Supplementary Data

Supplementary Materials and Methods

Reagents and antibodies

Vascular endothelial growth factor receptor 2 (VEGFR2) and cystathionine-γ-lyase (CTH) siRNA were purchased from Santa Cruz (Santa Cruz, CA). Vascular endothelial growth factor (VEGF) and CTH antibodies for Western blot were purchased from Santa Cruz. Erk, Akt, and Tubulin antibodies for Western blot were purchased from Cell Signaling (Danvers, MA). CTH antibody for immunofluorescence was purchased from Abcam (Cambridge, United Kingdom).

Chorioallantoic membranes assay

Sterile filter disks, which were presoaked with drug or phosphate-buffered saline, a negative control, were placed on the growing chorioallantoic membranes (CAM) of a 9-day-old chicken embryo. The drug was added every day for 3 days. The filter disks and the adherent CAM tissue were harvested and photographed.

Sponge implantation assay

C57BL/6 mice or Sprague–Dawley rats were anesthetized by 7% chloral hydrate (intraperitoneally) and dorsally implanted with polyester. Drug-treated mice or rats were injected intraperitoneally (50 mg/kg/day) every day for 7 days. The implanted sponge was removed for the photograph.

Burn wound assay

The burn wound assay was carried out as previously described (5) with some modification. In brief, C57BL/6 mice were anesthetized by 7% chloral hydrate (intraperitoneally) and burned with a heated metal stick to create an approximate 25 mm^2 scald wound on the abdominal surface of the animals. Drug-treated mice were injected intraperitoneally (50 mg/kg/day) every day for 9 days. The wound size was photographed every third day.

Determination of H_2S level and CTH activity

The determination of H_2S level and CTH activity was carried out using colorimetric assay as previously described (7).

Cloning, expression, and purification

The construction of plasmids was performed as previously described (3) with a slight modification. In brief, the murine tissue total RNA was extracted and purified with a GeneJET™ RNA Purification Kit (Fermentas, Vilnius, Lithuania), and then reverse transcribed to cDNA using a RevertAid™ First-Strand cDNA Synthesis Kit (Fermentas). A C-terminal fragment of the murine STAT3β cDNA, starting at residue G127, was amplified by PCR using PrimeSTAR® Max DNA Polymerase (TAKARA, Dalian, China) and the primers are as follows:

5′-dGGGATCTACTTCCATATGGGCCAGGCCAACCACC-3′
5′-dGGAATTCATCATTTCCAAACTGCATCAATGAATGGT GTCACACAGATGAACTTGGT-3′ (restriction sites underlined).

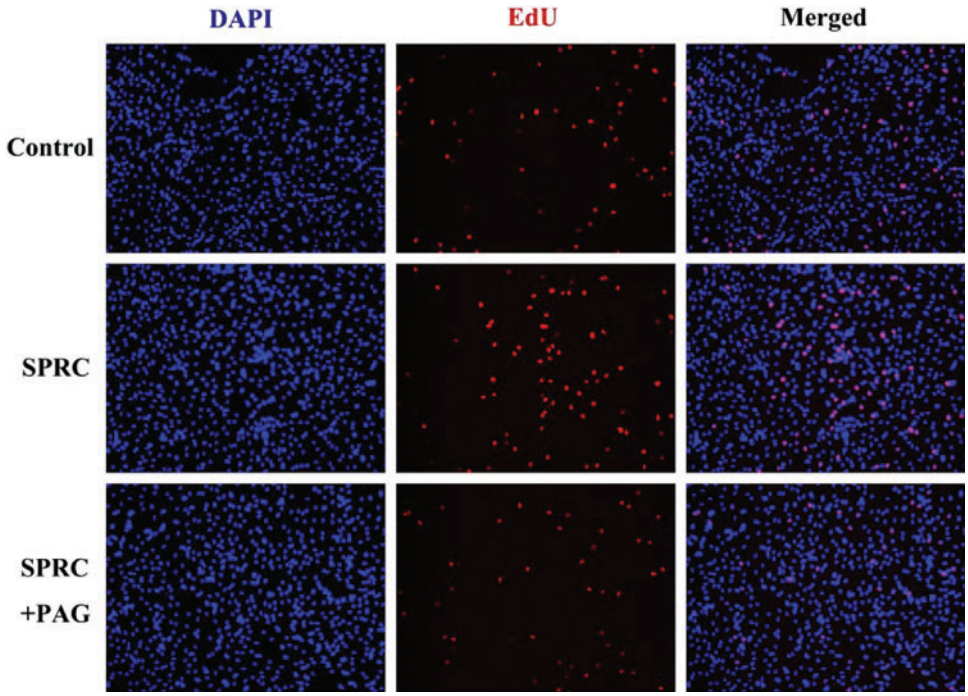
The PCR fragment was digested with NdeI/EcoRI (Fermentas) and cloned into the expression vector pET32a (Novagen, Billerica, MA). The resultant expression plasmid pET32aSTAT3 was transformed into the *Escherichia coli* strain BL21(DE3)TBK1 (Stratagene, Santa Clara, CA). The expression and purification of STAT3 protein were carried out as described in the same reference (3).

Crystallization and data collection

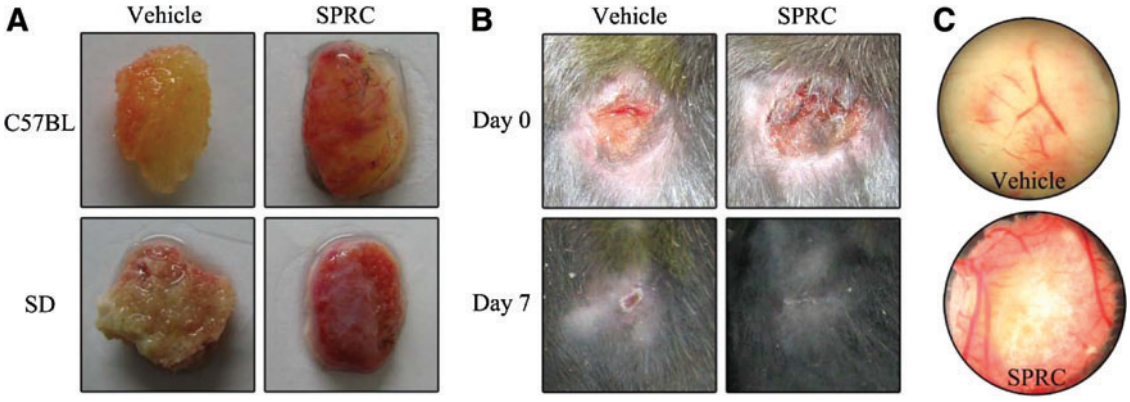
The purified STAT3 protein was incubated with hybridized oligonucleotides (5′-AAGATTTACGGGAAATGC-3′ and 5′-TGCATTTCCCGTAAATCT-3′) in an equal molar ratio before the crystallization set up. Afterward, the STAT3 protein solution at 6 mg/ml was mixed with an equal volume of reservoir buffer of MES (50 m*M*, pH6.1), Ammonium Acetate (100 m*M*), NaCl (200 m*M*), MgSO4 (5 m*M*), and 12.5% Glycerol, and crystallized against the same buffer by hanging-drop vapor diffusion method at 289 K. The crystals that appeared were stabilized in a reservoir buffer supplemented by 15% glycerol and SPRC (5 m*M*) for 6 h and stepwise transferred to a reservoir buffer supplemented by 25% glycerol as a cryoprotectant. The soaked crystals were then flash frozen in liquid nitrogen. Crystal diffraction data sets were collected at BL17U of the Shanghai Synchrotron Radiation Facility, and data were processed by iMOSFLM (2) in CCP4 package. The best crystal diffraction to 3.4 Å and structure was solved by MOLREP (6) using the published STAT3 model (PDB Code: 1BG1) as a search model. Manual model correction was performed in COOT (4), and the structures were further refined in PHENIX (1).

Supplementary References

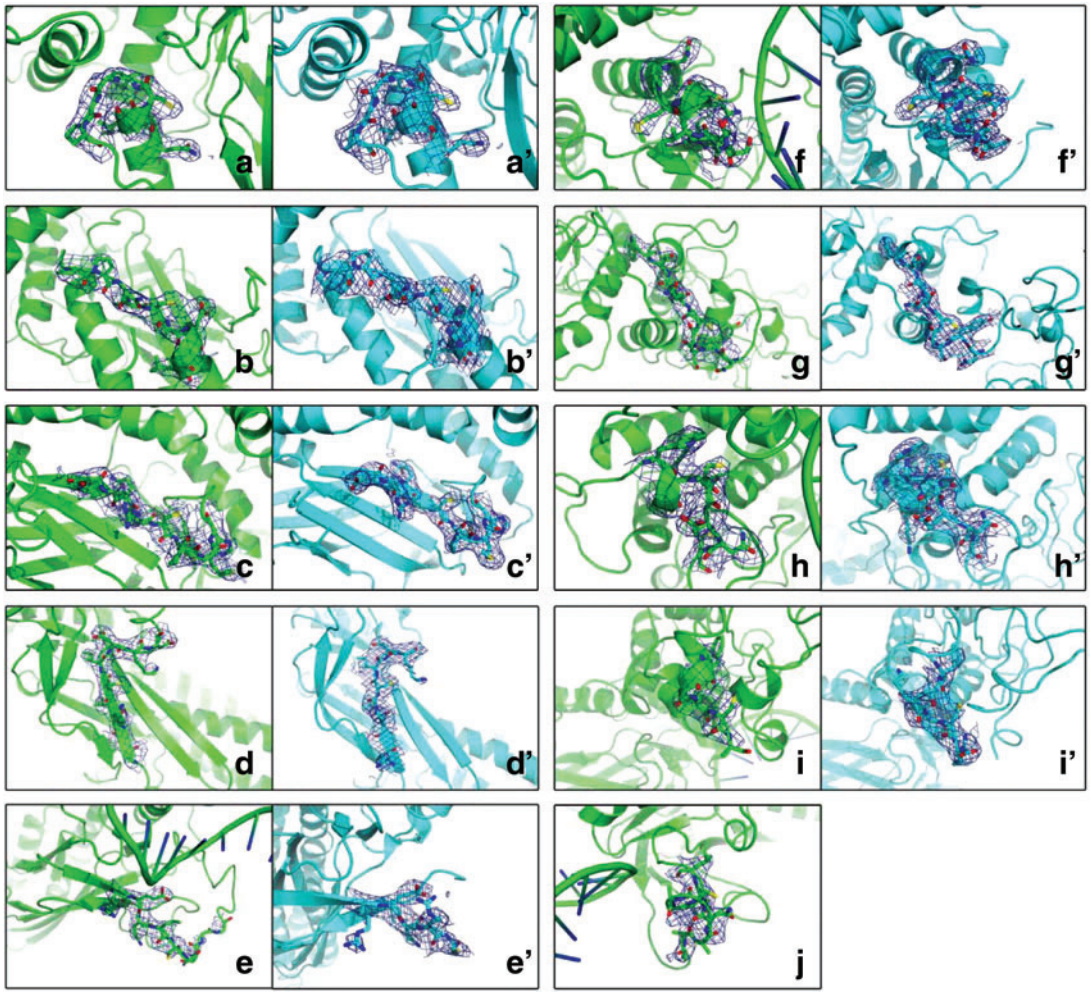
1. Adams PD, Afonine PV, Bunkoczi G, Chen VB, Davis IW, Echols N, Headd JJ, Hung LW, Kapral GJ, and Grosse-Kunstleve RW. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66: 213–221, 2010.
2. Battye TGG, Kontogiannis L, Johnson O, Powell HR, and Leslie AGW. iMOSFLM: a new graphical interface for diffraction-image processing with MOSFLM. *Acta Crystallogr D Biol Crystallogr* 67: 271–281, 2011.
3. Becker S, Corthals GL, Aebersold R, Groner B, and Müller CW. Expression of a tyrosine phosphorylated, DNA binding Stat3β dimer in bacteria. *FEBS Lett* 441: 141–147, 1998.
4. Emsley P, Lohkamp B, Scott WG, and Cowtan K. Features and development of Coot. *Acta Crystallogr D Biol Crystallogr* 66: 486–501, 2010.
5. Papapetropoulos A, Pyriochou A, Altaany Z, Yang GD, Marazioti A, Zhou ZM, Jeschke MG, Branski LK, Herndon DN, Wang R, and Szabo C. Hydrogen sulfide is an endogenous stimulator of angiogenesis. *Proc Natl Acad Sci U S A* 106: 21972–21977, 2009.
6. Vagin A and Teplyakov A. Molecular replacement with MOLREP. *Acta Crystallogr D Biol Crystallogr* 66: 22–25, 2009.
7. Wang Q, Liu HR, Mu Q, Rose P, and Zhu YZ. S-propargyl-cysteine protects both adult rat hearts and neonatal cardiomyocytes from ischemia/hypoxia injury: the contribution of the hydrogen sulfide-mediated pathway. *J Cardiovasc Pharmacol* 54: 139–146, 2009.



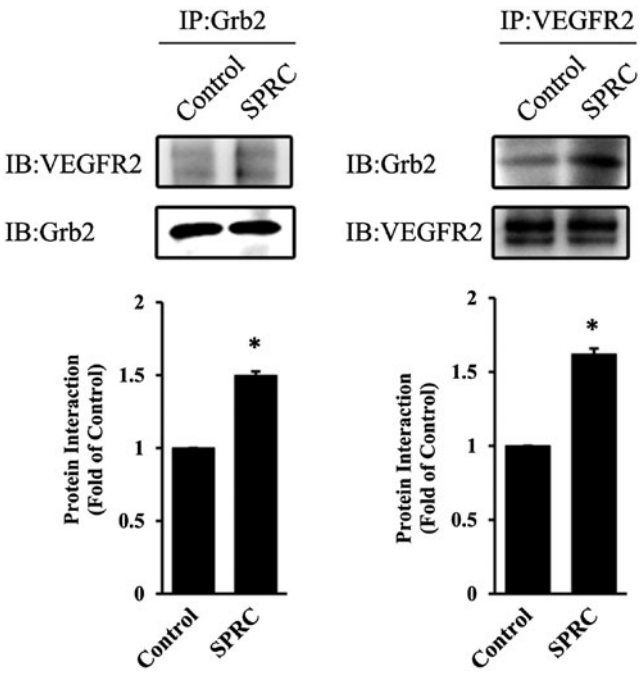
SUPPLEMENTARY FIG. S1. Representative micrographs of cell proliferation assay.



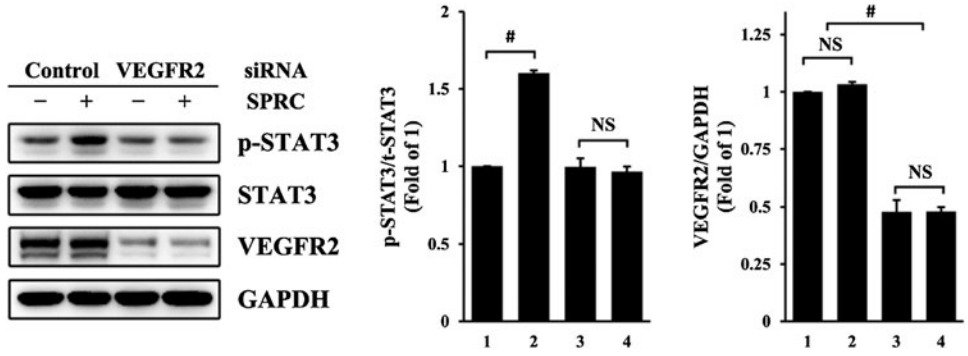
SUPPLEMENTARY FIG. S2. S-propargyl-cysteine (SPRC) promoted angiogenesis *in vivo*. (A) Representative photographs of sponge implantation assay. C57BL/6 mice or Sprague–Dawley rats were implanted with sponge and injected intraperitoneally with vehicle or SPRC (50 mg/kg/day) for 7 days. **(B)** Representative photographs of burn wound assay. C57BL/6 mice were burned to create a scald wound on their abdominal surface, and they were injected intraperitoneally with vehicle or SPRC (50 mg/kg/day) for 9 days. **(C)** Representative photographs of chorioallantoic membranes (CAM) assay. The CAM of a 9-day-old chicken embryo was covered with a filter disk on the surface, and treated with vehicle or SPRC (100 m*M*) for 3 days.



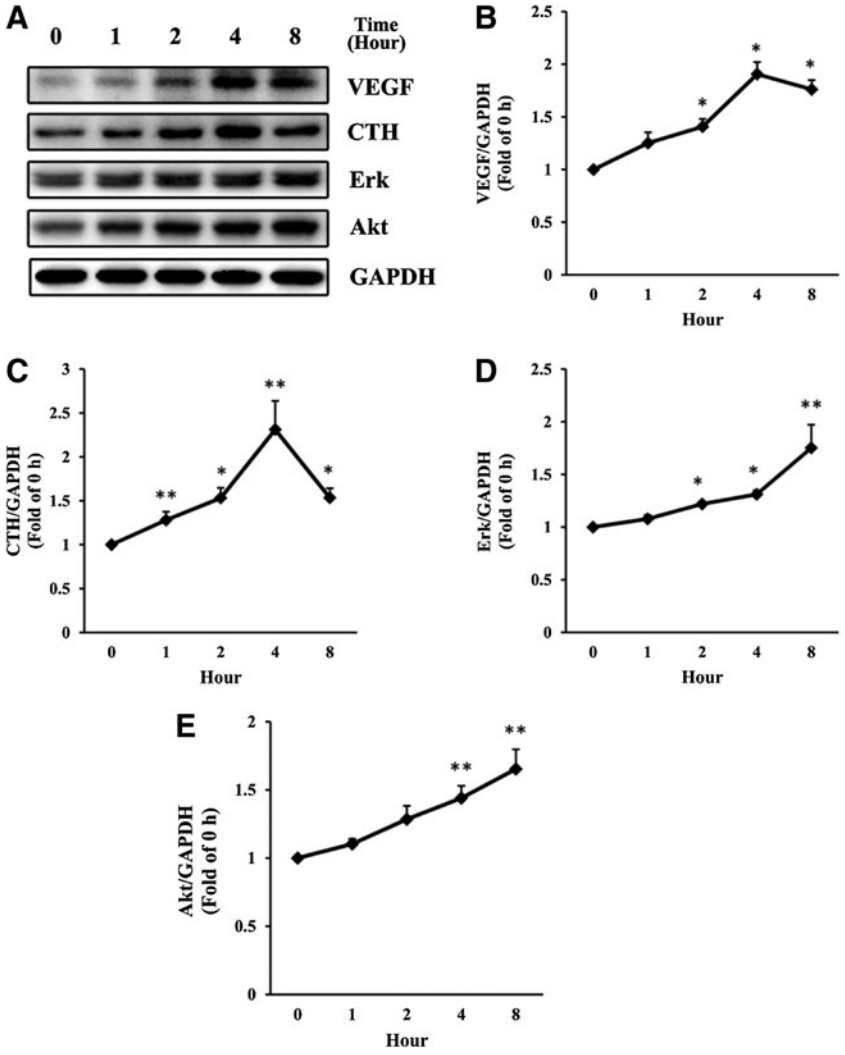
SUPPLEMENTARY FIG. S3. Cysteine residues with their electron densities in STAT3:DNA complex. Our model is colored in green, and a previously published STAT3 structure (PDB Code: 3CWG; cyan) is shown for a comparison. Cysteine residues of 251, 259, 328, 367, 418, 468, 542, 550, 687, and 426 were denoted in **(a–j, a′–i′)**, respectively. The 2Fo-Fc electron density maps (blue) that encompassed the cysteine residues and their particular preceding and succeeding residues were contoured at 1σ. Note that, since the residue cys426 is lacking in 3CWG structure, only the electron density for this residue in our model is shown.



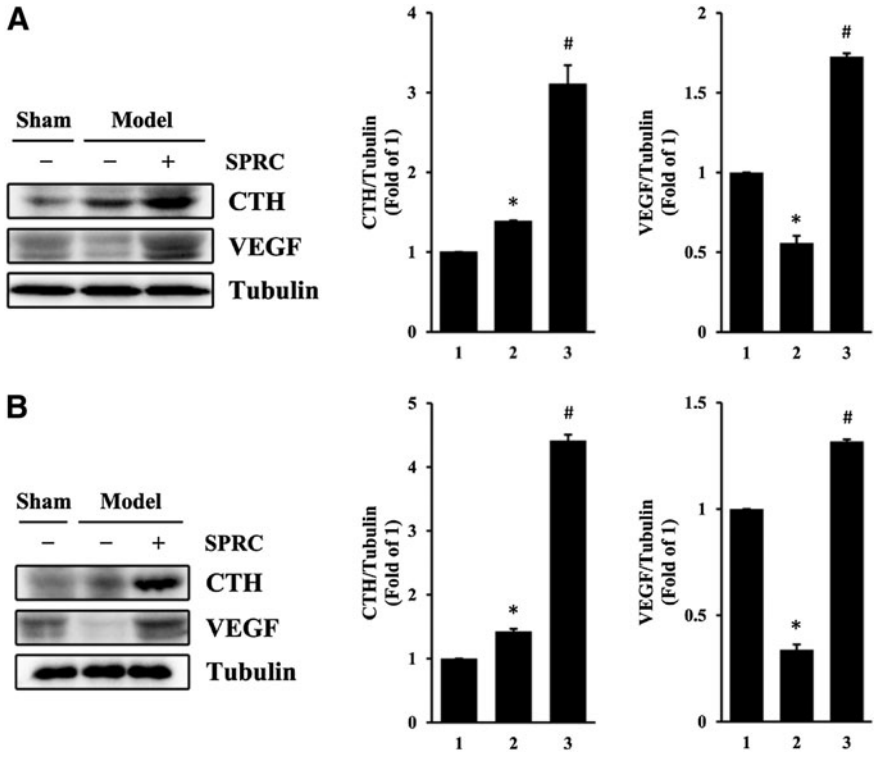
SUPPLEMENTARY FIG. S4. Protein level of vascular endothelial growth factor receptor 2 (VEGFR2) or Grb2 in complex immunoprecipitated with Grb2 or VEGFR2 antibody. Transformed cells of human umbilical vein endothelium (HUV-EC-C) were treated with SPRC (100 μM) for 20 min. $^{*}p < 0.01$ *versus* control group.



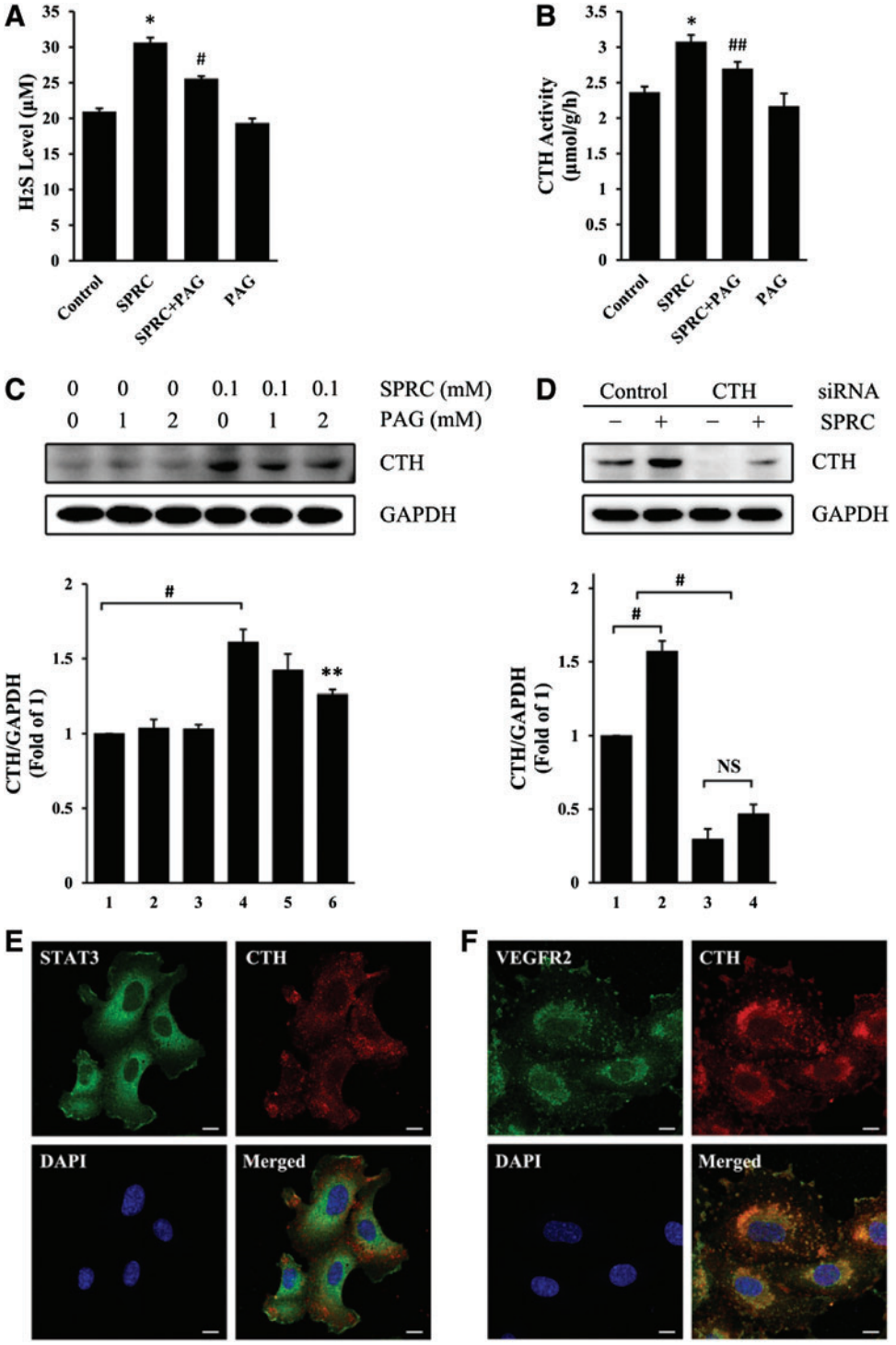
SUPPLEMENTARY FIG. S5. Primary human umbilical vein endothelial cells (HUVEC) were treated with SPRC (100 μM) for 20 min after VEGFR2 silencing, which was performed by siRNA transfection using an RNAiMAX kit for 72 h. Control siRNA containing a scrambled sequence was used as a negative control. $^{\#}p < 0.01$ between groups, as indicated. NS, nonsignificant ($p > 0.05$).



SUPPLEMENTARY FIG. S6. SPRC promoted protein synthesis. Primary HUVEC were treated with SPRC (100 μM) for 0, 1, 2, 4, and 8 h, and then, proteins were extracted and analyzed. **(B–E)** were statistical analyses of **(A)**. *$p <$ 0.01 *versus* 0 h, **$p < 0.05$ *versus* 0 h.



SUPPLEMENTARY FIG. S7. SPRC promoted protein synthesis *in vivo*. (A) After ligation of the left femoral artery, mice in the SPRC-treated group were injected intraperitoneally with SPRC (50 mg/kg/day) for 7 days. The protein was extracted from the homogenate of gastrocnemius muscle. **(B)** After ligation of the left coronary artery, rats in the SPRC-treated group were injected intraperitoneally with SPRC (50 mg/kg/day) for 42 days. The protein was extracted from the homogenate of ventricular myocardium. $^{*}p < 0.01$ *versus* group 1, $^{\#}p < 0.01$ *versus* group 2.



SUPPLEMENTARY FIG. S8. SPRC modulated the level of endogenous hydrogen sulfide *via* CTH. (A, B) Primary HUVEC were treated with SPRC (100 μM) for 6 h, and then, culture media were used to determine the level of hydrogen sulfide using colorimetric assay. The cells were homogenized to determine the activity of CTH. $^{*}p < 0.01$ *versus* control group, $^{\#}p < 0.01$ *versus* SPRC-treated group, and $^{\#\#}p < 0.05$ *versus* SPRC-treated control group. **(C)** Primary HUVEC were treated with PAG (1 or 2 m*M*), an inhibitor of CTH, for 2 h, followed by SPRC (100 μM) for 20 min. $^{**}p < 0.05$ *versus* group 4. $^{\#}p < 0.01$ between groups, as indicated. **(D)** Primary HUVEC were treated with SPRC (100 μM) for 20 min after CTH silencing, which was performed by siRNA transfection using an RNAiMAX kit for 72 h. Control siRNA containing a scrambled sequence was used as a negative control. $^{\#}p < 0.01$ between groups, as indicated. NS, nonsignificant ($p > 0.05$). **(E, F)** Primary HUVEC were fixed and specifically stained by STAT3 or VEGFR2 antibody (green) and CTH antibody (red). DAPI (blue) indicates the nucleus. Scale bar, 10 μm.