

SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Experimental Procedures

Mouse carotid artery ligation model

All experimental procedures were approved by Institutional Animal Care and Use Committee and the Ethic Committee of Chengdu Military General Hospital. Uncoupling protein 2 gene knockout (*Ucp2^{-/-}*) mice were purchased from Jackson Laboratory (Bar Harbor, ME, US). Littermate wild-type (WT) mice (C57BL/6 background) were used as controls. Eight-week-old male mice, housed under a 12h/12h day/night cycle, with *ad libitum* food and water, were used for experiments. Complete left common carotid artery (CCA) ligation was performed using aseptic techniques as previously described.¹ In brief, mice received a small midline incision in the neck area after anesthetized with ketamine (150mg/kg, intraperitoneal), xylazine (10mg/kg, intraperitoneal), and buprenorphine (0.1mg/kg, subcutaneous). The left CCA was dissected and ligated with 6-0 silk suture, and the right carotid artery served as unligated control. For postoperative analgesia, buprenorphine was added to the drinking water (6mg/L) for three days following the procedure. Mice were either given normal drinking water or water supplemented with 1mmol/L TEMPOL (4-hydroxy-2,2,6,6-tetramethyl-piperidinoxyl, Sigma-Aldrich, St. Louis, MO, US). At the end of the experiments, mice were deeply anesthetized with pentobarbital (100mg/kg, intraperitoneal) and perfused with saline. For histology, mice were fixed with perfusion. Carotid arteries were harvested for further experiments.

Rat and rabbit carotid artery balloon injury model

Sprague Dawley rats weighing 250–300g and New Zealand rabbits weighing about 2kg were purchased from the Animal Center of Dashuo Biotechnology Co. (Chengdu, Sichuan, China) and used for experiments.^{2, 3} Rats were anesthetized with ketamine (100mg/kg, intraperitoneal), xylazine (10mg/kg, intraperitoneal), and buprenorphine (0.05mg/kg, subcutaneous), while rabbits with ketamine (50mg/kg, intramuscular), xylazine (5mg/kg, intramuscular), and buprenorphine (0.05mg/kg, subcutaneous). After anesthesia, a median neck incision was performed and the left CCA was isolated. The internal carotid artery and

proximal CCA were temporary occluded with vascular clamps, and the external carotid artery was ligated. A 2F (for rat) or 3F (for rabbit) Fogarty balloon catheter was inserted to the left CCA through an arteriotomy of the external carotid artery proximal to the ligation knot. The left CCA was injured by three passages of the inflated Fogarty catheter. The sham operation involved simple ligation of the right external carotid artery without balloon injury. For gene transfer, 30 μ L (for rat) or 50 μ L (for rabbit) adenovirus, with a titer of 10^{11} PFU/mL, expressing UCP2 and GFP (Ad-UCP2) or GFP alone (Ad-GFP) was injected into the balloon injured CCA via the external carotid artery immediately after balloon injury and incubated for 30min. Then, the external carotid artery was ligated proximal to the puncture, and the clamps on internal carotid artery and proximal CCA were released to restore the blood flow. For postoperative analgesia, buprenorphine was added to the drinking water (6mg/L) for three days following the procedure. On 21 days after the procedure, rats and rabbits were deeply anesthetized with pentobarbital (100mg/kg, intraperitoneal) and perfused with saline and the injured CCA as well as the contralateral unmanipulated carotid arteries were collected.

Mini-pig coronary artery in-stent restenosis model

Male Guizhou mini-pigs (40-50kg, Dashuo Biotechnology) were used for the experiments. Pig coronary stenting was performed as previously described.⁴ Briefly, pigs received orally with aspirin and clopidogrel 24 hours prior to procedure and maintained on this dual antiplatelet therapy throughout the experiments to prevent in-stent thrombosis. Pigs were sedated by intramuscular injection of ketamine (10mg/kg) and buprenorphine (0.05mg/kg). General anesthesia was conducted by continuously intravenous injection of propofol (25 μ g/kg/min, Guorui Pharmaceutical Co., Sichuan, China). Vital signs were continuously monitored during the procedure as well as distress or pain. The femoral artery was surgically exposed and punctured directly, then a 6F sheath was introduced and the heparin solution (100U/kg) was injected. Under fluoroscopic guidance, a catheter was inserted and advanced to the coronary artery ostium, and the coronary angiography was performed by injecting the contrast agent (iohexol, GE Healthcare, Shanghai, China) prior to the

deployment of bare metal stents (Firebird™, Microport Scientific, Shanghai, China) to achieve a ratio of stent to artery diameter of 1.2:1. After stent implantation, a local drug infusion balloon catheter (ClearWay™ RX, Atrium Medical, Hudson, NH, US)⁵ was inserted into the stented segment. A total of 500µL of Ad-UCP2 or Ad-GFP (10¹¹ PFU/mL) diluted to 1mL in PBS was infused to inflate the balloon and held for 90 seconds. This special balloon has a microporous structure of tortuous channels allowing infused agent to weep out onto the vessel wall. For postoperative analgesia, buprenorphine (0.05mg/kg) was intramuscularly injected every 12 hours for three days following the procedure. Pigs received follow up coronary angiography via carotid artery 3 months after procedure and then were euthanized by intravenous overdose of pentobarbital (100mg/kg) and coronary vessels carefully removed.

Transcriptome array

Mice at 3 days after procedure were sacrificed and the ligated and contralateral unligated carotid arteries isolated and stored in RNA-later at -80 °C. The following microarray procedure and data analysis were performed by OE Biotech (Shanghai, China). Total RNA was extracted from mouse artery samples with RNAiso Plus reagent (9108, Takara, Shiga, Japan) and quantified using a NanoDrop ND2000 (Thermo Scientific, Waltham, MA, US). The quality of RNA was tested using the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, US). Samples with a RNA Integrity Number (RIN) greater than or equal to 7.0 and 28S/18S greater than or equal to 0.7 were subjected to microarray analysis. Briefly, total RNA was transcribed to double strand cDNA, then synthesized into cRNA and labeled with Cyanine-3-CTP. The labeled cRNAs were hybridized onto the microarray (Agilent SurePrint G3 Mouse GE 8×60K Microarrays, Design ID 028005). After washing, the arrays were scanned by the Agilent Scanner G2505C (Agilent Technologies). The data were processed with Feature Extraction software (version10.7.1.1, Agilent Technologies). The total data set included three biological replicates for both groups. Each consisted of three mouse carotid arteries. Differentially expressed genes were identified through analyzing fold change between the compared groups as well as *P* value calculated with Student *t*-test.

The threshold set for up- and down-regulated genes was a fold change greater than 2.0 and a *P* value less than 0.05. We analyzed 84 mitochondria function regulating genes, including *Aifm2*, *Aip*, *Bak1*, *Bbc3*, *Bcl2*, *Bcl2l1*, *Bid*, *Bnip3*, *Cdkn2a*, *Cox10*, *Cox18*, *Cpt1b*, *Cpt2*, *Dnajc19*, *Dnm1l*, *Fis1*, *Fxc1*, *Grpel1*, *Hsp90aa1*, *Hspd1*, *Imp1l*, *Imp2l*, *Lrpprc*, *Mfn1*, *Mfn2*, *Mipep*, *Msto1*, *Mtx2*, *Nefl*, *Opa1*, *Pmaip1*, *Rhot1*, *Rhot2*, *Sfn*, *Sh3glb1*, *Slc25a1*, *Slc25a10*, *Slc25a12*, *Slc25a13*, *Slc25a14*, *Slc25a15*, *Slc25a16*, *Slc25a17*, *Slc25a19*, *Slc25a2*, *Slc25a20*, *Slc25a21*, *Slc25a22*, *Slc25a23*, *Slc25a24*, *Slc25a25*, *Slc25a27*, *Slc25a3*, *Slc25a30*, *Slc25a31*, *Slc25a37*, *Slc25a4*, *Slc25a5*, *Sod1*, *Sod2*, *Stard3*, *Taz*, *Timm10*, *Timm17a*, *Timm17b*, *Timm22*, *Timm23*, *Timm44*, *Timm50*, *Timm8a*, *Timm8b*, *Timm9*, *Tomm20*, *Tomm22*, *Tomm34*, *Tomm40*, *Tomm40l*, *Tomm70a*, *Tp53*, *Tspo*, *Ucp1*, *Ucp2*, *Ucp3*, and *Uxt*.

Endothelial function assay

Carotid arteries were dissected and cut into 3-mm-long segments in cold Krebs solution containing (in mM): NaCl 119, NaHCO₃ 25, Glucose 11.1, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, and CaCl₂ 2.5, pH 7.4. Arterial rings were mounted in a 4-chamber wire myograph (Danish Myo Technology, Denmark) and maintained at 37°C in Krebs solution gassed with 95% O₂ and 5% CO₂. After equilibration, all rings were pre-contracted with 1μM phenylephrine and then relaxed with 10⁻⁹-10⁻⁵ M acetylcholine (ACh) to assess the endothelial relaxation.

Pathology

For histological analysis, mouse carotid arteries were fixed with perfusion and other vessels were fixed in 4% paraformaldehyde for 24 hours. Six serial sections from each animal were used for staining. Carotid arteries, saphenous veins, and distal sections to the stented porcine coronary arteries were dehydrated in graded ethanol, cleared in xylene, embedded in paraffin wax, sectioned into 5μm thick sections, and affixed to slides. Stented porcine coronary arteries were embedded in methyl methacrylate and cut at a thickness of 80μm using a hard tissue slicer with a rotating diamond-coated saw (Leica SP1600, Wetzlar, Germany). Sections were rehydrated and stained with H&E.

Immunohistochemistry

Six serial sections from each animal were used for staining. Paraffin sections were dewaxed and rehydrated. Tissue sections and chamber slides with a monolayer of cells were subjected to citric acid antigen retrieval and endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide. After blocking sections with 5% normal donkey serum in phosphate buffered saline (PBS), sections were incubated overnight at 4°C with mouse anti-smooth muscle (SM) α -actin (BM0002, Boster, Wuhan, China), rabbit anti-Ki67 (GTX16667, GeneTex, Irvine, CA, US), rabbit anti-NF- κ B p65 (ab16502, Abcam, Cambridge, UK), rabbit anti-CD31 (ab212709, Abcam), and rabbit anti-UCP2 (ab203244, Abcam) antibodies in 1% bovine serum albumin (BSA) in PBS. Sections were then incubated with appropriate horseradish peroxidase conjugated secondary antibody. Optimal visualization of staining was achieved using 3,3'-diaminobenzidine tetrahydrochloride (DAB, AR1022, Boster) or 3-amino-9-ethylcarbazole (AEC, AR1020, Boster) detection kits. Sections were counter-stained with Mayer's hematoxylin. All immunohistochemistry staining was quantified as the area of positive staining or the number of positive stained cells expressed as a percentage of total neointimal area.

Immunofluorescence

Frozen sections of vessel or chamber slides with a monolayer of cells were fixed in ice-cold acetone for 10min, blocked in 5% normal donkey serum in PBS for 60min, incubated at 4°C with rabbit anti-NF- κ B p65 (8242P, Cell Signaling, Danvers, MA, US) or rabbit anti-UCP2 (ab203244, Abcam) overnight. The p65 and UCP2 were visualized with Alexa Fluor 594-conjugated goat anti-rabbit IgG secondary antibody (1:500 dilution, A-11012, Invitrogen, Carlsbad, CA, US). Cell nuclei were stained with DAPI. Images were acquired on a Leica TCS-SP5 Confocal Microscope. The fluorescence intensity was calculated using ImageJ software.

Superoxide assay

To assess superoxide production, the fresh frozen sections of vessel or chamber slides with a monolayer of cells were incubated in dark with dihydroethidium (DHE; S0063, Beyotime,

Jiangsu, China) diluted in PBS (40 μ mol/l, stock solution prepared in dimethylsulphoxide) or a DHE-free solution for 30min at 37°C f. Images were acquired by using an inverted fluorescence microscope (Leica DMLB2; Leica Microsystems) outfitted with a rhodamine filter set.⁶ The fluorescence intensity was calculated using ImageJ software.

Reendothelialization assay

Reendothelialization of the carotid artery was determined by Evans blue dye (Sigma-Aldrich) staining 7 days after balloon injury in rat. Rats were anesthetized and 1mL of 5% Evans was injected into the tail vein, followed by fixation with a perfusion of 10% formalin. After rats were euthanized, the injured segments of the carotid arteries were harvested and examined under a light microscope. The ratio between the area stained in blue and the total carotid artery area was calculated.

TUNEL staining

Apoptotic cells in mouse carotid arterial sections were detected by using the fluorescein-dUTP TUNEL assay as per the manufacturer's instructions (MK1020, Boster). Briefly, slides were deparaffinized, washed, incubated with 20 μ g/mL of proteinase K, and then incubated with TUNEL reaction mixture at 37°C for 1 hour in a humidified chamber under parafilm coverslips. Cell nuclei were stained with DAPI. Images were acquired on a Leica TCS-SP5 Confocal Microscope. The positive cells in neointima were counted.

Cell culture

Human aortic smooth muscle cell (HA-SMC), purchased from ATCC (Manassas, VA, US), were grown in medium 231 with smooth muscle growth supplement (Gibco, Carlsbad, CA, US). The cells were incubated at 37°C in a humidified 5% CO₂ atmosphere. The experiments were performed on cells at passages 4 to 10 from the two different batches. For the knockdown of UCP2, cells were transfected with 20nmol/L of UCP2 specific siRNA or universal scrambled negative control siRNA (SR305019 and SR30004, Origene, Rockville, MD, US) by using Lipofectamine RNAiMAX Transfection Reagent (13778-075, Invitrogen) and incubated for 48 hours. For overexpression of UCP2, HA-SMCs cultured in 6-well plates were transfected with 6.2 μ L of Ad-UCP2 or Ad-GFP (10¹¹ PFU/mL) diluted

in 2 mL of medium in the presence of 4.0 $\mu\text{g}/\text{mL}$ hexadimethrine bromide (Sigma-Aldrich) for 48 hours. For specific purpose, 20 ng/mL of recombinated human PDGF (PeproTech, Rocky Hill, NJ, US), 1mmol/L of 4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL, 176141, Sigma-Aldrich, St. Louis, MO, US), or 10 $\mu\text{mol}/\text{L}$ of BAY 11-7085 (Santa Cruz Biotechnology, Santa Cruz, CA, US) were used to treat the cells.

Mouse aortic SMC isolation

Vascular SMCs were isolated from aortas of 5-week-old WT and *Ucp2*^{-/-} mice by collagenase-elastase digestion.⁷ SMCs were maintained in medium 231 with smooth muscle growth supplement. Cells between passages two and five were used for experiments.

Cell migration and proliferation assay

Cell migration assay was performed using a scratch wound healing assay.⁸ Confluent SMCs in six-well plates were growth arrested, scraped using sterilized 200- μL pipette tips, washed with PBS, and cultured for 24 hours. Images were captured by a phase-contrast microscope (CKX41; Olympus, Tokyo, Japan) and used to calculate the recovered area. Cell proliferation was measured using a cell counting kit-8 (CCK-8; Beyotime, Jiangsu, China).⁸

Real-time polymerase chain reaction (RT-PCR)

The RNA was extracted from the arterial tissue and SMCs using the RNAiso Plus reagent. The RT-PCR was performed using One Step SYBR Prime Scrip RT-PCR Kit II (RR086A, TaKaRa). The relative amount of mRNA was calculated by $2^{-\Delta\Delta\text{CT}}$ and was normalized to a housekeeping gene 18s rRNA. Each sample was run and analyzed in triplicate. PCR primer sequence is listed as follow: Mouse *Ucp2*: F, 5'-GCC ATT GTC AAC TGT GCT GA-3'; R, 5'-CGA TGA CGG TGG TGC AGA AG-3'; Human *UCP2*: F, 5'-GCC ATT GTC AAC TGT GCT GA-3'; R, 5'-CGA TGA CAG TGG TGC AGA AG-3'; 18s rRNA: F, 5'-CGC GGT TCT ATT TTG TTG GTT T-3'; R: 5'-GCG CCG GTC CAA GAA TTT-3'.

Antibody arrays

Cells were harvested and total protein was extracted. The antibody assays were performed with Human PDGF Phospho Antibody Array (PDG195, Full Moon BioSystems, Sunnyvale,

CA, US) according to the manufacturer's instructions. Three biological replicates were included in each group.

Western blotting

Total proteins were extracted from arterial tissue and SMCs using a protein extraction kit (Keygen Biotech, Nanjing, China) and quantified using an enhanced BCA Protein Assay Kit (Beyotime, Jiangsu, China). For specific experiments, cytoplasmic and nuclear extracts were separated by using a commercial kit (AR0106, Boster). Forty micrograms of extracted protein were loaded onto 8-12% SDS polyacrylamide gels. The separated proteins were then transferred to PVDF membranes (SLGP033RS, Millipore, Bedford, MA, US). Membranes were blocked with 5% bovine serum albumin (BSA) in TBS-T (Tris-buffered saline, 0.1% Tween 20) for 1 hour and then incubated with anti-UCP2 (ab203244, Abcam), anti-PDGF (ab23914, Abcam), anti-I κ B (ab32518, Abcam), anti-Phospho-I κ B (GTX50209, GeneTex), anti-IKK (ab32041, Abcam), anti-Phospho-IKK (ab38515, Abcam), anti-p65 (ab16502, Abcam), anti-p50 (BA1297-2, Boster), anti-GFP (GTX113617, GeneTex), anti-SM α -actin (BM0002, Boster), anti-calponin (ab46794, Abcam), anti-smooth muscle myosin heavy chain (SM MHC, ab53219, Abcam), anti-GAPDH (GTX627408-01, GeneTex), and anti-Histone H3 (bs-0349R, Bioss, Beijing, China) antibodies overnight. Membranes were rinsed three times with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour. Membranes were rinsed three times with TBS-T. Chemiluminescence detection reagent (BeyoECL Plus, P0018, Beyotime, Jiangsu, China) were dropwise added on the membranes. The luminescent signal was detected by exposure to x-ray film.

Mitochondrial membrane potential ($\Delta\Psi_m$) measurement

The $\Delta\Psi_m$ was measured using a 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1) $\Delta\Psi_m$ assay kit (Beyotime) according to the manufacturer's instructions. The increases in the ratios between fluorescence intensity in the red and green channels were interpreted as an increase in $\Delta\Psi_m$.

NF- κ B p65 transcription factor assay

Nuclear fractions of SMCs were isolated and the bound NF- κ B was detected using NF- κ B p65 Transcription Factor Assay Kit (ab133112, Abcam). The assay was carried out according to the kit manual.

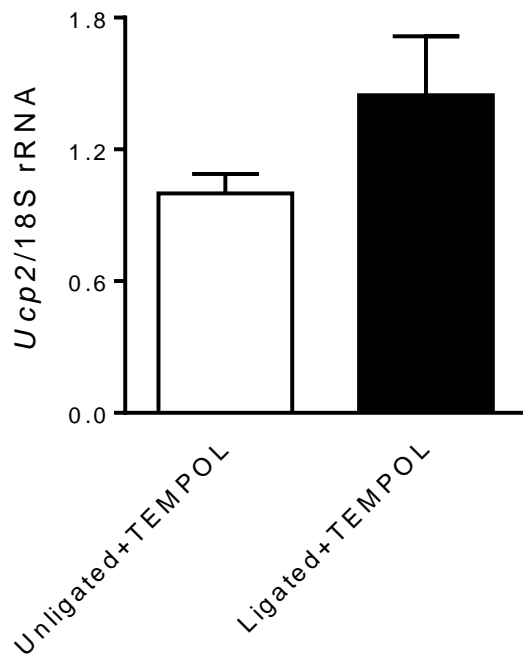
Human saphenous vein culture *ex vivo*

The experiment conformed to the principles outlined in the Declaration of Helsinki. Written informed consents and approval from the Ethics Committee of Chengdu Military General Hospital were obtained before tissue collection. Left over human saphenous vein tissue from surgery were collected and cultured as previously described.¹ Briefly, the veins were cut into 0.5 cm x 0.5 cm explants. Tissue explants were transfected with Ad-GFP or Ad-UCP2 and cultured with luminal surface facing up in RPMI 1640 medium supplemented with 20% fetal bovine serum and 10 ng/mL of PDGF for 14 days. Then, tissue explants were fixed and embedded in paraffin, and the paraffin sections were stained with H&E and Verhoeff–Van Gieson stain.

Statistical analysis

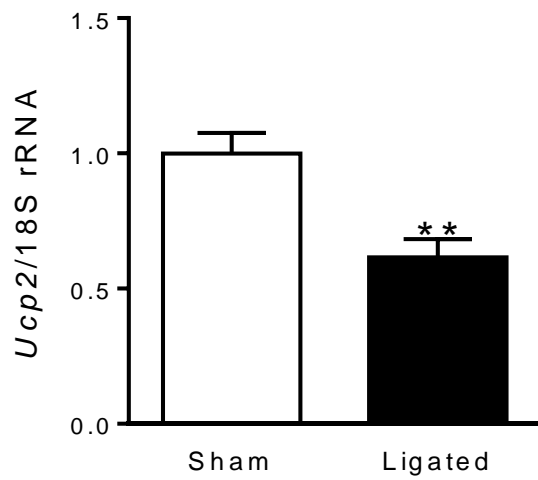
Continuous data are presented as mean \pm standard error. Normality was tested with the Kolmogorov-Smirnov test. Comparisons among groups were determined by analysis of variance with post hoc Tukey HSD test, while statistically significant differences between two groups were determined by using the Student's t-test (SPSS Inc, Chicago, IL, US). The nonparametric Mann-Whitney U test was used if data were not normally distributed. To compare means which involve two factors, we performed two-way ANOVA. $P < 0.05$ was considered statistically significant.

Figure S1. Effects of TEMPOL on the expression of *Ucp2*.



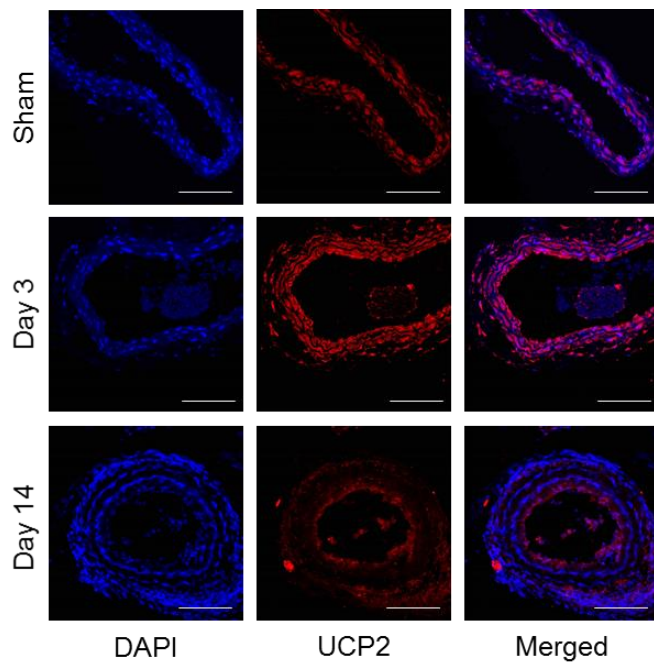
Expression of *Ucp2* in ligated and unligated carotid arteries harvested on day 3 after surgery from C57BL/6 mice treated with 1mmol/L TEMPOL in drinking water. n=6 mice.

Figure S2. Expression of *Ucp2* in sham and ligated mouse carotid arteries.



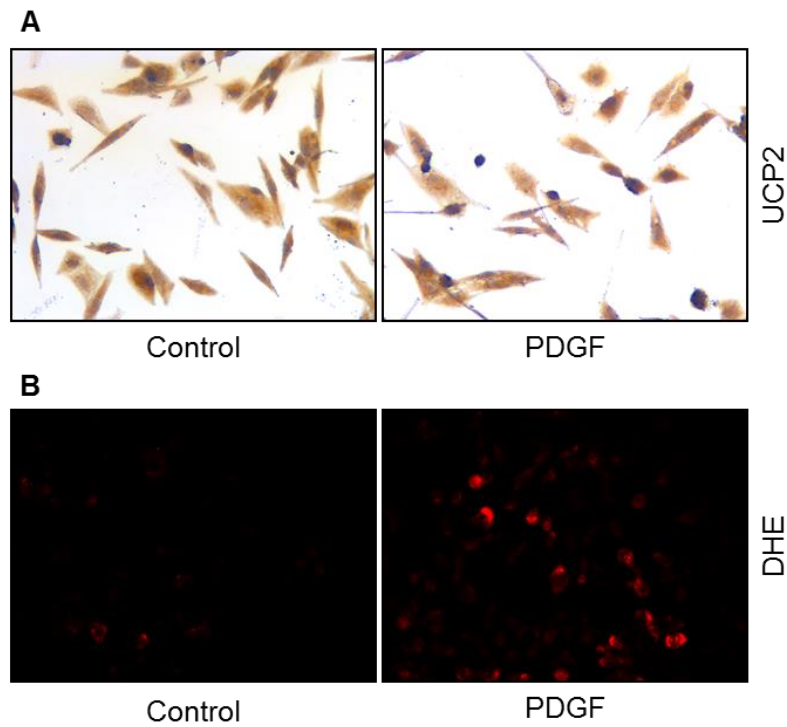
The RNA expression of *Ucp2* in sham and ligated carotid arteries of C57BL/6 mice on day 14 after surgery were measured by RT-PCR. n=6 mice in each group. ** $P < 0.01$ vs. sham group.

Figure S3. Expression of UCP2 in ligated mouse carotid arteries.



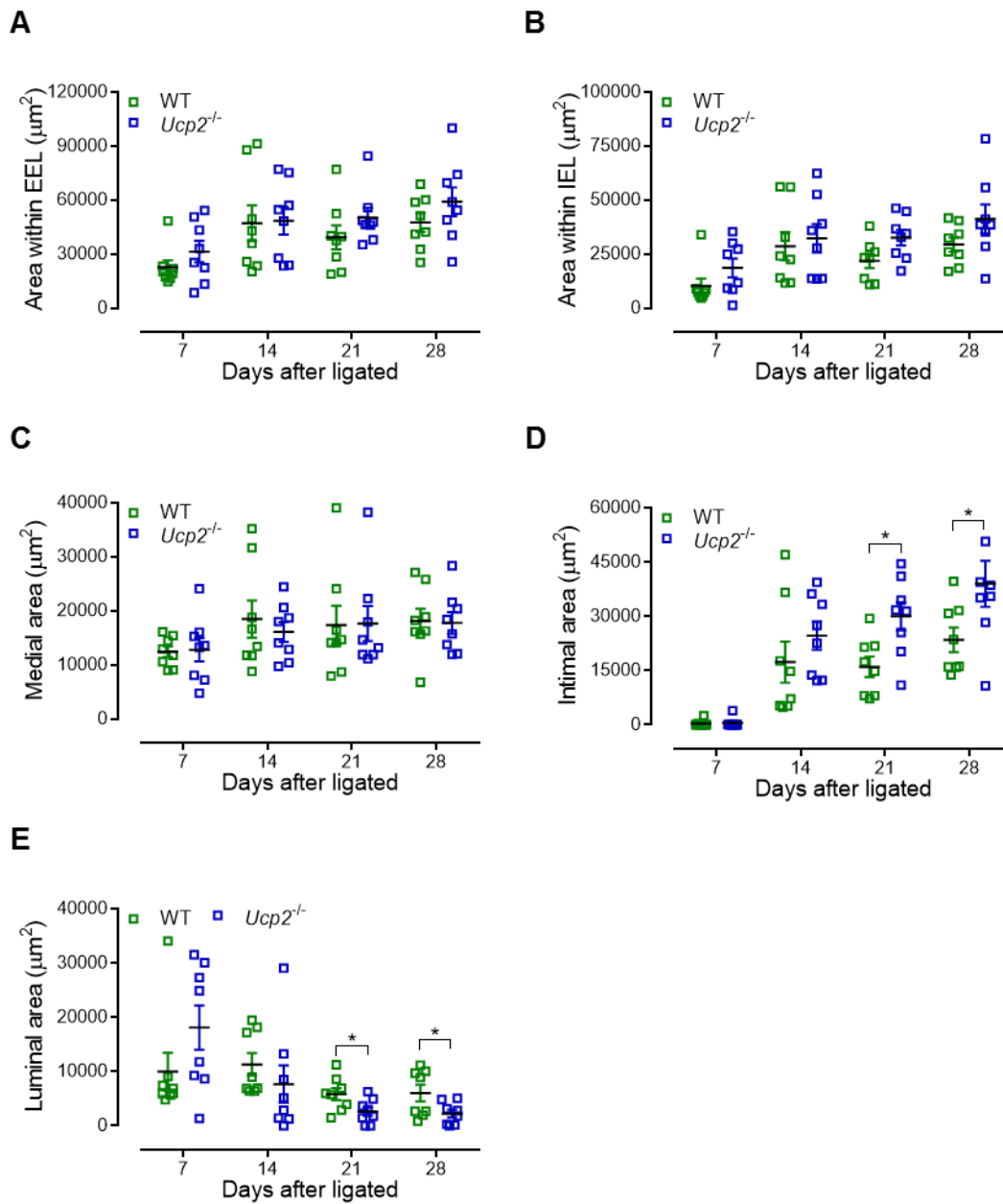
Representative immunohistochemistry staining of UCP2 in frozen section of C57BL/6 mouse carotid arteries harvested from sham and day 3 and day 14 after surgery. Scale bar: 50 μ m.

Figure S4. PDGF decreases the expression and function of UCP2.



(A) Immunohistochemistry staining of UCP2 in HA-SMCs treated with or without human recombinated PDGF (20ng/mL) for 48 hours. Brown indicates positive staining. (B) Detection of superoxide (red color) in HA-SMCs treated as above by staining with DHE dye.

Figure S5. UCP2 ablation exacerbates mouse myointimal hyperplasia.



Area within external elastic lamina (EEL) (A), area within internal elastic lamina (IEL) (B), medial layer area (C), intimal layer area (D), and luminal area (E) were calculated based on the H&E-stained sections of ligated carotid arteries from *Ucp2*^{-/-} mice and wild-type (WT) littermates 7, 14, 21, and 28 days after ligation. n=8 mice in each group. *P<0.05.

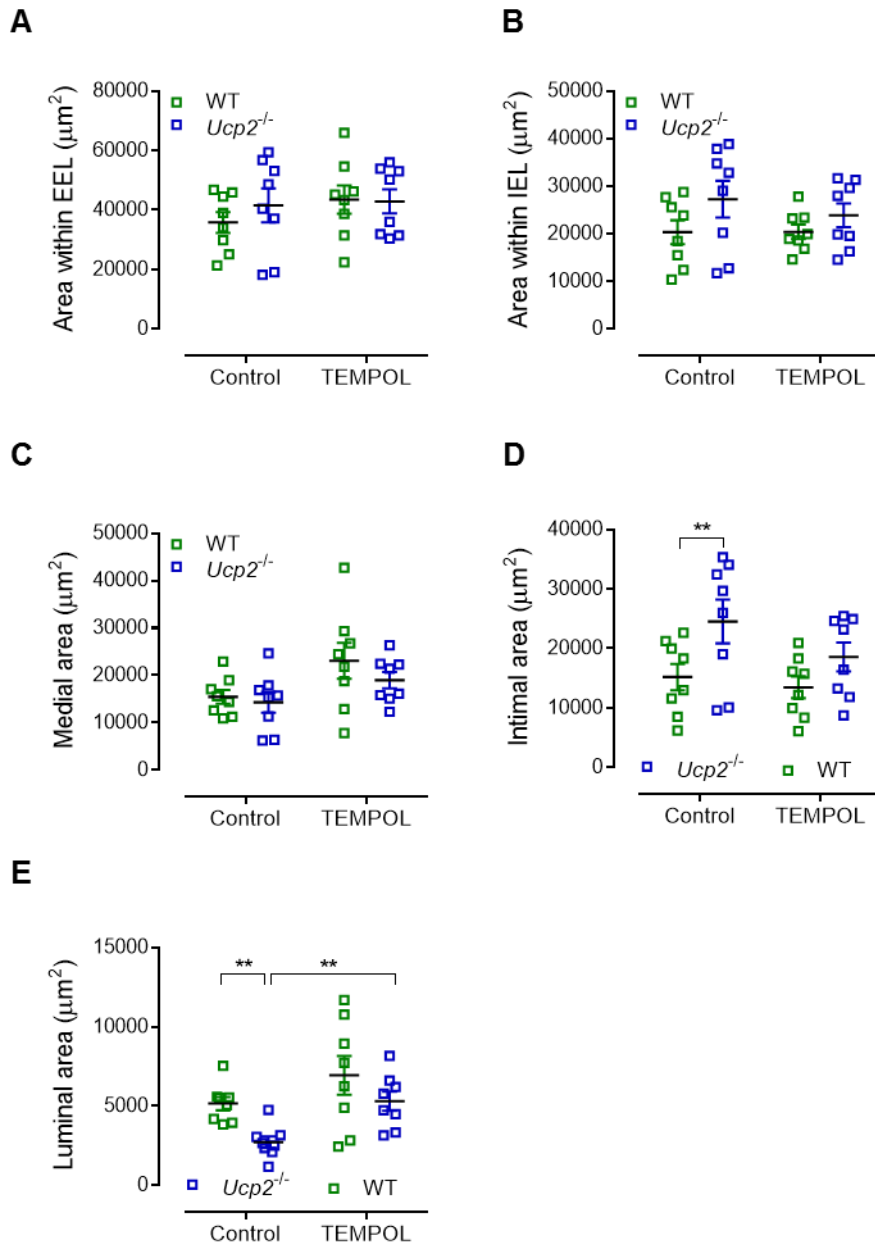


Figure S6. TEMPOL abolishes the UCP2 ablation-exacerbated myointimal hyperplasia. Area within external elastic lamina (EEL) (A), area within internal elastic lamina (IEL) (B), medial layer area (C), intimal layer area (D), and luminal area (E) were calculated based on the H&E-stained sections of ligated carotid arteries from $Ucp2^{-/-}$ mice and wild-type (WT) littermates treated with or without TEMPOL. $n=8$ mice in each group. $**P<0.01$.

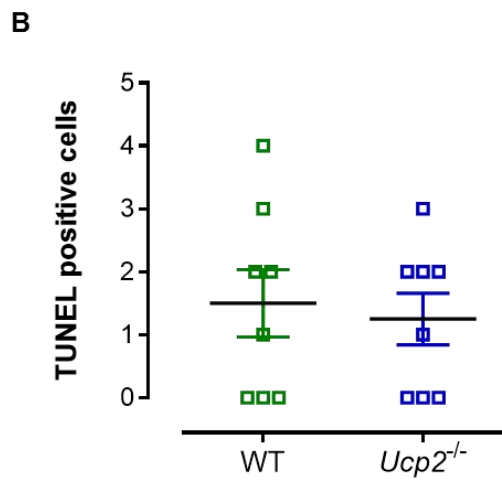
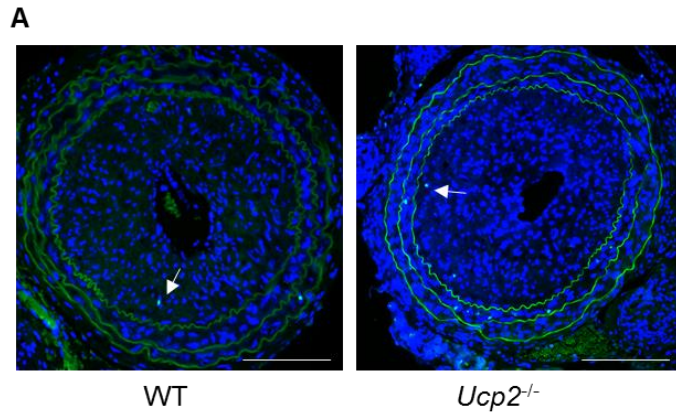


Figure S7. UCP2 ablation does not affect cell apoptosis. (A) Representative images of TUNNEL assay (green color). White arrows indicate positive cells. (B) The quantification of TUNEL-positive cells within the intima per section. Scale bar: 50 μ m. n=8.

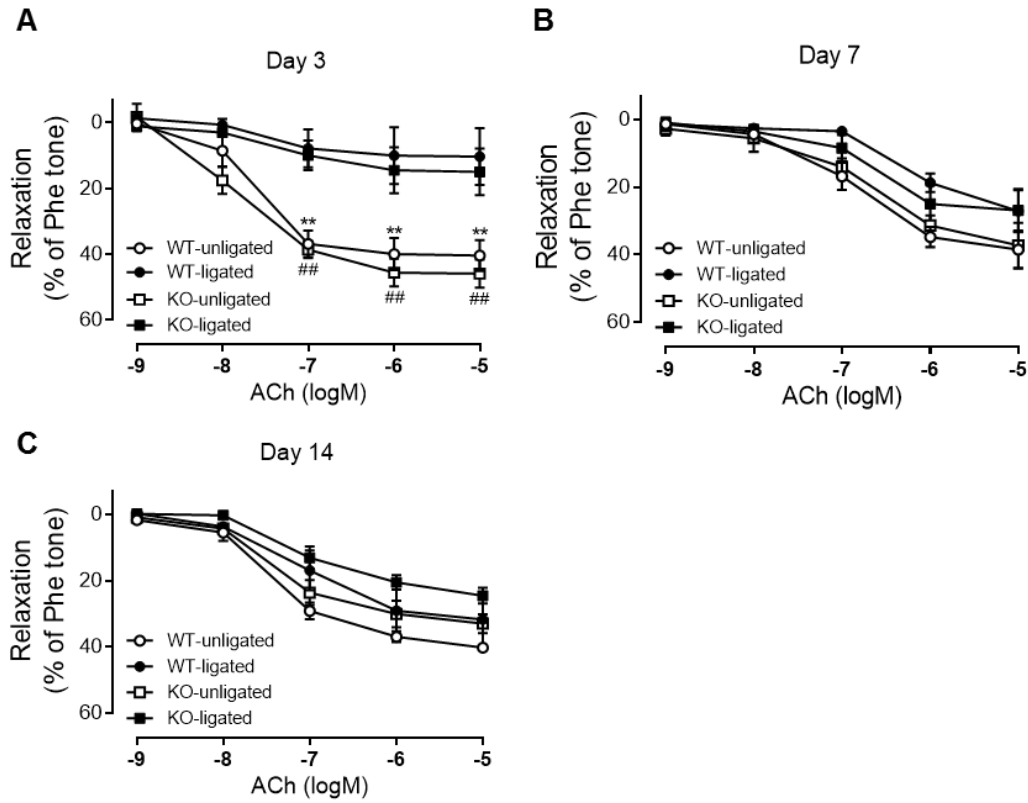


Figure S8. Endothelial function of mouse carotid artery. Acetylcholine (ACh)-induced relaxation of ligated and unligated carotid arteries from wild-type (WT) and UCP2 knockout (KO) mice on day 3 (A), day 7 (B), and day 14 (C) after the procedure. Arterial rings were pre-contracted with phenylephrine (Phe). Data are mean \pm s.e. of 5 animals. ** $P < 0.01$ vs. WT-ligated; ## $P < 0.01$ vs. KO-ligated.

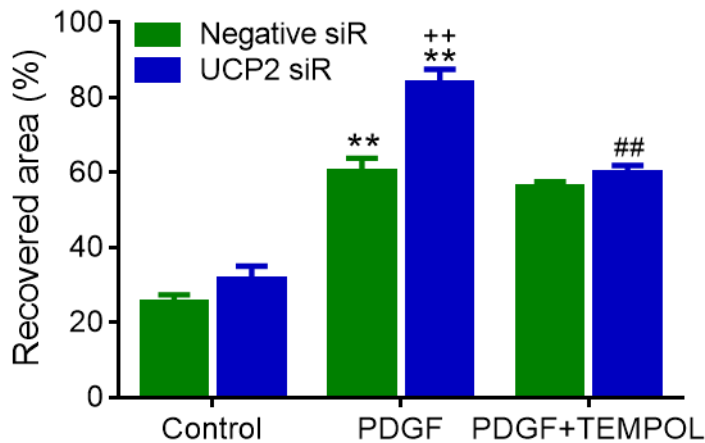


Figure S9. Knockdown of UCP2 promotes smooth muscle cell (SMC) migration. SMC migration was evaluated by scratch assay. UCP2 specific siRNA-2 (different from the one used in Figure 3C)- and negative siRNA-transfected SMCs were cultured in monolayer to confluency and then were scratched with a sterile 200- μ L pipette tip and treated with or without TEMPOL (1mmol/L). Images taken after 24 hours were used to calculate the recovered area. ** P <0.01 vs. control cells transfected with the same siRNA; ## P <0.01 vs. PDGF-treated cells transfected with UCP2 specific siRNA; ++ P <0.01 vs. PDGF-treated cells transfected with negative siRNA. n=9 independent experiments.

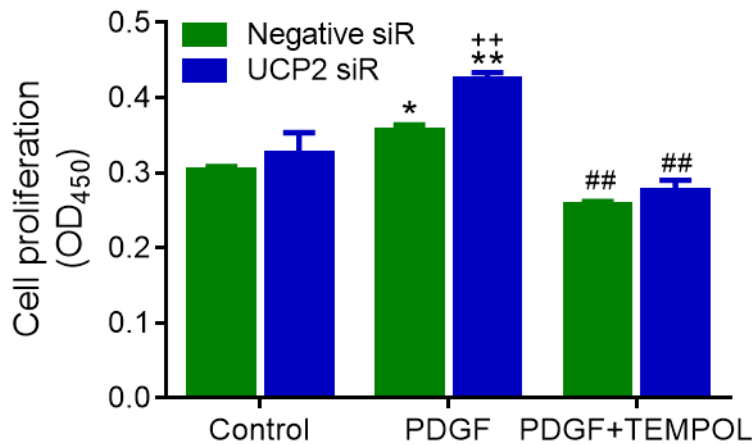


Figure S10. Knockdown of UCP2 promotes smooth muscle cell (SMC) proliferation. SMC proliferation was measured using a colorimetric assay kit (CCK-8). UCP2 specific siRNA-2 (different from the one used in Figure 3D)- and negative siRNA-transfected SMCs were treated with or without TEMPOL, incubated with CCK-8 reagent for 24 hours, and then the absorbance at 450 nm was read using a microplate reader. * $P < 0.05$, ** $P < 0.01$ vs. control cells transfected with the same siRNA; ^{##} $P < 0.01$ vs. PDGF-treated cells transfected with the same siRNA; ⁺⁺ $P < 0.01$ vs. PDGF-treated cells transfected with negative siRNA. n=8 independent experiments.

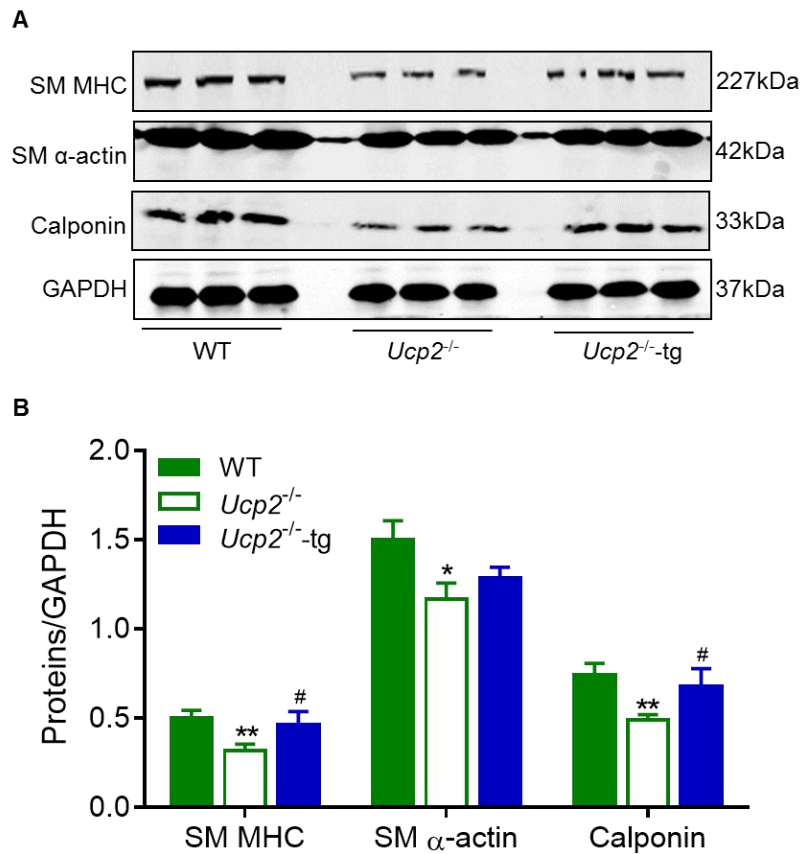


Figure S11. Effects of *Ucp2* overexpression on the expression of smooth muscle markers. The expression of SM MHC, SM α -actin, and calponin in primarily cultured smooth muscle cells which were isolated from WT and *Ucp2*^{-/-} mice and infected with Ad-*Ucp2* transgene to overexpress *Ucp2* (*Ucp2*^{-/-}-tg). Western blotting gel images (A) and quantitation (B). * $P < 0.05$, ** $P < 0.01$ vs. WT; # $P < 0.05$ vs. *Ucp2*^{-/-}.

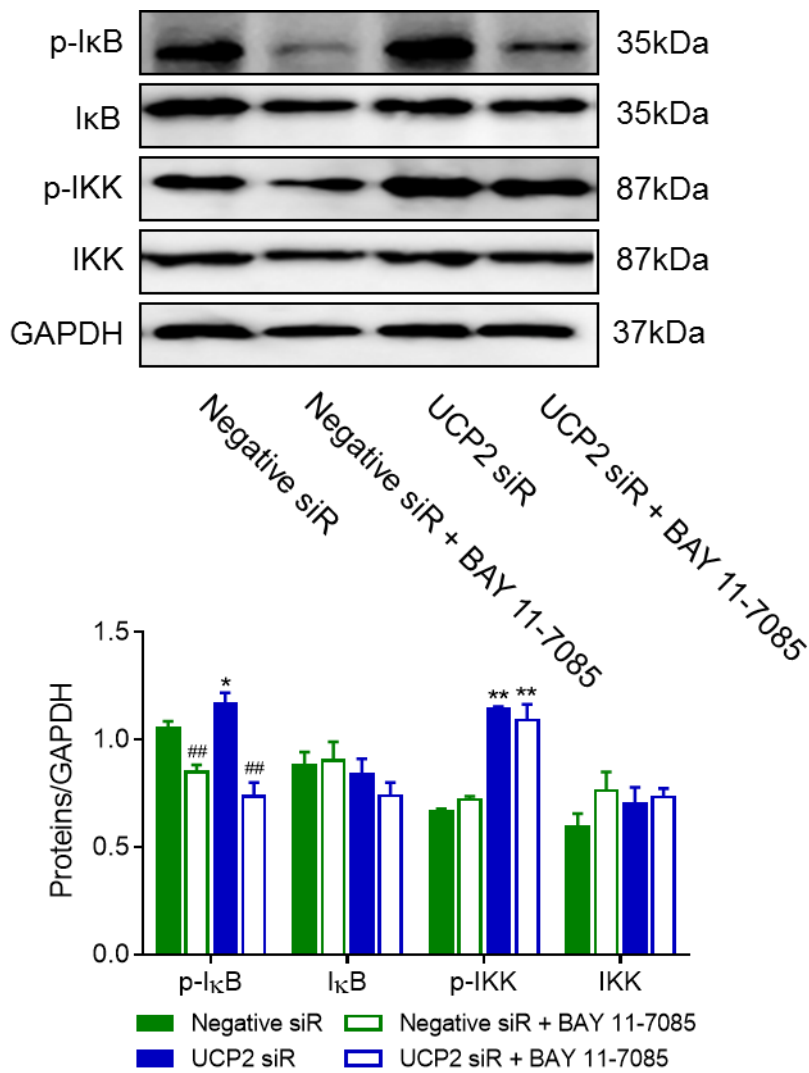


Figure S12. Expression of IKK/IκB. Western blots of phospho-IκB, total IκB, p-IKK, and IKK in HA-SMCs transfected with UCP2 specific or negative control siRNA (siR) and treated with or without BAY 11-7085. * $P < 0.05$, ** $P < 0.01$ vs. cells transfected with negative siR; ## $P < 0.01$ vs. cells transfected with the same siRNA but treated without BAY 11-7085.

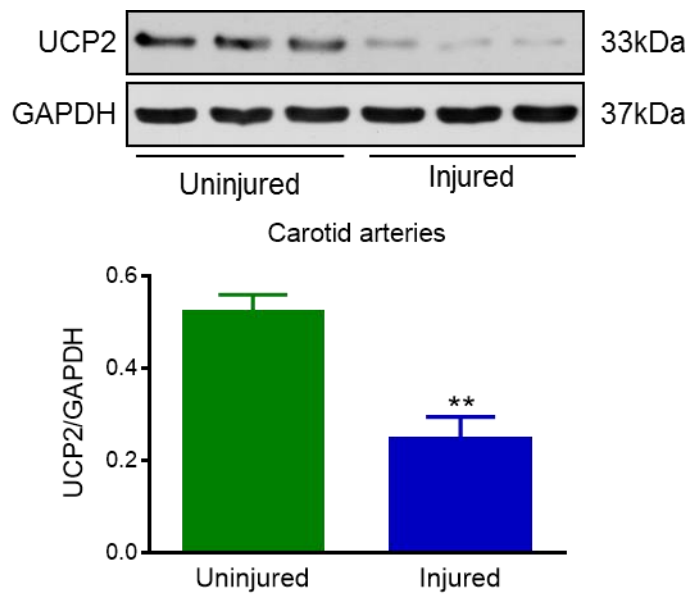


Figure S13. Protein expression of UCP2 in balloon injured rabbit carotid artery. Western blots of UCP2 in lysates from balloon injured and contralateral uninjured rabbit carotid arteries (upper panel) and the quantification of UCP2 level by normalizing to GAPDH. (lower panel). ** $P < 0.01$ vs. uninjured.

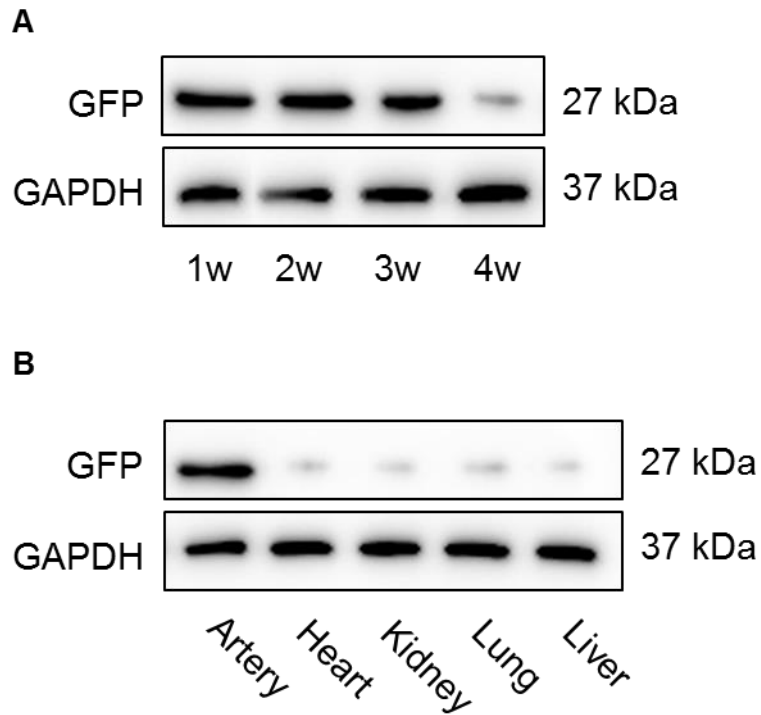


Figure S14. (A) Protein expression of GFP in balloon injured rat carotid artery in week 1, 2, 3, and 4 post infection with Ad-GFP-UCP2. (B) Protein expression of GFP in balloon injured rat carotid artery, heart, kidney, lung, and liver in week 1 post infection with Ad-GFP-UCP2.

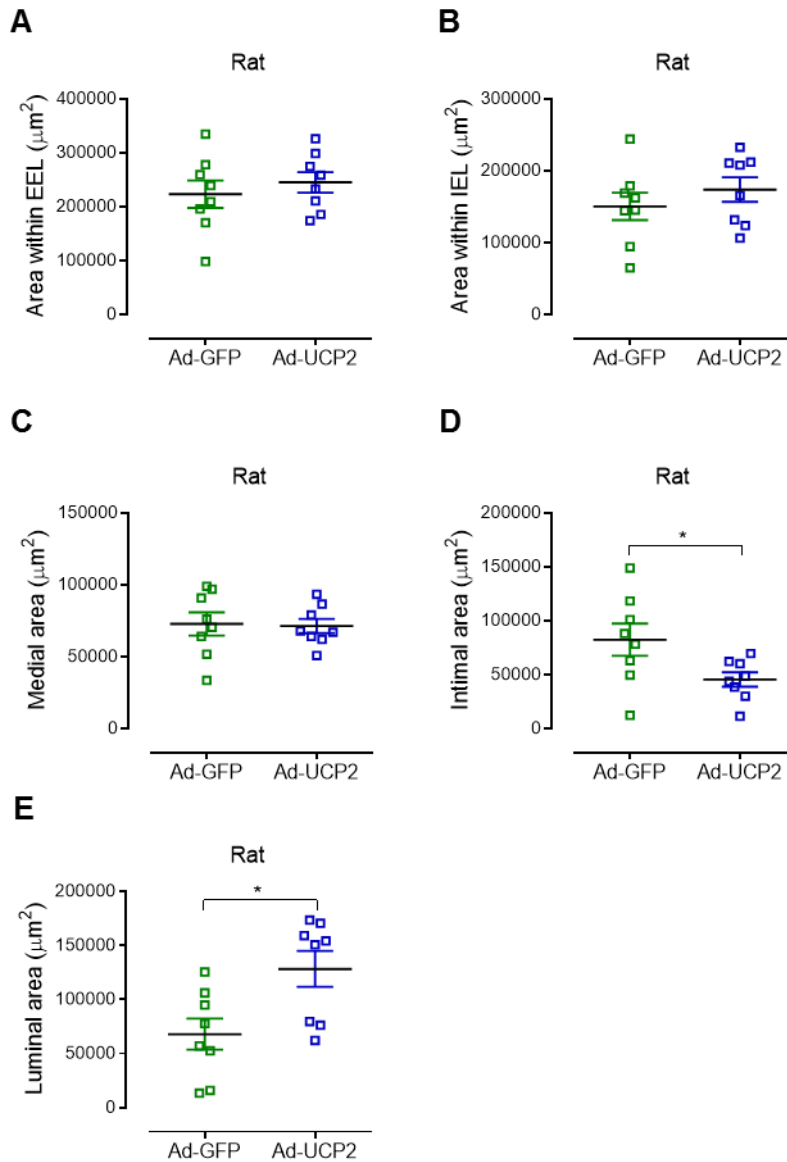


Figure S15. UCP2 ameliorates balloon injury-induced myointima hyperplasia. Carotid arteries of Sprague Dawley rats were injured with a balloon and locally infected with adenovirus expressing GFP alone (Ad-GFP) or both UCP2 and GFP (Ad-UCP2). Area within external elastic lamina (EEL) (A), area within internal elastic lamina (IEL) (B), medial layer area (C), intimal layer area (D), and luminal area (E) were calculated based on the H&E-stained sections of injured carotid arteries 21 days after injury and infection. $n=8$ animals in each group. $*P<0.05$.

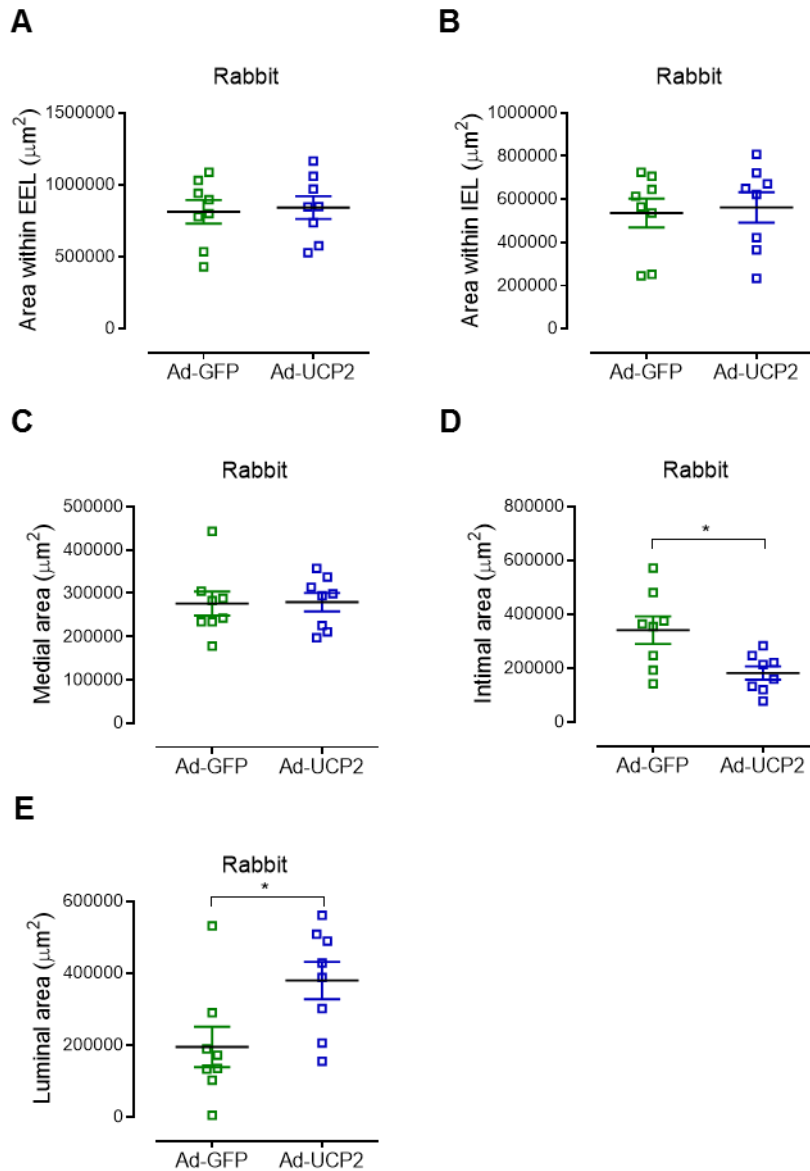


Figure S16. UCP2 ameliorates balloon injury-induced myointima hyperplasia. Carotid arteries of New Zealand rabbits were injured with a balloon and locally infected with adenovirus expressing GFP alone (Ad-GFP) or both UCP2 and GFP (Ad-UCP2). Area within external elastic lamina (EEL) (A), area within internal elastic lamina (IEL) (B), medial layer area (C), intimal layer area (D), and luminal area (E) were calculated based on the H&E-stained sections of injured carotid arteries 21 days after injury and infection. $n=8$ animals in each group. $*P < 0.05$.

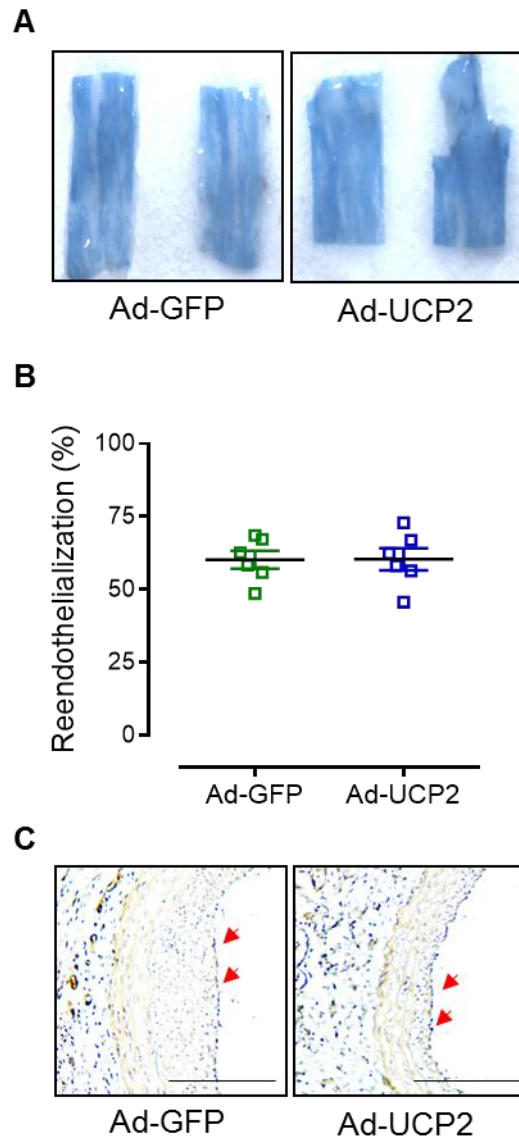


Figure S17. Reendothelialization of balloon injured rat carotid artery. (A) Representative en face Evans blue staining of rat carotid arteries on day 7 after balloon injury and local Ad-GFP or Ad-UCP2 infection. (B) Quantification of reendothelialization. n=7 animal in each group. (C) Immunohistochemistry staining of CD31 (brown) in rat carotid arteries on day 21 after balloon injury and local Ad-GFP or Ad-UCP2 infection. Red arrows indicate positive cells. Scale bar: 200µm.

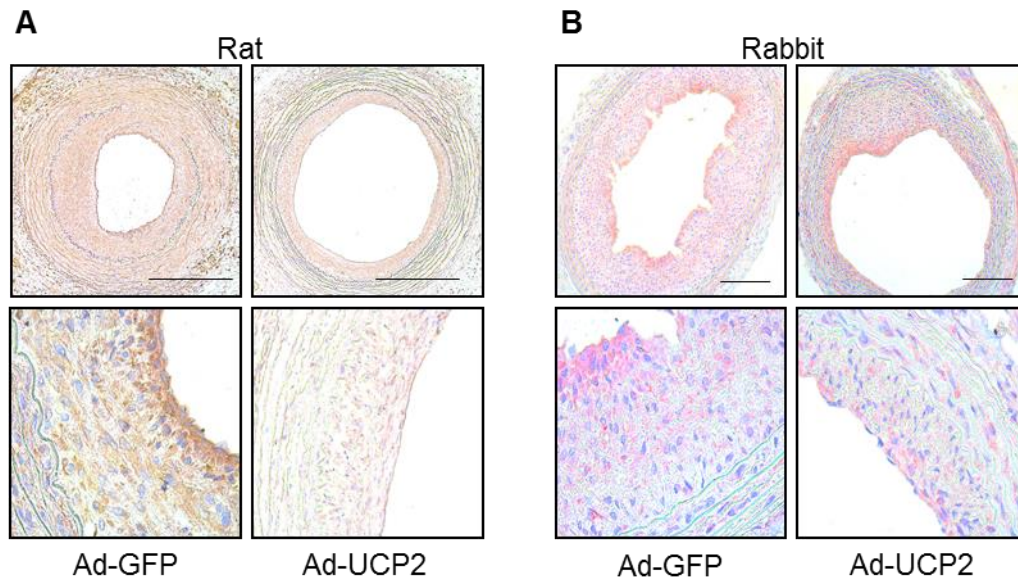


Figure S18. Expression of p53 in balloon injured artery. Immunohistochemistry staining of NF- κ B p53 in balloon injured rat (A) and rabbit (B) carotid arteries infected with Ad-GFP or Ad-UCP2. DAB (brown in A) and AEC (red in B) substrates were used for rat and rabbit artery, respectively. Scale bar: 200 μ m.

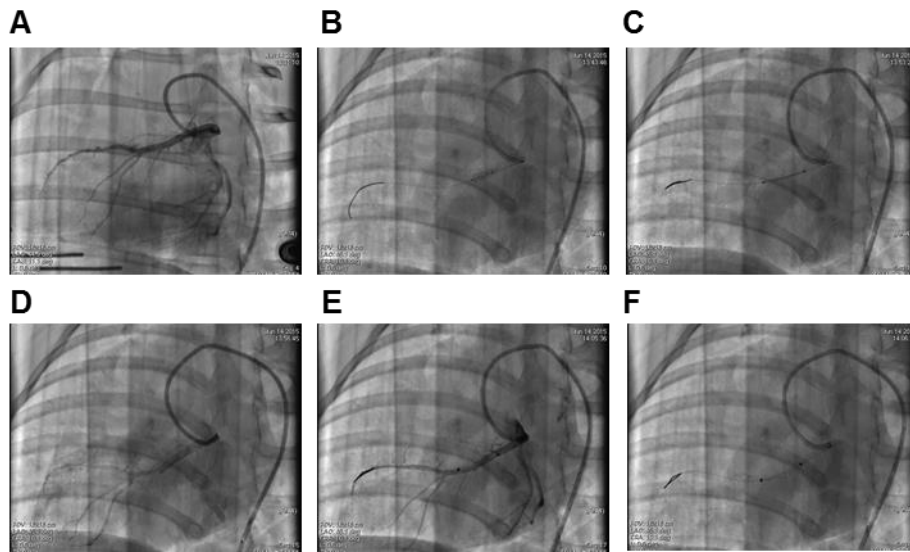


Figure S19. Stent implantation and adenovirus delivery of porcine coronary artery. (A) Coronary angiography of Guizhou mini-pig. (B) Balloon dilation of left anterior descending coronary artery. (C) Bare metal stent deployment. (D) Angiography after stent implantation. (E-F) ClearWay local drug infusion balloon in stented segment.

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