Supplemental Material
Supplemental Methods

Stereotactic body radiotherapy (SBRT) procedure for renal denervation (RDN)

Sedated animals were transported to image center and placed in lateral position. The computed tomography (CT) reference points were marked on the skin with three metallic radiopaque markers at the level of kidneys by use of wall-mounted lasers. A series of free-breathing CT scans including a native CT, a contrast-enhanced CT and a respiration-correlated 4-dimensional CT (4D-CT) were acquired on a Brilliance 16-slice CT scanner (Philips Medical Systems, Cleveland, USA) with 1 mm slice thickness. The native CT was for SBRT treatment planning. For enhanced scans to facilitate definition of target renal arteries, 50 ml contrast medium was injected (20 s delay; flow rate 2.5 ml/s, Omnipaque 350 mg I/ml, GE Healthcare, USA) through cannulated ear vein. 4D-CT obtained to assess respiration-related target motion was binned into ten respiratory phases (0-90%) with the maximum inspiration and expiration respectively corresponding to the 0% and 50% phases.

All CT images were imported to a contouring MIM workstation (MIM Software Corp., Beijing, China). The target structures defined as circumferential areas around the left and right renal arteries including aorticorenal ganglia and renal nerve were contoured in the axial planes for each slice of contrast-enhanced CT scan. These areas were combined to form a 3-dimensional clinical target volume (CTV). In addition,
organs at risk (OARs) such as kidneys, renal veins, aorta, spinal cord and bowels were delineated for protection from radiation. Contours on contrast-enhanced CT images were then transferred to 0% image sets of 4D-CT. Deformable image registration was automatically performed to propagate contours to each breathing phase with appropriate adjustment by a radiation oncologist and a cardiologist if necessary, following which the internal target volume (ITV) was generated to account for respiratory motion from maximum intensity projection image. An isotropic 3 mm expansion on ITV was used to derive the planning target volume (PTV) for setup and radiation delivery uncertainties.

The SBRT treatment plan was generated in the External Beam Planning station (Varian Medical Systems, California, USA) to deliver a total dose of 25 Gy in 2 consecutive fractions to cover most region of PTV. Doses to OARs were minimized without compromising PTV coverage or conformality. Based on the plan, radiation energy was designed to concentrate on renal arteries with rapid dose falloff away from the target.

SBRT was performed with an image-guided, volumetric modulated arc radiotherapy system (Varian Edge, Varian Medical Systems, California, USA) delivering 10-MV flattening filter-free photon beams at a dose rate of 2400 MU/min. On the treatment day, animals were initially aligned to the room lasers using skin tattoos. Before radiation delivery, a kilo-voltage cone beam CT was acquired and registered to planning CT. The registration process was performed automatically based on bony structures, followed by manual refining to ensure accurate beam
delivery as intended by the plan. The swine position was then corrected automatically according to the results of image registration, and the treatment was initiated.

**Standard semi-quantitative scoring system for histopathology**

All digitized histological slides were reviewed by two independent, experienced pathologists blinded to study procedure using CaseViewer version 2.0 (3DHISTECH Ltd, Budapest, Hungary). Treatment effects on renal nerves, vessels, arterioles and surrounding soft tissues were semiquantified using a standard scoring system of 0-4 proposed by Sakakura et al: 0 = none, 1 = minimal, 2 = mild, 3 = moderate, and 4 = severe. The type and extent of damage to periartrial organs were routinely evaluated.

Magnitude of nerve injury was determined based on the extent of both perineural changes including inflammation or fibrosis and endoneural changes including vacuolization, pyknotic nuclei, digestion chambers, and necrosis. To investigate the lesion depth and circumferential injury achieved with radioablation, the following parameters were analyzed: (1) maximum score of nerve change in separate regions of < 2, 2-4 and > 4 mm from arterial lumen; (2) maximum distance between arterial lumen and ablation zone; (3) number of quadrants with injured nerve fascicles (score ≥ 2)/number of quadrants with nerve fascicles; (4) number of quadrants with injured periartrial soft tissue.

Arterial and venous endothelium damage was evaluated circumferentially as 0 = no endothelial loss; 1 = endothelial loss < 25% of the vessel’s circumference; 2 = endothelial loss 25-50% of the vessel’s circumference; 3 = endothelial loss 51-75% of
the vessel’s circumference; 4 = endothelial loss > 75% of the vessel’s circumference.

Medial injury was assessed separately by the depth and circumference of the involved segments: 0 = no medial change; 1 = medial change < 25% of the medial depth/circumference; 2 = medial change 25-50% of the medial depth/circumference; 3 = medial change 51-75% of the medial depth/circumference; 4 = medial change > 75% of the medial depth/circumference. When evaluating the depth of arterial medial damage, medial thinning defined as thickness of media at the site of damage/unaffected media thickness < 0.5 was reported as absent (0) or present (1).

Meanwhile, the neointimal area of renal artery was quantitatively analyzed.

Arteriolar damage usually correlates with overall nerve damage. The scoring criteria were as follows: 0 = no injury; 1 = minimal perivascular inflammation and smooth muscle cell loss; 2 = mild perivascular inflammation and smooth muscle cell loss; 3 = moderate perivascular inflammation and segmental fibrinoid necrosis; 4 = severe perivascular inflammation and fibrinoid necrosis.

The extent of soft tissue injury characterized by the presence of denatured collagen, fat necrosis and granulation tissue was semiquantified as 0-4 according to the degree of fat necrosis, inflammation and fibrosis.

The maximum injury scores of all observational items were recorded in each section. After grading all histological sections, the maximum injury score in each vessel was determined. Mean value of scores from bilateral vessels per animal was used for statistical analysis.
| Case No. | Follow-up, month(s) | Treatment Time, min | Volume, ml | PTV (Left/Right Renal Artery) Maximum Dose, Gy | Minimum Dose, Gy | CI* | HI | Maximum/Mean Doses to Major OARs, Gy |
|----------|---------------------|--------------------|------------|-----------------------------------------------|----------------|-----|----|--------------------------------------|
|          |                     |                    |            | Left Kidney                                   | Right Kidney   | Bowel | Spinal Cord | Aorta |
| 1        | 1                   | 3.2                | 2.5/2.4    | 26.2/26.3                                    | 23.6/23.1      | 1.26 | 0.06/0.07 | 26.0/3.3 25.9/3.2 26.5/2.0 14.6/2.1 26.4/5.2 |
| 2        | 1                   | 2.5                | 2.6/3.1    | 25.8/26.0                                    | 20.8/21.3      | 0.79 | 0.12/0.09 | 25.0/3.1 25.1/3.0 25.6/1.8 17.1/3.2 25.8/5.2 |
| 3        | 1                   | 3.0                | 2.6/3.3    | 25.9/26.0                                    | 22.7/22.7      | 0.77 | 0.07/0.06 | 24.1/3.0 26.0/3.4 25.8/2.3 15.5/3.1 25.8/5.4 |
| 4        | 1                   | 3.8                | 2.8/3.4    | 26.9/27.1                                    | 23.8/22.9      | 0.77 | 0.06/0.09 | 26.9/4.3 27.1/4.2 26.5/3.7 14.4/3.3 26.1/6.3 |
| 5        | 6                   | 2.5                | 2.5/3.1    | 26.4/26.5                                    | 23.9/23.4      | 1.12 | 0.06/0.06 | 26.4/3.1 26.2/3.0 26.4/1.9 13.8/2.4 26.3/4.8 |
| 6        | 6                   | 3.7                | 2.8/2.4    | 26.2/25.9                                    | 23.2/19.9      | 0.75 | 0.06/0.06 | 25.9/4.4 25.5/3.6 25.6/1.3 8.9/1.5 25.7/8.5 |
| 7        | 6                   | 2.6                | 3.3/3.8    | 26.0/25.8                                    | 23.0/22.6      | 0.93 | 0.05/0.07 | 25.5/4.0 25.7/3.8 25.8/1.2 7.9/1.8 25.5/7.3 |
| 8        | 6                   | 5.5                | 3.5/3.6    | 27.7/27.1                                    | 24.4/23.5      | 1.48 | 0.07/0.07 | 27.0/5.1 27.1/5.1 26.6/1.8 17.9/5.5 26.9/8.7 |
| 9        | 6                   | 3.8                | 3.3/4.4    | 26.5/26.6                                    | 20.4/16.5      | 0.94 | 0.08/0.09 | 26.0/3.0 25.9/1.7 22.5/0.8 11.0/1.5 26.1/5.1 |

*CI is presented with a single value due to the same CI of radiation to bilateral renal arteries. CI, conformity index; HI, homogeneity index; OARs, organs at risk; PTV, planning target volume.
Table S2. Radiation-induced lesion in circumference and depth.

|                                | 1 month (n = 4) | 6 months (n = 5) | P value |
|--------------------------------|-----------------|------------------|---------|
| Number of injured tissue quadrants (0-4) | 3.8 ± 0.4       | 3.9 ± 0.2        | 0.633*  |
| Injured nerve (score≥2) quadrants per nerve quadrants (0-1) | 0.9 ± 0.1       | 1.0 ± 0.1        | 0.515*  |
| Maximum distance (mm)          | 11.6 ± 3.5      | 13.3 ± 0.9       | 0.221†  |

Values are expressed as mean ± SD. *P value was calculated from Mann-Whitney U test. †P value was calculated from unpaired Student t test.
Table S3. Renal artery diameters and kidney dimensions measured from CT images at 6 months.

|                             | Control (n = 5) | Treatment (n = 5) | P value |
|-----------------------------|----------------|-------------------|---------|
| Renal artery diameters      |                |                   |         |
| Proximal, mm                | 5.17 ± 0.67    | 4.67 ± 0.68       | 0.274   |
| Middle, mm                  | 4.66 ± 0.67    | 4.39 ± 0.52       | 0.495   |
| Distal, mm                  | 4.10 ± 0.37    | 4.23 ± 0.77       | 0.741   |
| Kidney dimensions           |                |                   |         |
| Craniocaudal, mm            | 106.66 ± 4.87  | 107.06 ± 6.08     | 0.911   |
| Transverse, mm              | 45.52 ± 1.93   | 44.37 ± 2.70      | 0.460   |
| Anteroposterior, mm         | 36.33 ± 3.72   | 33.67 ± 2.64      | 0.229   |

Values are expressed as mean ± SD. P value was calculated from unpaired Student t test. CT, computed tomography.
Figure S1. Renal function.

(A) Blood urea nitrogen (BUN) and (B) serum creatinine (sCr) at baseline, 1 month and 6 months follow-up in control and 6-month ablation groups (mean ± SD; n = 5; unpaired Student t test).
Figure S2. Terminal computed tomography (CT) examination.

Representative oblique coronal (A), axial (B) and sagittal (C) multiplanar reformatting CT images in control and treatment groups showing no obvious collateral damage within 6 months after radioablation with 25 Gy.
Figure S3. Radiation-induced collateral damage.

Representative images of damage to ureter (A) in 1-month group and kidney (B) in 6-month group stained with hematoxylin and eosin. Magnified micrographs of (C) focal vacuolization of ureteral epithelium (yellow square in A), (D) kidney injury including focal glomerular vitreous degeneration, disappearance of renal tubules with inflammatory cell infiltration, interstitial fibrous tissue hyperplasia and collagen degeneration (red square in B), and (E) uninjured part of the kidney (blue square in B).