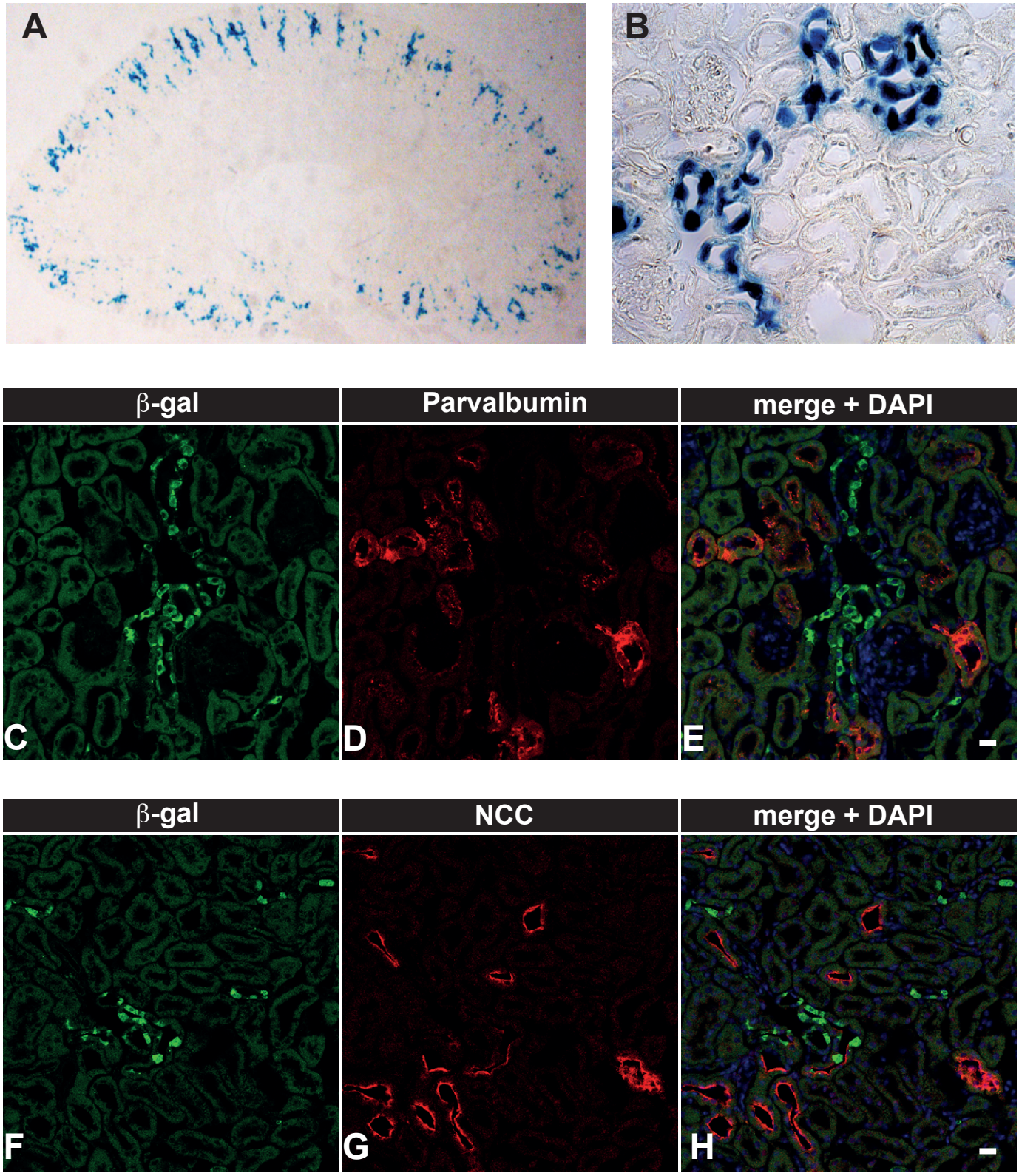
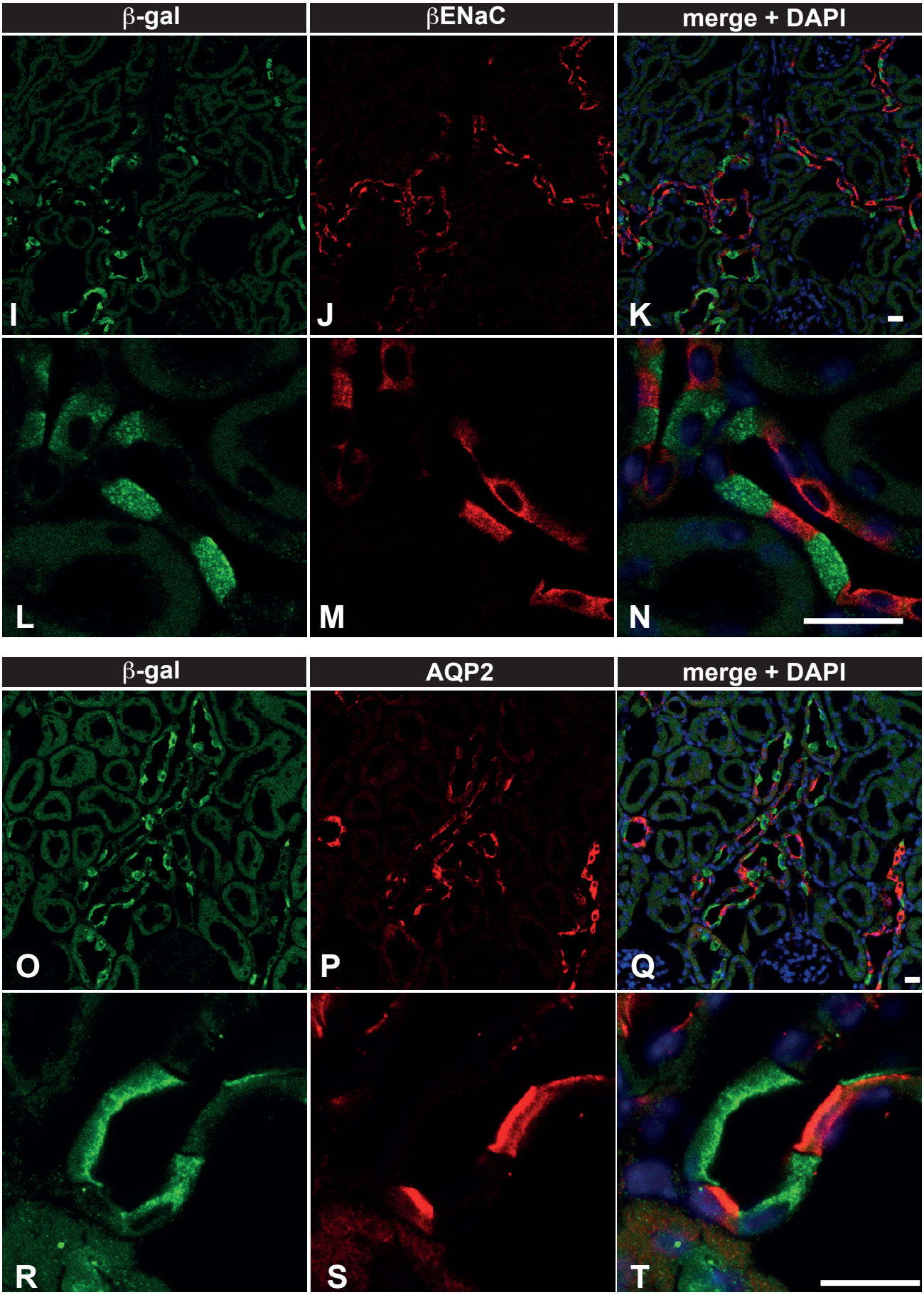
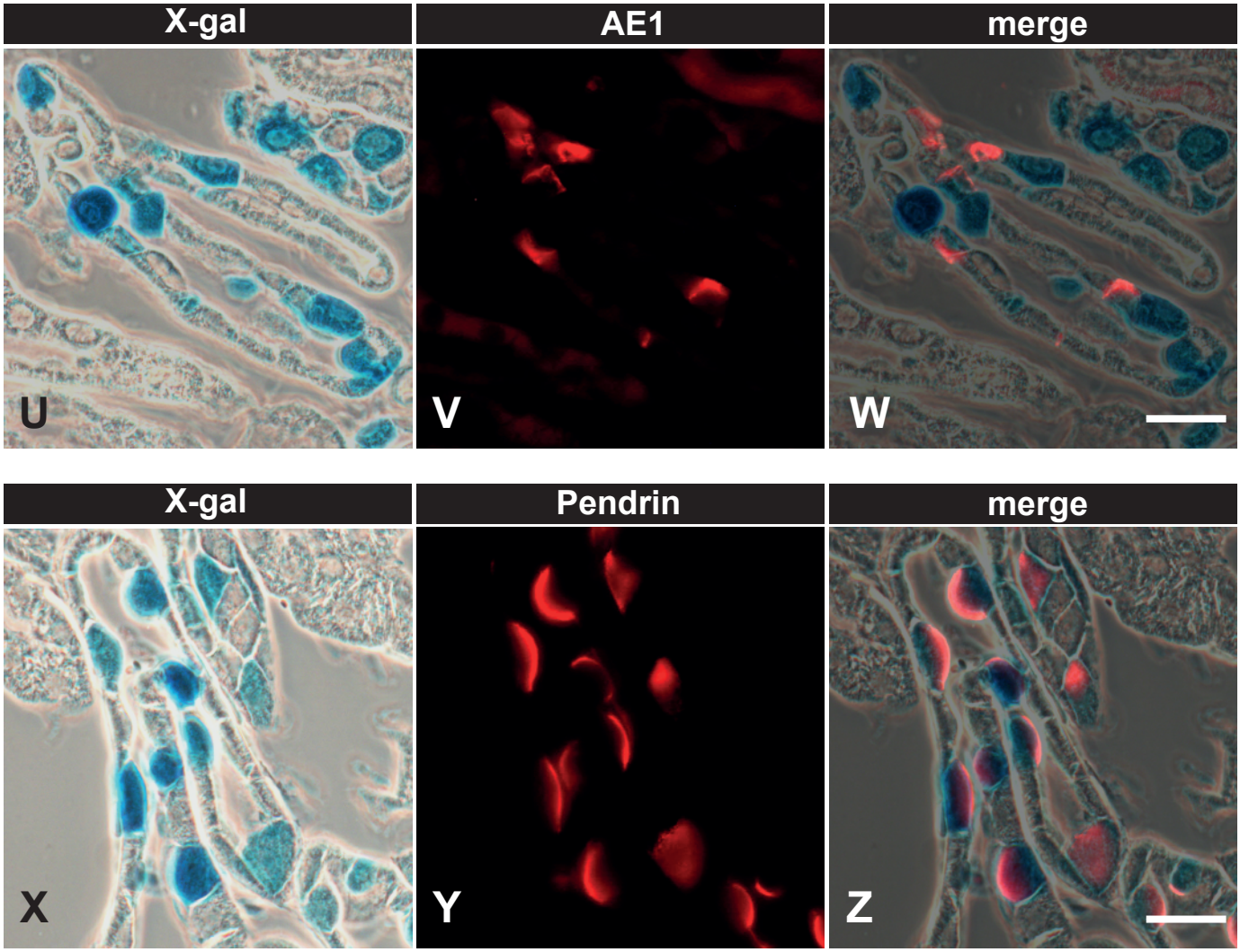


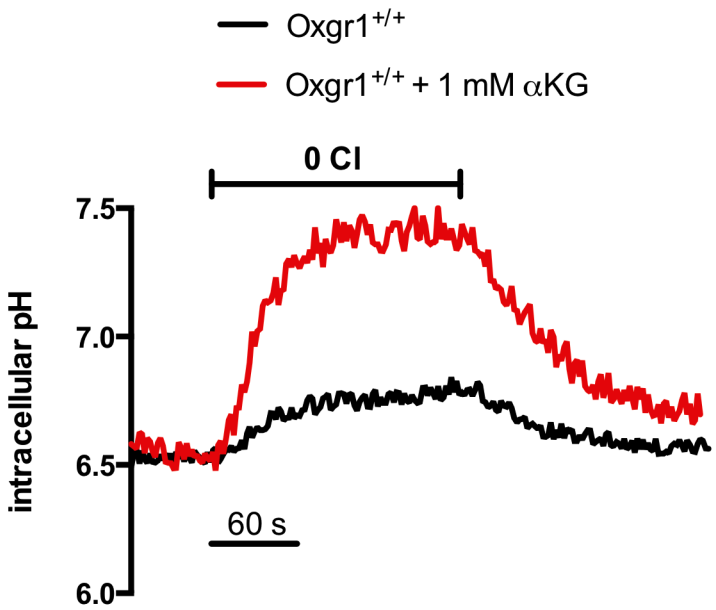
Supplementary Figure 1 (A-H). Oxgr1 expression in the kidney. **A**. Low magnification image of X-gal (Oxgr1) staining on frozen kidney section. This image demonstrates that Oxgr1 expression is limited to the renal cortex. **B**. High magnification image of (A) - demonstrates patchy distribution of Oxgr1-positive cells along the cortical nephron. **C** and **F**. Immunostaining with an antibody against β-galactosidase (green) confirms the patchy expression of Oxgr1 in the renal cortex. **D** and **G**. Immunostainig of two specific markers for the initial part of the distal convoluted tubule (DCT1), parvalbumin and the sodium-chloride cotransporter (NCC), respectively. **E** and **H**. Co-immunostaining of Oxgr1 with parvalbumin or NCC, respectively, demonstrates that Oxgr1 is not expressed in the DCT1. Scale bars: 50 μm.



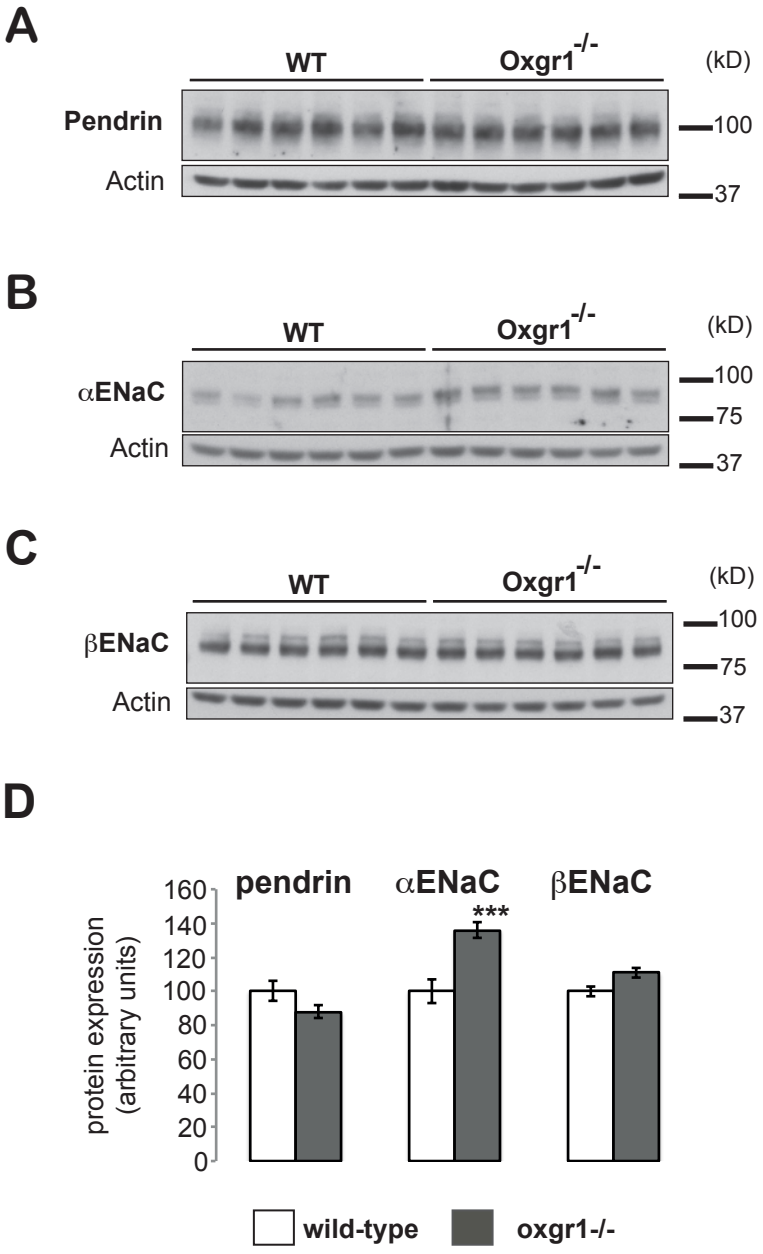
Supplementary Figure 1 (I-T). **Oxgr1 expression in the kidney.** **I** and **O**. Immunostaining with an antibody against β-galactosidase (green) in the renal cortex. This staining reveals patchy distribution of Oxgr1 in the cortical segments of the collecting system. **J** and **P**. Immunostaining of two specific markers for principal cells in the CNT and the CCD, beta subunit of the epithelial sodium channel (βENaC, red) and the aquaporin-2 water channel (AQP2, red), respectively. **K** and **Q**. Co-immunostaining of Oxgr1 with βENaC or AQP2, respectively, demonstrates that Oxgr1 is expressed in the same tubular segments, but not in principal cells. **L**, **M**, **N**, **R**, **S** and **T** are high magnification images of I, J, K, R, S and T, respectively. Scale bars: 50 μm.



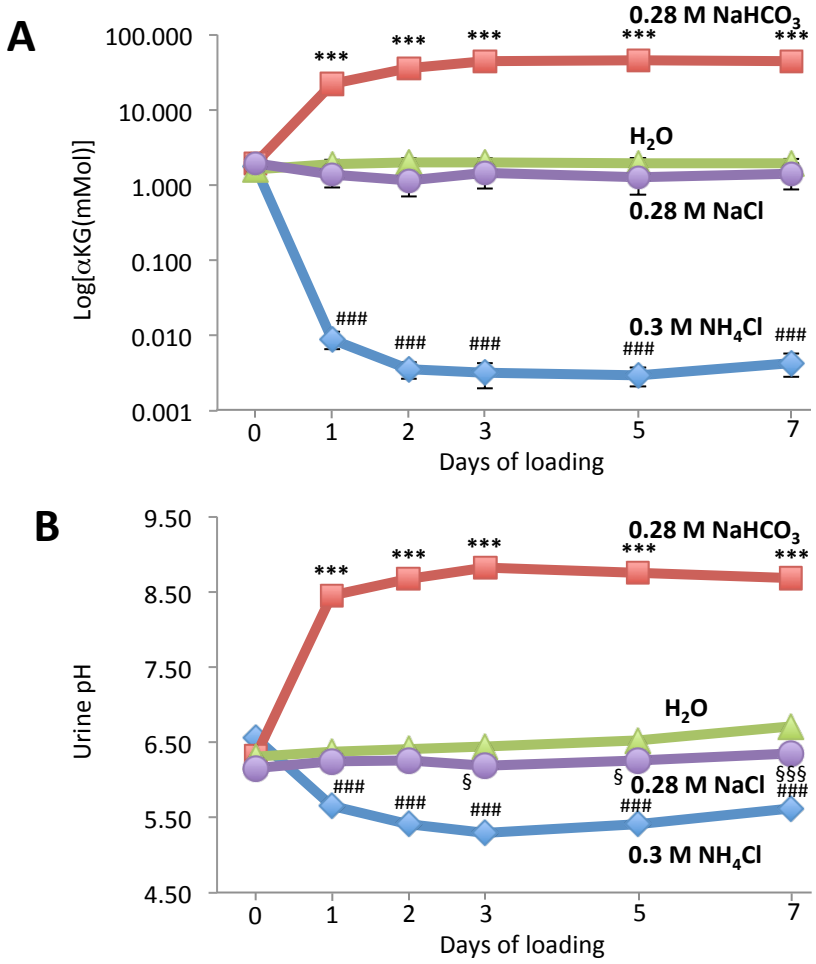
Supplementary Figure 1 (U-Z). Oxgr1 expression in the kidney. **U** and **X.** Oxgr1 (X-gal) staining (blue) in the renal cortex. **V**. Immunostaining of anion exchanger 1 (AE1, red) in the basolateral membrane of type-A intercalated cells. **W**. Co-staining of Oxgr1 and AE1 shows that Oxgr1 is expressed in the same tubular segments, but not in type-A intercalated cells. **Y**. Immunostaining of pendrin (red) in the apical membrane of type-B and non-A-non-B intercalated cells. **Z**. Co-staining of Oxgr1 with pendrin confirms that Oxgr1 is expressed in type-B and non-A-non-B intercalated cells. The Oxgr1(Xgal)/pendrin(Ab) co-staining also demonstrates that X-gal staining does not interfere with immunohistochemistry. Scale bars: 50 μm



Supplementary Figure 2. Representative traces of the changes in pHi elicited by luminal Cl^- removal and then re-addition, in the presence of extracellular HCO_3^- (25mM, pH 7.40) and in Na^+-free solutions. The traces have been recorded on 2 distinct tubules from $Oxgr1^{+/+}$ mice, in the absence (black trace) and in the presence (red trace) of 1 mM alpha-keto-glutarate in the lumen.

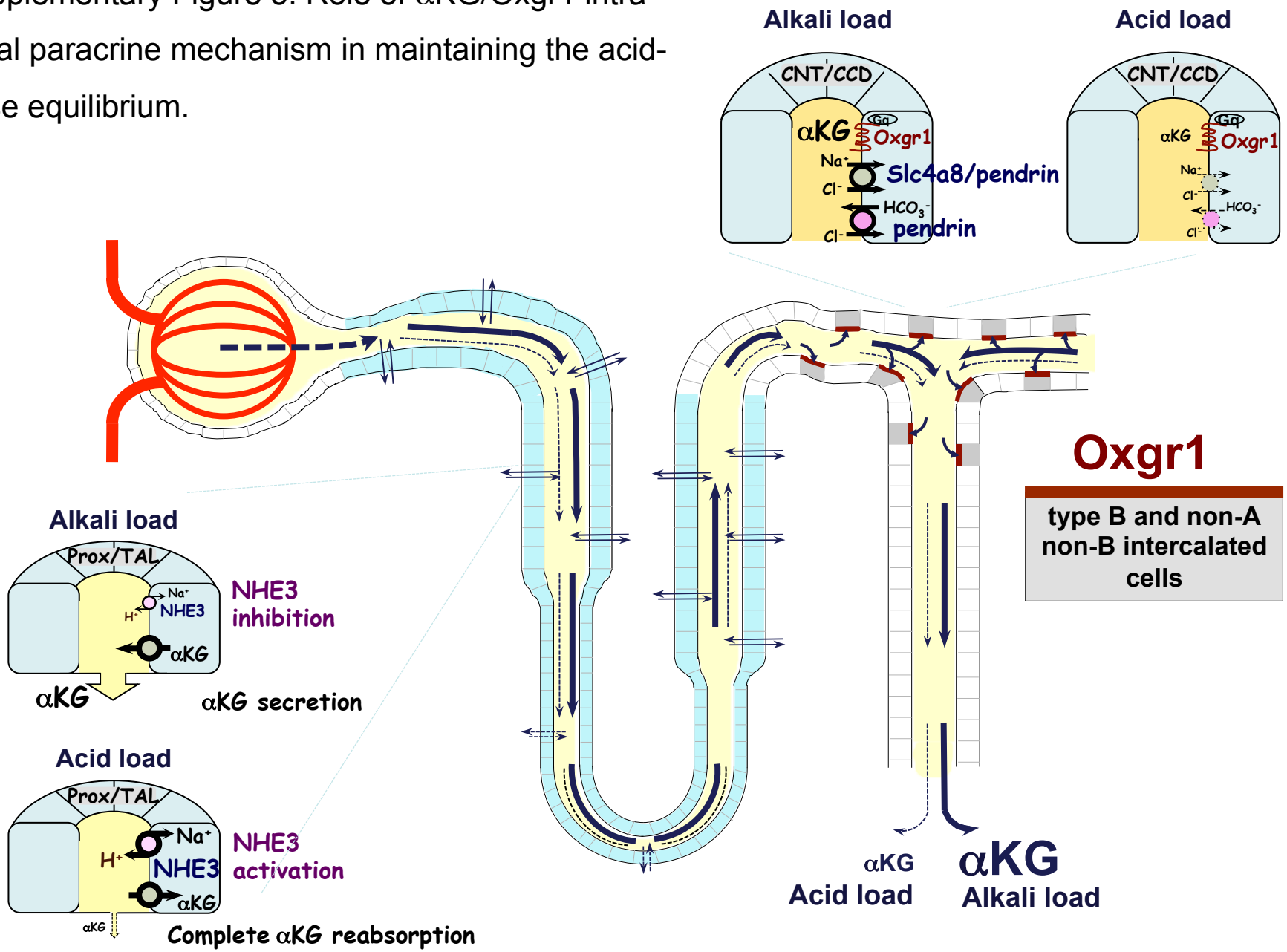


Supplementary Figure 3. Pendrin (**A**), αENaC (**B**) and βENaC (**C**) protein abundance in kidneys of wild-type and oxgr1**-/-** mice. Western blots were performed as described in Supplementary Methods. Acting was used as the loading control. (**D**) Densitometric quantitaion of Wester blot data presented in panels A, B and C. Values are means ± SEM. *** - significantly different compared to wild-type mice. Student's t-test.



Supplementary Figure 4. Effects of a dietary load of acid, base or NaCl on (**A**) urinary concentration of αKG, and (**B**) urine pH. These parameters were tested in urines collected from wild-type mice housed individually in metabolic cages. The 24-hour urine was collected from four groups of mice (6 mice/group): a control group receiving tap-water (green) and groups supplemented with acid (0.3 M NH_4Cl, blue), alkali (0.28 M $NaHCO_3$, red) or salt (0.28 M NaCl, purple) in drinking water. Values are means ± SEM. *, significantly different (*, $P<0.05$; **, $P<0.01$; ***, $P<0.005$) compared to 0.28 M NaCl group; #, significantly different (###, $P<0.005$) compared to H_2O group; §, significantly different (§, $P<0.05$; §§§, $P<0.005$) compared to H_2O group; Student's t-test.

Supplementary Figure 5. Role of αKG/Oxgr1 intra-renal paracrine mechanism in maintaining the acid-base equilibrium.



Supplementary Methods.

Identification of type B and non-A-non-B intercalated cells for pHi measurement.

Intercalated cells were identified by apical labeling with fluorescent peanut lectin (PNA, Vector Labs). Apical PNA labeling does not allow for discrimination between the different subtypes of intercalated cells. The type A cells were excluded from analysis on the basis of their bigger size and the lack of luminal Cl^--dependent change in intracellular pH. According to these criteria, approximately 55-65% of intercalated cells were classified as type A. Because BCECF was loaded from the peritubular fluid, the principal cells also displayed a significant fluorescence at the end of the BCECF exposure. They were excluded from the analysis on the basis of (i) the absence of PNA labeling and (ii) the lack of luminal Cl^--dependent change in intracellular pH. Use of fluorescein-coupled PNA allowed using the same set of filters for identification of cell types and pH measurements. However, PNA fluorescence did not interfere with BCECF fluorescence because it is restricted to the apical membrane and not the cytosol, and a much higher intensity of the excitation light was required to see the PNA fluorescence than the pH-sensitive BCECF fluorescence. Thus, after the cells labeled with PNA were identified, the illumination intensity was decreased and set to the BCECF requirements.

X-gal staining and Immunohistochemistry. Mice were anesthetized with ketamine/ xylazine and kidneys were perfused from the renal artery with 20 ml of 2% paraformaldehyde/PBS, then transferred to 30% sucrose/PBS and embedded to O.C.T. compound (Tissue-Tek). X-gal staining and immunostaining were performed on 8 μm-thick cryostat sections. For the X-gal staining, sections were incubated in the staining solution containing 5mM $K_3Fe(CN)_6$, 5mM $K_4Fe(CN)_6$, 2mM $MgCl_2$, 0.05% Tween-

20 in PBS followed by overnight incubation in the same staining solution to which the X-gal was added (1mg/ml, Invitrogen) (RT). Sections were washed with PBS, dehydrated and mounted in Roti-histokitt (Roth). For the immunostaining, sections were blocked with 10% normal donkey serum (Millipore), 0.05% Tween-20 in PBS, then incubated with primary antibody diluted in the blocking solution. After washing steps, sections were incubated with the secondary antibody: anti-rabbit alexa555 conjugated IgG or anti-chicken alexa488 conjugated IgG (Invitrogen) for 2 hours followed by the incubation with DAPI for nucleus staining. Immunostained sections were mounted in ProLong Gold antifade reagent (Invitrogen). For the double staining of X-gal and AE1, first, sections were stained with X-gal as described above. Then, the antigen-retrieval treatment was performed for AE1. Sections were microwaved for 15 minutes in the solution containing 10mM Na-citrate and 1mM EDTA and were cooled down at the room temperature. After washing steps, immunostaining was performed as described above. For double staining of X-gal and pendrin, the immunostaining (pendrin) was performed without antigen-retrieval. Images of X-gal staining, double immunofluorescence staining, and double staining of X-gal and immunofluorescence were obtained by stereomicroscope MZ16FA (Leica), confocal microscope SP5 (Leica) and Axovision (Zeiss) respectively. All images were processed with Adobe Photoshop (Adobe).

Western blotting Whole decapsulated kidneys (6 mice per genotype) were homogenized with a polytron in 3 ml RIPA buffer containing 50mM Tris-HCl (pH 7.2), 150mM NaCl, 0.1% SDS, 0.5% Na-deoxycholate, 1% NP40, 1mM PMSF and protease inhibitors. Protein extracts were sonicated and centrifuged for 5 minute at 1500rpm. The supernatant was recovered and the protein concentration was measured with Pierce BCA protein assay reagent (Thermo), then the concentration was adjusted

to 8mg/ml with RIPA buffer. Samples were mixed with Laemmli sample buffer (BIO-RAD) supplemented with 2-mercaptoethanol (SIGMA), and were heated for 15 minutes at 56 degrees C. 40μg of protein samples were migrated in the Mini-PROTEAN TGX gels, 4-20% (BIO-RAD), then transferred to the nitrocellulose membrane in the transfer buffer containing 20% Methanol. Membranes were stained with Ponseau S and then washed in TBS containing 0.2% NP40. Then, membranes were incubated with 5% skim milk in 0.2% NP40/TBS for 1 hour at the room temperature for the blocking, followed by the incubation with primary antibodies in the blocking solution, overnight (cold room). Membranes were washed and blocked as above, and incubated with anti-rabbit horseradish peroxidase conjugated IgG in 5% skim milk in 0.2% NP40/TBS for 1 hour at the room temperature. After the washing steps, SuperSignal west dura extended duration substrate (Thermo) was used and signals were visualized on Kodak Biomax XAR film (Kodak). Bands were digitalized by Epson Expression 1680 (EPSON) and were quantified with Quantity One 1-D Analysis software (BIO-RAD).