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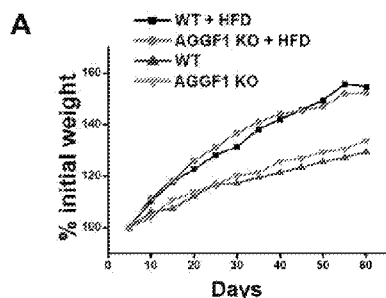
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(54) Title: AGGF1 AND AGGF1-PRIMED CELLS FOR TREATING DISEASES AND CONDITIONS



(57) Abstract: Provided herein are compositions, systems, kits, and methods for treating a subject with a disease or condition characterized by abnormal glucose metabolism (e.g., diabetes) or ischemic vascular disease (e.g., PAD or CAD) by administering a composition comprising AGGF1 peptides, or an expression vector encoding said peptides, or AGGF1-primed endothelial progenitor cells, to a subject. In certain embodiments, the AGGF1 peptide is full-length AGGF1, or a biologically active fragment or mutant thereof. In some embodiments, the AGGF1 peptides or expression vectors are administered around the same time insulin is administered to the subject.

AGGF1 AND AGGF1-PRIMED CELLS FOR TREATING DISEASES AND CONDITIONS

The present application claims priority to U.S. Provisional applications serial number
5 62/744,488 filed October 11, 2018, and serial number 62/842,283, filed May 2, 2019, both of
which are herein incorporated by reference.

GOVERNMENT FUNDING

This invention was made with government support under grant number HL077107
10 awarded by National Institutes of Health. The government has certain rights in the invention.

FIELD

Provided herein are compositions, systems, kits, and methods for treating a subject
with a disease or condition characterized by abnormal glucose metabolism (e.g., diabetes) or
15 ischemic vascular disease (e.g., PAD or CAD) by administering a composition comprising
AGGF1 peptides, or an expression vector encoding said peptides, or AGGF1-primed
endothelial progenitor cells, to a subject. In certain embodiments, the AGGF1 peptide is full-
length AGGF1, or a biologically active fragment or mutant thereof. In some embodiments,
the AGGF1 peptides or expression vectors are administered around the same time insulin is
20 administered to the subject.

BACKGROUND

Diabetes mellitus is a common and heterogeneous disease with high morbidity and
mortality worldwide. An estimated 422 million adults are affected with diabetes based on the
25 WHO's most recent 2016 data. The prevalence of diabetes, and in particular, of type 2
diabetes (T2DM), increases rapidly each year due to lifestyle habits and increased rates of
aging, inactivity, and obesity, and poses an important challenge to human health. However,
the treatment of diabetes remains suboptimal in a significant proportion of patients. Despite
intensive use of current antidiabetic drugs, >50% of T2DM patients have poor glycemic
30 control and 18% develop severe complications. Population HbA1c concentrations failed to
decline as much as expected due to poor medication compliance and other causes. To
address this grand challenge, much is needed to understand the pathophysiology of diabetes

and to develop new innovative therapies. In particular, long-lasting treatment can dramatically reduce poor medication compliance.

SUMMARY

5 Provided herein are compositions, systems, kits, and methods for treating a subject with a disease or condition characterized by abnormal glucose metabolism (e.g., diabetes) or ischemic vascular disease (e.g., PAD or CAD) by administering a composition comprising AGGF1 peptides, or an expression vector encoding said peptides, or AGGF1-primed endothelial progenitor cells, to a subject. In certain embodiments, the AGGF1 peptide is full-
10 length AGGF1, or a biologically active fragment or mutant thereof. In some embodiments, the AGGF1 peptides or expression vectors are administered around the same time insulin is administered to the subject.

 In some embodiments, provided herein are methods of treating a subject with a disease or condition characterized by abnormal glucose metabolism comprising:
15 administering a composition comprising: i) an AGGF1 peptide, or ii) an expression vector encoding the AGGF1 peptide, to the subject with the disease or condition. In particular embodiments, the disease or condition is Type I or Type II diabetes. In certain embodiments, the disease or condition is selected from the group consisting of: hyperglycemia, inadequate glucose clearance, and hyperinsulinemia. In particular embodiments, the methods further
20 comprise administering insulin to the subject. In other embodiments, the insulin is administered at about the same time as the composition.

 In certain embodiments, provided herein are methods of treating a subject comprising: administering a composition to a subject, wherein the subject has at least one of the following: a disease or condition characterized by abnormal glucose metabolism, ischemic
25 vascular disease, pulmonary hypertension, cardiac hypertrophy, and heart failure: wherein said composition comprises: i) an AGGF1 peptide, and/or ii) an expression vector encoding said AGGF1 peptide.

 In some embodiments, provided herein are methods of treating a subject with a cardiovascular disease or condition characterized comprising: administering a composition
30 comprising: i) an AGGF1 peptide that is no longer than 600 amino acids (e.g., 100-600 or 100-300 or 100-150 amino acids in length), or ii) an expression vector encoding the AGGF1 peptide, to the subject with the disease or condition. In certain embodiments, the disease or condition is selected from the group consisting of: coronary artery disease, myocardial

infarction, cardiac hypertrophy, heart failure, peripheral artery disease, restenosis and in-stent thrombosis after angioplasty and stenting, and pulmonary arterial hypertension.

In further embodiments, provided herein are methods of treating a subject with pulmonary arterial hypertension (PAH) comprising: administering a composition comprising:
 5 i) an AGGF1 peptide, or ii) an expression vector encoding the AGGF1 peptide, to the subject with the PAH.

In some embodiments, the subject in any of the methods herein is a human and: A) the composition comprises said AGGF1 peptide, and wherein the dosage is selected from the group consisting of: 0.1-15 mg/kg (e.g., 0.1 mg/kg ... 4.0 mg/kg ... 8.0 mg/kg ... 12.0 mg/kg ...
 10 and 15 mg/kg), 0.5-10 mg/kg, and 1-7 mg/kg; or B) said composition comprises said expression vector encoding said AGGF1 peptide, and wherein said dosage is about 1.0×10^{11} - 5.0×10^{13} (e.g., 1.0×10^{11} ... 1.0×10^{12} ... or 5.0×10^{13}).

In particular embodiments, provided herein are systems and kits comprising: a) a blood glucose monitoring device or article of manufacture; and b) a composition comprising:
 15 i) an AGGF1 peptide, or ii) an expression vector encoding the AGGF1 peptide. In some embodiments, the glucose monitoring device or article of manufacture is selected from the group consisting of: a blood glucose test strip, a reader for reading the glucose test strip, FreeStyle Libre, GlucoTrack, Eversense, GlucoWise, NovioSense, GlucoSense, and a smart contact lens.

20 In further embodiments, provided herein are systems and kits comprising: a) a composition comprising: i) an AGGF1 peptide, or ii) an expression vector encoding the AGGF1 peptide; and b) an implantable pump containing the composition, wherein the implantable pump is configured to deliver an amount of the composition into the bloodstream of a subject.

25 In certain embodiments, provided herein are compositions comprising: a) an AGGF1 peptide, or an expression vector encoding the AGGF1 peptide; and b) insulin peptide, or an expression vector encoding the insulin peptide.

In some embodiments, provided herein are methods of treating a subject comprising: administering a composition to a subject, wherein the subject has a disease or condition
 30 characterized by abnormal glucose metabolism and/or ischemic vascular disease, and wherein the composition comprises an AGGF1-primed endothelial progenitor cell. In certain

embodiments, the disease or condition is selected from the group consisting of peripheral artery disease (PAD), coronary artery disease (CAD), coronary heart disease (CHD), and myocardial infarction (MI). In other embodiments, the AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from the subject, wherein the EPC has been exposed to AGGF1 peptides ex vivo. In further embodiments, the AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from a relative of the subject, wherein the EPC has been exposed to AGGF1 peptides ex vivo. In additional embodiments, the AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from a non-relative of the subject, wherein the EPC has been exposed to AGGF1 peptides ex vivo.

In some embodiments, provided herein are compositions comprising: an AGGF1-primed endothelial progenitor cell. In further embodiments, the composition further comprises cell growth media. In other embodiments, the AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from a subject with a disease or condition characterized by abnormal glucose metabolism and/or ischemic vascular disease, wherein the EPC has been exposed to AGGF1 peptides ex vivo. In additional embodiments, the compositions further comprise a physiological tolerable liquid.

In other embodiments, provided herein are kits and systems comprising: a) an endothelial progenitor cell (EPC), and b) a composition comprising: i) an AGGF1 peptide, and/or ii) an expression vector encoding the AGGF1 peptide.

In certain embodiments, the AGGF1 peptide comprises full length human AGGF1 shown in SEQ ID NO:1. In other embodiments, the AGGF1 peptide has at least 97% sequence identity with SEQ ID NO:1 (e.g., 97 ... 98 .. 99 .. 99.5% identify). In other embodiments, the AGGF1 peptide is selected from any of SEQ ID Nos:2-16 or i) a peptide comprising the amino acid sequence from 574 to 614 of SEQ ID NO:1, ii) a peptide comprising the amino acid sequence from 574 to 624 of SEQ ID NO:1, or iii) a peptide comprising the amino acid sequence from 604 to 714 of SEQ ID NO:1. In further embodiments, the AGGF1 peptide has at least 97% sequence identity (e.g., 97 ... 98 .. 99 .. 99.5% identify) with any one of SEQ ID Nos:2-16. In certain embodiments, the administering reduces or eliminates at least one symptom of the disease or condition. In some embodiments, the subject is a human. In certain embodiments, the AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18). In certain embodiments, an expression vector encoding SEQ ID NO:18 is employed. In some

embodiments, the sequence encoding SEQ ID NO:18 is CAAAGAGAUGAUG CUCCUGCAUCUGUUC AU (SEQ ID NO:25). In certain embodiments, the expression vectors herein comprise SEQ ID NO:25.

5 DESCRIPTION OF THE FIGURES

The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

10 Fig. 1. AGGF1 regulates glucose metabolism and insulin resistance. (A) Body weight of AGGF1^{+/-} KO mice and littermate WT mice fed a standard chow diet or a high fat diet (HFD). (B) Fasting glucose levels in AGGF1^{+/-} KO mice and littermate WT mice fed HFD. (C) Fasting insulin levels in AGGF1^{+/-} KO mice and littermate WT mice fed HFD. (D) Glucose tolerance tests in AGGF1^{+/-} KO mice and littermate WT mice fed HFD. (E) Insulin
15 tolerance tests in AGGF1^{+/-} KO mice and littermate WT mice fed HFD. **P*<0.01, ***P*<0.005, n=8.

Fig. 2. AGGF1 protein therapy for diabetes. Intraperitoneal injection of AGGF1 protein dramatically lowers fasting glucose levels in HFD-induced diabetic mice (24-week-old male mice fed HFD for 16 weeks). **P*<0.01, ***P*<0.005, n=8.

20 Fig. 3. Therapeutic effects of chronic AGGF1 protein therapy on lowering fasting glucose levels. 24-week-old male HFD-induced diabetic mice were treated by intraperitoneal injection of 0.25 mg/kg AGGF1 protein or PBS control every 2 days for 36 days (chronic treatment). (A) Fasting serum glucose levels. (B) Body weight. **P*<0.01, ***P*<0.005, n=8.

Fig. 4. AGGF1 protein therapy is insulin-dependent. (A) Therapeutic effects of
25 insulin alone (1.00 ug/kg body weight) and in combination with AGGF1 (0.25 mg/kg body weight) in 24-week-old male HFD-induced diabetic mice. (B) No therapeutic effect for AGGF1 protein injection on fasting glucose levels in 16-week-old male diabetic mice induced by streptozotocin (STZ) for 4 weeks. (C) Therapeutic effect of AGGF1 protein injection is abolished in 24-week-old male HFD-induced diabetic mice treated with
30 somatostatin. **P*<0.01, ***P*<0.005, NS, not significant, n=8.

Figure 5a shows the amino acid sequence of full-length human AGGF1 (714 amino acid residues) (SEQ ID NO:1). Figure 5b shows the amino acid sequence of AGGF1-N1

(696 amino acids; 19 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:2). Figure 5c shows the amino acid sequence of AGGF1-N2 (629 amino acids; 86 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:3).

Figure 6a shows the amino acid sequence of AGGF1-N3 (550 amino acids; 165
5 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:4). Figure 6b shows the amino acid sequence of AGGF1-N4 (496 amino acids; 219 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:5). Figure 6c shows the amino acid sequence of AGGF1-N5 (431 amino acids; 284 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:6). Figure 6d shows the amino acid sequence of AGGF1-N6 (366 amino acids; 349
10 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:7).

Figure 7a shows the amino acid sequence of AGGF1-N7 (302 amino acids; 413 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:8). Figure 7b shows the amino acid sequence of AGGF1-N8 (244 amino acids; 472 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:9). Figure 7c shows the amino acid sequence of AGGF1-
15 N9 (193 amino acids; 522 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:10). Figure 7d show the amino acid sequence of AGGF1-N10 (141 amino acids; 574 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:11). Figure 7e shows the amino acid sequence of AGGF1-N101 (131 amino acids; 584 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:12). Figure 7f shows the amino acid sequence of
20 AGGF1-N102 (121 amino acids; 594 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:13).

Figure 8a shows the amino acid sequence of AGGF1-N103 (110 amino acids; 604 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:14). Figure 8b shows the amino acid sequence of AGGF1-C1 (664 amino acids; 50 amino acid deletion from the C-
25 terminus of AGGF1) (SEQ ID NO:15). Figure 8c shows the amino acid sequence of AGGF1-C2 (614 amino acids; 100 amino acid deletion from the C-terminus of AGGF1) (SEQ ID NO:16).

Figure 9 shows the full length human AGGF1 cDNA (SEQ ID NO:17), with arrows showing truncation mutants.

30 Figure 10 shows a diagram showing the full length AGGF1 (WT) and its serial N-terminal and C-terminal deletion mutant proteins.

Figure 11 shows results of purification of mutant AGGF1 proteins with serial N- and C-terminal deletions.

Figure 12 shows results of AGGF1-endothelial cell adhesion (binding) assays for serial N- and C-terminal AGGF1 deletion mutants.

5 Figure 13 shows results of Endothelial cell migration assays for serial N- and C-terminal AGGF1 deletion mutants.

Figure 14 shows results of angiogenesis assays for serial N- and C-terminal AGGF1 deletion mutants (capillary endothelial tube formation assays).

Figure 15. Micro-deletion analysis identified the short mutant form of AGGF1 with
 10 full functions of the full-length AGGF1 protein. (A) A diagram showing the full length AGGF1 (WT) and serial N-terminal deletion mutant proteins. (B) Endothelial cell adhesion assay with AGGF1 and its N-terminal deletion mutants. Coating buffer (CB) with and without BSA was used as a negative control. * $P < 0.05$. (C) Endothelial cell migration assay with AGGF1 and its N-terminal deletion mutants. BSA was used as a negative control.
 15 * $P < 0.05$. (D) Endothelial tube formation assay AGGF1 and its N-terminal deletion mutants. BSA was used as a negative control (left panel). * $P < 0.05$. (E) Western blot analysis of pAKTSer473 in HUVEC lysates. The cells were stimulated with AGGF1 and its N-terminal deletion mutants respectively. BSA was used as a negative control (left panel). * $P < 0.05$.

Figure 16. *Aggf1*^{+/-} KO mice spontaneously developed PAH. Male *Aggf1*^{Geo/+} KO
 20 mice (16-20 weeks) showed much higher mPAP (mean pulmonary arterial pressure) (A, B), total pulmonary resistance (TPR) (C), and right ventricular hypertrophy compared to WT mice (D). We assessed right ventricular hypertrophy by measuring the ratio of RV weight over the weight of the LV and septum, i.e. the RV/(LV + S) weight. ** $P < 0.01$; n=6.

Figure 17. *Aggf1*^{+/-} KO causes vascular remodeling. (A) Representative H&E of
 25 pulmonary arteries. (B) Mean medial wall thickness. (C) Resistance pulmonary arterial muscularization. For muscularization studies, lung sections (5 μ m) were co-immunostained with an α -smooth muscle actin antibody (α -SMA, VSMC marker) and a CD31 antibody (EC maker). Vessels were classified as fully (100%), partially, or non-muscularized (0%) vessels based on the percentage of CD31 signal surrounded by α -SMA signal. (D) PASMC
 30 proliferation (Ki67-positive dividing cells). ** $P < 0.01$; n=6.

Figure 18. AGGF1 attenuates the development of PAH in a hypoxic mouse model. Three-month-old male C567BL/6 mice were induced to develop PAH by hypoxia (10% oxygen for three weeks). Recombinant AGGF1 protein or control IgG (0.25 mg/KG body weight) was delivered by tail vein injection one day prior to hypoxia and continued twice a week for 3 weeks. RVSP, right-ventricular systolic pressure; RV/LV+S, RV hypertrophy ratio.

Figure 19. *Aggf1* is required for essential functions of EPCs in capillary tube formation, proliferation, transendothelial migration, and migration as in a diabetic mouse model (*db/db* mice). EPCs were isolated from bone marrow of WT, heterozygous *Aggf1*^{+/−} KO mice, *db/db* mice, and *Aggf1*^{+/−}*db/db* mice and characterized. (A) *Aggf1* haploinsufficiency inhibits angiogenesis mediated by EPCs. (B) Images from (A) were analyzed, quantified and plotted. (C) *Aggf1* haploinsufficiency inhibits EPC proliferation. (D) *Aggf1* haploinsufficiency inhibits transendothelial migration of EPCs in transwell assays. (E) Images from (D) were quantified and plotted. (F) *Aggf1* haploinsufficiency inhibits EPC migration in wound scratch migration assays. (G) Representative images from EPC migration assays. Data are shown as mean ± SD. **P*<0.05, ***P*<0.01, (n=6 mice per group).

Figure 20. AGGF1 dramatically improves essential functions of EPCs impaired by HG. (A) Western blot analysis showing increased AGGF1 expression in EPCs. (B) AGGF1 treatment reversed the impairment of angiogenic function of EPCs by HG. (C) Images from (B) were analyzed, quantified and plotted. (D) AGGF1 treatment reversed the reduced cell proliferation of EPCs by HG. (E) AGGF1 treatment reversed the HG-impaired transendothelial migration of EPCs. (F) Images from (E) were quantified and plotted. (G) AGGF1 treatment reversed the HG-impaired migration of EPCs in scratch wound migration assays. (H) Representative images from EPC migration assays. Data are shown as mean ± SD. **P*<0.05, ***P*<0.01, (n=6 mice per group).

Figure 21. AGGF1 protein treatment robustly potentiates the therapeutic effects of EPCs on peripheral vascular complications in a hindlimb ischemia model in *db/db* mice. A hindlimb ischemia model was created in *db/db* mice. (A) Transplantation of AGGF1-pretreated EPCs dramatically improved blood perfusion compared with EPCs without AGGF1 pretreatment in *db/db* mice. (B) Therapeutic effects of AGGF1-pretreated EPCs on necrosis compared with EPCs without AGGF1 pretreatment. (C) Therapeutic effects of AGGF1-pretreated EPCs on ambulatory impairment compared with EPCs without AGGF1

pretreatment. (D) Effects of AGGF1-pretreated EPCs on the density of CD31-positive capillary vessels compared with EPCs without AGGF1 pretreatment.

Figure 22. AGGF1 regulates the nuclear accumulation of Nrf2. (A) Western blot analysis for the effect of AGGF1, HG and HG+AGGF1 on the expression levels of nuclear Nrf2 (n-Nrf2) and its downstream signaling molecules, including HQ-1, NQO-1, and CAT. GAPDH was used as loading control. (B) AGGF1 protein treatment induces expression of *Nrf2* downstream anti-oxidative genes in EPCs. Data are shown as mean $\pm$ SD. * P <0.05, ** P <0.01, (n=3 per group).

Figure 23. AGGF1 activates AKT-Fyn-Nrf2 signaling in EPCs. (A-B) Western blot analysis showing the effect of siRNA for *AKT* (siAKT) (A) and wortmannin (B) on AGGF1-activated phosphorylation of AKT and nuclear accumulation of Fyn (n-Fyn). (C-E) Western blot analysis showing the effect of siAKT (C), wortmannin (D) and siRNA for *Nrf2* (siNrf2) (E) on AGGF1-induced increases of n-Nrf2 and its downstream signaling molecules. Data are shown as mean $\pm$ SD. * P <0.05, ** P <0.01, NS, not significant (n=3 per group).

Figure 24. Knockdown of *AKT* expression, wortmannin treatment, and knockdown of *Nrf2* expression attenuate the protective effects of AGGF1 on EPCs. (A) Western blot analysis for AGGF1 in EPCs. (B-E) Effects of *AKT* siRNA on AGGF1-mediated rescue of HG-impaired cell proliferation (B), tube formation (C), transendothelial migration (D), and cell migration (E) by EPCs. (F) Western blot analysis for AGGF1 in EPCs. (G-J) Effects of wortmannin on AGGF1-mediated rescue of HG-impaired cell proliferation (G), tube formation (H), transendothelial migration (I), and cell migration (J) by EPCs. (K) Western blot analysis for AGGF1 in EPCs. (L-O) Effects of *Nrf2* siRNA on AGGF1-mediated rescue of HG-impaired cell proliferation (L), tube formation (M), transendothelial migration (N), and cell migration (O) by EPCs. Data are shown as mean $\pm$ SD. * P <0.05, ** P <0.01, (n=6 mice per group).

Figure 25. Isolation and characterization of bone marrow derived EPCs from mice. Bone marrow mononuclear cells (MNCs) were washed out from the femurs and tibias of mice and isolated by density gradient centrifugation with histopaque-1083. MNCs were plated on gelatin-coated culture dishes and maintained in endothelial growth factor-supplemented media (EGM-2 bullet kit). Ten days after maintenance in endothelial-specific media, non-adherent MNCs were removed and the remaining adherent cells were subjected to immunostaining to verify their EPC identity. (A) Morphology of MNCs on day 4 and day 8 after plating on gelatin-coated dishes. Scale bar=50 μ m. (B) Representative images from assays for uptake of Dil-acLDL (red fluorescence) and FITC-UEA-1 (green fluorescence).

The isolated EPCs were Dil-acLDL and FITC-UEA-1 positive. Blue, staining of the nuclei by Hoechst. Scale bar=50 μm (upper), Scale bar=100 μm (lower). Three independent experiments were performed. (C) MNCs were cultured in the bullet kit medium in gelatin-coated cell culture dishes. Ten days after plating, adherent cells were further analyzed using flow cytometry with the cell surface antigen markers including antibodies for CD31, CD34, CD45 and CD144. Isotype IgG was used as a control antibody. Six mice per group (n=6/group) were studied each time.

Figure 26. *Aggf1* haploinsufficiency significantly reduces the number of bone marrow mononuclear cells (MNCs). MNCs were washed out from the femurs and tibias of WT and *Aggf1*^{+/-} KO mice and purified by density gradient centrifugation with histopaque-1083. MNCs were plated into gelatin-coated culture dishes and maintained in endothelial growth factor-supplemented media (EGM-2 bullet kit for ten days). Non-adherent MNCs were removed and the remaining adherent cells were subjected to immunostaining to verify their EPC identity. (A) Representative images from assays for uptake of Dil-Ac-LDL (red fluorescence) and FITC-UEA-1 (green fluorescence) are shown in (A). The isolated EPCs were Dil-ALDL- and FITC-UEA-1 positive. Blue, staining of the nuclei by Hoechst. (B) The number of the Dil-acLDL/FITC-UEA-1 (lectin) positive cells was plotted and compared between WT and *Aggf1*^{+/-} KO mice. Scale bar=100 μm . Three independent experiments were performed. Six mice per group (n=6/group) were studied each time. Data are shown as mean $\pm$ SD. **P*<0.05.

Figure 27. The expression level of the AGGF1 protein is increased in EPCs isolated from *db/db* mice or mice treated with HFD. (A) Western blot analysis showing increased AGGF1 expression in isolated EPCs from *db/db* mice. (B) Western blot analysis showing increased AGGF1 expression in isolated EPCs from HFD-induced T2DM mice. GAPDH was used as loading control. Data are shown as mean $\pm$ SD. ***P*<0.01, (n=6).

Figure 28. Increased glucose levels in mice fed with a high fat diet (HFD). C57BL/6J mice were fed with a high fat diet alone to induce T2DM. Mice fed with a chow diet were used as controls. After 16 weeks, blood glucose levels were measured using a glucometer, and then a hind-limb ischemia model was established with the mice. Three independent experiments (A, B, and C) were performed. Data are shown as mean $\pm$ S.D. ***P*<0.01 (n=6).

Figure 29. Analysis of the expression levels of CXCR4 (Fig. 29a), VEGF (Fig. 29b) and VEGFR2 (Fig. 29c) mRNA in EPCs from different groups of mice. Bone marrow-

derived EPCs were isolated and used for real-time RT-PCR analysis. Data are shown as mean $\pm$ SD. NS not significant, (n=3).

Figure 30. *Aggf1* is required for essential functions of EPCs in capillary tube formation, proliferation, transendothelial migration, and migration as in a HFD-induced diabetic mouse model. EPCs were isolated from bone marrow of wild-type (WT) and heterozygous *Aggf1*^{+/-} knockout (KO) mice fed with and without a high glucose diet (HFD), and characterized. (A) *Aggf1* haploinsufficiency inhibits angiogenesis mediated by EPCs. Angiogenic function of EPCs was assessed by a matrigel-based capillary tube formation. (B) Images from (A) were analyzed, quantified and plotted. (C) *Aggf1* haploinsufficiency inhibits EPC proliferation. Proliferation of EPCs was examined using the CCK-8 kit. (D) *Aggf1* haploinsufficiency inhibits transendothelial migration of EPCs in transwell assays. HUVECs (about 1×10^4 cells per well) were cultured in the upper chamber of a 24-transwell insert (8.0- μ m pores). EPCs were harvested, resuspended in basal culture medium (EBM-2, 0.5% BSA), and added to the upper chamber of the transwell plate (0.2 ml). Images were captured 24 hours after EPCs plating with an inverted Nikon Eclipse Ti microscope. (E) Images from (D) were quantified and plotted. (F) *Aggf1* haploinsufficiency inhibits EPC migration in wound scratch migration assays. EPCs monolayers in 6-well cell culture plates were wounded with a scratch by a 200 μ l pipette tip and examined 24 hours after wounding. Images were captured with an inverted Nikon Eclipse Ti microscope. (G) Representative images from EPC migration assays. Data are shown as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (n=6 mice per group).

Figure 31. Analysis of the expression levels of CXCR4 (Fig. 31a), VEGF (Fig. 31b) and VEGFR2 (Fig. 31c) mRNA in EPCs from mice treated with the AGGF1 protein and high glucose. EPCs were isolated, and used for real-time RT-PCR analysis. Data are shown as mean $\pm$ SD. NS not significant, (n=3).

Figure 32. AGGF1 protein treatment robustly potentiates the therapeutic effects of EPCs on angiogenesis and homing in a hindlimb ischemia model in *db/db* mice. (A) Representative images for H&E staining and immunostaining for CD31. Scale bar=100 μ m. (B) Representative images for immunostaining with an anti-GFP antibody. EPCs were infected with EGFP lentivirus, and transplanted into *db/db* mice with diabetic hind-limb ischemia. Scale bar=200 μ m. (n=12 mice per group).

Figure 33. AGGF1 protein treatment robustly potentiates the therapeutic effects of EPCs on peripheral vascular complications in a hindlimb ischemia model. A hindlimb ischemia model was created in mice. Two days after the surgery, the mice were injected with

EPCs, AGGF1-pretreated EPCs ($\sim 1 \times 10^6$), and control PBS via the tail vein. The therapeutic effects were evaluated using a Vevo 2100 High-Resolution Micro-Ultrasound System before the ischemic surgery and 7, 14 and 28 days after ischemia. (A) Transplantation of AGGF1-pretreated EPCs dramatically improved blood perfusion compared with EPCs without AGGF1 pretreatment. (B) Therapeutic effects of AGGF1-pretreated EPCs on necrosis compared with EPCs without AGGF1 pretreatment. (C) Therapeutic effects of AGGF1-pretreated EPCs on ambulatory impairment compared with EPCs without AGGF1 pretreatment. (D) Effects of AGGF1-pretreated EPCs on the density of CD31-positive capillary vessels compared with EPCs without AGGF1 pretreatment. Capillary density was measured in HFD-fed HLI mice. The gastrocnemius muscle from HLI mice were fixed in 4% paraformaldehyde for 24 hours and used for immunostaining analysis with an anti-CD31 antibody. (E) Representative images for H&E staining and immunostaining for CD31. Scale bar=100 μm . (F) Representative images for immunostaining with an anti-GFP antibody. EPCs were infected with EGFP lentivirus, and transplanted into mice with diabetic hind-limb ischemia. Four weeks after transplantation, the gastrocnemius muscle were fixed in 4% paraformaldehyde for 24 hours and then subjected to immunostaining analysis with an anti-EGFP antibody to determine whether EPCs were homed into the ischemic areas. Scale bar=100 μm . Data are shown as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$ ($n = 12$ mice per group).

Figure 34. EPCs were infected lentiviruses with GFP expression as an easily identifiable marker. The infection efficiency and the level of GFP expression were evaluated 72 h after infection. Scale bar=20 μm . Three independent experiments were performed and representative images were shown. Images were captured at the magnification of 40 $\times$.

Figure 35. AGGF1 inhibits HG-induced superoxide production by EPCs. Dichlorodihydrofluorescein diacetate (DCFH-DA, sigma) was used to detect superoxide in EPCs with flow cytometry (Beckman CytoFLEX). Fluorescence intensity was recorded, analyzed and plotted. Data are shown as mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, ($n = 6$ per group).

Figure 36. AGGF1 increases the level of nuclear Nrf2 in EPCs. EPCs were treated with a high glucose solution (HG) in the presence or absence of purified AGGF1 protein for 12 hours, and used for immunofluorescent staining with an anti-Nrf2 antibody to analyze nuclear translocation of Nrf2. Scale bar=200 μm .

Figure 37. AGGF1 does not affect the expression levels of Nrf2 and KEAP-1 in EPCs. EPCs were cultured in media with or without high glucose (HG, 30 mM) as well as a combination of high glucose and AGGF1 protein. (A) Total RNA was extracted with Trizol reagent and used for real-time RT-PCR analysis for the level of *Nrf2* mRNA. (B) Western

blot analysis with an anti-Nrf2 antibody. (C) Western blot analysis using an anti-KEAP-1 antibody. GAPDH was used as loading control. Three independent experiments were performed. Data are shown as mean $\pm$ S.D, (n=6).

Figure 38. Western blot analysis for nuclear accumulation of nNrf2 in EPCs treated with *Aggf1* siRNA and *Fyn* siRNA under the normal condition (Control) and under a high glucose condition (HG). GAPDH was used as loading control.

Figure 39. A schematic diagram showing the hypothetical molecular signaling pathway by which AGGF1 protein therapy potentiates the therapeutic effects of EPCs on diabetes-induced vascular complications. It is noted that the present invention is not limited to this particular mechanism, and an understanding of the mechanism is not necessary to practice the invention. AGGF1 activates AKT signaling, which inhibits Fyn-mediated export and degradation of nuclear Nrf2. Nrf2 binds to the promoter and regulatory region and activate the transcription of *CAT*, *HQ-1*, and *NQO-1*, which function as antioxidants to block generation of ROS, reducing oxidative stress and increasing function of EPCs. Overall, AGGF1 functions as an antioxidant regulator to protect the function of EPCs, and becomes a potential therapeutic target for improving the ischemia-reparative capacity of EPCs transplantation in DM.

Figure 40 shows the duration of the glucose-lowering effect of injection of a single dose of AGGF1 protein is more than 10 times longer than insulin in db/db diabetic mice. (A) Experimental design of the 48-hour AGGF1 treatment assay in Example 4. The time points for AGGF1 injection, glucose measurement, fasting period, and eating period are shown. (B) Therapeutic effects of the AGGF1 protein on blood glucose levels in db/db mice compared to IgG control after subcutaneous injection of IgG or AGGF1 (3 nmole/g body weight) over a time period of 48 hours. *P<0.05, **P<0.01, n=4. (C) Therapeutic effects of the AGGF1 protein on blood glucose levels in db/db mice compared to IgG control after subcutaneous injection of insulin (0.75 IU/kg body weight). *P<0.05, **P<0.01, n=4.

DEFINITIONS

To facilitate an understanding of the present technology, a number of terms and phrases are defined below. Additional definitions are set forth throughout the detailed description.

Throughout the specification and claims, the following terms take the meanings explicitly associated herein, unless the context clearly dictates otherwise. The phrase “in

some embodiments” as used herein does not necessarily refer to the same embodiment, though it may. Furthermore, the phrase “in another embodiment” as used herein does not necessarily refer to a different embodiment, although it may. Thus, as described below, various embodiments of the technology may be readily combined, without departing from the scope or spirit of the technology.

In addition, as used herein, the term “or” is an inclusive “or” operator and is equivalent to the term “and/or” unless the context clearly dictates otherwise. In addition, throughout the specification, the meaning of “a”, “an”, and “the” include plural references. The meaning of “in” includes “in” and “on.”

As used herein, the terms “subject” and “patient” refer to any animal, such as a mammal like a dog, cat, bird, livestock, and preferably a human (e.g., a human with a disease such as diabetes, cardiovascular disease, or related conditions).

As used herein, the term “administration” refers to the act of giving a drug, prodrug, or other agent, or therapeutic treatment to a subject. Exemplary routes of administration to the human body can be through the mouth (oral), skin (transdermal, topical), nose (nasal), lungs (inhalant), oral mucosa (buccal), inhaled, an implanted pump activation, by injection (e.g., intravenously, subcutaneously, intratumorally, intraocular, intraperitoneally, etc.), and the like.

DETAILED DESCRIPTION

Provided herein are compositions, systems, kits, and methods for treating a subject with a disease or condition characterized by abnormal glucose metabolism (e.g., diabetes) or ischemic vascular disease (e.g., PAD or CAD) by administering a composition comprising AGGF1 peptides, or an expression vector encoding said peptides, or AGGF1-primed endothelial progenitor cells, to a subject. In certain embodiments, the AGGF1 peptide is full-length AGGF1, or a biologically active fragment or mutant thereof. In some embodiments, the AGGF1 peptides or expression vectors are administered around the same time insulin is administered to the subject.

Similar to insulin, recombinant AGGF1 protein and its derivatives (e.g. short AGGF1 peptides or mutants with biological activity) can effectively lower glucose levels. However, different from insulin, therapies with AGGF1 and AGGF1 derivatives have a longer therapeutic effect, and do not cause hypoglycemia. Compared to typical insulin treatment,

which has a fast-acting effect in 30 minutes but lasts about 3 hours, work conducted during development of embodiments herein demonstrated AGGF1 treatment has a much longer therapeutic effect with a peak effect at 18-24 hours and lasting for >42 hours.

In certain embodiments, AGGF1 peptides (or expression vector encoding AGGF1) and insulin are administered at about the same time (e.g., within 1-25 minutes of each other). Combined treatment with both insulin and AGGF1 can quickly decrease the fasting glucose level within 30 minutes, but also significantly extend the effect of insulin. Therefore, the therapy with combined insulin and AGGF1 can have a sustained lowering effect on glucose levels for a much longer time than insulin alone. For insulin treatment, patients typically require insulin injections 3 times per day. Even the long-acting insulin such as insulin glargine (Lantus, Sanofi) requires injection once a day. In certain embodiments, the combined AGGF1 and insulin treatment reduces the frequency of injection to once per >2 days. The various embodiments described herein can enable more patients with type 1 and type 2 diabetes mellitus to reach better glucose targets by reducing the frequency of injections of drugs and enhancing medication compliance, and maintain a better quality of life.

AGGF1 is an angiogenic protein that has been proposed previously for use in treating cardiovascular diseases. In work conducted during the development of embodiments herein, it was shown that AGGF1 has activities similar to insulin. In AGGF1 KO mice, fasting glucose levels were significantly elevated when mice were fed a high fat diet and were insulin resistant. In diabetic mice, AGGF1 administration significantly lowered fasting glucose levels. In streptozotocin mice, the effects of AGGF1 seemed to depend on and potentiate insulins activities.

Benefits of AGGF1 therapy (e.g., protein or expression vector encoding protein) include, but are not limited to, AGGF1 peptides regulate glucose levels more precisely than insulin, no evidence of hypoglycemia, longer therapeutic window, peak glucose lowering at 18-24 hours, lasting for >42 hrs. In combination with insulin, AGGF1 peptide and expression vectors encoding AGGF1, can be used to better control glucose levels over extended periods of time compared with insulin alone.

The AGGF1 gene encodes a 714 amino acid angiogenic factor essential for embryonic development. As described in the Example below, it was found that heterozygous AGGF1 knockout (KO) mice develop diabetes with an increased fasting glucose level, impaired glucose tolerance, and insulin resistance, and that AGGF1 protein therapy dramatically

reduces the fasting glucose level in high fat diet (HFD)-induced diabetic mice. Compared to typical insulin treatment, which has a fast-acting effect in 30 minutes but lasts about 3 hours, AGGF1 treatment has a much longer therapeutic effect with a peak effect at 18-24 hours and lasting for >42 hours. It was found that AGGF1 protein therapy of one injection per two days
5 maintained the low glucose level constantly. In certain embodiments, where expression vectors are administered to a subject, long term expression can allow a subject to receive a single administration without further treatment of for a longer period of time (e.g., 2 months). In certain embodiments, diabetic patients are treated with AGGF1 therapy who also have coronary artery disease, at risk for heart attack, or heart failure, and receive a benefit for both
10 conditions.

In certain embodiments, the compositions according to the present disclosure (e.g., with an AGGF1 peptide or expression vector) comprises or consists of a pharmaceutically acceptable carrier, diluent, or excipient (including combinations thereof). Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art and are described, for
15 example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient, or diluent is selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical comprise as, or in addition to, the carrier, excipient, or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), and/or solubilizing agent(s).
20 This pharmaceutical composition will desirably be provided in a sterile form. It may be provided in unit dosage form and will generally be provided in a sealed container. A plurality of unit dosage forms may be provided.

In certain embodiments, the composition inside of pumps that are surgical embedded within a patient, such that the AGGF1 peptide or expression vector can be injected (e.g., in a
25 manner similar to insulin) to the subject's blood stream when needed (see, e.g., Shah et al., Int J Pharm Investig. 2016 Jan-Mar; 6(1): 1-9, herein incorporated by reference).

Pharmaceutical compositions within the scope of the present technology may include one or more of the following: preserving agents, solubilizing agents, stabilizing agents, wetting agents, emulsifiers, sweeteners, colorants, flavoring agents, odorants, and/or salts.
30 Compounds of the present technology may themselves be provided in the form of a pharmaceutically acceptable salt. In addition, embodiments may comprise buffers, coating

agents, antioxidants, suspending agents, adjuvants, excipients, and/or diluents. Examples of preservatives include sodium benzoate, sorbic acid, and esters of p-hydroxybenzoic acid.

They may also contain other therapeutically active agents in addition to compounds of the present technology (e.g., insulin). Where two or more therapeutic agents are used they
5 may be administered separately (e.g., at different times and/or via different routes) and therefore do not always need to be present in a single composition. Thus, combination therapy is within the scope of the present technology.

In certain embodiments, the compositions herein contain an expression vector encoding the AGGF1 peptide. In certain embodiments, the expression vectors is a plasmid,
10 an adenovirus-associated viruses, or other viruses. In certain embodiments, the expression vectors are delivered by nano-technologies and/or targeted delivery systems.

The routes for administration (delivery) include, but are not limited to, one or more of: oral (e.g. as a tablet, capsule, or as an ingestible solution), implantable pump, topical, mucosal (e.g. as a nasal spray or aerosol for inhalation), nasal, parenteral (e.g. by an
15 injectable form), gastrointestinal, intraspinal, intraperitoneal, intramuscular, intravenous, intrauterine, intraocular, intradermal, intracranial, intratracheal, intravaginal, intracerebroventricular, intracerebral, subcutaneous, ophthalmic (including intravitreal or intracameral), transdermal, rectal, buccal, via the penis, vaginal, epidural, sublingual. It is to be understood that not all of the agent need be administered by the same route. Likewise, if
20 the composition comprises more than one active component, then those components may be administered by different routes.

If the AGGF1 peptides and expression vectors described herein is administered parenterally, then examples of such administration include one or more of: intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally,
25 intrastemally, intracranially, intramuscularly, or subcutaneously administering the agent; and/or by using infusion techniques.

In some embodiments, pharmaceutical compositions adapted for oral administration are provided as capsules or tablets; as powders or granules; as solutions, food product, syrups or suspensions (in aqueous or non-aqueous liquids); as edible foams or whips; or as
30 emulsions. Tablets or hard gelatin capsules may comprise lactose, maize starch or derivatives thereof, stearic acid or salts thereof. Soft gelatin capsules may comprise vegetable oils, waxes, fats, semi-solid, or liquid polyols etc. Solutions and syrups may comprise water,

polyols and sugars. For the preparation of suspensions, oils (e.g., vegetable oils) may be used to provide oil-in-water or water-in-oil suspensions. An active agent intended for oral administration may be coated with or admixed with a material that delays disintegration and/or absorption of the active agent in the gastrointestinal tract (e.g., glyceryl monostearate or glyceryl distearate may be used). Thus, the sustained release of an active agent may be achieved over many hours and, if necessary, the active agent can be protected from being degraded within the stomach.

In certain embodiments, the AGGF1 peptides described herein may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. The agent of the present technology may also be dermally or transdermally administered, for example, by the use of a skin patch. For application topically to the skin, the agent of the present technology can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, it can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetaryl alcohol, 2-octyldodecanol, benzyl alcohol and water. If the AGGF1 peptides are administered parenterally, then examples of such administration include one or more of: intravenously, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intraurethrally, intrasternally, intracranially, intramuscularly or subcutaneously administering the agent; and/or by using infusion techniques.

Typically, a physician will determine the actual dosage of the AGGF1 peptides or expression vector which will be most suitable for an individual subject. The specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed; the metabolic stability and length of action of that compound; the age, body weight, general health, sex, diet, mode and time of administration; rate of excretion; drug combination; the severity of the particular condition; and the individual undergoing therapy. In some embodiments, the dosage is between 0.1 and 5 mg per kilogram of the subject (e.g., 0.1 ... 0.8 ... 1.5 ... 2.5 ... 4.0 ... 5.0 mg per kg). The agent and/or the pharmaceutical composition of the present technology may be administered in accordance with a regimen of from 1 to 10 times per day, such as once or twice per day, or only once every other day or every third day. For oral and

parenteral administration to human patients, the daily dosage level of the agent may be in single or divided doses.

EXAMPLES

5

EXAMPLE 1

AGGF1 may be regarded as the “the 2nd insulin” for treatment of diabetes. Compared to typical insulin treatment, which has a fast-acting effect in 30 minutes but lasts about 3 hours, AGGF1 treatment, in this Example, has a longer therapeutic effect with a peak effect at 18-24 hours and lasting for >42 hours. AAV-AGGF1 gene therapy can make its therapeutic effect last even for months.

10

Increased fasting glucose levels in heterozygous AGGF1^{+/-} KO mice.

In order to assess the effect of AGGF1 on diabetes, we characterized AGGF1 knockout mice developed by our laboratory¹⁰⁻¹³. Because homozygous AGGF1^{-/-} KO mice are embryonically lethal¹³, we studied heterozygous AGGF1^{+/-} KO mice. We found no significant differences in body weight and food intake between AGGF1^{-/-} KO mice and their littermate WT control mice fed a standard chow diet for up to 60 weeks ($P>0.05$, $n=8/\text{group}$) (Fig. 1A) or a high fat diet (HFD) ($P>0.05$, $n=8/\text{group}$) (Fig. 1A). Interestingly, when we measured the fasting glucose levels, we found that AGGF1^{+/-} KO mice showed a significantly higher level of fasting blood glucose than littermate WT mice fed HFD ($P<0.05$, $n=8/\text{group}$) (Fig. 1B). The fasting insulin level was also higher in AGGF1^{-/-} KO mice than in littermate WT mice fed HFD ($P<0.05$, $n=8/\text{group}$) (Fig. 1C).

15

20

Impaired glucose tolerance in heterozygous AGGF1^{+/-} KO mice.

We performed the glucose tolerance test (GTT). As shown in Fig. 1D, the GTT revealed significantly impaired glucose tolerance in AGGF1^{-/-} KO mice compared with littermate WT control mice ($P<0.05$, $n=8/\text{group}$).

25

Insulin resistance in heterozygous AGGF1^{+/-} KO mice.

We performed insulin tolerance tests (ITT). As shown in Fig. 1E, AGGF1^{-/-} KO mice showed characteristics of insulin resistance compared with littermate WT mice ($P<0.05$, $n=8/\text{group}$).

AGGF1 protein therapy dramatically lowers fasting glucose levels in diabetic mice.

Because AGGF1^{-/-} KO mice developed diabetes, we hypothesized that intraperitoneal injection of AGGF1 protein may inhibit diabetes. To test the hypothesis, AGGF1 protein (0.25 mg/kg body weight) was injected into HFD-induced hyperglycemic mice. Surprisingly, injection of AGGF1 protein dramatically reduced the fasting glucose level in HFD hyperglycemic mice compared with control PBS buffer (Fig. 2). The maximal glucose-lowering effect of AGGF1 was achieved at 18-24 hours after injection, and the sustained effect can be observed 42 hours after the injection (Fig. 2). Chronic AGGF1 treatment of HFD-induced hyperglycemic mice every 2 days for 36 days maintained the significantly low fasting glucose level for the entire treatment period (Fig. 3).

Effect of combined treatment with insulin and AGGF1.

HFD-induced hyperglycemic mice were administered with insulin alone (1 ug/kg body weight) or combination of insulin (1 ug/kg body weight) and AGGF1 protein (0.25 mg/kg body weight) by intraperitoneal injection. Insulin treatment quickly decreased the fasting glucose level within 30 minutes and the effect of insulin was sustained for only 150 minutes. AGGF1 protein treatment significantly extended the effect of insulin (Fig. 4A). These data suggest that the therapy with combined insulin and AGGF1 can have a sustained lowering effect on glucose levels for a much longer time than insulin alone.

Interestingly, we found that AGGF1 protein therapy is dependent on insulin. In a diabetic mouse model induced by streptozotocin (STZ, killing β -cells), AGGF1 protein therapy failed to lower blood glucose levels (Fig. 4B). Moreover, the glucose-lowering effect of AGGF1 was severely blunted when insulin secretion was inhibited by somatostatin (Fig. 4C). These data suggest that AGGF1 protein therapy lowers blood glucose levels in an insulin-dependent manner.

EXAMPLE 2

This Examples describes the identification of truncated AGGF1 peptides that function in a manner similar to the full length AGGF1 protein.

Identification of short, functional AGGF1 domain required for endothelial cell proliferation, migration, and angiogenesis between amino acids 574 and 624

In order to localize the functional domain of AGGF1, we created serial deletions of AGGF1 by systematic truncation of AGGF1 by 50 amino acids each time from the N-terminus or C-terminus (Figure 10). Successful creation of AGGF1 deletions are demonstrated by coomassie-blue staining of N-terminal and C-terminal serial deletion mutant proteins on SDS-PAGE gels shown in Figure 11.

The interaction domain between AGGF1 and endothelial cells is localized between amino acids 574 and 624.

To identify the AGGF1 domain responsible for binding to endothelial cells, endothelial cell adhesion assays were performed with human umbilical vascular endothelial cells (HUVECs) for full-length AGGF1 (WT) and serial N-terminal deletion mutant proteins. Significantly stronger binding (i.e. high fluorescence) was observed with WT-AGGF1 and deletion mutant proteins N1 to N10 than deletion mutant proteins N11 and N12 (Figure 12, left panel). These results suggest that the potential interaction domain of AGGF1 is between amino acid 574 and 624.

Endothelial cell adhesion assays were also performed with WT-AGGF1 and serial C-terminal deletion mutant proteins of AGGF1. Significantly higher binding (i.e. fluorescence) was observed with WT-AGGF1 and deletion mutants C1 and C2 than deletion mutants C3 to C13 (Figure 12, right panel). These results suggest that the potential interaction domain of AGGF1 with endothelial cells is between amino acid 564 and 614 (Figure 10). Together, these results narrow the AGGF1-endothelial cell interaction domain to a 40-amino acid region (amino acids 574-614) at the C-terminus of AGGF1.

The functional domain of AGGF1 responsible for endothelial cell migration is localized between amino acids 574 and 624.

The cellular mechanism of angiogenesis involves endothelial cell motility. Endothelial cell migration assays were performed with WT-AGGF1 and serial N- and C-terminal deletion mutant AGGF1 proteins. Significantly more HUVECs migrated into the 'wounded area' after 24 hours in presence of WT-AGGF1 and deletion mutant proteins N1 to N10 than deletion mutant proteins N11 and N12 (Figure 13). Significantly more HUVECs migration was also observed with deletion mutant proteins C1 and C2 than deletion mutant

proteins C3 to C13 (Figure 13). These endothelial cell migration assays demonstrate that the 40 amino acid AGGF1 binding domain (amino acids 574-614) is also responsible for inducing endothelial cell migration.

5 The functional domain of AGGF1 responsible for angiogenesis is localized between amino acids 574 and 624.

Angiogenesis assays were performed using the capillary endothelial tube formation assays. A significantly higher number of 'enclosed circles' (i.e. angiogenesis) were formed by HUVECs after 16 hours in presence of WT-AGGF1 and deletion mutant proteins N1 to
10 N10 than deletion mutant proteins N11 and N12 (Figure 14). Similarly, a significantly higher number of 'enclosed circles' were formed by HUVECs in presence of WT-AGGF1 and deletion mutant proteins C1 and C2 than deletion mutant proteins C3 to C13 (Figure 14). These endothelial tube formation assays demonstrate that the 40 amino acid AGGF1 binding domain (amino acids 574-614) is responsible for angiogenesis.

15

A short mutant AGGF1 protein with amino acids 604-714 has full functions of full-length AGGF1 in endothelial cell proliferation, migration, and angiogenesis

To further narrow down the amino acid residues involved in AGGF1 functions, we created four more serial N-terminal deletions, each truncating AGGF1 by 10 amino acids
20 between amino acids 574 and 624, referred to as N101, N102, N103, and N104 (Figure 15A)). Endothelial cell adhesion assays showed that WT-AGGF1 and deletion mutant proteins N101 to N103 can bind to endothelial cells, but not deletion N104 (Figure 15B). Similar findings were found for HUVEC migration (Figure 15C).

For endothelial tube formation (angiogenesis) assays (Figure 15D), WT-AGGF1 and
25 deletion mutant proteins N101 to N103 showed a significantly increased angiogenesis than deletion N104 and control BSA. N102 appeared to present with a stronger angiogenic activity than N103 (Figure 15D).

We also analyzed the WT and deletion mutant AGGF1 for their function in activation of the AKT signaling pathway involved in angiogenesis and insulin signaling. Compared to
30 control BSA, a significantly higher phosphorylation level of AKT (Ser473) was shown in

presence of WT-AGGF1. Similarly, a high phosphorylation level of AKT (Ser473) was shown with deletion mutant proteins N101 to N103, but a significantly less phosphorylation level of AKT (Ser473) was shown with deletion mutant protein N104 (Figure 15E).

In summary, by analyzing serial AGGF1 deletions, we have identified a short mutant form of AGGF1 spanning amino acids 604-714 (N103), which retains the full function of the full-length AGGF1 protein. This mutant AGGF1 is referred to as AGGF1₆₀₄₋₇₁₄. Because AGGF1₆₀₄₋₇₁₄ has full AGGF1 functions, we can utilize it for treatment of, for example, cardiac and vascular diseases, and the other diseases listed herein. Because AGGF1₆₀₄₋₇₁₄ has only 110 amino acid residues, it can be synthesized in vitro and used for clinical treatment of cardiac and vascular diseases. AGGF1₆₀₄₋₇₁₄ can be further optimized by many different modifications to increase its stability and functional activities in treatment of diseases. For example, AGGF1₆₀₄₋₇₁₄ can be further modified by removing more amino acids to identify the shortest form of AGGF1, but with full activities, which can be used for treatment of diseases (e.g., using the assays and procedures described in this Example). AGGF1₆₀₄₋₇₁₄ can be further modified by adding some amino acids to increase its stability and activities. AGGF1₆₀₄₋₇₁₄ can be further modified by adding some amino acids (e.g. mimotope) or by conjugating carriers for its targeted delivery to specific organs, tissues and cells for treatment of specific diseases. AGGF1₆₀₄₋₇₁₄ can be further modified by synthesizing as a circular form (cyclotide) to increase its stability for disease treatment.

Utilization of recombinant AGGF1 and AGGF1 derivatives to treat PAH

Pulmonary arterial hypertension (PAH) is characterized by an increased mean pulmonary arterial pressure (mPAP) of ≥ 25 mmHg, which is caused by functional and structural remodeling of pulmonary arteries, and in turn increases the burden on the right ventricle and eventually leads to right ventricular failure and premature death^{5,6}. The pathogenesis of PAH is complex and involves pulmonary endothelial dysfunction, proliferation of vascular smooth muscle cells (VSMCs), inflammation, fibrosis, as well as abnormal thickening and contraction of pulmonary arteries, thrombus formation, and genetic abnormalities, leading to incremental increases of pulmonary vascular resistance, increased right ventricular loading, and eventual right ventricular failure and death.⁷⁻¹¹ Accurate and early diagnosis of PAH is seriously lacking because PAH symptoms appear late. Hence, the best and early treatment for patients is delayed, resulting in a low survival rate.^{5,6} PAH

remains a fatal disease with 31-45% of patients dying within 3 years even with the best treatments.¹²⁻¹⁸ Moreover, current treatments cannot reverse vascular remodeling.^{8,9}

AGGF1 deficiency causes PAH:

5 We recorded the pulmonary artery pressure (PAP) from $Aggf1^{+/-}$ ($Aggf1^{-/-}$ is embryonically lethal) and wild type (WT) mice ($Aggf1^{+/+}$ littermates) at the age of 16-20 weeks (male mice, n=6/group) (Figure 16A). It was striking that mPAP in WT mice (16.4 $\pm$ 1.20 mmHg) falls within the normal range of 9-18 mmHg in humans, whereas mPAP in $Aggf1^{+/-}$ KO mice was significantly increased to 27.3 $\pm$ 1.90 mmHg ($P=0.007$, n=6/group),
10 which is higher than the threshold for the diagnosis of PAH in humans (Figure 16B). We conclude that $Aggf1^{+/-}$ KO mice develop spontaneous PAH. Compared with wild type mice, $Aggf1^{+/-}$ KO mice showed significantly higher total pulmonary resistance (TPR) (1.24 $\pm$ 0.17 mmHg/mL/min for KO mice vs. 0.72 $\pm$ 0.10 for WT, $P=0.001$, n=6/group) (Figure 16C). $Aggf1^{+/-}$ KO mice showed right ventricular (RV) hypertrophy because their RV/(LV+S)
15 weight is significantly higher than that from WT mice (32.8 $\pm$ 1.8 for KO vs. 26.1 $\pm$ 1.30 for WT, $P=0.003$, n=6/group) (Figure 16D). All of these findings are important features of PAH. We therefore conclude that $Aggf1^{+/-}$ KO mice develop spontaneous PAH.

We analyzed vascular remodeling associated with PAH in $Aggf1^{+/-}$ KO mice. The pulmonary arteries were isolated from $Aggf1^{+/-}$ KO and WT mice (16-20 weeks, male mice,
20 n=6/group), and stained with H&E. H&E staining showed that the pulmonary artery from $Aggf1^{+/-}$ KO mice had a significantly increased medial wall thickness compared to WT mice (KO 42.6 $\pm$ 2.1 vs. WT 31.7 $\pm$ 1.4, $P=0.002$, n=6/group) (Figure 17A-B). The pulmonary arteries of $Aggf1^{+/-}$ KO mice had a significantly increased full muscularization (see Figure 17 description for method of quantitation: KO 21.6 $\pm$ 4.4 vs. WT 12.4 $\pm$ 2.5 %, $P=0.002$, n=6/group) and partial muscularization (KO 34.3 $\pm$ 7.9 vs. WT 20.4 $\pm$ 5.7 %, $P=0.002$, n=6/group) compared to that of WT mice (Figure 17C).
25

We analyzed cell proliferation using immunostaining with an anti-Ki67 antibody, which identifies Ki67-positive proliferating or dividing cells. The density of positive Ki67 cells was much higher in pulmonary arteries from $Aggf1^{+/-}$ KO mice than that from WT
30 mice (6.87 $\pm$ 1.15 % for KO vs. 3.21 $\pm$ 0.62 for WT, $P=0.008$, n=6/group) (Figure 17D).

All of these data indicate that Aggf1^{+/-} KO mice develop spontaneous PAH and vascular remodeling that fully recapitulate human PAH pathology.

5 AGGF1 can block PAH:

Even with currently available therapies, the 3-year death rate for PAH patients is as high as 31%-45%.¹²⁻¹⁸ Therefore, there is an urgent medical need to develop new or improved therapeutic agents to manage PAH patients. We found that AGGF1 protein therapy can block PAH. Systemic injection of recombinant AGGF1 protein significantly reduced right
 10 ventricular systolic pressure (RVSP) in a hypoxia-induced mouse model for PAH compared with control IgG ($P=0.019$) (Figure 18). AGGF1 protein therapy also reduced RV hypertrophy (RV/LV+S) compared with IgG control (Figure 18) although additional mice are needed to achieve statistical significance.

We have created a new mutant form of AGGF1, AGGF1₆₀₄₋₇₁₄, which has the full
 15 activities of the full-length AGGF1, and can be utilized for treatment of PAH. The AGGF1 protein therapy for PAH has several advantages. First, because our study indicates that Aggf1 KO and deregulation are directly related to the pathogenesis of PAH in mice and humans, AGGF1 treatment can be a specific therapy for PAH. On the other hand, other VSMC proliferation blocking agents (proteasome inhibitors bortezomib BTZ and carfizomib
 20 CFZ) have off-target effects (causing cardiac apoptosis in RV and LV).¹⁹ Existing therapy for PAH such as calcium channel blockers causes hypotension, but AGGF1 treatment does not affect blood pressure under a normal physiological condition.

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EXAMPLE 3

In Vivo Treatment with AGGF1-Primed EPCs

20 Hyperglycemia-triggered vascular abnormalities are the most serious complications of diabetes mellitus (DM). The major cause of vascular dysfunction in DM is endothelial injury and dysfunction associated with the reduced number and dysfunction of endothelial progenitor cells (EPCs). However, the major regulators and underlying molecular mechanism are poorly characterized. In this Example, it is shown that EPCs from
25 heterozygous knockout (KO) *Aggf1*^{+/-} mice showed impairment of proliferation, migration, angiogenesis, and transendothelial migration as in hyperglycemic mice fed with a high fat diet (HFD) or *db/db* mice. The number of EPCs from *Aggf1*^{+/-} mice was significantly

reduced. *Ex vivo*, AGGF1 protein can fully reverse damaging effects of hyperglycemia on EPCs. *In vivo* transplantation of AGGF1-primed EPCs successfully restores blood flow, and blocks tissue necrosis and ambulatory impairment in HFD-induced hyperglycemic mice or *db/db* mice with diabetic hind-limb ischemia. While not limited to any particular mechanism, it is believed that AGGF1 activates AKT and reduces nuclear localization of Fyn, which increases the nuclear level of Nrf2 and expression of anti-oxidative genes, and inhibits ROS generation. These results suggest that *Aggf1* is required for essential function of EPCs, AGGF1 reverses the damaging effects of hyperglycemia on EPCs, and AGGF1-priming of EPCs is a useful treatment modality for vascular complications in DM.

Diabetes mellitus (DM) is a chronic metabolic disorder, however, hyperglycemia-triggered vascular abnormalities, including cardiovascular disease and diabetic peripheral arterial disease (PAD), are the most serious complications, contributing to numerous deaths, diabetic foot wounds and lower-extremity amputation (1-8). The major causes of vascular dysfunction in DM are endothelial injury and dysfunctions associated with the reduced number and dysfunction of endothelial progenitor cells (EPCs) (9-12). EPCs are progenitors of endothelial cells (ECs) and have the potential to proliferate, migrate, home into the disrupted endothelium and differentiate into ECs to maintain endothelium integrity, restore endothelial dysfunction, promote neovascularization and repair damaged vessels (11; 13-15). Cell-based therapy based on implantation of EPCs has emerged as a potential therapy for myocardial ischemia, brain ischemia and pulmonary embolism (16; 17). However, the efficacy of a therapy using EPC implantation for diabetic vascular complications is uncertain (18). In particular, autologous EPCs transplantation may not work efficiently because of the impaired function of such EPCs in DM by hyperglycemia (9; 11; 18).

DESIGN AND METHODS

Animals

Wild-type (WT) C57BL/6J mice and *db/db* mice (Jackson Lab) were used in the study. *Aggf1*^{+/-} KO mice with exons 2-11 deleted were described previously (20). Because homozygous *Aggf1*^{-/-} KO mice die before E8.5, heterozygous *Aggf1*^{+/-} KO mice were studied. A high fat diet (HFD)-induced mouse model for type 2 diabetes mellitus (T2DM) was previously described (25-27). Male C57BL/6J mice fed with HFD or *db/db* mice were used to create a hind-limb ischemia model associated with T2DM by ligation of the femoral artery with 6/0 Ethilon sutures as described previously by us and others (28-30).

Isolation and Culture of Bone Marrow-Derived EPCs

EPCs were isolated from the bone marrow of WT, HFD-induced diabetic mice, and db/db mice, and cultured as described (11; 31). Mice were anesthetized with chloral hydrate (3%, m/v) via intraperitoneal injection. Bone marrow mononuclear cells (MNCs) were washed out from the femurs and tibias of mice with phosphate buffer saline (PBS), and then purified by density gradient centrifugation (2000 r/min) with histopaque-1083 (Sigma-Aldrich, St. Louis, MO) for 30 min at room temperature with a horizontal rotor centrifuge (Anke, LCJ-2B, Shanghai, China). The volume ratio of single cell suspension and histopaque-1083 separation liquid was 2:1. The cells were then washed twice using PBS, resuspended gently, plated in 0.1% (m/v) gelatin-coated cell culture dishes, and maintained in endothelial growth factor-supplemented media (EGM-2 bullet kit; Lonza, Switzerland) with 10% fetal bovine serum (FBS). Cells were cultured at 37°C with 5% CO₂ in a humidified water jacket incubator (Thermo Fisher Scientific, MA, USA). The EGM-2 medium was replaced every 3 days after first plating, and cellular morphology was monitored every day.

Characterization of Bone Marrow-Derived MNCs

Characterization of MNCs was performed as described (11; 31). Seven days after maintenance in endothelial-specific media and the removal of non-adherent MNCs, the remaining cells were subjected to immunostaining analysis. Cells were plated on gelatin-coated slide (ibidi) for one day, and then incubated with 10 µg/mL of acetylated DiI lipoprotein from human plasma (DiI-Ac-LDL, Thermo Fisher Scientific, MA, USA) at 37°C for 4 hours. The cells were washed 3 times with PBS, and incubated with 10 µg/mL of fluorescein isothiocyanate-labeled ulex europaeus lectin-1 (Sigma-Aldrich, St. Louis, MO, USA) for 1 hour at 37°C. The cells were rinsed 3 times with PBS again, and visualized under a confocal microscopy.

MNCs were also characterized by flow cytometry analysis. Cells were incubated with 5% BSA (Sigma-Aldrich, St. Louis, MO) for 15 minutes for to block non-specific binding, and then stained with anti-mouse CD34-phycoerythrin (PE), CD31-PE, CD45-PE or CD144-PE (BD Biosciences, San Jose, CA, USA) at room temperature for 1 hour, respectively. The same fluorescein-labeled isotype IgG served as negative control. Cells were analyzed using a Beckman CytoFLEX, and data were analyzed using FlowJo software version 10 (TreeStar, Ashland, OR, USA).

Immunostaining for CD31

In brief, muscle samples were fixed overnight in paraformaldehyde (4%), embedded in paraffin and sectioned. Sections were deparaffinized, dehydrated and rehydrated after being sectioned. After microwave antigen retrieval, endogenous peroxidase blocking and normal goat serum blocking in equilibration buffer, sections were subject to immunohistochemical analysis using a rabbit polyclonal antibody against CD31 (1:200, Proteintech). DAB (3, 30-diaminobenzidine) was used as a chromogenic substrate, and the sections were counterstained with hematoxylin. All ready sections were sealed with clear nail polish or neutral balsam (mounting medium). Images were photographed with an inverted Nikon Eclipse Ti microscope (Nikon USA, Melville, NY, USA) and further analyzed using the Image-Pro Plus version 6.0 software (Media Cybernetics Inc., Bethesda, MD, USA).

Cell Therapy with Implantation of EPCs Primed with and without AGGF1

Recombinant AGGF1 protein was purified as described previously (21; 33). EPCs isolated from mice were treated with purified AGGF1 protein (0.5 µg/ml) or negative control elution buffer for purification at 37°C for 12h. Two days after the diabetic hind-limb ischemia surgery, AGGF1-primed EPCs (~1×10⁶) were injected into mice via the tail vein. Blood flow in both legs was measured in mice anesthetized with chloral hydrate (3%, m/v) using a Vevo 2100 High-Resolution Microultrasound System (Visualsonics Inc) immediately before the ischemic surgery and at time-points of 7, 14 and 28 days after ischemia. We measured the peak systolic velocity (Vs), the minimal end diastolic flow velocity (Vd), and the temporal average velocity per cardiac cycle (Va) in the femoral artery. The blood flow ratio was computed using the formula of (Vs-Vd)/Va using four continuous cardiac cycles (22; 34).

Production of Lentiviruses and Infection

Human embryonic kidney 293 cells (HEK293) were transfected with a lentiviral vector with EGFP, the packaging plasmid psPAX2 and the envelope plasmid pMD2.G using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Viral supernatants were produced from the transfected HEK293 as described (11). HEK293 cells were maintained at 37°C in the high-glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% (V/V) FBS (Gibico Life Technologies, New York, USA) in a humidified water jacket

incubator with 5% CO₂. The supernatant was harvested 72 hours after transfection, filtered through 0.45-µm polyvinylidene fluoride filters, and stored at -80°C until use.

EPCs were infected with the GFP-lentiviruses overnight at a multiplicity of infection of 50 with 2.5 µg/mL polybrene supplemented, and the culture medium was replaced with the fresh growth medium 24 hours after infection. After transfection for 72 hours, the infection efficiency was determined by GFP expression under a Nikon EclipseTi microscopy (Nikon, Japan).

AGGF1 protein

The open reading frame of AGGF1 cDNA was cloned into pET-28b (Novagen) and then transformed into Escherichia coli BL21 (DE3) Star. 6xHis-AGGF1 protein was overexpressed, purified and quality of purification was examined by SDS-PAGE with coomassie blue staining and Western blot analysis (35).

siRNAs and inhibitors of PI3K

siRNAs for Nrf2 and AKT negative control siRNAs were chemically synthesized by Ribobio. The control siRNA is the silencer scrambled negative control siRNA (siNC) having no significant homology to any known gene sequences from the mouse genome. The sequences of siRNAs are as follows: Nrf2 sense: 5'-CGAGAAGUGUUUGACUUUATT-3' (SEQ ID NO:19), Nrf2 antisense: 5'-UAAAGUCAAAACA-CUUCUCGTT-3' (SEQ ID NO:20); AKT sense: 5'-UGCCCUUCUACAACCAGGATT-3' (SEQ ID NO:21), AKT antisense: 5'-UCCUGGUUGUAGAAGGGCATT-3' (SEQ ID NO:22); siNC sense: 5'-UUCUC-CGAACGUGUCACGUTT-3' (SEQ ID NO:23), and siNC antisense: 5'-ACGUGACAC-GUUCGGAGAATT-3' (SEQ ID NO:24). EPCs were transfected with siRNA in the Opti-MEM medium overnight using transfection reagent (Santa Cruz Biotechnology, Dallas, TX) as described (36-38). The knockdown efficiency was determined by real time RT-PCR analysis described below. Wortmannin, inhibitors of PI3K was purchased from Selleck Chemicals (Houston, TX).

Tube Formation Assays

Fourteen days after maintenance in endothelial-specific media, adherent cells were subjected to tube formation assays. The matrigel basement membrane matrix (BD Biosciences, San Jose, CA, USA) was thawed and pre-coated into 48-well plate wells with pre-chilled tips (100 µl/well). The plates were centrifuged at room temperature at 1500 rpm

for 30 minutes to make a flat surface, and incubated at 37°C for another hour to make the matrix gel solidified. Purified AGGF1 protein (0.5 µg/mL) or D-glucose (30 mM, Sigma-Aldrich, St. Louis, MO, USA, control, L-glucose) was added into wells, and incubated at 37°C for 12 hours. Images were visualized and captured at 40 × magnification under an inverted Nikon Eclipse Ti microscope (Nikon, Japan). For quantification, the number of enclosed circles formed by the endothelial tube-like structures was counted in each field.

Cell Proliferation Assays

Cells were plated into 96-well plates for 48 hours and analyzed for proliferation using a CCK-8 kit according to the manufacturer's instruction (Dojindo Laboratories, Japan). EPCs were seeded into 96-well cell culture plates, and treated with AGGF1 (0.5 µg/mL) or a solution with high D-glucose (30 mM, Sigma, St. Louis, MO, control, L-glucose). Then, 10 µl/well of CCK-8 solution was added, and the plates were incubated for 1-4 hours in dark. Cell proliferation was determined by the measurement of absorbance at 450 nm using a Molecular Devices VERSA max microplate reader as described (39; 40).

Transendothelial Migration Assays

We evaluated transendothelial migration (TEM) capabilities of EPCs with a transwell assay as described (11). Human umbilical vein endothelial cells (HUVECs) (about 1×10^4 cells per well) were cultured in the upper chamber of a 24-transwell insert (8.0 µm pores; BD Falcon, San Jose, CA, USA) to confluency. Monolayer confluency was confirmed under an inverted fluorescence microscopy before each experiment. EPCs were treated with AGGF1 vs. elution buffer, a high glucose solution vs. control, and siRNAs, and cultured for 24 hours. The EPCs were harvested and resuspended in the basal culture medium (EBM-2, 0.5% BSA). EPC suspension (~0.2 mL) was added to the upper chamber of Transwell, and 0.5 ml of EBM-2 medium was added to the lower chamber. Cells were cultured for 24 hours at 37°C, and EPCs that migrated from the upper chamber to the lower chamber of Transwell were visualized. Images were captured at 40× magnification under an inverted Nikon Eclipse Ti microscope (Nikon, Japan). The number of cells was counted and analyzed.

Scratch-Wound Cell Migration Assays

EPCs in cell culture dishes were trypsinized and resuspended in growth media and allowed to grow to a confluent monolayer in 6-well plates. EPCs were starved for 6 hours by replacing the regular growth media with serum-free growth media. EPC monolayers were

then wounded with a 200 µl pipette tip gently through the cell sheet to remove some cells and make a wound. The growth medium was aspirated out and wells washed twice with PBS to remove cell debris. Media supplemented with AGGF1 (0.5 µg/ml) were added to the wells in a total volume of 1 ml of serum-free growth media. Cells were cultured for 24 hours by incubating the plate at 37°C and 5% CO₂. Images were captured at 40X magnification under an inverted Nikon Eclipse Ti microscope (Nikon, Japan). The movement of cells into the scraped area was measured.

Western Blot Analysis

Protein extracts were prepared from cultured EPCs with Western-IP lysis buffer (Beyotime, Beijing, China) supplemented with a proteinase inhibitor cocktail (Roche, Basel, Switzerland), separated by 12% SDS-PAGE, and transferred to polyvinylidene-fluoride membranes. The membranes were blocked with skim milk and incubated with appropriate primary antibodies overnight at 4°C with gentle shaking. The membranes were then incubated with appropriate secondary antibodies for 2 hours at room temperature after standard TBST washing procedures. The primary antibodies against Nrf2 (1:1000 dilution), heme oxygenase-1 (HQ-1, 1:2000), NAD(P)H dehydrogenase quinone 1 (NQO-1, 1:1000), catalase (1:1000), and GAPDH (1:5000) were purchased from ProteinTech (Wuhan, China). Antibodies for total protein kinase B or AKT (tAkt) and phosphorylated AKT (p-Akt, Ser473; 1:1000) were purchased from Cell Signaling Technology (Danvers, MA, USA). Images from Western blot analysis were developed using a ChemiDoc XRS (Bio-Rad Laboratories, Richmond, CA, USA) with the SuperSignal West Pico Chemiluminescent Substrate (Pierce Chemical Co., Rockford, IL, USA), and further analyzed with a Gel-Pro analyzer. GAPDH was used as the loading control.

Quantitative Real-Time RT-PCR Analysis

Total RNA samples were isolated from EPCs using Trizol reagent (TaKaRa Biotech, Japan), and reversed transcribed into cDNA with M-MLV reverse transcriptase (Promega, Madison, WI) according to the manufacturer's instruction. DNase I (Thermo Fisher Scientific, MA, USA) was used to remove contaminating genomic DNA before reverse transcription. Quantitative real-time RT-PCR analysis was performed in triplicate with a 10 µl reaction system using the FastStart Universal SYBR Green Master (Roche, Basel, Switzerland) and a 7900 HT Fast Real-Time PCR System (ABI, US). The PCR profile was 94°C for 5 min, and 40 cycles of 94°C for 10 s and 60°C for 15 s. Fold differences between

samples were assessed with a comparative cycle time (Ct) method. GAPDH served as an internal standard. The data were analyzed using $2^{-\Delta\Delta C_t}$ relative expression quantity as reported (41).

5 Statistical Analysis

Data are presented as mean $\pm$ standard deviation (SD). The comparison of means of two groups was made by a Student's t test or a nonparametric Wilcoxon rank test when the sample size was small and/or the distribution was not normal. To compare the means of more than 2 groups, one-way ANOVA or the generalized linear regression approach was employed for data with normal distribution and the Kruskal–Wallis test for non-normal distribution data or small samples. $P < 0.05$ was considered to be statistically significant.

RESULTS

15 Aggf1 Haploinsufficiency (Heterozygous Aggf1 $\pm$ KO) Significantly Reduces the Number of Bone Marrow–Derived EPCs

We isolated and characterized bone marrow–derived mononuclear cells (MNCs) from wild type (WT) and heterozygous Aggf1 $\pm$ KO mice. The isolated MNCs appeared to be centrally rounded EPCs with a peripheral spindle shape four days after culture (Fig. 25A). The cells appeared to be spindle-shaped adherent EPCs and some showed cobblestone-like morphology at day 8 (Fig. 25A). The isolated EPCs were confirmed by a DiI-Ac-LDL endocytosis assay (red fluorescence) and a UEA-1 binding assay (green fluorescence). The isolated EPCs were able to endocytose Ac-LDL and bind UEA-1 (Fig. 25B), suggesting that they are endothelial lineage cells with characteristics of EPCs as previously described (32; 42; 43). Previous studies indicated that early EPCs (<14 days) were positive for CD34, CD31, CD14, CD45, Scal-1, c-Kit, and VEGFR2, but mostly negative for CD144 (11). Our flow cytometry analysis showed that the isolated EPCs were positive for CD31, CD34, and CD45, and negative for CD144 at day 10 of culture (Fig. 25C). We compared the number of DiI-Ac-LDL/FITC-UEA-1 positive cells from heterozygous Aggf1 $\pm$ KO mice with the cells from WT mice. The number of DiI-Ac-LDL and FITC-UEA-1 positive cells isolated from heterozygous Aggf1 $\pm$ KO mice was moderately but significantly reduced compared with WT mice ($P < 0.05$; $n = 6$ /group) (Fig. 26). These data suggest that Aggf1 may be involved in differentiation of EPCs.

Aggf1 Is Required for Angiogenic and Other Functions of EPCs

Western blot analysis showed that AGGF1 expression was significantly increased in EPCs isolated from db/db mice and HFD-induced T2DM mice (Fig. 27). Interestingly, we also found that Aggf1 is required for angiogenic and other functions of EPCs. We studied db/db mice and a mouse model for type 2 DM (T2DM) by treating C57BL/6J mice with HFD. HFD treatment successfully induced a significant increase of blood glucose levels in mice (Fig. 28). The EPCs isolated from db/db mice showed a significantly decreased angiogenic function in a matrigel-based capillary tube formation assay (Fig. 19A-B), decreased proliferation in a CCK8 assay (Fig. 19C), decreased transendothelial migration required for homing of EPCs to sites of endothelial disruption for repair and neovascularization in a transwell assay (Fig. 19D-E), and reduced migration in a scratch-wound assay (Fig. 19F-G) compared with EPCs isolated from wild type mice (WT). Interestingly, Aggf1^{+/-} showed similar effects as db/db mice (Fig. 19A-G). Aggf1^{+/-} db/db double KO mice showed exacerbated effects compared with Aggf1^{+/-} KO mice (Fig. 19A-G). We analyzed blood glucose levels between WT mice and Aggf1^{+/-} KO mice at baseline, but no significant difference was detected (WT: 6.54±0.78 mM vs. Aggf1^{+/-} KO: 7.14±0.92 mM). We analyzed the expression levels of VEGF, VEGFR2 and CXCR4 mRNA in EPCs isolated from different groups of mice. Our real-time RT-PCR analysis showed that Aggf1 haploinsufficiency did not affect the expression of VEGF, VEGFR2 and CXCR4 in EPCs (Fig. 29), suggesting that the effect of Aggf1 haploinsufficiency is independent of VEGF, VEGFR2 and CXCR4. In addition, similar results were obtained in the HFD-induced mouse model for T2DM (Fig. 30). These data suggest that Aggf1 is required for important functions of EPCs.

AGGF1 Protein Treatment Reverses Hyperglycemia-Impaired Angiogenic and Other Functions of EPCs

We found that the AGGF1 protein treatment reversed the impaired angiogenesis, proliferation, migration and transendothelial migration of EPCs induced by high glucose (HG). When cultured in HG media (30 mM D-glucose mimicking the pathological environment of diabetic dyslipidemia or hyperglycemia; 30 mM L-glucose as negative control), mouse EPCs showed significantly decreased angiogenic function (Fig. 20B, C), proliferation (Fig. 20D), transendothelial migration (Fig. 20E, F), and migration (Fig. 20G, H) compared with the EPCs cultured in control regular media. AGGF1 is an angiogenic factor that can be secreted outside of cells (21), therefore, we treated EPCs with the purified human AGGF1 protein in culture to determine the effects of AGGF1 on functions of EPCs

(Fig. 20A). All the defects by high glucose on EPC functions were blocked by AGGF1 treatment (Fig. 20B-H). We analyzed the expression levels of VEGF, VEGFR2 and CXCR4 mRNA in EPCs treated with or without AGGF1 in combination with or without high glucose. AGGF1 did not affect the expression levels of VEGF, VEGFR2 and CXCR4 in EPCs (Fig. 31). These data suggest that AGGF1 can counter the damaging effects of hyperglycemia on EPCs, however, its effects appear independent of CXCR4, VEGF or VEGFR2.

AGGF1 Boosts EPC-Mediated Angiogenesis and Blood Perfusion in T2MD

In a T2DM mouse model (db/db mice), AGGF1 was found to greatly boost the therapeutic effects of cell therapy with EPC implantation on vascular complications under ischemia in vivo. As AGGF1 can reverse the damaging effects of T2DM on EPCs, we hypothesized that AGGF1-primed EPCs can serve as a successful treatment tool for vascular complications in T2MD in vivo. We pre-treated EPCs with recombinant AGGF1 and transplanted the AGGF1-primed EPCs into db/db mice after a hind-limb ischemia surgery. Notably, AGGF1-primed EPCs were significantly more effective than elution-buffer-pretreated EPCs in increasing blood flow in diabetic mice with ischemia (Fig. 21A). The rate of tissue necrosis was significantly decreased by AGGF1-primed EPCs compared with elution-buffer-pretreated control EPCs (Fig. 21B). Similar significant inhibitory effect on ambulatory impairment was detected for AGGF1-primed EPCs (Fig. 21C). Immunostaining showed that the density of CD31-positive vessels was significantly higher for AGGF1-primed EPCs than for elution-buffer-pretreated control EPCs at day 28 after transplantation (Fig. 21D and Fig. 32A). Similar therapeutic efficacy for AGGF1-primed EPCs was obtained in HFD-induced hyperglycemia mice (Fig. 31A-E). Together, these data demonstrate that AGGF1-primed EPCs improve blood perfusion and angiogenesis in T2MD mice with hind-limb ischemia, indicating that AGGF1 can boost the therapeutic effects of cell therapy with EPCs.

The therapeutic effect of AGGF1-primed EPCs may be related to the possibility that AGGF1 increases the homing of transplanted EPCs to the ischemic areas. To test the hypothesis, we infected EPCs with EGFP lentivirus (Fig. 34) and performed EPC transplantation into db/db mice. Four weeks after transplantation, the gastrocnemius muscle of DM mice with hind-limb ischemia was characterized by immunostaining with an anti-GFP antibody. As shown in Fig. 32B, EPCs labeled by overexpression of EGFP and primed by AGGF1 induced significantly more homing or mobilization of GFP-labeled EPCs in the

vascular injury site in the muscle than elution-buffer-pretreated control EPCs. Similar results were obtained in HFD-induced hyperglycemia mice (Fig. 33F).

AGGF1 Attenuates Hyperglycemia-Induced Oxidative Stress in EPCs

5 To identify the molecular mechanism by which AGGF1 boosts the therapeutic effects of EPCs, we analyzed the effect of AGGF1 on oxidative stress induced by hyperglycemia in EPCs. Oxidative stress is a key factor accounting for endothelial dysfunction in diabetic EPCs (44-47). Therefore, we analyzed the effect of AGGF1 on high glucose-induced oxidative stress in EPCs. The level of reactive oxygen species (ROS) in EPCs after treatment
10 with a high glucose solution was significantly increased, however, the effect was attenuated by treatment with AGGF1 (Fig. 35). However, AGGF1 treatment did not fully reverse the high glucose-induced ROS increase ($P < 0.05$ between the control group and HG+AGGF1 group) (Fig. 35). The data suggest that AGGF1 can attenuate the production of ROS in EPCs, which may be a factor for its effects on boosting the therapeutic effects of EPCs on
15 endothelial dysfunction in DM.

AGGF1 Activates Nuclear Localization of Nrf2 via the AKT/ Fyn/Nrf2 Signaling Pathway in EPCs

To identify the molecular mechanism by which AGGF1 inhibits ROS generation, we
20 characterized the effect of AGGF1 on anti-oxidative transcription factor Nrf2 (NF-E2 p45-related factor 2) (43-46). We found that AGGF1 promoted nuclear accumulation of Nrf2, thereby activating the Nrf2 pathway in EPCs. When cultured in high glucose media, the nuclear accumulation of Nrf2 was significantly reduced, however, the effect was abolished by treatment with AGGF1 (Fig. 22). Nrf2 activates the transcription from many downstream
25 genes, including HQ1, NQO-1, and CAT (11; 49). Western blot analysis showed that AGGF1 increased the expression levels of HQ1, NQO-1, and CAT (Fig. 22A). Real-time RT-PCR analysis showed that AGGF1 increased the expression levels of HQ1, NQO-1, and CAT mRNA (Fig. 22B). Furthermore, immunostaining with an anti-Nrf2 antibody confirmed the finding of increased nuclear immunofluorescence/localization for Nrf2 by AGGF1 (Fig.
30 36). Interestingly, real-time RT-PCR analysis showed that AGGF1 did not affect the expression level of Nrf2 mRNA (Fig. 37A). Western blot analysis also showed that AGGF1 did not affect the expression level of Nrf2 and KEAP-1 (binding to Nrf2 to facilitate its ubiquitination) (50) (Fig. 37B-C). These data demonstrate that AGGF1 increases the nuclear

accumulation of Nrf2, but not the expression levels of Nrf2 and KEAP-1, and results in transcriptional activation of Nrf2 downstream target genes NQO-1 and CAT.

It was previously shown that AGGF1 can activate PI3K and AKT signaling in endothelial cells and in zebrafish (19; 20). PI3K and AKT were shown to regulate phosphorylation and nuclear localization of Fyn (19; 20). Fyn was shown to phosphorylate Nrf2 at Y568, resulting in nuclear export and degradation of Nrf2 (19; 20). Therefore, we hypothesized that AGGF1 regulates ROS generation and EPC functions via an AGGF1-AKT-Fyn-Nrf2 signaling pathway. When cultured in high glucose media, EPCs showed a significantly reduced level of phosphorylation of AKT, however, the effect was reversed by AGGF1 treatment (Fig. 23A). On the other hand, the effect of AGGF1 was blocked by siRNA for AKT or wortmannin, a specific inhibitor of PI3K upstream of AKT (Fig. 23). EPCs treated with high glucose showed a significantly increased level of nuclear Fyn (n-Fyn), however, the effect was reversed by AGGF1 treatment (Fig. 23A-B). The effect of AGGF1 was blocked by siRNA for AKT or wortmannin (Fig. 23A-B). Similarly, siRNA for AKT, wortmannin or siRNA for Nrf2 blocked the rescue effects of AGGF1 on the HG-induced decrease of Nrf2 nuclear accumulation and down-regulation of Nrf2 downstream genes NQO-1, and CAT (Fig. 23C-E). Furthermore, the nuclear accumulation of nNrf2 was decreased by siRNA for AGGF1 under either a normal or HG condition, however, Fyn knock down with siRNA blocked the effect of AGGF1 (Fig. 38). These data suggest that AGGF1 promotes Nrf2 nuclear localization and activates Nrf2 downstream target genes by regulating the AKT-Fyn signaling pathway.

To further demonstrate that AGGF1 regulates the function of EPCs by Nrf2, we studied the effects of wortmannin and siRNAs for AKT and Nrf2 on EPCs treated with AGGF1 (Fig. 24A, F, K). Treatment of EPCs with AGGF1 reversed the impaired angiogenesis (Fig. 24C, H, M), proliferation (Fig. 24B, G, L), transendothelial migration (Fig. 24D, I, N), and migration by hyperglycemia (Fig. 24E, J, O). However, these effects of AGGF1 were attenuated by wortmannin, knockdown of AKT, and knockdown of Nrf2 (Fig. 24). These data further indicate that AGGF1 regulates the functions of EPCs by affecting Nrf2.

DISCUSSION

While the results presented above, and discussed below, discuss possible mechanisms, the present invention is not limited to any particular mechanism and an understanding of the mechanism is not necessary to practice the invention. We show that AGGF1 activates the

AKT-Fyn-Nrf2 signaling pathway in EPCs (Fig. 39). Nrf2 is a transcription factor important to cellular defense against oxidative stress. Nrf2 needs to be translocated into the nucleus to execute its function, and its nuclear localization is regulated by AKT-Fyn signaling. AGGF1 activates AKT, which leads to de-phosphorylation of Fyn, resulting in reduced translocation of Fyn into the nucleus and decreased nuclear Fyn (n-Fyn). Decreased n-Fyn leads to decreased phosphorylation of Nrf2 and inhibits the nuclear export, ubiquitination and degradation of Nrf2, increasing the expression levels of nuclear Nrf2 and downstream cytoprotective genes such as NQO-1 and CAT (Fig. 22). This blocks hyperglycemia-induced ROS generation in EPCs and potentiates the functions of EPCs. Oxidative stress was considered to be a critical factor accounting for EPC dysfunction in T2DM (44; 45). Our results suggest that AGGF1 protects EPCs partly by inhibiting hyperglycemia-induced ROS generation through a novel anti-oxidative stress signaling pathway (Fig. 39).

Endothelial dysfunction is a major problem in T2DM, and lack of endothelial regeneration and impaired angiogenesis by EPCs are responsible for the vascular abnormalities in DM (10; 11). Hence, there is an unmet need for therapeutic interventions to accelerate the repair of dysfunctional endothelium and restore blood flow by EPCs in treatment of DM patients. The data in this Example establish AGGF1 as an important molecule to repair diabetic EPC dysfunction induced by hyperglycemia or dyslipidemia in db/db mice and HFD-induced hyperglycemia mice (Fig. 21, Fig.32 and Fig. 33). Robust therapeutic effects were observed for AGGF1-primed EPCs in boosting angiogenesis, restoring blood flow, reducing tissue necrosis and ambulatory impairment in vivo in a diabetic hind-limb ischemia model in db/db mice and HFD-induced hyperglycemia mice (Fig. 21, Fig. 32 and Fig. 33). Although some hypoglycemic agents (e.g. metformin), lipid lowering drugs (e.g. statins) and renin-angiotensin system inhibitors were shown to increase circulating EPC levels, however, the increases were moderate as compared to the levels in healthy study subjects (53; 54). Moreover, EPC functions in DM patients are severely impaired, therefore, the therapeutic effects may be limited even with the increased level of autologous EPCs by these drugs. We showed that AGGF1 fully reversed hyperglycemia-impaired functions of EPCs (Fig. 20), therefore, one potential advantage of the AGGF1-based EPC therapy is that dysfunctional EPCs from DM patients may be repaired and/or functions enhanced, which may achieve a maximum efficacy for the treatment for DM-associated vascular abnormalities.

An AGGF1-based EPC therapy for diabetic ischemia may have additional benefits for DM patients who are often affected with CAD/MI, heart failure, peripheral vascular disease,

and other conditions. Furthermore, DM can also cause many other diseases including diabetic cardiomyopathy, end-stage renal disease (ESRD) and vascular aging, which are all associated with abnormal angiogenesis and vascular dysfunction. Based on our data, it is likely that AGGF1-primed EPC therapy may serve as a new strategy not only for treating DM-associated vascular abnormalities, and those with vascular abnormalities without DM, but also for many other diabetes mellitus complications such as diabetic cardiomyopathy, ESRD and vascular aging. This Example showed that in db/db mice or HFD-induced diabetic mice with dysfunctional EPCs, AGGF1-primed EPCs attenuated vascular complications by increasing blood flow and CD31-positive capillary density. Because AGGF1-primed EPCs showed increased capillary formation capability, they may be able to increase blood flow and capillary density in lean and non-diabetic mice with functional EPCs, too.

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EXAMPLE 4**In Vivo Treatment with AGGF1 Peptides**1. AGGF1 protein therapy reduces plasma glucose levels in db/db diabetic mice.

AGGF1 protein therapy is effective in lowering fasting glucose levels in HFD-induced diabetic mice. To further conform this, we examined the efficacy of AGGF1 protein therapy in another independent diabetic mouse model. The *db/db* mice are a well-established murine model for diabetes. We administered recombinant His-tagged AGGF1 protein (3 nmole or 0.25 ug/g body weight) vs. IgG negative control (3 nmole/g body weight) to 4 db/db mice via subcutaneous injection over a time period of 48 hours. The overall study design is shown in Figure 40A. As shown in Figure 40B, compared to IgG control, AGGF1 significantly lowers the plasma glucose levels for more than 24 hours. The effect was lost at the time point of 30 hours. These data confirm that subcutaneous injection of recombinant AGGF1 protein is an effective therapy for lowering plasma glucose levels in diabetic mice.

2. The therapeutic effects of AGGF1 is more than 10 times longer than insulin treatment.

We performed a similar treatment study for insulin in *db/db* mice. As shown in Figure 40C, subcutaneous injection of insulin (0.75 IU/kg body weight) also lowers plasma glucose levels in *db/db* diabetic mice. The maximum effect of insulin was found to be achieved at 1.0-1.5 hours after injection, however, the effect was lost 2.5 hours after injection. Comparing Figure 40B and 40C, it is clear that the therapeutic effect of AGGF1 is more than 10 times longer than insulin in *db/db* diabetic mice. It is also important to note that AGGF1 therapy did not cause hypoglycemia, one of the major adverse effect of insulin treatment.

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All publications and patents mentioned in the above specification are herein incorporated by reference in their entirety for all purposes. Various modifications and variations of the described compositions, methods, and uses of the technology will be apparent to those skilled in the art without departing from the scope and spirit of the technology as described. Although the technology has been described in connection with specific exemplary embodiments, it should be understood that the technology as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the technology that are obvious to those skilled in pharmacology, biochemistry, medical science, or related fields are intended to be within the scope of the following claims.

CLAIMS

We Claim:

1. A method of treating a subject comprising: administering a composition to a subject, wherein said subject has at least one of the following: a disease or condition characterized by abnormal glucose metabolism, ischemic vascular disease, pulmonary hypertension, cardiac hypertrophy, and heart failure:
wherein said composition comprises:
 - i) an AGGF1 peptide, and/or
 - ii) an expression vector encoding said AGGF1 peptide.
2. The method of Claim 1, wherein said AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18).
3. The method of Claim 1, wherein said expression vector comprises the nucleic acid sequence in SEQ ID NO:25.
4. The method of Claim 1, wherein said disease or condition is selected from the group consisting of: pulmonary hypertension, peripheral artery disease (PAD), coronary artery disease (CAD), coronary heart disease (CHD), myocardial infarction (MI), Type I diabetes, Type II diabetes, hyperglycemia, inadequate glucose clearance, and hyperinsulinemia.
5. The method of Claim 1, wherein said method further comprises administering insulin to said subject.
6. The method of Claim 1, wherein said AGGF1 peptide comprises full length human AGGF1 shown in SEQ ID NO:1 or wherein said AGGF1 peptide has at least 97% sequence identity with SEQ ID NO:1.
7. The method of Claim 1, wherein said AGGF1 peptide is selected from any of SEQ ID Nos:2-16 or i) a peptide comprising the amino acid sequence from 574 to 614 of SEQ ID NO:1, ii) a peptide comprising the amino acid sequence from 574 to 624 of SEQ ID NO:1, or iii) a peptide comprising the amino acid sequence from 604 to 714 of SEQ ID NO:1.

8. The method of Claim 1, wherein said AGGF1 peptide has at least 97% sequence identity with any one of SEQ ID Nos:2-16.
9. The method of Claim 1, wherein said administering reduces or eliminates at least one symptom of said disease or condition.
10. The method of Claim 1, wherein said composition comprises said AGGF1 peptide.
11. The method of Claim 1, wherein said composition comprises said an expression vector encoding said AGGF1 peptide.
12. The method of Claim 1, wherein said subject is a human.
13. The method of Claim 12, wherein said composition comprises said AGGF1 peptide, and wherein the dosage is selected from the group consisting of: 0.1-15 mg/kg, 0.5-10 mg/kg, and 1-7 mg/kg.
14. The method of Claim 12, wherein said composition comprises said an expression vector encoding said AGGF1 peptide, and wherein said dosage is about 1.0×10^{11} - 5.0×10^{13} .
15. A system or kit comprising:
 - a) a blood glucose monitoring device or article of manufacture; and
 - b) a composition comprising: i) an AGGF1 peptide, or ii) an expression vector encoding said AGGF1 peptide.
16. The system or kit of Claim 15, wherein said composition comprises said AGGF1 peptide.
17. The system of kit of Claim 1, wherein said glucose monitoring device or article of manufacture is selected from the group consisting of: a blood glucose test strip, a reader for reading said glucose test strip, FreeStyle Libre, GlucoTrack, Eversense, GlucoWise, NovioSense, GlucoSense, and a smart contact lens.

18. A system or kit comprising:
- a) a composition comprising: i) an AGGF1 peptide, or ii) an expression vector encoding said AGGF1 peptide; and
 - b) an implantable pump containing said composition, wherein said implantable pump is configured to deliver an amount of said composition into the bloodstream of a subject.
19. The system or kit of Claim 18, wherein said composition comprises said AGGF1 peptide.
20. A composition comprising:
- a) an AGGF1 peptide, or an expression vector encoding said AGGF1 peptide; and
 - b) insulin peptide, or an expression vector encoding said insulin peptide.
21. The composition of Claim 21, wherein said AGGF1 peptide comprises full length human AGGF1 shown in SEQ ID NO:1, or wherein said AGGF1 peptide has at least 97% sequence identity with SEQ ID NO:1.
22. The composition of Claim 21, wherein said AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18).
23. The composition of Claim 21, wherein said AGGF1 peptide is selected from any of SEQ ID Nos:2-16.
24. The composition of Claim 21, wherein said AGGF1 peptide has at least 97% sequence identity with any one of SEQ ID Nos:2-16.
25. A method of treating a subject with a cardiovascular disease or condition comprising: administering a composition comprising: i) an AGGF1 peptide that is no longer than 600 amino acids, ii) an expression vector encoding said AGGF1 peptide, to said subject with said disease or condition, or iv) the AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18).

26. The method of Claim 25, wherein said disease or condition is selected from the group consisting of: coronary artery disease, myocardial infarction, cardiac hypertrophy, heart failure, peripheral artery disease, restenosis and in-stent thrombosis after angioplasty and stenting, and pulmonary arterial hypertension.

27. The method of Claim 25, wherein, wherein said AGGF1 peptide is selected from any of SEQ ID Nos:2-16.

28. The method of Claim 25, wherein said subject is a human and: A) said composition comprises said AGGF1 peptide, and wherein the dosage is selected from the group consisting of: 0.1-15 mg/kg, 0.5-10 mg/kg, and 1-7 mg/kg; or B) said composition comprises said expression vector encoding said AGGF1 peptide, and wherein said dosage is about 1.0×10^{11} - 5.0×10^{13} .

29. The method of Claim 25, wherein said AGGF1 peptide is no longer than 300, or no longer than 150, amino acids in length and said peptide comprises, consists essentially of, or consists of: i) the amino acid sequence from 574 to 614 of SEQ ID NO:1, ii) the amino acid sequence from 574 to 624 of SEQ ID NO:1, iii) the amino acid sequence from 604 to 714 of SEQ ID NO:1, or iv) the AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18).

30. The method of Claim 25, wherein said AGGF1 peptide has at least 97% sequence identity with any one of SEQ ID Nos:2-16.

31. A method of treating a subject with pulmonary arterial hypertension (PAH) comprising: administering a composition comprising: i) an AGGF1 peptide, or ii) an expression vector encoding said AGGF1 peptide, to said subject with said PAH.

32. The method of Claim 31, wherein said AGGF1 peptide is no longer than 300, or no longer than 150, amino acids in length and said peptide comprises, consists essentially of, or consists of: i) the amino acid sequence from 574 to 614 of SEQ ID NO:1, ii) the amino acid sequence from 574 to 624 of SEQ ID NO:1, iii) the amino acid sequence from 604 to 714 of

SEQ ID NO:1; or iv) the AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18).

33. The method of Claim 31, wherein said AGGF1 peptide has at least 97% sequence identity with any one of SEQ ID Nos:2-16.

34. A method of treating a subject comprising: administering a composition to a subject, wherein said subject has a disease or condition characterized by abnormal glucose metabolism and/or ischemic vascular disease, and wherein said composition comprises an AGGF1-primed endothelial progenitor cell.

35. The method of Claim 34, wherein said disease or condition is selected from the group consisting of peripheral artery disease (PAD), coronary artery disease (CAD), coronary heart disease (CHD), and myocardial infarction (MI).

36. The method of Claim 34, wherein said disease or condition is selected from the group consisting of: Type I diabetes, Type II diabetes, hyperglycemia, inadequate glucose clearance, and hyperinsulinemia.

37. The method of Claim 34, wherein said AGGF1 peptide comprises full length human AGGF1 shown in SEQ ID NO:1.

38. The method of Claim 34, wherein said AGGF1 peptide has at least 97% sequence identity with SEQ ID NO:1.

39. The method of Claim 34, wherein said AGGF1 peptide is selected from any of SEQ ID Nos:2-16 or i) a peptide comprising the amino acid sequence from 574 to 614 of SEQ ID NO:1, ii) a peptide comprising the amino acid sequence from 574 to 624 of SEQ ID NO:1, iii) a peptide comprising the amino acid sequence from 604 to 714 of SEQ ID NO:1, or iv) the AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18).

40. The method of Claim 34, wherein said AGGF1 peptide has at least 97% sequence identity with any one of SEQ ID Nos:2-16.
41. The method of Claim 34, wherein said administering reduces or eliminates at least one symptom of said disease or condition.
42. The method of Claim 34, wherein said composition comprises a said AGGF1 peptide.
43. The method of Claim 34, wherein said composition comprises said an expression vector encoding said AGGF1 peptide.
44. The method of Claim 34, wherein said subject is a human.
45. The method of Claim 34, wherein said AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from said subject, wherein said EPC has been exposed to AGGF1 peptides ex vivo.
46. The method of Claim 34, wherein said AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from a relative of said subject, wherein said EPC has been exposed to AGGF1 peptides ex vivo.
47. The method of Claim 34, wherein said AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from a non-relative of said subject, wherein said EPC has been exposed to AGGF1 peptides ex vivo.
48. A composition comprising: an AGGF1-primed endothelial progenitor cell.
49. The composition of Claim 48, wherein said AGGF1-primed endothelial progenitor cell comprises an endothelial progenitor cell (EPC) from a subject with a disease or condition characterized by abnormal glucose metabolism and/or ischemic vascular disease, wherein said EPC has been exposed to AGGF1 peptides ex vivo.
50. The composition of Claim 48, further comprising a physiological tolerable liquid.

51. A kit or system comprising:
- a) an endothelial progenitor cell (EPC), and
 - b) a composition comprising:
 - i) an AGGF1 peptide, and/or
 - ii) an expression vector encoding said AGGF1 peptide.
52. The kit or system of Claim 51, wherein said AGGF1 peptide consists of, consists essentially of, or comprises QRDDAPASVH (SEQ ID NO:18).
53. The kit or system of Claim 51, wherein said expression vector comprises the nucleic acid sequence in SEQ ID NO:25.

FIG. 1

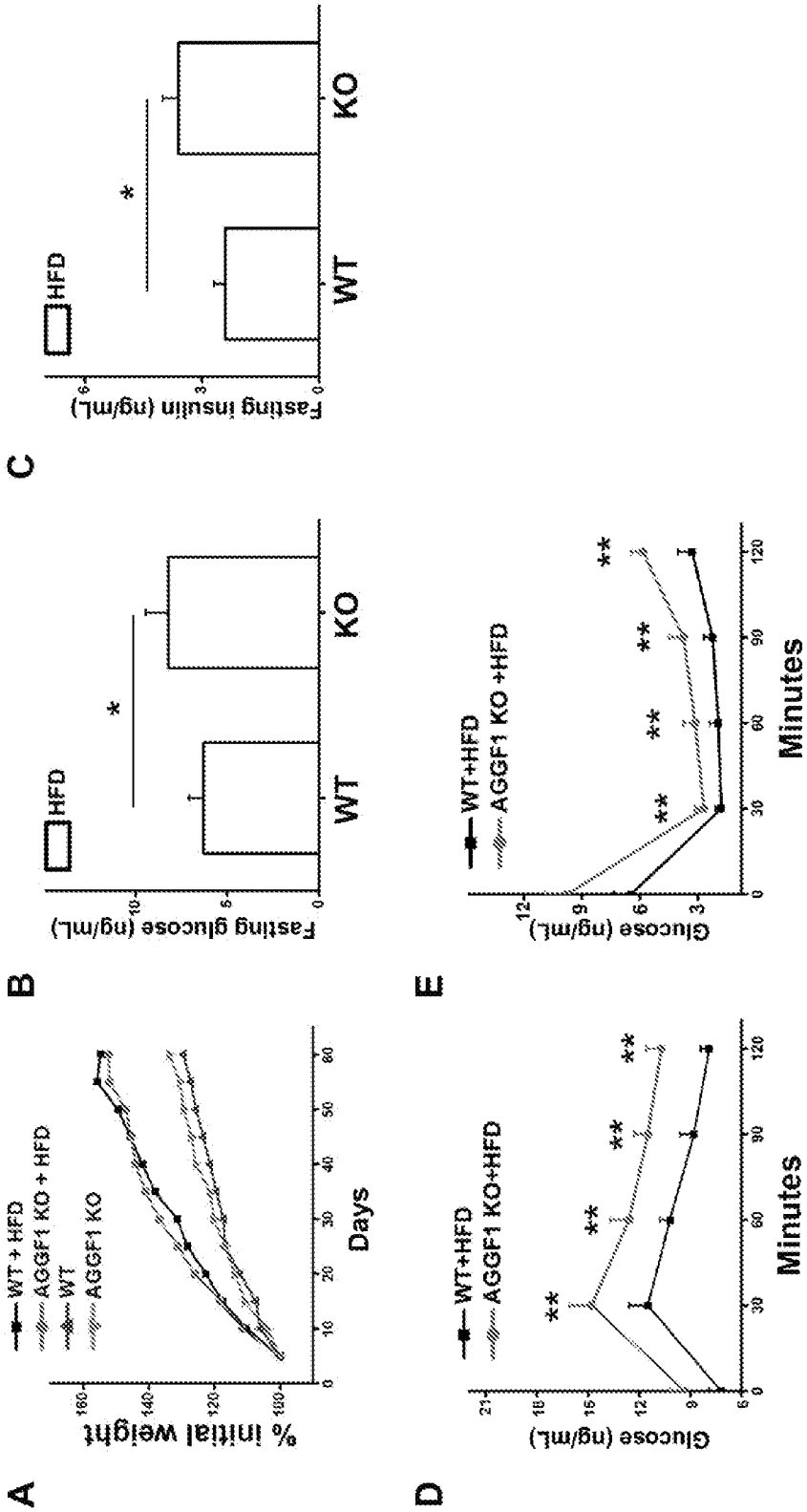


FIG. 2

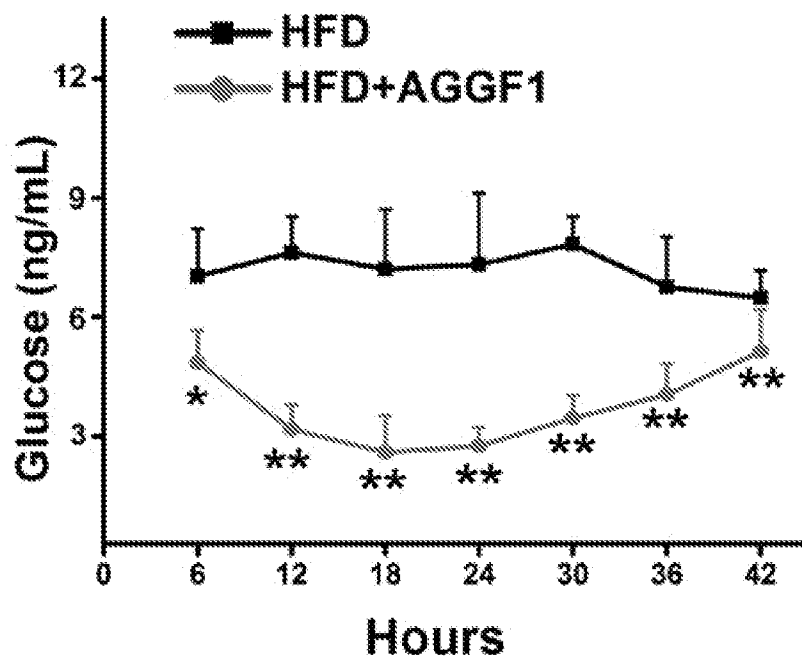


FIG. 3

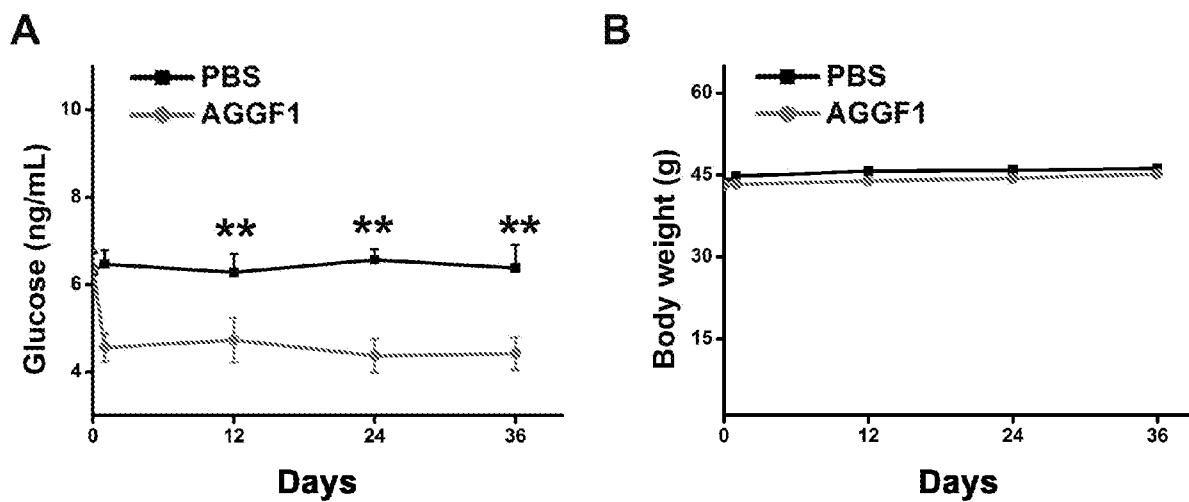


FIG. 4

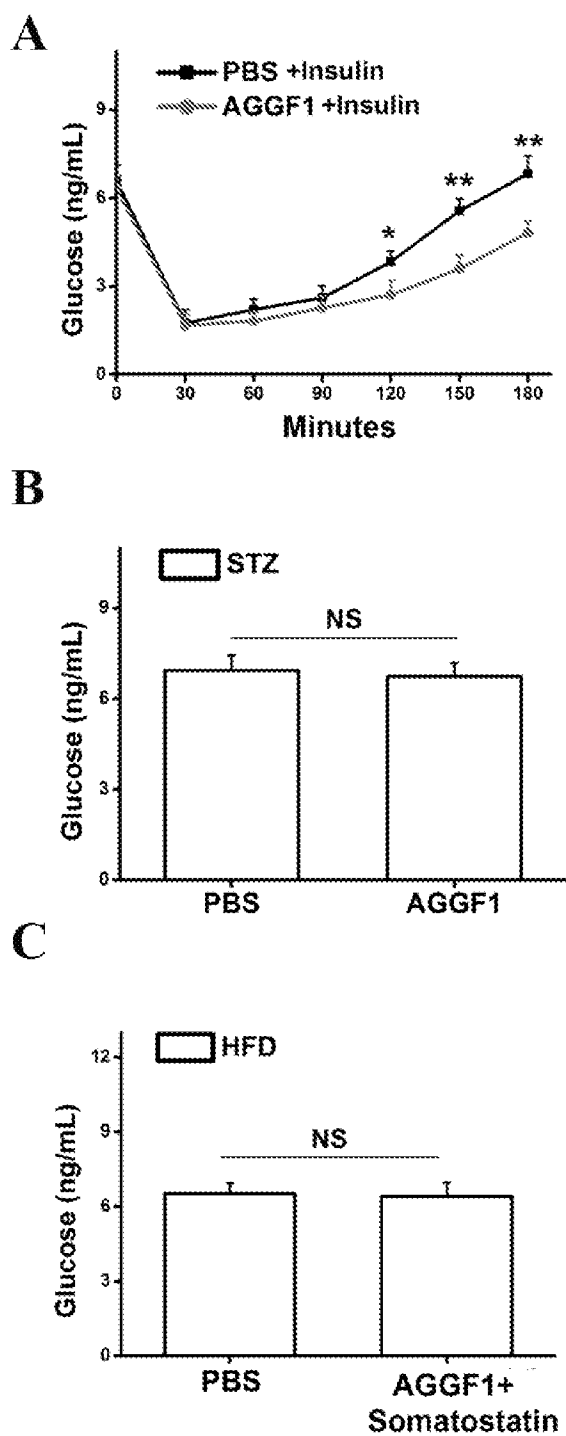


FIG. 5

A. Full-length AGGF1 (714 amino acid residues) (SEQ ID NO:1)

MASEAPSPPRSPPPPPTSPEPELAQLRRKVEKLERELRSCRKQVREIEKLLHHTERLYQNAESNNQELRTQ
VEELSKILQGRNEDNKKSDVEVQ TENHAPWSISDYFYQTYNDVSLPNKVTELS DQQDQAIETSILNSK
DHLQVENDAYPGTDRTE NVKYRQVDHFASNSQEPASALATEDTSLEGSSLAESLRAAAEA AVSQTGFSYD
ENTGLYFDHSTGFFYDSENQLYYDPSTGIYYCDVESGRYQFHSRVLDLPYPTSSTKQSKDKKLKKKRKD
PDSSATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVP TSGNTIESPLHENI
SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
QIGSLFIITAVNPATIGREKDM EHTLRIP EVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
KTKCDPYVLEHGDEVKIGETVLSFHIHPGSDTC DGCEPGQVRAHLRLDKKDES FVGPTLSKEEKELERRK
ELKKIRVKYGLQNT EYEDEKTLKNPKYKDRAGKRREQVGSEGT FQRDDAPASVHSEITDSNKGRKMLEKM
GWKKGEGLGKDGGMKTP IQLQLRRTHAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
DDPGTMPWVKGTLE

B. AGGF1-N1 (696 amino acids; 19 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:2)

MPELAQLRRKVEKLERELRSCRKQVREIEKLLHHTERLYQNAESNNQELRTQ
VEELSKILQGRNEDNKKSDVEVQ TENHAPWSISDYFYQTYNDVSLPNKVTELS DQQDQAIETSILNSK
DHLQVENDAYPGTDRTE NVKYRQVDHFASNSQEPASALATEDTSLEGSSLAESLRAAAEA AVSQTGFSYD
ENTGLYFDHSTGFFYDSENQLYYDPSTGIYYCDVESGRYQFHSRVLDLPYPTSSTKQSKDKKLKKKRKD
PDSSATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVP TSGNTIESPLHENI
SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
QIGSLFIITAVNPATIGREKDM EHTLRIP EVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
KTKCDPYVLEHGDEVKIGETVLSFHIHPGSDTC DGCEPGQVRAHLRLDKKDES FVGPTLSKEEKELERRK
ELKKIRVKYGLQNT EYEDEKTLKNPKYKDRAGKRREQVGSEGT FQRDDAPASVHSEITDSNKGRKMLEKM
GWKKGEGLGKDGGMKTP IQLQLRRTHAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
DDPGTMPWVKGTLE

C. AGGF1-N2 (629 amino acids; 86 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:3)

MKKSDVEVQ TENHAPWSISDYFYQTYNDVSLPNKVTELS DQQDQAIETSILNSK
DHLQVENDAYPGTDRTE NVKYRQVDHFASNSQEPASALATEDTSLEGSSLAESLRAAAEA AVSQTGFSYD
ENTGLYFDHSTGFFYDSENQLYYDPSTGIYYCDVESGRYQFHSRVLDLPYPTSSTKQSKDKKLKKKRKD
PDSSATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVP TSGNTIESPLHENI
SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
QIGSLFIITAVNPATIGREKDM EHTLRIP EVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
KTKCDPYVLEHGDEVKIGETVLSFHIHPGSDTC DGCEPGQVRAHLRLDKKDES FVGPTLSKEEKELERRK
ELKKIRVKYGLQNT EYEDEKTLKNPKYKDRAGKRREQVGSEGT FQRDDAPASVHSEITDSNKGRKMLEKM
GWKKGEGLGKDGGMKTP IQLQLRRTHAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
DDPGTMPWVKGTLE

FIG. 6

A. AGGF1-N3 (550 amino acids; 165 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:4)

MHFASNSQEPASALATEDTSLEGSSLAESLRAAAEAAVSQTGFSYD
 ENTGLYFDHSTGFYYDSENQLYYDPSTGIYYCDVESGRYQFHSRVDLQPYPTSSTKQSKDKKLKKRKRK
 PDSSATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVPVPTSGNTIESPLHENI
 SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
 QIGSLFIITAVNPATIGREKDMETLRIPEVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
 KTKCDPYVLEHGDEVKIGETVLSFHIHPSDTCDGCEPGQVRAHLRLDKKDESFVGPTLSKEEKELERRK
 ELKKIRVKYGLQNTYEYDEKTLKNPKYKDRAGKRREQVGSEGTQORDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKKNKNWDKARERFTENFPETKPKQK
 DDPGTMPWVKGTLE

B. AGGF1-N4 (496 amino acids; 219 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:5)

MSTGFYYDSENQLYYDPSTGIYYCDVESGRYQFHSRVDLQPYPTSSTKQSKDKKLKKRKRK
 PDSSATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVPVPTSGNTIESPLHENI
 SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
 QIGSLFIITAVNPATIGREKDMETLRIPEVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
 KTKCDPYVLEHGDEVKIGETVLSFHIHPSDTCDGCEPGQVRAHLRLDKKDESFVGPTLSKEEKELERRK
 ELKKIRVKYGLQNTYEYDEKTLKNPKYKDRAGKRREQVGSEGTQORDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKKNKNWDKARERFTENFPETKPKQK
 DDPGTMPWVKGTLE

C. AGGF1-N5 (431 amino acids; 284 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:6)

MATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVPVPTSGNTIESPLHEI
 SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
 QIGSLFIITAVNPATIGREKDMETLRIPEVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
 KTKCDPYVLEHGDEVKIGETVLSFHIHPSDTCDGCEPGQVRAHLRLDKKDESFVGPTLSKEEKELERRK
 ELKKIRVKYGLQNTYEYDEKTLKNPKYKDRAGKRREQVGSEGTQORDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKKNKNWDKARERFTENFPETKPKQK
 DDPGTMPWVKGTLE

D. AGGF1-N6 (366 amino acids; 349 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:7)

MI
 SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
 QIGSLFIITAVNPATIGREKDMETLRIPEVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
 KTKCDPYVLEHGDEVKIGETVLSFHIHPSDTCDGCEPGQVRAHLRLDKKDESFVGPTLSKEEKELERRK
 ELKKIRVKYGLQNTYEYDEKTLKNPKYKDRAGKRREQVGSEGTQORDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKKNKNWDKARERFTENFPETKPKQK
 DDPGTMPWVKGTLE

FIG. 7

A. AGGF1-N7 (302 amino acids; 413 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:8)

MVIRSPVL

QIGSLFIITAVNPATIGREKDMHTLRIPEVGVSKFHAEIYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
 KTKCDPYVLEHGDEVKIGETVLSFHIHPGSDTCDGCEPGQVRAHLRLDKKDESFVGPTLSKEEKELEERRK
 ELKKIRVKYGLQNTHEYEDKTLKNPKYKDRAGKRREQVGSEGTQFQDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
 DDPGTMPWVKGTLE

B. AGGF1-N8 (244 amino acids; 472 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:9)

MVDQGSQNGTIVNGKQILQP

KTKCDPYVLEHGDEVKIGETVLSFHIHPGSDTCDGCEPGQVRAHLRLDKKDESFVGPTLSKEEKELEERRK
 ELKKIRVKYGLQNTHEYEDKTLKNPKYKDRAGKRREQVGSEGTQFQDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
 DDPGTMPWVKGTLE

C. AGGF1-N9 (193 amino acids; 522 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:10)

MCDGCEPGQVRAHLRLDKKDESFVGPTLSKEEKELEERRK

ELKKIRVKYGLQNTHEYEDKTLKNPKYKDRAGKRREQVGSEGTQFQDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
 DDPGTMPWVKGTLE

D. AGGF1-N10 (141 amino acids; 574 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:11)

MEYEDEKTLKNPKYKDRAGKRREQVGSEGTQFQDDAPASVHSEITDSNKGRKMLEKM

GWKKGEGLGKDGGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
 DDPGTMPWVKGTLE

E. AGGF1-N101 (131 amino acids; 584 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:12)

MPKYKDRAGKRREQVGSEGTQFQDDAPASVHSEITDSNKGRKMLEKM

GWKKGEGLGKDGGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
 DDPGTMPWVKGTLE

F. AGGF1-N102 (121 amino acids; 594 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:13)

MREQVGSEGTQFQDDAPASVHSEITDSNKGRKMLEKM

GWKKGEGLGKDGGGMKTPIQLQLRRTAGLGTGKPSSFEDVHLLQNKNNKNWDKARERFTENFPETKPQK
 DDPGTMPWVKGTLE

FIG. 8

A. AGGF1-N103 (110 amino acids; 604 amino acid deletion from the N-terminus of AGGF1) (SEQ ID NO:14)

MQRDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGGMKTPIQLQLRRTHAGLTGKPSSEFEDVHLLQNKKNKNDKARERFTENFPETKPK
 DDPGTMPPWVKGTLE

B. AGGF1-C1 (664 amino acids; 50 amino acid deletion from the C-terminus of AGGF1) (SEQ ID NO:15)

MASEAPSPPRSPPPPPTSPEPELAQLRRKVEKLERELRSCKRQVREIEKLLHHTERLYQNAESNNQELRTQ
 VEELSKILQGRNEDNKKSDVEVQTENHAPWSISDYFYQTYNDVSLPNKVTELSQQDQAIETSIILNSK
 DHLQVENDAYPGTDRTEENVKYRQVDHFASNSQEPASALATEDTSLEGSSLAESLRAAAEAAVSQTGFSD
 ENTGLYFDHSTGFYYDSENQLYYDPSTGIYYYCDVESGRYQFHSRVLDQPYPTSSTKQSKDKKLKKRKRK
 PDSSATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVPVPTSGNTIESPLHENI
 SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
 QIGSLFIITAVNPATIGREKDMETLRIPEVGVSKEFHAIEYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
 KTKCDPYVLEHGDEVKIGETVLSFHIHPSGSDTCGCEPGQVRAHLRLDKKDES FVGPTLSKEEKELERRK
 ELKKIRVKYGLQNTYEDEKTLKNPKYKDRAGKRREQVGSEGTQRRDDAPASVHSEITDSNKGRKMLEKM
 GWKKGEGLGKDGGGMKTPIQLQLRRTHAGLTGK

C. AGGF1-C2 (614 amino acids; 100 amino acid deletion from the C-terminus of AGGF1) (SEQ ID NO:16)

MASEAPSPPRSPPPPPTSPEPELAQLRRKVEKLERELRSCKRQVREIEKLLHHTERLYQNAESNNQELRTQ
 VEELSKILQGRNEDNKKSDVEVQTENHAPWSISDYFYQTYNDVSLPNKVTELSQQDQAIETSIILNSK
 DHLQVENDAYPGTDRTEENVKYRQVDHFASNSQEPASALATEDTSLEGSSLAESLRAAAEAAVSQTGFSD
 ENTGLYFDHSTGFYYDSENQLYYDPSTGIYYYCDVESGRYQFHSRVLDQPYPTSSTKQSKDKKLKKRKRK
 PDSSATNEEKDLNSEDQKAFSVEHTSCNEEENFANMKKKAKIGIHHKNSPPKVTVPVPTSGNTIESPLHENI
 SNSTSFKDEKIMETDSEPEEGEITDSQTEDSYDEAITSEGNVTAEDSEDEDEDKIWPPCIRVIVIRSPVL
 QIGSLFIITAVNPATIGREKDMETLRIPEVGVSKEFHAIEYFDHDLQSYVLVDQGSQNGTIVNGKQILQP
 KTKCDPYVLEHGDEVKIGETVLSFHIHPSGSDTCGCEPGQVRAHLRLDKKDES FVGPTLSKEEKELERRK
 ELKKIRVKYGLQNTYEDEKTLKNPKYKDRAGKRREQVGSEGTQRRDDAPASVH

FIG. 9

Full Length Human AGGF1 cDNA (SEQ ID NO:17), with arrows showing truncation mutants

362	AUGGCCUCGGAGGCGCCGUCCCCGCCGGUCGCCGCCG	421
	M A S E A P S P P R S P P	13
422	CGCCCACCUCSCCGAGCCUGAGCUGGCCAGCUAAGGCGGAAGGUGGAGAAGUUGGAA	481
	P P T S P E P E L A Q L R R K V E K L E	33
		
	AGGF1-N1	
482	CGUGAACUGCGGAGCUGCAAGCGGCAGGUGCGGGAGAUCGAGAAGCUGCUGCAUCACACA	541
	R E L R S C K P Q V R E I E K L L R H T	53
542	GAACGGCUGUACCAGAACGCAGAAAGCAACAACCAGGAGCUCCGCACGCAGGUGGAAGAA	601
	E R L Y Q N A E S N N Q E L P T Q V E E	73
602	CUCAGUAAAUAUCUCCAACGUGGGAGAAAUGAAGAUAAAAGUCUGAUGUAGAAGUA	661
	L S K I L Q R G R N E D N K K S D V E V	93
		
	AGGF1-N2	
662	CAAACAGAGAACCAUGCUCCUUGGUCAAUCUCAGAUUUUUUAUCAGACGUACUACAAU	721
	Q T E N H A P W S I S D Y F Y Q T Y Y N	113
722	GACGUUAGUCUCCAAAUAAGUGACUGAACUGUCAGAUCAACAAGAUCAAGCUAUCGAA	781
	D V S L P N K V T E L S D Q Q D Q A I E	133
782	ACUUCUAUUUUGAAUUCUAAAGACCAUUUACAAGUAGAAAAUGAUGCUUACCCUGGUACC	841
	T S I L N S K D H L Q V E N D A Y P G T	153
842	GAUAGAACAGAAAAUGUAAAUAUAGACAAGUGGACCAUUUUGCCUCAAAUUCACAGGAG	901
	D R T E N V K Y R Q V D R F A S N S Q E	173
		
	AGGF1-N3	
902	CCAGCAUCUGCAUUAGCAACAGAAGAUACCUCCUAGAAGGCUCAUCAUUAGCUGAAAGU	961
	P A S A L A T E D T S L E G S S L A E S	193
962	UUGAGAGCUGCAGCAGAAGCGGCUGUAUCACAGACUGGAUUUAGUUAUGAUGAAAAUACU	1021
	L R A A A E A A V S Q T G F S Y D E N T	213
1022	GGACUGUAUUUUGACCACAGCACUGGUUUUAUUUAUGAUUCUGAAAAUCAACUCUAUUUAU	1081
	G L Y F D H S T G F Y Y D S E N Q L Y Y	233
		
	AGGF1-N4	
1082	GAUCCUCCACUGGAUUUUUUACUAUUGUGAUGUGGAAAGUGGUCGUUAUCAGUUUCAU	1141
	D P S T G I Y Y Y C D V E S G R Y Q F H	253
1142	UCUCGAGUAGAUUUGCAACCUUAUCCGACUUCUAGCACAAAACAAAGUAAAGAUAAAAAA	1201
	S R V D L Q P Y P T S S T K Q S K D K K	273

FIG. 9 (cont.)

1202 UUGAAGAAGAAAAGAAAAGAUCCAGAUUCUUCUGCAACAAAUGAGGAAAAGGAUUUGAAC 1261
L K K K R K D P D S S A T N E E K D L N 293



AGGF1-N5

1262 UCAGAGGAUCAAAAAGCCUUCAGUGUUGAACAUACAAGCUGCAAUGAGGAAGAAAUUUC 1321
S E D Q K A F S V E H T S C N E E E N F 313

1322 GCAAAUAUGAAAAAGAAGGCCAAAUAAGGCAUUCACACAAAAUAGUCCCCCAAAGUC 1381
A N M K K K A K I G I H H K N S P P K V 333

1382 ACUGUCCAACUAGUGGAAAUACUAUAGAGUCUCCUCUUAUGAAAACAUCUCUAAUUA 1441
T V P T S G N T L E S P L H E N I S N S 353



AGGF-N6

1442 ACAUCAUUUAAAGAUGAGAAAAUCAUGGAGACUGAUAGUGAACCAGAGGAAGGUGAAAUU 1501
T S F K D E K I M E T D S E P E E G E I 373

1502 ACAGACUCUCAGACUGAGGAUAGUUAUGACGAAGCCAUUACCAGUGAAGGCAAUGUAAU 1561
T D S Q T E D S Y D E A I T S E G N V T 393

1562 GCAGAAGAUAGUGAGGAUGAAGAUGAAGACAAAUUUGGCCCCCAUGUAAUAGAGUAAU 1621
A E D S E D E D E D K I W P P C I R V I 413



AGGF1-N7

1622 GUCAUUAGAUACCUGUGUUGCAGAUAGGAUCACUCUUUAUCAUUACUGCUGUAAACCCU 1681
V I R S P V L Q I G S L F I I T A V N P 433

1682 GCUACAAUUGGAAGAGAAAAGGAUAUGGAACAUACUCUCCGAAUCCUGAAGUUGGUGUC 1741
A T I G R E K D M E H T L R I P E V G V 453

1742 AGUAAGUUUCAUGCAGAAAUUUUAUUUGACCAUGACUUACAAAGUUAUGUCCUUGUGGAU 1801
S K F H A E I Y F D H D L Q S Y V L V D 473



AGGF1-N8

1802 CAAGGCAGUCAAAAUGGCACAAUUGUUAUUGGAAAACAGAUUCUUCAGCCGAAAACUAAA 1861
Q G S Q N G T I V N G K Q I L Q P K T K 493

1862 UGUGACCCUACGUACUUGAGCAUGGAGAUGAAGUCAAAAUUGGAGAAACUGUCUUAUCC 1921
C D P Y V L E R G D E V K I G E T V L S 513

1922 UUUCACAUUAUCCUGGCAGUGAUACCUGUGAUGGCUGUGAACCAGGGCAGGUUAGAGCC 1981
F H I H P G S D T C D G C E P G Q V R A 533



AGGF1-N9

1982 CACCUUCGCCUUGAUAAAGAAAGAUAAUCUUUUGUUGGUCCAACACUAAGUAAGGAGGAA 2041
H L R L D K K D E S F V G P T L S K E E 553

FIG. 9 (cont.)

2042 AAAGAGUUGGAAAGAAGAAAAGAAUUAAGAAAAUACGAGUAAAAUAUGGUUUACAGAAU 2101
K E L E R P K E L K K I R V K Y G L Q N 573

2102 ACAGAAUACGAAGAUGAAAAGACAUUGAAGAAUCCAAAAUAUAAAGAUAGAGCUGGAAAA 2161
T E Y E D E K T L K N P K Y K D R A G K 593
AGGF1-N10 AGGF1-N101

2162 CGUAGGGAGCAGGUUGGAAGUGAAGGAACUUUCCAAAGAGAUGAUGCUCUGCAUCUGUU 2221
R R E Q V G S E G T F Q R D D A P A S V 613
AGGF1-N102 AGGF1-N103

2222 CAUUCUGAAAUUACUGAUAGCAACAAAGGUCGGAAGAUGUUGGAGAAGAUGGGUUGGAAG 2281
H S E I T D S N K G P K M L E K M G W K 633
AGGF1-C2

2282 AAAGGAGAGGGCCUGGGGAAGGAUGGUGGAGGAUGAAAACGCCGAUCCAGCUUCAGCUU 2341
K G E G L G K D G G G M K T P I Q L Q L 653

2342 CGGCGAACACAUGCAGGCUUGGGGACAGGCAAACCAUCCUCAUUUGAAGAUGUUCACCUU 2401
P R T H A G L G T G K P S S F E D V R L 673
AGGF1-C1

2402 CUCCAAAACAAGAACAAAAAAACUGGGACAAAGCACGAGAGCGGUUUACUGAAAACUUC 2461
L Q N K N K K N W D K A P E P F T E N F 693

2462 CCAGAAACUAAGCCUCAAAAAGAUGACCCAGGGACCAUGCCUUGGUAAAAGGGACUUUA 2521
P E T K P Q K D D P G T M P W V K G T L 713

2522 GAGUGA
E *

FIG. 10

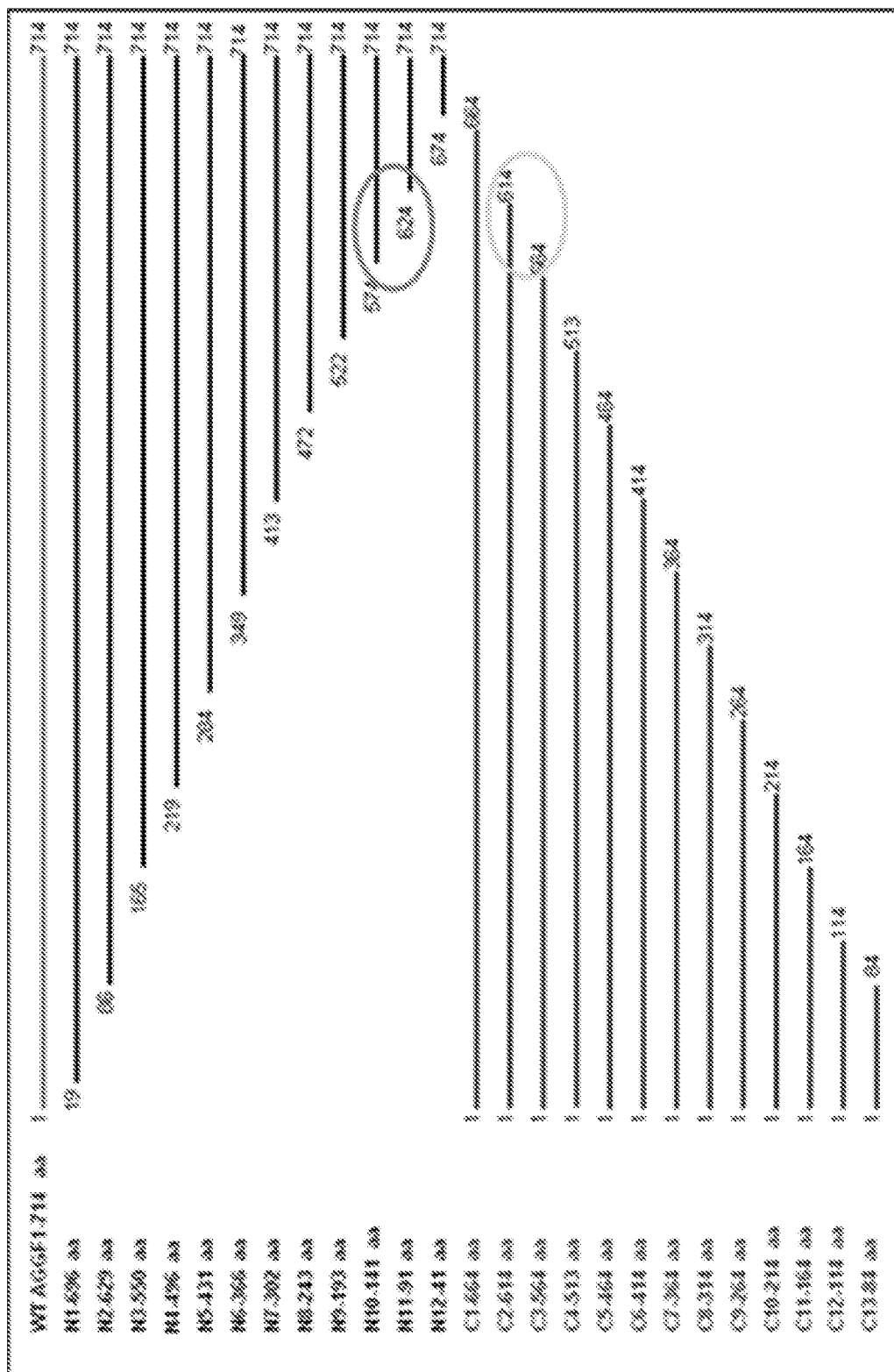


FIG. 11

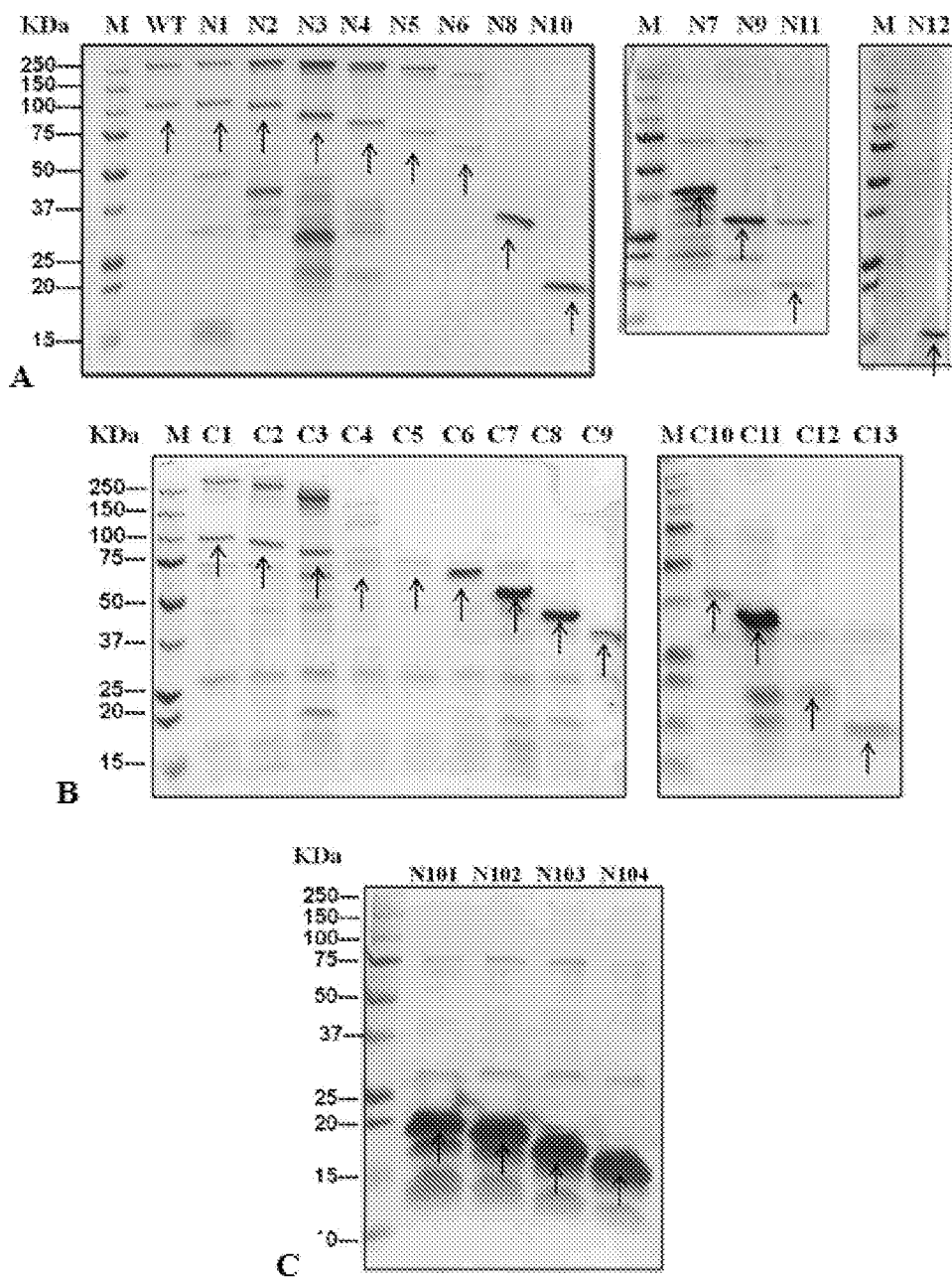


FIG. 12

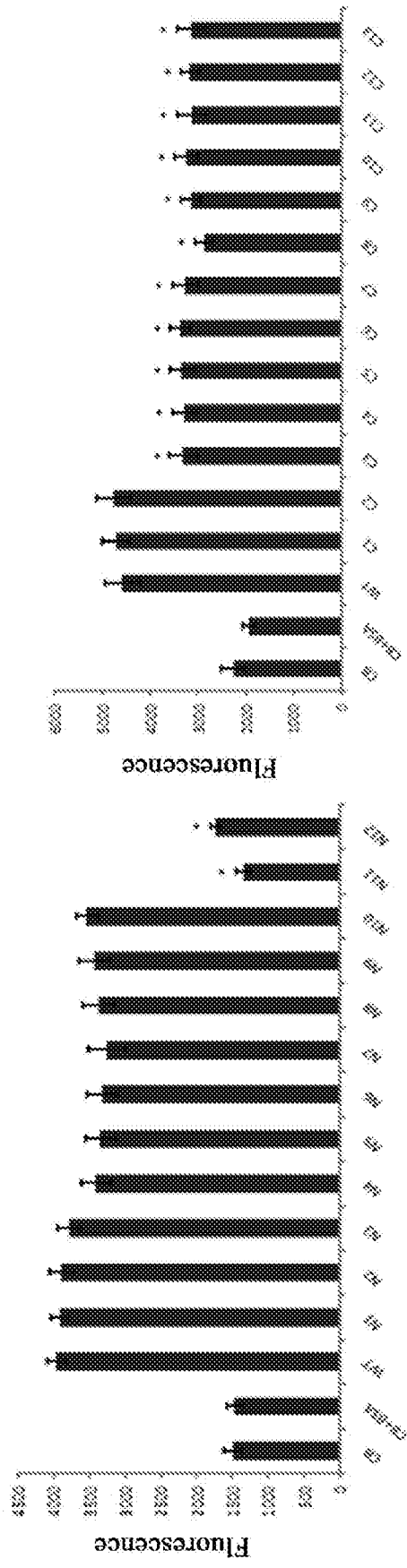


FIG. 13

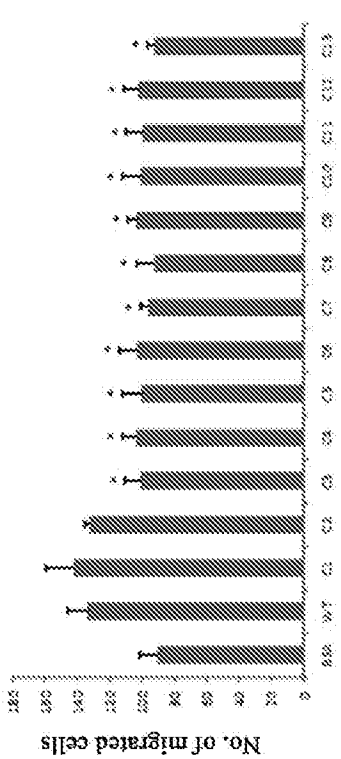
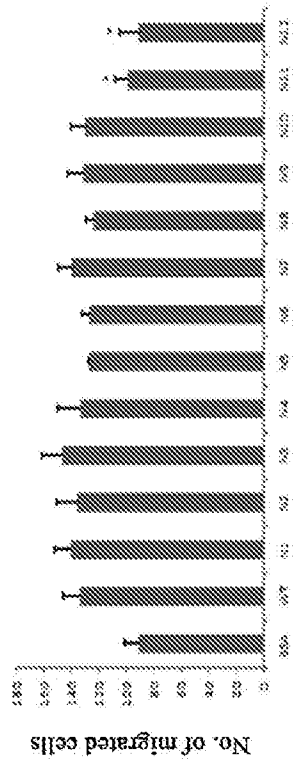
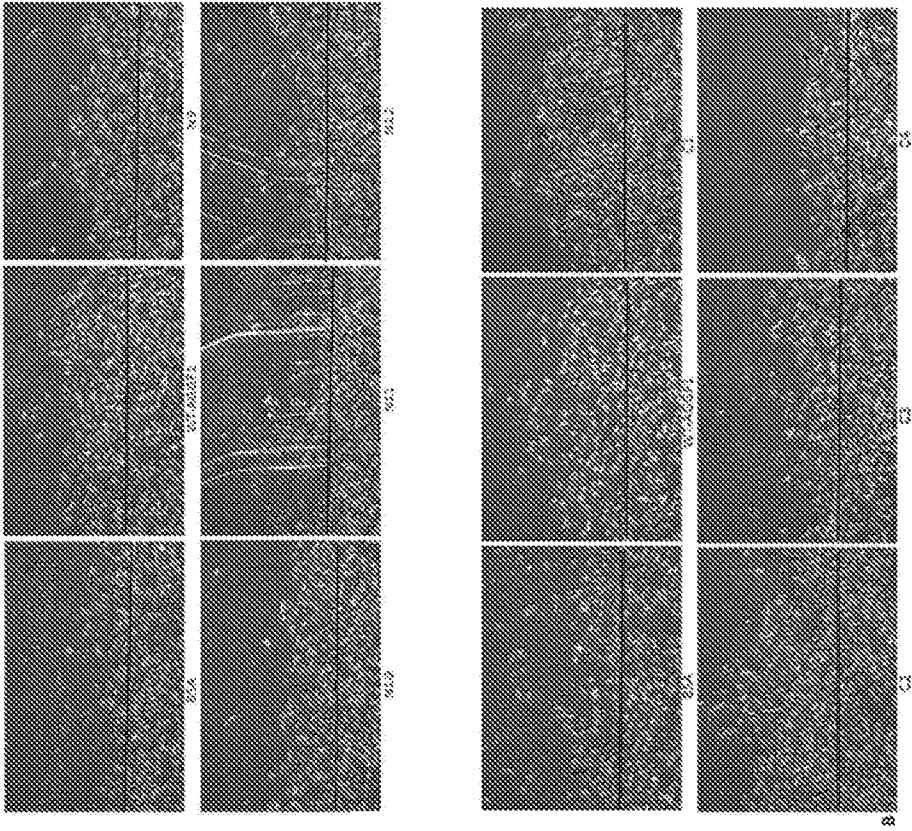


FIG. 14

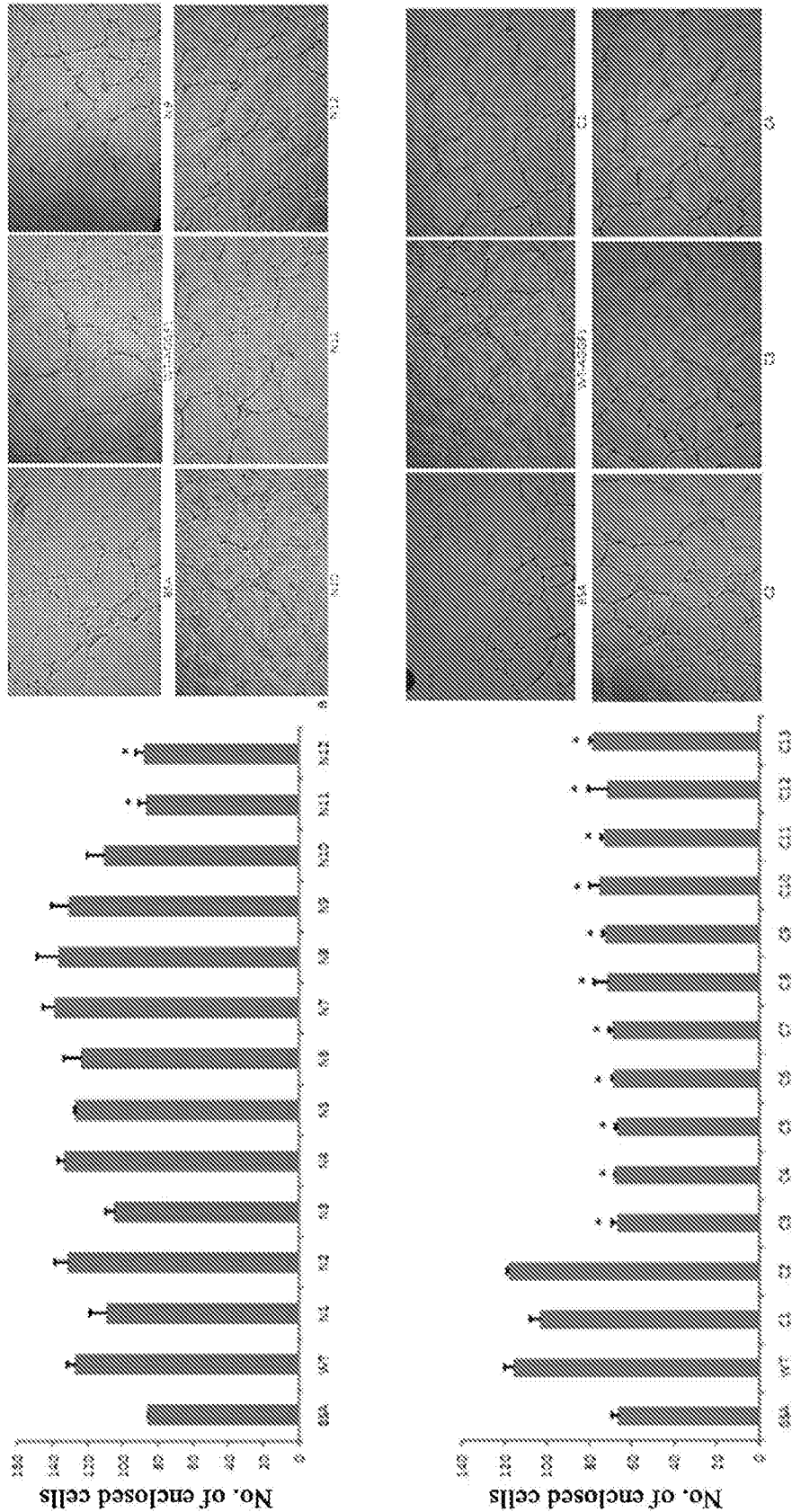


FIG. 15 (cont.)

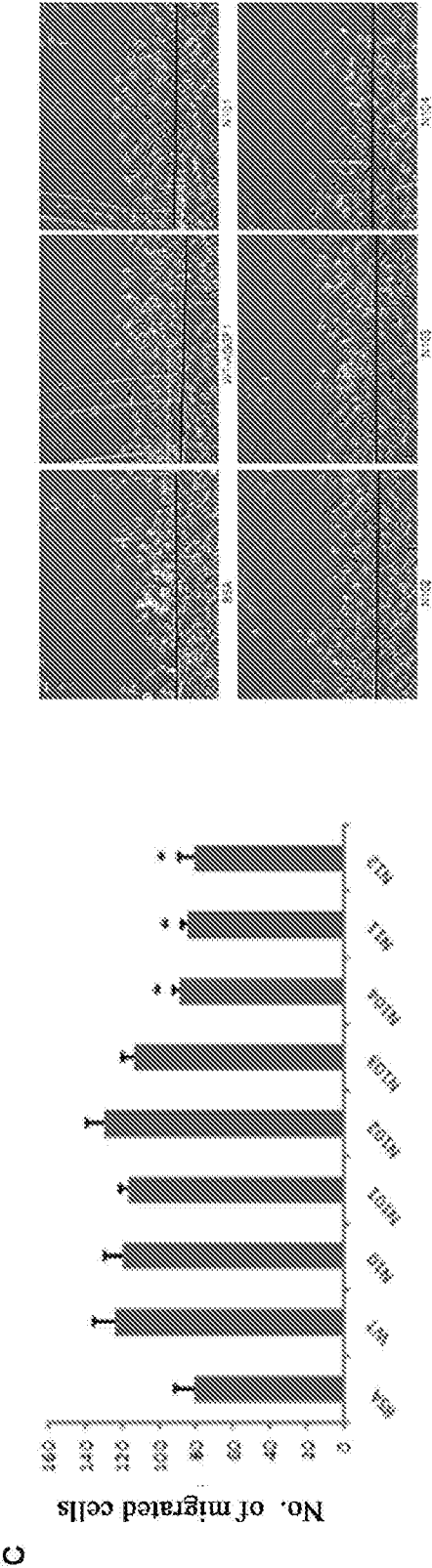


FIG. 15 (cont.)

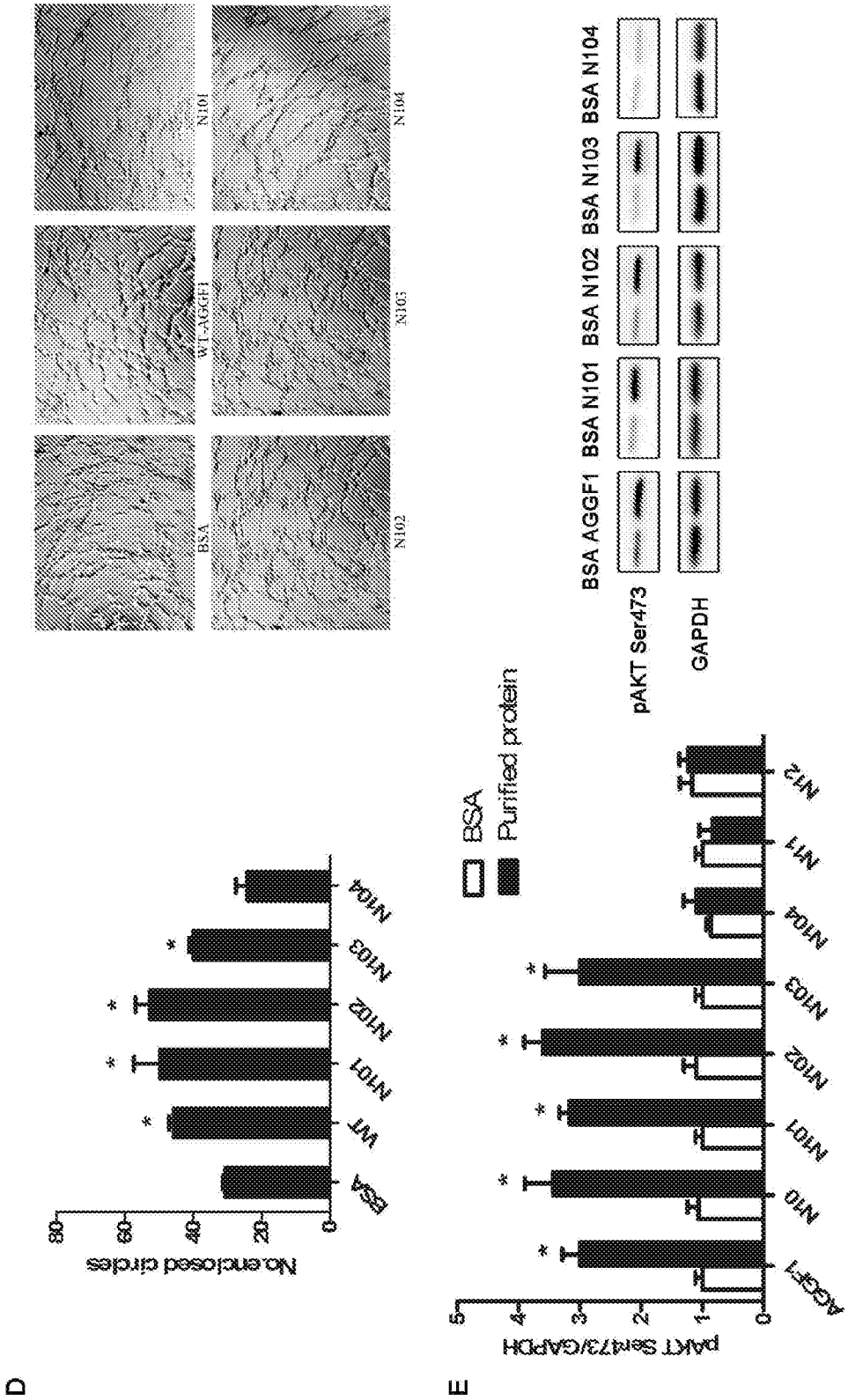


FIG. 16

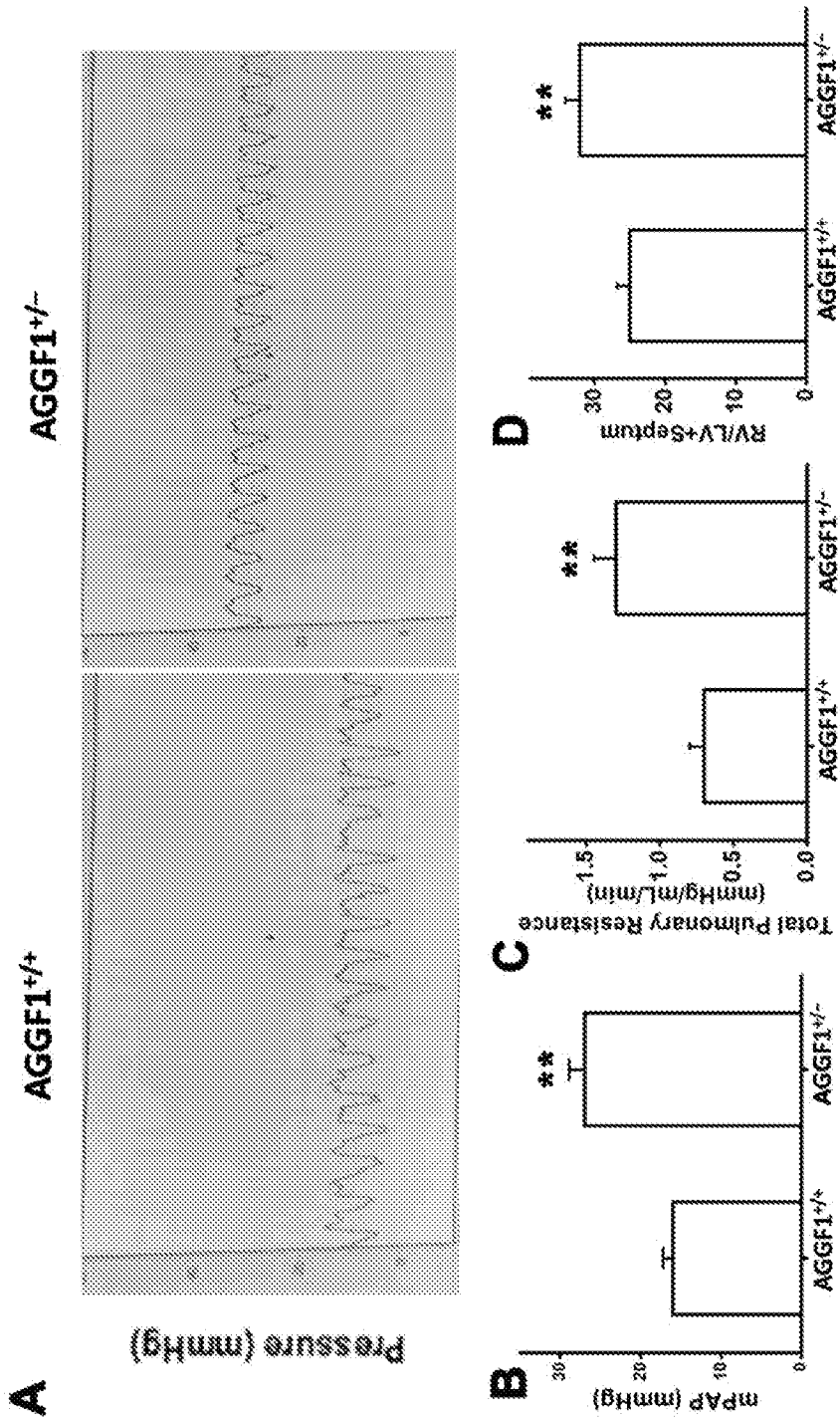


FIG. 17

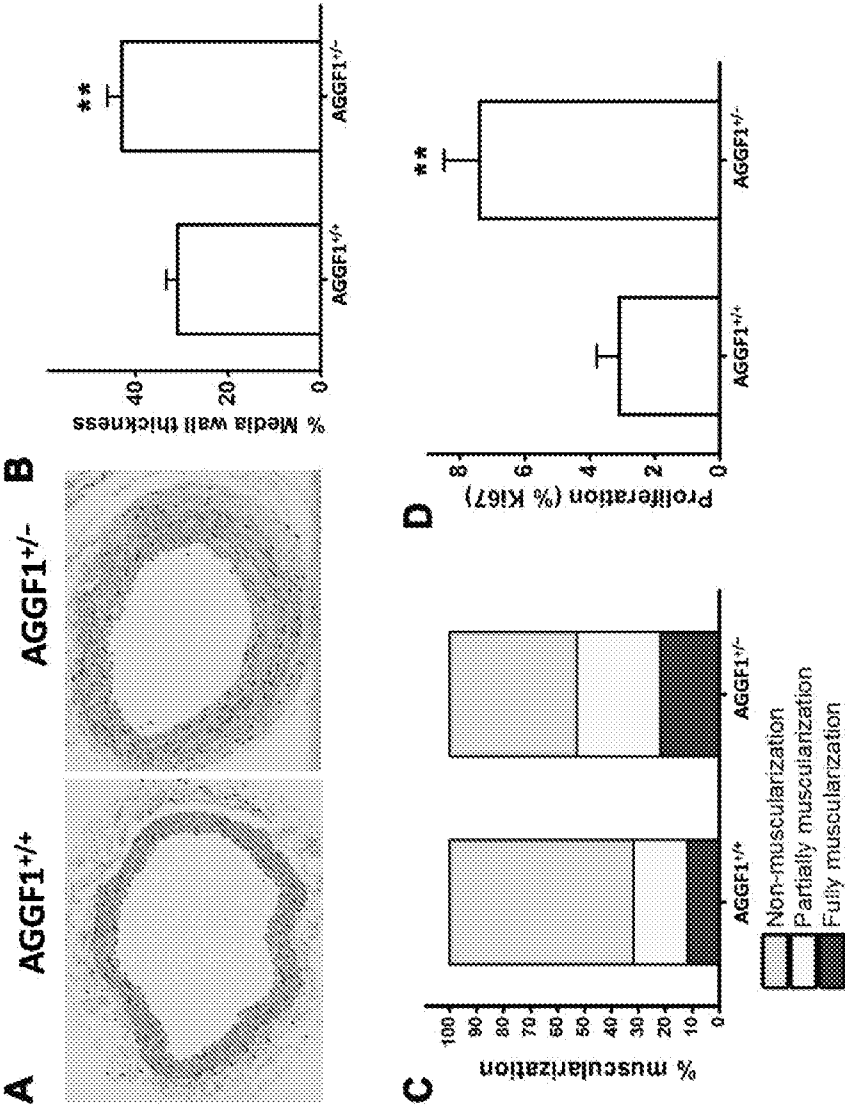


FIG. 18

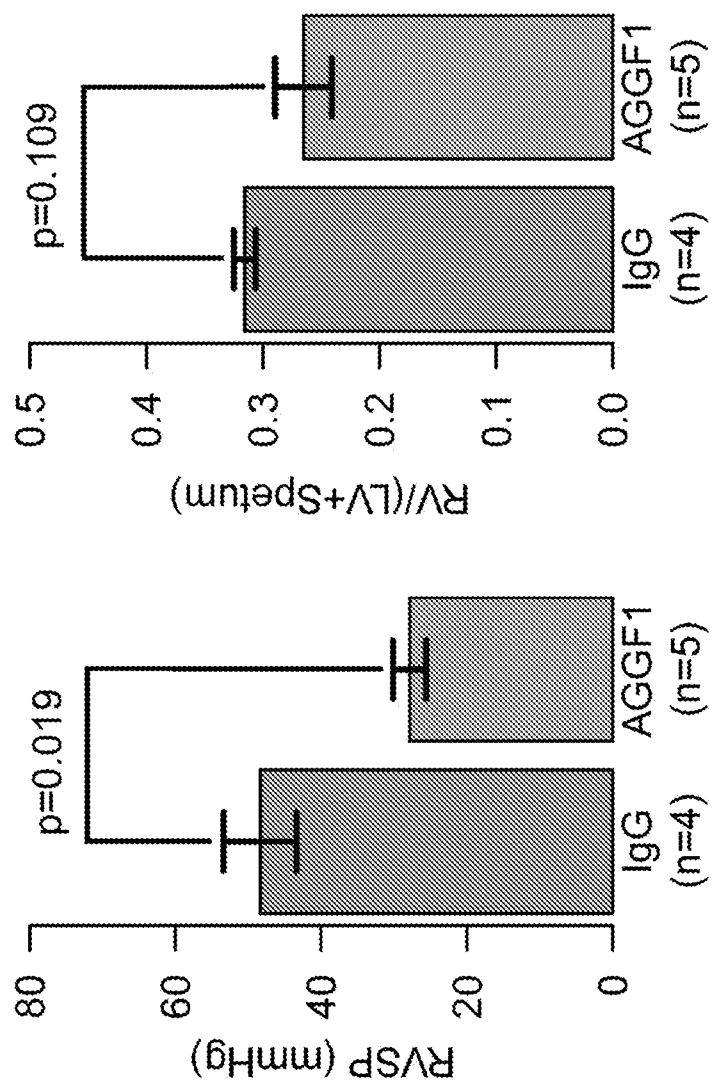


FIG. 19

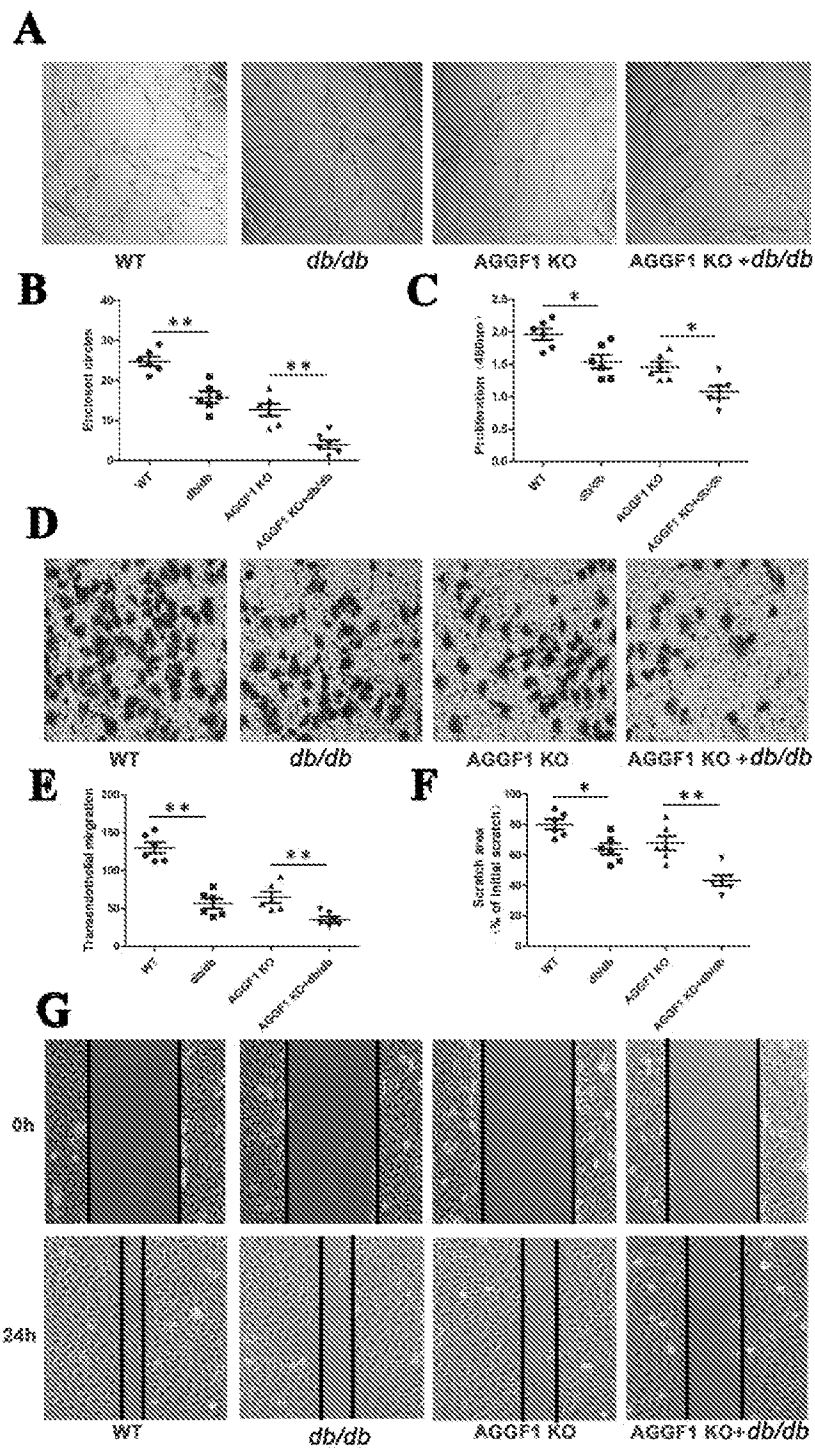


FIG. 20

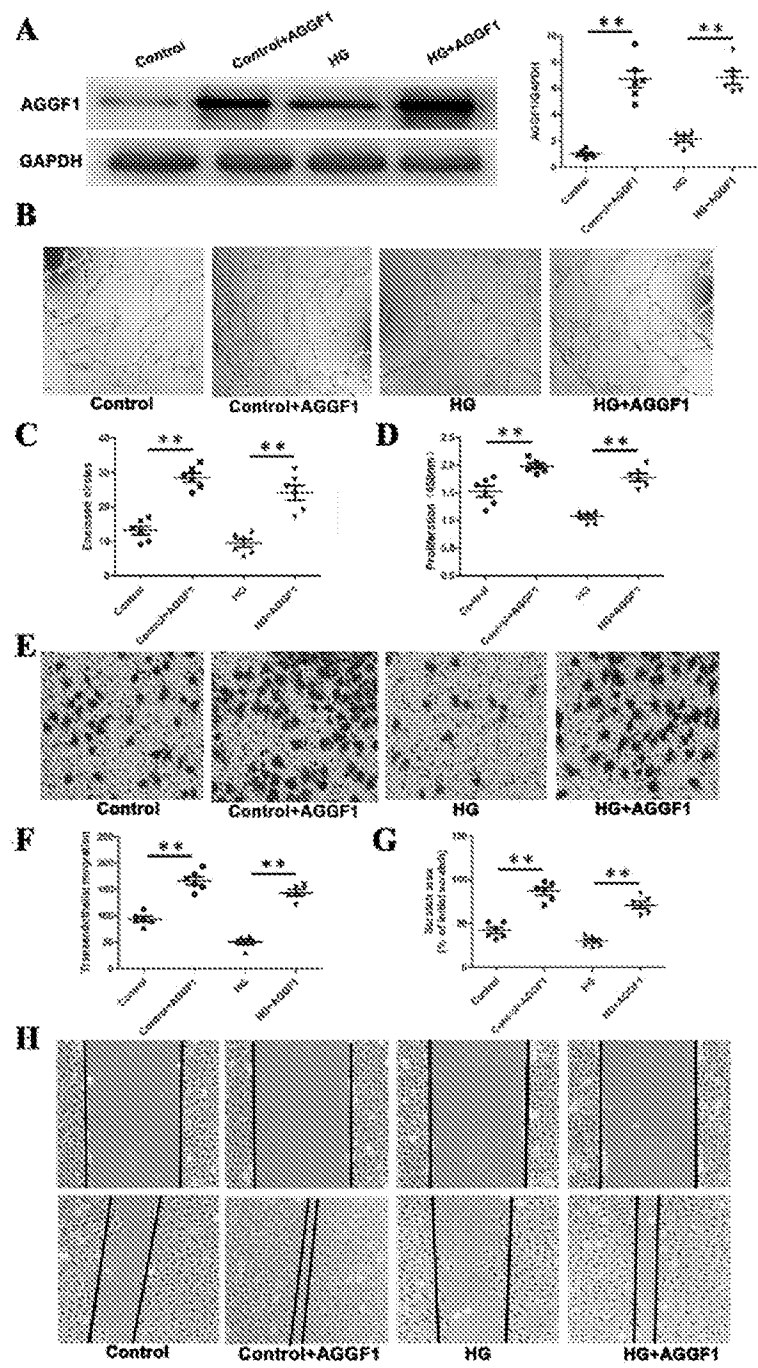


FIG. 21

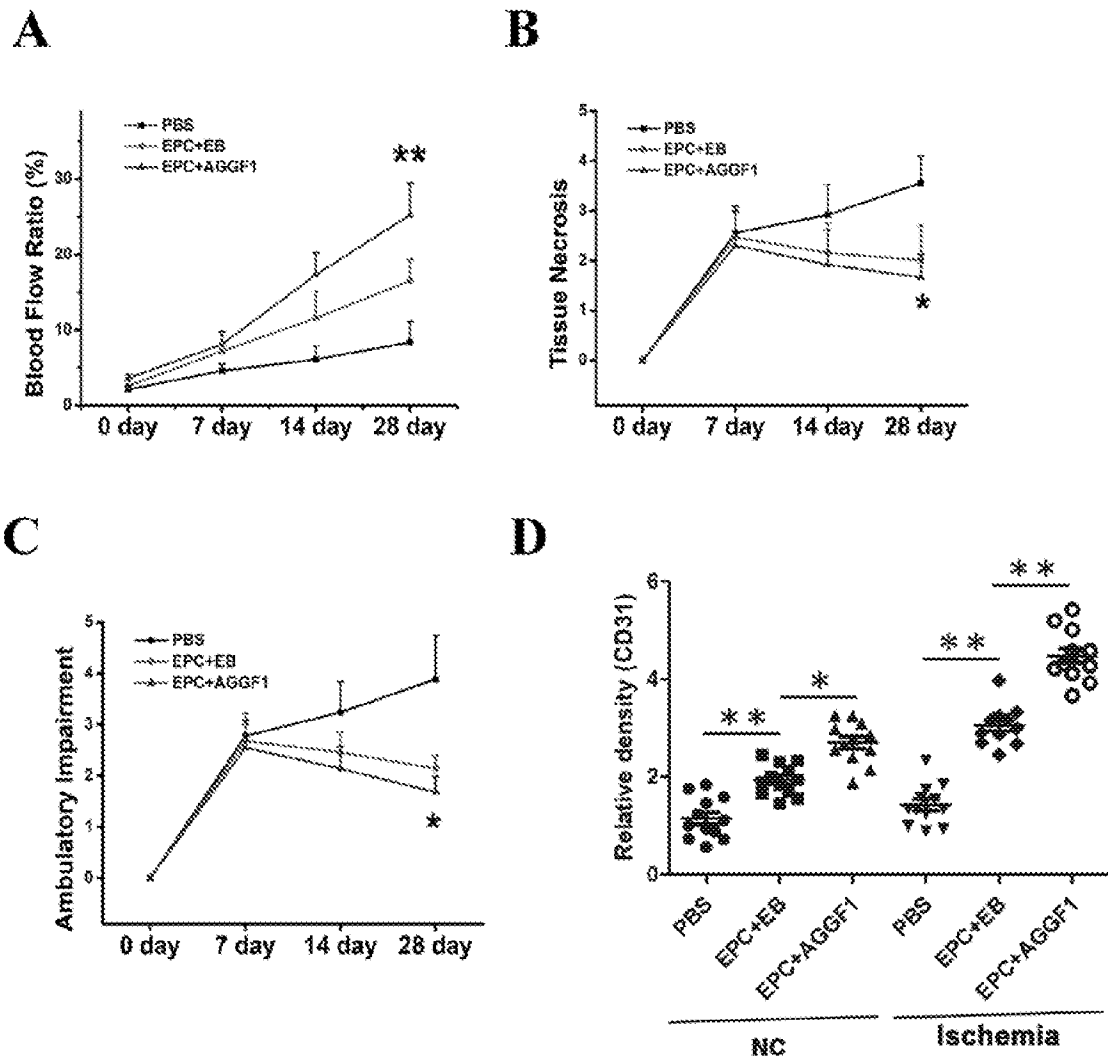


FIG. 22

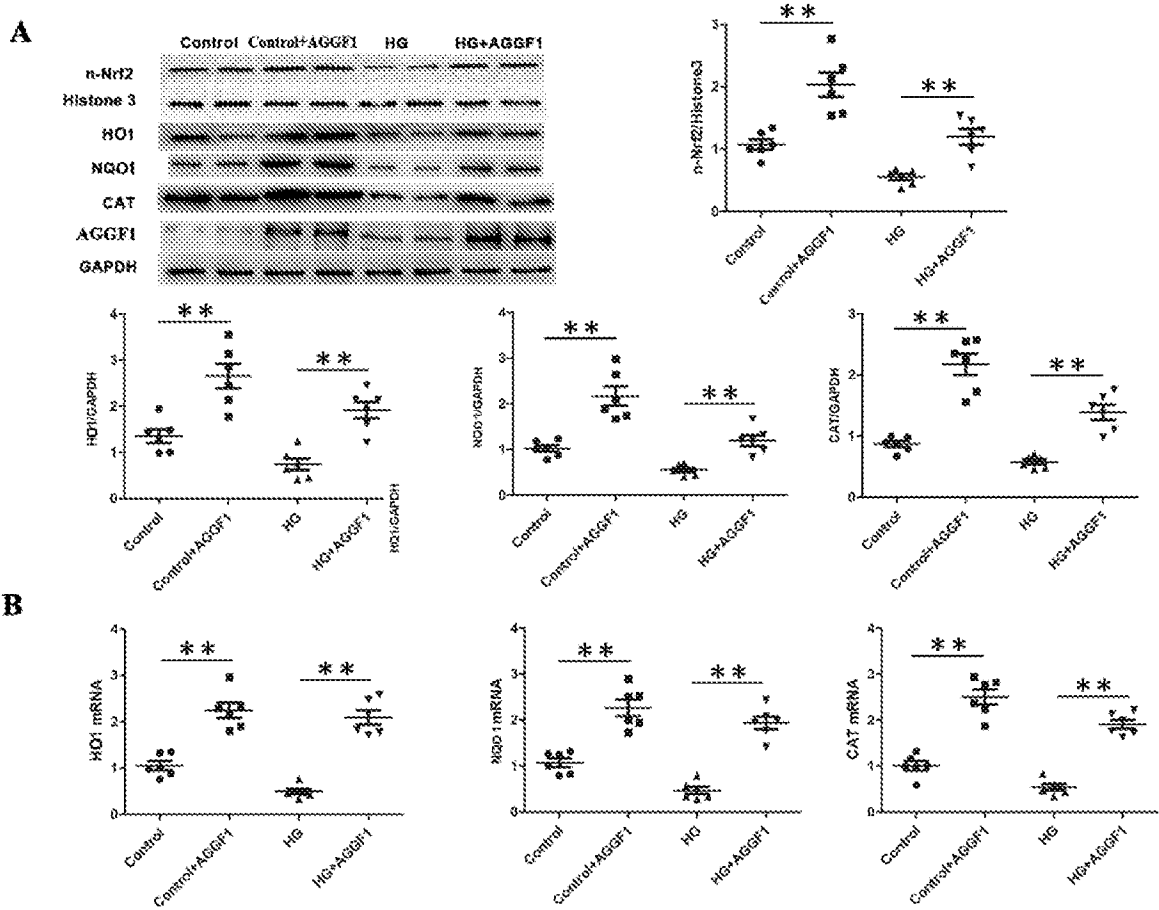


FIG. 23 (cont.)

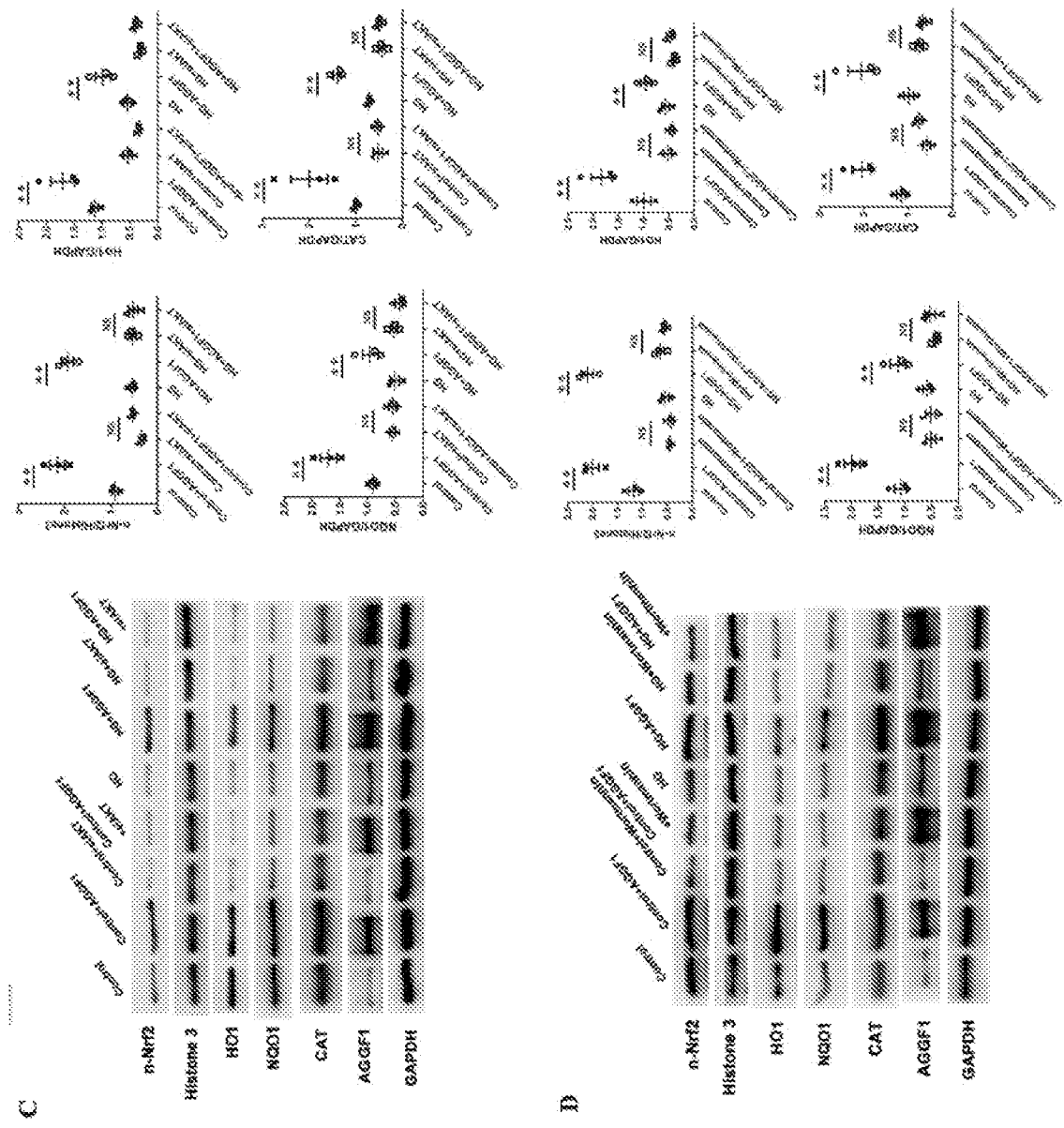


FIG. 23 (cont.)

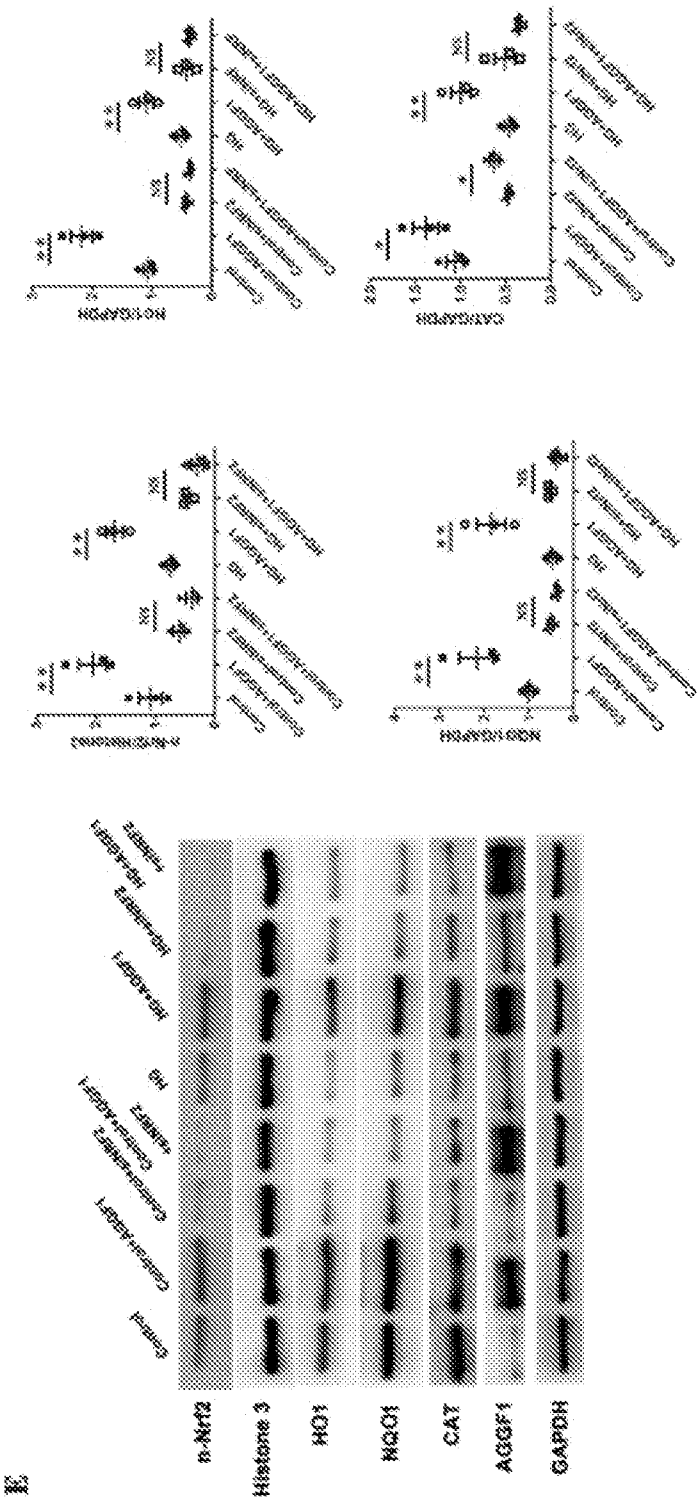


FIG. 24

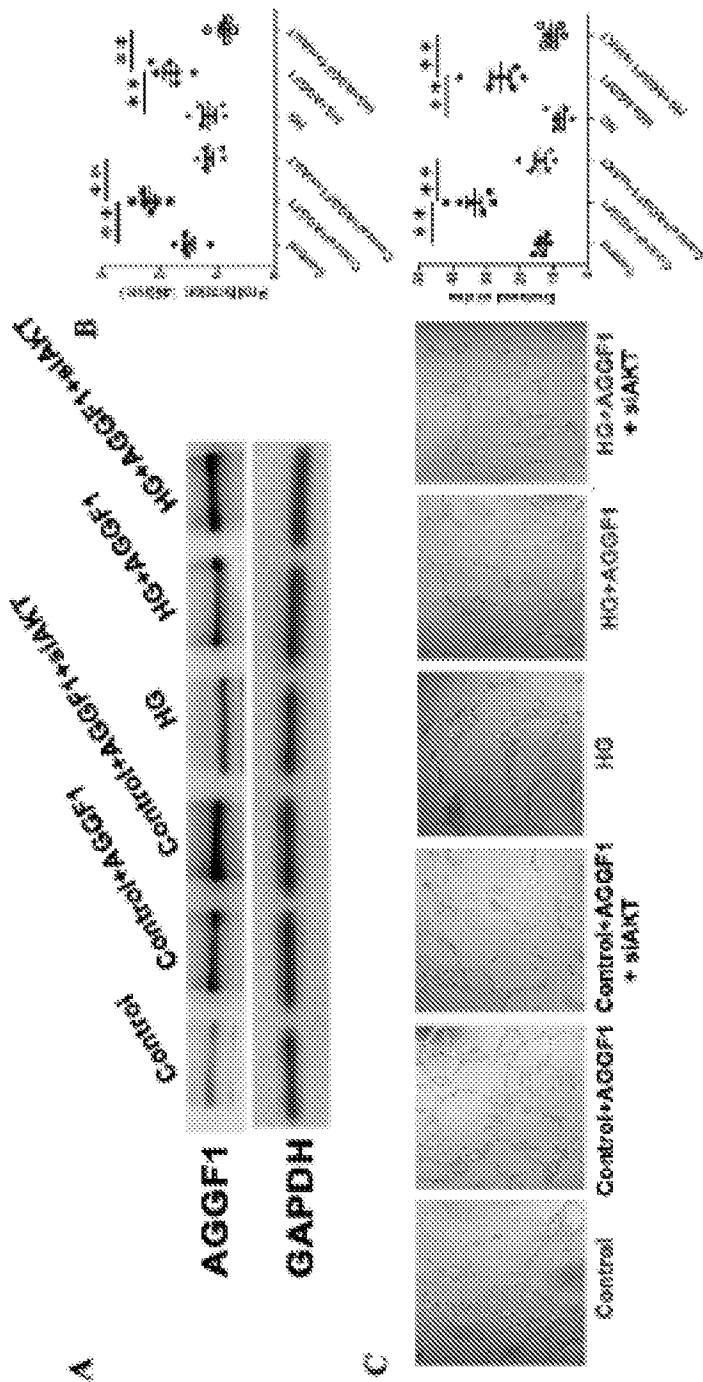


FIG. 24 (cont.)

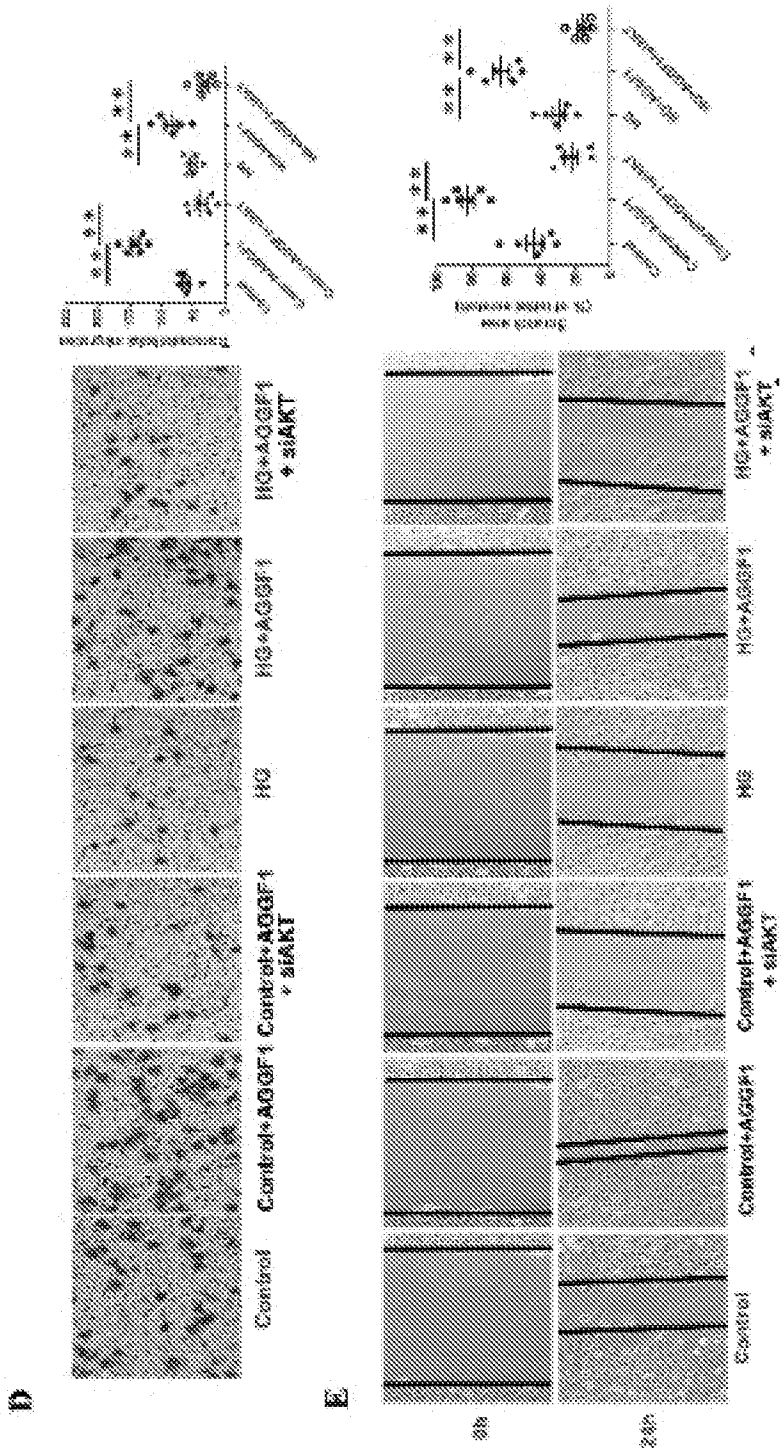


FIG. 24 (cont.)

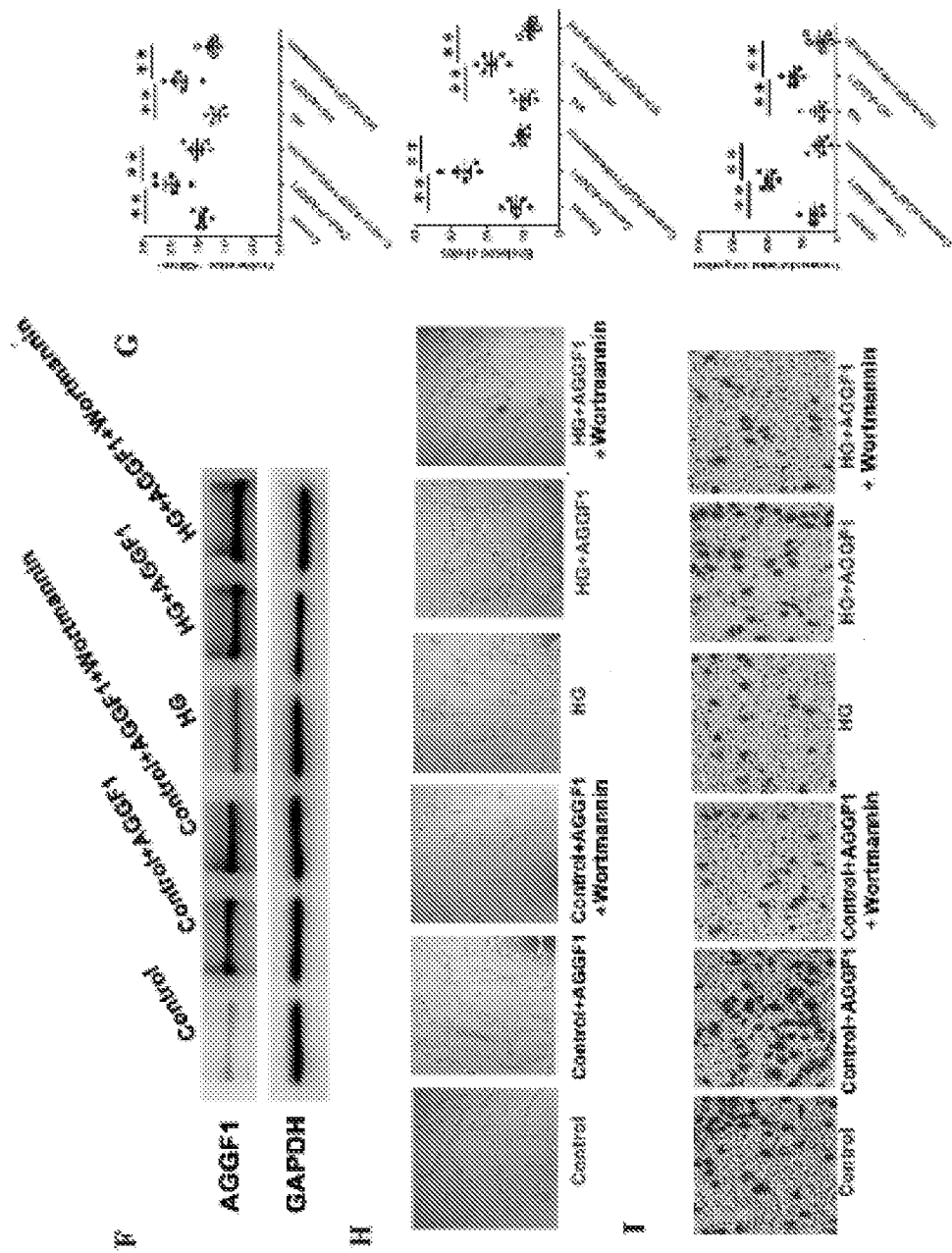


FIG. 24 (cont.)

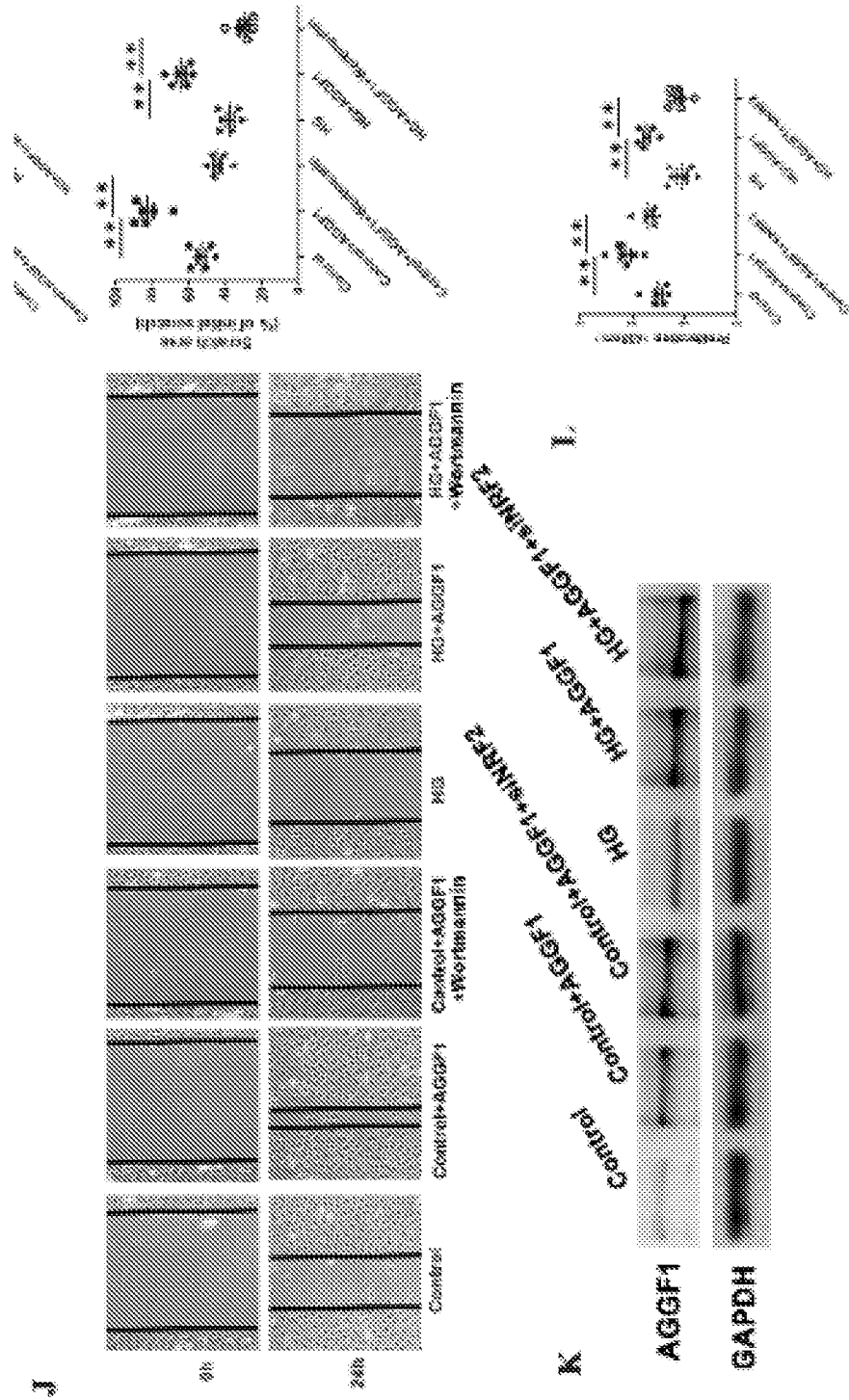


FIG. 24 (cont.)

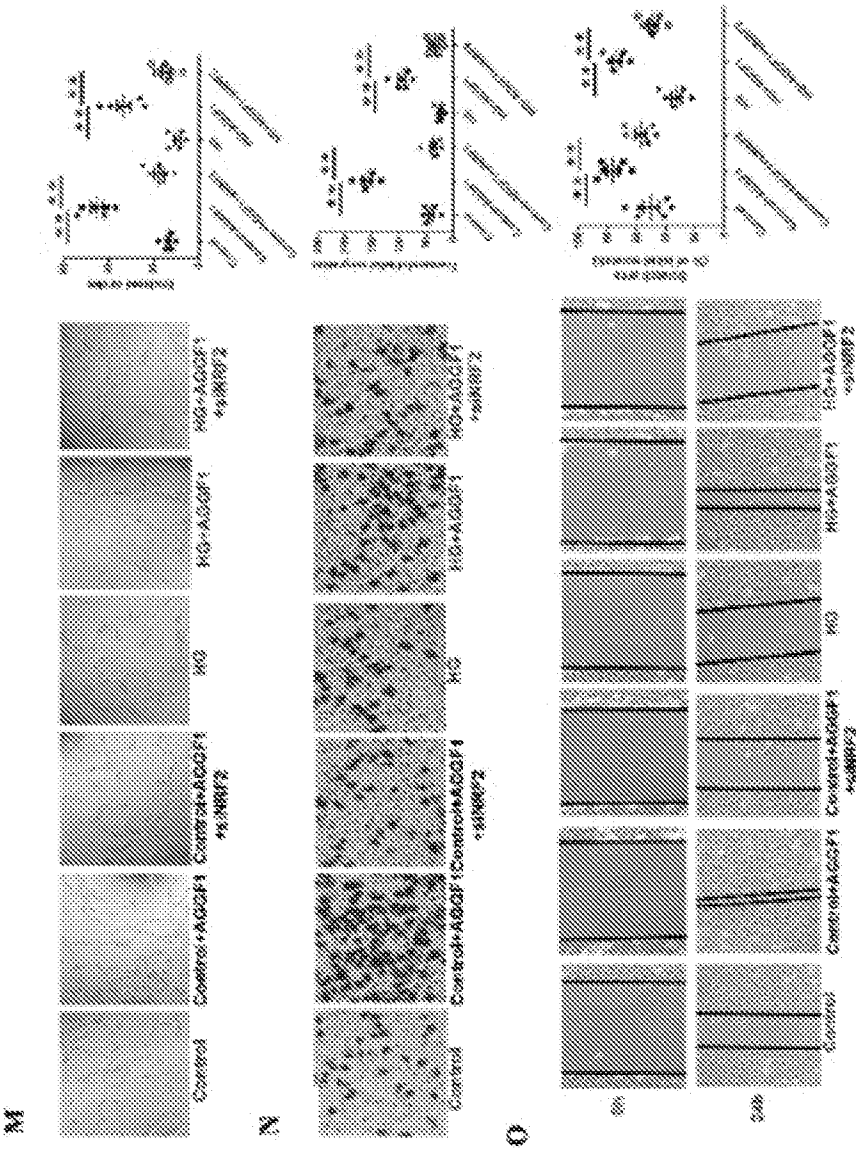


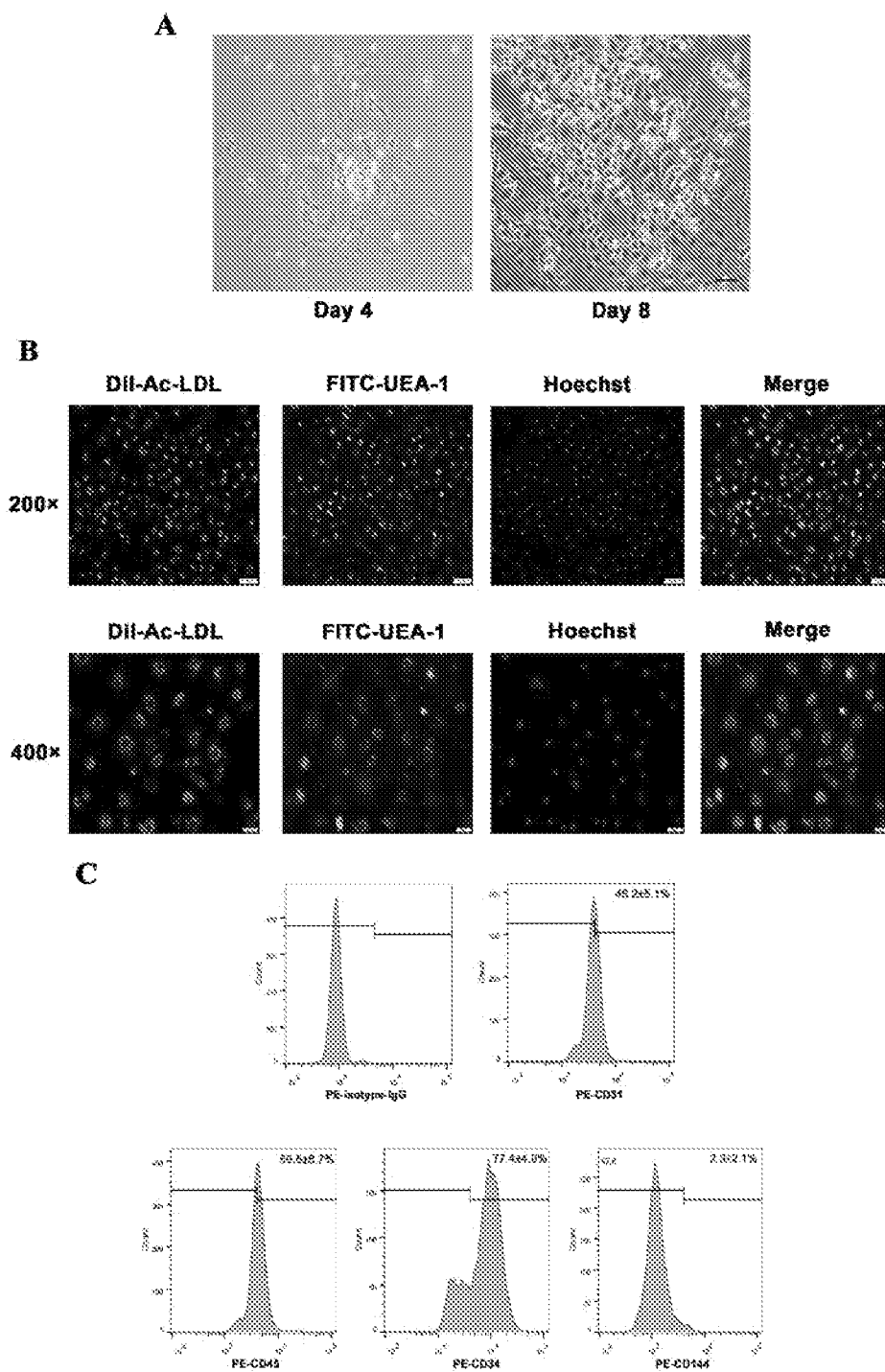
FIG. 25

FIG. 26

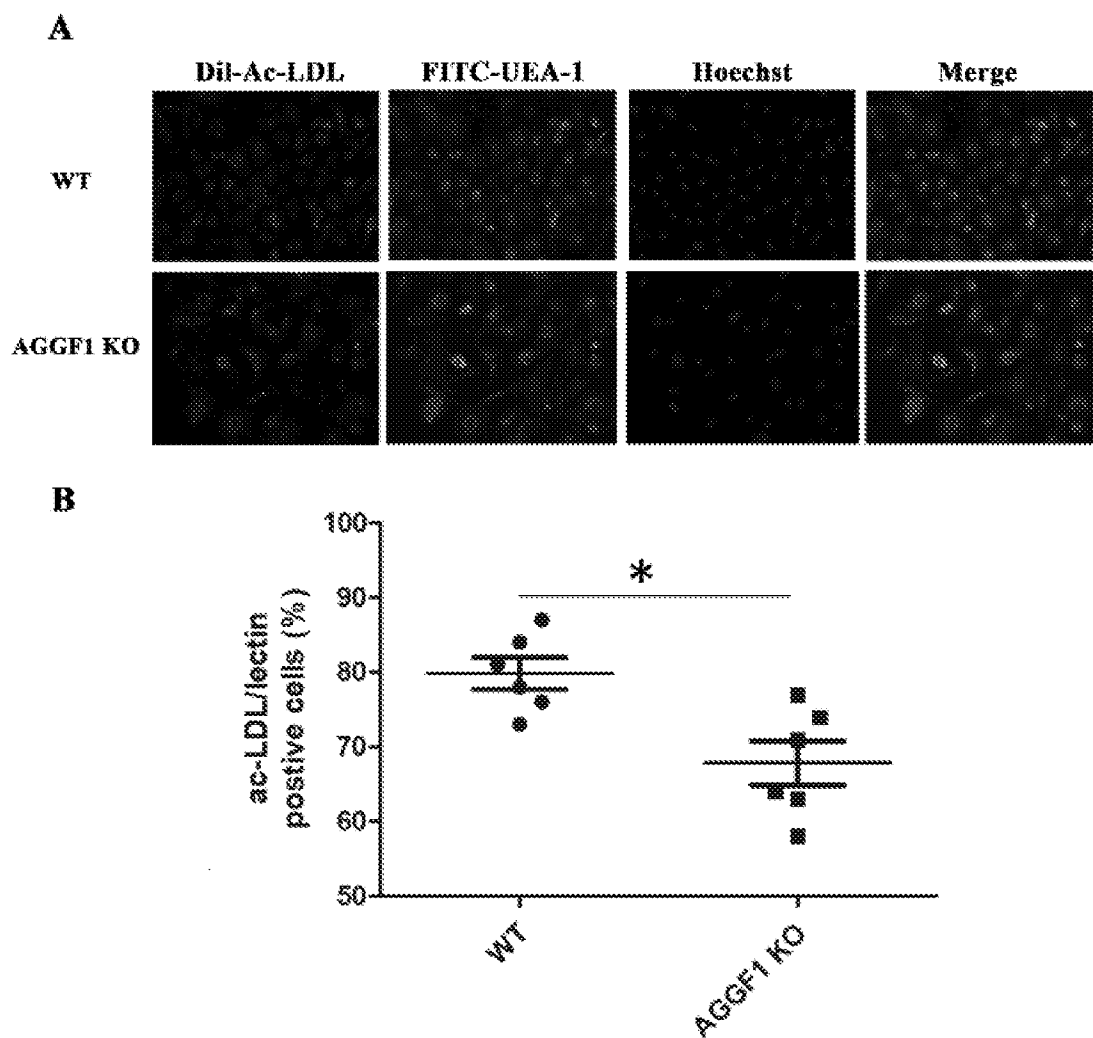


FIG. 27

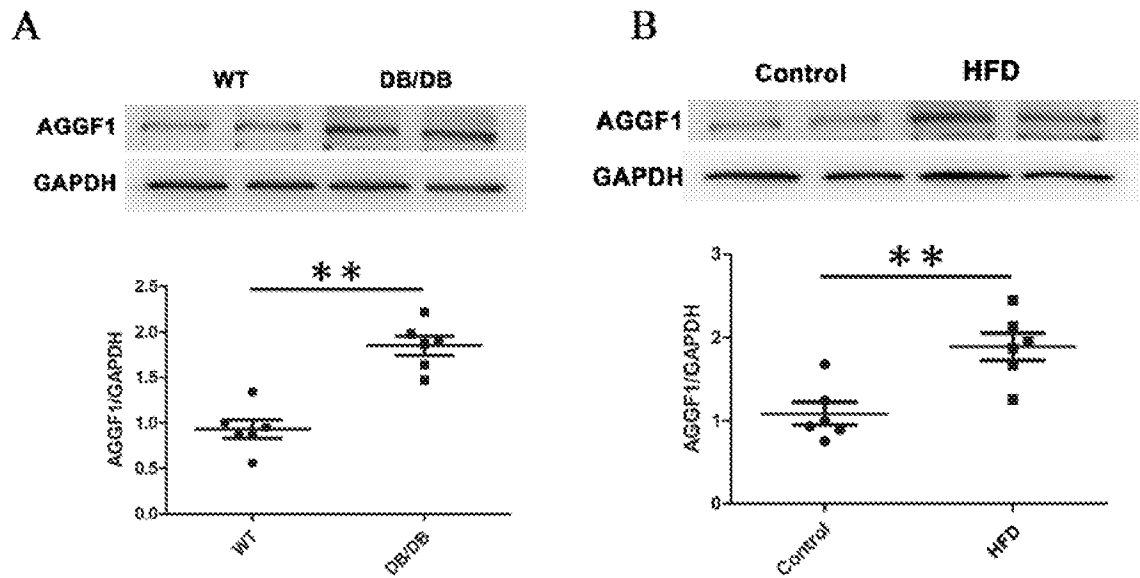


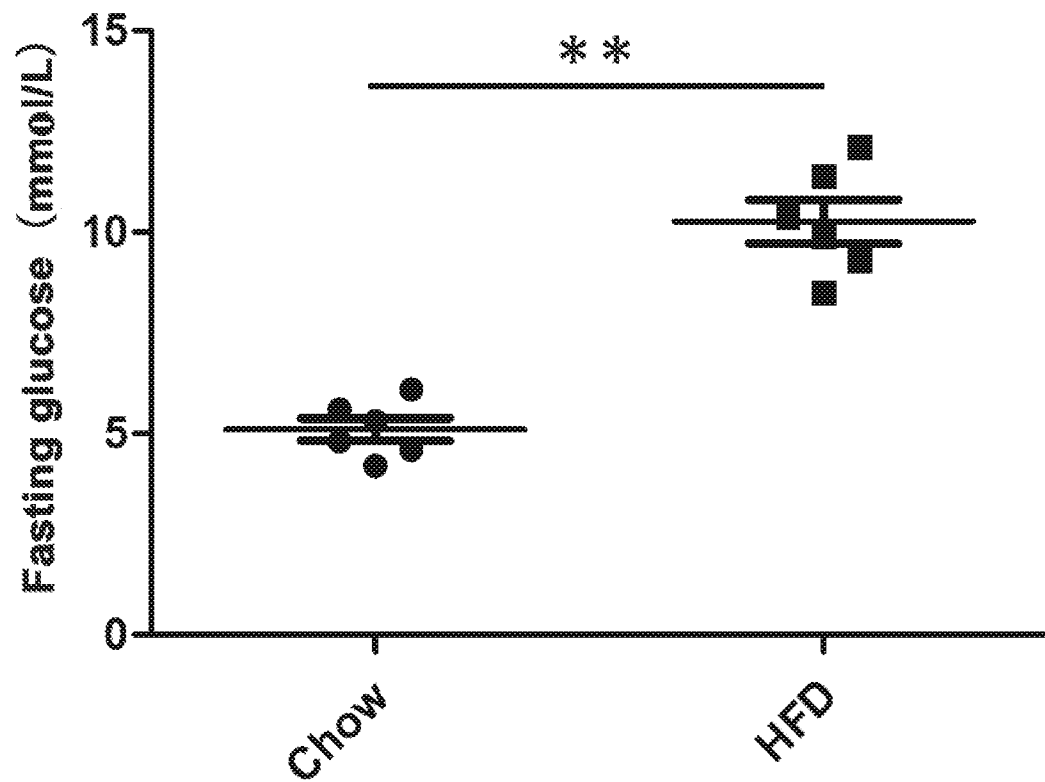
FIG. 28

FIG. 29

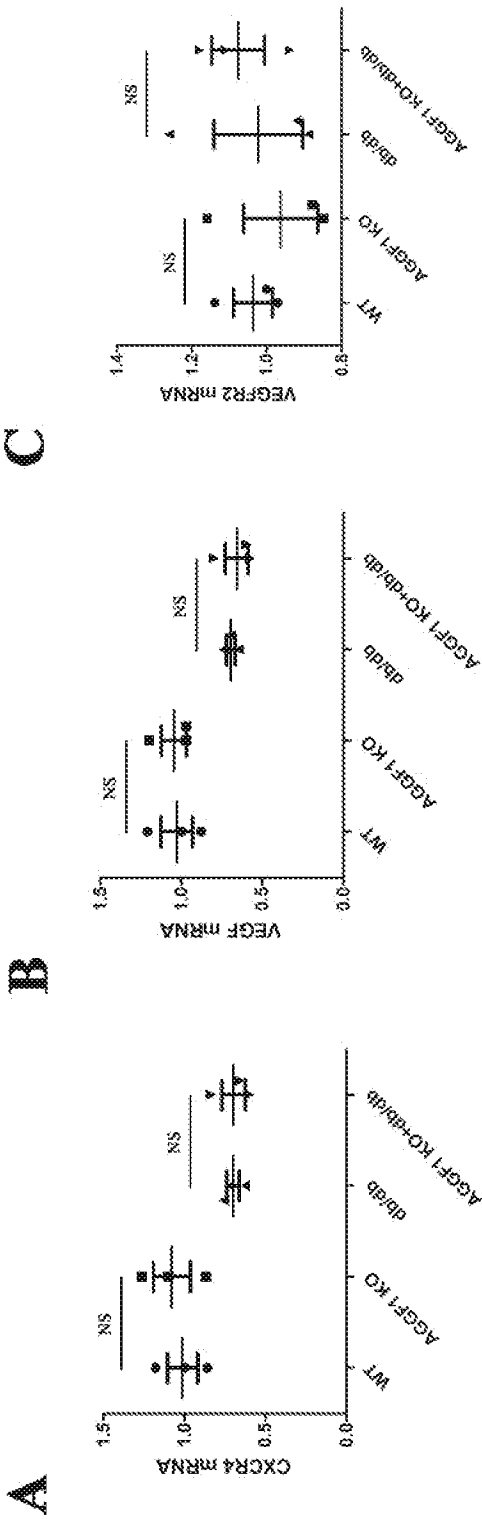


FIG. 30

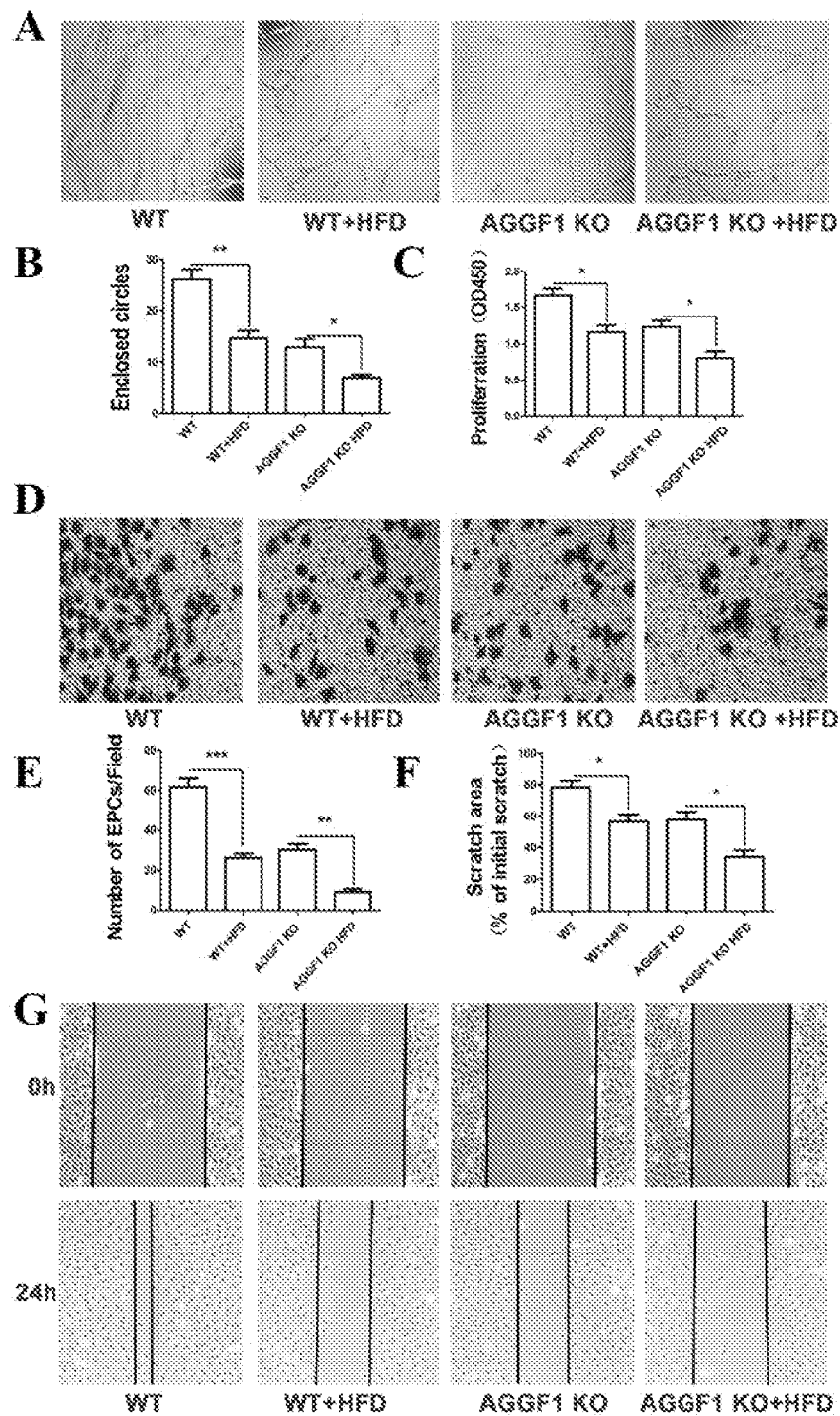


FIG. 31

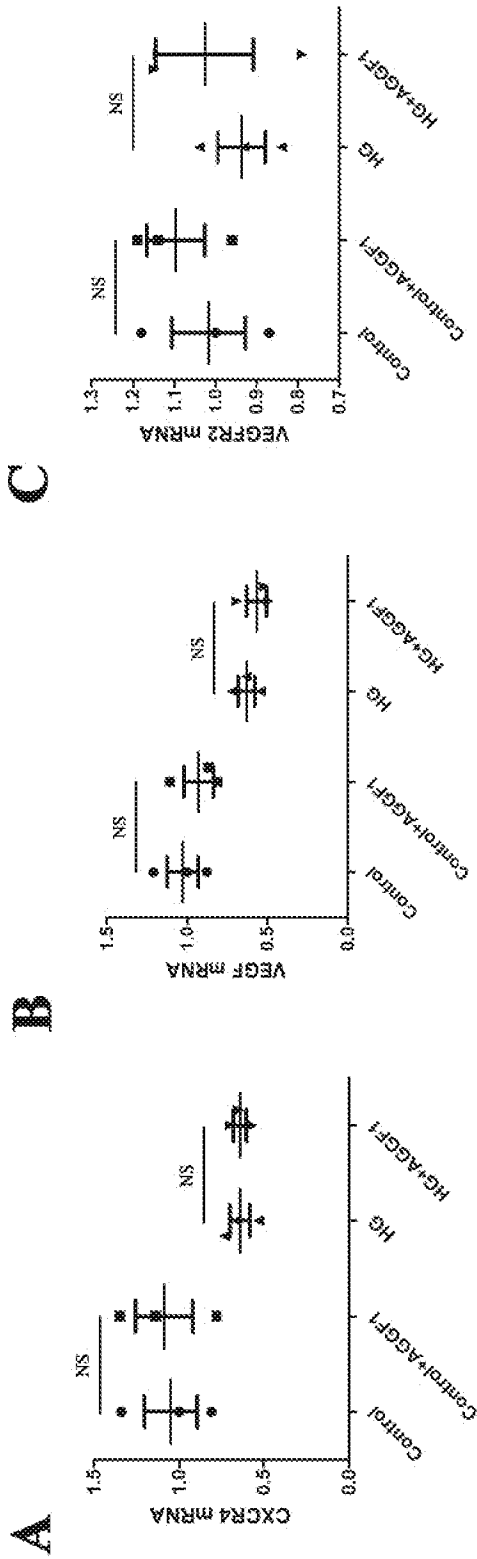


FIG. 32

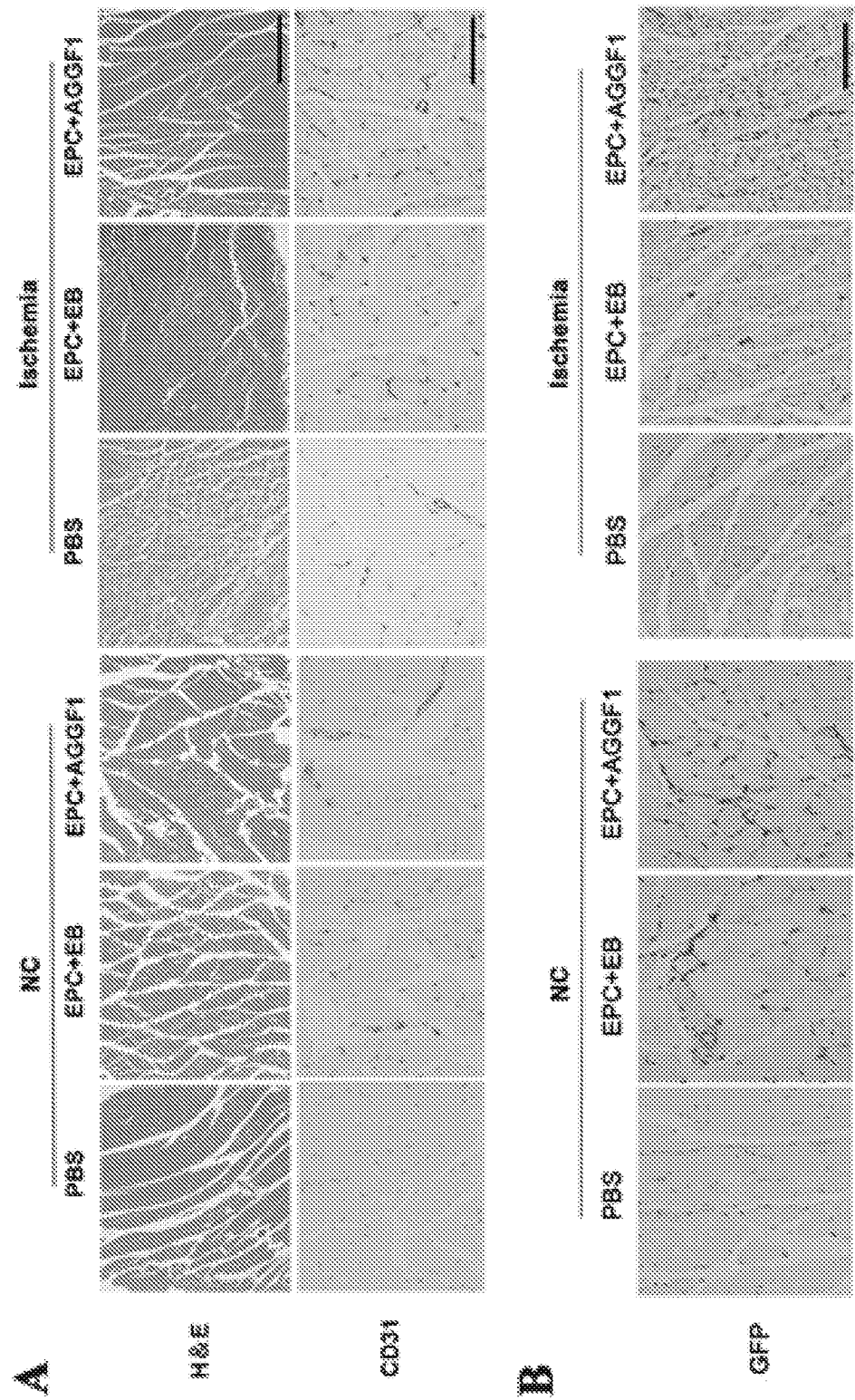


FIG. 33

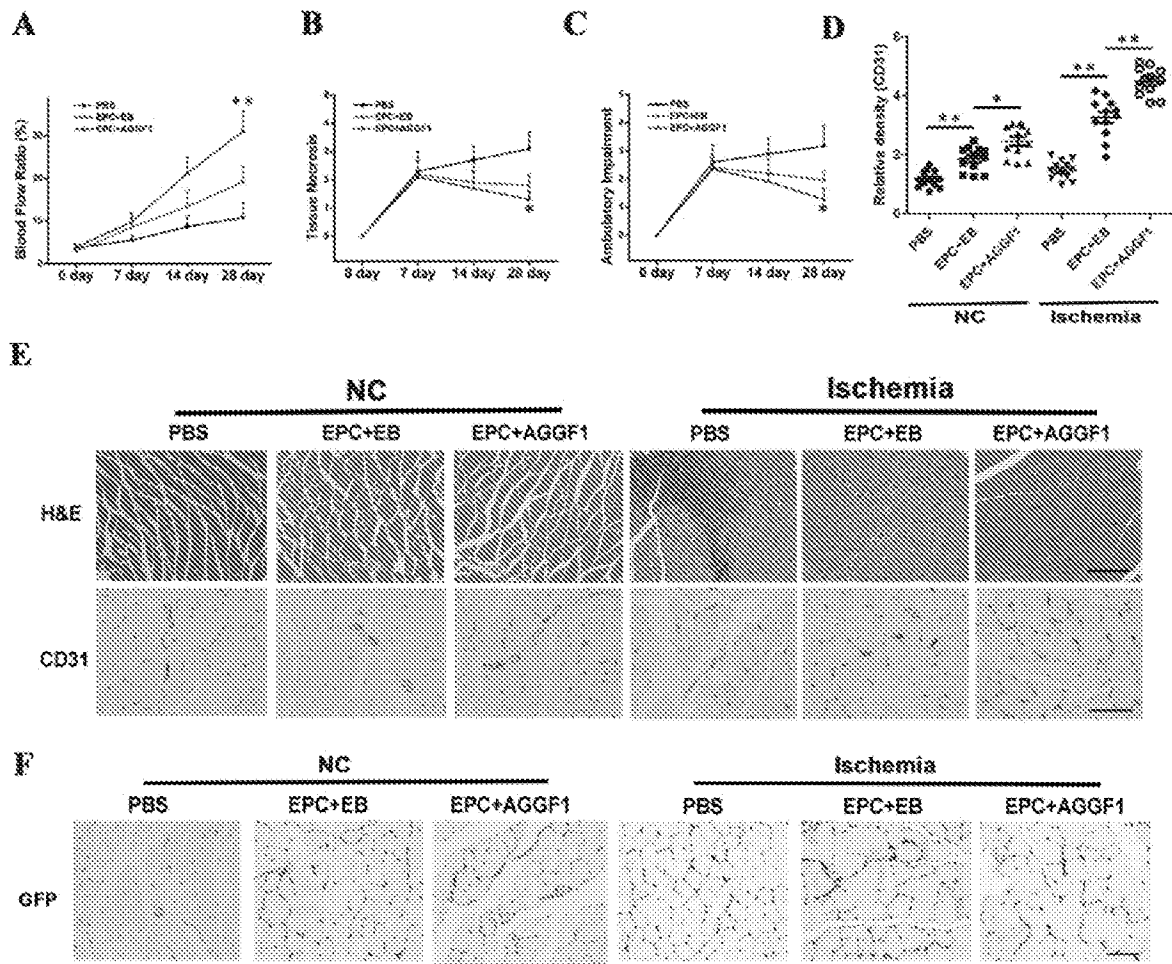
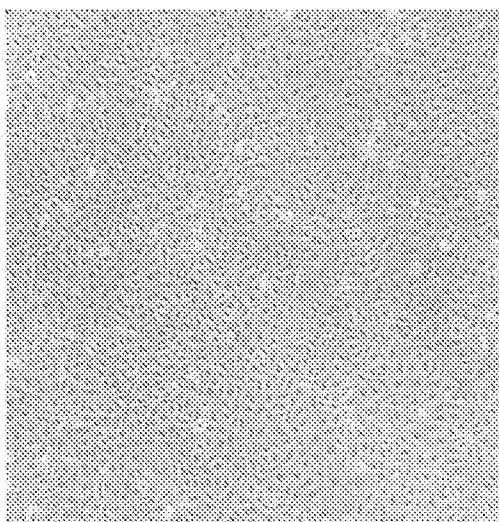


FIG. 34

White



GFP

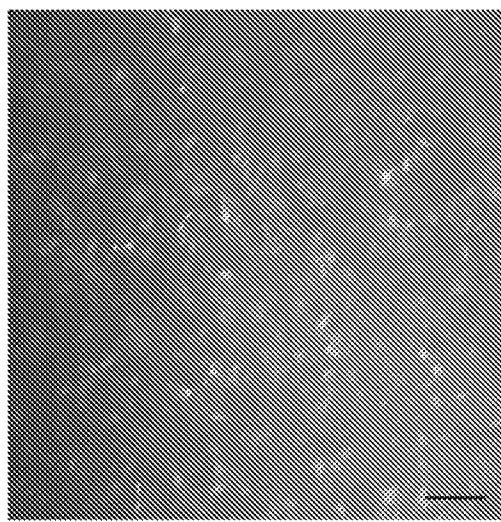


FIG. 35

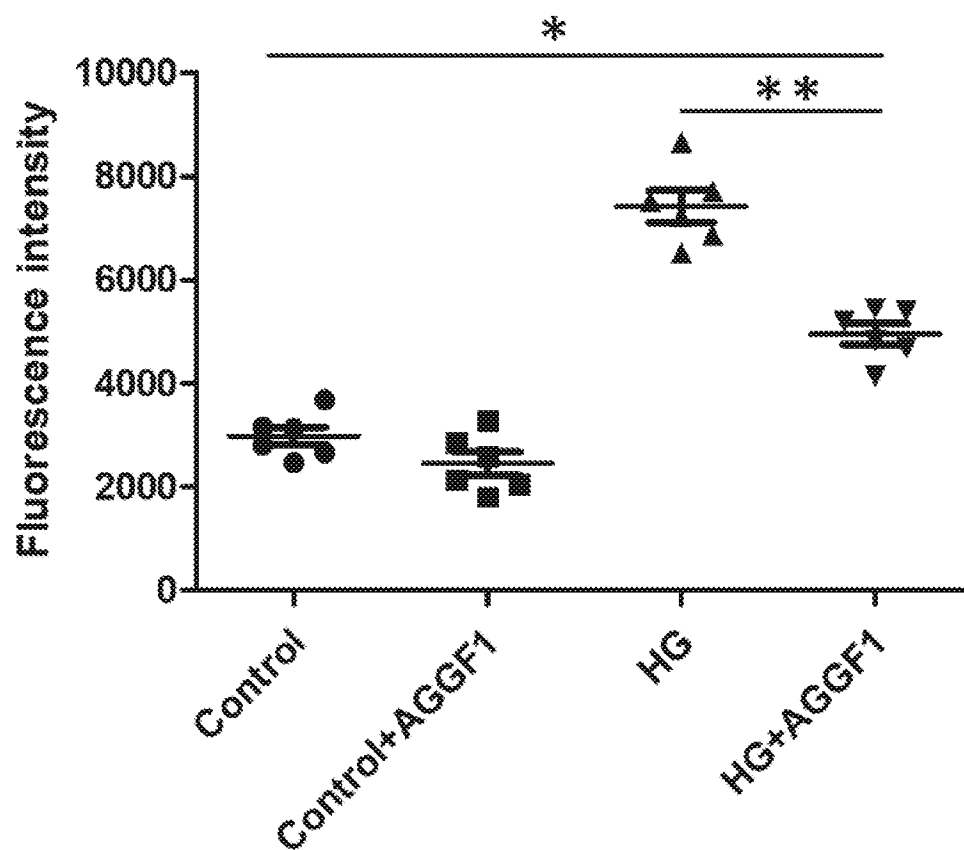


FIG. 36

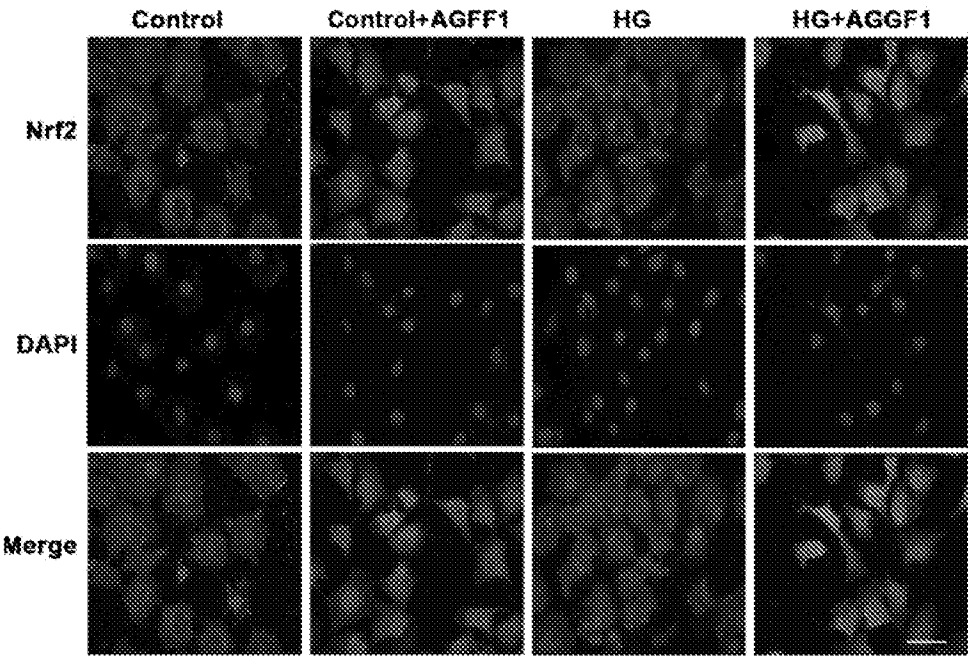


FIG. 37

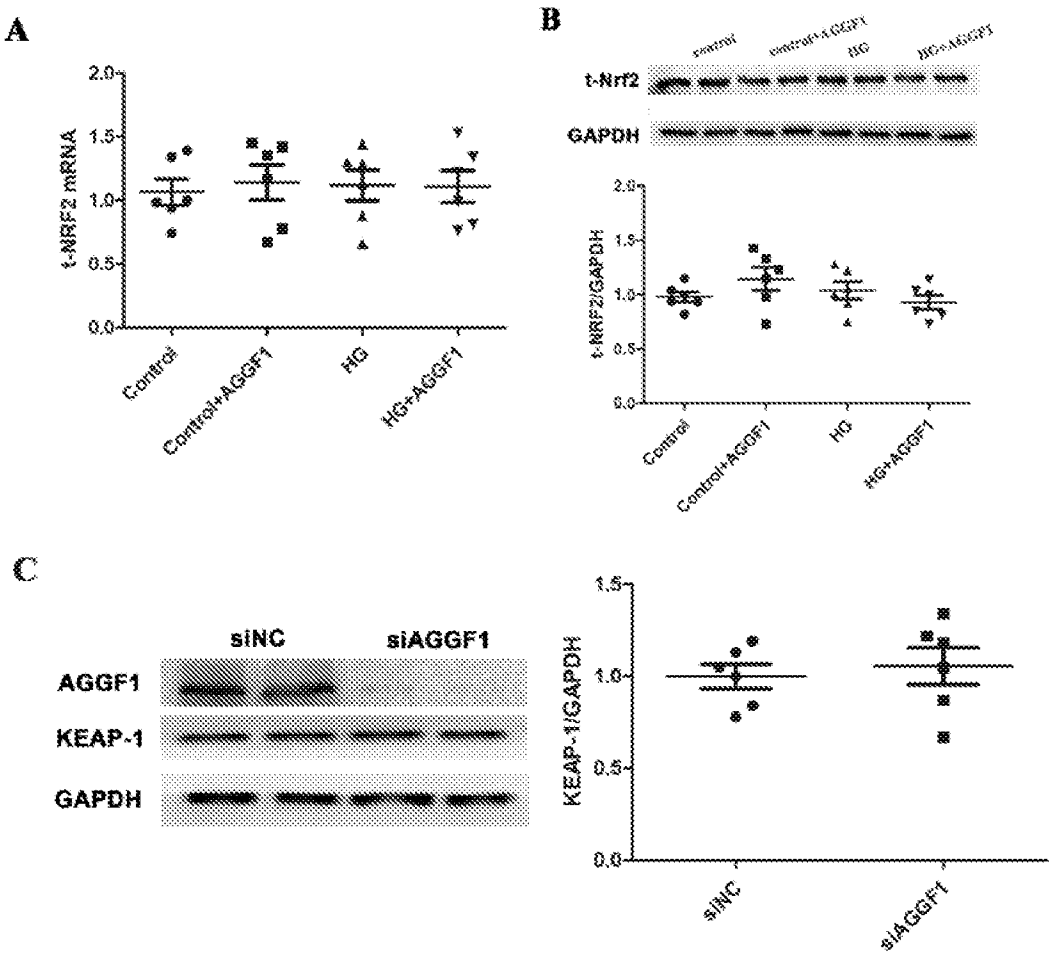


FIG. 38

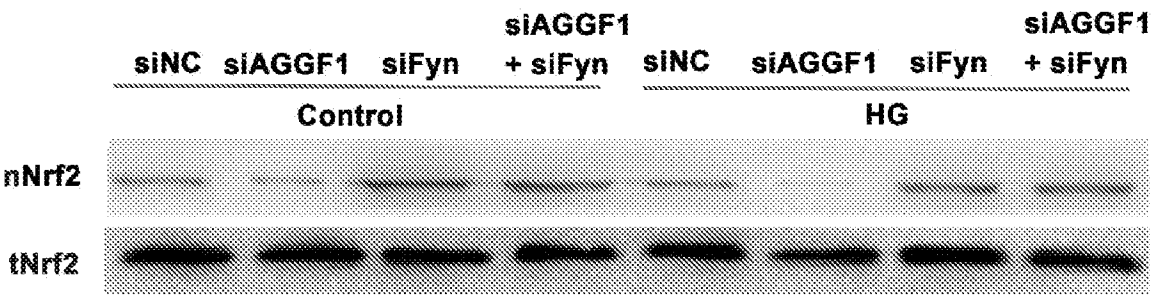


FIG. 39

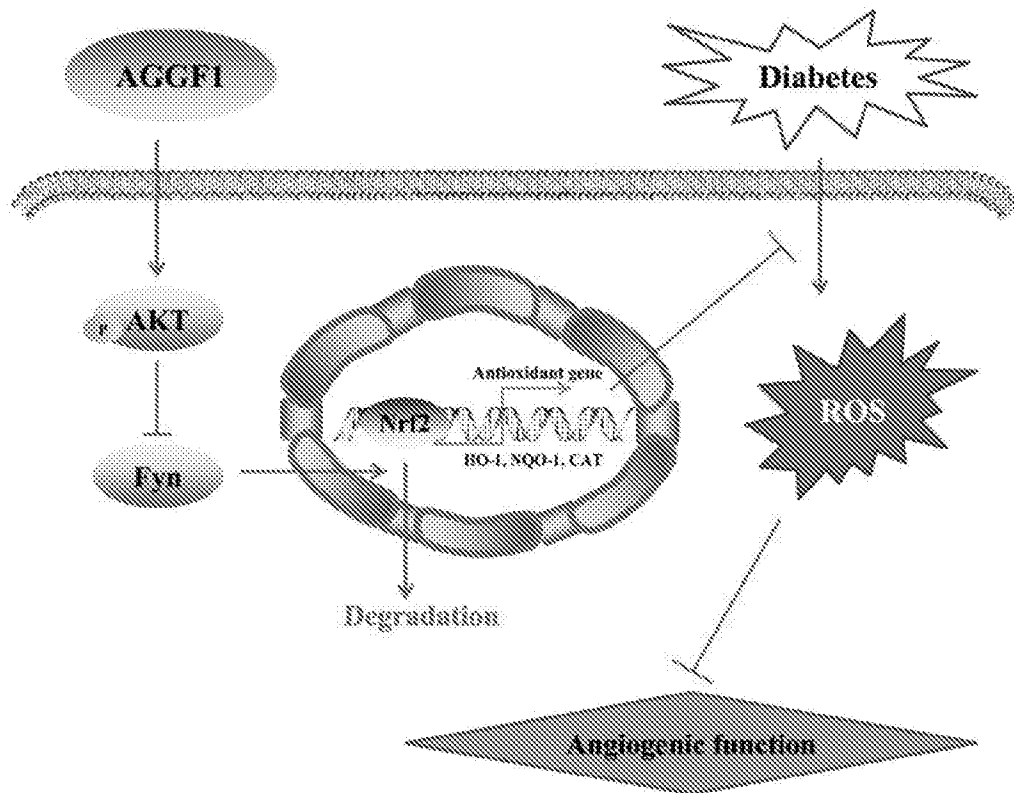
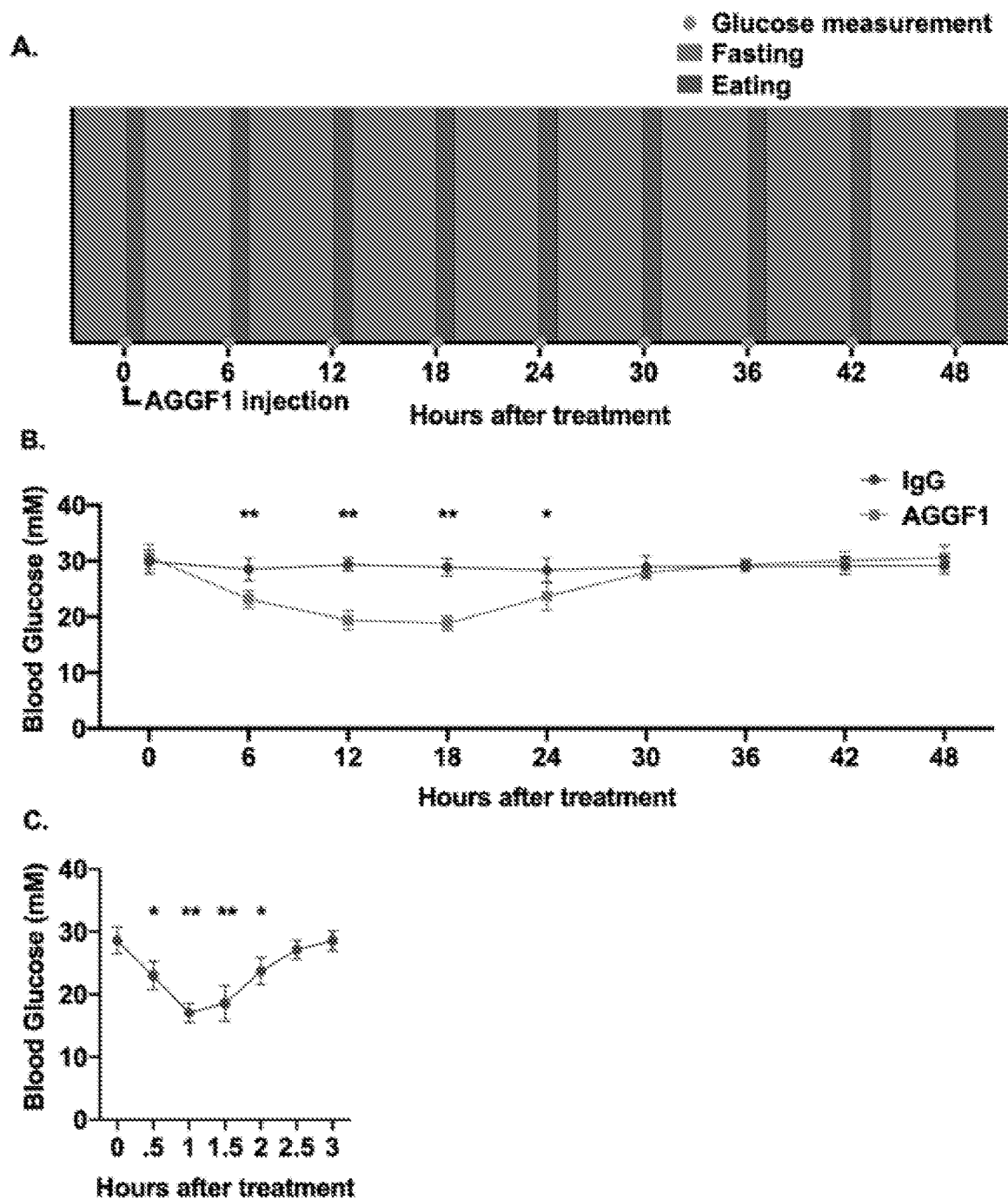


FIG. 40



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/055513

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/00; A61K 38/18; A61P 9/00; A61P 9/10; C07K 14/475; C12N 5/10 (2019.01)

CPC - A61K 38/00; A61K 38/18; C07K 14/475; C07K 14/515 (2019.08)

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

USPC - 435/375; 514/7.6; 514/13.3; 530/350; 536/23.5 (keyword delimited)

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	US 2013/0058940 A1 (WANG et al) 07 March 2013 (07.03.2013) entire document	1-4, 6-14, 25-28, 30
Y		5, 15-24, 31, 33-53
Y	US 2017/0087217 A1 (MANNKIND CORPORATION) 30 March 2017 (30.03.2017) entire document	5, 20-24
Y	US 2012/0122227 A1 (WIEGEL et al) 17 May 2012 (17.05.2012) entire document	15-17
Y	US 2006/0074404 A1 (STRUBLE) 06 April 2006 (06.04.2006) entire document	18, 19
Y	US 2017/0312317 A1 (STEWART et al) 02 November 2017 (02.11.2017) entire document	31, 33
Y	US 2007/0059288 A1 (DINSMORE et al) 15 March 2007 (15.03.2007) entire document	34-47, 49, 51-53
Y	WO 2007/049096 A1 (INSERM (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE) et al) 03 May 2007 (03.05.2007) entire document	45-50
P, X	YAO et al. "Angiogenic Factor AGGF1-Primed Endothelial Progenitor Cells Repair Vascular Defect in Diabetic Mice," Diabetes, 15 May 2019 (15.05.2019), Vol. 68, Pgs. 1635-1648. entire document	1-53
A	BARKER et al. "Is the E133K allele of VG5Q associated with Klippel-Trenaunay and other overgrowth syndromes?," J Med Genet, 27 January 2006 (27.01.2006), Vol. 43, Pgs. 613-614. entire document	1-53

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

13 December 2019

Date of mailing of the international search report

09 JAN 2020

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/055513

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIU et al. "AGGF1 protects from myocardial ischemia/reperfusion injury by regulating myocardial apoptosis and angiogenesis," Apoptosis, 04 June 2014 (04.06.2014), Vol. 19, Pgs. 1254-1268. entire document	1-53

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/055513

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:
☒ in the form of an Annex C/ST.25 text file.
☐ on paper or in the form of an image file.
- b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-16, 18, and 25 were searched.