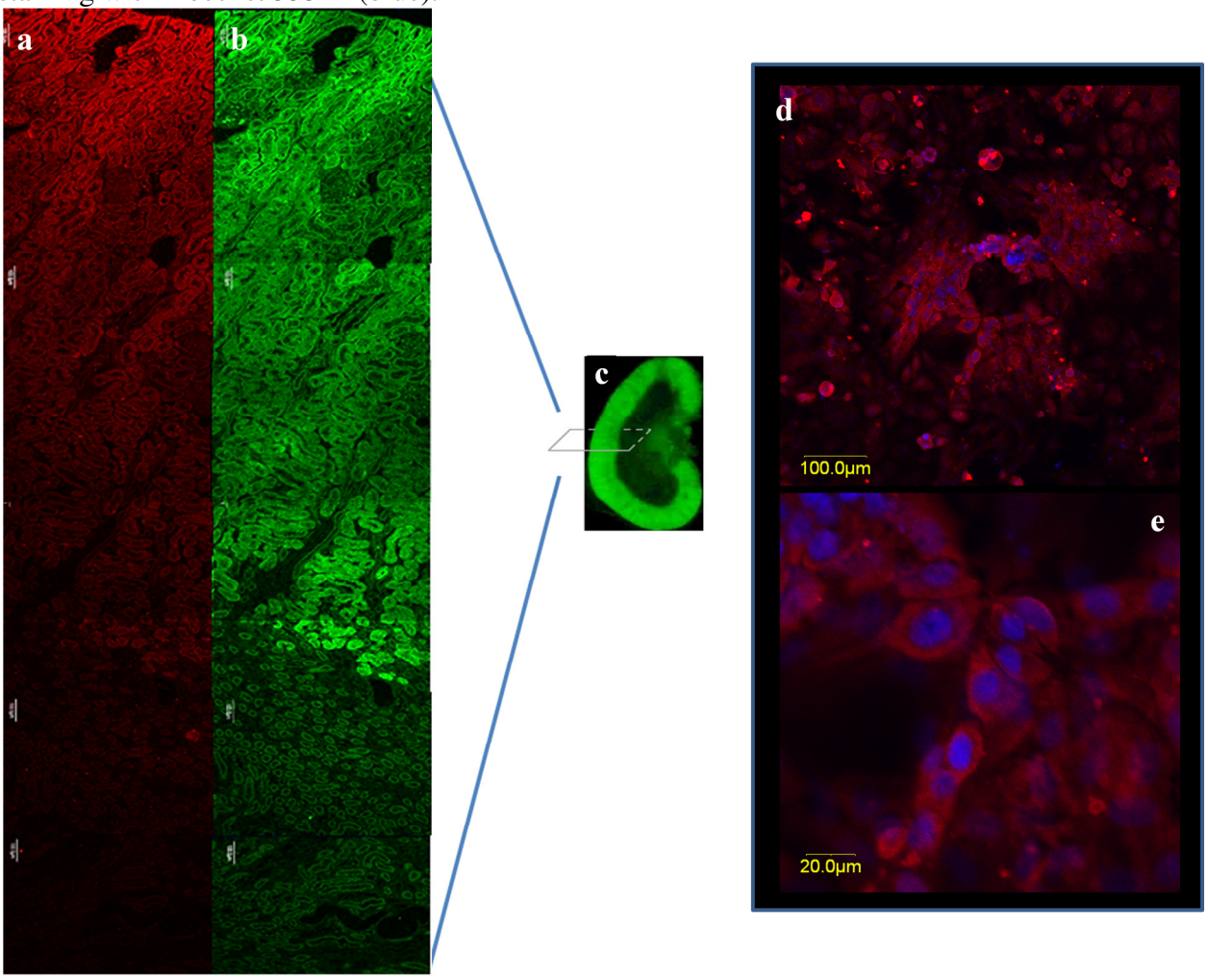
Supplementary Materials

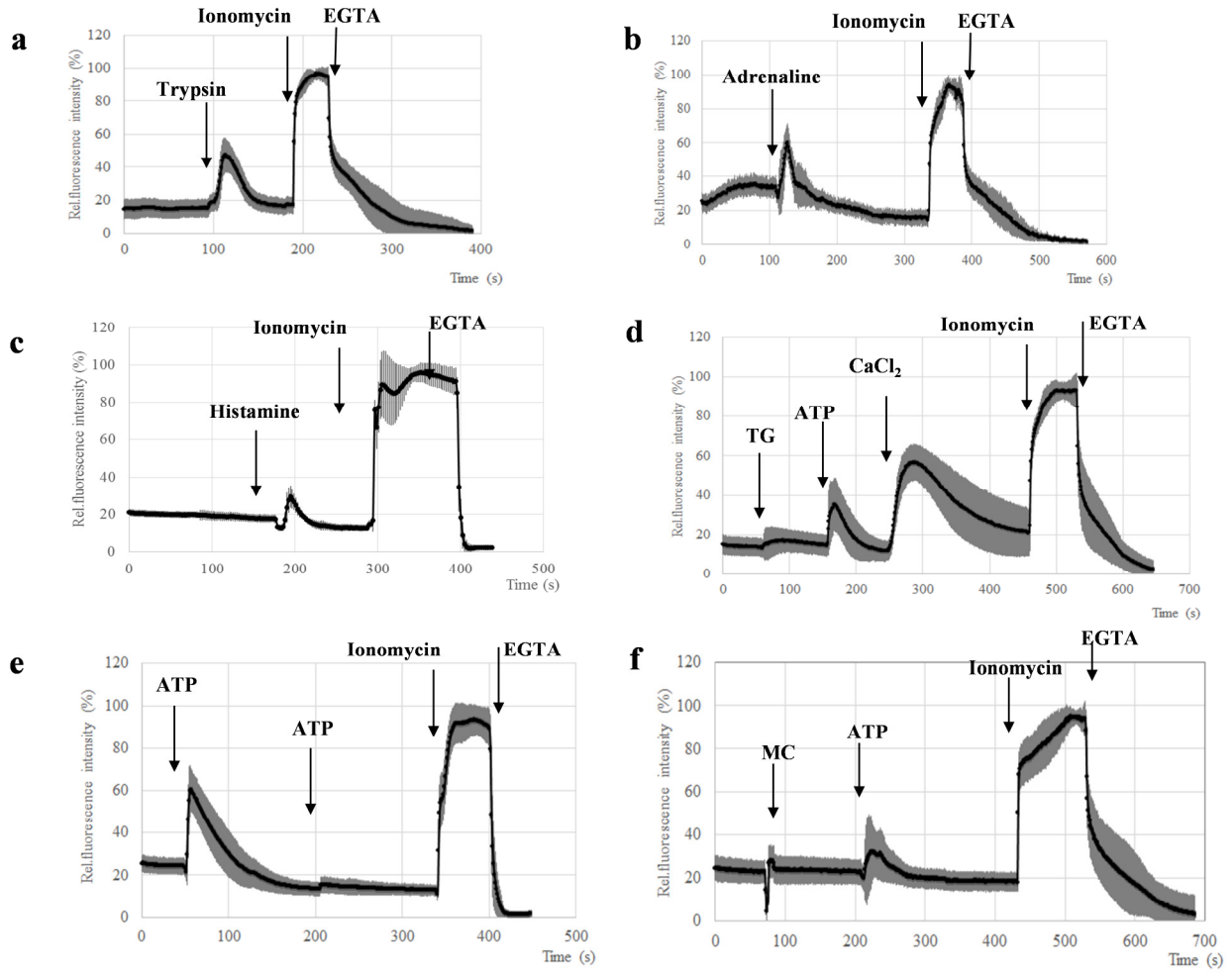
for the paper: **"Visualization of calcium dynamics in rat kidney proximal tubules",** by Szebényi et al.

Supplementary Figure 1. Frozen, fixed and antibody-stained horizontal section of the GCaMP2 expressing transgenic rat kidney cortical area. **(a)**: anti-GGT1 immunostaining and **(b)**: anti-GFP immunostaining. Single images were taken by a 20x objective from neighboring areas and were overlapped to produce a low-magnification image of the kidney cross-section. **(c)**: schematic localization of the horizontal cross-section area in the cortical area of freshly removed kidney of a GCaMP2 expressing transgenic rat (live GCaMP2 fluorescence, image was taken with a fluorescent in vivo imaging system LT-9MACIMSYSPLUSC, Lightools Research, South Encinitas, CA, USA). **(d-e)**: Isolated cultures of PT epithelial cells stained by monoclonal antibody against ATP binding cassette transporter G2 (ABCG2, red) and nuclei were visualized by nuclear DNA staining with Hoechst 33342 (blue).



Supplementary Figure 2. Ligand-induced changes in free calcium levels in cultured proximal tubule epithelial (PTE) cells, reflected by GCaMP2 fluorescence. Analysis of fluorescence confocal microscopy experiments. In each case a calibration of the cellular calcium level was performed by the addition of ionomycin (5 µM), followed by the addition of EGTA, sufficient to remove extracellular calcium.

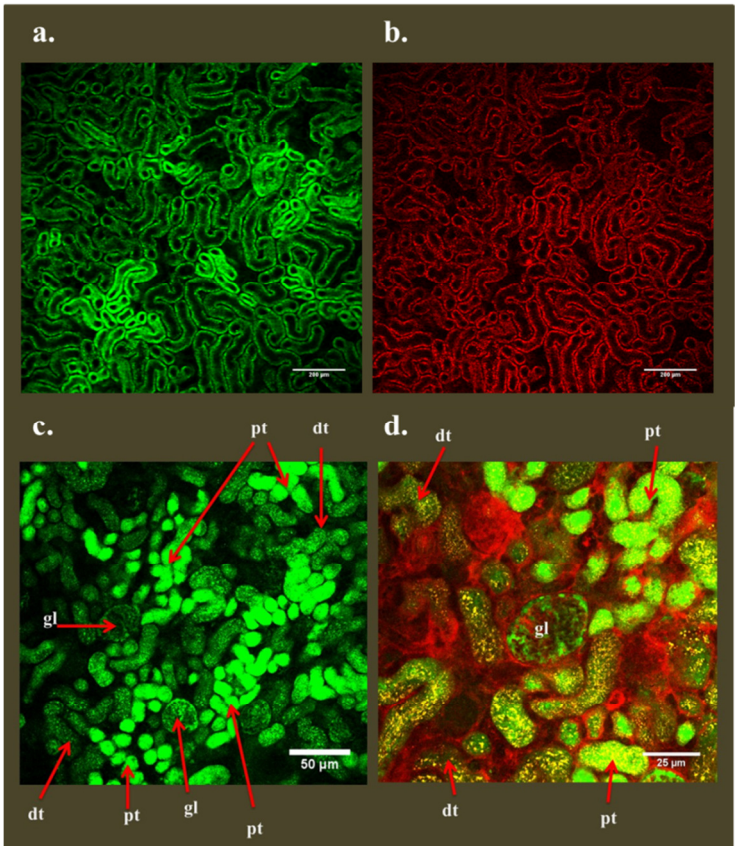
(a): addition of 2.5 µg/ml trypsin, **(b)**: addition of 10 µg/ml adrenalin, **(c)**: addition of 100 µM histamine, **(d)**: addition of 10 µM thapsigargin (a SERCA pump inhibitor) and 100 µM ATP in the absence of external calcium, and readdition of calcium (final concentration 1.5 mM) to the medium. **(e)**: addition of ATP (100 µM) and a second addition of 100 µM ATP, **(f)**: effect of medium change and a following addition of ATP (100 µM) on calcium-dependent fluorescence.



Supplementary Figure 3 and Online video 1. In vivo two-photon microscopy studies in GCaMP2 transgenic rat kidney cortical areas.

(a): Variable calcium-dependent GCaMP2 fluorescence intensity in a live cortical kidney area, containing mostly tubular structures. **(b)**: Autofluorescence of the same live cortical kidney area as shown in (a). Strongly autofluorescent structres are proximal tubules; distal convoluted tubules, glomeruli and cortical collecting ducts are only weakly autofluorescent. **(c)**: Variable calcium-dependent GCaMP2 fluorescence intensity in a live cortical kidney area, containing both glomeruli and tubular structures. **(d)**: Calcium-dependent GCaMP2 fluorescence (green) and rhodamine-dextran fluorescence (red) measurement in a kidney cortical area containing both glomeruli and tubular structures. pt: proximal tubule, dt: distal tubule, gl: glomerulus. To obtain images from the deeper regions of the cortex containing glomeruli, a thin tissue layer was removed from the surface of the kidney (c-d). Excitation was at 850nm (a-d).

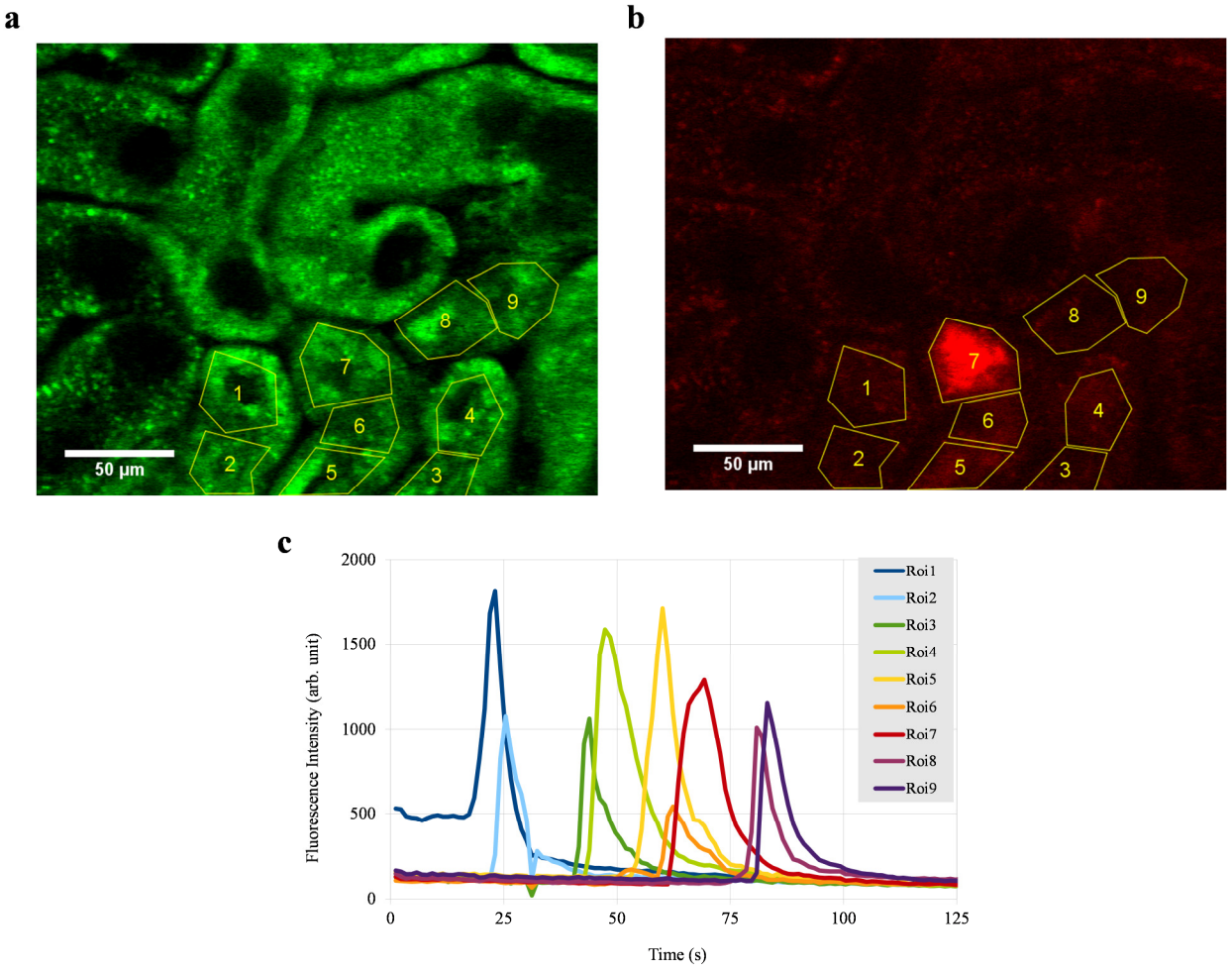
Online video 1: Cross-sections of a GCaMP2 transgenic rat kidney cortical areas – calcium-dependent green fluorescence in the proximal tubules.



Supplementary Figure 4, and Online video 2. In vivo two-photon microscopy studies in rhodamine-dextran injected GCaMP2 transgenic rat kidney.

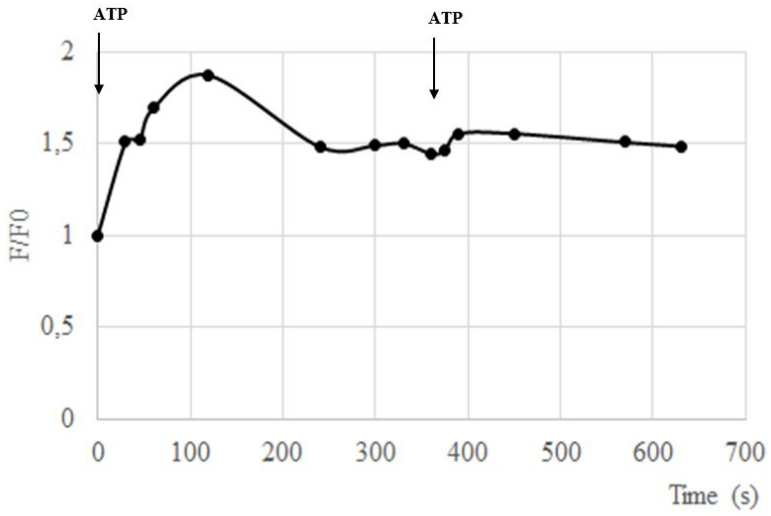
(a) GCaMP2 (green) and **(b)** rhodamine-dextran (red) fluorescence measurements in the renal proximal tubular area. **(c)** Time dependent appearance of Rhodamine in selected neighboring (labeled as regions of interest), connected proximal tubule lumens.

Online video 2. In vivo video of a two-photon microscopy measurement of calcium-dependent GCaMP2 fluorescence (green) and Rhodamine fluorescence (red) in the kidney proximal tubules of GCaMP2 transgenic rat.

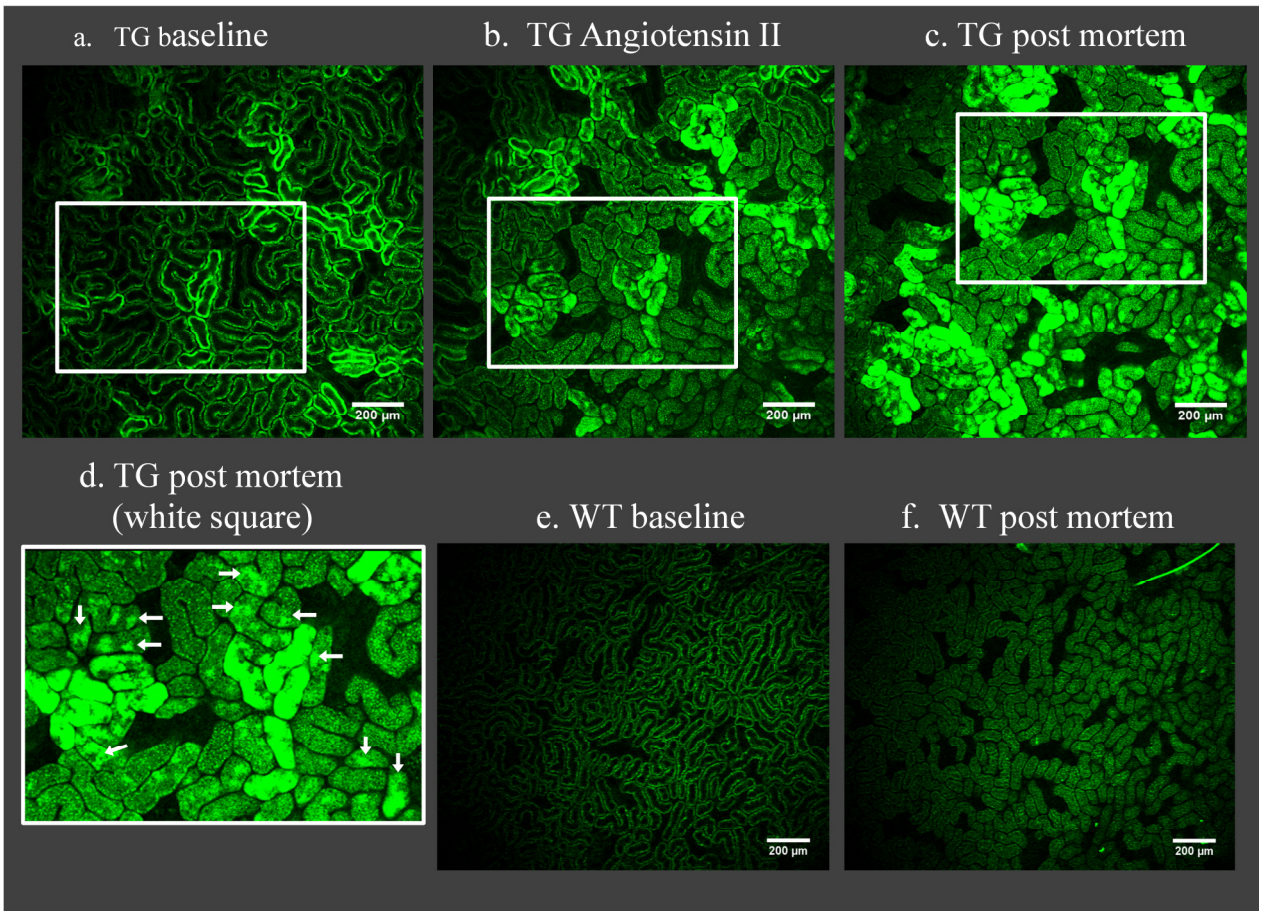


Online video 3.: In vivo video of a two-photon microscopy measurement of GCaMP2 fluorescence (green) showing spontaneous calcium-dependent fluorescence oscillations in the PTE cells. Calcium oscillations were detected by 1sec/frame, horizontal resolution was 730 pixels/line, with a pixel width of 0.4µm.

Supplementary Figure 5. In vivo two-photon microscopy measurement of calcium-dependent GCaMP2 fluorescence in rat kidney cortical proximal tubules. Effects of the first and second intraarterial addition of 100 µM ATP.



Supplementary Figure 6. (a-d) In vivo two-photon microscopy measurement of calcium-dependent GCaMP2 fluorescence in rat kidney cortical proximal tubules. Effect of intraarterial addition of 1 µM Angiotensin II and the death of the animal caused by the addition of a lethal dose of anesthetics. Rectangles indicate the same areas during the measurement. The white arrows show specific regions with high GCaMP2 signal in post mortem kidney (the indicated regions showed autofluorescence-like GCaMP2 signal in the baseline image, suggesting that even apparently non-GCaMP2 expressing PT-segments express the GCaMP2 protein) (d). **(e-f)** In vivo two-photon microscopy measurement of autofluorescence of PTs in wild type rat and after the death of the animal.



Supplementary Methods:

1. For immunochemistry studies the tissue slices were transferred to microscope slides and were incubated with 10 mM EGTA for 5 minutes before fixation with methanol. Tissue sections were then washed in PBS, blocked with 2% bovine serum in PBS (1 h) and incubated with a rabbit polyclonal antibody (1:500) targeting GFP (Abcam, ab290) and with a goat polyclonal antibody (1:100) raised against γ-glutamyltranspeptidase 1 and 2 (GGT1/2) (Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-23823) in 0.5% bovine serum in PBS overnight at 4°C. After a 90 min PBS wash, tissue slices were incubated (1 h) with matching secondary antibodies: Alexa Fluor 488- or 568-conjugated secondary antibody against rabbit IgG (1:200; Invitrogen, Life Technologies, Carlsbad, CA, USA) or Alexa Fluor 568-conjugated secondary antibody against goat IgG (1:200; Invitrogen, Life Technologies, Carlsbad, CA, USA), washed in PBS and nuclei stained with 5 µM Hoechst 33342 (Sigma, Hungary) for 10 min. Stained sections were mounted with ProLong Gold (Invitrogen).

2. For the preparation of isolated tubular cells, the animals were sacrificed by overdosing a 4-component anesthetic mixture (zolazepam, xylazine, butorphanol, tiletamine) and the kidneys were removed, weighted and washed twice in ice cold PBS. The kidney was decapsulated, cortex and medulla were separated with fine scissors and the cortex was chopped into ~1mm^3 pieces. The pieces were transferred to a 50 ml conical tube containing 20 ml enzyme mixture of 200 U/ml type IV collagenase and 0,6 U/ml dispase (Gibco, Life Technologies, Carlsbad, CA, USA), and digested for 2 hours at 37°C with continuous stirring. The cell suspension was filtered through a 40 micron nylon filter insert (BD Biosciences, Franklin Lakes, NJ, USA), centrifuged for 5 min on 300×g, the supernatant was removed and the pellet was dissolved in complete RPMI (RPMI + 3% penicillin/streptomycin + 1% glutamine + 20% fetal bovine serum).