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Supplementary Information for

Vitexin Inhibits APEX1 to Counteract the Flow-Induced Endothelial Inflammation

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Supplemental Materials and Methods

Cell culture. Human umbilical vein endothelial cells (HUVECs) were isolated from umbilical cords from healthy patients after full-term deliveries. Umbilical cords were obtained with the agreement of the patients and approved by the Peking University People's Hospital Medical Ethics Committee (2015PHB024). HUVECs within passages 5–8 were maintained in Medium 199 supplemented with 10% fetal bovine serum (FBS) (Gemini 900-008), 4 μg/mL of endothelial cell growth factor (ECGF) (Sigma E1388), 1% penicillin/streptomycin at 37 °C in an incubator with 95% humidified air and 5% CO_2 and passaged every 3 days.

Flow experiments. Monocultured HUVECs seeded on collagen I (50 μg/mL)-coated glass slides were subjected to shear stress in a parallel-plate flow apparatus as described previously (1). The flow channel in the chamber was created by a silicon gasket with dimensions of 2.5 cm in width (w), 5.0 cm in length, and 0.025 cm in height (h). The chamber containing the cell-seeded glass slide fastened with the gasket was connected to a perfusion loop system, kept in a constant-temperature controlled enclosure, with pH maintained at 7.4 by continuous gassing with a humidified mixture of 5% (vol/vol) CO_2, 20% (vol/vol) O_2, and 75% (vol/vol) N_2. The shear stress (τ) generated on the HUVECs was estimated as 6Qμ/wh2, where Q is flow rate, w is dimension in width and μ is perfusate viscosity. The flow of pulsatile shear (PS, 12 ± 4 dynes/cm^2) or oscillatory shear (OS, 0.5 ± 4 dynes/cm^2) is composed of mean flow with shear stress at 12 dynes/cm^2 or 0.5 dynes/cm^2 supplied by a sinusoidal oscillation using a piston pump with a frequency of 1 Hz and a peak-to-peak amplitude of ± 4 dynes/cm^2. HUVECs were perfused in M199 medium containing 2% FBS for the indicated time.

Connectivity Map (CMap)-based drug screening. The CMap database, composed of 7,056 gene expression profiles induced by 1,309 small molecule compounds, was utilized to search for compounds that produce similar gene signature as induced by PS vs. OS. The gene expressions of HUVECs subjected to PS (12 ± 4 dyn /cm^2, 1 Hz) or OS (0.5 ± 4 dyn/cm^2, 1 Hz) for 24 hours were analyzed and the differentially expressed genes were classified into up-regulated and down-regulated groups ($|\log_2$ Fold Change$| \geqslant 0.58$). These genes from the two groups were uploaded to the CMap online tool for calculating. The enrichment scores (also termed as the connectivity scores) were obtained. The connectivity score, assuming values from -1 to +1, reflects the closeness or connection between the expression profiles. A positive value of the score denotes the degree of similarity and a negative value represents an inverse similarity between a query signature and a reference profile. A null score occurs when the up- and downregulated genes are randomly distributed over the reference profile.

Drugs. Vitexin (>98%, HPLC) was purchased from Sangon Biotech and was diluted in DMSO with a storage concentration of 100 mmol/L for in vitro test. Vitexin was dissolved in normal saline (NaCl, 0.9%) for animal intraperitoneal injection (2, 3). E3330, a known APEX1 redox inhibitor, was from Selleck and was diluted in DMSO with a storage concentration of 100 mmol/L. 100×Histone deacetylase inhibitor cocktail (40 μmol/L Trichostatin A, 1 mmol/L EX-527, 400 mmol/L nicotinamide and 200 mmol/L sodium butyrate in 70% DMSO) was from Beyotime and was used 1×in each experiment.

Transcriptome Analysis. HUVECs pre-incubated with DMSO or vitexin for 24 hours were subjected to either PS or OS for 6 hours. Total RNA from the cells was extracted using the RNeasy kit with DNase treatment (Qiagen) and RNA integrity (RIN > 9) was checked by a Bioanalyzer 2100 system (Agilent Technologies). cDNA libraries were constructed using the NEBNext UltraTM RNA Library Prep Kit according to the manufacturer's instructions (Illumina). Cluster was generated using cBot Cluster Generation System with TruSeq PE Cluster Kit v3-cBot-HS (Illumia). After cluster generation, sequencing was performed on an Illumina Novaseq platform and 150 bp paired-end reads were generated. Index of the reference genome was built using Hisat2 v2.0.5 and paired-end clean reads were aligned to the human genome using Hisat2 v2.0.5. FeatureCounts v1.5.0-p3 was used to count the reads numbers mapped to each gene. FPKM of each gene was calculated based on the length of the gene and reads count mapped to

this gene. Differential expression analysis was performed using the DESeq2 R package (1.20.0). The resulting P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. In total, 2,377 genes were differentially expressed in any of the pairwise comparisons (n = 3, | log2 Fold Change | >0, P-value < 0.05). Pathway over-representation analysis was performed using clusterProfiler R package to test the statistical enrichment of differential expression genes in KEGG database (http://www.genome.jp/kegg/).

RNA Extract and Quantitative real-time PCR. RNA was extracted by using Trizol Reagent (Applygen) according to the manufacturer's protocol. The cDNA was obtained by reverse transcription using M-MLV Reverse transcriptase (Invitrogen) with oligo-dT as primers. Quantitative PCR was performed using SYBR Select (YEASEN) following the manufacturer's protocol. Primers used for PCR are shown in Table S2. The initial denaturation step of PCR amplification was 95℃ for 10 minutes, followed by 40 cycles of 95℃ for 15 seconds and 55℃ for 1 minute, then melting at 95℃ for 15 seconds and 60℃ for 1 minute, and last at 95℃ for 1 second. Gene expressions were normalized against GAPDH.

Western blotting. Cells were homogenized in cold RIPA lysis buffer (25 mM HEPES, pH 7.4, 1% Triton X-100, 1% deoxycholate, 0.1% SDS, 125 mM NaCl, 5 mM EDTA, 50 mM NaF) supplemented with complete protease inhibitors cocktail. Equal amounts of protein were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to nitrocellulose membranes. Non-specific binding was blocked in 5% skim milk in Tris-buffered saline (TBS) containing 0.1% Tween-20. The membrane was incubated with specific primary antibodies overnight at 4°C: VCAM-1(Cell Signaling Technology, 1:1000), ICAM-1 (Proteintech, 1:1000), SELE (Proteintech, 1:1000), APEX1 (Proteintech, 1:1000), NFκB-p50/105 (Huabio, 1:1000), NFκB-p65/c-Rel (Huabio, 1:1000), Pan Acetyl-Lysine (ABclonal, 1:1000), total-p300 (Affinity, 1:1000), phospho-p300 (S1834) (Affinity, 1:1000), GAPDH (Santa Cruz Biotech, 1:1000). Bound antibodies were detected by horseradish-peroxidase-conjugated secondary antibody and visualized by enhanced chemiluminescence.

Monocyte adhesion assay. HUVECs were pre-incubated with DMSO or vitexin for 24 hours then exposed to either PS or OS for 6 hours or stimulated with TNFα for 6 hours. Human peripheral blood mononuclear leukocytes (THP-1 cells) were cultured in RPMI 1640 medium (Invitrogen) and were labeled with CM-Dil (Invitrogen, 1 μg/ml) at 37 °C for 30 minutes, followed by wash in phosphate-bufferd saline (PBS) for 3 times. Resuspended in serum free 1640 medium, about 1×10^5 THP-1 cells were added onto the treated HUVECs and then incubated for 1 hour at 37℃. Followed by PBS wash for 3 times, the adherent monocytes were fixed with 4% paraformaldehyde for 20 minutes. Cell were counted in 10 randomly selected microscopic fields under an inverted epi-fluorescence microscope.

Cell permeability assay. Collagen-precoated culture inserts containing a polytetrafluoroethylene membrane with 0.4 μm diameter pores and a growth area of 1 cm^2 were placed in 12-well plates. At least 1×10^5 HUVECs in 0.5 ml of complete medium were seeded on the upper side of the membrane, while 1.5 ml medium was added into the lower compartment. Cultures were grown for 3 days to confluence. DMSO or vitexin in complete medium were added into the upper compartment of the transwell and incubated for 24 hours at 37°C, followed by TNFα stimulation for 6 hours. For detection of macromolecular passage across the filter inserts, the upper side medium was replaced with a tracer solution containing FITC-Dextran (mol weight 40, 000, 1 mg/ml) in complete medium, whereas medium in the lower compartment was replaced with 1.5 ml of fresh medium. After 1 hour-incubation, 50 μl of medium in the lower compartment were diluted with 50 μl of PBS, followed by measurement on a fluorospectrophotometer at emission/excitation wavelengths 495/520 nm. The fluorescence intensity of fresh medium diluted with PBS (1:1) served as a negative control, which was defined as 0%. While the 50 μl medium in the lower compartment without cells in the membrane, diluted with 50 μl of PBS served as a positive control, which was defined as 100%.

Detection of ROS production. ROS production was detected with DHE kit (KeyGEN BioTECH) according to the manufacturer's instructions. In brief, cells were pre-incubated for 24 hours with DMSO or vitexin, subjected for 6 hours to either PS or OS, and were then incubated with DHE (25 μM in M199 basal medium) for 30 minutes at room temperature. After being washed 3 times with PBS, the fluorescence of cells was measured by flow cytometry.

Cell fractionation. Cells were washed with PBS and lysed by gentle pipetting with buffer A, containing 10 mM Tris (pH 7.5), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5% NP-40 and proteinase inhibitor. Cytoplasmic and membrane proteins in the supernatant were acquired by centrifugation at 7,000 rpm for 5 minutes. Nuclear proteins were extracted at 4 ℃ by gentle resuspension with buffer B, containing 20 mM Tris (pH 7.5), 1.5 mM $MgCl_2$, 420 mM NaCl, 10% Glycerol, 0.2 mM EGTA and proteinase inhibitor, followed by platform incubation for 30 minutes. Nuclear proteins were then obtained by centrifugation at 13,000 rpm for 15 minutes. All the fractions were analyzed by western blotting assay.

Cellular thermal shift assay (CETSA). The procedures were modified from the previous report (4). Briefly, intact HUVECs were pretreated with DMSO, vitexin or E3330 for 24 hours and collected and rinsed with PBS for 2 times. After being resuspended in PBS with complete protease inhibitor cocktail, the cell suspensions were divided into seven equal aliquots and centrifuged at 1,000 rpm for 5 minutes. The supernatants were discarded. The cell pellets were heated to different temperatures, incubated for three minutes, and then resuspended in PBS containing complete protease inhibitor cocktail. After quickly frozen and thawed twice in liquid nitrogen, the supernatants containing the soluble proteins were collected by centrifugation at 20,000 g for 20 minutes. For the cell lysate CETSA experiments, HUVECs were harvested and suspended in PBS supplemented with complete protease inhibitor cocktail. The cell suspensions were freeze-thawed three times and the soluble fractions (lysate) were separated from the cell debris by centrifugation at 20,000 g for 20 minutes at 4°C. The cell lysates were diluted with appropriate buffers and divided into two aliquots, with one treated with DMSO and the other aliquot with vitexin. After incubation at room temperature, the respective lysates were divided into smaller aliquots (50 μL) and heated individually at different temperatures for three minutes followed by cooling for 3 minutes at room temperature. The heated lysates were centrifuged at 20,000 g for 20 minutes at 4°C in order to separate the soluble fractions from precipitates. The supernatants were transferred to new microtubes and analyzed by western blotting experiment. The band intensities were quantified using ImageJ-win 64 (Fiji) and normalized to the loading controls. The melting curve were fitted using IC50 nonlinear regression curve in GraphPad Prism. The dose-response curves were fitted using EC50 nonlinear regression curve in GraphPad Prism.

Surface plasmon resonance (SPR). The Biacore T200 system (GE Healthcare, Uppsala, Sweden) was used for measuring the binding affinities (5). 100 μl of N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N-ethyl carbodiimide hydrochloride (EDC) were injected at a flow rate of 10 μL/min twice for 600 seconds to activate the dextran on CM5 sensor chip surface. Human recombinant APEX1 proteins (SinoBiological) were dissolved with sodium acetate solution pH 5.5 to a concentration of 20 μg/ml and total 400 μL immobilized on the CM5 sensor chip by amino coupling method. 200 μl of ethanolamine hydrochloride (ETH) were used to seal dextran that did not bind to the protein on the chip surface. APEX1 was immobilized on a CM5 sensor chip (GE Healthcare) by amine coupling to reach target densities of 7000 resonance units (RU). The running buffer contained PBS-P (20 mM sodium phosphate, 137 mM NaCl, 2.7 mM KCl, 0.05% Surfactant P20, pH 7.4) and 5% DMSO. Data were recorded at 25 °C. After solvent calibration and testing with 4.5% DMSO and 5.8% DMSO in PBS-P, the regeneration conditions were explored. The regeneration solution in this experiment is 2.5 mmol/L sodium hydroxide solution.

Vitexin (1.5625 μM to 100 μM) was injected at a flow rate of 30 μL/min. The contact time and dissociation time were set both at 60 seconds. Following each binding cycle, the surface was

regenerated with a 60 s injection of 2.5 mmol/L sodium hydroxide solution, removing the bound. E3330 (12.5 μM to 200 μM) was employed as a positive control. Ka (the association constant), Kd (the dissociation constant), and KD (the equilibrium dissociation constant) were calculated by the Biacore T200 Evaluation Software. The bound ability of compounds to APEX1 protein was evaluated by KD.

Immunoprecipitation and co-immunoprecipitation assays. Cells were trypsinized and lysed with Lysis Buffer containing 25 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 0.5% NP-40, 0.5% sodium deoxycholate, 5 mmol/L EDTA and a protease inhibitor Cocktail (Baidiebio). The supernatant containing soluble proteins was collected by centrifugation at 13,000 rpm for 15 minutes. Protein concentration of the lysate was adjusted to be 1.5-2 μg/μL. 1-1.5 μg of antibody against APEX1 or control rabbit IgG were added into 100 μl of cell lysate, followed by an overnight incubation at 4℃ on a rotator. The immune complexes were pulled down with protein A/G Sepharose beads (Santa) for 1-4 hours at 4℃ and washed with the lysis buffer for 3 times. 50 μl of 2 × SDS buffer were added into each sample, which was then subjected to Western blotting analysis. The blot intensities were quantified using ImageJ-win 64 (Fiji) and normalized to the respective internal controls.

SiRNA-mediated gene silencing. HUVECs were transfected in suspension using the Lonza nucleofector. For loss-of-function studies of APEX1 and NFκB, cells at 80% confluence were transfected with siRNAs specific for APEX1, IκBα, or the scrambled siRNA (Shown in Table S2). 10^7 cells were mixed with 500 μl nucleofection buffer and the pre-defined HUVEC nucleofection protocol on the instrument was used for electroporation of 50 nmol/L siRNA, in agreement with standard nucleofection protocols. After electroporation, the nucleofection buffer with cells was added to fresh culture media of endothelial cells without growth factors. The cells were further cultured for 48 hours.

Plasmid transfection and luciferase activity assay. HEK293T cells cultured to sub-confluence in 6-well plates were transfected with the firefly luciferase reporter plasmid of NFκB containing a TA promoter (pNF-κB-TA-luc, Beyotime Biotechnology) along with a β-galactosidase reporter plasmid (Progema) and siAPEX1 or scrambled siRNA by using lipofectamine reagent (Invitrogen) as instructed. 48-hours later, the cells were treated with TNFα (10 ng/ml) for another 6 hours. The luciferase activity in cell lysates was measured by a dual luciferase reporter assay system (Progema).

MTT cell viability assay. HUVECs were seeded into 96-well plates at a density of 5× 10^3 cells/well. After being pretreated with DMSO or vitexin (25 μM to 200 μM) for 24 hours, MTT (Applygen) reagents were added into the cell culture media to a concentration of 5 mg/ml. After incubation with the media for 4 hours, the supernatant was removed, and 150 μl of DMSO were added into each well. The optical density (OD) at 490 nm was measured.

Animals. All animal studies were performed in accordance with the approved protocol of the Animal Care and Use Committee of Peking University and approved by the Ethics Committee of Peking University Health Science Center (LA2019262). $Apex1^{flox/flox}$ mice were generated at the Model Animal Research Center of Nanjing University as described previously (6). In brief, following integration to generate the floxed allele and subsequent removal of the selectable marker via Flp recombination, expression of the Cre recombinase drived deletion of exon 3, a major coding region of Apex1. $Apex1^{flox/flox}$ mice were maintained in a C57BL/6 background on standard chow and crossed with vascular endothelial-cadherin Cre recombinase-positive mice (Cdh5(PAC)-Cre^{ERT2}) (7), to generate the $Apex1^{flox/flox}$ Cdh5-Cre^{ERT2+} ($Apex1^{ECKO}$) mice. $Apex1^{flox/flox}$ Cdh5-Cre^{ERT2-} ($Apex1^{WT}$) littermates were used as the controls. Male apolipoprotein E deficient ($ApoE^{-/-}$) mice and C57BL/6 wild type (WT) mice were obtained from the Experimental Animal Center at Peking University Health Science Center (Beijing, China). Mice were housed in specific pathogen-free cages, 12-hour light-dark cycle, controlled temperature and humidity, and

had water and food ad libitum. Anesthetization and euthanasia were performed by intraperitoneal injection of sodium pentobarbital (50 mg/kg and 150 mg/kg, respectively).

En face Analysis of Aortic Endothelium. C57BL/6 wild-type mice (12 weeks old, 22-25 g) were anesthetized and fixed with 4% paraformaldehyde in PBS buffer for 10 minutes by perfusion through left cardiac ventricle under physiological pressure. Aortas were harvested, further fixed with 4% paraformaldehyde in PBS buffer for 20 minutes and were then longitudinally dissected with microdissecting scissors. The luminal surfaces of the aortas were exposed, blocked with 3% bovine serum albumin in PBS for 1 hour at room temperature, and were incubated with primary antibodies against APEX1 (Proteintech,1:100), CD31 (Santa Cruz,1:100) at 4℃ overnight. The aortas were washed three times with PBS and were then probed with secondary antibodies including Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:500) or Alexa Fluor 555-conjugated goat anti-mouse IgG (1:500). Nuclei were counterstained with DAPI for 5 minutes at room temperature. Image acquisition was performed using a laser-scanning confocal microscopy (Leica TCS SP8).

Partial carotid ligation. Partial ligation of carotid artery in mice was performed as we previously reported (8, 9). Briefly, the left carotid bifurcation of mice was exposed following a neck incision. Three branches (external carotid, internal carotid, and occipital) of the left carotid artery were ligated with a 6-0 silk suture, and the superior thyroid artery was left intact. The right carotid arteries were served as controls. All animals were divided into two groups of 10 animals each, and vitexin (5 mg/kg) or saline was injected intraperitoneally twice a week for 1 or 4 weeks. At one-week after the operation, three animals from each group were sacrificed and the vessels were perfused with a fixative (4% paraformaldehyde in PBS) under a pressure of 100 mmHg. The carotid arteries were harvested for immunofluorescent staining. The remaining animals were sacrificed at 4-weeks after the operation and their carotid arteries were harvested for Hematoxylin and Eosin staining.

Tail-cuff measurement of blood pressure. Blood pressure levels were recorded by using a CODA Mouse & Rat Tail-Cuff Blood Pressure System (Kent Scientific Co., Connecticut, USA) (10). Mice were placed in the restraint corridor and allowed at least 10 minutes of acclimation. The area was warmed with a heating pad and a quiet, dark environment was maintained to ensure reliable measurements within the parameters of this technology. The mice underwent 7 consecutive days of training sessions from 1 to 5 PM each day to become accustomed to the tail-cuff procedure. Five measurements were daily performed on each mouse and the blood pressure was the mean value of all successful measurements.

Immunofluorescent staining. Tissues were first washed with PBS buffer and adventitia was removed carefully, and then they were fixed with 4% paraformaldehyde and embedded in 20% sucrose solution before being frozen in TissueTek cutting medium (Sakura Finetek). 7-μm sections were processed for immunofluorescent analysis. The sections were further fixed with 4% paraformaldehyde for 20 minutes. For immunostaining of attached cells, HUVECs were fixed in 4% PFA for 20 minutes and permeabilized with 0.1% Triton X-100 (in PBS) for 5 minutes and rinsed for 3 times. Nonspecific binding was blocked by 3% BSA in PBS for 1 hours. Tissues/cells were incubated at 4 °C overnight in incubation buffer containing 3% BSA and the primary antibody including APEX1 (Proteintech, 1:200), VCAM-1 (Abcam, 1:200), ICAM-1 (Proteintech, 1:200), SELE (Proteintech, 1:200), CD31 (Santa Cruz, 1:100). After being washed in PBS for 3 times, the specimens were incubated with Alexa-Fluor 488-, Alexa-Fluor 555-conjugated secondary antibodies (ThermoFisher, 1:500) for 1 hour at room temperature. The fluorescent signals were detected by fluorescence microscopy (Leica DMI6000B; Leica TCS SP8).

Hematoxylin and Eosin staining. After rehydration, 7-μm sections were stained with hematoxylin solution for 20 seconds followed by 2 dips in 1% acid ethanol (1% HCl in 70% ethanol) and then rinsed in running water. Then the sections were stained with eosin solution for 3 min and followed by dehydration with graded alcohol (70%, 80%, 90%, 95%, 100%) and clearing in xylene.

Atherosclerosis model and oil red O staining. Male apolipoprotein E deficient ($ApoE^{-/-}$) mice (12 weeks, 22-27 g) on C57BL/6 background were randomly divided into two groups (n = 12, 24 mice in total), fed on a Western diet (Research Diets, D12108C, High fat rodent diet with 1.25% cholesterol), and vitexin (5 mg/kg) or saline was injected intraperitoneally twice a week for 12 weeks. The mice were sacrificed after 12 weeks of Western diet and atherosclerotic plaque formation was determined by Oil Red O and H&E staining. Mouse aortas were dissected in cold PBS and cut open to expose the atherosclerotic plaques. After fixation in 4% formaldehyde for 24 hours at 4 °C, the tissues were first rinsed with PBS for 10 minutes and then rinsed with 60% isopropanol at room temperature. The aortas were stained with Oil Red O (0.2% in 60% isopropanol) for 10 minutes with gentle shaking and rinsed again with 60% isopropanol and then with water for three times. For heart outflow tract sections, they were counterstain performed with Mayer's hematoxylin, washed and coverslipped with glycogelatin. The plaque areas were determined using ImageJ software and calculated by expressing the plaque area relative to the total vascular area.

In vivo delivery of virus. $ApoE^{-/-}$ and C57BL/6 wild type mice (10-12 weeks old, 20-23 g) were anesthetized and were then subjected to partial ligation of carotid artery. The mice were divided into 3 groups, one group was subjected to control adenovirus and control injection, one group was subjected to control adenovirus and vitexin injection, and the other group was subjected to Ad-APEX1 adenovirus (Vigene) and vitexin injection. For intraluminal incubation with adenovirus, the left common carotid artery was exposed by blunt dissection. The internal carotid and occipital arteries were ligated with 6–0 silk suture. The heart proximal end of the left common carotid artery was clamped temporarily. 20 μl of adenovirus (1×10^{12} particles) were injected into each common carotid artery through external carotid artery with a catheter and was retained intraluminal in the artery for 30 minutes. After that the external carotid artery was ligated while the superior thyroid artery was left intact. The clamp was then removed. Immediately after the surgery, mice were injected intraperitoneally twice per week with vitexin (5 mg/kg) or with control DMSO in saline. $ApoE^{-/-}$ mice were fed with high fat diet immediately after surgery. One or four weeks after ligation, the mice were sacrificed and fixed with 4% paraformaldehyde in PBS for 10 minutes by perfusion through the left cardiac ventricle under physiological pressure. The ligated carotid arteries were harvested and subjected to histology and immunostaining analyses of the vessels.

Genotyping. The genotypes of mice swabbed for genomic DNA were determined using the standard tail-clipping method. The distal 0.2 cm of the tail was removed from 3-week-old mice and the tissue digested in Buffer A (25 mmol/L NaOH,0.4% EDTA (0.5 mol/L, pH=8.0)) at 95 ℃ for 60 minutes, and then in Buffer B (4% Tris-HCl (1 mol/L, pH=8.0)). The genomic DNA was then extracted from the supernatant after centrifugation at 10,000 rpm for 5 minutes and may be stored at –80 °C. The Apex1 floxed (flanked by loxP site) genomic region was determined by PCR using appropriate 1 μg of genomic DNA in a 20-μl reaction. The initial denaturation step of PCR amplification was 95℃ for 5 minutes, followed by 20 cycles of 95℃ for 30 seconds, 65℃ for 30 seconds, 95℃ for 30 second, 55℃ for 30 seconds, 72℃ for 30 seconds, and last at 95℃ for 5 minutes. 10 μL of each reaction were run on a 2% agarose gel and stained with DiGreen Safe DNA Dye (DiNing). The forward and reverse primers for each gene analyzed are given in Table S2.

AAV9-PCSK9 production and purification. AAV9-PCSK9 viruses were produced and purified as described previously (11). The gain-of-function murine PCSK9 mutant plasmid (pAAV/D377Y-mPCSK9) was a gift from Jacob Bentzon (Addgene plasmid # 58376). AAV-plasmids were cloned and propagated in the DH5α E. coli strain (Life Technologies) in medium with ampicillin. Shuttle plasmid pAAV-D377Y mPCSK9 was packaged into capsids AAV9, using helper plasmids p-helper (providing the three adenoviral helper genes) and plasmid pAAV2/9 (providing rep and cap viral genes). The amplified AAV shuttle and helper plasmids were co-transfected into HEK293T cells by polyethyleneimine (PEI). A total of 420 μg of plasmid DNA (mixed in an equimolar ratio)

were used for 30 × 100-mm plates seeded with 1×10^7 cells per plate. 72 hours post-transfection, cell culture media and transfected cells were harvested separately. The cell pellet was suspended in 1 ml of PBS and 10% sodium deoxychalate, and then 50 µl of DNAase were added, sonicated with a 15-45 seconds cycle for 10 times. Cell debris was pelleted by spinning at 12,000g for 30 minutes at 4°C. The lysate and supernatant were sequentially filted through 0.45 µm filter and 0.22 µm filter. 40% polyethylene glycol (PEG) in 2.5 mol/L NaCl was added to the liquid to a final concentration of 8% and precipitated for 4 hours. The PEG-precipitated viruses were pelleted by centrifugation at 2,500g for 20 minutes at 4°C. The precipitations were washed by with 8% PEG, and then 20 ml of PBS with 4% sucrose was added to resuspend the pellets. The mixture was centrifuged at 2,500g for 15 minutes at 4°C to obtain the supernatant, which was then dialysised through an Amicon Ultra 100,000 MWCO concentration unit (Merck-Millipore) to acquire purified virus. The viruses were aliquoted and stored at −80°C.

Statistical analysis. Data are presented as mean ± SEM of the mean from at least three independent experiments as indicated. For in-vitro experiments, as each experimental data set is an average of a large number of cultured cells, we assumed the data was normally distributed based on the central limit theorem. For in-vivo experiments, the n value represents independent repeats or the numbers of animals. All analysis was performed using GraphPad Prism version 7.00. Normality of data distribution (raw or following logarithmic transformation) was tested with a D'Agostino-Pearson or Shapiro-Wilk test. For normally distributed data, differences between treatment groups were determined using paired or unpaired t-test for two groups of data and one-way or two-way ANOVA for multiple groups of data. Statistical significance among multiple groups was determined by post hoc analysis (Tukey honestly significant difference test). Nonparametric tests were used when data were not normally distributed, the Kruskal-Wallis test was conducted to test statistical significance for experiments with $\geqslant$ 3 groups followed by Dunn's post-hoc test, and the Mann-Whitney test was used to examine statistical significance between 2 groups. Values of $P < 0.05$ were considered statistically significant.

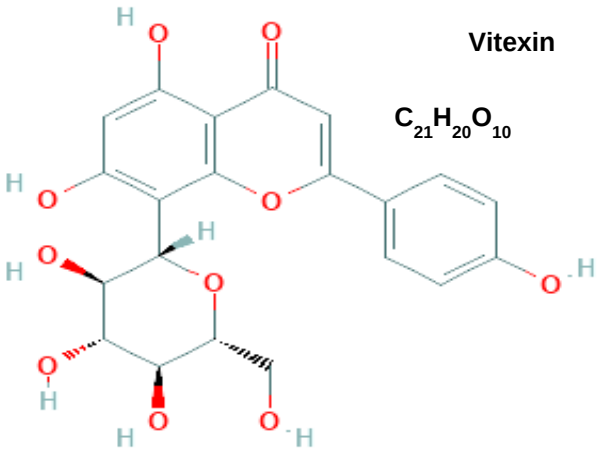

Fig. S1. The chemical structure of vitexin.

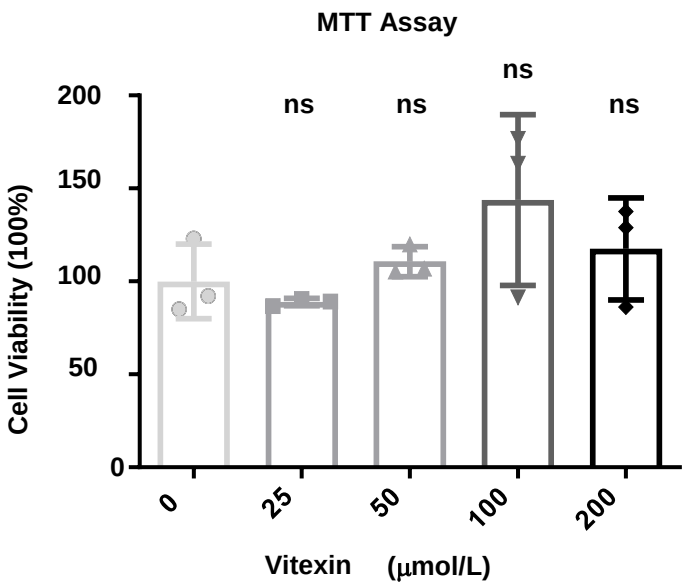

Fig. S2. Assay for cell viability in vitexin-treated cells. MTT cytotoxicity assay was performed to measure the viability of ECs treated with vitexin for 24 hours at the indicated concentrations (n=3). *P<0.05 by one-way ANOVA followed by Tukey's test.

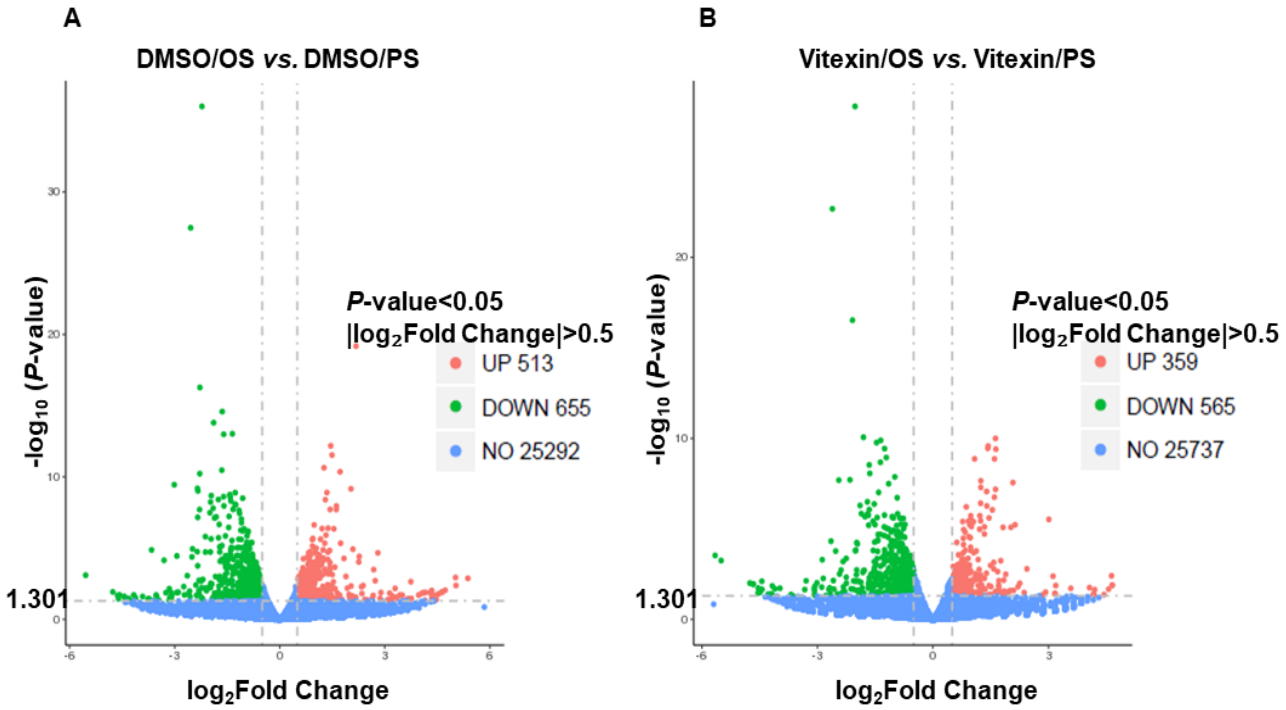

Fig. S3. Volcano plots showing the distribution of gene expression fold changes and P-values. (A) The transcriptome in ECs pretreated with DMSO for 24 hours and exposed for 6 hours to PS or OS. The x-axis indicates the $\log_2$ Fold Change and the y-axis represents the $-\log_{10}$ (P-value). Broken vertical and horizontal lines reflect the filtering criteria ($\log_2$ Fold Change=±0.5 and P-value=0.05). Red and green dots represent probe sets for transcripts expressed at significantly higher (gene number=513) or lower (gene number=655) levels of OS compared to PS, respectively. Blue dots indicate genes with no significant changes. (B) The transcriptome of ECs pretreated with vitexin (50 μmol/L) for 24 hours and exposed for 6 hours to PS or OS. Red and green dots represent probe sets for transcripts expressed at significantly higher (gene number=359) or lower (gene number=565) levels of OS compared to PS, respectively.

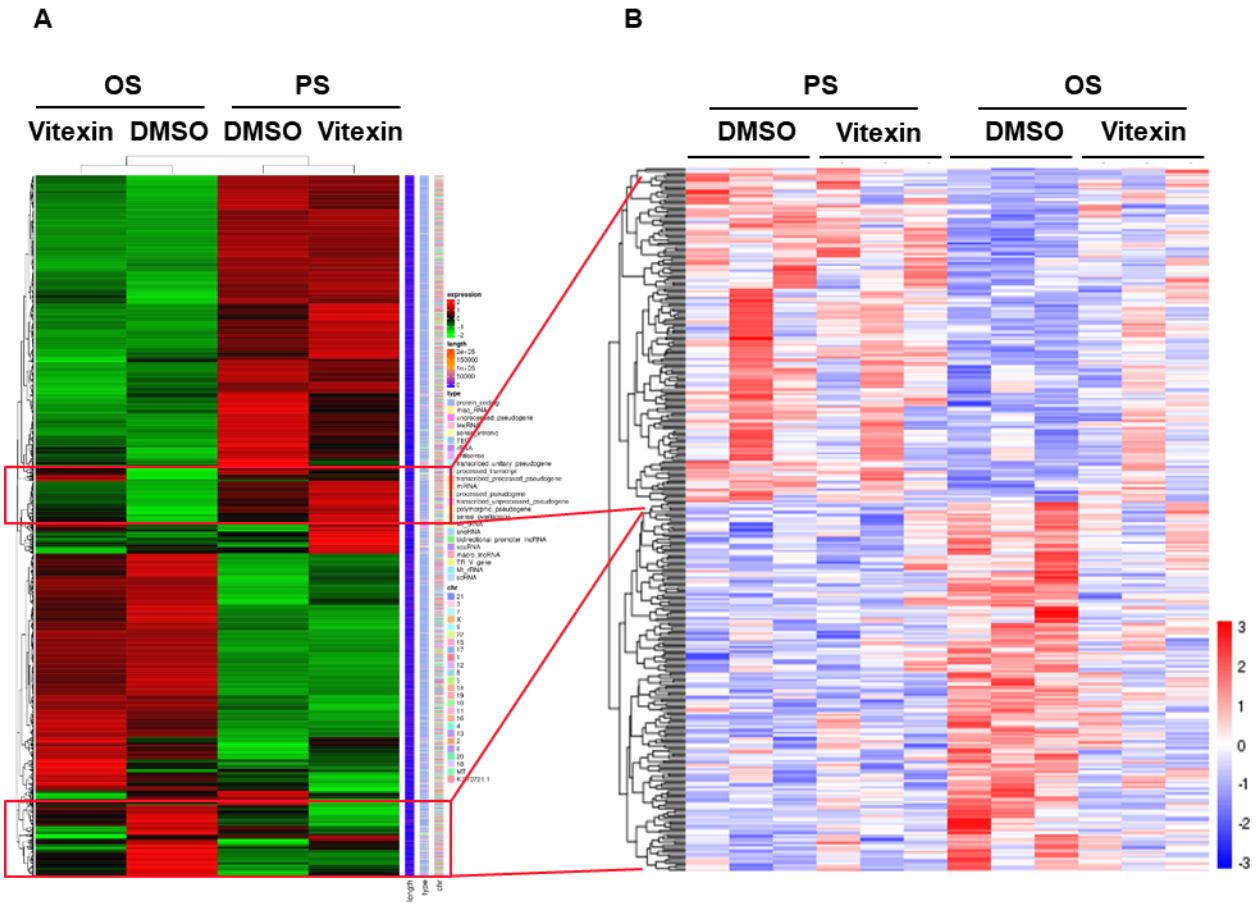

Fig. S4. The differentially expressed genes in DMSO- or vitexin-treated ECs with shear stress exposure. (A) Clustering and heatmap showing 2,377 genes differentially expressed in any of the pairwise comparisons (n=3, $|\log_2$ Fold Change$|$ >0, *P*-value<0.05). (B) Clustering and heatmap showing the expression of 322 genes selected from A. The inclusion criteria are specified as follows: The up- or down-regulation of gene expression (in DMSO groups) by OS vs. PS can be attenuated or reversed by the treatment with vitexin.

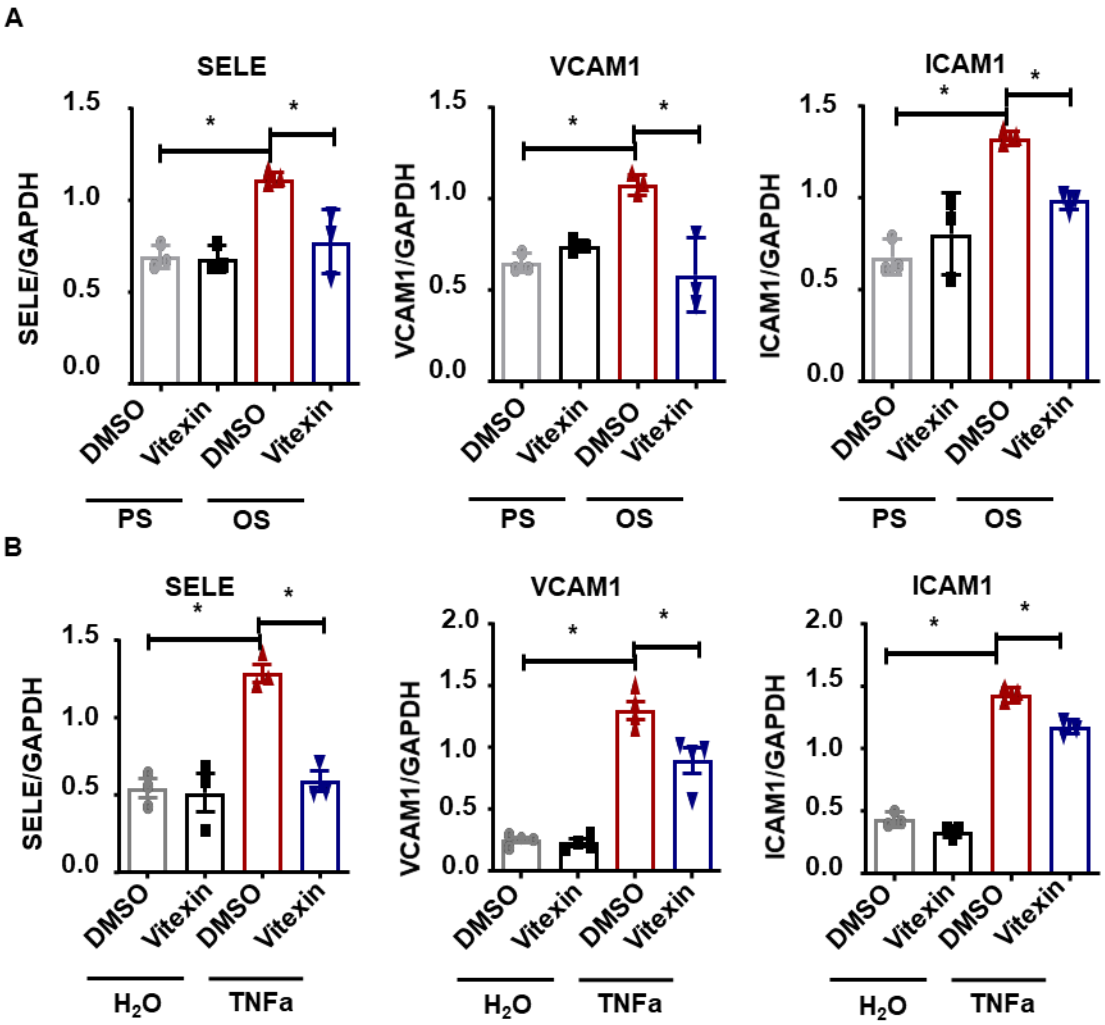

Fig. S5. Semi-quantification of the Western blots in Figure 1D and F. (A) The expressions of pro-inflammatory and adhesion molecules in cells in Figure 1D (n=3-4). (B) The expressions of pro-inflammatory and adhesion molecules in cells in Figure 1F (n=3-4). **P*<0.05 by two-way ANOVA followed by Tukey's test.

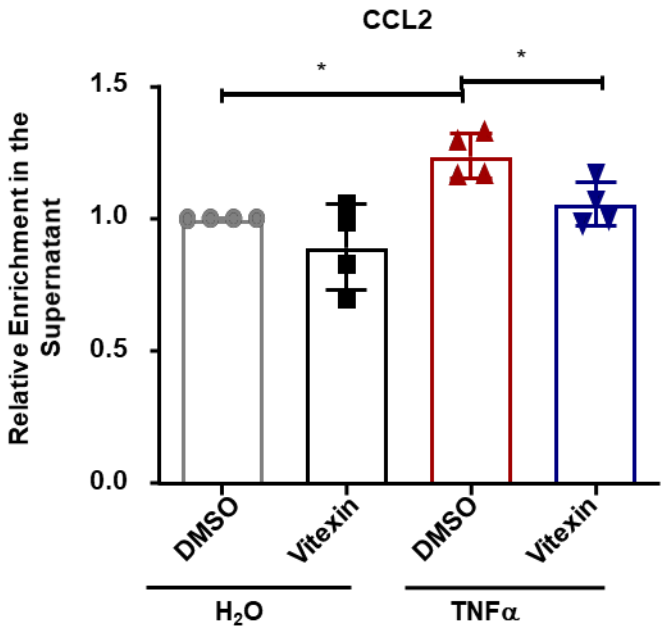

Fig. S6. The enrichment of CCL2 in the EC-conditioned media. Cells were pretreated with DMSO or vitexin (50 μmol/L) for 24 hours, stimulated with TNFα (10 ng/ml) for 6 hours, and then the enrichment of CCL2 in the cell culture-supernatant was detected by enzyme-linked immunosorbent assay (ELISA) (n=4). *P<0.05 by two-way ANOVA followed by Tukey's test.

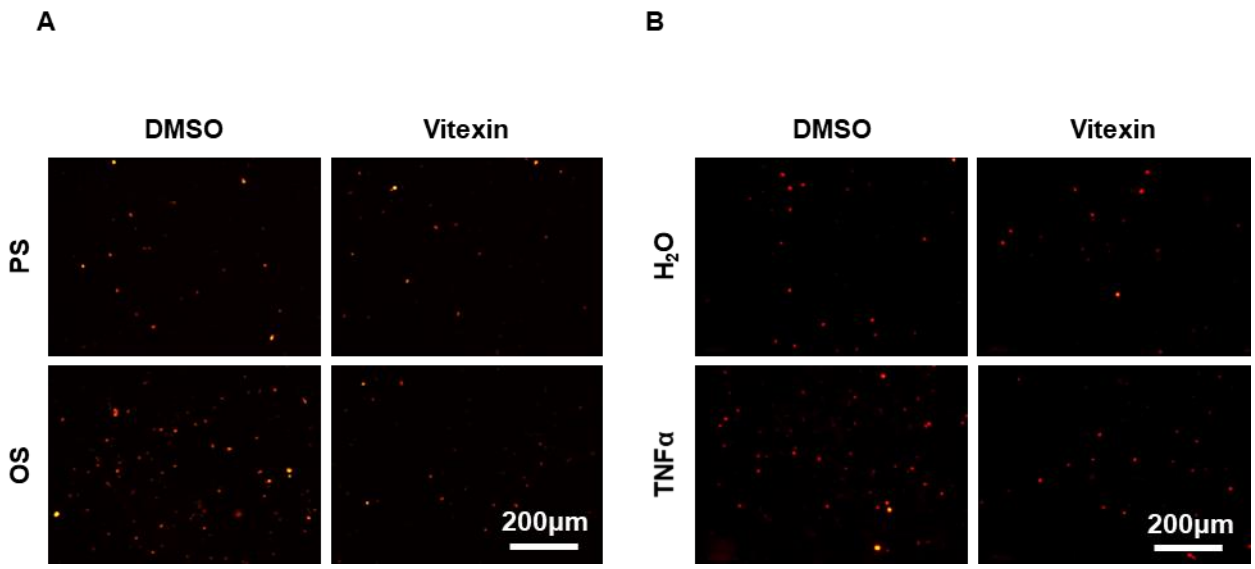

Fig. S7. Representative images of the monocyte adhesion assay. (A) Representative images of DiI (red)-labeled THP-1 monocytes adhering to ECs that have been pretreated with vitexin at 50 μmol/L or DMSO for 24 hours and then exposed to PS or OS for 6 hours (n=3). (B) Representative images of DiI (red)-labeled THP-1 monocytes adhering to ECs that have been pretreated with vitexin at 50 μmol/L or DMSO for 24 hours and then exposed to TNFα or the solvent for 6 hours (n=3).

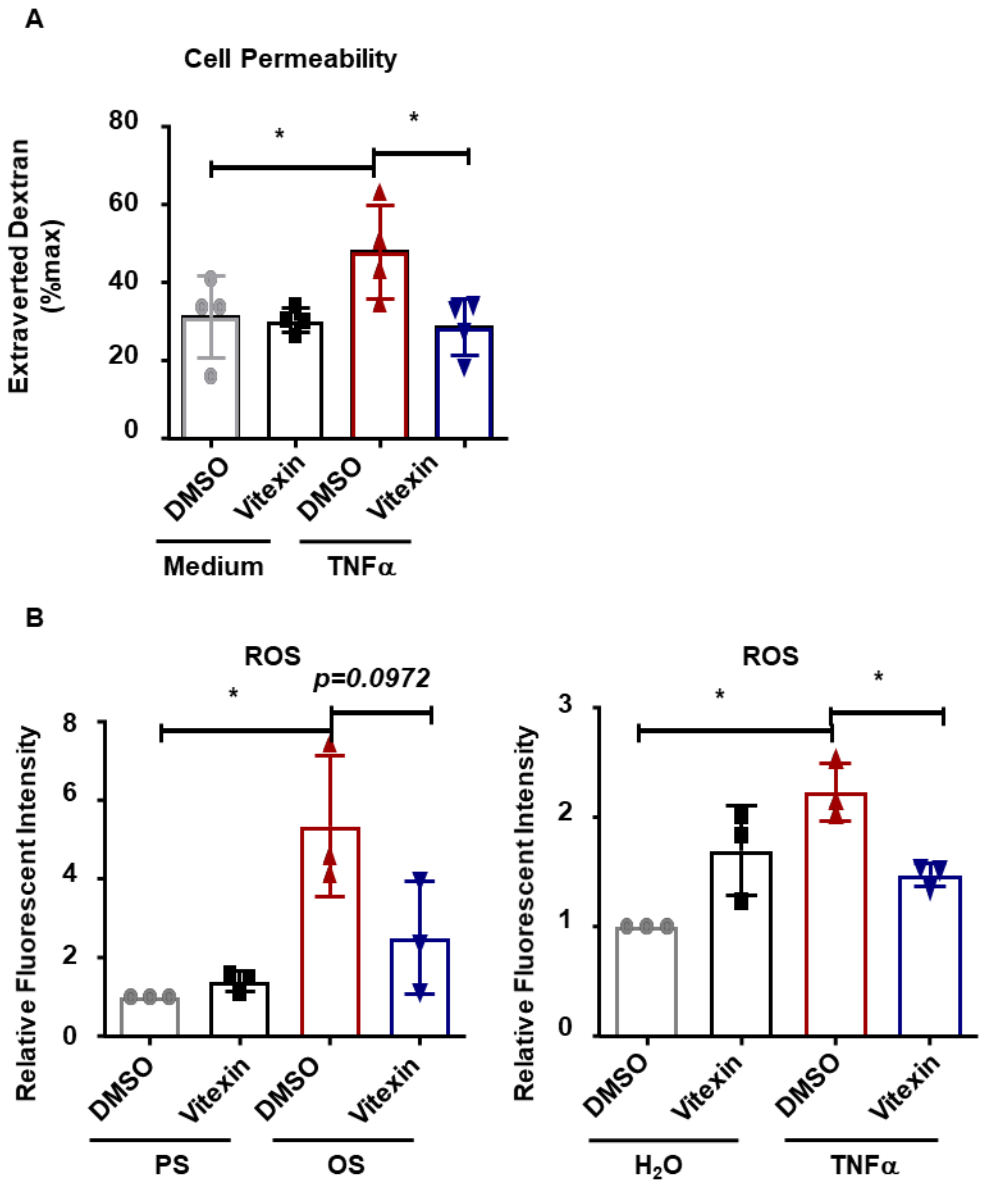

Fig. S8. The protective effects of vitexin on EC function. (A) Treatment (24 hours) with vitexin inhibits the TNFα (6 hours)-induced increase in endothelial cell permeability (n=4). In the permeability assay, the fluorescence labeled dextran were placed in the upper chamber of a transwell with treated endothelial cells seeded on and the fluorescence intensity of the culture medium in the subcompartment penetrating the endothelial layer was measured by spectrophotometer. (B) The effects of vitexin treatment on ROS production in endothelial cells subjected to shear or TNFα stimulation (n=3). Cells were pretreated with DMSO or 50 μmol/L vitexin for 24 hours and were then exposed to PS/OS or TNFα stimulation for 6 hours. After stained with DCFH-DA, ROS production was analyzed by flow cytometry. *P<0.05 by two-way ANOVA followed by Tukey's test.

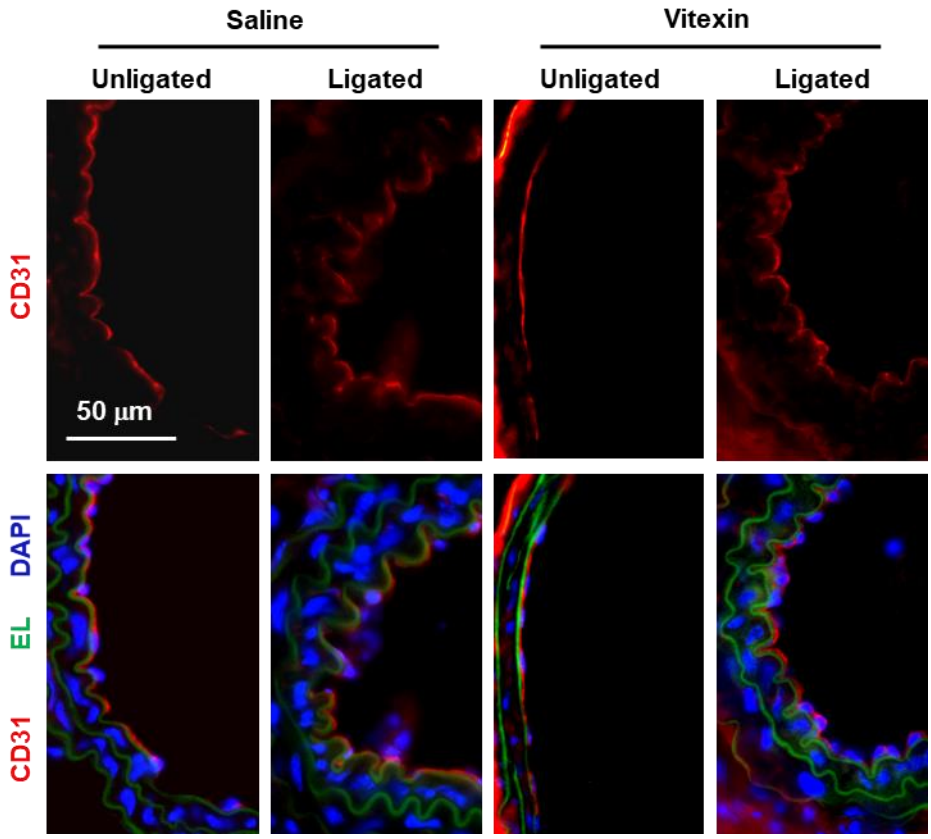

Fig. S9. The effects of disturbed flow and administration of vitexin on the endothelial integrity. Representative immunofluorescence staining of CD31 in cross sections of ligated (the left side) or unligated (the right side) common carotid arteries from mice subjected to vitexin (5 mg/kg) or saline administration for 1 week. DAPI: to stain the nucleus; EL: elastic lamina.

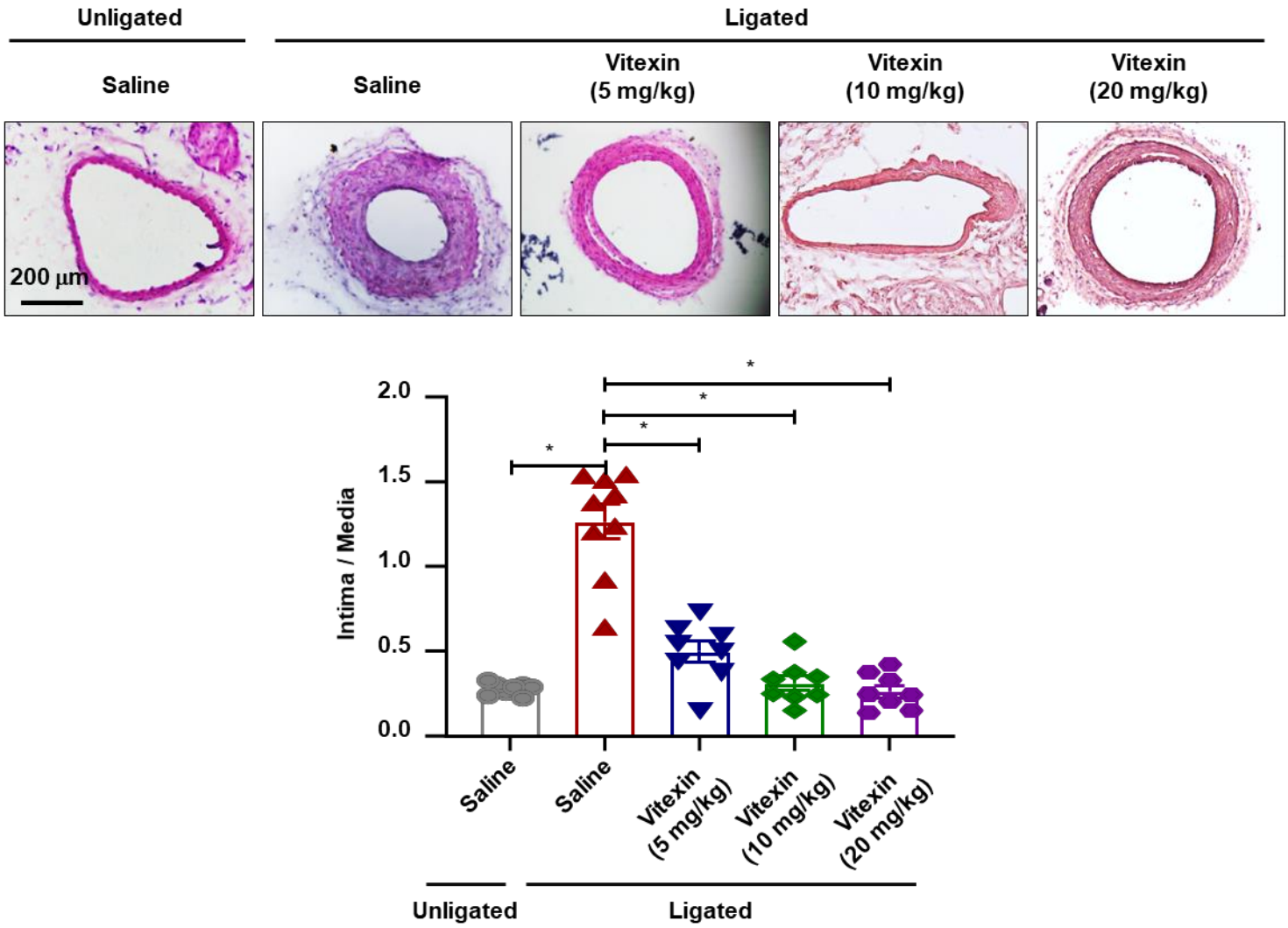

Fig. S10. The dose effect of vitexin on the disturbed flow-induced neointima formation. Representative images and quantification of H&E-stained intima/media. The mice were subjected to partial ligation and saline or vitexin (5, 10, 20 mg/kg) intraperitoneal injection twice a week for 4 weeks. Results are mean ± SEM from 8/9 animals in each group. *P<0.05 by one-way ANOVA followed by Tukey's test.

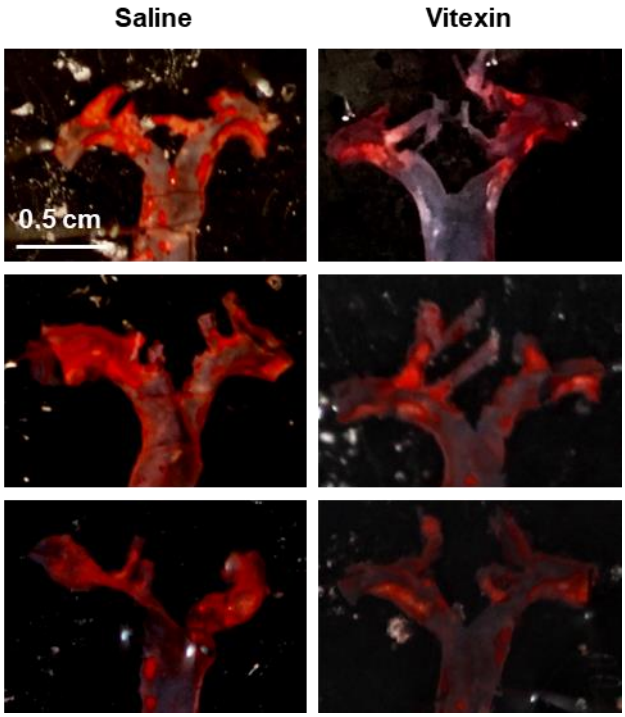

Fig. S11. Representative images of Oil red O staining of aortic arch in ApoE$^{-/-}$ mice with vitexin or saline injection. The mice were subjected to saline or vitexin (5 mg/kg) intraperitoneal injection twice a week for 12 weeks.

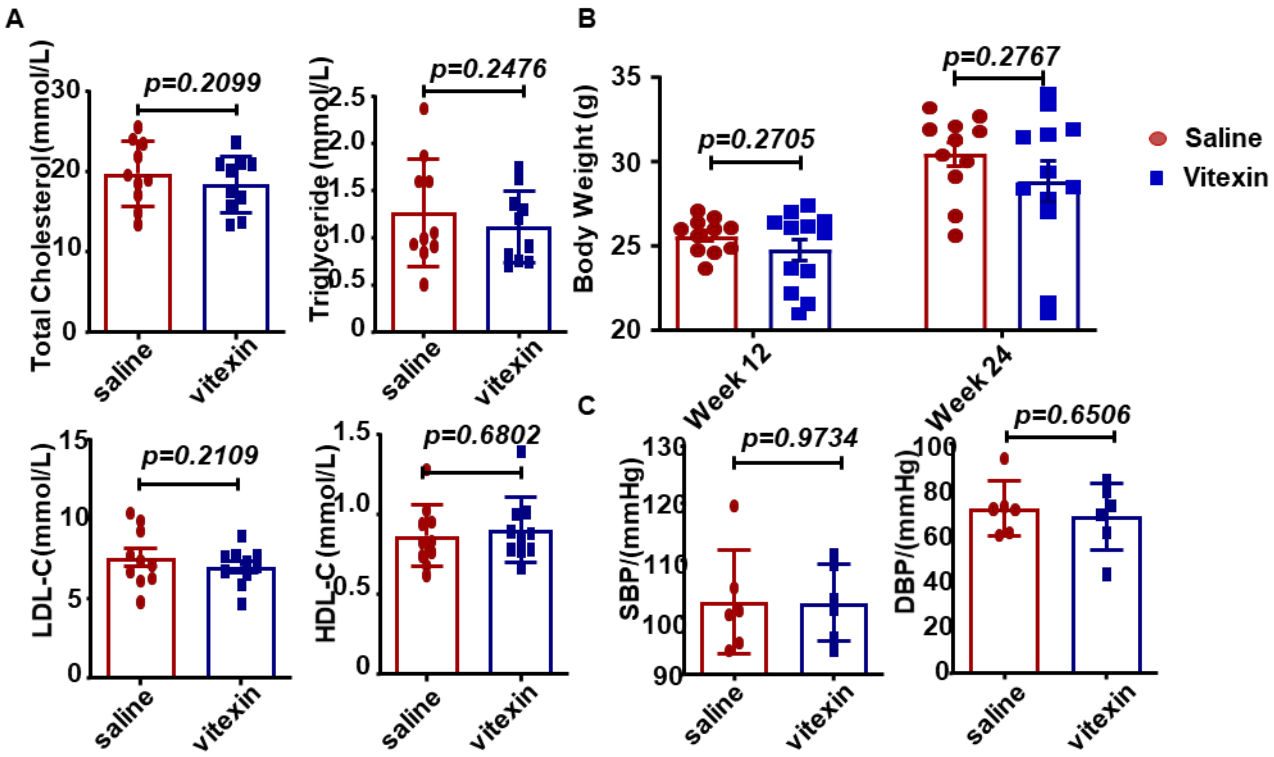

Fig. S12. The effects of vitexin on body weight, blood pressure and serum lipids in ApoE$^{-/-}$ mice. (A) Total serum cholesterol, triglyceride, LDL-C, and HDL-C level in the vitexin- or saline-treated mice at week-24 (n = 10). (B) Body weight at week-12 and week-24 (n = 11). (C) Systolic and diastolic blood pressures at week-24 (n = 6). SBP: systolic blood pressure; DBP: diastolic blood pressure. Data are represented as means ± SEM. *P*-value is calculated by Student-t test.

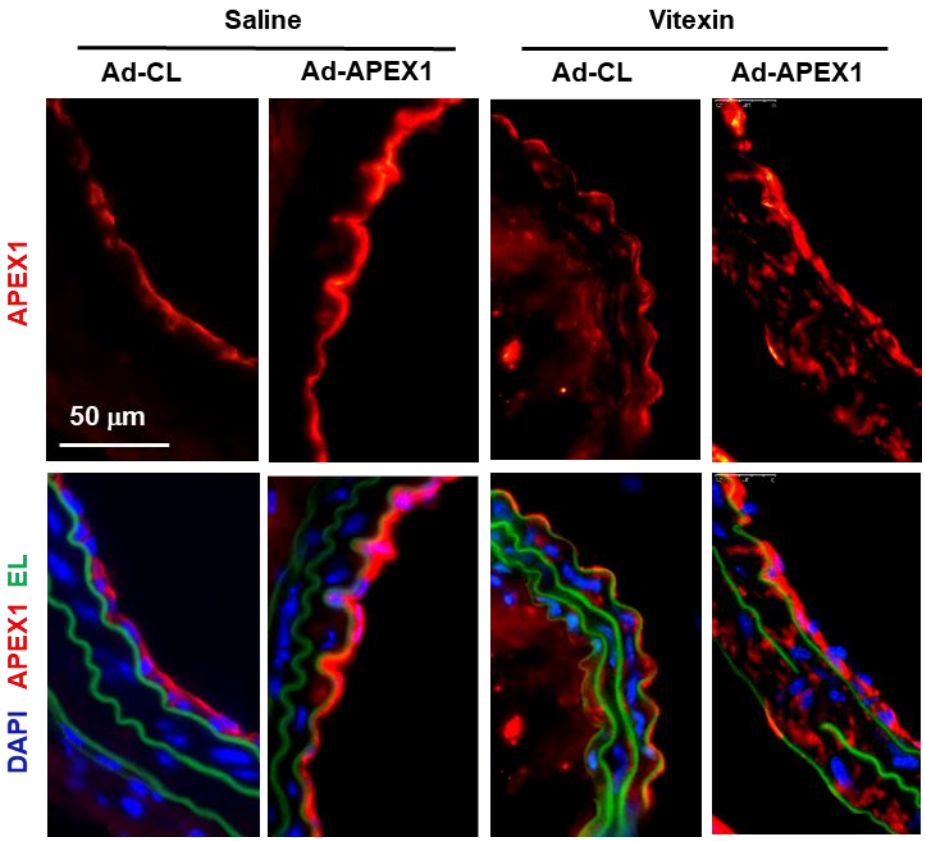

Fig. S13. Test for efficacy of intraluminal overexpression of APEX1. Representative immunofluorescent staining of APEX1 in carotid artery of mice at 1 week after partial ligation combined with intraluminal incubation with adenovirus overexpressing APEX1 (Ad-APEX1) or control virus (Ad-CL). EL: elastic lamina.

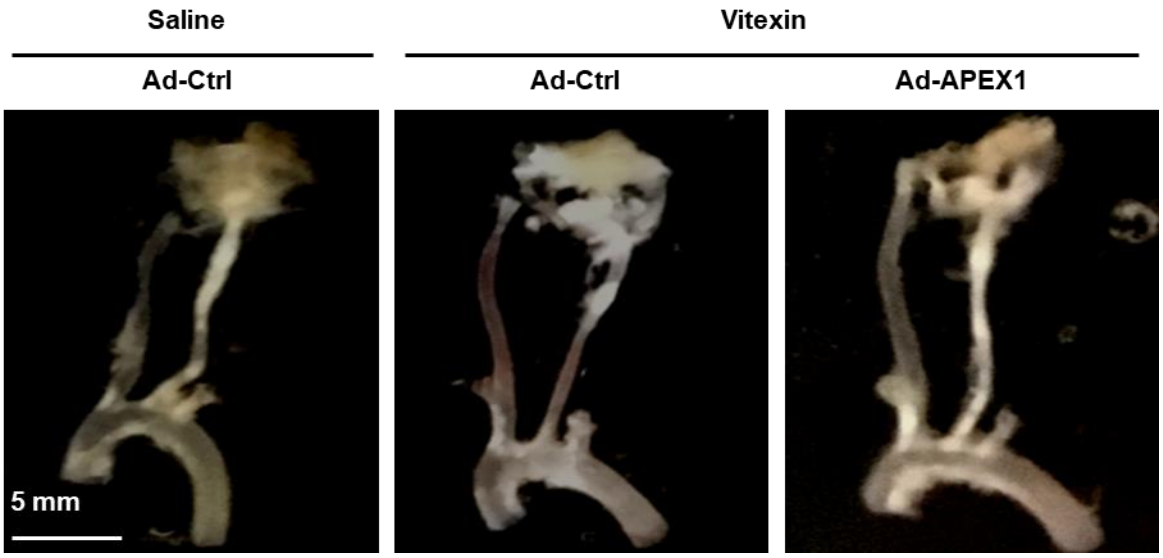

Fig. S14. Representative gross images of carotid arteries from the indicated mice. Shown are ApoE$^{-/-}$ mice at 4 weeks after partial ligation and intraluminal incubation with adenovirus overexpressing APEX1 (Ad-APEX1) or control virus (Ad-Ctrl).

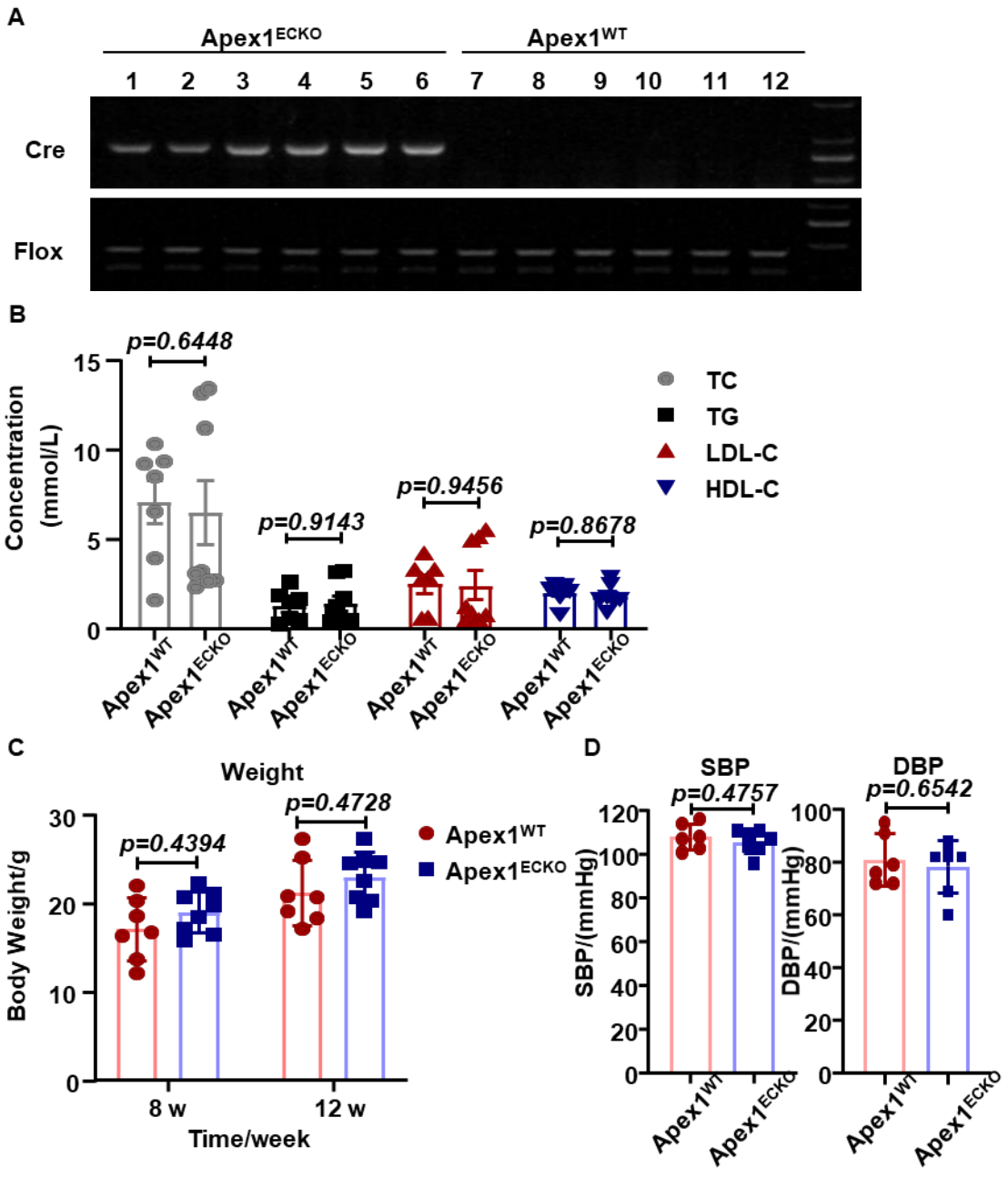

Fig. S15. The effects of endothelial-specific depletion of Apex1 on body weight, blood pressure and serum lipids in mice. (A) Representative gel images of PCR products demonstrative of successful generation of Apex1^{ECKO} and Apex1^{WT} mice. (B) Total serum cholesterol, triglyceride, LDL-C, and HDL-C level in the Apex1^{ECKO} and Apex1^{WT} mice at week-12 (n = 7/8). (C) Body weight at week-8 and week-12 (n = 7/8). (D) Systolic and diastolic blood pressures at week-12 (n = 6). SBP: systolic blood pressure; DBP: diastolic blood pressure. Data are represented as means ± SEM. *P*-value is calculated by Mann-Whitney test.

Table S1. Binding parameters of the interaction between Vitexin or E3330 and APEX1 obtained from SPR. The affinity data were analyzed using Biacore T200 Evaluation Software. Ka means the association constant, Kd means the dissociation constant, KD stands for the equilibrium dissociation constant, and KD = Kd/Ka.

Compound	Ka (1/Ms)	Kd (1/s)	KD (M)
Vitexin	**373.0**	**0.008742**	**2.344×10^{-5}**
E3330	**222.2**	**0.01982**	**8.921×10^{-5}**

Table S2. Primers and siRNAs used in this study.

Primers (q-PCR)	SELE	F	TGGTGAGGTGTGCTCATTCC
		R	TGATCTTTCCCGGAACTGCC
	VCAM1	F	GGACCACATCTACGCTGACA
		R	TTGACTGTGATCGGCTTCCC
	ICAM1	F	AGCCAACCAATGTGCTATTCAAAC
		R	CACCTGGCAGCGTAGGGTAA
	CCL2	F	CCCAAAGAAGCTGTGATCTTCA
		R	TCTGGGGAAAGCTAGGGGAA
	NFKB1 （NFκB-p50）	F	TGAAAAGAACAAGAA GTCCTACCC
		R	TCACATGAAGTATACCCAGGTTTG
	RelA （NFκB-p65）	F	CTGCAGTTTGATACTGATGAGGAC
		R	GAGTTATAGCCTCAGGGTACTCCA
	GAPDH	F	CATACCAGGAAATGAGCTTG
		R	ATGACATCAAGAAGGTGGTG
siRNAs	siAPEX1	1-F	CUGGUACGACUGGAGUACCTT
		1-R	GGUACUCCAGUCGUACCAGAC
		2-F	CUCCAGUCGUACCAGACCUTT
		2-R	AGGUCUGGUACGACUGGAGUA
	siIκBα	1-F	CUCCGAGACUUUCGAGGAATT
		1-R	UUCCUCGAAAGUCUCGGAGTT
		2-F	GAAAAGGCACUGACCAUGGTT
		2-R	CCAUGGUCAGUGCCUUUUCTT
	scrambled siRNA	F	UUCUCCGAACGUGUCACGUTT
		R	ACGUGACACGUUCGGAGAATT
Primers (Genotyping)	CDH5-CreERT2	F	CCGGTCGATGCAACGAGTGATGAGG
		R	GCCTCCAGCTTGCATGATCTCCGG
	Apex1-flox	F	AAGTCGGGTAAGCGTGCCCA
		R	GCAGATTTGCCACTGGGTGA

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