Supplemental Material for

Endothelial UCP2 is a mechanosensitive suppressor of atherosclerosis

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Expanded Materials & Methods

Online Supplemental Figures S1 – S11

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Materials & Methods

Chemicals and reagents

Simvastatin (#10010344), Rosuvastatin (#12029) and Resveratrol (#10004235) were purchased from Cayman chemicals (Ann Arbor, Michigan, USA). IL-1β (201-LB) were from R&D Systems (Minneapolis, USA). L-α-Lysophosphatidylcholine (LPC, #L4129) and Phorbol 12-myristate 13-acetate (PMA, # P8139) were from Sigma (St. Louis, MO, USA). AS1842856 (HY-100596) was from MedChemExpress. Tempol (ALX-430-081-G001) was from Enzo Life Sciences (Farmingdale, New York). The antibodies used in this study includes: anti-UCP2 (AF4739, R&D Systems; #89326, CST), anti-KLF2 (#09-820, Merck), anti-Myc tag (ab9132, Abcam), anti-eNOS (#610297, BD bioscience), anti-FoxO1(#2880, CST), anti-VCAM-1 (ab134047, Abcam), anti-MCP-1 (ab25124, Abcam), anti-IL-6 (sc-57315, Santa Cruz), anti-p-AKT S473 (#4060, CST), anti-p-AKT T308 (#4056, CST), anti-p-FoxO1 S256 (#9461, CST), anti-AKT (#9272, CST), anti-p-AMPKα T172 (#2535, CST), anti-AMPKα (#2532, CST), anti-IκBα (#4814, CST), anti-LDLR (# PA5-22976, Invitrogen), and anti-GAPDH (#2118, CST). For more detailed information, please refer to Major Resources Table.

Cell culture

Human aortic endothelial cells (HAECs) were purchased from ThermoFisher Scientific (Catalog #: C0065C) and cultured in Medium 200 supplemented with Low Serum Growth Supplement Kit (Catalog #: S003K). Human umbilical vein endothelial cells (HUVECs) were from Lonza Bioscience (Catalog #: CC-2519) and cultured in Endothelial cell Growth Medium supplemented with EGS and 20% Fetal bovine serum (FBS) at 37 °C in incubator with 95% humidified air and 5% CO_2. HAECs and HUVECs were subcultured every six and three days, respectively. Cells before passage 8 were used in this study. HEK293A and HEK293T cells used for virus packaging were purchased from ATCC and cultured in high glucose DMEM supplemented with 10% FBS. For more detailed information, please refer to Major Resources Table.

Adeno-associated virus (AAV) generation

For generation of EC-specific AAV vector, the CMV promoter in the pAAV-MCS (Cell Biolabs) vector was replaced with a 2500 bp mouse Cdh5 promoter[54] and the new vector was named as pAAV-Cdh5. The mouse Ucp2 cDNA was PCR amplified from the cDNA template obtained from mouse liver and was cloned to pAAV-Cdh5 vector to generate recombinant pAAV-Cdh5-Ucp2 vector. The pAAV-Cdh5 was used as the control during virus packaging. RGDLRVS-AAV9-cap plasmid (a gift from Dr. O.J. Müller, University Hospital Heidelberg, Germany) was used to package recombinant endothelial enhanced AAV owing to its higher infection efficiency in endothelial cells[55]. The pAAV-Cdh5-Ucp2 or pAAV-Cdh5-Vector, RGDLRVS-AAV9-cap, and pAAV-Helper were co-transfected into HEK293 T cells. 72 hours post-transfection, the AAV viral particles were harvested and purified by ultracentrifuge. The titer of AAV was determined by AAV real-time PCR titration kit provided by Takara (#6233). To generate AAV-Pcsk9, the pAAV/D377Y-mPCSK9 (Addgene, #58376) was used to co-transfect with pAAV-DJ (Cell Biolabs) and pAAV-Helper to HEK293T cells. The virus was harvested and purified using the same protocol as described above.

Adenoviral vectors

Human KLF2 cDNA was PCR amplified and cloned to pAdtrack-CMV plasmid. The resultant plasmid Adtrack-CMV-KLF2 linearized by Pme I was co-transformed with pAdEasy-1 (adenoviral backbone plasmid) for homologous recombination in E. coli BJ5183 strain cells. Positive recombinants were digested with Pac I and then transfected into HEK293A cells for adenovirus packaging. The pAdtrack-CMV vector was used as the control vector during virus packaging. Cells and culture medium were harvested until cytopathic effect became obvious. Three cycles of freeze and thaw method were performed to release adenovirus to the culture medium. After centrifuge, the virus-containing supernatant was subsequently precipitated with 5 X PEG buffer. The virus was then suspended in PBS containing 4% sucrose and aliquoted to vials before storage at -80 °C. To generate adenovirus short hairpin RNAs (shRNA) targeting human KLF2 (Ad-shKLF2, CGGCACCGACGACGACCTCAA) and human UCP2 (Ad-shUCP2, GCCTGTATGATTCTGTCAAAC), the shRNA against KLF2 and UCP2 and the scrambled shRNA were cloned to pAdtrack-U6 vector and the recombinant adenovirus was generated according to the above method. For generation of EC-specific adenoviral vector, the CMV promoter in the pAdtrack-CMV vector was replaced with mouse Cdh5 promoter (2500 bp) [1] and the new vector was named as pAdtrack-Cdh5. The mouse Klf2 cDNA was cloned to pAdtrack-Cdh5 vector to generate recombinant pAdtrack-Cdh5-Klf2 vector. Ad-FoxO1-AAA (three Akt phosphorylation sites are altered from Serine/Threonine to Alanine) was generated using the shuttle vector pAdTrack FKHR AAA (Addgene, #1199). For more detailed information, please refer to Major Resources Table.

Lentiviral vectors

The PLKO.1 plasmid containing shRNAs targeting human FoxO1, PGC-1α and SMAD4 were purchased from GeneChem (Shanghai, China). Lentivirus encoding these shRNAs was generated by co-transfection of PLKO.1, PMD2.0 and PSPAX2 to HEK293T cells for 72 hours. The supernatant containing recombinant lentivirus was collected and purified. The shRNA sequences were available upon request. For more detailed information, please refer to Major Resources Table.

Animals

Both the male and female mice were used in the present study. C57BL/6 mice were provided by The Chinese University of Hong Kong (CUHK) Laboratory Animal Services Center and maintained at controlled temperature (22-23°C, 55 ± 5% humidity) with a 12-hour light/dark cycle with free access to standard mice diet (Research Diet Inc., USA) and water. All animal procedures were approved by the CUHK Animal Experimentation Ethics Committee (Approval number: Ref No. 16-029-MIS). Animals were randomly assigned to the experimental groups and the analysis of animal experiments were blinded whenever possible by numerical coding of samples. The present study did not use G*power software to determine the animal group sizes. Sample size were determined according to our previous experience with mouse model of atherosclerosis. To account for potential loss of mice due to illness or sudden death, group size of 7-10 mice were chosen. All the mice and samples were included for analysis unless unsuccessful surgical operation or sample processing happens. Partial ligation of carotid artery was not successful in one mouse of the *Ucp2*$^{f/f}$ group and the data from this mouse was excluded. In all other cases, no animals were excluded from analysis. *Ucp2* floxed mice (B6;129S-Ucp2tm2.1Lowl/J) were from Jackson laboratory (Stock No: 022394 | Ucp2lox) and crossbred with Ve-Cadherin Cre mice {B6;129-Tg (Cdh5-cre)1Spe/J; Stock No: 017968} to achieve endothelium-selective deletion of UCP2 (*Ucp2*$^{\triangle EC}$). The mice were genotyped according to a protocol provided by Jackson laboratory. The LDLR downregulation in liver was achieved by a single dose injection of AAV-Pcsk9 (1 x 10^{12} vg/mouse) via tail vein to the *Ucp2*$^{f/f}$ and *Ucp2*$^{\triangle EC}$ mice, which were fed on a western diet (Cat# D12336, Research Diet Inc., USA) for induction of atherosclerosis. For more detailed information, please refer to Major Resources Table.

Lung endothelial cell isolation

To isolate endothelial cells from mouse lungs, the perfused lung lobes were cut into tiny pieces before Collagenase I (450 U/mL) digestion at 37°C for 30 minutes. The digestion was stopped by adding the FACS buffer (2 mM EDTA, 2% FBS). The samples were transferred to 70 μm cell strainer and the cell suspension is incubated with mouse CD31 microbeads on ice for 15 minutes before being sorted using LS column (Miltenyi Biotec) on the magnet. Column was washed with FACS buffer for several times before transferring to a 15 Ml-tube for collection of $CD31^+$ cells by

pushing syringe plug. The cell suspension was then centrifuged, and the cell pellet was subjected to RNA isolation.

In vitro shear stress experiment

Ibidi flow system (Ibidi, Germany) connected with home-made flow chambers were used to generate unidirectional shear stress (USS) and oscillatory shear stress (OSS) (12 dyn/cm^2 for USS and 0.5 ± 6 dyn/cm^2, 1 Hz for OSS). The setup of flow system and computer control program were followed by manufacturer's instructions. HUVECs and HAECs were seeded at a density of 5×10^5 cells to fibronectin (50 μg/ml, Sigma)-coated glass slides (75 mm × 38 mm; Corning). 24 hours post-seeding, HUVECs and HAECs on the glass slide was mounted to the flow chambers and connected to the Ibidi flow system. The perfusion solution is EGM supplemented with 2% FBS for HUVECs, or Medium 200 supplemented with 2% FBS for HAECs.

Quantitative real-time PCR

HUVECs, HAECs or mouse lung ECs were homogenized and lysed in RNAiso plus (TaKaRa, #9109) and isolated according to manufacturer's protocol. Total RNA (1 μg) was reverse transcribed into cDNA using PrimeScript RT Master Mix (TaKaRa, RR036A). For detection of genes of interest, 0.25 μM primers, 0.5 μL cDNA with 2 × SYBR Green reagent (Vazyme, # Q411), were mixed and loaded to 384-well plate for RT-PCR analysis on the ViiA^{TM}7 real time PCR system (ThermoFisher). The comparative C^T method was applied to analyze the relative level of gene expression. The expression of *GAPDH* level was used as internal control for normalization. The sequences of the primers used in the present study were listed in Supplementary Table S1 and S2.

RNA sequencing

HAECs were transduced with Ad-SCR and Ad-shUCP2 for 120 hours and total RNA was isolated by using RNeasy Mini Kit (Qiagen, # 217004). The extracted RNA samples were sent to Novogene Co., Ltd (Beijing, China) for RNA sequencing analysis. FDR<0.05 was used as a threshold for differentially expressed genes (DEGs). DAVID tools were used for the KEGG pathways

enrichment analysis. The analyzed sequencing data (Upregulation and downregulation of DEGs) is available as Online Dataset 1-3. Heatmap was generated using an online tool named phantasus which can be accessed via the link https://artyomovlab.wustl.edu/phantasus/.

Western blotting

Whole lysate isolated from cellular and tissue samples was obtained by using RIPA buffer supplemented with protease inhibitors and phosSTOP phosphatase inhibitors (Roche, 11836153001). Protein concentrations were determined by Bradford Assay (Bio-Rad). Equal amount of proteins was loaded and separated by SDS–PAGE and transferred to PVDF (Millipore, USA) membranes. After blocking at room temperature for 1 hour, all antibodies were incubated overnight at 4 °C in 1 X TBST buffer. Membranes were washed three times and incubated with secondary antibodies at room temperature for 1 h. Membranes were visualized by enhanced chemiluminescence (WBKLS0500, Millipore) and the protein bands were visualized by using Bio-Rad ChemiDoc™ Imaging System.

Measurement of mitochondrial ROS production in *en face* endothelium of mouse aortas

After isolation, aortic segments were incubated with MitoSOX™ Red (ThermoFisher) at 5 μmol/L for 30 minutes at 37 ℃ in extracellular medium (in mmol/L: 121 NaCl, 5 $NaHCO_3$, 10 Na-HEPES, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 2 $CaCl_2$, 10 glucose; pH=7.4). Then the segments were washed twice in extracellular medium and cut open longitudinally. The endothelium was facing upward before cover slipping and examined under a confocal microscope (FV1000, Olympus, Japan). The images showing mitochondrial ROS signals were taken at excitation 510 nm and emission 580 nm (autofluorescence of elastin excitation: 488 nm, emission 520 nm). For determination of intracellular mitochondrial ROS in vitro, HAECs were incubated with MitoSOX™ Red (5 μmol/L) for 20 min at 37 °C, protected from light. Fluorescent images were analyzed by FV10 ASW viewer software (Version 4.2, Olympus, Japan).

Partial ligation of carotid artery

Partial ligation of carotid artery was performed as described previously[56,57]. Briefly, *Ucp2*$^{f/f}$ and *Ucp2*$^{\triangle EC}$ mice pre-injected with AAV-Pcsk9 were anesthetized by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (80 mg/kg) mixture. A ventral midline incision (4–5 mm) was made in the neck to expose the left common carotid artery. Left external carotid, internal carotid, and occipital artery were ligated while the superior thyroid artery was left intact. After surgery, the mice were monitored until recovery in a chamber with a heating pad. Post-surgery, the mice were immediately fed on western diet for 3 weeks. For evaluation of the effect of FoxO1 inhibitor AS1842856 on disturbed flow-induced atherosclerosis, the *Ucp2*$^{f/f}$ and *Ucp2*$^{\triangle EC}$ mice pre-injected with AAV-Pcsk9 were orally administered with AS1842856 (30mg/kg daily) one-week post-surgery and lasted for two weeks. For assessment of the effect of UCP2 overexpression in endothelial cells on disturbed flow-induced atherosclerosis, the *ApoE*$^{-/-}$ mice were injected with AAV-vector (1×10^{12} vg/mouse) and AAV-Cdh5-Ucp2 (1×10^{12} vg/mouse) one day before partial ligation of carotid arteries.

UCP2 promoter cloning

Human UCP2 promoters were cloned into pGL3-basic vector (Promega). The PCR primers to clone human UCP2 promoters with different lengths are listed in the following. The genomic DNA template for PCR was extracted from HUVECs genome. The forward primers (Xho I as the restriction enzyme) used for cloning the promoters with different lengths are listed below: length 3558 bp Fwd primer: CCGCTCGAGCTACAGGCAAGCACCACCACAC; the 2302 bp Fwd primer: CCGCTCGAGGTCCACAGGACATCTTATGACTT; the 581 bp Fwd primer: CCGCTCGAGACGCTGTTAGAAACCGTCCTGGC; the 308 bp Fwd primer: CCGCTCGAGAAGTAGGAGCTGGCAGGCC; the 227 bp Fwd primer: CCGCTCGAGTGCGCGGAGCCCCACTGCGAA. All the above forward primers were used together with the same following reverse primer to amplify different sizes of UCP2 promoters. The Reverse (Hind III as the restriction enzyme) primer: CCCAAGCTTTGGGTGGGAGAGAAGGTAAATGGAA.

Luciferase assay

One day prior to transfection, HEK293A cells were seeded to a 24-well plate at a density of 1×10^5 cells/well. 200 ng luciferase reporter plasmid, 5 ng internal control plasmid renilla and 200 ng the control plasmid were mixed in 100 μl low serum OMEM (Gibco, USA). The plasmid cocktail was gently mixed and transfected to HEK293A cells with 0.8 μl lipofectamine 2000 (Invitrogen, USA). 24 hours post-transfection, the cells were washed with PBS twice and lysed in 100 μl 1× Passive lysis buffer for 15 min at room temperature with mild shaking. 10 μl cell lysate were transferred to 1.5 ml EP tube for measurement of relative luminescence using dual-luciferase reporter gene assay system (Promega, USA). The assay was done in GloMaxR-20/20 single-Tube Luminometer (Promega, USA) via sequentially mixing the cell lysate with 50 μl LAR II buffer and 50 μl Stop & Glow buffer. The data was expressed as relative luciferase activity by dividing the value of firefly luciferase by that of renilla luciferase. For luciferase activity assay done in HUVECs, the plasmids were transfected to the cells using an electroporation system from Lonza (Amaxa® HUVEC Nucleofector® Kit).

Monocyte adhesion assay

THP-1 cells were cultured in RPMI 1640 medium. HAECs in 6-well plate pre-transduced by the Ad-shUCP2 or Ad-SCR for 96 hours were washed twice in PBS to remove cell debris, and the 2 mL THP-1 monocytes suspension (10^5 cells/mL) in full EGM were added to monolayers of HAECs and incubated for 40 min under normal cell culture condition. Nonadherent THP-1 cells were removed following by gentle washing of the cells for three times with EGM and cultured in full EGM medium during visualization under microscope. The THP-1 bound HAECs were visualized under a bright-field microscopy. For quantification of bound THP-1 cells, all the cells were lysed with 200 μl lysis buffer (0.1% sodium hydroxide/0.01% SDS) and quantified for fluorescence intensity using SpectraMax i3x Multi-Mode Detection Platform (Molecular devices, USA).

Determination of cellular ADP/ATP ratio

ADP/ATP ratio in HUVECs was determined by an ADP/ATP Ratio Assay Kit (ab65313, abcam), following the manufacturer's protocol. Briefly, HUVECs were washed and harvested after mixing with nucleotide releasing buffer and incubated at room temperature with gentle shaking for 5 min. Subsequently, 100 μl prepared reaction mix was dispensed to control wells and the background luminescence (SpectraMax i3x Multi-Mode Detection Platform) was recorded (Data A). Then 50 μl sample was added and the luminescence was detected after 2 min (Data B). To determine ADP levels in HUVECs, the luminescence of samples was read again (Data C), followed by the addition of 10 μl 1 X ADP converting enzyme and measurement of luminescence after approximately 2 min (Data D). ADP/ATP ratio = [Data D-Data C]/[Data B-Data A].

Quantification of mitochondrial membrane potential

HUVECs were incubated with MitoTracker CMXRos (50 nM; Thermo Fisher Scientific) as specific dye of mitochondrial membrane potential at 37^{o}C for 30 min in DMEM/F12 medium. The cells were later washed three times in PBS and counterstained with DAPI. Fluorescence signals were detected by the Olympus Fluoview FV1000 laser scanning confocal system (Olympus).

Chromatin immunoprecipitation

Chromatin was prepared from HUVECs seeded on 15 cm petri dish using ChIP-IT Express Enzymatic kit (#53035, Active Motif) according to manufacturer's instruction. Briefly, HUVECs were fixed in formaldehyde for cross-linking the DNA-binding proteins to the DNA. Next, the chromatin was sheared into small fragments by enzymatic shearing and immunoprecipitated using antibodies against Myc tag (ab9132, Abcam) and p300 (ab10485, Abcam). Then the chromatin was eluted, reversed cross-links and treated with proteinase K before PCR analysis. Multiple primer pairs were used for the optimal PCR amplification of UCP2 promoter. The primer pair used for DNA gel electrophoresis is: Forward 5′- TGACCATCTCGGGGAACGAA -3′ and Reverse 5′- GGCCTGCCAGCTCCTACTT -3′. For ChIP-qPCR assay, chromatin was prepared from HUVECs using the similar method, the kit used was from Cell Signaling Technology (SimpleChIP® Enzymatic Chromatin IP Kit, Magnetic Beads, #9003). The antibody used for

262 immunoprecipitation is anti-KLF2 ((#09-820, Merck). The information of primers used was listed
263 in Supplemental Table S3.

264

265 **Immunofluorescence staining**

266 The HAECs transduced with Ad-shUCP2 or Ad-SCR for 120 hours were fixed in 4%
267 paraformaldehyde for 15 minutes and permeabilized by 0.1% Triton X-100. The cells were
268 blocked by 5% normal donkey serum at room temperature for 1 hour, and then incubated with the
269 primary antibody against FoxO1 (1:100) overnight at 4 ℃. Next day, the cells were washed and
270 followed by incubation with secondary antibody (Alexa Fluor 546, 1:500) for 2 hours at room
271 temperature in dark. Nuclei were counterstained with DAPI (Invitrogen) in PBS for 5 min. For
272 immunofluorescence staining in carotid arteries, the sectioned vessel segments were fixed and
273 blocked by 5% bovine serum albumin at room temperature for 2 hours, and then incubated with
274 antibody against VCAM-1 (1:100) and CD68 (1:100) overnight at 4 ℃, followed by AlexaFluor
275 secondary antibodies (1:500) for 2 hours at room temperature in dark. Images were captured by
276 Fluoview FV1000 laser scanning confocal system (Olympus, Tokyo, Japan).

277

278 **Cytoplasmic and nuclear fractionation**

279 NE-PER Nuclear and Cytoplasmic Extraction Kit (#78833, ThermoFisher Scientific) was used for
280 the fractionation of cytoplasmic and nuclear contents of HUVECs. Briefly, HUVECs (SCR and
281 *UCP2*^{KD}) were harvested with trypsin-EDTA and then centrifuged to obtain the cell pellet, which
282 was washed with ice-cold PBS. The cell pellet in the 1.5 mL microcentrifuge tube was sequentially
283 subjected to lysis using CER I, CER II and NER reagents (all containing protease inhibitors). The
284 cytoplasmic extract and nuclear extract were analyzed using western blotting to determine FoxO1
285 level. β-tubulin and Histone H3 were used to monitor the successful fractionation.

286

287 **Serum lipid profile**

288 Mouse blood was collected through the celiac vein and the serum was obtained by centrifugation
289 at 3000 rpm for 10 minutes at room temperature. The serum lipid level was determined by a
290 commercial assay kit (Stanbio, Boerne, Texas, USA) specialized for serum total cholesterol (TC),

triglycerides (TG) and high-density lipoprotein (HDL) cholesterol. To separate the HDL from whole serum, HDL precipitating reagent (Stanbio) was added to the serum (1:10), followed by centrifugation at 1000 g for 10 minutes. Lipid profile was measured according to the manufacturer's instructions and the data were obtained by reading the absorbance at wavelength 500 nm on a plate reader (Bio-Rad). The amount of non-HDL cholesterol was calculated using the following formula: non-HDL cholesterol = TC – (TG/5) – HDL.

Immunohistochemical Analysis

To examine atherosclerotic lesions, hearts of *Ucp2*$^{f/f}$ and *Ucp2*$^{\Delta EC}$ mice were harvested and rinsed in ice-cold phosphate-buffered saline, followed by overnight fixation in 4% paraformaldehyde. Later, the paraffin-embedded hearts were sectioned horizontally to the aortic axis towards the aortic arch. The collagen content in the aortic roots was assessed by Masson's trichrome staining following standard protocols. In brief, the deparaffinized and rehydrated aortic roots were stained in Weigert's iron hematoxylin working solution for 10 min, followed by staining in Biebrich scarlet-acid fuchsin solution for 10 min. The aortic roots were subsequently differentiated in phosphomolybdic-phosphotungstic acid solution for 10 min or until the collagen was not red. Before mounting, the sections were transferred to aniline blue solution for 5 min prior to a 2-min differentiation in 1% acetic acid solution. For H&E staining of carotid arteries subjected to partial ligation, the tissues were fixed with 4% formaldehyde, embedded in paraffin and cut into 5 μm sections which were then baked at 65°C overnight, dewaxed, hydrated in distilled water, stained with hematoxylin (1min), differentiated in hydrochloric acid alcohol, blued in ammonia water, counterstained with eosin (7s), dehydrated with ethanol at different concentrations (75%, 90% and anhydrous ethanol), transparentized with xylene I and xylene II, and finally mounted in neutral gum.

Oil Red O staining for atherosclerotic aortas

The *Ucp2*$^{f/f}$ and *Ucp2*$^{\triangle EC}$ mice were euthanized by CO_2 asphyxiation. Mouse aortas were dissected in cold KHS solution and cut open to expose the atherosclerotic plaques. The exposed aortas were pinned in a matric gel and fixed in 4% formaldehyde overnight at 4 °C. Next day, the aortas were

first rinsed in water for 10 minutes and then in 60% isopropanol. The aortas were stained with Oil Red O for 15 minutes with gentle shaking and rinsed again in 60% isopropanol and then in water for three times. The aortas were fixed on the cover slides with endothelium facing upwards. The images were recorded using HP Scanjet G4050. The plaque areas were determined using the NIH ImageJ software and calculated by expressing the plaque area relative to the total vascular area.

Human artery samples and Immunohistochemical staining of UCP2

Human renal arteries (n=6) were dissected from patients subjected to nephrectomy. These samples were collected from Department of Surgery, Prince of Wales Hospital, CUHK. Samples were fixed in paraformaldehyde solution (4% in PBS) and subsequently embedded in paraffin. The paraffin-embedded samples were sliced into cross sections (5 μm) for Masson's trichrome staining and immunohistochemistry staining. The study was approved by the ethics committee of clinic research, The Chinese University of Hong Kong (Ref. NO.: 2014.468). All participants have signed an informed consent prior to inclusion into the study.

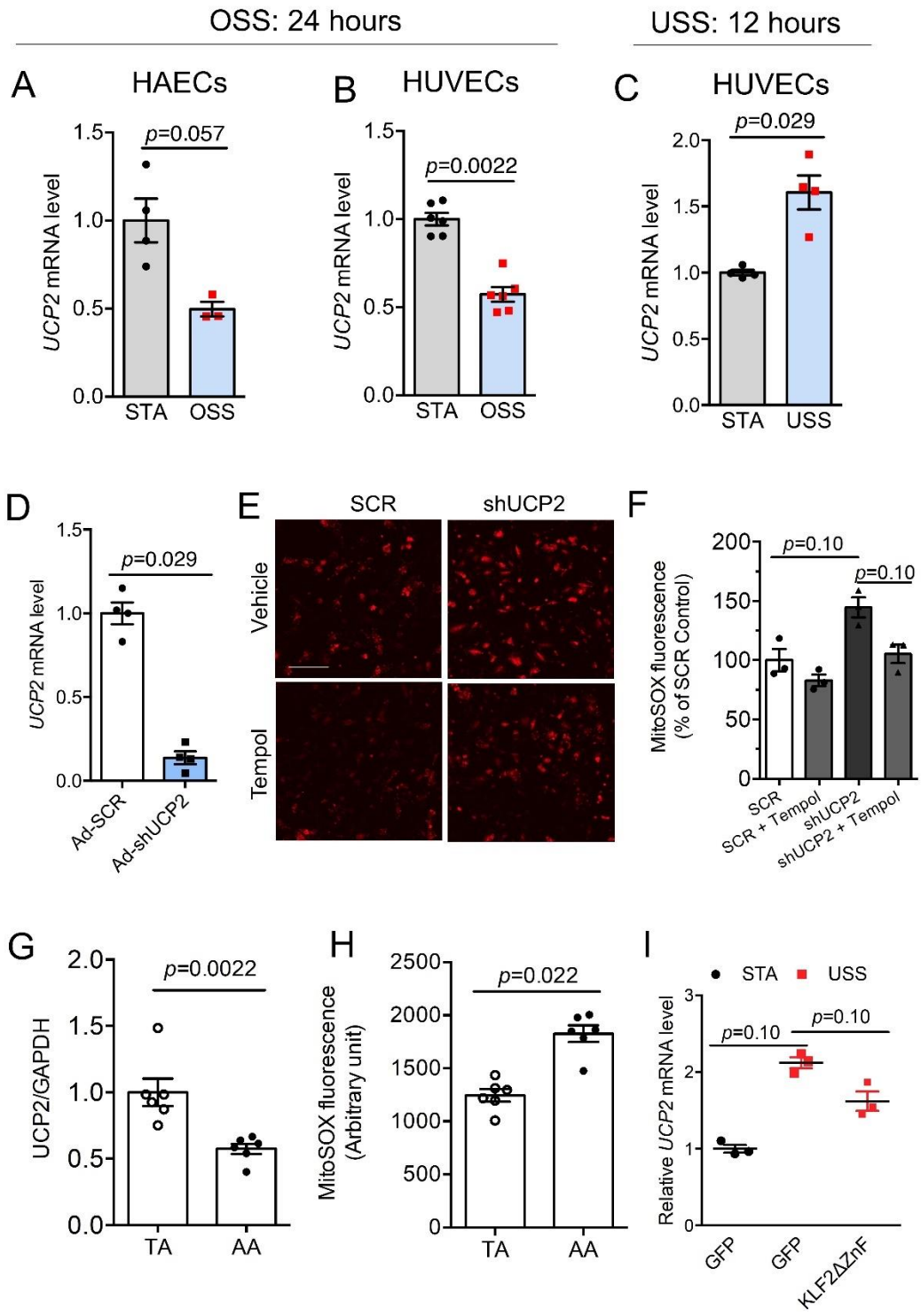
Paraffin sections of human renal arteries were used for the immunohistochemistry for UCP2. Antigen retrieval was achieved in 0.01 M citrate buffer (pH 6.0). After a 2-h blocking by 5% BSA at room temperature, the sections were incubated with the primary antibody anti-UCP2 (1:100; sc-390189; Santa Cruz Biotechnology) at 4 ℃ overnight. Signals were developed by applying the UltraSensitiveTM SP kit (Fuzhou MAXIM Biological Technology Development Co., Ltd., China) following the manufacturer's protocols. The nuclei of the samples were counterstained in hematoxylin.

Statistical analysis

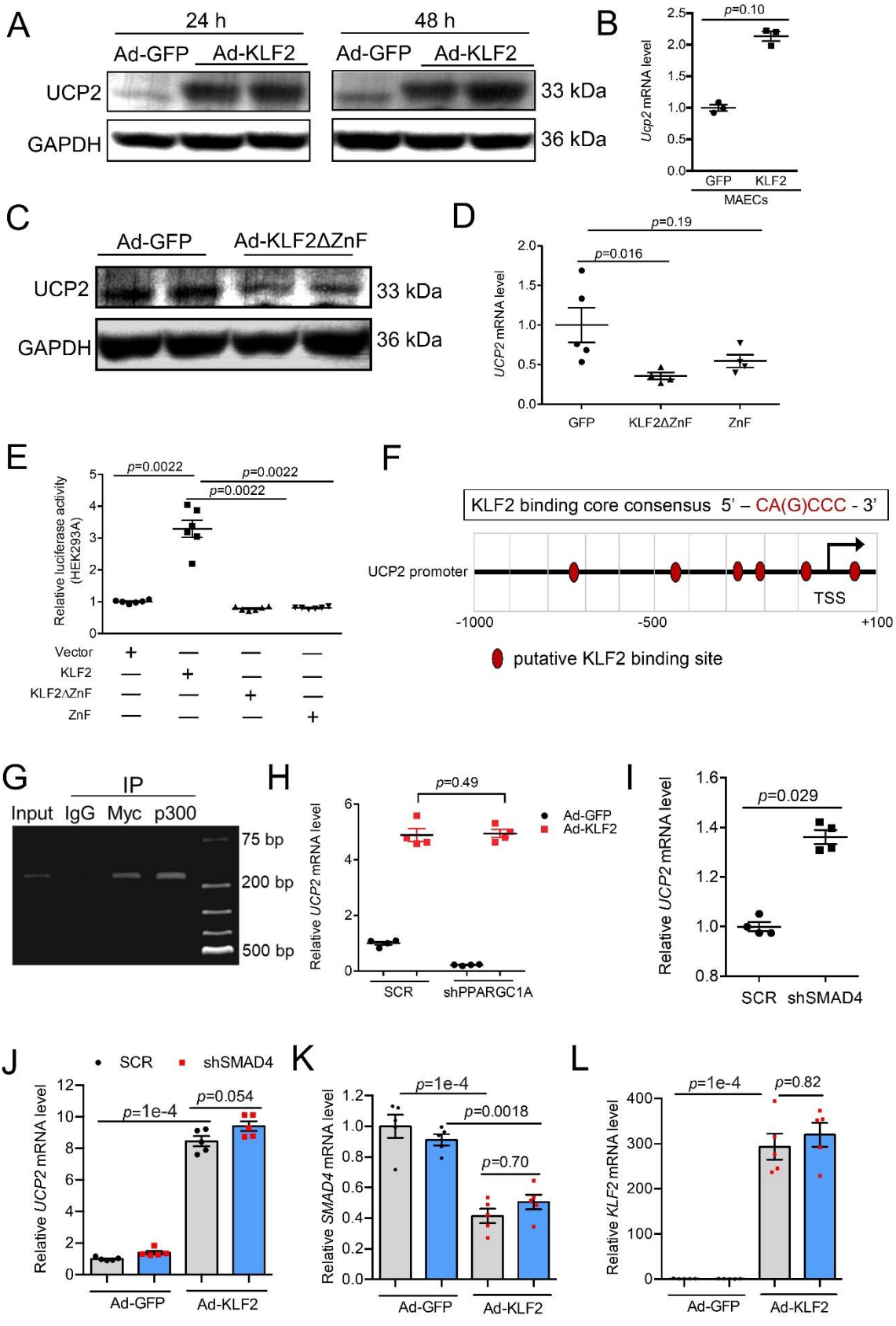
Data are expressed as means ± SEM. Gene and protein expression were normalized to the expression level of GAPDH and then expressed as relative to control. Column graphs were constructed using GraphPad Prism software (Version 6.0, San Diego, CA, USA). Normality and equal variance tests were performed for all variables before further analysis. For data passed equal variance test, unpaired *t* test was used to analyze differences between 2 groups and One-way

ANOVA followed by Tukey multiple comparison test was used for analyzing the differences between multiple groups. If a normal distribution could not be achieved, nonparametric tests were used. Mann-Whitney test was applied for 2 independent groups and Kruskal-Wallis test followed by Dunn's multiple comparison test, was used for comparing multiple groups. $p<0.05$ was considered as statistically different between groups.

Supplemental Figures



Supplemental Figure S1. Regulation of UCP2 by shear stress and UCP2 knockdown increased production of mitochondrial reactive oxygen species (ROS). Exposure to oscillatory shear stress **(**OSS) for 24 hours inhibited *UCP2* mRNA expression in **(A)** HAECs and **(B)** HUVECs. **(C)** Exposure to unidirectional shear stress (USS) for 12 hours increased *UCP2* mRNA expression in HUVECs. **(D)** Verification of the successful knockdown of *UCP2* mRNA expression by UCP2-shRNA using qPCR. **(E, F)** ROS scavenger Tempol revered UCP2 knockdown-induced production of mitochondrial ROS indicated by MitoSOX staining. Scale bar: 50 μM. **(G)** Summarized data showing reduced expression of UCP2 protein in AA compared to that in TA detected by western blotting. **(H)** Summarized data showing increased mitochondrial ROS generation in AA compared to that in TA indicated by MitoSOX staining. (**I**) DNA binding defective KLF2 mutant KLF2**Δ**ZnF attenuated USS-induced *UCP2* mRNA expression in HUVECs. **A-D, F, G, H and I:** Mann-Whitney test.


A
24 h
48 h
Ad-GFP
Ad-KLF2
Ad-GFP
Ad-KLF2
UCP2
GAPDH
33 kDa
36 kDa
B
p=0.10
Ucp2 mRNA level
GFP
KLF2
MAECs
C
Ad-GFP
Ad-KLF2ΔZnF
UCP2
GAPDH
33 kDa
36 kDa
D
p=0.19
p=0.016
UCP2 mRNA level
GFP
KLF2ΔZnF
ZnF
E
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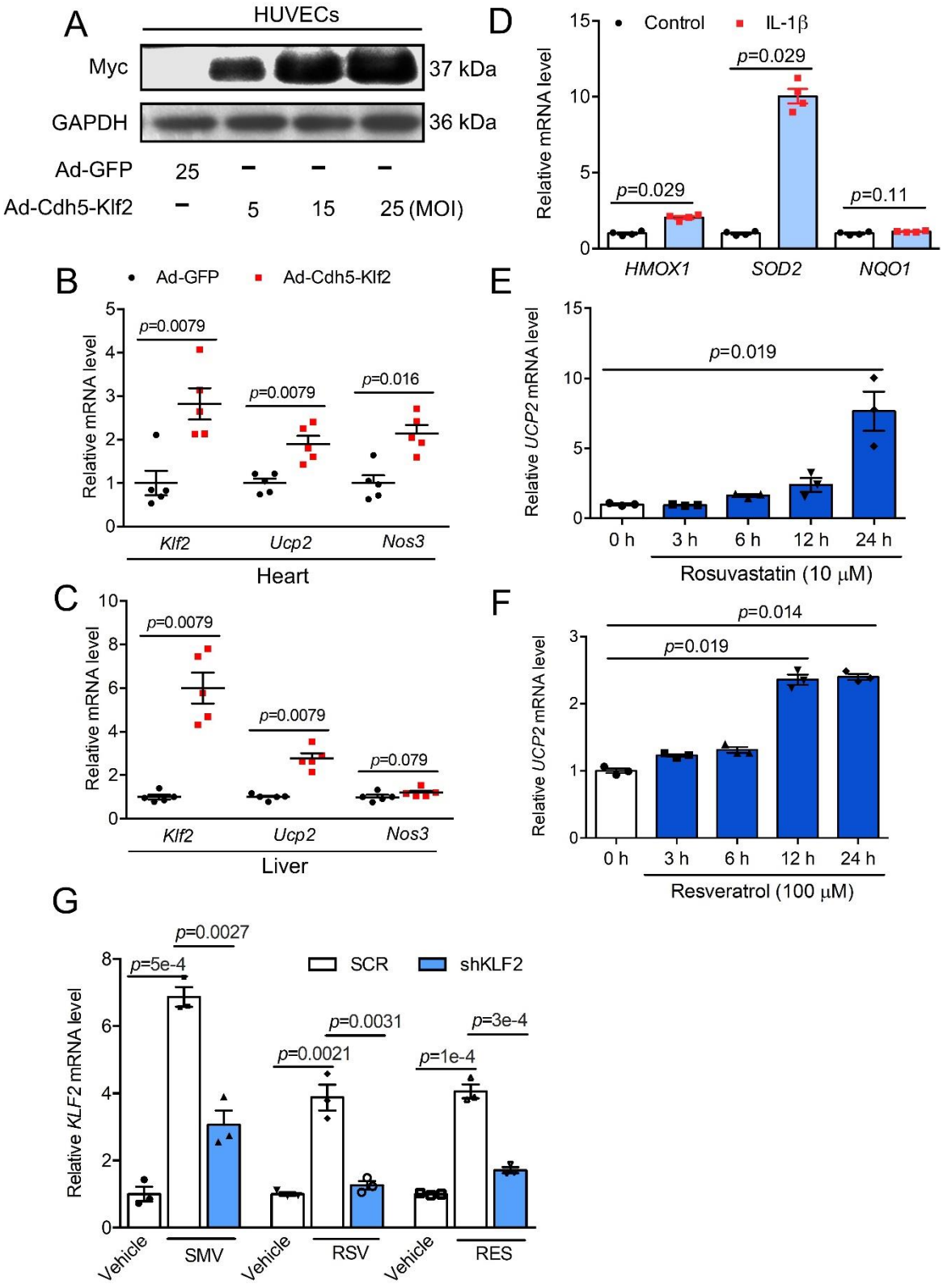
p=0.0022
p=0.0022
Relative luciferase activity (HEK293A)
Vector
KLF2
KLF2ΔZnF
ZnF
F
KLF2 binding core consensus 5' – CA(G)CCC - 3'
UCP2 promoter
TSS
-1000
-500
+100
putative KLF2 binding site
G
IP
Input
IgG
Myc
p300
75 bp
200 bp
500 bp
H
p=0.49
Relative UCP2 mRNA level
Ad-GFP
Ad-KLF2
SCR
shPPARGC1A
I
p=0.029
Relative UCP2 mRNA level
SCR
shSMAD4
J
SCR
shSMAD4
p=1e-4
p=0.054
Relative UCP2 mRNA level
Ad-GFP
Ad-KLF2
K
p=1e-4
p=0.0018
p=0.70
Relative SMAD4 mRNA level
Ad-GFP
Ad-KLF2
L
p=1e-4
p=0.82
Relative KLF2 mRNA level
Ad-GFP
Ad-KLF2

368

Supplemental Figure S2. KLF2 regulates UCP2 expression. (A) Ad-KLF2 overexpression for 24 and 48 hours increased UCP2 protein expression in HUVECs. **(B)** KLF2 overexpression upregulated *Ucp2* mRNA level in mouse aortic ECs (MAECs), Mann-Whitney test. **(C)** Ad-KLF2ΔZnF overexpression for 48 hours downregulated UCP2 protein expression in HUVECs. **(D)** Overexpression of Ad-KLF2ΔZnF and Ad-ZnF for 24 hours downregulated *UCP2* mRNA expression in HUVECs. Mann-Whitney test. **(E)** Dual luciferase reporter assay showing KLF2 increases whereas KLF2ΔZnF and ZnF inhibit UCP2 promoter activity in HEK293A cells. Mann-Whitney test. **(F)** Schematic diagram showing putative KLF2 binding sites in UCP2 promoter (-1000/+100 bp), TSS: transcription start site. **(G)** DNA gel electrophoresis image showing the result of ChIP-PCR assay of the UCP2 promoter in HUVECs transduced with Ad-Cdh5-Klf2 (Myc tagged). The image is representative of triplicate experiments. **(H)** Knockdown of PGC-1α using shRNA downregulated *UCP2* mRNA expression but did not affect KLF2-induced upregulation of *UCP2* mRNA level. 2 Way ANOVA, Tukey post hoc. **(I)** Knockdown of SMAD4 upregulated basal *UCP2* mRNA expression in HUVECs. Mann-Whitney test. **(J-L)** qPCR results showing KLF2-induced UCP2 expression is independent of SMAD4. Mann-Whitney test.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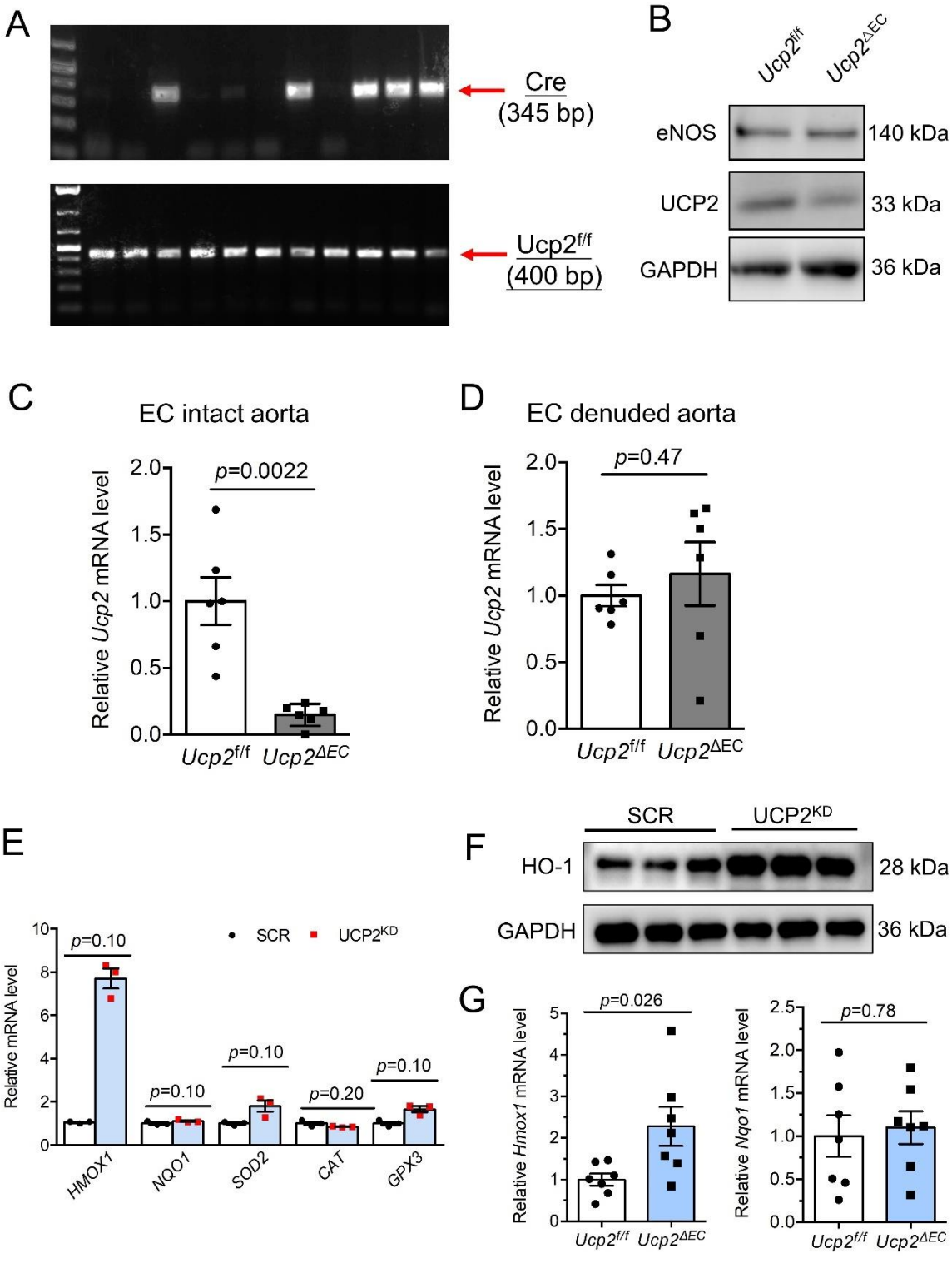
HUVECs
Myc
37 kDa
GAPDH
36 kDa
Ad-GFP
25
Ad-Cdh5-Klf2
5
15
25 (MOI)
B
Ad-GFP
Ad-Cdh5-Klf2
Relative mRNA level
p=0.0079
p=0.0079
p=0.016
Klf2
Ucp2
Nos3
Heart
C
Relative mRNA level
p=0.0079
p=0.0079
p=0.079
Klf2
Ucp2
Nos3
Liver
D
Control
IL-1β
Relative mRNA level
p=0.029
p=0.029
p=0.11
HMOX1
SOD2
NQO1
E
Relative UCP2 mRNA level
p=0.019
0 h
3 h
6 h
12 h
24 h
Rosuvastatin (10 μM)
F
Relative UCP2 mRNA level
p=0.014
p=0.019
0 h
3 h
6 h
12 h
24 h
Resveratrol (100 μM)
G
Relative KLF2 mRNA level
p=0.0027
p=5e-4
SCR
shKLF2
p=0.0031
p=3e-4
p=0.0021
p=1e-4
Vehicle
SMV
Vehicle
RSV
Vehicle
RES

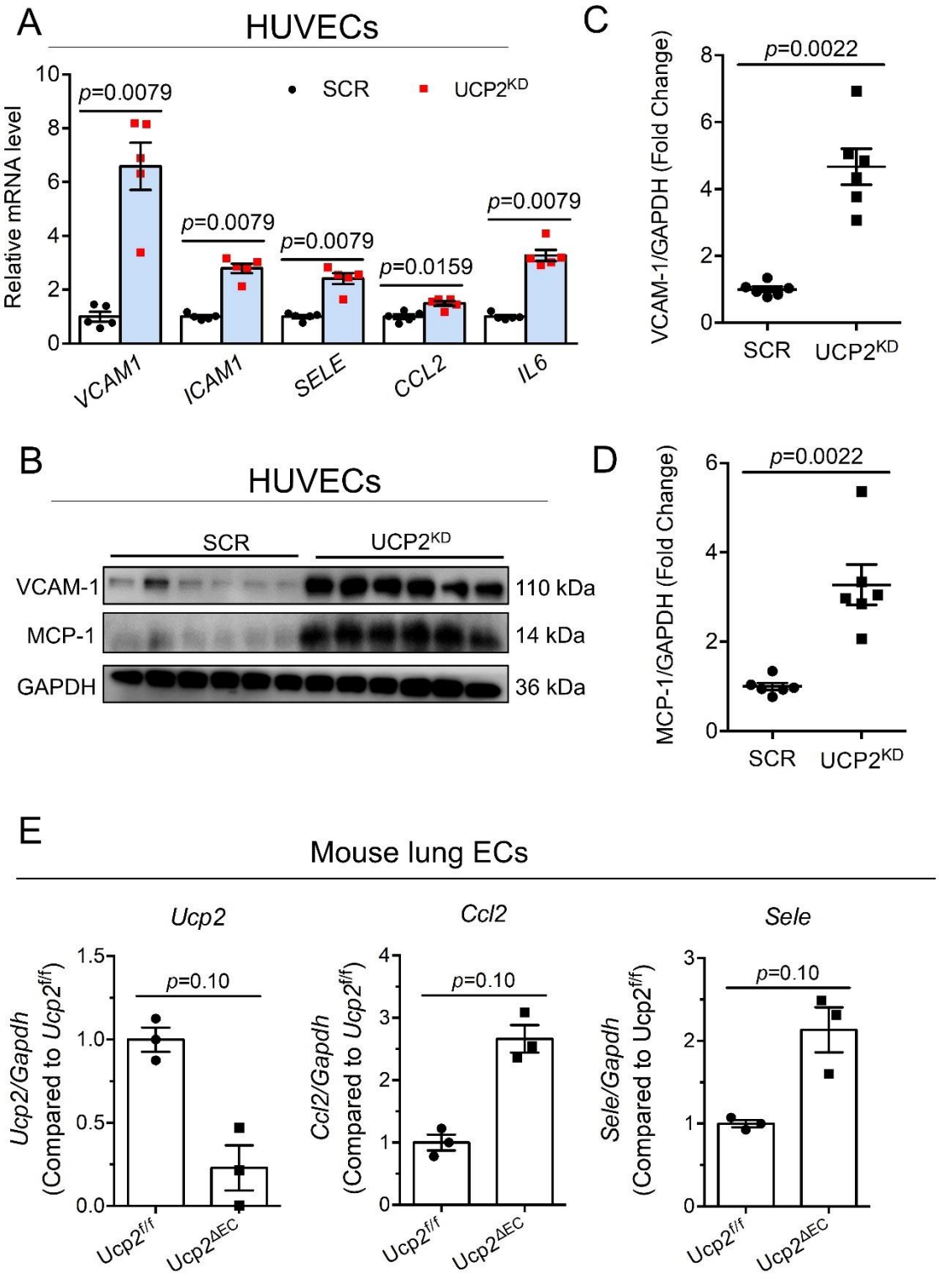
384

Supplemental Figure S3. EC-specific adenoviral Klf2 overexpression vector and induction of UCP2 mRNA expression by rosuvastatin and resveratrol. (A) Titer-dependent increase of exogenous KLF2 expression by Ad-Cdh5-Klf2 (Myc-tagged) in HUVECs. Ad-Cdh5-Klf2 increased *Klf2* and *Ucp2* mRNA expression in hearts **(B)** and livers **(C)** from mice. Mann-Whitney test. **(D)** IL-1β (10 ng/ml) treatment for 24 hours induced *HMOX1* and *SOD2* expression in HUVECs. Mann-Whitney test. Time-dependent upregulation of *UCP2* mRNA by **(E)** rosuvastatin (10 μM) and **(F)** resveratrol (50 μM) in HUVECs. Kruskal-Wallis test. **(G)** KLF2-shRNA inhibited the effect of simvastatin (SMV), rosuvastatin (RSV) and resveratrol (RES) on induction of *KLF2* mRNA in HUVECs. 2 Way ANOVA, Tukey post hoc.


A
Cre
(345 bp)
Ucp2f/f
(400 bp)
B
Ucp2f/f
Ucp2ΔEC
eNOS
140 kDa
UCP2
33 kDa
GAPDH
36 kDa
C
EC intact aorta
p=0.0022
Relative Ucp2 mRNA level
Ucp2f/f
Ucp2ΔEC
D
EC denuded aorta
p=0.47
Relative Ucp2 mRNA level
Ucp2f/f
Ucp2ΔEC
E
SCR
UCP2KD
Relative mRNA level
p=0.10
p=0.10
p=0.10
p=0.20
p=0.10
HMOX1
NQO1
SOD2
CAT
GPX3
F
SCR
UCP2KD
HO-1
28 kDa
GAPDH
36 kDa
G
p=0.026
Relative Hmox1 mRNA level
Ucp2f/f
Ucp2ΔEC
p=0.78
Relative Nqo1 mRNA level
Ucp2f/f
Ucp2ΔEC

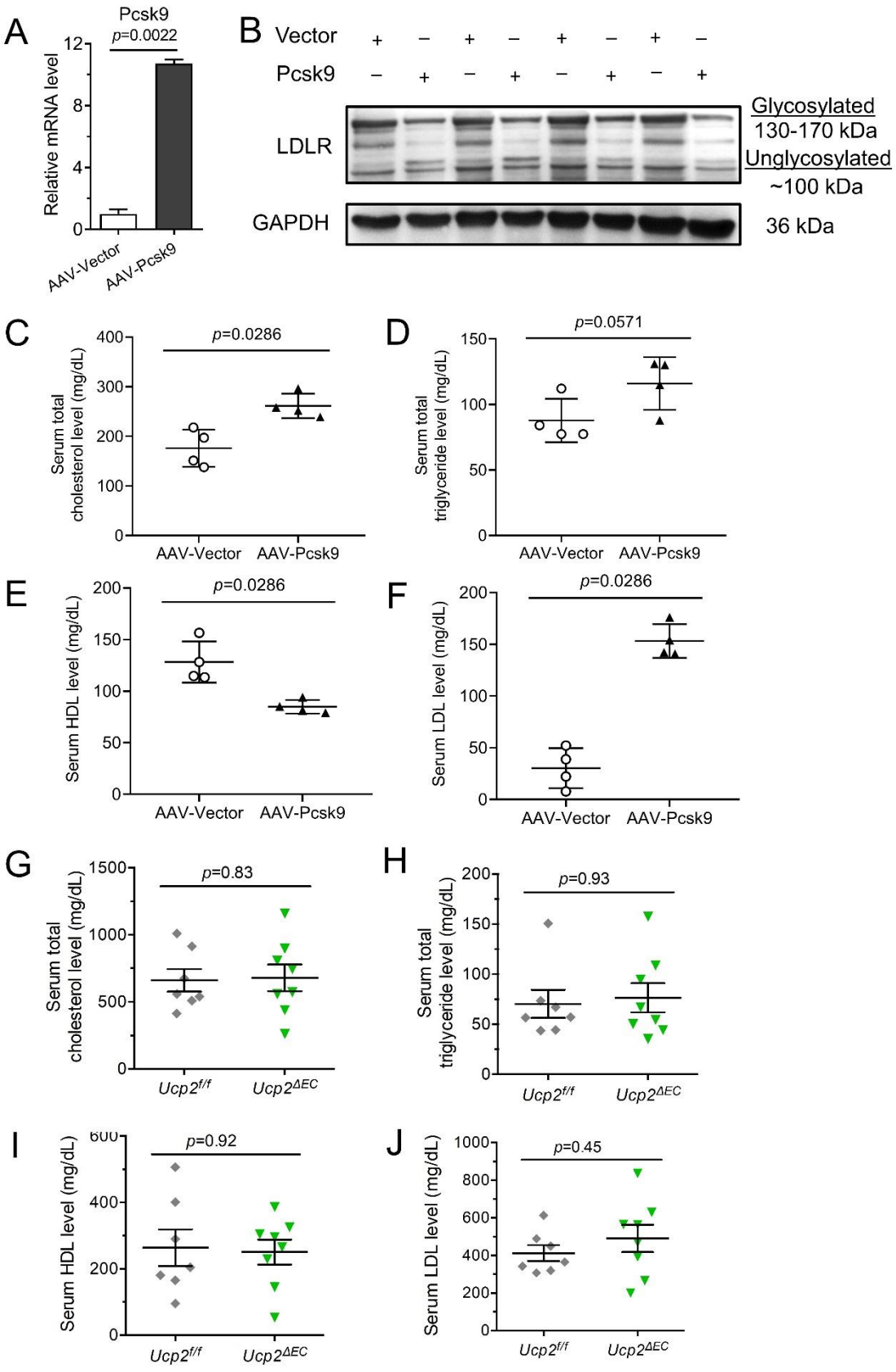
394

Supplemental Figure S4. Validation of EC-specific *Ucp2* knockout in mice and *UCP2* knockdown induces activation of antioxidant pathway. (A) DNA gel image showing the genotyping results of $Ucp2^{f/f}$ and $Ucp2^{\Delta EC}$ mice. **(B)** Reduced aortic UCP2 protein expression in $Ucp2^{\Delta EC}$ mice. Reduced *Ucp2* mRNA expression in EC intact aortas **(C)** but not in EC denuded aortas **(D)** from $Ucp2^{\Delta EC}$ mice. Mann-Whitney test. **(E)** The effect of $UCP2^{KD}$ on antioxidant gene expression in HAECs. Mann-Whitney test. **(F)** Upregulated HO-1 protein expression in $UCP2^{KD}$ HAECs. **(G)** Upregulated mRNA expression of *Hmox1* but not *Nqo1* in aortas from $Ucp2^{\Delta EC}$ mice. Mann-Whitney test.


A
HUVECs
SCR
UCP2KD
Relative mRNA level
p=0.0079
p=0.0079
p=0.0079
p=0.0159
p=0.0079
VCAM1
ICAM1
SELE
CCL2
IL6
C
VCAM-1/GAPDH (Fold Change)
p=0.0022
SCR
UCP2KD
B
HUVECs
SCR
UCP2KD
VCAM-1
MCP-1
GAPDH
110 kDa
14 kDa
36 kDa
D
MCP-1/GAPDH (Fold Change)
p=0.0022
SCR
UCP2KD
E
Mouse lung ECs
Ucp2
Ccl2
Sele
Ucp2/Gapdh (Compared to Ucp2f/f)
Ccl2/Gapdh (Compared to Ucp2f/f)
Sele/Gapdh (Compared to Ucp2f/f)
p=0.10
p=0.10
p=0.10
Ucp2f/f
Ucp2ΔEC

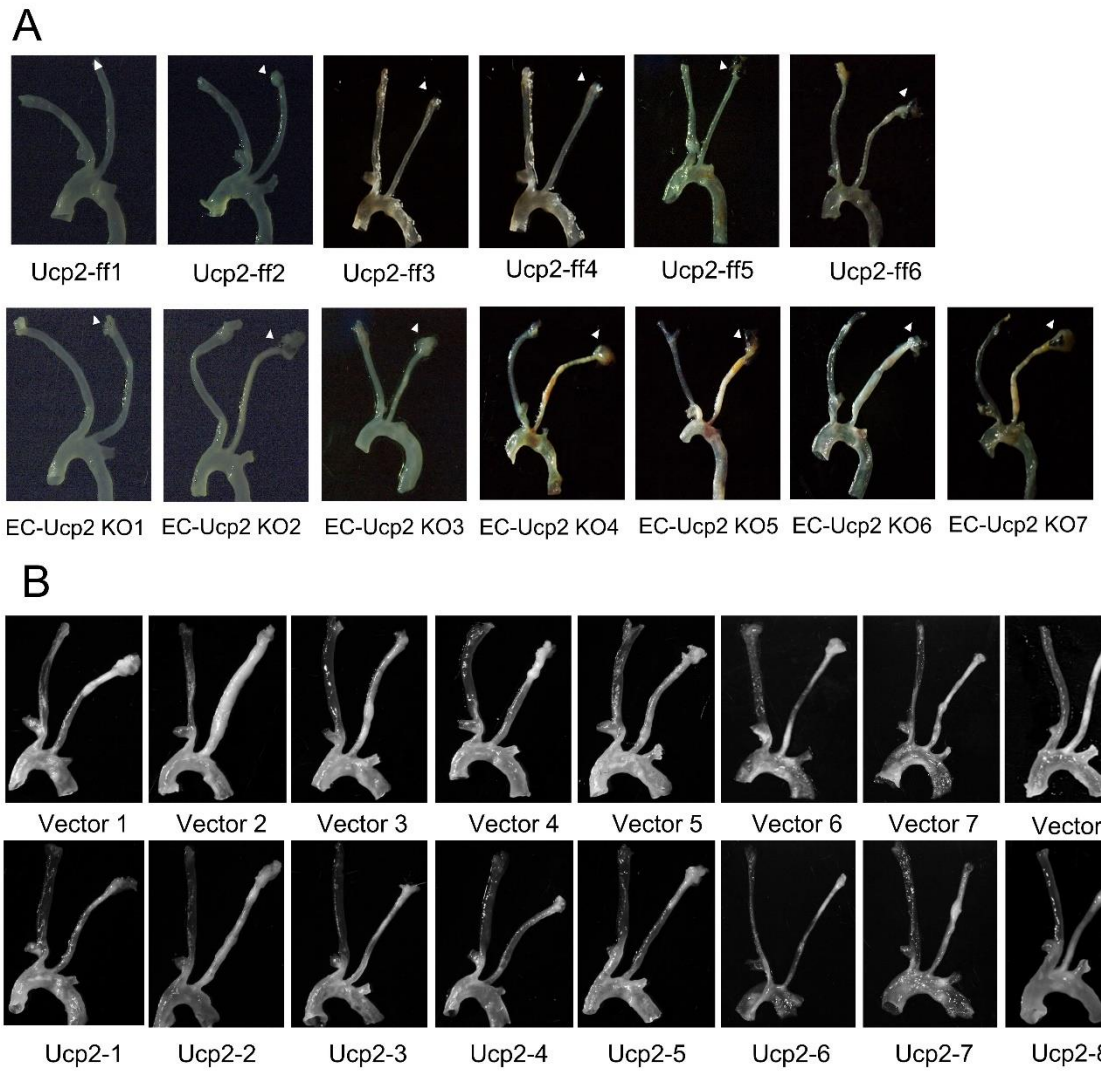
403

Supplemental Figure S5. The proinflammatory effect of UCP2 deficiency in HUVECs and in mouse lung ECs. (A) Increased expression of *VCAM1*, *ICAM1*, *SELE*, *CCL2* and *IL6* in $UCP2^{KD}$ HUVECs detected by qPCR. Mann-Whitney test. (B) Increased protein expression of VCAM-1 and MCP-1 in $UCP2^{KD}$ HUVECs. Summarized data for VCAM-1 (C) and MCP-1 (D) protein expression in $UCP2^{KD}$ HUVECs. Mann-Whitney test. (E) Reduced expression of *Ucp2* but increased expression of *Ccl2* and *Sele* in mouse lung ECs from $Ucp2^{\Delta EC}$ mice. Mann-Whitney test.


A
Pcsk9
p=0.0022
Relative mRNA level
12
8
4
0
AAV-Vector
AAV-Pcsk9
B
Vector
Pcsk9
LDLR
GAPDH
Glycosylated
130-170 kDa
Unglycosylated
~100 kDa
36 kDa
C
p=0.0286
Serum total cholesterol level (mg/dL)
400
300
200
100
0
AAV-Vector
AAV-Pcsk9
D
p=0.0571
Serum total triglyceride level (mg/dL)
150
100
50
0
AAV-Vector
AAV-Pcsk9
E
p=0.0286
Serum HDL level (mg/dL)
200
150
100
50
0
AAV-Vector
AAV-Pcsk9
F
p=0.0286
Serum LDL level (mg/dL)
200
150
100
50
0
AAV-Vector
AAV-Pcsk9
G
p=0.83
Serum total cholesterol level (mg/dL)
1500
1000
500
0
Ucp2f/f
Ucp2ΔEC
H
p=0.93
Serum total triglyceride level (mg/dL)
200
150
100
50
0
Ucp2f/f
Ucp2ΔEC
I
p=0.92
Serum HDL level (mg/dL)
600
400
200
0
Ucp2f/f
Ucp2ΔEC
J
p=0.45
Serum LDL level (mg/dL)
1000
800
600
400
200
0
Ucp2f/f
Ucp2ΔEC

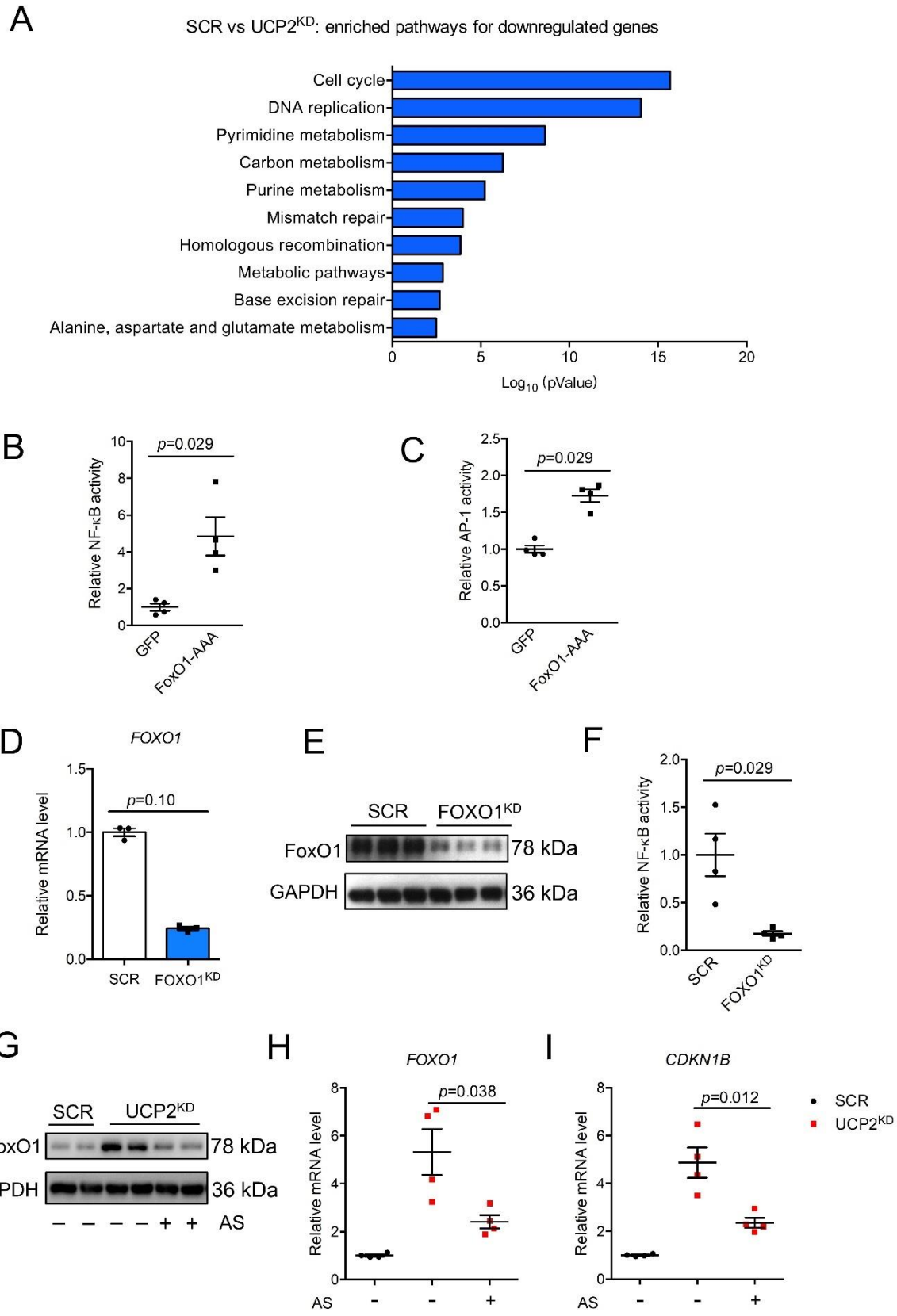
410

Supplemental Figure S6. AAV-Pcsk9-mediated knockdown of LDL receptor and serum lipid profile in mice. (A) Compared to AAV-vector group, upregulated mRNA expression of *Pcsk9* and **(B)** decreased LDLR protein expression in livers from C57 mice injected with AAV-Pcsk9 for 7 days. Mann-Whitney test. **(C, D, E, F)** Elevated serum lipid level in C57 mice injected with AAV-Pcsk9 for 7 days. Mann-Whitney test. **(G, H, I, J)** EC-specific *Ucp2* knockout does not affect serum lipid profile. Mann-Whitney test.


A
Ucp2-ff1
Ucp2-ff2
Ucp2-ff3
Ucp2-ff4
Ucp2-ff5
Ucp2-ff6
EC-Ucp2 KO1
EC-Ucp2 KO2
EC-Ucp2 KO3
EC-Ucp2 KO4
EC-Ucp2 KO5
EC-Ucp2 KO6
EC-Ucp2 KO7
B
Vector 1
Vector 2
Vector 3
Vector 4
Vector 5
Vector 6
Vector 7
Vector 8
Ucp2-1
Ucp2-2
Ucp2-3
Ucp2-4
Ucp2-5
Ucp2-6
Ucp2-7
Ucp2-8
C
AAV-vector
AAV-Cdh5-Ucp2
UCP2
33 kDa
GAPDH
36 kDa
D
p=0.0022
p=0.0022
Ucp2 mRNA level
AAV-vector
AAV-Cdh5-Ucp2
Liver
Aorta
E
p=0.029
p=0.029
p=0.057
Relative mRNA level
UCP2
KLF2
NOS3
SCR
UCP2KD
F
p=0.0067
Relative mRNA level
Ad-GFP
Ad-UCP2
Time
0 24 48 72
0 24 48 72 h
KLF2
NOS3

417

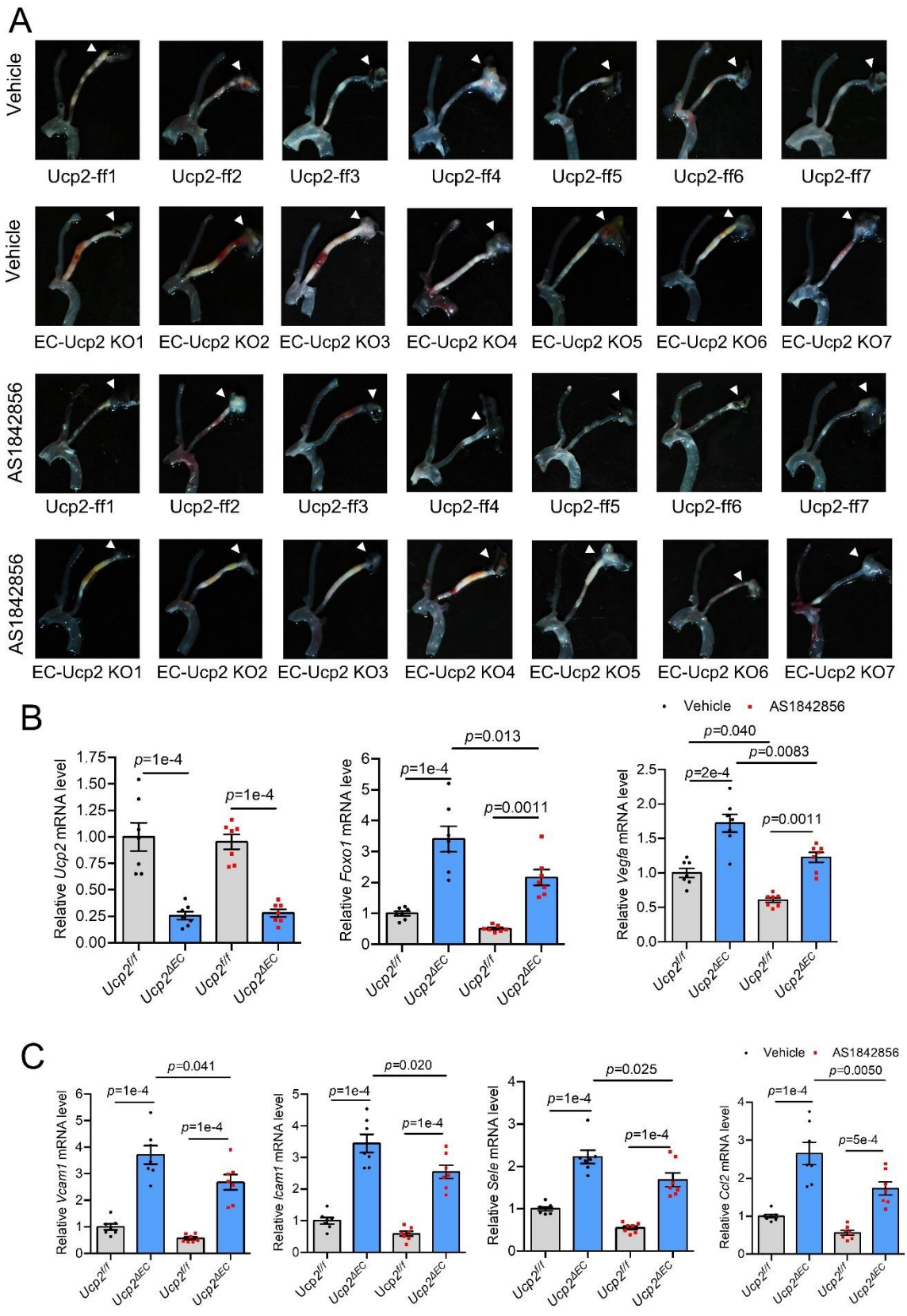
Supplemental Figure S7. Effect of UCP2 gain- and loss-of-function on disturbed flow-associated atherosclerosis and UCP2 regulates KLF2 expression in HUVECs. Pictures showing the atherosclerotic plaques in carotid arteries received partial ligation **(A)** EC-specific *Ucp2* KO mice **(B)** AAV-Cdh5-Ucp2 overexpression in *ApoE*$^{-/-}$ mice. **(C)** Upregulated UCP2 protein level by AAV-Cdh5-Ucp2 in HUVECs. **(D)** Upregulated *Ucp2* mRNA level in mouse liver and aorta by AAV-Cdh5-Ucp2. Mann-Whitney test. **(E)** Reduced *KLF2* mRNA level in UCP2-silenced HUVECs. Mann-Whitney test. **(F)** Ad-UCP2 overexpression time-dependently increased *KLF2* and *NOS3* mRNA expression in HUVECs. Kruskal-Wallis test.


A
SCR vs UCP2KD: enriched pathways for downregulated genes
Cell cycle
DNA replication
Pyrimidine metabolism
Carbon metabolism
Purine metabolism
Mismatch repair
Homologous recombination
Metabolic pathways
Base excision repair
Alanine, aspartate and glutamate metabolism
0
5
10
15
20
Log10 (pValue)
B
p=0.029
Relative NF-κB activity
GFP
FoxO1-AAA
C
p=0.029
Relative AP-1 activity
GFP
FoxO1-AAA
D
FOXO1
p=0.10
Relative mRNA level
SCR
FOXO1KD
E
SCR
FOXO1KD
FoxO1
78 kDa
GAPDH
36 kDa
F
p=0.029
Relative NF-κB activity
SCR
FOXO1KD
G
SCR
UCP2KD
FoxO1
78 kDa
GAPDH
36 kDa
– – – – + + AS
H
FOXO1
p=0.038
Relative mRNA level
AS - - +
I
CDKN1B
p=0.012
Relative mRNA level
AS - - +
SCR
UCP2KD

426

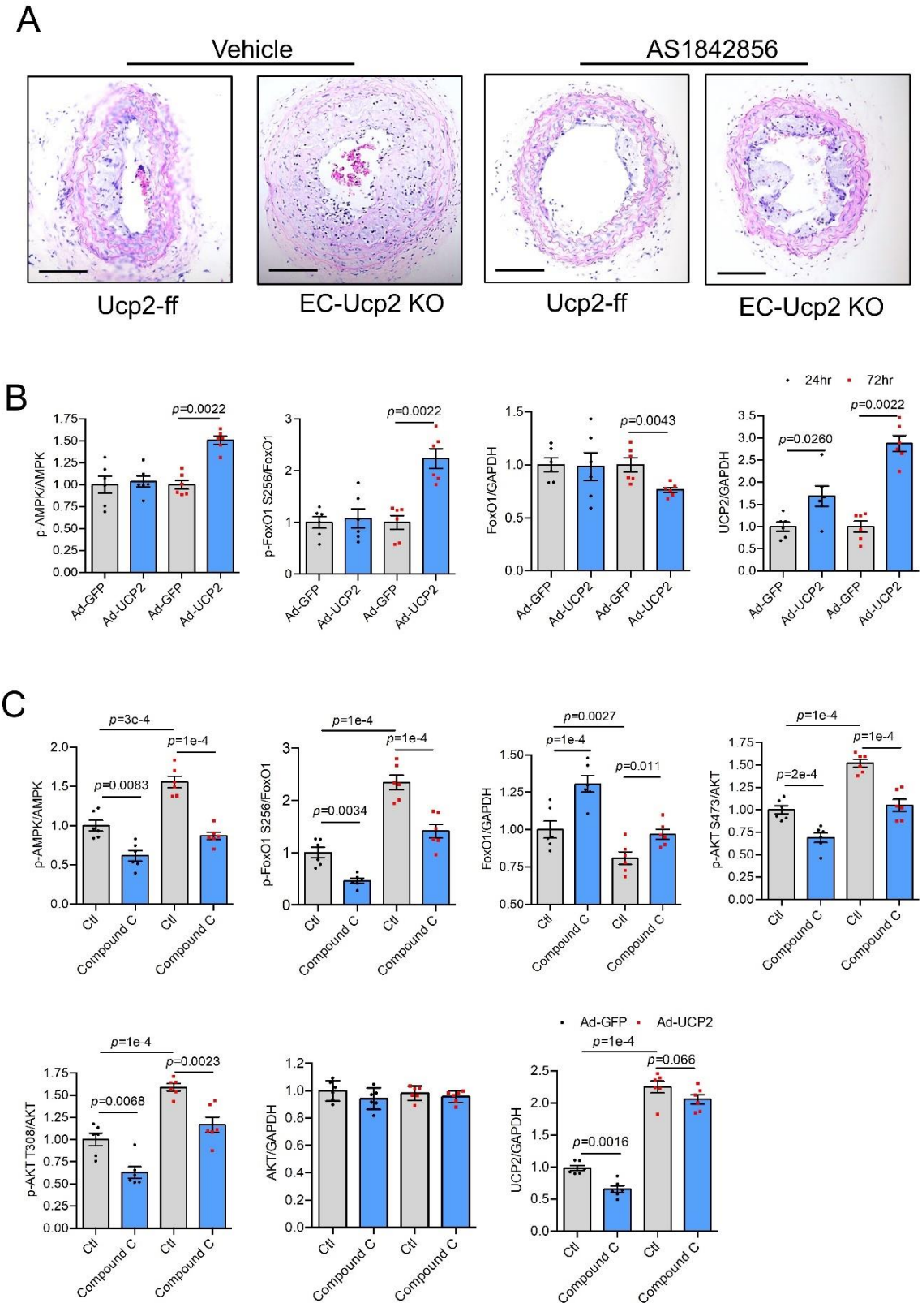
Supplemental Figure S8. FoxO1 is proinflammatory in ECs.

(A) KEGG enrichment pathway analysis of downregulated genes in *UCP2*^{KD} HAECs. Overexpression of FoxO1-AAA increased transcriptional activity of **(B)** NF-κB and **(C)** AP-1 in HUVECs detected by dual luciferase assay. Mann-Whitney test. FOXO1 mRNA **(D)** and **(E)** protein downregulation by FOXO1-shRNA in HAECs. Mann-Whitney test. **(F)** Knockdown of FoxO1 by FOXO1-shRNA inhibits transcriptional activity of NF-κB in HUVECs detected by dual luciferase assay. Mann-Whitney test. **(G)** AS1842856 treatment inhibited UCP2^{KD}-induced FoxO1 protein expression. AS1842856 abolished *UCP2*^{KD}-induced mRNA expression of FOXO1 **(H)** and FoxO1 target gene *CDKN1B* **(I)** in HAECs. 2 Way ANOVA, Tukey post hoc.


A
Vehicle
Ucp2-ff1
Ucp2-ff2
Ucp2-ff3
Ucp2-ff4
Ucp2-ff5
Ucp2-ff6
Ucp2-ff7
Vehicle
EC-Ucp2 KO1
EC-Ucp2 KO2
EC-Ucp2 KO3
EC-Ucp2 KO4
EC-Ucp2 KO5
EC-Ucp2 KO6
EC-Ucp2 KO7
AS1842856
Ucp2-ff1
Ucp2-ff2
Ucp2-ff3
Ucp2-ff4
Ucp2-ff5
Ucp2-ff6
Ucp2-ff7
AS1842856
EC-Ucp2 KO1
EC-Ucp2 KO2
EC-Ucp2 KO3
EC-Ucp2 KO4
EC-Ucp2 KO5
EC-Ucp2 KO6
EC-Ucp2 KO7
B
Vehicle
AS1842856
Relative Ucp2 mRNA level
p=1e-4
p=1e-4
Relative Foxo1 mRNA leve
p=0.013
p=1e-4
p=0.0011
Relative Vegfa mRNA level
p=0.040
p=0.0083
p=2e-4
p=0.0011
Ucp2f/f
Ucp2ΔEC
C
Relative Vcam1 mRNA level
p=0.041
p=1e-4
p=1e-4
Relative Icam1 mRNA level
p=0.020
p=1e-4
p=1e-4
Relative Sele mRNA level
p=0.025
p=1e-4
p=1e-4
Relative Ccl2 mRNA level
p=0.0050
p=1e-4
p=5e-4

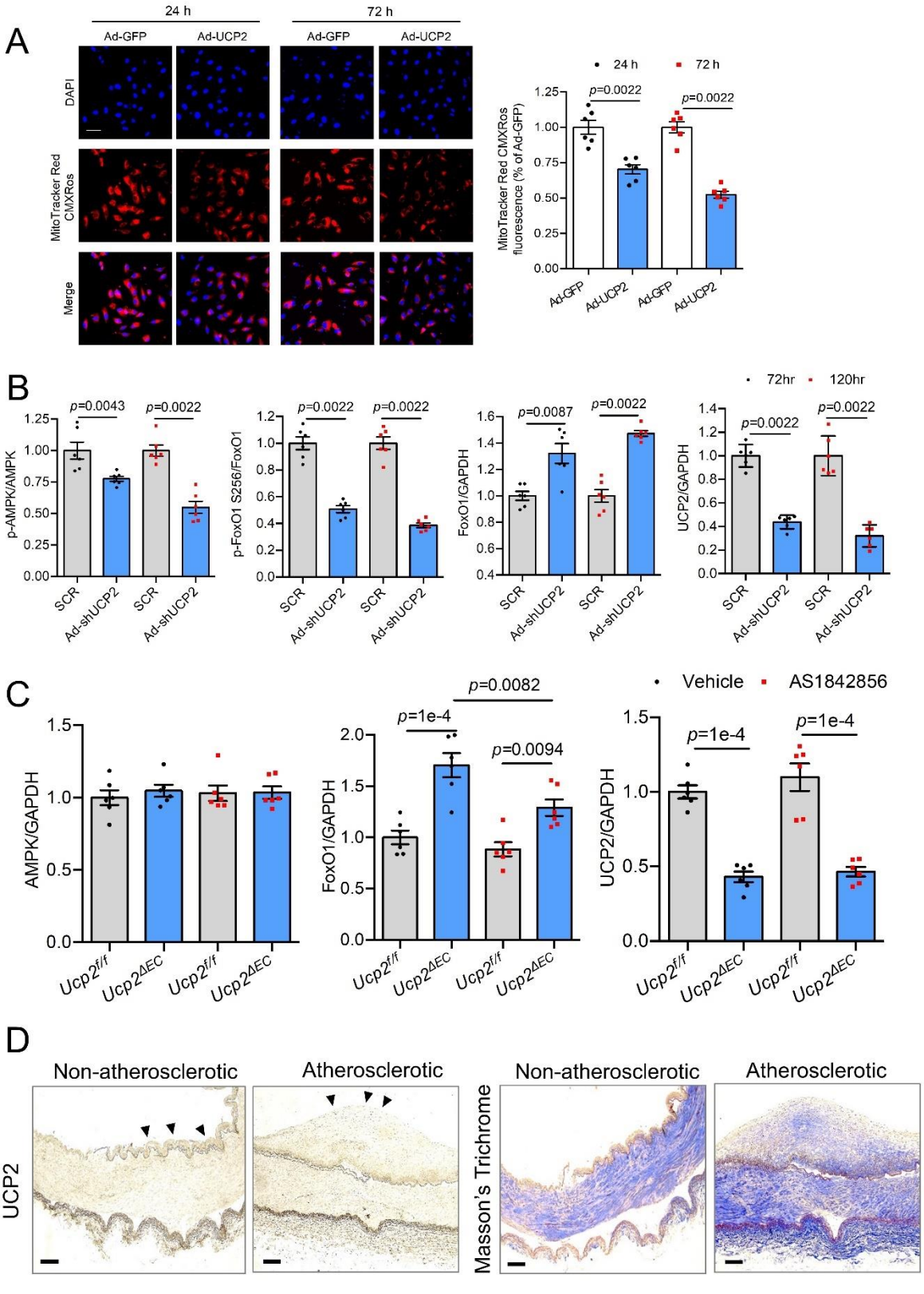
436

Supplemental Figure S9. FoxO1 inhibition by AS1842856 attenuated disturbed flow-enhanced atherosclerotic plaque formation in carotid arteries. (A) Pictures showing the atherosclerotic plaques in carotid arteries received partial ligation in EC-specific *Ucp2* KO mice treated with vehicle and AS1842856. **(B)** qPCR results showing AS1842856 inhibited the mRNA expression of *Foxo1* and FoxO1 target gene *Vegfa* in aorta of EC-specific *Ucp2* KO mice. 2 Way ANOVA, Tukey post hoc. **(C)** Reduced mRNA expression level of proinflammatory genes (*Vcam1, Icam1, Sele* and *Ccl2*) in aorta of EC-specific *Ucp2* KO mice treated with AS1842856. 2 Way ANOVA, Tukey post hoc.


A
Vehicle
AS1842856
Ucp2-ff
EC-Ucp2 KO
Ucp2-ff
EC-Ucp2 KO
B
24hr
72hr
p-AMPK/AMPK
p-FoxO1 S256/FoxO1
FoxO1/GAPDH
UCP2/GAPDH
p=0.0022
p=0.0022
p=0.0043
p=0.0260
p=0.0022
Ad-GFP
Ad-UCP2
C
p-AMPK/AMPK
p-FoxO1 S256/FoxO1
FoxO1/GAPDH
p-AKT S473/AKT
p-AKT T308/AKT
AKT/GAPDH
UCP2/GAPDH
Ctl
Compound C
Ad-GFP
Ad-UCP2
p=0.066
p=0.0016

445

Supplemental Figure S10. UCP2 overexpression activates AMPK to inhibit FoxO1. (A) H&E staining results showing AS1842856 suppressed the carotid wall thickening in EC-specific *Ucp2* KO mice subjected to partial ligation of carotid arteries. **(B)** Summarized western blotting data showing UCP2 overexpression for 72 hours increased level of p-AMPK and p-FoxO1 but inhibited FoxO1 expression in HUVECs. Mann-Whitney test. **(C)** Summarized western blotting data showing inhibition of AMPK by Compound C abolished the effect of UCP2 overexpression on Akt and FoxO1 activity in HUVECs. 2 Way ANOVA, Tukey post hoc.


A
24 h
72 h
Ad-GFP
Ad-UCP2
DAPI
MitoTracker Red CMXRos
Merge
MitoTracker Red CMXRos fluorescence (% of Ad-GFP)
p=0.0022
B
72hr
120hr
p-AMPK/AMPK
p-FoxO1 S256/FoxO1
FoxO1/GAPDH
UCP2/GAPDH
p=0.0043
p=0.0087
SCR
Ad-shUCP2
C
Vehicle
AS1842856
AMPK/GAPDH
p=1e-4
p=0.0082
p=0.0094
Ucp2f/f
Ucp2ΔEC
D
Non-atherosclerotic
Atherosclerotic
UCP2
Masson's Trichrome

453

Supplemental Figure S11. UCP2 regulates AMPK activity HUVECs and in mouse aortas. (A) UCP2 overexpression time-dependently reduced mitochondrial membrane potential in HUVECs. Scale bar=50 μM. **(B)** UCP2 knockdown reduced levels of p-AMPK and p-FoxO1 but increased FoxO1 expression in HUVECs. Mann-Whitney test. **(C)** Summarized western blotting data showing EC-specific *Ucp2* knockout did not affect total AMPK expression but increase FoxO1 protein expression in aortas. 2 Way ANOVA, Tukey post hoc. **(D)** Immunohistochemical staining of UCP2 for diminished UCP2 expression, and Masson's Trichrome staining for elevated collagen level in plaque region of human renal arteries. Scale bar: 200 μM.

462 **Supplemental Table S1. Real time qPCR primers for detection of mouse genes**

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Gene name	Primer sequences	Species
Gapdh	F: AGGTCGGTGTGAACGGATTTG R: TGTAGACCATGTAGTTGAGGTCA	Mouse
Klf2	F: CTCAGCGAGCCTATCTTGCC R: CACGTTGTTTAGGTCCTCATCC	Mouse
Ucp2	F: ATGGTTGGTTTCAAGGCCACA R: CGGTATCCAGAGGGAAAGTGAT	Mouse
Pcsk9	F: GAGACCCAGAGGCTACAGATT R: AATGTACTCCACATGGGGCAA	Mouse
Nos3	F: TGTGACCCTCACCGCTACAA R: GCACAATCCAGGCCCAATC	Mouse
Vcam1	F: GTTCCAGCGAGGGTCTACC R: AACTCTTGGCAAACATTAGGTGT	Mouse
Icam1	F: GTGATGCTCAGGTATCCATCCA R: CACAGTTCTCAAAGCACAGCG	Mouse
Sele	F: ATGCCTCGCGCTTTCTCTC R: GTAGTCCCGCTGACAGTATGC	Mouse
Ccl2	F: TTAAAAACCTGGATCGGAACCAA R: GCATTAGCTTCAGATTTACGGGT	Mouse
Il6	F: TTCAGCCCTTGCTTGCCTC R: ACACTTTTACTCCGAAGTCGGT	Mouse
Hmox1	F: AAGCCGAGAATGCTGAGTTCA R: GCCGTGTAGATATGGTACAAGGA	Mouse
Nqo1	F: TGGCCGAACACAAGAAGCTG R: GCTACGAGCACTCTCTCAAACC	Mouse
Angpt2	F: CAGCCACGGTCAACAACTC R: CTTCTTTACGGATAGCAACCGAG	Mouse

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Supplemental Table S2. Real time qPCR primers for detection of human genes

Gene name	Primer sequences	Species
GAPDH	F: CCACTCCTCCACCTTTGAC R: ACCCTGTTGCTGTAGCCA	Human
KLF2	F: CTACACCAAGAGTTCGCATCTG R: CCGTGTGCTTTCGGTAGTG	Human
UCP2	F: CCCCGAAGCCTCTACAATGG R: CTGAGCTTGGAATCGGACCTT	Human
SMAD4	F: ACGAACGAGTTGTATCACCTGG R: TGCACGATTACTTGGTGGATG	Human
PPARGC1A	F: TCTGAGTCTGTATGGAGTGACAT R: CCAAGTCGTTCACATCTAGTTCA	Human
NOS3	F: TGATGGCGAAGCGAGTGAAG R: ACTCATCCATACACAGGACCC	Human
IL6	F: CCTGAACCTTCCAAAGATGGC R: TTCACCAGGCAAGTCTCCTCA	Human
VCAM1	F: CAGTAAGGCAGGCTGTAAAAGA R: TGGAGCTGGTAGACCCTCG	Human
ICAM1	F: TTGGGCATAGAGACCCCGTT R: GCACATTGCTCAGTTCATACACC	Human
SELE	F: TGTGGGTCTGGGTAGGAACC R: AGCTGTGTAGCATAGGGCAAG	Human
CCL2	F: CAGCCAGATGCAATCAATGCC R: TGGAATCCTGAACCCACTTCT	Human
SERPINE1	F: AGTGGACTTTTCAGAGGTGGA R: GCCGTTGAAGTAGAGGGCATT	Human
F3	F: CCCAAACCCGTCAATCAAGTC R: CCAAGTACGTCTGCTTCACAT	Human
MMP1	F: GGGGCTTTGATGTACCCTAGC R: TGTCACACGCTTTTGGGGTTT	Human
MMP2	F: GATACCCCTTTGACGGTAAGGA R: CCTTCTCCCAAGGTCCATAGC	Human
COL1A1	F: GTGCGATGACGTGATCTGTGA R: CGGTGGTTTCTTGGTCGGT	Human
COL1A2	F: GGCCCTCAAGGTTTCCAAGG R: CACCCTGTGGTCCAACAACTC	Human
COL3A1	F: TTGAAGGAGGATGTTCCCATCT R: ACAGACACATATTTGGCATGGTT	Human

COL4A1	F: GGGATGCTGTTGAAAGGTGAA R: GGTGGTCCGGTAAATCCTGG	Human
COL4A2	F: GGTTTCTACGGAGTTAAGGGTG R: GCCAGGGTAACCCCTCAGT	Human
COL5A1	F: TACCCTGCGTCTGCATTTCC R: GCTCGTTGTAGATGGAGACCA	Human
COL5A2	F: GACCAGAACATGTAGGTCCCC R: CTGACATGACAAAAGCGTGCAT	Human
HMOX1	F: GCCATGAACTTTGTCCGGTG R: TTTCGTTGGGGAAGATGCCA	Human
NQO1	F: GAAGAGCACTGATCGTACTGGC R: GGATACTGAAAGTTCGCAGGG	Human
SOD2	F: TTTCAATAAGGAACGGGGACAC R: GTGCTCCCACACATCAATCC	Human
GPX3	F: CCATGGTGGCATAAGTGGCA R: ATGACCAGACCGAATGGTGC	Human
FOXO1	F: TCGTCATAATCTGTCCCTACACA R: CGGCTTCGGCTCTTAGCAAA	Human
CDKN1B	F: TAATTGGGGCTCCGGCTAACT R: TGCAGGTCGCTTCCTTATTCC	Human

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Supplemental Table S3. ChIP-qPCR primers for detection of KLF2 enrichment in UCP2 promoter.

Primer name	Primer sequences	Species
UCP2 (-889/-691)	F: GGCCCAATTGTTGGCTGTTC R: GTGACCTCACGCTCCTACAC	Human
UCP2 (-314/-200)	F: AGACTTGTTTCTGGCGGTCA R: TACTCCTTCGTTCCCCGAGA	Human
UCP2 (-228/-138)	F: GAGTGACCATCTCGGGGAAC R: CCGCCTAGGGTTCTCTCCAT	Human
UCP2 (-157/+42)	F: ATGGAGAGAACCCTAGGCGG R: TGGACAGTCAATCCCAAGGC	Human