

Renal denervation exacerbates LPS- and antibody-induced acute kidney injury, but protects from pyelonephritis in mice

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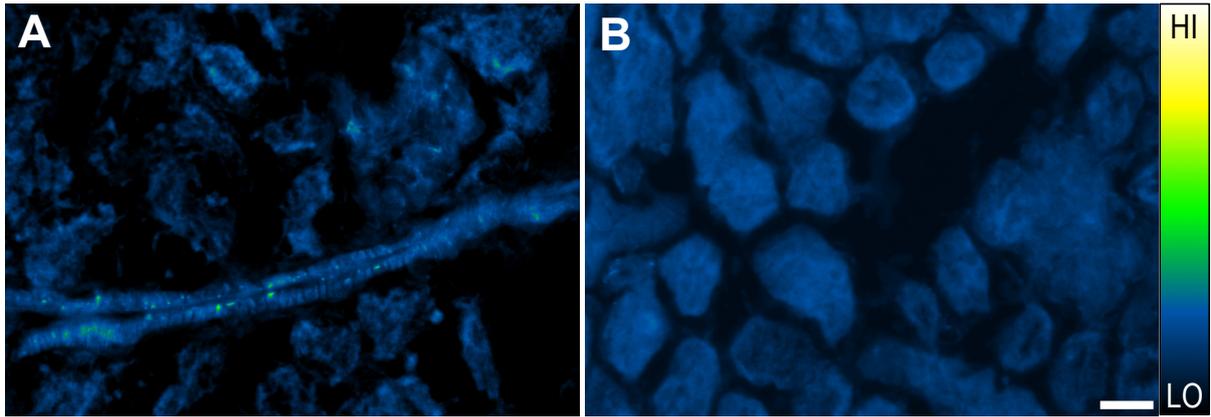
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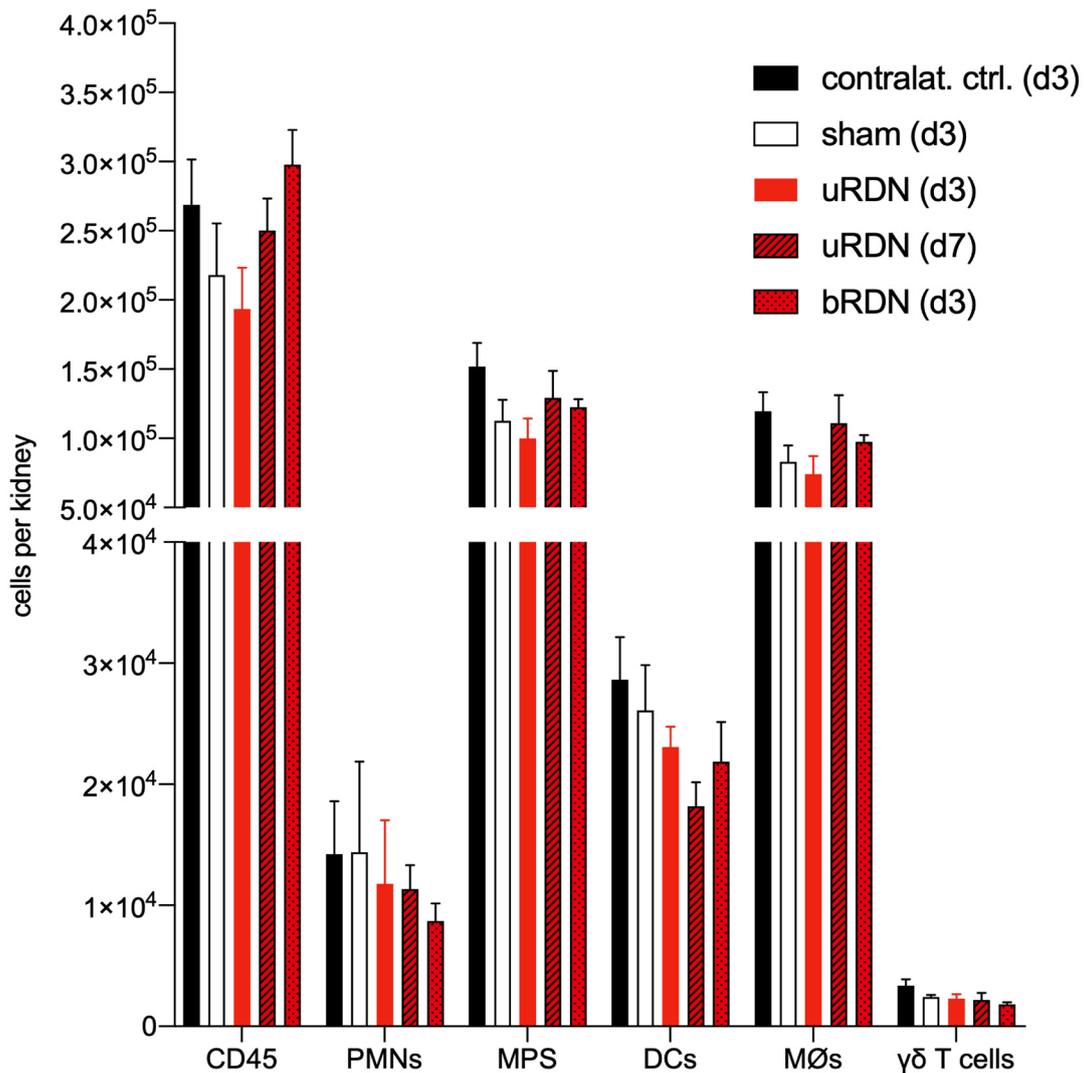
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Suppl. Fig. 1: Verification of denervation using immunofluorescence.

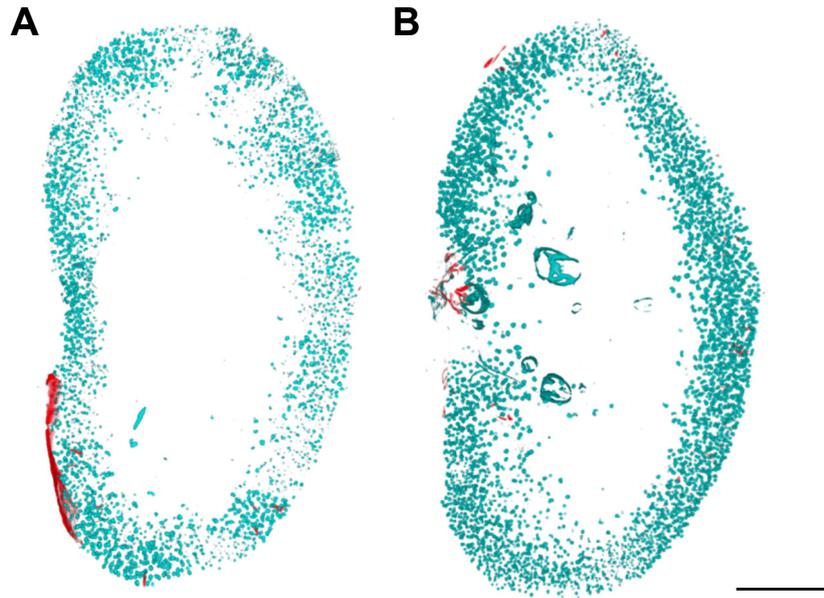
Representative images of kidney cryo-sections stained with anti-Tyrosine Hydroxylase (TH) to confirm effect of RDN. A) Control kidney. B) RDN-treated kidney three days after treatment. Color-scheme for TH signal intensity on the right side. Scale bar is 20 μ m for both images.



Suppl. Fig. 2: Enumeration of leukocyte subsets after renal denervation.

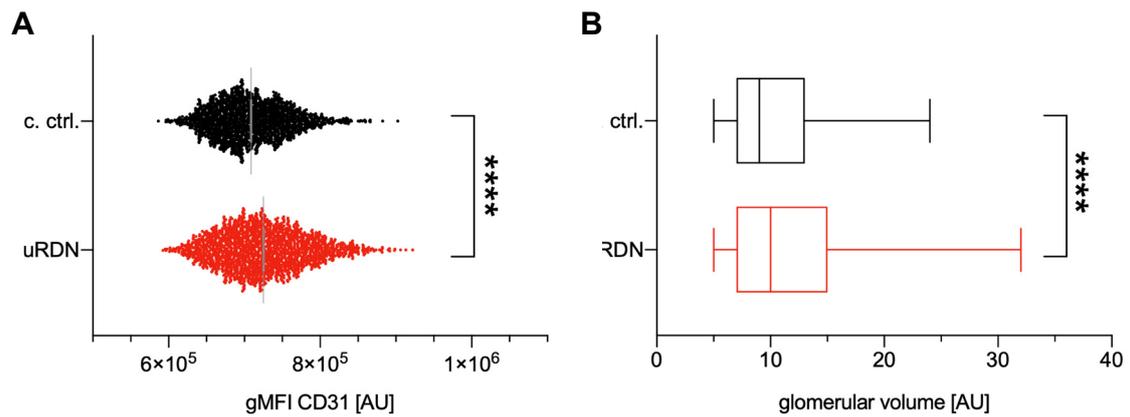
Black bars indicate contralateral kidneys from unilaterally denervated mice after 3 days, white bars indicate kidneys from sham-operated mice after 3 days, red bars indicate denervated kidneys from unilaterally denervated animals after 3 days, red hatched bars indicate denervated kidneys from unilaterally denervated animals after 7 days. Red dotted bars indicate kidneys from bilaterally denervated animals after 3 days. Error bars indicate S.E.M. Experiment was performed with at least 5 animals per group in three individual experiments.

Data shown were obtained using flow cytometry of 5% of the kidney after digestion of half a kidney. Single cells were further gated on living cells by exclusion of DAPI⁺ dead cells.



Suppl. Fig. 3: Control kidneys analyzed with LSM.

A, B) Virtual 3D figures of optically cleared kidneys of an unchallenged uRDN-treated control kidney (A) 3 days after denervation and a sham-treated kidney (B) 3 days after NTN induction. Virtual coronary sections of 200 μ m thickness. Vasculature stained with CD31-AF647 in cyan. Dysfiltrated albumin-TxRed in red, scale bar is 2mm.

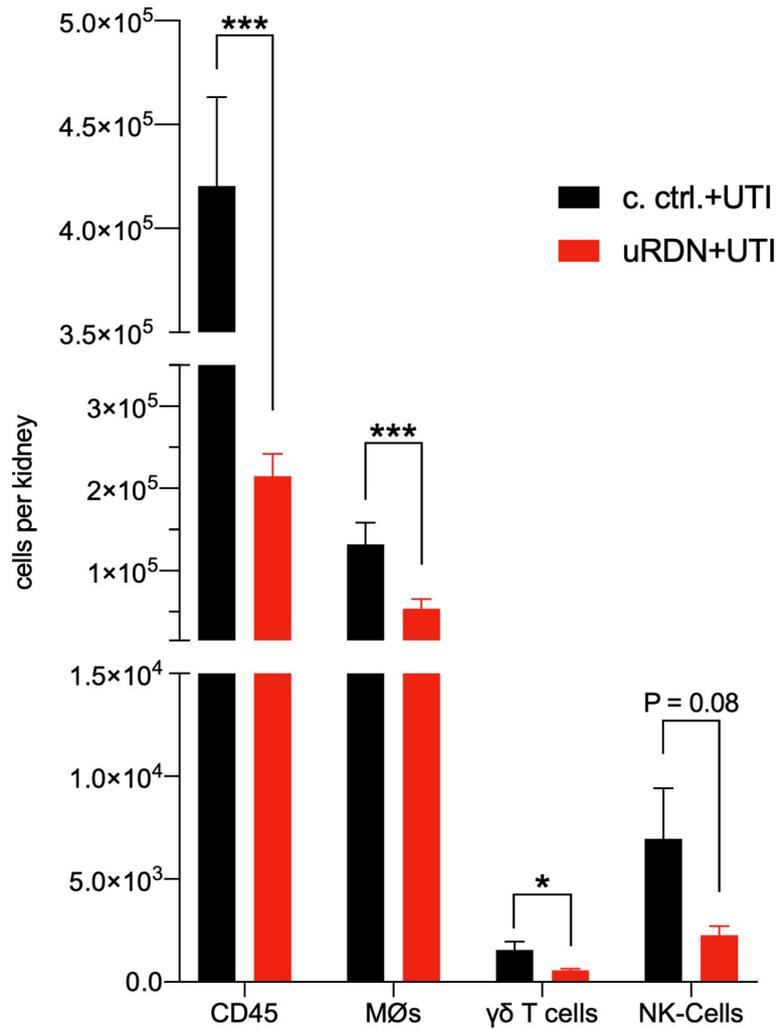


Suppl. Fig. 4: Consequences of uRDN for CD31 deposition and glomerular volume.

A) Geometric mean fluorescence intensity of 2000 glomeruli per kidney 3 days after uRDN. Mean and S.E.M. with gray lines on top of data points. P-value was calculated using unpaired t-test. **** P<0.0001

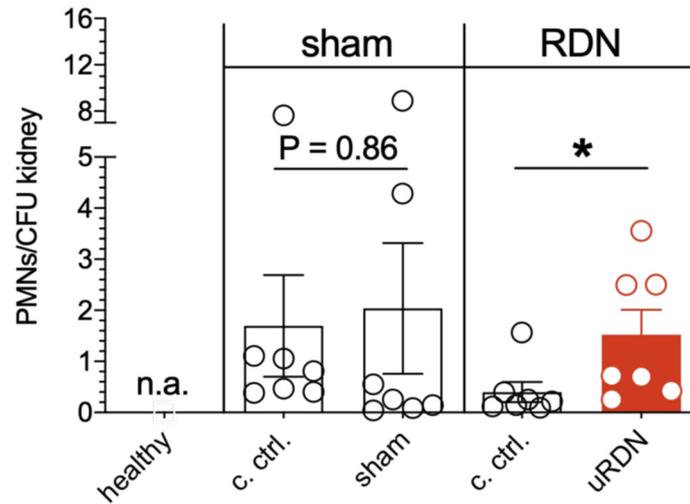
B) Glomerular volumes of 2000 glomeruli 3 days after denervation. Boxes show the 95% CI. P-value was calculated using unpaired t-test. **** P<0.0001

Kidneys intravitally stained for CD31 were optically cleared and imaged with LSFM. Glomeruli were segmented by CD31 signal intensity and investigated using a macro in Fiji.



Suppl. Fig. 5: Reduced leukocyte influx into uRDN kidneys compared to their contralateral controls (c. ctrl.) during pyelonephritis.

Experiment was performed with 10 mice per group. Error bars show S.E.M. P-values were calculated using paired t-test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Data shown was obtained using flow cytometry of 5% of the cells after digestion of half a kidney. Single cells were further gated on living cells by exclusion of DAPI+ dead cells.



Suppl. Fig. 6: Ratio between neutrophils and UPECs (CFU) per kidney at d1 after UTI induction.

First panel represents the ratio of healthy uninfected kidneys (here not applicable). The second panel (sham) compares the contralateral control (c. ctrl.) kidneys to the sham operated kidneys. The third panel (RDN) compares the contralateral control (c. ctrl.) kidneys to the denervated (uRDN) kidneys.

Experiments were performed with at 7 mice per group. Error bars show S.E.M. P-values were calculated using paired t-tests.