Materials and Methods
Isolation and in vitro culture of HUVECs
Primary human umbilical cord vein endothelial cells (HUVECs) were isolated using collagenase digestion as described previously. Cells were cultured in M199 medium with 20% fetal bovine serum (FBS, Gibco, 16000-044), 50 mcg/ml Endothelial Cell Growth Supplement (ECGS, Biomedical Technologies, BT-203), 100 U/ml Penicillin-Streptomycin (Gibco, 15140-148) and 100 mcg/ml Heparin (Sigma-Aldrich, H4784) in 0.1% gelatin-coated culture dish. When subcultures reached 80–90% confluence, serial passaging was performed by trypsinization, and the number of population doubling level (PDLs) was monitored for further experiments. PDLs were calculated as described previously; briefly, the number of population doublings (PD) that occurred between passages was calculated according to the equation PD=\log_2(CH/CS), where CH is the number of viable cells at harvest and CS is the number of cells seeded. We termed HUVECs in PDLs<10 as “young cells” and HUVECs in PDLs>40 as “senescent cells”.

Animal Model
The studies were performed in 2 groups of male Wistar Kyoto (WKY) rats: old rats (22 months old, 382 ± 31g body wt) and young rats (2 months old, 172 ± 17g body wt). The temperature was held constant at 24 °C in the animal housing. All animal procedures were approved in accordance with the institutional guidelines established by the Committee of Ethics on Animal Experiments at the Chinese Academy of Sciences.

Determination of SA-β-gal activity
Cytochemical staining for senescence-associated-β-galactosidase (SA-β-gal) was detected as described previously, using the Senescence β-Galactosidase Staining Kit (Cell Signaling Technology, 9860). Cells with cytosolic blue staining were counted from random fields, and the percentage of SA-β-Gal-positive cells was calculated.

Enzyme-linked immunosorbent assay (ELISA)
Cell culture supernatants were collected at the end of treatment and stored at −20 °C until analyzed by ELISA for IL-6, MCP-1, and PAI-1. Samples were assayed according to the manufacturer’s instructions (R&D Systems).

In vitro angiogenesis analysis using matrigel
The matrigel assay was used to assess the spontaneous formation of capillary like structures in vitro, was performed as previously described. HUVECs were plated at a density of 5 × 10^4 cells/well in 48-well plates previously coated with growth factor-reduced matrigel matrix (BD Bioscience, 356234). After 6 h of incubation, tube formation was observed with a computer-assisted microscope (Nikon). Loops number and branch number were manually counted in random microscopic fields from each well.
**Endothelial migration assay**

Cell migration assays were performed using 6.5 mm diameter and 8.0 um pore size Transwells (Costar) coated with 0.5% gelatin as previously described. The lower chamber contained 50 ng/ml VEGF as a chemoattractant. Endothelial cells were prepared in serum-free medium, and 4 × 10⁴ cells were added to the upper chamber in migration buffer (M199 containing 0.1% BSA). After 4 h of incubation at 37 °C, cells were removed from the upper surface of the membranes with a cotton swab, and cells that migrated to the lower surface were fixed with 4% paraformaldehyde for 30 minutes and then stained with 0.1% crystal violet (Sigma, C6158) for 10 minutes. Migrated cells were then counted under a microscope.

**Cytotoxicity tests**

Cell viability was assessed using Cell Counting Kit-8 assay (CCK-8) (Dojindo Laboratories, CK04). In total, 2×10⁴ cells (100 μl) were seeded on 96-well plates. 24h after the treatment of drugs, 10 μl CCK-8 solution was added to each well followed by 4h incubation at 37 °C. Next, the OD value for each well was read at a wavelength of 450 nm to determine cell viability using a microplate reader. The wells containing only medium were used as a control.

**Quantitative PCR with reverse transcription**

Total RNA was extracted from different samples using an RNeasy Plus Mini Kit (QIAGEN, 74134) following the manufacturer's instructions and treated with DNase I (Promega, Z3588-C) for 15 min to eliminate the potential contamination of genomic DNA. cDNA was generated by reverse-transcribed total RNA (1 μg) using oligo (dT) primer and ReverTra Ace reverse transcriptase (Toyobo, FSQ-101). Q-PCR was performed and analyzed by kinetic real-time PCR using the ABI PRISM 7900 system (Applied Biosystems, Foster City, CA, USA) with SYBR Green Realtime PCR Master Mix plus (Toyobo, QPK-212) for relative quantification of the indicated genes. The sequence of primers used in the experiments was shown in Supplemental Table I . The transcript of GAPDH was used for internal normalization.

**Western blot analysis**

Briefly, Cells were lysed in RIPA lysis buffer (Millipore, 20-188) with phosphatase inhibitor (1 mM sodium vanadate, 1 mM sodium fluoride) and protease inhibitor (1 mM PMSF, 1 ug/ml leupeptin , 1 ug/ml pepstatin) for 30 min on ice. After centrifugation for 15 min at 12,000 g (4 °C), the protein concentration of the samples was determined using the Pierce BCA Protein Assay Kit (Thermo Scientific, 23225). The proteins were subjected to SDS-PAGE and immunoblotted with primary antibodies: DRP1 (32898), CD31 (376764), and GAPDH (365062) were purchased from Santa Cruz Biotechnology, p21Waf1/Cip1 (AP0713) and p16Ink4a (BS6431) were purchased from Bioworld, phosopho-eNOS (Ser1177) (9571), eNOS (9572) and LC3A/B (4108) were purchased from Cell Signaling Technology, p62 (P0067) was purchased from Sigma-Aldrich (1:1000 dilutions). The specific reactive bands were detected using horseradish peroxidase-conjugated secondary antibodies (Santa Cruz, 2313& 2314).
Bands were visualized using the Western Lightning® Plus-ECL (PerkinElmer, NEL104001EA) in an Odyssey Imager and quantified with Image J.

**Mitochondrial morphology staining**
Mitochondrial morphology was detected by MitoTracker Mitochondrion Selective Probes (Molecular Probes, M7514). Living cells were incubated with 50 nmol/L Mito-tracker green for 30 minutes at 37 °C. After the incubation, the cells were washed three times with phosphate-buffered saline (PBS). Fluorescence at excitation/emission wavelength of 590/516 nm was observed using Zeiss inverted fluorescence microscope.

**Construction of recombinant adenoviruses**
Recombinant adenoviruses encoding human Drp1 (Ad-Drp1), empty vector (Ad-EV), short hairpin RNA of Drp1 (Ad-shRNA-Drp1), and nontemplate shRNA (Ad-NC) were prepared as described previously using the pAdEasyTM vector system (Qbiogene). Briefly, human Drp1 was cloned into pShuttle-CMV (Qbiogene, USA), the short heparin Drp1 sequence (forward: AATTCGGAATTCAGGTCCGAGTCGCTCTCAGTGTCTGTCTGAGGAGCTGGATGATGTCCG; reverse: GATCCCTAAAGACATCATCCTGCAGCTGGATGTCTGTCTGAGGAGCTGGATGATGTCCG) and nontemplate shRNA sequence (forward: GATCTTTCTCCGAGGCAGCTGGATGTCTGTCTGAGGAGCTGGATGATGTCCG; reverse: AATTCAAAAAATTCTCCGAGGCAGCTGGATGTCTGTCTGAGGAGCTGGATGATGTCCG) was cloned into reconstituted pShuttle-U6 (Qbiogene, USA) and homologously recombined in bacteria BJ5183 with pAdeasy-1. The recombinant plasmid was transfected to 293A cells, and recombinant adenoviral plaques were isolated and further purified by two rounds of plaque assays as described previously. The titers of stocks measured by TCID50 assay on HEK293 were 2 × 10^10 pfu/mL for Ad-EV and Ad-NC, 6 × 10^9 pfu/mL for Ad-Drp1, and 1 × 10^10 pfu/mL for Ad-shRNA-Drp1.

**Immunofluorescence imaging**
To monitor the various stages of autophagy, the tandem mCherry-GFP-LC3 (tf-LC3) adenovirus construct was used in this study. GFP fluorescence is quenched in the acidic pH of the lysosomal compartment, thereby limiting the use of GFP-LC3 to the identification of autophagosomes. However, mCherry continues to fluoresce, and mCherry-LC3 can be used to identify both autophagosomes and autolysosomes. By using tf-LC3 and determining the number of red dots that overlay green dots and appear yellow in merged images, the number of autophagosomes can be evaluated. The red dots that do not overlay green dots and appear red in merged images indicate autolysosome formation. In brief, to perform image-based analysis for autophagy, simply infected HUVECs with the tf-LC3 adenovirus for 24 hours, and then the cells were treated and imaged for GFP and RFP by using confocal fluorescence microscopy. Images were acquired and analyzed using Zeiss Axiovision software. Punctate fluorescent tagged LC3 dots were counted and expressed as number per cell.
HUVECs were identified by the expression of endothelial specific-surface markers CD31. Briefly, after washing with PBS, cells were fixed with 4% paraformaldehyde for 30 minutes, blocked for 30 minutes with 5% BSA in PBS and then incubated with primary antibodies against CD31 (1:200 dilution) and fluorescence-conjugated secondary antibody (1:200 dilution, Invitrogen, A-11062). Nuclei were stained with Hoechst. Images were acquired and analyzed by Zeiss inverted microscope.

**En face immunofluorescence staining**

Immunofluorescence staining of rat aortic ECs were performed as described previously. Briefly, rats were anesthetized with pentobarbital sodium (30 mg/kg body weight). Subsequently, the whole thoracic aorta was dissected, stripped of adventitial tissue carefully, cut open longitudinally, permeabilized with 0.4% Triton X-100 in PBS for 10 min and blocked with 10% Normal Goat Serum in Tris-buffered saline (TBS) containing 0.1% Tween-20 for 1 h at room temperature. Next, aortas were incubated rabbit anti-DRP1 or rabbit anti-LC3 or rabbit anti-p62 and mouse anti-CD31 in the blocking buffer overnight at 4 ℃. After rinsing with washing solution 3 times, fluorescence-conjugated secondary antibodies (1:500 dilutions, Alexa Fluor 555 labeled anti-rabbit IgG, Invitrogen, A-31572; Alexa Fluor 488 labeled anti-mouse IgG, Invitrogen, A-10680) were applied for 1 h at room temperature. Finally, after another 3 rinses in the washing solution, aortas were mounted in the Fluorescence Mounting Medium (Dako, S302380). Aortas were examined by a laser-scanning confocal microscope (Zeiss) with 40x lens.

**Ex vivo Aortic ring assay**

To examine the migration and proliferation of rat aortic endothelial cells in an ex vivo condition, an aortic ring assay was performed using type I Collagen (BD Bioscience, 354236) as described previously. The whole thoracic aorta was dissected from rats, stripped of adventitial tissue, and cut into 1-mm ring segments. The recombinant adenovirus was isolated from 293 cells and purified by CsCl centrifugation. The titers of stocks were 1 × 10^{11} pfu/mL for Ad-NC and Ad-shRNA-Drp1. Pieces of aortas were immediately exposed or not to recombinant adenoviruses at 5 × 10^9 plaque-forming units (pfu) in 0.5ml DMEM in 24-well plate at 37 ℃ in 5% CO₂. After one day of infection, aortic rings were extensively rinsed with DMEM, placed in 150ul type I collagen coated plates (48-well), and cultured with MCDB131 (Gibco, 10372019) medium containing 2.5% FBS and 30ng/ml VEGF (Peprotech, 40031). Quantify microvessel growth at day 9 by Zeiss inverted microscope.

**In vivo adenoviral gene transfer**

The in vivo adenoviral gene transfer in rat common carotid artery was performed as described previously. Wistar Kyoto rats weighing 250g to 350g were anesthetized with pentobarbital sodium. The right common carotid artery was isolated through vascular clamps to expose a 1.5-cm-segment of the artery from the bifurcation. Adenoviral solution containing Ad-NC or Ad-shRNA-Drp1 (2X10^9 pfu) in PBS was introduced into the common carotid through a syringe placed in the external carotid
artery and incubated for 10 minutes. After the viral solutions were withdrawn, the external carotid artery was ligated, and blood flow was restored. The common carotid arteries were excised at day 3 for further study.

**Measurement of mitochondrial oxidative stress**
Mitochondrial superoxide was detected by MitoSOX™ Red Mitochondrial Superoxide Indicator (Molecular Probes, M36008). Living cells were incubated with 100 nmol/L MitoSOX Red for 30 minutes at 37 °C. After the incubation, the cells were washed three times with PBS. Fluorescence at excitation/emission wavelength of 510/580 nm was observed using Zeiss inverted fluorescence microscope. Fluorescence intensity was analyzed in independent fields with Image J.

**Statistical analysis**
Data was expressed as means ± standard error of the mean (SEM). Significant differences between the two groups were estimated using Student's t-test. A P value < 0.05 was considered statistically significant.

**References for the supplement**
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