µm PVDF membranes (Bio-Rad). Membranes were blocked for 1 hour with a 5% BSA solution (Sigma Aldrich) in TBST (Cell Signaling). For immunoblotting, membranes were incubated overnight with primary antibodies at 4°C and for 1 hour at room temperature with secondary antibodies. The blots were developed using an ECL substrate (Thermo Fisher).

[00233] For co-immunoprecipitation studies, Dynabeads™ Protein A Immunoprecipitation Kit (Invitrogen) was used. All steps were carried out according to the manufacturer's protocol. After completing the protocol, beads were removed with a magnet and the resultant supernatant was collected for analysis.

Immunofluorescence staining

[00234] Cell monolayers and OCT embedded tissue sections were fixed with 4% paraformaldehyde (PFA) for 15 minutes and blocked for 1 hour with 5% BSA at room temperature. Cells and tissue sections were incubated overnight at 4 °C with primary antibodies followed by incubation with secondary antibodies) for 1 hour at room temperature. Primary and secondary antibody dilutions were made with 5% BSA, based on the manufacturers' recommended concentrations for each antibody. Cell nuclei were stained with DAPI (Thermo Fisher) and coverslips were secured with mounting media (Thermo Fisher) and allowed to cure for 48 hours Images were acquired with inverted Leica SP8 confocal or DMi8 widefield microscopes.

Synthetic peptides

[00235] Thermo Fisher Scientific was commissioned to produce the synthetic traC peptide using the following sequence: [FITC][Ahx]-CSNSSSFSMPLFLLLLLVLLLLLEDAGA(SEQ ID NO:8)-[COOH]. Mass spectrum and HPLC data reported a molecular mass of 3483.18 Da and purity of >98%. Due to the hydrophobic properties of the peptide, traC was reconstituted in DMSO, aliquoted to avoid freeze-thaw cycles, and stored at -80°C. For experiments, aliquots were diluted and vortexed before application, ensuring even distribution in aqueous media and preventing areas of localized concentration during treatment. FITC-L10 and FITC-scramble peptide, with a sequence of [FITC][Ahx]-LLLLLLLLLLEDAGA(SEQ ID NO:16)-[COOH] and [FITC][Ahx]-LSLESALMLFLCLDLSGVFLSLPLSNL(SEQ ID NO:10)-[COOH] respectively, were obtained from Thermo Fisher Scientific. Mass spectrum and HPLC data reported a molecular mass of 2094.58 Da and 3412.10 Da for FITC-L10 and FITC-scramble respectively and a purity of >98% for both. Antennapedia peptide was obtained from GeneScript, with mass spectrometry and HPLC analysis reporting a molecular mass of 2749.28 Da and a purity of 96.6%.

Matrigel plug angiogenesis assay

[00236] One μmol of traC, with or without 2 μg/ml VEGF, was mixed into 300 μl of growth factor-reduced Matrigel (Corning) at 4°C. The mixture was subcutaneously injected into the opposite iliac regions of 8–10-week-old C57BL/6J WT mice. Five days post-injection, mice were euthanized and Matrigel plugs were collected and embedded in OCT. The plugs were frozen, sectioned, and stained with goat polyclonal anti-CD31 (R&D Systems) overnight at 4°C, followed by Alexa Fluor 594-labeled chicken anti-goat IgG (Thermo Fisher) for 1 hour at room temperature. Sections were imaged using a widefield microscope (Leica DMi8). When imaging, areas containing the highest CD31 staining at the edge of the sections were imaged and the percentage of the CD31-positive area relative to the total imaged area was quantified blindly and reported.

Pellet preparation and corneal micro-pocket angiogenesis assay

[00237] Pellets were prepared 24 hours before implantation. Initially, 5 μl of a 12% (w/v) solution of poly(2-hydroxyethyl methacrylate) (poly-HEMA; Sigma Aldrich) dissolved in ethanol and 1 μl of a 10% (w/v) sucralfate solution (Cayman Chemical) dissolved in PBS were mixed.

[00238] Subsequently, either 4 μl of FITC-traC (0.75 μg/μl), 4 μl of recombinant human VEGF (1 μg/μl, R&D Systems), or 4 μl of a solution containing both FITC-traC (0.75 μg/μl) and VEGF (1 μg/μl) was added and mixed. Vehicle controls consisted of 4 μl of the diluted base solvent used to dissolve FITC-traC or VEGF. From the resulting 10 μl mixture, 0.2 μl of the solution was pipetted onto parafilm to create pellets and allowed to dry. The resulting pellets contained either 60 ng of FITC-traC, 80 ng of VEGF, a combination of both, or the vehicle controls. Implantation of the pellets was performed on 12-week-old C57BL/6J mice (Jackson Laboratory). Briefly, using a micro-knife (Ambler Surgical), a 1 mm incision was made on the cornea, approximately 1 mm from the limbus, into which a pellet was inserted. Each mouse was implanted with two pellets, one under each cornea. 13 days post implantation, corneas were harvested and fixed in 4% paraformaldehyde on ice for 1 hour, followed by permeabilization and blocking with a 5% BSA solution containing 0.1% Triton X-100 (Sigma Aldrich). Goat polyclonal anti-CD31 (R&D systems) was prepared in blocking buffer and incubated with the corneas overnight, with gentle agitation at 4℃. For secondary antibody labeling, corneas were incubated with Alexa Fluor 594-labeled chicken anti-goat IgG (Thermo Fisher) for 2 hours at room temperature. Finally, corneas were flattened, whole-mounted onto glass slides, and imaged by confocal microscopy using the tile scan function

with a z-stack; maximum projections were used for the quantification. Images were thresholded to distinguish CD31-positive staining from the background. To reduce sampling bias, the entire flat-mounted cornea was analyzed blindly. The total cornea area was defined with the innermost vessel of the limbus arcade as the border. The CD31 positive area was quantified and reported as a percentage of the ROI.

Hindlimb ischemia

[00239] For Hindlimb Ischemia, 12-week-old C57BL/6J mice (Jackson Laboratory) were subjected to femoral artery ligation and analyzed by Laser Doppler imaging to measure blood flow in the hindlimb. Briefly, the femoral artery was ligated proximal to the superficial epigastric artery branch and all branches between the two ligatures were ligated. The arterial segment between the ligatures was then excised.

[00240] Osmotic mini pumps (Alzet) were primed and prepared to deliver 1.74mg/day of traC for 7 days to the ligated limb based on a pumping rate of 1.05ml/hr, according to the manufacturer's instructions. Following ligation, the osmotic mini pump was subcutaneously inserted into the back of the mouse, and a catheter (Alzet) connected to the pump was guided and secured adjacent to the ligated thigh. The animals were randomly assigned to treatment or control groups. Laser Doppler flow images of the foot were acquired before, immediately after surgery, and on days 3, 5, and 7 post-surgery. Images were recorded blinded to treatments with an infrared laser doppler imager (LDI; Moor Instruments) at 37.0-37.5 °C under anesthesia with ketamine/xylazine (100/10 mg/kg). The data were analyzed with the Moor LDI image processing software V5.00 and the results were reported as a ratio between the ischemic and non-ischemic foot.

[00241] After 7 days of treatment, the mice were euthanized and the thigh and the leg were collected in OCT, frozen, and sectioned. Four sections, approximately 2 mm spaced apart, from each thigh were stained for CD31. The percentage of the CD31-positive area was calculated for each of the four sections relative to the total area of each thigh and the results were averaged to obtain a representative percentage of the CD31-positive area within each thigh. Quantification of *cdh5* gene expression was performed using RT-PCR with *Gapdh* used as the housekeeping gene. Results were reported as $2^{-\Delta Ct}$. Fifty sections, each 5 μm thick, were collected at 8 locations along the thigh or the leg at 1 mm intervals and pooled for each animal. Total mRNA was isolated using the RNeasy kit (Qiagen), and reverse transcription (RT) was carried out with the QuantiTect RT kit (Qiagen). Real-time PCR was

conducted in triplicates using cDNA with TaqMan gene expression assays, following the manufacturer's instructions. Tissue analysis was performed blinded to treatment status.

Statistical analysis

[00242] Results are expressed as the mean ± standard deviation. All statistical analyses were performed using GraphPad Prism 10. Data were checked for normality with the Shapiro-Wilk test. For data that did not pass the Shapiro-Wilk test, non-parametric tests were used. Statistical significance was determined by a two-way analysis of variance (ANOVA, followed by Tukey's post hoc test, for multiple groups, and a student t-test or a Mann-Whitney U test for comparison between two groups. A p-value<0.05 was considered significant.

Study approval

[00243] All studies were performed under protocols approved by Yale University and Veterans Affairs Connecticut Healthcare System Institutional Animal Care and Use and Human Investigational Committees.

Example 2-3: The DCBLD2 SS traC subdomain is involved in DCBLD2 interaction with VEGFR2

[00244] Distinct functional and structural units of DCBLD2 include an unusually long SS, which in tandem with CUB, LCCL, and Discoidin domains form the extracellular segment. A transmembrane domain and an intracellular segment comprise the rest of the molecule. To identify the key domain(s) of DCBLD2 that interact with VEGFR2, the present study generated several plasmids expressing various combinations of different DCBLD2 domains linked to a C-terminal hemagglutinin (HA) tag (Fig. 15A I-V. Upon co-transfection with VEGFR2 in HEK 293T cells each of these constructs expressed the appropriate-size protein (Fig. 19). Surprisingly, co- immunoprecipitation with an anti-VEGFR2 antibody showed that all these products complex with VEGFR2 (Fig. 15B). Furthermore, VEGF signaling was diminished in *Dcbld2*-deficient murine lung endothelial cells (MLEC), all these DCBLD2 constructs enhanced VEGF-induced ERK phosphorylation upon transfection in *Dcbld2*$^{-/-}$ MLEC (Fig. 15C). This led us to speculate that a common component of the constructs, i.e., the SS, might be involved in its interaction with VEGFR2 and enhancing VEGFR2 signaling.

[00245] To test this possibility, and to rule out HA as the VEGFR2 interacting component, the present study constructed additional plasmids expressing DCBLD2 SS, Shrew-1 SS

(another unusually long SS with a similar 'NtraC' domain structure) or no SS in tandem with C-terminal green fluorescent protein (GFP)-HA tags (Fig. 15A VI-VIII). Upon co-transfection of these constructs with VEGFR2 in HEK 293T cells and HA immunoprecipitation, VEGFR2 could be pulled down by GFP-HA-tagged DCBLD2 SS, but not Shrew-1 SS or GFP-HA without any SS (Fig. 15D). In addition, despite a similar predicted size, the product of DCBLD2 SS-GFP-HA plasmid appeared larger on western blots than the products of Shrew-1 SS-GFP-HA or GFP-HA constructs (Fig. 15D). This raised the possibility that the Shrew-1 SS but not the DCBLD2 SS is cleaved in the final product. Indeed, the SignalP 6.0 server, which can predict the location of the signal peptide cleavage site, suggested that unlike Shrew-1, the N-terminal SS is not cleaved in DCBLD2 (Figs. 20A-20B). To confirm this experimentally, the present study transfected HEK 293T cells with a dually tagged DCBLD2 plasmid with N-terminal FLAG and C-terminal HA tags. Immunoprecipitation of the product with an anti-HA antibody followed by FLAG and DCBLD2 immunoblotting showed a single (non-cleaved) product (Figs. 18A-18E). Replacing one or both alanine residues in the putative A- X-A signal peptidase cleavage site of DCBLD2 with glycine led to similarly sized products, indicating that the DCBLD2 SS remains uncleaved (Fig. 21). Finally, VEGF-induced ERK phosphorylation in *Dcbld2*$^{-/-}$ MLEC was enhanced upon transfection with the DCBLD2 SS-GFP- HA construct (Fig. 22). Taken together, these data suggest the DCBLD2 SS remains an integral component of the mature DCBLD2 and is a key domain in the DCBLD2 interaction with VEGFR2 that modulates downstream VEGF signaling.

[00246] Based on the 'NtraC' model, the DCBLD2 SS 'N' subdomain contains 38 amino acid residues, and the 24 amino acid C-terminal residues constitute the 'C' subdomain, with those two separated by a short 'tra' subdomain (Fig. 15E). Hydrophobicity analysis by ProtScale (Fig. 235) and the transmembrane helix prediction by DeepTMHMM (Fig. 24) indicated significant differences between different DCBLD2 SS subdomains, with the 'C' subdomain adjacent to 'tra' predicted to be hydrophobic and transmembrane. To identify the subdomain of DCBLD2 SS that interacts with VEGFR2, the present study synthesized additional constructs IX-XI expressing different DCBLD2 SS subdomains (Fig. 15F), which were co-transfected with VEGFR2 into HEK 293T cells. Co-immunoprecipitation studies indicated that the 'traC' subdomain, but not the 'N' subdomain, is involved in the DCBLD2-VEGFR2 interaction (Fig. 15G).

Example 2-4: FITC-TraC enhances VEGF/VEGFR2 signaling in EC

[00247] Given the interaction between the DCBLD2 SS traC fragment and VEGFR2, the present study sought to investigate whether a synthetic traC peptide, labeled with FITC for tracking purposes, can modulate VEGF signaling in EC. As a first step in evaluating the effects of FITC-traC in EC, the present study followed by fluorescence imaging the fate of the labeled peptide added to EC culture media.

[00248] Interestingly, FITC-traC was rapidly taken up by HUVEC with the peptide detected in the cytoplasm as early as 1 minute after its addition to the medium. The cytoplasmic accumulation of the peptide increased over time and by 15 minutes it was also detectable in the cell membrane (Fig. 16A), where VEGFR2 is also located (Fig. 16B). Accordingly, the 15-minute time point was selected for the next set of studies.

[00249] Next, the present study evaluated the effect of synthetic DCBLD2 FITC-traC on VEGF signaling. Pretreatment of HUVEC with FITC-traC peptide (1μM in 1% DMSO, 15 minutes), but not a scrambled homologue, significantly increased VEGF-induced VEGFR2, ERK, and AKT phosphorylation compared to HUVEC pre-treated with vehicle control (Fig. 16C and 25). A dose-response study showed that the effect of FITC-traC peptide on VEGF signaling is concentration-dependent (Fig. 26). Pre-heating of FITC-traC peptide (120 °C, 50 minutes) abrogated its effect on VEGF signaling (Fig. 27). To explore the molecular mechanisms of this observation, the present study first determined whether the effect of FITC-traC is simply related to its cell penetrating property. A similar set of experiments showed that unlike FITC-traC, pre-treatment of HUVEC with a classical cell penetrating peptide, antennapedia, does not affect downstream VEGF signaling (Fig. 28). Next, the present study sought to determine whether FITC-traC peptide can reverse the inhibitory effect of DCBLD2 downregulation on VEGF signaling in EC. DCBLD2 siRNA significantly reduced VEGF-induced VEGFR2 and ERK phosphorylation in HUVEC. This impaired VEGF/VEGFR2 signaling was fully restored upon pre-treatment of cells with FITC-traC peptide (Fig. 16D). Importantly, VEGFR2, but not PDGFRβ, could be co-immunoprecipitated with FITC-traC, when HUVEC were pretreated with the peptide for 15 minutes (Fig. 16E), indicating that like endogenous DCBLD2, the exogenous peptide interacts with VEGFR2. The specificity of VEGFR2 interaction with FITC-traC was confirmed following co-transfection of different tyrosine kinase receptors in HEK 293 cells (Fig. 29). As the effect of DCBLD2 in regulating VEGF signaling has been linked to regulation of VEGFR2-protein tyrosine phosphatase interaction, the present study evaluated the effect of synthetic FITC-traC peptide on VEGFR2 interactions with protein tyrosine phosphatase 1B (PTP1B) and T cell protein tyrosine phosphatase (TCPTP). Addition of

FITC-traC peptide to HUVEC markedly reduced PTP1B and TCPTP co-precipitation with VEGFR2 (Fig. 15F), suggesting that the effect of FITC-traC on VEGF signaling parallels, at least in part, the effect of endogenous DCBLD2 on VEGF signaling.

Example 2-5: DCBLD2 SS FITC-traC peptide promotes VEGF and ischemia-induced angiogenesis *in vivo*

[00250] The effect of DCBLD2 SS FITC-traC peptide on in vivo response to exogenous VEGF was evaluated in matrigel plug and cornea micropocket angiogenesis assays. Visual inspection of matrigel plugs collected 5 days after implantation showed apparently more neovascularization in the presence of FITC-traC and VEGF, compared to VEGF alone (Fig. 17A). This was confirmed by immunostaining which showed significantly more CD31 in matrigel plugs containing FITC- traC and VEGF compared to VEGF alone, while in the absence of VEGF, there was no difference between FITC-traC and control matrigel plugs (Fig. 17B). Similarly, in the cornea micropocket assay, FITC-traC enhanced angiogenesis in the presence of VEGF. This difference was readily detectable on visual inspection of corneas and was confirmed by CD31 immunostaining (Figs. 17C-17D).

[00251] Next, the present study evaluated the effect of FITC-traC on blood flow recovery and angiogenesis following femoral artery ligation in mice. Evaluation of hindlimb blood flow by laser Doppler imaging showed improved blood flow recovery in animals continuously treated with FITC-traC, detectable as early as 5 days after femoral artery ligation, compared to control animals (Fig. 17E). Histological analysis of the hindlimb muscles collected 7 days after femoral artery ligation showed significantly higher thigh and calf muscle capillary density, as assessed by immunostaining (for CD31) and quantitative RT-PCR (for VE-cadherin) in FITC-traC treated mice relative to control animals at 7 days (Figs. 17F and 17G). As expected, no difference was found in the non-ligated hindlimb VE-cadherin expression between the two groups of animals (Figs. 30A-30B).

Example 2-6: A poly leucine decapeptide mimics the effect of DCBLD2 SS traC on VEGF signaling

[00252] As a prelude to future evaluation of related signal sequences in therapeutic applications, the present study sought to identify its smallest sequence that interacts with VEGFR2. The DCBLD2 SS traC was split into two parts with an N-terminal part 1 (CSNSSSFSMPLF, SEQ ID NO:17), and a C-terminal part 2 (LLLLLVLLLLLEDAGA, SEQ ID NO:1) encompassing the poly-leucine sequence. Following co-transfection of

plasmids expressing VEGFR2 with GFP-HA-tagged part 1 or part 2 into HEK 293 cells, part 2, but not part 1 could be co-immunoprecipitated with VEGFR2 (Fig. 18A). Part 2 was then split into two sub-parts: LLLLLVLLLLL (L5VL5) (SEQ ID NO:5) and EDAGA (SEQ ID NO:3), each connected to a C-terminal GFP-HA tag. Following co-transfection with VEGFR2 in HEK 293 cells, only the sequence L5VL5, and not EDAGA, could co-precipitate with VEGFR2, indicating that this segment is involved in the DCBLD2 SS interactions with VEGFR2 (Fig. 18B).

[00253] A comparison of the rat, mouse, and human DCBLD2 amino acid sequences showed that while the three proteins share a poly-L segment in their N-terminal, the L5VL5 is replaced by L9 in murine and rat proteins (Figs. 31A-31B). The present study therefore generated a set of plasmids that express a 9 or 16 leucine peptide connected to a C-terminal GFP-HA tag. Following co-transfection with VEGFR2 in HEK 293 cells, co-immunoprecipitation studies showed that the removal of the valine in the L5VL5 sequence does not affect its interaction with VEGFR2. Interestingly however, elongating the polyleucine moiety to 16 amino acids, hampered VEGFR2 co- immunoprecipitation (Fig. 18C). Evaluation of additional constructs encompassing 7 to 12 leucine residues showed that the 9 to 11-leucine sequences appear to have the strongest interaction with VEGFR2, and this interaction diminishes as the number of leucine residues increases or decreases (Fig. 18D). Finally, to evaluate the functional significance of this interaction, the effect of a L10 synthetic peptide, FITC-L10EDAGA (connected to EDAGA to increase water solubility) on VEGF signaling was evaluated. Like FITC-traC, the FITC-L10 peptide enhanced VEGF signaling in HUVEC (Fig. 18E).

Example 2-7:

[00254] This study uncovered a new non-canonical function for the unusually long signal sequence of DCBLD2. DCBLD2 regulates developmental and adult angiogenesis by engaging in a macromolecular complex with VEGFR2, which promotes VEGF signaling by dissociating VEGFR2 away from its negative regulators of downstream signaling, VE-cadherin and protein tyrosine phosphatases, PTP1 and TC-PTP. The present study identified the hydrophobic 'traC' subdomain of the DCBLD2 SS, and more specifically, its L5VL5 segment, as a key component of the DCBLD2 interaction with VEGFR2. The SS is not excised in the mature ESDN protein and potentially functions as a second transmembrane domain. Notably, the synthetic FITC-traC peptide specifically interacted with VEGFR2 and

enhanced downstream signaling in a concentration-dependent manner, promoting VEGF-stimulated and ischemia-induced angiogenesis.

[00255] The data indicate that the DCBLD2 SS is not cleaved and therefore, may function as a signal anchor sequence. This would indicate that DCBLD2 is a double-pass transmembrane protein with cytosolic N and C terminals. Transmembrane domains are often engaged in protein-protein interactions and regulate the activity of the parent molecule. Similarly, the DCBLD2 SS and more specifically, its traC subdomain is involved in DCBLD2-VEGFR2 interaction and modulates VEGF signaling.

[00256] The present study further determined that the smallest unit in the DCBLD2 SS that can interact with VEGFR2 is the L5VL5 sequence. Most short ploy-leucine transmembrane domains containing single amino acid substitutions can bind to and activate PDGFRβ. Minor changes in the composition of these sequences (e.g., changing the placement of a single side chain methyl group) can alter their partner selectivity. Interestingly, even after removing the central valine, the L10 sequence could also interact with VEGFR2. However, shorter or longer poly-leucines were less effective in this regard, raising the possibility that the size of the poly-leucine sequence is one of the fundamental principles that determine such interactions.

[00257] Notably, the exogenous FITC-traC and its derivative polyleucine peptide mimicked the effect of endogenous DCBLD2 in interacting with VEGFR2 and regulating VEGF signaling.

[00258] VEGFR2 is a main receptor for VEGF, the prototypic pro-angiogenesis growth factor, in endothelial cells. Angiogenesis is a key process during development, growth, and adulthood. Inadequate neovessel formation is a major pathologic feature in ischemia-related cardiovascular diseases and contributes to poor wound healing. In other pathological settings, e.g., diabetic retinopathy and malignancy, angiogenesis is improperly exaggerated. As such, there is considerable interest in regulators of VEGF signal transduction. FITC-traC promoted angiogenesis, not only when induced by exogenous VEGF, but also in the presence of ischemia, suggesting that it may be of value in treating ischemic diseases. Notably, FITC-traC also improved hindlimb blood flow recovery within a few days after femoral artery ligation in mice. This is in line with the finding that deletion of DCBLD2 impairs blood flow recovery in the same animal model, and may indicate that in addition to regulating angiogenesis, DCBLD2 and its SS derivatives may regulate collateral recruitment or arteriogenesis. The role of DCBLD2 SS in related signaling pathways and cell types will be

the subject of future studies, which could also identify the specific VEGFR2 sequences/domains that interact with traC.

[00259] In conclusion, the DCBLD2 SS has a novel post-targeting function in interacting with and regulating VEGFR2 signaling and promoting angiogenesis through its transmembrane traC subdomain. This effect is narrowed down to the L5VL5 and L10 sequences.

Enumerated Embodiments:

[00260] In some aspects, the present invention is directed to the following non-limiting embodiments:

[00261] Embodiment 1: A method of promoting neovascularization and/or hair growth in a subject in need thereof, the method comprising: administering to the subject a compound comprising a polypeptide, wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, optionally wherein the polypeptide comprise a plurality of independently selected first peptides, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues, or

(b) the polypeptide comprises the amino acid set forth in SEQ ID NO:5, 6, 8, or 9.

[00262] Embodiment 2: The method of Embodiment 1, wherein the compound is soluble in water or insoluble in water.

[00263] Embodiment 3: The method of any one of Embodiments 1-2, wherein the compound is soluble in water, and wherein the compound further comprises a hydrophilic motif.

[00264] Embodiment 4: The method of Embodiment 3, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

[00265] Embodiment 5: The method of any one of Embodiments 1-4, wherein the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

[00266] Embodiment 6: The method of any one of Embodiments 1-5, which is a method of promoting neovascularization, and wherein the method promotes in the subject at least one selected from the group consisting of angiogenesis and arteriogenesis.

[00267] Embodiment 7: The method of any one of Embodiments 1-6, which is a method of promoting neovascularization, wherein at least one of the following applies:

(a) the subject suffers from a chronic wound healing disorder, and wherein the method promotes neovascularization in or near at least one chronic wound of the subject;

(b) the subject suffers from an acute wound, and wherein the method promotes neovascularization which speeds up the healing of the acute wound.

[00268] Embodiment 8: The method of Embodiment 7, wherein the chronic wound comprises at least one of the following:

(a) a nonhealing wound;

(b) an infected wound, optionally an infected surgical wound or an infected traumatic wound;

(c) an ulcer, optionally a diabetic ulcer such as a diabetic foot ulcer, an arterial ulcer, a venous ulcer, a pressure ulcer, or an ischemic ulcer; or

(d) a wound associated with immunosuppression or radiation.

[00269] Embodiment 9: The method of any one of Embodiments 1-8, wherein the compound reduces phosphatase activity exerted by protein-tyrosine phosphatase 1B (PTP1B) and/or T-cell protein tyrosine phosphatase (TC-PTP) on vascular endothelial growth factor receptor 2 (VEGFR2).

[00270] Embodiment 10: The method of any one of Embodiments 1-9, wherein the method further comprises administering to the subject an effective amount of vascular endothelial growth factor (VEGR).

[00271] Embodiment 11: A method of promoting wound healing in a subject in need thereof, the method comprising:

administering to the subject a compound comprising a polypeptide,

wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, optionally wherein the polypeptide comprise a plurality of independently selected first peptides, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues, or

(b) the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

[00272] Embodiment 12: The method of Embodiment 11, wherein the compound is soluble in water or insoluble in water.

[00273] Embodiment 13: The method of any one of Embodiments 11-12, wherein the compound is soluble in water, and wherein the compound further comprises a hydrophilic motif.

[00274] Embodiment 14: The method of Embodiment 13, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

[00275] Embodiment 15: The method of any one of Embodiments 11-14, wherein the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

[00276] Embodiment 16: The method of any one of Embodiments 11-15, wherein the method promotes wound healing by promoting in the subject at least one selected from the group consisting of angiogenesis and arteriogenesis.

[00277] Embodiment 17: The method of any one of Embodiments 11-16, wherein the wound is a chronic wound or an acute wound.

[00278] Embodiment 18: The method of Embodiment 17, wherein the chronic wound comprises at least one of the following:

(a) a nonhealing wound;

(b) an infected wound, optionally an infected surgical wound or an infected traumatic wound;

(c) an ulcer, optionally a diabetic ulcer such as a diabetic foot ulcer, an arterial ulcer, a venous ulcer, a pressure ulcer, or an ischemic ulcer; or

(d) a wound associated with immunosuppression or radiation.

[00279] Embodiment 19: The method of any one of Embodiments 11-18, wherein the compound reduces phosphatase activity exerted by protein-tyrosine phosphatase 1B (PTP1B) and/or T-cell protein tyrosine phosphatase (TC-PTP) on vascular endothelial growth factor receptor 2 (VEGFR2).

[00280] Embodiment 20: The method of any one of Embodiments 11-19, wherein the method further comprises administering to the subject an effective amount of vascular endothelial growth factor (VEGR).

[00281] Embodiment 21: A compound comprising a polypeptide and a hydrophilic motif, wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are

leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues,

(b) the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

or a salt or solvate thereof.

[00282] Embodiment 22: The compound of Embodiment 21, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

[00283] Embodiment 23: The compound of Embodiment 21 or 22, wherein the polypeptide comprise a plurality of independently selected first peptides.

[00284] Embodiment 24: A wound dressing, comprising a base dressing material, and a compound comprising a polypeptide,

wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues,

(b) the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

[00285] Embodiment 25: The wound dressing of Embodiment 24, wherein the compound is soluble in water or insoluble in water.

[00286] Embodiment 26: The wound dressing of any one of Embodiments 24-25, wherein the compound is soluble in water, and wherein the compound further comprises a hydrophilic motif.

[00287] Embodiment 27: The wound dressing of any one of Embodiments 24-26, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

[00288] Embodiment 28: The wound dressing of any one of Embodiments 24-27, wherein the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

[00289] Embodiment 29: The wound dressing of any one of Embodiments 24-28, wherein the base dressing material comprises at least one selected from the group consisting of an

alginate dressing material, a biosynthetic dressing material, a collagen dressing material, a composite dressing material, a contact layer dressing material, a gauze, a hydrocolloid dressing material, a hydrofiber dressing material, a hydrogel, a hydropolymer dressing material, a polyurethane foam dressing material, a skin substitute dressing material, a superabsorbent dressing material, a transparent film dressing material, a wound filler, a wound pouch.

[00290] Embodiment 30: The wound dressing of any one of Embodiments 24-29, wherein the compound comprising the polypeptide is on a surface of or impregnated into the base dressing material.

[00291] Embodiment 31: The wound dressing of any one of Embodiments 24-30, further comprises an adhesive substrate.

[00292] The foregoing outlines features of several embodiments so that those skilled in the art may better understand the aspects of the present disclosure. Those skilled in the art should appreciate that they may readily use the present disclosure as a basis for designing or modifying other processes and structures for carrying out the same purposes and/or achieving the same advantages of the embodiments introduced herein. Those skilled in the art should also realize that such equivalent constructions do not depart from the spirit and scope of the present disclosure, and that they may make various changes, substitutions, and alterations herein without departing from the spirit and scope of the present disclosure.

CLAIMS

What is claimed is:

1. A method of promoting neovascularization and/or hair growth in a subject in need thereof, the method comprising:

administering to the subject a compound comprising a polypeptide,

wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, optionally wherein the polypeptide comprises a plurality of independently selected first peptides, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues, or

(b) the polypeptide comprises the amino acid set forth in SEQ ID NO:5, 6, 8, or 9.

2. The method of claim 1, wherein the compound is soluble in water or insoluble in water.

3. The method of any one of claims 1-2, wherein the compound is soluble in water, and wherein the compound further comprises a hydrophilic motif.

4. The method of claim 3, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

5. The method of any one of claims 1-4, wherein the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

6. The method of any one of claims 1-5, which is a method of promoting neovascularization, and wherein the method promotes in the subject at least one selected from the group consisting of angiogenesis and arteriogenesis.

7. The method of any one of clams 1-6, which is a method of promoting neovascularization, wherein at least one of the following applies:

(a) the subject suffers from a chronic wound healing disorder, and wherein the method promotes neovascularization in or near at least one chronic wound of the subject;

(b) the subject suffers from an acute wound, and wherein the method promotes neovascularization which speeds up the healing of the acute wound.

8. The method of claim 7, wherein the chronic wound comprises at least one of the following:

(a) a nonhealing wound;

(b) an infected wound, optionally an infected surgical wound or an infected traumatic wound;

(c) an ulcer, optionally a diabetic ulcer such as a diabetic foot ulcer, an arterial ulcer, a venous ulcer, a pressure ulcer, or an ischemic ulcer; or

(d) a wound associated with immunosuppression or radiation.

9. The method of any one of claims 1-8, wherein the compound reduces phosphatase activity exerted by protein-tyrosine phosphatase 1B (PTP1B) and/or T-cell protein tyrosine phosphatase (TC-PTP) on vascular endothelial growth factor receptor 2 (VEGFR2).

10. The method of any one of claims 1-9, wherein the method further comprises administering to the subject an effective amount of vascular endothelial growth factor (VEGF).

11. A method of promoting wound healing in a subject in need thereof, the method comprising:

administering to the subject a compound comprising a polypeptide,

wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, optionally wherein the polypeptide comprises a plurality of independently selected first peptides, and the compound does not comprise a peptide

sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues, or

(b) the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

12. The method of claim 11, wherein the compound is soluble in water or insoluble in water.

13. The method of any one of claims 11-12, wherein the compound is soluble in water, and wherein the compound further comprises a hydrophilic motif.

14. The method of claim 13, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

15. The method of any one of claims 11-14, wherein the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

16. The method of any one of claims 11-15, wherein the method promotes wound healing by promoting in the subject at least one selected from the group consisting of angiogenesis and arteriogenesis.

17. The method of any one of clams 11-16, wherein the wound is a chronic wound or an acute wound.

18. The method of claim 17, wherein the chronic wound comprises at least one of the following:

(a) a nonhealing wound;

(b) an infected wound, optionally an infected surgical wound or an infected traumatic wound;

(c) an ulcer, optionally a diabetic ulcer such as a diabetic foot ulcer, an arterial ulcer, a venous ulcer, a pressure ulcer, or an ischemic ulcer; or

(d) a wound associated with immunosuppression or radiation.

19. The method of any one of claims 11-18, wherein the compound reduces phosphatase activity exerted by protein-tyrosine phosphatase 1B (PTP1B) and/or T-cell protein tyrosine phosphatase (TC-PTP) on vascular endothelial growth factor receptor 2 (VEGFR2).

20. The method of any one of claims 11-19, wherein the method further comprises administering to the subject an effective amount of vascular endothelial growth factor (VEGF).

21. A compound comprising a polypeptide and a hydrophilic motif,

wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues,

(b) the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

or a salt or solvate thereof.

22. The compound of claim 21, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

23. The compound of claim 21, wherein the polypeptide comprises a plurality of independently selected first peptides.

24. A wound dressing, comprising a base dressing material, and a compound comprising a polypeptide,

wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% of the amino acid residues as leucine residues,

(b) the polypeptide comprises an amino acid set forth in SEQ ID NO:5, 6, 8 or 9.

25. The wound dressing of claim 24, wherein the compound is soluble in water or insoluble in water.

26. The wound dressing of any one of claims 24-25, wherein the compound is soluble in water, and wherein the compound further comprises a hydrophilic motif.

27. The wound dressing of any one of claims 24-26, wherein the hydrophilic motif is at least one selected from the group consisting of:

(a) a second peptide which is hydrophilic; and

(b) one or more polyethylene glycol (PEG) groups.

28. The wound dressing of any one of claims 24-27, wherein the peptide comprises residues 1-66, residues 39-66, residues 51-66, or residues 51-61 of the sequence set forth in SEQ ID NO:15.

29. The wound dressing of any one of claims 24-28, wherein the base dressing material comprises at least one selected from the group consisting of an alginate dressing material, a biosynthetic dressing material, a collagen dressing material, a composite dressing material, a contact layer dressing material, a gauze, a hydrocolloid dressing material, a hydrofiber dressing material, a hydrogel, a hydropolymer dressing material, a polyurethane foam dressing material, a skin substitute dressing material, a superabsorbent dressing material, a transparent film dressing material, a wound filler, a wound pouch.

30. The wound dressing of any one of claims 24-29, wherein the compound comprising the polypeptide is on a surface of or impregnated into the base dressing material.

31. The wound dressing of any one of claims 24-30, further comprises an adhesive substrate.

Att

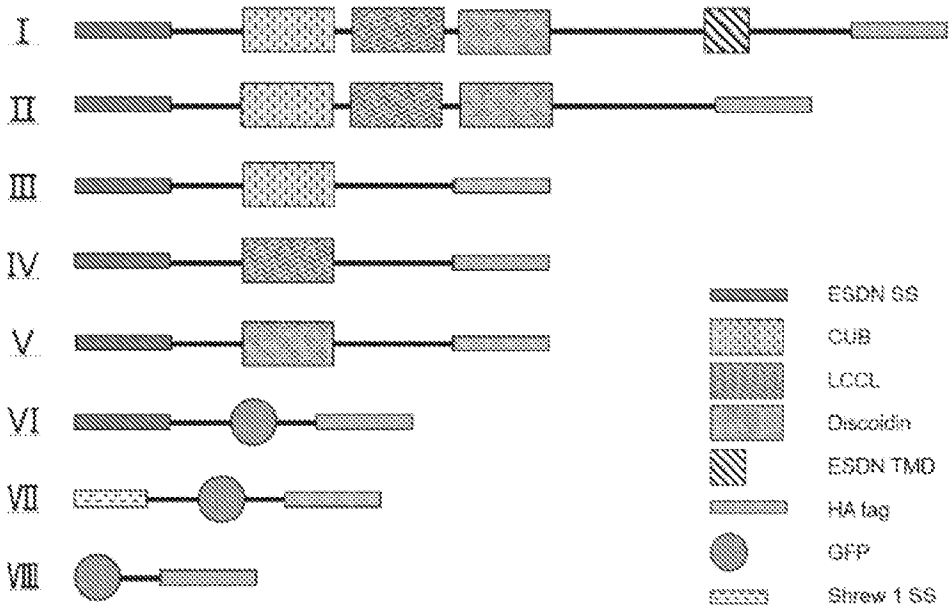


Fig. 1A

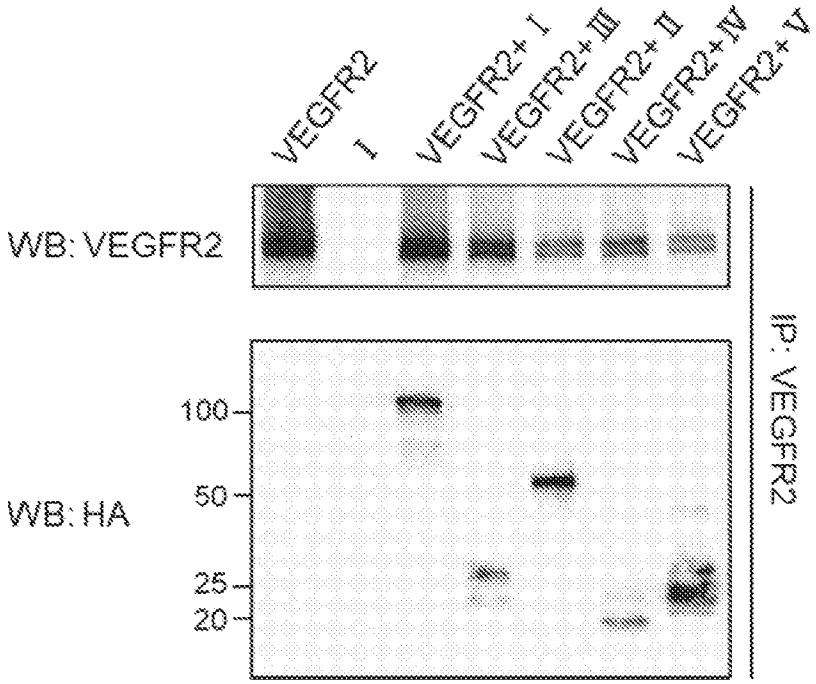
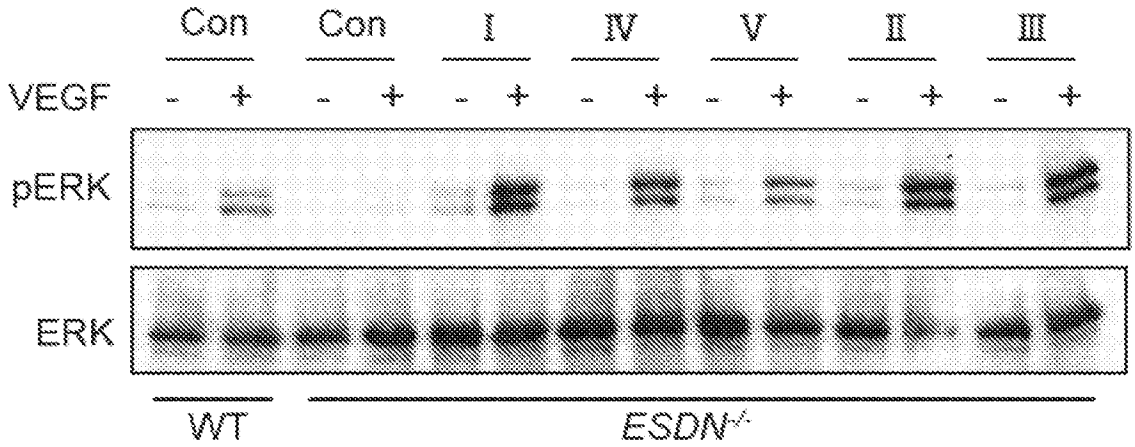


Fig. 1B

Att


Con
Con
I
IV
V
II
III
VEGF
- + - + - + - + - + - + - +
pERK
ERK
WT
ESDN-/-

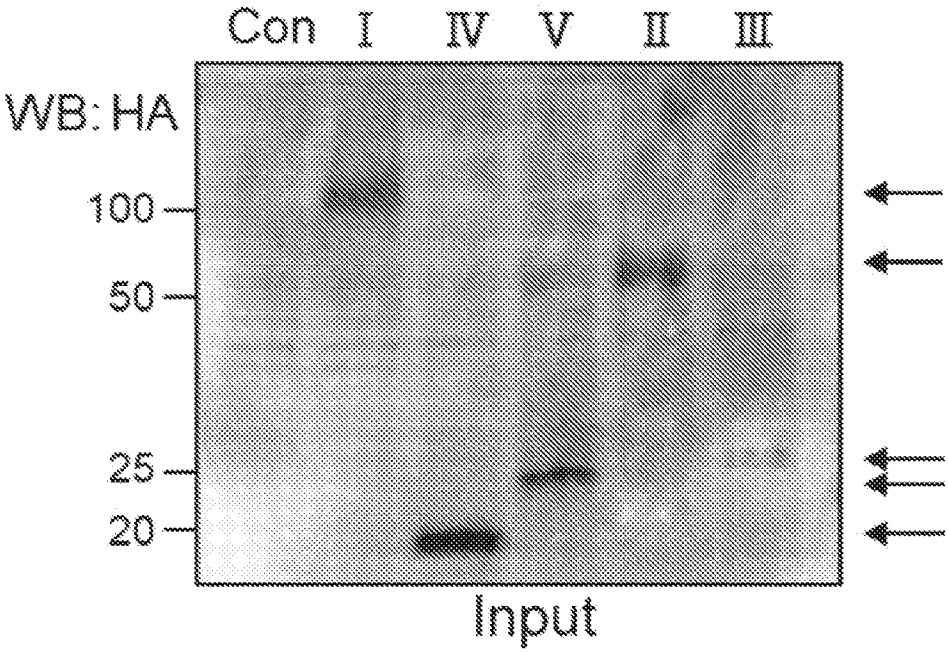

Con I IV V II III
WB: HA
100
50
25
20
Input

Fig. 1C

Att

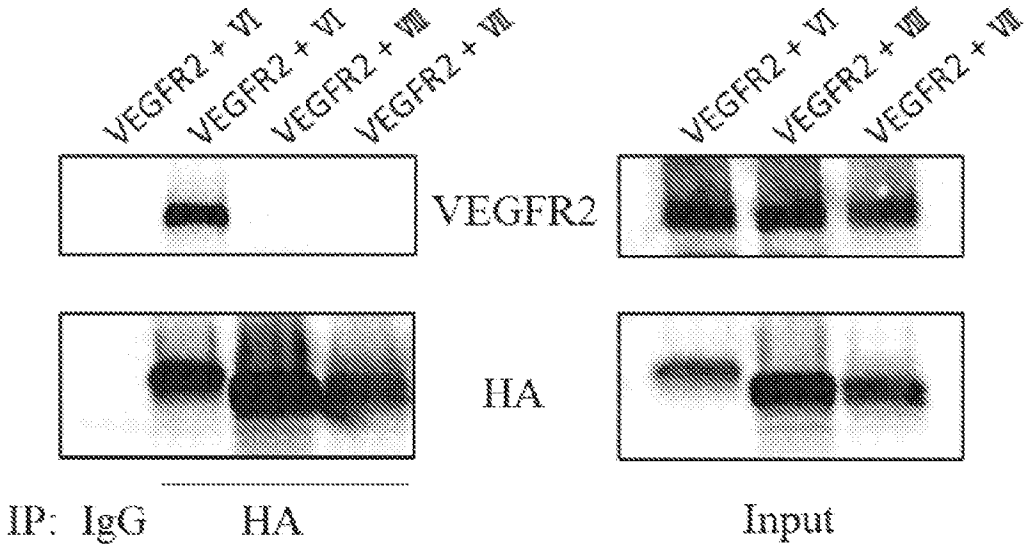


Fig. 1D

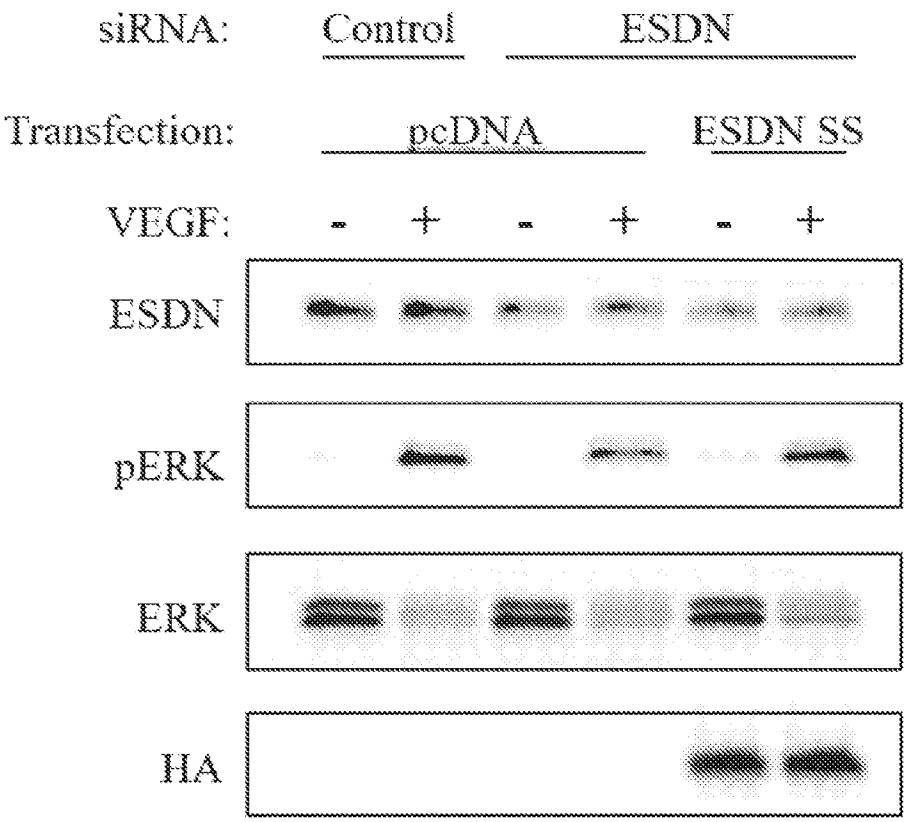


Fig. 1E

Att

Fig. 2A

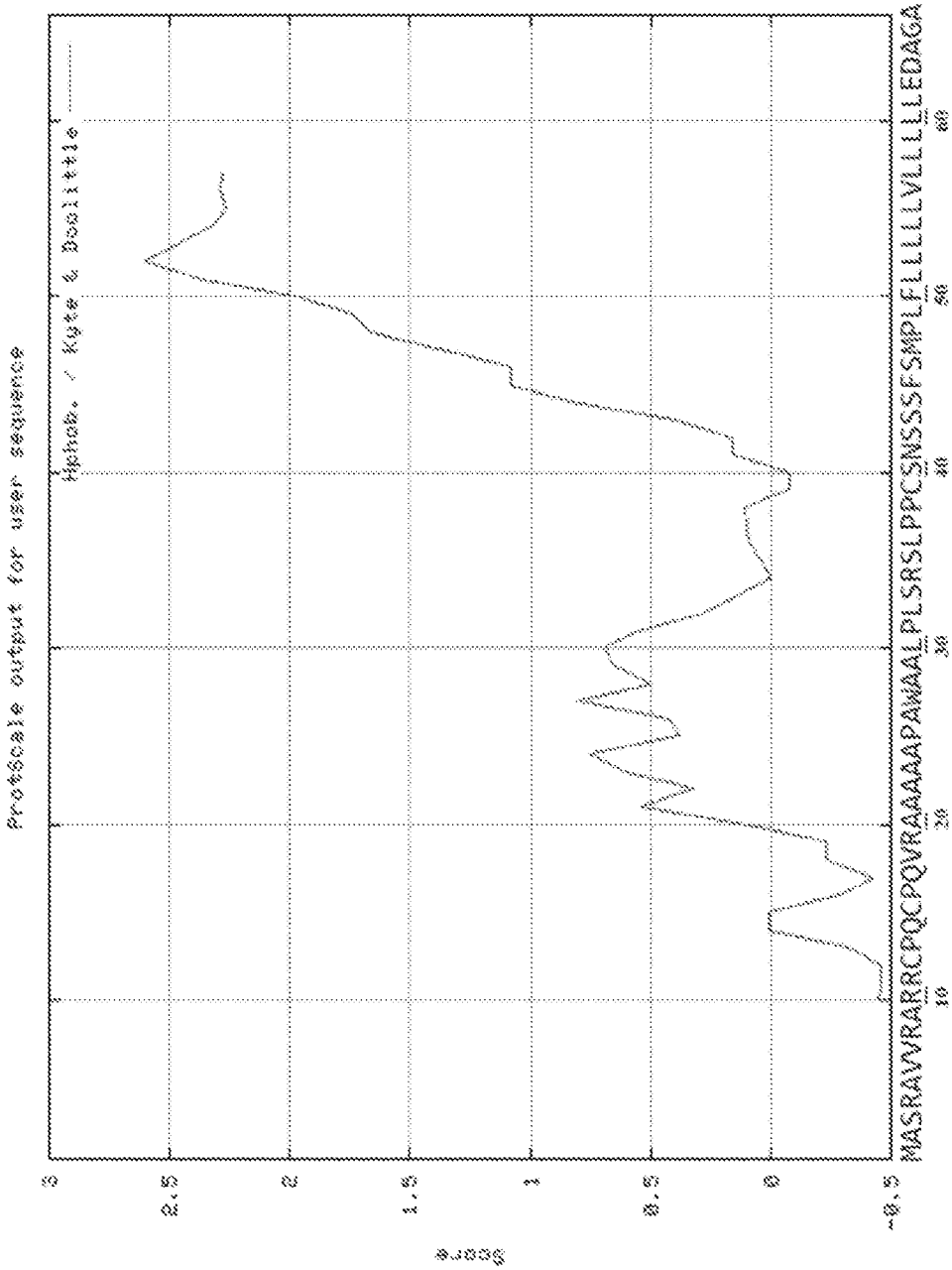


Fig. 2B

Att

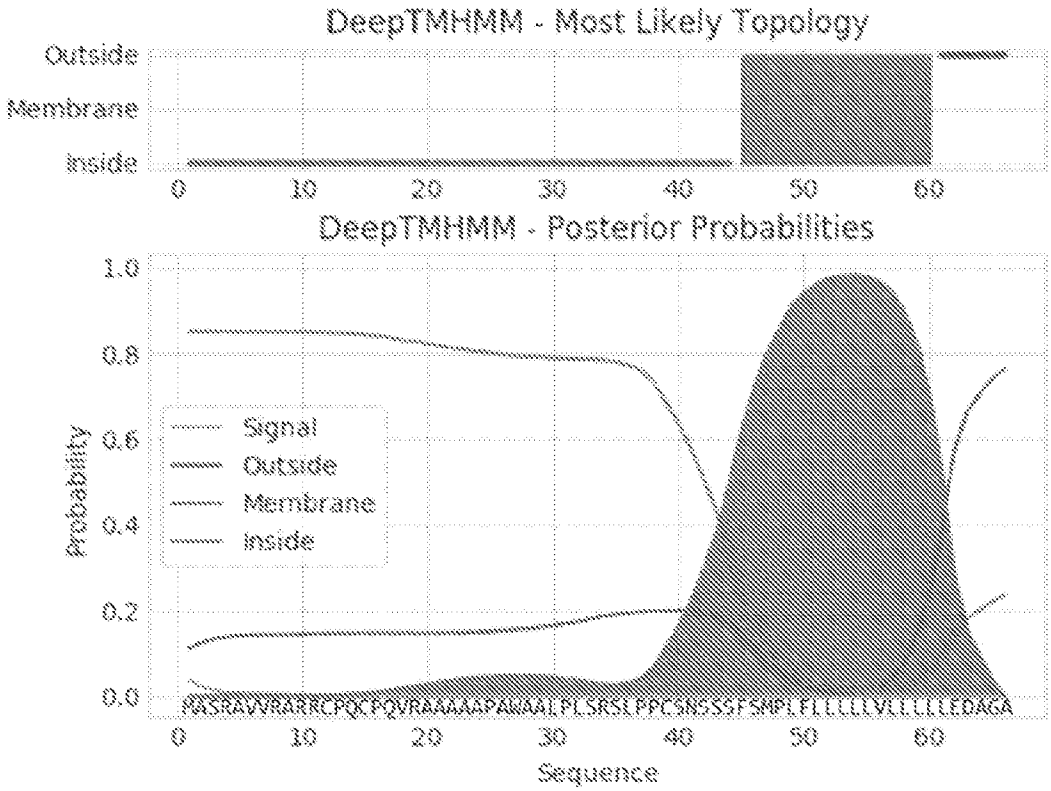


Fig. 2C

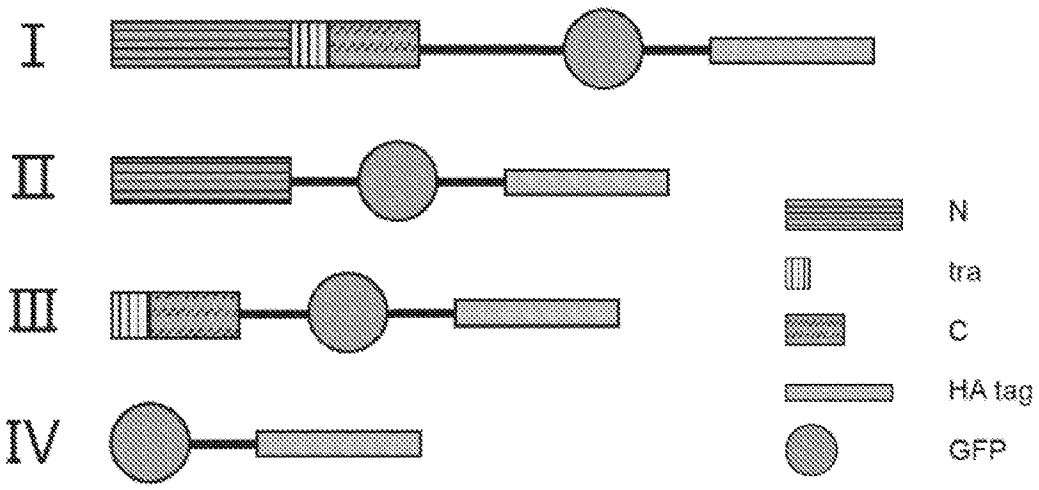


Fig. 2D

Att

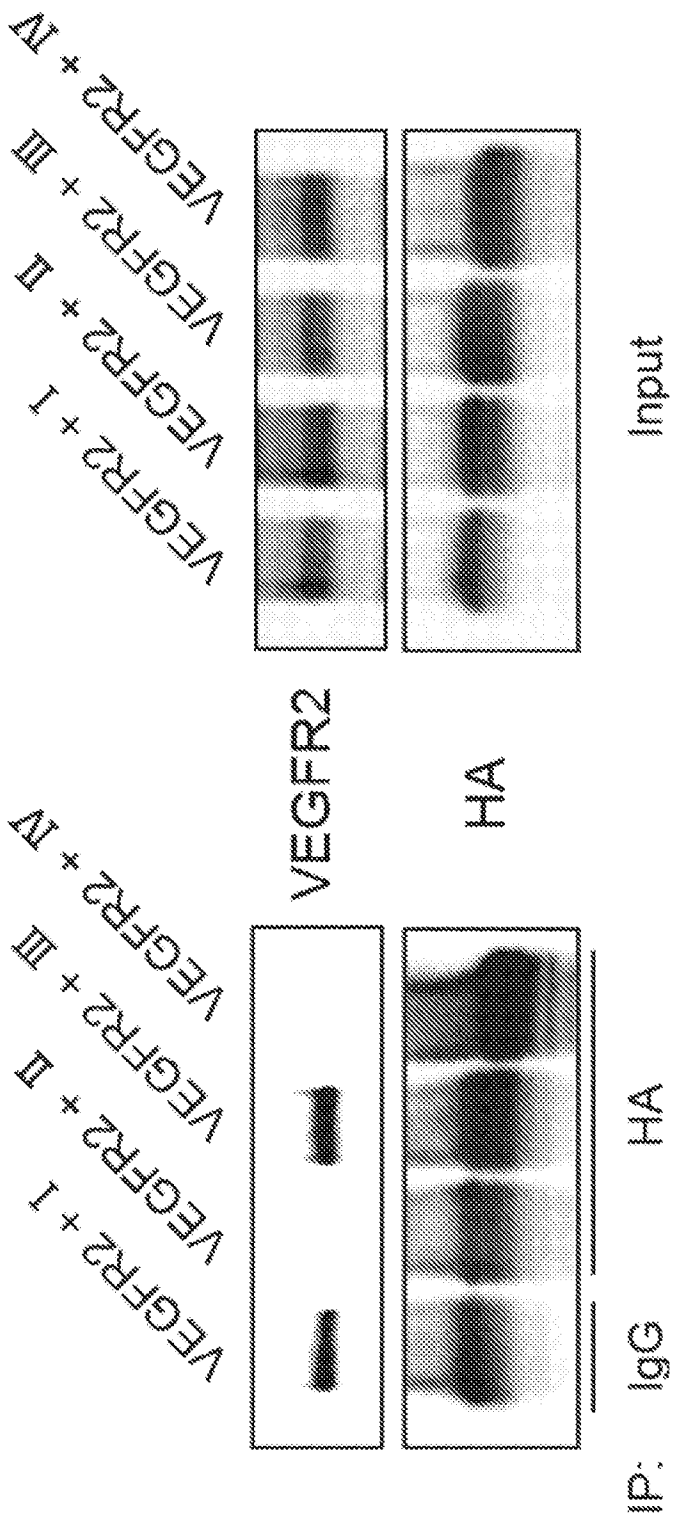

VEGFR2 + I
VEGFR2 + II
VEGFR2 + III
VEGFR2 + IV
Input
HA
VEGFR2
IP: IgG
HA

Fig. 2E

Att

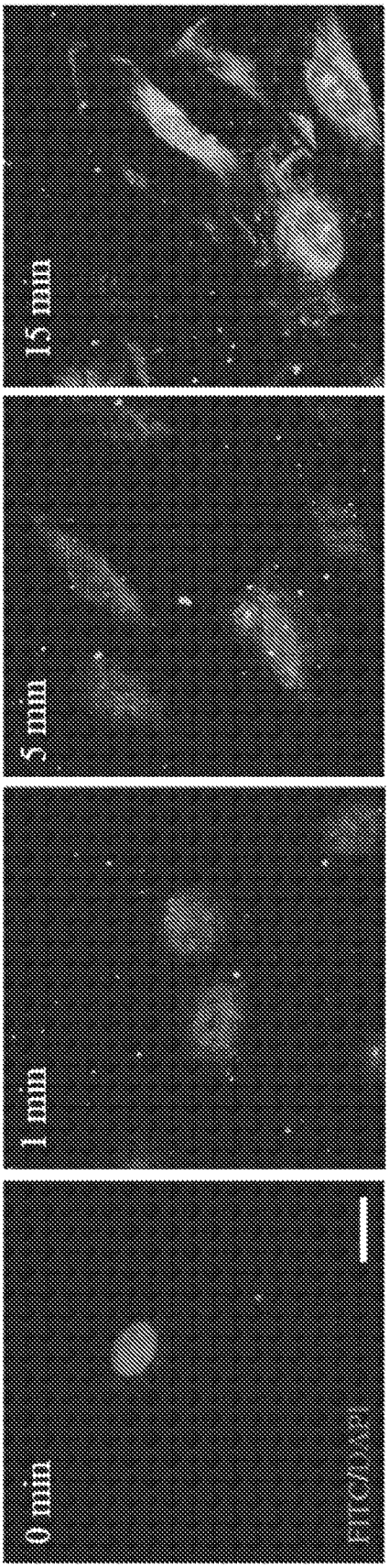


Fig. 3A

Fig. 3B

Att

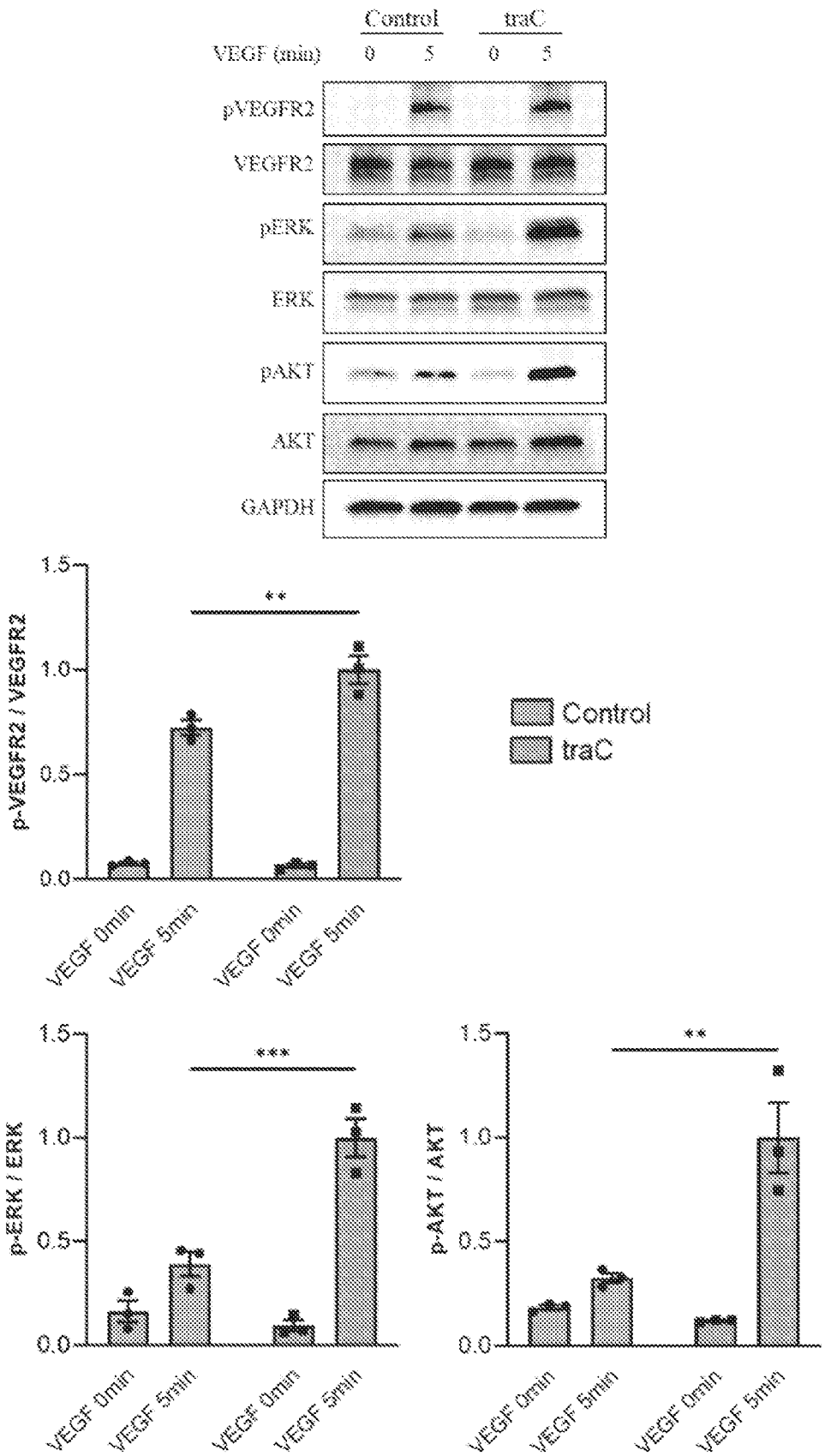

Control
traC
VEGF (min)
0
5
0
5
pVEGFR2
VEGFR2
pERK
ERK
pAKT
AKT
GAPDH
p-VEGFR2 / VEGFR2
1.5
1.0
0.5
0.0
**
Control
traC
VEGF 0min
VEGF 5min
VEGF 0min
VEGF 5min
p-ERK / ERK
***
p-AKT / AKT
**

Fig. 3C

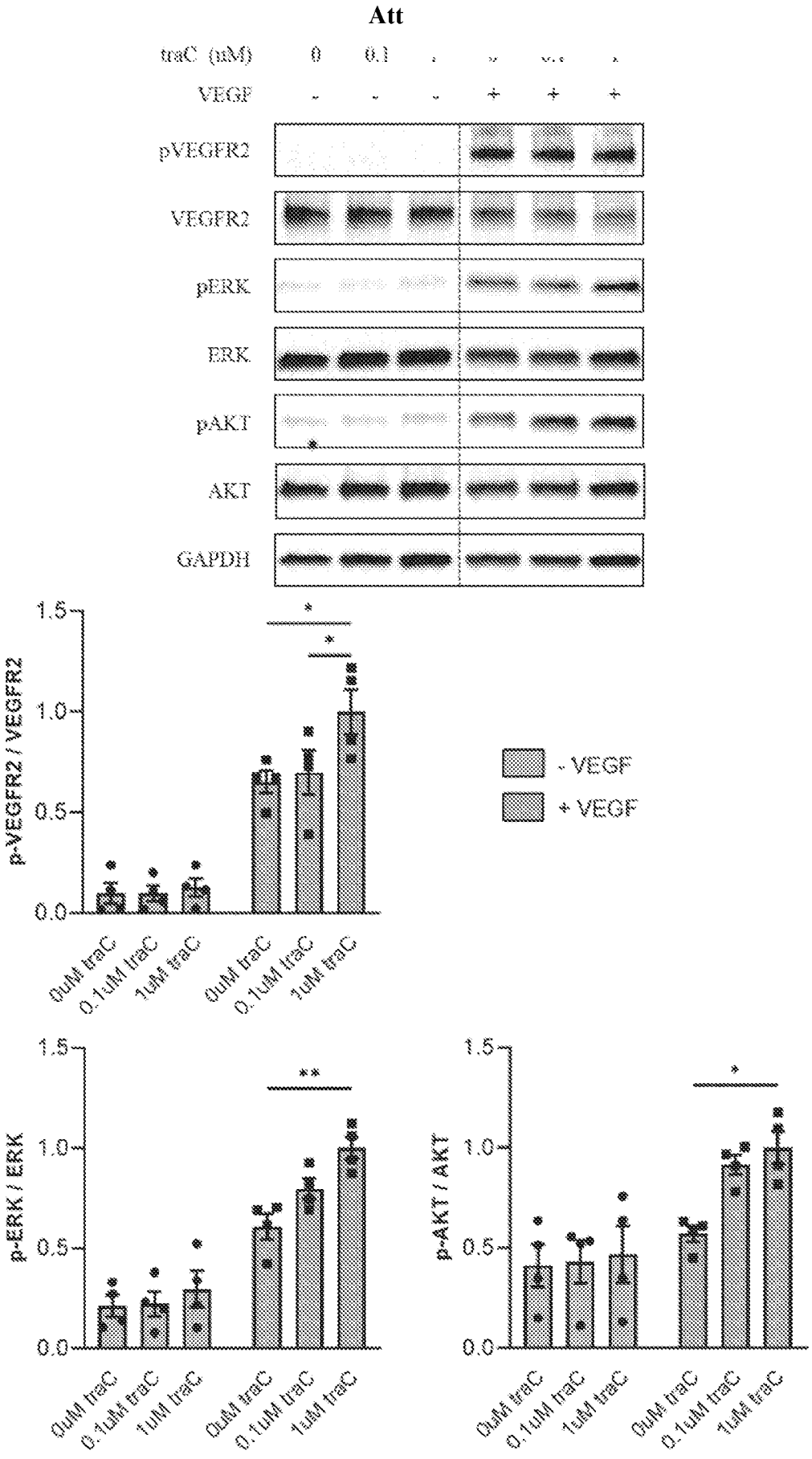

Att
traC (nM)
VEGF
pVEGFR2
VEGFR2
pERK
ERK
pAKT
AKT
GAPDH
p-VEGFR2 / VEGFR2
- VEGF
+ VEGF
0uM traC
0.1uM traC
1uM traC
p-ERK / ERK
p-AKT / AKT

Fig. 3D

Att

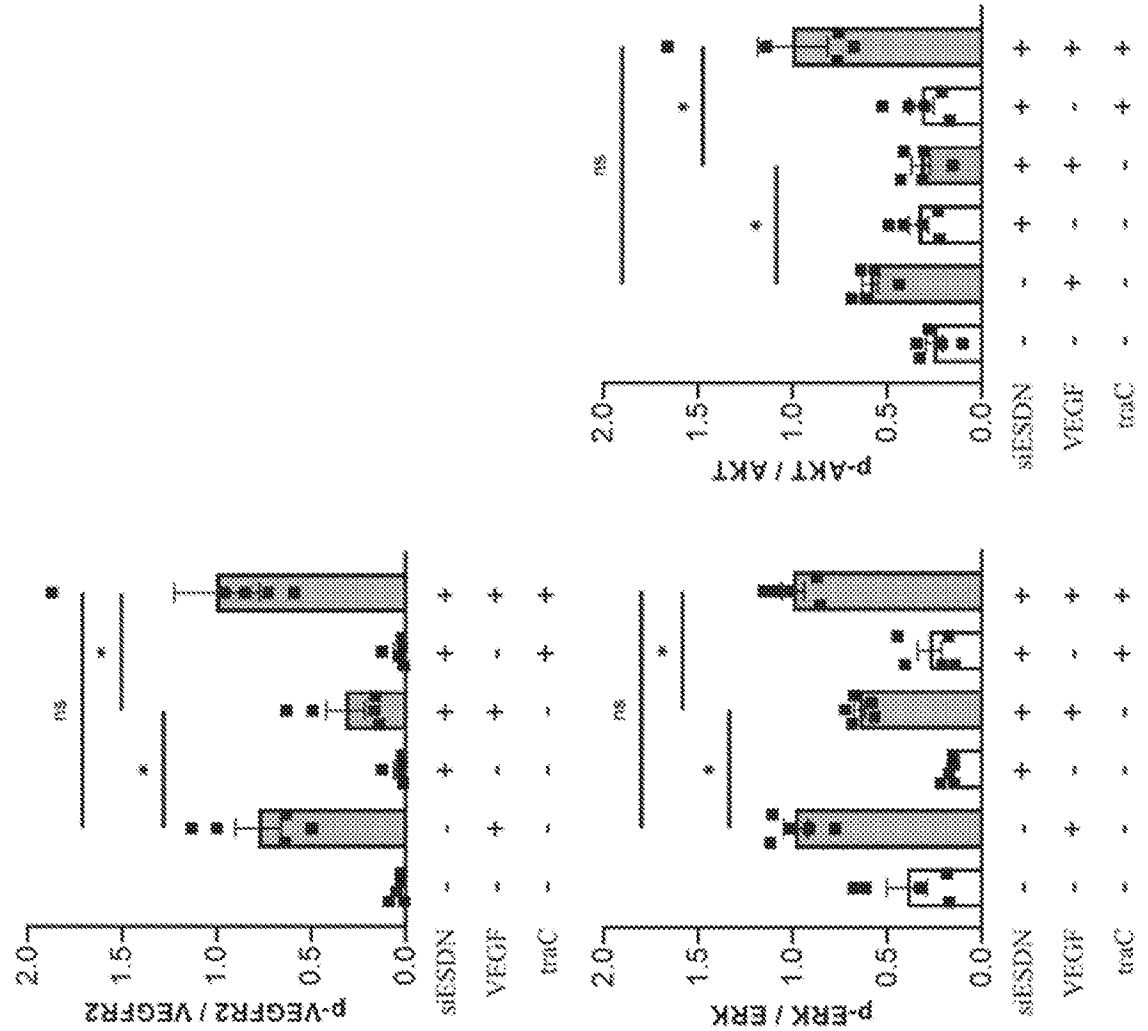

siCon
siESDN
tmC
VEGF
pVEGFR2
VEGFR2
pERK
ERK
pAKT
AKT
FITC
GAPDH
p-VEGFR2 / VEGFR2
p-ERK / ERK
p-AKT / AKT
siESDN
VEGF
tmC
ns
*

Fig. 3E

Att

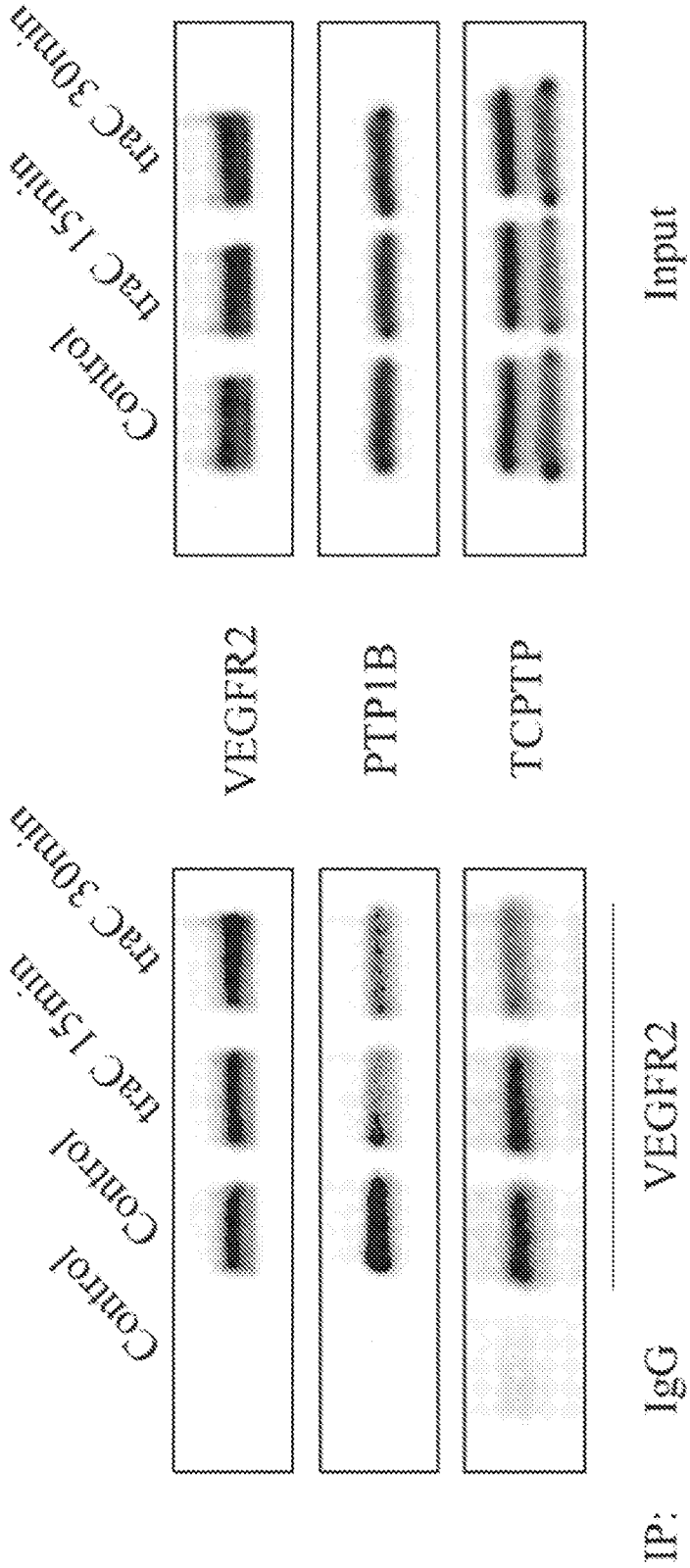

IP:
IgG
VEGFR2
Control
Control
tiaC 15min
tiaC 30min
VEGFR2
PTP1B
TCPTP
Input
Control
tiaC 15min
tiaC 30min

Fig. 3F

Att

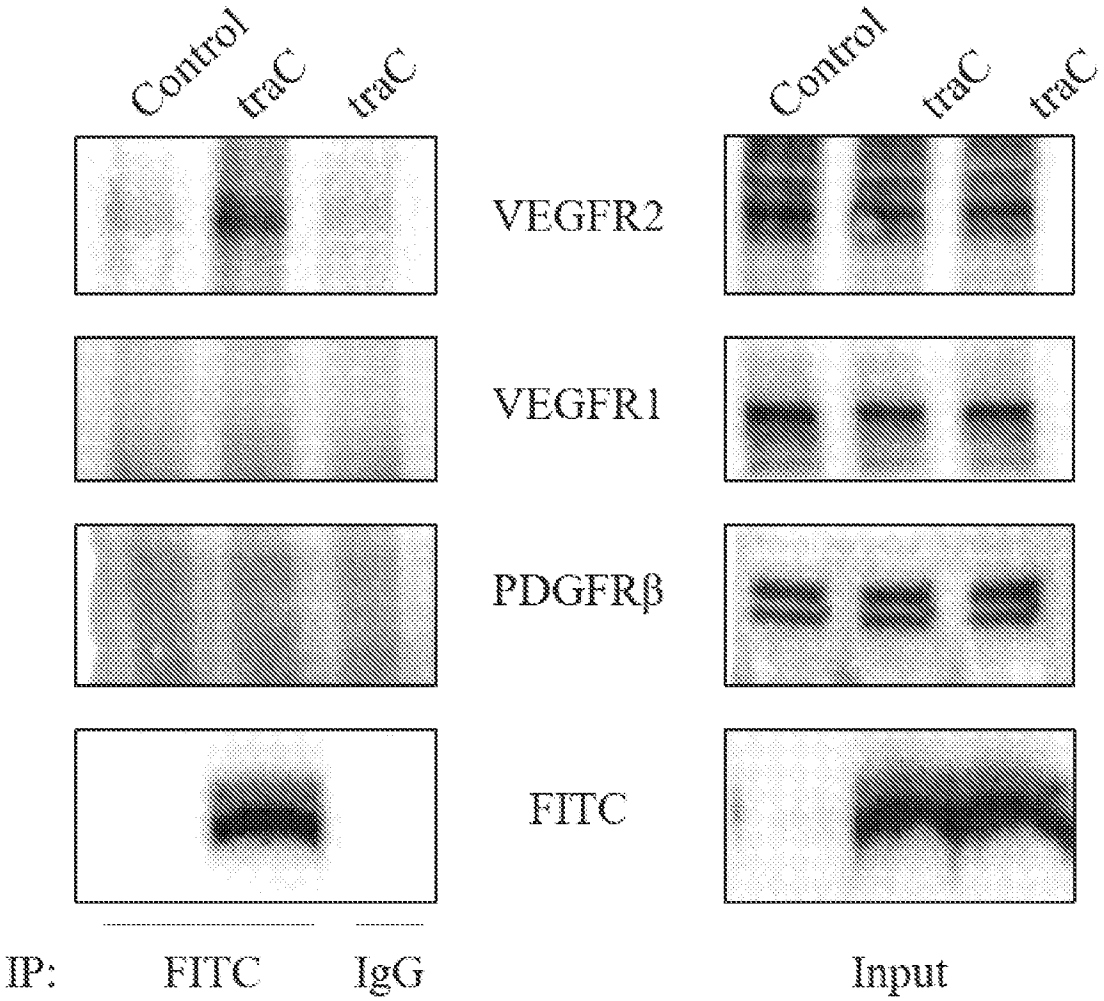

Control
traC
traC
Control
traC
traC
VEGFR2
VEGFR1
PDGFRβ
FITC
IP:
FITC
IgG
Input

Fig. 4

Att

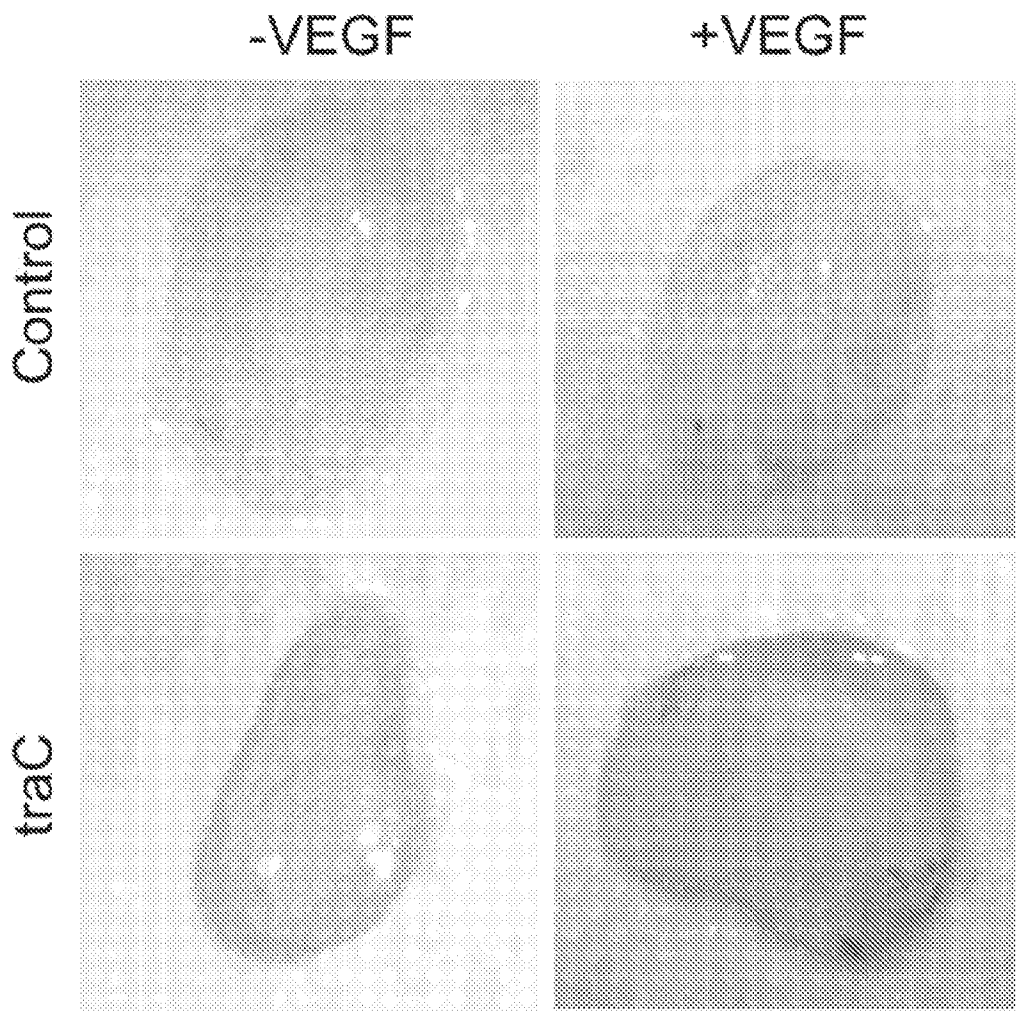


Fig. 5A

Att

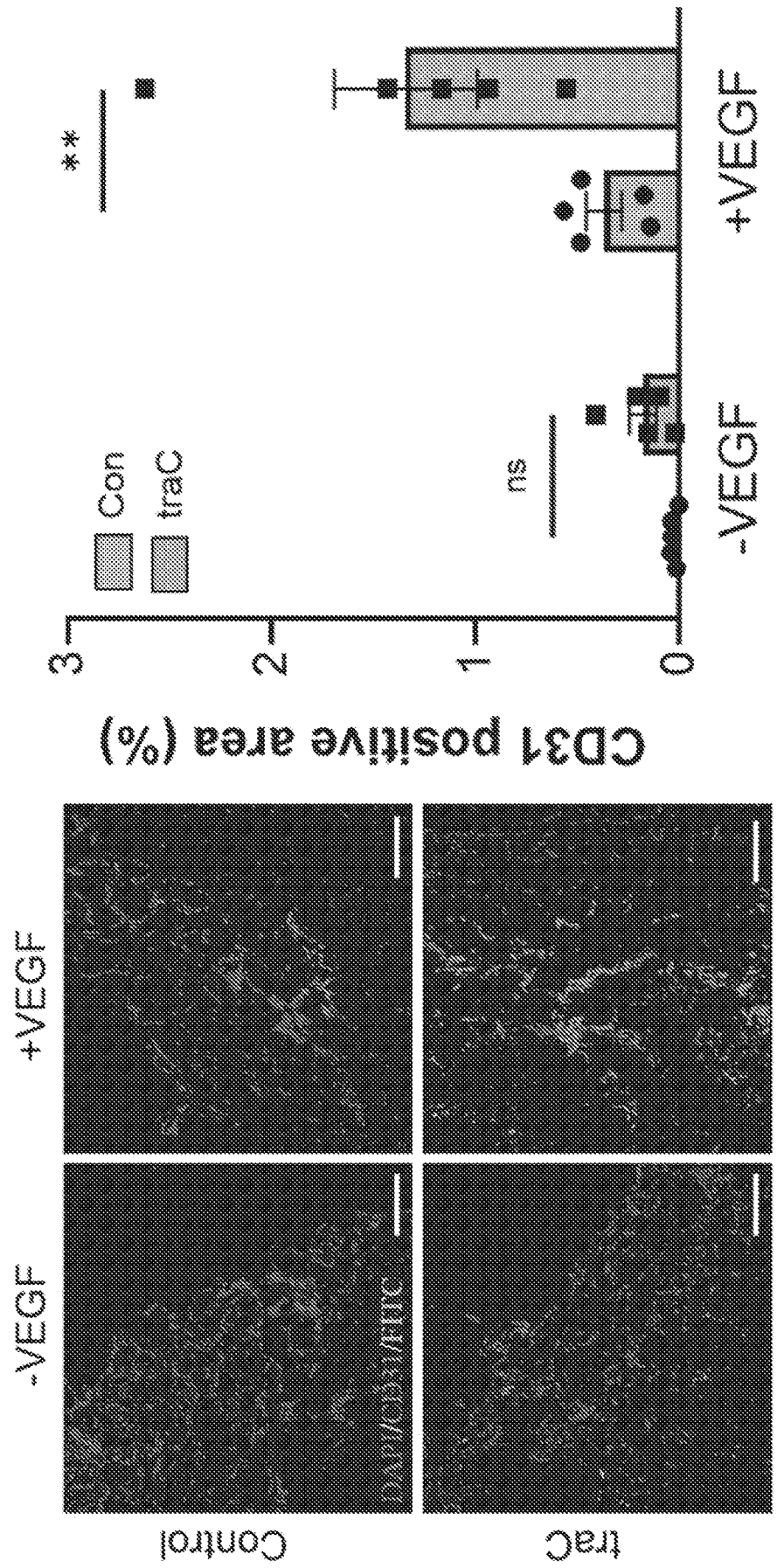

Control
traC
-VEGF
+VEGF
CD31 positive area (%)
Con
traC
ns
**
0
1
2
3
-VEGF
+VEGF

Fig. 5B

Att

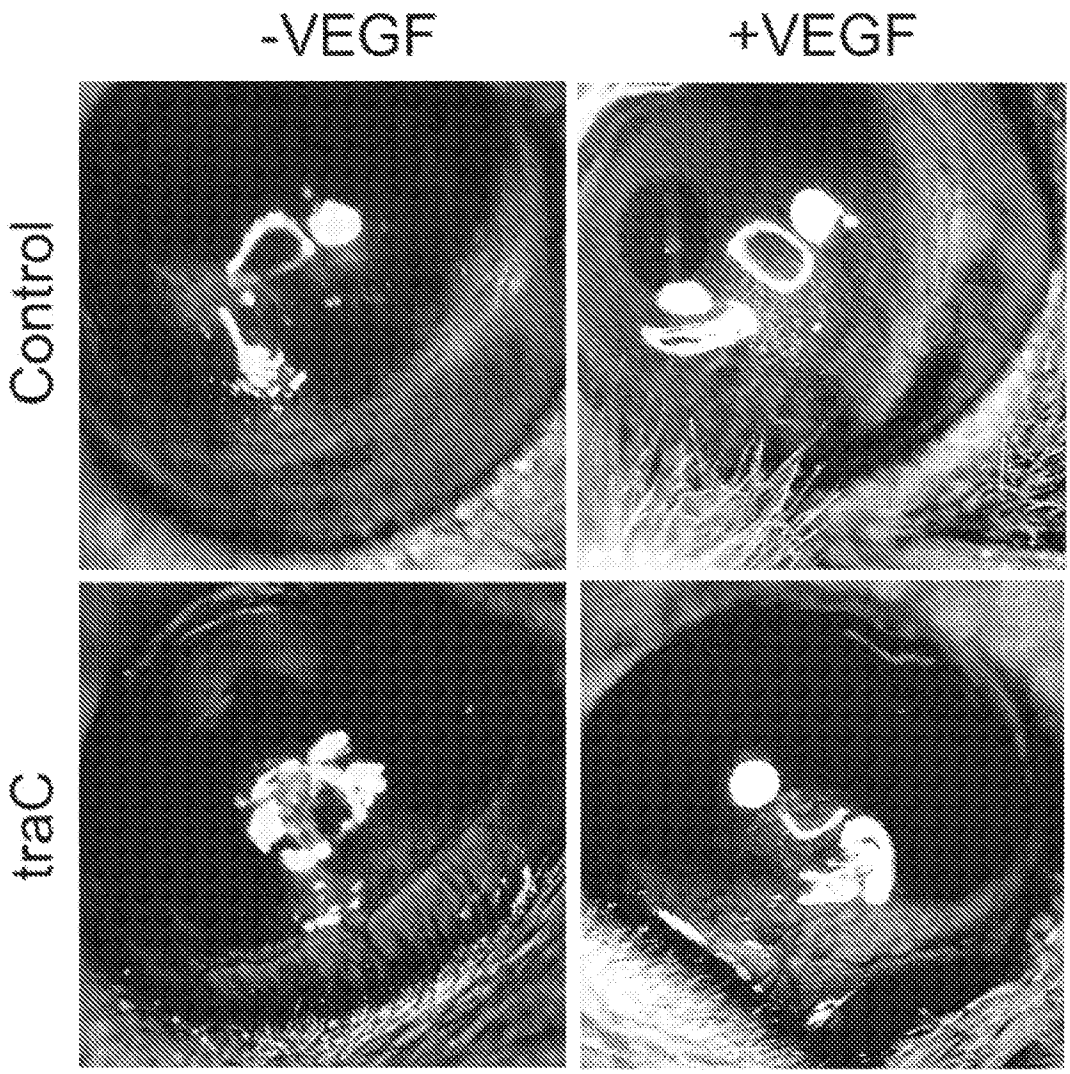


Fig. 6A

Att

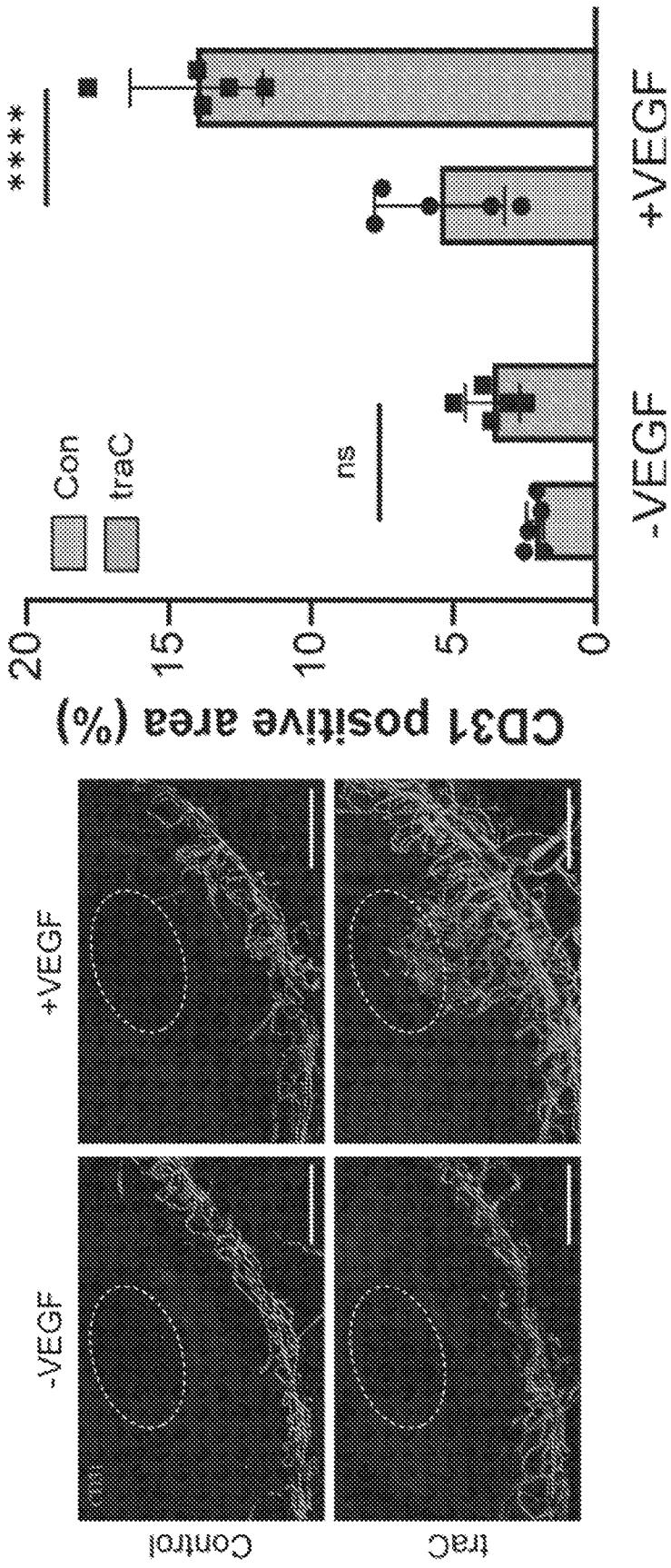

CD31 positive area (%)
20
15
10
5
0
Con
traC
ns
****
-VEGF
+VEGF
-VEGF
+VEGF
Control
traC

Fig. 6B

Att

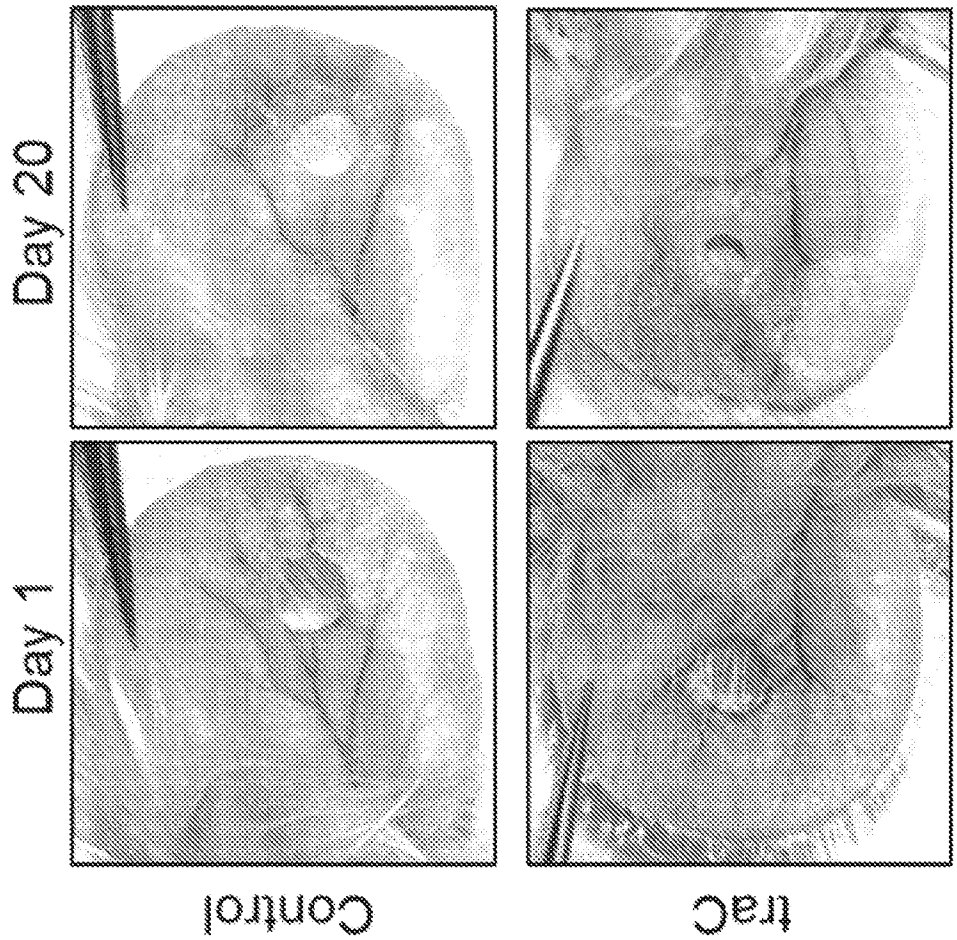


Fig. 7B

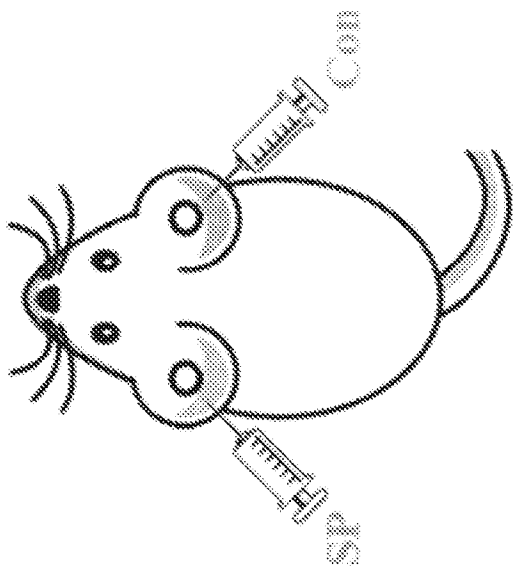


Fig. 7A

Att

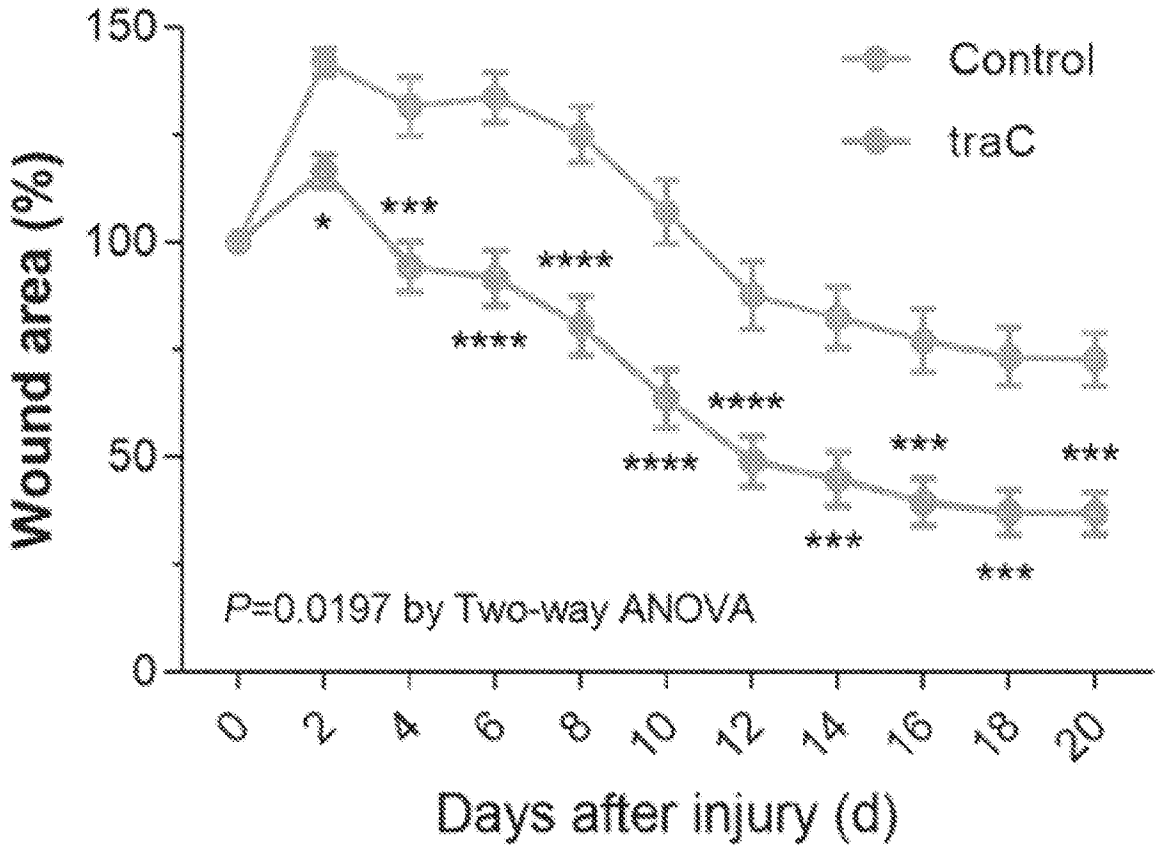

Wound area (%)
150
100
50
0
Control
traC
*
***
****
****
****
****
***
***
***
***
P=0.0197 by Two-way ANOVA
0
2
4
6
8
10
12
14
16
18
20
Days after injury (d)

Fig. 7C

Att

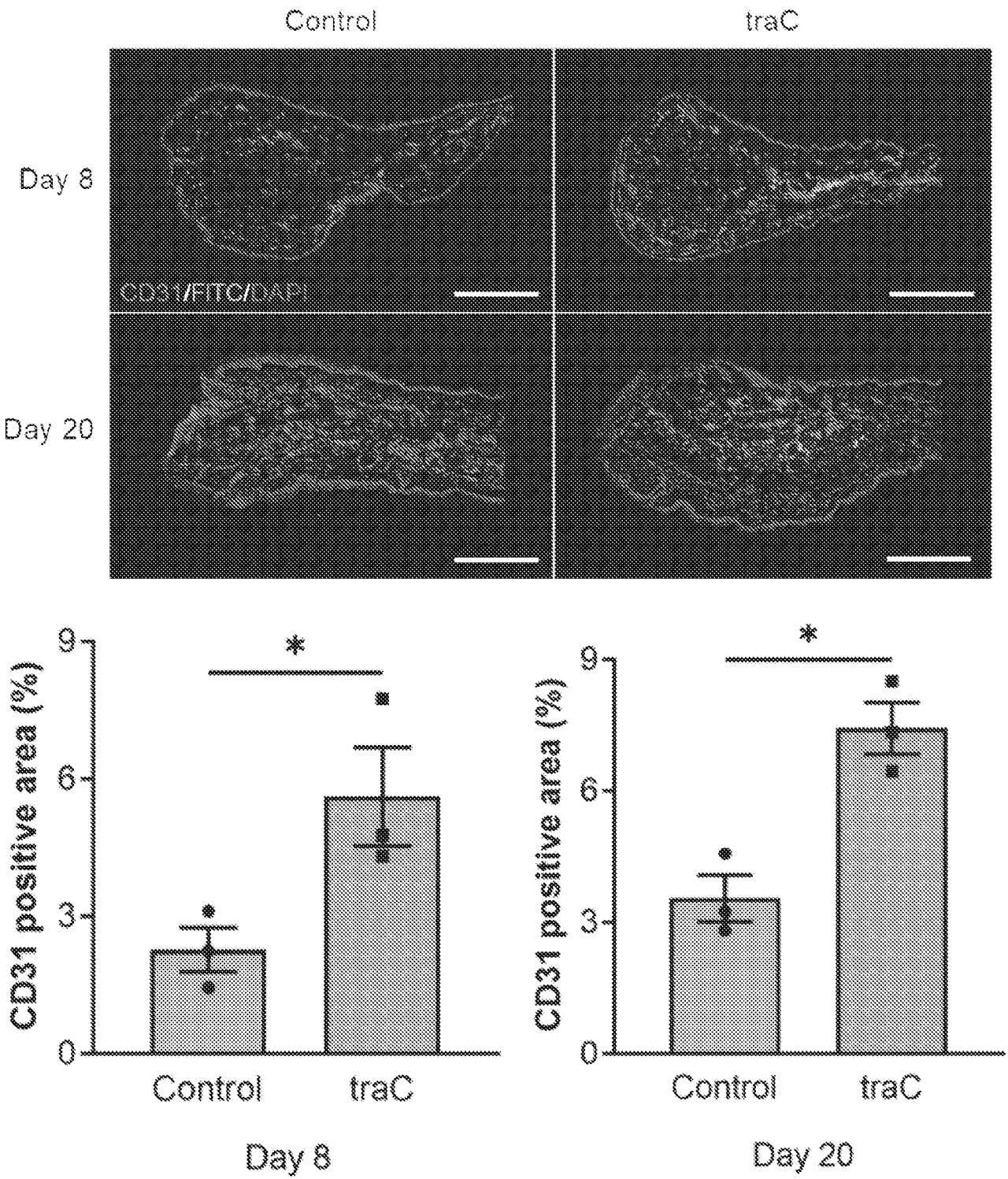

Control
traC
Day 8
Day 20
CD31/FITC/DAPI
CD31 positive area (%)
9
6
3
0
*
Control
traC
Day 8
Day 20

Fig. 7D

Att

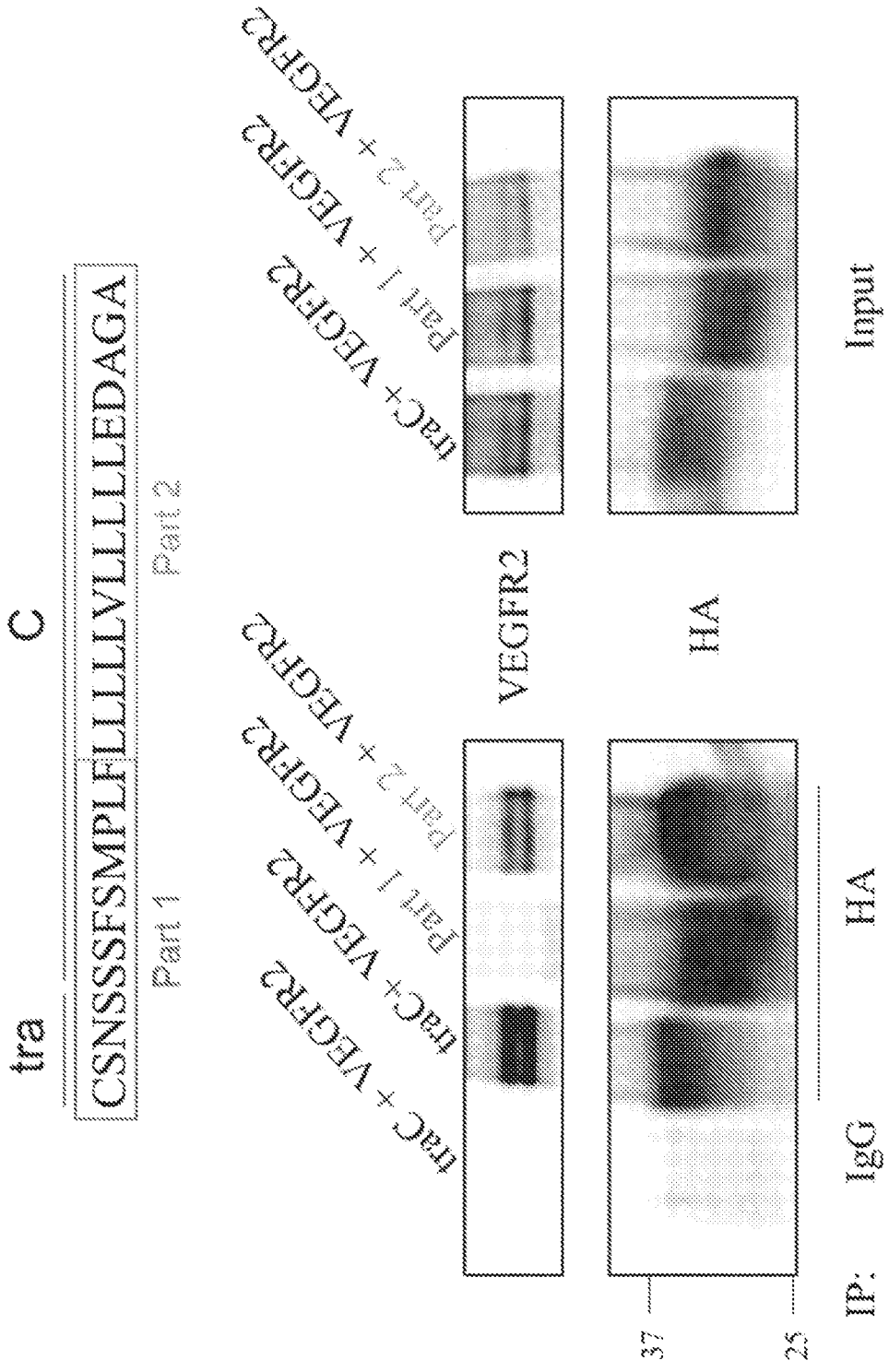

C
tra
CSNSSSFSMPLFLLLLLVLLLLLEDAGA
Part 1
Part 2
mAC+ VEGFR2
Part 1 + VEGFR2
Part 2 + VEGFR2
VEGFR2
HA
Input
IP:
IgG
HA
37
25

Fig. 8A

Att

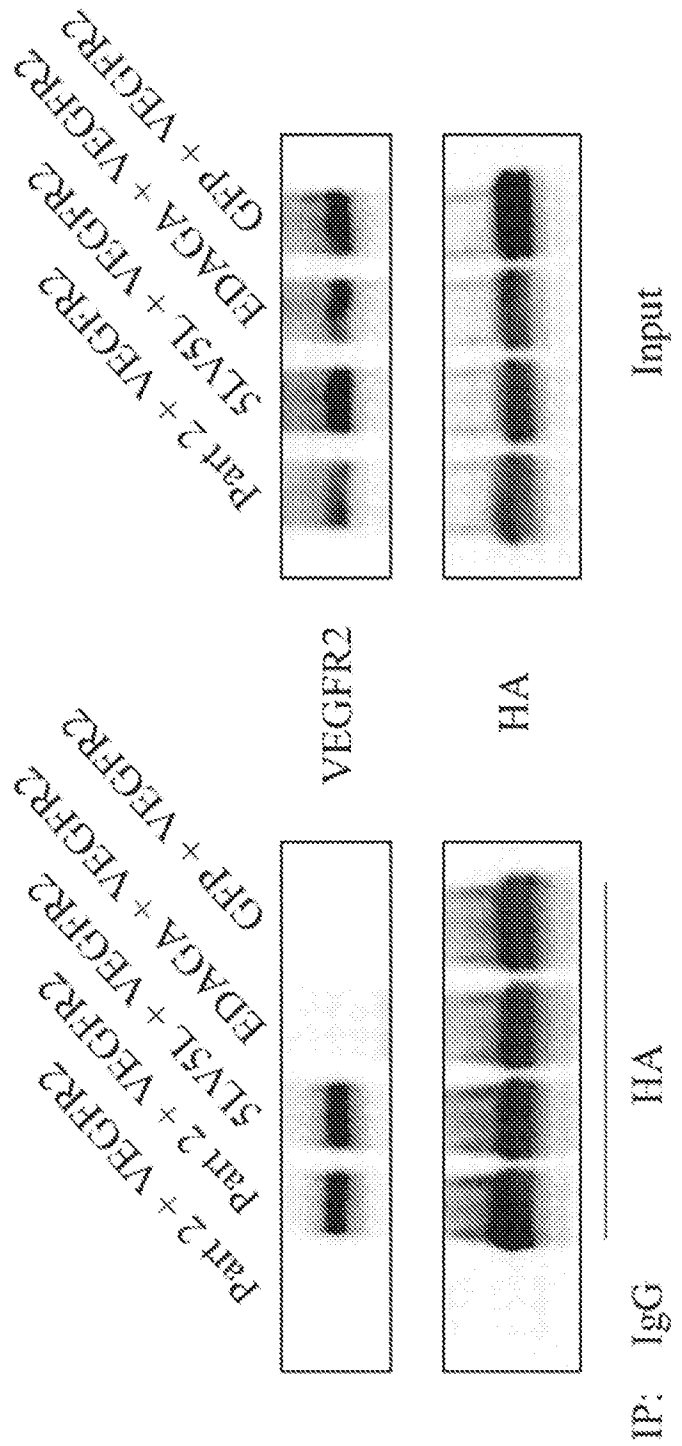

IP: IgG HA
VEGFR2
HA
Input
Pan 2 + VEGFR2
SLV5L + VEGFR2
EDAGA + VEGFR2
GFP + VEGFR2
Pan 2 + VEGFR2
SLV5L + VEGFR2
EDAGA + VEGFR2
GFP + VEGFR2

Fig. 8B

Att

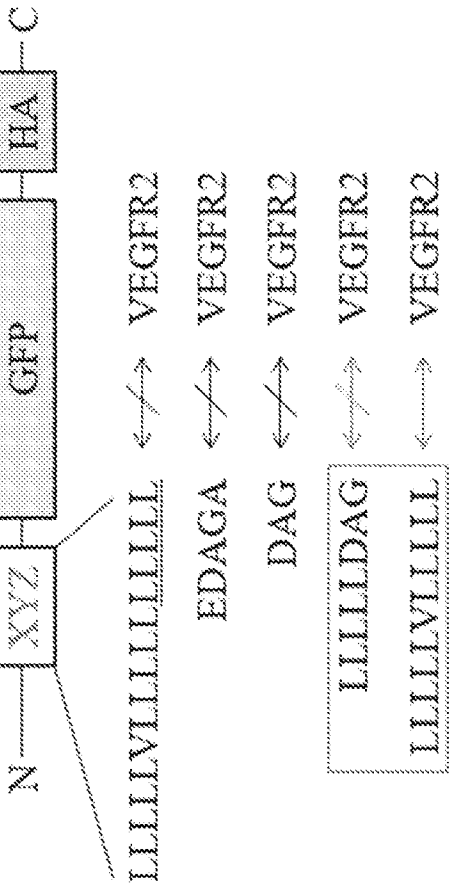

N
XYZ
GFP
HA
C
LLLLLVLLLLLLLL
VEGFR2
EDAGA
VEGFR2
DAG
VEGFR2
LLLLLDAG
VEGFR2
LLLLLVLLLLL
VEGFR2
traC part 2
LLLLLVLLLLEDAGA

Fig. 8C

Att

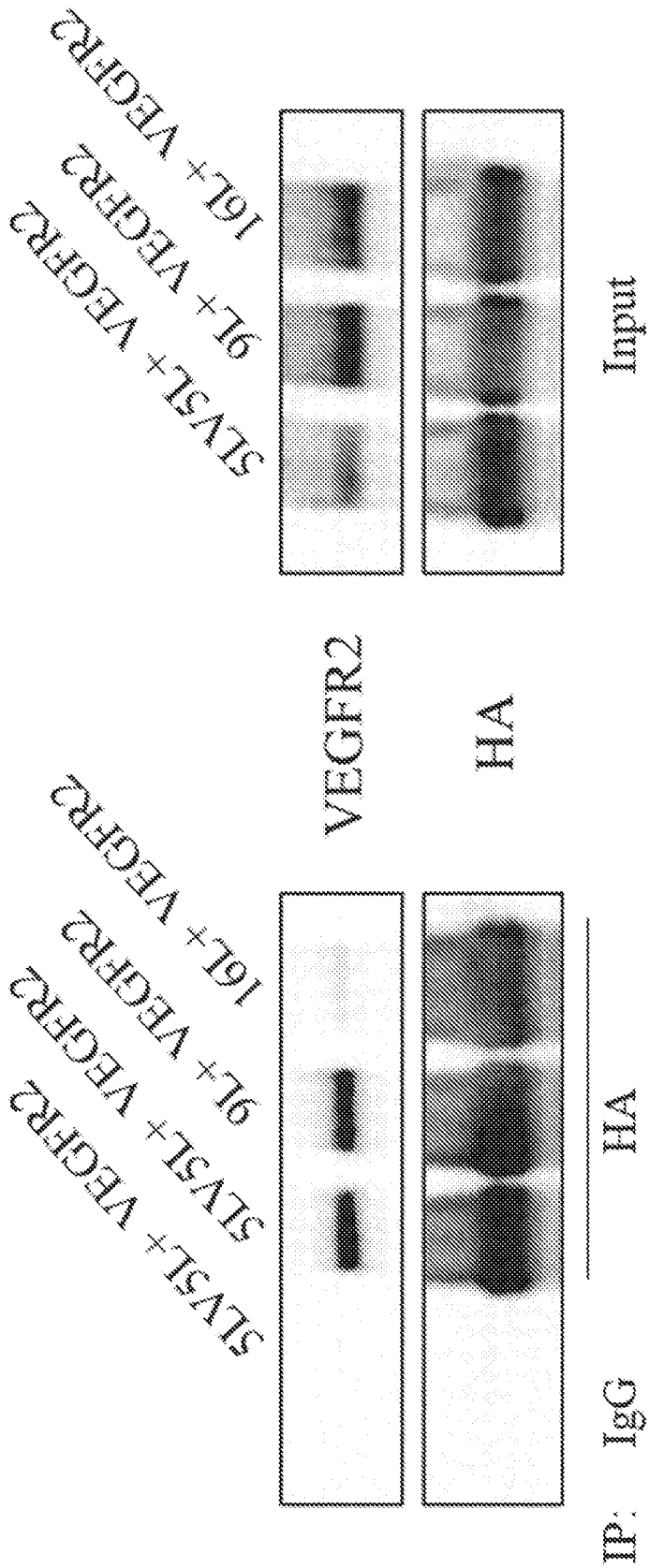

IP:
IgG
HA
Input
VEGFR2
HA
5LV5L+ VEGFR2
5LV/5L+ VEGFR2
9L+ VEGFR2
16L+ VEGFR2
5LV5L+ VEGFR2
5LV/5L+ VEGFR2
9L+ VEGFR2
16L+ VEGFR2

Fig. 8D

Att

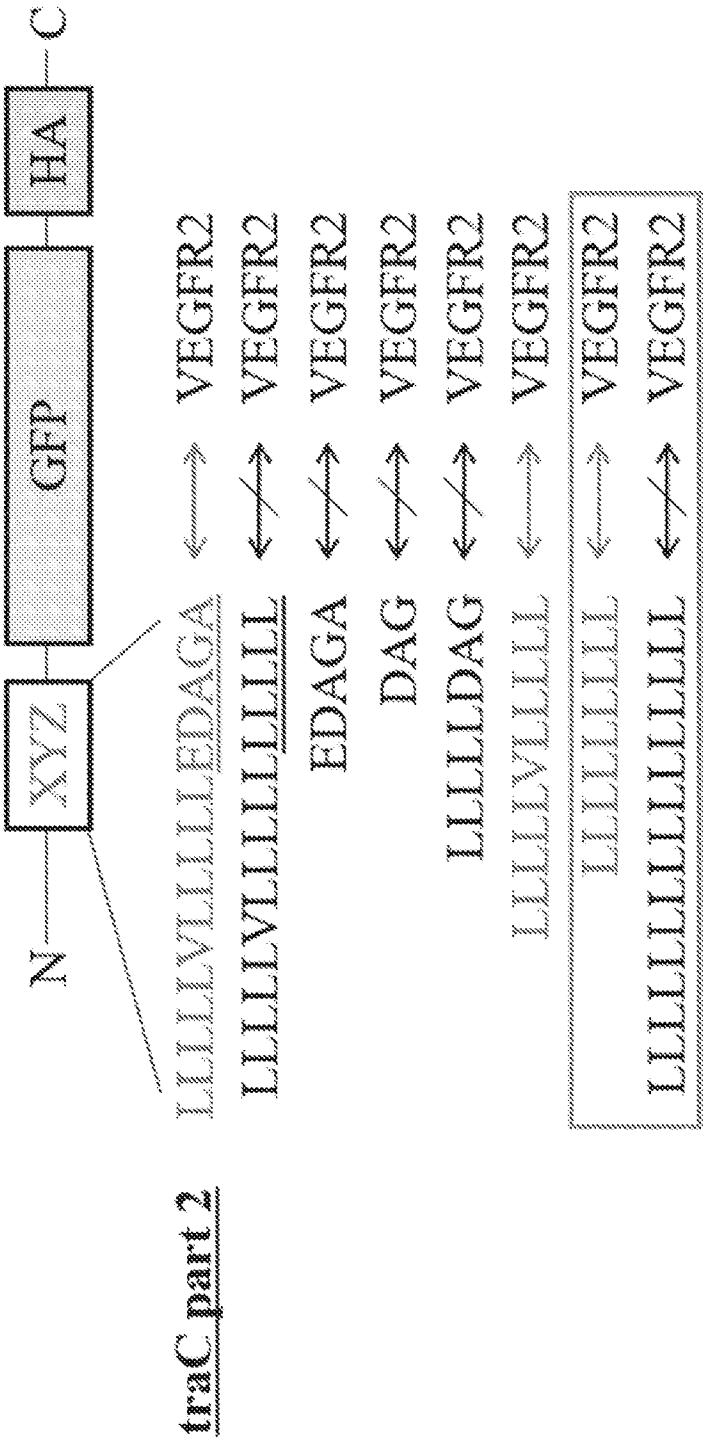

N
XYZ
GFP
HA
C
traC part 2
LLLLLVLLLLLEDAGA
LLLLLVLLLLLLLL
EDAGA
DAG
LLLLLDAG
LLLLLVLLLLL
LLLLLLLLL
LLLLLLLLLLLLLL
VEGFR2
VEGFR2
VEGFR2
VEGFR2
VEGFR2
VEGFR2
VEGFR2
VEGFR2

Fig. 8E

Att

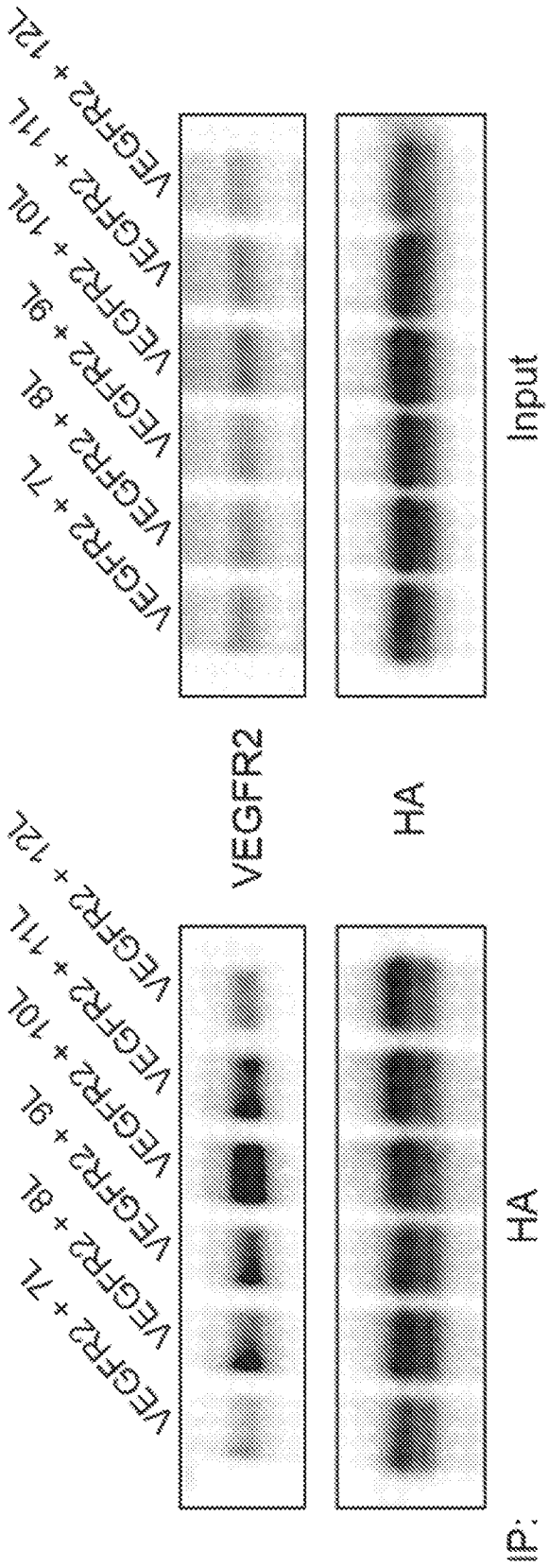

VEGFR2 + 7L
VEGFR2 + 8L
VEGFR2 + 9L
VEGFR2 + 10L
VEGFR2 + 11L
VEGFR2 + 12L
Input
HA
VEGFR2
IP:
HA

Fig. 8F

Att

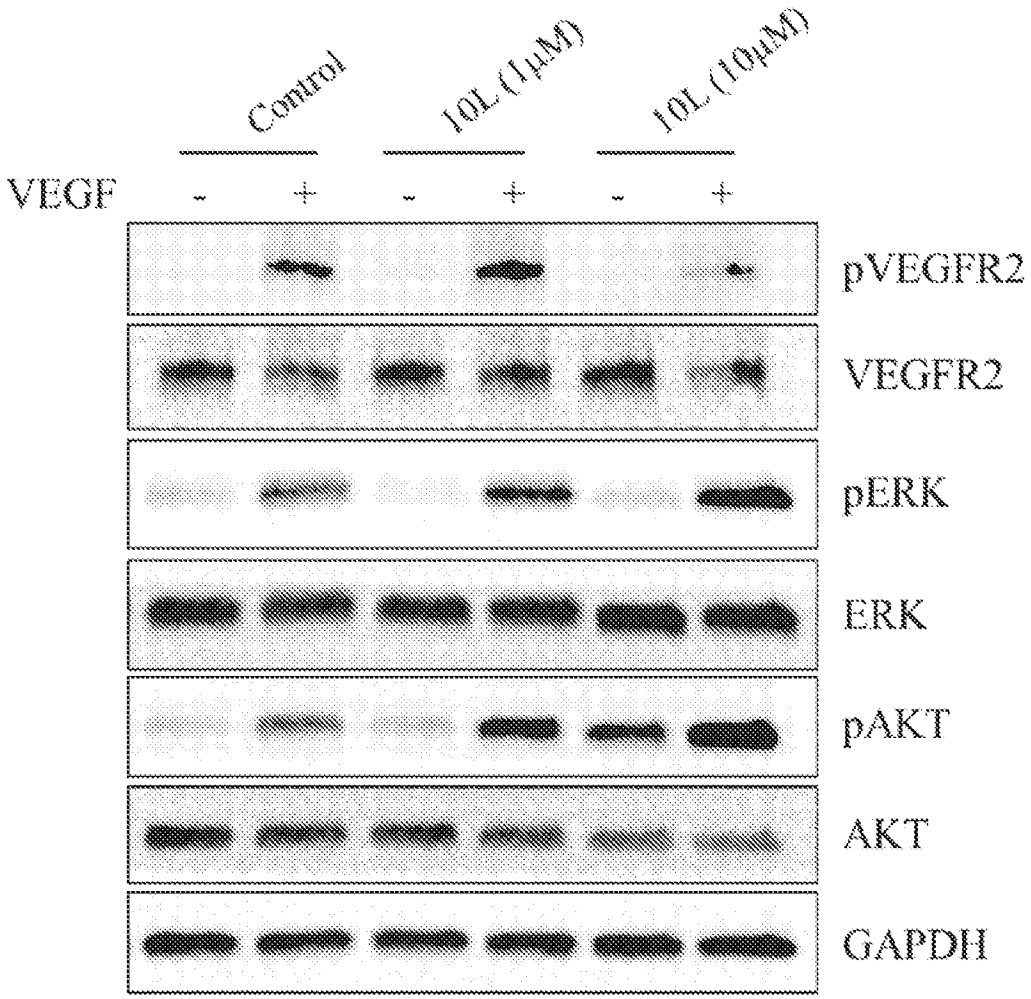

Control
10L (1μM)
10L (10μM)
VEGF
-
+
-
+
-
+
pVEGFR2
VEGFR2
pERK
ERK
pAKT
AKT
GAPDH

Fig. 8G

Att

Peptide Name	Sequence
FITC-traC A	CSNSSSFSMPLFLLLLLVLLLLLEDAGA
FITC-control I	CSNSSSFSMPIFIIIIIVIIIIIEDAGA
FITC-scramble	LSLESALMLFLCLDLSGVFLSLPLSNL

Fig. 9

Att

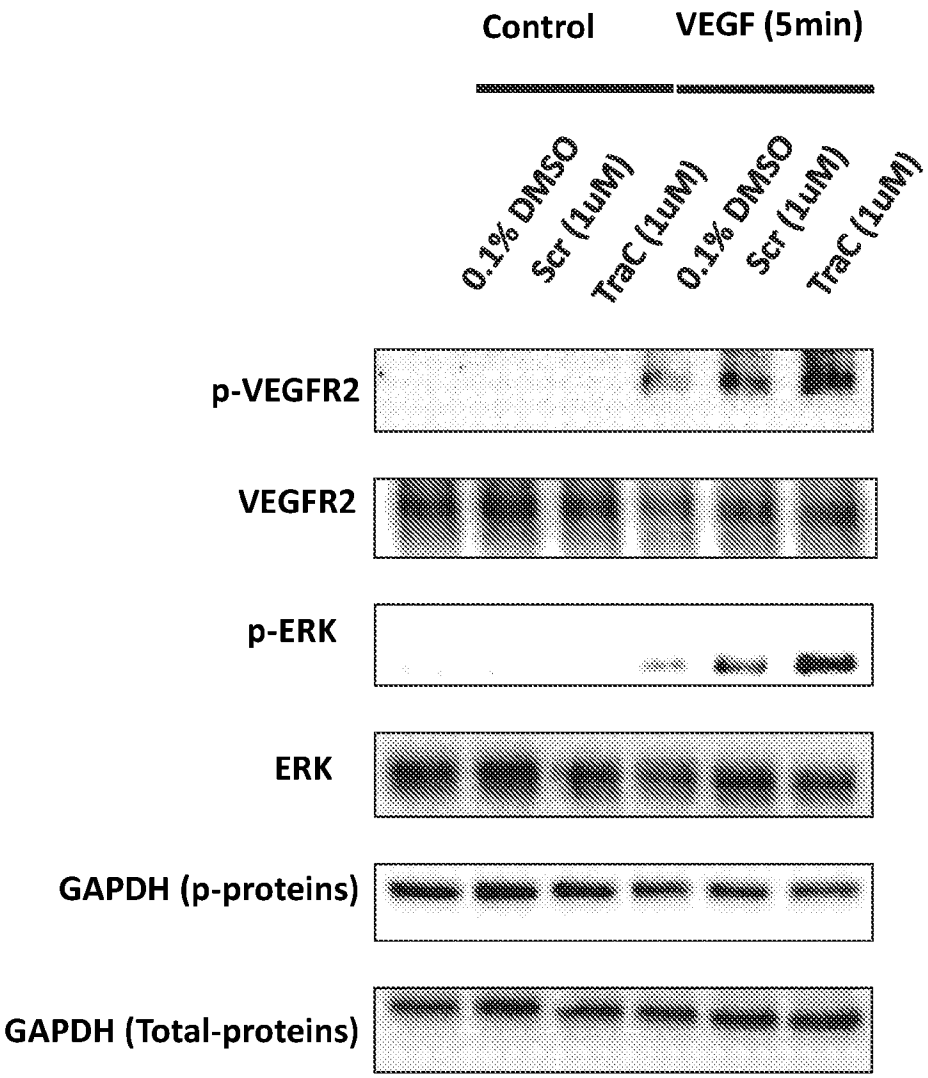

Control
VEGF (5min)
0.1% DMSO
Scr (1uM)
TraC (1uM)
0.1% DMSO
Scr (1uM)
TraC (1uM)
p-VEGFR2
VEGFR2
p-ERK
ERK
GAPDH (p-proteins)
GAPDH (Total-proteins)

Fig. 10A

Att

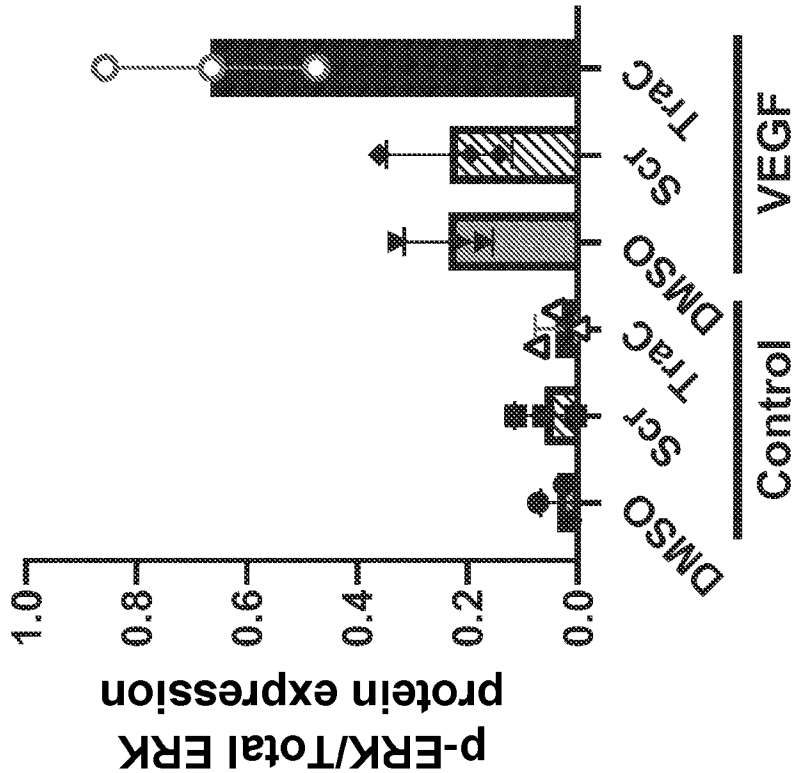


Fig. 10C

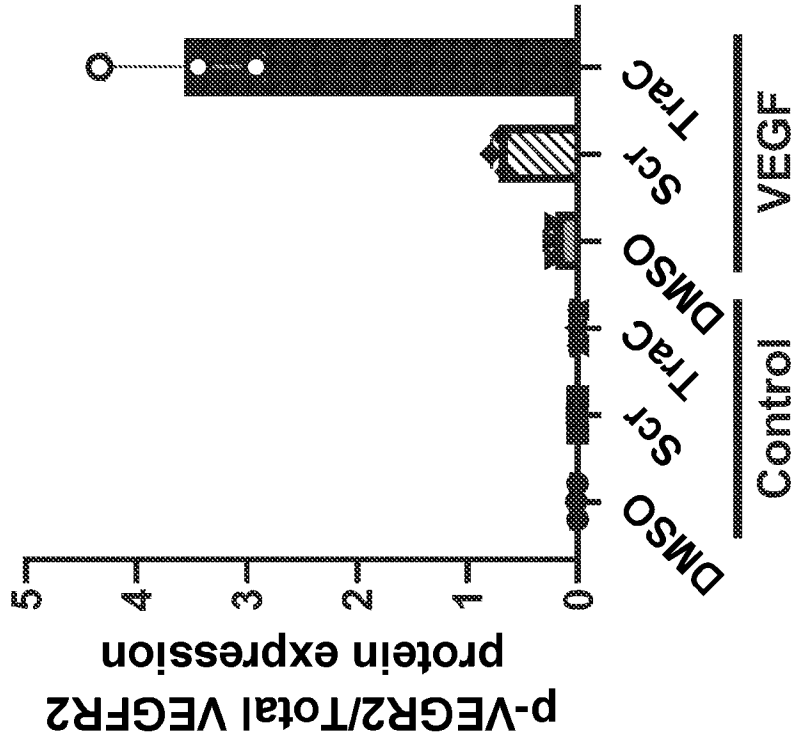


Fig. 10B

Att

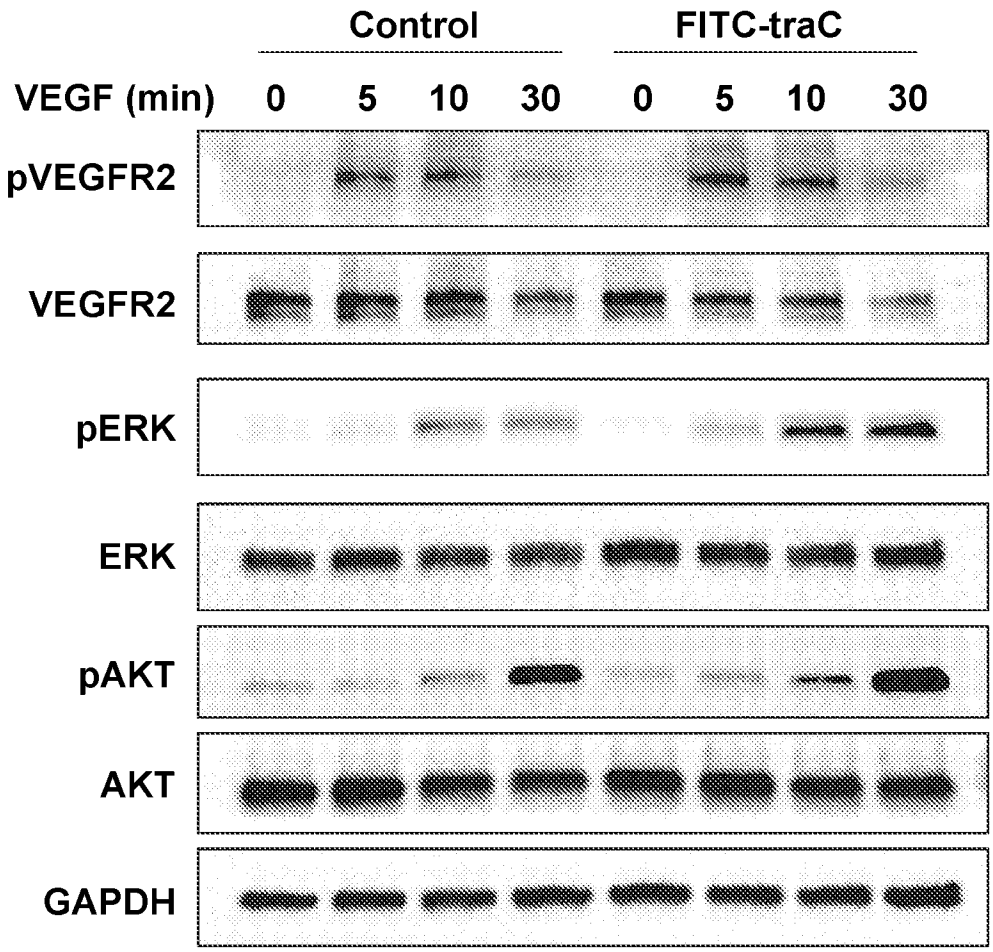

Control
FITC-traC
VEGF (min)
0
5
10
30
0
5
10
30
pVEGFR2
VEGFR2
pERK
ERK
pAKT
AKT
GAPDH

Fig. 11A

Att

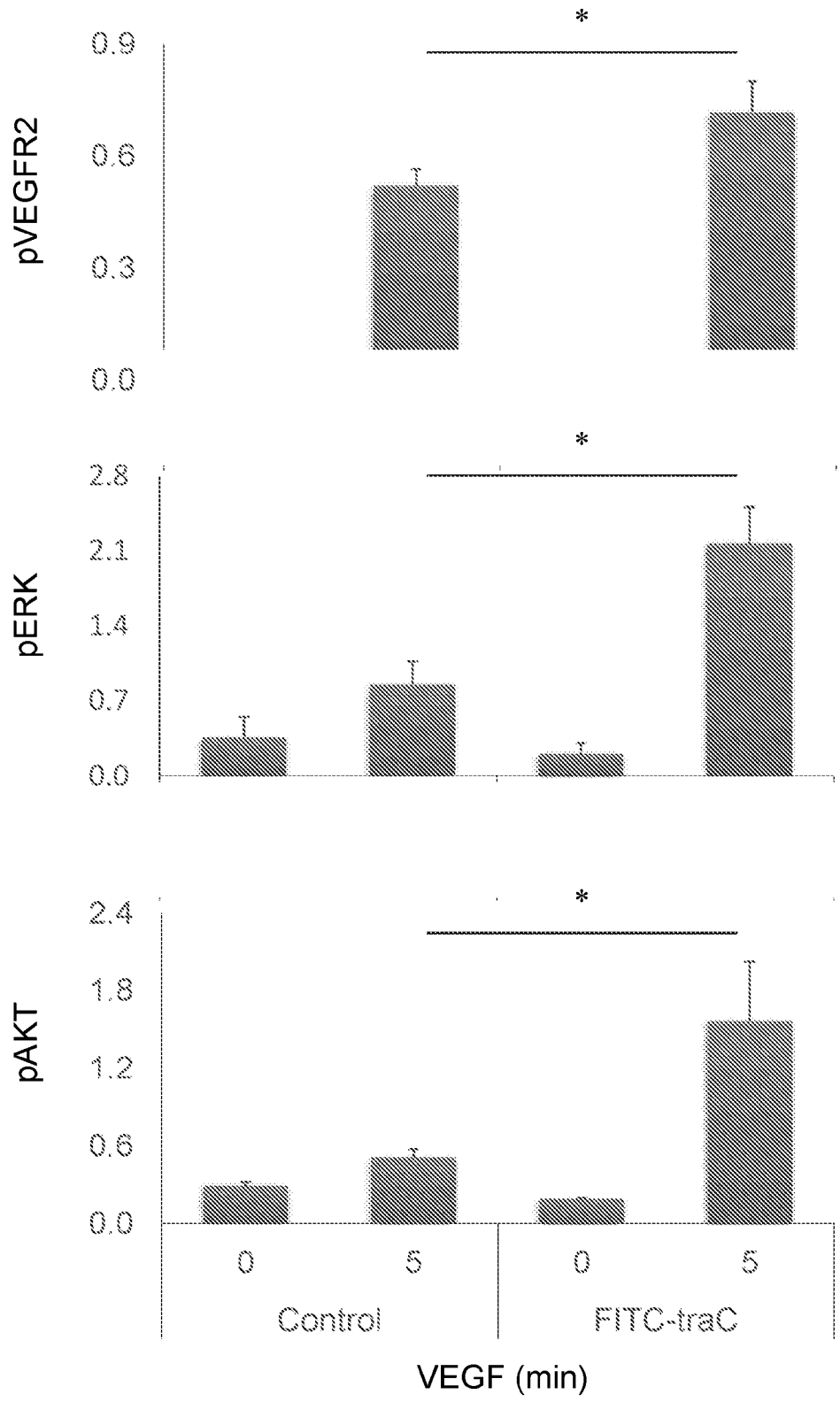

pVEGFR2
0.9
0.6
0.3
0.0
*
pERK
2.8
2.1
1.4
0.7
0.0
*
pAKT
2.4
1.8
1.2
0.6
0.0
*
0
5
0
5
Control
FITC-traC
VEGF (min)

Fig. 11B

Att

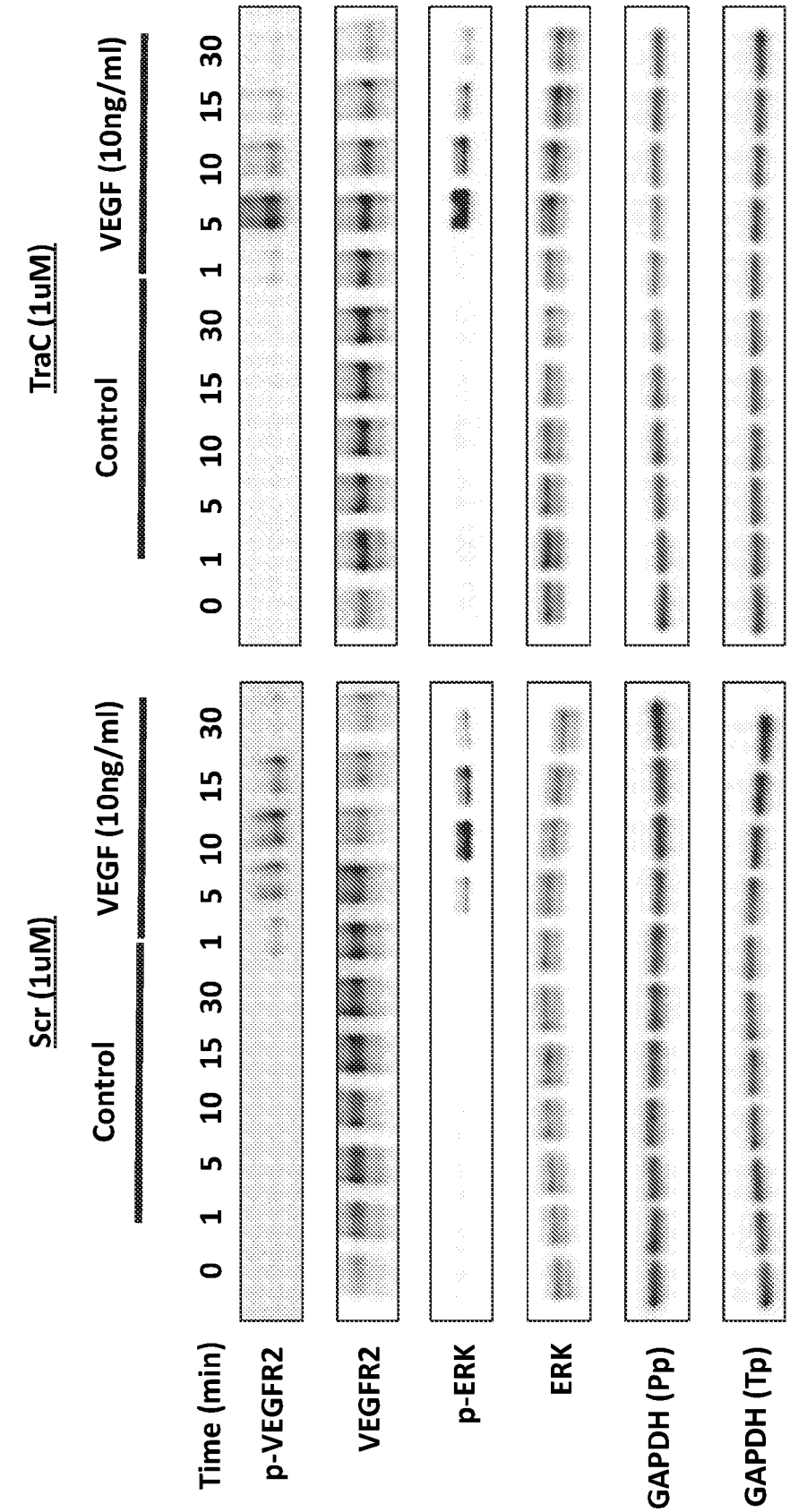

Scr (1uM)
Control
VEGF (10ng/ml)
TraC (1uM)
Control
VEGF (10ng/ml)
Time (min)
0 1 5 10 15 30 1 5 10 15 30
p-VEGFR2
VEGFR2
p-ERK
ERK
GAPDH (Pp)
GAPDH (Tp)

Fig. 12

Att

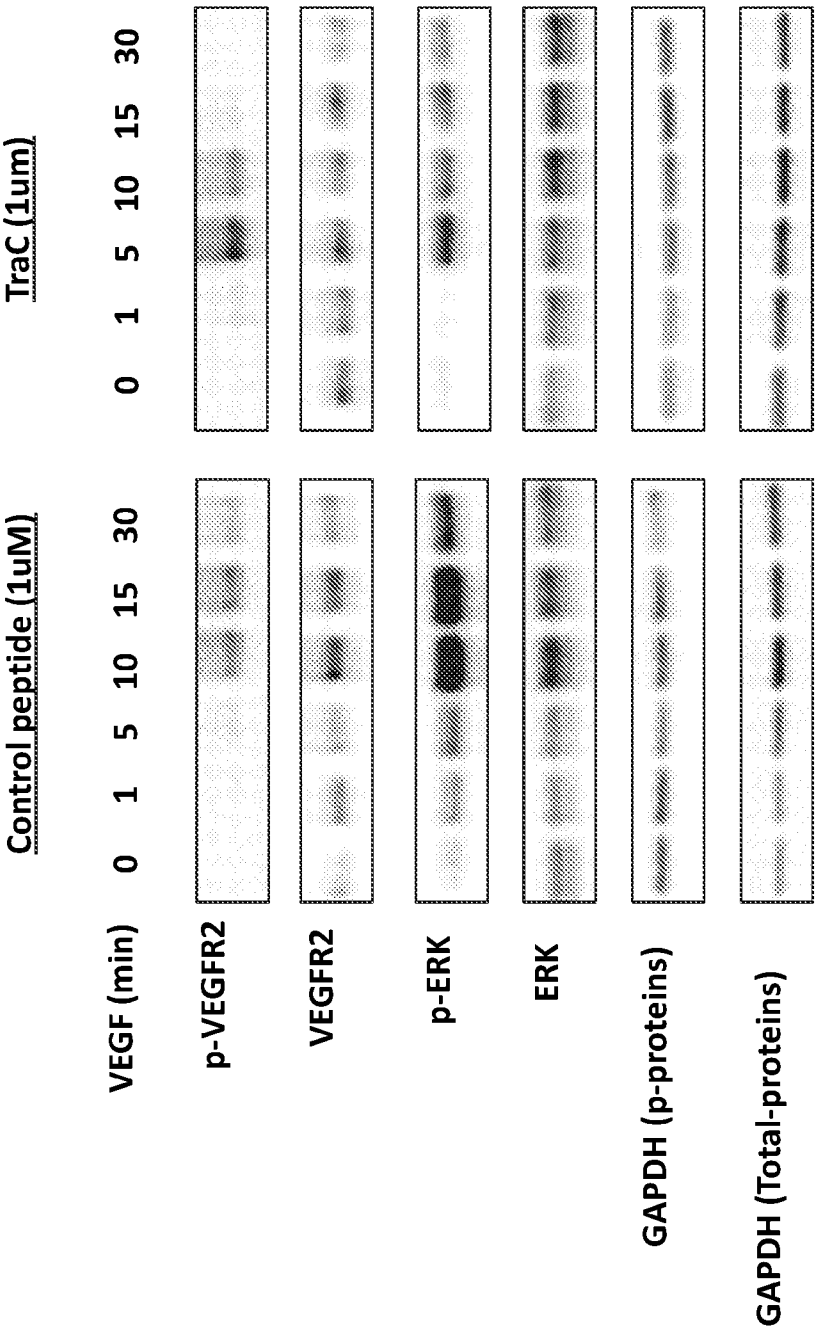

Control peptide (1uM)
TraC (1um)
VEGF (min)
0 1 5 10 15 30
0 1 5 10 15 30
p-VEGFR2
VEGFR2
p-ERK
ERK
GAPDH (p-proteins)
GAPDH (Total-proteins)

Fig. 13A

Att

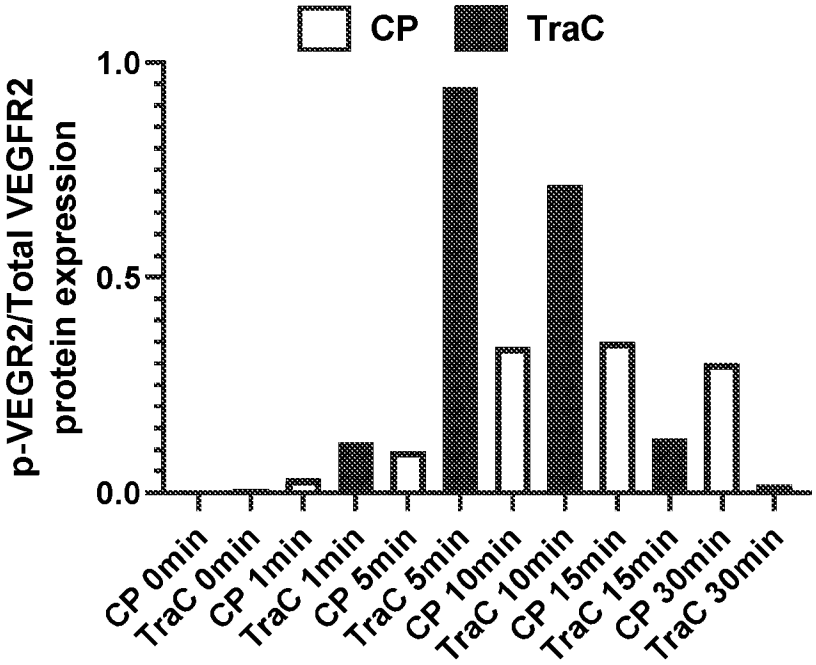


Fig. 13B

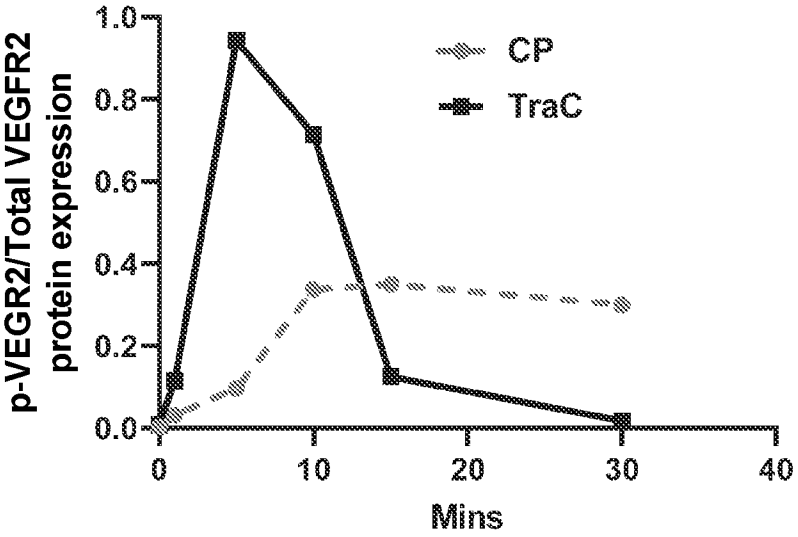


Fig. 13C

Att

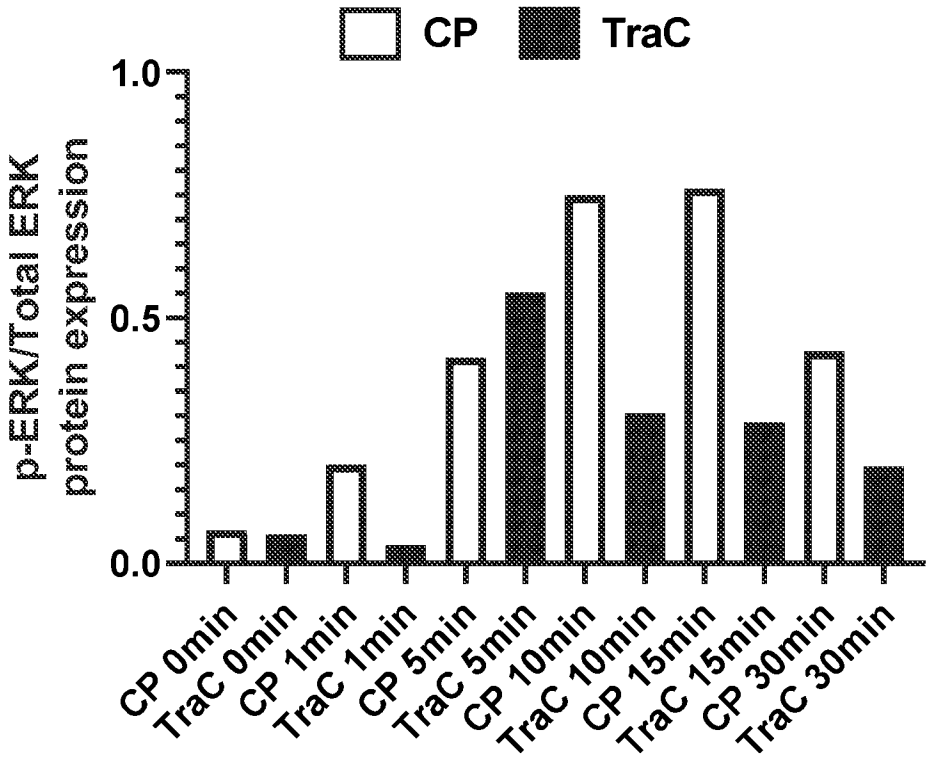


Fig. 13D

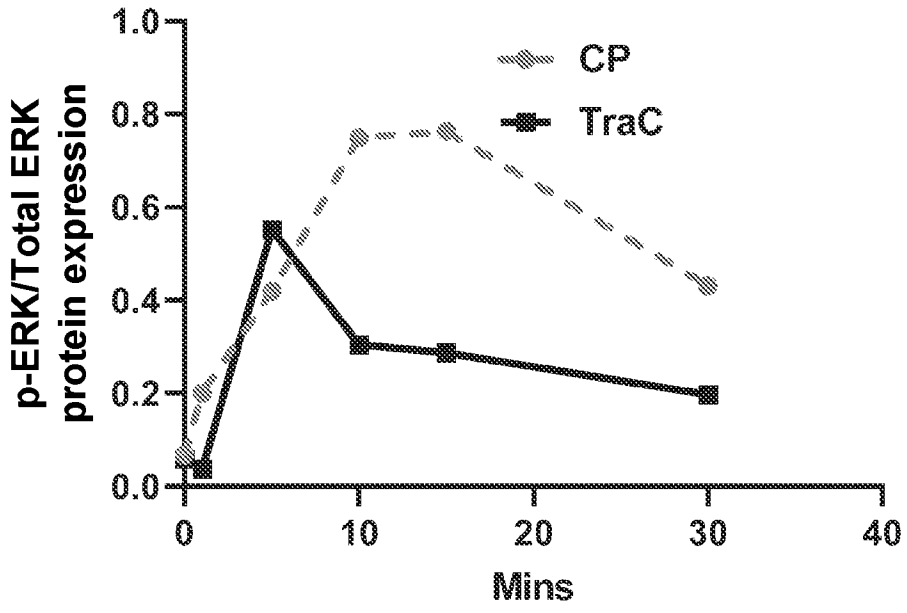


Fig. 13E

Att

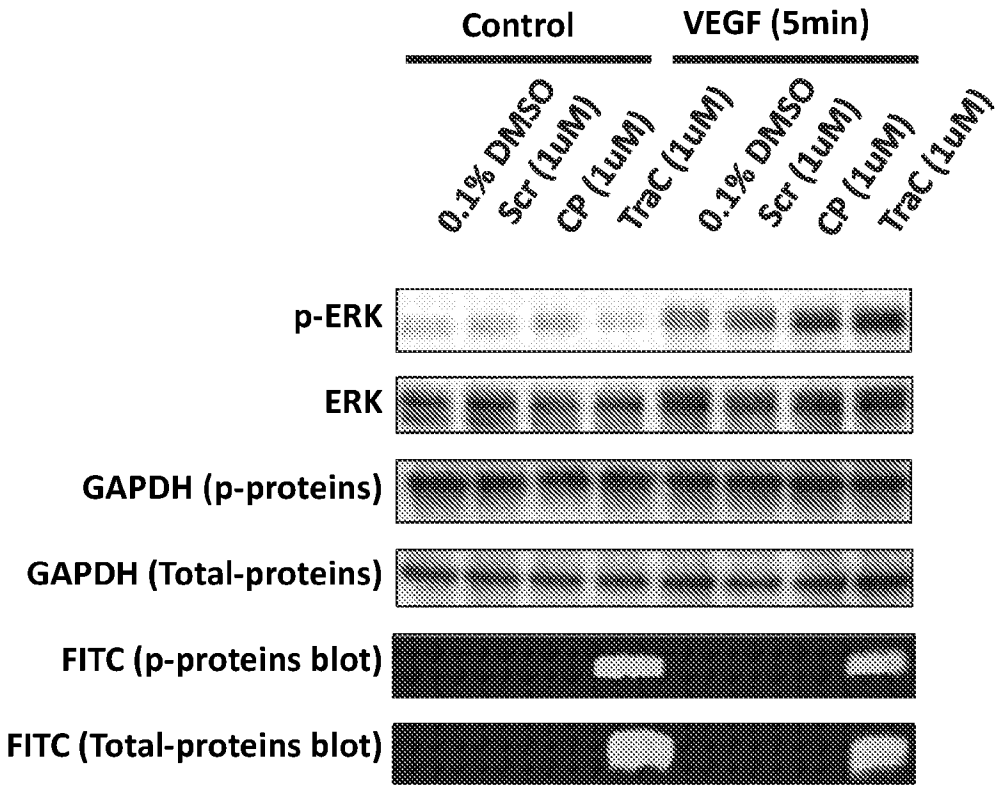
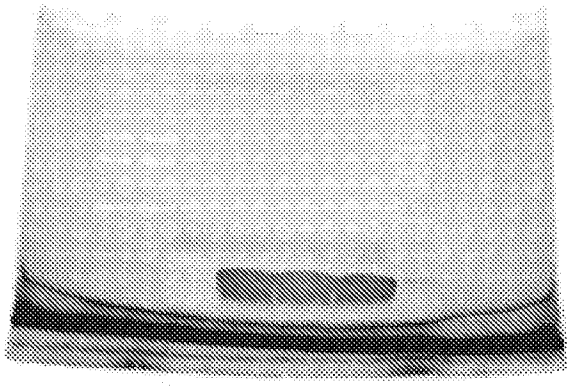

Control
VEGF (5min)
0.1% DMSO
Scr (1uM)
CP (1uM)
TraC (1uM)
0.1% DMSO
Scr (1uM)
CP (1uM)
TraC (1uM)
p-ERK
ERK
GAPDH (p-proteins)
GAPDH (Total-proteins)
FITC (p-proteins blot)
FITC (Total-proteins blot)

Fig. 14A

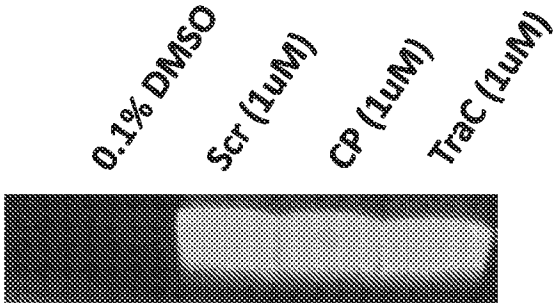
Att

Gel: 0.2uM FITC-Scr/CP/traC (in M199)

Stain free gel (UV):



Gel (488):



Blot (488):

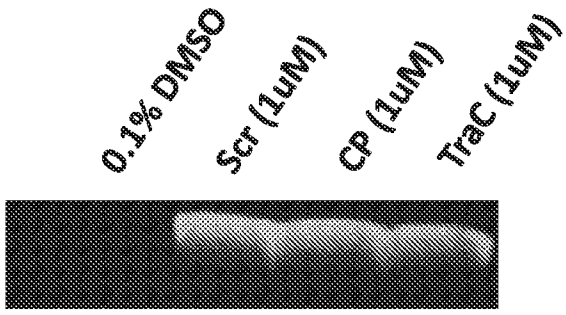


Fig. 14B

Att

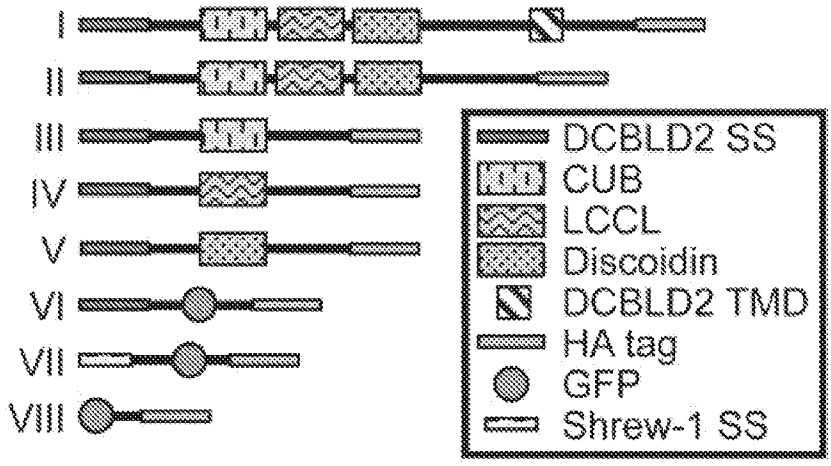


Fig. 15A

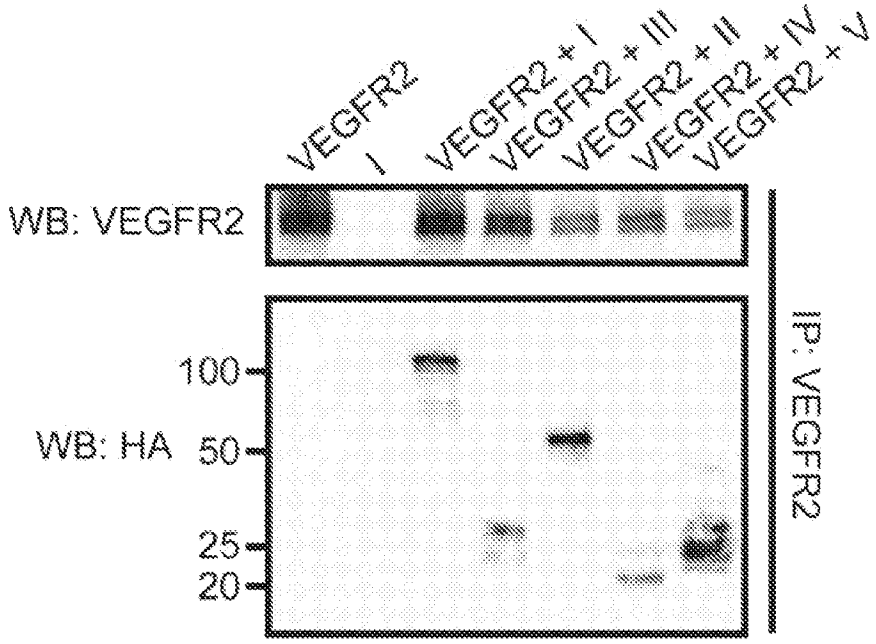


Fig. 15B

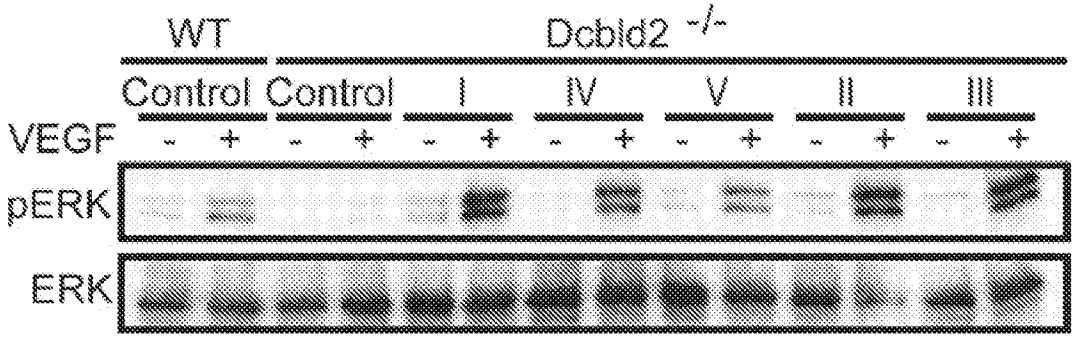


Fig. 15C

Att

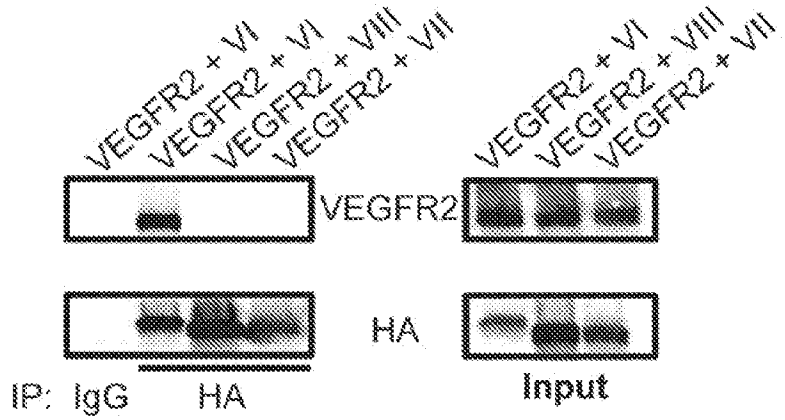


Fig. 15D

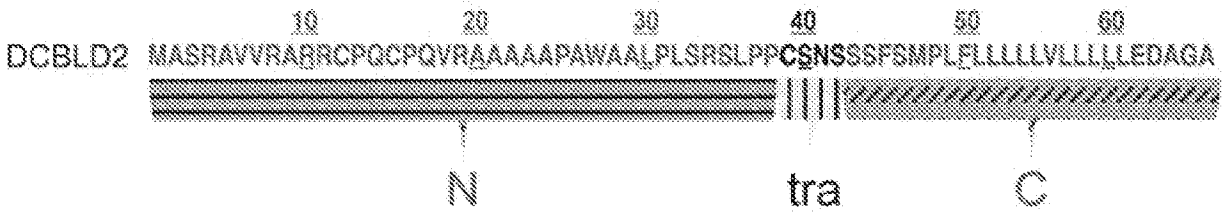


Fig. 15E

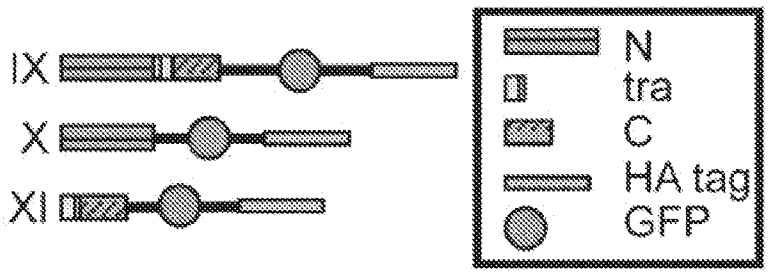


Fig. 15F

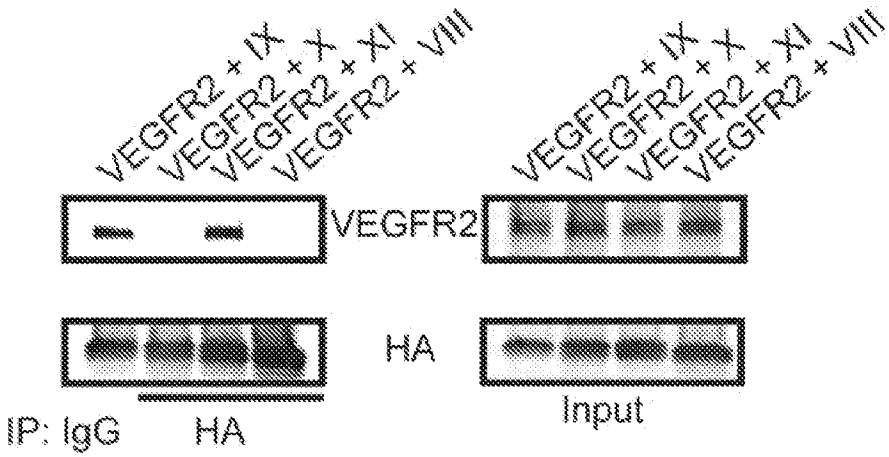


Fig. 15G

Att

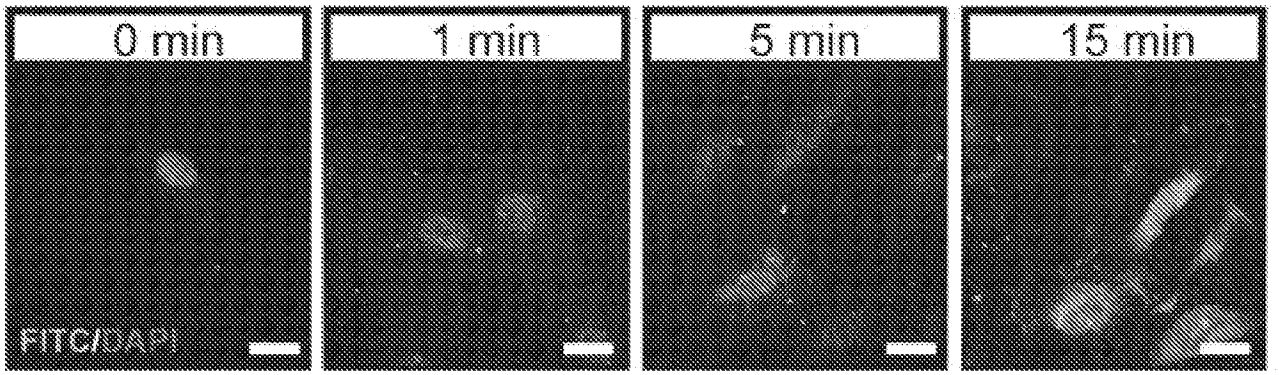


Fig. 16A

Fig. 16B

Att

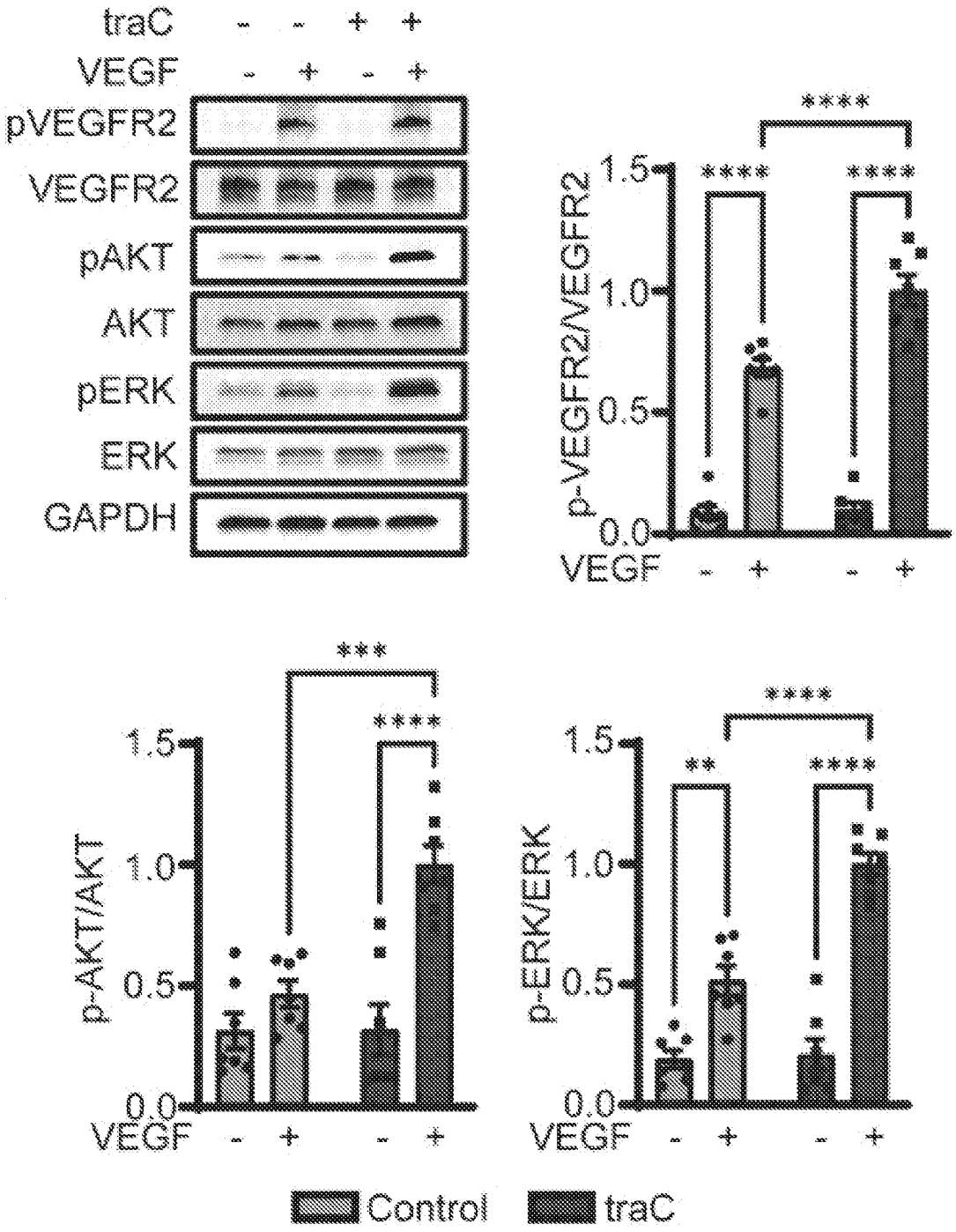

traC - - + +
VEGF - + - +
pVEGFR2
VEGFR2
pAKT
AKT
pERK
ERK
GAPDH
p-VEGFR2/VEGFR2
1.5
1.0
0.5
0.0
****
****
****
VEGF - + - +
p-AKT/AKT
***
****
p-ERK/ERK
**
****
Control
traC

Fig. 16C

Att

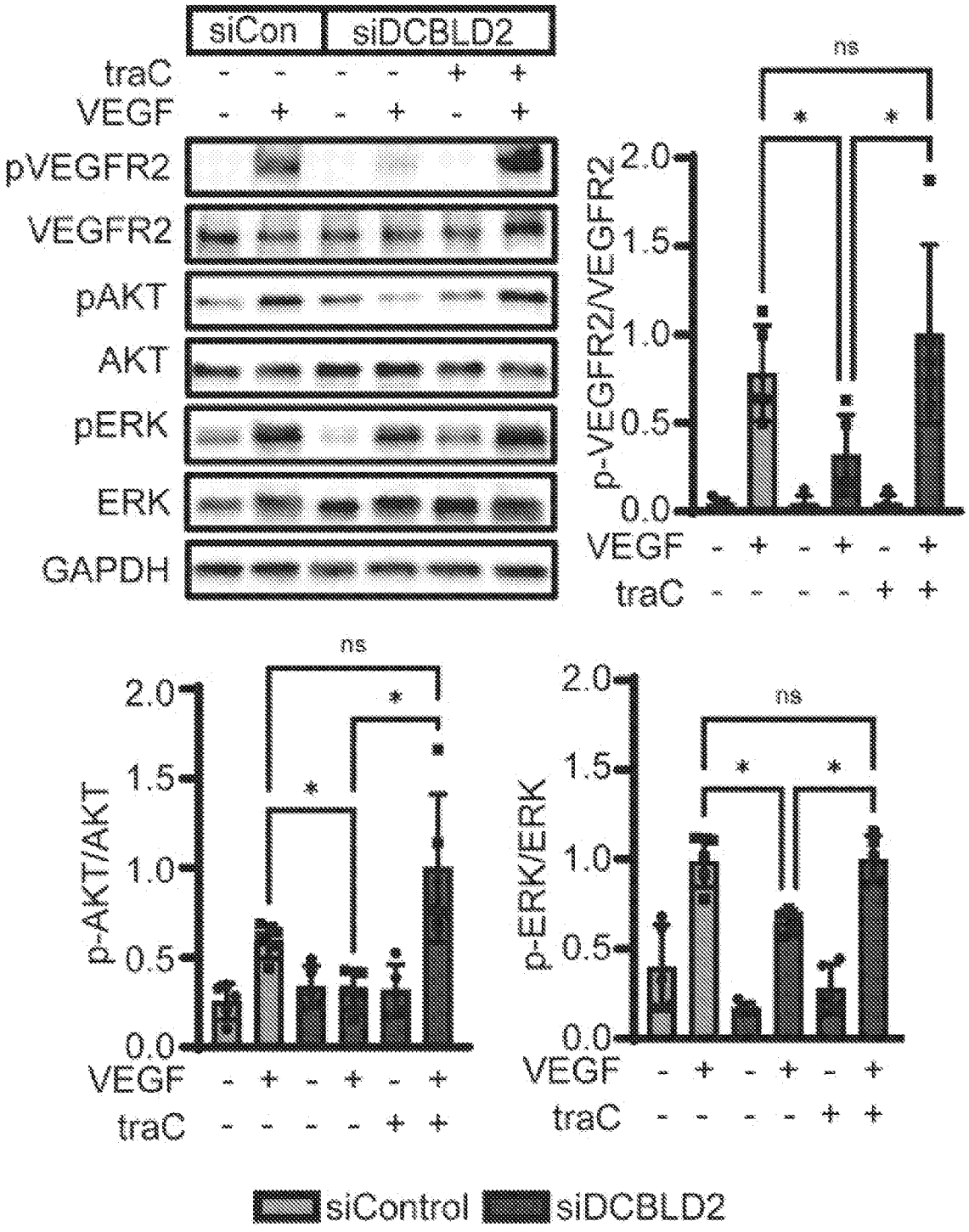

siCon
siDCBLD2
traC
VEGF
pVEGFR2
VEGFR2
pAKT
AKT
pERK
ERK
GAPDH
p-VEGFR2/VEGFR2
p-AKT/AKT
p-ERK/ERK
ns
*
siControl
siDCBLD2

Fig. 16D

Att

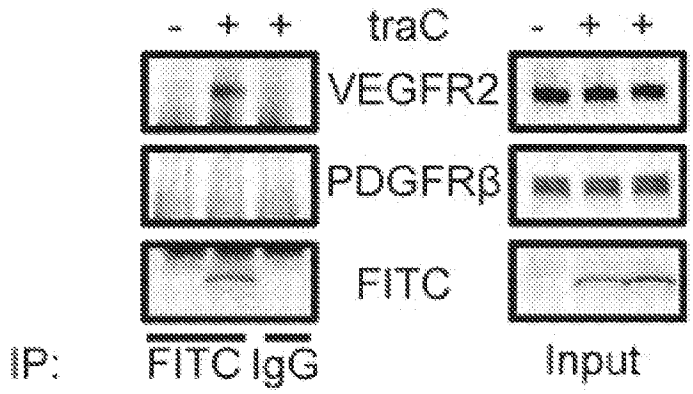


Fig. 16E

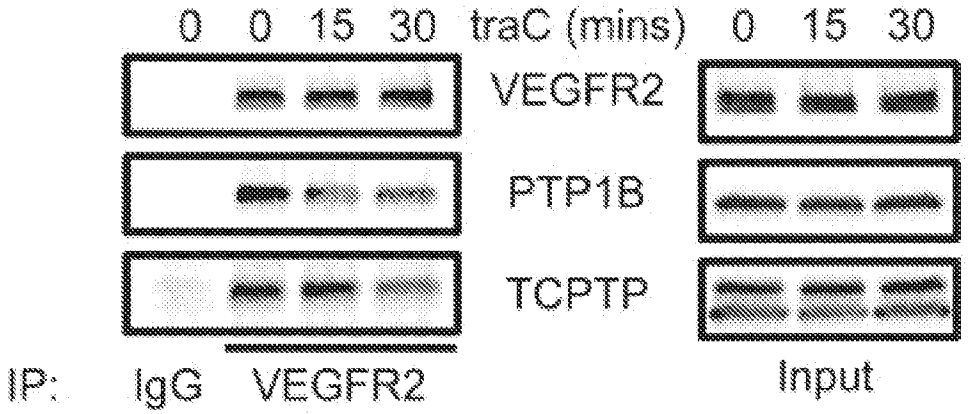


Fig. 16F

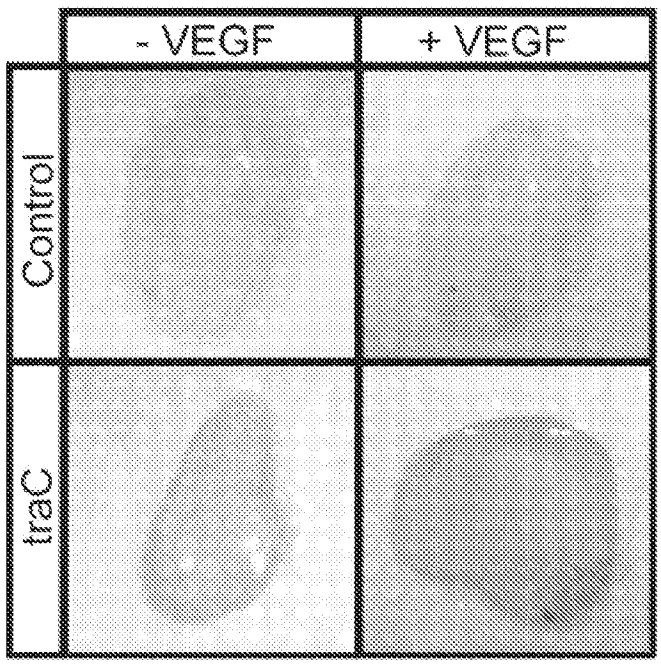


Fig. 17A

Att

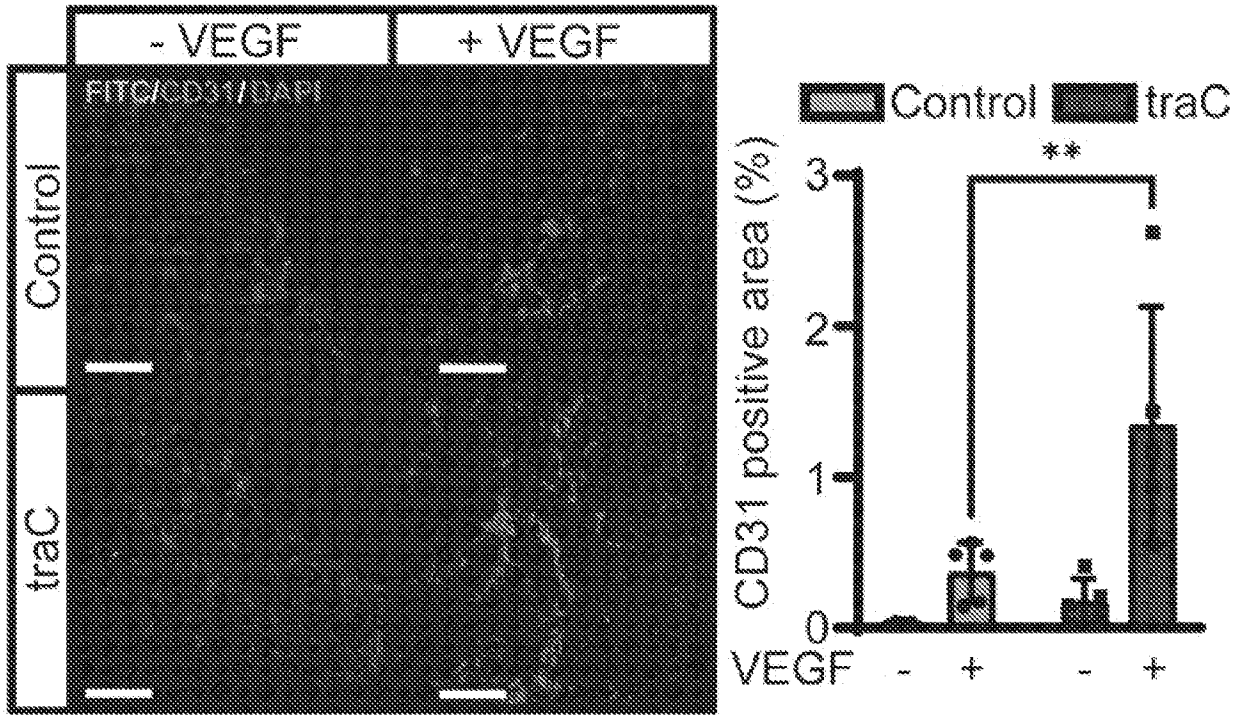


Fig. 17B

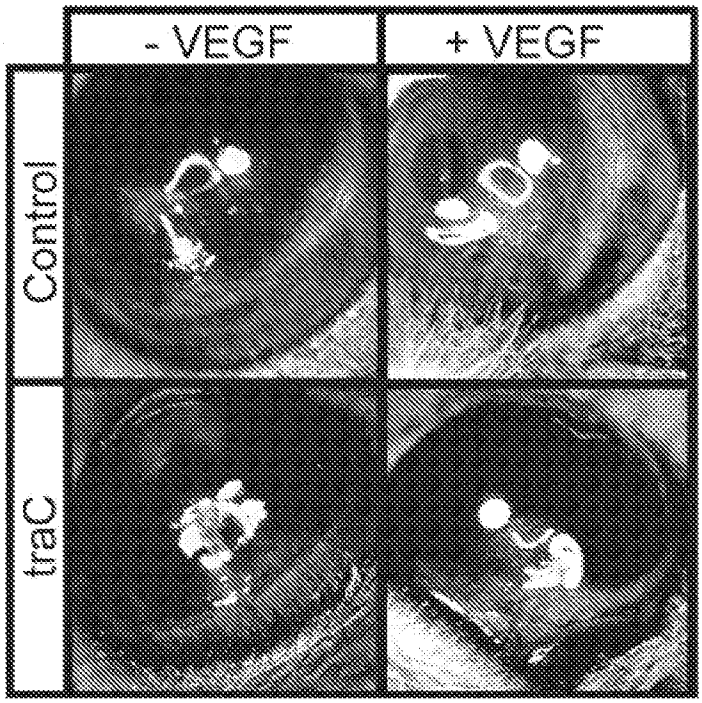


Fig. 17C

Att

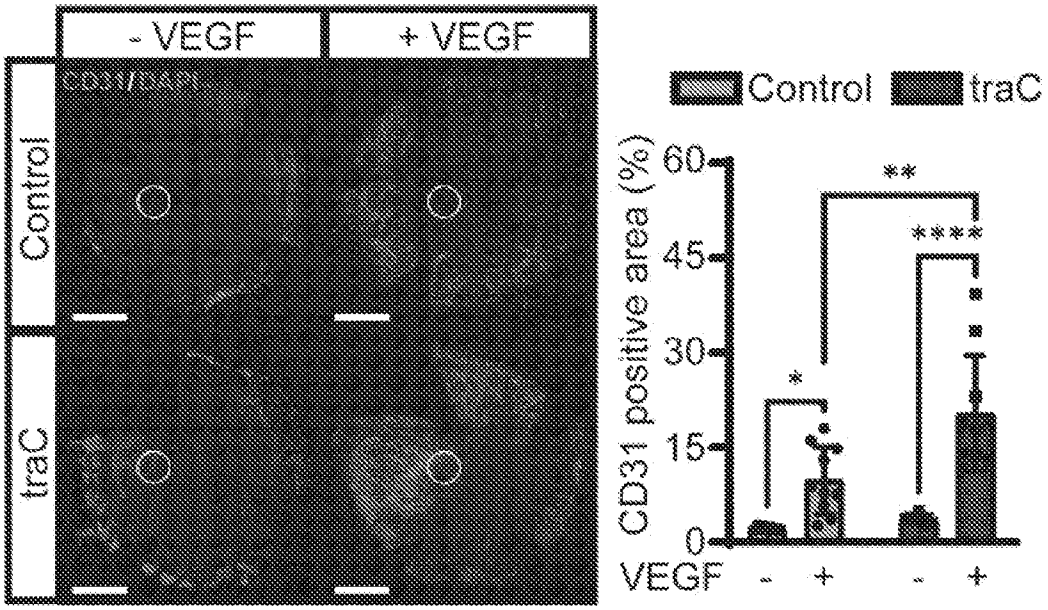


Fig. 17D

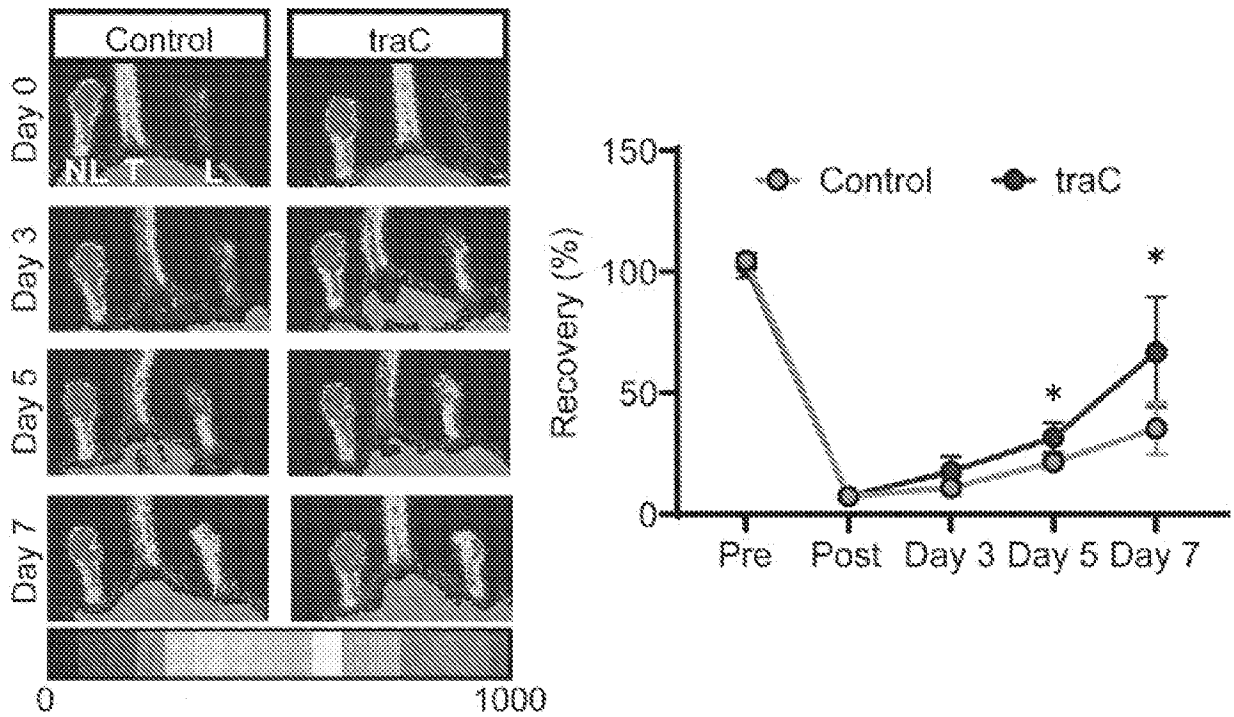


Fig. 17E

Att

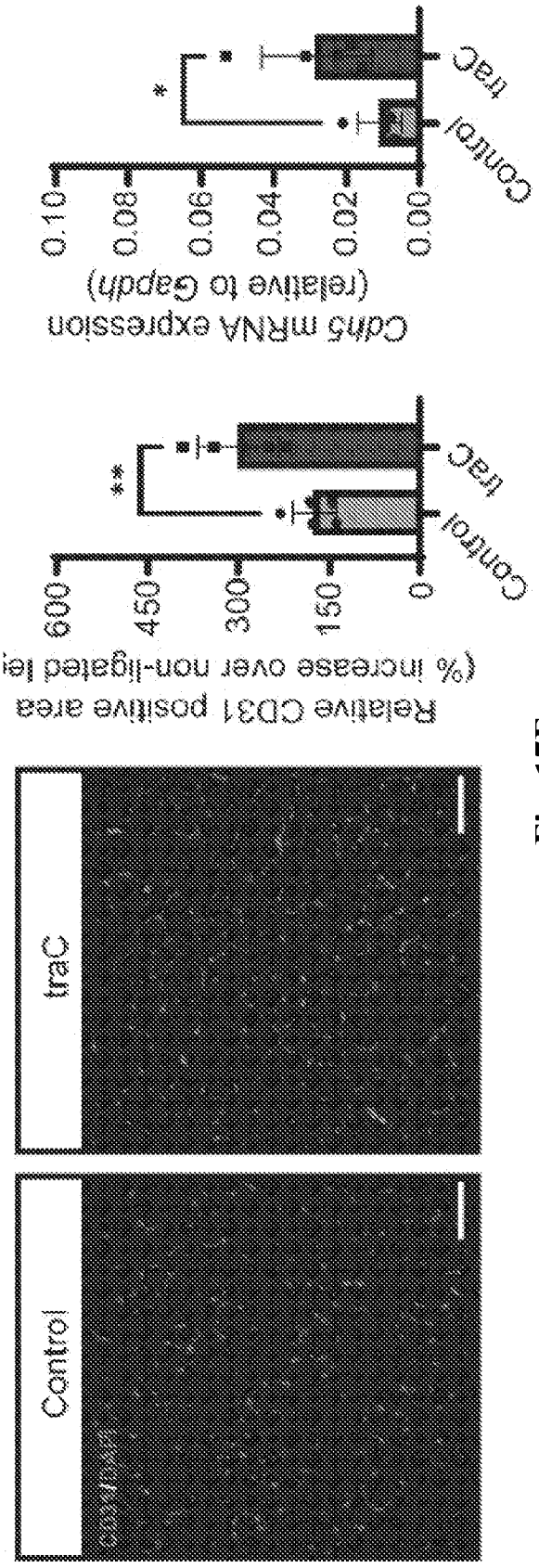


Fig. 17F

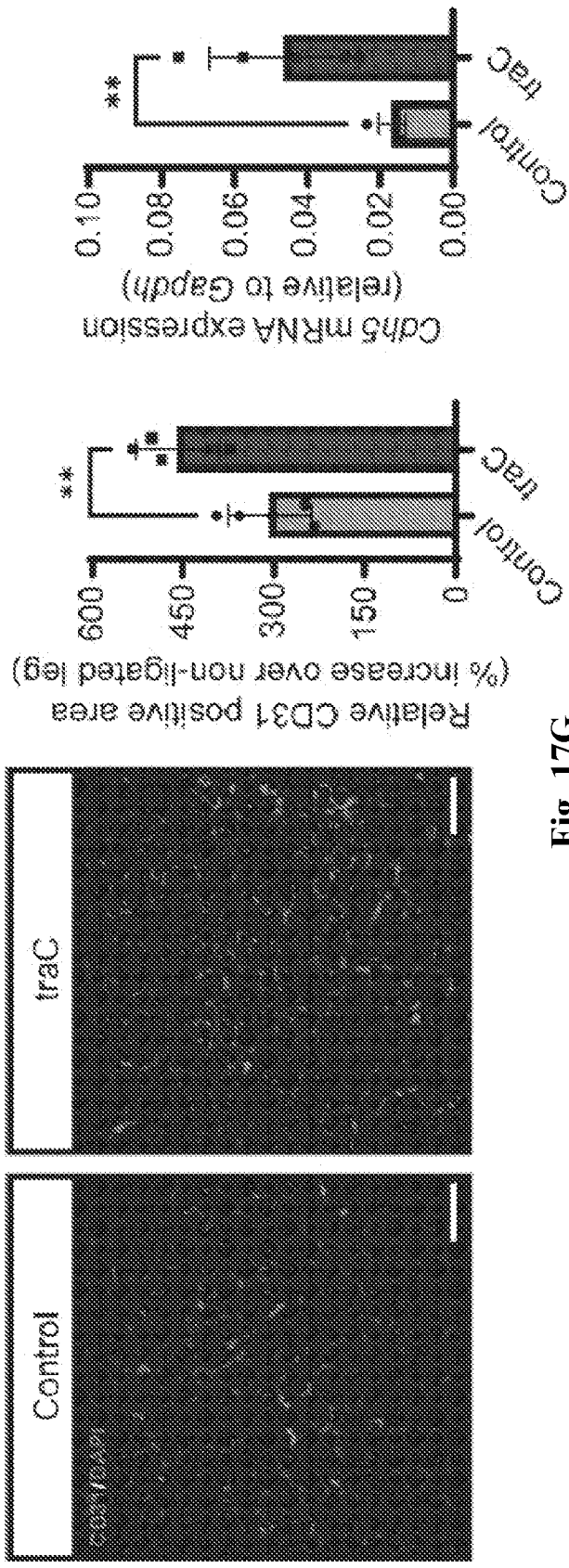


Fig. 17G

Att

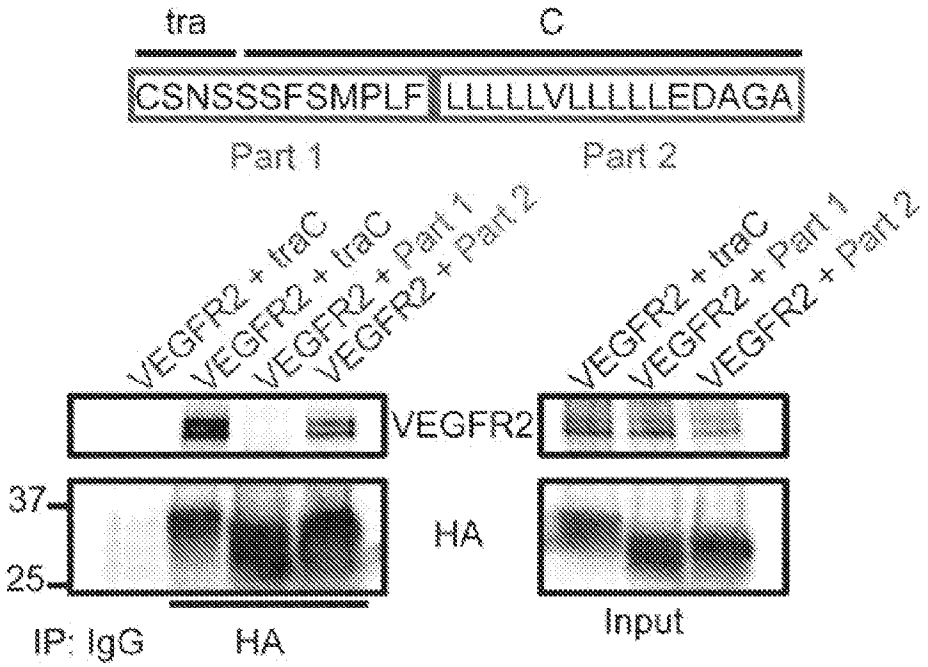


Fig. 18A

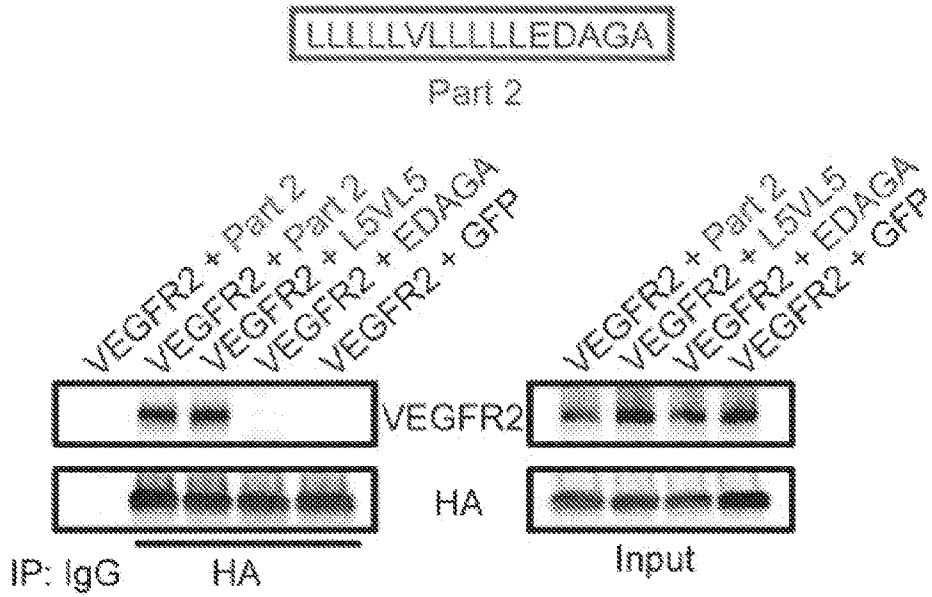


Fig. 18B

Att

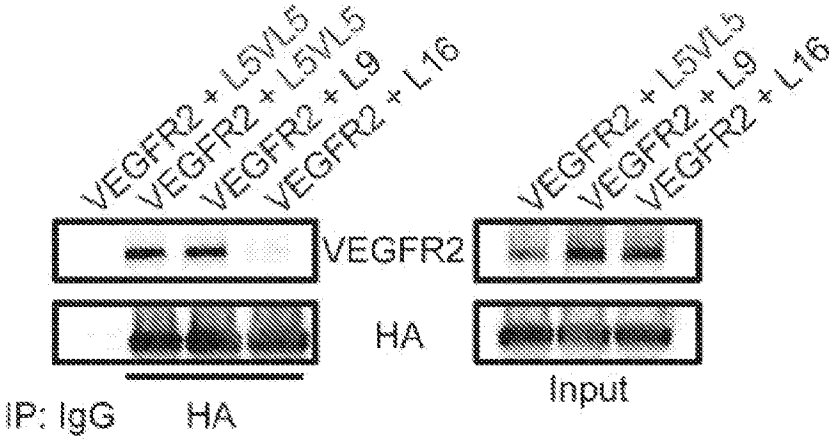


Fig. 18C

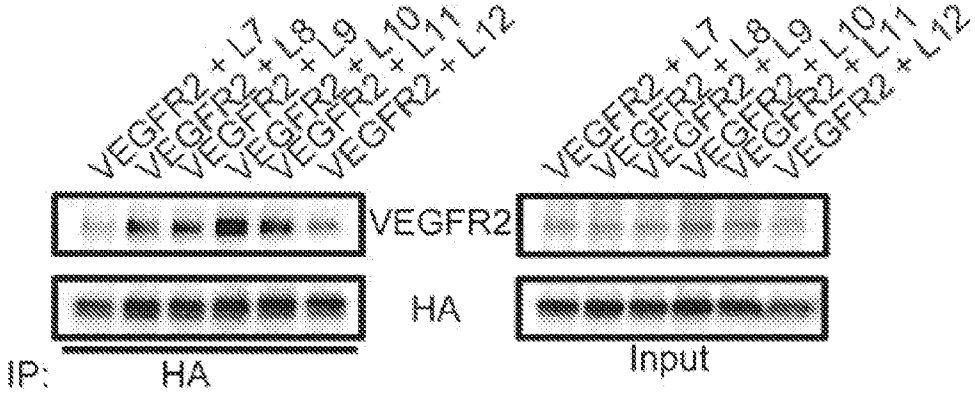


Fig. 18D

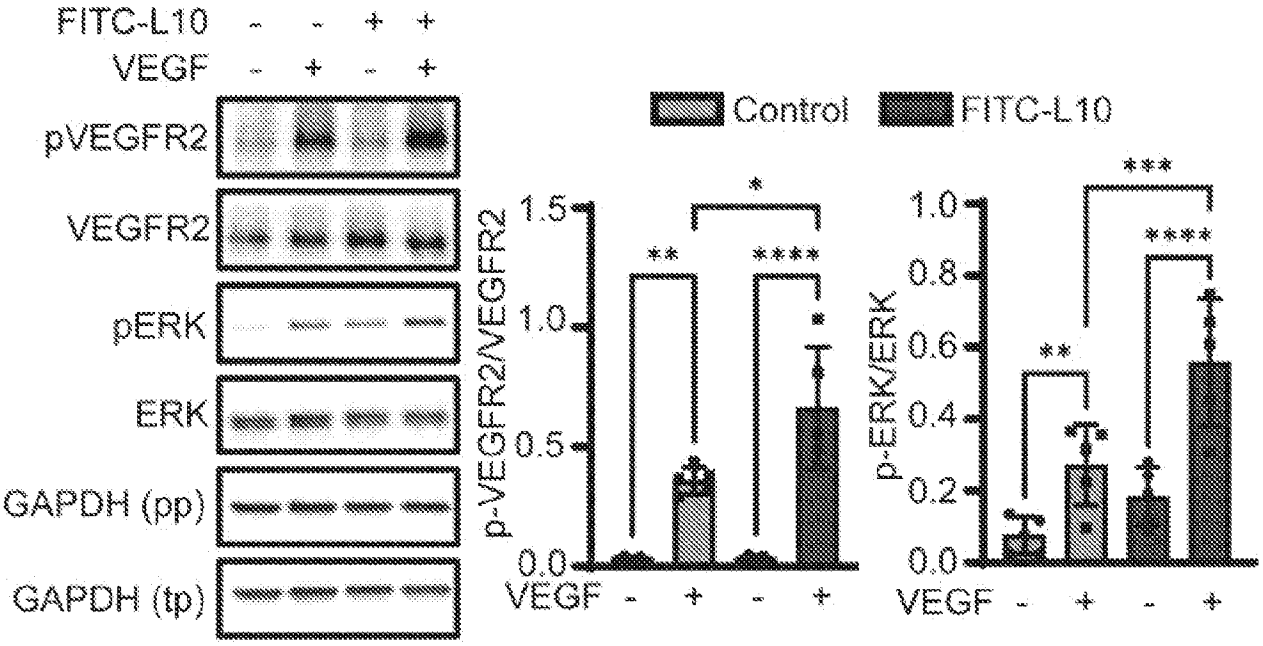


Fig. 18E

Att

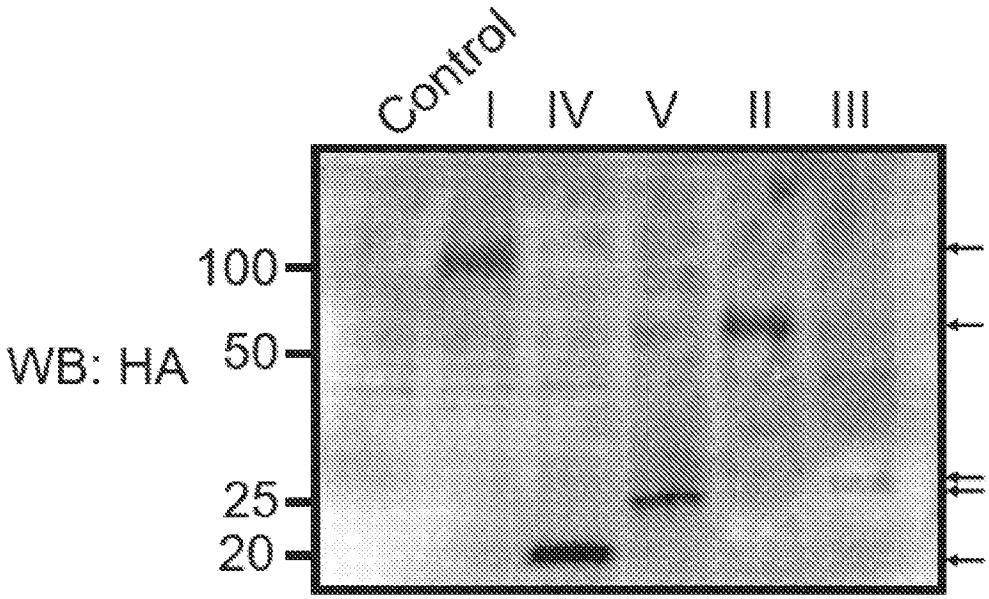


Fig. 19

Att

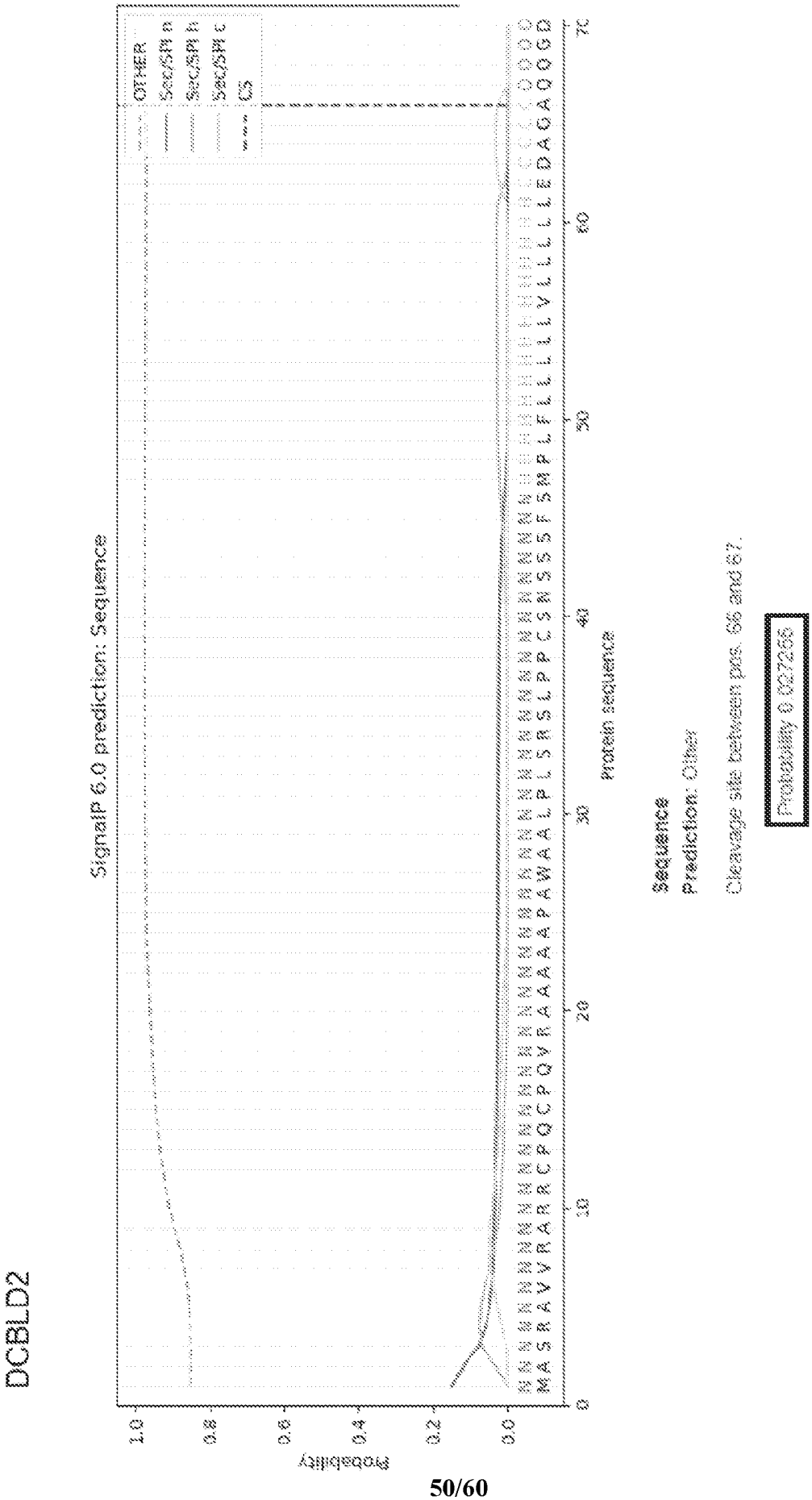

DCBLD2
SignalP 6.0 prediction: Sequence
OTHER
Sec/SPI n
Sec/SPI h
Sec/SPI c
CS
Probability
Protein sequence
Sequence
Prediction: Other
Cleavage site between pos. 66 and 67:
Probability 0.007256

Fig. 20A

Att

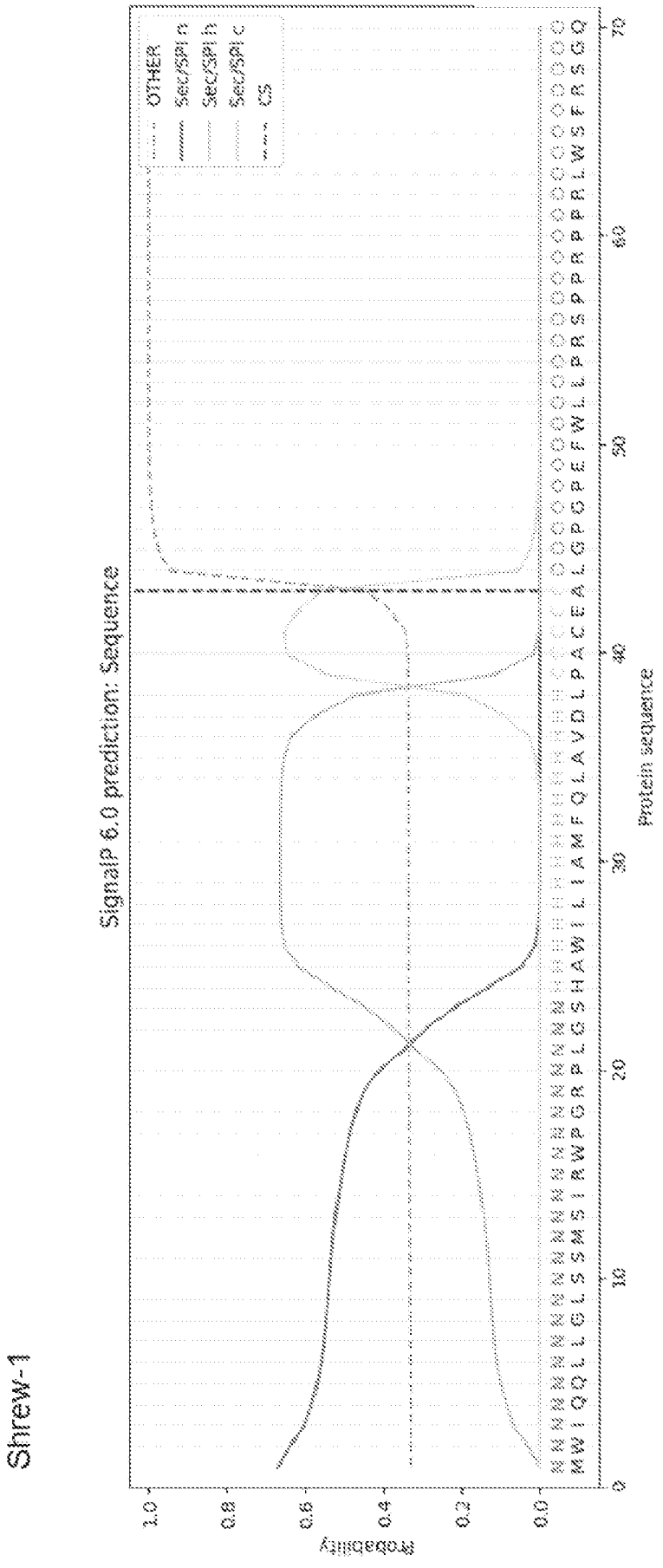

Shrew-1
SignalP 6.0 prediction: Sequence
OTHER
Sec/SPI n
Sec/SPI h
Sec/SPI c
CS
Probability
Protein sequence
Sequence
Prediction: Signal Peptide (Sec/SPI)
Cleavage site between pos. 43 and 44:
Probability 0.560366

Fig. 20B

Att

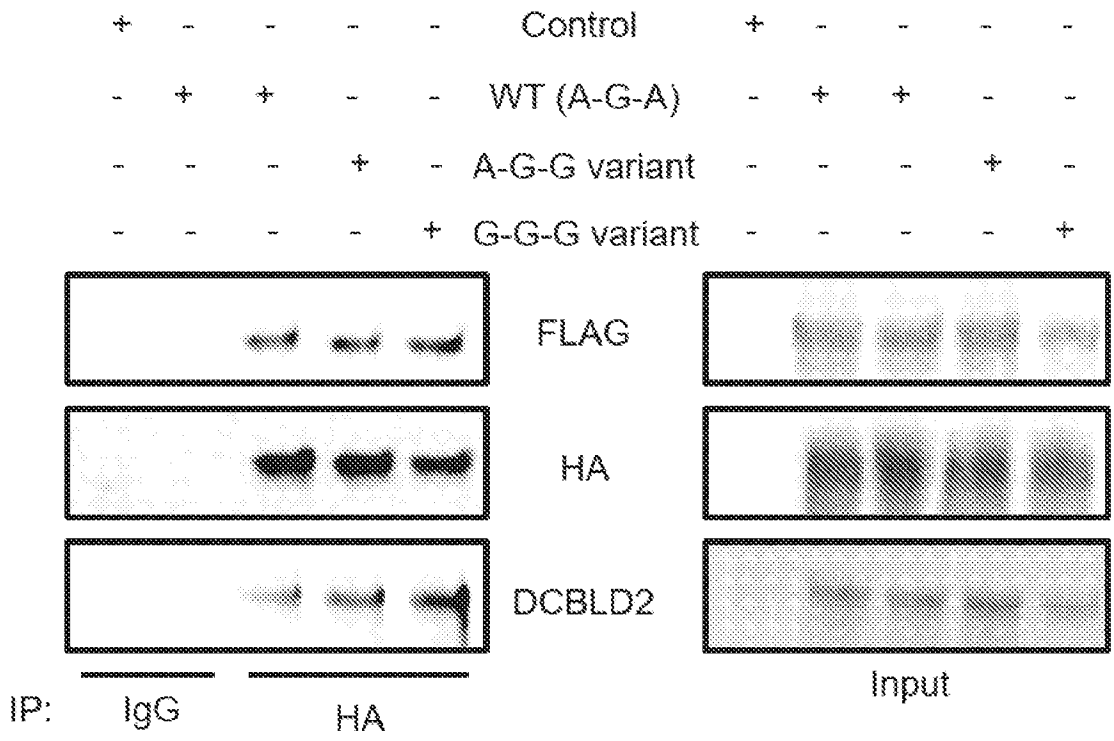


Fig. 21

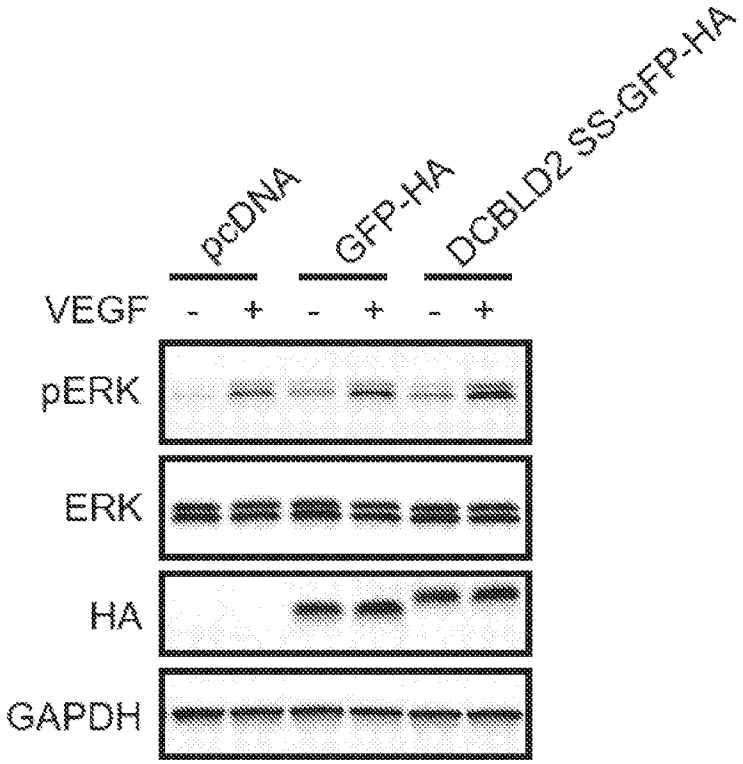


Fig. 22

Att

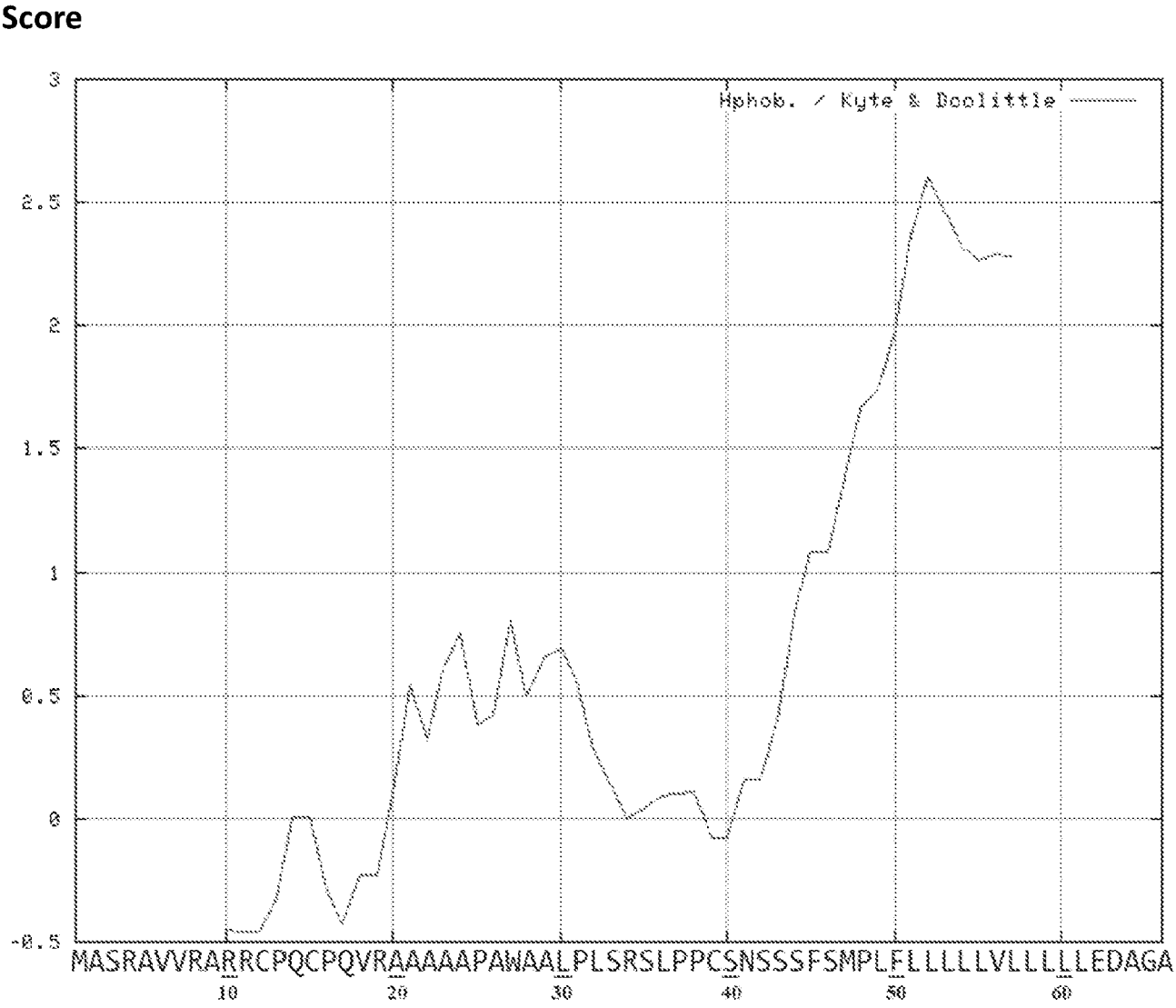

Score
Hphob. / Kyte & Doolittle
3
2.5
2
1.5
1
0.5
0
-0.5
MASRAVVRARRCPQCPQVRAAAAAAPAWAALPLSRSLPPCSNSSSFSMPLFLLLLLVLLLLLLEDAGA
10
20
30
40
50
60

Fig. 23

Att

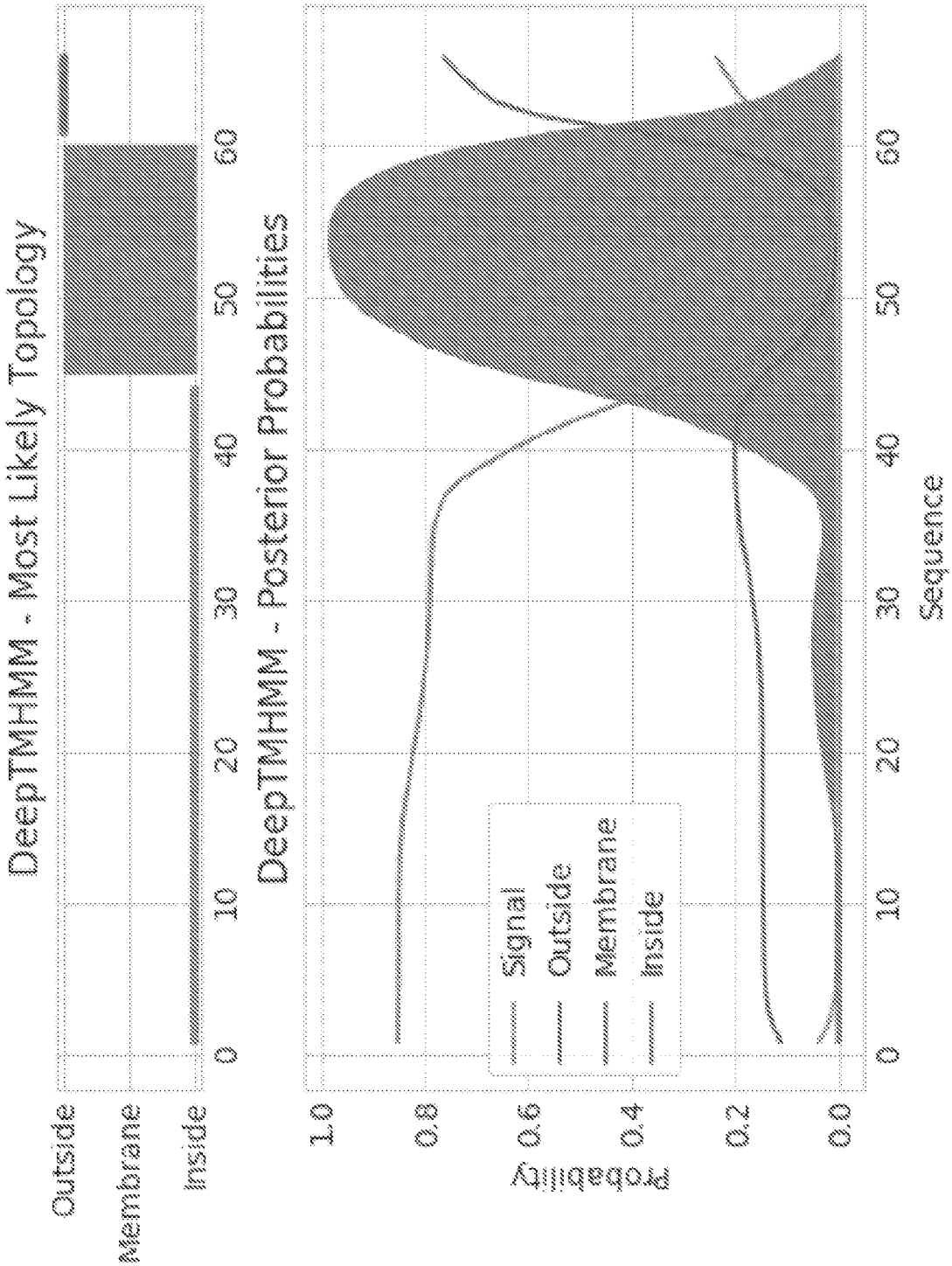

DeepTMHMM - Most Likely Topology
Outside
Membrane
Inside
0
10
20
30
40
50
60
DeepTMHMM - Posterior Probabilities
Signal
Outside
Membrane
Inside
Probability
1.0
0.8
0.6
0.4
0.2
0.0
Sequence

Fig. 24

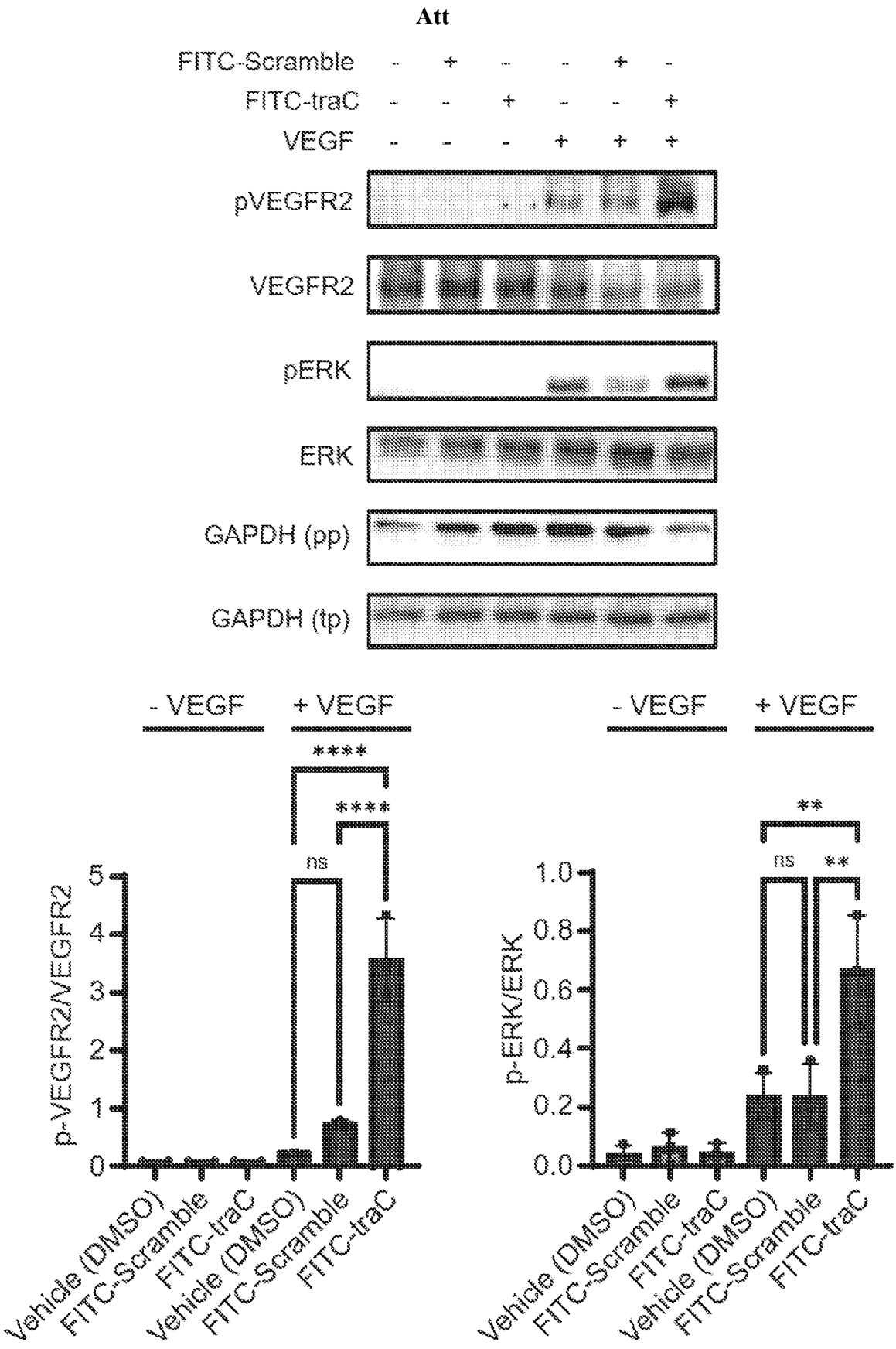

Att
FITC-Scramble - + - - + -
FITC-traC - - + - - +
VEGF - - - + + +
pVEGFR2
VEGFR2
pERK
ERK
GAPDH (pp)
GAPDH (tp)
- VEGF
+ VEGF
****
****
ns
p-VEGFR2/VEGFR2
0
1
2
3
4
5
Vehicle (DMSO)
FITC-Scramble
FITC-traC
- VEGF
+ VEGF
**
ns
**
p-ERK/ERK
0.0
0.2
0.4
0.6
0.8
1.0
Vehicle (DMSO)
FITC-Scramble
FITC-traC

Fig. 25

Att

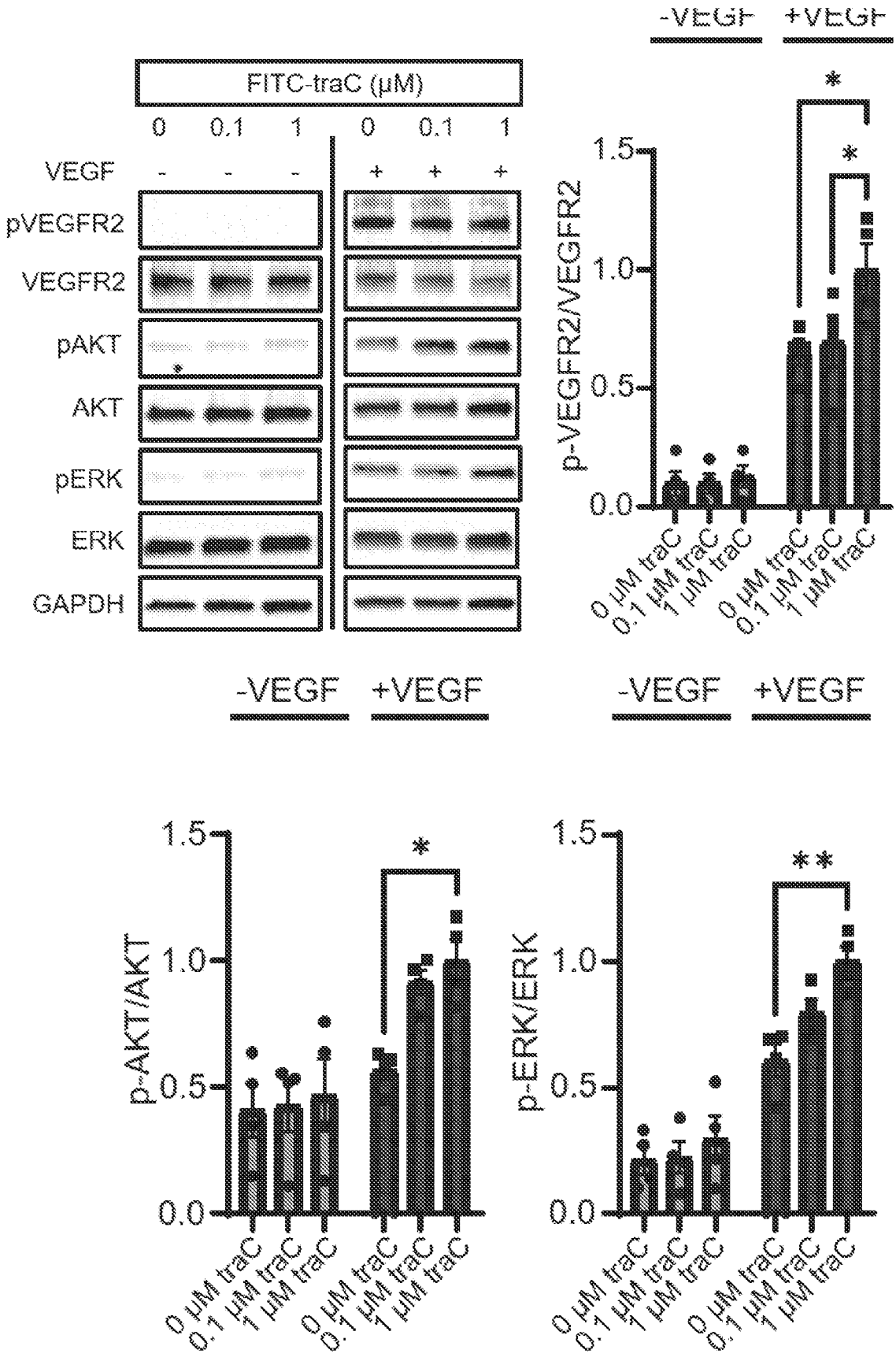

FITC-traC (μM)
0 0.1 1 0 0.1 1
VEGF - - - + + +
pVEGFR2
VEGFR2
pAKT
AKT
pERK
ERK
GAPDH
-VEGF +VEGF
p-VEGFR2/VEGFR2
1.5
1.0
0.5
0.0
*
*
0 μM traC
0.1 μM traC
1 μM traC
-VEGF +VEGF
p-AKT/AKT
*
p-ERK/ERK
**

Fig. 26

Att

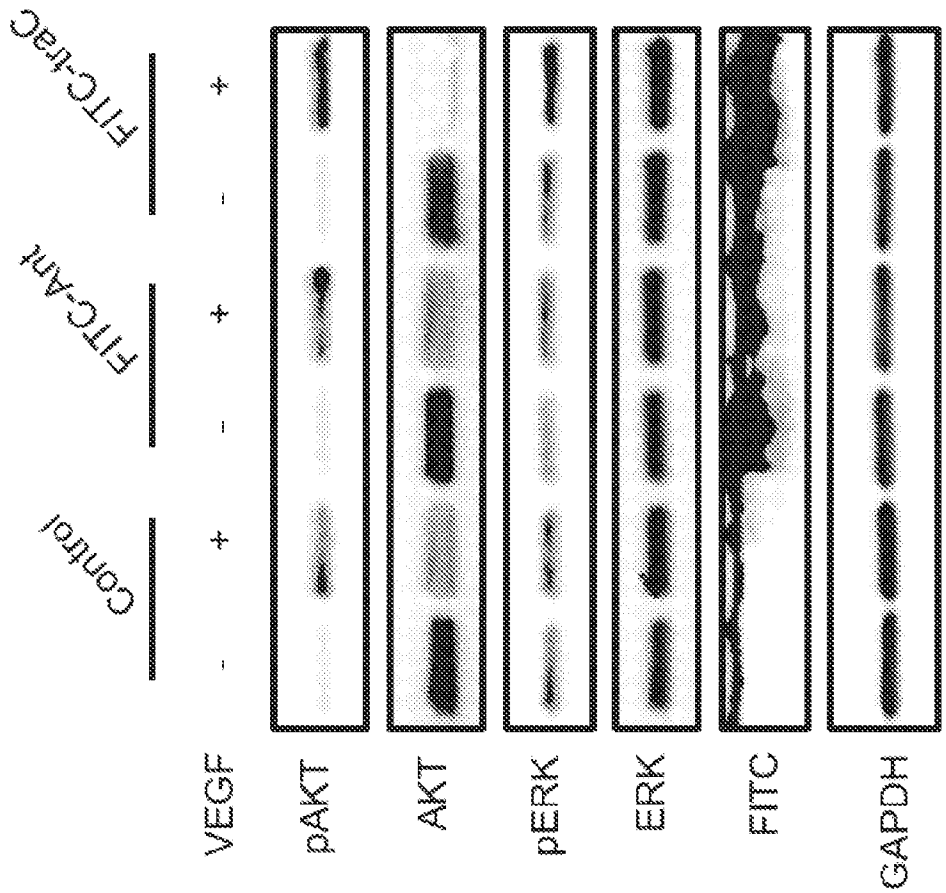


Fig. 28

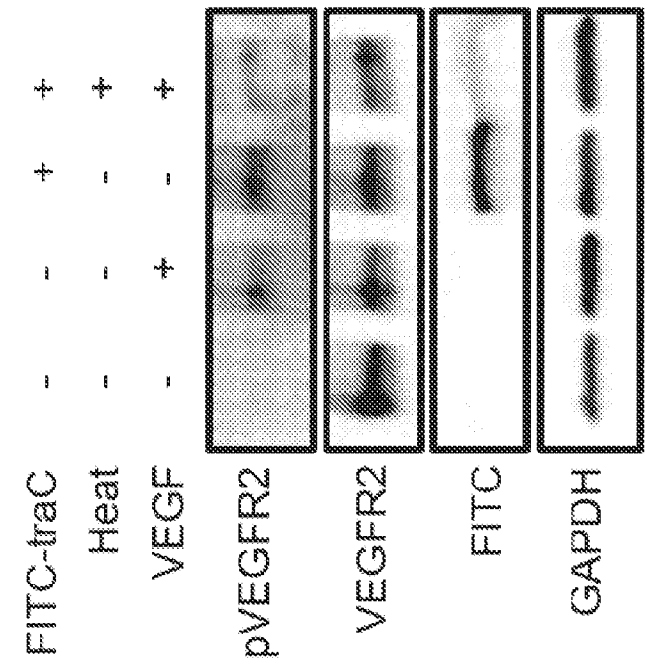


Fig. 27

Att

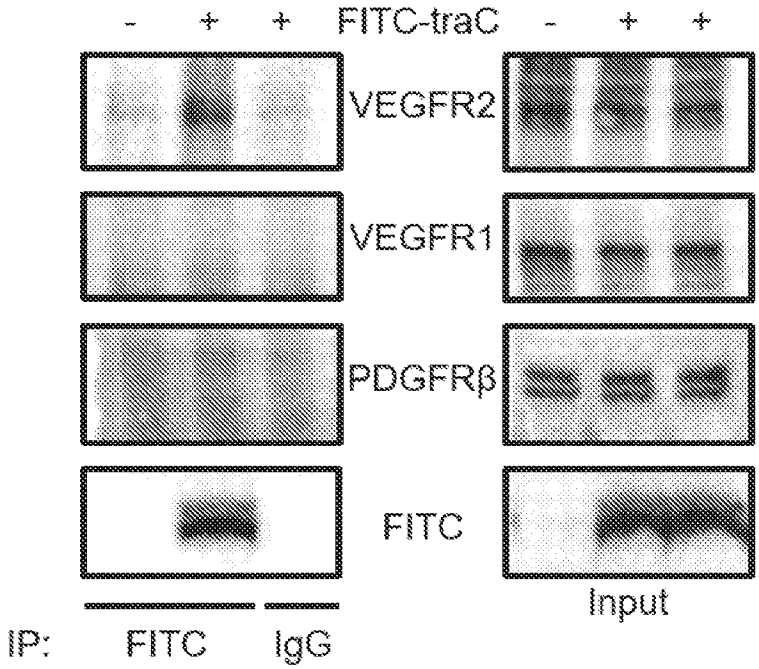


Fig. 29

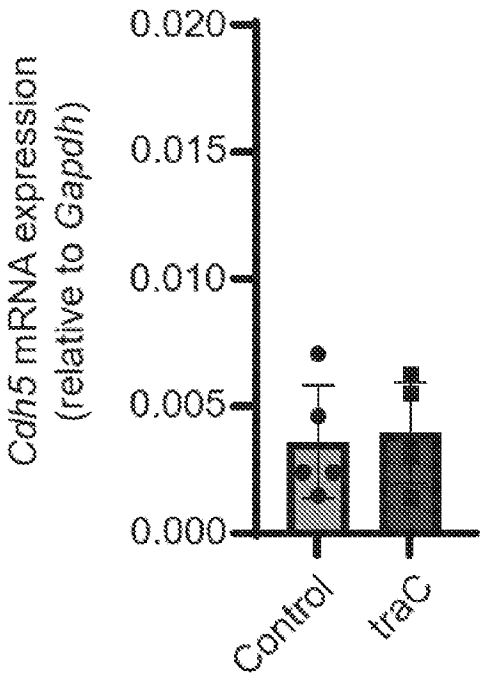


Fig. 30A

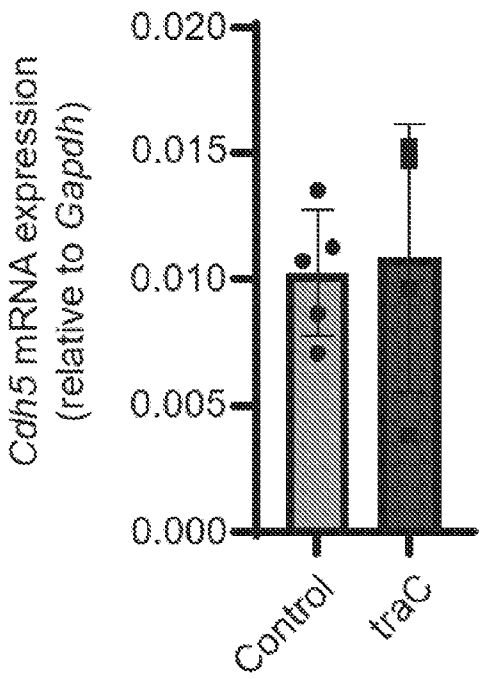


Fig. 30B

Att

Rat
Mouse
Human

Fig. 31A

Att

Rat
Mouse
Human

```
396  GAAQDKIFQGNKDYHKDVRNNFLPPIIARFIRVNPVQWQQKIAMKVELLGCQFTLKGRLPKLTQPPPPRNSNNLKNTTVH  475
396  GVDQDKIFQGNKDYHKDVRNNFLPPIIARFIRVNPVQWQQKIAMKVELLGCQFTLKGRLPKLT--PPPRNGNNLRNTTAR  473
399  GVEQDKIFQGNKDYHQDVRNNFLPPIIARFIRVNPTQWQQKIAMKMELLGCQFIPKGRPPKLTQPPPPRNSNDLKNTTAP  478

476  PKLG--RAPKFTQALQPRSRNDLPLLPAQTTATPDVKNTTVTPSVTKDVALAAVLVPVLVMALTTLILILVCAWHWRNRK  553
474  PKLGKGRAPKFTQVLQPRSRNELPVQPAETTTTPDIKNTTVTPSVTKDVALAAVLVPVLVMALTTLILILVCAWHWRNRK  553
479  PKIAKGRAPKFTQPLQPRSSNEFPAQTEQTTASPDIRNTTVTPNVTKDVALAAVLVPVLVMVLTTLILILVCAWHWRNRK  558

554  KKAEGTYDLPHNDRAGWNKGVKQLLPAKSVEHEETPVRYSNSEVSHLSPREVTTVLQADSAEYAQPLVGGIVGTLHQRST  633
554  KKTEGAYDLPHNDRAGWNKGMKQLLPAKSVDHEETPVRYSTSEVSHLSAREVTTVLQADSAEYAQPLVGGIVGTLHQRST  633
559  KKTEGTYDLPYWDRAGWWKGMKQFLPAKAVDHEETPVRYSSSEVNHLSPREVTTVLQADSAEYAQPLVGGIVGTLHQRST  638

634  FKPEEGKEASYADLDPYNAPVQEVYHAYAEPLPVTGPEYATPIVMDMSGHSTASVGLPSTSTFRTAGNQPPALVGTYNTL  713
634  FKPEEGKEAGYADLDPYNSPMQEVYHAYAEPLPVTGPEYATPIVMDMSGHPTASVGLPSTSTFKTAGTQPHALVGTYNTL  713
639  FKPEEGKEAGYADLDPYNSPGQEVYHAYAEPLPITGPEYATPIIMDMSGHPTTSVGQPSTSTFKATGNQPPPLVGTYNTL  718

714  LSRTDSCSSGQAQYDTPKGGKPAA-APEELVYQVPQSTQEASGAGRDEKFDAFKETL  769
714  LSRTDSCSSGQAQYDTPKGGKSAA-TPEELVYQVPQSTQELSGAGRDEKFDAFKEIL  769
719  LSRTDSCSSAQAQYDTPKAGKPGLpAPDELVYQVPQSTQEVSGAGRDGECDVFKEIL  775
```

Fig. 31B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/056265

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***A61K 35/36*** (2025.01); ***A61K 35/44*** (2025.01); *A61L 24/10* (2025.01)

CPC: ***A61K 35/36; A61K 35/44; C07K 14/435;*** *A61L 26/0028; C07K 4/12*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2002/022815 A1 (HYSEQ, INC.) 21 March 2002 (21.03.2002) pg 13, ln 26-27; pg 15, ln 1-4; pg 17, ln 21-23pg 54, ln 28-29; pg 58, ln 14-18; pg 66, ln 21-22; pg 78, ln 21-23; pg 78, ln 29-30, pg 103, ln 30; SEQ ID NO: 7	1-4, 11-14
A	US 2005/0202481 A1 (HONJO et al.) 15 September 2005 (15.09.2005) Entire document	1-4, 11-14

☐ 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

"D" document cited by the applicant in the international application

"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	Date of mailing of the international search report
09 January 2025 (09.01.2025)	**19 March 2025 (19.03.2025)**

Name and mailing address of the ISA/US	Authorized officer
COMMISSIONER FOR PATENTS **MAIL STOP PCT, ATTN: ISA/US** **P.O. Box 1450** **Alexandria, VA 22313-1450** **UNITED STATES OF AMERICA**	**KARI RODRIQUEZ**
Facsimile No. **571-273-8300**	Telephone No. **PCT Help Desk: 571-272-4300**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/056265

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed.
   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),
      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/056265

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☑ Claims Nos.: **5-10, 15-20, 27-31**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I: Claims 1-4 and 11-14, directed to a method of promoting neovascularization, hair growth or wound healing, the method comprising administering to the subject a compound comprising a polypeptide, wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, 80% or more of the amino residues of the first peptide are leucine residues, optionally, the polypeptide comprises a plurality of first peptides, and the compound does not comprise a peptide that is longer than about 14 amino acids or that has more than about 80% leucine amino acid residues, or

(b) the polypeptide comprises the amino acid set forth in SEQ ID NO:5, 6, 8, or 9.

Group II: Claims 21-26, directed to a composition comprising a compound comprising a polypeptide and a hydrophilic motif, e.g., a wound dressing with a base dressing material, and a compound comprising a polypeptide, wherein at least one of the following applies:

(a) the polypeptide comprises a first peptide comprising from about 6 amino acid residues to about 14 amino acid residues, wherein 80% or more of the amino residues are leucine residues, and the compound does not comprise a peptide sequence that has a length longer than about 14 amino acids and that has more than about 80% leucine residues, (b) the polypeptide comprises an amino acid set forth in SEQ ID NO: 5, 6, 8 or 9.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2024/056265

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1-4, 11-14**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.