Supplementary material.

Method

Surgical procedure

The surgical procedure was as follows: anesthesia was induced in 8-week-old male Sprague–Dawley rats (SD) rats using 5% isoflurane and was maintained during surgery using 1.5% to 2% isoflurane. The abdomen was disinfected and opened through an incision, a cotton bud was used to remove fat from the right kidney and blood vessels, the blood vessels entering the kidney were ligated, and the kidney was removed. A cotton bud was used to locate the left renal blood vessels, and blood flow was stopped using a bulldog clamp. Renal ischemia was maintained for 30, 45, 60, 75, or 90 minutes. After removal of the bulldog clamp and confirming the return of blood flow through the left kidney, the surgical wound was closed. After suturing, the animals were awoken from anesthesia and were moved to the rearing room. After the abdominal cavity was reopened, the left renal blood vessels were ligated, the left kidney was removed, blood was collected from the heart, and the animal was euthanized. One-half of the removed kidney was stored and refrigerated in 4% formaldehyde (Biosesang, Seongnam, Korea) before preparing paraffin blocks for histological analysis. One-quarter of the kidney was stored in liquid nitrogen for protein analysis, and the other one-quarter was stored in liquid nitrogen for RNA analysis. The blood collected from the heart was transferred to EDTA-coated tubes and centrifuged (3,000 rpm, 10 minutes, 4°C).