# Supporting Information

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### SI Results

**Serum Lipid Levels.** The serum concentrations of total cholesterol (TC) and triglycerides (TGs) did not differ significantly among the three animal groups before or after treatment (Table S1).

### SI Materials and Methods

**Preparation of ACE2 Ad Vector.** The murine ACE2 cDNA was amplified by RT-PCR from RNA of mouse kidney with the following primers: ACE2 F: 5′-GAAAGTTGCTCAGTGGATGGGAT-3′; ACE2 R: 5′- TTTGCTAAAAGGAAGTCTGAGCATC-3′. The amplified product was first cloned into a pMD18-T vector (Invitrogen), then subcloned into pDC316 with the EcoRI and SalI sites. The ACE2 cDNA sequence in the pDC316-ACE2 plasmid was confirmed by sequencing. A recombinant adenoviral (Ad) vector carrying the murine ACE2 (Ad-ACE2) or a control transgene (Ad-EGFP) was prepared as described (1) by using the AdMax system (Microbix Biosystems).

Ad vectors were amplified and produced in 293 cells (Chinese Academy of Sciences, Shanghai) and purified by anion chromatography. The concentration of viral particles was determined by the TCID50 method.

**Animal Model and Gene Transfer.** Sixty-six male New Zealand White rabbits (≈1.5 kg, 8–10 wk old) were housed individually and fed an atherogenic chow containing 1% cholesterol and 5% peanut oil. All animal care and experimental protocols complied with the animal management regulations of the Ministry of Public Health, China, and the Animal Care Committee of Shandong University. All animal research protocols were approved by the Animal Care Committee of Shandong University.

After 4 wk of acclimation, rabbits underwent balloon injury of the abdominal aorta by using a 4F Fogarty embolectomy catheter as described (2). This procedure was performed with rabbits under general anesthesia by intramuscular injection of ketamine (35 mg/kg)/xylazine (7 mg/kg) and topical anesthesia of the inguinal region by lidocaine. Balloon injury accelerates atheroma formation, renders lesions more uniform in size and distribution, and produces plaques with a smooth muscle–rich fibrous cap overlying a layer of lipid-laden macrophages.

Rabbits were randomly divided into three groups ($n$ = 22 per group). Rabbits in Ad-ACE2, Ad-EGFP, and nontransduced groups received a suspension of Ad-ACE2 ($2.5 \times 10^9$ pfu), a suspension of Ad-EGFP ($2.5 \times 10^9$ pfu), and no treatment, respectively. Rabbits were maintained on a high-cholesterol diet for an additional 4 wk. One rabbit in each group died because of anesthetic accident. At the end of week 8, the remaining rabbits were anesthetized and the atherosclerotic lesions from abdominal aortas were isolated for analysis.

**HUVEC and VSMC Culture and Gene Transfer.** VSMCs were obtained from human umbilical arteries and grown in DMEM/F12 medium containing 10% FCS under standard culture conditions. Cultured VSMCs from passages 3–5 were used for all experiments. Briefly, adhering connective tissue was removed by blunt dissection from the umbilical artery, and the vessel was opened longitudinally. The artery was then minced into 1-mm pieces, incubated for an additional 1.5–2 h, and rinsed twice with PBS to remove the cells, which were counted and seeded at $10^4$ cells in plastic culture flasks. Cells were harvested for passaging at 2-wk intervals, and cells between passages 3 and 5 were used. The cells exhibited the typical "hill and valley" growth morphology of VSMCs and reacted with a monoclonal antibody that selectively recognizes muscle forms of actin. HUVECs derived from the human umbilical cord vein were cultured in M199 medium, and VSMCs were cultured in DMEM/F12 medium supplemented with 20% FCS. Subconfluent HUVECs or VSMCs were selected for experiments.

HUVECs or VSMCs were incubated with AngII for 24 h before they were divided into Ad-ACE2, Ad-EGFP, and nontransduced groups. Ad-ACE2 ($1 \times 10^6$ pfu per well) or Ad-EGFP ($1 \times 10^6$ pfu per well) was transfected into cells in a six-well plate, and cells were harvested 24, 48, and 72 h after gene transfection for Western blot analysis.

SB203580 (10 μM; an inhibitor of ERK1/2 MAPK), PD98059 (20 μM; an inhibitor of p38 MAPK), AG-490 (10 mM; Cell Signaling Technology; an inhibitor of JAK2), and SH-6 (10 μM; Alexis Biochemicals; an inhibitor of Akt) were added 1 h before AngII stimulation.

**Measurement of ACE2 and ACE Activity.** The enzymatic activities of ACE2 for converting AngII to Ang(1-7) and those of ACE for converting AngI to AngII were evaluated by surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS; ref. 3) to differentiate the mass of small peptide substances as peaks at distinct positions.

Membrane proteins from abdominal aortic plaque tissue were isolated by using an NE-PER nuclear and cytoplasmic extraction reagents kit (Pierce). Protein concentrations were determined by the bicinchoninic acid method, and membrane protein lysates were analyzed by 14% SDS/PAGE. The components of the reaction mixture were 25 μL of Mes buffer (50 mM, pH 6.75), 2 mM PMSF, 10 μM bestatin (an inhibitor of aminopeptidase), atherosclerotic lesion tissue extract, recombinant (r) human ACE2 or ACE, and 10 μM AngII or AngI. The incubation time was from 30 to 120 min at 37 °C. Recombinant human ACE2 or ACE served as a positive control.

For SELDI-TOF-MS, normal phase exchange (NP20) ProteinChips were used for the retention and analysis of substrate and peptide enzyme products. NP20 ProteinChip spots were first outlined by using a hydrophobic marker (pap-pen; RPI) and air-dried. The chip surface was activated with 10 μL of HPLC-grade water and then washed three times with 10 μL of deionized water. After incubation, 10 μL of the reaction mixture was spotted onto the ProteinChip NP20 and analyzed as described (3).

To evaluate the possible impact of ACE2 overexpression on ACE activity, the ACE2 inhibitor DX600 (1 μM; Phoenix Pharmaceuticals) was added 20 min before the addition of AngI peptides to prevent ACE2 from converting AngII to Ang(1-7), and then the ACE efficiency in converting AngI to AngII was measured.

**Serum Lipid Measurement.** Blood samples were collected at the end of weeks 4 and 8, and serum concentrations of total cholesterol and triglycerides were determined by enzymatic assays (Sigma Diagnostics).

**Histopathological Analysis.** Consecutive segments of the abdominal aorta were embedded in paraffin, sectioned, mounted on glass slides, and conventionally stained with H&E and Oil-red O. Serial sections with the maximal amount of lesions were chosen for quantification by a single observer blinded to the experimental protocol. All images were captured and analyzed by computer-assisted morphometric analysis by using an automated image analysis system (Image-Pro Plus 5.0; Media Cybernetics). The area of intima and media of the abdominal aorta was obtained by tracing the perimeter of the lumen and the internal and

external elastic laminae. The mean area of the intima and media was measured by tracing multiple sections, and the ratio of the area of intima to media (I/M area) was calculated.

**Immunohistochemical Analysis.** Paraffin-embedded arteries were cut into 4-μm-thick cross-sections, dewaxed, and rehydrated. Macrophages were identified by using a monoclonal antibody for rabbit macrophages (RAM-11, 1:250; DAKO); LOX-1 by a purified polyclonal goat anti-mouse antibody (1:100; Santa Cruz Biotechnology); MCP-1 by a polyclonal goat anti-human-MCP-1 antibody (1:50; Immunogenex); Ang(1-7) by a polyclonal rabbit anti-human Ang(1-7) antibody (Phoenix Pharmaceuticals); α-actin of SMCs and CD31 by monoclonal mouse anti-human antibodies (Abcam); PCNA by a polyclonal goat anti-human antibody (Abcam); and the $AT_1R$ by an $AT_1R$-specific antibody (Cell Signaling Technology). After incubation with biotinylated secondary antibody followed by avidin-biotin amplification, the slides were incubated with 3,3′-diaminobenzidine (DAB) and counterstained with hematoxylin. Sections reacted with nonimmune IgG, secondary antibody only, and no primary and secondary antibodies were used as negative controls.

Quantification of macrophages and MCP-1, LOX-1, and $AT_1R$ expression involved use of an automated image analysis system (Image-Pro Plus 5.0; Media Cybernetics), and the positively stained area was measured and expressed as mean percentage of the lesion area in at least 10 high-power fields (400× magnification). Cells with positive PCNA staining were determined by double-labeled immunocytochemistry as reported (4).

**Quantification of MCP-1, ICAM-1, and AngII Levels by ELISA.** HUVECs were transfected in vitro with Ad-ACE2 or Ad-EGFP at 100 pfu per cell for 2 h, then cells were divided into three groups for treatment: (*i*) AngII+AdACE2, (*ii*) AngII+AdEGFP, and (*iii*) AngII only (nontransduced group). AngII (Sigma) was added to the fresh culture medium of HUVECs at a final concentration of 1 μM, and the supernatants were collected after cells were stimulated for 24 h. Levels of MCP-1 and ICAM-1 protein in the supernatant were measured by using an ELISA kit (R&D Systems), and the concentration of AngII in the HUVEC lysates was also measured by using an ELISA kit (SPI-BIO).

**Real-Time RT-PCR.** Quantitative gene expression analysis was performed by using SYBR Green technology. ACE2 primers were F5′-GCAGCTAAGTATAATGGTTCTCTG-3′ and R5′-AGTGTTCCACCCCACAAAA-3′. Total RNA was isolated from freshly isolated atherosclerotic lesions by the TRIzol method. Purified RNA was DNase-pretreated and then reverse-transcribed (RevertAid M-MulV Reverse Transcriptase), and one-step real-time RT-PCR was performed by using the Applied Lightcycler 2.0 detection system (Roche Applied Science) according to manufacturer's instructions. Relative mRNA expression was calculated by a comparative method described in the Applied Biosystems user bulletin. The data were analyzed by the $2^{[\text{minus}]\triangle\triangle CT}$ method.

**Western Blot Analysis.** ACE2 and ACE protein expression from membrane protein preparations, and AngII, $AT_1R$, MCP-1, LOX-1, Ang(1-7), ERK, p38, JAK2, and STAT3 expression from total protein preparations, was detected in rabbit atherosclerotic lesions and VSMCs by Western blot analysis. Samples (100 μg of protein) underwent 14% SDS/PAGE and were transferred to nitrocellulose membranes. After an incubation in blocking solution (4% nonfat milk, Sigma-Aldrich Chemie), membranes were incubated overnight at 4 °C with a 1:200 dilution of the primary antibodies anti-ACE2 (Santa Cruz Biotechnology), anti-ACE (Abcam), anti-$AT_1R$ (Abcam), anti-MCP-1 (Immunogenex), anti-LOX-1 (1:100; Abcam), anti-Ang(1-7) (Phoenix Pharmaceuticals), anti-ERK (Cell Signaling Technology), anti-p38 (Cell Signaling Technology), anti-JAK (Cell Signaling Technology), and anti-STAT (Cell Signaling Technology). Membranes were washed, incubated with a 1:1,000 dilution of second antibodies for 1 h, and incubated with enhanced chemiluminescence reagent (Pierce). Relative band intensities were analyzed with an MSF-300G Scanner (Microtek Lab, Nikon). For measurement of small peptides such as Ang(1-7), the electrophoretic separation and transfer speed and time were optimized to obtain satisfactory results.

**Terminal dUTP Nick-End Labeling Staining.** Segments of abdominal aorta were stained for apoptotic nuclei by using an in situ cell death detection kit as reported (5). Paraffin-embedded tissues were treated with proteinase K, then endogenous peroxidase activity was blocked, and tissues were subjected to avidin-biotin blocking with antigen retrieval. Tissues were incubated with TUNEL reaction mixture and treated with converter-peroxidase solution. The TUNEL-positive cells were analyzed by light microscopy.

**Measurement of Endothelial Function.** Rabbits were killed, and 2- to 4-mm-long aortic segments were mounted in a wire myograph for measurement of the isometric tension. Vessel segments were equilibrated in aerated Krebs-Henseleit solution for 1 h at 37 °C under a resting tension of 1,000 mg. Aortic segments were preconstricted with 1 μM phenylephrine, and relaxation curves in response to acetylcholine (1 nM to 10 μM) were generated. The extent of vasorelaxation was expressed as a percentage of the phenylephrine-induced maximal response.

**VSMCs or HUVECs Proliferation Assay.** VSMCs or HUVECs were seeded at $1 \times 10^4$ cells per well in 96-well plates and treated with Ad-ACE2 or Ad-EGFP at 100 pfu per cell or PBS for 24 h. AngII (Sigma) was added to the culture medium of VSMCs at a final concentration of 1 μM. After the incubation period, BrdU labeling mixture was added to each well, and cultures were incubated for 6 h at 37 °C. VSMC DNA replication was quantitated by BrdU incorporation by using an ELISA (R&D Systems).

**Thymidine Incorporation Assay.** VSMCs or HUVECs were plated at $2.5 \times 10^4$ cells per well in 24-well tissue culture dishes and allowed to grow to confluence in DMEM/F12 medium (VSMCs) or Medium 199 (HUVECs) containing 10% FCS under standard culture conditions. AngII (Sigma) was added to the culture medium of VSMCs at a final concentration of 1 μM. VSMCs or HUVECs were transfected with Ad-ACE2 or Ad-EGFP at 100 pfu per cell or PBS, serum-starved for 48 h, and then serum-stimulated for 24 h with 1 μCi/mL $^3$H-TdR (Amersham Biosciences). The experiment was terminated by washing the cells twice with Dulbecco's PBS and twice with ice-cold 10% trichloroacetic acid. The precipitate was dissolved in 500 mL of 0.3 M NaOH and 0.1% SDS after incubation at 50 °C for 2 h. Aliquots from four wells for each treatment with 10 mL of scintillation fluid were counted in a liquid scintillation counter (5).

**Migration Assay.** VSMC or HUVEC migration was assayed by a modified Boyden's chamber method. The filters were coated with Matrigel (Sigma Chemical) suitable for VSMC invasion assay.

VSMCs or HUVECs were plated at $2.5 \times 10^4$ cells per well and transfected in vitro with Ad-ACE2 or Ad-EGFP at 100 pfu per cell. AngII was added to the culture medium at a final concentration of 1 μM. VSMCs or HUVECs at $2.5 \times 10^4$ cells per group were aliquoted onto the upper surface of an 8-μm-pore size ChemoTx plate (Neuro Probe). DMEM/F12 medium containing 5% serum (VSMCs) or Medium 199 containing 5% serum (HUVECs) was aliquoted into the lower chamber of the plate. Cells were allowed to migrate for 24 h at 37 °C. Cells on the upper surface were removed, and migrated cells on the lower surface were fixed and stained with Crystal violet. The extent of migration was determined by using Image-Pro Plus 5.0 (Media Cybernetics). The

number of cells from six high-power fields was counted to calculate the mean, which was used to quantitate the extent of migration.

For analyzing VSMC migration and related signaling pathways, VSMCs were incubated with AngII for 24 h before they were divided into six groups to receive AngII, AngII+ERK inhibitor, AngII+p38 inhibitor, AngII+JAK inhibitor, AngII+MMP inhibitor (GM6001), and no treatment (control group), and cells were harvested to analyze cell migration.

**Tube-Like Structure Formation Assay.** Matrigel (200 μL) was added to each well of 12-well plates and allowed to polymerize at 37 °C for 30 min. HUVECs ($5 \times 10^5$ cells/mL) were added on top of the Matrigel, suspended in Medium 199 with 10% FCS, and incubated at 37 °C for 16 h. The cumulative tube length was measured after 18 h by using Image-Pro Plus 5.0 and evaluated in six random fields (6).

**Zymography.** MMP-2 and MMP-9 activity was evaluated by zymography. Proteins with gelatinolytic or caseinolytic activity were identified by electrophoresis in the presence of SDS in 10% or 12% discontinuous polyacrylamide gels containing 1 mg/mL gelatin. Briefly, VSMC extractions for SDS/PAGE were not boiled before electrophoresis under nonreducing conditions. After electrophoresis, the substrate gels were soaked twice with Triton X-100 solution (2.5%) for 30 min at room temperature to remove SDS. The gels were then incubated in 50 mM Tris·HCl (pH 7.4), 0.15 M NaCl, 5 mM $CaCl_2$, 0.02% $NaN_3$, and 0.05% Brij 35 for 24 h at 37 °C. The lysis of the substrates in the gels was visualized by staining with 2.5% Coomassie brilliant blue (Sigma).

**Measurement of Reactive Oxygen Species (ROS) Levels.** To measure ROS levels, HUVECs were first stimulated by AngII at a final concentration of 1 μM for 24 h and were then transfected with Ad-ACE2 or Ad-EGFP at 100 pfu per cell. Then, HUVECs were harvested and incubated for 20 min with 6-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H2DCFDA; Genmed Scientifics). ROS levels were assessed by measuring fluorescence at excitation and emission wavelengths of 490 and 530 nm, respectively.

**Detection of NF-κB p65 Activity.** To examine NF-κB DNA-binding activity, HUVECs were first stimulated by AngII and were then transfected with Ad-ACE2 or Ad-EGFP. Nuclear protein was extracted from the collected HUVECs and transcription factor assays were performed. Thereafter, HUVECs were harvested, and NF-κB p65 activity in nuclear extracts was detected by using the TransAM-NF-κB p65 Transcription Factor Assay Kit (Active Motif) according to the manufacturer's instructions as reported (7).


1. Ng P, Parks RJ, Cummings DT, Evelegh CM, Graham FL (2000) An enhanced system for construction of adenoviral vectors by the two-plasmid rescue method. *Hum Gene Ther* 11:693–699.
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3. Elased KM, Cunha TS, Gurley SB, Coffman TM, Morris M (2006) New mass spectrometric assay for angiotensin-converting enzyme 2 activity. *Hypertension* 47:1010–1017.
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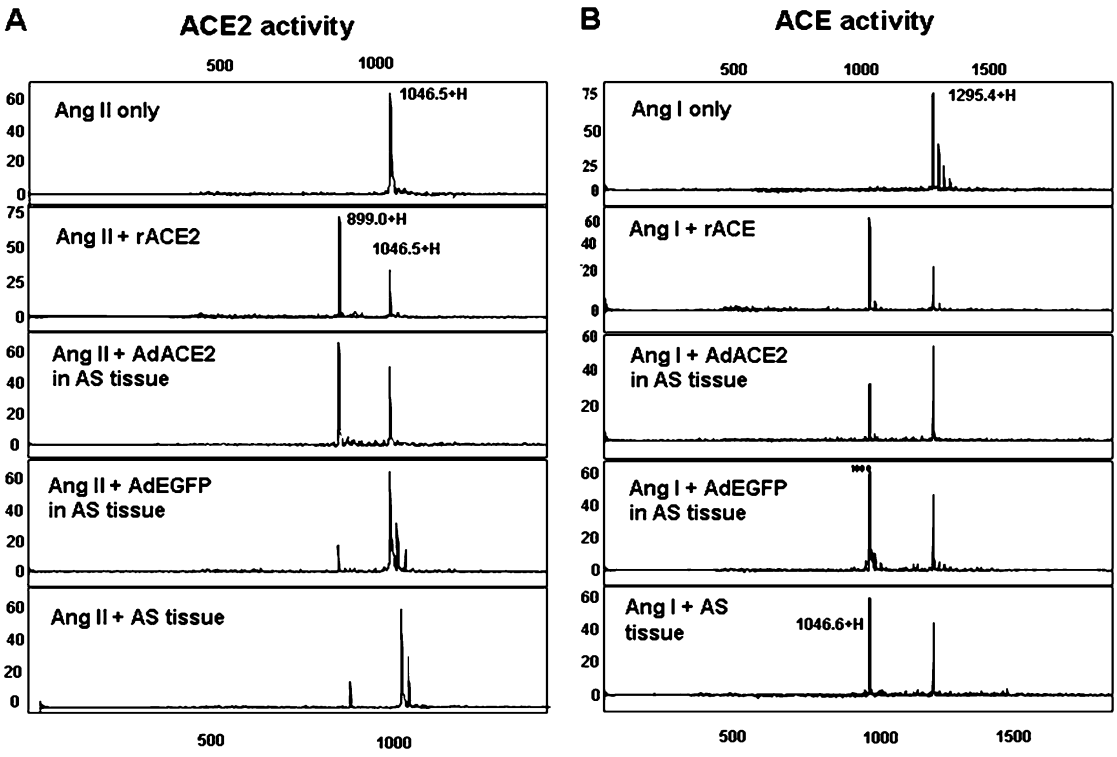


**Fig. S1.** ACE2 and ACE activity in rabbit aortic tissue evaluated by SELDI-TOF-MS analysis. (*A*) ACE2 activity in cleaving AngII. (*B*) ACE activity in cleaving AngI. AngII or AngI (10 μM) was added as a substrate in extracts of aortic tissue from Ad-ACE2, Ad-EGFP, and nontransduced groups, and the peptide peaks were measured by MS. At the top, each panel had a no-tissue negative control group and a recombinant-protein positive control group. $n = 12$ in each group.

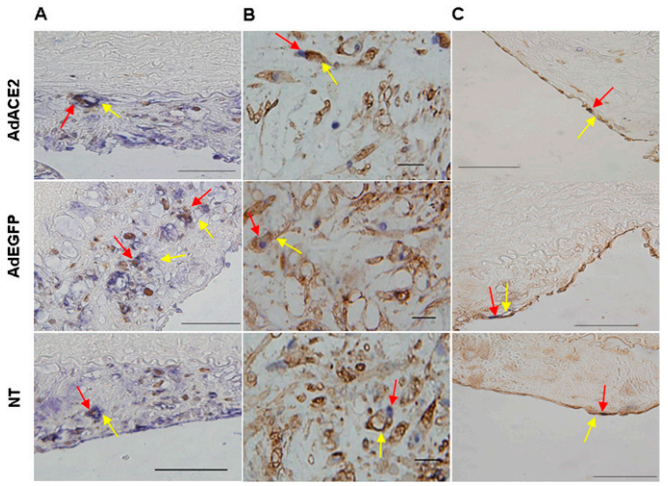


**Fig. S2.** Double-labeled immunocytochemistry of cells with positive PCNA staining. (*A*) Double immunolabeling for PCNA (yellow peroxidase product, red arrows) and macrophages (blue alkaline phosphatase product, yellow arrows); oil-immersion microscopy. (Magnification: 100×.) (Scale bars: 50 μm.) (*B*) Double immunolabeling for PCNA (blue alkaline phosphatase product, red arrows) and SMCs (α-actin staining, yellow peroxidase product, yellow arrows). (Magnification: 40×.) (Scale bars: 50 μm.) (*C*) Double immunolabeling for PCNA (blue alkaline phosphatase product, red arrows) and ECs (CD31 staining, yellow peroxidase product, yellow arrows); oil-immersion microscopy. (Magnification: 100×.) (Scale bars: 50 μm.)

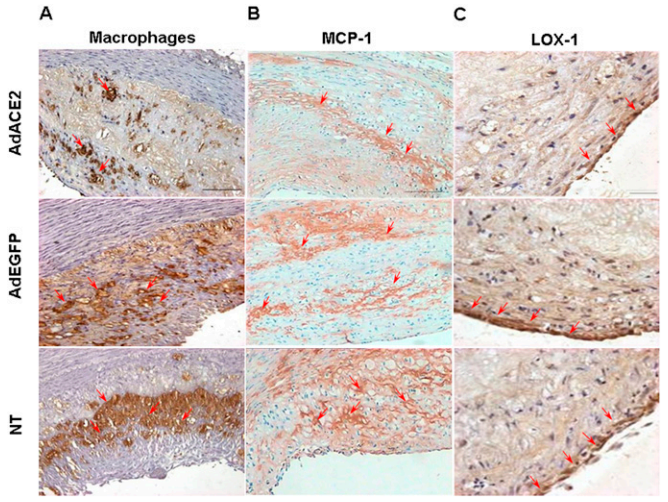


**Fig. S3.** Immunohistochemistry of atherosclerotic lesions. Macrophage infiltration (*A*) and expression of MCP-1 (*B*) and LOX-1 (*C*) protein (red arrows). (Magnification: 20×.) (Scale bars: 200 μm.) $n = 12$ in each group.

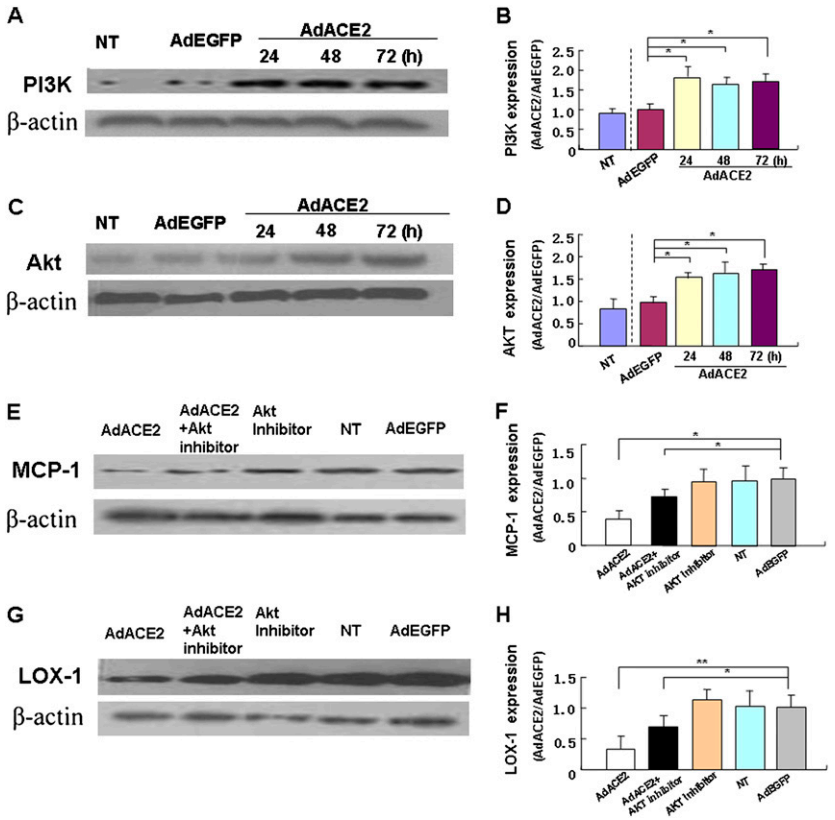


**Fig. S4.** Expression of PI3K, Akt, MCP-1, and LOX-1 protein in HUVECs by Western blot analysis. (*A*) PI3K protein expression in nontransduced, Ad-EGFP, and Ad-ACE2 groups (24, 48, and 72 h after transfection). (*B*) Quantitative analysis of results in *A*. $*P < 0.05$ vs. nontransduced or Ad-EGFP group. (*C*) Akt protein expression in nontransduced, Ad-EGFP, and Ad-ACE2 groups (24, 48, and 72 h after transfection). (*D*) Quantitative analysis of results in *C*. $*P < 0.05$ vs. nontransduced or Ad-EGFP group. (*E*) MCP-1 protein expression 48 h after Ad-ACE2 transfection. (*F*) Quantitative analysis of results in *E*. $*P < 0.05$ vs. nontransduced, AdACE2+Akt inhibitor, Akt inhibitor, or Ad-EGFP group. (*G*) LOX-1 protein expression 72 h after Ad-ACE2 transfection. (*H*) Quantitative analysis of results in *G*. $*P < 0.05$; $**P < 0.01$ vs. nontransduced, AdACE2+Akt inhibitor, Akt inhibitor, or Ad-EGFP group.

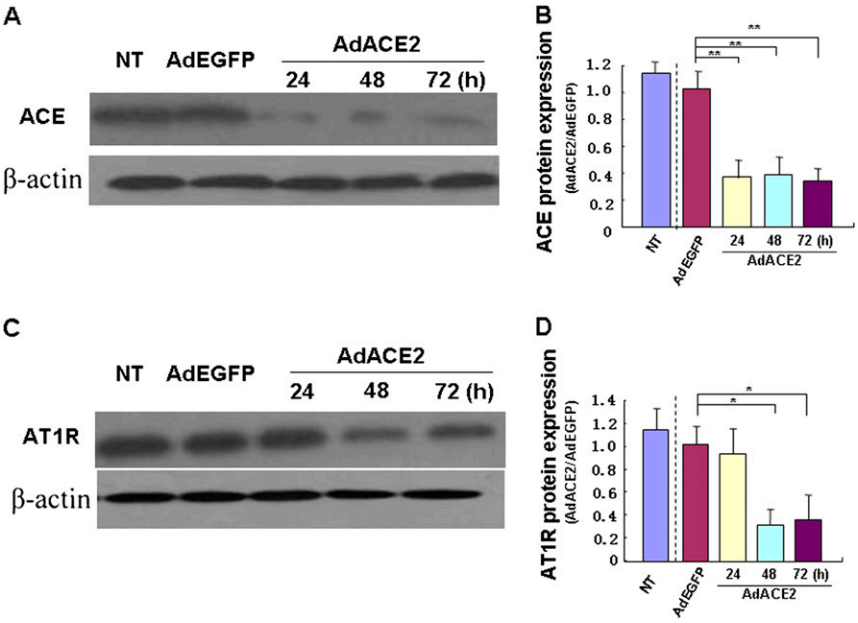


**Fig. S5.** ACE and $AT_1R$ protein expression in cultured HUVECs by Western blot analysis. (*A*) ACE protein expression in nontransduced, Ad-EGFP, and Ad-ACE2 groups of HUVECs (24, 48, and 72 h after transfection). (*B*) Quantitative analysis of results in *A*. $**P < 0.01$ vs. nontransduced or Ad-EGFP group. (*C*) $AT_1R$ protein expression in nontransduced, Ad-EGFP, and Ad-ACE2 groups (24, 48, and 72 h after transfection). (*D*) Quantitative analysis of results in *C*. $**P < 0.01$ vs. nontransduced, Ad-EGFP, or Ad-ACE2 group (24 h after transfection).

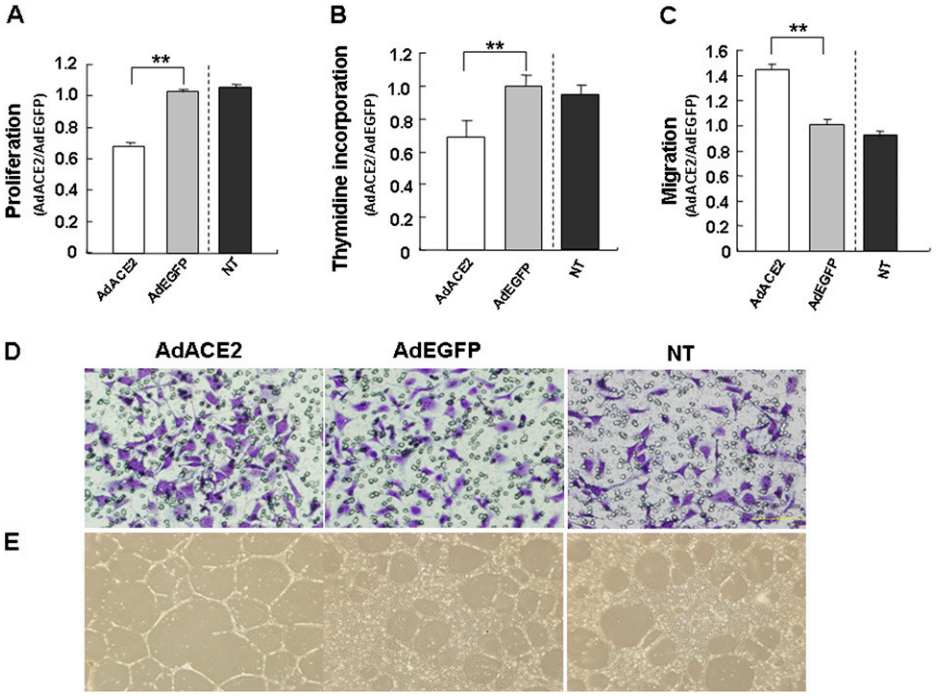


**Fig. S6.** HUVEC proliferation and migration in vitro. (*A*) Quantitative analysis of HUVEC proliferation measured by BrdU incorporation. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. (*B*) Quantitative analysis of HUVEC proliferation by thymidine incorporation assay. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. (*C*) Quantitative analysis of HUVEC migration. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. (*D*) HUVEC migration assay. (*E*) Formation of tube-like structure in HUVECs induced by ACE2 overexpression. (Magnification: 20×.) (Scale bars: 200 μm.)

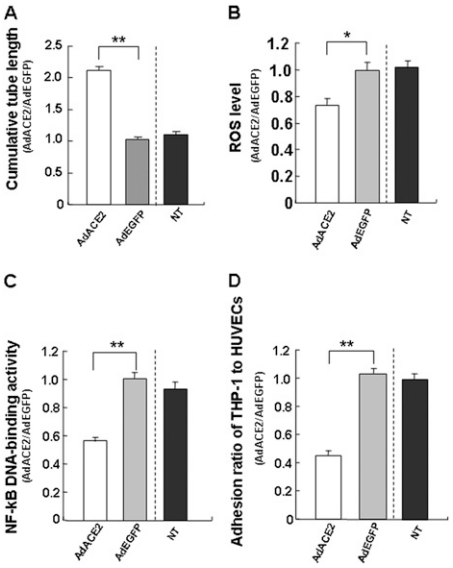


**Fig. S7.** Quantitative analysis of tube length, ROS levels, NF-κB p65 activity, and adhesion of monocytes to HUVECs in vitro. (*A*) Comparison of cumulative tube length. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. (*B*) Comparison of ROS levels. $^{*}P < 0.05$ vs. nontransduced or Ad-EGFP group. (*C*) Comparison of NF-κB p65 activity. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. (*D*) Comparison of THP-1 adhering to endothelial cells. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group.

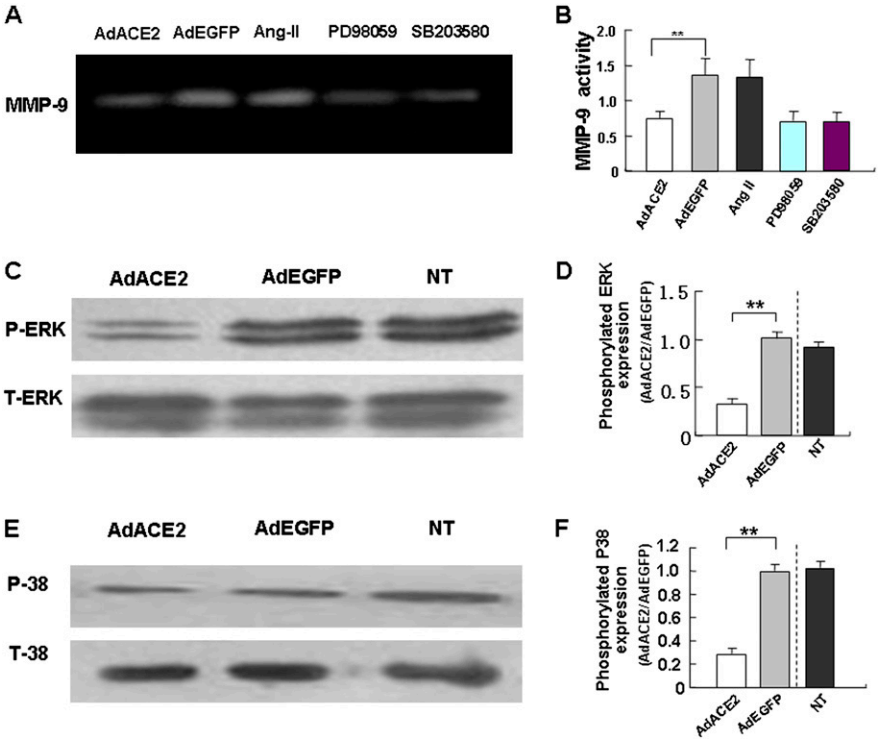


**Fig. S8.** MMP-9 activity and MAPK signal pathway. (*A*) MMP-9 activity measured by zymography in VSMCs with AngII treatment and ACE2 gene transfer. Treatment with p38 MAPK and ERK1/2 MAPK with SB203580 (10 μM) and PD98059 (20 μM), respectively. (*B*) Quantitative analysis of MMP-9 activity in *A*. $^{**}P < 0.01$ vs. AngII or Ad-EGFP group. (*C*) ERK protein expression in atherosclerotic lesions. (*D*) Quantitative analysis of results in *C*. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. (*E*) p38 protein expression in atherosclerotic lesions. (*F*) Quantitative analysis of results in *E*. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. $n = 12$ in each group.

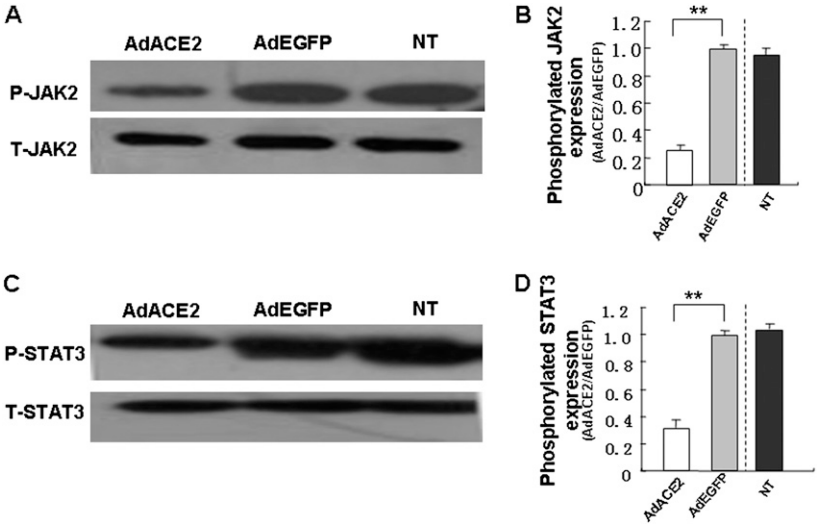


**Fig. S9.** JNK-STAT signal pathway by Western blot analysis of atherosclerotic lesions. (*A*) JAK2 protein expression in atherosclerotic lesions. P-JAK2, phosphorylated JAK2; T-JAK-2, total JAK2. (*B*) Quantitative analysis of results in *A*. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. (*C*) STAT3 protein expression in atherosclerotic lesions. P-STAT3, phosphorylated STAT3; T-STAT3, total STAT3. (*D*) Quantitative analysis of results in *C*. $^{**}P < 0.01$ vs. nontransduced or Ad-EGFP group. $n = 12$ in each group.

**Table S1. Serum total cholesterol (TC) and triglyceride (TG) levels in the three experimental groups of rabbits**

| | TC, mM | | | TG, mM | | |
|---|---|---|---|---|---|---|
| Group (*n*) | Week 0 | Week 4 | Week 8 | Week 0 | Week 4 | Week 8 |
| Ad-ACE2 (21) | 0.77 ± 0.17 | 35.82 ± 4.71 | 36.06 ± 5.56 | 0.56 ± 0.10 | 2.09 ± 0.61 | 2.12 ± 0.62 |
| Ad-EGFP (21) | 0.79 ± 0.19 | 35.45 ± 4.66 | 35.99 ± 5.11 | 0.60 ± 0.09 | 1.93 ± 0.50 | 2.02 ± 0.54 |
| NT (21) | 0.76 ± 0.14 | 34.38 ± 4.55 | 34.85 ± 5.47 | 0.58 ± 0.10 | 2.04 ± 0.46 | 2.05 ± 0.54 |

**Table S2. Pathology parameters of atherosclerotic lesions in the three experimental groups of rabbits**

| Groups (*n*) | Intimal area | Medial area | I/M ratio |
|---|---|---|---|
| Ad-ACE2 (12) | 0.77 ± 0.32** | 1.21 ± 0.29 | 0.62 ± 0.18* |
| Ad-EGFP (12) | 1.88 ± 0.30 | 1.40 ± 0.23 | 1.37 ± 0.26 |
| NT (12) | 1.89 ± 0.27 | 1.39 ± 0.23 | 1.39 ± 0.31 |

*$P < 0.01$ vs. nontransduced or Ad-EGFP group.

**Table S3. Effects of ACE2 gene transfer on MCP-1 and ICAM-1 levels in HUVECs**

| Parameters | Ad-ACE2 | Ad-EGFP | NT |
|---|---|---|---|
| MCP-1 (12 h), pg/mL | 238.46 ± 17.28* | 349.12 ± 22.02 | 357.89 ± 21.03 |
| MCP-1 (24 h), pg/mL | 438.46 ± 21.90* | 802.93 ± 22.14 | 810.19 ± 21.92 |
| ICAM-1 (12 h), ng/mL | 109.38 ± 14.72* | 181.23 ± 14.26 | 186.69 ± 21.03 |
| ICAM-1 (24 h), ng/mL | 180.23 ± 18.85* | 276.09 ± 12.44 | 267.65 ± 16.52 |

*$P < 0.01$ vs. nontransduced or Ad-EGFP group at the same time point.

**Table S4. Effects of ACE2 transfection on endothelium-dependent vasorelaxation by acetylcholine**

| | Acetylcholine dose | | | | |
|---|---|---|---|---|---|
| Group (*n*) | 1 nM | 10 nM | 100 nM | 1 μM | 10 μM |
| Ad-ACE2 (12) | 10.18 ± 1.26* | 32.35 ± 2.56* | 58.53 ± 2.17* | 76.49 ± 2.92* | 88.26 ± 4.76* |
| Ad-EGFP (12) | 3.58 ± 1.22 | 11.14 ± 1.79 | 35.32 ± 3.35 | 45.64 ± 3.71 | 39.67 ± 4.92 |
| NT (12) | 3.64 ± 1.22 | 10.60 ± 2.02 | 35.73 ± 2.88 | 46.79 ± 3.43 | 40.28 ± 4.55 |

*$P < 0.01$ vs. nontransduced or Ad-EGFP group at the same dose.