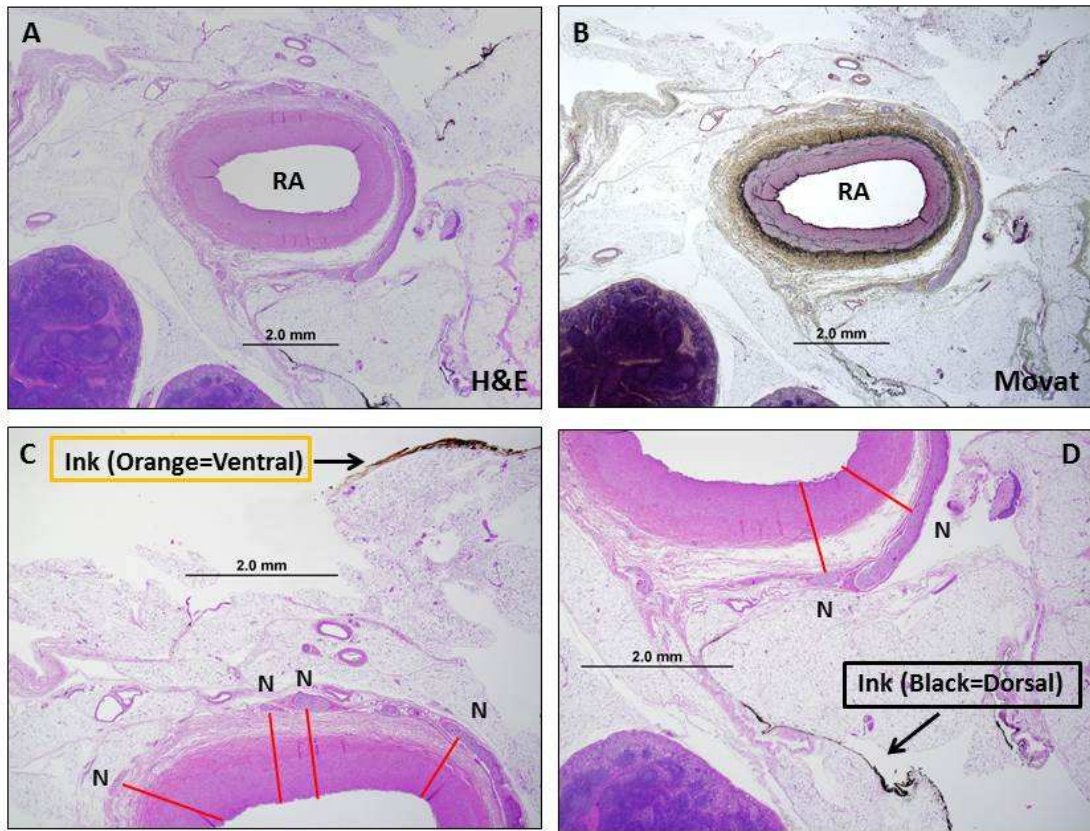


## **Supplemental methods**

### **Methods of fixation and tissue dissection**

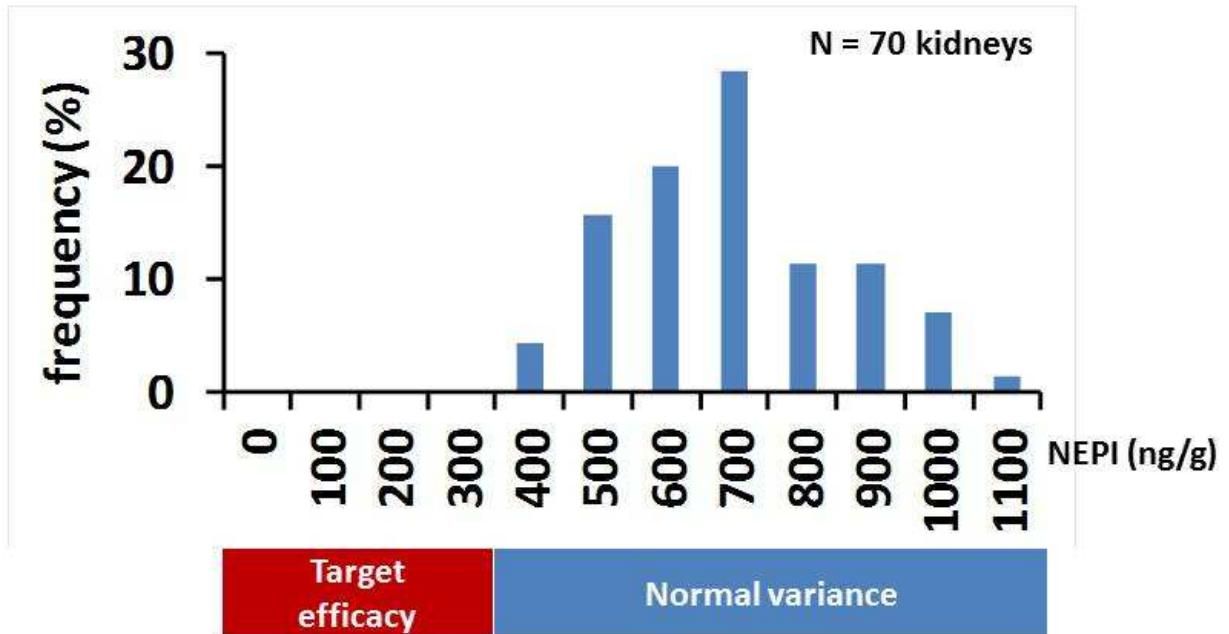
Fixation is important for the preservation of arterial and peri-arterial tissues. Physiological pressure perfusion (80-100 mm Hg) is important to determine reliably the dimension of the renal arteries and the distances from renal lumen to nerve location before and following thermal injury. Complete and rapid fixation can only be obtained when immersion fixation is used if there is sufficient volume and space for the tissue. Poor fixation not only results in deterioration of the histopathologic quality (autolysis) but also adversely affects immunohistochemical reactions. Tissue must be adequately fixed especially in the presence of renal fat tissue, which is difficult to fix and enhancing techniques such as microwave fixation may be utilized. Tissue should be accompanied by color-coded ink labeling of the arterial wall to facilitate circumferential orientation. After fixation, each renal artery with surrounding soft tissue must be cut approximately at 4-5 mm intervals, resulting in 5 to 8 segments of the renal artery. Tissues are adequately labeled and dehydrated in graded series of alcohol and paraffin embedded.

Supplemental Figure 1



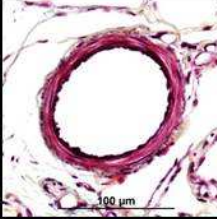
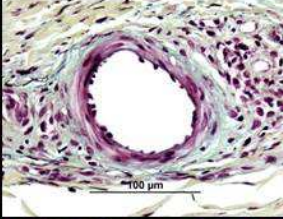
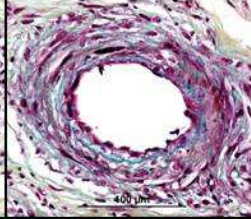
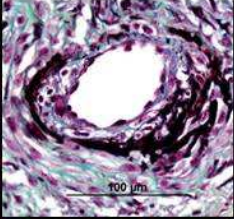
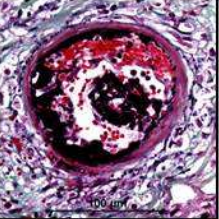
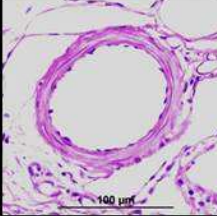
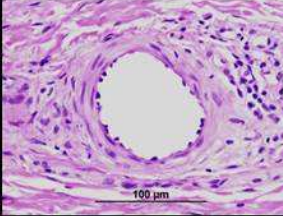
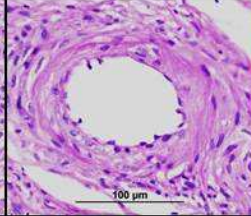
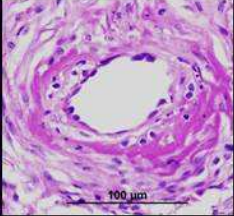
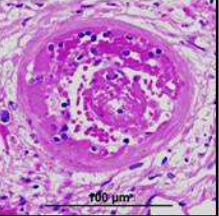
**Supplemental Figure 1. Representative images of distance measurements. A and B** are low-magnification images of pig renal artery (RA) and surrounding tissues stained by H&E and Movat pentachrome stains. **C and D** show the method of nerve distance measurements from the luminal surface of renal artery (RA) to each nerve (N) edge. Marking by the orange and black ink represent ventral and dorsal locations, of the nerves, respectively.

Supplemental Figure 2





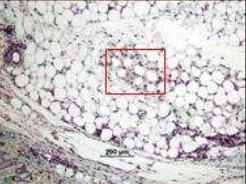
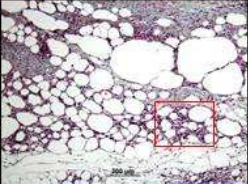
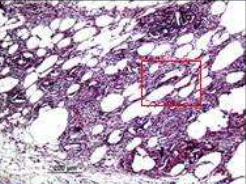

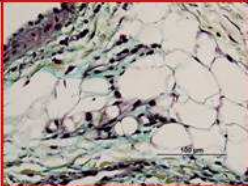
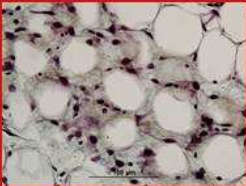
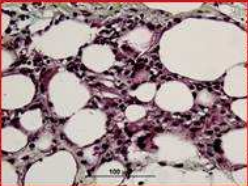
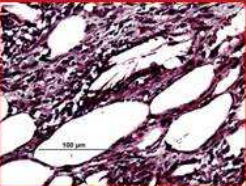
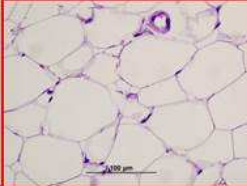
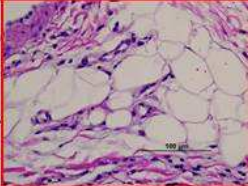
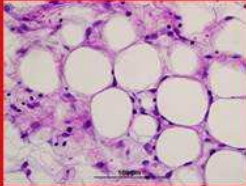
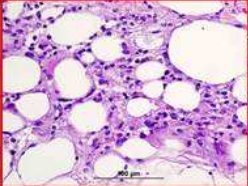
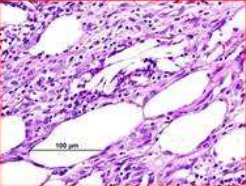
**Supplement Figure 2. Variance of norepinephrine level in 70 control kidneys.** Of 70 untreated kidneys, mean tissue norepinephrine concentration is  $649 \pm 161$  ng/g. Level of 700 ng/g is greatest, and followed by level of 600 ng/g.

Supplemental Figure 3

Injury Absent	Minimal Injury	Mild Injury	Moderate Injury	Severe Injury
Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
No damage to intima, media, and adventitia	Minimal perivascular inflammation. Minimal smooth muscle cell loss.	Mild perivascular inflammation. Mild smooth muscle cell loss.	Moderate perivascular inflammation. Segmental fibrinoid necrosis.	Severe perivascular inflammation. Fibrinoid necrosis.
				
				

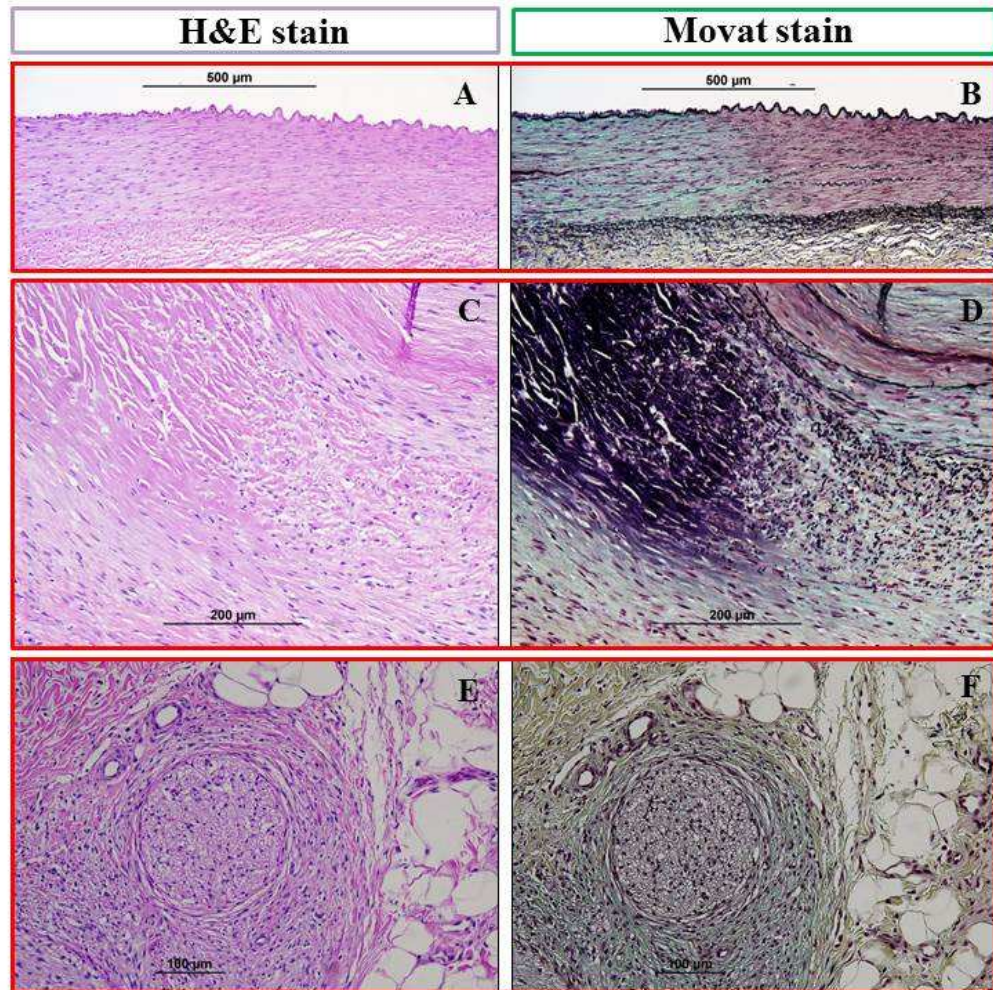
**Supplemental Figure 3. Semi-quantitative scoring scheme for arteriolar injury.** Each photomicrograph shows each grade of arterioles damage. Upper panel is stained with Movat pentachrome, whereas the lower panel is stained by H&E stain.

Supplemental Figure 4

Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Normal	Minimal fat necrosis & inflammation/fibrosis	Mild fat necrosis & inflammation/fibrosis	Moderate fat necrosis & inflammation/fibrosis	Severe fat necrosis & inflammation/fibrosis
				
				
				

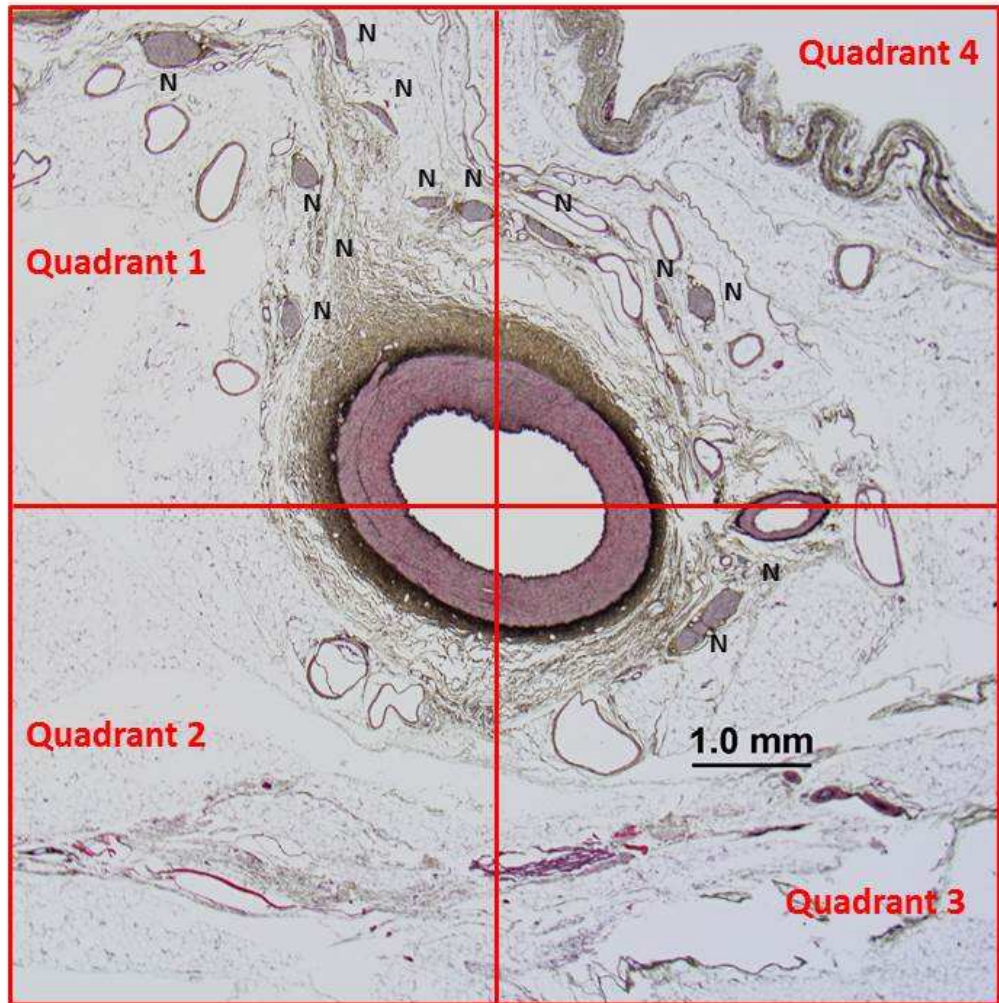
**Supplemental Figure 4. Semi-quantitative scoring scheme for fat necrosis.** Each panels represents changes observed in the perirenal fat by the grade of injury. Upper and middle panels shows low- and high-power images of injured fat stained with Movat pentachrome. The lower panels shows high-power images of injured fat stained with H&E stain.

Supplemental  
Figure 5



**Supplemental Figure 5. Images of arterial medial wall (A and B); intima, medial, and adventitia wall (C and D), and perineural fibrosis (E and F) stained by H&E (left panel) and Movat (right panel) pentachrome stains. A and B are high-power images of injured arterial media. Proteoglycan replacement (green) is observed within media wall in B. C and D are High-power image of intimal thickening, thinned media and adventitial. denatured collagen (black in D) is easily identified by Movat stain. E and F are high-power images of perineural fibrosis which is better appreciated by Movat stain (F).**

Supplemental  
Figure 6



**Supplemental Figure 6. Low-power image of the renal artery and perirenal tissues to illustrate quadrants analysis.** The distribution of nerves (N) not uniform in all quadrants. Note, absence of nerves in Quadrant 2, and very few nerves in Quadrant 3.