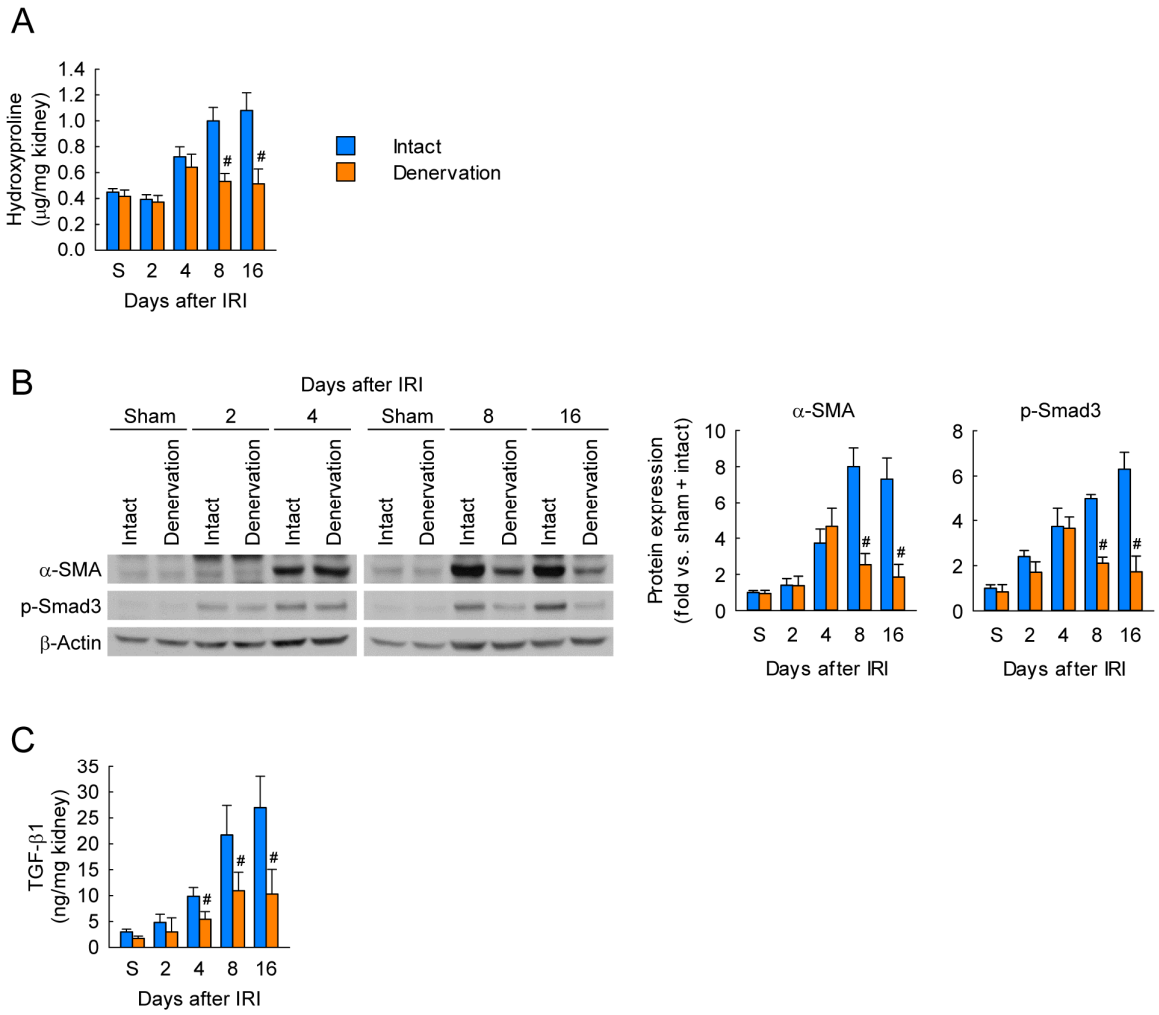
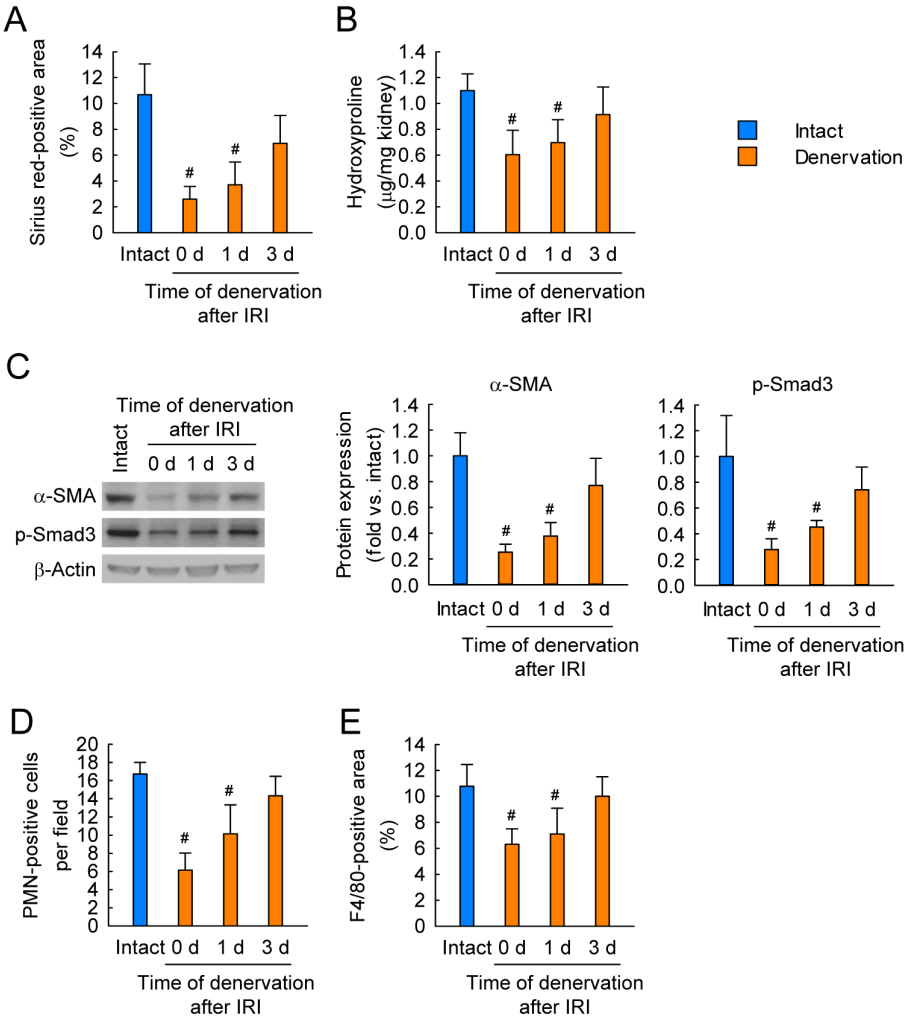


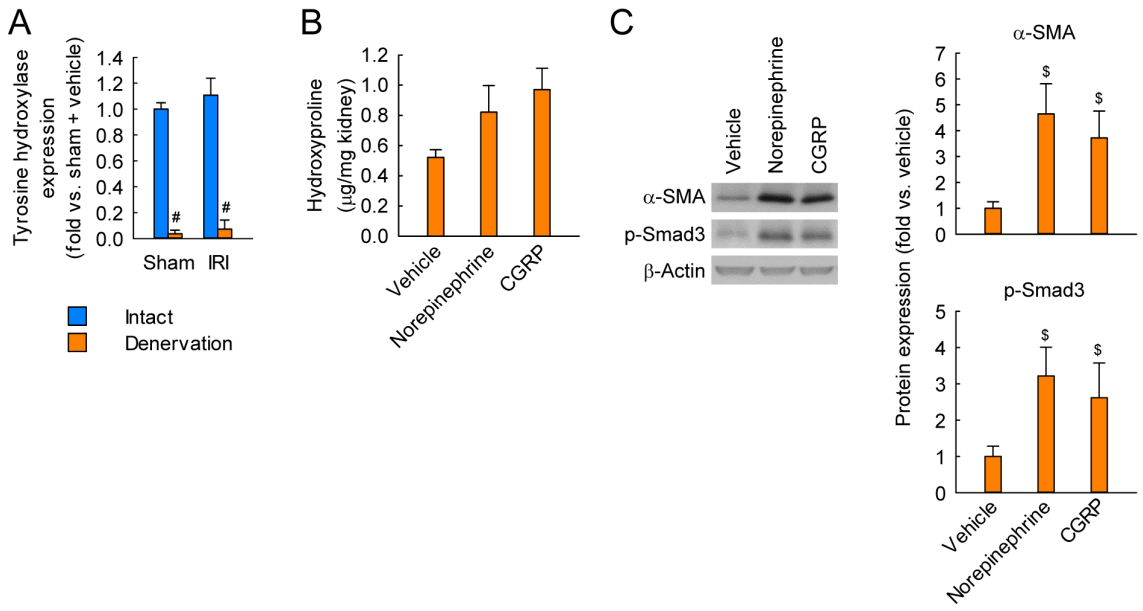

**Supplementary Figure 1: Renal denervation inhibits interstitial fibrosis induced by IRI.**

Two days after denervation in left kidneys of mice, IRI or sham operation (S) in the left kidneys was carried out (n = 5). (A) Collagen content represented by hydroxyproline level in the kidneys. (B) Expression levels of α-SMA and p-Smad3 in the kidneys. The Western blot bands were quantified using Lab Works analysis software. (C) Level of TGF-β1 protein in the kidneys using multiplex immunoassay kit. Error bars represent SD. $^{\#}P<0.05$ versus intact.



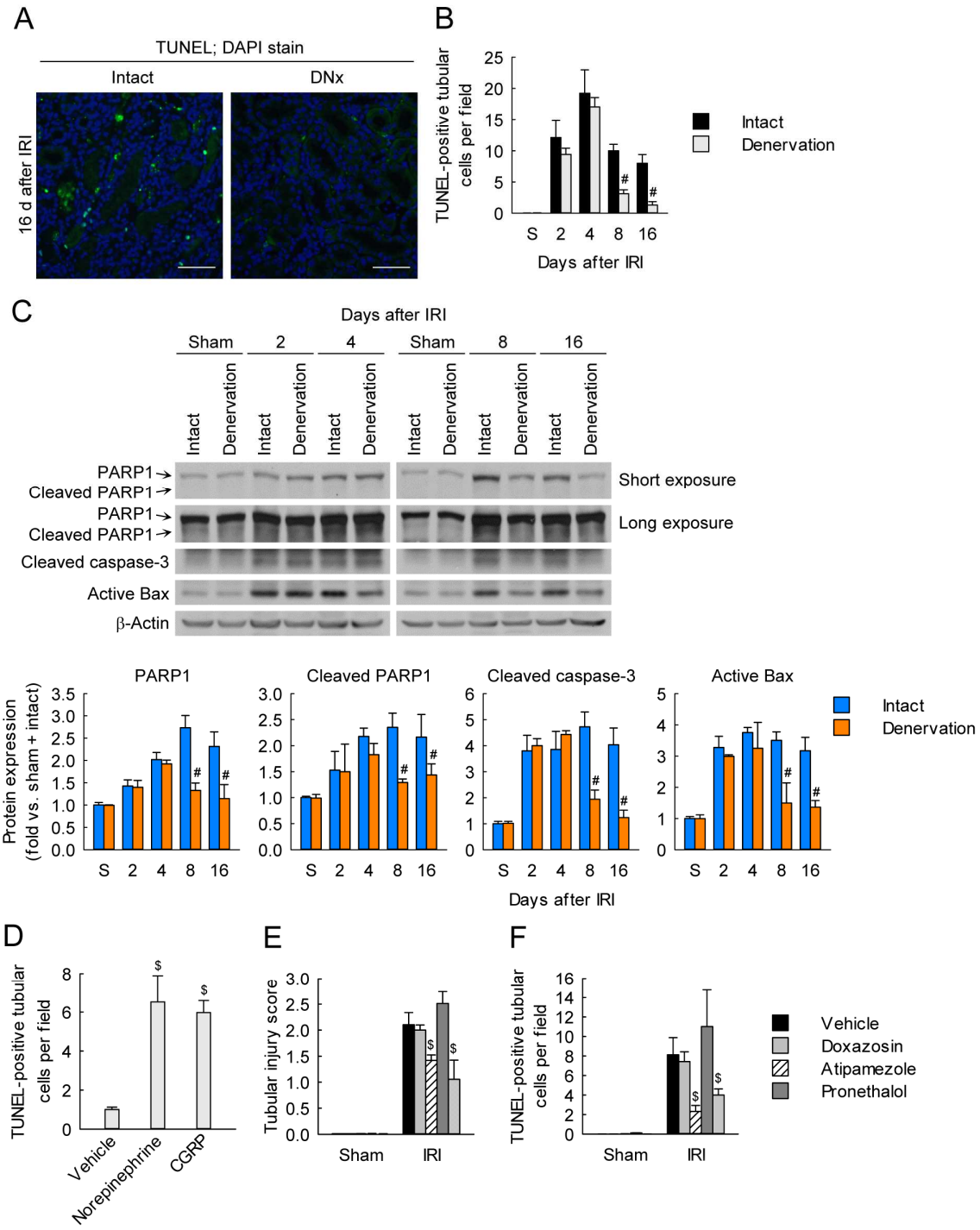

**Supplementary Figure 2: Postconditioning of renal denervation inhibits interstitial fibrosis and inflammation induced by IRI.**

Mice were subjected to left kidney ischemia followed by 16 days of reperfusion. Denervation in the left kidneys was carried out at 0, 1 and 3 days after IRI (n = 4). (A) Percentage of Sirius red-positive area on the kidney sections. (B) Collagen content represented by the level of hydroxyproline in the kidneys. (C) Expression of α-SMA and p-Smad3 protein using Western blot analysis in the kidneys. Anti-β-actin antibody served as a loading control. (D) Neutrophil infiltration represented by the number of PMN-positive cells on immunohistochemically stained sections on the kidney sections. (E) Macrophage infiltration represented by percentage of F4/80-positive area on immunohistochemically stained kidney sections. Error bars represent SD (n = 4). $^{\#}P<0.05$ versus intact.



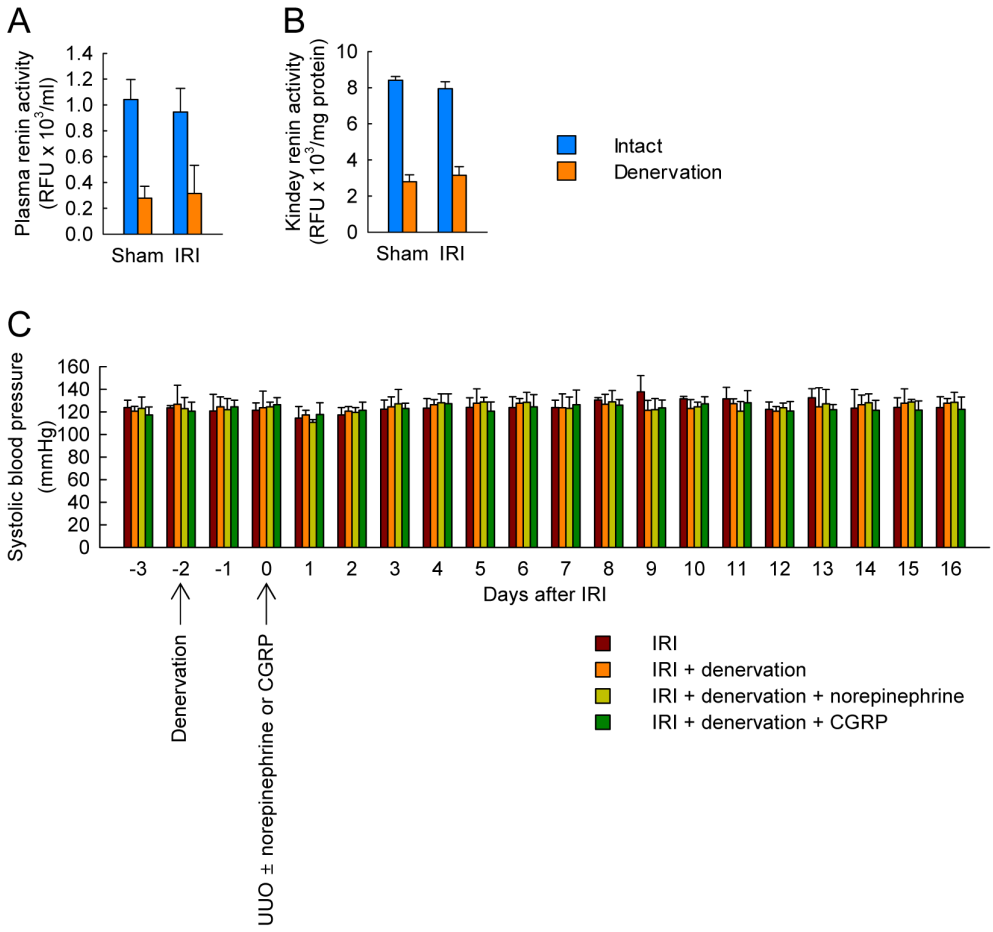

**Supplementary Figure 3: Exogenous norepinephrine or CGRP enhanced interstitial fibrosis induced by IRI in denervated kidneys.**

(A) Two days after denervation in left kidneys of mice, IRI or S in the left kidneys was carried out. Kidney expression of tyrosine hydroxylase at 16 days after IRI or sham. The Western blot bands were quantified using Lab Works analysis software. (B and C) Denervation in left kidneys of mice was carried out; 2 days after the denervation, norepinephrine, CGRP or vehicle was continuously infused into the denervated kidneys via mini-osmotic pump. The kidneys were subjected to 30 min of ischemia followed by 16 days of reperfusion. (B) Collagen content represented by the level of hydroxyproline in the kidneys. (C) Expression levels of α-SMA and p-Smad3 in the kidneys. The Western blot bands were quantified using Lab Works analysis software. Error bars represent SD (n = 4). $^{\#}P<0.05$ versus intact; $^{\$}P<0.05$ versus vehicle.



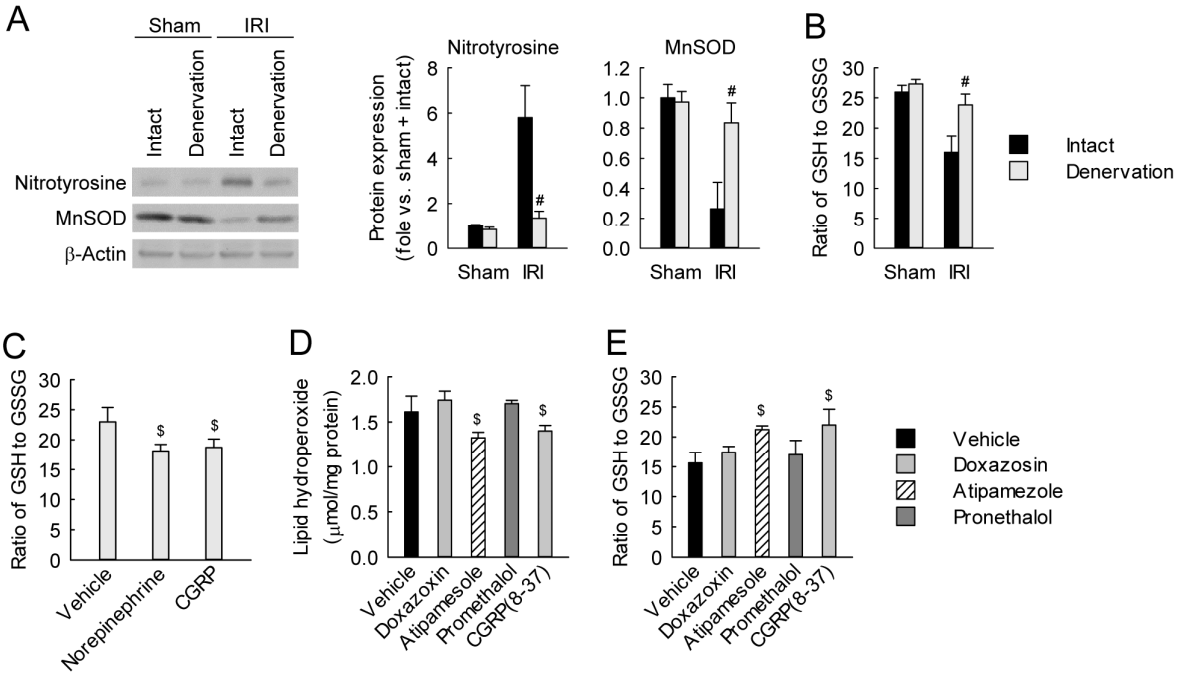

**Supplementary Figure 4. Norepinephrine and CGRP signaling contribute to tubular cell death during a period of interstitial fibrosis after IRI.**

(A) TUNEL assay on the kidney sections. Scale bars indicate 50 μm. (B, D and F) Apoptosis in tubular cells represented by counting TUNEL-positive tubular cells on kidney sections. (C) Expression of PARP1, cleaved PARP1, cleaved caspase-3 and active Bax in the kidneys using Western blot analysis. (E) Tubular injury score measured on PAS-stained kidney sections. Error bars represent SD. $^{\#}P<0.05$ versus intact and $^{\$}P<0.05$ versus vehicle.



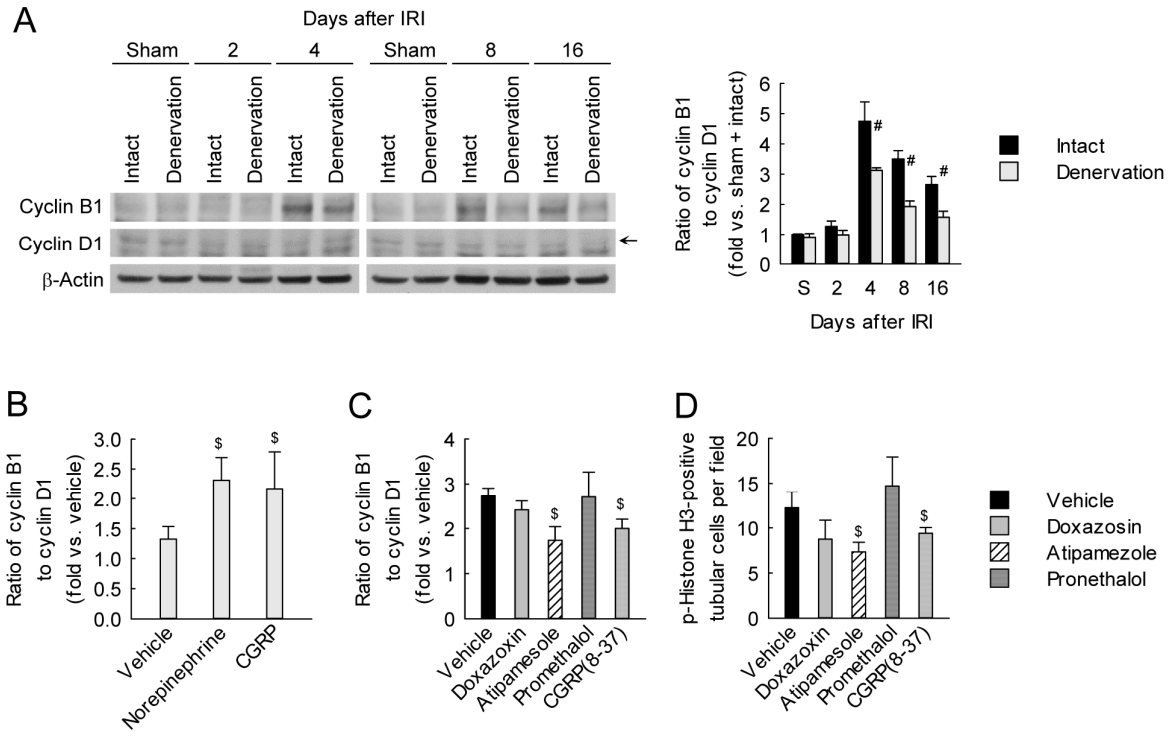

**Supplementary Figure 5: Renin activity and blood pressure in interstitial fibrosis induced by IRI.**

Two days after denervation in left kidneys of mice, IRI or sham in the left kidneys was carried out (n = 5). In some mice, 2 days after the denervation, norepinephrine or CGRP was continuously infused into the denervated kidneys via mini-osmotic pump; and the kidneys were subjected to 30 min of ischemia (n = 4). (A and B) Renin activity in plasma and kidneys at 16 days after IRI or sham was measured by a mouse renin assay kit. (C) Systolic blood pressure of mice was measured by a noninvasive taill cuff method.



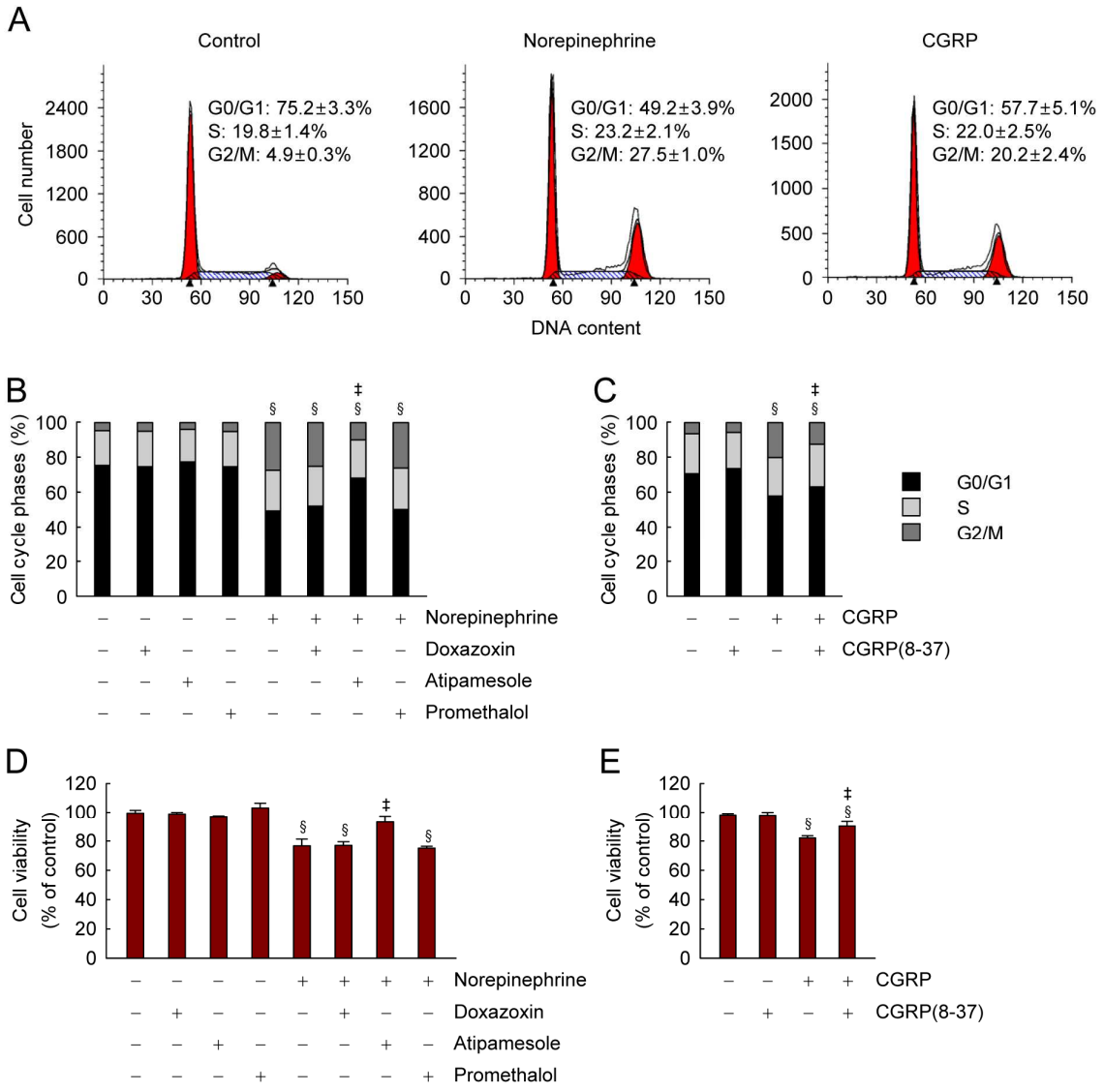

**Supplementary Figure 6. Renal denervation reduces oxidative stress in kidneys after IRI.**

(A and B) Two days after denervation in left kidneys of mice, IRI or sham in the left kidneys was carried out; then the kidneys were harvested at 16 days after reperfusion (n = 5). (C) Denervation in left kidneys of mice was carried out; 2 days after the denervation, norepinephrine, CGRP or vehicle was continuously infused into the denervated kidneys via mini-osmotic pump; and the kidneys were subjected to 30 min of ischemia followed by 16 days of reperfusion (n = 4). (D and E) Mice were continuously treated with doxazosin, atipamezole, pronethalol or vehicle via an intraperitoneal implantation of mini-osmotic pump immediately before 30 min of left kidney ischemia and 16 days of reperfusion (n = 5). (A) Nitrotyrosine levels and expression level of MnSOD in the kidneys. The Western blot bands were quantified using Lab Works analysis software. (B, C and E) The ratio of GSH to GSSG in kidneys. (D) Lipid peroxidation represented by level of lipid hydroperoxide in kidneys. Error bars represent SD. $^{\#}P<0.05$ versus intact.




**Supplementary Figure 7. Renal denervation inhibits tubular cell cycle arrest in a norepinephrine and CGRP-dependent manner in kidneys after IRI.**

(A) Two days after denervation in left kidneys of mice, IRI or sham in the left kidneys was carried out (n = 5). (B) Two days after denervation in left kidneys of mice, norepinephrine, CGRP or vehicle was continuously infused into the denervated kidneys via mini-osmotic pump and the kidneys were subjected to 30 min of ischemia followed by 16 d of reperfusion (n = 4). (C and D) Mice were continuously treated with doxazosin, atipamezole, pronethalol, CGRP(8-37) or vehicle via an intraperitoneal implantation of mini-osmotic pump immediately before 30 min of left kidney ischemia and 16 d of reperfusion (n = 5). (A-C) Ratio of cyclin B1 to cyclin D1 by quantifying expressions of cyclin B1 and cyclin D1 protein in the kidneys using Western blot analysis. Anti-β-actin antibody served as a loading control. (D) The number of phosphorylated histone H3 (p-H3)-positive tubular cells on immunohistochemically stained kidney sections. Error bars represent SD. $^{\#}P<0.05$ versus intact and $^{\$}P<0.05$ versus vehicle.




**Supplementary Figure 8. Norepinephrine and CGRP signaling induces cell cycle arrest in kidney proximal tubule epithelial cells.**

(A) Cell cycle analysis of HK-2 cells treated with vehicle, norepinephrine or CGRP. (B) The percent of HK2 cells in different cell cycle phases after treatment with norepinephrine or AR antagonists using cell cycle analysis. $^{\S}P<0.05$ versus no treatment and $^{\ddagger}P<0.05$ versus treatment with norepinephrine alone. (C) The percent of HK2 cells in different cell cycle phases after treatment with CGRP or its receptor antagonist. $^{\S}P<0.05$ versus no treatment and $^{\ddagger}P<0.05$ versus treatment with CGRP alone. (D) Cell viability after treatment with norepinephrine or AR antagonists using MTT assay. $^{\S}P<0.05$ versus no treatment and $^{\ddagger}P<0.05$ versus treatment with norepinephrine alone. (E) Cell viability after treatment with CGRP or its receptor antagonist. $^{\S}P<0.05$ versus no treatment and $^{\ddagger}P<0.05$ versus treatment with CGRP alone.

## SUPPLEMENTARY METHODS

### Mice and surgical preparation

Male 129S1/SvImJ mice aged 8-10 weeks were purchased from Jackson Laboratories (Bar Harbor, ME). All mouse experiments were performed in accordance with the animal protocols approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center. Renal denervation was carried out, as described previously.[1] Two days before or 0, 1 or 3 days after the denervation, mice were subjected to 30 minutes of left renal ischemia followed by either 1, 2, 4, 8 or 16 days of reperfusion or sham operation under anesthetization with an intraperitoneal injection of a cocktail containing ketamine (200 mg/kg body weight) and xylazine (16 mg/kg body weight), as previously described.[2] Sham-operated mice underwent the same surgical procedure except for the ischemia. For local treatment of neurotransmitters, norepinephrine (Sigma, St. Louis, MO; 30 ng/kg body weight per day), CGRP (R&D Systems, Minneapolis, MN; 30 ng/kg body weight per day), or vehicle (0.9% saline) was continuously infused into the cortical region of the denervated kidney via an intrathecal catheter attached to a mini-osmotic pump (Alzet, Palo Alto, CA) that was inserted immediately in the cortical region after IRI or sham operation.[1] Severe damage to tubular segments and glomeruli was not found on PAS-stained sections in catheter-inserted kidneys. Some left kidneys were removed and weighted.[3] For treatment with respective antagonists of AR subtypes, doxazosin (R&D Systems, 12 mg/kg body weight per day) against $\alpha_1$-AR, atipamezole (R&D Systems, 2.4 mg/kg body weight per day) against $\alpha_2$-AR, pronethalol (R&D Systems, 2.4 mg/kg body weight per day) against $\beta$-AR, or vehicle (10% DMSO in PBS) was intraperitoneally administered via the mini-osmotic pump (Alzet) immediately before IRI or sham operation.[1] For inhibition of CGRP, mice were given CGRP(8-37) (R&D Systems, 120 $\mu$g/kg body weight per day) or 0.9% saline via intraperitoneal implantation of the mini-osmotic pump (Alzet) immediately before IRI or sham operation.[1]

### Collagen deposition

Collagen deposition was assessed by both Sirius red staining and hydroxyproline assay as previously described.[4]

### Histology

Immunohistochemical staining of the kidneys was performed on paraffin-sections as previously described.[1, 5] Briefly, 4% paraformaldehyde-fixed kidney sections were rehydrated and labeled with antibodies against F4/80 (Abcam, Cambridge, MA), PMN (Accurate, Westbury, NY), p-H3 and CGRP (Santa Cruz, Santa Cruz, CA). The sections were then incubated to peroxidase-conjugated secondary antibodies (Vector Laboratories, Burlingame, CA). The F4/80-positive area were measured in 5 randomly chosen high-power (× 200 magnification) fields per kidney using NIH Image J software. The respective numbers of PMN-positive and p-

H3-positive cells were counted in 5 randomly chosen high-power (× 200 magnification) fields per kidney. PAS-stained sections were used for tubular injury score as described previously.[6] TUNEL assay on the kidney sections was carried out using In Situ Cell Death Detection kit, Fluorescein (Roche, Mannheim, Germany).[1]

**Western blot**

We performed electrophoresis of protein extracts derived from whole kidneys using tris-glycine buffer systems and subsequent blotting as described.[1, 6] Membranes were incubated with antibodies against α-SMA (Sigma), p-Smad3, PARP1, caspase-3, Cyclin B1 and Cyclin D1 (Cell Signaling, Beverly, MA), tyrosine hydroxylase (Abcam, Cambridge, MA), Bax (Santa Cruz), Bid (BD Biosciences, San Jose, CA), Nitrotyrosine (Cayman, Ann Arbor, MI) or MnSOD (Millipore, Bedford, MA). Peroxidase-conjugated secondary antibodies (Vector Laboratories) were applied, and a chemiluminescence reagent (PerkinElmer, Boston, MA) was used to detect proteins. Anti-β-actin antibody (Sigma) was used for loading controls on stripped membranes. The bands were quantified using Lab Works analysis software (Ultra-Violet Products, Cambridge, UK).

**ELISA**

The levels of cytokine/chemokine TGF-β1, MIP-2, KC and MCP-1 in whole kidneys were measured using a multiplex immunoassay (Millipore, Bedford, MA). The contents of kidney norepinephrine and CGRP were measured using an ELISA kit from ALPCO Diagnostics (Windham, NH) and Cayman (Ann Arbor, MI), respectively.[1] The glutathione and lipid hydroperoxide assays were performed in the kidneys using an ELISA kit (BioVision, Mountain View, CA).[6] Renin activity in plasma and kidneys was determined using a Fluorometric Sensolyte 520 Mouse Renin Assay kit (AnaSpec, San Jose, CA) following the manufacturer's protocol.

**Cell culture and treatment**

HK-2 human kidney proximal tubule epithelial cells were maintained in Keratinocyte-SFM medium supplemented with recombinant epidermal growth factor 5 ng/ml and bovine pituitary extract 0.05 mg/ml (Gibco, Grand Island, NY) at 37°C with 5% $CO_2$. The cells were grown until 70% confluence on culture plates and then either norepinephrine or CGRP was added to the culture at a final concentration of 1 nM in PBS (vehicle) for 48 h. Either doxazosin mesylate (1 μM in DMSO), atipamezole hydrochloride (100 nM in DMSO), pronethalol (100 nM in DMSO) or CGRP(8-37) (100 nM in PBS) was co-treated to the culture (n = 4). For cell cycle analysis, the cells were prepared for propidium idodide (Sigma) staining using a Telford method[7], and DNA content was determined with FACSCaliber (BD Biosciences). The cell viability was measured using MTT assay.[8] After 48 hours of treatments in 24-well plates, 300 μl of MTT solution (5 mg/ml) was added to wells, and the plates were incubated at 37°C for 2

hours. To solubilize the formazan crystals, 200 μl of DMSO was added to the cells and incubated with gentle shaking at RT for 2 hours. The absorbance of the formazan-solubilized DMSO was measured at 595 nm (test wavelength) and 690 nm (reference wavelength) using a microplate reader (SpectraMax Paradigm; Molecular Devices, Sunnyvale, CA).

**Renal function**

Renal function was assessed by creatinine clearance considered as GFR. To obtain urine samples, some mice were placed in mouse-specific metabolic cages for 18 hours beginning at 6 hours after contralateral nephrectomy. Blood samples were taken from the retroocular vein plexus. Urinary and plasma concentrations of creatinine were measured using QuantiChrom™ Creatinine Assay kit (BioAssay Systems, Hayward, CA). The formula for measurements of GFR was as follows: GFR = urinary concentration of creatinine x urine volume/plasma concentration of creatinine/time of urine collection/body weight.[9]

**Blood Pressure Measurement**

Systolic blood pressure of mice was measured by a noninvasive tail cuff method (CODA; Kent Scientific, Torrington, CT). Mice were placed on a heated platform (30°C) in an isolated chamber, and systolic blood pressure levels were obtained. Mice were trained for 5 days before denervation followed by daily recording for experimental days as previously described.[1, 10]

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